INVESTIGATION OF THE POPULATION STRUCTURE OF BARLEY LANDRACES (*Hordeum vulgare* L.) FROM DIFFERENT ECO-GEOGRAPHICAL ENVIRONMENTS IN SYRIA

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Abstract

The population structure of accessions of barley landraces (BLR) (*Hordeum vulgare* L ssp. *vulgare*) from Syria was investigated using genetic markers. Barley landraces are old, diverse cultivars of barley which have evolved under cultivation without much directed selection. BLRs have been shown to outperform modern cultivars under low input conditions in Syria due to the level of local adaptation and population buffering (i.e. the ability to compensate the failure of a component by a genetically different component of the same population) based on a high level of diversity within landraces.

The purpose of the present study was to identify suitable genetic markers, to use these to characterise the population structure and diversity of barley landraces, and to investigate how population structure is influenced by environmental conditions. Results were compared with those achieved for accessions of wild barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch) and modern cultivars in order to investigate the influence of domestication and cultivation on mating system and population structure. Implications of the results for breeding strategies in low input agriculture and for conservation strategies were investigated.

Two recent accessions of wild barley and 15 recent accessions of BLRs were available from Syria, where landraces are still under cultivation. Seven earlier accessions of the same landraces from Syria were available from gene banks. Modern cultivars and progeny of known pedigree were available from barley breeders. Genetic indices of accessions were analysed using three contrasting types of genetic markers, morphological, isoenzyme and molecular markers, respectively. Standard methods of population genetics were used to analyse the population structure of accessions. Environmental conditions of the sampling sites were analysed using the geographical information system ArcView.

Results showed that BLRs are predominantly inbreeding populations with a low outcrossing rate of 1.7%. The same outcrossing rate has been reported for wild barley,

so that it can be assumed that domestication has not influenced the outcrossing rate. Recently collected BLRs showed a high level of diversity and a regionally localised and geographically variable population structure. Harsh environmental conditions seem to increase diversity of BLRs. Genetic diversity may have been lost through the process of domestication. More diversity was found within populations of wild barley, while BLRs showed a higher proportion of diversity between accessions, for two of the three molecular markers used. Little diversity was found between modern cultivars, and no diversity within them. This may imply that modern breeding methods have led to a loss of diversity in the current gene pool. Gene flow between accessions within regions was high, but low between more distant regions, implying little seed exchