THE CHARACTERISATION OF HERITAGE VEGETABLES

by

JENNIFER PRESTON

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ABSTRACT

A collection of heritage variety accessions were characterised using Amplified Fragment length Polymorphisms (AFLPs) (200 accessions) and multivariate analysis of morphological characters (366 accessions); key features of interest for the conservation of Plant Genetic Resources were the identification of diversity within and between accessions. Motivations and practices of heritage variety growers were explored using questionnaires.

Heritage varieties are herein defined as traditional crop varieties that have a historical origin of over 40 years, are non-hybrid and non-GMO and are of cultural/heritage value to their users; they are part of the suite of plant genetic resources currently utilised by growers and of potential use to plant breeders in the future.

A large range of morphological and genetic diversity was present between accessions in all crops; in addition, diversity was found within accessions, particularly in *Vicia faba*, *Daucus carota* and *Cucumis sativus*. Comparisons between data sets were made for diversity, relationships, comparisons with commercial standards and identifying potential duplicates. The synthesis of both data sets highlighted the 13 potential duplicates for further investigation by HSL.

The findings highlight the importance of heritage varieties and the Heritage Seed Library, both culturally and in terms of conservation for present and future use.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
CBD	Convention on Biological Diversity
CGAIR	Consultative Group on International Agricultural research
CWR	Crop wild relative
DEFRA	Department for Environment, Food and Rural Affairs
DUS	Distinct, Uniform and Stable
EMR	Brogdale and East Malling Research
FAO	United Nations Food and Agriculture Organisation
FERA	The Food and Environment Research Agency
GO	Garden Organic
HSL	Heritage Seed Library
IPCC	Intergovernmental Panel on Climate Change
IPGRI	International Plant Genetic Resources Institute
JIC	John Innes Centre
NFC	National Fruit Collection
PGR	Plant Genetic Resources
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SASA	Science and Advice for Scottish Agriculture
SG	Seed Guardian
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
UNCED	United Nations Conference on Environment and Development
UNEP	United Nations Environment Program
WHRI	Warwick Horticultural Research International

CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

The characterisation of heritage vegetables in this project is an important aspect of the broader need to characterise as much of the potentially shrinking pool of plant genetic resources (PGR) as possible. This is in order to enable their conservation and maintenance, as well to gain information to facilitate their use in the present by growers and in the future, by those growers and potentially by breeders.

1.2 What are plant genetic resources?

Plant genetic resources comprise the variation in crop plant genetic material that is available for present and potential future utilisation (FAO, 1996). This variation includes diversity at the level of nucleotide sequences, alleles and genotypes, and is necessary for the development of new cultivated varieties as well as contributing to the resilience of current varieties (Hammer *et al*., 2003).

PGR include material that can be classified into seven groups (Hawkes *et al*., 2000): primitive forms of cultivated plants and land races, modern cultivars, obsolete cultivars, breeding lines and genetic stock, weed races, related wild species and other wild species (Hawkes *et al*., 2000). Of these, crop wild relatives, weedy races and land races have been less exploited in breeding programs (Maxted *et al*., 2008). The former of these, crop wild relatives, can be defined in the broad sense as closely related wild taxa, including progenitors of crop species and closely related species from the same genus as the domesticated crop species; CWR are of use in agriculture due to their close genetic relationship to crop species (Maxted *et al*., 2006;

Heywood *et al*., 2007). Landraces have been widely reviewed and defined, including Harlan (1975), Zeven (1998), Friis-Hansen and Sthapit (2000), Saxena and Singh (2006), Negri (2007), Camacho Villa *et al*. (2005), Tiranti and Negri (2007) and Berg (2009). In his paper on the threats to plant genetic resources, Harlan (1975) described crop evolution through history, particularly the close relationship between artificial and natural selection pressures, which resulted in "variable, adapted populations called landraces" (p618). He described the genetic variability of these populations that were adapted to pests and diseases and local climate, and highlighted the replacement of traditional crop populations with modern cultivars and the risk this posed to genetic variation. These defining features of genetic diversity and long cultivation history, which in turn confer adaptation to local conditions though natural and human selection, are common features in many of the definitions mentioned above. They are summarised in the definition of Camacho Villa *et al*. (2005), which states that land races are dynamic populations that have some or all of the characters of high genetic diversity, adaptation to local conditions, a long cultivation history, a lack of formal improvement and an association with traditional farming systems.

No formal definition of heritage varieties has been published; a review of the relevant literature and a putative definition will be offered as part of the current project, along with how they sit relative to land races.

1.3 Utilisation of PGR

PGR are an important source of genetic diversity and contribute towards food security. Plant breeding is based on the exploitation of genetic diversity within and between crop species and

varieties; genetic diversity is important for both the resilience of crop varieties and the creation of new varieties.

For the former point, the CBD (Secretariat of the CBD, 2001) states that high genetic variability increases the flexibility of species, whereas low variability increases the risk of extinction. In agriculture, this lack of variability can be due to a low number of varieties being grown, or a genetic uniformity within a variety (FAO, 1996). The impoverishment of particular crops and the effects of monoculture have been seen for example in the Irish potato famine of the 1840s, and southern corn leaf blight in *Zea mays* in the 1970s (Hammer and Teklu, 2008), where outbreaks of disease led to widespread crop failure.

For the latter point, with a loss of unique diversity and alleles, the ability of breeders to adapt and breed new varieties to combat pests, diseases and environmental stresses is reduced. The UK government commissioned Foresight report on The Future of Food and Farming (Foresight, 2011), and the Royal Society report, 'Reaping the Benefits' (Royal Society, 2009), highlight the importance of conservation of plant genetic resources, including landraces and crop wild relatives, and the development of new crop varieties, with a view to increasing the adaptability and resilience of the global food system. Food security is affected by many complex and interrelated factors of which crop improvement is a key part (Figure 1.1).

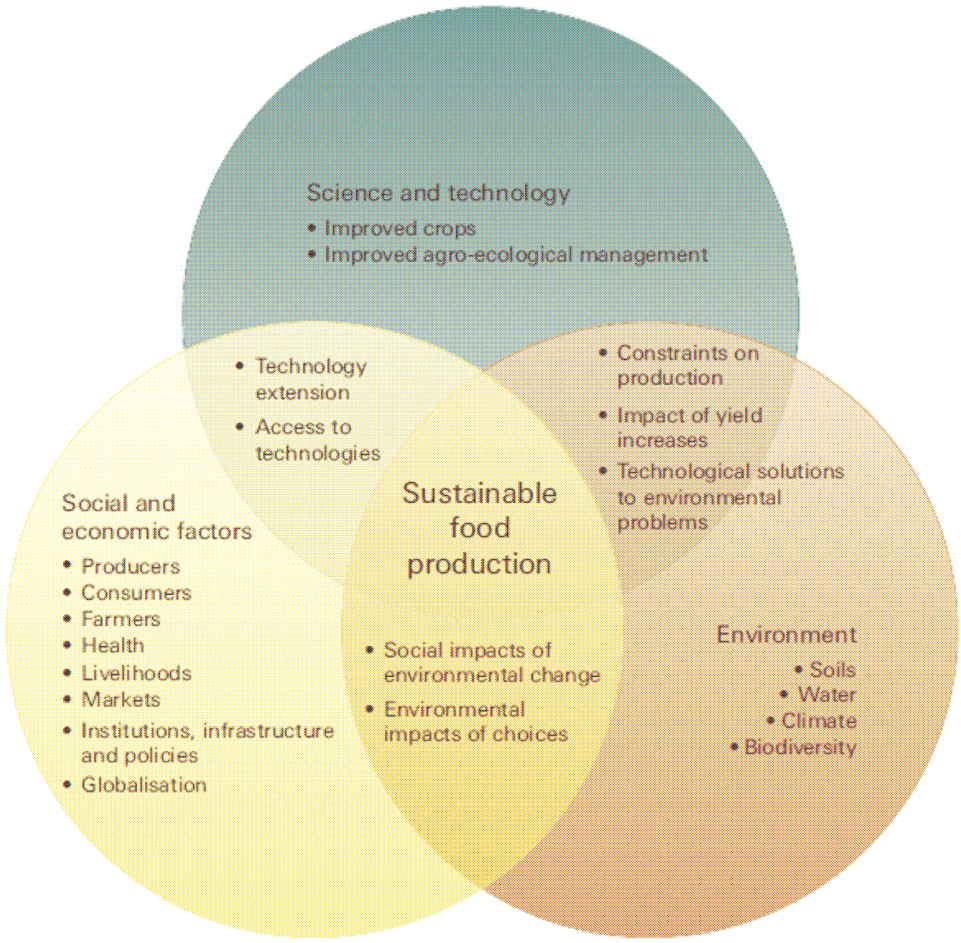

Figure 1.1 The complexity of agricultural systems. Graphic reproduced from the Royal Society report: Reaping the Benefits (page 5) (2009).

Not only do new varieties need to be bred for current problems, two major problems on the horizon are set to be a challenge for future crop development – namely the growing human population and climate change – both of which are aspects of food security.

The first challenge for food security is human population growth and the limitations of finite resources. Since the Green Revolution of the 1960s, world food production has increased by 138%, from 1.84 billion tonnes in 1961 to 4.38 billion tonnes in 2007 (Royal Society, 2009).

However, the human population is projected to increase to eight billion by 2030 and to around nine billion by 2050 (based on a model assuming 'medium' fertility levels) (United Nations, 2010). This increase, combined with the use of finite resources in agriculture such as fossil fuels, petrochemical based fertilizers, land area suitable for cultivation and water, mean that more food will need to be produced from a declining amount of land and resources. The nutritional value of food also needs to increase in many areas (Foresight, 2011); worldwide, an estimated 925 million people suffer from hunger, and an additional 1 billion may be lacking essential micronutrients such as vitamins and minerals (Foresight, 2011).

The second challenge is climate change. The earth's temperature increased by 0.74°C between 1906 and 2005 (Intergovernmental Panel on Climate Change (IPCC), 2007) and is projected to increase in the range of 1.8°C to 4°C by the end of the 21st century (IPCC, 2007). The projected impacts of climate change, some of which are already occurring, include increased temperatures, sea level rise, changes in precipitation patterns and higher frequency extreme weather events (IPCC, 2007). Crops will have to be bred that are adapted to deal with these conditions, as well as associated shifting climatic ranges and changes in seasonality. Crop performance will be affected by climate change directly, by water logging, drought, pest and disease range shifts, salinisation, soil erosion and physical damage due to changes in rainfall and extreme weather events (such as tropical cyclones) (IPCC, 2007; Foresight, 2011).

For continued crop improvement in the face of the above challenges there is a need for diverse plant genetic resources (Royal Society, 2009). CWR and landraces have been used as a source of material for crop improvement.

Examples of crop wild relative germplasm being used as a source of improvement include the use of *Beta* wild material (*Beta maritima*) to confer resistance to Rhizomania, Erwinia root rot and *Cercospora beticola* leaf spot resistance in sugar beet (*Beta vulgaris*) (Doney and Whitney, 1990); yield improvement using QTL loci taken from *Glycine soja* into *Glycine max* (soybean) (Concibido *et al*., 2003); and the creation of a line of *Hordeum vulgare* for use in elite barley breeding with improved performance on multiple agronomic traits using introgression from wild barley (*H. vulgare* ssp. *vulgare*) (Schmalenbach *et al*., 2009).

Examples of landrace materials being used in modern varieties include: resistance to angular leaf spot (*Phaeoisariopsis griseola*) and anthracnose (caused by *Colletotrichum lindemuthianum*) in *Phaseolus vulgaris* using landrace material from Mexico (Singh *et al*., 2003); the state of the world report (FAO, 1996) reviews the inclusion of landrace material including the use of Daruma/Norin 10 which was used as a donor of dwarfing genes in wheat and part of the 'Green Revolution'.

As well as being important for breeding, landraces and traditional varieties (including heritage varieties) are a valuable part of home gardening, low input and organic agriculture (Negri *et al*., 2000; Andreatta, 2000; Jordan, 2007; Bailey *et al*., 2009); the Slow Food Movement, started in Italy in 1986 by Carlo Petrini to campaign for a slower pace of life (Slow Food, 2012a) values landraces as part of its Ark of Taste initiative, which includes conserving traditional, local, vegetable species and varieties (Slow Food, 2012b); niche markets, such as those relating to traditional uses in a limited geographic range, such as the Italian *Phaseolus vulgaris* landrace 'a pisello' (Negri, 2003); and traditional farming, particularly in marginal

areas, and subsistence agriculture where they provide yield stability and food security (Brush *et al*., 1981; Cleveland *et al*., 1994).

1.4 Why are PGR at risk?

Diversity in PGR is at risk due to genetic erosion brought about by a number of largely interrelated factors. Genetic erosion can be the loss of genes, alleles or genotypes from crop species, or more broadly the loss of varieties (FAO, 1996). It may be brought about by the replacement of cultivation of a large number of genetically diverse landraces and traditional varieties, with a small number of genetically similar modern varieties (Tanksley and McCouch, 1997). Concerns regarding the genetic erosion in PGR have been increasing since the early part of the twentieth century; from Baur (1914, in Hammer and Teklu, 2008), through Harlan and Martini (1936, cited in Brush, 1999) and Frankel and Bennett (1970, cited in Brush, 1999) in the 1960s and 1970s when the transformative power of crop improvement was increasingly being demonstrated (Brush, 1999).

Large changes in agriculture - moving from 'traditional' to 'modern' techniques - are of key importance. Traditional methods of agriculture include: fewer inputs (such as fertilizers), repeated cycles of selection of diverse populations of land races and the use of suites of varieties with desirable features (particularly yield stability) (as described by Wright and Turner, 1999; Zeven, 2002; Camacho Villa *et al*., 2005). It can also include mass selection and the adaptation of varieties to local conditions over time, and classical selection and varietal crosses for desirable traits (Gepts, 2002; Moose and Mumm, 2008). This is being replaced with intensive agriculture and the purchase of seed each year from private seed companies. These are seeds of 'modern' varieties (also known as 'improved' or often 'high-

yielding' varieties) and are developed using techniques such as the systematic breeding of pure lines or F1 hybrids, or using molecular methods such as transgenic technology (Gepts, 2002; Moose and Mumm, 2008).

1.5 Evidence for genetic erosion

The picture concerning the genetic diversity of crop plants is complex and hindered by the lack of long time-series data (Brush, 1999); also, because limited initial baseline data are available for comparison, the amount of diversity already lost often cannot be measured directly (FAO, 1996). Methods employed to estimate genetic erosion include molecular genetic diversity studies, quantification of changes in the number of species or cultivated varieties grown over time, using a genetic assessment model or using a check list of risk factors (Guarino, 1999; de Oliveira and Martins, 2002; Hammer and Teklu, 2008).

The first FAO State of the World's Plant Genetic Resources for Food and Agriculture (FAO, 1996) states examples of changes in the number of cultivated varieties being grown. For example, there has been a reduction in wheat varieties used in production in China from 10,000 in 1949 to around 1000 in the 1970s; of these, in the 1950s, 81% of production used local varieties; by the 1970s this figure was 5%. The replacement of local varieties and landraces was reported for other crops including *Zea mays* and *Phaseolus vulgaris* in Costa Rica and *Triticum aestivum* in what was then Yugoslavia (FAO, 1996). This is also the approach of Hammer *et al*. (1996), who found substantial reduction in the number of landrace samples collected in Albania (72.4%) and southern Italy (72.8%), over a 30-50 year period.

The level of genetic diversity that remains in landraces can also be directly assessed. No significant change in genetic diversity levels present in *Oryza sativa* landraces collected for *ex situ* conservation in South and Southeast Asia, between 1962 and 1995, were found (Ford-Lloyd *et al*., 2008). The study posited possible reasons for this lack of change including the effects of historical collection area selection (with collectors only visiting high diversity areas), the interaction of collection size and the inbreeding nature of the crop (such that all alleles can be captured in a relatively small sample size), and that changes in the area of cultivation of each of the landraces were not included in the analysis.

A distinction may be made between the genetic bottleneck caused by the replacement of a large number of diverse landraces with modern varieties, and a general reduction in current genetic diversity due to modern breeding techniques (Koebner *et al*., 2003). The former bottleneck was investigated in northern European *Hordeum vulgare*, using SSRs (Russell *et al*., 2000). This study compared genetic diversity between landraces and modern cultivated varieties and found quantitative and qualitative shifts. A large proportion of the variation in alleles present within the dataset (72%) could be accounted for in the 19 ‘foundation genotypes’ (landraces and key progenitors of modern varieties) surveyed, and a lower level of genetic diversity in post-1985 cultivars than ‘foundation genotypes’. The bottleneck from landraces to cultivated varieties was also observed in the genetic diversity of *Pisum sativum* (Martin-Sanz *et al*., 2011).

Both trends were observed in the Reif *et al* (2005) study comparing *Triticum tauschii* accessions, with modern cultivars and landraces of *T. aestivum*, with a reduction in genetic diversity between domestication and landraces, between landraces and modern varieties and

over time in modern varieties. The most recent cultivars examined, however, increased in diversity due to introgression of exotic material including landraces and wild relatives to increase the environmental sustainability and resistance of wheat (Reif *et al*., 2005).

For the second trend of reduction, over time within varieties, a large number of studies have examined the genetic diversity of cultivars released in different time periods, using crops including *Triticum aestivum* (Donini *et al*., 2000 Srinivasan *et al*., 2003; Khlestkina *et al*., 2004; Fu and Somers, 2009), *Hordeum vulgare* (Koebner *et al*., 2003), *Zea mays* (Le Clerc *et al*., 2005a), *Pisum sativum* and *Z. mays* (Le Clerc *et al*., 2006) and multiple crops including *H. vulgare*, *T. aestivum*, *P. sativum*, *Oryza sativa* and *Avena sativa* (van de Wouw *et al*., 2010). The main trends in these studies have been: diversity was greater within decade groups than between, that genetic diversity between decades overlapped, and that that most recent varieties encompassed most of the diversity found in the earlier ones (new varieties were developed from previous ones). Generally, no overall narrowing was measured but a slight reduction in the 1970s was often observed, from which genetic diversity levels then recovered. The main finding was that significant changes relate to qualitative shifts in the alleles present. Van de Wouw *et al*. (2010), also highlighted the potential for regional and species differences, and the importance of being aware of conserving what diversity is extant now and looking for other novel material, which is vital for future breeding.

1.6 Causes of genetic erosion

Related changes in agriculture and breeding have led to concerns regarding the potential erosion of plant genetic resources.

As mentioned above, one potential source of genetic erosion is the replacement of cultivation of a large number of diverse landraces with that of, fewer, potentially less diverse, modern cultivars. These cultivars are different due to changes in crop improvement methods (Secretariat of the Convention on Biological Diversity, 2001; Rao and Hodgkin, 2002). New varieties and cultivars have been developed using pure inbred lines and F1 hybrids that have desirable characters for farmers, for example morphological uniformity, pest resistance or high yield (Secretariat of the CBD, 2001). By their nature, pure lines are genetically homogeneous, and saved seeds from F1 varieties are not true breeding. Regardless of whether this potential narrowing of the genetic base of crops has occurred yet, or has occurred previously and been recovered from (using introgression from diverse material), the conservation of a broad range of PGR in order to maintain genetic diversity for future use is necessary, to prevent or remedy future bottlenecks and challenges (van de Wouw *et al*., 2010).

Changes in agriculture including mechanisation, irrigation and use of fertilizers can also lead to loss of landraces through changes in variety choice (van de Wouw *et al*., 2009). Increased mechanisation and larger-scale farming mean that farmers favour varieties that are of uniform size that can be picked with machines and are robust enough to withstand this process; additionally, the system of food distribution is more centralised (with fewer and larger retailers) so food has to be able to travel further (Cebolla-Cornejo *et al*., 2007).

For heritage varieties, many of which are ex-commercial, changes in seed and variety legislation are also of relevance. In the UK, all major agricultural and vegetable crops grown were covered by The Seeds (National List of Varieties) Regulations, 1973; varieties were

placed on a national catalogue and in turn on to a European Common Catalogue. It was illegal to sell seed that was not on the National List. At its establishment, registration on the National Lists was free for older vegetable varieties, and many varieties of open-pollinated crops were added to a secondary, 'B list'. Before 1980 no DUS or maintainer fees were charged. Obsolete varieties and varieties that did not have a maintainer were conserved in WHRI or SASA's *ex situ* collections (SASA also acted as maintainer for varieties that were still being sold but which had no maintainer) (Niall Green, Personal communication).

The current legislation is the Seeds (National Lists of Varieties) Regulations 2001 and the Seeds (National List of Varieties) (amendment) Regulations 2011. To be accepted onto the National List a variety has to meet the 'DUS' criteria: it has to be distinct (in character from any other listed varieties), uniform (taking into account breeding system) and stable (remain true to its defined characteristics after successive multiplications or propagations). In the case of agricultural crops (not vegetables), a new variety has to offer improved cultivation characteristics (Food and Environmental Research Agency (FERA), 2010). The legislation was intended to standardise variety names and protect consumers and breeders; however, it has reduced the access of gardeners to older vegetable varieties because they are not on the list and therefore easily accessible (Negri *et al*., 2009), including heritage vegetables, although access can be available on request to the holder of the seed (such as SASA or previously WHRI), if a variety does not have a maintainer, or for sale if it does have a maintainer and there is sufficient demand. The number of varieties available in seed catalogues changes over time; as the 1973 legislation was introduced, seed lists underwent some rationalisation; a large number of variety names were removed from seed catalogues

after field trials because they were synonyms; further varieties were discovered to be homonyms, and so were registered as new varieties (Niall Green, Personal communication). In addition, varieties with no maintainer were not registered. Further changes occur as the number of companies and individuals maintaining seed reduces (due to consolidation of breeding companies), and with the replacement of varieties with new cultivars with improved characters.

Recent changes to EU legislation (Commission Directive 2008/62/EC and Commission Directive 2009/145/EC) have altered the legislation to allow derogations for 'Agricultural Conservation varieties', which are landraces and locally adapted varieties that are threatened by genetic erosion, and 'Vegetable Conservation Varieties' and 'Amateur Vegetable Varieties' which are varieties intended specifically for amateur gardeners and for sale in small seed packets. Both Directives allow reduced requirements for registration on a National List and for marketing of seed. Member States may adopt their own registration provisions. For example, for Conservation Varieties must be sourced from their region of origin in order to protect population diversity resulting from local cultivation and environmental factors from contamination; in the UK the region of origin may be as broad as the UK (Niall Green, Personal communication).

1.7 Conservation legislation and organisations

In response to the above challenges faced by Plant Genetic Resources, various conferences and legislative structures have been established (Negri *et al*., 2009). The Convention on Biological Diversity (CBD) (UNCED, 1992) was a response to the growing threat to all biological diversity, including plant genetic resources for food and agriculture. Continuing on

from the work of the CBD, the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGR) (FAO, 2001) came into force in 2004 and was in recognition of the specific threats posed to plant genetic resources by genetic erosion. Its objectives relate to plant genetic resources for food and agriculture (PGRFA) and include its survey, inventory and monitoring (FAO, 2001).

As well as legislation, several international bodies have been established to face the threat of genetic erosion in crop species. The Consultative Group for International Agricultural Research (CGIAR) was set up in 1971 by the World Bank in response to concerns about food supply in developing countries (www.cgiar.org). CGIAR scientists play an important role in the collection, characterisation and conservation of PGR; 11 of the CGIAR research centres are international gene banks, preserving and making available 650,000 samples of crops, forage and agroforestry PGR worldwide.

One of the CGIAR centres is Bioversity International (formerly the International Plant genetic Resources Institute (IPGRI)) (www.boversityinternational.org). Its current mandate is to promote the conservation and sustainable use of PGR for present and future generations through research and the provision of training and advice. Of particular relevance to the current study is the extensive role of Bioversity in the development of documentation standards for germplasm (Ford-Lloyd and Maxted, 1997); this includes the development of crop descriptor lists (used for many of the crops in the current study).

1.8 Conservation approaches

The threats to plant genetic resources mean that conservation steps are necessary; these can be *in situ* or *ex situ*, and these techniques should be utilised in a complementary manner (UNCED, 1992).

The CBD defines *in situ* conservation as: "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties" (UNCED, 1992, p147). *In situ* conservation can be further subdivided into genetic reserves (wild species) and on-farm conservation (crops) (Maxted *et al*., 1997a).

Advantages of the *in situ* approach include: continued interaction of plants with their natural environment, including exposure to local conditions and selection pressures, such as climatic changes and pathogens, and evolution (Maxted *et al*., 1997a); maintenance of wild relatives and crop weedy forms (Maxted *et al*., 1997a); and maintenance of species interactions, such as with animals for pollination and seed dispersal (Prance, 1997).

However, there are situations where *in situ* conservation is not possible or where support is required from *ex situ* methods. The CBD defines *ex situ* conservation as: "the conservation of components of biological diversity outside their natural habitats" (UNCED, 1992, p146). *Ex situ* conservation involves the removal of plant material, in the form of seeds or germplasm, from its original location to another place to conserve it; *ex situ* techniques include seed storage, DNA storage, *in vitro* storage, field gene banks and botanical gardens (Maxted *et al*.,

1997a). The relative advantages of each technique (as described by Hawkes *et al*., 2000) include ease of access to material for characterisation and evaluation (all methods), low cost (DNA and pollen storage), low maintenance (seed storage), feasible for medium-long term storage (seed storage and *in vitro* storage) and easy access for utilisation (seed storage, *in vitro* storage and field gene bank). A major challenge to *ex situ* conservation as a whole is the risk of suspension of evolutionary processes in stored samples, as they are no longer exposed to selection pressures (Hawkes *et al*., 2000). Other general disadvantages associated with the techniques include the risks of genetic diversity loss due to regeneration (seed storage), high technology and maintenance costs (*in vitro* storage), susceptibility to pests and disease (field gene bank) and requirement for large land areas (field gene banks and botanical gardens) (Hawkes *et al*., 2000). Characterisation of seed bank resources is a vital step in the utilisation of PGR (Tanksley and McCouch, 1997; Hawkes *et al*. 2000).

Of particular relevance to heritage varieties are home gardens, which involve conservation on a smaller-scale in the home, kitchen garden or back yard gardens (Maxted *et al*., 1997a). Home gardens are found in rural or urban settings, and are structurally complex and multi-functional spaces (Galluzzi *et al*., 2010). From a topological point of view home gardens are proximal to human dwellings and are delimited from surrounding areas by barriers such as hedges or fences (Galluzi *et al*., 2010), they may or may not be directly connected to larger agro-ecosystems, and their size can range hugely: from 186m² in the UK (Hessayon and Hessayon, 1973 cited in Smith *et al*., 2006) to 6000m² in Venezuela (Quiroz *et al*., 2002). The sizes are context-dependent relating to socio-economic and agro-ecological factors, as urban gardens tend to be more fragmented than gardens associated with farms (Gaston *et al*., 2005;

Galluzzi *et al*., 2010). Home gardens are important locations for genetic diversity in crop species; studies of home gardens as important reserves for biodiversity, landraces and traditional varieties (including heritage varieties) include: conservation of unique crop species diversity such as *Opuntia sp.* (cactus pears) in Mexican home gardens (Reyes-Aguero and Rivera, (2011), conservation of landraces in mountainous regions (Volg-Lukasser and Volg, 2004), urban home gardens in Brazil (Winkler-Prins and de Souza, 2005) and the Netherlands (van de Schans, 2010), tropical home gardens (Kumar and Nair, 2004) and European home gardens (Bailey *et al*., 2009). Home gardens have been found to be refuges for heritage and heirloom varieties (Eyzaguirre and Watson, 2002), as well as landraces (for example, *Vigna unguiculata* subsp. *unguiculata* (cowpea) in Umbria, Italy, (Negri and Polegri, 2009)), can contribute general ecosystem services, such as soil enrichment (Eyzaguirre and Watson, 2002), pollination and seed dispersal (Goddard *et al*., 2010), and fuel (Sileshi *et al*., 2007). As well as *in situ* conservation of PGR (where fruit and vegetable diversity has been seed-saved for generations (Maxted and Scholten, 2006)), home gardens have a vital role to play in *ex situ* conservation via seed saving networks. These are often implemented by non-governmental organisations (NGOs) that facilitate the conservation and use of varieties that may not be maintained within commercial seed trade by distributing seed from varieties, as mentioned above, that have been rationalised or removed from National Lists, and are therefore no longer available to growers, or those heirloom varieties that have never been available (Qualset *et al*., 1997; Hawkes *et al*., 2000; Sherman, 2009). Examples of seed saver schemes include the Garden Organic Heritage Seed Library (UK), Irish Seed Savers (Republic of Ireland), Dyfi Valley Seed Savers (Wales), Seed Savers Exchange (USA) and Arche Noah (Austria).

1.9 Plant genetic resources in Europe and the UK

The process of agricultural intensification and extent of use of modern varieties is at different stages around the world (Qualset *et al*., 1997). In Europe, it is arguable that the transition is almost total. Landraces are still grown in Europe, although their extent and situation is not yet fully understood (Negri, 2005). In additional to legislation restrictions, in Europe, the factors affecting the conservation of PGR include: decreasing and aging rural populations and a related risk of loss of skills and knowledge; problems transmitting knowledge between generations; and a reduction in seed saving (due both to the ease of purchasing seeds and as a result of limited space, equipment or knowledge for seed saving) (Negri, 2005; Vetelainen *et al*., 2009).

In the UK, landraces and traditional varieties (including heritage varieties and heirlooms) are maintained *in situ* by small seed companies, growers and gardeners, and to a lesser extent on-farm in marginal areas or for niche markets (Kell *et al*., 2009; DEFRA, 2010). As well as the reasons for PGR loss outlined above, Kell *et al*. (2009) highlight the problem of the increasing age of variety maintainers.

The United Kingdom Country Report on PGRFA (DEFRA, 2010) for the second State of the World report (FAO, 2010) states that *ex situ* conservation in the UK is undertaken by gene bank institutions, including the Vegetable Genetic Resources Unit at Warwick Crop Research Centre (formerly Warwick Horticultural Research Institute (WHRI)), Science and Advice for Scottish Agriculture (SASA) and the John Innes Centre (JIC); private organisations such as the Garden Organic Heritage Seed Library (HSL) and the National Council for the

Conservation of Plants and Gardens (NCCPG); and by field gene bank institutions, the National Fruit Collection (NFC) and Brogdale and East Malling Research (EMR).

Initiatives to increase the conservation of PGR in the UK include Seed Search schemes, such as those run by HSL and Dyfi Valley Seed Savers, to trace regional, heirloom and historic varieties (Garden Organic, 2011; Dyfi Valley Seed Savers, 2009), and the Scottish Landrace Protection Scheme (SLPS), which provides seed security for growers in the event of a poor harvest (Green *et al*., 2009).

Approximately 78% of wild taxa in the UK are classified as CWR, and the UK Inventory contains 1955 species (although not all are native) (Maxted *et al*., 2007). Potential threats to CWR in the UK include declining habitat and species richness, and agricultural intensification (DEFRA, 2010).

Although some varieties that could be classified as heritage varieties are also stored elsewhere (including SASA and JIC), they are not classified as such and their numbers are unknown; the HSL conserves and maintains 800 heritage variety accessions.

1.10 Garden Organic Heritage Seed Library

Garden Organic (formerly the Henry Doubleday Research Association, HDRA) is a British charity based at Ryton Gardens, near Coventry. It was founded by Lawrence Hills in 1954 as a membership organisation for experimenting gardeners, and became a charitable organisation in 1958. The Garden Organic Heritage Seed Library (HSL) was started in 1975 (Stickland, 1998), in response to the 1973 Seed (National List of varieties) Act. HSL were particularly concerned, firstly, with conservation of varieties removed from the National list that were

identified as synonyms, as HSL argued that this was not the case and maintained the varieties in order that they not be lost (Stickland, 1998). A smaller number were removed as they do not pass/have not been through DUS testing. Secondly, HSL were concerned to facilitate use of these varieties by gardeners by ensuring that they could get access to seed.

HSL currently holds approximately 800, mostly European, heritage variety accessions. Members can join HSL, paying a member fee, and receive up to six varieties of seed per year for free. HSL actively seek out UK heritage varieties to conserve and make available, which would otherwise not be readily accessible to growers (as they are not in seed catalogues). These are both varieties that have been removed from catalogues and those that have never been available, such as those heirlooms developed by gardeners and handed down through families.

HSL maintain seeds for each variety at Ryton; small numbers of seeds are grown up each year by HSL at Ryton and by Seed Guardians. Seed Guardians are volunteers who regenerate seed for distribution to members. Varieties are grown up in rotation to check and maintain seed viability and the interaction of the accession with the environment. Of the 800 accessions held, around 200 are available to HSL members; this number is limited due to insufficient seed and information regarding the accessions.

1.11 Rationale

The conservation of traditional, local, varieties of crops for current and future use is a significant challenge to UK plant genetic resources, to maintain the pool of diversity available to breeders and growers. Maintenance of the fullest possible range of diversity is essential,

bearing in mind the potential pressures of climate change and population growth and the requirement for novel genetic diversity to breed new cultivated varieties and to sustain grower choice. The genetic diversity of UK heritage vegetable varieties has not been assessed; the discussion of landraces and PGR highlights the problem that we do not know where to place heritage varieties in the scale from modern to landraces - are heritage varieties landraces? Where do heirlooms fit in? These questions may be tackled by a review of relevant literature.

As mentioned above, HSL currently conserves approximately 800 accessions of mainly vegetable crops with the aim of ensuring that these varieties remain available to members for cultivation. However, many of these accessions, originally donated by members, have not been fully described, some have little passport information, and others may be duplicates (entered into the collection under different synonyms); this hinders their management by HSL and their utilisation by HSL members, and limits their conservation value. To address this problem, 366 accessions from 11 crop species were morphologically characterised using standard crop descriptors (see appendix two) and 200 were further characterised using molecular techniques.

For these varieties to continue to be conserved in the future, HSL is dependent on growers for contributions, and on select members for regeneration of seed. If these relationships are to continue and strengthen, and to attract other participants, it is important to know what motivates people to become involved, and to place heritage varieties in a broader context of home gardening in the UK.

1.12 Project aims

The overall aims of the project were to facilitate the conservation and utilisation of UK heritage vegetable varieties by morphological and molecular characterisation of the Garden Organic Heritage Seed Library collection, to evaluate the importance of heritage varieties as a source of genetic and morphological diversity, and the importance of their conservation in the UK, particularly with reference to genetic erosion and the conservation of plant genetic resources. This was achieved by:

1. The proposal of a definition of a heritage variety
    a. Review of literature with reference to heritage varieties, heirloom varieties and landraces.
2. A genetic diversity study using molecular markers to answer the following questions:
    a. What genetic diversity is present within and between HSL accessions?
    b. Are there any groups of similar accessions?
    c. Are there any duplicate accessions?
    d. How does diversity in the HSL accessions compare with that in commercial varieties?
3. A morphological characterisation study to answer the following questions:
    a. What variation/diversity is present within and between accessions?

b. Are there groups of similar accessions within any of the crops?

c. Are there any duplicate accessions in the HSL collection?

d. How does the diversity of the HSL collections compare to those that are commercially available?

4. A survey of HSL growers and Seed Guardians to investigate:

    a. With regard to Seed Guardians:

        i. What are the motivations of people volunteering to become Seed Guardians?

        ii. How do they regenerate seed for HSL?

    b. With regard to members:

        i. What are the motivations of members for involvement in heritage vegetable growing?

        ii. How do heritage seeds fit into a larger picture of home vegetable gardening?

        iii. How can HSL encourage reporting of variety performance and explore possible regional differences?

        iv. To what uses are end produce (vegetables and seeds) put?

CHAPTER 2 A CLOSER LOOK AT HERITAGE VARIETY DEFINITION[1]

2.1 Introduction

The term heritage variety is part of the array of terminology used to refer to traditional crop varieties, which includes: landraces, primitive, folk, obsolete, farmer and heir-loom varieties (Camacho Villa *et al*., 2005). Although some terms are eponymous (such as farmer variety) or functional (such as obsolete variety) others are used without clear definition, and many are used inter-changeably, both in the formal literature (for example, Rodriguez-Burruezo *et al*., 2005, p. 453 refer to 'heirloom (traditional)' tomato varieties) and the less formal literature (for example, Thorness, 2009, The Royal Horticultural Society, 2010 and Fedor, 2010, each use the terms heritage and heirloom variety interchangeably).

Communication, conservation prioritisation and the search for 'useful' genetic information/diversity for breeding requires a clarification of the terminology applied to specific sets of plant genetic resources (PGR) with characteristics held in common, so time and money may be directed effectively (Hawkes *et al*., 2000). An artificial distinction where none exists in reality is not useful; however, if terms are not synonymous, characters identified under each term may affect potential use, for example, if the genetic profiles of the groups differ. These terms arguably refer to

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different sections of the suite of crop types that are each cultivated by humans, they have a distinct set of characteristics that define them, although some potential overlap is evident between certain terms.

In discussing the definition of a landrace, Zeven (1998), Camacho Villa *et al*. (2005), Tiranti and Negri (2007) and Berg (2009) highlighted the usefulness of term-clarification. The purpose of this chapter is to discuss and propose a definition of the term 'heritage variety' and its relationship to the term 'heirloom' with which it is sometimes considered a direct synonym. These two terms are used widely by charities and seed-saving organizations, such as Garden Organic (UK), Seed Savers Exchange (USA), Irish Seed Savers (Ireland), gardeners as recorded by Watson (1997) and Stickland (1998), and seed companies like Thompson and Morgan (2011) and Thomas Etty Esq. (no date).

It is proposed that when we refer to heritage varieties we are referring to a specific subset of traditional crop varieties that are identified by users via consistently applied characteristics, namely historical origin, open pollination, and cultural/heritage value. The heritage variety will be discussed with reference to: historical origin, mode of breeding, genetic diversity, local genetic adaptation, and association with traditional farming systems.

2.2 Definitions and Terminology

Of the terminology used in association with traditional crop varieties, the definition of a landrace is the most explored. Recent papers (including Zeven, 1998; Camacho Villa *et*

al., 2005; Tiranti and Negri, 2007; Berg, 2009) have proposed definitions of the term landrace, with the view to aiding conservation of landrace diversity. Camacho Villa *et al*. (2005, p. 381) proposed the following definition:

"A landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems"

This definition encompasses all of the traits included in alternative definitions, with the exception of the emphasis on cultural importance stressed by Tiranti and Negri (2007). Tiranti and Negri (2007) highlight the close association of landraces with the people who develop and grow them, and their role in traditions and culture. Camacho Villa *et al*. (2005) emphasize that the presence of all six (seven if local cultural importance is added) characteristics is not necessary to define a landrace, as the exact mix of characteristics will differ between crops and contexts.

Some of the terms are functional definitions, such as 'obsolete' variety, which refers to those varieties that are no longer commercially available and have been superseded by 'elite' varieties (Hawkes *et al*., 2000; Skovmand *et al*., 2001). Identity of breeder is often used in the nomenclature; for example, 'farmer's' variety (where the farmer may be breeding for his/her own personal use or for commercial purposes) (Zeven, 2000) or 'garden race' where the gardener is the putative breeder (Zeven, 1998, 2002). Perhaps the broadest term is that of 'traditional variety' itself, this being anything that is not a 'modern variety' and that is associated with traditional cultivation practice, seed management and breeding techniques (Rhoades and Nazarea, 1999; Camacho Villa *et*

al., 2005). A modern variety is then one that is genetically definable and results from commercial breeding strategies.

2.3 Elements that define heritage varieties

2.3.1 Mode of breeding

Heritage varieties are likely to be of non-homogeneous breeding origin and, as their custodianship has changed over time, the precise origin of many of these varieties has been lost. However, many heritage varieties are ex-commercial, for example those UK varieties not commercially traded following the implementation of the Seed (National List of Varieties) Act of 1973 (Stickland, 2008). They have been subject to definite human selection through directed seed-saving (from plants with desired characters) or crossing to select for specific phenotypic characters such as colour, size and shape (Science and Advice for Scottish Agriculture, no date). This selection for particular crop types distinguishes heritage varieties from other traditional crop varieties, where human selection is at a very low level (Zeven, 2000), using mass selection, or, more stringently, where selection is absent (Berg, 2009), with landraces being simply seed-saved each year, and new adapted genotypes mixed in. Berg (2009) uses degree of selection to distinguish between landraces and 'folk varieties'; the latter is subject to human selection, including for particular traits, resulting in a narrower definition of a landrace that would exclude many entities and varieties included in both the Camacho Villa *et al*. (2005) and Zeven (1998) definitions. Heritage varieties, as described here, would not be landraces according to the Berg (2009) definition; they would, however, be included in

the Camacho Villa *et al*. (2005) definition, as the latter states that not all characteristics in the definition have to be present in order to be recognized as a landrace

2.3.2 Historical origin

Historical origin encompasses both temporal and spatial aspects of landrace development (Camacho Villa *et al*., 2005). Stickland's (1998) research and variety summaries suggest that many heritage varieties were developed and popularised in the 1800s. The exact length of cultivation history required to classify a variety as heritage is not standardized; for example Thorness (2009) states that these varieties have been grown since before World War I; often authors speak generally about 'older' varieties. A commonly used length of cultivation period is a minimum of 40–50 years (Stickland, 2008; Thompson and Morgan, 2011). This is in contrast to the length of cultivation period of other traditional crop varieties, which is relatively long; those have been grown 'since time immemorial' or 'for many centuries' (von Runker, 1908, and Cholton, personal communication, both in Camacho Villa *et al*., 2005, p. 375).

The spatial aspect of historical origin relates to the cultivation of that landrace in a specific geographic location. Heritage varieties are often developed in one particular location and then distributed elsewhere: if developed by a breeder through an associated seed company (Stickland, 1998), by a farmer through family or other local farmers (Zeven, 1998, 1999), or by a home gardener or allotment holder to family and friends (Stickland, 1998). In the case of seed from companies, the seed origin would be the area

the company is located in, rather than the location at which the varieties are actually grown by customers (Kell *et al*., 2009).

2.3.3 Open pollination

Open pollination is proposed here to be one of the three main characters of heritage varieties, as identified by users (Stickland, 1998; Thorness, 2009; Dyfi Valley Seed Savers, 2010; Garden Organic, 2010; Irish Seed Savers, 2011). Open-pollination in this context includes out-breeding (cross pollinating) crops as well as inbreeding crops which may have historical origin and be maintained by mass selection, and excludes modern varieties bred as F1 hybrids, complex hybrids or by single seed descent. In common with most traditional crop varieties, heritage varieties are open-pollinated, meaning that they are not hybrids and breed true, except where gene flow has unintentionally occurred from another variety, and thus can be seed-saved. Although this is the same as for other traditional crop varieties, it is a key feature identified by users to distinguish heritage varieties from modern varieties, and it is important because it provides further distinction between modern and more traditional breeding techniques. Some heritage varieties may originally have been early hybrids but have since been stabilized and continue as open-pollinated varieties (Watson, 1997).

2.3.4 Level of genetic diversity

There are concerns regarding the loss of plant genetic resource (PGR) diversity due to: replacement of traditional crop varieties with modern cultivars (Hawkes *et al*., 2000; Negri *et al*., 2009); a reduction in the number of varieties relied upon for food; along

with legislation prohibiting the sale of unlisted varieties, which has resulted in a reduction in the availability of some varieties, particularly heritage varieties (Stickland, 2008). However, meta-analyses suggest that genetic diversity rates in crop cultivars have recovered since a decrease in the 1960s and 1970s, and overall no reduction in regional genetic diversity has been found (van de Wouw *et al*., 2010). Yet the importance of traditional crop varieties, including heritage varieties, as potential sources of genetic diversity and rare alleles for future breeding must be recognized. Previous studies have found traditional crop varieties to contain high levels of genetic diversity, such as in *Phaseolus vulgaris* (Tiranti and Negri, 2007), *Phaseolus coccineus* (Sicard *et al*., 2005), *Solanum lycopersicum* (Terzopoulos and Bebeli, 2010) and *Daucus carota* (Shim and Jorgensen, 2000).

Genetic diversity is proposed as one of the characters that can be used to distinguish heritage varieties from other traditional crop varieties, finding heritage varieties on the spectrum in between landraces and modern varieties. Landraces can have the appearance of highly variable populations, such that they may not be strictly referred to as 'cultivars' (Zeven, 1998; Camacho Villa *et al*., 2005); the application of some breeding, particularly for selection of desired characters (Astley and Munro, personal communication, in Camacho Villa *et al*., 2005, p. 376) in heritage varieties, means that heritage varieties may not demonstrate this attribute. The genetic diversity of heritage varieties can be problematical to unearth in the literature, due to the past uses of the term or lack thereof. Varieties, fitting the heritage variety definition proposed here, have been investigated, often as ex-commercial varieties or by date of cultivation. For

example, Shim and Jorgensen (2000, p. 228) compared 'old' varieties of *D. carota* (carrot) to wild and modern varieties. These were open-pollinated varieties released between 1976 and 1978 and were found to have relatively high within-population genetic diversity compared with recent cultivars, which could be attributed to breeding history. Archak *et al*. (2002, p. 1140) referred to 'old local cultivars' of *S. lycopersicum* (tomato) from India, which were found to be more genetically diverse than varieties released since the 1990s, due to breeding for uniformity of specific plant and fruit types. Although few studies were found that specifically investigated heritage variety genetic diversity, there are accounts of heritage varieties being used as the basis of improved varieties, such as *Phaseolus coccineus* (runner bean) variety Prizewinner, introduced by Suttons of Reading in 1892; it has since been improved for disease resistance and released as the modern variety Enorma (Stocks, 2008).

2.3.5 Local genetic adaptation

Although local adaptation is not proposed as a defining character of heritage varieties, some users do highlight as an important feature that seed be adapted to local climatic or edaphic conditions (Dyfi Valley Seed Savers, 2010; Irish Seed Savers, 2011). Since the adoption of National Lists, many heritage varieties, previously supplied by 'local' seed companies with their own selection criteria, are now seed-saved by individuals and seed-saving organizations.

Local genetic adaptation arises as a result of repeated cycles of planting, harvesting and selection over extended periods of time, particularly in marginal environments

(Camacho Villa *et al*., 2005). Local adaptation is cited as a character of some heritage varieties (Stickland, 1998; Dyfi Valley Seed Savers, 2010; Irish Seed Savers, 2011); due to the necessity of extensive field trials to determine the evaluative characters of crops, much of the evidence for adaptation is anecdotal.

Franks *et al*. (2007) found that genetic diversity allows crop adaptation to environmental change to occur in very few generations. This suggests that the length of cultivation proposed here for heritage varieties (40–50 years) is sufficient time for varieties to be under selection pressure and adapt if grown in a particular location. However, quantifying these changes is problematical: details of seed sources can be lost and conserved seed samples small (and thus vulnerable to genetic bottlenecks and founder effects (Prada, 2009), so masking adaptation); long-term seed storage in *ex situ* collections can lead to genetic drift (Hawkes *et al*., 2000; Prada, 2009); and evaluation trials in different locations over time would be necessary to explore this further, but it would be a valid avenue to explore (Prada, 2009).

2.3.6 Association with traditional grower/gardener systems

Heritage varieties are identified by users of the term as being of heritage or cultural value (Stickland, 1998; Irish Seed Savers, 2011). Similarly, the association between people and landraces can be related to the use of the variety in specific personal traditions and habits, or preference for characters not found in modern varieties and hence for the landrace itself, rather than with the farming system (Camacho Villa *et al*.,

2005). The importance of this tight intertwining of biological and cultural heritage is strongly argued by Negri (2005).

Heritage varieties provide important links with the past (Stickland, 1998) such as local customs/festivals and family recipes, and can be connected specifically with places or names. For example Brighstone bean, is a variety of *P. vulgaris* (french bean) grown by gardeners on the Isle of Wight, which has its own local story of origin as it is said to have washed ashore from a shipwreck in the late 1800s (Stickland, 2008).

Heritage value can be associated with personal or common good value. For example, for varieties gardeners have grown in the past or for particular traits they value, a variety has personal value. The most prominent of these traits is taste preference of heritage over modern varieties (Russo, 2008; Kell *et al*., 2009), but a wide range of other characters such as unusual colours/shapes and diversity of maturation time, to avoid gluts, are also valued (Kell *et al*., 2009). While for the common good value, many growers find the concept of conserving heritage for historical/ cultural value to be of importance (Negri, 2003; Kell *et al*., 2009) and so grow with the aim of being directly involved in the conservation of these varieties. People often start growing heritage varieties for personal reasons, then become interested in the biological diversity conservation aspects (Jordan, 2007).

2.4 Definition

The discussion of characteristics associated with the term heritage variety, in the context of traditional crop varieties, has confirmed the importance of three key traits most often

identified by users with heritage varieties: open pollination, cultivation history of 40–50 years or more, and the heritage and cultural value of the varieties to growers. The discussion also highlighted that some characteristics identified in landrace definitions may be absent or not yet adequately assessed in heritage varieties (degree of formal improvement, level of genetic diversity and local adaptation). It can be argued therefore that heritage varieties are a subset of traditional crop varieties that can be consistently identified with the proposed definition:

'A traditional crop variety that has historical origin of over 40 years, is open-pollinated and is of cultural/heritage value to its users.'

2.5 The Case of Heirloom Varieties

This chapter has so far focused on the term heritage variety; however, the terms heritage variety and heirloom variety are often used interchangeably. The term heirloom is particularly used in the USA, and it is for this reason it has been omitted from discussion thus far in this chapter, as definitions in the Europe and USA appear to differ. Many sources use the term heirloom to describe varieties that would fit the above definition of heritage variety; for example, Taylor's Guide to Heirloom Vegetables (Watson, 1996) defines heirloom using very similar characteristics to those identified above for a heritage variety (open-pollinated, cultivated for over 50 years, with a history of its own). Some sources offer no description, such as Gonclaves *et al*. (2008, p. 1289) who refer to 'traditional (heirloom) seeds'. There is certainly considerable overlap between the two terms: both refer to open-pollinated varieties, derived from moderate levels of classical breeding (not modern-bred or genetically engineered), and are of

significant cultural importance, these characters being highly valued by users. However, it could be argued that heirlooms have the additional character of never having been available in seed catalogues, as they are closely tied to family members or close family associates, being bred by gardeners, and are exchanged along these lines, outside of the commercial seed trade (Watson, 1997; DeMuth, 1998). These heirlooms have a strong identity often linked with the breeder (or selector) by a name or the locality of development.

Both Watson (1996) and DeMuth (1998) recognize the dilemma of inclusion of commercial (or ex-commercial) varieties within the definition of an heirloom and recommend using the wider definition for general use (tallying with the one proposed for heritage varieties, above) as it is more inclusive, with 'true' heirlooms being those that have not been sold and are handed down in families or communities. Watson (1996) opts for the broader definition (tallying with that of heritage variety) as he argues to do otherwise ignores the valuable contributions of professional breeders and explorers; DeMuth (1998) argues that since many varieties are poorly documented and changes arise in the plants over time, the origin of varieties can be impossible to determine.

This suggests that heritage variety and heirloom are used widely as direct synonyms; however, it can be useful to distinguish between the two as their genetic profiles may differ. The genetic character of 'true' heirloom varieties is unknown and may be different to that of heritage varieties. The original source of seed for heirlooms is usually unknown; many will originally have been commercial varieties seed-saved and

possibly selected from by gardeners. This could potentially represent a significant bottleneck. Others may have been developed from landraces and undergone selection for specific characters. With time and genetic diversity (and restrictions in reproductive biology (Zeven, 1998)), both heirlooms and heritage varieties that are not maintained or selected can lose their improvement (reflected in changing allele and genotype frequencies), through forces such as outcrossing, mutation and natural selection (Parlevliet, 2007), potentially becoming secondary landraces (also known as creole varieties) (Mayr, 1937 in Zeven, 1998).

A proposed definition of an heirloom variety therefore, is simply an extension of the heritage variety definition:

'A traditional crop variety that has historical origin of over 40 years, is open pollinated, is of cultural/heritage value to its users, that has been developed, maintained and transferred through families and communities rather than commercial seed trade.'

2.6 Discussion

We have proposed that heritage varieties are part of a suite of important Plant Genetic Resources and constitute a subset of traditional crop varieties that at least partially overlap with the broad definition of heirlooms. Kell *et al*. (2009) state when reporting their UK landrace survey that it is prudent to use the widest definition of a landrace to encompass as much diversity for conservation as possible; therefore even though heritage varieties may be less heterogeneous than other traditional crop varieties and have some formal improvement, they are still an important constituent of traditional

crop diversity. Conservation of both heritage varieties and heirlooms is important for cultural reasons (such as growers' choice and conservation as cultural artefacts) and, in the face of potential genetic erosion, as a source of novel genetic material for breeders to use.

The application of the traditional crop variety terminology matters to users of the seed (conservationists, growers, breeders) and may have legislative implications in the future (such as with reference to European seed legislation). Therefore, we suggest a classification of crop varieties based on terminology usage. It attempts to distinguish between, and indicates the relationship between, traditional and modern crop varieties (including obsolete crop varieties and current crop varieties), and within traditional crop varieties between commercial/farm varieties (including landrace, heritage and farmer's varieties) and non-commercial/garden (including heirloom and garden varieties) (see Figure 2.1). The classification is proposed as an aid to clearer terminology use and it is suggested that clearer usage of agreed terminology might help promote conservation of traditional crop varieties themselves. However, as implied in the title of this chapter, if the Shakespearian quotation is continued, 'What's in a name? That which we call a rose by any other name would smell as sweet', definitions of heritage varieties and heirlooms are merely a tool to assist distinction, and counter examples of usage are likely to exist. But it is hoped that by agreeing a more concrete definition of terminology it will be easier to plan strategically and implement necessary traditional crop variety conservation actions before diversity is lost and definitions themselves become superfluous.

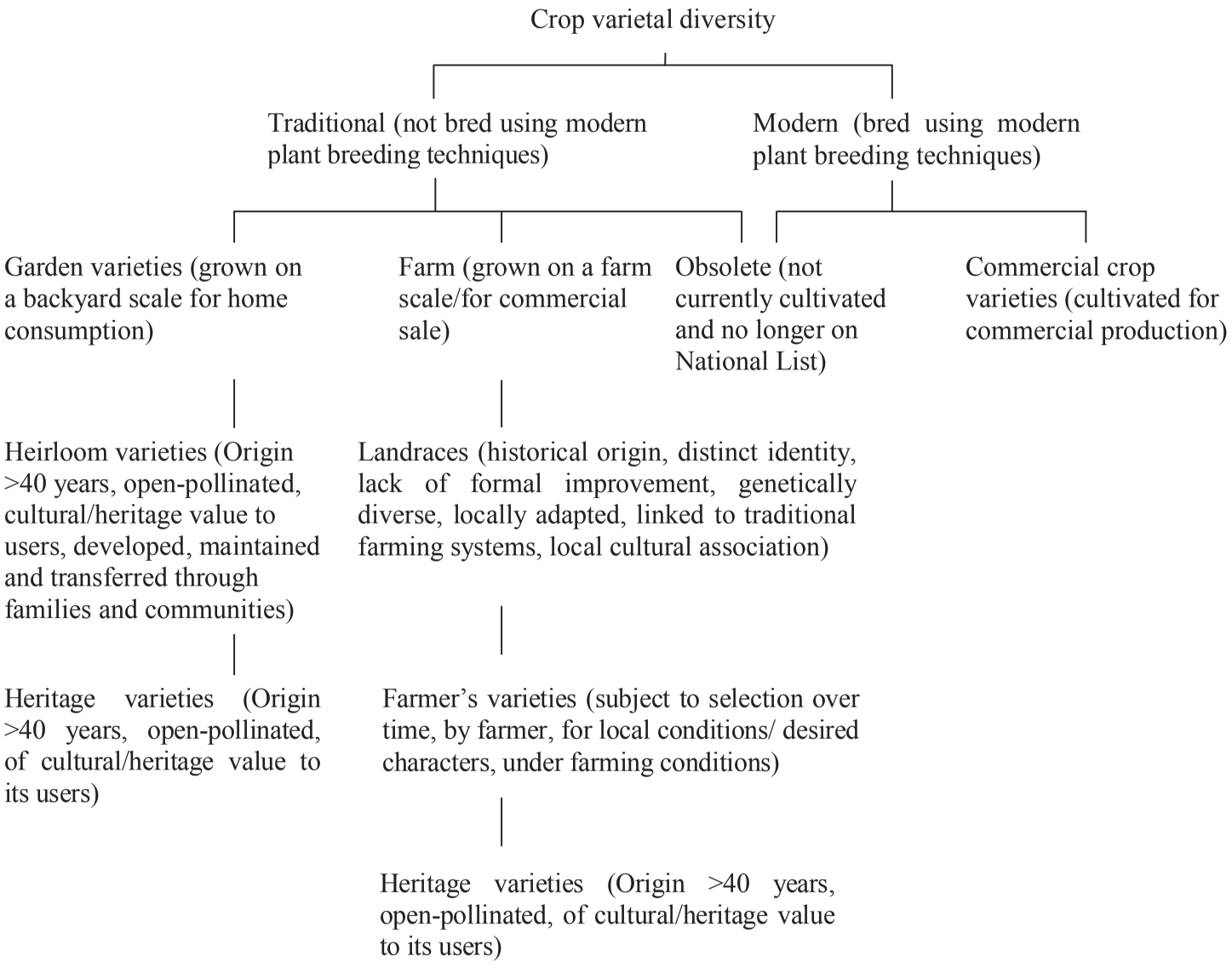

Figure 2.1 Proposed artificial classification of traditional crop variety terminology.

CHAPTER 3 GENETIC CHARACTERISATION OF HERITAGE VARIETIES USING AMPLIFIED FRAGMENT LENGTH POLYMORPHSMS (AFLPs)

3.1 Introduction

3.1.1 Genetic diversity

Genetic diversity comprises the total genetic variation present in a population or species; it is the differences within and between species or varieties in genes, alleles and genotypes, caused by mutation and recombination. Genetic diversity is the basis of selection in crop plants; it is vital for the development of new varieties by using novel combinations and traits. In the field, genetic diversity among and between individuals and varieties is vital for resistance to pests and diseases, as well as tolerance and adaptation to climatic conditions and changing climate.

Maintenance of the range and magnitude of genetic diversity present within a taxon is a primary aim of plant conservation (Newbury and Ford-Lloyd, 1997). To facilitate the conservation of genetic variation for present and future use, and to establish a baseline, the diversity of plant genetic resources, such as those held at HSL, needs to be characterised.

3.1.2 Characterisation of genetic diversity

Genetic diversity has been explored using, predominantly, three marker types: morphology, proteins and DNA-based methods.

Before the advent of modern genetic technology, morphological markers were the classical method for characterisation or estimation of genetic diversity. These comprised a diversity of

traits and measurements that were recorded at all stages of plant development. Using morphological markers has many advantages and morphological studies are often the first step in species studies and plant genetic resource activities serving to inform molecular studies as to where diversity may exist (Karp *et al*. 1997). Advantages include the low cost and level of technology required, and the relation of markers to traits of agronomic importance (Newbury and Ford-Lloyd, 1997). However, there remain limits to the usefulness or applicability of morphological markers, many of which are met by molecular methods. These include: the limited number of informative characters available, some of which may show little variation over material; the quantitative nature of their inheritance (being jointly influenced by genetics and environmental conditions of growth, because of this, some traits cannot be reliably isolated); related to this is the effect of environment that may mask the genetic co-ordinate, and therefore influence genetic diversity estimates based on morphology (Spooner *et al*., 2005).

3.1.3 Molecular markers

Molecular markers can be subdivided into protein-based and DNA-based methods. Protein-based methods pre-date DNA-based methods, and the most widespread method used banding patterns on non-denaturing gels to distinguish differing allele products (isozymes and allozymes) between specimens. Since these markers are based on gene products, they suffer from similar limitations to morphological markers, namely a limitation on the number of informative markers (proteins) for use, and differential expression due to plant developmental stage or growth conditions (Newbury and Ford-Lloyd, 1997; Smykal *et al*., 2008). DNA-based techniques identify polymorphisms at DNA sequence level and so are both independent

of environmental influence and show high levels of variation, and therefore potentially a large number of markers dispersed across the genome.

DNA-based markers can be obtained using a variety of methods, including arbitrary sequence techniques that utilize the Polymerase Chain Reaction (PCR) (including Random Amplified Polymorphic DNA (RAPDs) and Amplified Fragment Length Polymorphisms (AFLPs)), those that use hybridisation (Restriction Fragment Length Polymorphisms (RFLPs)), and site targeted PCR (including microsatellites (SSRs).

Random Amplified Polymorphic DNA (RAPD) are dominant markers which generate a high number of informative markers using the PCR (Williams *et al*., 1990); they do not require *a priori* sequence knowledge, however, the replicability of this technique is variable to sensitivity to reaction conditions (Vos *et al*, 1995; Mueller and Wolfenbarger, 1999). Restriction Fragment length polymorphism (RFLP) are co-dominant markers that use Southern hybridisation and probes to obtain high resolution data (Botstein *et al*., 1980), however, RFLPs are expensive, require a high level of expertise, require prior knowledge of sequences to be cloned and result in a comparatively fewer number of informative markers (Newbury and Ford-Lloyd, 1993; Mueller and Wolfenbarger, 1999). Simple Sequence Repeats (SSRs or microsatellites) are tandem repeat sequences of DNA, usually of between two and six nucleotides in length, which show high levels of polymorphism (repeat number) between individuals, are co-dominant and highly reproducible (Mueller and Wolfenbarger, 1999; Ellis and Burke, 2007). SSRs require a long run-up time and *a priori* knowledge of sequences for primer design (Newbury and Ford-Lloyd, 1997). AFLPs are dominant markers, with a high number of informative markers derived from the selective PCR amplification of

restriction fragments, visualised using gel electrophoresis or capillary electrophoresis (Vos *et al*., 1995). AFLP uses arbitrary primers therefore no *a priori* sequence knowledge is required (Vos *et al*., 1995; Mueller and Wolfenbarger, 1999).

3.1.4 Amplified Fragment Length Polymorphisms

The present study employed AFLPs, for a number of reasons. Firstly, the project required estimations of genetic diversity and relationships between a relatively large number of accessions (200), for seven different crops, but not necessarily with high numbers of accessions in individual crops. Secondly, AFLPs generate a large number of markers per experiment facilitating the high resolution required for distinguishing between closely related taxa such as crop varieties and allows a broad sweep of a large number of accessions to look for general patterns of diversity and relationships. Thirdly, AFLPs are cost-effective in both time and resources; this is partly because it is not necessary to design primers beforehand (Spooner *et al*., 2005), compared to microsatellites and SNPs (Bensch and Akesson, 2005), and can be used in any species and any genome size (Spooner *et al*., 2005; Semagn *et al*., 2006). Fourthly, relatively small amounts of DNA are required compared to RFLPs (Spooner *et al*., 2005; Semagn *et al*., 2006).

Brief AFLP methodology

Vos *et al.* (1995) describe AFLP as a DNA fingerprinting technique; as stated above it is a PCR-based molecular marker. DNA is digested by two restriction enzymes (a rare cutter and a frequent cutter; *Mse*I and *Eco*RI respectively); double stranded AFLP adapters are ligated to these. The end of each adapted fragment now consists of an adapter sequence and the

remaining part of the restriction sequence, which then serves as a priming site, and then the fragments are amplified using PCR. The amplified products are then run through gel electrophoresis (the fragments are visible as bands on the gel) or using capillary electrophoresis and separate out according to their length (Vos *et al.*, 1995). Fragment lengths differ when DNA sequences differ; this can be due to mutation (insertion or deletion of nucleotide bases) or recombination. AFLPs are a dominant marker as fragments are scored as present or absent; heterozygotes appear as present and cannot normally be distinguished from homozygotes.

3.1.5 Characterisation of plant genetic resources

The level of genetic diversity present in populations is influenced by many factors including life form, breeding system, seed dispersal and geographic range. The overall result of this is a higher level of genetic diversity within populations and lower level of differentiation between populations in outbreeding species, and a lower level of genetic diversity within populations and higher between population differentiation in inbreeding species (Hamrick and Godt, 1996). Genetic patterns of diversity may be variable across time, such as in crop species due to changes in agriculture (see chapter 1), and such as in *ex situ* collections (due to genetic drift, cross pollination and selective effects during regeneration, which can result in changes in genetic diversity and allele frequencies (van Hintum *et al*., 2007; Negri and Tiranti, 2009; Cieslarova *et al*., 2011).

3.1.6 Current project background, aims and rationale

There is a need for the characterisation of plant genetic resources using molecular methods to enable a baseline of genetic diversity for resources to be established, to allow seed bank managers to know where to focus their attention and look for accessions of interest for future use. Additionally, the genetic diversity of heritage varieties (compared to both landraces and commercial varieties) is untested. The present study is concerned with the Heritage Seed Library (HSL) at Garden Organic, which has never been characterised using genetic techniques; thus the level of genetic diversity, both within and between accessions, is unknown. The aim of the present study is to generate a picture of the current level of diversity held within the collection, using a sample of 200 accessions. AFLPs have been chosen, in order to allow a broad view of many crops and accessions, due to the large number of informative markers the method generates.

The main research questions to be answered are: what genetic diversity is there within the HSL collection, what diversity exists within and between HSL accessions, how does diversity in the HSL accessions compare with that in commercial varieties, are there any groups of similar accessions, and are there any duplicate accessions?

3.2 Materials and methods

3.2.1 Crop selection

The HSL collection holds approximately 800 accessions; funding was available to characterise 200 of these using AFLPs. The numbers of accessions of each crop are not

distributed evenly across the collection. For example, tomatoes and french beans constitute 197 and 177 accessions, respectively; each would take most of the allocated resources, whereas a small number of different crop studies would be more informative about the diversity present within the collection as a whole. Other crops in the collection are only present in very small numbers so as to make statistical implications unreliable. Therefore, broad bean *Vicia faba* (broad bean), Phas*eolus coccineus* (runner bean), *Pisum sativum* (pea), *Daucus carota* (carrot), *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce) and *Brassica oleracea* var. *acephala* (kale) were chosen, having 33, 26, 77, 12, 13, 22 and 14 accessions respectively; this included two commercial varieties grown for each crop for comparison. Brief crop backgrounds are given in Appendix three.

Five samples for each accession were analysed, resulting in 980 individual samples in total, with replicate individuals totalling 10% for each crop also processed to allow calculation of error rate. The number of replicates needed was determined following the methodology in Bonin *et al*. (2007), which states that a minimum of 5-10% of samples should be replicated.

3.2.2 Plant cultivation

Material was harvested from new leaves, from plants in the field trials where possible, and from additional material grown in glasshouses where necessary. Leaf samples were taken and stored as individual plant samples, in tubes, flash frozen in liquid nitrogen then stored at -20^{o}C.

3.2.3 Primer selection

The use of combinations of primers allows screening of a representative fraction of the genome (Mueller and Wolfenbarger, 1999). Six primer pairs (with three selective nucleotides per primer) were optimised for eight individuals from each crop (Table 3.1), at the Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth, with two primer pairs ultimately used for each crop (see Table 3.2). Primer pairs were selected from those identified in previous studies (see Table 3.1). Final primer pair selection was based on trace quality, number of polymorphisms and clarity of informative peaks (Nowosielski *et al*., 2002).

Table 3.1 AFLP primer combinations optimised.

Crop	*Eco*RI	*Mse*I	Source
Brassica oleracea var. *acephala*	AAC	CAA	Seyis *et al*., 2003
	AAC	CTT	Seyis *et al*., 2003
	AAG	CTT	Seyis *et al*., 2003
	AAC	CTA	Srivastava *et al*., 2001
	ACC	CAG	Srivastava *et al*., 2001
	CAG	AGG	Hansen *et al*., 2001
Daucus carota	AGG	CTG	Shim and Jorgensen, 2000
	CAC	ACG	Shim and Jorgensen, 2000
	ACA	CAA	Nakajima *et al*., 1998
	ACA	CTG	Nakajima *et al*., 1998
	AAG	CAT	Nakajima *et al*., 1998
	ACC	CTC	Grzebelus *et al*., 2001
Pisum sativum	AAC	CAA	Simioniuc *et al*., 2002
	ACA	CAG	Simioniuc *et al*., 2002
	ATC	CAC	Simioniuc *et al*., 2002
	ATC	CAT	Simioniuc *et al*., 2002
	ATG	CAA	Simioniuc *et al*., 2002
	ATG	CAG	Simioniuc *et al*., 2002

Crop	*Eco*RI	*Mse*I	Source
Phaseolus coccineus	CAC	ACT	Negri and Tosti, 2002
	CAC	ACA	Negri and Tosti, 2002
	CAG	ATA	Negri and Tosti, 2002
	ACA	CAG	Nowosielski *et al*., 2002
	ACT	CTG	Nowosielski *et al*., 2002
	ACA	CTC	Nowosielski *et al*., 2002
Vicia faba	ACC	CAG	Zong *et al*., 2009
	AGG	CTT	-
	AGG	CTC	Zeid *et al*., 2003
	ATT	CAA	Zong *et al*., 2009
	ACG	CTT	Zong *et al*., 2009
	AAC	CAC	Zeid *et al*., 2003
Cucumis sativus	AAA	CCA	Yashiro *et al*., 2006
	ACA	CTC	Garcia-mas *et al*., 2000
	AAA	CCT	Yashiro *et al*., 2006
	AAC	CTC	Garcia-mas *et al*., 2000
	ACG	CTA	Garcia-mas *et al*., 2000
	AAG	CAT	Garcia-mas *et al*., 2000
Lactuca sativa	AAC	CAC	Yang *et al*., 2007
	ACA	CAG	Yang *et al*., 2007
	AGG	GCT	Hill *et al*., 1996
	ACA	CAC	Koopman *et al*., 2001
	CCT	CCT	Jeuken *et al*., 2001
	ACG	CTA	Yang *et al*., 2007

Table 3.2 AFLP primer pairs applied.

Crop	Primer pair 1 (*Eco*RI/*Mse*I)	Primer pair 2 (*Eco*RI/*Mse*I)
Vicia faba	ACG/CTT	ACC/CAG
Phaseolus coccineus	ACT/CTG	ATA/CAG
Pisum sativum	ATG/CAG	ACA/CAG
Cucumis sativa	AAC/CTC	ACG/CGA
Brassica oleracea var. *acephala*	AAC/CTA	ACC/CAG
Lactuca sativa	ACG/CTA	ACA/CAG
Daucus carota	ACA/CTG	ACC/CTC

3.2.4 Amplified Fragment Length Polymorphism method

DNA isolation

Plant tissue was extracted for leaf tissue using the Qiagen DNeasy 96 plant kit as per manufacturer's instructions (Qiagen, 2006).

AFLP method

AFLPs, as described by Vos (1995), were processed at the Institute of Biology, Ecology and Rural Science (IBERS), in Aberystwyth, following their set protocol (Skot *et al*., 2005).

The digestion ligation step (DIG/LIG) comprised a total genome digest, performed using the restriction enzymes MseI and EcoRI, along with ligation of oligonucleotide adapters. The DIG/LIG mix was produced according to the IBERS protocol, each sample contained 0.57 µl sterile distilled water, 1.10 µl 10 x T4DNA ligase buffer, 1.10 µl 0.5M NaCl, 0.55 µl BSA (1mg/ml), 1.00 µl Mse adaptor, 1.00 µl Eco adaptor, 0.1 µl Mse1 (10 units/ µl), 0.05 µl EcoR1 (100 units/ µl), and 0.3 µl T4 DNA ligase (30 Weiss U/ µl).

5.5 µl of DIG/LIG were mixed into each well of a 96 well PCR plate, followed by 5.5 µl of DNA (approximately 20ng/ µl) in each well. Samples were spun briefly to ensure the sample was at the bottom of the well. Samples were incubated in the PCR machine for 2 hours at 37°C. Each sample was diluted with 29 µl $T_{10}E_{0.1}$ to obtain a volume of 40 µl.

Samples were run 10 µl out on an agarose gel, and were then ready for pre-selective amplification.

AFLP Polymerase Chain Reaction (PCR) amplification proceeds in two stages. The first stage is pre-amplification; this is performed with a single selective nucleotide (in order to reduce smearing in electrophoresis due to a too high number of restriction fragments, and to reduce primer mismatching); the second stage is selective amplification with three base pair extensions.

Preamplification

Pre-amplification of sets of restriction fragments using 1 µl EcoR1+MseI pre-amp primer mix, 15 µl AFLP core mix, 4 µl diluted DIG/LIG DNA. Pipette 16 µl of pre-selective amplification mix into each well and add 4 µl of diluted DIG/LIG DNA.

The plate was spun briefly to ensure samples were at the bottom of the wells, set up PCR reaction (Table 3.3).

Table 3.3 PCR reaction and timings used.

HOLD	CYCLES			HOLD	HOLD
	20 cycles				
72°C	94°C	56°C	72°C	60°C	4°C
2 minutes	20 sec	30 sec	2 min	45 min	∞

The pre-amplification product was diluted as follows in a microtiter plate: 10 µl pre-amplification product plus 190 µl $TE_{0.1}$. This was mixed well and stored in fridge until used.

The remaining 10 µl was run out on a gel to check there was a product before continuing to selective amplification.

Selective amplification

For selective amplification core mix was made on the IBERS site for use on day: for 750 µl of core mix: 578 µl sterile distilled water, 100 µl 10 x Amplitaq buffer, 60 µl $MgCl_2$ (25mM), 8 µl dNTP's (25mM), 4 µl Amplitaq Gold (5U/ µl).

The following selective amplification mix was prepared: 0.5 µl MseI primer-Cxx (5 µM) per sample, 0.5 µl EcoRI primer-Axx (1 µM) per sample, 7.5 µl core mix (as above) per sample, and 1.5 µl diluted pre-amp product per sample.

The following selective PCR reaction was performed:

Table 3.4 PCR program and timings

HOLD	CYCLE			Number of cycles
95°C 10 min	94°C 20 sec	66°C-56°C 30 sec (-0.7 per cycle)	72°C 2 min	13
	94°C 20 sec	56°C 30 sec	72°C 2 min	20
60°C 30 min				1
4°C ∞				1

Samples were next run on the ABI3730xl 16 capillary system. These were stored in the refrigerator short-term and in the freezer long-term. Selective amplification products were treated at 60°C for 45 minutes, cooled down to 25°C, and set up to run on the ABI3730xl with 1 µl sel-amp product plus 10 µl Hi-Di Formamide/size standard.

3.2.5 Genotyping

Resulting information was displayed and genotyping performed using GeneMapper (version 4.0). Profiles were normalised in GeneMapper and settings were standardised to include peaks with an average RFU over 50, in the range of 50-500 base pairs (bp). All individual sample traces were verified manually to correct GeneMapper omissions, off-centre peak bin locations, or bin misclassifications (following Whitlock *et al*., 2008). Only clear sample traces were retained; noisy sample traces were removed, as were peak shoulders, overlapping peaks, peaks that were unclear due to low peak intensity in some accessions, and traces that contributed multiple unique peaks (as this was suggestive of potential contamination) (Whitlock *et al*., 2008).

3.2.6 Statistical analysis

Genetic diversity metrics were calculated using AFLP SURV version 1.0 (Vekemans *et al*., 2002). AFLPs are dominant markers, and therefore to calculate genetic diversity metrics allele frequencies have to be estimated (Bonin *et al*., 2007). The method used for this in AFLP SURV was method 4 ('a Bayesian method with non-uniform prior distribution of allele frequencies', Zhivotovsky, 1999; Vekemans, 2002), which calculates separate allele frequency distributions for each population (accession) using the sample size and number of individuals in the sample that lack the allele (peak) to calculate the frequency of the null allele. The two methods for measuring genetic diversity deployed in the present study were the proportion of polymorphic loci at the 5% confidence level (PLP or P) and Nei's gene diversity (or expected heterozygosity – Hj) (Lynch and Milligan, 1994, Vekemans, 2002).

The proportion of polymorphic loci (expressed as a percentage) is an estimate of allele richness that measures to the total number of alleles or genotypes in a population (Mohammadi and Prasanna, 2003); it is the number of polymorphic loci divided by the total number of loci sampled (Laurentin, 2009). Nei's Gene Diversity (Nei, 1973), or expected heterozygosity, is the probability that an individual will be heterozygous at a given locus. It is a measure of allele evenness, which is a function of both the number and frequency of alleles in a population (Mohammadi and Prasanna, 2003), and is based on allele frequencies estimated from the proportion of heterozygous loci in an individual, and the number of individuals that are heterozygous for the loci. The proportion of polymorphic loci as a measurement is more vulnerable to sample size effects (Mohammadi and Prasanna, 2003), however it can be more useful when the plant breeding system is unclear.

Since both metrics rely on the estimation of allele frequencies, the Bayesian method used (Zhivotovsky, 1999) enables the user to specify whether the populations are in Hardy Weinberg equilibrium (Vekemans, 2002). In the current study values for PLP and Hj are most likely conservative estimates for the outbreeding crop species. The software used in the analysis (AFLP SURV version 1.0, Vekemans *et al*., 2002) allows the user to specify the species position on a scale from completely outbreeding (FIS = 0), to completely inbreeding (FIS = 1); as the current study is of crop plants a large degree of inbreeding (for type etc) is inevitable, even in open-pollinated varieties, therefore the analyses were run with three scenarios: complete out breeding, half and half, and complete inbreeding. Results between scenarios show the same relationships between accessions, only with increased distance between relationships and higher genetic diversity. Because the study plants are crop species,

the scenario of complete inbreeding (FIS = 1) is presented here, which is the more conservative estimate.

Genetic distance is an estimate of nucleotide substitution over time, and hence of the similarity between two populations or species based on sequence divergence, which increases over time. The current study calculated Nei's genetic distance (D) (Nei, 1972) in conjunction with the software AFLP SURV (Vekemans *et al*., 2002; Lynch and Milligan, 2004), for between-accession analysis, and GenAlEx (Peakall and Smouse, 2006), for between-individuals analysis.

For each crop Nei's genetic distance measures, calculated in AFLP SURV, were used to construct a UPGMA dendrogram in Phylip (Felsenstein, 1989), using Neighbor and Consense to construct the tree and perform bootstrap analyses, and TreeView (Page, 1996) to visualise it.

GenAlEx version 6.0 (Peakall and Smouse, 2006) was used to create pair-wise genetic distances from the binary data matrix for measuring genetic distance between individuals (following Huff *et al*., 1993, in Peakall and Smouse, 2006) and to visualise patterns of groupings in a Principal Coordinates Analysis (PCoA).

GenAlEx was also used to create PCoA plots for Nei's genetic distance, from a similarity matrix using measures calculated in AFLP SURV for between-accession distances. PCoA and cluster analysis were both chosen as complementary methods to display genetic distance measures, as cluster analysis allows the calculation of bootstrap values (Felsenstein 1985; Felsenstein, 1989) and PCoA is informative regarding distances between major groups,

whereas cluster analysis is sensitive to closely related individuals (Hauser and Crovello, 1982 in Sun *et al*., 2001).

Potential total redundancy was estimated by dividing the number of duplicate pairs by the total number of accessions and multiplying by 100. Those accessions identified as potential duplicates using the AFLP genetic distance data will be re-examined in the context of the morphological data (where available) in the final discussion chapter (chapter 6).

3.2.7 Potential sources of error

AFLP is a dominant molecular marker method that generates a large number of informative markers, distributed across the genome. It is particularly applicable for investigating genetic diversity and relations between closely related individuals, such as crop varieties (Meudt and Clarke, 2007), and with comparatively few *a priori* resources (Bensch and Akesson, 2005). However, there are several potential sources of error in the AFLP process (Bonin *et al*., 2004; Pompanon *et al*., 2005; Bonin *et al*., 2007).

Potential sources of error can be broadly split into two areas; each will be discussed with reference to the steps taken in the current study to avoid them. In the first, errors attributable to the experimental portion of the method include those due to human error and those resulting in missing peaks, which can have multiple causes, and are indistinguishable from genuine allele absence (null-allele homoplasy) (Pompanon *et al*., 2005). Causes include low quality DNA, and have been addressed in the current study through the use of fresh, young plant material, flash freezing, and use of the standard pre-amp step in AFLP. Additional measures taken to limit these errors included the employment of highly skilled specialised

laboratory and staff at IBERS, using an established protocol, the use of blank control wells on each plate to ensure removal of artefact peaks, and quality control measures such as on DNA quantity and quality (following Pompanon *et al*., 2005). The second set of errors is found during genotyping, namely homoplasy and scoring errors (Bonin *et al*., 2007). To reduce the occurrence of size homoplasy, Bonin *et al*. (2007) recommend the following steps, which were taken in the current study: firstly, all crops were analysed separately (avoiding intra-specific analyses, as homoplasy increases with taxonomic distance); secondly, only primer combinations that generated clearly readable traces, along which bands were evenly distributed were used (facilitated by primer optimisation and removal from datasets of poor traces); and thirdly, preference was given to markers representing longer bands, where sufficient markers were available (over 100 bp in length).

To reduce errors due to scoring, genotyping was automated using GeneMapper (version 4.0) and checked manually, and replicates (10% of total number of individuals) were run to track genotyping errors and allow estimation of an error rate (Pompanon *et al*., 2005; Bonin *et al*., 2007). Replicates were run independently (i.e. on separate plates to the main experiments, and analysed blind (separately from the main analysis) (following Pompanon *et al*., 2005).

Error rates were calculated both as error rate per locus (the ratio between number of loci and the number of mismatches), and the average error rate per replicates sample pair (the average of the ratios between number of loci and number of mismatches between each replicate pair) (Pompanon *et al*., 2005). This facilitated the removal of error-prone loci and samples and reduction in the number of errors in the dataset (Bonin *et al*., 2004; Pompanon *et al*., 2005).

3.3 Results

Satisfactory traces were retrieved for both primer pairs for five crops (*P. sativum, D. carota, C. sativus, L. sativa and B. oleracea* var. *acephala*), and one primer pair for *V. faba*. No traces for either primer pair of *P. vulgaris* were of sufficient quality for analysis.

3.3.1 Vicia faba

One primer pair combination (ACG/CTT) was used to analyse 26 HSL *V. faba* accessions and one commercial variety (The Sutton). A total of 335 loci were generated, of which 76 were both polymorphic and of sufficient quality for analysis. 42 samples were removed due to poor trace quality (including all of the samples for accessions Bonny Lad, Mr Jones, Mr Lenthall's and Standard 1 (Bunyard's Exhibition); Canner's 45 and The Shippam were not included due to lack of sufficient seed. The error rate for the data set was 2.98% (based on 9.1% of samples being repeated) which is well within the threshold suggested by Bonin *et al*. (2007); however due to the low number of markers available the error rate of individual loci was over 0.1 for eight loci (0.13 for seven loci and 0.2 for one locus).

Population genetic structure was Fst = 0.32 (standard error = 0.09) which indicates very large population differentiation.

Genetic diversity

The percentage of polymorphic loci ranged from 10.5%, in Beryl, to 76.3% in Red Bristow's (Table 4.5). Expected heterozygosity ranged from 0.07, in Beryl, to 0.36 in Brown. The

average PLP for all accessions was 57.55%, and average expected heterozygosity was 0.25 (standard error = 0.012).

Table 3.5 Genetic diversity measures for *Vicia faba*. Genetic diversity measures for 26 *V. faba* HSL accessions and one commercial variety; calculated using AFLP SURV method 4 (Bayesian method with non-uniform prior distribution of allele frequencies), using 76 loci, based on one AFLP primer pair ACG/CTT; FIS=1.

Accession name	Sample size	PLP	Expected heterozygosity (Hj)	S.E. (Hj)
Bacardi	5	65.80	0.29	0.03
Beryl	5	10.50	0.07	0.01
Bossingham Long Pod	4	61.80	0.28	0.03
Bowland's Beauty	5	56.60	0.24	0.03
Brown	5	73.70	0.36	0.03
Chak'rusga	4	65.80	0.32	0.03
Cretian	5	73.70	0.33	0.02
Crimson Flowered	5	50.00	0.14	0.02
Estonian	5	57.90	0.22	0.03
Gloucester Champion	5	63.20	0.27	0.03
Jack Gedes	5	59.20	0.27	0.03
Jonah's	5	50.00	0.21	0.03
Londonderry	4	56.60	0.23	0.03
Martock	5	51.30	0.20	0.03
Mr Townend's	5	50.00	0.18	0.03
Painswick Wonder	5	44.70	0.14	0.02
Perovka	3	57.90	0.28	0.03
Red Bristow's	5	76.30	0.34	0.02
Rent Payer	5	64.50	0.24	0.03
Seville	3	64.50	0.25	0.03
Somerset	4	51.30	0.24	0.03
Stafford	5	61.80	0.28	0.03
Standard 2 - The Sutton	3	52.60	0.21	0.03
Sweet Lorraine	4	47.40	0.23	0.03
White Continental	5	63.20	0.27	0.03
Canadian Purple	4	61.80	0.30	0.03
Gloucester Bounty	5	61.80	0.24	0.03

Clustering within accessions

Principal Coordinate Analysis using binary data revealed a wide distribution of accessions (Figure 3.1), with one large cluster and two smaller clusters. Principal Co-ordinates one and two accounted for 21.00% and 19.8% of the variance, respectively. Individuals from Painswick wonder (coded as 20) were slightly separated from the other accessions by both principal coordinates; accessions Estonian (11), Rent Payer (23) and Seville (24) were separated by principal coordinate 1 from the main mass of accessions, and were widely spread suggesting high diversity; individuals from the accession Beryl (2) were clustered closely together; individuals from the remaining accessions were widely spread across the plot.

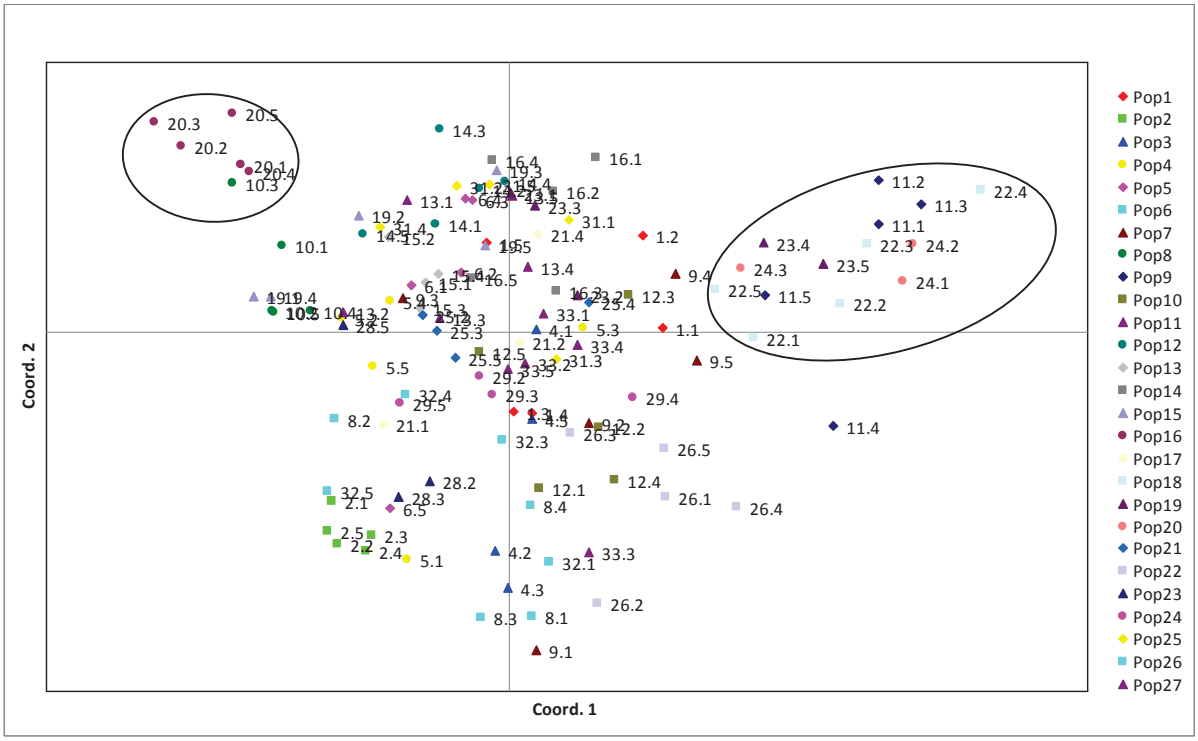

Figure 3.1 Principal Coordinates Analysis (PCoA) plot of *Vicia faba* individuals. Scatter diagram of first two principal co-ordinates, explaining 40.80% of cumulative variance. Derived using AFLP binary data, creating a pair-wise distance matrix in GenAlEx, for 26 HSL *V. faba* accessions and one commercial variety, using primer pair ACG/CTT, 76 polymorphic loci, between three and five individuals sampled per accession. Numbers represent accessions Bacardi (1), Beryl (2), Bossingham Long Pod (4), Bowland's Beauty (5), Brown (6), Chak'rusga (8), Cretian (9), Crimson Flowered (10), Estonian (11), Gloucester Champion (12), Jack Gedes (13), Jonah's (14), Londonderry (15), Martock (16), Mr Townend's (19), Painswick Wonder (20), Perovka (21), Red Bristow's (22), Rent Payer (23), Seville (24), Somerset (25), Stafford (26), Standard 2 - The Sutton (28), Sweet Lorraine (29), White Continental (31), Canadian Purple (32), Gloucester Bounty (33). Circles highlight individuals from two possible clusters indicating similarity.

Genetic distance and relationships between accessions

UPGMA cluster analysis, using Nei's genetic distance, revealed one large cluster (Figure 3.2), with several smaller clusters outside; however, none of these were supported by bootstrap values over 50%, reflecting both the high genetic diversity within and between accessions, and possible overlaps in genetic variation between accessions. A relationship was suggested between Chak'rusga (8) and Cretian (9), and particularly between Red Bristow's (22) and Seville (24), which implied that these accessions could be duplicates. The loose cluster of Rent payer (23), Estonian (11), Red Bristow's (22) and Seville (24), seen in the PCoA above, was present.

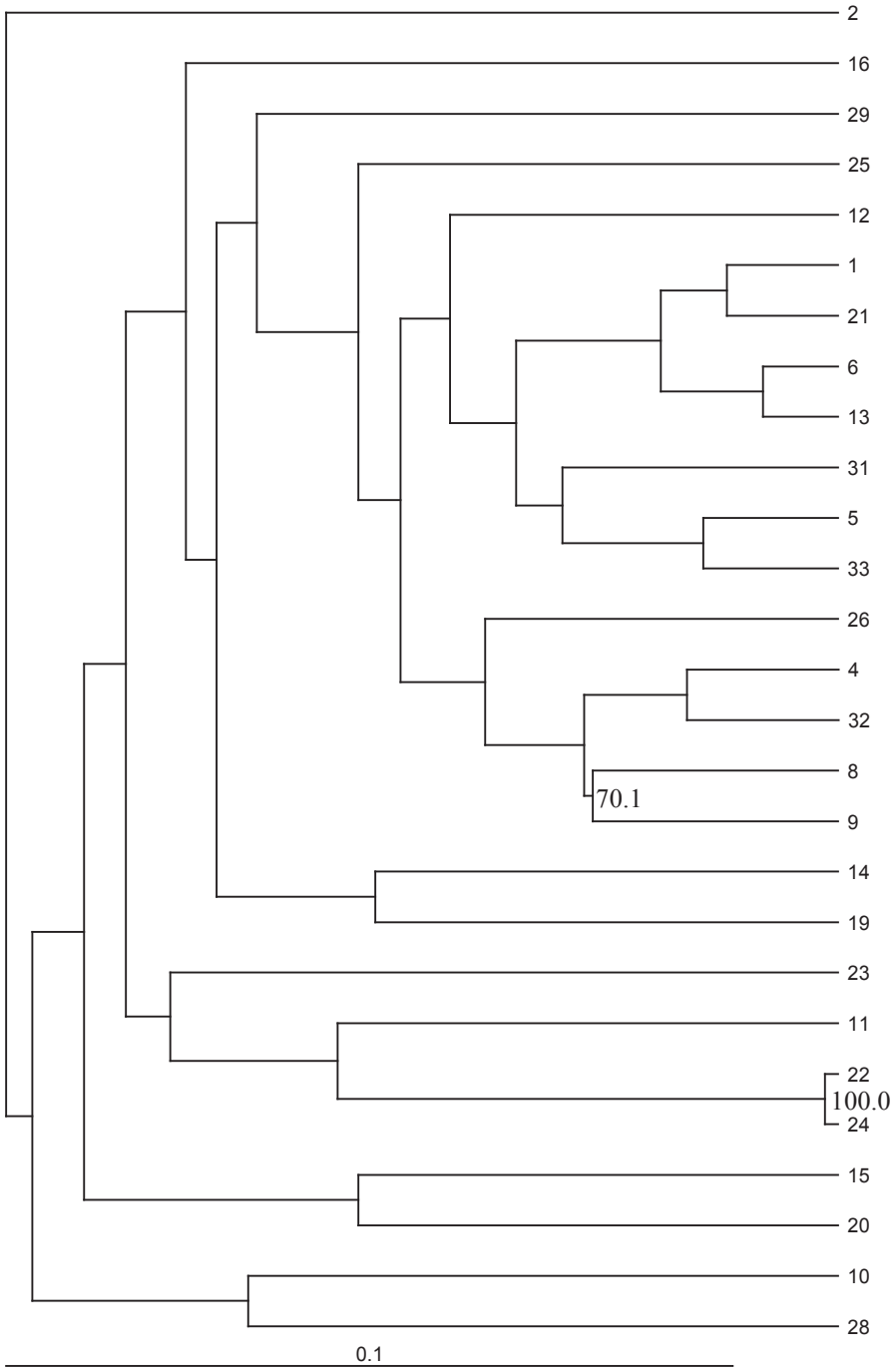

Figure 3.2 UPGMA genetic distance dendrogram for *Vicia faba* accessions. 26 HSL *V. faba* accessions and one commercial variety were analysed using AFLP primer pair ACG/CTT, 76 polymorphic loci, between three and five individuals sampled per accession. Derived using Nei's genetic distance calculated from AFLP SURV output, using Neighbour and Consense in Phylip, and TreeView. Numbers represent accessions (see above figure) and bootstrap values.

Principal coordinates analysis of clustering between accessions using Nei's genetic distance (Figure 3.3) showed the very broad spread of *V. faba* accessions, indicating large genetic distance between accessions. The first two co-ordinates explained 25.62% and 21.89% of the variance, respectively. The most genetically distant were Seville (24), Red Bristow's (22) and Estonian (11), as in the previous analyses, which formed a loose cluster separated from the other accessions on the basis of the first principal coordinate. A second, tighter, cluster (indicating a lower genetic distance) was comprised of accessions White Continental (31), Jack Gedes (13), Jonah's (14), Londonderry (15) and Mr Townend's (19). Rent Payer (23), Painswick Wonder (20) and Stafford (26) were slightly outlying from the other accessions.

Figure 3.3 Principal Coordinates Analysis (PCoA) plot of *Vicia faba* accessions. Principal Coordinates Analysis (PCoA) plot of clustering between 26 *V. faba* accessions and one commercial variety, derived from similarity matrix, in GenAlEx, obtained using Nei's genetic distance (calculated in AFLP SURV). Data from 76 loci from one AFLP primer pair ACG/CTT. Between three and five individuals were sampled per accession. Cumulative variation explained within the first two co-ordinates was 25.62% and 47.51%. Numbers represent accessions (see figure above). Circles highlight potential clusters of similar accessions.

3.3.2 Daucus carota

Two primer combinations (ACA/CTG and ACC/CTC) were used to analyse ten HSL *Daucus carota* accessions and two commercial varieties (F1 Nelson and F1 Maestro). A total of 257 loci were generated, of which 178 were both polymorphic and of sufficient quality for analysis. Eight samples were removed due to poor trace quality; error rate for the data set was 5.6% (based on 21.6% of samples being repeated), however individual locus error rates were higher for 20 loci (17 at 0.18, and three at 0.27).

Fst for the dataset was 0.35 (standard error = 0.077), which indicates a very large differentiation between populations.

Genetic diversity

The percentage of polymorphic loci ranged from 50.6%, in standard 1, to 59.0%, in Giant improved flak and Manchester Table (Table 3.6). Expected heterozygosity ranged from 0.15, in standard 1, to 0.28, in Giant improved flak. The average PLP for all accessions was 56.18%, and average expected heterozygosity was 0.20 (standard error = 0.0097). Giant improved flak is of much higher genetic diversity than all other accessions, although a small sample size was used, standard error shows this accession is higher than the others. Orange rooted HSL accessions were more heterozygous than white or purple rooted accessions.

Commercial varieties were low in genetic diversity compared to most HSL accessions; standard 1 was lowest in both expected heterozygosity (0.15) and PLP (50.6%), standard 2 was near the group average for PLP (56.7%), and one of the lowest expected heterozygosities compared to HSL accessions (0.16).

Table 3.6 Genetic diversity measures for *Daucus carota*. Genetic diversity measures for ten HSL *D. carota* accessions and two commercial varieties; calculated using AFLP SURV method 4 (a Bayesian method with non-uniform prior distribution of allele frequencies), using 178 loci, based on two AFLP primer pairs ACA/CTG and ACC/CTC; FIS=1.

Accession	Sample size	Proportion of polymorphic loci	Expected Heterozygosity (Hj)	S.E. (Hj)
Afghan Purple	5	58.4	0.1928	0.0157
Altringham	4	56.7	0.2128	0.0168
Beta III	4	56.2	0.1989	0.0165
Egmont Gold	3	53.9	0.2120	0.0177
Giant Improved Flak	2	59	0.2790	0.0203
John's Purple	5	52.8	0.1833	0.0159
Manchester Table	4	59	0.2179	0.0170
Red Elephant	4	57.9	0.2298	0.0176
Scarlet Horn	5	57.3	0.2045	0.0166
Standard 1 – F1 Nelson	5	50.6	0.1513	0.0149
Standard 2 – F1 Maestro	5	56.7	0.1614	0.0145
White Belgium	5	55.6	0.1759	0.0150

Clustering within accessions

Four clusters were defined by the first and second principal co-ordinates, which cumulatively explained 47.76% of the total variance (Figure 3.4). John's Purple and Afghan Purple (accessions 6 and 1) group together and are distinct from the other accessions (based on the first principal co-ordinate); individuals from accession John's Purple were more widely spread suggesting greater diversity. The second cluster was composed of Altringham and Red Elephant (accessions 2 and 8) and was separated from the third cluster on the second principal co-ordinate. The spread of these individuals also suggested diversity. The individuals from standard 2 (accession 11) were clustered slightly apart from the third group (consisting of the

remaining HSL accessions and commercial standards). Individuals from the other commercial standard, accession 10, clustered closely together. Individual 5.1 (from Giant Improved Flak) was positioned outside all of the clusters.

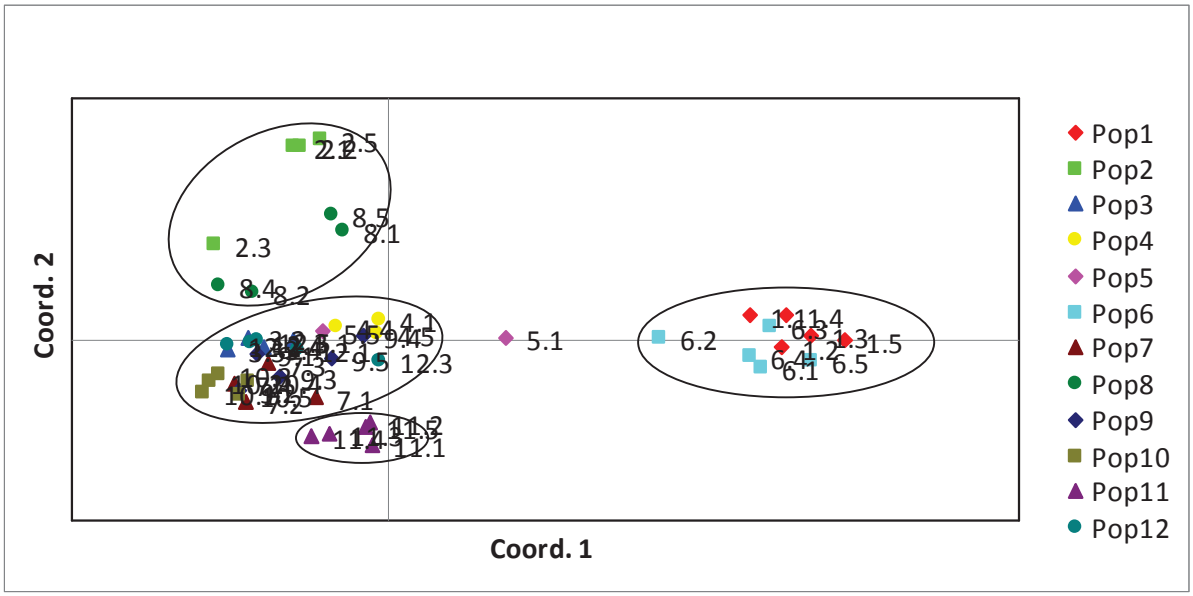

Figure 3.4 Principal Coordinates Analysis (PCoA) plot of *Daucus carota* individuals. Scatter diagram of the first two principal coordinates explaining cumulative variation of 47.76% (the third co-ordinate increased it to 62.66%) of individuals from 10 HSL *D. carota* accessions and 2 commercial varieties, between 2 and five individuals per accession, derived in GenAlEx obtained using presence/absence data from 178 loci from two AFLP primer pairs ACA/CTG and ACC/CTC. Numbers represent accessions Afghan Purple (1), Altringham (2), Beta III (3), Egmont Gold (4), Giant Improved Flak (5), John's Purple (6), Manchester Table (7), Red Elephant (8), Scarlet Horn (9), Standard 1 (10), Standard 2 (11), White Belgium (12). Circles highlight clustering of individuals.

Genetic distance and relationships between accessions

Three of the clusters seen above were also represented in the genetic distance dendrogram (Figure 3.5). Few branches were well supported by bootstrap values; the first cluster with Afghan purple and John's purple, was well supported (96.2%); bootstrap values for the second cluster (Altringham and Red elephant) were over 50%. The standards and orange accessions were together in the main cluster; bootstrap values did not suggest any particularly decisive relationships. Branch lengths were long, suggesting large genetic distance between all accessions. White Belgium clustered in with the orange accessions.

Bootstrap values were also calculated for a Hardy Weinberg assumed scenario, in this bootstrap values were higher but gave the same pattern of results.

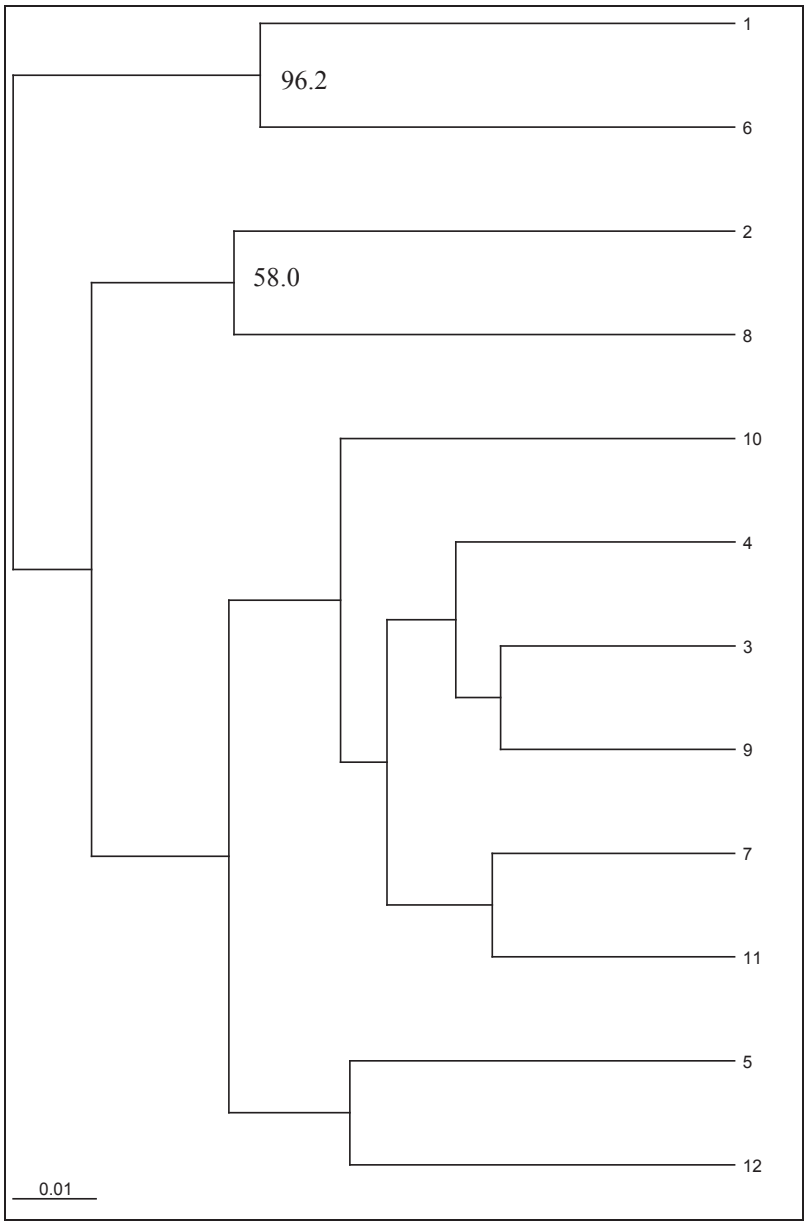

Figure 3.5 UPGMA Genetic distance dendrogram displaying *Daucus carota* relationships. UPGMA genetic distance tree displaying relationships for ten HSL *Daucus carota* accessions and two commercial varieties, between two and five individuals sampled per accession; derived from 178 AFLP marker loci, resulting from two primer pairs (ACA/CTG and ACC/CTC). Similarity matrix using Nei's genetic distance calculated in AFLP-SURV, assuming complete deviation from Hardy Weinberg (FIS=1). Bootstrap values were calculated in Phylip using Consense, tree visualised using TreeView software. Numbers represent accessions (see figure above).

Using Nei's Genetic Distance measure, PCoA indicated two loose clusters defined by the first and second principal co-ordinates, which explained 50.17% of the cumulative variance (Figure 3.6). As in previous analyses Afghan purple and John's Purple clustered together based on the first principal co-ordinate; Altringham and Red Elephant were separated from the main bulk of accessions on the second principal co-ordinate, although they were also separated from each other, indicating a large genetic distance. Beta III, Manchester Table, Scarlet Horn, White Belgium and Standard 1 clustered together, suggesting smaller genetic distance between these accessions; Giant Improved Flak, Egmont Gold and Standard 2 were genetically distant from other accessions in that cluster. The same analysis was carried out assuming Hardy Weinberg equilibrium, and gave the same results, although with greater genetic distances and hence separation between the accessions already identified above.

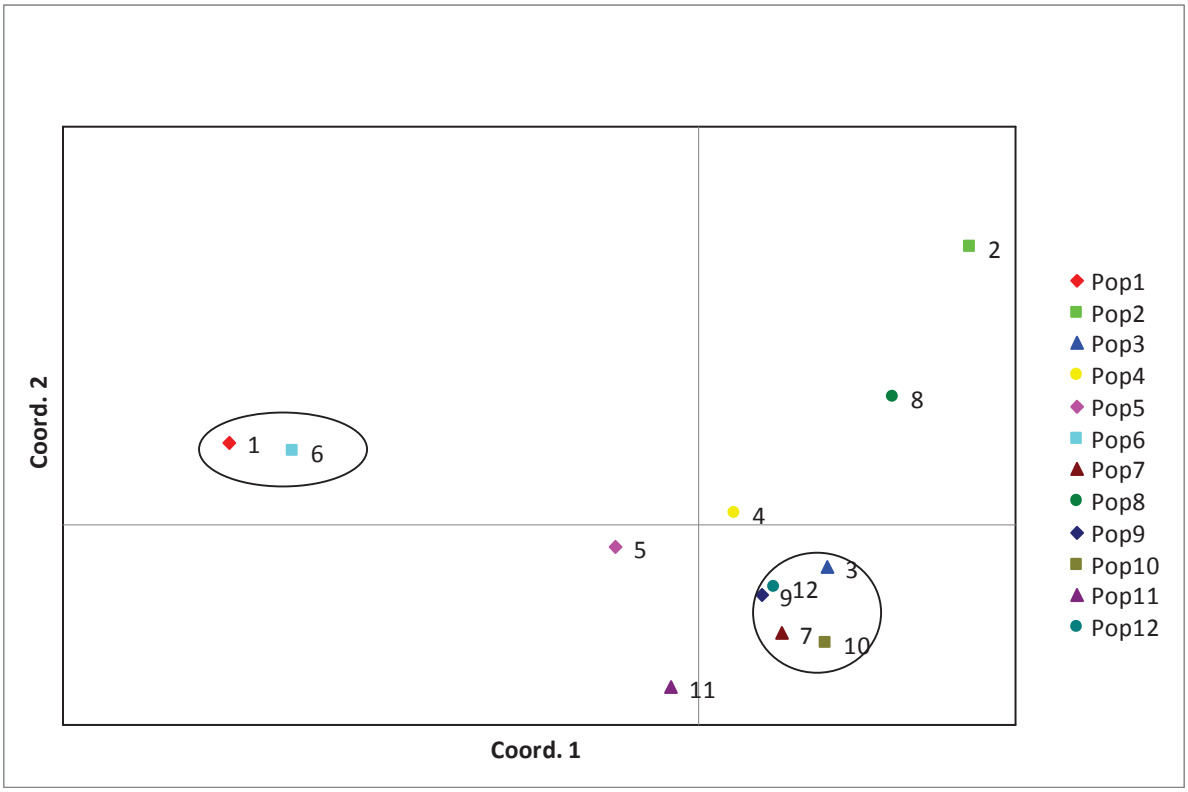

Figure 3.6 Principal Coordinates Analysis (PCoA) plot of *Daucus carota* accessions. Principal Coordinates Analysis (PCoA) plot of clustering between 10 HSL *D. carota* accessions and two commercial standards, derived from similarity matrix calculated in GenAlEx, obtained using Nei's genetic distance (calculated in AFLP SURV). Data from 178 loci, from two AFLP primer pairs ACA/CTG and ACC/CTC. Between two and five individuals were sampled per accession. Cumulative variation explained within the first three co-ordinates is 28.28%, 50.17% and 66.25% respectively. Numbers represent accessions: Afghan Purple (1), Altringham (2), Beta III (3), Egmont Gold (4), Giant Improved Flak (5), John's Purple (6), Manchester Table (7), Red Elephant (8), Scarlet Horn (9), Standard 1 (10), Standard 2 (11), White Belgium (12). Circles highlight potential clusters of similar accessions.

3.3.3 Pisum sativum

Two primer pairs ACA/CAG and ATG/CAG, 322 loci were reported for 75 HSL *P. sativum* accessions and two commercial standards, of which 120 were both polymorphic and of sufficient clarity and quality for analysis. Nineteen samples were removed due to poor trace quality. The error rate was 0.1%, based on the replication of 9.84% of samples.

Fst for *P. sativum* was 0.78 (SE = 0.02), which indicates high differentiation between populations.

Genetic diversity

The percentage of polymorphic loci ranged from 0% in Harold Idle, Holland Capucijner's, Lancashire lad, Newick, Stokesley, Sutton's Harbinger and Table Talk to 55.8% in Latvian (Table 3.7). Expected heterozygosity ranged from 0.021 in Newick to 0.17 in Latvian Grey Pea. Average PLP for all accessions was 11.2%; average expected heterozygosity for all accessions was 0.059 (SE = 0.003).

The genetic diversity of the commercial standards was PLP 16.7 (standard 1) and 2.5 (standard 2), and expected heterozygosity 0.1 for standard 1 and 0.05 for standard 2. This means that Standard 1 was above average for PLP and expected heterozygosity, and Standard 2 was below average for PLP and slightly below average for expected heterozygosity.

Table 3.7 Genetic diversity measures for *Pisum sativum*. Genetic diversity measures for 75 HSL *P. sativum* accessions and two commercial standards; calculated using AFLP SURV method 4 (a Bayesian method with non-uniform prior distribution of allele frequencies), using 120 loci, based on two AFLP primer pairs (ACA/CAG and ATG/CAG); FIS=1.

Accession name	Population	n	PLP	Expected heterozygosity (Hj)	Standard error (Hj)
Alex	1	5	3.3	0.055	0.008
Bijou	2	5	10	0.084	0.014
Carlin	3	5	10	0.088	0.015
Carruther's Purple Podded	4	4	0.8	0.049	0.006
Champion of England	5	5	1.7	0.045	0.007
Clarke's Beltony Blue	6	5	3.3	0.046	0.007
Commander	7	5	5	0.060	0.010
Cooper's Bean Pea	8	5	2.5	0.049	0.009
Doug Bray of Grimsby	9	5	1.7	0.043	0.007
Duke of Albany	10	5	2.5	0.051	0.007
Dun	11	4	1.7	0.056	0.007
Dwarf Defiance/John Lee	12	5	0.8	0.041	0.005
Early Capucijner	13	4	0.8	0.042	0.006
Eat All	14	5	3.3	0.054	0.009
Epicure	15	5	3.3	0.051	0.008
Espoir de Gemboux	16	5	3.3	0.046	0.008
Forty First	17	5	0.8	0.038	0.005
Frueher Heinrich	18	5	5	0.058	0.009
Giant Stride	19	5	1.7	0.047	0.007
Gladstone	20	5	1.7	0.044	0.007
Glory of Devon	21	5	13.3	0.090	0.012
Golden Sweet (India)	22	5	0.8	0.043	0.006
Gravedigger	23	5	1.7	0.040	0.006
Harold Idle	24	5	0	0.026	0.003
Holland Capucijner’s	25	5	0	0.024	0.003
Hugh's Huge	26	5	2.5	0.048	0.006
Irish Preans	27	5	0.8	0.038	0.006
Jeyes	28	5	1.7	0.042	0.006
Kent Blue	29	5	0.8	0.036	0.005
Lancashire Lad	30	5	0	0.024	0.003

Accession name	Population	n	PLP	Expected heterozygosity (Hj)	Standard error (Hj)
Large Grey	31	5	11.7	0.094	0.015
Latvian	32	5	55.8	0.135	0.015
Latvian Grey Pea	33	5	57.5	0.178	0.019
Latvian Large Grey	34	5	3.3	0.048	0.009
Laxton's Exquisite	35	4	45	0.066	0.010
Magnum Bonum	36	5	3.3	0.052	0.008
McPartlin	37	4	50	0.154	0.020
Moldova	38	5	2.5	0.050	0.008
Mr Bethell’s Purple Podded	39	5	3.3	0.053	0.009
Mr Bound's Bean Pea	40	5	1.7	0.040	0.006
Mummy's	41	5	2.5	0.049	0.008
Ne Plus Ultra	42	4	0.8	0.042	0.006
Newick	43	5	0	0.021	0.003
Ostgotaart	44	4	40.8	0.055	0.008
Panthers	45	5	5.8	0.065	0.010
Parsley	46	5	18.3	0.112	0.014
Pilot	47		11.7	0.088	0.012
Poppet	48	5	8.3	0.078	0.011
Prean	49	4	40.8	0.063	0.010
Prew's Special	50	5	3.3	0.049	0.007
Prince of Prussia	51	5	0.8	0.040	0.006
Purple Flowered Russian	52	3	40.8	0.069	0.010
Purple Mangetout	53	3	40.8	0.065	0.009
Purple Pod	54	3	40	0.064	0.009
Purple Podded	55	4	41.7	0.064	0.010
Raisin Capucijner’s	56	4	1.7	0.052	0.007
Robinson	57	5	4.2	0.061	0.009
Salmon Flowered	58	5	0.8	0.039	0.005
Simpson's Special	59	5	5	0.055	0.009
Standard 1 (Early Onward)	60	5	16.7	0.100	0.013
Standard 2 (Kelvedon Wonder)	61	5	2.5	0.050	0.006
Stenu	62	4	40.8	0.068	0.011
Stephens	63	5	49.2	0.121	0.015
Stokesley	64	5	0	0.029	0.003
Suttons Achievement	65	5	2.5	0.044	0.006
Suttons Harbinger	66	5	0	0.028	0.003
Suttons Purple Podded	67	5	1.7	0.043	0.007

Accession name	Population	n	PLP	Expected heterozygosity (Hj)	Standard error (Hj)
Table Talk	68	5	0	0.026	0.003
Telephone	69	5	12.5	0.085	0.012
Time Out of Mind	70	5	1.7	0.047	0.006
Turner's Spring	71	5	11.7	0.099	0.014
Tutankhamun	72	5	3.3	0.049	0.008
Ultra U	73	5	1.7	0.046	0.007
Veitch's Western Express	74	5	7.5	0.068	0.009
Victorian Purple Podded	75	5	2.5	0.044	0.006
Wieringen White	76	4	42.5	0.063	0.009
Winfreda	77	4	40	0.050	0.007

Clustering within accessions

Due to the large number and wide distribution of accessions, delineation of clusters for *P. sativum* individuals was complex. The most clearly defined clusters in the principal coordinate analysis are highlighted in Figure 3.7, and were one large cluster and many small clusters that were defined by the first two principal coordinates, which explained 52.34% of the variance. Many small clusters were visible some consisted of individuals from one accession (namely Clarke's Beltony Blue (6), Standard 1 (60), Espoir de Gemboux (16), Winfreda (77), Laxton's Exquisite (35) and Raisin Capucijner's (56)), others were more than one, and individuals from the same accession clustered closely together in most cases; the exceptions were Cooper's Bean Pea, Pilot, Carlin, Latvian Grey Pea, Large Grey, Poppet, Parsley, Stephens and Standard 1 the individuals of which were more dispersed, suggesting higher genetic diversity.

Accession clustered included Tutankhamun (72), Prew's Special (50), Mummy's (41), Doug Bray of Grimsby (9); Irish Prean's (27), Mr Bound's Bean Pea (40), Cooper's Bean Pea (8),

Prean (49), with Purple Pod (54) just to the right; Prince of Prussia (51), Coopers Bean Pea (individual 8.1), Ostgotaart (44) and Jeyes (28); Ultra U (73) and Ne Plus Ultra (42); Dun (11) and individuals from Carlin (3.4 and 3.2) (other individuals of Carlin were just outside; Commander (7), Sutton's Purple Podded (67) and Purple Mangetout (53); and the much larger cluster which included a densely packed region consisting of Telephone (69), Epicure (15), Hugh's Huge (26), Turner's Spring (71), Robinson (57), Alex (1), Veitch's Western Express (74), Champion of England (5), Stokesley (64), Duke of Albany (10), Glory of Devon (21), Carruther's Purple Podded (4), Dwarf Defiance/John Lee (12) and Time Out of Mind (70), and a looser region with additional accessions standard 2(61), Giant Stride (19), McPartlin (37), Glory of Devon (21), Telephone (61), Standard 1 (60), Pilot (47), Simpson's Special (59), Table Talk (68), Parsley (46), Harold Idle (24) and Panther's (45); the outer edge definitions of these clusters may be relatively arbitrary, as the distribution of accessions was fairly continuous.

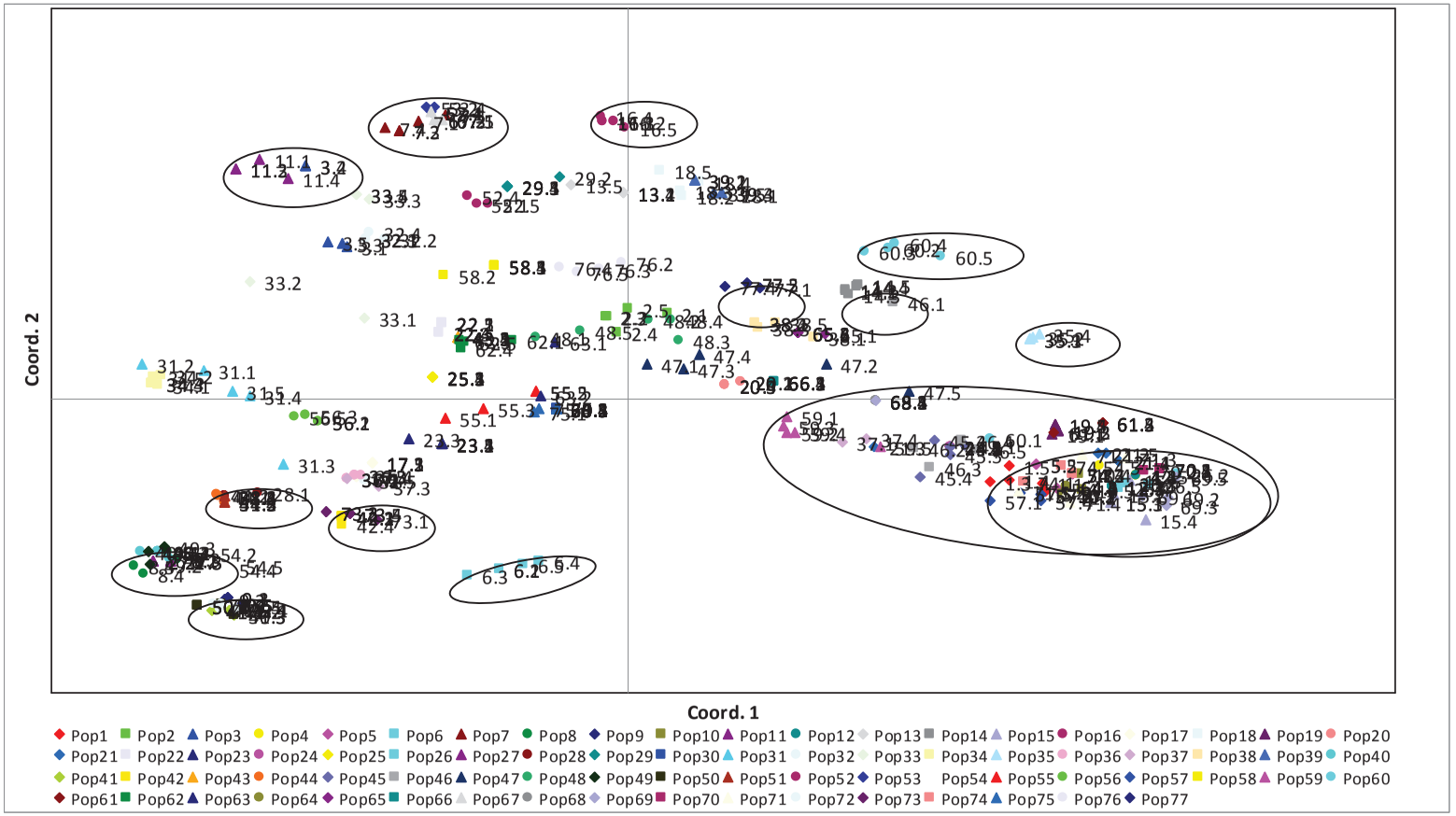

Figure 3.7 Principal Coordinates Analysis (PCoA) plot of *Pisum sativum* individuals. First two principal coordinates explaining cumulative variation of 52.34% for individuals from 75 *P. sativum* accessions and 2 commercial standards; 3-5 individuals per accession, derived in GenAlEx, using presence/absence data from 120 loci from two AFLP primer pairs ACA/CAG and ATG/CAG. Numbers represent accessions see overleaf. Circles highlight potential clusters of similar individuals.

Alex (1), Bijou (2), Carlin (3), Carruther's Purple Podded (4), Champion of England (5), Clarke's Beltony Blue (6), Commander (7), Cooper's Bean Pea (8), Doug Bray of Grimsby (9), Duke of Albany (10), Dun (11), Dwarf Defiance/John Lee (12), Early Capucijner (13), Eat All (14), Epicure (15), Espoir de Gemboux (16), Forty First (17), Frueher Heinrich (18), Giant Stride (19), Gladstone (20), Glory of Devon (21), Golden Sweet (India) (22), Gravedigger (23), Harold Idle (24), Holland Capucijner's (25), Hugh's Huge (26), Irish Preans (27), Jeyes (28), Kent Blue (29), Lancashire Lad (30), Large Grey (31), Latvian (32), Latvian Grey Pea (33), Latvian Large Grey (34), Laxton's Exquisite (35), Magnum Bonum (36), McPartlin (37), Moldova (38), Mr Bethell's Purple Podded (39), Mr Bound's Bean Pea (40), Mummy's (41), Ne Plus Ultra (42), Newick (43), Ostgotaart (44), Panthers (45), Parsley (46), Pilot (47), Poppet 48), Prean (49), Prew's Special (50), Prince of Prussia (51), Purple Flowered Russian (52), Purple Mangetout (53), Purple Pod (54), Purple Podded (55), Raisin Capucijner's (56), Robinson (57), Salmon Flowered (58), Simpson's Special (59), Standard 1 (60), Standard 2 (61), Stenu (62), Stephens (63), Stokesley (64), Suttons Achievement (65), Suttons Harbinger (66), Suttons Purple Podded (67), Table Talk (68), Telephone (69), Time Out of Mind (70), Turner's Spring (71), Tutankhamun (72), Ultra U (73), Veitch's Western Express (74), Victorian Purple Podded (75), Wieringen White (76), Winfreda (77).

Genetic distance and relationships between accessions

UPGMA cluster analysis using Nei's genetic distance did not present any large clusters that were highly supported by bootstrap values, however many of the smaller clusters were well supported (Figure 3.8). Well supported branches were found between: Commander and Purple Mangetout, which then also linked to Sutton's Purple Podded and Mr Bethell's Purple

Podded; Champion of England and Veitch's Western Express (74); Carlin and Dun; Bijou and Eat All; Large Grey and Latvian Large Grey; Irish Preans was linked to a group consisting of Prean, Cooper's Bean Pea and Mr Bethell's Bean Pea; Prince of Prussia, Jeyes and Ostgotaart were closely related; Mummy's and Prew's Special, which were in turn linked to Doug Bray of Grimsby and Tutankhamun and then in turn to Purple Pod; Latvian and Latvian Grey Pea; Magnum Bonum and McPartlin; Harold Idle and Panther's, which in turn were linked to Parsley (46); Forty First (17) linked to Purple Podded (55), which in turn was clustered with Victorian Purple Podded (75), Lancashire Lad (30) and Stephen's (63); Carruther's Purple Podded (4) and Dwarf Defiance/John Lee (12); Turner's Spring (71) was in a cluster with Robinson (57), Alex (1) and Stokesley (64).

Very short branch lengths indicate a very short genetic distance, and may indicate duplicate accessions. Potential duplicates were: Alex (1) and Stokesley (64) (genetic distance 0.0074); Carruther's Purple Podded (4) and Dwarf Defiance/John Lee (12) (genetic distance 0); Victorian Purple Podded (75), Lancashire Lad (30) and Stephen's (63) (genetic distance 0); Champion of England (5) and Veitch's Western Express (74) (genetic distance 0); Harold Idle (24) and Panther's (45) (genetic distance 0.001); Prince of Prussia (51), Jeyes (28) and Ostgotaart (44) (genetic distance 0); Prean (49), Cooper's Bean Pea (8) and Mr Bethell's Bean Pea (40) (genetic distance between Prean and Coopers Bean Pea 0, between Cooper's Bean Pea and Mr Bethell's Bean Pea 0.0055; between Prean and Mr Bethell's Bean Pea 0.0016); and finally Commander (7) and Purple Mangetout (53) (genetic distance 0.036).

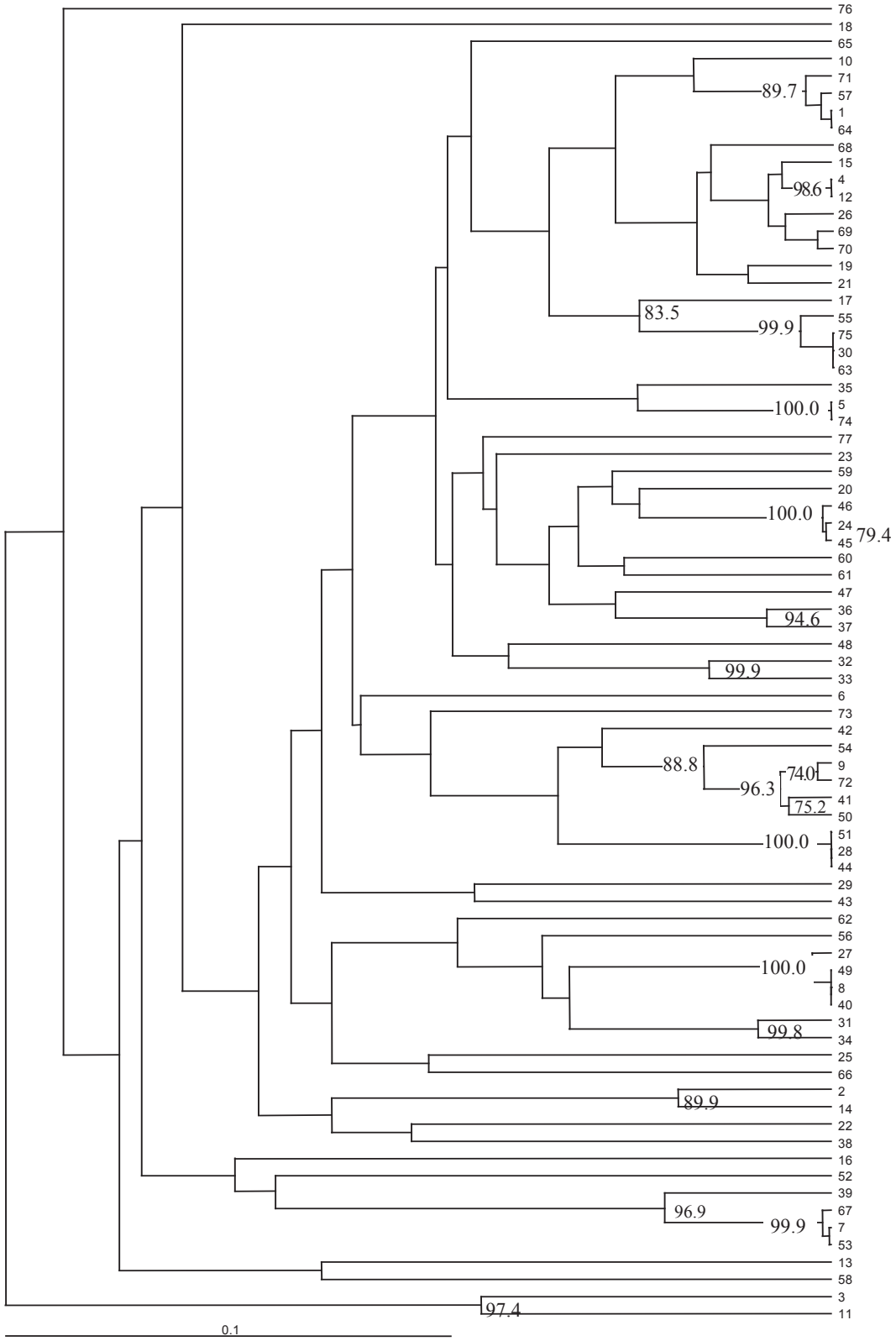

Figure 3.8 UPGMA genetic distance dendrogram for *Pisum sativum* accessions. UPGMA dendrogram for 75 HSL *Pisum sativum* accessions and two commercial standards, using AFLP primer pairs ACA/CAG and ATG/CAG, 120 polymorphic loci, between three and five individuals sampled per accession. Derived using Nei's genetic distance calculated from AFLP SURV output, using Neighbour and Consense in Phylip; bootstrap values were calculated in Phylip using Consense, tree visualised using TreeView. Numbers represent accessions (see above).

Using Nei's genetic distance measure, a PCoA indicated two principal coordinates accounting for 51.90% of the variance. *P. sativum* accessions were seen to be distributed widely across the PCoA plot, with one large cluster formed and several small.

The large cluster contained loosely clustered accessions and a sub-cluster of more densely grouped accessions (Figure 3.9). The more loosely grouped accessions were Laxton's Exquisite (35), Standard 1 (60), Eat All (914), Sutton's Achievement (65), Moldova (38), Pilot (47), Gladstone (20), Sutton's Harbinger (66), Simpson's Special (59), Table Talk (68), Parsley (46), Harold Idle (24) and Panther's (45); within this cluster the more closely assembled were Giant Stride (19), Glory of Devon (21), Standard 2(61), Alex (1), Robinson (57), Epicure (15), Hugh's Huge (26), Time Out of Mind (70), Carruther's Purple Podded (4), Telephone (69), Dwarf Defiance/John Lee (12), Stokesley (64), Duke of Albany (10), Veitch's Western Express (74), Champion of England (5) and Turner's Spring (71). The accessions present in the tighter cluster were similar to those seen in the tighter cluster of the PCoA between individuals (using binary data). Those accessions that are absent from this cluster in the between individual PCoA (Laxton's Exquisite, Eat All, Sutton's Achievement, Moldova, Gladstone, and Sutton's Harbinger) were located just outside of the cluster. The accessions in the tighter PCoA cluster were also seen in the UPGMA cluster analysis, although were not well supported with bootstrap values. In this PCoA Giant Stride (19), Standard 2 (61) and Glory of Devon (21) were in the more tightly clustered group than in the PCoA between individuals.

Smaller clusters of accessions were composed of: Lancashire Lad (30) and Victorian Purple Podded (75); Commander (7), Sutton's Purple Podded (67) and Purple Mangetout (53); Bijou

(2) and Poppet (48); Mr Bethell's Purple Podded (39) and Frueher Heinrich (18); Cooper's Bean Pea (8), Prean (49), Mr Bound's Bean Pea (40) and Irish Preans (27); Stenu (62), Golden Sweet (India) (22) and Newick (43); Magnum Bonum (36) and Forty First (17); Jeyes (28), Ostgotaart (44) and Prince of Prussia (51); Mummy's (41), Prew's Special (50), Tutankhamun (72) and Doug Bray of Grimsby (9).

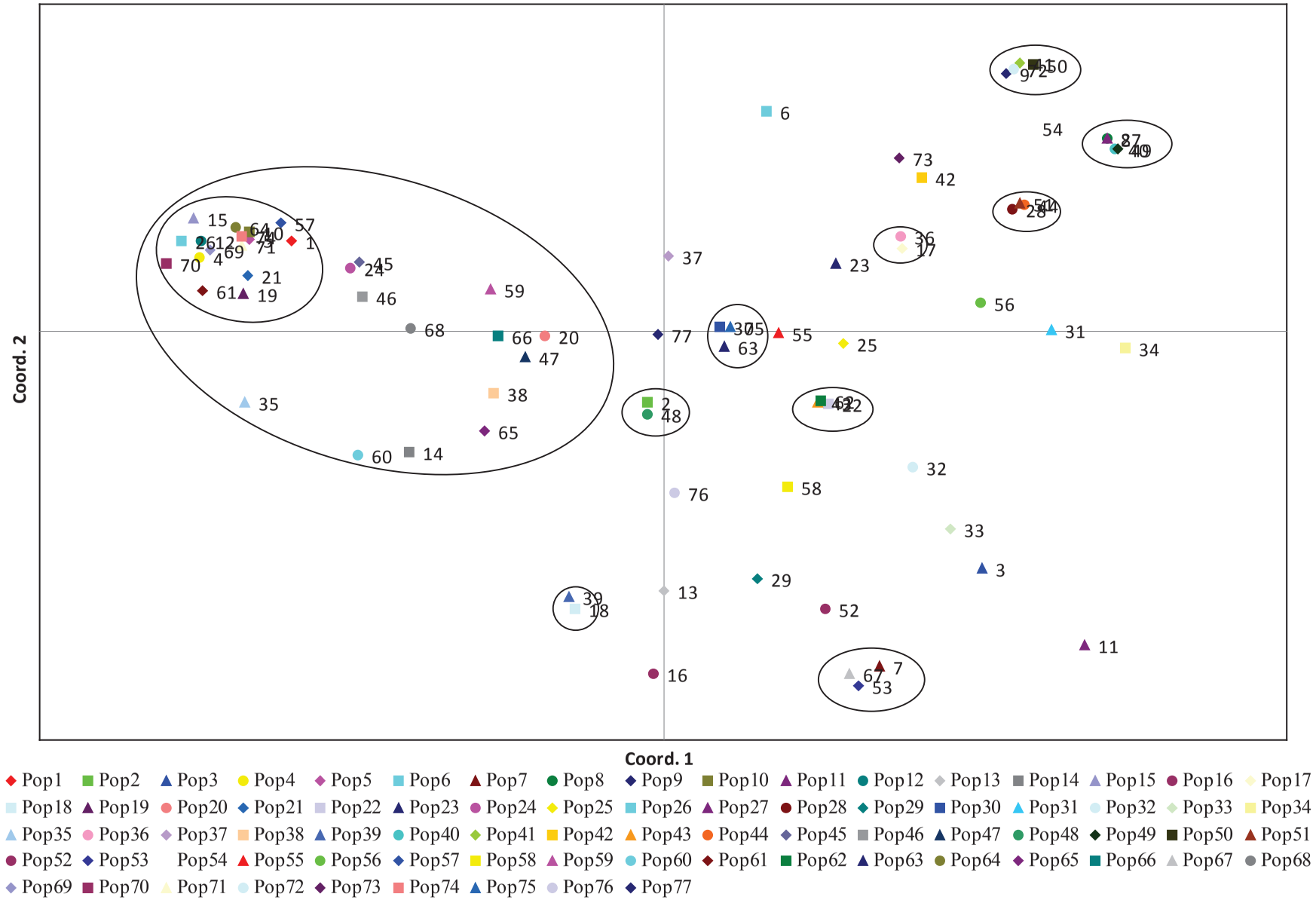

Figure 3.9 Principal Coordinates Analysis (PCoA) plot of *Pisum sativum* accessions. PCoA plot of clustering between 75 HSL *P. sativum* accessions and two commercial standards, PCoA performed in GenAlEx, obtained using Nei's genetic distance calculated in AFLP SURV. Data from 120 loci, from two AFLP primer pairs ATG/CAG and ACA/CAG. Between three and five individuals were sampled per accession. Cumulative variation explained within the two principal co-ordinates is 51.90%. Numbers represent accessions: see above. Circles highlight potential clusters of similar accessions.

3.3.4 Lactuca sativa

Two primer combinations (ACG/CTA and ACA/CAG) were used to analyse 20 HSL *L. sativa* accessions and two commercial standards (Iceberg and Corsair). A total of 286 loci were generated, of which 103 were both polymorphic and of sufficient quality for analysis. Five samples were removed due to poor trace quality; error rate for the data set was 3.88% (based on 15.89% of samples being repeated). In five loci an individual error rate of 0.2 occurred, however these were retained due to their important information content.

Fst for the dataset was 0.7 (standard error = 0.024), which indicates a very high level of differentiation.

Genetic diversity

The level of genetic diversity varied widely across accessions in the collection. The percentage of polymorphic loci ranged from 2.9%, in Black seeded samara, to 59.9%, in Soulie (Table 3.7). Expected heterozygosity ranged from 0.05, in Black seeded samara, to 0.18 in Soulie. The average PLP for all accessions was 24.63%, and average expected heterozygosity was 0.10 (standard error = 0.001).

Commercial standards were below the average for PLP and expected heterozygosity, and were towards the lower end of the range of genetic diversity for all accessions analysed.

Table 3.8 Genetic diversity measures for *Lactuca sativa*. Proportion of polymorphic loci and heterozygosity for 20 HSL *Lactuca sativa* accessions and two commercial standards, based on two AFLP primer pairs (ACG/CTA and ACA/CAG), 103 loci; calculated in AFLP SURV using method 4 (a Bayesian method with non-uniform prior distribution of allele frequencies), assuming FIS = 1.

Accession name	Sample size	Proportion of polymorphic loci (PLP)	Expected heterozygosity (Hj)	S.E. (Hj)
Asparagus	5	11.7	0.09	0.01
Bath Cos	5	5.8	0.07	0.01
Black Seeded Samara	5	2.9	0.05	0.01
Bronze Arrow	5	16.5	0.12	0.01
Brown Bath Cos	5	4.9	0.06	0.01
Brown Goldring	5	6.8	0.07	0.01
Bunyard's Matchless	4	61.2	0.15	0.02
Burpee's Iceberg	5	5.8	0.06	0.01
George Richardson	4	61.2	0.13	0.02
Liller	5	9.7	0.08	0.01
Laitue Cracoviensis	4	60.2	0.14	0.02
Loos Tennis Ball	5	9.7	0.09	0.01
Maroulli Cos	5	62.1	0.14	0.02
Mescher	5	9.7	0.08	0.01
Northern Queen	5	11.7	0.10	0.01
Rouge D'Hiver	5	68.9	0.16	0.02
Soulie	5	69.9	0.18	0.02
Standard 1 – Iceberg	5	9.7	0.08	0.01
Standard 2 – Corsair	5	11.7	0.09	0.01
Stoke	5	7.8	0.07	0.01
White Seeded Samara	5	10.7	0.09	0.01
Windermere	5	23.3	0.16	0.02

Clustering between individuals

PCoA of the distribution of variation between individuals (within accessions) resulted in *L. sativa* accessions being separated into multiple small groups on the first two principal coordinates (Figure 10). There was a broad distribution of individuals and many small clusters

that contained one or two accessions. Accessions were regularly distributed along the length of the first co-ordinate, with the second co-ordinate separating out Soulie (17), Windermere (22) and Standard 1 (18) in particular. Single individuals from Asparagus, Bronze Arrow, Soulie, Standard 1, George Richardson and Bunyard's Matchless were separated from their main clusters. For most varieties individuals clustered tightly together, suggesting a degree of genetic homogeneity, including: Loos Tennis Ball and Mescher; Black Seeded Samara and Northern Queen; Brown Bath Cos and Brown Goldring; Bunyard's Matchless, Burpee's Iceberg and George Richardson. Individual 17.4 (Soulie) was a large distance from the rest of the Soulie individuals; as were individuals 7.1 (Bunyard's Matchless) and 9.4 (George Richardson). Accession Rouge D'Hiver was very broadly distributed suggesting high heterogeneity; Black Seeded Samara and Northern Queen were next to each other, tightly clustered within accession but not overlapping, as were Stoke and Asparagus. Liller, Standard 1, Windermere, Soulie and White Seeded Samara individuals clustered together but were distant from other clusters. This was reflected also in the clustering between varieties derived using Nei's genetic distance (Figure 3.10).

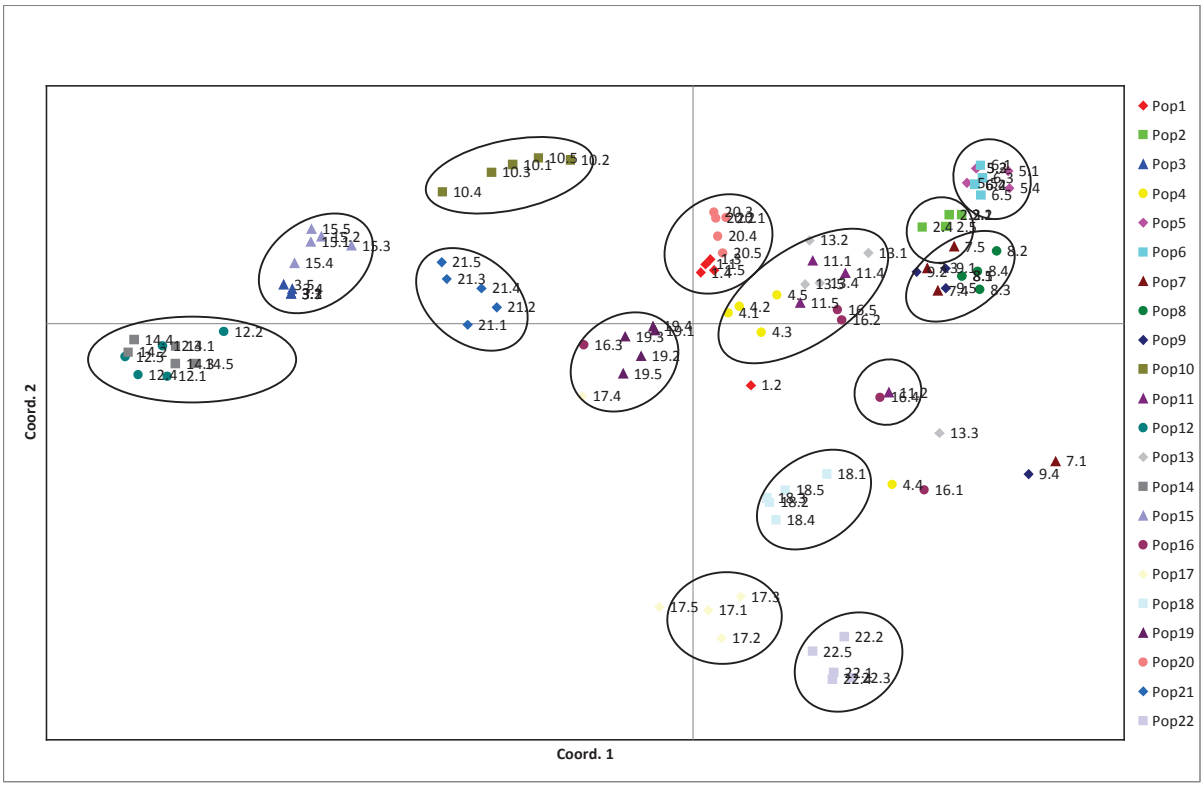

Figure 3.10 Principal Coordinates Analysis (PCoA) plot of *Lactuca sativa* individuals. Principal Coordinate Analysis (PCoA) plot clustering between individuals of 20 HSL *Lactuca sativa* accessions and two commercial standards, using between four and five individuals per accession. Based on AFLP markers, using two primer pairs (ACG/CTA and ACA/CAG), 103 loci, using presence/absence data analyses in GenAlEx. Cumulative variation present in the first three principal coordinates was 27.22%, 48.00% and 64.99% respectively. Numbers represent accessions: Asparagus (1), Bath Cos (2), Black Seeded Samara (3), Bronze Arrow (4), Brown Bath Cos (5), Brown Goldring (6), Bunyard's Matchless (7), Burpee's Iceberg (8), George Richardson (9), Laitue Cracoviensis (11), Liller (10), Loos Tennis Ball (12), Maroulli Cos (13), Mescher (14), Northern Queen (15), Rouge D'Hiver (16), Soulie (17), Standard 1 (Iceberg) (18), Standard 2 (Corsair) (19), Stoke (20), White Seeded Samara (21), Windermere (22). Circles highlight potential clusters of similar individuals.

Genetic distance and relationships between accessions

From the UPGMA cluster analysis using Nei's genetic distance, *L. sativa* accessions were split into many small clusters, on long branches indicating large genetic distance (this reflected the above PCoA result). Several of the branches on the genetic distance dendrogram (Figure 3.11) were well supported, with the splitting off from the rest of the accessions by Asparagus (1) and Laitue Cracoviensis (11). Very low genetic distance was indicated between accessions: Brown Bath Cos and Brown Goldring; Bunyard's Matchless and George Richardson; and Loos Tennis Ball and Mescher, suggesting that these accessions may be duplicates. The relationship between Bronze Arrow and Rouge D'Hiver was well supported. Low supporting values for other branches may suggest either low resolution between accessions, or that accessions may not be distinct entities and they have overlap in genetic variation between accessions. These very short genetic distances between the accessions will be explored more fully with reference to morphological data that may clarify whether these accessions are distinct or possible duplicates. The branch separating Maroulli Cos, Brown Bath Cos and Brown Goldring from other accessions was also over 50%.

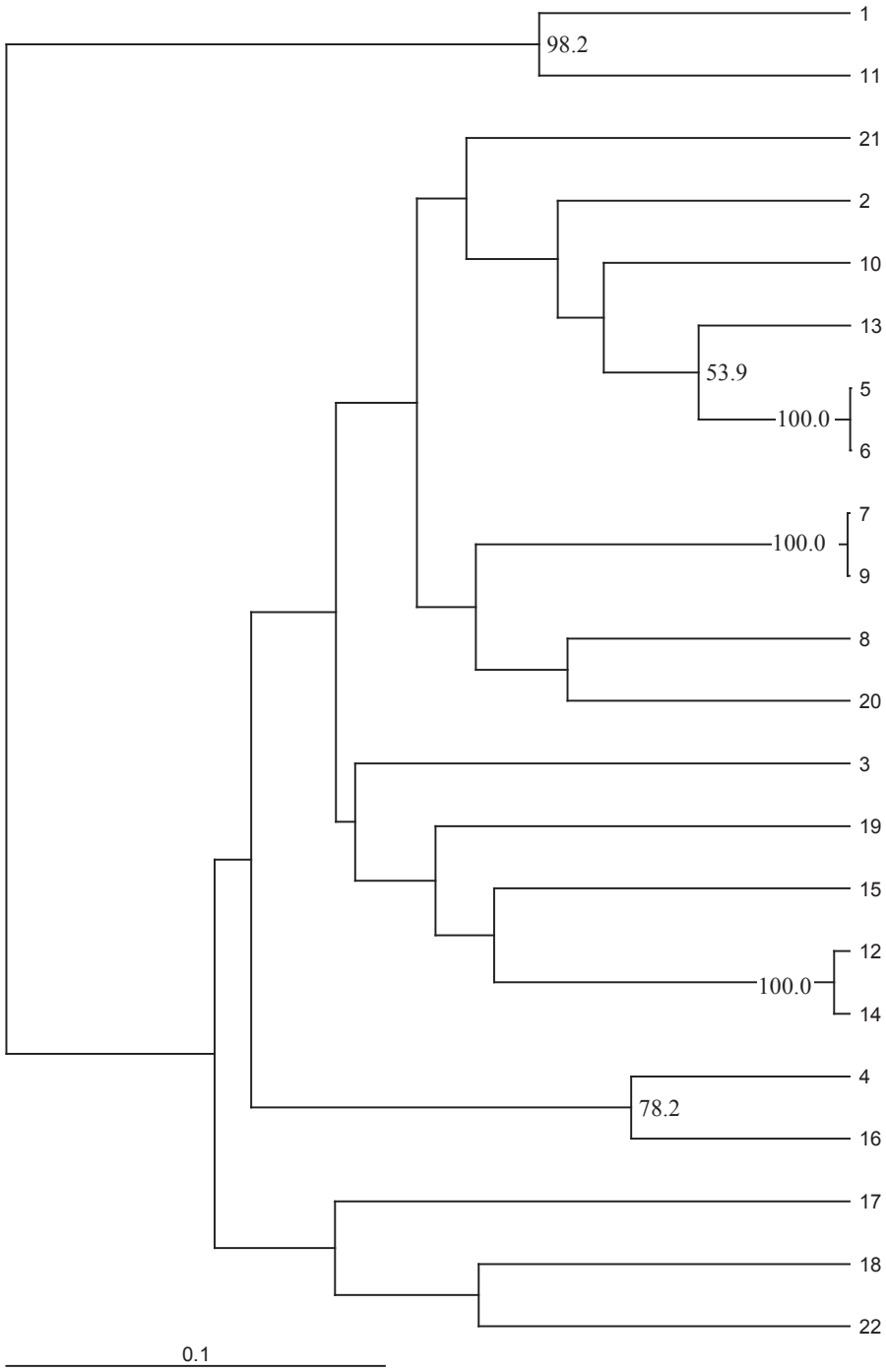

Figure 3.11 UPGMA genetic distance dendrogram for *Lactuca sativa* accessions. UPGMA dendrogram showing Nei's genetic distance relationships for 20 HSL *L. sativa* accessions and two commercial standards, four to five individuals sampled per accession; derived from two AFLP primer pairs ACG/CTA and ACA/CAG), using 103; FIS = 1. Genetic distance calculated in AFLP SURV; bootstrap values calculated in Phylip using Neighbour and Consense; tree visualised in TreeView. Numbers represent accessions see figure above.

Principal coordinate analysis examining variation and genetic distance between accessions showed most accessions widely separated. Three small clusters were defined by the first and second principal co-ordinates, which explained 49.17% of the cumulative variance (Figure 3.12). Accessions clustering together (reflecting results above) were Bunyard's Matchless, Burpee's Iceberg and George Richardson; Bath Cos, Brown Bath Cos and Brown Goldring; Loos Tennis Ball and Mescher. Other accessions were distributed fairly widely.

The same analysis was carried out assuming Hardy Weinberg equilibrium, and gave the same results, although with smaller genetic distance between accessions Standard 1 (18) and Windermere (22).

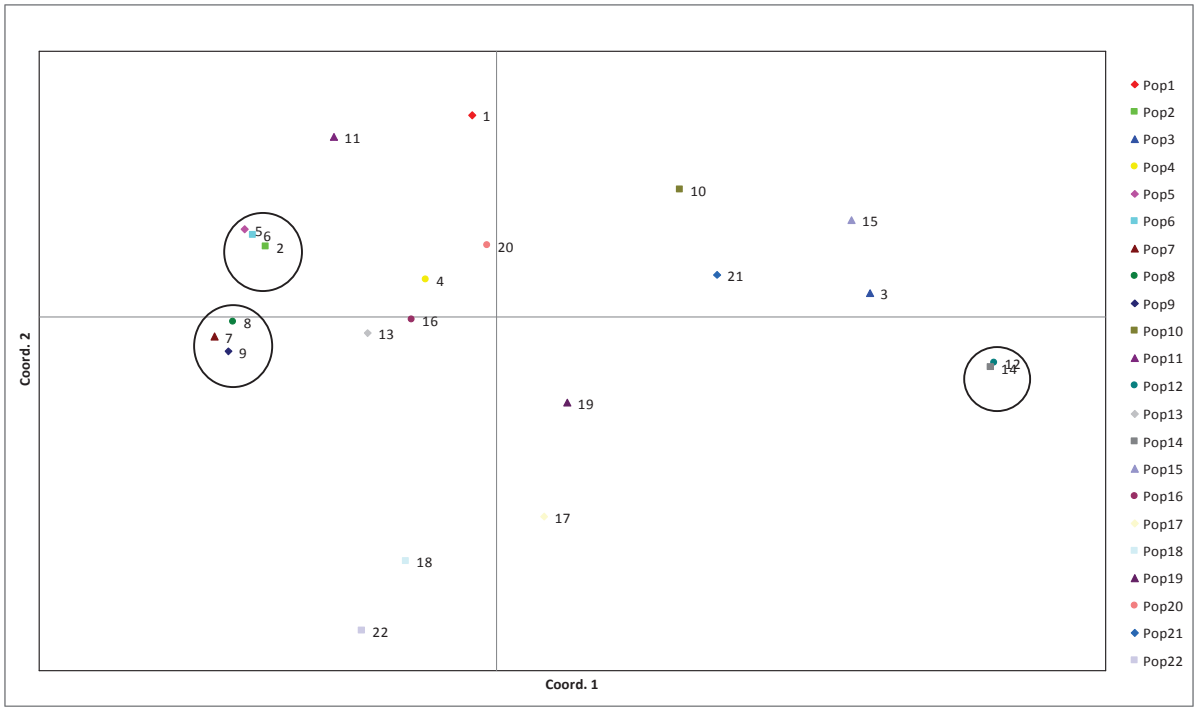

Figure 3.12 Principal Coordinates Analysis plot of *Lactuca sativa* accessions. Principal Coordinate Analysis (PCoA) plot of clustering between 20 HSL *L. sativa* accessions and two commercial standards, between four and five individuals were sampled per accession. Nei's genetic distance calculated in AFLP SURV using non-Hardy Weinberg equilibrium assumption (FIS=1); derived from two AFLP primer pairs (ACG/CTA and ACA/CAG), 103 loci. PCoA performed in GenAlEx. Cumulative variation present in the first three principal coordinates was: 27.99%, 49.17% and 66.66% respectively. Numbers represent accessions, see figure above. Circles highlight potential clusters of similar accessions.

3.3.5 Cucumis sativus

Two primer combinations (AAC/CTC and ACG/CGA) were used to analyse eleven HSL *C. sativus* accessions and two commercial standards (Telegraph Improved and Burpless Tasty Green F1 Hybrid). A total of 399 loci were generated, of which 286 were both polymorphic

and of sufficient quality for analysis. Three accessions (Striped and Sweet, Kiwano African Horned and West India Burr Gherkin) were found to be too different from other accessions to be included in the AFLP analysis, as they caused severe data bias. Their names suggest that these latter two accessions are most likely *Cucumis metuliferus* (species Kiwano) and *Cucumis anguria* (species West Indian gherkin) respectively (Meglic *et al*., 1996). From the final selection of eight *C. sativus* HSL accessions and two standards, 139 loci were used; two samples were removed due to poor trace quality; error rate for the final data set was 2.77%, based on 15.5% of samples being repeated. Error rate for individual loci was below 0.1 for all loci except five (error rate of 0.2).

Fst for the dataset was 0.32 (standard error = 0.14), which indicates very high differentiation between populations.

Genetic diversity

The level of genetic diversity varied widely across accessions; the percentage of polymorphic loci ranged from 18.0% in standard 2, to 77.7%, in Izjastnoi (Table 3.9). Expected heterozygosity ranged from 0.12 in standard 2 to 0.37 in Izjastnoi. The average PLP for all accessions was 57.76%, and average expected heterozygosity was 0.22 (standard error = 0.02).

There was a large difference between genetic diversity levels for the two commercial standards; Standard 2 was the lowest of all accessions/varieties sampled, for both PLP and expected heterozygosity, however Standard 1 had PLP of 68.3% and expected heterozygosity of 0.24, which is of a comparable level to HSL accessions.

Table 3.9 Genetic diversity measures for *Cucumis sativus*. Proportion of polymorphic loci and heterozygosity for eight HSL *Cucumis sativus* accessions and two commercial standards, based on two AFLP primer pairs (AAC/CTC and ACG/CGA), 139 loci; calculated in AFLP SURV using method 4 (a Bayesian method with non-uniform prior distribution of allele frequencies), assuming FIS = 1.

Accession name	Sample size	Proportion of polymorphic loci (PLP)	Expected heterozygosity (Hj)	Standard error (Hj)
741 Peking China	5	40.3	0.15	0.02
Jordanian	5	52.5	0.21	0.02
Butcher's Disease Resisting	5	58.3	0.21	0.02
Dekah	4	69.8	0.27	0.02
Izjastnoi	4	77.7	0.37	0.02
Boothby's Blond	5	64.7	0.22	0.02
King of the Ridge	5	56.1	0.16	0.02
Sigmadew	5	71.9	0.22	0.02
Standard 1 – Telegraph Improved	3	68.3	0.24	0.02
Standard 2 – Burpless Tasty Green F1 Hybrid	5	18	0.12	0.01

Clustering between individuals

The principal coordinate analysis of *C. sativus* individuals separates accessions into four main clusters (Figure 3.13). 741 Peking China (accession 1) was the most clearly separated from all other accessions on both principal co-ordinates. Principal coordinate one (explaining 42.11% of the variance) separated clusters comprising King of the Ridge and Standard 1, from other accessions. Principal coordinate two (explaining 26.31% of the variance) and principal coordinate one separated the cluster comprising three individuals of Jordanian and two of Izjastnoi (accessions 2 and 5). The remaining individuals and accessions were separated on principal coordinate two. Many accessions were very widely distributed and had individuals in with clusters of other accessions, namely Jordanian, Izjastnoi, Dekah and Butcher's Disease Resisting. Accessions with widely spread individuals (but still clustered together) were 741

Peking China, Boothby's Blond, King of the Ridge, Butcher's Disease Resisting. Tightly clustered individuals were seen in Standard 2.

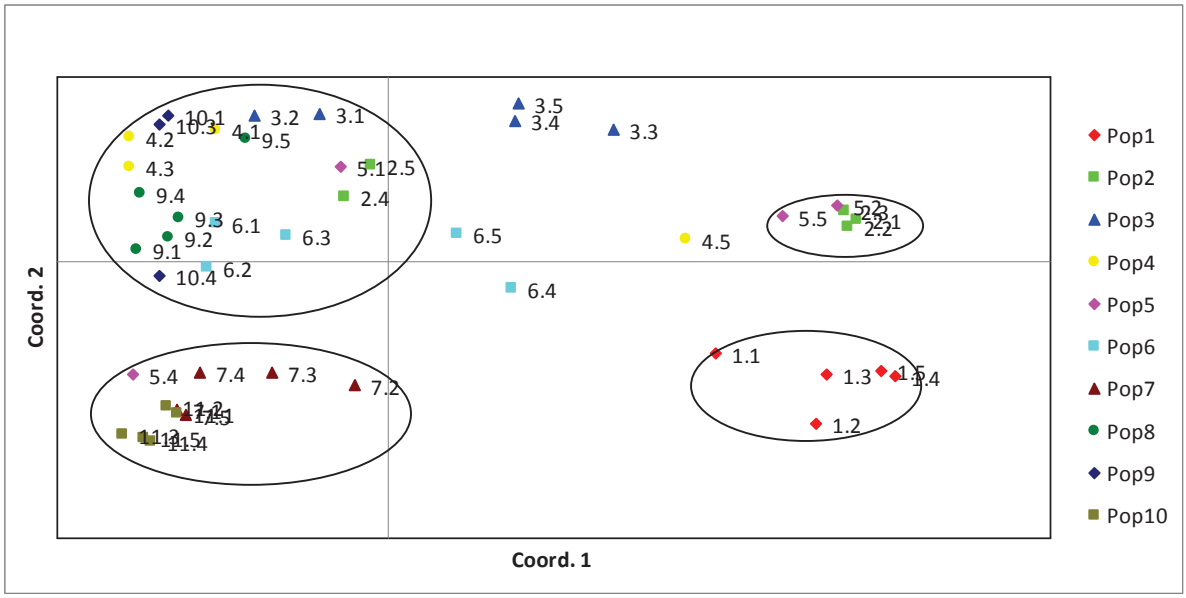

Figure 3.13 Principal Coordinates Analysis (PCoA) plot of *Cucumis sativus* individuals. Principal Coordinates Analysis (PCoA) plot clustering of individuals from eight HSL *C. sativus* accessions and two commercial standards, between two and five individuals sampled per accession, derived in GenAlEx, obtained using presence/absence data from 139 loci from two AFLP primer pairs. Numbers represent accessions. 741 Peking China (1), Jordanian (2), Butcher's Disease Resisting (3), Dekah (4), Izjastnoi (5), Boothby's Blond (6), King of the Ridge (7), Sigmadew (9), Standard 1 (10), Standard 2 (11). Circles highlight potential clusters of similar individuals.

Genetic distance and relationships between accessions

UPGMA analysis, using Nei's Genetic Distance measure, separated accessions into three clusters (Figure 3.14). 741 Peking China was the accession most genetically distant to the others, with a moderately well supported bootstrap value (67.9%). The first cluster comprised

King of the Ridge and Standard 2 and was well supported with a bootstrap value of 81.5%. The cluster comprising Dekah, Sigmadew and standard 1, was also well supported (with bootstrap values of 84.1% and 89.0%). The branch lengths between Sigmadew and standard 1 were the shortest, indicating a lower genetic distance.

When this analysis was run assuming Hardy Weinberg equilibrium, the relationship between Sigmadew and standard 1 was maintained; however, no other clusters were visible.

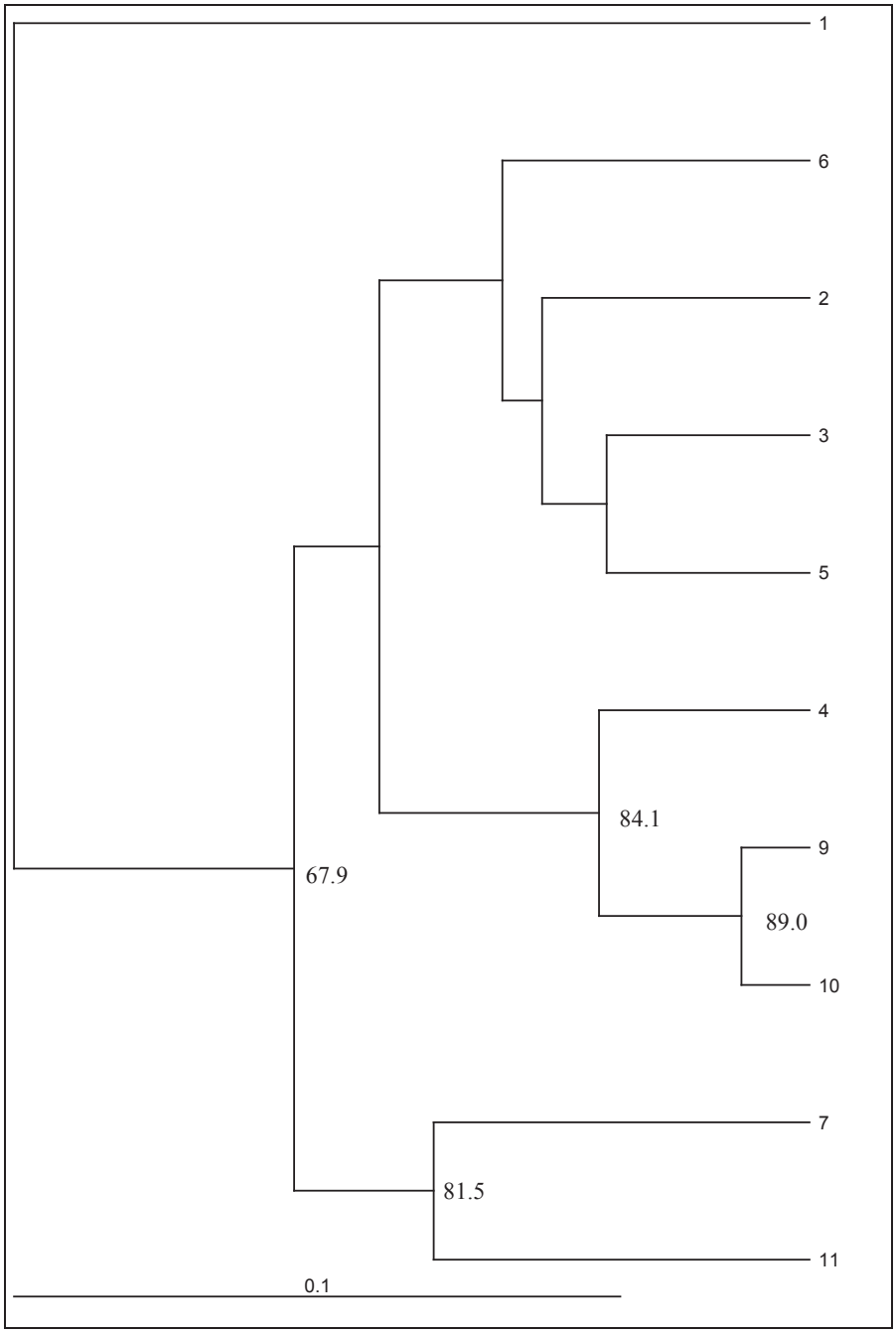

Figure 3.14 UPGMA genetic distance dendrogram for *Cucumis sativus* accessions. UPGMA genetic distance tree for 8 HSL *C. sativus* accessions and two commercial standards, using AFLP markers. Two primer pairs were used (AAC/CTC and ACG/CGA) providing 139 polymorphic loci. Nei's genetic distance calculated AFLP SURV using method 4 ('a Bayesian method with non-uniform prior distribution of allele frequencies'); FIS = 1; bootstrap values calculated in Phylip using Neighbor and Consense; tree visualised in TreeView. Numbers represent accessions, see figure above.

In a PCoA derived from Nei's genetic distance (Figure 3.15), accession 741 Peking China was again separated from the other accessions; Sigmadew and Standard 1 clustered together; King of the Ridge and Standard 2 were nearer to one another than to other accessions, and the rest were distributed in between. The variance explained by the first three principal coordinates was 42.11%, 26.31% and 10.34%, respectively.

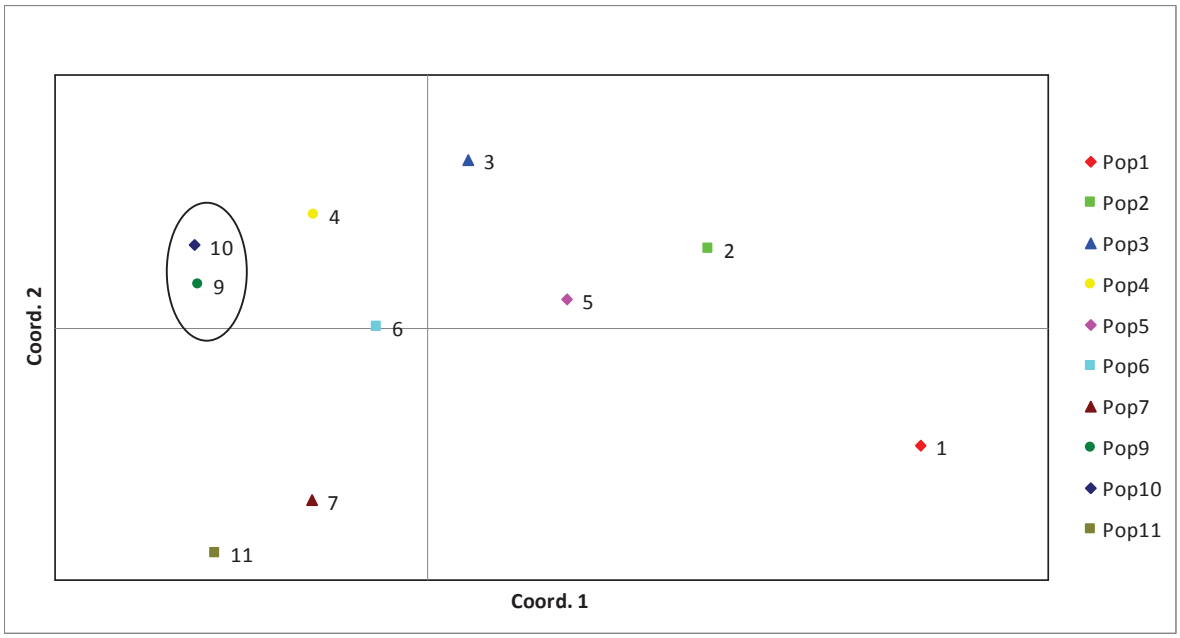

Figure 3.15 Principal Coordinates Analysis (PCoA) plot of *Cucumis sativus* accessions. Principal Coordinates Analysis (PCoA) plot clustering between 8 HSL *C. sativus* accessions and 2 commercial standards, using Nei's genetic distance, derived using two AFLP primer pairs (AAC/CTC and ACG/CGA), 139 loci. Genetic distance calculated in AFLP SURV using method 4 ('a Bayesian method with non-uniform prior distribution of allele frequencies'), PCoA in GenAlEx. FIS=1. Cumulative variation explained in the first three principal coordinates was: 42.11%, 68.42% and 78.76% respectively. Numbers represent accessions, see figure above. Circles highlight potential clusters of similar accessions.

3.3.6 Brassica oleracea var. *acephala*

Two primer pair combinations (AAC/CTA and ACC/CAG) were used to analyse 12 HSL *B. oleracea* var. *acephala* accessions and two commercial standards (F1 Redbore and Dwarf Green Curled), resulting in 242 loci, of which 118 were both polymorphic and of sufficient quality for analysis. Nine samples were removed due to poor trace quality, of these four each were from accessions Asparagus and Uncle Bert's Purple, leaving only one sample for each; these accessions were therefore included in the GenAlEx presence/absence principal coordinates analysis, but were excluded from AFLP SURV genetic diversity and genetic distance calculations. The error rate (including all 14 accessions) was 1.3% (based on the replication of 21.3% of samples). Two individual loci had an error rate of 0.13 and two had a rate of 0.23.

Fst was 0.7 (standard error = 0.03) indicating very high differentiation. This is reflected in all analyses below, with a split in the collection between two groups composed of two separate species: *Brassica oleracea* var. *acephala* (kale) and *Brassica napus* var. *pabularia* (leaf rape, Siberian kale or rape kale) (Cartea *et al*., 2005) (all are listed as *Brassica oleracea* var. *acephala* (kale) in the HSL collection.

Genetic diversity

There was a very large difference in genetic diversity between the *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions analysed, with a higher level of genetic diversity in the *B. oleracea* var. *acephala* accessions, than the *B. napus* var. *pabularia* (with the exception of the accession Russian/Hungry Gap, which is higher in diversity than other *B. napus* var.

pabularia accessions) (Table 3.10). The proportion of polymorphic loci ranged from 1.7% in Asparagus Marshal Curtis Mill to 58.5% in Spis Bladene; expected heterozygosity ranged from 0.05 in Asparagus, Marrowfat Greens and Ragged Jack, to 0.2 in Spis Bladene. The average proportion of polymorphic loci for all accessions was 25.7%, and the average expected heterozygosity was 0.1 (standard error = 0.01); however, due to the large difference in genetic diversity levels between the two groups these averages do not accurately represent the genetic diversity of either group. The averages for the *B. oleracea* var. *acephala* group were 45.62% and 0.15 for PLP and Hj, respectively. The averages for the *B. napus* var. *pabularia* group were 11.49% and 0.06 for PLP and Hj, respectively. The two commercial standards were in the *B. oleracea* var. *acephala* group with higher genetic diversity with PLP of 55.1% and 49.2% and expected heterozygosity of 0.14 and 0.15 respectively. Asparagus and Uncle Bert's Purple did not have enough individuals to be included in the analysis at this stage.

Table 3.10 Genetic diversity measures for *Brassica oleracea* var. *acephala* and *Brassica napus* var. *pabularia*. AFLP SURV calculations for proportion of polymorphic loci and heterozygosity (Hj) for 10 *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions and 2 commercial standards, based on two AFLP primer pairs (AAC/CTA and ACC/CAG), 118 loci, analyses performed on accessions with between two and five individuals sampled per accession using method 4 (a Bayesian method with non-uniform prior distribution of allele frequencies).

Accession name	*Species (and population number in brackets)*	Pop number	Sample size	Proportion of polymorphic loci (PLP)	Expected heterozygosity (Hj)	Standard error (Hj)
Georgia Southern Collard	*B. oleracea* var. *acephala*	4	5	45.8	0.14	0.02
Spis Bladene	*B. oleracea* var. *acephala*	11	5	58.5	0.20	0.02

Standard 1- F1 Redbore	*B. oleracea* var. *acephala*	12	5	55.1	0.14	0.02
Standard 2 – Dwarf Green Curled	*B. oleracea* var. *acephala*	13	5	49.2	0.15	0.02
Tall Kale	*B. oleracea* var. *acephala*	14	5	19.5	0.12	0.02
Asparagus	*B. napus* var. *pabularia*	1	1			
Asparagus Marshall Curtis Mill	*B. napus* var. *pabularia*	2	5	1.7	0.05	0.01
Canadian Ragged Jack	*B. napus* var. *pabularia*	3	5	5.1	0.06	0.01
Madeley	*B. napus* var. *pabularia*	5	5	5.9	0.06	0.01
Marrowfat Greens	*B. napus* var. *pabularia*	6	5	4.2	0.05	0.01
Ragged Jack	*B. napus* var. *pabularia*	7	5	2.5	0.05	0.01
Red Russian	*B. napus* var. *pabularia*	8	5	8.5	0.07	0.01
Russian/Hungry Gap	*B. napus* var. *pabularia*	9	4	52.5	0.11	0.01
Uncle Bert's Purple	*B. napus* var. *pabularia*	15	1			

Clustering of individuals within accessions

Four clusters were visible using binary presence/absence data to group accessions (Figure 3.16). The two smaller clusters consisted of individuals from Marrowfat Greens (16), and individuals from Tall Kale (14); the first larger cluster (*B. napus* var. *pabularia*), separated on principal coordinates one and two, had accessions and individuals tightly clustered together, suggesting relative homogeneity, the second larger cluster (*B. oleracea* var. *acephala*), separated on principal coordinate two were more widely distributed, indicating heterogeneity. Asparagus (individual 1.1) sat within the individuals from Asparagus Marshall Curtis Mill (2), suggesting a close relationship, and Uncle Bert's Purple (15.1) sat in the *B. napus* var. *pabularia* cluster near populations of Ragged Jack (7) and Red Russian (8).

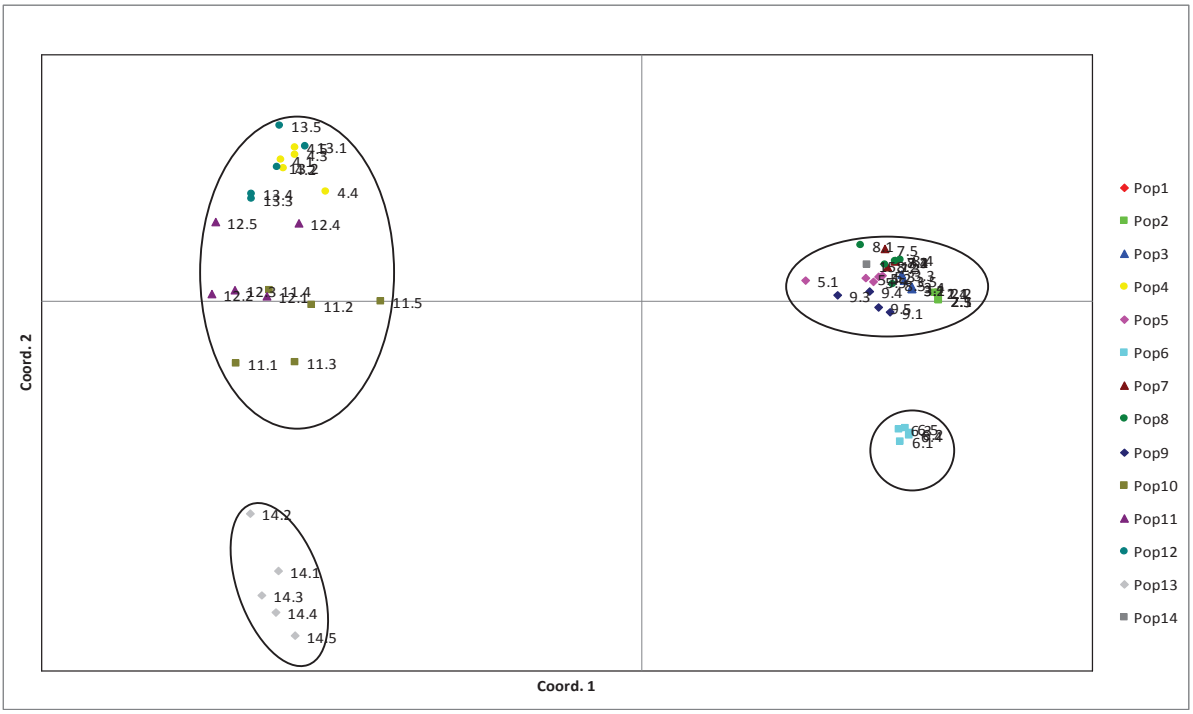

Figure 3.16 Principal Coordinates Analysis (PCoA) plot of *Brassica oleracea* var. *acephala* and *Brassica napus* var. *pabularia* individuals. Principal Coordinates Analysis (PCoA) plot with clustering between individuals from 12 *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions and 2 commercial standards, between one and five individuals per accession, derived in GenAlEx obtained using presence/absence data from 118 loci from two AFLP primer pairs (AAC/CTA and ACC/CAG). Cumulative variation explained within the first three co-ordinates is 59.57%, 70.94% and 80.50% respectively. Numbers represent accessions: Asparagus (1) Asparagus Marshall Curtis Mill (2) Canadian Ragged Jack (3) Georgia Southern Collared (4) Madeley (5) Marrowfat Greens (6) Ragged Jack (7) Red Russian (8) Russian/Hungry Gap (9) Spis Bladene (11) Standard 1 (12) Standard 2 (13) Tall Kale (14) Uncle Bert's Purple (15). Circles highlight potential clusters of similar individuals.

Genetic distance and relationships between accessions

The UPGMA analysis separated *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions into two distinct clusters (Figure 3.17). The first cluster comprised Tall Kale (14), Georgia Southern Collard (4), Spis Bladene (11), Standard 1 (12) and Standard 2 (13), with long branch lengths indicating higher genetic distance, and many well supported nodes. The most distant accession was Tall Kale (14), which reflected the results above. The second cluster had much shorter branches, indicating less genetic distance; the separation of Marrowfat Greens (6) was well supported, indicating greater genetic distance, as was the separation of accessions Asparagus Marshall Curtis Mill (2) and Madeley (5) from the others, and the very close relationship between Ragged Jack (7) and Red Russian (8).

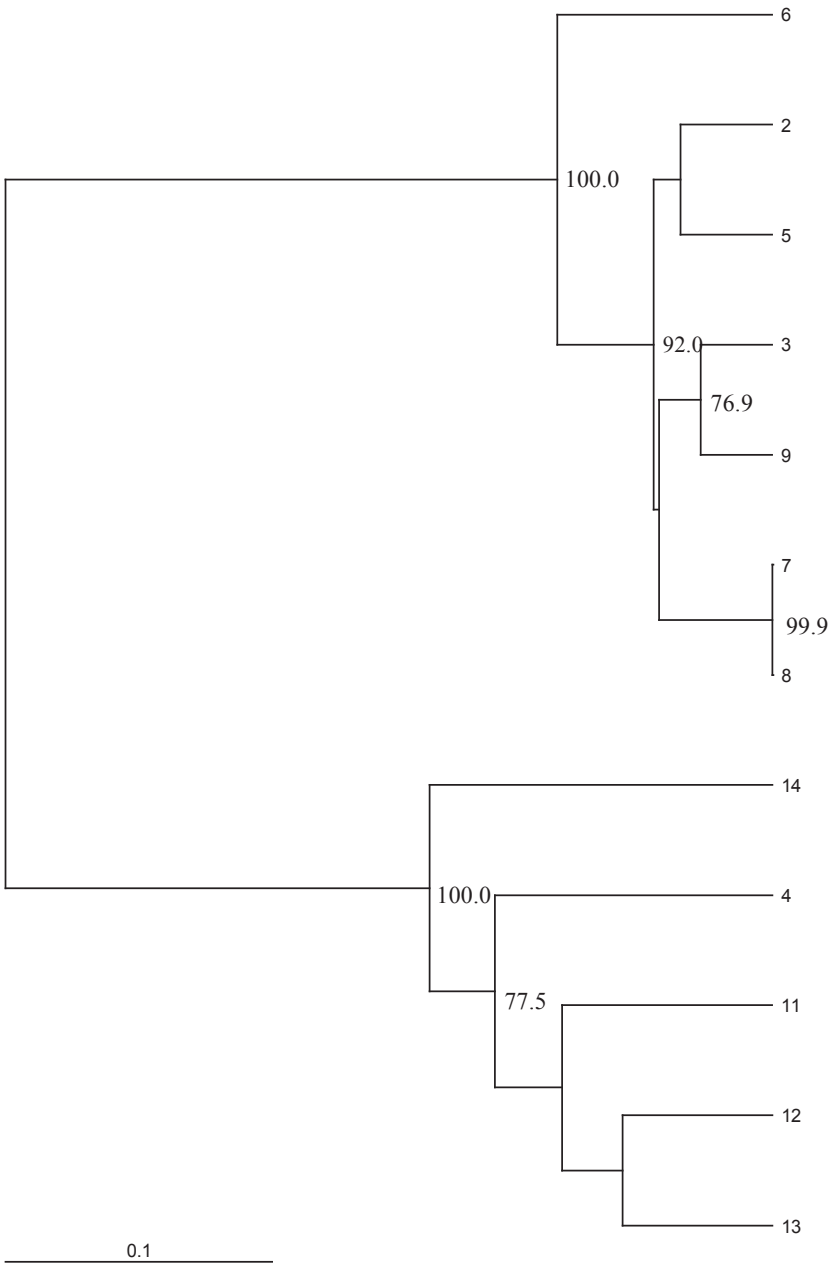

Figure 3.17 UPGMA genetic distance tree for *Brassica oleracea* var. *acephala* and *Brassica napus* var. *pabularia* accessions. UPGMA genetic distance tree of 10 HSL *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions and two commercial standards, two to five individuals sampled per accession, derived using AFLP markers with two primer pairs (AAC/CTA and ACC/CAG), 118 loci. Nei's genetic distance calculated in AFLP SURV, FIS = 1; bootstrap values calculated in Phylip using Neighbor and Consense and tree visualised using TreeView. Numbers represent accessions as in above figure.

The first two principal coordinates, using Nei's genetic distance, explained 65.66% and 10.45% of the variance present within the *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions (Figure 3.18). Accessions showed clear clustering or separation, with Marrowfat Greens (6) separated on the second principal coordinate; Spis Bladene (11) and Standard 1 (12) were separated on the first principal coordinate; and Tall Kale (14) was separated on both the first and second principal coordinates. Accessions Georgia Southern Collard (4) and Standard 2 (13) were also separated on the first principal coordinate. Remaining accessions clustered tightly, with low genetic distance between them.

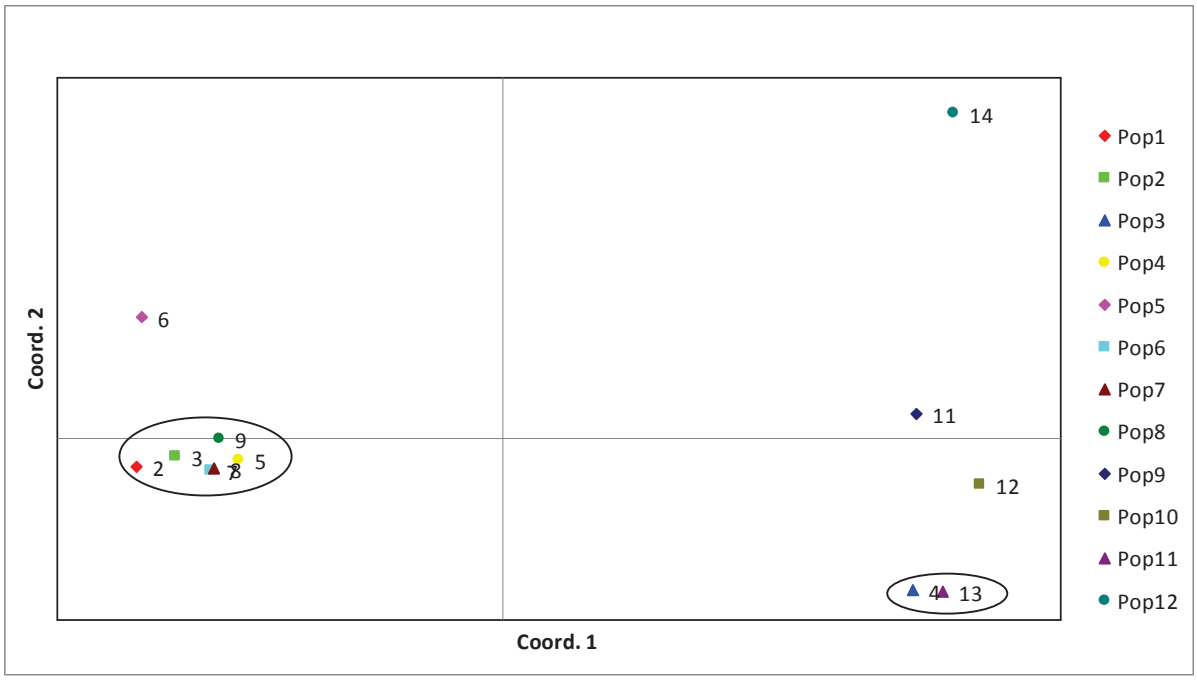

Figure 3.18 Principal Coordinates Analysis (PCoA) plot of *Brassica oleracea* var. *acephala* and *Brassica napus* var. *pabularia* accessions. Principal Coordinates Analysis (PCoA) plot for clustering between 10 *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions and 2 commercial standards (between two and five individuals per accession), from 118 AFLP loci over two primer pairs (AAC/CTA and ACC/CAG), using Nei's genetic distance calculated in AFLP SURV using method 4, and visualised using GenAlEx. Cumulative percentage of variation explained within the first three principal coordinates was 65.66%, 76.11% and 84.11% respectively. Numbers represent accessions, see figure above. Circles highlight potential clusters of similar accessions.

3.4 Discussion

The primary questions addressed in the AFLP portion of the present study were: what genetic diversity is there within the HSL collection, what diversity exists within and between HSL accessions, how does diversity in the HSL accessions compare with that in commercial

standards, are there any groups of similar accessions, and are there any duplicate accessions? These will be discussed below.

3.4.1 Genetic diversity

Genetic diversity was found to be generally high in *Vicia faba*, to be low in *Lactuca sativa* and *Pisum sativum*, and ranges of genetic diversity levels were measured in *Daucus carota*, *Cucumis sativus* and *Brassica oleracea* var. *acephala*.

The results of the *Vicia faba* analysis demonstrated that there was a great deal of genetic diversity present within and between HSL accessions; average genetic diversity and differentiation between accessions was high. Genetic diversity was lowest in Beryl and highest in Red Bristow's and Brown. Although the inclusion of different accessions and different numbers of accessions between studies can make comparisons difficult (Sanz *et al*., 2007), these are comparable to those found in *V. faba* by Ouji *et al*. (2011) studying isozymes in Tunisian material. Average expected heterozygosity was 0.25 (standard error = 0.012); this was above the average found by Zong *et al*. (2009), and was comparable to the values given in Sanz *et al*. (2007) (although this study again utilized SSAP transposons) and Zong *et al*. (2010) (AFLPs) and Ouji *et al*. (2011) (isozymes).

The HSL *D. carota* accessions contained a large amount of genetic diversity both within and between accessions, with high genetic diversity and differentiation. There was a narrow range of genetic diversity, which was lowest in Standard 1, and highest in Giant Improved Flak. There are no comparable AFLP genetic diversity studies in *D. carota*; however the higher genetic diversity found in many of the heritage varieties reflect the general patterns found in

Shim and Jorgensen (2000), who reported a division between wild species, old cultivars (equivalent to heritage varieties) and commercial varieties, with genetic diversity decreasing respectively. Isozyme studies in carrot (St Pierre and Bayer, 1991) give comparable levels of genetic diversity for both expected heterozygosity and PLP, including lower estimates for F1 hybrid lines (as would be expected). Lower expected heterozygosity seen in the accessions John's Purple, Afghan Purple and White Belgium, may stem from their unique colouring, which may have led to more rigorous roguing, as genetic deviants may be more noticeable (Le Clerc *et al*., 2005).

Genetic diversity was generally low in *Pisum sativum* accessions and differentiation between accessions was very high (suggesting little gene flow between accessions). Genetic diversity was lowest in Harold Idle, Holland Capucijner's, Lancashire lad, Newick, Stokesley, Sutton's Harbinger and Table Talk and highest in Latvian and Latvian Grey Pea. Comparable studies were not available for *P. sativum* (other studies used different measure of diversity, such as observed heterozygosity of Polymorphic Information Content (PIC), however these levels are consistent with *P. sativum* being a strict in-breeder (Simioniuc *et al*., 2002).

For *Lactuca sativa*, a very high level of varietal differentiation was measured (again, potentially indicating little gene flow between accessions). The level of genetic diversity varied widely across accessions in the collection, particularly in PLP, but was generally low. Levels were lowest in Black Seeded Samara and highest in Soulie. The broad range of PLP values encompass those found in previous studies (Yang *et al*., 2007). Average expected heterozygosity is lower than that found in the overall total of the AFLPs in van Treuren and van Hintum (2009), however the individual expected heterozygosities of each lettuce type are

of comparable range. *L. sativa* is predominantly self-fertilizing (although some cross pollination may take place and lead to heterozygotes (van Treuren and van Hintum, 2010), therefore low heterozygosity is in accordance with expectations (Jensen *et al*., 2006).

Varietal differentiation was high in *Cucumis sativus*. The level of genetic diversity varied widely across accessions; it was lowest in Standard 2 and highest in Izjastnoi. No previous AFLP studies were available for comparison. Staub *et al*. (2002) investigated 11 allozyme loci in cucumber germplasm from various sources including geographic regions, various dated cultivars and landraces; expected heterozygosity for the current study was slightly higher than the average found but covered a very similar range.

B. oleracea var. *acephala* accessions analyses showed a split into two groups, which can be recognised as two separate species *B. oleracea* var. *acephala* and *B. napus* var. *pabularia*. These groups were observed in all genetic distance analyses, and overall population differentiation was very high (Fst = 0.7, standard error = 0.03). The two species groups had very different genetic diversity levels, with the *B. oleracea* var. *acephala* group much higher than the other, this may reflect the relative outbreeding and inbreeding nature of each crop (Gowers, 2010). *B. oleracea* var. *acephala* is an outbreeding species that suffers readily from inbreeding depression, whereas *Brassica napus* species are self-fertile and tolerant of inbreeding (Gowers, 2010). The most genetically diverse accession was Spis Bladene and the least diverse were Asparagus Marshall Curtis Mill, Marrowfat Greens and Ragged Jack.

The genetic diversity (PLP and Hj) for *Daucus carota*, *Cucumis sativus*, *Brassica oleracea* var. *acephala* and *Vicia faba* accessions may be underestimated when assuming that FIS=1,

when using AFLP SURV, since these crops are out-breeding (with the exception of the commercial standards from *D. carota* and Standard 2 for *C. sativus*, which are F1 hybrids). However, the assumption of complete inbreeding still shows the marked difference between accessions, and the same genetic relationships (see below).

The genetic diversity found generally reflects that found in other studies for these crops (see references above), and also reflects the background of crops relating to breeding system and cultivation history, for example the high genetic diversity in outbreeding crops *D. carota* and *V. faba*, and lower relative genetic diversity in inbreeding *P. sativum* and *L. sativa*, and the narrowing of the genetic base of cultivated *C. sativus* and some *D. carota*.

Varietal differentiation levels (Fst) are also broadly consistent with previous studies, with relative levels less for out breeding crops and more distinction between populations for inbreeding crops (Hamrick and Godt, 1996). The exception was for *B. oleracea* var. *acephala*, which is an allogamous crop, which in the current study demonstrated very high differentiation. Levels of differentiation between populations were proportionally higher for all crops compared to previous studies (Loveless and Hamrick, 1984; Hamrick and Godt, 1996).

3.4.2 Groups of similar accessions

In *Vicia faba* no large clusters formed, had few highly supported nodes and had large genetic distances between most accessions. This reflects the general high level of genetic diversity and low genetic structure found in previous AFLP *V. faba* studies due to out crossing and plant breeding methods (Hamrick and Godt, 1996; Zeid *et al*., 2003; Zong *et al*., 2009; Duc *et*

al., 2010; Zong *et al*., 2010), with individuals forming a continuum of points rather than discrete clusters. Clusters were formed between Red Bristow's and Seville and between Chak'rusga and Cretian.

For *Daucus carota*, accessions were separated into three main clusters. These were composed of purple rooted accessions, a cluster composed of accessions Altringham and Red Elephant, and the remaining accessions including orange rooted and white rooted accessions. The current study was unable to resolve orange rooted relationships; this may be due to the overlapping nature of the diversity present. Clotault *et al*. (2010) discuss the split in carrot accessions between eastern and western carrots with reference to microsatellites (SSRs) and Single Nucleotide Polymorphisms (SNPs), and found purple carrots tend to cluster in the eastern group, the clusters found in the present study may reflect this; however since detailed backgrounds are not available this cannot be confirmed.

In *Pisum sativum*, accessions were distributed broadly, with around a third of accessions not part of a cluster. A further third of accessions, that clustered into small, tight groups indicating genetic similarity, and the remaining third were clustered loosely together, with a sub-cluster within of more closely related accessions.

For *Lactuca sativa*, accessions were very broadly distributed, in many small clusters based on individuals from one, two or three accessions. Three sets of accessions clustered closely: Bath Cos, Brown Bath Cos and Brown Goldring; Bunyard's Matchless, Burpee's Iceberg and George Richardson; Loos Tennis Ball and Mescher. The basis of larger clusters in the dendrogram does not tally completely with lettuce type as was found in previous studies

(Yang *et al*., 2007), however all highly supported nodes (mentioned above) are within types, except for Rouge D'Hiver and Bronze Arrow which are cos and leafy respectively. Yang *et al*. (2007) also did not separate leafy and cos accessions. Two of the three crisphead accessions clustered together. The lettuce type butterhead are all located within one cluster, except Liller (which is in a predominantly cos cluster), and for the presence of Standard 2 (Corsair), which is a cos lettuce. The practice of mixing lettuce types to obtain new varieties may have led to genuine blurring of these lettuce-type boundaries and increasing complexity of these relationships (van Treuren and van Hintum, 2000).

In *Cucumis sativus* accessions, 741 Peking China is the most distant accession; results suggest a relationship between Standard 1 and King of the Ridge, which are consistently separated from both 741 Peking China and other accessions, with the other accessions (particularly Sigmadew, Standard 1 and Dekah) forming a cluster. Since the history of the accessions is not known, it is only possible to speculate as to the reasons for these clusters. 741 Peking China could conceivably originate from China and therefore could represent a different gene pool; in previous studies Chinese germplasm has been compared to others and has grouped separately (Staub *et al*., 1999).

As discussed above, accessions identified in the HSL collection as *Brassica oleracea* var. *acephala* accessions split into two large clusters (consisting of *B. oleracea* var. *acephala* and *B. napus* var. *pabularia* accessions) and the accessions Marrowfat Greens and Tall Kale. Previous studies have found that *Brassica oleracea* var. *acephala* accessions cluster based on geographic origin, for example Okumus *et al*. (2007) and Zeven *et al*. (1998) (in closely related perennial kale (*Brassica oleracea* var. *ramosa*)). Both of these studies found relatively

low levels of intra-varietal variation. These studies differ to those results from Christensen *et al*. (2010), who found a continuous range of genetic diversity, with higher levels in those accessions still widely in cultivation. This was attributed to gene flow due to either cross-pollination or farmer seed exchange. Since detailed background information for many HSL *Brassica oleracea* var. *acephala* accessions is unavailable, a geographical component cannot be investigated. No geographic groupings were identified in this latter study, and the broad split in the accessions can be attributed to the presence of accessions of the species *B. napus* var. *pabularia*.

3.4.3 Comparing HSL accessions and commercial standards

Previous genetic studies comparing modern cultivars with landraces have found higher levels of genetic diversity in the former. Although the background of many heritage varieties is unknown (as discussed in chapter 3), it has been proposed that the genetic diversity of heritage varieties is on a spectrum, in between that of landraces *sensu stricto* and modern varieties (see chapter 3). This is consistent with the limited number of studies that examine heritage variety diversity (or the diversity of varieties that fit the definition of heritage varieties proposed by the current study, see chapter 3) (Shim and Jorgensen, 2000; Archak *et al*., 2002).

In the present study average genetic diversity was higher in HSL accessions than in commercial standards in *Vicia faba*. However, diversity levels were still high in the standard. This is consistent with previous studies of commercial and modern *V. faba* germplasm, which

support the suggestion that the commercial *V. faba* gene pool still retains much genetic diversity (for example Zeid *et al*., 2003; Duc *et al*., 2010).

Daucus carota standards were lower in genetic diversity than HSL accessions, which may be due to their being F1 hybrid varieties. Standard 1 was low in both PLP and Hj, and Standard 2 was low in Hj and below average in PLP.

In *Pisum sativum* the genetic diversity of the commercial standards was above average in Standard 1, and below average for standard 2.

Genetic diversity in commercial standards was towards the higher end of the range presented in the present study in *Brassica oleracea* var. *acephala* (no standards for *B. napus* var. *pabularia* were included as their present was unknown *a priori*).

In *Cucumis sativus* there was a large disparity between the levels of genetic diversity in the standards. Standard 2 was the lowest of all accessions/varieties sampled, for both PLP and Hj, however Standard 1 was of a comparable level to HSL accessions. Possible reasons for this disparity are firstly, that Standard 2 is a hybrid variety, and secondly, that Standard 1 is related to the original open-pollinated 'Telegraph' variety (Suttons, 2008).

In *Lactuca sativa,* commercial standards were below the average for PLP and expected heterozygosity, and were towards the lower end of the range of genetic diversity for all accessions analysed.

3.4.4 Potential duplicates

UPGMA Cluster Analysis, using Nei's genetic Distance was used to identify potential duplicate accessions, with short branch lengths between accessions identifying similar accessions. No duplicate accessions were identified for *Cucumis sativus* or *Daucus carota*.

In *Vicia faba* possible duplicates identified were Red Bristow and Seville.

No potential duplicates were identified in *Brassica oleracea* var. *acephala*; in *B. napu*s var. *pabularia* potential duplicate accessions were Ragged Jack and Red Russian.

In *Lactuca sativa*, Brown Bath Cos and Brown Goldring; Bunyard's Matchless and George Richardson; and Loos Tennis Ball and Mescher, were on very short branches, indicating that these accessions may be duplicates.

In *Pisum sativum* potential duplicates were: Alex and Stokesley; Carruther's Purple Podded and Dwarf Defiance/John Lee; Victorian Purple Podded, Lancashire Lad and Stephen's; Champion of England and Veitch's Western Express; Harold Idle and Panther's; Prince of Prussia, Jeyes and Ostgotaart; Prean, Cooper's Bean Pea and Mr Bethell's Bean Pea; and Commander and Purple Mangetout.

This gives a potential total redundancy in the collection of 10.6%, the majority of which is accounted for by *Pisum sativum* accessions (which is also the crop with the largest number of accessions). Those accessions identified as potential duplicates using the AFLP genetic distance data will be re-examined in the context of the morphological data (where available) in the final discussion chapter (chapter 6).

3.4.5 Methodological issues

AFLP markers were found to be an effective method of obtaining a high number of informative markers for the crops of the present study. Steps were taken to reduce potential errors that were due to the AFLP method itself, relating to homoplasy and scoring errors, as discussed in the materials and methodology section. The range of samples sizes found in the literature is quite broad; Simioniuc *et al*. (2002) used 20 samples per population; Kiambi *et al*. (2005) used 5-10 individuals; Terzopoulos and Bebeli (2008) used 10 individuals per population. The sample sizes used in the current study were smaller than those mentioned above, however the aim of the study was to survey a breadth of accessions, rather than depth, with indications of genetic diversity and distance being the goal. Due to small sample sizes, allele frequency estimates and Fst values should be treated with caution (Bonin *et al*., 2007), however the method using non-uniform prior distribution of alleles is more accurate at the 95% level than previous studies (Zhivotovsky, 1999), and levels within the same study can be compared without being affected by bias (Bonin *et al*., 2007). PCoA between individuals in GenAlEx is a band-based analysis, i.e. simple matching, which is therefore not reliant on allele frequency calculations and is less susceptible to sample size effects (Bonin *et al*., 2007) and could therefore be used to independently verify the later Nei's genetic distance results, confirming trends.

Both of the genetic diversity metrics used, proportion of polymorphic loci and expected heterozygosity, have drawbacks; PLP is vulnerable to differences in sample sizes between populations and heterozygosity is vulnerable to small sample sizes but they were used complementarily to gain overall relative trends (Mohammadi and Prasanna, 2003).

Two commercial standards were included in the analysis for each crop (with the exception of *Vicia faba*, for which the traces of the second standard were of insufficient quality, and so were removed); although this may not be sufficient to give a view of the full range of genetic diversity present in commercial crop varieties, it should give a broad indication of relative diversity. This is particularly applicable to the crops with larger numbers of accessions (especially *Pisum sativum*), where analysis of a larger number of standards relative to the HSL accessions may have been beneficial in order to capture a fuller range of diversity. The inclusion of known landrace material, or material from other gene banks (such as accessions with the same name) for comparison may also have been informative; however, given cost and time restraints this was not possible for the current project.

3.4.6 Conclusions

If the backgrounds of these varieties are unknown, then their genetic backgrounds, including how they were bred, whether they have been through a recent bottleneck, and the number of seeds/individuals from which they were bred, is also unknown. The work done here is a snapshot of where these accessions are now; small sample size, bottlenecks and founder effects are all relevant issues to *ex situ* collections. The genetic characterisation of accessions has three main implications for HSL. Firstly, the results of this analysis can be used to make judgements when resources are limited, a range of diverse and genetically distinct accessions from different groups could be conserved. Secondly, for the low diversity accessions identified, where information exists, HSL can explore potential causes of this low diversity, and can consider actions that may be taken to increase or maintain remaining diversity. Thirdly, potential duplicates are highlighted for further investigation by HSL.

The next chapter will characterise and investigate the morphology of the HSL collection, with five of the crops surveyed here (*Pisum sativum*, *Daucus carota*, *Cucumis sativus*, *Vicia faba* and *Lactuca sativa*) with additional supplementary crops to obtain an overview of morphological variation across a wide section of the collection.

CHAPTER 4 MORPHOLOGICAL CHARACTERISATION OF HERITAGE VARIETIES

4.1 Introduction

Having explored the genetic diversity and relationships present in the Garden Organic Heritage Seed Library (HSL) using Amplified Fragment Length Polymorphisms (AFLPs) in the previous chapter, this chapter will examine the morphological variation present both within and between accessions, and in comparison with commercial standards.

Although genetic studies of diversity now take a more central role in measurement and characterisation of Plant Genetic Resource (PGR) diversity, morphological studies are still of importance and relevance (Newbury and Ford-Lloyd, 1997). With the increased use and decreased cost of molecular marker technologies, and the move from searching for phenotypes to searching for genes (Tanksley and McCouch, 1997), morphological studies are increasingly incorporated into predominantly genetic studies. Although morphological studies can only provide an indirect measure of genetic diversity (Newbury and Ford-Lloyd, 1997), and may be limited in number (especially when compared to molecular markers (Karp *et al*., 1997; Laurentin, 2009), morphological characterisation is still an important step. Newbury and Ford-Lloyd (1997) state that the advantages of morphological analysis include the ease of use without the need for expensive technology, direct relevance of some characters to agronomy, and the visibility of characters that can be identified immediately and can assist in accession identification. The results of characterisation allow gene bank managers to identify duplicated accessions within collections, to identify accessions of particular interest to users, to match accessions to particular characters, and potentially to streamline collections through the establishment of core collections (Brown, 1995).

At a day-to-day level, organisations such as HSL need morphological characters to identify and prioritise accessions. This morphological work can also be used to verify or further explore AFLP study findings, such as identifying similar clusters and investigating potential duplicate accessions. The conservation and utilisation of plant genetic resources includes the vital step of characterisation of those accessions stored within field/gene/seed banks (Ford-Lloyd and Maxted, 1997).

Characterising collections such as the HSL, which maintain varieties that are no longer commercially available, means that a baseline is being established against which changes in future morphological characters (and the availability thereof) can be compared.

Currently a large proportion of the HSL is not available to members or other interested parties, either due to incomplete characterisation or insufficient seed stocks. The former of these problems is the rationale behind the current study: to characterise a substantial proportion of the collection, both to facilitate the identification by HSL of accessions that may be of interest to growers, based on their experience, such as particular traits of interest or varieties with unique traits, and also, in a wider context, to assess whether a collection of heritage varieties collected on an *ad hoc* basis over time, are not duplicates, are different to commercial standards and hold characters of potential interest for future breeding strategies.

The following chapter aims to answer the following wider thesis questions: what variation/diversity is present within and between accessions?; are there any duplicate accessions in the HSL collection?; are there groups of similar accessions within any of the crops?; and, how does the diversity of the HSL collections compare to those commercially available? The question of how these results relate to the findings of the AFLP study will then be addressed in chapter six.

This chapter will discuss the crops *Vicia faba*, *Pisum sativum*, *Daucus carota*, *Cucumis sativus*, *Lactuca sativa*, which were analysed using AFLPs in the previous chapter, along with additional crops *Solanum lycopersicum*, *Allium porrum*, *Brassica napobrassica*, *Brassica rapa* var. *rapa*, *Capsicum annuum* and *Raphanus sativus*, which were not included in the AFLP analysis. Brief crop backgrounds are given in Appendix three.

4.2 Materials and Methods

4.2.1 Crop selection

The HSL contains around 800 accessions from 30 crop species; selection of the crop species for inclusion in the current study was based on the number of accessions held, so for crops with only one accession, no comparisons could be made so they were not included. In the second year, remaining crops with fewer than four accessions were excluded. Accession selection was based on seed availability from HSL. Accessions included for each crop are listed in Appendix one.

Over two years 366 accessions were grown from 11 crops (Table 4.1). *Solanum lycopersicum* was grown in both years, due to the large number of accessions.

Table 4.1 Crop species grown for morphological characterisation. Crop species grown over two years (2008-9 and 2009-10) in glasshouses at the University of Birmingham Elms road site. Initial planting of seeds was in glass houses, with crops transplanted into larger grow bags or outside as appropriate to species. n/a refers to plants sown in place.

Crop	Number of plots/ accessions	Sowing depth (mm)	Sowing date	Transplant date	Year grown
Solanum lycopersicum block 1	101	0-5	11/04/2008	28/04/2008	2008-9
Solanum lycopersicum block 2	101	0-5	22/04/2008	20/05/2008	2008-9
Solanum lycopersicum block 3	101	0-5	23/04/2008	28/05/2008	2008-9
Capsicum annuum	11	0	10/04/2008	19/05/2008	2008-9
Cucumis sativus	13	10	10/04/2008	28/04/2008	2008-9
Vicia faba	33	50	17/04/2008	14/05/2008	2008-9
Brassica rapa var. *rapa*	5	10	16/06/2008	15/08/2008	2008-9
Daucus carota	12	10	12/05/2008	24/06/2008	2008-9
Allium porrum	7	10	02/06/2008	01/09/2008	2008-9
Solanum lycopersicum block 4	79	0-5	21/03/2009		2009-10
Solanum lycopersicum block 5	79	0-5	08/05/2009		2009-10
Solanum lycopersicum block 6	17	0-5	11/03/2009	03/04/2009	2009-10
Lactuca sativa	22	12	29/09/2009	n/a	2009-10
Raphanus sativus	12	10	10/03/2009	24/03/2009	2009-10
Brassica napobrassica	4	10	11/03/2009	02/04/2009	2009-10
Pisum sativum block 1	77	50	08/04/2009	13/05/2009	2009-10
Pisum sativum block 2	77	50	20/05/2009	02/06/2009	2009-10

4.2.2 Experimental design

In the first growing season, the accessions were planted in plots of five individuals, with three replicate plots per accession; in the second growing season accessions were planted in plots of five individuals again, but with two replicate plots per accession. Three seeds were planted in each pot in case of germination/seedling failure; plants were thinned down to one per pot when large enough to handle. Two commercial varieties for each crop were grown as a control and for comparison (Table 4.2).

Table 4.2 Commercial varieties grown for morphological characterisation. Commercial varieties grown as standards and for comparison.

Crop	Variety name	Seed company
Allium porrum	Lyon	Thompson and Morgan
Allium porrum	Blue green autumn Neptune	Suttons seeds
Brassica napobrassica	Angela	Suttons seeds
Brassica napobrassica	Virtue	Thompson and Morgan
Brassica rapa var. *rapa*	Purple top melon	Suttons seeds
Brassica rapa var. *rapa*	Oasis	Thompson and Morgan
Capsicum annuum	World beater	Suttons seeds
Capsicum annuum	F1 Gypsy	Suttons seeds
Cucumis sativus	Telegraph improved	Suttons Seeds
Cucumis sativus	Burpless tasty green F1 hybrid	Thompson and Morgan
Daucus carota	F1 Nelson	Suttons seeds
Daucus carota	F1 Maestro	Suttons seeds
Lactuca sativa	Iceberg	Suttons seeds
Lactuca sativa	Corsair	Thompson and Morgan
Pisum sativum	Early onward	Suttons seeds
Pisum sativum	Kelvedon wonder	B and Q
Raphanus sativus	Saxa 2	B and Q
Raphanus sativus	Scarlet globe	Suttons seeds
Solanum lycopersicum (indeterminate)	Ailsa Craig	Suttons seeds
Solanum lycopersicum (indeterminate)	Tamina	Suttons seeds
Solanum lycopersicum (determinate)	Legend	Thompson and Morgan
Solanum lycopersicum (determinate)	Red alert	Thompson and Morgan
Vicia faba	Bunyard's exhibition	Thompson and Morgan
Vicia faba	The Sutton	Thompson and Morgan

A fully randomised design was used for most crops (following IPGRI, 2001): all plots were blinded (the accession name was removed and a number was assigned as an identifier) and randomised using the random number generator function in MS Excel; the original, unblinded, tables were kept in electronic and paper format. For crops with a larger number of accessions (*Solanum lycopersicum* and *Pisum sativum*) a Randomised Complete Block Design was used (following IPGRI, 2001): three (in the first year) or two (in the second year) experimental blocks were set up for each crop, with one replicate plot from each accession in each block, each block was blinded and randomised separately (Figure 4.1).

A row of guard plants was placed around the perimeter of experiments to standardise conditions.

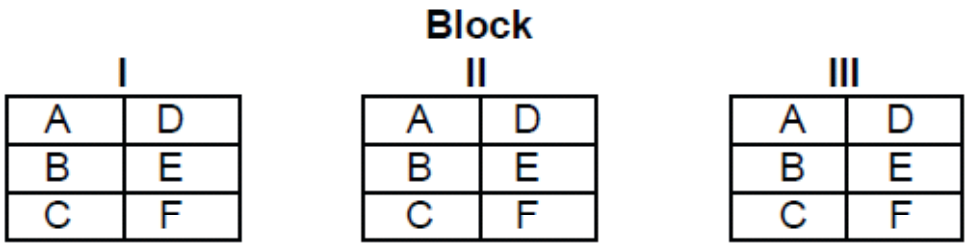

Figure 4.1a A randomised complete block design before randomisation. Graphic based on IPGRI (2001), Figure 6.1a, page 16.

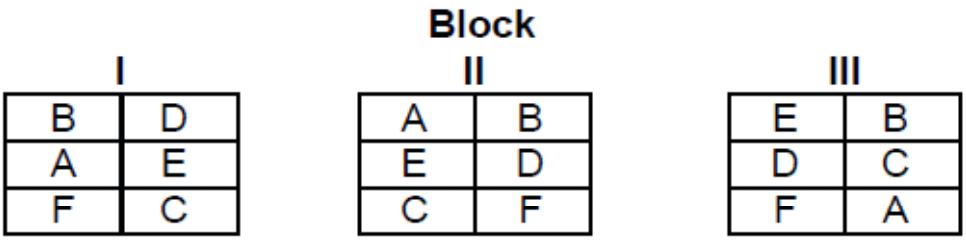

Figure 4.1b The same randomised complete block design after randomisation. Graphic based on IPGRI (2001), Figure 6.1b, page 16.

4.2.3 Cultivation

Glasshouses

Crops grown and numbers of accessions (plots) per crop are shown in Table 4.1. Seeds were sown in 1.5-inch pots of Humax multi-purpose compost at the University of Birmingham, School of Biosciences greenhouses, at Elms Road; seeds were planted at a depth as indicated by the commercial seed packet instructions (see Table 4.1). When large enough to handle,

seeds were potted up into 5 litre bags, which were placed in rows on flood benches. Bench space did not allow the potting up of guard perimeter plants around the whole perimeter, so these were only present indoors until this stage, and then at the ends of the experimental benches.

In year two, the same practices were followed, however, two plots were sown per crop instead of three as three was too substantial a number to be feasible, particularly as more accessions were grown in year two.

Greenhouse temperatures were kept at an average of 22°C. Fertilizer was added when necessary (Vitax Vitafeed 101) and greenfly infestations in the *Capsicum annuum* and *Lactuca sativa* experiments were treated with Majestik (Certis).

Plant characters were recorded *in situ*; fruit characters were recorded after harvest in the laboratory.

Field

Outdoor crops were planted as above, and, when large enough to handle, were hardened off in frames and transplanted into the field at Elms Road, Birmingham, UK, in 2008 and 2009. Seedlings were planted out in 50 m and 65 m rows (in 2008 and 2009 respectively) at spacing of 5 per meter for legumes and *Allium porrum*, and 3 per metre for *brassicas*; a perimeter of guard plants surrounded each crop. Weeds were controlled by hand with no application of herbicides, pesticides or fungicides. Fertilizer was added when necessary, depending on plant condition (Vitax Vitafeed 101). During the growing period, plant characters were recorded in the field for legumes; fruit/pod/seed characters (and whole plant characters in the case of *Brassica* sp.) were recorded in the laboratory after harvest.

4.2.4 Descriptors

The accessions were morphologically characterised using standard crop descriptors. Descriptors are available for the majority of crops from Bioversity (formerly the International Plant Genetic Resources Institute, IPGRI). HSL also use descriptors based on IPGRI; however, additional descriptors are also identified by HSL for some crops, these were also included. For *Pisum sativum* no IPGRI descriptors were currently available so descriptors were compiled from HSL sources (derived from John Innes Centre and UPOV descriptors) and scientific literature (Sardana *et al*., 2007; N. Munro, personal communication). Descriptors for each crop are listed in Appendix two.

4.2.5 Statistical analysis

Analysis was carried out using Statistical Package for the Social Sciences (SPSS) 17. Multivariate techniques used were Principal Components Analysis (PCA) and Cluster Analysis using the Unweighted Pair-group method using Arithmetic Average (UPGMA) algorithm (first outlined by Sokal and Michener, 1958). Accessions clustering closely in PCA and Cluster Analysis were compared using Mann-Whitney U to further explore whether they might be duplicates.

PCA was carried out in order to classify groups of accessions and elucidate variables of importance in each crop and to examine the distribution of variation between variables, in crops for which more than six quantitative variables (minimum) were recorded. The correlation matrix setting in SPSS automatically standardises variables to zero mean and unit variance. Variables with a coefficient of greater than 0.5 were considered to be relevant to the Component (Munoz-Falcon *et al*., 2008). For highly correlated variables ($r > 0.9$) one of the

pair was excluded to reduce bias. Variables in the Component Matrix with a loading above 0.7 were considered to contribute most to that Principal Component. The Kaiser-Meyer-Olkin (KMO) statistic was used as a guide to assess whether the solution gained was due to chance or mathematical artefact (KMO > 0.6). Rotated and non-rotated solutions were tested for each crop, with the best solution shown. The rotations were carried out using Promax rotation, which is a non-orthogonal method that allows correlation between the final Components and can provide more clearly defined Components (variables load more highly on to single factors). Where PCA was not possible (for example due to small variable number or high correlation of variables) scatter plots of quantitative variables were examined for general trends such as clusters and outlying plots/accessions.

For UPGMA, quantitative variables were standardized using z-scores (zero mean and unit variance), and qualitative variables were coded in a binary system (present = 1, absent = 0). UPGMA was carried out using the following variable combinations: scale variables only, scale and ordinal variables, all variables (quantitative and qualitative), and binary variables only, to explore the effect this had on the clustering patterns.

Duplicates

Le Clerc *et al*. (2005b) use the criterion of the presentation of one clearly distinctive character to classify an accession as morphologically distinguished; the present study will also adopt this criterion, with the weight of preference being on qualitative, non-evaluative, characters, where relevant, as these are often less influenced by environmental conditions. Mann-Whitney U test was used to test for significance in quantitative variables due to the unequal sample sizes and small samples sizes that therefore are unlikely to be normally distributed. Although many variables will be technically taken from normally distributed data, due to

small sample sizes this cannot be confirmed, so the weaker non-parametric test was used as a precautionary measure.

4.3 Results

In total 366 accessions from 11 crops were grown and character data collected (Table 4.3). Accessions not accounted for were those that either did not germinate or those that did not produce measurable organs (such as roots or fruits).

Table 4.3 Characterised accessions, by crop.

Crop	Number of HSL accessions
Vicia faba	31
Daucus carota	10
Cucumis sativus	11
Allium porrum	5
Lactuca sativa	20
Pisum sativum	75
Capsicum annuum	9
Raphanus sativus	10
Brassica napobrassica	2
Solanum lycopersicum	190
Brassica rapa var. *rapa*	3
Total	366

Variation within accessions was assessed using PCA and Cluster Analysis (data not shown). Due to the amount of variation present within accessions and the number of replicates per accession, insufficient data was present to draw reliable conclusions, other than the general observation that variation was generally greater within accessions in outbreeding crops, than in inbreeding crops.

4.3.1 Vicia faba

Data for 33 morphological descriptors were collected for the 31 HSL *V. faba* accessions and two commercial standards (Bunyard's Exhibition and The Sutton), of which 12 quantitative variables were included in the Principal Components Analysis and 27 variables in the Cluster Analysis.

Distribution of variation between accessions

PCA was used to investigate distribution of variation in the *V. faba* accessions; Table 4.4 shows the variables included in the PCA solution shown below. The variable days to 50% flowering was excluded due to the high number of missing values; seed height was excluded

PCA with a non-rotated solution was optimal, as the Component correlation was not highly significant; this was confirmed by running a rotated solution and checking the solutions were sufficiently similar. KMO was 0.68 and so was acceptable. Extraction was lowest for number of branches at basal nodes. The first inflection of the scree plot was at three Components; however this was below an eigenvalue of one, suggesting a two-Component solution (Figure 4.2). The third Component represented number of branches at basal node.

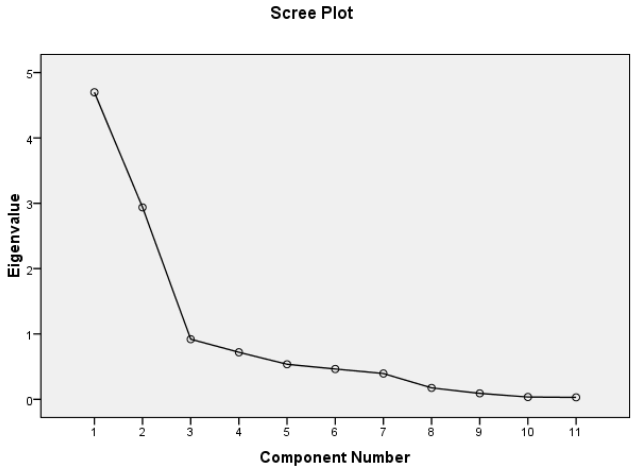

Figure 4.2 Principal Components Analysis scree plot. Scree plot for Principal Components Analysis of *Vicia faba* accessions using 11 morphological variables. Derived using SPSS. Two components above an eigenvalue of one were found.

The first two Principal Components explained 42.72% and 26.7% of the variance respectively. The Component matrix is shown in Table 4.4. Component one related to pod and seed characters, with highly loading variables pod length, pod width and seed weight); Component two related to plant characters, with plant height being the highest loading variable.

Table 4.4 Principal Components Analysis component matrix. Component matrix for *Vicia faba* unrotated solution. Derived using SPSS. Variables with loadings greater than 0.7 are highlighted in bold.

	Component	
	1	2
Mean pod length (cm)	**.947**	
Mean pod width (cm)	**.837**	
100 dry seed weight (g)	**.821**	
Mean number seeds per pod	**.749**	
Mean dry seed length (mm)	**.740**	
Mean number pods per node	-.665	
Mean number of branches at basal nodes	-.533	
Mean plant height (cm)		**.874**
Mean stem thickness (mm)		**.748**
Mean height lowest pod bearing node (cm)		.668
Mean number of leaflets per leaf	.518	.571

A scatter plot of the two Principal Components (Figure 4.3) separates out Cretian, Chak'rusga, Beryl, Martock and Sweet Lorraine; these are the smaller seeded accessions with short pod lengths; their relative positions along the PC2 axis shows that Cretian had the shortest plant height and Sweet Lorraine the tallest. Closely clustering accessions that needed further exploration to determine whether they are duplicates were Canadian Purple and Estonian, Standard 1 and Red Bristow's, Londonderry and Mr Lenthall's, and Mr Jones and Brown. These accessions will be highlighted in the Cluster Analysis and explored using the addition of qualitative variables.

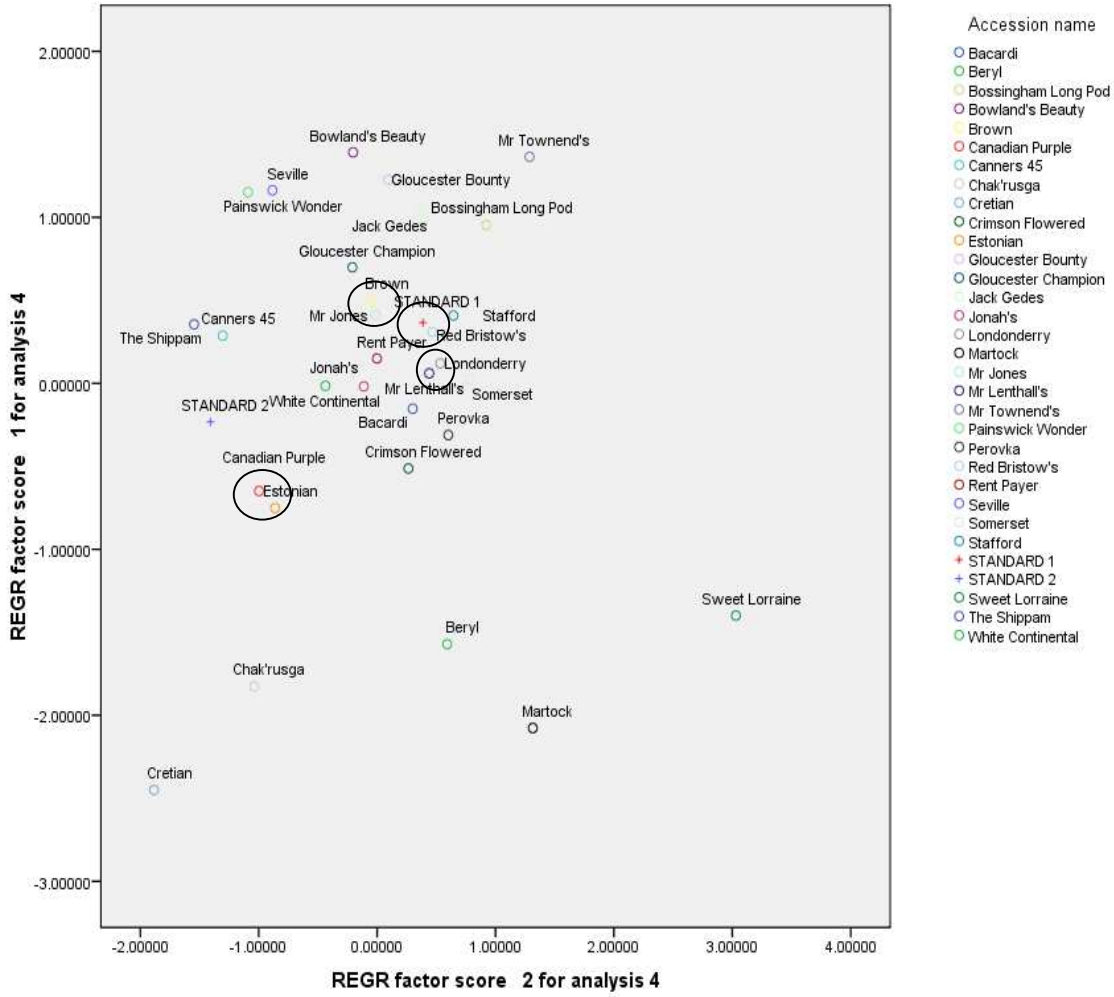

Figure 4.3 Scatter plot of first two Principal Components. Principal Components Analysis of *Vicia faba* accessions produced using 11 morphological variables; Principal Components 1 and 2 explained 42.72% and 26.7% of the variance respectively. Derived using SPSS. Circles highlight closely clustering accessions, indicating morphological similarity.

Clustering between accessions

Plot replicates were clustered using the UPGMA algorithm. Excluded variables were stem colour, red pigment, testa pattern, colour at maturity and seed shape as these variables either showed little or no variation, or because of low data quality.

Twelve scale variables were used; definitively identifying the basis of all clusters was not possible due to the high level of variability in all variables. The variables used to derive the UPGMA dendrogram (Figure 4.4) are listed in Table 4.5. Possible reasons for clustering for the first separation was seed size (Sweet Lorraine, Cretian, Chak'rusga, Martock and Beryl were distinct from each other and the rest of the accessions. This corresponded to accessions with smaller seeds, the larger cluster being those with larger seeds); a cluster formed of accessions with low heights of the lowest pod bearing node; remaining accessions were separated by pod lengths or pod width. Accessions that clustered on short braches, suggesting morphological similarity, included: Rent Payer, Bunyard's Exhibition (Standard 1)) and Stafford; Perovka and Crimson Flowered; Brown and Mr Jones; Estonian and Canadian Purple; Canner 45 and The Shippam; Mr Lenthall's and Red Bristow; Crimson Flowered and Perovka; and Bowland's Beauty and Gloucester Bounty.

Table 4.5 Scale variables included in UPGMA cluster analysis of *Vicia faba* accessions

Variable
100 seed weight dry (g)
Mean stem thickness (mm)
Mean height lowest pod bearing node (cm)
Mean number of branches at basal nodes
Mean number of leaflets per leaf
Mean number pods per node
Mean plant height (cm)
Mean pod width (cm)
Mean pod length (cm)
Mean number seeds per pod
Mean seed height (mm)
Mean dry seed length (mm)

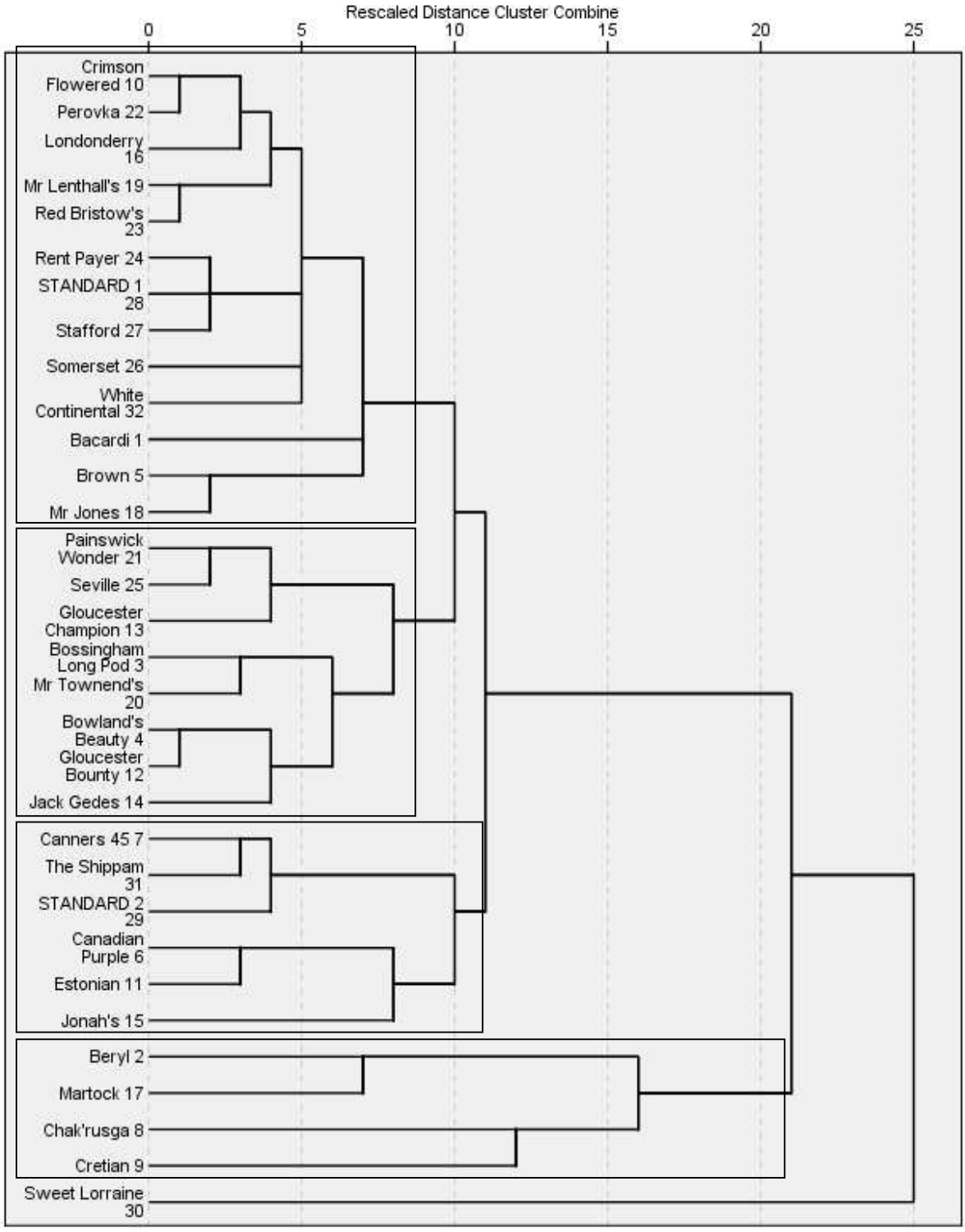

Figure 4.4 UPGMA Cluster Analysis dendrogram for *Vicia faba* accessions. UPGMA cluster analysis of *Vicia faba* accessions using 12 quantitative (scale) variables. Derived using SPSS. Boxes highlight accession clustering potentially based on seed size, height of lowest pod bearing node, pod width and pod length.

Many of these branches increased in length (and hence dissimilarity) and fewer clusters formed when qualitative variables were included (Table 4.6 and Figure 4.5). Crimson Flowered was separated (based on flower colour) along with Beryl, Sweet Lorraine, Martock, and Chak'rusga (separated by seed size and pod and plant characters); Canners 45 and Red Bristow were separated due to seed colour. The cluster indicated (below) consisted of accessions with green seed testa colour, flattened, non-constricted, pod shape, large seeds, white flowers with black streaks and a white spot on the wing petals. The shortest branches were between Perovka and Mr Lenthall's and then white continental; Jack Gedes and Mr Townend's; and Gloucester champion and Stafford. Accessions identified in the PCA as being proximal were Mr Lenthall's and Londonderry, Red Bristow's and Standard 1, Mr Jones and Brown, and Canadian Purple and Estonian; the only pairing visible in the Cluster Analysis using all variables, is Canadian Purple and Estonian. Low clustering of many accessions suggests high variation (dissimilarity) between accessions.

Table 4.6 Variables included in UPGMA cluster analysis of *Vicia faba* accessions.

Variable	Data type
100 dry seed weight (g)	Quantitative (scale)
Mean stem thickness (mm)	Quantitative (scale)
Mean height lowest pod bearing node (cm)	Quantitative (scale)
Mean number of branches at basal nodes	Quantitative (scale)
Mean number of leaflets per leaf	Quantitative (scale)
Mean number pods per node	Quantitative (scale)
Mean plant height (cm)	Quantitative (scale)
Mean pod width (cm)	Quantitative (scale)
Mean pod length (cm)	Quantitative (scale)
Mean number seeds per pod	Quantitative (scale)
Mean seed height (mm)	Quantitative (scale)
Mean dry seed length (mm)	Quantitative (scale)
Intensity of flag streaks	Quantitative (ordinal)
Leaflet size	Quantitative (ordinal)
Resistance to lodging	Quantitative (ordinal)
Flag petal colour	Qualitative
Wing colour pattern	Qualitative
Growth habit	Qualitative
Branching from higher nodes	Qualitative (presence/absence)
Leaflet shape	Qualitative
Stipule spot pigmentation	Qualitative (presence/absence)
Pod attitude	Qualitative
Pod distribution	Qualitative
Pod shape	Qualitative
Ground colour of testa	Qualitative
Hilum colour	Qualitative

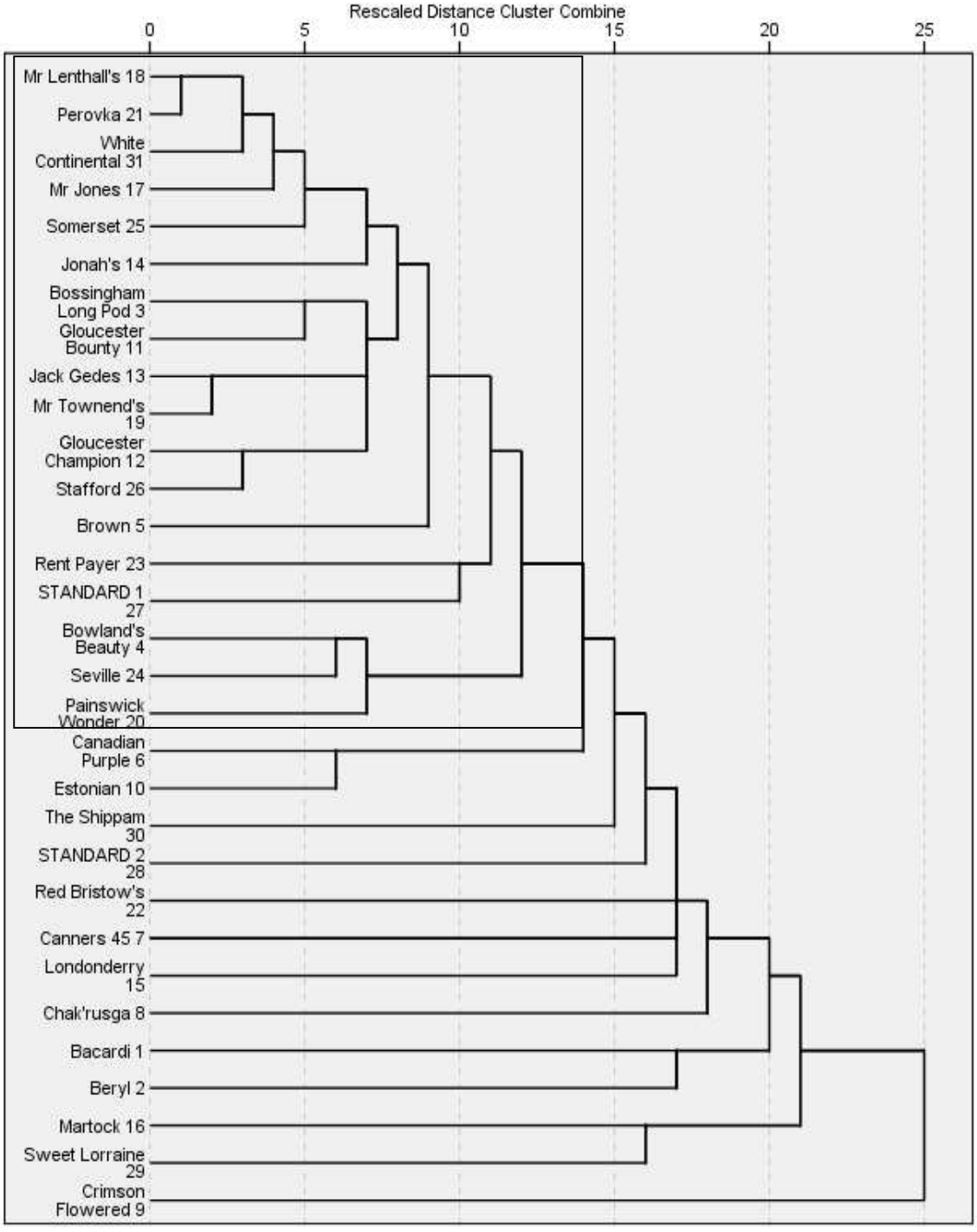

Figure 4.5 UPGMA Cluster Analysis dendrogram for *Vicia faba*. UPGMA cluster analysis of *Vicia faba* accessions using 26 variables. Derived using SPSS. Lack of large clusters suggested high dissimilarity between accessions; box highlights cluster of accessions with the following variables in common: green seed testa colour, flattened, non-constricted, pod shape, large seeds, white flowers with black streaks and a white spot on the wing petals.

When binary variables only were examined (dendrogram not shown) the short branches mentioned above were maintained, suggesting that there were quantitative variable differences between these accessions that otherwise were similar qualitatively. Other branch lengths were long and no large clusters were visible.

Duplicates

Results suggest that there were effectively no morphological duplicates within the *V. faba* accessions. Accessions identified in the analyses above can be distinguished on multiple characters (see Table 4.7).

Canadian Purple and Estonian had significant differences in height of lowest pod bearing node (U = 16.5, p = 0.005, r = 0.6), number of seeds per pod (U = 62.0, p = 0.034, r = 0.39), seed length (U = 26.0, p = 0.00, r = 0.66) and seed height (U = 41.00, p = 0.003, r = 0.54). They also had differences in stem colour (dark green and light green respectively) and leaf shape (sub-elliptic and mixed respectively). Results for pod length and number of seeds pod were not significant, however effect sizes were medium, suggesting that a larger sample size may show significant results (r= 0.35 and r = 0.39, respectively).

Perovka and Mr Lenthall's displayed significant differences in height of lowest pod bearing node (U = 25.5, p = 0.01, r = 0.51), pod width (U = 45.5, p = 0.005), r = 0.51), pod length (U = 22.5, p = 0.00, r = 0.68) and seed length (U = 16.0, p = 0.00, r = 0.73). Number of pods per node had a medium effect size, suggesting that a larger sample size may show significant effects.

Jack Gedes and Mr Townend's measured significant differences in stem thickness (U = 27.5, p = 0.048, r = 0.42), plant height (U = 42.5, p = 0.02, r = 0.45), number of branches at basal

node (U = 34.5, p = 0.02, r = 0.47) and number of leaflets per leaf (U = 20.0, p = 0.001, r = 0.45), and had medium effect size in seed height (r = 0.34) which suggest larger sample sizes will show significant differences in this variable. Differences were also observed in flag petal streak intensity (moderate intense respectively).

Gloucester Champion and Stafford were distinguishable on plant and flower characters, there were significant differences measured in pod width (U=2.0, p=0.00, r = 0.7), number of seeds per pod (U = 39.5, p = 0.035, r = 0.42), seed length (U = 49.5, p = 0.009, r = 0.49) and seed height (U = 38.5, p = 0.002, r = 0.56). They also had different results for resistance to lodging (medium and high respectively), stem colour (light and dark) and leaflet size (large and medium respectively).

Table 4.7 Distinguishing potential *Vicia faba* duplicates. Differences in quantitative and qualitative variables between *Vicia faba* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Quantitative characters	Qualitative characters
Canadian purple and Estonian	Height of lowest pod bearing node, number of seeds per pod, seed length, seed height	Stem colour, leaflet shape
Perovka and Mr Lenthall's	Height of lowest pod bearing node, pod width, pod length, seed length	
Jack Gedes and Mr Townend's	Stem thickness, Plant height, number of branches at basal node, number of leaflets per leaf	Flag petal streak intensity
Gloucester Champion and Stafford	Pod width, number of seeds per pod, seed length, seed height	Resistance to lodging, stem colour, leaflet size

4.3.2 Daucus carota

Despite extensive soil preparation, soil quality was challenging for the *Daucus carota* crop, and led to much root curling, branching and splitting. Roots that did not fully develop were photographed and then removed from the dataset; data cleaning for this crop included removal of extreme values (outliers), using SPSS.

Nineteen descriptors were recorded for ten HSL *Daucus carota* accessions and two commercial standards (F1 Nelson and F1 Maestro). Six variables were used in the Principal Components Analysis (PCA) and 14 in the Cluster Analysis.

Variation in more evaluative characters, such as root splitting, may be potentially informative regarding accession tolerances of non-ideal conditions. The percentage of roots split was highest in standard 2 (F1 Maestro), percentages were low in Giant Improved Flak, Scarlet Horn and White Belgium, and root splitting was absent in Afghan Purple. Root branching was absent to sparse in most accessions at the plot level. At accession level branching was most severe in Afghan Purple, Altringham and Egmont Gold (measured on scale of 0 to 7 as 5 or 'intermediate'), with no plots recorded as having dense branching.

Distribution of variation between accessions

PCA was used to investigate distribution of variation in the *D. carota* accessions; Table 4.11 shows the variables included in the PCA solution shown below. Root weight without foliage was removed due to high correlation with root weight with foliage (r = 0.9).

PCA with a rotated solution (using Promax) was optimal, as the Component correlation was significant for correlation between Components (0.32), although result from an unrotated solution was the same. KMO was 0.42, which is below the acceptable threshold; this suggests

that the result found may be a mathematical artefact, rather than a true representation. The low value may be due to a number of contributing factors such as small number of items or small number of variables in the analysis. Extraction was high for all variables. The first inflection, above an eigenvalue of 1, on the scree plot (Figure 4.6) was at two Components, suggesting a two-Component solution.

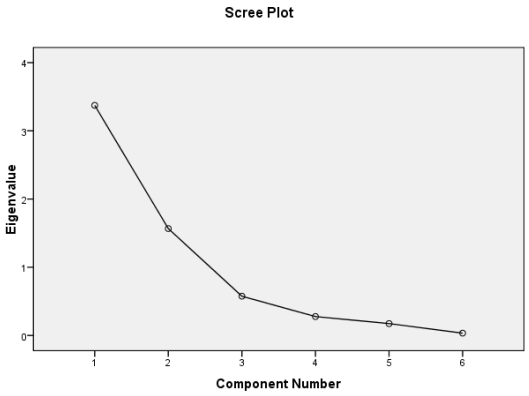

Figure 4.6 Principal Components Analysis scree plot. Scree plot for Principal Components Analysis of *Daucus carota* accessions using 6 morphological variables. Derived using SPSS. The first inflection, above an eigenvalue of 1 was at two Components, suggesting a two-Component solution.

The first two Components explain 56.23% and 26.11% of the variance, respectively. PC1 had root weight with foliage, leaf length and leaf width loading most heavily; PC2 had width of core (Table 4.8).

Table 4.8 Principal Components Analysis Component matrix. Component matrix for *Daucus carota*. Derived in SPSS using a Promax rotation. Variables with loadings above 0.7 are highlighted in bold.

	Component	
	1	2
Mean root weight with foliage	**.909**	
Mean leaf length	**.872**	
Mean leaf width	**.840**	
Mean root diameter	**.710**	
Mean width of core		**.861**
Mean root length		-.685

The pattern matrix (Table 4.9; derived from the Promax rotation and containing information about the unique contribution of a variable to a factor and forces the key variables to load onto a single Component) and the structure matrix (Table 4.10; which allows account to be taken of the relationships between the factors and is the product of the pattern matrix and the correlation coefficients between factors) also had the same four highest loading variables contributing to PC1 (in varying order) and also had width of core as the main loading variable on PC2.

Table 4.9 Principal Components Analysis pattern matrix. Pattern matrix for *Daucus carota*. Derived in SPSS using a Promax rotation. Variables with loadings above 0.7 are highlighted in bold.

	Component	
	1	2
Mean root length	**.949**	
Mean leaf length	**.933**	
Mean root weight with foliage	**.702**	
Mean leaf width	.694	
Mean width of core		**1.030**
Mean root diameter		**.762**

Table 4.10 Principal Components Analysis structure matrix. Structure matrix for *Daucus carota*. Derived in SPSS using a Promax rotation. Variables with loadings above 0.7 are highlighted in bold.

	Component	
	1	2
Mean leaf length	**.941**	
Mean root weight with foliage	**.827**	.618
Mean root length	**.820**	
Mean leaf width	**.790**	
Mean width of core		**.956**
Mean root diameter		**.831**

A scatter plot of PC1 against PC2 (Figure 4.7) showed three main clusters with Scarlet Horn on its own; Altringham, Afghan Purple, John's Purple and Red Elephant clustered; and the remaining accessions fairly loosely clustered, with Egmont Gold and Giant Improved Flak being closest together. PC2 was mostly accounted for by width of core (and root diameter); this reflected the Scarlet Horn individuals' larger overall size (in the top right position); the cluster separated by component 2 was large core width and with smaller leaves, as PC1 was largely composed of leaf characters (and root length, which loaded on both Components to varying degrees); with the distribution of accessions within the final cluster showing the positive correlation between the Principal Components.

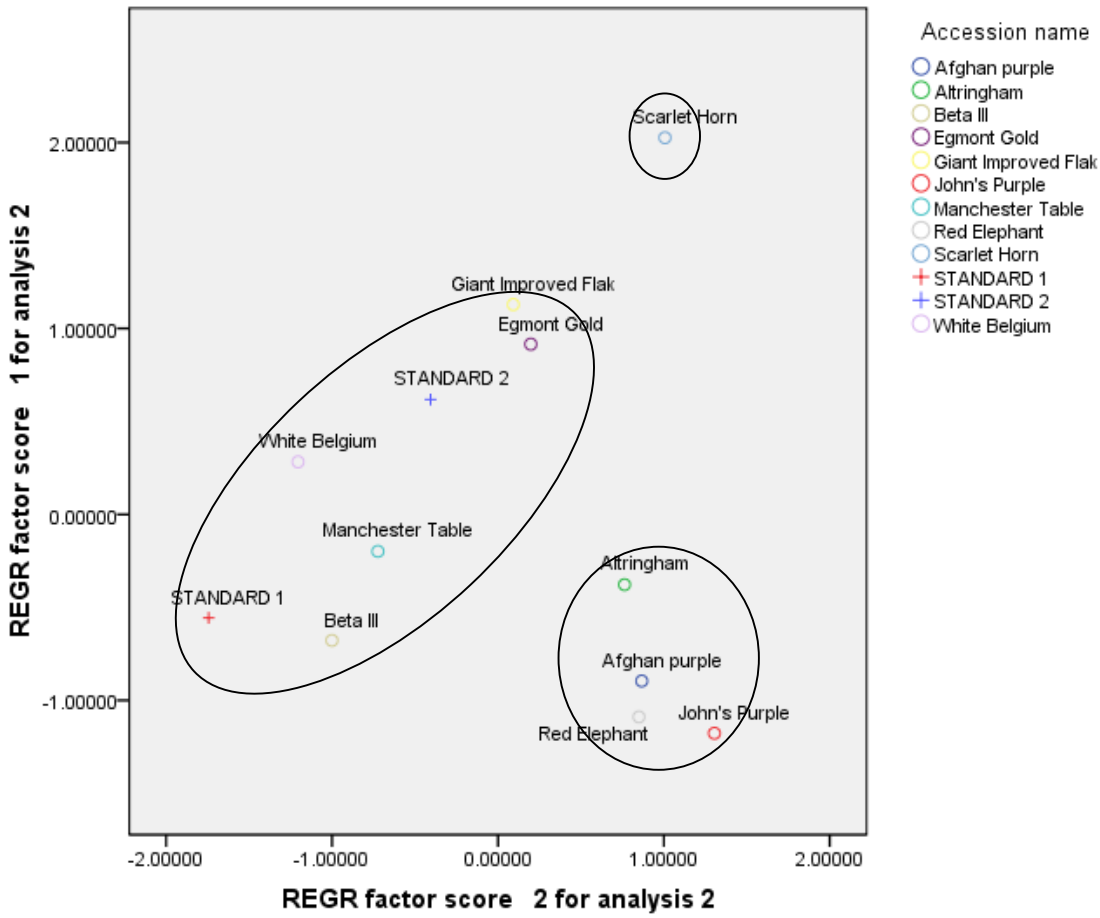

Figure 4.7 Scatter plot of first two Principal Components. Principal Components Analysis of *Daucus carota* accessions produced using 6 morphological variables; Principal Components 1 and 2 explained 56.25% and 26.11% of the variance respectively. Derived using SPSS. Circles highlight clustered accessions.

Clustering between accessions

When using scale variables only (dendrogram not shown), three main clusters were visible: Afghan Purple, John's Purple, Altringham and Red Elephant (seen in the PCA); Egmont Gold, Giant Improved Flak and Scarlet Horn; and the remaining accessions. Cluster branches were quite long suggesting a general morphological dissimilarity between accessions; the shortest branches were between John's Purple and Red Elephant.

When applying all variables (Table 4.11), branch lengths are long between clusters, suggesting an overall low level of similarity between the clusters (Figure 4.8); clusters were formed on the basis of root colour. Within the orange cluster were two groups, with Manchester Table and Standard 1 on the shortest branches. The sub-groupings within the orange cluster may have been based on root width and weight (accessions within the top cluster were not as wide or heavy as the second orange root cluster). Red Elephant and Altringham were similar in many of the descriptors particularly red shoulder.

Table 4.11 Variables included in UPGMA cluster analysis of *Daucus carota* accessions.

Variable	Data type
Mean root length (cm)	Quantitative (scale)
Mean root diameter (cm)	Quantitative (scale)
Mean width of core (cm)	Quantitative (scale)
Mean leaf length (cm)	Quantitative (scale)
Mean leaf width (cm)	Quantitative (scale)
Mean root weight without foliage (g)	Quantitative (scale)
Mean root weight with foliage (g)	Quantitative (scale)
Median root branching	Quantitative (ordinal)
Median extent of green shoulder	Quantitative (ordinal)
Root skin colour	Qualitative
Red shoulder	Qualitative (presence/absence)
Flesh colour distribution in trans-section	Qualitative
Colour inner core	Qualitative
Colour tissue surrounding core	Qualitative

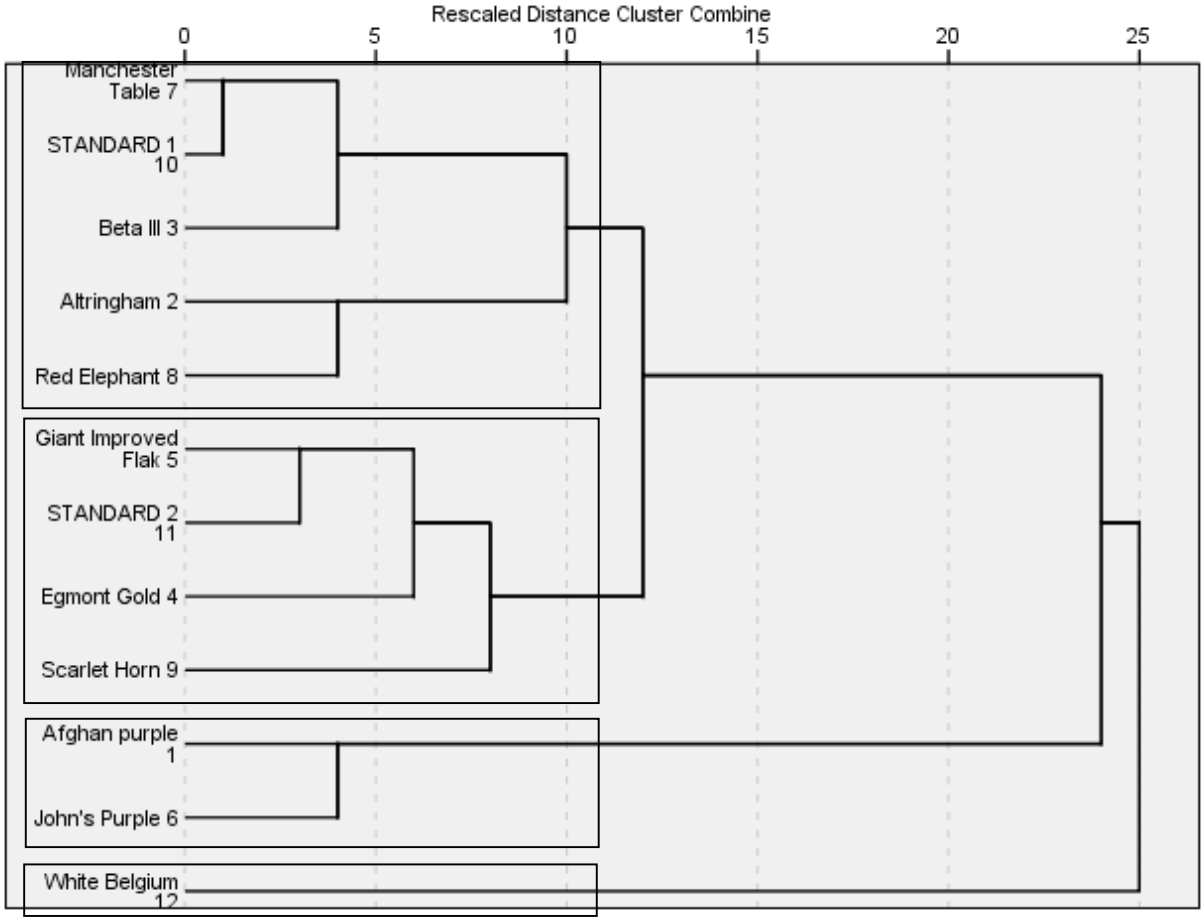

Figure 4.8 UPGMA Cluster Analysis dendrogram of Daucus carota accessions. UPGMA cluster analysis of *Daucus carota* accessions using 14 variables. Derived using SPSS. Boxes highlight accession clusters, based predominantly on root colour (top to bottom: orange, purple and white), with orange rooted accessions further subdivided by root weight.

Duplicates

Although clustering together using quantitative variables only (dendrogram not shown), Afghan Purple and Red Elephant could be distinguished as they were purple and orange rooted (respectively).

Red Elephant and Altringham were significantly different for root core width ($U = 21.0$, $p = 0.01$, $r=0.55$). Although results for root diameter and leaf width were non-significant, the

effect sizes were medium (U=35.5, p=0.12, r=0.35, and U=38.0, p=0.15, r=0.31 respectively) suggesting that with a larger sample size significant differences may be observed. Differences were also noted in qualitative variables (Table 4.19).

Between Egmont Gold and Giant Improved Flak, no significant differences were found in quantitative variables between the accessions; however, as above, the variable weight with foliage was non-significant but with a medium effect size (U=52.5, p=0.062, r=0.36) suggesting a larger sample may show significant differences. These accessions were dissimilar in evaluative characters (see Table 4.12), with Giant Improved Flak performing 'better' in each case.

There were insufficient cases to compare Afghan Purple and John's Purple using the Mann-Whitney U test. The two purple rooted accessions had different results for crown shape and root branching; although of these characters, the former was not included in the analyses due to potential user bias, and the latter was an evaluative character and therefore potentially more vulnerable to environmental effects. For this reason, these accessions were highlighted as potential duplicates for further study by HSL.

Standard 1 and Manchester Table were the most difficult accessions to distinguish, with root splitting being the only character with notable differences between them (not enough data to perform a significance test). No significant differences were found between quantitative variables using Mann-Whitney U test, even when taking effect size into account. Confirmation of the lack of duplicates may not be possible using these morphological characters only.

Table 4.12 Differences between potential *Daucus carota* duplicate accessions. Differences in quantitative and qualitative variables between *Daucus carota* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Quantitative characters	Qualitative characters
Afghan Purple and Red Elephant		Root colour
Egmont Gold and Giant Improved Flak		Root splitting, green shoulder, root branching
Standard 1 and Manchester Table		Root splitting
Red Elephant and Altringham	Root core width	Crown shape, root branching
John's Purple and Afghan Purple		Crown shape, root branching

4.3.3 Pisum sativum

Sixteen morphological descriptors were collected for 75 HSL *Pisum sativum* accessions and two commercial standards (Kelvedon Wonder and Early Onward). Of these variables, six quantitative were available for PCA, and 12 were used in the Cluster Analysis.

Distribution of variation between accessions

A non-rotated PCA solution was found to be optimal, as Principal Components were not correlated (confirmed by running a Promax rotation). Only a small number of variables (six) were available for PCA; this may contribute to the low KMO for this analysis (0.59). Again, a non-rotated solution is shown, due to low correlation between Principal Components. KMO was again low (0.59). Extraction was very low (0.12) for number of tendrils, however this was not a major loading variable on any Component above an eigenvalue of 1. Analysis was performed both with and without the variable to confirm it had no substantial effect. The scree plot (Figure 4.9) suggested a two-Component solution.

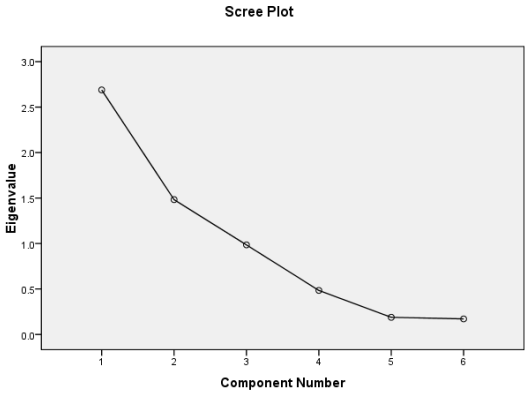

Figure 4.9 Principal Components Analysis scree plot. Scree plot for Principal Components Analysis of *Pisum sativum* accessions using 6 morphological variables. Derived using SPSS. The scree plot suggests a two-component solution.

The first two Components explained variances of 44.82% and 24.71% respectively (a third Component with an eigenvalue of 0.99 with number of tendrils loading highly additionally explained 16.42% bringing the cumulative variance explained to 85.95%).

Component 1 was composed of pod width and seed length (Table 4.13); Component 2 was composed of number of seeds per pod. Component 3 (below an eigenvalue of 1) had number of tendrils loading highly.

Table 4.13 Principal Components Analysis component matrix. Component matrix for *Pisum sativum*. Derived in SPSS. Variable loadings above 0.7 highlighted in bold.

	Component		
	1	2	3
Mean pod width	**.866**		
Mean seed length	**.840**		
Dry 100 seed weight	**.776**		
Mean pod length	**.719**	.551	
Mean number of seeds per pod		**.886**	
Mean number of tendrils			**.916**

There were no obvious clusters when PC1 (seed length and pod width) PC2 (number of seeds per pod) were plotted (Figure 4.10), suggesting morphological similarity for the current variable set, however, separated out from main cluster in PC1 against PC2 were Raisin Capucijner, Carlin and Poppet, Prean, and a small cluster of Salmon Flowered, Eat All and Early Capucijner. Those very closely clustered were Eat All and Salmon Flowered, Kent Blue and Espoir de Gemboux, and Laxton's Exquisite and Newick. Commercial standards (circled in blue) were part of the main cluster.

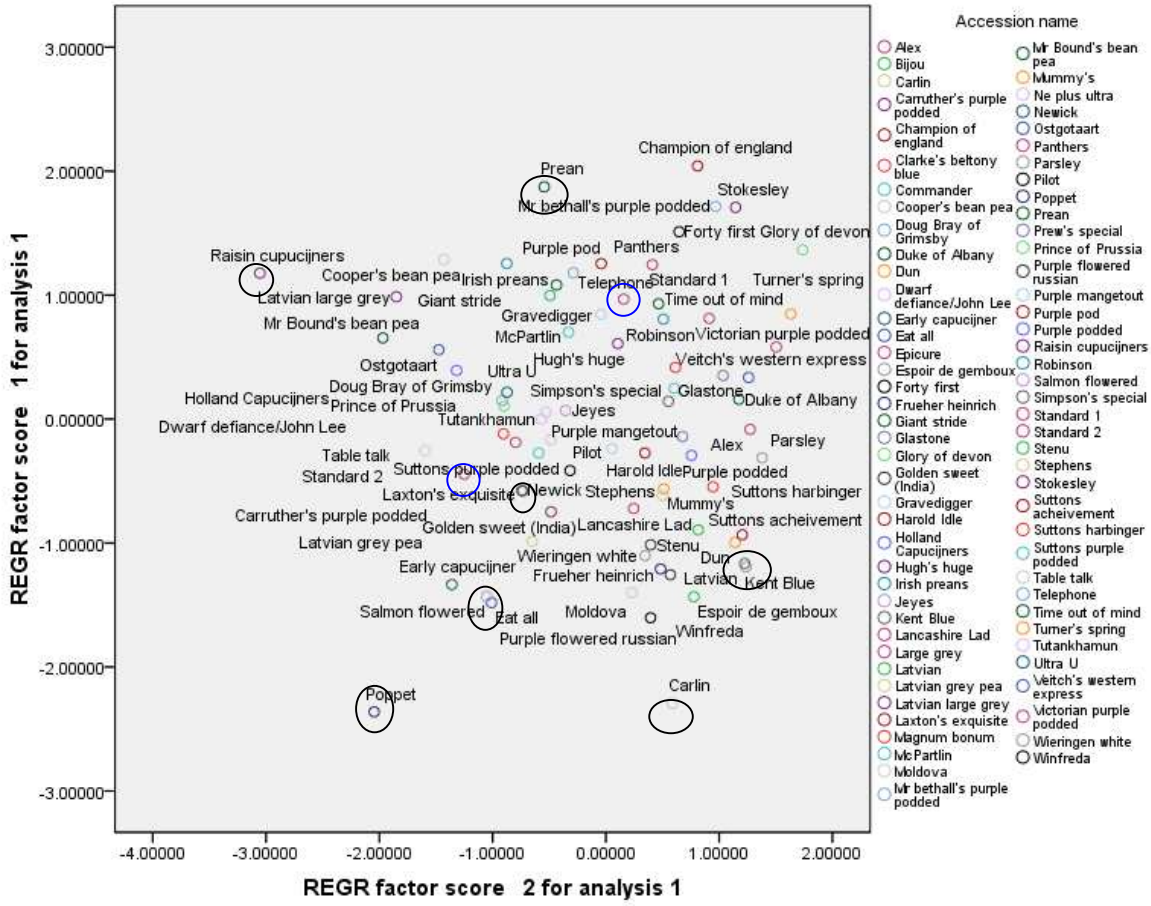

Figure 4.10 Scatter plot of first two Principal Components. Principal Components Analysis of *Pisum sativum* accessions produced using 6 morphological variables; Principal Components 1 and 2 explained 44.82% and 24.71% of the variance respectively. Derived using SPSS. Black circles highlight outlying or closely clustered accessions; blue circles indicate commercial standards.

Clustering between accessions

Overall branch lengths were short, indicating a degree of morphological similarity between accessions. The large number of accessions and low number of variables means that resolution was limited.

In the UPGMA Cluster Analysis using scale variables only (dendrogram not shown), because of their different tendril numbers, Poppet and Parsley cluster separately, as in the PCA. Using quantitative and qualitative characters (Table 4.14) accessions clustered into three groups based on pod colour (yellow, purple and green) (Figures 4.1a and 4.11b). In addition Poppet was separated out due to the dissimilarity if its tendril number from other accessions. The yellow-podded cluster contains only the accession, Golden Sweet. As in the plots analysis above, overall branch lengths are short, indicating a high degree of morphological similarity.

Table 4.14 Variables used in UPGMA analysis of *Pisum sativum* accessions.

Variable	Data type
Mean pod width (cm)	Quantitative (scale)
Mean pod length (cm)	Quantitative (scale)
Mean number of seeds per pod	Quantitative (scale)
Dry 100 seed weight (g)	Quantitative (scale)
Mean seed length (mm)	Quantitative (scale)
Mean number of tendrils	Quantitative (scale)
Brown marbling	Qualitative (presence/absence)
Anthocyanin	Qualitative (presence/absence)
Mature pod colour	Qualitative
Young pod colour	Qualitative
Flower presence of anthocyanin	Qualitative (presence/absence)

Within the cluster composed of purple-podded accessions (Figure 4.11a), two further sub-clusters were based on the presence or absence of anthocyanin in the seed coat.

Within the cluster composed of green-podded accessions, two sub-clusters had brown seed coat marbling present (Figure 4.11b) and one sub cluster had anthocyanin present in the seed coat. All green-podded accessions with purple flowers were clustered together (Figure 4.10a), with the exception of Forty First and Purple Flowered Russian, which were in clusters with white flowered accessions.

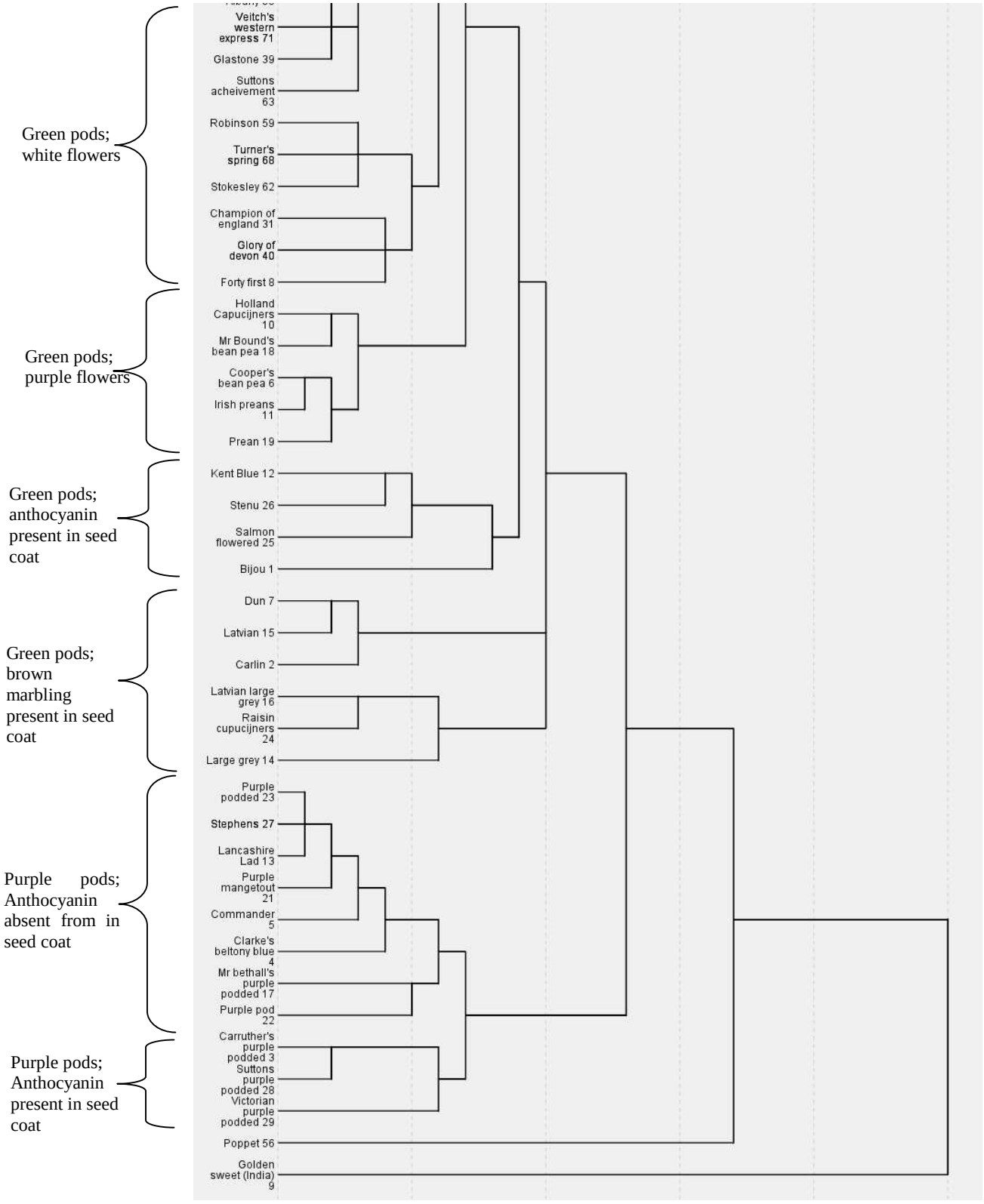

Figure 4.11a First section of UPGMA dendrogram for *Pisum sativum* accessions. UPGMA cluster analysis of *Pisum sativum* accessions Derived in SPSS using 11 variables. Brackets highlight accession clusters, based predominantly on pod colour and seed coat patterns.

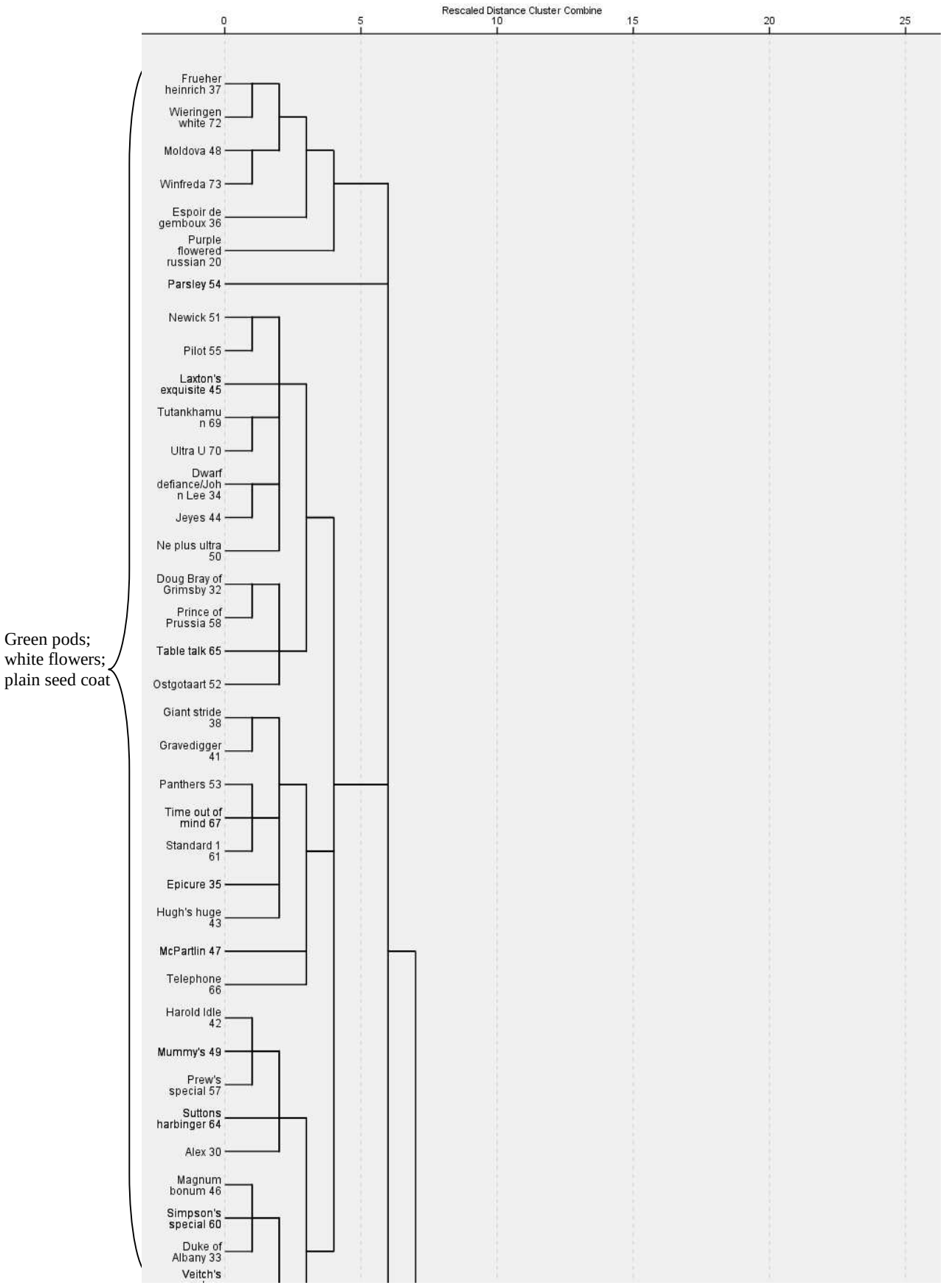

Figure 4.11b Second section of UPGMA dendrogram for *Pisum sativum* accessions. UPGMA cluster analysis of *Pisum sativum* accessions showing the clustering of those accessions with green pods, white flowers and plain seed coat. Derived in SPSS using 11 variables.

Duplicates

Accessions identified in the Principal Components Analysis or Cluster Analysis as being similar (either closely distributed in the PCA or on short branches in the Cluster Analysis) were further examined a comparison of qualitative results between accession pairs and using Mann-Whitney U to test of significant differences.

No significant differences in quantitative variables were found between Eat All and Salmon Flowered, however flower colour was different between accessions (Table 4.15), which can be used to adequately distinguish between the accessions. In addition, effect sizes in Pod width ($r = 0.42$), pod length ($r = 0.39$), number of seeds per pod ($r = 0.35$), dry seed weight ($r = 0.71$), seed length ($r = 0.41$) and number of tendrils ($r = 0.5$) also suggested a larger sample size may show significant difference.

As well as being distinguishable using seed coat and flower characters, Kent Blue and Espoir De Gemboux were significantly different in pod width ($U = 0$, $p = 0.007$, $r = 0.85$). Effect size was also medium in pod length ($r = 0.37$), large in dry seed weight ($r = 0.71$), seed length ($r = 0.53$), and number of tendrils ($r = 0.61$).

Laxton's Exquisite and Newick showed significant difference in seed length ($U = 2.5$, $p = 0.006$, $r = 7.2$) and large effect sizes in dry seed weight ($r = 0.71$) and number of tendrils ($r = 0.82$).

Purple Podded and Stephen's were not significantly different in any quantitative variables, and no differences were recorded in the qualitative descriptors collected.

No differences in quantitative or qualitative variables were recorded between Purple Podded and Lancashire Lad, however effect size was medium in pod width ($r = 0.41$).

No qualitative differences or significant quantitative differences were found between Lancashire Lad and Stephen's; however, effect sizes were medium for pod width (r = 0.37), suggesting a larger sample size may reveal significant results.

Mummy's and Prew's Special could not be distinguished using the present data.

Panther's and Time Out Of Mind did not have any quantitative variables that showed significant differences, however effect size was medium for seed length (r=0.38). No qualitative differences were recorded.

Jeyes and Ne Plus Ultra pod width did not show any significant differences in quantitative variables, but effect sizes were medium in pod width (r = 0.45), and seed length (r = 0.38) and large in dry seed weight (r = 0.71) and number of tendrils (r = 0.5).

Doug Bray and Prince of Prussia did not show any significant differences in quantitative variables, effect sizes were medium for pod length (r = 0.3) and seed length (r = 0.32). No differences in qualitative characters were observed.

Moldova and Winfreda had significant differences in pod width (U = 21, p = 0.04, r = 0.47). No qualitative differences were observed.

Magnum Bonum and Simpson's Special had medium effect size in pod width (r = 0.31), but no significant differences in other quantitative variables, or observed differences in the descriptors recorded.

Harold Idle and Prew's Special had no significant differences however effect size was medium for Seed Length (r = 0.44). No quantitative differences, so for the present study these are potential duplicates.

Panthers and Standard 1 had no quantitative or qualitative differences with the present set of variables, and therefore are so far duplicates. However, effect size was medium for number of tendrils (r = 0.5), and therefore a larger sample size may give a significant result.

Between Time Out Of Mind and Standard 1 no quantitative differences found were statistically significant, however effect sizes were medium for length of pod (r = 0.32) and dry seed 100 weight (r = 0.39) and large for number of tendrils (r = 0.81), suggesting a larger sample size may have yielded significant results. No qualitative differences were found.

Harold Idle and Mummy's and Frueher Heinrich and Wieringen White had no significant quantitative differences and no qualitative differences, therefore were for the sake of this study, duplicates.

Magnum Bonum and Duke of Albany did not show any significant differences but had medium effect sizes for pod length (r = 0.36) and seed length (r = 0.39), suggesting larger sample sizes are needed to confirm significant difference. No qualitative differences were found in the variables recorded.

Simpson's Special and Duke of Albany had significant differences in seed length (U = 16, p = 0.02, r = 0.54) and medium effect size in pod length (r = 0.42). No differences were found in the qualitative descriptors recorded.

Tutankhamun and Ultra U had significant difference in seed length (U = 7.5, p = 0.001, r = 0.72), and medium effect size in dry seed 100 weight (r = 0.39). No differences in retained qualitative variables were recorded.

Dwarf Defiance/John Lee and Jeyes had no significant quantitative differences, however effect size was medium for number of seeds per pod (r = 0.31); no differences in retained qualitative variables were recorded.

No significant differences were recorded in quantitative variables between Giant Stride and Gravedigger, however effect size was large in Dry seed 100 weight (r = 0.71), suggesting a larger sample size may yield significant results. No qualitative differences were found.

No significant quantitative differences were found between Cooper's Bean Pea and Irish Preans, however effect sizes were medium for dry seed 100 weight (r = 0.39) and number of tendrils (r = 0.5). No differences in qualitative variables were observed.

Table 4.15 Differences between potential *Pisum sativum* duplicate accessions. Differences in quantitative and qualitative variables between *Pisum sativum* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Quantitative variables	Qualitative variables
Eat All and Salmon Flowered	-	Flower colour
Kent blue and Espoir de Gemboux	-	Seed coat anthocyanin, flower colour
Laxton's Exquisite and Newick	Seed length	-
Purple Podded and Stephen's	-	-
Purple Podded and Lancashire Lad	-	-
Stephen's and Lancashire Lad	-	-
Harold Idle and Mummy's	-	-
Harold Idle and Prew's Special	-	-
Mummy's and Prew's Special	-	-
Panther's and Time Out of Mind	-	-
Jeyes and Ne Plus Ultra	-	-
Doug Bray and Prince of Prussia	-	-
Moldova and Winfreda	Pod width	
Magnum Bonum and Simpson's Special	-	-
Magnum Bonum and Duke of Albany	-	-
Simpson's Special and Duke of	Seed length	-

Accession names	Quantitative variables	Qualitative variables
Albany Panthers and Standard 1	-	-
Time Out of Mind and Standard 1	-	-
Frueher Heinrich and Wieringen White	-	-
Tutankhamun and Ultra U	Seed length	-
Dwarf Defiance/John Lee and Jeyes	-	-
Giant Stride and Gravedigger	-	-
Cooper's Bean Pea and Irish Preans	-	-

For the purposes of the current variable set and sample size those accessions with no qualitative or quantitative differences were duplicates, even if they had medium-large effect sizes for quantitative variable differences.

4.3.4 Lactuca sativa

12 morphological descriptors were recorded for the 20 HSL *Lactuca sativa* accessions and two commercial standards (Corsair and Iceberg). However, plants were harvested before they were completely mature, due to bolting in many accessions. Plants were grown under glasshouse conditions, which can cause *Lactuca sativa* to be prone to bolting. Accessions that bolted were Asparagus, Bath Cos, Brown Bath Cos, Brown Goldring, Burpee's Iceberg (one plot), Laitue Cracoviensis, Maroulli Cos, Rouge D'Hiver and Soulie. For this reason some measurements may not be comparable to plants grown in other conditions; for example, lettuce type, leaf shape and leaf margin dissection of outer leaves may be static, however leaf length, lettuce weight and other quantitative characters will only be comparable within the present project.

Four lettuce types were recorded in the collection (plus the two standard varieties Iceberg and Corsair (the second of these did not produce sufficient material for measurement)): crisphead (iceberg), butterhead, cos (romaine) and leafy. White Seeded Samara and Bronze Arrow were

leafy (non-heading) accessions; Burpee’s Iceberg, Windermere and Standard 1 were crisphead type; Black Seeded Samara, Liller, Loos Tennis Ball, Mescher, and Northern Queen were butterhead type; and Asparagus, Bath Cos, Bronze Bath Cos, Brown Goldring, Bunyard's Matchless, George Richardson, Laitue Cracoviensis, Maroulli Cos, Rouge D’Hiver, Soulie and Stoke were cos type.

Distribution of variation between accessions

There were insufficient quantitative variables recorded to perform a Principal Components Analysis, therefore differences between accessions were visualised using scatter plots (Figure 4.12). At accession level scatter plots between variables showed positive correlations between the variables (positive correlations were statistically significant in leaf length and leaf width, using Spearman’s Rank). The scatter plot of leaf length by width showed Asparagus as an outlier, and possibly two main broad clusters. Leaf length by weight gave one main cluster with two outliers, Asparagus and Maroulli Cos. Leaf width against weight again gave one outlier, Maroulli Cos, and two main clusters.

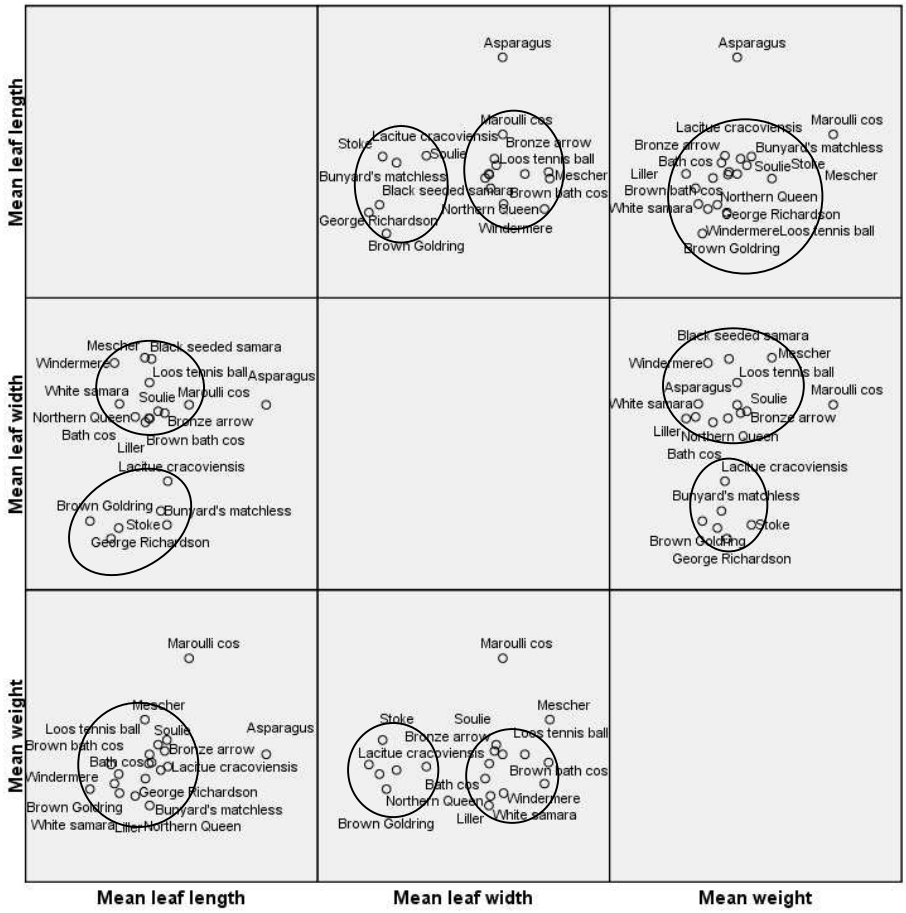

Figure 4.12 Scatter plot of quantitative variables for *Lactuca sativa*. Scatter plot of quantitative variables mean lettuce weight, mean leaf width (cm) and mean leaf length (cm) for *Lactuca sativa* accessions. Circles highlight accession clusters. Derived in SPSS.

Clustering between accessions

Using scale variables alone (dendrogram not shown) clusters reflected the results found in the scatter plots, with Maroulli Cos and Asparagus the last to agglomerate. The shortest branches, suggesting highest morphological similarity were between George Richardson and Rouge D'Hiver; Bunyard's Matchless and Laitue Cracoviensis; Northern Queen and White Seeded Samara; Bath Cos and Brown Bath Cos; Black Seeded Samara and Loos Tennis Ball; Bronze Arrow and Soulie.

Using both quantitative and qualitative variables (Table 4.16) increased the length of the branches compared to analysis using qualitative variables alone, suggesting low morphological similarity (Figure 4.13). Cos and butterhead accessions clustered according to type, although clusters were not well defined, with high internal branch lengths suggesting low internal similarity. Outside of these clusters were White Seeded Samara and Liller, which were leafy and butterhead types respectively; Windermere as the only crisphead type and Bronze Arrow, which was the last to agglomerate suggesting it was the most dissimilar. The shortest branches were between Bunyard's Matchless and Stoke; and George Richardson and Laitue Cracoviensis.

Using qualitative branches alone produced very long branches, with few structured clusters (dendrogram not shown), reflecting the variation between accessions in qualitative variables.

Table 4.16 Variables used in Cluster Analysis of *Lactuca sativa* accessions.

Variable	Data type
Leaf folding	Qualitative
Leaf margin dissection	Qualitative
Leaf shape	Qualitative
Leaf texture	Qualitative
Lettuce type	Qualitative
Median leaf colour intensity	Quantitative (ordinal)
Mean leaf length	Quantitative (scale)
Mean leaf width	Quantitative (scale)
Mean weight	Quantitative (scale)

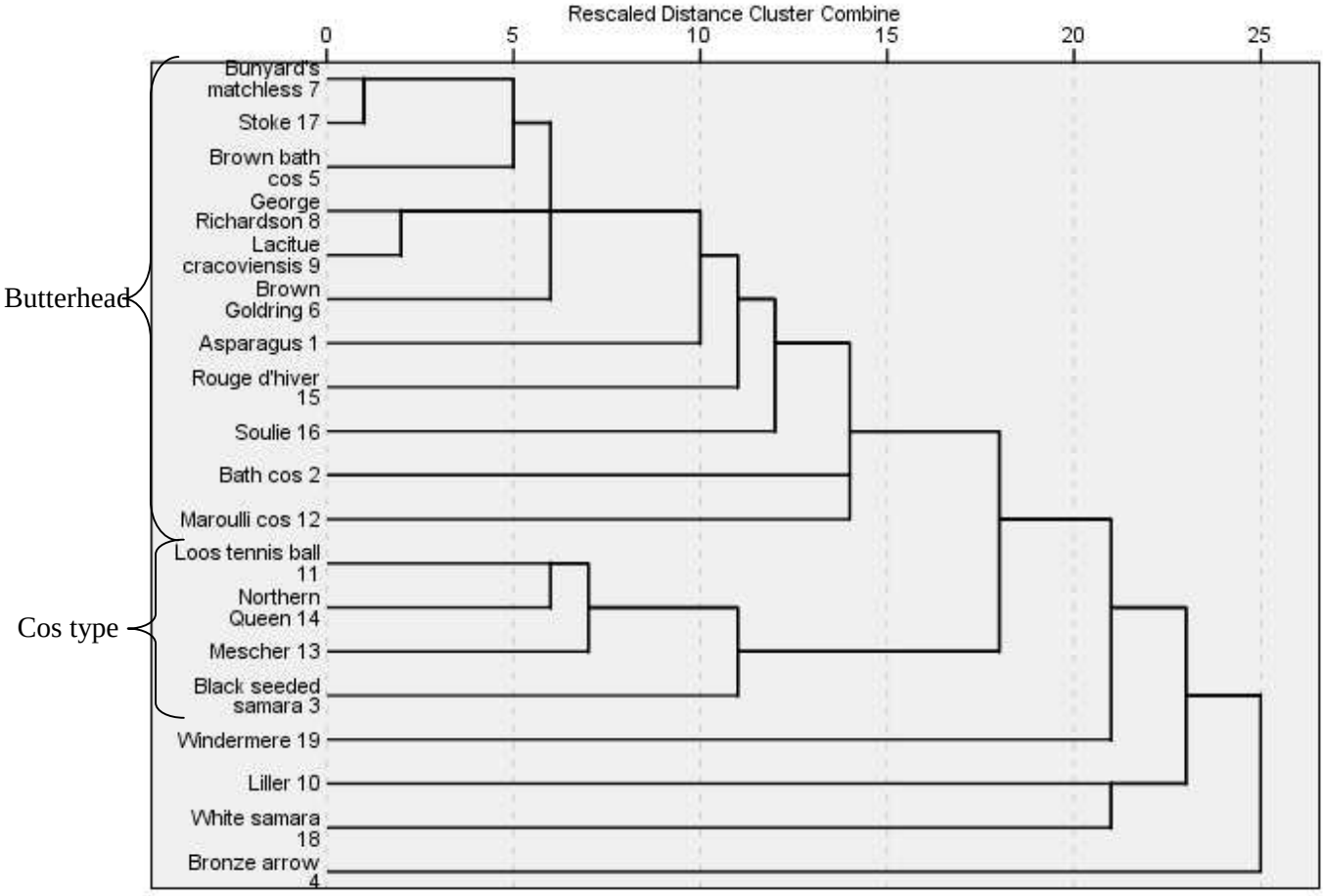

Figure 4.13 UPGMA Cluster Analysis dendrogram of *Lactuca sativa* accessions. Cluster Analysis of *Lactuca sativa* accessions. Butterhead and cos lettuce type clusters highlighted with brackets. Derived in SPSS using nine variables.

Duplicates

Due to the incomplete maturation of many accessions, it was not possible to determine accurately whether duplicates are present within the accessions studied.

4.3.5 Cucumis sativus

Eleven HSL accessions and two commercial standards (Telegraph Improved and Burpless Tasty Green F1 Hybrid) were grown for *C. sativus*. One accession, Striped and Sweet, did not germinate well and all plants died; two accessions, Kiwano African Horned and West Indian Burr Gherkin, produced no fruit and so were removed from the analysis. These latter two accessions are most likely not *Cucumis sativa*, but relate to *Cucumis metuliferus* (Kiwano) and *Cucumis anguria* (West Indian Gherkin) respectively. Eight quantitative variables were recorded and 12 qualitative.

Distribution of variation between accessions

After the removal of highly correlated variables (leaf length and leaf width) and variables with large amounts of missing data (mature fruit characters) insufficient variables remained to perform a PCA, therefore relationships were visualised using scatter plots. The scatter plots of quantitative variables (Figure 4.14) showed multiple correlations, and outlying accessions were Jordanian (red circles) and Standard 1 (red crosses), which were the smallest and largest accessions, respectively. Standard 2 (blue crosses) was clustered with the main group of accessions.

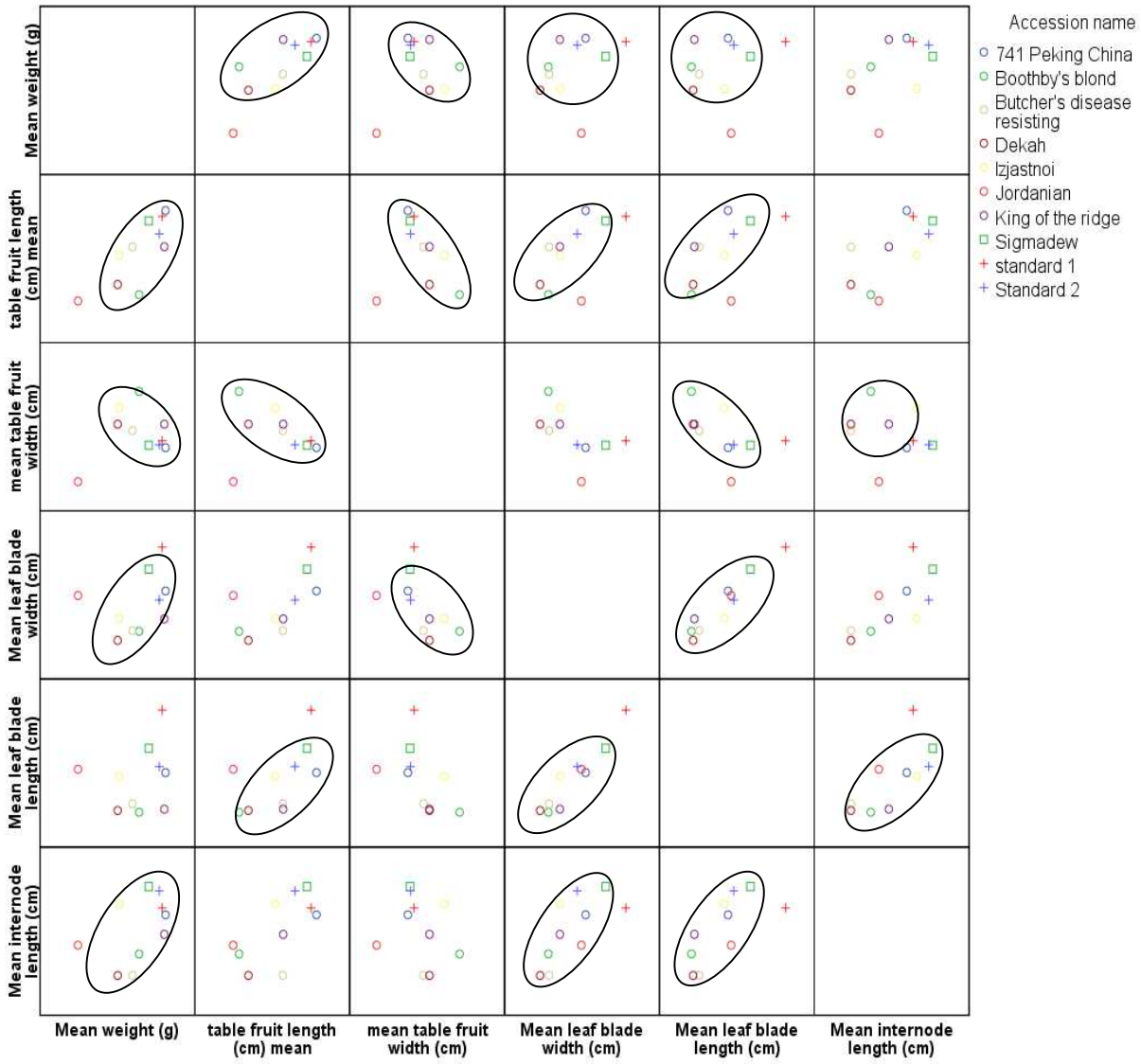

Figure 4.14 Scatter plots of quantitative variables for *Cucumis sativus*. Scatter plot matrix of quantitative variables for *Cucumis sativus* accessions. Derived in SPSS. Circles highlight accession clusters.

Clustering between accessions

In a UPGMA Cluster Analysis using quantitative variables only (dendrogram not shown), two clusters were formed; this grouped the accessions predominantly based on fruit characters. One cluster was composed of 'conventional' shaped cucumbers (those with long and narrow

fruits – 741 Peking China, Standards 1 and 2 and Sigmadew), and the other cluster was composed of smaller or wider fruited accessions. Jordanian was the least similar accession due to the small size of the fruits (this reflects what was seen in the scatter plots above). The closest clustering accessions were 741 Peking China and Standard 2, and Butcher's Disease Resisting and Dekah.

Using all variables (Table 4.17) two clusters were formed (Figure 4.15). Due to the high degree of variation in many variables both within and between accessions (reflected in the long branch lengths), the basis of the clusters was not clearly defined. Accessions 741 Peking China, Standards 1 and 2 and Izjastnoi had stripes over one third of their length and the longest internodes recorded. In this cluster, Izjastnoi was the last to agglomerate; it had a shorter average length than the other clustered accessions. In the second cluster, accessions present had no stripes, with the exception of Dekah, which had stripes over more than two-thirds of the fruit length. Jordanian was the most different in this cluster being of shorter fruit length and having smooth skin texture. Sigmadew was outlying in this analysis, due to its white skin colour. King of the Ridge was also outlying, being the last to agglomerate due to its different skin colour (orange and green), spine colour (black) and shape/dimensions.

Table 4.17 Variables used in UPGMA Cluster Analysis of *Cucumis sativus* accessions.

Variable	Data type
Fruit length (at table readiness)(cm)	Quantitative (scale)
Fruit width (at table readiness) (cm)	Quantitative (scale)
Fruit weight (at table readiness) (g)	Quantitative (scale)
Mean leaf blade width (cm)	Quantitative (scale)
Mean leaf blade length (cm)	Quantitative (scale)
Mean internode length (cm)	Quantitative (scale)
Stem end shape IPGRI	Qualitative
Stem end shape HSL	Qualitative
Spine colour	Qualitative
Skin colour (at table readiness)	Qualitative
Shape at blossom end	Qualitative
Fruit shape	Qualitative
Stripe extent	Qualitative
Stripe colour	Qualitative
Skin texture	Qualitative

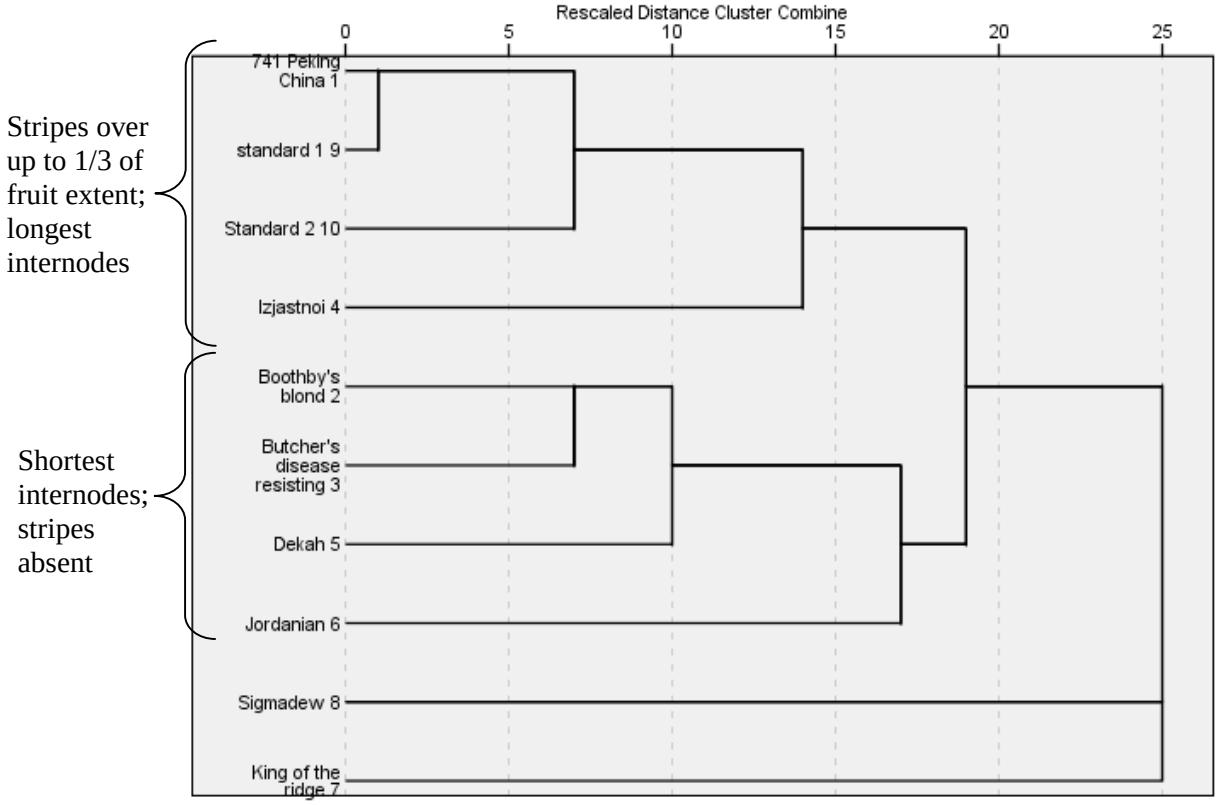

Figure 4.15 UPGMA Cluster Analysis dendrogram for *Cucumis sativus* accessions. UPGMA dendrogram for *Cucumis sativus* accessions. Derived in SPSS using 15 variables. Accession clusters based on stripe presence/absence and internode length highlighted with brackets.

When qualitative variables only were utilized (dendrogram not shown), no large clusters were formed and branch lengths were very long suggesting high morphological dissimilarity between accessions in these variables.

Duplicates

All accessions are morphologically distinct and therefore none of the accessions characterised in the present study are duplicates. Cluster Analysis identified 741 Peking China and standard 1 as being potentially morphologically similar, however these can be clearly distinguished as

741 Peking China has prominent white spines on frequent ‘warts’, whereas standard 1 has few to absent warts (predominantly smooth skin) with low frequency-absent spines.

4.3.6 Capsicum annuum

Sixteen descriptors were recorded for nine HSL accessions and two commercial standards (Worldbeater and F1 Gypsy).

Distribution of variation between accessions

There were insufficient quantitative variables to perform a PCA therefore scatter plots were used (Figure 4.16). Scatter plots of the three quantitative variables indicated positive correlations between fruit weight and fruit width, other variables were uncorrelated. Accessions Skinny and Trifetti, Standard 1 and Californian Bell, Standard 2 and Soror Sarek (occasionally with Macedonian Sweet), and Nardello formed small clusters or outliers.

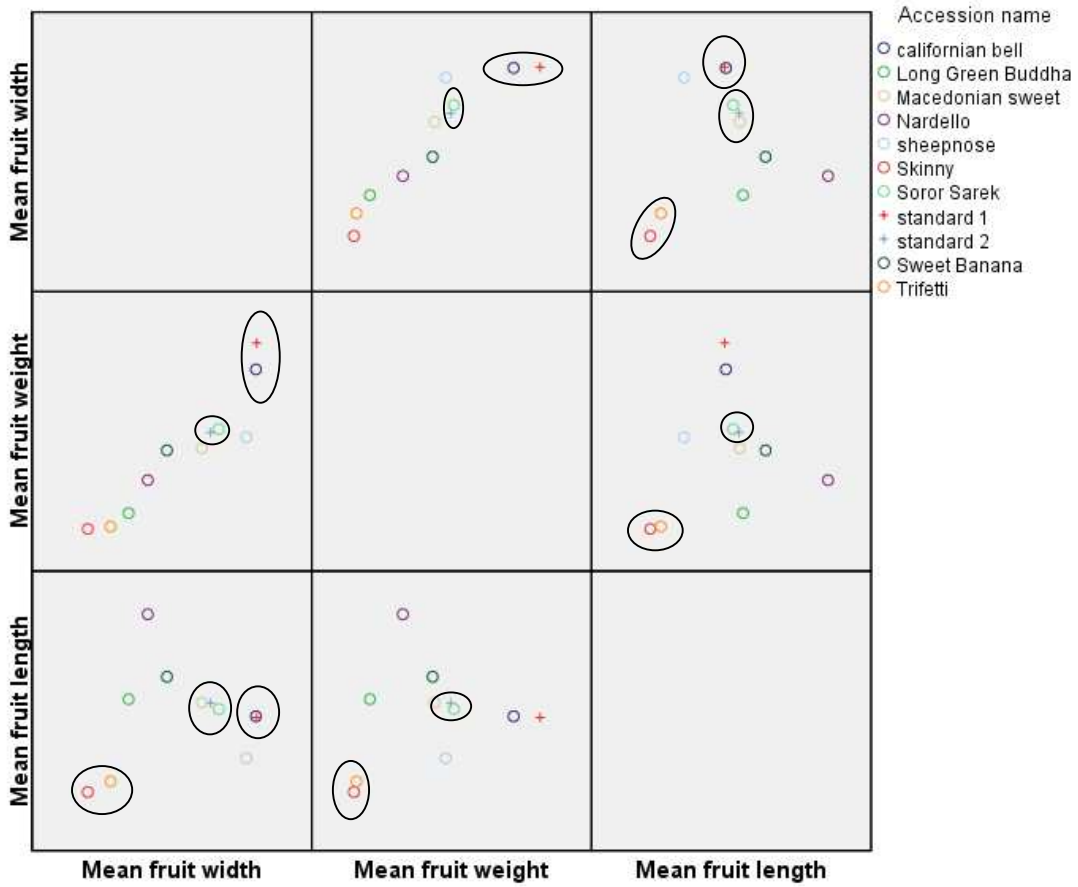

Figure 4.16 Scatter plots of quantitative variables for *Capsicum annuum*. Scatter plot matrix of quantitative variables mean fruit length, mean fruit weight and mean fruit width for *Capsicum annuum* accessions. Derived in SPSS. Circles highlight closely clustering accessions.

Clustering between accessions

Characters for which data were collected but that were subsequently not included in the analysis were excluded due to multiple factors including: possible distortion from environmental effects from overcrowding in the glasshouses (growth habit), subjectivity (stem shape, flower position), or absence of variation (mature fruit exterior colour, anther

colour, neck at base of fruit). Ultimately, three quantitative variables, one ordinal and five qualitative variables were used to perform the UPGMA Cluster Analysis.

At the accession level of analysis, accessions clustered predominantly on size and shape characters; using quantitative variables only (mean fruit width, mean fruit weight and mean fruit length) (Figure 4.17) the three main clusters observed were small fruited accessions, long and narrow fruited types, and larger and wider fruited types (bell peppers).

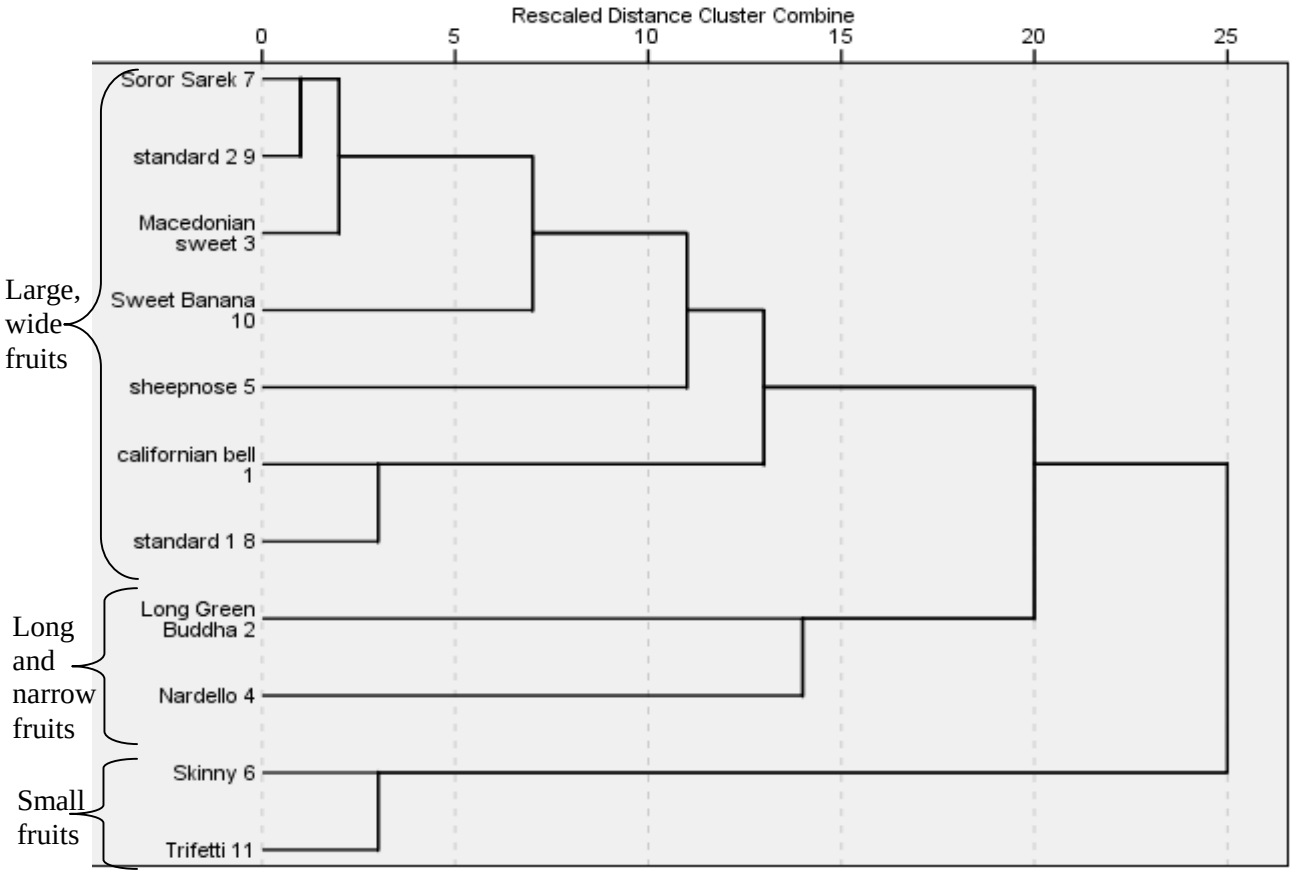

Figure 4.17 UPGMA Cluster Analysis dendrogram of *Capsicum annuum* **accessions.** UPGMA dendrogram for *Capsicum annuum* accessions. Derived in SPSS using three quantitative variables. Clusters based on fruit dimensions highlighted with brackets.

In the analysis utilizing all variables (Table 4.18), accessions clustered according to overall shape (Figure 4.18), with differences in quantitative variables (i.e. actual size) adding finer detail and longer branch lengths. For example, Skinny was the same overall shape as the other members of its cluster (long and narrow), however was a fraction of the size. The three main clusters were 'blocky' accessions, 'elongate' accessions, and 'triangular' accessions. Intermediate stage colour and corolla colour were of importance in identifying Trifetti as the most dissimilar accession. Shortest branch lengths were between Standard 2, Californian Cell and Macedonian Sweet, and Sweet Banana and Nardello.

Table 4.18 Variables used in UPGMA Cluster Analysis of *Capsicum annuum* accessions.

Variable	Data type
Mean fruit width	Quantitative (scale)
Mean fruit weight	Quantitative (scale)
Mean fruit length	Quantitative (scale)
Cross section corrugation	Quantitative (ordinal)
Corolla colour	Qualitative
Fruit colour intermediate stage	Qualitative
Fruit shape	Qualitative
Fruit shape at blossom end	Qualitative
Blossom end appendage	Qualitative (presence/absence)

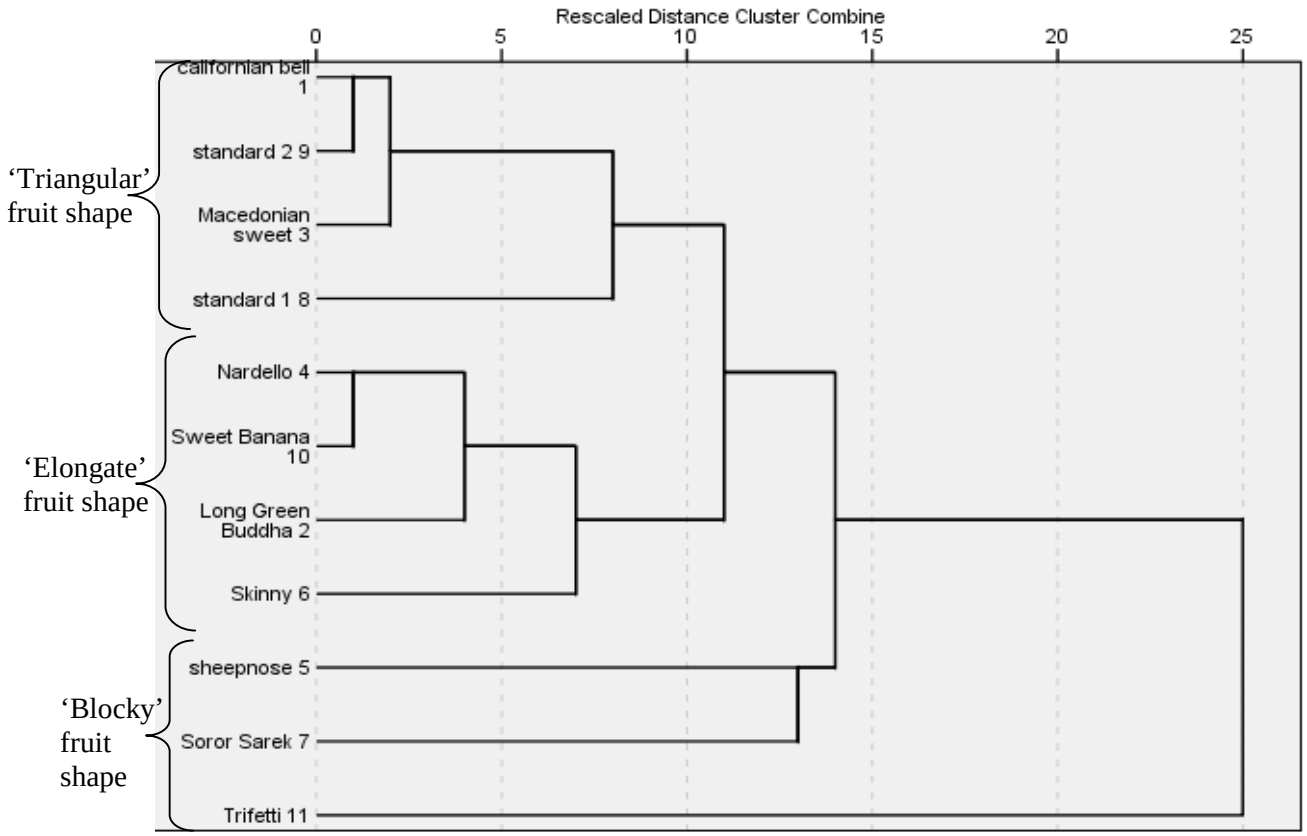

Figure 4.18 UPGMA Cluster Analysis dendrogram of *Capsicum annuum* accessions. UPGMA dendrogram for *Capsicum annuum* accessions. Derived in SPSS using nine variables. Clusters based on fruit dimensions highlighted with brackets.

Using just qualitative variables (dendrogram not shown) served to separate Soror Sarek from the 'triangular' fruit shape cluster due to the presence of a blossom end appendage (absent in other accessions); Standard 1 and Sheepnose formed a small cluster based on blossom end shape, remaining accessions clustered according to fruit shape.

Duplicates

C. annuum accessions highlighted as similar by the UPGMA or PCA were further examined to check for duplicate accessions (Table 4.19). All accessions had either statistically significant differences in quantitative variables, or one qualitative variable difference.

Californian Bell and Macedonian Sweet were significantly different in fruit width (U=46.5, p=0.00, r=0.6) and fruit weight (U=69.5, p=0.02, r=0.49), as well as having differences recorded in fruit shape (Californian Bell had ‘blocky’ and ‘elongate’ fruits, compared to ‘elongate’ and ‘triangular’ fruits in Macedonian Sweet).

Accessions Nardello and Sweet Banana clustered closely in the accession level analysis using all variables. Statistically significant differences were present between these accessions in fruit length (U = 100.5, p = 0.00, r = 0.7) and fruit width (U = 306.0, p = 0.03, r = 0.37). Sweet Banana also had a higher frequency of ‘triangular’ shaped fruits than Nardello.

Soror Sarek and Macedonian Sweet showed significant differences in fruit cross section corrugation (U=40, p=0.03, r=0.57), as well as in fruit shape (with ‘elongate’ and ‘triangular’ fruits in Macedonian Sweet, and ‘blocky’ and ‘triangular’ fruits in Soror Sarek).

No statistically significant differences were recorded in quantitative variables between Standard 1 and Californian Bell. However, a third of Standard 1 fruits were blunt ended, this shape was not recorded in any Californian Bell individuals.

Californian Bell and Standard 2 had significant differences in fruit length (U = 230.0, p = 0.03, r = 0.3), fruit width (U = 120.0, p = 0.00, r = 0.56) and fruit weight (U = 183.5, p = 0.03, r = 0.41).

Macedonian Sweet and Standard 2 had no significant quantitative differences recorded, but qualitative differences in fruit shape and fruit blossom end shape were observed. The former being Macedonian sweet had higher frequency of 'elongate' fruits at plot level, which were absent in Standard 2. Fruit blossom end shape was predominantly 'pointed' in Macedonian sweet, with 'pointed' and 'sunken' seen in Standard 2 ('sunken' was not recorded in Macedonian Sweet).

Standard 2 and Soror Sarek were statistically significantly different in cross section corrugation ($U = 118.5$, $p = 0.038$, $r = 0.39$) and also had qualitative differences in the variable blossom end appendage (present in Soror Sarek but absent in Standard 2).

Accessions Skinny and Trifetti frequently clustered together in analyses using quantitative variables, as both were very small-fruited accessions, however differences were recorded in four qualitative variables (Table 4.26).

Table 4.19 Differences between potential *Capsicum annuum* duplicate accessions. Differences in quantitative and qualitative variables between *Capsicum annuum* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Quantitative variables	Qualitative variables
Californian Bell and Macedonian Sweet	Fruit width, fruit weight	Fruit shape
Nardello and Sweet Banana	Fruit length, fruit width	
Soror Sarek and Macedonian Sweet	Cross section corrugation	Fruit shape
Standard 1 and Californian Bell	Fruit length, fruit width, fruit weight	Fruit blossom end shape
Standard 2 and Californian Bell		
Standard 2 and Macedonian Sweet		Fruit shape, fruit blossom end shape
Standard 2 and Soror Sarek	Cross section corrugation,	Blossom end appendage
Trifetti and Skinny		Intermediate stage fruit colour, flower colour, fruit shape, fruit blossom end shape

All accessions could be distinguished using at least one descriptor; by this criterion the collection contained no duplicate accessions.

4.3.7 Raphanus sativus

Twenty nine morphological descriptors were collected for the 10 HSL accessions and two commercial standards (Saxa 2 and Scarlet Globe), of which eight were used for the PCA and 20 were used for the Cluster Analysis. These variables gave enough information to separate out all accessions.

Distribution of variation between accessions

Eight quantitative variables were utilized in the PCA, of these leaf length and leaf width were highly correlated (r = 0.93), however were retained for the analysis due to potentially important variation between accessions, and root width was low scoring in anti-image correlation, however was retained, again, because of potentially important within and between accession variation.

An unrotated factor solution was found to be optimal for *R. sativus*, as Principal Components were not correlated (confirmed by running the Promax rotation), which had a KMO of 0.62, which is low but acceptable; extraction was lowest in root length (0.65). As in the above analysis leaf length and leaf width were highly correlated (r = 0.92), but were retained. The scree plot indicated a two-Component solution (Figure 4.19), with the main inflection, above an eigenvalue of one, at two Components. The first two Components explained 51.29% and 23.77% of the variance; the third Component above an eigenvalue 1 explained a further 12.95% (cumulative variance of the three Components was therefore 88.00%).

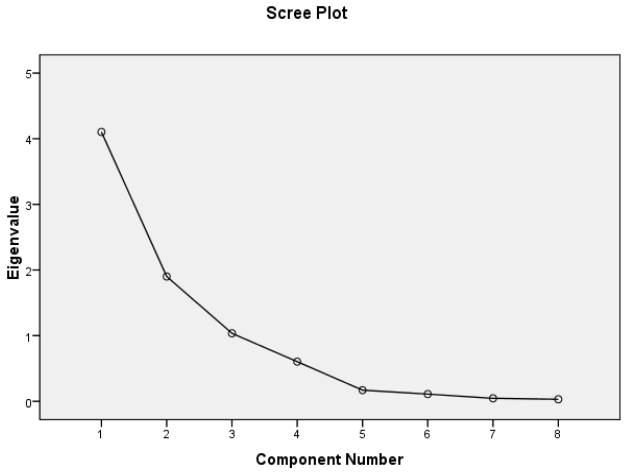

Figure 4.19 Principal Components Analysis scree plot. Scree plot for Principal Components Analysis of *Raphanus sativus* accessions using eight variables. The scree plot indicated a two-Component solution, with the main inflection, above an eigenvalue of one, at two Components. Derived using SPSS.

The first Principal Component, as in the previous analysis, was defined by the highly loading variables leaf length, weight, leaf width and petiole width (Table 4.20); the second Principal Component was defined by root width; and the third had number of leaves loading at a low score.

Table 4.20 Principal Components Analysis components matrix. Component matrix for *Raphanus sativus*. Derived in SPSS. Variables with loadings over 0.7 are highlighted in bold.

	Component		
	1	2	3
Mean leaf length	**.950**		
Mean weight	**.948**		
Mean leaf width	**.933**		
Mean petiole width	**.839**		
Mean root length	.669		
Mean root width		**.809**	
Mean petiole length		**-.798**	
Mean number of leaves		.661	.670

In a scatter plot projecting PC1 and PC2 (Figure 4.20) accessions were distributed broadly, with one main cluster and two outlying accessions (Munchen Bier and Rat's Tail). No variables were tightly clustered, suggesting the presence of morphological variation between accessions in the variables measured.

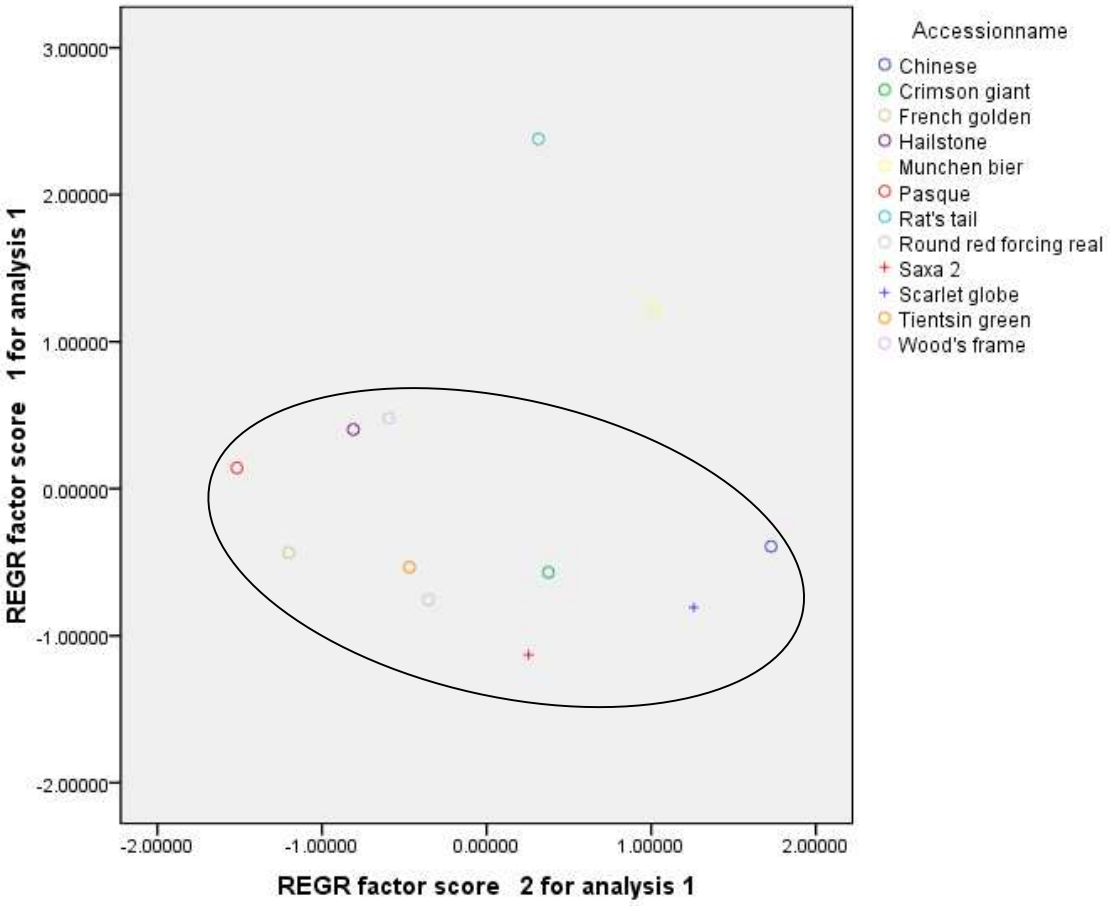

Figure 4.20 Scatter plot of first two Principal Components. Principal Components Analysis of *Raphanus sativus* accessions produced using eight morphological variables; Principal Components 1 and 2 explained 51.29% and 23.77% of the variance respectively. Circle highlights accession cluster. Derived using SPSS.

In a scatter plot of Components one and three (not shown), accessions were evenly distributed along the axis of Component three, with Rat's Tail and Munchen Bier separated out by

Component 1. A scatter plot of Components two and three (Figure 4.21), indicted a different distribution, with: one loose cluster, a close association between Saxa 2 and Rat's Tail, and Hailstone, Standard 2 (Scarlet Globe) and Tientsin Green outlying.

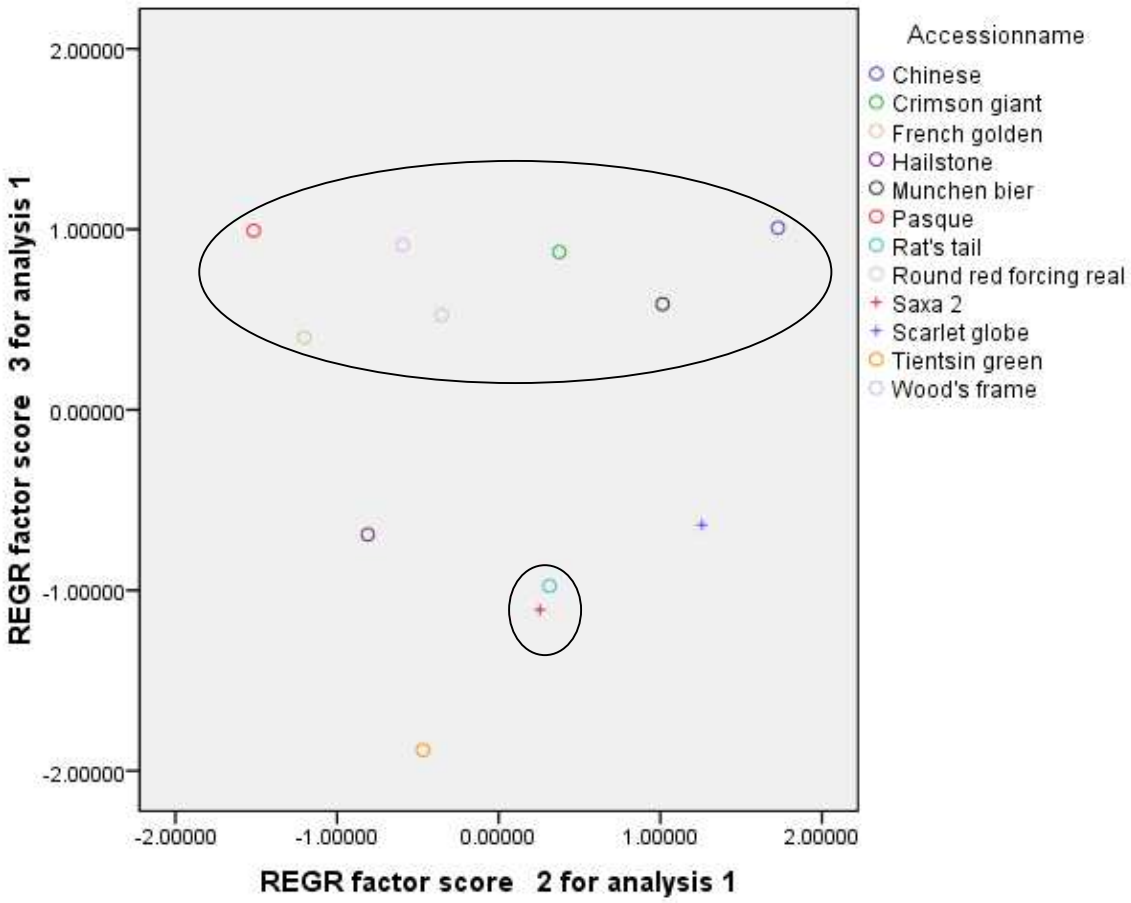

Figure 4.21 Scatter plot of second and third Principal Components. Principal Components Analysis of *Raphanus sativus* accessions produced using eight morphological variables; Principal Components 3 and 2 explained 12.95% and 23.77% of the variance respectively. Circles highlight accession clusters. Derived using SPSS.

Clustering between accessions

In a UPGMA Cluster Analysis using quantitative variables only (dendrogram not shown) no large clusters were formed. Munchen Bier and Rat's Tail were the most dissimilar. These accessions had the largest leaves and were similar in root length.

Long branch lengths suggested low morphological similarity between most accessions when using all variables (Table 4.21; Figure 4.22). In the top cluster, there were short branches between Round Red Forcing Real and Saxa 2; also in this cluster were Crimson Giant and Scarlet Globe, all of these accessions have an absence of lighter exterior colour, are predominantly 'spheric' in shape, with purple exterior colour. Tientsin Green is also 'spheric', however, differed in root shape at base and shoulder, as well as in leaf apex shape.

Table 4.21 Variables used in UPGMA Cluster Analysis of *Raphanus sativus* accessions.

Variable	Data type
Mean weight (g)	Quantitative (scale)
Mean number of leaves	Quantitative (scale)
Mean leaf length (cm)	Quantitative (scale)
Mean leaf width (cm)	Quantitative (scale)
Mean petiole length (cm)	Quantitative (scale)
Mean petiole width (cm)	Quantitative (scale)
Mean root length (cm)	Quantitative (scale)
Mean root width (cm)	Quantitative (scale)
Lateral root emergence on bulb	Quantitative (ordinal)
Leaf blade shape outline	Qualitative
Leaf division margin	Qualitative
Leaf division incision	Qualitative
Leaf apex shape	Qualitative
Petiole colour	Qualitative
Root shape long section	Qualitative
Root shape shoulder	Qualitative
Root shape base tip	Qualitative
Root exterior colour pattern	Qualitative
Exterior root colour darker	Qualitative
Exterior root colour lighter	Qualitative

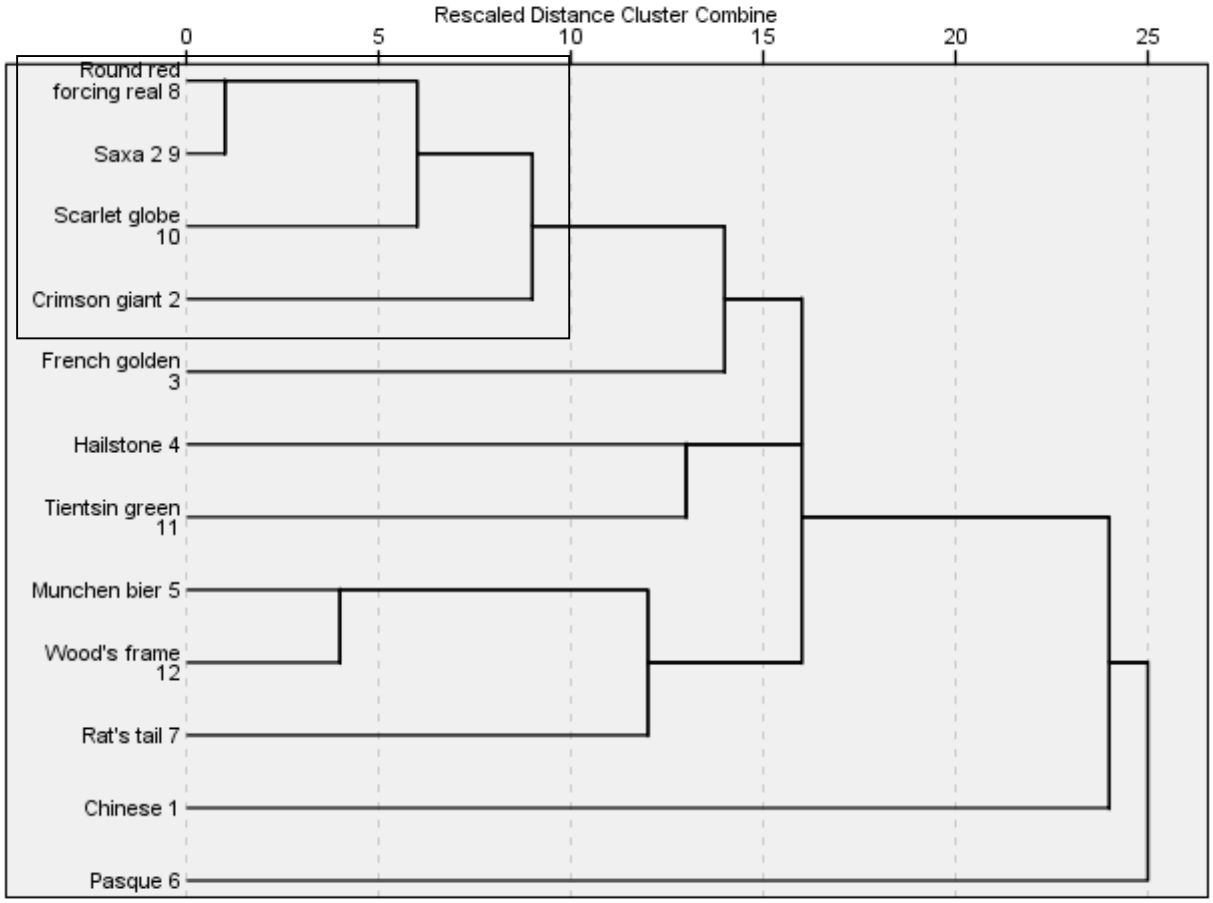

Figure 4.22 UPGMA Cluster Analysis dendrogram of *Raphanus sativus* accessions. UPGMA dendrogram for *Raphanus sativus* accessions. Box highlights clustering of accessions based on the morphological characters absence of lighter exterior colour, 'spheric' shape, and with purple exterior colour. Derived in SPSS using 20 variables.

With qualitative variables only (not shown) the Saxa 2, Scarlet Globe and Round Red Forcing Real cluster and Crimson Giant cluster is maintained.

Duplicates

The accessions highlighted by the Cluster Analysis as morphologically similar were Round Red Forcing Real, Crimson Giant and the two commercial standards (Saxa 2 and Scarlet Globe). These accessions were further examined using Mann-Whitney U to test whether the two HSL accessions were duplicates, and also that the two accessions highlighted above as being the most similar (Round Red Forcing Real and the standard Saxa 2) were significantly different.

Although none of the differences in quantitative variables were significant between Round Red Forcing Real and Crimson Giant, the effect sizes were medium for root weight (r=0.42) and number of leaves and leaf scars (r=0.43), suggesting that a larger sample size may demonstrate significant differences. In qualitative characters, root shapes were more diverse in Round Red Forcing Real with many ‘cylindric’ individuals observed as well as ‘spheric’, in Crimson giant all individuals were ‘spheric’; lateral root emergence was higher in Round Red Forcing Real; leaf division margin was crenate in Crimson giant and dentate in Round Red Forcing Real. These differences suggest that the two accessions may not be duplicates (Table 4.22).

No significant differences were found in quantitative variables between Round Red Forcing Real and Saxa 2, although petiole width and leaf length had large effect sizes (r=0.55 and r=0.5 respectively) therefore a larger sample may show significant results. For qualitative variables also, a larger sample would be informative; variation was present in both accessions in root colour, with both recording a replicate plot each for different shades of pink/purple; there were very slight differences in root shape and root shape at base between the accessions. Both accessions showed a mixture of ‘spheric’ and ‘cylindric’ root shapes; however, some

'inverse triangle' shaped individuals were also present rarely in Saxa 2. Both accessions had predominantly convex root base shape, however in Round Red Forcing Real occasional individuals had obtuse root shape at base. Lateral root emergence was much greater on Round Red Forcing Real than on Saxa 2.

Table 4.22 Differences between potential *Raphanus sativus* duplicate accessions. Differences in quantitative and qualitative variables between *Raphanus sativus* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Qualitative variables	Quantitative variables
Round Red Forcing Real and Crimson Giant	-	Leaf division margin, root shape, lateral root emergence on bulb
Round Red Forcing Real and Standard 1 (Saxa 2)	-	Root shape, root shape at base, lateral root emergence on bulb

4.3.8 Brassica napobrassica

Two HSL *Brassica napobrassica* accessions and two commercial standards (Angela and Virtue) were characterised; all accessions could be distinguished from one another using the descriptors collected. No leaves were available for characterisation. 11 morphological descriptors were recorded.

Distribution of variation between accessions

There were not enough variables to perform a PCA. Scatter plots for each variable combination (Figure 4.23) demonstrated that all variables were positively correlated, and showed accessions widely distributed across the plot, with no clusters.

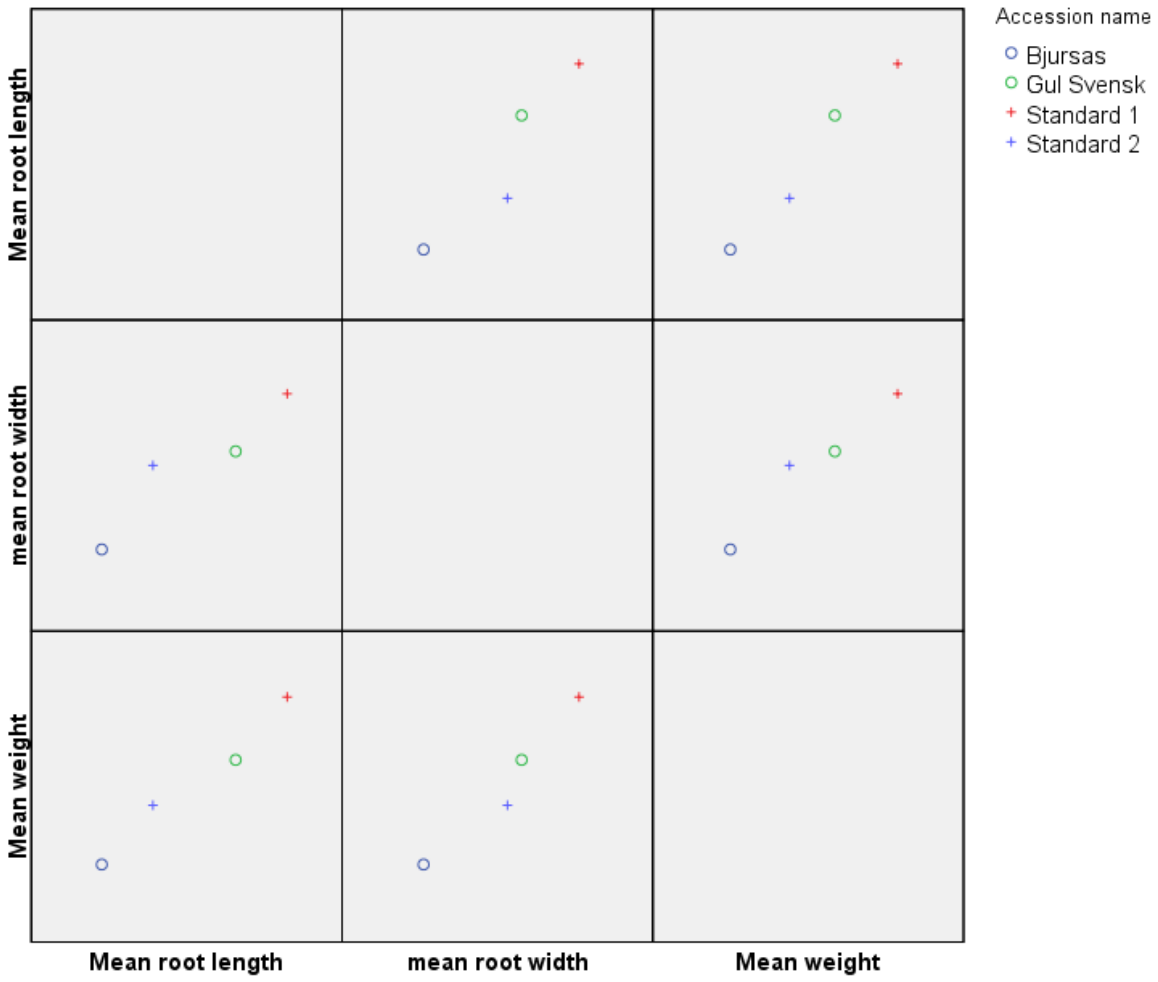

Figure 4.23 Scatter plots of quantitative variables for *Brassica napobrassica*. Scatter plots of quantitative variables mean root weight, mean root length and mean root width for *Brassica napobrassica* accessions. Derived in SPSS.

Clustering between accessions

In the by accession Cluster Analysis there were not enough accessions to form large clusters. Figure 4.24 shows the dendrogram for all variables (Table 4.23). Bjursas was consistently most different from the other accessions, using the different types of variables. Gul Svensk was always on short branches clustered with either standard 1 or standard 2, however which standard changed with variable combinations. This may be due to the general high level of

morphological variability in root shape within accessions being as high as between. Bjursas was consistently be the last to agglomerate, due to its difference in size to the other accessions (it was smaller) and had a white interior whereas the other three were yellow.

Table 4.23 Variables used in UPGMA Cluster Analysis of *Brassica napobrassica* accessions.

Variable	Data type
Mean weight	Quantitative (scale)
Mean root length	Quantitative (scale)
Mean root width	Quantitative (scale)
Lateral root emergence	Quantitative (ordinal)
Root shape in long section	Qualitative
Root shape at base tip	Qualitative
Root exterior colour pattern	Qualitative
Exterior root colour	Qualitative
Interior root colour	Qualitative
Flesh colour distribution trans section	Qualitative

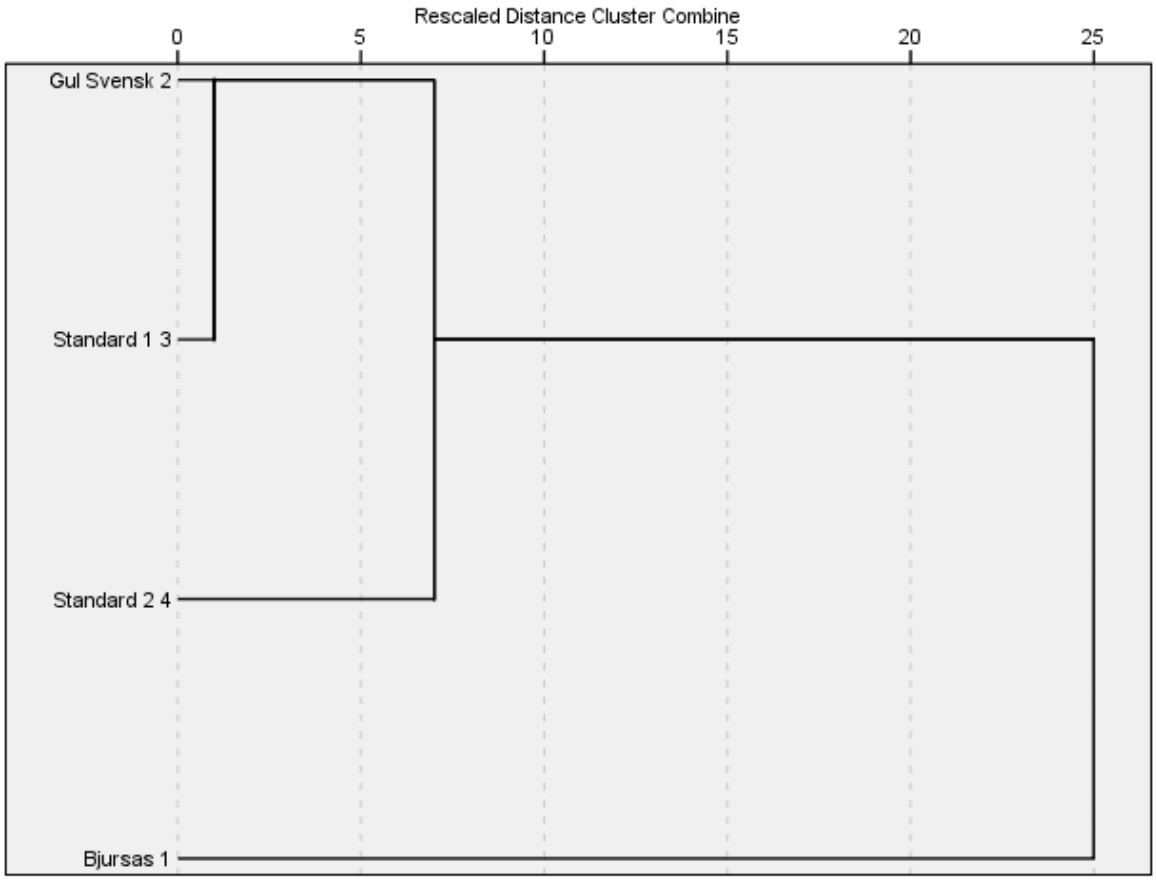

Figure 4.24 UPGMA Cluster Analysis dendrogram for *Brassica napobrassica* accessions. UPGMA dendrogram for Cluster Analysis of *Brassica napobrassica* accessions. Derived in SPSS, using 10 variables.

Duplicates

All accessions could be distinguished using the present variables. Standard 1 and Gul Svensk, which clustered closely in the above analysis, were observed to have different root colours (Gul Svensk being predominantly green and standard 1 being predominantly purple) and different root shapes (Gul Svensk had individuals that were either ‘apically bulbous’ or ‘inverse triangular’ shaped, and Standard 1 also had these in addition to ‘cylindric individuals’).

4.3.9 Brassica rapa var. *rapa*

Three HSL accessions and two commercial standards (Purple Top Melon and Oasis) were characterised, using 28 morphological descriptors; all accessions could be distinguished.

Distribution of variation between accessions

Due to the small number of accessions and low number of uncorrelated quantitative variables a PCA was not applicable. This was reflected in the low KMO value obtained (0.33) and low anti-image correlation scores. Using scatter plots to display quantitative variables for accessions, positive correlations were observed between mean root weight (without leaves) and root width and between leaf width and root length, and negative correlation were observed between root length and root width (confirmed using Spearman's correlation, significant to the 0.05 level) (Figure 4.25). Different accessions were outlying, depending on the variable combination. Standard 2 and Kaskinauris Stock frequently clustered together, as did Black Sugarsweet and Gammel Svensk. Standard 1 was outlying in root weight (without leaves) plots.

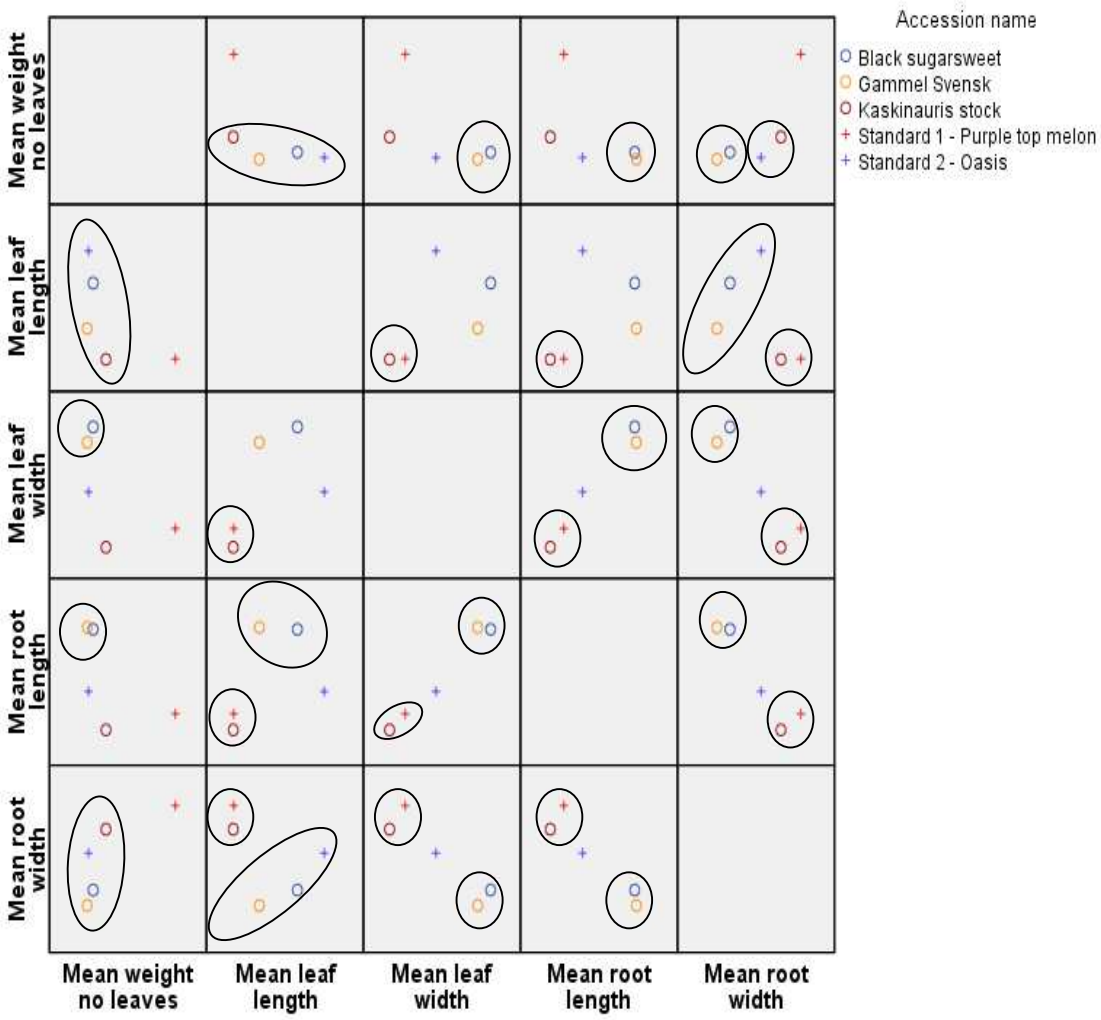

Figure 4.25 Scatter plot of quantitative variables for *Brassica rapa* var. *rapa*. Scatter plot matrix of quantitative variables mean weight without leaves, mean leaf length, mean leaf width, mean root width and mean root length for *Brassica rapa* var. *rapa* accessions. Derived in SPSS. Circles highlight clusters of accessions.

Clustering between accessions

Using either quantitative variables (dendrogram not shown) or quantitative and qualitative variables (Table 4.24; Figure 4.26), relationships between accessions were the same as in the above plots analysis, with Kaskinauris Stock and Standard 1 clustered together, and Black Sugarsweet and Gammel Svensk clustered together (Figure 4.26). Branch lengths were shorter

within those clusters, and were long between clusters, indicating morphological dissimilarity. The two larger clusters again may be based on root dimensions, with long and narrow ('horn-cylindric'), 'inverse triangular-elliptic', and 'transverse elliptic-elliptic' shaped accessions, or other root characters including colour and colour pattern. Employing all variables allowed all accessions to be distinguished. Although Gammel Svensk and Black Sugarsweet were on short branches, they were different colours and so were not duplicates.

Kaskinauris Stock and Standard 1 were extremely similar in root characters. However, results for the present study suggest that they may be distinguished using leaf characters, including petiole colour, leaf division, leaf colour and blade shape.

Table 4.24 Variables used in UPGMA Cluster Analysis of *Brassica rapa* var. *rapa* accessions.

Variable	Data type
Mean weight	Quantitative (scale)
Mean number of leaves and scars	Quantitative (scale)
Mean leaf length	Quantitative (scale)
Mean leaf width	Quantitative (scale)
Mean root length	Quantitative (scale)
Mean root width	Quantitative (scale)
Median leaf angle	Quantitative (ordinal)
Median position of bulb in soil	Quantitative (ordinal)
Median leaf apex shape	Quantitative (ordinal)
Median leaf blade blistering	Quantitative (ordinal)
Median leaf hairiness	Quantitative (ordinal)
Median lateral root emergence on bulb	Quantitative (ordinal)
Leaf division margin	Qualitative
Leaf division incision	Qualitative
Petiole and midvein colour	Qualitative
Root shape in long section	Qualitative
Root shape of shoulder	Qualitative
Root shape at base tip	Qualitative
Root exterior colour pattern	Qualitative
Exterior root colour	Qualitative
Interior root colour	Qualitative
Root flesh colour distribution in transverse section	Qualitative

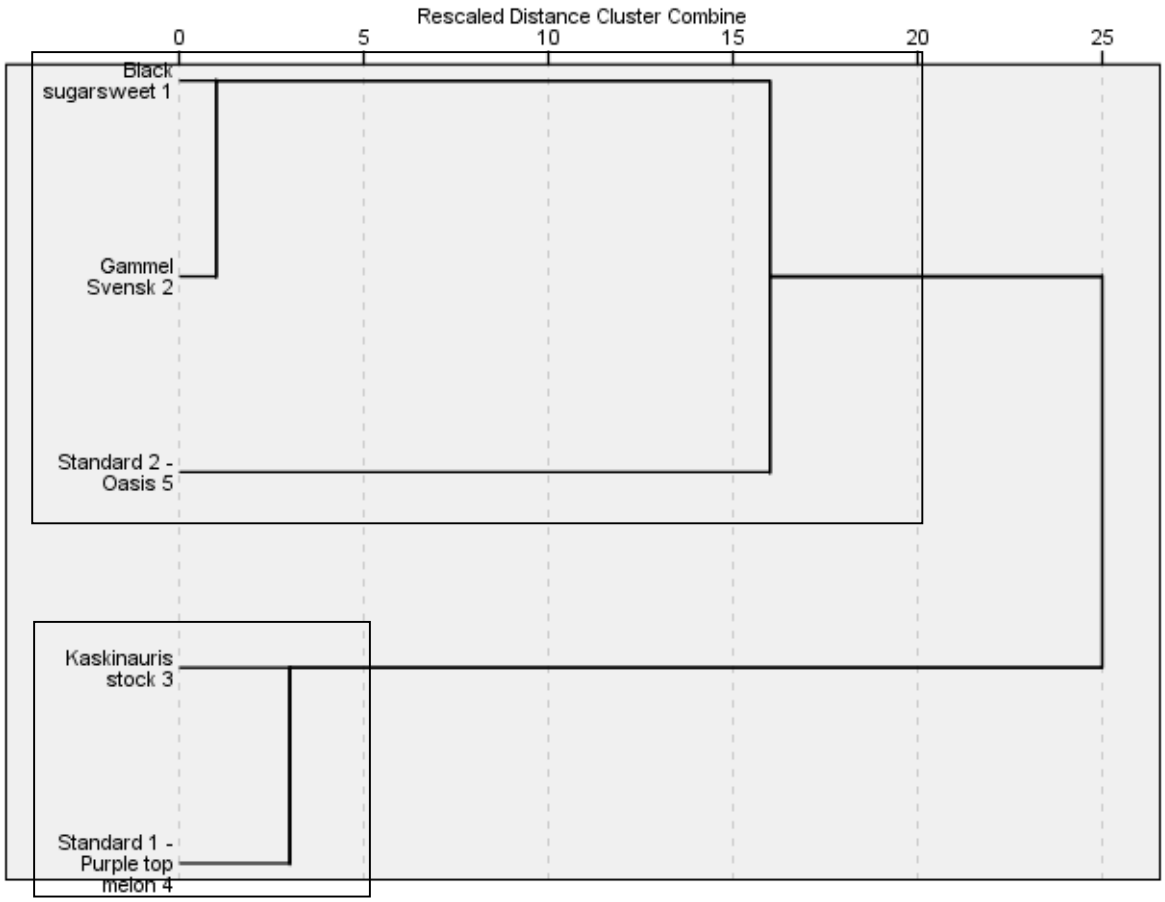

Figure 4.26 UPGMA Cluster Analysis dendrogram for *Brassica rapa* var. *rapa*. Dendrogram for UPGMA Cluster Analysis of *Brassica rapa* var. *rapa* accessions. Boxes highlight two larger clusters potentially based on root dimensions, with long and narrow ('horn-cylindric'), 'inverse triangular-elliptic', and 'transverse elliptic-elliptic' shaped accessions, or other root characters including colour and colour pattern. Derived in SPSS, using 22 variables.

Duplicates

All accessions could be distinguished using the current descriptors. The Cluster Analysis above identified Gammel Svensk and Black Sugarsweet as being potentially most morphologically similar; closer investigation showed differences in root shape (being 'cylindric' and 'horn-shaped' respectively), root exterior colour (being yellow and black

respectively), and root interior colour distribution (being ‘split into cortex and cambium’ and ‘uniform’ respectively). There were no significant differences between quantitative variables; however, there was a medium effect size for leaf length (r = 0.34), suggesting a larger sample size may show significant effects.

Standard 1 and Kaskinauris stock were also on relatively short branches, these accessions differed in petiole colour (being purple and white respectively) and interior root colour (being white and yellow respectively) (Table 4.25), as well as having significant differences in number of leaves and leaf scars (U = 3.0, p = 0.03, r = 0.66) and root width (U = 1.0, p = 0.01, r = 0.78).

Table 4.25 Differences between potential *Brassica rapa* var. *rapa* duplicate accessions. Differences in quantitative and qualitative variables between *Brassica rapa* var. *rapa* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accession names	Quantitative variables	Qualitative variables
Black Sugarsweet and Gammel Svensk	-	Root shape, root exterior colour, root interior colour distribution
Standard 1 (purple top melon) and Kaskinauris Stock	Number of leaves and leaf scars, root width	Petiole/midvein colour, interior root colour

4.3.10 Allium porrum

As with *L. sativa*, the developmental stage at which the *Allium porrum* plants were characterised was uncertain; they had been planted out a sufficient time to reach maturity, however, they were not very large.

Twelve morphological characters were collected for *A. porrum*. Overall morphological variation was low both within and between accessions.

Distribution of variation between accessions

There were too few quantitative variables and insufficient morphological variation to perform a PCA. In scatter plots of quantitative variables (Figure 4.27), positive correlations were observed between leaf length and weight, leaf width and shaft diameter, leaf length and leaf width, leaf width and shaft length, weight and shaft diameter. Negative correlations were found between shaft length and weight, shaft length and shaft diameter, and shaft length and leaf width. Accessions clustered generally across the scatter plots, with outlying replicates from Standard 1, Kelvedon King, Hannibal, Colossal and Sim Seger.

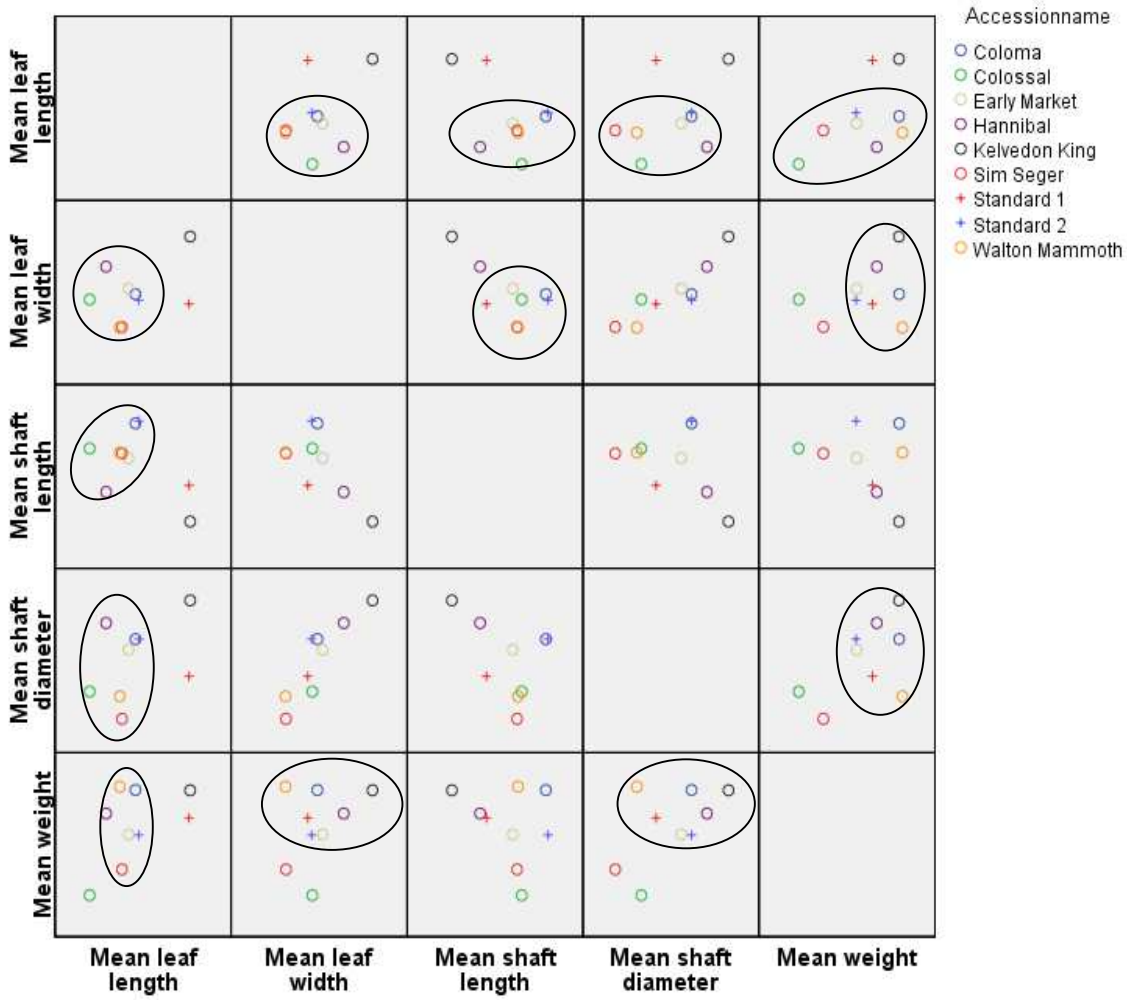

Figure 4.27 Scatter plots of quantitative variables for *Allium porrum*. Scatter plot matrix of quantitative variables mean leaf length, mean leaf width, mean shaft length, mean shaft diameter and mean weight for *Allium porrum* accessions. Derived in SPSS.

Clustering between accessions

UPGMA analysis of *A. porrum* accessions using all variables (Table 4.26; Figure 4.28) had no well-defined clusters. Coloma and standard 2 were the two most morphologically similar accessions, with very short branch lengths in all variable combinations.

Table 4.26 Variables utilised in UPGMA Cluster Analysis of *Allium porrum* accessions.

Variable	Data type
Mean leaf length	Quantitative (scale)
Mean leaf width	Quantitative (scale)
Mean weight	Quantitative (scale)
Mean shaft length	Quantitative (scale)
Mean shaft diameter	Quantitative (scale)
Median foliage cracking	Quantitative (ordinal)
Median foliage attitude	Quantitative (ordinal)
Leaf density	Quantitative (ordinal)
Foliage colour	Qualitative
Shape mature bulb	Qualitative

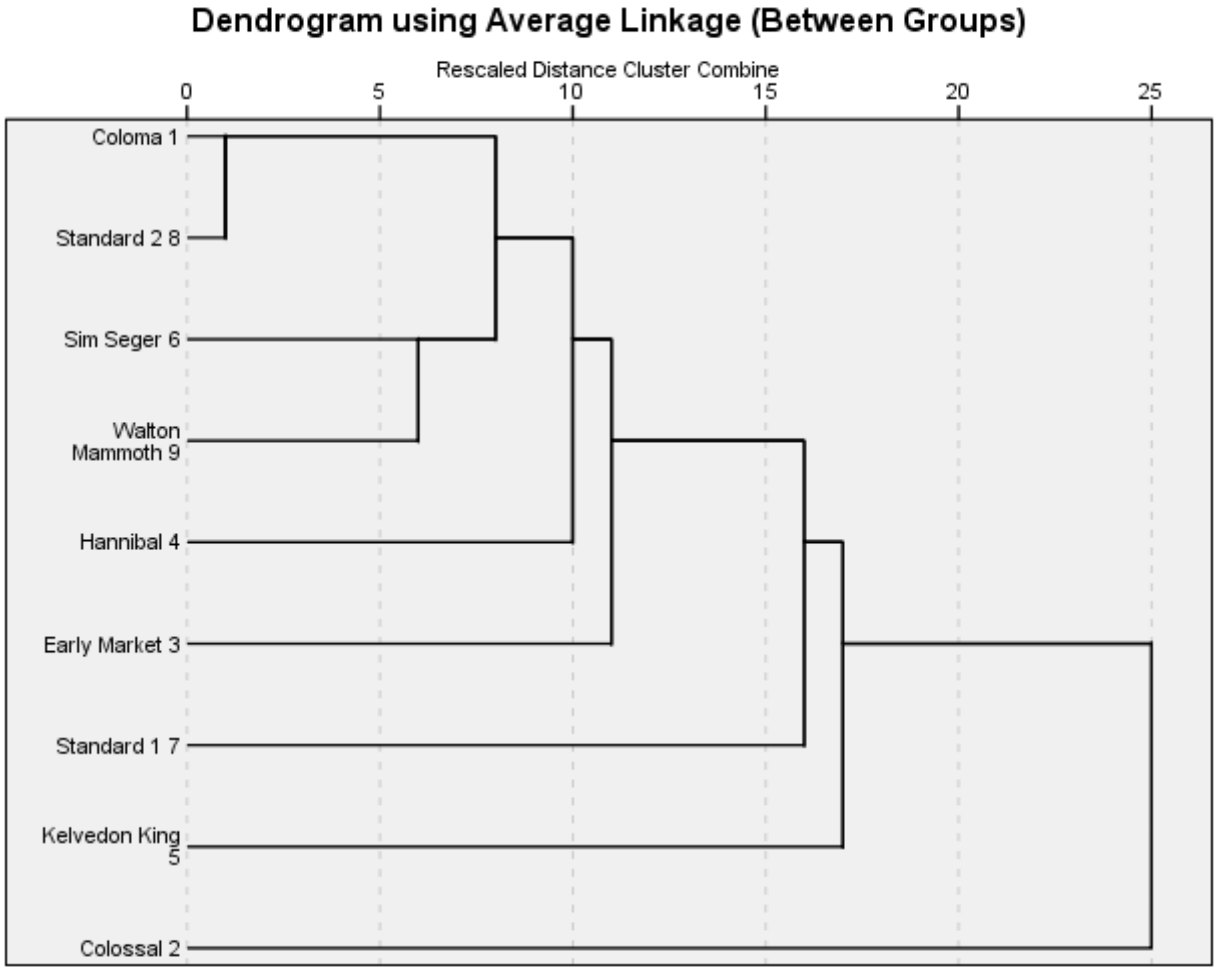

Figure 4.28 UPGMA Cluster Analysis dendrogram for *Allium porrum* accessions. UPGMA Cluster Analysis dendrogram of *Allium porrum* accessions. Derived in SPSS using 10 variables.

Duplicates

Due to the low level of morphological variation between any of the *A. porrum* accessions characterised, absence of duplicates cannot be confirmed with the current descriptors employed.

4.3.11 Solanum lycopersicum

Five quantitative variables were recorded for *S. lycopersicum*. In addition six ordinal variables and 12 qualitative variables were scored.

Distribution of variation between accessions

A rotated solution was used due to correlation between Principal Components (0.47). KMO was 0.77. Weight and width were correlated (r = 0.92) but were left in due to the potentially important variation they potentially held. The scree plot (Figure 4.29) showed a two-Component solution (the first inflection is at 2), the first Component explained 66.26% of the variance, and the second Component 21.28%.

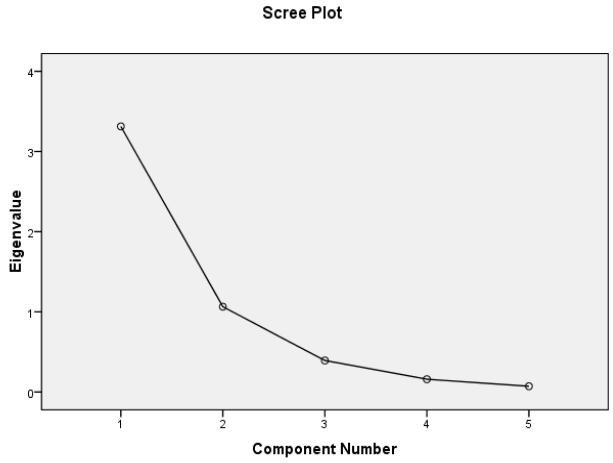

Figure 4.29 Principal Components Analysis scree plot. Scree plot for Principal Components Analysis of *Solanum lycopersicum* plots, indicating a two-Component solution (the first inflection is at 2). Derived in SPSS.

The matrices (Tables 4.27 to 4.29) all indicated fruit weight, width and number of locules as highly loading on Component one (as was indicated by the by plot analysis), and pedicel length and fruit length on Component two.

Table 4.27 Principal Components Analysis component matrix. Component matrix for Principal Components Analysis of *S. lycopersicum* accessions. Variables with loadings over 0.7 highlighted in bold. Derived using SPSS.

	Component	
	1	2
Weight (g)	**.957**	
Width (cm)	**.939**	
Number of locules	**.793**	-.513
Length (cm)	**.766**	
Pedicel length (cm)	.548	**.738**

Table 4.28 Principal Components Analysis pattern matrix. Pattern matrix for Principal Components Analysis of *S. lycopersicum* accessions. Variables with loadings over 0.7 highlighted in bold. Derived using SPSS.

	Component	
	1	2
Number of locules	**1.037**	
Width (cm)	**.898**	
Weight (g)	**.862**	
Pedicel length (cm)		**.991**
Length (cm)		**.749**

Table 4.29 Principal Components Analysis structure matrix. Structure matrix for Principal Components Analysis of *S. lycopersicum* accessions. Variables with loadings over 0.7 highlighted in bold. Derived using SPSS.

	Component	
	1	2
Width (cm)	**.955**	.543
Weight (g)	**.953**	.597
Number of locules	**.916**	
Pedicel length (cm)		**.905**
Length (cm)	.583	**.858**

A scatter plot of Component one and Component two (Figure 4.30) displayed dense clustering of accessions, with the two graded axes of accessions visible (seen in the previous analysis) (outlined by dashed circles). The long, narrow (plum, pear or oblong) fruited accessions and small fruited (currant or cherry tomatoes) were separated by PC1. Larger fruited accessions were separated by PC2.

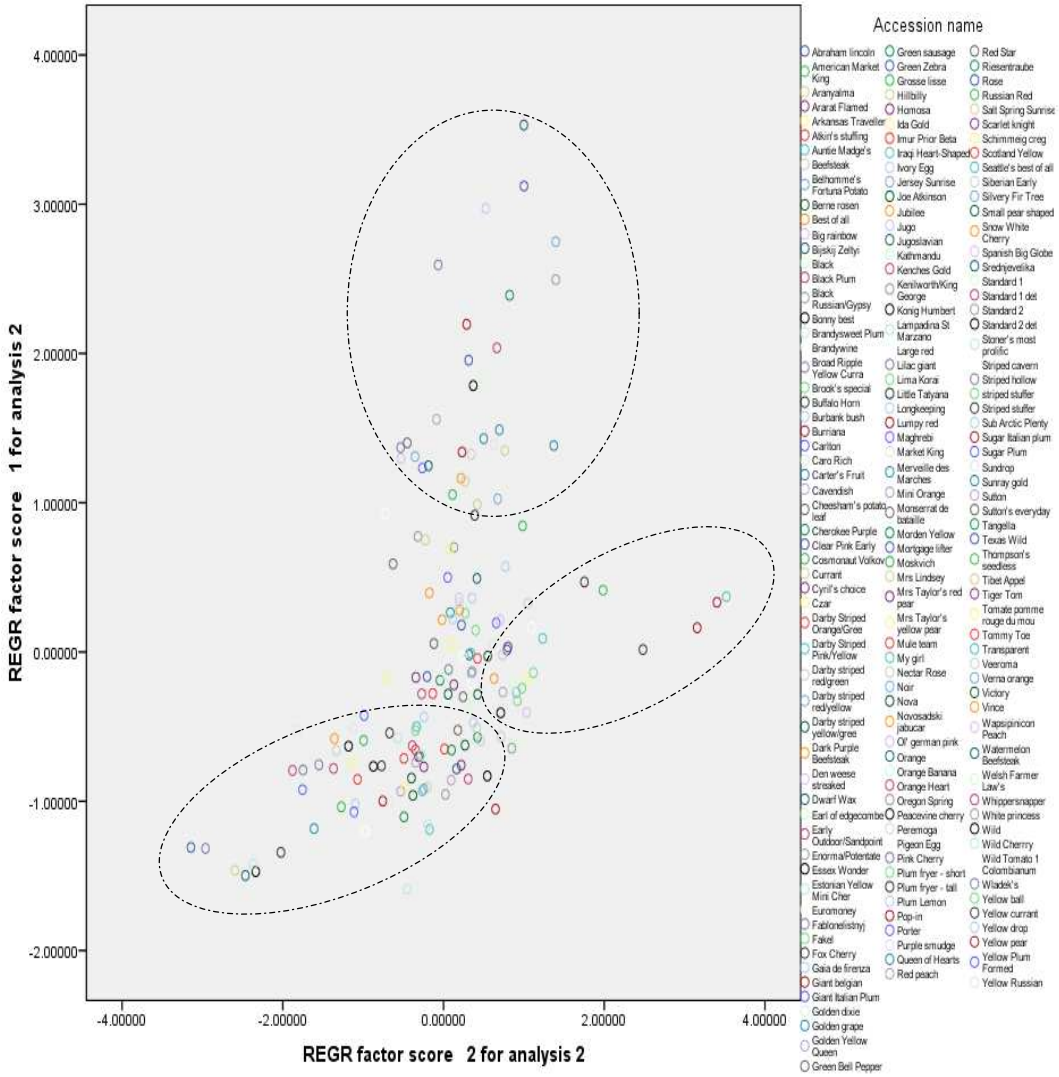

Figure 4.30 Scatter plot of first two Principal Components. Scatter plot of Principal Components 1 and 2, for *Solanum lycopersicum* accessions. The first two components explained 66.26% and 21.28% of the variance, respectively. Dashed circles indicate accession clusters. Derived in SPSS.

Clustering between accessions

Due to the large number of accessions dendrograms for *S. lycopersicum* were too large to display. When all variables were employed (Table 4.30), clusters formed primarily based on skin colour or stripes, and on fruit shape. The most morphologically dissimilar accessions (those last to cluster) are Den Weese Streaked and Dwarf Wax (both being unique in colour,

and the only two multicoloured flesh accessions); Green Sausage and Green Zebra; Watermelon Beefsteak (the only dwarf accession); and Iraqi Heart-shaped (unique fruit shape). The largest clusters were then based on fruit shape (with clusters for pyriform (pear-shaped), ellipsoid (plum-shaped) and oblate (flattened or beefsteak)), exterior fruit colour at maturity (with clusters for red, yellow and orange), presence of stripes and colourless peeled skin.

Considering the large number of accessions, very few were on very short branches (see duplicate section below), suggesting a large amount of diversity in both qualitative and quantitative variables.

Table 4.30 Variables used in UPGMA Cluster Analysis of *Solanum lycopersicum* accessions.

Variable	Data type
Weight (g)	Quantitative (scale)
Length (cm)	Quantitative (scale)
Width (cm)	Quantitative (scale)
Pedicel length from abscission layer (cm)	Quantitative (scale)
Number of locules	Quantitative (scale)
Shoulder shape	Quantitative (ordinal)
Ribbing at calyx end	Quantitative (ordinal)
Radial cracking	Quantitative (ordinal)
Concentric cracking	Quantitative (ordinal)
Puffiness	Quantitative (ordinal)
Pedicel scar width	Quantitative (ordinal)
Plant growth habit	Qualitative
Green shoulder	Qualitative (presence/absence)
Colour of stripes	Qualitative
Predominant shape	Qualitative
Fruit blossom end shape	Qualitative
Abscission layer	Qualitative (presence/absence)
Peeled skin colour	Qualitative
Skin stripe colour	Qualitative
Flesh colour	Qualitative
Fruit shape in cross section	Qualitative
Exterior colour mature fruit	Qualitative
Shape of pistil scar	Qualitative

Duplicates

Kenches Gold and Yellow Ball had no significant quantitative differences, nor any qualitative differences (Table 4.31).

Kenches Gold and Golden Yellow Queen had significant differences in fruit weight (U = 119.0, p= 0.021, r = 0.36), fruit length (U = 134.5, p = 0.012, r = 0.37), fruit width (U = 145.5, p = 0.024, r = 0.34) and number of locules (U = 137.5, p = 0.00, 0.52). No qualitative differences were found with the current variable set.

Yellow Ball and Golden Yellow Queen had significant quantitative differences in fruit weight (U = 70.5, p = 0.00, r = 0.55), fruit length (U = 50.0, p = 0.00, r = 0.63), fruit width (U = 80.0, p = 0.001, r =0.51) and number of locules (U = 99.0, p = 0.001, r = 0.54). No qualitative differences were found.

Yellow Pear and Yellow Drop had significant quantitative differences in fruit length (U = 112.0, p = 0.006, r = 0.43) and medium effect size was present in pedicel length from abscission layer (r = 0.31). No qualitative differences were found.

Yellow Pear and Mrs Taylor's Yellow Pear had significant quantitative difference in fruit length (U = 116.5, p = 0.005, r = 0.43). No qualitative differences were found.

Yellow Drop and Mrs Taylor's Yellow Pear had significant differences in fruit weight (U = 114.0, p = 0.004, r = 0.44), fruit length (U = 67.5, p = 0.00, r = 0.61) and pedicel length (U = 94.0, p = 0.014, r = 0.40). No qualitative differences were found.

Mrs Taylor's Red Pear and Small Pear Shaped had significant differences in fruit weight (U = 76.0, p = 0.001, r = 0.53) and fruit width (U = 87.5, p = 0.002, r = 0.48). No qualitative differences were found.

Best of All and Brook's Special had no statistically significant differences in quantitative characters. No qualitative differences were found.

Best of All and Spanish Big Globe were significantly different in pedicel length (U = 12.0, p = 0.006, r = 0.53). No qualitative differences were found.

Brooks' Special and Spanish Big Globe were not significantly different in quantitative characters. Medium effect size was estimated for pedicel length (r = 0.36), suggesting that a larger sample size may yield significant differences. No qualitative differences were found.

Enorma/Potentate and Maghrebi had no significant quantitative differences. No qualitative differences were found.

Ararat Flamed and Peacevine Cherry were significantly different in pedicel length (U = 152.0, p = 0.02, r = 0.35). No qualitative differences were found.

Sugar Plum and Thompson's Seedless were significantly different in fruit weight (U = 88.0, p = 0.02, r = 0.38) and pedicel length (U = 87.0, p = 0.001, r = 0.53). No qualitative differences were found.

Cavendish and Welsh Farmer Law's no significant differences in quantitative variables. No qualitative differences were found.

Cavendish and Cheetham's Potato Leaf were significantly different in pedicel length (U = 44.00, p = 0.04, r = 0.38), and had a medium effect size (r = 0.32) in fruit length. No qualitative differences were found.

Cavendish and Red Star had no significant differences in quantitative variables, however there were medium effect sizes in fruit weight (r = 0.31), fruit length (r = 0.37) and fruit width (r = 0.38). No qualitative differences were found.

Welsh Farmer Laws and Cheetham's Potato Leaf had significant difference in pedicel length (U = 93.5, p = 0.00, r = 0.54). No qualitative differences were found.

Welsh Farmer Laws and Red Star had significant differences in fruit weight (U = 118.5, p = 0.01, r = 0.38), fruit length (U = 120.0, p = 0.008, r = 0.4), fruit width (U = 126.0, p = 0.012, r = 0.38) and pedicel length (U = 96.0, p = 0.035, r = 0.35). No qualitative differences were found.

Cheetham's Potato Leaf and Red Star were significantly different in locule number (U = 136.5, p = 0.041, r = 0.34), and medium effect size in fruit width (r = 0.3). No qualitative differences were found.

American Market King and Fox Cherry were significantly different in fruit width (U = 247.0, p = 0.046, r = 0.27) and pedicel length (U = 133.5, p = 0.001, r = 0.48). No qualitative differences were found.

Market King and Stonor's Most Prolific were significantly different in fruit length (U = 206.5, p = 0.03, r = 0.29). No qualitative differences were found.

Market King and Cyril's Choice were significantly difference in fruit weight (U = 103.0, p = 0.03, r = 0.35), fruit width (U = 80.5, p = 0.004, r = 0.47) and number of locules (U = 77.0, p = 0.00, r = 0.62). No qualitative differences were found.

Market King and Kathmandu were significantly different in pedicel length (U = 106.0, p = 0.05, r = 0.32), and medium effect size in fruit width (r = 0.31). No qualitative differences were found.

Stonor's Most Prolific and Cyril's Choice were significantly different in fruit weight (U = 156.0, p = 0.00, r = 0.49), fruit length (U = 120.5, p = 0.00, r = 0.57), fruit width (U = 131.0, p = 0.00, r = 0.54), pedicel length (U = 179.0, p = 0.03, r = 0.32) and number of locules (U = 127.0, p = 0.00, r = 0.68). No qualitative differences were found.

Stonor's Most Prolific and Kathmandu were significantly different in fruit weight (U = 167.0, p = 0.004, r = 0.39), fruit length (U = 129.5, p = 0.00, r = 0.49), fruit width (U = 160.0, p = 0.003, r = 0.41) and pedicel length (U = 148.0, p = 0.05, r = 0.30). No qualitative differences were found.

Cyril's Choice and Kathmandu are significantly different in pedicel length (U = 95.5, p = 0.014, r = 0.4) and number of locules (U = 63.5, p = 0.00, r = 0.65). No qualitative differences were found.

Joe Atkinson and Seattle's Best of All did not have any significant quantitative differences. No qualitative differences were found.

Joe Atkinson and Berne Rosen were significantly different in fruit weight (U = 51.5, p = 0.03, r = 0.4) and fruit length (U = 52.5, p = 0.04, r = 0.4). No qualitative differences were found.

Joe Atkinson and Scarlet Knight were significantly different in fruit weight (U = 35.0, p = 0.01, r = 0.52), fruit length (U = 32.0, p = 0.003, r = 0.55) fruit width (U = 38.0, p = 0.01, r = 0.5) and number of locules (U = 58.5, p = 0.01, r = 0.48), and medium effect size in pedicel length differences (r = 0.33). Scarlet Knight also had some concentric cracking, which was absent in Joe Atkinson.

Seattle's Best of All and Berne Rosen had no significant quantitative differences. No qualitative differences were found.

Seattle's Best of All and Scarlet Knight were significantly different in locule number (U = 97.5, p = 0.003, r = 0.46). Scarlet Knight had some concentric cracking which was absent in Seattle's Best of All.

Berne Rosen and Scarlet Knight were significantly different in fruit width (U = 48.0, p = 0.04, r = 0.4) and number of locules (U = 45.5, p = 0.004, r = 0.55). Scarlet Knight had some concentric cracking which was absent in Berne Rosen.

Currant and Wild were significantly different in fruit weight (U = 139.5, p = 0.02, r = 0.37). No qualitative differences were found.

Queen of Hearts and Silvery Fir Tree were significantly different in locule number (U = 54.5, p = 0.03, r = 0.41). No qualitative differences were found.

Lumpy Red and Mortgage Lifter had no statistically significant quantitative differences. A difference was recorded in fruit cross-section shape, which were ‘round’ and ‘irregular’ respectively.

Buffalo Horn and Italian Plum had no statistically significant differences. Effect size was medium for fruit weight (r = 0.31) and fruit length (r = 0.31). Puffiness was slight in Buffalo horn and absent in Sugar Italian Plum. Fruit cross-section shape was ‘round’ in Buffalo Horn and ‘irregular’ in Sugar Italian Plum.

Table 4.31 Differences between potential *Solanum lycopersicum* duplicate accessions. Differences in quantitative and qualitative variables between *Solanum lycopersicum* accessions suggested by analyses to be similar. Accessions are distinct if they have one qualitative difference or one statistically significant quantitative variable (using Mann-Whitney U tests).

Accessions	Quantitative	Qualitative
Kenches Gold and Yellow Ball	-	-
Kenches Gold and Golden Yellow Queen	Fruit weight, fruit length, fruit width, number of locules	-
Yellow Ball and Golden Yellow Queen	Fruit weight, fruit length, fruit width, number of locules	-
Yellow Pear and Yellow Drop	Fruit length	-
Yellow Pear and Mrs Taylor’s	Fruit length	-

Accessions	Quantitative	Qualitative
Yellow Pear		
Yellow Drop and Mrs Taylor's Yellow Pear	Fruit weight, fruit length, pedicel length	-
Mrs Taylor's Red Pear and Small Pear Shaped	Fruit weight, fruit width	-
Best of All and Brook's Special	-	-
Best of All and Spanish Big Globe	Pedicel length	-
Brooks' Special and Spanish Big Globe	-	-
Enorma/Potentate and Maghrebi	-	-
Ararat Flamed and Peacevine Cherry	Pedicel length	-
Sugar Plum and Thompson's Seedless	Fruit weight, pedicel length	-
Cavendish and Welsh Farmer Laws	-	-
Cavendish and Cheetham's Potato Leaf	Pedicel length	-
Cavendish and Red Star	-	-
Welsh Farmer Laws and Cheetham's Potato Leaf	Pedicel length	-
Welsh Farmer Laws and Red Star	Fruit weight, fruit length, fruit width, pedicel length	-
Cheetham's Potato Leaf and Red Star	Number of locules	-
American Market King and Fox Cherry	Fruit width, pedicel length	-
Market King and Stoners Most Prolific	Fruit length	-
Market King and Cyril's Choice	Fruit weight, fruit width, number of locules	-
Market King and Kathmandu	Pedicel length	-
Stoner's Most Prolific and Cyril's Choice	Fruit weight, fruit length, fruit width, pedicel length, number of locules	-
Stoners Most Prolific and Kathmandu	Fruit weight, fruit length, fruit width and pedicel length	-
Cyril's Choice and Kathmandu	Pedicel length, number of locules	-
Joe Atkinson and Seattle's Best of All	-	-
Joe Atkinson and Berne Rosen	Fruit weight, fruit length	-
Joe Atkinson and Scarlet Knight	Fruit weight, fruit length, fruit width, number of locules	Concentric cracking
Seattle's Best of All and Berne Rosen	-	-
Seattle's Best of All and Scarlet Knight	Number of locules	Concentric cracking
Berne Rosen and Scarlet Knight	Fruit width, number of locules	Concentric cracking
Currant and Wild	Fruit weight	-
Queen of Hearts and Silvery Fir Tree	Number of locules	-

Accessions	Quantitative	Qualitative
Lumpy Red and Mortgage Lifter	-	Fruit cross-section shape
Buffalo Horn and Italian Plum	-	Puffiness, fruit cross-section shape

4.4 Discussion

The goals of the morphological study were to explore the questions: what morphological variation was present within and between HSL accessions, were any similar groups of accessions observed, how did HSL accessions compare with commercial standards, and were there any duplicates?

4.4.1 Morphological variation present within and between HSL accessions

The characterisation and conservation of morphological diversity is necessary to facilitate use and potential future breeding, as well as to identify a baseline for stocks currently held against future losses (Hawkes *et al*., 2000; Lorenzetti and Negri, 2009).

Morphological variation was present between accessions for all crops and within accessions in seven crops (*Daucus carota*, *Cucumis sativus*, *Raphanus sativus*, *Capsicum annuum*, *Brassica rapa* var. *rapa*, *Brassica napobrassica* and *Vicia faba*). *Solanum lycopersicum* and *Pisum sativum* demonstrated the least variation within accessions, with the exception of *Allium porrum* and *Lactuca sativa*, which are not comparable due to the potential immaturity of the specimens when harvested. Morphological variation within accessions was assessed using the positioning of replicates in the PCA and Cluster Analyses. Distance between replicates was judged using branch lengths as an indicator of similarity, therefore replicate plots that clustered together on short branches were deemed to have higher within accession similarity.

A large amount of diversity was observed both within and between *Vicia faba* accessions, such that no large clusters were found when all variables were employed, and low similarity was implied within all accessions; UPGMA dendrogram branch lengths were generally long. High morphological variability is consistent with the findings of previous studies (Zeid *et al*., 2003; Terzopoulos *et al*., 2003; Ouji *et al*., 2011).

Daucus carota accessions were recorded as having differences both within and between accessions. The accessions with the most similar replicates (suggesting lower variation within varieties) were Standard 1 and Standard 2; this may be expected as they are F1 hybrids. Variability in *Daucus carota* root dimensions (in the case of the present study large amounts of variation were seen within accessions in root width and length) is common in open-pollinated varieties, and increased uniformity has been a goal for F1 hybrid breeding strategies (Stein and Nothnagel, 1995).

In *Pisum sativum* variation was recorded between accessions; variation within accessions was more limited. Many accessions had replicates that were within the same small cluster; accessions with the highest internal similarity for all variable combinations were Ostgotaart, Prince of Prussia and Standard 1. Previous morphological studies of *Pisum sativum* collections have found a large amount of variation between varieties (Tar'an *et al*., 2005; Nisar *et al*., 2008; Sarikamis *et al*., 2010).

The range of *Cucumis sativus* diversity present within the accession studied was large, with differences noted both within and between accessions, as seen in the by-plot and by-accession cluster analyses. A large range of morphological types was observed including shapes, colours, spines and sizes. High diversity within and between accessions is consistent with findings from other studies of *Cucumis sativus* landraces or local varieties (Ah-Rawahi *et al*.,

2011). The most internally similar accessions were Butcher's Disease Resisting, 741 Peking China and Standard 1.

Variation was found between *Capsicum annuum* accessions in qualitative and quantitative characters, particularly size, shape and cross-section corrugation. No accessions had consistently clustering plots across all variable types. Variation within accessions was lower in quantitative variables, than in qualitative variables such as fruit shape.

Raphanus sativus accessions were found to be very diverse both within and between accessions, with differences in root shape in particular. Root shape, including elongation, is controlled by a combination of genetic, environmental and physiological factors (Zaki *et al*., 2011). The general root variability found in the present study reflects the open pollinated and outbreeding nature of *Raphanus sativus*, and its cultivation history, which has likely avoided bottlenecks and increased in morphological diversity as its use has spread from the Mediterranean into South and East Asia (Wang *et al*., 2008; Ullah *et al*., 2010). Accessions Rat's Tail and French Golden were the most internally similar accessions, although both of these accessions had high diversity when considering quantitative characters only.

Brassica rapa var. *rapa* and *Brassica napobrassica* both showed high diversity within and between accessions, in both qualitative and quantitative characters. In *Brassica rapa* var. *rapa* Gammel Svensk and Standard 2 are the most internally similar, but show variation in qualitative and quantitative characters, respectively. In *B. napobrassica* Standard 2 came out most consistently as similar between plots; Bjursas was most similar in qualitative characters. *Brassica rapa* var. *rapa* is generally more diverse than *Brassica napobrassica* due to the former being outbreeding and the latter predominantly inbreeding (McNaughton, 1995a;

McNaughton, 1995b). This is difficult to assess in the current study due to small number of accessions.

Solanum lycopersicum showed large amounts of morphological diversity between accessions. Replicate plots cluster together frequently in the quantitative analyses, and branch lengths are short in many accessions, suggesting that morphological similarity within accessions was greater than between. Branch lengths increased when qualitative variables are included, suggesting that diversity is greater in qualitative characters both within (namely shape characters) and between accessions (shape and colour characters). Previous studies of *Solanum lycopersicum* collections have found higher inter-varietal than intra-varietal variation in quantitative characters (Mazzucato *et al*., 2010). Due to the large amount of diversity between accessions, many plot replicates clustered together. However, accessions identified as having the most similarity within accession were Wild, Wild Cherry, Yellow Plum Formed, Riesentraube, Plum Fryer (Short), Auntie Madge's and Darby Red and Yellow Striped.

In summary, these results reflect the broad range of diversity held in most heritage variety crops within the HSL, as well as diversity within accessions that may be of interest both to growers, who value diversity, and for conservation for future. It also highlights the differences between inbreeding and breeding crops, the latter of which have higher heterogeneity within accessions.

4.4.2 Groups of similar accessions

Groups of accessions by morphological characters were noted in most crops; these were root colour and shape in *Raphanus sativus,* fruit shape in *Capsicum annuum,* pod colour in *Pisum*

sativum, fruit shape and colour, in *Solanum lycopersicum*, root colour in *Daucus carota*, root shape in *Brassica rapa* var. *rapa* and seed size in *Vicia faba*. These will be further discussed below.

The lack of large clusters is consistent with previous studies of *Vicia faba*, and is due to high morphological variation, and overlaps between accessions (Zeid *et al*., 2003; Terzopoulos *et al*., 2003). When quantitative variables only were used, clusters were based on seed size with one large group of the larger seeded varieties and Martock, Sweet Lorraine, Beryl and Cretian, which were of significantly smaller seed size, outside. Bacardi, Sweet Lorraine, Cretian, Beryl and Martock weighed less than 55g 1000 seed weight consistent with the minor *Vicia faba* group (Duc, 1997). These accessions also have erect pod attitude and short pods, consistent with minor types (Duc, 1997).

The three main morphological clusters in *D. carota* were based on root colour: orange, white and purple. Domesticated *D. carota* can be grouped into two types: 'eastern' (yellow or purple roots) and 'western' (orange or white roots), with eastern types giving rise to the western types; white rooted varieties in turn may be selected from these (Riggs, 1995; Clotault *et al*. 2010).

Clusters observed in *Pisum sativum* were based on flower colour and differences in tendril number. Tendril number in *P. sativum* has been at the forefront of breeding efforts to reduce biomass (including plant size and leaf size and number) that in turn results in higher yield (Cousin, 1997). Further data would be needed to confirm the loci present in HSL accessions, including leaf number and stipule size, although combinations of 'afila' ('*af*') and 'tendril-less' ('*tl*') genes, do result in a phenotype similar to that observed in parsley ('af tl' double mutant, Cousin, 1997; Gourlay *et al*., 2000), the recessive '*af*' mutant (Gourlay *et al*., 2000)

also bears a resemblance to HSL poppet type with many-branched rachides terminating in tendrils phenotype. Testa marbling (observed in the cluster containing the Grey, Latvian and Dun accessions) and testa anthocyanin (observed in some accessions) are related to the alleles '*M'* and *F* or *Fs* respectively (Ambrose, 2008). Groups were also observed based on pod colour, determined by the alleles *Pa* and *Vim* (green), *Gp* (yellow), *Dp* (blue-green) and *Pu* or *Pur* (purple) (Ambrose, 2008).

In *Cucumis sativus*, groups of accessions were observed but, due to the large morphological differences between accessions, the basis for the groups was not clear and may have been fruit characters, such as shape or stripes, or internode length.

Capsicum annuum show diversity in fruit shape and size, previous studies have found *Capsicum annuum* accessions cluster by fruit morphotypes, namely bell-shaped (blocky/triangular), elongated, and small elongated fruits (Geleta *et al*., 2005; Portis *et al*., 2006). This reflects the findings of the current study. There were two main morphological groups observed in the *Capsicum annuum* accessions, defined by fruit shape. The first group consisted of accessions producing long and narrow fruits; the second consisted of accessions producing bell-shaped fruits. Two of the accessions were chilli *Capsicum annuum*, of which Trifetti separated from the sweet, and Skinny sat in the elongated cluster. Further research by Ortiz *et al*. (2010) has suggested that genetic variation in the bell-shaped *Capsicum annuum* group tends to be less than other clusters; the difficulty distinguishing *Capsicum annuum* of this morphology in the present study perhaps reflects this. Bozokalfa *et al*. (2009) also found similar groupings, however they also included capsaicin levels, fruit wall thickness and plant characters such as leaf width and plant height (excluded from the current study due to concerns over high environmental influence in the glasshouse conditions).

In *Raphanus sativus* a group of accessions (Standards 1 and 2, and HSL accessions Round Red Forcing Real and Crimson Giant) formed on the basis of qualitative root characters; this was defined most notably by red root exterior colour, white flesh and spheric root shape. The main economic types of European *Raphanus sativus* are small-rooted 'garden' radishes (Crisp, 1995). These are under strong directional selection for preferred market traits (red root colour and white flesh) (Muminovic *et al*., 2004).

In *Brassica napobrassica* and *Brassica rapa* var. *rapa*, possibly due to the small number of accessions and the large morphological differences between them, no groups of accessions were observed.

In *Solanum lycopersicum* fruit phenotype is controlled by Quantitative Trait Loci (QTLs). Genes identified, some of which give phenotypes of the same appearance as fruit in some HSL accessions, include *ovate* pear-shaped fruits, and multigenic effects of *fasciated*, *locule-number*, *fw*1.1, *fw*2.2, and *fw*3.1 to give some giant beefsteak varieties (Tanksley, 2004). Accessions in the current study grouped based on fruit shape and size (clusters observed in the PCA were based on small, large and plum/pear shaped fruit) and in the cluster analysis finer detail was added with yellow, red, pink and striped fruit colours.

Lactuca sativa and *Allium porrum* accessions were of incomplete maturity; therefore any conclusions are tentative. Four of the seven *Lactuca sativa* morphotypes (Kristkova *et al*., 2008) were present within the HSL accessions characterised: crisphead, butterhead, cos and leafy.

Of the characters gathered, leaf colour and shaft length are key characters in *Allium porrum* classification (De Clercq *et al*., 1999). Using the classification of De Clercq *et al*. (1999) the leaf colour results of the present study would suggest that all accessions grown were

‘Autumn’ or ‘Winter’ varieties (having green or blue-green leaves); this does not correspond with what is known of Coloma, Early Market, and possibly Sim Seger and Walton Mammoth, which are ‘Early’ or ‘Summer’ types; this discrepancy maybe due to the incomplete maturation of the crops at harvest/characterisation or variation in foliage colour expression due to environmental conditions (such as low temperature or dryness). No clusters were observed for shaft length also, due to variability in this character (shaft lengths were also distinctly shorter than given in De Clercq *et al*. (1999), also suggesting incomplete maturation).

The above groupings may be due to the selection pressure of valued crop traits, such as root or fruit shape and colour; this may result in the morphological convergence of different accessions, or may be due to breeding from common ancestor material (Muminovic *et al*., 2004; Hu *et al*., 2010). For the groups identified in *V. faba*, *D. carota*, *C. sativus* and *P. sativum*, a comparison of the groups formed from AFLP analysis will be presented in the general discussion (Chapter 6).

4.4.3 Comparison of morphological diversity of HSL accessions to that in commercial standards

Concerns regarding the loss of diversity within varieties since the advent of modern breeding (Brush, 1999), has led to studies comparing the genetic diversity of cultivated varieties over time, and comparing landraces and modern varieties. Most of these studies use genetic techniques, such as molecular markers, to compare varieties. It may be expected that, since the focus of breeding in many crops is on uniformity, for a number of reasons such as for mechanised handling and consumer preference (Esquinas-Alcazar, 2005), that the standards utilised in the present study may be less morphologically diverse than the heritage accessions.

In order to compare the diversity of commercial standards with that of HSL accessions, the clustering patterns of accession replicate plots was examined using PCA and Cluster Analysis. Insufficient plots were available for comparison for *Raphanus sativus*. As above, *Allium porrum* and *Lactuca sativa* were not compared. No differences in clusters or variation were observed between HSL accessions and commercial standards (for example replicate plots did not cluster together more frequently, and branch lengths were not shorter, which may have suggested a greater homogeneity) in the following crops: *Vicia faba* (both equally diverse), *Capsicum annuum* (both equally homogeneous as part of the bell-pepper cluster), *Solanum lycopersicum* (of similar branch lengths and clustering as seen in HSL accessions), *Brassica rapa* var. *rapa* (equally diverse) and *Solanum lycopersicum* (equally homogeneous). *Daucus carota* standards presented slightly shorter branches between two or more accession replicates, suggesting that they were more homogeneous than HSL accessions, which is consistent with the standards being F1 hybrid varieties. Standard 1 in *Pisum sativum* was less diverse than many HSL accessions. In *Cucumis sativus*, Standard 1 presented shorter branches, comparable to those of 741 Peking China and Butcher's Disease Resisting (in quantitative variable scatter plots, two out of three plots clustered closely together); Standard 2 had missing data so was not available for comparison). In *Brassica napobrassica* Standard 1 was comparable and Standard 2 was less diverse. In the PCA standards were more diverse than Bjursas, but not in the cluster analysis.

4.4.4 Potential duplicate accessions

The identification of duplicate accessions in *ex situ* collections allows managers to focus resources on unique material, such as prioritising regeneration (Le Clerc *et al*., 2005b). Few candidates for duplication were found for all crops, with most potential duplicates having

significant quantitative variable differences, or observable differences in qualitative variables, or else effect size was sufficient to suggest further investigation be merited, either in terms of larger sample size, or through the recruitment of additional crop descriptors. Crops with the largest numbers of putative duplicates were *Solanum lycopersicum* and *Pisum sativum*, which were also the crops with the largest numbers of accessions characterised. Accessions from *Capsicum annuum*, *Vicia faba*, *Cucumis sativus*, *Brassica napobrassica*, *Brassica rapa* var. *rapa*, *Raphanus sativus* were all morphologically distinct. Assessment of duplicates was not attempted for *Allium porrum* or *Lactuca sativa* due to the incomplete maturation of these crops. Possible duplicates will be further discussed below.

In *Daucus carota*, the two purple accessions, Afghan Purple and John's Purple could not be distinguished with the currently used morphological descriptors. This represents one pair out of 10 HSL accessions or a potential redundancy of 10%.

In *Solanum lycopersicum*, accessions that could not be distinguished by any characters recorded were Kenches Gold and Yellow Ball, Best of All and Brooks Special, Brooks Special and Spanish Big Globe, Enorma/Potentate and Maghrebi, Cavendish and Welsh Farmer Law's, Cavendish and Red Star, Joe Atkinson and Seattle's Best of All, and Seattle's Best of All and Berne Rosen. This represents eight pairs out of 180 accessions, or a potential redundancy of 4.55%.

For *Pisum sativum* the main limitation was the small number of variables collected; this was due to limited time and resources, and related to the relative larger size of this crop group, so not all variability present may have been observed (Tar'an *et al*. 2005). Duplicates therefore cannot be entirely eliminated, and more data - both larger sample sizes and a greater variety of characters - are required for comparison. For the current study variable set and sample sizes,

four sets of accessions were potential duplicates (Purple Podded and Stephens, Harold Idle and Mummy's, Frueher Heinrich and Wieringen White, and Mummy's and Prew's Special). In addition, 13 further pairs were not distinguishable using the current data, but effect sizes suggested larger sample sizes might yield statistically significant differences in at least one quantitative variable. This represents four pairs out of 75 HSL accessions (or a potential redundancy of 5.33%) in the first case, or 17 pairs out of 75 HSL accessions (or a potential redundancy of 22.67%).

As mentioned above, some convergence of morphological traits may occur due to strong selection pressure to type (Le Clerc *et al*., 2005b). These duplicates will be further examined with reference to the Amplified Fragment Length Polymorphism study of the previous chapter, in the general discussion (chapter 6).

4.4.5 Conclusions

The present characterisation study has confirmed a wide variety of characters are present in the HSL collection, and had provided an estimate for redundancy of 8.1%, with the most potential morphological duplicates identified in *Pisum sativum*; hence heritage varieties contain a spectrum of morphological traits and diversity. The characterisation of these accessions has three main implications for HSL. Firstly, it provides information on the range of characters available, for both conservation and use. Secondly, the list of characters for each accession can be added to the HSL database and enable HSL to manage and more fully use the accessions they are holding and to filter this information to enable informed choice by users, in the longer term increasing access to the HSL, and freeing up staff time from characterisation. Thirdly, it has highlighted potential duplicate accessions for further investigation by HSL.

The accessions that were identified as potential duplicates highlight the main limitations of the current study. Firstly, due to limited time and resources the sample sizes collected were necessarily small. Secondly, the numbers of morphological descriptors used was determined very much by the number of accessions being characterised and the time available, so in some cases was necessarily low in number (leading to reduced resolution).

Finally, having considered the morphological characters of a large proportion of the HSL collection, a further analysis is to compare the available morphological data with molecular genetic variation. Results from the subset of crops that were analysed in both morphology and genetics, will be compared in the general discussion (chapter 6).

CHAPTER 5 INVESTIGATING THE MOTIVATIONS AND PRACTICES OF HERITAGE SEED LIBRARY SEED GUARDIANS AND MEMBERS

5.1 Introduction

The conservation of heritage varieties has been discussed, thus far, in terms of molecular and morphological characterisation. The current chapter will focus on the role of communities and individuals in the process of heritage variety conservation, through investigation of the motivations and practices of Garden Organic members and Heritage Seed Library (HSL) Seed Guardians.

5.1.1 Conservation and home gardens

Home gardens are an effective way of conserving both ex-commercial varieties and varieties that have never been commercially available (heirlooms) (Qualset *et al*., 1997). The role of home gardens as refuges for crop genetic diversity has been widely reviewed, particularly with reference to subsistence agriculture, (for example Watson and Eyzaguirre, 2002; Brush, 2004; Bailey *et al*., 2009) along with the importance of home gardens in the context of the conservation of landraces, heritage and heirloom varieties (Qualset *et al*., 1997; Galluzzi *et al*., 2010) and highlight the importance of home gardens, and therefore gardeners, in light of genetic erosion (Qualset *et al*., 1997). The distribution of diversity in home gardens can be examined in terms of "richness", "evenness" and "distinctness"; the richness of home gardens reflects the number of different crops or varieties grown, evenness reflects their distribution, and distinctness refers to how different the crop types are (Hodgkin, 2002). Averages for home garden richness vary between countries; Gebauer (2005) found an average of three species per garden in an arid region of Sudan; Leiva *et al*. (2001) found 6 crops per garden in

Guatemala; Yongneng *et al*. (2006) found an average of 18 species per garden in China; Birol *et al*. (2006) found an average of 18 species per garden in Hungary, Sunwar *et al*. (2006) found 33 cultivated species per garden in Nepal. Diversity is also seen intra-species, for example Castineiras *et al*. (2002) found up to 13 varieties of *P. vulgaris* per garden in Cuban home gardens, with a co-existence of modern and traditional varieties.

5.1.2 Community and individual participation

The interest of gardeners in plant conservation can be discussed in the context of community/individual participation, which can be described on two levels (Hawkes *et al*., 2000), firstly, conservation within a local area for historical or personal reasons (and to their own benefit); secondly, as part of a collaboration with professional conservationists or organisations, that can influence conservation at a larger scale and have broader implications to society (Hawkes *et al*., 2000). The interaction between various individuals (see below) and Garden Organic shows the expression of interest in conservation for an individual. Examples of conservation from an individual/community level include shows, informal sector botanic gardens and seed saver schemes (Hawkes *et al*., 2000).

5.1.3 Seed saving

Seed saving can be performed at multiple levels, from individuals saving for their own use, to organisations such as the HSL (UK), Seed Savers Exchange (US), Arche Noah (Austria), Irish Seed Savers (Ireland), and international organisations such as the International Centre for Agricultural Research in the Dry Areas (ICARDA), which has collecting missions in multiple countries.

Seed saving can be undertaken for a multitude of reasons including saving seed from varieties that are to be discontinued, trying something different, saving money, personal connections to specific varieties (such as inherited heirlooms or varieties with historical or cultural connections), or a wider view that encompasses genetic erosion and conservation (Stickland, 2008). Seed saving may contribute to the continued use of varieties that are no longer available commercially, and to conservation of heirloom varieties that may be lost due to the discontinuation of maintenance by their breeder (Stickland, 2008).

Saving seed has a number of advantages including the feasibility of medium-long-term storage, easy access for characterisation and utilisation and low maintenance once material is in storage (Hawkes *et al*., 2000). However, the genetics of seed saving are of relevance to long-term conservation. The continuance of genetic diversity in a conserved population is affected by such factors as sample size, sample selection, and during regeneration the effects of genetic drift (after multiple regenerations), contamination and natural selection. The potential risks reflect those in more formal *ex situ* conservation environments such as seed banks. Van Hintum *et al*. (2007) found changes in allele frequencies between *Brassica oleracea* gene bank accessions of a comparable magnitude to differences between initially similar accessions. Cieslarova *et al*. (2010) found changes in *P. sativum* allele frequencies and genetic composition during regeneration cycles, with both increases and decreases in genetic diversity levels found.

5.1.4 Heritage Seed Library

The HSL is comprised of around 800 accessions of diverse backgrounds including landraces, ex commercial varieties and heirlooms. HSL seed is regularly grown by GO Members and HSL Seed Guardians. Seed Guardians work with the Garden Organic (GO) Heritage Seed

Library (HSL) to regenerate heritage vegetable seed as part of the HSL seed regeneration rotation. Each Seed Guardian is usually assigned two varieties per year to grow, which they choose from an “orphans list”. Advice is distributed in the form of Seed Saving Guidelines (HSL, 2008); these include information on how to grow each crop (including cultivation, pollination and isolation distance), and how to clean and store seed.

GO Members pay a subscription fee to GO and can pay extra to join the HSL; in return they choose up to six varieties of heritage vegetable seed each year.

5.1.5 Rationale

No in depth research has been performed into the experiences of SG and GO Members regarding any knowledge they may have on varieties, nor have any studies examined these groups that contribute towards the maintenance of the HSL (SG), their practices or motivations. The importance of heritage vegetables to Members and why they are interested is of importance for the future engagement of Members and Seed Guardians. Exploring the knowledge of these two groups also complements and supplements the present genetic and morphological analyses, as it may provide a source of additional information on the HSL varieties.

The Seed Guardians are an important asset to HSL in order to maintain seed viability by assisting with seed regeneration as required (every five years minimum; Neil Munro, personal communication) and to generate enough to be distributed to members. Seed Guardian practices influence genetic quality of accessions and the practices they use are of interest to HSL. The close relation of Seed Guardians to the material would also put them in a position to observe variation within accessions. Some information is reported back in the Seed

Guardian return forms (Neil Munro, personal communication) including plant yield, germination details, and problems with pest/disease and isolation details. There is also a general field asking for any other comments about the varieties. However a general survey, including specific questions about variation and practices, has never been conducted.

The gardening practices and motivations of members are unknown and, unlike Seed Guardians, no formal system is in place with HSL to report back any variation/points of interest in varieties. GO Members, geographically widely distributed, grow locally named varieties; investigations into their experiences of growing these in different areas could potentially contribute to this aspect of heritage varieties. Testing this formally and rigorously would be a field trial project in its own right; however, if Members have noticed any variation in performance in those accessions with local names it might be an interesting starting point for such information collection.

Examining the role of heritage variety growing in peoples' everyday lives is important in informing how to encourage people to become more involved in their conservation.

5.1.6 Aims and objectives

The overarching aim was to elucidate the motivations, practices and experiences of Garden Organic members and HSL Seed Guardians. This was accomplished by the presentation of two surveys. One survey was targeted to Seed Guardians, and one was to GO members. Due to both the geographically dispersed nature of the groups and time and cost limitations questionnaires were chosen as the most efficient and effective way to collect the data.

The aims of the Seed Guardian survey were to investigate: 1) the motivations of people volunteering to become Seed Guardians; 2) how they select which orphan (accession) to

grow; 3) to investigate the practices of Seed Guardians including whether they were following provided guidelines, whether they found these guidelines sufficient and if any additional measures were taken (including soil preparation); 4) to report any variation they have noted; 5) to examine seed saving practices (including seed destination); 6) to discover whether Seed Guardians are satisfied with their relationship with HSL.

The aims of the GO Member survey were: 1) to investigate the motivation of Members for involvement in heritage vegetable growing; 2) to explore how heritage seeds fit into a larger picture of home vegetable gardening (including organic gardening techniques and the growing of standard varieties); 3) to encourage reporting of variety performance and explore possible regional differences; 4) to explore alternative seed destinations for HSL seed (such as seed swaps); and 5) to enquire about the uses to which end produce is put.

5.2 Methods

5.2.1 Sampling, publicity and distribution

There are approximately 200 Seed Guardians and around 10,000 Garden Organic Members. The comparatively small number of Seed Guardians permitted questionnaires to be printed and posted along with a regular mailing they receive from HSL. Questionnaires were posted directly to each individual inside a regular HSL mailing with an introductory letter. A stamped-addressed envelope was included.

Due to the large number of Members a paper mail strategy would have been prohibitively expensive; online surveys are an effective and efficient way of reaching a large number of people (Kaye and Johnson, 1999). With this in mind the Member questionnaire was primarily

Internet based and was posted online via Survey Monkey (http://www.surveymonkey.com/member-grower-survey).

Survey Monkey was chosen because it offers a low-cost platform that is easy to manipulate for researcher and respondent. Questionnaires can be split into smaller sections and a large range of answer format options are available. A monthly fee is paid. The URL can be personalised to the survey so a straightforward name can be chosen to increase the number of respondents (as a list of numbers is very hard to type in) and unlimited questions and replies can be posted. The survey was available from the 30th of September 2009 to the 31st of January 2010. It was brought to the attention of Members via Garden Organic's 'Organic Way' magazine. The article included background information regarding the project and an estimate of how long it would take to complete. It publicised the web link and also offered email and postal details to widen the opportunities for response to those without Internet access.

5.2.2 Survey design

Both questionnaires were designed in pencil and paper format (Kaye and Johnson, 1999); the Members' questionnaire was then adapted to fit the Survey Monkey format.

As time and money were limiting factors in both surveys, and access to participants would only be possible once, the surveys were pre-tested on five colleagues. Fowler (1995) recommends pre-testing (for example using a subset test re-test strategy or with focus groups and interviews). Good question design is paramount so questions were rigorously designed according to the criteria outlined below and consulted with those five colleagues, as well as

input from previous questionnaires provided by Nigel Maxted (Cardoso and Maxted, 2008) and Shelagh Kell (Kell *et al., 2*008).

The Member questionnaire (Appendix four) consisted of 23 questions, and was split into six sections that related to overall questionnaire objectives (heritage vegetable growing, gardening practices, variety choice, variety traits, seeds and a free text box for any other comments). The Survey Monkey format allows different pages per section and this was an advantage as it broke the survey down into more accessible segments.

The Seed Guardian questionnaire (Appendix five) consisted of 11 questions, and was not split into sections as it was fairly concise and pages provided natural breaks.

Survey length in both questionnaires was kept to a minimum to encourage participation, completion and accuracy.

5.2.3 Question construction

Fowler (1995) highlights the importance of questionnaire validity and reliability in minimising error, and identifies key principles for survey design. These include, firstly, unambiguous wording so that all participants' understanding allows the answering of the same question and in the same format. Secondly, participants are only asked questions to which they are capable of knowing the answer. Thirdly, respondents should wish to give accurate answers. This final point supports the inclusion of introductory contextual text to the survey, as does the exclusion of questions that participants may feel put them in a negative light (Fowler, 1995). Fowler (1995) also advises limiting answers to a set time period which encourages specific answers and can aid memory recall; both of these points increase answer accuracy.

General principles (*sensu* Fowler, 1995) were applied to question construction for both questionnaires. Questions were designed to be not leading; to be unambiguous; the language used was non-technical with key terms defined in the relevant question or more complex questions explained. An assumption was made that both Seed Guardians and GO Members had a specific interest in heritage vegetables so would know what they were, rather than including a long definition of terms.

The response detail level was implied to the respondents using tick boxes, text boxes and lines. Multiple-choice answers were given where outcomes could be anticipated or a small number were involved (Fowler, 1995). Narrative answers were used when outcomes could not be anticipated. Fowler (1995) recommends closed questions are preferable where possible, to reduce the number of answer options to improve analysis; however, as opinions and practices were being sought a large number of answer categories were possible and motivations are unknown, open-ended questions and narrative answers were used where applicable.

Skip questions were avoided in the Seed Guardian questionnaire and kept to a minimum in the Members survey to simplify completion.

5.2.4 Personal information

No personal details were requested from any of the respondents. This was both to avoid data protection issues and to encourage unbiased responses (Fowler, 1995). Characterisation of opinion and practice was considered to be more important than demographic analyses.

5.2.5 Statistical analyses

Analyses included descriptive summaries of numerical data and content analysis of open-ended/qualitative data.

5.3 Results

For the purposes of this chapter, scientific genus and species names are not presented, and the word ‘variety’ is used instead of ‘accession’, in order to remain consistent with the terminology used by questionnaire respondents.

5.3.1 Data collection and responses

A total of 54 Seed Guardian questionnaires were received by post.

Online responses to the Members’ questionnaires consist of 43 completed; in addition two postal questionnaires were returned, and one email request responded to and completed questionnaire received.

5.3.2 Seed Guardian questionnaire results

For question 1 (what are the main reasons that you became a Seed Guardian?) responses were categorised by theme under ten headings: Conservation (split further into Biodiversity, Food security/gene pool/breeding, Help conserve heritage varieties), Educational value, Intrinsic interest/gardening interest, Anti-commercial/anti-control, Helping HSL specifically, Try new/different varieties, Because I can and Seed access/seed swap/saving seed (Table 5.1).

Table 5.1 Response categories for Seed Guardian questionnaire question 1: What are the main reasons that you became a Seed Guardian? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Answers classified by keyword.

Category	Number of responses	Percentage of responses
Biodiversity	23	16.31
Food supply	7	4.96
Conservation of heritage varieties	36	25.53
Education	6	4.26
Intrinsic/gardening interest	34	24.11
Anti-commercial-anti-control	12	8.51
Helping HSL	23	16.31
Try new/different varieties	13	9.22
Because I can	7	4.96
Seed swap/access to seed/seed saving	11	7.80

The category of conservation was broadly subdivided into three overlapping subheadings: biodiversity, food security/gene pool/breeding, and conservation specifically of varieties which were defined as heritage, old, rare or “off-list”. 16.31% of responses included reasons that were related to what I have classed ‘Biodiversity’. This heading broadly encompasses sustainable development, conserving the broadest range of diversity for current and future use, and I have left it to include diversity at the general levels (biodiversity as a whole and general term), and also species and genetic diversity. Statements such as “to help maintain biodiversity”, “concern about decrease in biodiversity”, “maintain variety diversity” and “maintaining all that is good for future generations” have been included. This category overlaps with the second subheading, (4.96% of responses) food security/gene pool/breeding, as this is concerned with sustainable use and conservation. Statements such as “world food security”, “because I am a genetic engineer and understand about maintaining the gene pool” and “preservation of diverse varieties for possible future use” were included under this heading. This third subdivision, the largest category with answers from 25.53% of respondents was conservation of varieties that were specifically labelled as heritage/old/rare

or “off-list”. Statements in this category, which again has overlap with the previous two sections, were “conservation of heritage seed”, “like to collect heirloom and native varieties” and “to keep old seeds going”. Statements that referred to the history of the varieties were also included in this category such as “history behind the seeds” and one respondent who grows *Vicia faba* variety Martock due to a family connection to Somerset. An example of a statement that overlaps these categories is “I am convinced bio-diversity can only be maintained by maintaining heritage varieties”, and demonstrates the connectedness of the issues. This latter category also has overlap with the category (see below) of Intrinsic interest/gardening interest, as people stated they are interested in heritage varieties for different reasons including for conservation (perhaps for the variety’s own sake), for interest (to themselves) or for the future.

The next category identified that the reason people became a Seed Guardian was for educational value or purposes. 6 responses (4.26%) came under this category, and included statements such as “the educational value”, “to augment my horticultural studies” and “to grow as an educational resource at our community orchard”. Educational targets included the respondents themselves and/or members of their community.

The next category identified was named Intrinsic interest/gardening interest, and reflected respondents interests in heritage varieties as a part of their gardening interests or as something that was of interest in and of itself. 24.11% of responses mentioned something that would fit into this category. Exemplar statements include “because it’s a fun thing to do”, “interest in growing vegetables as a hobby”, “would rather not use F1 varieties” and “I like scientific observation and data gathering”. There was overlap with other groups, most often conservation, for example “interest in growing and saving seed from “off-list” varieties” and

“to grow+save+pass on unusual foods” which was placed in this category, conservation and trying something different (see below).

8.51% of responses included statements that could be grouped as against commercial growing, the increased prevalence of F1 varieties and their replacement of traditional varieties, or the general control of seed production, out of the hands of garden growers. These were placed in a category defined as Anti-commercial/Anti-control. Statements included “dislike of agribusiness”, “to help conserve non-commercial varieties”, “to preserve varieties bred for small gardens, not commercial growers” and “do not want to see GM crops. Want to control crosses and hand pollinate”.

16.31% of respondents specifically mentioned a desire to help HSL, and/or a belief in their goals. Exemplar statements include, “I believe in the work of HSL”, “to help the HSL”, and “Support GO/HSL”. Other more general statements included “Lend support to a worthy cause” and statements about the continuance of sufficient seed stocks: “Assist the maintenance of seed stock for Garden Organic”, with overlap between other categories including conservation, “it seems very sensible to maintain genetic diversity for the future!…putting a bit back after a lifetime in horticulture” and intrinsic interest/gardening interest “To aid my own food security + by giving away seed to help others” and Because I can category (see below) “ time and energy to do something worthwhile”.

Similar to the Intrinsic interest/gardening interest category above, this category (Try new/different varieties), reflects the use of the heritage varieties themselves, and as such has overlap with intrinsic interest/gardening interest and conservation (subsection conservation of heritage varieties), but was specifically separated out to highlight the importance of the perceived “different-ness” of the varieties. 9.22% of responses contained sentiments that fitted

into this category. Exemplar statements include: “I like growing new varieties”, “interest in growing something different” and “to try new vegetables”. The overlap is observed in statements such as “to grow + save+pass on unusual foods” (already mentioned above).

Many respondents (4.96%) included phases that stated they were Seed Guardians because they had the time and/or space to do so, and seemed generally to fit into the category ‘Because I can’. Statements to this effect included “Time and space available in garden” and the eponymous “Because I can”. Many of the statements in this category overlapped with the above category of aiming to help HSL specifically, such as “An organisation is asking for help and I am in the fortunate position that I am able to offer help” and “Time and energy available to do something worthwhile”.

The final category is a broad catchall category, and encompasses the 7.8% of comments that included statements about seed saving, access to seed and seed swapping, and has strong overlap with many of the other categories. Statements range from “free seed”, “to aid seed distribution in my locality” and “only way to obtain the seeds”, to statements that overlap with above categories such as conservation and difference “to gain access to different/old varieties not available in shops”, intrinsic interest/gardening interest “to save some myself & swap with other seed guardians & potential seed guardians” and “to save seed for my garden”, and food security “to aid my own food security + by giving away seed help others”.

For question 2 (how many different crops do you grow (as a Seed Guardian) on average, per year?) the largest proportion of recipients reported growing two crops per year (33%) (Figure 5.1), the second largest category was three crops per year (30%). 2% of respondents did not complete this question (noted as missing data).

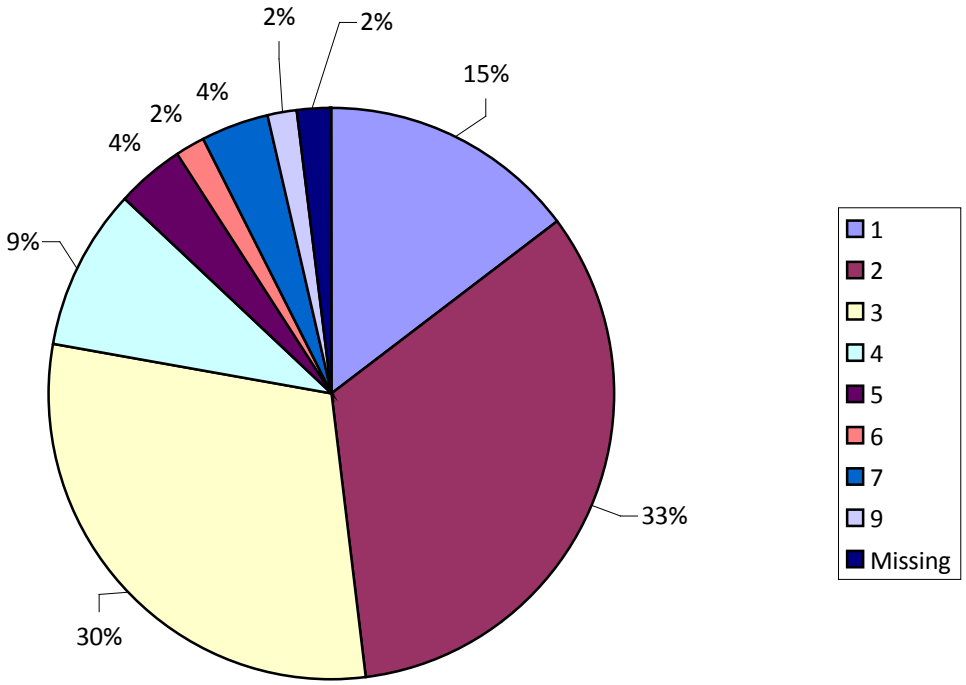

Figure 5.1 Seed Guardian questionnaire question 2: How many different crops do you grow (as a Seed Guardian) on average, per year? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Respondents who left this section blank are noted as missing.

For question 3 (how many varieties do you grow (as a seed guardian) on average, per year?) the largest response category for question three was two varieties (33%), followed by one variety and three varieties (both from 20% of respondents) (Figure 5.2).

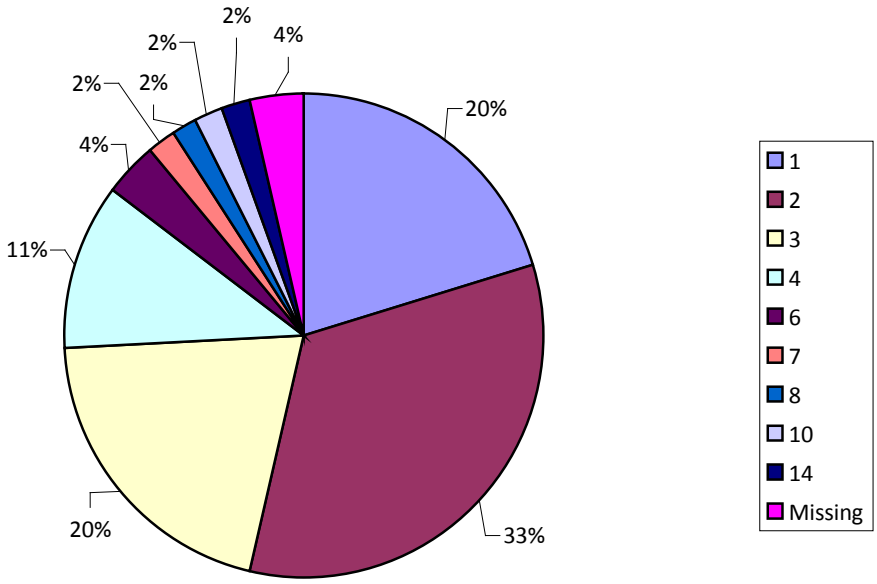

Figure 5.2 Seed Guardian questionnaire question 3: How many varieties do you grow (as a seed guardian) on average, per year? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Respondents who left this section blank are noted as missing.

For question 4 (are there any varieties that you like to grow regularly? Why do you choose these varieties?) analysis was split into three parts: the crops people prefer, the frequency and the reasons.

121 responses were given in total, with 15 specific crop types returned (Figure 5.3), the crop most respondents stated that they like to grow regularly was french bean (31%) (this includes dwarf and climbing french bean), followed by pea (20%) and tomato (15%). Three respondents (2.5%) replied 'bean', which could be broad, runner or french or all of these, and one responded 'pulses' (0.8%). Three respondents said they had no preference (2.5%). Two respondents said they like to grow a different crop every year (1.7%). The 'other' category

includes three first time growers, one second-time grower, one that stated they would grow any variety that 'stood out' and one with indecipherable handwriting.

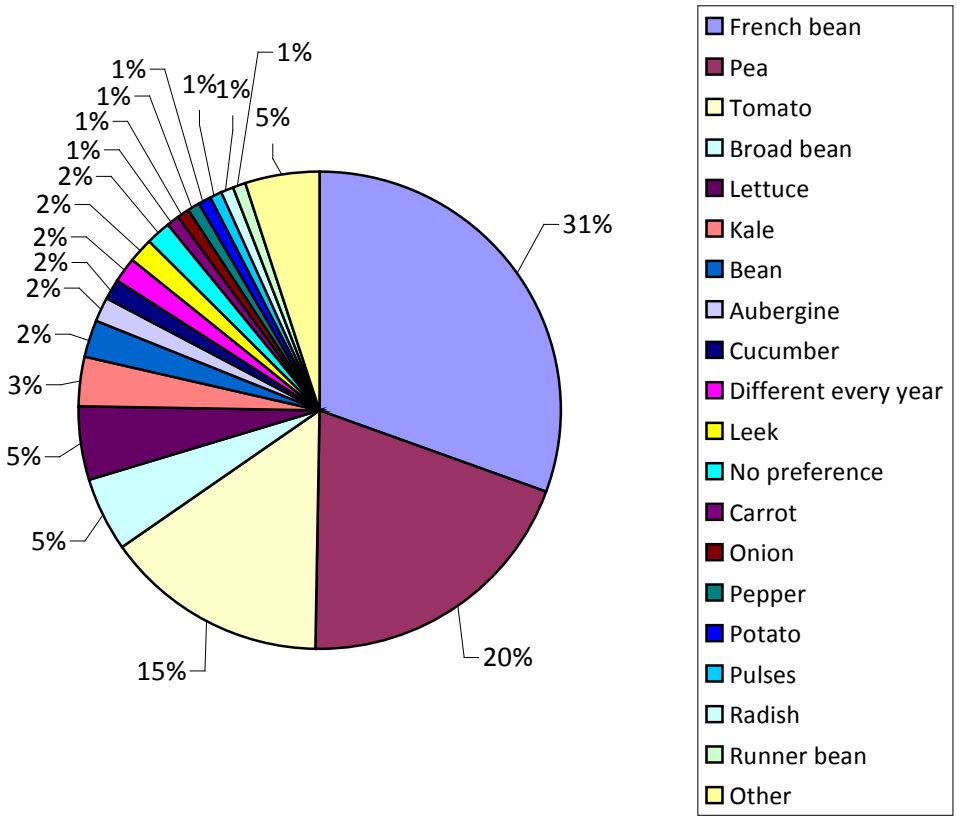

Figure 5.3 Seed Guardian questionnaire question 4: Are there any varieties that you like to grow regularly? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Responses given sorted by crop type.

Asked which varieties they like to regularly grow, 71 varieties were given (Figure 5.4). 57 respondents gave different varieties, with three varieties (Asparagus (kale), Stoke (lettuce) and Blue Coco (french bean)) mentioned by three people, and 11 varieties named by three people.

Asked, how regularly do you grow this variety 102 responses were given for this question (Figure 5.4). Most Seed Guardians grow the variety/crop every year (71%). The 'Other' category included responses that were not frequencies ("if offered by HSL", "all year", "most of the year" and "the last two years"). 10% of responses indicated first time growers.

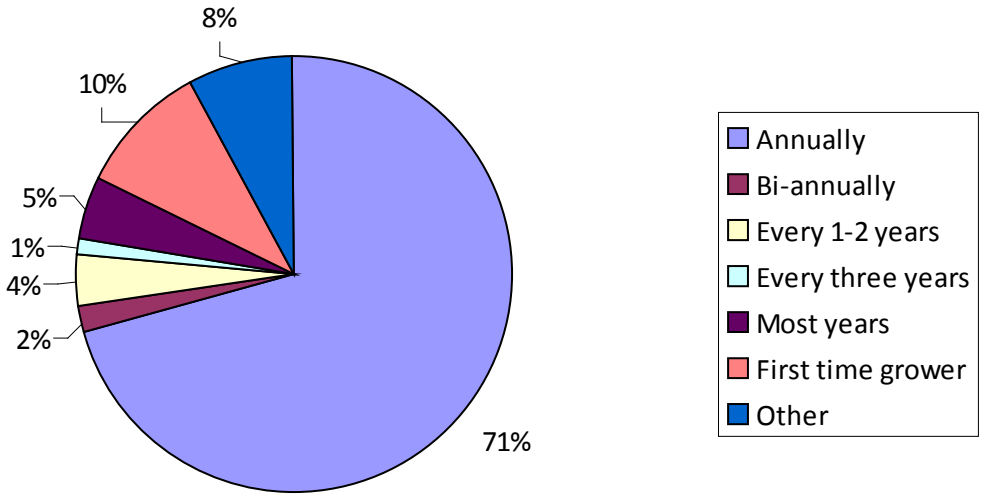

Figure 5.4 Seed Guardian questionnaire question 4: How regularly do you grow this variety? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned.

For the final part of this question (what is the reason you chose this variety?) the 113 responses were summarised under 11 categories: Appearance, Flavour/texture, Ease of growing, Cropping, Something different/unusual, Seed Guardian, Use, Cross-pollination, Personal link to variety, Environmental, Personal preference, Other (Table 5.2). As before, some comments fitted into more than one category, therefore the total responses adds up to more than 100%.

Table 5.2 Seed Guardian questionnaire question 4: What is the reason you choose this variety? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Responses categorised by keyword.

Category	Number of responses	Percentage
Flavour/texture	27	23.89
Seed Guardian	22	19.47
Cropping	18	15.93
Personal preference	18	15.93
Use	17	15.04
Appearance	15	13.27
Cross pollination	13	11.50
Something different/unusual	11	9.73
Environmental	8	7.08
Personal link to variety	9	7.96
Growing ease	7	6.19
Other	11	9.73

Six categories (Flavour/texture, Appearance, Ease of growing, Cropping, Cross pollination and Environmental) can be further clustered, as they all refer to aspects relating to varietal traits. The most popular reason given was Flavour/texture, with 29.3% of responses mentioning this trait. Phrases used included “delicious”, “flavour”, “great taste” and “flavour and texture”. Appearance of variety was mentioned in 13.3% of responses, including “attractive”, “pretty plants” and “fascinated by purple pods”.

The second most popular reason (19.5% of responses) related to answers pertaining to the actual Seed Guardian scheme, phrases such as “to guard the seed”, “my original Seed Guardian variety”, “available on Orphan’s list” and “to preserve and enlarge collection”. Included in this category were comments about how easy the seed was to save, which had some overlap with the varietal trait categories above, and also the personal links category (see below), as a respondent stated a personal history of cultivating varieties for the scheme.

16% of responses included mention of how well the variety cropped, with comments including “heavy cropper”, “easy to crop” and “reliable”. Closely related to this were comments such as “easy to grow” and “easy to grow and crop”, which were placed in the category Ease of growing (6.2% of responses mentioned this). This category is in turn closely related to cross-pollination. Many respondents stated that they chose specific crops that would not cross-pollinate (11.5% of responses) (for example if they knew there were no other beans in the vicinity; this makes growing easier as no isolation is necessary).

7.1% of responses mentioned a reason for growing the variety that was specifically related to an environmental trait, including hardiness, the ability to grow well at high latitudes and “reliable outdoors”.

15% of responses included comments relating to how crops/varieties were used; of these many related to beans or peas: “good for drying” and “can use for fresh or dried beans”, or tomatoes “good all round tomato” and “an excellent tomato”.

As in question one, the appeal to grow something unusual or different was recorded as a reason people liked particular varieties (9.7%); key phrases included “creates interest on allotment” and included in this category were responses that implied the sense of choice at HSL including “enjoy trying out different varieties” and “there are so many types”.

The category Personal link to variety (7.9% of responses) demonstrates the importance of heritage varieties to individuals and included a respondent who grows Martock broad bean due to a family link to Somerset (as mentioned in question 1), a respondent who lived in Gladstone so grows Gladstone pea, and a respondent who grows Gravedigger pea as they are located next to a graveyard. Included in this category were general comments about the appeal of variety names, for example “liked the name”.

Personal preference (15.9% of responses) was a broad category that included comments that predominantly referred to crops as a whole; exemplar statements include “love broad beans”, “I like growing peas” and “they are fun”, with some specific varieties mentioned, such as “have kept my own Stoke [lettuce] seed for years”.

The ‘Other’ category (9.7%) was composed of a broad range of comments that were stand alone and so could not be grouped into larger, generalised categories. It included the responses “because I can reliably do so”, “I lost all peas to mice this year”, “to enhance and preserve my private collection” and “because they are 6 ft tall and out of reach of my snails” (in reference to Gladstone pea).

For question 5 (how closely do you follow Seed Saving Guidelines? Any additional measures used?) analysis was performed by growing stage. For pre-treatment (to seed before it’s sown) the largest proportion of Seed Guardians responded that the followed HSL Seed Saving Guidelines exactly (43%), followed by mostly (35%) (Figure 5.5). Additional measures given were pre-germination on damp kitchen towel, application of GA3 hormone to old seed, pre-soaking and warm in a saucer. One respondent stated that they were not aware of the Seed Saving Guidelines.

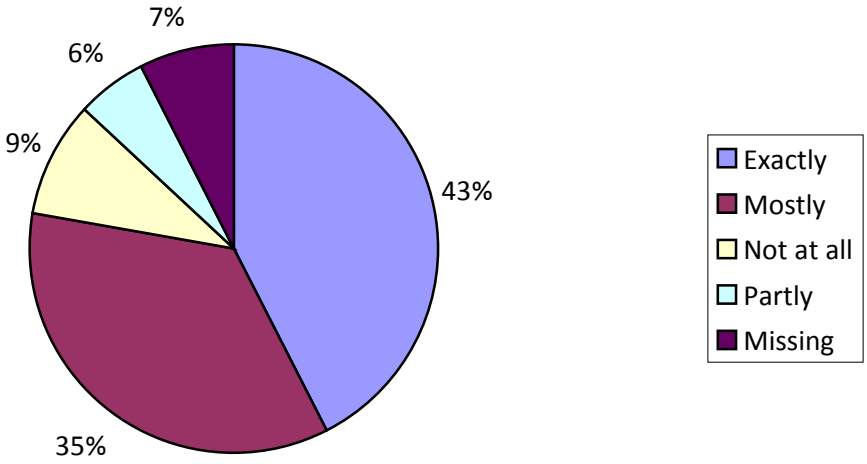

Figure 5.5 Seed Guardian questionnaire question 5: How closely do you follow Seed Saving Guidelines in regard to treatment of seed before it is sown? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned.

With regard to distances between varieties during cultivation the largest proportion of respondents stated that they 'Mostly' followed HSL Seed Saving Guidelines (48%) (Figure 5.6), followed by 'exactly' followed Guidelines (37%). Additional measures given were separation in time not distance, grow varieties that won't cross pollinate, "only grow one variety at a time", "grow seeds in deep beds so can be sown closer together", and "depends on available room".

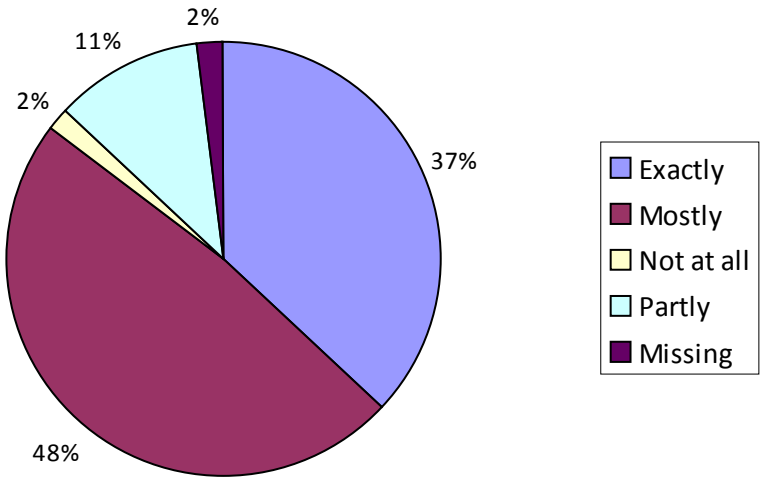

Figure 5.6 Seed Guardian questionnaire question 5: How closely do you follow HSL Seed Saving Guidelines with reference to distance between varieties during cultivation? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned.

The largest proportions were again ‘exactly’ and ‘mostly’ followed Seed Saving Guidelines for harvesting (42% and 41% respectively) (Figure 5.7). Only one respondent gave an additional measure, which was leaving the seed longer than recommended to ensure it is ready.

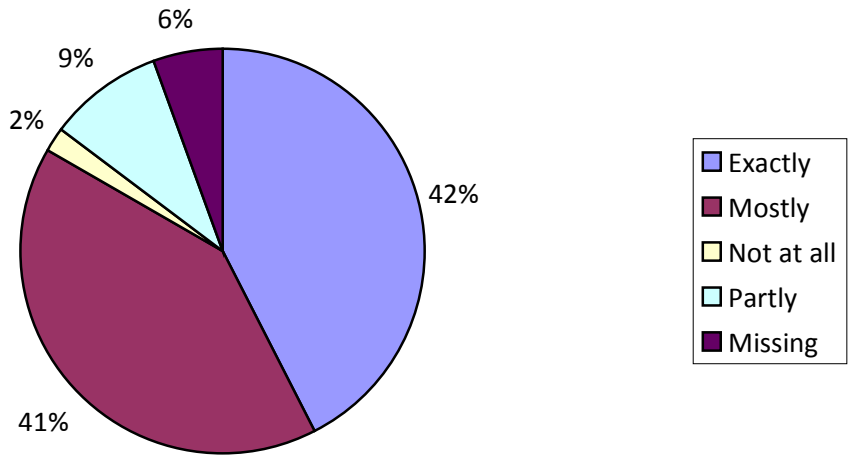

Figure 5.7 Seed Guardian questionnaire question 5: How closely do you follow HSL Seed Saving Guidelines with reference to harvesting? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned.

For post-harvest seed treatment, the largest proportion of respondents stated that they followed Seed Saving Guidelines ‘exactly’ (54%), followed by ‘mostly’ (37%) (Figure 5.8). Measures added were drying seed (three respondents, including one with silica gel), and one respondent stated they performed germination tests before returning seed to HSL. One respondent stated Seed Saving Guidelines were “spot on”.

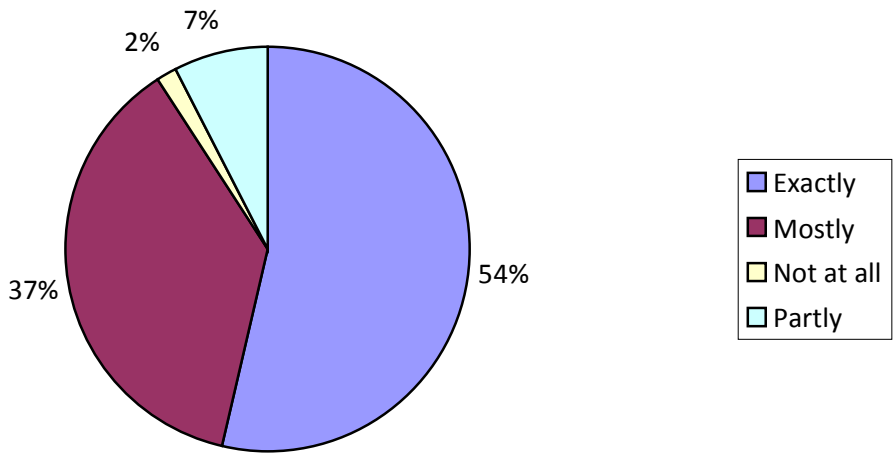

Figure 5.8 Seed Guardian questionnaire question 5: How closely do you follow Seed Saving Guidelines with regard to post-harvest seed treatment? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned.

For question 6 (do you have any practices that you apply before you sow the seed?) 55.6% of respondents ticked the box that they applied compost before they sow seed. 27.8% of respondents ticked the box to indicate that they applied manure before sowing the seed. 16.7% of respondents ticked both boxes. A free text space was also available for any addition practices used. Responses given were seaweed (five respondents), blood/fish/bone (four respondents), leaf mould (two respondents), rock dust (two respondents), nettles (one respondent), 'organic fertilizer' (one respondent) and wood ash (one respondent). Three respondents specified that they used homemade fertilizers, including comfrey feed, potash, seaweed and compost. Time periods mentioned in responses were 'occasionally' for application of blood/fish/bone and seaweed, and manure every four-five years. 11 respondents out of the 54 did not tick either box and made no additional comments. One respondent

specifically said no treatment. More general treatments included two respondents said they used ‘general’ preparations, other treatments included starting plants off in pots, Propapaks, propagators, under cover or in greenhouses, using a no-digging raised bed system, digging and covering the ground with plastic to deter weeds.

Question 7 (where and how do you store seeds?) was a free text box; only one respondent left the box blank. Answers generally followed the format of stating the immediate container in which seed was stored (envelopes (40.7%) and/or paper bags (22%) were the most common stated), then these seeds were stored in a larger container (a box or tin (24.1%), airtight plastic tubs (18.5%) or jars (3.7%)) and then the room in the house/garage (in house (48%), in garage/shed/greenhouse/utility room/pantry (27.8%)).

Comments relating to temperature were made by 44% of respondents, of these all but three stated they kept seeds in a “cool” or “unheated” place, the remaining three said “frost-free”, “room temperature” and “slightly heated”.

Five respondents specified that they kept seeds dry (9.3%); four respondents mentioned keeping seeds somewhere dark (7.4%) and three (5.6%) said they used silica gel to keep seeds dry or to dry them out.

Question 8 (how do you choose which seeds to send back to HSL?) was also a free text box; all respondents completed this section. Answers were along common themes, with the main categories mentioned being plant selection (44%), seed selection (53.7%), number of plants (9.3%) and seed quantity (35.2%).

Plant selection category included the selection of healthy/strong plants (22.2% of responses); the removal of rogues (18.5% of responses), and single respondents chose tallest pea plants,

slowest bolters, fullest pods, and plants from the middle of the row. 9.3% of respondents made reference to the number of plants they select from, these ranged from “several rows go to seed” and “harvest from several plants”, to “as many plants as possible”.

Seed selection included seed quality and selection based on features, and included comments such as “seed is checked for uniformity of appearance”, “Large and best. Discard small misshapen ones”, “good quality seed”, “disease free seeds” and “seed that looks like that which was sent. No small seed”. 5.6% of respondents said they used no selection, one respondent said it depended on the crop, for tomato no selection, for bean just the “best marked”.

Seed quantity (35.2%), most respondents in this category stated they sent back two-thirds, “the majority” or “all seed”, with three responses received being for keeping enough seed to grow again next year and three keeping some for themselves.

For question 9 (if you have any spare after returning seeds to HSL, how do you use them?) 32 respondents (59.3%) ticked the box to indicate that they shared seed with others if they had any spare after returning seed to HSL. Forty-seven respondents (87.0%) ticked the box to indicate that they used seed for their own retention. No other options were added, however many respondents added more detail; popular additions were seed swaps, seeds were retained to be consumed or grown for next year, three respondents mentioned the use of seeds in schools or university, either as a teaching aid or to grow with school children or students. Seed swaps were either with friends, work colleagues or through local seed swaps (garden society or university), or seed swaps over the Internet (Cottage Garden Seed Exchange or on Grapevine Forum), or HSL seed swaps.

There were 15 respondents to question 10 (have you noticed any varieties that do not breed true or show unexpected variation?), two of whom gave two varieties, totalling 17 responses (Table 5.3).

Table 5.3 Seed Guardian questionnaire question 10: Have you noticed any varieties that do not breed true or show unexpected variation, for example in shape/size/colour? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing. Seventeen responses were given from 15 respondents. Varietal differences have been simplified to show common themes.

Crop	Variety	Year grown	Variation observed
Climbing french bean	Alice White's	2009	Plant colour differences; seed coat differences
Climbing french bean	Major Cooke	2009	Seed and pod colour differences
Dwarf French bean	French horticultural	2006	Plant stature differences (climbed)
Dwarf French bean	Pewitt	2004	Seed colour
French bean	Bird's egg	2001	Seed colour
Leek	Coloma	2008	Flower colour
Pea	Frueher Heinrich		Seed coat wrinkling
Pea	Pilot	2009	Plant stature differences (very tall)
Pea	Salmon flowered	2008	Flower colour
Pea	Victoria purple podded	2008	Flower colour
Radish	Rat's tail	2009	Seed pod variation
Tomato	Buffalo horn	1996	Colour and shape of fruit
Tomato	Earl of Edgecombe	2005	Colour of fruit
Tomato	Madame Jardel Black	2008	Colour and shape of fruit
Tomato	Purple Calabash	2008	Leaf shape
Tomato	Snow white cherry	2009	Fruit size and shape
Tomato	Sub-arctic plenty	2004	Plant growth (weak)

For question 11 (are there any services or supports that you feel HSL could provide to better meet your needs?) the total number of responses was 129, including additional suggestions from the 'Other' free text area. Feedback on seed return to HSL was the most popular service respondents identified (28%), followed by a regular newsletter/e-newsletter (16%) and local Seed Guardian networks (14%) (Figure 5.9).

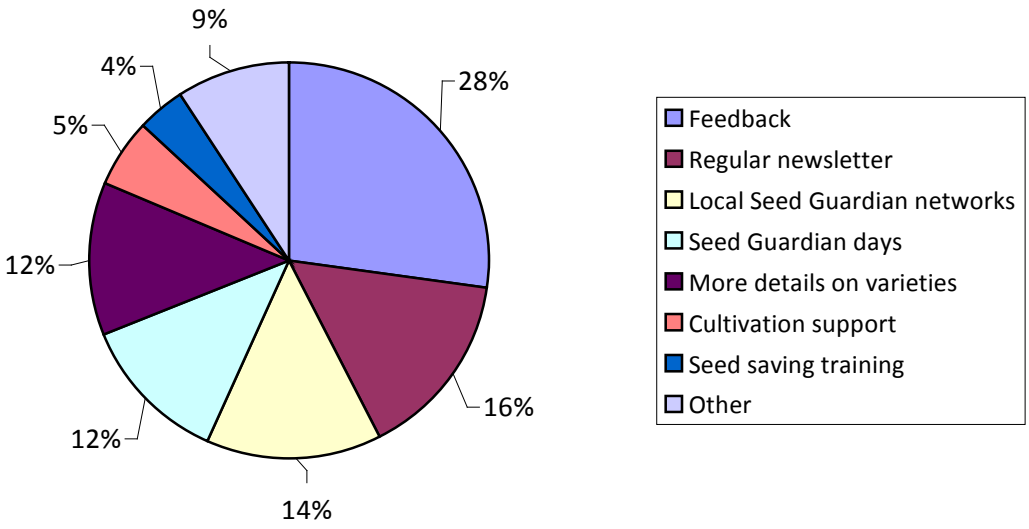

Figure 5.9 Are there any services or supports that you feel HSL could provide to better meet your needs? Paper questionnaires were sent out to Seed Guardians with a regular HLS mailing, 54 were returned. Percentages shown are percentage of total number of responses, not respondents, as many respondents ticked multiple boxes.

The 'other' section was used by respondents to say that no other support was necessary (four respondents) or to elaborate on the boxes already ticked, such as stressing that local SG days, training and networks would be appreciated (one respondent for each of these) and more history for the varieties, or how to research, (one respondent). Another suggestion added here was increased email contact, particularly for first time growers (two respondents), which may come under cultivation support or feedback on seed return to HSL. Real-time seed swapping, perhaps via an Internet forum, was also suggested, although this is probably beyond the remit of the Seed Guardian Scheme (one respondent).

5.3.3 Member questionnaire

Heritage vegetables

Respondents had been growing vegetables for an average of 23.2 years (standard deviation = 12.26 years), answers ranged from 3 to 45 years. The average for growing heritage vegetables in particular was 9.7 years (standard deviation = 6.31 years), answers ranged from 1 to 30 years.

The reasons people gave for growing heritage vegetables can be seen below in Table 5.4.

Table 5.4 Member questionnaire question 1.3: Reasons stated for growing heritage vegetables. Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed. Responses grouped by keyword.

Overall category	Number of responses	Percentage of responses
Varietal traits	37	31.90
Conservation – Heritage varieties	26	22.41
Conservation – Biodiversity	16	13.79
Intrinsic interest	15	12.93
Availability of something different	10	8.62
Help a good cause	6	5.17
Anti-agribusiness	6	5.17

The reasons respondents gave for growing heritage varieties could be grouped into seven categories: Varietal traits, Conservation (Biodiversity and Heritage varieties), Intrinsic interest, Availability of something different, Help a good cause and Anti-agribusiness.

The largest category was that of varietal traits (31.9%). The largest response for this category included taste or flavour; remaining statements related to traits including disease resistance, yield and varieties that are specifically aimed at gardeners.

Reasons for growing heritage varieties including statements regarding the conservation of heritage varieties featured in 22.4% of responses, the second largest category, and included statements that explicitly mentioned the conservation of heritage varieties, including “important to keep old varieties going”, “keep varieties alive” and “protect heritage”.

Conservation – Biodiversity, was featured in 14.3% of responses and included the reasons of respondents for growing heritage varieties that mentioned biodiversity, conservation or protecting genetic diversity; exemplar statements included “believe in preserving biodiversity”, “ensure diversity in gene pool” and “food security linked to diversity of veg varieties”.

The Help a good cause category was composed of responses that mentioned helping HSL (5.2%), and exemplar statements include “supporting a worthwhile endeavour” and “want to do my bit”.

Anti-agribusiness or anti-commercial (5.2%) mainly included responses such as “it’s nice to grow things you can’t buy in the shop”, and “I am completely against big business and what it stands for – particularly being told what to buy and eat”.

The next category was Intrinsic interest. 12.9% of responses included statements to the effect that they chose heritage varieties because they are interesting, different or unusual. Exemplar statements include “it adds interest to growing food”, “fun and attractive” and “enjoy the unusual varieties”. This category is related to the first category of Conservation- Heritage varieties.

The final category was availability of something different (8.6% of responses), and related to the availability of heritage varieties; respondents grew them because they were “not usually available to buy”, they had access to seed or “not available commercially”.

In question four, 87% of respondents reported that they also grew standard varieties alongside the heritage ones (4.3% responded that they did not, 8.7% left this question blank); their reasons for growing modern varieties also can be seen in Table 5.5.

Table 5.5 Member questionnaire question 1.5: why do you grow standard varieties? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed. Responses grouped by keyword.

Category	Number of responses	Percentage of responses
Range or availability	37	44.05
Preference for a variety	12	14.29
Improved characteristics	11	13.10
Seed saving problems	8	9.52
Modern breeding	3	3.57
Suits site	4	4.76
Other	12	14.28

The largest response category was range or availability (44% of responses). This category was composed from responses that stated that particular varieties or crops were not available from HSL, that they wanted to grow more than the six varieties that they can get from HSL, or that availability was easier and larger choice was available, or just that they liked them. This overlaps with the next category that was composed of responses from growers who had a known variety that they have grown successfully before or have found to be reliable (14.3%).

The improved characteristics of some modern varieties was given as the reason they were grown in 13.1% of responses; specific traits mentioned were disease resistance and yield. This is related to the category of Modern breeding (3.6% of responses), in which growers stated

that they specifically grow some modern varieties to support varieties that are bred for gardens or the new diversity resulting from modern breeding.

The category Suits site (4.8%) related to the environment in which the varieties are grown, and included statements such as “some varieties are more reliable on our soil or in our garden” and so also overlaps with the personal preference for a known variety category above.

Some respondents (9.5%) specifically stated that they grew modern varieties because they could not seed save due to reasons such as limited space (for allowing plants to go to seed), that saving seed was too difficult or that buying seed was more convenient.

The Other category includes a broad range of statements that did not form an overall theme, such as price, or more generally about growing such as “I like to grow as much of our food as possible” and “I want to cut down on food miles”.

Respondents were asked what proportion of the vegetables that they grew were heritage varieties. Figure 5.10 shows that the majority (52%) of respondents reported growing about 50/50 heritage and modern varieties, with no respondents stating that they grow all heritage varieties.

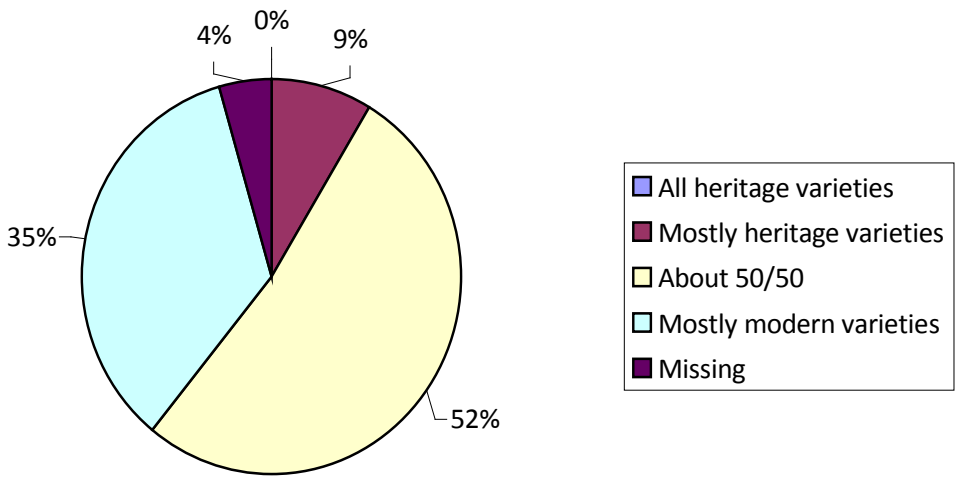

Figure 5.10 Member questionnaire question 1.6: roughly what proportion of the vegetables that you grow are modern/heritage varieties? questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Some people answered in more than one category as people who had tubs also had a garden or allotment, and two respondents ticked allotment and garden (Figure 5.11).

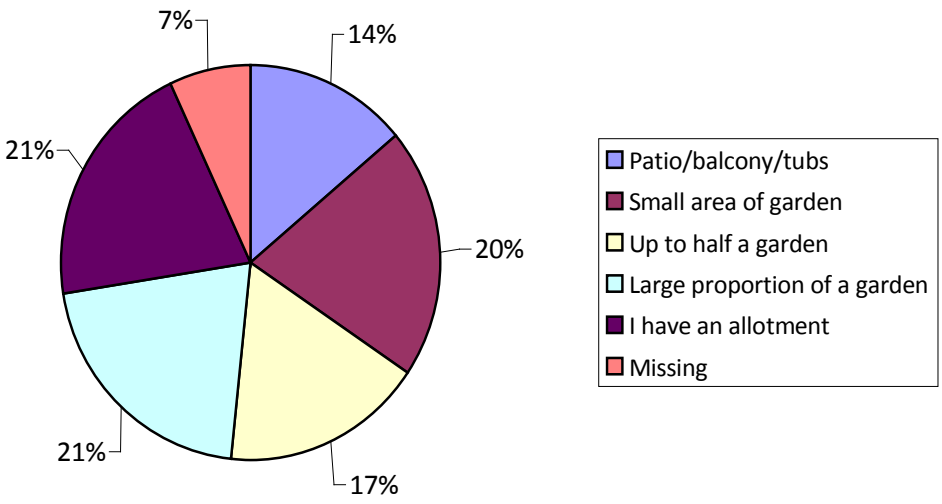

Figure 5.11 Member questionnaire question 2.1: How much space do you have allocated for vegetable growing? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

When asked about which organic gardening practices they used, most participants reported that they used most if not all of the practices options listed (see Figure 5.12).

The six organic practices were employed by at least 60% of respondents. The practices most widely adopted were bee-friendly gardening and encouraging predators of pests (76.1% and 78.3% respectively).

Practices reported in the 'Other' category were 11 respondents who made their own compost, including from nettles, comfrey and garden and kitchen waste; three respondents used green manures (*Phacelia* and red clover were noted by one respondent); other measures stated were crop rotation, water conservation, slug barriers, poultry waste, cow manure, guinea pig waste and rock dust (one respondent each).

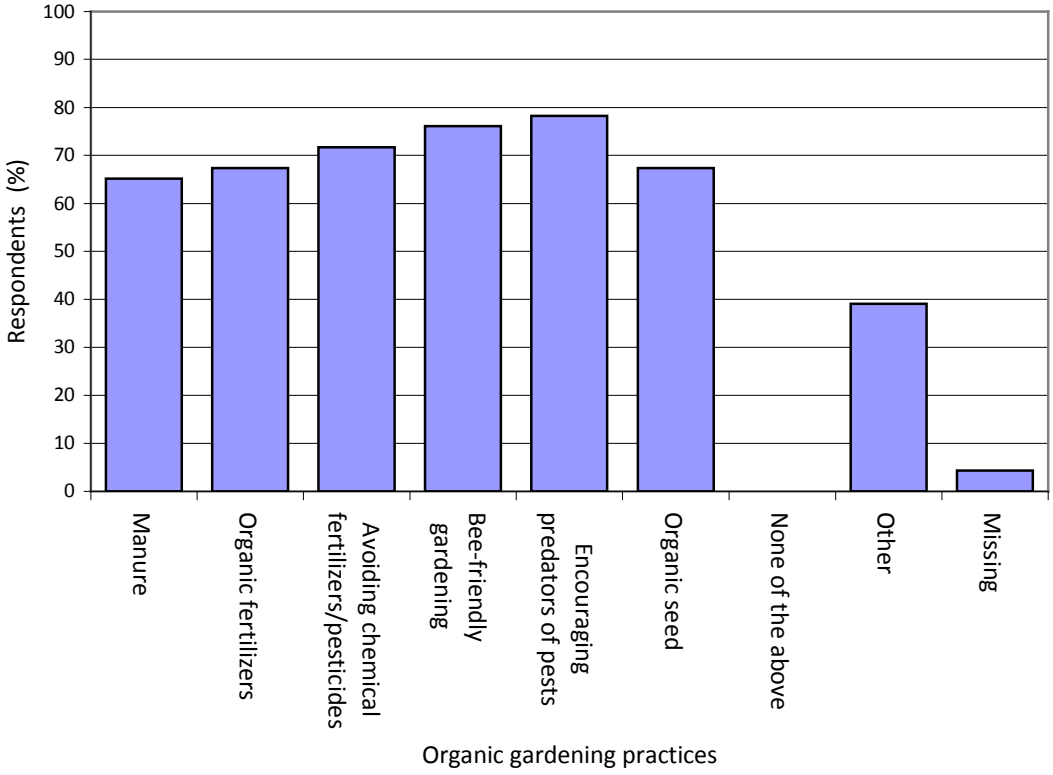

Figure 5.12 Member questionnaire question 2.2: Which of the following organic practices do you regularly use? Percentage of respondents reporting each organic gardening practice. Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Figure 5.13 shows that 92% of respondents chose to grown mostly the same crops each year. None reported growing completely different crops each year. Figure 5.14 shows that 70% of respondents reported growing mostly the same varieties each year with 2% proportions growing all the same or all different varieties from year to year. 24% of respondents said that they grew mostly different varieties each year. HSL informs members of the seed that is available each year. The implications of these results are twofold; firstly, that there may be pressure on certain accessions in the library, and secondly, that people may need to be

encouraged to grow new varieties. The information on popular traits in this survey may be able to assist this.

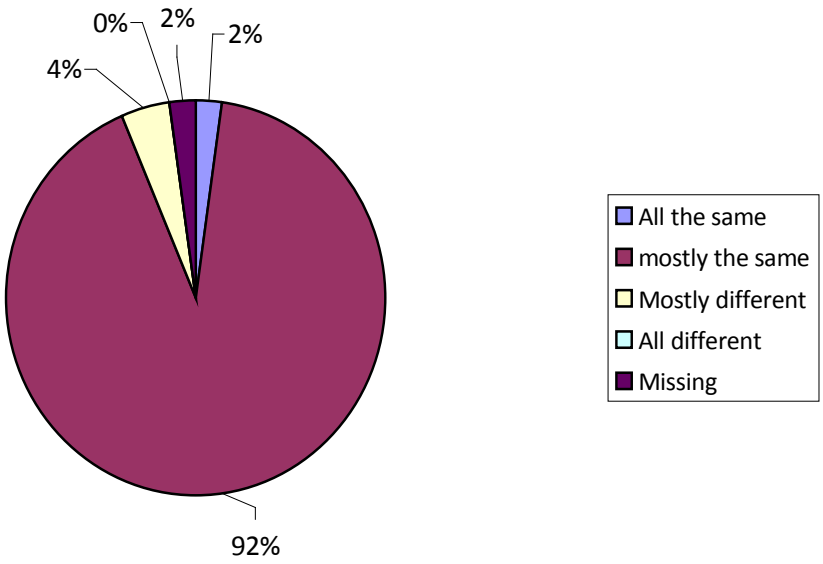

Figure 5.13 Member questionnaire question 3.1: Do you grow the same crops each year? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

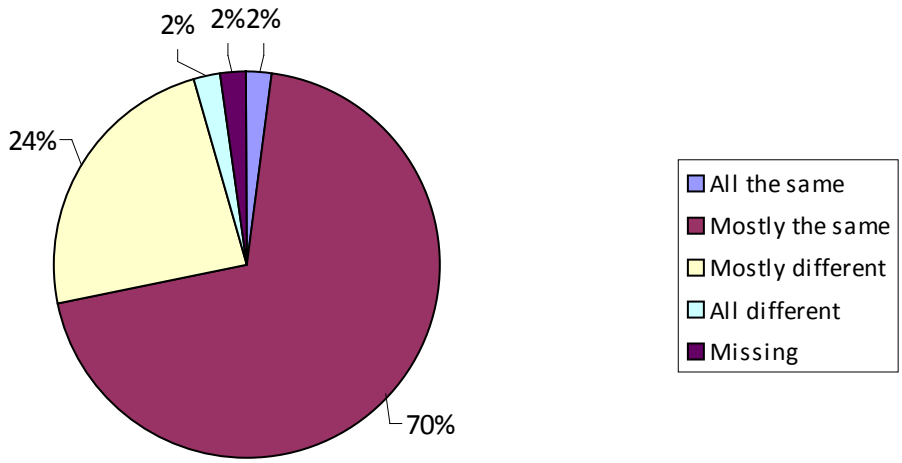

Figure 5.14 Member questionnaire question 3.2: Do you grow the same varieties each year? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Respondents were asked to identify specific traits that they look for when choosing which varieties to grow; Table 5.6 shows the responses reported. These responses are very similar to those reported in tables 4 and 5 regarding choosing which type of vegetables to grow and suggests a general level of importance for these traits.

Responses were grouped into ten categories. The most important trait looked for by respondents when choosing which variety to grow was taste (28.3%), followed by pest/disease resistance (16%) and appearance (15.1%). Aspects of appearance mentioned were colour, attractiveness, novelty and that people looked for "unusual" traits, for example: "unusual traits e.g. no red tomatoes".

The next most popular criterion for variety selection was yield /cropping (13.2%); this included statements such as “productivity”, “ease of cropping” and “heavy cropping”.

The Other category (8.5%) included a broad range of statements, from “variation” and “to get a good selection”, which relate to the overall suite of varieties that people grow, to “low watering requirements” and “historical connections”.

Respondents also looked for traits relating to suitability of the variety to growing conditions/hardiness (7.6%), these were predominantly statements relating to environmental (weather) or soil conditions.

Further traits looked for were earliness of maturity (3.8%), plant architecture requirement (e.g. compact habit) (2.8%) and End use suitability (3.8%). This latter category included general statements such as “good for the kitchen”, “ease of use” and “quality for exhibiting”.

Table 5.6 Member questionnaire question 3.3: When you are choosing which varieties to grow are there any particular traits that you look for? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed. Responses grouped by keyword.

Code	Number of responses	Percentage of responses
Flavour	30	28.30
Pest/Disease resistance	17	16.04
Appearance	16	15.09
Yield/cropping	14	13.21
Other	9	8.49
Suitability to growing conditions/hardiness	8	7.55
Ease of cultivation/reliability	5	4.72
Earliness of maturity	4	3.77
Plant architecture requirements	3	2.83
End use suitability	4	3.77

89.1% of respondents reported growing more than one variety of each crop. Participants were asked to rank their reasons for growing more than one variety for each crop; Figure 5.15 shows that having a larger variety choice was the most important reason overall. A free text option was also permitted; the other main reasons given were to extend or stagger the cropping season (six responses), curiosity/trying new things (four responses), seasonality (three responses), to grow a mixture of heritage and standard varieties (two responses), and to get a range of colours, shapes and flavours (one response).

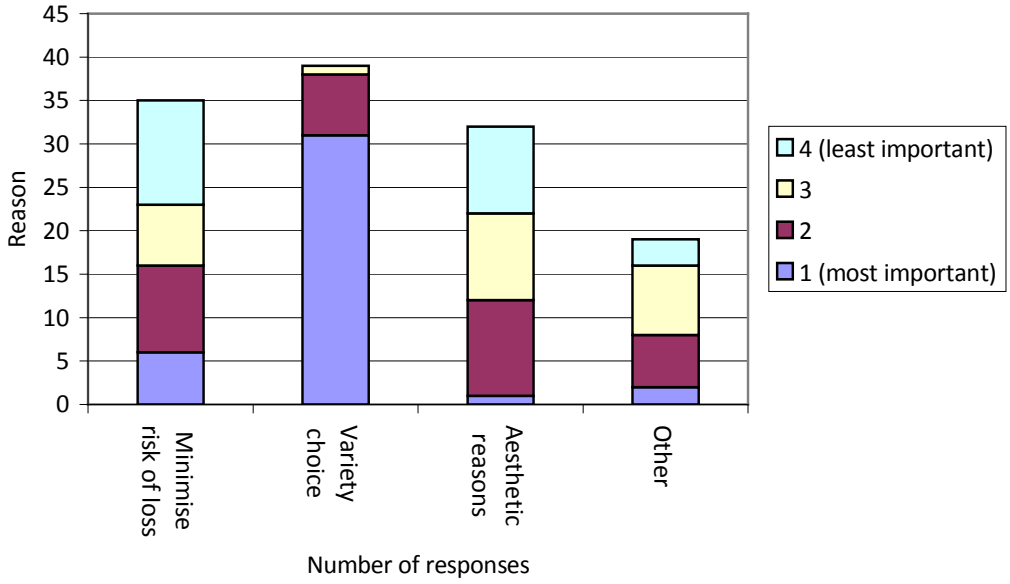

Figure 5.15 Member questionnaire question 3.5: Please rank your reasons for growing more than one variety from 1 (most important) to 4 (least important). Rankings and number of responses for reasons for growing more than one variety of each crop. Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Questions 6 and 7 were not analysed in detail. In the former, most respondents identified different varieties of interest and reasons given for varietal preference reflected those already discussed above. Question 7 did not reveal any special uses for the heritage varieties grown; all uses stated were general (such as freezing, bottling and drying).

Evaluation of the varieties is beyond the scope of the characterisation phase of the present study; however many heritage varieties are thought to have traits, such as disease resistance, that may not have been formally recorded. Respondents were asked whether they had seen any such occurrences; Table 5.7 shows the responses; four responses were discarded as either the variety name or the specific resistance was not given.

Table 5.7 Member questionnaire question 4.1: Have you found any varieties that have a particular pest/disease/environmental (weather etc) resistance? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Crop	Variety	Resistance
Broad bean	Purple-Flowered broad bean	No blackfly or mosaic
Carrot	Yellowstone	Carrot root fly
Courgette	Astra	Downy/powdery mildew
French bean	Early Warwick	Stands cool and wet weather
Kale	Uncle Bert's Purple	Tolerant of temperatures below zero degrees
Leek	Mammoth	Weather/rust/pest
Lettuce	Bronze Arrowhead	Adverse weather, slug and bolt resistant
Lettuce	Bronze Arrowhead	Slug and bolt resistant
Squash	Sucrette	Cropped well in poor squash summer
Tomato	Broad Ripple Yellow Currant	Lasts until heavy frosts
Tomato	Broad Ripple Yellow Currant	Always the last to get blight
Tomato	Ferline	Blight resistant
Tomato	Red Russian	Variable temperatures and rain don't bother it
Tomato	Tangella	Last to get blight

Participants were asked whether any of the varieties that they had experience of growing showed unexpected variation (or they suspected of not breeding true); Table 5.8 lists the reported varieties; all were reported once apart from Crimson-flowered broad bean which was reported by four respondents as having occasional white flowers. Three responses were discarded as either variety name or character was not specified.

Table 5.8 Member questionnaire question 4.2: Have you noticed any varieties that do not breed true or show unexpected variety, for example in shape or colour? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Crop	Variety	Number of respondents	Characteristic
Broad bean	Crimson Flowered	4	White flowers
Tomato	Black Plum	1	Changed shape
French bean	Cherokee Trail of Tears	1	Some round and some flat pods
Pea	Forty First	1	Variation in pod colour, some green and some flushed with varying amounts of purple
Dwarf french bean	Black Valentine	1	Gives some variation in seed colour and habit
French bean	Bird's Egg	1	Occasional sport of red bean with white splashes

Another question that could imply routes for investigations for the future, or a place to look for comparisons with the current characterisation study is the occurrence of duplicates in the collection. Only a few affirmative responses were reported and can be found in Table 5.9. No varieties were reported more than once.

Table 5.9 Member questionnaire question 4.3: Have you grown and varieties that you think may be the same but with different names? Varieties reported. Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Crop	Variety 1	Variety 2
Climbing french bean	District Nurse	Bridgwater Bean
Kale	Ragged Jack	Red Russian
Kale	Westphalen Kale	Asparagus Kale
Pea	Jeyes	Duke of Albany
Tomato	Scotland Yellow	Kenches Gold

Table 5.10 shows the results of the question that intended to elucidate the relationship, if any, between varieties bred in a locality and its performance in similar versus different

geographical areas. As no location information was formally collected from the participants answers relied on participants proving this; responses relating to Shirley, Stoner's Exhibition and Essex Wonder were received however no comment on the location where it had been grown was left so response had to be discarded. The complexity of this issue made the question very difficult to phrase and word, giving the Lancashire Lad pea as an example clearly influenced responses. Perhaps if more names had been listed a greater response would have been received.

Table 5.10 Member questionnaire question 4.4: Varieties may do well close to the area where they were bred. If you have grown any seeds that have a local name (e.g. Lancashire Lad or Southampton Wonder) have you noticed any variation in performance compared to other varieties (including poor performance particularly if you live far from the place of origin)? Reported performances of varieties with local names. Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

Crop	Variety	Outcome
Lettuce	Bunyard's Exhibition	Local, do seem to grow well
Lettuce	Stoke	Local, do seem to grow well
Pea	Kent Blue	Disaster in Yorkshire (tiny hard peas)
Pea	Lancashire Lad	Did not grow well (postmark Norwich)
Pea	Lancashire Lad	Grows well in Oxford
Pea	Lancashire Lad	Does well in Yorkshire
Pea	Robinson's	Robinson's may have been local to East Leicestershire? but it is about as good as Centre of England
Tomato	Scotland yellow	Does not do any better than other varieties in Scotland

Seeds were obtained from a number of sources; Figure 5.16 shows that 37% of respondents identify Garden Organic as their main seed source. The smallest proportions were supermarkets (3%). 14% of respondents use seeds of their own retention. This is an area of interest of HSL as their members organise seed swaps, and seed swaps can be an important source of heritage vegetable seed (Stickland, 1998). Of the "other" responses received four

identified seed catalogues by mail order or online, four purchased seed from a catalogue via a garden society or allotment and one used a seed merchant.

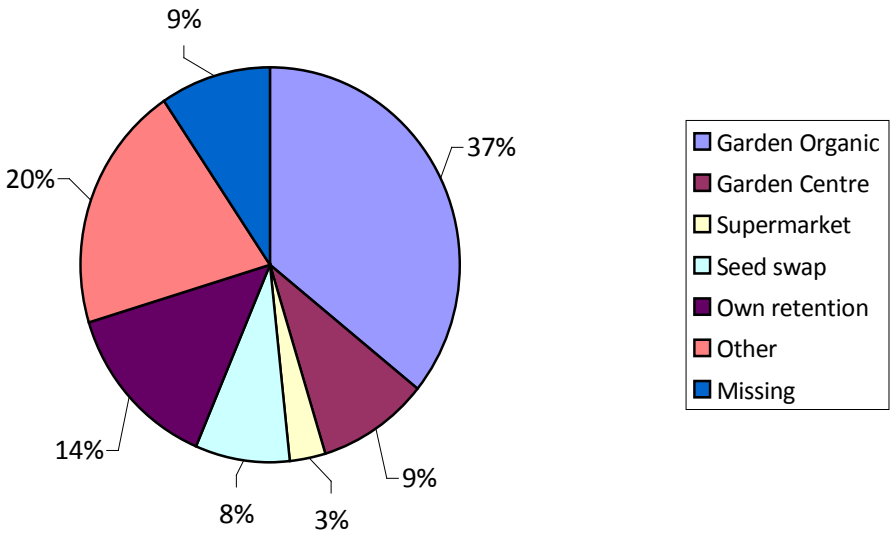

Figure 5.16 Member questionnaire question 5.1: What is the main source of the seeds that you grow? Online questionnaires were advertised in Garden Organic The Organic Way magazine and were published using the Survey Monkey website, 46 questionnaires were completed.

When asked specifically about saving their own seed 82.6% of respondents reported that they do save their own seed. This is perhaps consistent with the earlier stated practices of people sowing the same crops and varieties each year (figures 5.13 and 5.14). 63% of respondents reported that they share seeds with other growers.

5.4 Discussion

The overarching aims of both surveys were to elucidate the motivations, practices and experiences of Garden Organic Members and HSL Seed Guardians. This was accomplished by the presentation of two questionnaires; one survey targeted to Seed Guardians, and one

targeted to GO Members. The results can be summarised under four main themes: motivations, practices, performance and variation, and seed saving and end use of products.

5.4.1 Motivations

Seed Guardians were asked *what are the main reasons that you became a Seed Guardian*? Members were asked *why do you grow heritage vegetable varieties*? Answers for these questions contained a large degree of overlap between both surveys. For Seed Guardians the most popular responses related specifically to the conservation of heritage varieties, and the second most popular related to intrinsic interest in the varieties or gardening. For Members, people grew heritage varieties firstly, because of specific traits that the varieties possessed, such as disease resistance or spreading the yield, and secondly, as above, specifically for the conservation of heritage varieties. Reasons given for conserving heritage varieties were similar in both questionnaires: to keep the varieties themselves going, to conserve or because of an interest in their history. Both questionnaire results link the importance of conservation and heritage with practical elements such as desired traits and interest in growing. Other common themes were the conservation of biodiversity for the future and a resistance to a corporate or big business role in breeding and seed supply. These themes of conservation, choice and traits of interest to gardeners are reflected in those reasons given by Stickland (2008) for why people save seed, and there is also overlap with the reasons given by farmers who grow landraces (Negri, 2003), namely because of a unique or better taste, or suitability to a particular environment. Tradition is often a reason that landrace farmers state for growing landraces (Negri, 2003). Although this was mentioned by some respondents in the current study more often the history and heritage associated with heritage varieties and heirlooms is

not necessarily based on a personal connection, but a cultural value of these aspects in a general sense.

In addition, Members were asked why they grew modern varieties alongside heritage varieties (this question was not raised with Seed Guardians as the focus of the study was on Seed Guardian-related duties). The most popular answers related to the greater range and availability of modern varieties. The supply of seed available for the HSL to distribute to Members is necessarily limited to the amount of seed they can regenerate (aided by Seed Guardians). This limit also affects the variety choice available, as does lack of agronomic information about varieties held within the collection.

5.4.2 Practices

Questions about how respondents select which varieties to grow were put to both groups; Seed Guardians were asked how they select which orphans to grow, and Members were asked which traits were important to them when they were selecting varieties to grow.

For Seed Guardians, the most popular reason for selecting which variety to grow was flavour/texture, with the second most popular response being reasons that related directly to the Seed Guardian scheme; some overlapped with why they became involved in being a Seed Guardian, such as to keep the HSL going, and personal reasons such as it being the varieties they have always guarded under the scheme.

Members also identified taste as the most important trait they desired. This is a popular reason stated for growing heritage varieties, and the lack of taste of modern varieties is widely discussed (Stickland, 2008; Jordan, 2007; Galluzzi *et al*., 2010). The next popular answer was

pest/disease resistance, which concords with the reason many people stated for growing both heritage varieties and modern varieties.

For Seed Guardians, questions about the practices that they used related to the extent that they followed HSL Seed Saving Guidelines (Garden Organic, 2008) before, during and after cultivation; for Members gardening practice questions related to organic gardening.

For Seed Guardians, over three quarters of respondents stated that they grow one, two or three crops as a Seed Guardian each year, and the same proportions for varieties grown each year. This is consistent with the assignation of up to three varieties on average per Seed Guardian of varieties by HSL each year. Those who grow more do so from their own stocks, and perhaps every year; this is consistent with the reporting that almost three quarters of respondents grew the same crop every year.

For Members, the majority grew a 50/50 split between heritage and modern varieties, with the next largest response category being ‘mostly modern’ (perhaps linking back to the availability of heritage varieties, or some other selection choice such as varietal traits desired). The amount of space given over to vegetable growing was very evenly split between options given (between ‘small area of garden’, ‘up to half a garden’ and ‘large proportion of a garden’). Around a fifth of respondents had an allotment. For Members the questionnaire showed that most people grow the same crops every year and three quarters grow the same varieties every year. This suggests that once people find a variety or crop they like/have a use for, they stick with it, and indeed this was mentioned in some of the reasons people gave. Around a fifth of respondents grew mostly different varieties each year, which also may concord with the reasons for selection given above, being that people like to try something different.

The details of Seed Guardian growing practices related directly to the seed saving guidelines distributed by HSL (Garden Organic, 2008). These are species-specific guidelines, that, as well as providing brief botanical background of each species, advises guardians on how to save seed. The main focus of the document is preserving varietal purity through isolation and prevention of cross-pollination and through roguing. It also advises on harvesting, seed cleaning and how to store seeds to make them last longer.

For all four stages (pre-treatment, isolation, harvest and post-harvest), three-quarters to four-fifths of all respondents said that they followed Seed Saving Guidelines exactly or mostly. The proportion that stated ‘not at all’ was low (around 2%) in most stages; it is unclear whether these respondents were simply not aware of the guidelines (as one stated directly), whether they took additional measures or whether they took no measures. This is important to know, and would be useful to explore in more detail, due to the potential impact on varietal purity.

This is also the case for the seed storage question; answers were given in a very broad range, from respondents stating they kept the seeds in an envelope, to some only stating a room, and others giving exact details including temperature and light levels. Most respondents however had in common cool, dark, conditions, and most in paper envelopes and airtight containers. This is in accordance with the Seed Saving Guidelines (Garden Organic, 2008), and should be sufficient to preserve seed quality until it reaches longer-term storage. The implications of incorrect seed storage are loss of seed viability and longevity, which in turn leads to a reduction in the availability of seed both for future growth and regeneration and increased challenge in the long term conservation of accessions (Rao *et al*., 2006).

For Members, cultivation practices focus on organic gardening options; since they are Members of an organic gardening charity, it is perhaps not surprising that all options were employed by at least 60% of respondents.

5.4.3 Performance and variation – crop and variety, specifics

Seed Guardians and Members were asked whether they had seen any unexpected variation in varieties. This question could provide information about varietal uniformity and genetic variation in characters as well as environmental effects. For example, in peas the anthocyanin colouration in purple-podded varieties can be unstable depending on the alleles present, with colour ranging from entirely purple to entirely green on the same plant (Niall Green, Personal communication). 17 varieties were reported by Seed Guardian and six by Members, of which the french bean Bird's Egg was the only one to appear in both lists. The variety reported the most was broad bean Crimson Flowered, which was noted as displaying white flowers by four Members. Seed Guardians currently fill in a report for HSL regarding variety performance; no official mechanism for this exists for Members.

Members were additionally asked whether there were any varieties they thought were duplicates. Five pairs were returned, none of which were repeated between respondents. Ragged Jack and Red Russian were included in the AFLP analysis of the current study (see chapter 3), and were found to be potential duplicate accessions of *Brassica napus* var. *pabularia*. Members were also asked to mention any particular disease/pest resistances noted, of which 12 varieties were named, with resistances including to slugs, blight and adverse weather. Finally, Members were also asked whether they had noted any regional variations in variety performance. Due to time and space limitations this question was presented as a free-text space. Six varieties were reported. Most answers were fairly tentative; three people

mentioned Lancashire Lad, which was mentioned as an example in the question, which highlights that if this question had been posed more specifically as part of a larger survey about varietal differences between areas, more information may have been forthcoming. Due to concerns about data protection, respondents were not asked to specifically identify their locality, which perhaps hampered the question more than anticipated.

5.4.4 Seed selection and saving

Seed Guardians were asked about how they selected seed to send back to HSL. This is vital to know as this directly affects the genetic integrity of the collection. Responses also included selection of plants and seeds, with the general activities of roguing (removal of plants or pods that are markedly atypical (Garden Organic, 2008)) and removal of unhealthy or diseased plants being in keeping with the Seed Saving Guidelines. The guidelines state that seeds (particularly peas and beans) should be constant between generations in size, shape, colour and markings and this was reflected in many responses. A small number of responses implied selection that may be slightly beyond that included in the guidelines, such as the selection of the tallest plants and largest seeds, which could be an extension of the health or vigour of plants, but could have longer-term consequences if maintained over repeated cycles of selection.

The final destination of seed was asked of Seed Guardians and Members. For Seed Guardians 87% of respondents said that they kept spare seed for their own retention, and 59% said that they shared seed with others if they had any left over. The importance of seed swaps – both locally organised ones and with friends and colleagues – was also evident from the number of people who added this in comments. Comparable figures were available from Members; 83% of respondents saved their own seed and 63% share seed with other growers.

5.4.5 Additional services

Seed Guardians were asked whether there were any additional services that they would like HSL to provide. The most popular item was feedback on seed returned, followed by a regular newsletter. The options given highlight the importance of interaction between the volunteers and the organisation, both for training and support (Hawkes *et al*., 2000).

5.4.6 General discussion

The implications of the questionnaire studies for HSL comprise two main aspects relating firstly to Seed Guardians specifically, and secondly to members in general.

Firstly, it was found that the majority of Seed Guardians adhere closely to the Seed Saving Guidelines; therefore these should continue to be publicised. If SSGs are adhered to, varietal purity is being maintained through isolation and rouging, and seeds are appropriately stored, then this method of bulking is an appropriate method of maintaining broad accession characters for use by HSL, to provide seed for members. The study also provides HSL with data regarding preferences for potential ways of increasing contact with SGs, such as in the form of a dedicated newsletter or organised days.

Secondly, with reference to members, the implication for HSL is the highlighted importance of heritage varieties to gardeners, with key motivations for involvement being the intrinsic value of heritage varieties and the sense of public good associated with conserving and growing these varieties.

More widely, these results relate back to the importance and role of individuals, both through growing and conserving heritage varieties so that they can be conserved for the future, and in involvement in seed saving schemes. These are closely linked, as in the case of HSL this is

where people obtain the seed to grow, as well as saving their own. They are also linked to the importance of home gardens as refuges for varieties that are at risk of extinction (Galluzzi *et al*., 2010). The reasons given by people for getting involved are in accordance with those reasons outlined by Stickland (2008). This information is of use to Garden Organic, so that they can encourage people to become involved both by appealing to them through common values and to show them practically how their actions help. The importance of seed saving schemes as part of *ex situ* conservation is highlighted by Hawkes *et al*. (2000), and again reiterates that home gardens are a source of diversity and refuge (Hammer and Diederichsen, 2009; Galluzzi *et al*., 2010).

The results are also vital in the context of the genetic integrity and viability of the HSL collection. From the viewpoint of varietal purity, the importance of this is expressed by HSL to Seed Guardians as an absolute priority, and the comments received seem to indicate that for the majority, roguing and seed selection criteria (as well as temporary seed storage) are in line with recommendations from HSL (and in turn wider references for long-term gene bank maintenance such as Engels and Visser, 2003; Rao *et al*., 2006; Dulloo *et al*., 2008).

The main limitations of the study relate to sample size and question composition. Both of these elements were conducted to the best that circumstances could allow. The questionnaires were advertised to all Members and sent to all Seed Guardians; more specific targeting would have increased time and expenditure, outweighing the number of responses gained. In reference to questionnaire design, again as part of a larger study more pre-testing would have allowed fewer open-ended questions (as the current study demonstrated these are more complex to interpret and can be misunderstood by the respondents). However this was not possible. Also, the questionnaire attempted to cover a very broad range of subjects, from

gardening practices to interrogation of varietal performance and seed swapping, each of which could be a study in its own right, which meant that less detail was available for each individual subject. Again this was unavoidable, as a larger survey would have placed a greater demand on respondents' time and probably their willingness to become involved; therefore this was traded for the responses could be collected.

The disappearance of some heritage varieties from seed catalogues in the UK means that access to seed may be reduced (Negri *et al*., 2009), (although varieties are available on request from *ex situ* collections, namely SASA and WHRI, for genetic resource purposes). The work of HSL to conserve these varieties and make them available is supported by Seed Guardians and Members and in order for it to continue and expand in the future, and for these varieties to continue to be conserved and made available, individual and community involvement including growing and seed saving are vital. The results of these surveys may enable HSL to further explore ways of encouraging people to become and stay involved, such as feedback for Seed Guardians, the encouragement of local networks whether for Seed Guardians or for seed swapping, and have highlighted the importance of the Seed Saving Guidelines and adherence to them by Seed Guardians, in order for the continuance of these varieties in the HSL, the diversity of which has been confirmed in the previous chapters, to be maintained and utilised in the future.

CHAPTER 6 GENERAL DISCUSSION

6.1 Comparison of morphological and genetic characterisation

The main questions addressed in each chapter (diversity, groupings, potential duplicates and the comparison to standards) are revisited below with reference to both datasets.

6.1.1 General comments regarding diversity

The morphological and genetic characterisations gave the same overall patterns in diversity for the crops studied by both methods (*Vicia faba*, *Daucus carota*, *Pisum sativum* and *Cucumis sativus*). Diversity was generally higher in out-breeding crops (*Vicia faba*, *Daucus carota* and *Cucumis sativus*) compared to inbreeding crops (*Pisum sativum*) (*Lactuca sativa* morphological results could not be utilised for this purpose due to the immaturity of the morphological specimens).

6.1.2 Accession clusters

In the morphological analysis, no large clusters were presented for *Vicia faba*; the main identifiable cluster was based on accessions with small seeds (Cretian, Chak'rusga, Beryl, Martock and Sweet Lorraine). In the AFLP analysis, only Cretian and Chak'rusga clustered together.

In the *Pisum sativum* morphological analysis, clusters were observed based predominantly on pod colour, with finer detail added by flower colour and seed coat patterning. In the AFLP analyses, two groups of purple-podded accessions were observed, but the two sets were not close to one another. The accessions in one cluster were Sutton's Purple Podded, Purple Mangetout, Commander and Mr Bethell's Purple Podded. The other cluster was composed of

Purple Podded, Victorian Purple Podded and Stephen's. In the AFLP analysis most green-podded, purple flowered accessions still clustered together (Holland Capucijner, Mr Bound's Bean Pea, Irish Prean, Cooper's Bean Pea and Prean). No clustering of accessions with anthocyanin in the seed coat was seen. Accessions with brown marbling did not cluster closely together, however they were in the same region of the PCoA (Latvian large Grey, Raisin Capucijner, Latvian Carlin and Large Grey).

The clustering of *Lactuca sativa* accessions by type was seen broadly in both data sets, although morphological data was insufficient to draw firm conclusions. Although the basis of larger clusters in the dendrogram does not tally completely with lettuce type; all highly supported nodes were within types, except for Rouge D'Hiver and Bronze Arrow which are cos and leafy respectively. Two of the three crisphead accessions clustered together. The lettuce type butterhead are all located within one cluster, except Liller (which is in a predominantly cos cluster), and for the presence of Standard 2 (Corsair), which is a cos lettuce.

The sub-division of *Daucus carota* accessions into clusters based on root colour was seen clearly in both morphological and genetic analyses. This suggests a convincing separation in the gene pool.

For *Cucumis sativus*, in the AFLPs, King of the Ridge and Standard 2 clustered. These accessions are morphologically very different in shape, size and colour. 741 Peking China was the most different genetically, however in morphology all accessions were very distinct. AFLP clustered Jordanian and Izjastnoi, which were morphologically very different in both size and skin texture. Sigmadew and Standard 1 also clustered in the AFLP analysis, however can be distinguished by skin colour.

6.1.3 Comparing HSL accessions and commercial standards

Looking at the general differences in diversity between standards and HSL accessions, in *Vicia faba*, diversity levels were high in both characterisations, with standards being around average in diversity, but still high overall. This is consistent with other studies, as already noted.

Daucus carota standards presented slightly shorter branches between two or more accession replicates, suggesting that they were more homogeneous than HSL accessions; this was also seen in the AFLP study.

Standard 1 in *Pisum sativum* was less diverse than many HSL accessions in morphology. Standard 2 was average in both. This is the opposite of the AFLP results for Standard 1, where Standard 1 being lower is not reflected in the genetic results, with this standard being above average in genetic diversity.

In *Cucumis sativus*, Standard 1 presented shorter branches, comparable to those of 741 Peking China and Butcher's Disease Resisting (in quantitative variable scatter plots, two out of three plots clustered closely together). In the AFLP analysis there was a large disparity between the levels of genetic diversity in the standards; Standard 1 was of comparable genetic diversity to HSL accessions. The low level of genetic diversity seen in 741 Peking China is also seen in the genetic diversity results, but Butcher's Disease Resisting was high in diversity. Standard 2 had the lowest diversity of all accessions/varieties sampled, however morphological results were not available for comparison due to missing data.

In *Lactuca sativa*, commercial standards were below the average were towards the lower end of the range of genetic diversity for all accessions analysed, morphological data was not of sufficient reliability for comparison.

It may also be noted that, due to time and space restrictions, the number and choice of standards may not be fully representative of the full spectrum of diversity present in commercial crop varieties; therefore the conclusions drawn are limited to representing only the varieties used here.

6.1.4 Potential duplicate HSL accessions

In the morphological study, no *Vicia faba* accessions were proposed as potential duplicates. Close relations were tested between accessions Canadian purple and Estonian, but there were found to be statistically significant differences in quantitative characters between the accessions; their distance from one another in the AFLP study supports this. Jack Gedes and Mr Townend's were also tested as potential duplicates in the morphological characterisation, and although they were in the same cluster in the AFLP PCoA analysis, they were not proximal. This is also true for Gloucester Champion and Stafford, which are near in both analyses but not fully overlapping. This supports the theory of there being a broad spectrum of overlapping genetic diversity between *Vicia faba* accessions. In the AFLP analysis possible duplicates identified were Red Bristow and Seville, however these were morphologically distinct.

In the *Daucus carota* AFLP analysis no potential duplicates were identified. The accessions identified as similar in the morphology analysis were Afghan Purple and John's Purple. These accessions did cluster together in the AFLP analysis, however branch lengths in the UPGMA were long, and the individual PCoA suggests a similar situation to that of *Vicia faba*, that the

diversity is adjacent, and maybe slightly overlapping, but they may still be separate accessions. The other accessions that were similar but not duplicates in the morphology analysis were Egmont Gold and Giant Improved Flak, and Red Elephant and Altringham. The first pair did not cluster in the accession analysis; again individual points were proximal, but not overlapping, suggesting similarity but not duplication. The latter pair did cluster together and away from all other orange rooted accessions, however again, diversity was very broad and not overlapping.

Lactuca sativa duplicates cannot be determined due to the lack of morphological data. Potential duplicates identified by the AFLP analysis were Brown Bath Cos and Brown Goldring; Bunyard's Matchless and George Richardson; and Loos Tennis Ball and Mescher. All that can be confirmed is that Brown Bath Cos and Brown Goldring are both cos lettuces; Bunyard's Matchless and George Richardson are also both cos types and clustered closely morphologically as well, so would be worth investigation by an expert. Loos Tennis Ball and Mescher are both butterhead lettuce, but fairly long branch lengths in all cluster analysis suggest there is morphological dissimilarity between them, although again data is poor.

No duplicates were observed in *Cucumis sativa* for either characterisation, and diversity was found to be high in both.

In the *Pisum sativum* AFLP analysis potential duplicates were: Alex and Stokesley; Carruther's Purple Podded and Dwarf Defiance/John Lee; Victorian Purple Podded, Lancashire Lad and Stephen's; Champion of England and Veitch's Western Express; Harold Idle and Panther's; Prince of Prussia, Jeyes and Ostgotaart; Prean, Cooper's Bean Pea and Mr Bethell's Bean Pea; and Commander and Purple Mangetout. None of these close duplicates in the AFLP analysis were in the short-list of duplicates for morphology, however Lancashire

Lad and Stephens were in the long list. Although Cooper's Bean Pea, Irish Prean, Mr Bound's Bean Pea and Prean did not appear in both lists, they do form a cluster morphologically and genetically, suggesting a close relationship in both data types.

The diversity in genetic and morphological characters is of a different source. AFLPs measure neutral variation, whereas the genes in morphological characters measured are under selection, due to pressure from the breeding of desirable characteristics into a variety; growers of garden and ex commercial varieties maintain this pressure even after they are removed from official sale, in order to maintain their characters (Parlevliet, 2007). In traditional varieties, selection pressure is only high for those desirable characters, therefore genetic diversity may remain in other characters (Zeven, 2002). Diversity levels may be different using molecular markers and morphology. The current study showed that patterns of diversity may be different between the two measurement types (also discussed in Karhu *et al*., 1996). However, relative levels between crops of different breeding systems are visible. *Pisum sativum* is inbreeding and accessions were highly similar in both characterisation types. In *Daucus carota* and *Vicia faba*, which are largely outbreeding, variation was higher.

6.2 Implications for HSL and conservation

The implications of the present study for HSL have been considered separately for each chapter; however, from a synthesis of the four chapters several wider implications can be drawn. As stated in chapters three and four, this information can be used to manage the collection, conserving accessions representing diversity and distinctness, the highlighting of potential duplicates for further investigation by HSL, and information for database on morphological characters for 572 accessions.

The present study suggests that the HSL collection holds a broad spectrum of morphological and genetic diversity, with levels of genetic diversity comparable to that found in previous studies (see chapter discussions for details). Many accessions have been collected and maintained by HSL since its beginning in 1975, including ex-commercial varieties and heirloom varieties which have never been commercially available (Stickland, 2008), it is likely that some of the diversity held may be unique, particularly the heirloom varieties that are unlikely to be held in other genetic resource collections.

In the wider context the current study of this collection of heritage varieties contributes to a better understanding of the importance of varieties developed by both small-scale breeders, including local firms (more prominent in the past and responsible for the breeding of many of these heritage varieties) and individual heirloom growers, and thus the importance of maintenance of varieties, either *in situ*, with the person/company that developed it, or *ex situ* (Kell *et al*., 2009). The study also highlights the potential importance of those varieties removed from the National List (if the cost of staying on the list is not outweighed by revenue from seed sale) or seed catalogues (either due to competition from other varieties, including the emergence of improved varieties or due to small circulation), and their conservation to maintain a broad range of diversity for present and future use.

The diversity found within the collection is greatly valued by its users; particularly the collective ‘heritage value’ of accessions and their conservation; a sense of contribution towards things termed of greater importance (a ‘common good’); the conservation of a broad range of genetic diversity and biodiversity for sustainable future use; and for their traits, which are often perceived as superior to newer varieties (in terms of taste, some evaluative characters in some cases, in terms of uniqueness/difference/unusualness, designed with

gardeners in mind and seed saved varieties are perceived by growers as being adapted to local environment (as yet untested), and sometimes are varieties people have grown which have been removed from seed catalogues.

6.2.1 Wider implications

There are two further implications of this study; first is the importance of heritage varieties in the context of genetic erosion, and second is the importance of *ex situ* conservation resources.

The wider picture of genetic erosion encompasses the potential replacement of diverse landraces by a small number of elite cultivars. Chapter 2 argued that heritage varieties are, in the broad definition, landraces. Due to the diverse histories of heritage varieties, some may be genetically diverse where others are not. The current study investigated the genetic diversity of heritage varieties in the collection held by HSL, and found a broad range of genetic diversity held between accessions in all crops, and with some crops showing generally high levels of diversity within accessions. In identifying which taxa (in this case accessions) to conserve, it is optimal to conserve as broad a range as possible, with outliers being of particular importance, with representative samples from highly diverse accessions, genetically distant and distinct accessions to allow choice and option value for the future challenges and continuing use (Negri and Tiranti, 2010).

It is important to monitor this diversity in light of challenges faced by HSL and *ex situ* collections in general, in terms of genetic drift, sample size and regeneration risks, however at present there is diversity within the collection, and a broad range of accessions to sample from and maintain. This serves to highlight the importance of the HSL collection and the risks associated with genetic erosion if these crops are replaced in the market by more homogeneous versions (as well as the social aspects discussed previously). In Chapter 5,

respondents stated that they are involved with GO as Members or as Seed Guardians because they understand the importance of genetic diversity and wish to help conserve it; these results potentially enable Members to confirm the importance of their roles. The present study found that people grow heritage varieties for their heritage value and to conserve them for the future. The option value of heritage varieties is important both to growers/gardeners and potentially to breeders.

This research also serves to highlight the importance of small seed-saving organisations in conservation and scientific research (as suggested by Gepts, 2006) and the importance of charities and grassroots organisations in affecting biodiversity and conservation in the 'informal' seed sector (Galluzzi *et al*. 2010) as well as home gardens as potential reservoirs of agrobiodiversity, including landraces, relics and heirlooms (Galluzzi *et al*., 2010). The role of *ex situ* collections is important as a complementary measure to *in situ*, particularly in varieties that are already no longer in agricultural use (Esquinas-Alcazar, 2005). As stated by Negri and Tiranti (2010) since it is not known which alleles will be needed in the future, it is preferable to conserve as much diversity as possible and to account for qualitative and regional losses of alleles (Le Clerc *et al*., 2006; van de Wouw *et al*. (2010).

6.3 Further work

Characterisation is an important first step in the utilisation of PGR, as well as establishing a baseline against which future change can be measured (Hawkes *et al*., 2000). This project, now having characterised a portion of the collection and identified accessions of interest, work can be done both to further investigate duplicates (perhaps through the use of experts or background information where available, such as from HSL) and to evaluate varieties for traits of interest (van Treuren and van Hintum, 2010). As mentioned above, the next step

towards the utilisation of plant genetic resources is the evaluation of germplasm, such as for disease resistance. Future exploration of the collection could include measurement of evaluative features. This would be of particular interest both to growers and breeders and would be of interest in light of climate change and the breeding of future varieties for resistance to pests and diseases.

Further work may also be valuable in examining the diversity and importance of variety/accession names, and whether they can be used as possible highlighters or identifiers, whether of history, diversity or duplication (Appa Rao *et al*., 2002; Reedy *et al*., 2009). This emerged from the current study, for example in the grouping of *Pisum sativum* accessions Mr Cooper's Bean Pea, Mr Bound's Bean Pea, Irish Preans and Prean clustering consistently together.

If further background information were available on HSL accessions, the identification of accessions as 'landraces' *sensu stricto* (Berg, 2009), 'heritage varieties' (Preston *et al*., 2012 (Chapter 2 in this thesis)) or 'heirlooms' (Preston *et al*., 2012 (Chapter 2 in this thesis)) and a comparison of genetic diversity would be very informative, to investigate whether these show different levels of genetic diversity, are any of them related (for example are more recent varieties selected from/diverged from landraces), and whether material of unexpectedly low genetic diversity be explained(such as from small initial sample sizes or bottleneck events (e.g. disease/environmental effects causing temporarily more inbreeding).

In many *ex situ* collections regeneration is kept to a minimum, in the HSL this is not possible due to the need to supply seed to members. Investigation into how this regeneration affects both genetic diversity levels and genetic relationships between samples from different generations could be informative.

6.4 Conclusions and the future of heritage varieties

The scientific importance of the HSL is shown by the presence of accessions of high genetic diversity and distinctness, and of accessions of genetic distance from the commercial standards measured. In order to fully explore the value of the HSL and heritage varieties in a UK and even global context, further work that examines the HSL collection alongside material from *in situ* and other *ex situ* collections would be invaluable (particularly landrace material, *sensu stricto*). This could be both in terms of distinctness, how similar synonymous accessions are (particularly after regeneration over time) and in terms of genetic diversity.

Additional importance of the collection can also be highlighted through its accessibility to members (other heritage varieties in other *ex situ* collections are not identified as heritage), and although available on request, the presentation of identifiable heritage varieties in a catalogue makes them accessible. For the future, access to a greater number of accessions in the collection for growers is desirable, to which this project has contributed through characterisation.

Genetic diversity and distinctness present within the HSL accessions above may contribute to the already extant practices of grower-based breeding using traditional varieties, both through seed saving, selection over time, and accidental or deliberate cross-pollination (as discussed in Kell *et al*., 2009). Landraces have been used in the past as sources of genetic diversity, as well as for traits such as disease resistance (Esquinas-Alcazar, 2005). With characterisation and evaluation data, the conservation and use of HSL accessions and other landraces would be of great benefit both to gardeners and breeders looking for diversity and adaptation to climate change and increasing food supply for the growing human population in the future.

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APPENDIX 1 LIST OF ACCESSIONS FOR EACH CROP

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Vicia faba

Accession name	HSL batch no.	Plot	Characterisation
Bacardi	HSL07	19, 31,	Yes
Beryl	HSL04	3, 42, 64	Plot 64 3 germinated
Bonny lad	BB9	29, 71,	Plot 73 none germinated
Bossingham long pod	HSL05	38, 44,	Yes
Bowland's beauty	2716J	17, 21,	Plot 17 all plants died
Brown	HSL05	34, 43,	Plot 43 1 plant died
Canners 45	HSL06	14, 39,	Plot 39 1 plant died
Chak'rusga	HSL01	5, 68, 92	Yes
Cretian	HSL02	40, 80,	Plot 40 3 plants died
Crimson Flowered	2300H	62, 78,	Yes
Estonian	2113H	9, 33, 50	Yes
Gloucester champion	HSL04	46, 56,	Yes
Jack Gedes	HSL06	15, 20,	Plot 22 1 plant died
Jonah's	HSL03	11, 24,	Plot 24 1 plant died
Londonderry	2002	6, 58, 88	6 1 plant germinated Plot 58 4
Martock	3240J/PRE2002	16, 41,	Plot 67 1 germinated
Mr Jones	HSL05	27, 35,	Yes
Mr Lenthall's	3177J	4, 18, 45	Yes
Mr Townend's	HSL05	51, 53,	Yes
Painswick wonder	3341K	28, 30,	Yes
Perovka	56D/HSL06	65, 70,	Yes
Red Bristow's	HSL04	36, 37,	Yes
Rent payer	HSL05	12, 76,	Yes
Seville	698	8, 10, 23	Yes
Somerset	KB2006	49, 52,	Yes
Stafford	5599	2, 26, 85	Plot 26 1 plant died
Bunyard's exhibition (standard)		1, 89, 93	Yes
The Sutton (standard)		7, 48, 54	Yes
Sweet Lorraine	17246/HSL04	25, 61,	Yes
The shippam	HSL05	32, 60,	Plot 32 all plants died
White continental	HSL98	13, 47,	Yes
Canadian purple	HSL07	94, 95,	Plot 95 1 plant died Plot 96 1 plant
Gloucester bounty	HSL04	97, 98,	Yes

Daucus carota

Accession name	Characterisation	HSL seed batch	Plot number
Afghan purple	Yes	504F	14,23,35
Altringham	Yes	HSL05	11,18,21
Beta III	Yes	3312K	9,16,26
Egmont gold	Yes	2198H	1,33,36
Giant improved flak	Yes	HSL05	13,15,19
John's purple	Yes	HSL04	4,5,17
Manchester table	Yes	HSL06	3,24,25
Red elephant	Yes	1336G	6,22,30
Scarlet horn	Yes	HSL06	20,27,28
Standard 1	Yes	-	10,31,32
Standard 2	Yes	-	12,29,34
White belgium	Yes	HSL05	2,7,8

Cucumis sativus

Accession name	Characterisation	HSL seed batch	Plot number
741 Peking China	Yes	HSL2002	23,35,38
Boothby's blond	Yes	PS07	16,19,20
Butcher's disease resisting	Yes	STOLKHSL01	5,6,39
Dekah	Yes	HSL06	13,26,30
Izjastsnõi	Yes	2517H	17,28,37
Jordanian	Yes	3609K	2,12,18
King of the ridge	Yes	3169J	9,13,36
Kiwano African horned	No fruit produced	J	7,15,29
Sigmadew	Yes	3184J	10,25,33
Standard 1 (name)	Yes	-	1,32,34
Standard 2 (name)	Yes	-	3,8,14
Striped and sweet	Seed did not germinate	STOCK866F	11,21,22
West India burr gherkin	No fruit produced	2005	4,24,27

Allium porrum

Accession name	Characterisation	HSL seed batch	Plot number
Coloma	Yes	HSL 04	1,15,20
Colossal	Yes	GS 2004	11,13,17
Early martket	Yes	1671g	3,12,21
Hannibal	Yes	HSL 06	2,16,23
Kelvedon king	Yes	3608K	8,18,25
Sim seger	Yes	3701H	4,9,24
Standard 1	Yes	-	5,7,27
Standard 2	Yes	-	10,19,22
Walton mammoth	Yes	3353J	6,14,26

Lactuca sativa

Accession name	Characterisation	HSL seed batch	Plot number
Asparagus	Yes	865F	3,5
Bath cos	Yes	1204g 2004	10,42
Black seeded samara	Yes	HSL05	31,32
Bronze arrow	Yes	472F	15,30
Brown bath cos	Yes	3607K	12,39
Brown Goldring	Yes	LV07	6,14
Bunyard's matchless	Yes	2284H	28,41
Burpees Iceberg	Part	1870g	19,38
George Richardson	Yes	HSL05	4,17
Lacitue cracoviensis	Yes	HSL04	22,35
Liller	Yes	HSL06	11,27
Loos tennis ball	Yes	2577h	18,44
Maroulli cos	Yes	HSL05	2,20
Mescher	Yes	3508k	26,29
Northern Queen	Yes	2333H	25,34
Rouge d'hiver	Yes	HSL04	8,37
Soulie	Yes	3363k	21,36
Standard 1-Iceberg	Part	-	9,43
Standard 2 – Corsair	Yes	-	13,23
Stoke	Yes	2583H	1,33
White samara	Yes	HSL03	24,40
Windermere	Yes	HSL03	7,16

Pisum sativum

Accession	Characterisation	HSL seed batch	planting 1	planting 2
Alex		AP04S	42	21
Bijou		3934L	56	63
Carlin		2586H	8	17
Carruther's purple podded		3403K	66	36
Champion of england		3478K	57	25
Clarke's beltony blue		2758J	30	29
Commander		3886L	50	3
Cooper's bean pea		HSL07	45	60
Doug Bray of Grimsby		HSL07	54	2
Duke of Albany		3516K	63	33
Dun		2840J	7	61
Dwarf defiance/John Lee		2204H	12	53
Early capucijner		HSL07	23	66
Eat all		3636K	52	16
Epicure		3217J	74	27
Espoir de gemboux		HSL07	15	24
Forty first		2625J	13	59
Frueher heinrich		2642J	58	37
Giant stride		2686J	51	15
Glastone		3401K	21	56
Glory of devon		04S	61	73
Golden sweet (India)		3206J	17	74
Gravedigger		3848L	39	52
Harold Idle		HSL08	16	76
Holland Capucijners		3376K	43	65
Hugh's huge		3116J	55	6
Irish preans		4078L	65	28
Jeyes		3732L	53	35
Kent Blue		3213J	60	57
Lancashire Lad		SHORT 488	27	64
Large grey		HSL08	1	20
Latvian		3296K	3	22
Latvian grey pea		HSL05	48	40
Latvian large grey		HSL04	49	43
Laxton's exquisite		4103	18	10
Magnum bonum		3546K	34	11
McPartlin		3404K	36	67

Moldova		HSL05	5	68
Mr bethall's purple podded		4004L	46	48
Mr Bound's bean pea		3038J	11	30
Mummy's		HSL08	22	18
Ne plus ultra		3570K	19	7
Pilot		3113J	28	75
Raisin cupucijners		2162H	68	69
Newick		3186J	71	5
Ostgotaart		3425K	40	42
Panthers		2573H	76	47
Parsley		3918J	2	50
Poppet		HSL08	75	26
Prean		HSL07	10	46
Prew's special		3142J	59	34
Prince of Prussia		2882J	72	62
Purple flowered russian		3612K	69	13
Purple mangetout		HSL05	77	1
Purple pod		4111L	47	31
Purple podded		3949L	38	41
Robinson		3020J	32	55
Salmon flowered		2585H	20	12
Simpson's special		HSL04	73	44
Standard 1		-	14	71
Standard 2		-	25	39
Stenu		3610K	64	4
Stephens		HSL05	33	77
Stokesley		2661J	44	8
Suttons acheivement		HSL07	24	58
Suttons harbinger		PA06	9	19
Suttons purple podded		HSL07	41	9
Table talk		3235J	29	14
Telephone		1991H	70	51
Time out of mind		3739L	35	45
Turner's spring		2930J	4	23
Tutankhamun		HSL07	37	38
Ultra U		HSL05	31	49
Veitch's western express		3277K	26	54
Victorian purple podded		3600K	62	72
Wieringen white		HSL07	67	32
Winfreda		04S	6	70

Raphanus sativus

Accession name	Characterisation	HSL seed batch	Plot number
Chinese	Yes	3124J	4,10
Crimson giant	Yes	3596K	3,13
French golden	Yes	HSL06	2,5
Hailstone	Yes	HSL97	9,12
Munchen bier	Yes	HSL08	19,23
Pasque	Yes	3158J	21,22
Rat's tail	Yes	HSL04	6,11
Round red forcing real	Yes	2671J	16,24
Standard 1: Saxa 1	Yes	-	8,20
Standard 2: Scarlet globe	Yes	-	7,17
Tientsin green	Yes	3615J	1,15
Wood's frame	Yes	3210J	14,18

Brassica napobrassica

Accession name	Characterisation	HSL seed batch	Plot number
Bjursas	Yes	562F	1,3
Gul Svensk	Yes	HSL06	5,6
Standard 1	Yes	-	4,7
Standard 2	Yes	-	2,8

Solanum lycopersicum

Indeterminate accessions

Year 1

Accession name	**Characterisation**	**HSL seed batch**	**T1**	**T2**	**T3**
American Market King		2045H	7	27	57
Amish Yellow	No fruit produced	3183J	61	85	11
Aranyalma		HSL05	64	101	77
Ararat Flamed		GS04	23	54	84
Arkansas Traveller		HSL06	82	78	3
Auntie Madge's		3342K	69	75	85
Belhomme's Fortuna Potato Leaf		HSL05	24	60	101
Berne rosen		1678G	67	93	21
Big White	No fruit produced	HSL07	13	69	16
Bijskij Zeltyi		2582H	11	86	5
Black Plum		2329H	59	89	36
Brandysweet Plum		HSL07	73	90	59
Broad Ripple Yellow Currant		1279G	84	34	46
Brook's special		1868G	93	72	70
Buffalo Horn		3380K	74	71	53
Burpee's Jubilee	No fruit produced	3222J	53	33	63
Carlton		3171J	56	49	35
Caro Rich		3498K	10	2	17
Carter's Fruit		2752J	15	73	32
Cavendish		2028H	79	65	55
Cherokee Purple		2453G	92	83	98
Clear Pink Early		HSL06	55	25	23
Darby Striped Orange/Green		2580H	4	38	68

Darby Striped Pink/Yellow		3644K	36	15	33
Dark Purple Beefsteak		HSL05	17	97	73
Early Outdoor/Sandpoint		1337G	80	45	39
Essex Wonder		3650K	77	87	78
Estonian Yellow Mini Cherry		HSL05	42	3	89
Fablonelistnyj		2599H	101	66	95
Fox Cherry		2041H	37	20	7
Giant Italian Plum		HSL07	19	80	44
Giant Tree Tomato	No fruit produced	3580K	21	6	58
Golden Yellow Queen		2138H	41	23	82
Greek	No fruit produced	HSL05	54	58	41
Green Bell Pepper		3557K	95	52	25
Green Zebra		3544K	71	99	51
Hillbilly		2194H	26	74	62
Hugh's	No fruit produced	2598H	30	46	75
Ida Gold		HSL06	52	61	65
Imur Prior Beta		HSL07	76	9	9
Iraqi Heart-Shaped		HSL04	27	56	52
Ivory Egg		THALIA07	70	59	76
Jersey Sunrise		1319G	9	67	34
Jugoslavian		2176H	86	16	96
Kathmandu		HSL04	35	82	10
Kenches Gold		HSL07	75	88	60
Kenilworth/King George		HSL05	99	30	31
Little Tatyana		HSL06	6	1	30
Longkeeping		HSL05	58	55	45
Madame Jardel's Black	No fruit produced	2401H	8	100	69
Mammoth German Gold	No fruit produced	2973J	18	91	14
Market King		2042H	87	81	64

Merveille des Marches		1903G	1	12	66
Mini Orange		HSL05	3	37	86
Mr Novak	No fruit produced	3535J	43	92	2
Mrs Lindsey		HSL05	89	47	90
Nectar Rose		HSL06	44	4	37
Noir		3182J	34	95	94
Novosadski jabucar		HSL07	28	48	79
Orange Banana		2481G	66	5	87
Orange Heart		3180J	14	41	26
Oregon Spring		HSL07	49	36	71
Peremoga		1324G	88	96	12
Pigeon Egg		HSL07	94	63	8
Pink Cherry		HSL07	29	43	48
Plum Lemon		HSL07	100	42	50
Potato Leaf White	No fruit produced	2924J	33	11	67
Prudens Purple	No fruit produced	HSL06	57	51	15
Queen of Hearts		HSL05	16	35	20
Red Peach		1948G	91	17	22
Red Star		HSL06	50	40	43
Riesentraube		1466G	20	39	47
Rose		HSL06	97	22	99
Russian Red		HSL07	81	14	97
Ryder's Midday Sun	No fruit produced	3190J	32	44	74
Sandul Moldovan	No fruit produced	HSL07	38	77	72
Scotland Yellow		2006H	45	26	91
Siberian Early		HSL07	40	21	28
Silvery Fir Tree		HSL07	2	8	4
Snow White Cherry		1882G	31	68	1
Spanish Big Globe		HSL07	5	32	49

Standard 1			48	31	54
Standard 2			83	7	24
Stoner's most prolific		HSL05	98	53	42
Sugar Plum		HSL05	96	28	27
Sundrop		HSL07	47	64	93
Tangella		2240H	65	13	18
Tiger Tom		2562H	46	70	40
Tomate pomme rouge du montpellier		P507	90	62	81
Tommy Toe		1886G	78	94	38
Victory		1958G	22	24	88
Vince		HSL07	60	76	83
Wapsipinicon Peach		HSL07	68	57	80
Watermelon Beefsteak		HSL07	12	98	92
Welsh Farmer Law's		1875G	51	84	13
White Queen	No fruit produced	HSL07	62	18	56
Wild		HSL07	85	50	29
Wild Cherrry		HSL07	39	79	6
Yellow Oxheart	No fruit produced	2123H	25	10	100
Yellow Plum Formed		3605K	72	29	19
Yellow Russian		HSL04	63	19	61

Year 2

Accession name	Characterisation	HSL seed batch	T4 plot number	T5 plot number
Abraham Lincoln		2732J	23	64
Atkin's stuffing		3317K	1	18
Berne rosen		1678G	58	80
Best of all		HSL08	47	24
Big rainbow		3139J	10	9
Black		HSL04	27	45
Black Russian/Gypsy		1868G	64	30
Bonny best		2897J	61	47
Brandywine		HSL05	42	13
Brook's special		1868G	71	61
Burbank bush		3840L	12	8
Burriana		HSL05	81	48
Cavendish		2028H	63	70
Cheesham's potato leaf		1628G	25	78
Cyril's choice		3769L	16	54
Czar		HSL08	36	42
Darby striped red/green		HSL08	74	74
Darby striped red/yellow		2581H	55	21
Darby striped yellow/green		3201J	62	7
Den weese streaked		HSL08	14	56
Earl of edgecombe		1996	65	75
Enorma/Potentate		4035L	5	19
Euromoney		3238J	13	22
Fakel		2533H	44	23
Gaia de firenza		HSL06	26	1
Garden peach		1991	21	67
Giant belgian		HSL04	4	29
Golden grape		3127J	80	25
Green sausage		HSL06	17	10
Grosse lisse		3181J	19	38
Homosa		3835Z	33	43
Joe Atkinson		2903J	11	2
Jubilee		HSL01	72	6
Jugo		HSL05	40	77
Konig Humbert		HSL08	49	11
Lampadina St Marzano		4065L	51	17
Large red		HSL06	69	37
Lilac giant		HSL06	28	51

Lumpy red		HSL06	45	32
Maghrebi		2097H	7	68
Maltese plum		2035H	67	76
Monserrat de bataille		HSL06	60	65
Mortgage lifter		2454G	38	49
Moskvich		HSL06	59	39
Mrs Taylor's red pear		3972L	35	15
Mrs Taylor's yellow pear		2078H	2	53
Mule team		HSL06	30	35
My girl		4038L	52	41
Ol' german pink		HSL06	70	55
Orange		HSL08	18	26
Peacevine cherry		GILBERT05	53	14
Plum fryer - short		2006	3	50
Plum fryer - tall		2006	48	5
Pop-in		2047H	6	33
Porter		2348H	46	34
Purple smudge		HSL06	22	57
Red peach		4104L	20	63
Scarlet knight		2067H	31	20
Schimmeig creg		HSL08	29	16
Seattle's best of all		HSL06	54	81
Small pear shaped		HSL08	8	4
Srednjevelika		3830J	43	12
Standard 1		-	56	58
Standard 2		-	79	72
Striped cavern		HSL08	57	79
Striped hollow		HSL06	37	73
Striped stuffer		HSL06	77	46
Sugar Italian plum		HSL08	50	40
Sunray gold		HSL08	41	36
Sutton		HSL06	34	66
Sutton's everyday		2029H	39	62
Thompson's seedless		HSL08	68	52
Transparent		3231J	15	59
Veepro paste		3809L	73	31
Verna orange		HSL07	32	28
White princess		HSL08	66	3
Wladek's		3931L	24	27
Yellow ball		2895J	9	44

Yellow currant		HSL06	78	71
Yellow drop		HSL08	76	60
Yellow pear		HSL06	75	69

Determinate accessions

Name	**Characterisation**	**HSL seed batch**	**T6 plot number**
Beefsteak		HSL06	22,34
Cosmonaut Volkov		HSL06	13,25
Currant		HSL08	7,30
Dwarf wax		2005H	6,23
Golden dixie		3107J	8,26
Lima korai		HSL04	27,32
Morden yellow		1843G	16,31
Nova		4041L	10,33
Salt Spring sunrise		3541K	2,11
Standard 1		-	9,18
Standard 2		-	17,20
Sub arctic plenty		3281K	19,28
Texas wild		HSL05	12,21
Tibet appel		3364K	1,15
Veeroma		HSL06	5,14
Whippersnapper		HSL06	4,29
Wild tomato 1 colombianum		HSL05	3,24

Capsicum annuum

Accession name	Characterisation	HSL seed batch	Plot number
Californian bell	Yes	HSL06	1,13,21
Long green Buddha	Yes	14856/1776G	18,20,28
Macedonian sweet	Yes	1406/HSL06	8,14,32
Nardello	Yes	3233J	6,16,29
Sheepnose	Yes	2005 seed	3,26,30
Skinny	Yes	3211J	10,12,19
Soror sarek	Yes	HSL04/2565J	22,24,31
Standard 1	Yes	-	4,11,23
Standard 2	Yes	-	17,25,27
Sweet banana	Yes	3537J	7,9,33
Trifetti	Yes	HSL05	2,5,15

Brassica rapa var. rapa

Accession name	HSL seed batch	Plot number	Characterised

Black Sugarsweet	HSL05	6,7,13	Yes
Gammel Svensk	3221J	5,9,15	Yes
Kaskinauris stock	HSL99	4,8,10	Yes
Standard 1	-	1,3,14	Yes
Standard 2	-	2,11,12	Yes

APPENDIX 2 LIST OF DESCRIPTORS USED FOR EACH CROP

Cucumis sativus

Descriptor	Source	Data capture	Character states											
Leaf intensity of green colour	IPGRI	At physiological maturity (from centre of plant)	3	light	5	medium	7	dark						
Leaf hairiness	HSL		a	no hairs	b	sparse hairs	c	intermediate	d	dense hairs				
Leaf length (cm)	IPGRI/HSL	At physiological maturity (from centre of plant)												
Leaf width (cm)	IPGRI/HSL													
Fruit length (cm)	IPGRI/HSL	At table use maturity												
Fruit width (cm)	IPGRI/HSL	Widest point at table use maturity												
Fruit weight (g)	HSL													
Internode length (cm)	HSL													
Fruit shape at stem end	HSL	At ready to eat stage	a	Depressed	b	Flattened	c	Rounded	d	Pointed				
Fruit shape at stem end	IPGRI	At table use maturity	1	Necked	2	Acute	3	Obtuse	99	other				
Spine colour	IPGRI/HSL	At table use maturity	0	No spines	1	Black	2	Brown	3	White				
Predominant skin colour	IPGRI/HSL	At table use maturity	1	White	2	Yellow	3	Green	4	Orange	99	Green to orange		
Predominant skin colour	IPGRI/HSL	At physiological maturity	1	White	2	Yellow	3	Green	4	Orange	5	Brown	99	Other
Flower colour	HSL		a	White	b	Yellow	c	Orange	d	Other				
Fruit shape	HSL		a	Elongated	b	Squat	c	Round	d	Stem-end tapered	e	Blossom-end tapered		
Fruit shape at blossom end	HSL		a	Depressed	b	Flattened	c	Rounded	d	Pointed	e			
Fruit mottling	HSL		0	Absent	1	Present								
Fruit striping	HSL		a	No stripes	b	Stripes over less than 1/3 fruit length	c	Stripes over about 1/2 length	d	Stripes over more than 2/3 of the length				
Fruit stripe colour	HSL		a	No stripes	b	White	c	Green	d	Yellow	e	Other		
Fruit spines	HSL		0	Absent	1	Present								
Skin dull/glossy	HSL		a	Dull	b	Glossy								
Skin texture	HSL		a	Smooth	b	Wrinkled	c	Netted	d	Covered in small warts	e			

Discarded descriptors	Source	Reason discarded
Plant growth habit	IPGRI/HSL	No variation - all indeterminate
Tendrils present/absent	HSL	No variation - present in all
Parthenocarpy	IPGRI	Not recorded
Reproductive system	IPGRI	Not recorded
Monoecious/gynoecious	IPGRI	Not recorded
Dry seed colour		Not recorded
Number of seeds		Time restraint
Seed length		Time restraint
Mature fruit width		Recorded but not used in analysis as market stage descriptors of more relevance
Mature fruit length		Recorded but not used in analysis as market stage descriptors of more relevance
Mature fruit weight		Recorded but not used in analysis as market stage descriptors of more relevance

Pisum sativum

Descriptor	Source	Timing	Character states															
Colour of standard petal	HSL		a	white	b	other												
Colour of wing petals	HSL		a	white	b	other												
What shape is young flat pod	HSL		a	curved	b	straight	c	mixed	d	other								
Fibrousness of young flat pod	HSL		a	not stringy	b	intermediate	c	stringy										
Pod colour	HSL	When full but not dry	a	green	b	purple	c	yellow	d	other								
Pod width (cm)	HSL	When full but not dry, measure 5 pods																
Pod length (cm)	HSL	When full but not dry, measure 5 pods																
Number of seeds per pod	HSL	Average of 5, when dry, hilum to base																
Is dry seed smooth/wrinkled	HSL	When dry	a	smooth and round	b	wrinkled												
Colour of seed coat	HSL	When dry	a	green	b	grey green	c	parchment/green	d	parchment	e	light brown	f	dark brown	g	black	h	other

Presence of brown marbling	UPOV/JIC	Seed coat pattern - when dry	p	presence	a	absence													
Presence of anthocyanin	UPOV/JIC	Seed coat pattern - when dry	p	presence	a	absence													
Number of tendrils	HSL	At maturity, average of 5 plants																	
Seed weight (g)	JP	100 seeds when dry																	
Seed length (mm)	JP	Measure 5 seeds, when dry																	
Seed size		When fresh																	
Discarded descriptors		Reason for discard																	
Pod wall fleshy/fibrous	HSL	Not quantifiable																	
Pod distribution		Time restraint																	
Plant height		Time restraint																	
Fresh seed coat colour		No variation																	
Hilum colour	UPOV/JIC	Data not collected for all experiment 1 plots plus no variation																	

Lactuca sativa

Descriptor	Source	Timing	Character states																
Lettuce type	HSL		a	Butterhead	b	Cos (Romaine)	c	Curled (lollo rosso)	d	Crisphead (iceberg)	e	Leafy (non-heading)	f	Mixed					
Leaf colour	HSL		a	Blue green	b	Dark green	c	Grey green	d	Green	e	Grey green	f	Pale green	g	Red	h	Yellow green	I	Other
Leaf shape	HSL		a		b		c		d		e		f						
Leaf texture	HSL		1	Limp	2		3		4		5		6	Crisp					
Leaf folding	HSL		a	Almost flat	b	Intermediate	c	Intermediate	d	Tightly folded									
Leaf margin dissection	HSL		a	Not dissected	b	Intermediate	c	Intermediate	d	Very dissected									
Leaf width	HSL																		
Leaf length	HSL																		
Bolting	HSL																		
Outer leaf colour	ECPG		1	Yellow green	2	Green	3	Grey green	4	Blue green	5	Red green							

	R																				
Outer leaf colour intensity	ECPGR		3	Light	5	Medium	7	Dark													
Descriptor discarded		Reason for discard																			
Head shape	ECPGR	Too many bolted																			
Heart formation	ECPGR	Too many bolted																			
Homogeneity	ECPGR	Not recorded																			

Brassica napobrassica

Descriptor	Source	Timing	Character states																					
Lateral root emergence	IPGRI	On bulb at harvest	0	Absent	3	Lower portion	5	Lower half	7	More than half														
Root length (cm)	IPGRI	Measure storage portion																						
Root width (cm)	IPGRI	At widest point																						
Root weight (g)	IPGRI	Without leaves																						
Exterior root colour	IPGRI	At harvest	1	White	2	Yellow	3	Green	4	Pink	5	Red	6	Purple	7	Bronze	8	Brown	9	Black	10	Other		
Interior root colour	IPGRI	At harvest	1	White	2	Yellow	3	Green	4	Pink	5	Red	6	Purple	7	Other								
Root exterior colour pattern	IPGRI	At harvest	0	Uniform	1	Bicolour	2	Multicolour	4	Mixed colours	5	Lateral root grooves of different colour to root	6	Other										
Root flesh colour distribution	IPGRI	In transverse section	1	Uniform	2	Colour in cortex and cambium	3	Colour radially distributed in stellate pattern	4	Concentric rings of colour	5	Irregular distribution	6	Other										
Root shape at base	IPGRI	At harvest, tip	1	Acute	3	Obtuse	5	Convex	7	Plane	9	Concave												

Root shape	IPGRI	In long section	1	Taproot	2	Triangular	3	Cylindric	4	Elliptic	5	Spheric	6	Transverse elliptic	7	Inverse triangle	8	Apically bulbous	9	Horn	10	Branched	11	Other
Descriptor discarded		Reason discarded																						
Lateral root-groove tissue scars	IPGRI	No variation																						
Leaf Length	IPGRI	Leaves all died																						
Leaf width	IPGRI	Leaves all died																						
Petiole length	IPGRI	Leaves all died																						
Petiole width	IPGRI	Leaves all died																						
Root shape of shoulder	IPGRI	No variation																						
Weight of harvest organ (including leaves)	IPGRI	Leaves all died																						

Solanum lycopersicum

Descriptor	Source	Timing	Character states																			
Concentric cracking	IPGRI	At maturity	1	Corky lines	3	Slight	5	Intermediate	7	Severe												
Exterior colour of mature fruit	IPGRI/HSL	At maturity	1	Green	2	Yellow	3	Orange	4	Pink	5	Red	6	Other								
Stripe colour	JP	At maturity	1	Green	2	Yellow	3	Orange	4	Pink	5	Red	6	Other								
Fruit shoulder shape	IPGRI/HSL	At maturity	1	Flat	3	Slightly depressed	5	Moderately depressed	7	Strongly depressed												
Fruit weight (g)	IPGRI/HSL	At maturity																				
Predominant fruit shape	IPGRI/HSL	At maturity	1	Flattened (oblate)	2	Slightly flattened	3	Rounded	4	High rounded	5	Heart-shaped	6	Cylindrical (long oblong)	7	Pyriform	8	Ellipsoid (plum-shaped)	9	Other		
Radial cracking	IPGRI	At maturity	1	Corky lines	3	Slight	5	Intermediate	7	Severe												

Ribbing at calyx end	IPGRI/HSL	At maturity	1	Very weak	3	Weak	5	Intermediate	7	Strong										
Exterior colour of immature fruit	IPGRI/HSL	Average 10 fruits from different plants before maturity	1	Greenish-white	3	Light green	5	Green	7	Dark green	9	Very dark green								
Corolla colour	IPGRI/HSL	Observe the 2nd and 3rd truss of at least 10 plants	1	White	2	Yellow	3	Orange	4	Other										
Style protruding/retracted to anther cone	IPGRI/HSL	on 2nd or 3rd truss																		
Flesh colour of pericarp	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage	1	Green	2	Yellow	3	Orange	4	Pink	5	Red	6	Other						
Fruit blossom end shape	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage	1	Indented	2	Flat	3	Pointed												
Fruit cross-sectional shape	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage	1	Round	2	Angular	3	Irregular												
Number of locules	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage																		
Presence of green shoulders on fruit	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage	0	Absent	1	Present														
Shape of pistil scar	IPGRI/HSL	on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage	1	Dot	2	Stellate	3	Linear	4	Irregular										
Skin colour of ripe fruit	IPGRI/HSL	Peeled at maturity	1	Colourless	2	Yellow														
Skin stripe colour	JP	Peeled at maturity	1	Green	2	Yellow	3	Orange	4	Pink	5	Red	6	Other						
Puffiness appearance	IPGRI	Presence of cavity at maturity	3	Slight	5	Intermediate	7	Severe												
Fruith width (cm)	IPGRI/HSL	Recorded at the largest diameter of cross-sectioned fruits to one decimal place at maturity																		
Width of pedicel scar	IPGRI	Recorded at the widest part on 10 randomly selected fruits from different plants	3	Narrow (covered by calyx)	5	Medium (slightly apparent around the calyx)	7	Wide (very apparent around the calyx)												
Presence of pedicel	JP		0	Absent	1	Present														

abscission layer																				
Pedicel length from abscission layer (cm)	IPGRI	Recorded from abscission layer to calyx. Average of 10 pedicels																		
Fruit length (cm)	IPGRI/HSL	Recorded from stem end to blossom end, to one decimal place, at maturity																		
Plant growth type	IPGRI/HSL	When fruits of 2nd and 3rd truss are ripened	1	Dwarf	2	Determinate	3	Semi-determinate	4	Indeterminate										
Discarded descriptors		Reason for discard																		
Fruit firmness (after storage)	IPGRI	No time or space																		
Number of flowers per inflorescence	IPGRI/HSL	Not consistently recorded																		
Fruit size	IPGRI/HSL	Not necessary due to actual measurements taken																		
Foliage density	IPGRI/HSL	Not reliable due to greenhouse overcrowding																		
Plant size	IPGRI	Not reliable due to greenhouse overcrowding																		
Fruit size homogenity	IPGRI/HSL	Not consistently recorded																		
Inflorescence type	Just IPGRI	Not consistently recorded																		
Leaf type	IPGRI/HSL	Photographs taken, no time to analyse																		
Number of days to maturity	IPGRI	Not consistently recorded																		

Capsicum annuum

Descriptor	Source	Timing		Characters		States																						
Fruit surface	IPGRI/HSL	Recorded on mature fruits																										
Cross	IPGRI	Recorded on	3	Slightly	5	Intermediate	7	Corrugate																				

section corrugation	/HSL	mature fruits (average of 10 fruits 1/3 from pedicel end)		corrugated				d																									
Number of flowers per axil	IPGRI /HSL	Recorded on fully open flowers in the first fresh flowering	1	One	2	Two	3	Three or more	4	Many flowers in bunches but each in individual axil (fasciculate growth)	5	Other (I.e. cultivars with two flowers in the first axil and with one only in the other)																					
Fruit length (cm)	IPGRI /HSL	Recorded on mature fruits (average of 10 fruits)																															
Fruit width (cm)	IPGRI /HSL	Recorded on mature fruits (average of 10 fruits)																															
Fruit weight (g)	IPGRI /HSL	Recorded on mature fruits (average of 10 fruits)																															
Plant growth habit	IPGRI	Observed when 50% of the plants bear ripe fruits	3	Prostrate	5	Intermediate (compact)	7	Erect	9	Other																							
Corolla colour	IPGRI	Recorded on fully open flowers in the first fresh flowering	1	White	2	Light yellow	3	Yellow	4	Yellow-green	5	Purple with white base	6	White with purple base	7	White with purple margin	8	Purple	9	Other													
Anther colour	IPGRI	Observed immediately after blooming before anthesis	1	White	2	Yellow	3	Pale blue	4	Blue	5	Purple	6	Other																			

Fruit colour at intermediate stage	IPGRI	Recorded on fruits just before the ripening stage	1	White	2	Yellow	3	Green	4	Orange	5	Purple	6	Deep purple	7	Other																	
Fruit colour at mature stage	IPGRI	Recorded on mature fruits	1	White	2	Lemon-yellow	3	Pale orange-yellow	4	Orange-yellow	5	Pale orange	6	Orange	7	Light red	8	Red	9	Dark red	10	Purple	11	Brown	12	Black	13	Other					
Fruit shape	IPGRI /HSL	Recorded on mature fruits (average of 10 fruits)	1	Elongate	2	Almost round	3	Triangular	4	Campanulate	5	Blocky	6	Other																			
Stem shape	IPGRI /HSL	Observed at plant maturity	1	Cylindrical	2	Angled	3	Flattened																									
Flower position	IPGRI /HSL	Recorded on fully open flowers in the first fresh flowering	3	Pendant	5	Intermediate	7	Erect																									
Shape at blossom end	IPGRI /HSL	Recorded on mature fruits (average of 10 fruits)	1	Pointed	2	Blunt	3	Sunken	4	Sunken and pointed	5	Other																					
Blossom end appendage	IPGRI /HSL	Recorded on mature fruits	0	Absent	1	Present																											
Descriptor discarded		Reason discarded																															
Life cycle	IPGRI /HSL	Not measureable over 1 year, little variation probably																															
Yield per planting	HSL	Not possible to collect all fruit due to time restraints																															
Days to fruiting	IPGRI	Not possible to measure																															

		reliably due to crop harvesting as and when																												
Male sterility	IPGRI	Not recorded																												
Plant height	IPGRI	Not reliable measure due to greenhouse overcrowding																												
Hypocotyl colour	IPGRI /HSL	Not variation																												
Days to flowering	IPGRI /HSL	Time restraint meant could not be reliably recorded																												
1000 seed weight	IPGRI /HSL	Time restraints																												
Number of seeds per fruit	IPGRI /HSL	Time restraints																												
Seed colour	IPGRI /HSL	No variation (all RHS code 18c)																												
Neck at base of fruit		No variation – all absent																												
Stem colour	IPGRI /HSL	Collected at maturity, should have been juvenile	1	Green	2	Green with purple stripes	3	Purple	4	Other																				

Allium porrum

Descriptor	Source	Timing		Character		States																	
Foliage attitude	IPGRI	At harvest	3	Prostrate	5	Intermediate	7	Erect															
Foliage cracking	IPGRI/ HSL	At harvest	3	Weak	5	Medium	7	Strong															
Leaf length (cm)	IPGRI/ HSL	Record the average length of longest leaf of 5 fully developed plants																					
Leaf width (cm)	IPGRI/ HSL	record the maximum width of the longest leaf																					
Shaft diameter (cm)	IPGRI/ HSL	Measured on mature harvested plants at the median point after the removal of dead and dying leaves																					
Shaft length (cm)	IPGRI/ HSL	Measured on 5 plants from base to first spltting leaf (cross)																					
Weight (g)	JP	At harvest																					
Foliage colour	IPGRI/ HSL	At harvest	1	Light green	2	Yellow green	3	Green	4	Grey-green	5	Dark green	6	Bluish green	7	Purplish-green	99	Other					
Shape of bulb	IPGRI	When fresh	1	Flat	2	Flat globe	3	Rhomboid	4	Broad oval	5	Globe	6	Broad eliptic	7	Ovate (elongated oval)	8	Spindle	9	High top	99	Other	
Descriptors discarded	IPGRI/ HSL	Reason for discard																					
Cross-section	IPGRI/ HSL	No variation present	1	Circular	2	Semi-circular	3	Square	4	Pentagonal	5	V-shaped	6	Flat	7	Triangular	8	Concave	99	Other			

shape of leaf																					
Degree of leaf waxiness	IPGRI/ HSL	No variation present	3	Weak	5	Medium	7	Strong													
Leaf density	IPGRI/ HSL	No variation present	3	Low	5	Medium	7	High													

Raphanus sativus

Descriptor	Source	Timing		Character		States															
Lateral root emergence on bulb	IPGRI	After harvest																			
Weight of harvested organ (g)	IPGRI	After harvest																			
Number of leaves and leaf scars	IPGRI	After harvest																			
Leaf length (cm)	IPGRI	Measured on outermost fully expanded leaf, largest leaf including petiole																			
Leaf blade width (cm)	IPGRI	Measured on outermost fully expanded leaf, widest point of largest leaf																			
Petiole length (cm)	IPGRI	After harvest, measured where blade intercepts with petiole																			
Root length (cm)	IPGRI	After harvest, measure storage portion																			
Root width (cm)	IPGRI	After harvest, at widest point																			
Petiole width (cm)	IPGRI	After harvest, widest point of widest leaf																			

Leaf angle	IPGRI	Pre-harvest, angle of petiole with horizontal	1	Erect >87o)	2	Open (~67o)	3	Semi-prostrate (~45o)	4	Prostrate (<30o)	5	Horizontal	6	Oblique (>-10o)																						
Leaf blade shape	IPGRI	After harvest, in outline including lobes	1	Orbicular	2	Elliptic	3	Obovate	4	Spathulate	5	Ovate	6	Lanceolate	7	Oblong	8	Other																		
Leaf division (margin)	IPGRI	Measured on outermost fully expanded leaf	0	Entire	1	Crenate	2	Dentate	3	Serrate	4	Undulate	5	Doubly dentate	6	Other																				
Leaf division (incision)	IPGRI	Measured on outermost fully expanded leaf	1	Entire	2	Sinuate	3	Lyrate	4	Lacerate	5	Other																								
Leaf apex shape	IPGRI	Measured on outermost fully expanded leaf	2	Acute	4	Intermediate	6	Rounded	8	Broadly rounded																										
Leaf blade blistering	IPGRI	Measured on outermost fully expanded leaf	0	None	3	Low	5	Intermediate	7	High																										
Leaf tip attitude	IPGRI	Pre-harvest, measured on outermost fully expanded leaf	3	Curving upwards	5	Straight	7	Drooping																												
Leaf colour	IPGRI	After harvest, measured on outermost fully expanded leaf	1	Yellow green	2	Light green	3	Green	4	Dark green	5	Purple green	6	Purple	7	Other																				
Petiole and/or midvein colour	IPGRI	After harvest	1	White	2	Light green	3	Green	4	Purple	5	Red	6	Other																						
Stem width at crown	IPGRI	After harvest	3	Narrow	5	Intermediate	7	Wide																												
Root shape	IPGRI	After harvest, long section	1	Nonswollen taproot	2	Triangular	3	Cylindric	4	Elliptic	5	Spheric	6	Transverse elliptic	7	Inverse triangle	8	Apically bulbous	9	Horn	10	Branched	11	Other												
Root shape of shoulder	IPGRI	After harvest	3	Concave	5	Plane	7	Convex																												
Root shape at base (tip)	IPGRI	After harvest	1	Acute	3	Obtuse	5	Convex	7	Plane	9	Concave																								
Root exterior	IPGRI	After harvest	0	Uniform	1	Bicolour	2	Multicolour	4	Mixed colours	5	Lateral root grooves of	6	Other																						

colour pattern													different colour to root																			
Exterior root colour	IPGRI	After harvest	1	White	2	Yellow	3	Green	4	Pink	5	Red	6	Purple	7	Bronze	8	Brown	9	Black	10	Other										
Interior root colour	IPGRI	After harvest	1	White	2	Yellow	3	Green	4	Pink	5	Red	6	Purple	7	Other																
Root flesh colour distributio n	IPGRI	After harvest, in transverse section	1	Uniform	2	Colour in cortex and cambium	3	Colour radially distributed in stellate pattern	4	Concentric rings of colour	5	Irregular distribution	6	Other																		
Position of bulb in soil	IPGRI	Pre-harvest	1	Buried	3	Mostly buried	5	Half buried	7	Largely above the soil line	9	Above the soil line																				
Descriptor discarded		Reason discarded																														
Plant growth habit	IPGRI	No variation																														
Harvest index	IPGRI	part not recorded																														
Weight of entire plant except fibrous roots	IPGRI	Some had no leaves																														
Plant height	IPGRI	Time restriction																														
Plant diameter	IPGRI	Time restriction																														
Plant height/dia meter ratio	IPGRI	Time restriction																														
Leaf hairiness	IPGRI	No variation																														
Stem axis elongation and	IPGRI	No variation																														

enlargement																						

Vicia faba

Descriptor	Source	Timing		Character		States																					
Growth habit	IPGRI/HSL		1	Determinate	2	Semi-determinate	3	Indeterminate																			
Stem pigmentation	IPGRI/HSL	at flowering time	0	Absent	3	Weak	5	Intermediate	7	Strong	X	Mixed															
Branching from basal nodes	IPGRI/HSL	Mean number of branches (to the nearest whole number) per plant taken from 5 representative plants in late flowering stage	0	Absent	1	Present																					
Branching from higher nodes	IPGRI/HSL	late flowering stage	0	Non-branching	+	Branching	X	Mixed																			
Plant height (cm)	IPGRI/HSL	Measured at near maturity from ground to the tip of the plant. Average of 10 plants																									
Days to flowering	IPGRI/HSL	Days from sowing to 50% of plants in flower.																									
Flower ground (flag petal) colour	IPGRI/HSL		1	White	2	Violet	3	Dark brown	4	Light brown	5	Pink	6	Red	7	Yellow	8	Other	X	Mixed							
Intensity of streaks	IPGRI/HSL		0	No streaks	3	Slight	5	Moderate	7	Intense																	
Wing petal background colour	HSL																										
Wing petal colour pattern	IPGRI/HSL		1	Uniformly white	2	Uniformly coloured	3	Spotted	X	Mixed																	
Pod shape	IPGRI/HSL		1	Sub-cylindrical	2	Flattened constricted	3	Flattened non-constricted	X	Mixed																	

Pod colour	IPGRI/HSL	At maturity	1	Light (yellow)	2	Dark (brown/black)	X	Mixed																					
Pod length (cm)	IPGRI/HSL	Mean of 5 dry pods																											
Pod width (cm)	JP	Mean of 5 dry pods																											
Number of seeds per pod	IPGRI/HSL	Mean of 5 dry pods																											
Ground colour of testa	IPGRI/HSL	Observed immediately after harvest (within month after harvest)	1	Black	2	Dark brown	3	Light brown	4	Light green	5	Dark green	6	Red	7	Violet	8	Yellow	9	White	10	Grey	11	Other	X	Mixed			
Hilum colour	IPGRI/HSL		1	Black	2	Colourless	3	Other	X	Mixed																			
Seed shape	IPGRI/HSL		1	Flattened	2	Angular	3	Round	X	Mixed																			
Seed length (cm)	JP	Mean of 5 dry seeds																											
Seed Width (cm)	JP	Mean of 5 dry seeds																											
Leaflet shape	IPGRI/HSL	To be observed on middle leaflet of fully expanded leaf at the intermediate flowering nodes of the plant. See Fig. 1	1	Narrow (elongate)	2	Intermediate (sub-elliptic)	3	Rounded (sub-orbicular)																					
Number of leaflets per leaf	IPGRI/HSL	Mean of 5 leaves (1 from each of 5 separate plants) observed on fully expanded leaves at the median flowering node																											
Leaflet size	IPGRI	To be observed on fully expanded leaves at the intermediate flowering nodes	3	Small	5	Medium	7	Large																					
Stem colour	IPGRI	At maturity	1	Light	2	Dark																							
Pod angle/attitud	IPGRI/HSL	At maturity (on second or third pod-	1	Erect	2	Horizontal	3	Pendent	X	Mixed																			

e		bearing node)																									
100 seed weight (fresh) (g)	IPGRI																										
100 seed weight (dry) (g)	JP																										
Stipule spot pigmentation	IPGRI		0	Absent	1	Present																					
Stem thickness (mm)	IPGRI	Mean stem thickness of single representative tiller from 10 representative plants. Measured as width of one side of stem at mid-height of plant at early podding stage																									
Resistance to lodging	IPGRI		3	Low	5	Medium	7	High																			
Height of lowest pod-bearing node (cm)	IPGRI	At harvest Mean of 5 plants																									
Number of pods per node	IPGRI/HSL	Mean number of pods on the second pod-bearing node of 5 plants																									
Pod distribution on the stem	IPGRI		1	Uniform	2	Mainly basal	3	Mainly terminal	4	Central																	
Discarded descriptors		Reason for discard																									
Date of harvest		Would not reflect actual changes as some harvested at later stage of maturity																									
Days to		Probably not reliable																									

maturity		as for harvest date																								
Maximum number of ovules per pod		Not recorded																								
Seed yield [g/m2]		Not possible to harvest all seeds																								
Pod shattering		No variation detected (no shattering)																								
Testa pattern	IPGRI	No variation detected (no patterns)	1	Plain	2	Speckled	3	Ringed																		
Number of flowers per inflorescence	IPGRI	Not consistently collected																								
Pod surface reflectance	IPGRI	Hard to measure, subjective and subject to age of pod																								

Daucus carota

Descriptor	Source	Timing		Character		States								
Extent of green shoulder	HSL/IPGRI	At harvest	a	Absent/very little	b	Small	c	Medium	d	Large	e	Very large		
Root branching	IPGRI	At harvest	0	Absent	3	Sparse	5	Intermediate	7	Dense				
Leaf length (cm)	HSL/IPGRI	Mature, including petiole												
Leaf width (cm)	HSL/IPGRI	Mature, at widest point												
Root weight (g) with foliage	HSL	At harvest												
Root weight (g) without foliage	HSL/IPGRI	At harvest												
Root length (cm)	HSL/IPGRI	At harvest												
Root width (cm)	HSL/IPGRI	At harvest, at widest point												
Split/misshapen?	HSL	Notes whether unfavourable conditions present												
Width of core (mm)	HSL/IPGRI	At shoulder												

Leaf colour	HSL/IPGRI	At harvest	1	Yellow green	2	Green	3	Grey green	4	Purple green	99	Other		
Root colour	HSL/IPGRI	At harvest	1	White	2	Yellow	3	Orange	4	Red	5	Purple	99	Other
Root shape	IPGRI	In long section	1	Round	2	Obovate	3	Obtriangular	4	Oblong	5	Tapering	99	Other
Root shape	HSL	In long section	A	Circular	B	Obovate	C	Obtriangular	D	Oblong	E	Mixed		
Root tip shape	HSL/IPGRI		1	Blunt	2	Rounded	3	Pointed	99	Other				
Typical shape of crown	HSL/IPGRI		A	Flat	B	Flat-rounded	C	Rounded	D	Rounded/conical	E	Conical		
Colour of core	HSL/IPGRI		1	White	2	Yellow	3	Orange	4	Red	99	Other		
Colour of tissue surrounding core	HSL/IPGRI		1	White	2	Yellow	3	Orange	4	Red	99	Other	E	Purple
Flesh colour distribution	IPGRI	In transverse section	1	Indistinctly uniform throughout outer and inner cores	2	Colour in two disticnt outer and inner cores	3	Colour radially distributed in stellate pattern	4	Colour radially distributed from inner core	99	Other		
Discarded descriptors		Reason discarded												
Carrot fly	HSL	No incidents reported												
Carrot motley dwarf virus?	HSL	No incidents reported												
Root size uniformity within accession	IPGRI	Redundant												
Skin texture	HSL	No variation												
Root curling	HSL	Recorded, but nearly all curled due to field conditions												
Bolting	HSL	No incidents reported												
Leaf dissection	IPGRI	No variation												
Leaf colour	HSL	No variation												

APPENDIX 3 CROP BACKGROUNDS

APPENDIX 3 CROP BACKGROUNDS

Vicia faba L. Sp. Pl. 2:737 (1753)

Vicia faba (broad bean, horse bean, field bean, faba bean) (Leguminosae) is an annual, herbaceous plant. It has a large genome relative to those of other legumes (13000 Mbp) (Young *et al*., 2003), due to large numbers of retrotransposon copies (Duc *et al*., 2010), and has 12 chromosomes. The species is largely allogamous, with high levels of outcrossing generally reported (Bond, 1995). Pollination is entomophilous, usually by bees. *Vicia faba* is part of subgenus *Vicia*, and has four infra-specific divisions: subspecies *faba* (var. *minor*, var. *equina*, var. *faba*) and subspecies *paucijuga* (Maxted, 1995; Maxted and Kell, 2009). No wild relative for *V. faba* is currently known (Maxted and Kell, 2009).

Vicia faba was probably first cultivated in the Near East in the Neolithic, around 4800 BC, expanding outwards into Europe, along the North African coast to Spain, through Egypt to Ethiopia and from Mesopotamia to India (Bond, 1995; Duc *et al*., 2010). *V. faba* var. *minor*, from southwestern Asia (Duc *et al*., 2010) is thought to be the more primitive form (Bond, 1995), with *V. faba* var. *major* arising later in the West, around 500 AD (Duc *et al*., 2010). The main centre of diversification of *V. faba* is the Mediterranean, with secondary centres in South America, North America and southern Siberia (Maxted, 1995; Maxted and Kell, 2009). *V. faba* has undergone several significant changes during domestication including pod indehiscence, reduction in seed dormancy, changes in seed colour (from all black to the multiple forms found today), increased yield through selection for plant height, increased numbers of flowers per node and fewer stems per plant (Bond, 1995).

Cultivation of *Vicia faba* is widespread throughout the northern temperate zone and in higher altitudes of some sub-tropic areas (Bond, 1995). World production was 4.17 million tonnes in 2009 (FAOSTAT, 2009), the largest producer was China (1.65 million tonnes), and the UK produced 100,000 tonnes (2.4% of world total) (FAOSTAT, 2009, marked as unofficial figures). The seeds are the consumed part of the plant, with uses both as human food (fresh, dried or canned) and for animal feed (Torres *et al*., 2006). *V.*

faba is an important source of protein, which ranges from 27-34% of seed dry matter depending on variety (Duc, 1997).

The preferred growing conditions of *V. faba* are cooler conditions, however breeding efforts have developed varieties with adaptation to warmer temperatures, higher latitudes and winter conditions (Duc, 1997).

Current levels of genetic diversity are found to be high due to a number of factors including high levels of outcrossing, maintenance of many varieties as open pollinated, and its wide geographical cultivation, leading to adaptation to many environments and conditions; this is reflected in the findings of both phenotypic and molecular marker studies (Duc *et al*., 2010).

Current breeding objectives include yield stability, reduced flower loss and disease (including *Ascochyta fabae* (Ascochyta blight), *Uromyces viciae-fabae* (rust) and *Botrytis fabae* (chocolate spot)) drought and frost resistance (Bond, 1995; van de Wouw *et al*., 2001; Duc *et al*., 2010).

Brassica oleracea var. acephala (DC.) Alef. Ldw. Fl. (1866) 234 (this is citation as in literature or IPNI says B.oleracea L subsp. acephala (DC.) Metzq. Syst. Beschr. Kohlart. 14. 1833)

B. oleracea var. *acephala* (kale) (Brassicaceae) is an outbreeding, annual, herbaceous crop plant, with 18 chromosomes (2n = 18). It is an outbreeding crop with strong self-incompatibility (Brown *et al*., 1991). In the Triangle of U, which describes the genetic relationships of *Brassica* species as developed from the three progenitor diploid species (*B. rapa*, *B. oleraceae* and *B. nigra*) to the hybrid (allotetraploid) forms (*B. juncea*, *B. carinata* and *B. napus*) *Brassica oleracea* var. *acephala* is genotype CC (Morinaga, 1934 and U, 1935, both cited in Williams and Hill, 1986). *Brassicas* are generally grown in temperate regions, and are of particular importance in Europe (Hodgkin, 1995). The centre of origin for cultivated *Brassicas* is thought to be the Mediterranean, with *B. oleracea* being domesticated after *B. rapa*, *B. nigra* and *B. juncea* (Hodgkin, 1995; Prakash *et al*., 2012). Gomez-Campo and Prakash (1999, in Allender *et al*.,

2007), suggest that *B. oleracea* was first cultivated as kale (or an n=9 chromosome wild progenitor of kale), originating from Atlantic coastal wild populations and spreading then to the Mediterranean, where diversification occurred. It has not been confirmed as to whether any of the wild kale populations found are truly wild, or whether they are escapee cultivars, and so whether *B. oleracea* truly exists in the wild (Allender *et al*., 2007). In the UK most populations may be introduced and naturalized (Watson-Jones *et al*., 2006). Populations of *B. oleracea* (currently recorded as wild) are found in the UK (as wild cabbage or sea cabbage), and are found predominantly on steep maritime cliffs of limestone or chalk, or below the cliffs in scree (Mitchell, 2008; Lanner-Herrera *et al*., 1996; BSBI, 2011). Due to this preference, populations are often isolated, being separated by other, unsuitable habitats, such as sandy soils or woodland, resulting in high differentiation between populations in the UK (Lanner-Herrera *et al*., 1996). High genetic diversity is also found within *B. oleracea* wild populations in the UK (Watson-Jones *et al*., 2006).

Kale is adapted to cooler temperatures and is grown throughout the year, with winter and spring harvests (Hodgkin, 1995; Velasco *et al*., 2007), including in the UK.

Kale is grouped with cabbages and other brassicas in FAOSTAT, therefore only these can be given. World total production was 64.4 million tonnes in 2009, with the largest production in China (30.2 million tonnes) (FAOSTAT, 2009). Production in the UK was 250,000 tonnes (0.39% of world production) (FAOSTAT, 2009). The predominant part of *B. oleracea* var. *acephala* that is eaten is the leaves, and to a lesser extent, for animal fodder, the leaves and stems. Accessions of kale have been reported as being of importance in terms of the nutrient content; omega-3 fatty acids (linoleic acid and α-linolenic acid) make up 66% of total fatty acid content in dry leaves; macro and micro nutrients include 19.7 mg per gram of dry weight of calcium, 13.5 mg per gram of dry weight to potassium and 72.6 μg of iron per gram of dry weight (Ayaz *et al*., 2006). Kale is also reported to have all essential amino acids and is high in vitamins C, E and A. Recent research into the anti-oxidant and potentially anti-carcinogenic properties of chemicals in kale including polyphenols, flavonoids, isoflavones and glucosinolates (Kural *et al*., 2011).

Due to the strong self-incompatibility in *Brassica oleracea* var. *acephala*, development of F1 hybrids has been slow (Hodgkin, 1995). Future breeding strategies may focus on crop uniformity (Hodgkin, 1995), disease resistance, in particular *Plasmodiophora brassicae* (club root) resistance (Laurens and Thomas, 1993), and optimising nutritional content (Vilar *et al*., 2008), with landraces and the CWR *brassica oleracea* as possible sources of desirable traits (Branca and Cartea, 2011).

Pisum sativum L. Sp. Pl. 2: 727 (1753)

Pisum sativum (pea) (Leguminosae) is a diploid species, with 14 chromosomes (2n = 14). It is an herbaceous annual, and is self-pollinating, with cleistogamous flowers, although low levels of cross pollination – 1% in most cultivated varieties – can occur (Gritton, 1980). The taxonomy of the genus *Pisum* is unsettled, however recent studies tend to use the taxonomy determined by Maxted and Ambrose (2001, in Ambrose, 2008; Smykal *et al*., 2011). The study adopted three species in the genus *Pisum*: *P. sativum*, *P. fulvum* and *P. abyssinicum*. *P. sativum* has two subspecies *sativum* (which includes var. *sativum* and var. *arvense*) and subspecies *elatius* (which includes var. *elatius*, var. *brevipedunculatum* and var. *pumilio*).

The domestication of *P. sativum* is also contentious. Recent studies suggest that the progenitors of cultivated *P. sativum* may have arisen in the Middle East (possibly wild *P. elatius*), and dispersed eastwards and westwards. From this western progression lineages arose, in the eastern Mediterranean, which then dispersed further west into west and/or central Mediterranean, and gave rise to another lineage which spread east and southwards into Asia minor and from which came modern cultivated pea (Kosterin *et al*., 2010; Jing *et al*., 2010; Smykal *et al*., 2011). *P. sativum* is a cool, temperate crop. Wild representatives of *P. sativum* have a current geographic range that includes Iran and Turkmenistan through anterior Asia, northern Africa and southern Europe (Maxted *et al*., 2010; Smykal *et al*., 2011). There are no *P. sativum* wild relatives in the UK.

Current uses include for human consumption as dry seeds or a vegetables and animal feed. 2009 production levels for dry and green peas were world total 10.5 million tonnes

dry and 16.0 million tonnes green; the largest producers were Canada, producing 3.4 million tonnes dry and China, producing 9.6 million tonnes green; 2009 UK production was 141,000 tonnes dry and 263,360 tonnes green (FAOSTAT, 2009).

The main constituent of seeds is starch, occurring as round starch granules in smooth-seeded varieties and as composite granules in wrinkle-seeded varieties (Cousin, 1997). Protein levels also vary according to genotype, with double recessive wrinkle-seeded varieties having lower protein, and smooth varieties having the highest (Cousin, 1997).

Domestication traits include larger seed size, more compact habit, and changes in seed coat texture.

One of the key improvements in pea cultivation has arisen from the fact that yield increases as total plant biomass decreases, therefore genetic mutations, such as *afila* and *tendriless*, have been developed to convert leaves in to tendrils and hence to increases yield (due to reduced competition between plants) (Ambrose, 2008). Current breeding targets include increasing yield, disease resistance (including *Fusarium*, *Peronospora* and *Erysiphe* (mildews)) and cold resistance (Cousin, 1997).

Cucumis sativus L. Sp. Pl. 2: 1012. 1753

Cucumis (Cucurbitaceae) is a genus of around 52 species (Renner *et al*., 2007), of which four are crop species (*C. sativus* (cucumber), *C. melo*, *C. anguria*, *C. Metuliferus*) (Bates and Robinson, 1995). *C. sativus* is an annual, diploid species, with 14 chromosomes (2n = 14); *C. melo*, *C. anguria* and *C. metuliferus* are 2n = 24. The centre of diversity for *C. sativus* is most likely Asia (Sebastian *et al*., 2010). There are no wild relatives in the UK.

Worldwide production of cucumber and gherkins in 2009 was 60.6 million tonnes; the largest producer was China at 44.3 million tonnes, and the UK produced 48,925 tonnes (0.08% of world total production) (FAOSTAT, 2009). This estimate is likely to be conservative due to the large proportion of this crop grown in home gardens (Bates and Robinson, 1995).

Parts of the plants most utilsed are the edible fruits, however some medicines, cosmetics and confectionary use the seed oil (Bates and Robinson, 1995).

Current breeding efforts include reduction in plant stature, disease resistances, plant architecture, sex expression (to maximise fruit production), and production of hybrid seeds (Bates and Robinson, 1995).

Capsicum annuum L. Sp. Pl. 1: 188. 1753

Capsicum annuum (pepper) is a member of the Solanaceae family; *Capsicum* is a genus of 25-30 species, five of which are domesticated: *C. annuum*, *C. baccatum*, *C. frutescens*, *C. chinense* and *C. pubescens*. *C. annuum* is 2n = 24. *C. annuum* is self compatible, an annual and appears to breed mostly through selfing (Heiser, 1995)).

The centre of diversity of Capsicum is South Americam and the of origin of *C. annuum* is Central America, in upland central-eastern Mexico (Loaiza-Figueroa *et al*., 1989; Clement *et al*., 2010). Domestication traits include pendent fruits, increased fruit size, fruit colour variation, and change from outbreeding to inbreeding (Clement *et al*., 2010). There are no wild relatives in the UK.

In 2009 world production of *C. annuum* (comprising fresh chillies and peppers) was 28.1 million tonnes; the largest producer was China at 14.5 million tonnes; the UK produced 15,340 tonnes in 2009, which represented 0.05% of the world total (FAOSTAT, 2009). Uses include the edible fruit and spices, use as a colouring agent, in medicine, ornamental shrub (Heiser, 1995).

Breeding efforts in the past have focussed on pest and disease resistance (Picklersgill, 1997). Current and future breeding efforts include disease resistance for diseases such as *Phytophthora capsici* (Oelke *et al*., 2003) and improvements in fruit taste, colour and texture (Pickersgill, 1997).

Solanum Lycopersicum L. Sp. Pl. 1: 185. 1753

Solanum (Solanaceae) is a genus of around 1500 species (Taylor, 1986). *S. lycopersicum* is a self fertile, inbreeding annual, with 2n = 24. The name for tomato is not fully resolved and is also known as *Lycopersicon esculentum* Miller. and *Lycopersicon lycopersicum* (L.) Karsten (Taylor, 1986; Peralta and Spooner, 2007).

The centre of origin is uncertain, however the Andes is most likely, including areas now in Chile, Bolivia, Ecuador, Peru and Columbia (Bai and Lindhout, 2007), with it being a descendent of var. *cerasiforme* (cherry tomato) (Taylor, 1986). The centre of domestication was in either Peru or Mexico (Peralta and Spooner, 2007). Domestication traits include change from exserted to inserted stigma and increased fruit size. There are no wild relatives in the UK.

In 2009 world production of *S. Lycopersicum* was 153.0 million tonnes; the largest producer was the United States of America at 14.1 million tonnes; the production in the UK was 91,000 tonnes, which represents 0.01% of the world total (FAOSTAT, 2009). *S. lycopersicum* is used for its edible fruits, and is high in vitamins A and C (Peralta and Spooner, 2007).

Recent improvements include determinate and compact habit, high productivity, disease resistance, fruit size/shape/structure to withstand mechanised handling, breeding of CWR material into tomatoes for disease resistance, and improved fruit quality (Bai and Lindhout, 2007). Diseases of particular relevance to tomato include Gemini viruses including tomato yellow leaf curl, bacterial wilt (*Pseudomonas solancearum*) and powdery mildew (*Leveillula taurica*). Also of interest to breeders is arthropod resistance and stress tolerance.

Raphanus sativus L. Sp. Pl. 2: 669. 1753

Raphanus sativus (radish) (Brassicaceae) is an annual or biennial, outbreeding, self-incompatible, insect pollinated plant. It is a diploid species with 18 chromosomes. *R. sativus* can be divided into two morphological sizes (based on commercial development

not taxonomical), the small-rooted, short-seasoned European types, grown in temperate regions; and large-rooted Asian types, grown in temperate and sub-tropical regions (Crisp, 1995). The origins of cultivated radish are as yet unclarified and evidence suggests multiple domestications may have occurred (Yamane *et al*., 2009). *R. sativus* is thought to have two possible centres of origin – Asia and Europe – with gene flow between the two (Crisp, 1995; Wang *et al*., 2008), and a likely centre of domestication in the Mediterranean (Rabbani *et al*., 1998; Ullah *et al*., 2010).

Wild taxa occur throughout Europe and Asia, and are introduced weeds in America. Wild relatives are *R. raphanistrum* subsp. *raphanistrum*, *R. maritimus*, *R. rostras* and *R. landra*, of which the former two occur in the UK. *R. raphanistrum* subsp. *raphanistrum* occurs as a weed in cultivated fields and along roadsides; *R. maritimus* occurs in coastal areas, in coastal grassland, shingle and maritime cliffs (Botanical Society of the British Isles, BSBI, 2011).

World production is estimated to be 7 million tonnes a year (Schippers, 2004). Uses include consumption of the roots as salad crops and large rooted varieties are often pickled for winter food; sprouted seeds are also eaten.

Breeding foci are different between the two radish types, with small rooted varieties focussed more on physiological traits such as earliness, bulbing under different seasons and high temperatures without bolting. In large rooted varieties, breeding has focussed on disease resistances including *Fusarium*, *Albungo candida*, *Peronospora parasitica* (Crisp, 1995). Targets for the future include further disease resistance.

Lactuca sativa L. Sp. Pl. 2: 795. 1753

Lactuca sativa (lettuce) (Asteraceae) is an annual, herbaceous, plant. It has nine chromosome pairs and is inbreeding (self-fertilizing). The three most common wild relatives of *L. sativa* are *L. serriola*, *L. saligna* and *L. virosa*, with some contention regarding whether *L. serriola* is a part of *L. sativa* (Koopman *et al*., 2001). *L. sativa* types can be split into seven classes: cos (romaine), crisphead (iceberg), butterhead, romaine, leaf (cutting), latin, oilseed and stem (De Vries, 1997).

Several candidate centres of domestication have been posited: Egypt, the Mediterranean or Kurdistan-Mesopotamia (Ryder and Whitaker, 1995; De Vries, 1997). De Vries (1997) argues that lettuce cultivation began in Kurdistan-Mesopotamia, spread into Egypt and from there into Europe. The origin of cultivated lettuce is uncertain (Ryder and Whitaker, 1995). Three scenarios for its origin are posited. Firstly, that *L. sativa* arose from *L. serriola* (as all variants of *L. sativa* except extreme head variation are present in *L. serriola*). Secondly, that both *L. sativa* and *L. serriola* arose from a hybrid that diverged into manmade and weedy types (*L. sativa* and *L. serriola* respectively). Thirdly, that *L. sativa* ancestors may be hybrids between *L. serriola* and another unknown species or *L. serriola* might be a product of hybridisation between *L. sativa* and another (Ryder and Whitaker, 1995; Yang *et al*., 2007). Early cultivars were narrow leaved, with erect rosettes, and these may have led to cos lettuce types in southern Europe. Cultivar development in northern Europe and North America then emphasised head formation through butterhead and then crisphead types (Ryder and Whitaker, 1995).

The distribution of *L. sativa* wild relatives in the UK is as follows: *L. saligna* is a native, lowland plant, listed as Nationally Rare in the UK (occurring in fewer than 15 hectads in Great Britain) (Cheffings and Farrell, 2006) and occurs on disturbed coastal land including shingle and old sea wall (BSBI, 2011); *L. serriola* is an archeophyte, which grows on disturbed ground including roadsides, waste ground and gravel-pits (BSBI, 2011). *L. virosa* is also a native, lowland species, occurring on coastal cliffs, outcrops, calcareous grassland, woodland margins and rough ground (BSBI, 2011). All three are listed as Least Concern on the IUCN Red List (Bilz *et al*., 2011).

Domestication included the development of non-shattering seed heads, and late flowering (slow bolting), the loss of spines, a reduction in latex content, and increased seed size and hearting (Ryder and Whitaker, 1995).

World production of lettuce and chicory is 23.6 million tonnes (FAOSTAT, 2010); the greatest producer is China, at 12.6 million tonnes (FAOSTAT, 2010). The UK produces 133,900 tonnes (FAOSTAT, 2010). It is grown as a salad crop in north temperate or sub-tropical regions. In Europe butterhead and romaine types were developed for winter cultivation in the Mediterranean. Butterheads in Europe are summer cultivated (Ryder

and Whitaker, 1995). Crisphead types are suited to large-scale irrigated culture, and long distance transportation.

Breeding has included disease resistance, colour, size, weight and bolting, as well as the use of crop wild relatives *L. serriola* (as a source of resistance for Downy mildew) and *L. virosa* (as a source of dark leaf colour and leaf texture improvements) and *L. saligna* (for leaf crispness) (Ryder and Whitaker, 1995).

Future breeding aims include resistance to diseases and insects, including downy mildew, lettuce mosaic virus, big vein, sclerotina, corky root rot, aphids, lepidoptera, caterpillars and whitefly (Ryder and Whitaker, 1995).

Brassica napobrassica Mill. Gard. Dict., ed. 8. n. 2. 1768

The nomenclature for swede is applied inconsistently, with *Brassica napobrassica*, *Brassica oleracea* var. *napobrassica*, Brassica napus, *Brassica napus* subsp. *napobrassica* and *Brassica napus* subsp. *rapifera* all used in the literature.

Brassica napobrassica (Brassicaceae) is a biennial crop with 38 chromosomes. It is self-fertile and tolerant of inbreeding. It is an allotetraploid formed from the hybridisation of *Brassica rapa* var. *rapa* and *Brassica oleracea* (McNaughton, 1995a; Gowers, 2010). In the Triangle of U (see above), *B. napobrassica* is identified as genotype AACC.

The origin of *B. napobrassica* is relatively recent; it may have formed in European gardens as a hybrid between *Brassica oleracea* or *Brassica oleracea* var. *acephala* and *Brassica rapa* var. *rapa*. It is uncertain if it exists in true wild form; if wild specimens are not escapees, then it is a European-Mediterranean species (McNaughton, 1995a). *B. napobrassica* was introduced into UK from Sweden in around 1775-80 (McNaughton, 1995a; Gowers, 2010). Wild specimens in the UK are thought to be cultivated escapees, with subspecies *oleifera* (oil seed rape) widely naturalised, particularly along roadsides and cultivated ground (BSBI, 2011). Wild relatives are *Brassica oleracea* (see above) and *Brassica rapa* (see below).

In the UK the combined area of cultivation for *B. napobrassica* and *B. rapa* var. *rapa* is approximately 30,000 hectares (Gowers, 2010). The utilised part of the plant is an enlarged hypocotyl, eaten cooked as a vegetable, particularly as a winter crop. It is also used for animal fodder (McNaughton, 1995a). The nutritional content of *B. napobrassica* includes high levels of vitamins A, B□ and C, fibre, calcium, niacin and iron (Gowers, 2010). *Brassica napobrassica* roots also contain glucosinolates, including gluconasturtiin (2-phenylethylglucosinolate) (Gowers, 2010), which are anti-carcinogenic.

Improvements in *B. napobrassica* by mass selection and pedigree breeding include resistance to fungal diseases such as powdery mildew (*Erisephe cruciferarum*), dry rot (*Leptosphaeria maculans*) and clubroot (*Plasmodiophora brassicae*), the physiological disorder internal browning, higher uniformity for mechanical harvesting and introgression of desirable traits from other B*rassicas* (including dry rot and club root from *Brassica rapa* var. *rapa*) (McNaughton, 1995a). Future breeding aims include improvement of storage qualities (preventing fungal rots and insects such as flea beetles, root flies and aphids), as well as the enhancement of flavour, nutrition and texture (McNaughton, 1995a; Gowers, 2010).

Brassica rapa L. Sp. Pl. 2: 795. 1753

Brassica rapa var. *rapa* (turnip) (Brassicaceae) is a biennial crop, with 20 chromosomes. It is out breeding, self-incompatible and suffers from inbreeding depression (McNaughton, 1995b). In the Triangle of U (see above), the genotype of *Brassica rapa* var. *rapa* is AA. It is part of a complex of crops which includes true turnips (*Brassica rapa* var. *rapa*, previously known as *Brassica campestris* subsp. *rapifera*), oil seeds (including subsp. *oleifera* (turnip rape)) and leafy forms (including subsp. *chinensis* (pak-choi) (McNaughton, 1995b).

Two main centres of origin have been proposed. Firstly, in the Mediterranean for European forms. Secondly, in eastern Afghanistan and the adjoining part of Pakistan as the primary centre for other forms, with Asia Minor, Transcaucasus and Iran as

secondary centres (McNaughton, 1995b). The wild relative of *Brassica rapa* var. *rapa* is *Brassica rapa* subsp. *campestris* (previously known as *Brassica campestris* subsp. *campestris*, which is an annual (McNaughton, 1995b)). In the UK, *B. rapa* subsp. *campestris* is an archeophyte, occurring on river and canal banks, roadsides and in arable fields (BSBI, 2011).

In *B. rapa* var. *rapa* the part of the plant eaten is the hypocotyl. They are eaten as vegetables in Northern Europe and New Zealand, and are used as cattle forage (McNaughton, 1995b). *B. rapa* var. *rapa* is high in vitamins B□, B□ and C, calcium, copper, potassium and dietary fibre (Gowers, 2010). Turnip roots also contain glucosinolates, including gluconasturtiin (2-phenylethylglucosinolate) (Gowers, 2010), which are anti-carcinogenic.

Progress has been made in breeding for disease resistance including in club root, dry rot and powdery mildew, although resistance and disease outbreaks are regional, therefore breeding efforts continue (Gowers, 2010). Further goals include increased yield, increased nutritional value, manipulation of glucosinolate levels and resistance to insects (McNaughton, 1995b; Gowers, 2010).

Daucus carota L. Sp. Pl. 1: 242. 1753

Daucus carota (carrot) (Apiaceae) is an out-breeding (with andromonoecious flowers with protandry and some geitonomy), biennial herbaceous plant, with 18 chromosomes, and a genome of 480 Mb (Riggs, 1995; Lorrizzo *et al*., 2011). The primary centre of origin for *D. carota* is believed to be Afghanistan, with the main centres of diversity in the Anatolian region of Asia Minor (Turkey) and Iran (Vavilov, 1951, ctied in Stolarczyk and Janick, 2011). Domesticated *D. carota* can be divided into two groups: anthocyanin or eastern carrot, with yellowish or purple roots, originated in Afghanistan, and carotene or western carrot, with orange, yellow or white roots, which arose from eastern carrots (Banga, 1963). European carrot improvement began with purple material from Arab countries by way of Turkey, north Africa and Spain in the 13th to 14th centuries, was followed by yellow carrot in the 16th and orange in the Netherlands in the

17th century; because of these later developments Turkey and temperate Europe may be considered as secondary centres of origin for *D. carota* (Banga, 1963; Riggs, 1995; Clotault *et al*., 2010; Stolarczyk and Janick, 2011). All present carotene carrot varieties are from the four original Netherlands varieties: Long Orange, Late Half Long, Early Scarlet Horn and Early Half Long. White carrots were probably selected from yellow (western types) (Banga, 1963). Most varieties were open-pollinated until the 1960s, with inbreeding depression preventing greater uniformity (Riggs, 1995).

The colour of the carrot root has been identified as being controlled by single dominant loci P□ and Y□ (purple and yellow respectively) (Simon, 1996).

The most well known wild relative of *D. carota* (or *D. carota* subsp. *sativa*) in temperate regions is *Daucus carota* subsp. *carota* (Shim and Jorgensen, 2000). In the UK, *D. carota* subsp. *carota* is a native, biennial herb, occurring in well-drained, often calcareous soils, including roadsides, grassland and disturbed ground (BSBI, 2011). In the UK *Daucus carota* subsp. *gummifer* is also native, occurring on clifftop grasslands and stable sand dunes (BSBI, 2011).

D. carota is grown worldwide, in temperate climates (and tropics and sub-tropics as a winter crop or at high elevations) (Riggs, 1995). World production of *D. carota* (figures also include *Brassica rapa* var. *rapa* figures) was 33.7 million tonnes in 2010 (FAOSTAT, 2010). The largest producer was China (15.9 million tonnes) (FAOSTAT, 2010). The UK produced 747,900 tonnes in 2010 (FAOSTAT, 2010). The part of the plant that is eaten is the fleshy storage root, which is high in vitamins A and C, and is a source of fibre and sugars. Uses include salads and cooked, and also canned, frozen, dehydrated and juiced (Riggs, 1995).

Domestication traits include comparatively thicker and shorter tap roots in cultivated than wild species; roots that are unbranched, brittle, pigmented and palatable; fewer and more erect leaves; and floral differences (Riggs, 1995).

Previous breeding has included introgression of carrot fly resistance (*Psila rosae*) from *Daucus capillifolius*, improved yield and improved root colour (Riggs, 1995; Stein and Nothnagel, 1995). Current breeding objectives include further increases in yield and

root uniformity, improved appearance (shape, exterior and interior colour, smoothness), disease and pest resistance, bolting resistance, quality (taste, nutrition), seed yield of female lines and a reduction of terpinoids for improved taste (Riggs, 1995; Jagosz, 2011).

Allium porrum L. Sp. Pl. 1: 295. 1753

Allium porrum (Amaryllidaceae) (synonym *Allium ampeloprasum* or *Allium ampeloprasum* var. *porrum*) is an out-breeding, tetraploid species, with 2n = 4x =32 chromosomes and a genome of size 16,366 Mbp (Ricroch *et al*., 2005). Crop *A. porrum* can be functionally split by harvest time into summer, autumn and winter leek types (De Clerq *et al*., 1999). Other species of commercial importance within the *Allium* genus are *A. cepa* (bulb onion), *A. schoenoprasum* (chive) and *A. sativum* (garlic). The wild progenitor of leek may be *A. ampeloprasum*, or more recently *A. iranicum* and *A. atroviolaceum* have been proposed as close relatives (Hirshegger *et al*., 2010). In the UK *A. ampeloprasum* (wild leek) is a perennial, archeophyte species occurring in rank vegetation of sandy and rocky coastal sites, including paths, fields and sheltered cliff slopes (BSBI, 2011).

Western Europe as a whole is the largest producer and consumer of leeks in the world (De Clerq *et al*., 1999). In terms of countries, the largest producer of ‘leeks and other alliaceous veg’ in 2009 was Indonesia (549,365 tonnes); the UK produced 36200 tonnes (FAOSTAT, 2009). Most of the plant is composed of edible leaves, the bases of which are the commonly consumed portion. *Allium porrum* is high in vitamins B□ and C, as well as in quercetin and kaempferol which have anti-carcinogenic properties (Galeone *et al*., 2006; Filjushin *et al*., 2011).

Previous breeding efforts have focussed on yield, uniformity, bolting resistance and resistance to yellow stripe *potyvirus* (Havey, 1995). Future breeding targets include increased uniformity (De Clerq *et al*., 2003) as well as research into the potential medicinal properties of *Alliums* generally (Havey, 1995).

APPENDIX 4 GARDEN ORGANIC MEMBER QUESTIONNAIRE

Member grower survey

1. Heritage vegetables

1. For how many years have you been growing vegetables generally?

2. For how many years have you been growing heritage vegetables in particular?

3. Why do you grow heritage vegetables? Please list up to three reasons in the space below.

4. Do you also grow standard (non-heritage or "modern") vegetable varieties?

- No (Please go to the next page)
- Yes (Please go to question 5)

5. Why do you grow these? Please list up to three reasons in the space below.

6. Roughly what proportion of the vegetables that you grow are modern/heritage varieties?

- All heritage varieties
- Mostly heritage varieties
- About 50/50
- Mostly modern varieties

Member grower survey

2. Gardening practices

1. How much space do you have allocated for vegetable growing (tick all that apply)

- Patio/balcony/tubs
- Small area of a garden
- Up to half a garden
- large proportion of a garden
- I have an allotment

2. Which of the following organic practices do you regularly use (please tick all that apply)?

- Peat-free compost
- Manure
- Organic fertilizers
- Avoiding chemical fertilizers/pesticides
- Bee-friendly gardening
- Encouraging predators of pests
- Organic seed
- None of the above
- Other (please specify)

Member grower survey

3. Variety choice

1. Do you grow the same crops each year?

- All the same
- mostly the same
- Mostly different
- All different

2. Do you grow the same varieties each year?

- All the same
- Mostly the same
- Mostly different
- All different

3. When you are choosing which varieties to grow are there any particular traits that you look for? Please list up to three main reasons in the space below.

4. Do you grow more than one variety of a crop?

- No (Please go to question 6)
- Yes

5. In the boxes provided, please rank your reasons for growing more than one variety from 1 (most important) to 4 (least important).

Minimise risk of loss	
Variety choice	
Aesthetic reasons	
Other (please state)	

Member grower survey

6. In the spaces below please indicate any heritage vegetables you regularly grow, your favourite variety for each and why you like it.

Tomato: Favourite variety

Why do you like it?

Cucumber: Favourite variety

Why do you like it?

Pepper: Favourite variety

Why do you like it?

Squash: Favourite variety

Why do you like it?

Cabbage: Favourite variety

Why do you like it?

Onion: Favourite variety

Why do you like it?

Carrot: Favourite variety

Why do you like it?

Pea: Favourite variety

Why do you like it?

Runner bean: Favourite variety

Why do you like it?

French bean: Favourite variety

Why do you like it?

Any other favourite varieties (please specify)

Why do you like it?

7. Do you have any special uses for any of the heritage varieties that you grow (for example jam or wine making)?

Member grower survey

4. Variety traits

1. Have you found any varieties that have a particular pest / disease / environmental (weather etc) resistance? Please state variety name and resistance.

2. Have you noticed any varieties that do not breed true or show unexpected variety, for example in shape or colour (if so please specify)

Variety 1	
Observed differences	
Variety 2	
Observed differences	
Variety 1	
Observed differences	

3. Have you grown any varieties that you think may be the same but with different names? Please give both names.

4. Varieties may do well close to the area where they were bred. If you have grown any seeds that have a local name (e.g. Lancashire Lad or Southampton Wonder) have you noticed any variation in performance compared to other varieties (including poor performance particularly if you live far from the place of origin)?

Member grower survey

5. Seeds

1. What is the main source of the seeds that you grow? Please tick the box below.

- ☐ Garden Organic
- ☐ Garden Centre
- ☐ Supermarket
- ☐ Seed swap
- ☐ Own retention
- ☐ Other (please specify)

2. Do you save seed?

- ☐ Yes
- ☐ No

3. Do you share seeds with other growers?

- ☐ Yes
- ☐ No

Member grower survey

6. Any other comments?

1. Is there anything else you would like to add about your experiences of growing heritage vegetables?

Thank you very much for completing this questionnaire: please email jxp707@bham.ac.uk if you have any queries.

APPENDIX 5 SEED GUARDIAN QUESTIONNAIRE

APPENDIX 5 SEED GUARDIAN QUESTIONNAIRE

As seed guardians, you perform a vital role growing seed for the Heritage Seed Library (HSL), helping to maintain seed stocks and safeguard rare varieties. We are interested in learning about your experiences as a seed guardian, such as why you chose to take on the role, what crops you grow, and the ways in which you grow them. We would be very grateful if you could spare a few minutes of your time to complete the following questions.

Completion of this questionnaire is entirely voluntary. If you decide to complete the questions below, please be assured that your answers are completely anonymous: you do not need to provide any personal information. The information given will be used as part of a Birmingham University PhD project contributing to the conservation of heritage varieties. Completed questionnaires will be kept and reviewed at Birmingham University with a summary of results sent to HSL.

Q1. What are the main reasons that you became a Seed Guardian? (Please list up to three)

1 _____

2 _____

3 _____

Q2. How many different crops do you grow (as a Seed Guardian) on average, per year?

Q3. How many varieties do you grow (as a Seed guardian) on average, per year?

Q4. Are there any varieties that you like to grow regularly? Why do you choose these varieties from the Orphans list? Please list:

	Variety	How regularly do you grow this variety?	Reason chosen?
1			
2			
3			
4			
5			

Q5. The HSL publishes Seed Saving Guidelines to advise growers on seed saving techniques (such as how to avoid cross-pollination). We are interested in how seeds are handled at different growing stages, how closely the Seed Saving guidelines are followed, and if you have any additional practices you have found useful, for example to improve germination etc.

Stage of development	How closely do you follow SSGs?				Any additional measures used
	Exactly	Mostly	Partly	Not at all	
Treatment to seed before it is sown	☐	☐	☐	☐	
Distance between varieties during cultivation	☐	☐	☐	☐	
Harvesting	☐	☐	☐	☐	
Post-harvest seed treatment	☐	☐	☐	☐	

Q6. How do you prepare the soil before/during cultivation?

Do you sterilise the soil?	Yes/No	What do you use to do this?	
Do you use composts/manures	Yes/No	When and how do you apply them?	
Do you use mulches?	Yes/No	If so, what do you use?	
How do you deal with weeds?			
Do you sterilise the soil?	Yes/No	What do you use to do this?	

Q7. Where and how do you store seeds?

Q8. As a Seed Guardian, how do you choose which seeds to send back to the HSL? (E.g., do you select the strongest plants, or plants with a particular character, etc?)

Q9. If you have spare after returning seeds to HSL, how do you use them?

- ☐ Share seeds with other growers
- ☐ Own retention
- ☐ Other (Please state): _____

Q10. Have you noticed any varieties that do not breed true or show unexpected variation, for example in shape/size/colour (if so please specify)?

Crop	Variety	Year grown	Variation observed

Q11. Are there any services or supports that you feel HSL could provide to better meet your needs? (Please tick any that apply)

- ☐ Cultivation support
- ☐ Feedback on seed returned to HSL
- ☐ Regular newsletter/ e-newsletter
- ☐ Training on seed saving
- ☐ Other (please specify)
- ☐ Seed Guardian Days
- ☐ More details on varieties
- ☐ Local Seed Guardian Networks

Thank you very much for completing this questionnaire; please email jxp707@bham.ac.uk if you have any queries.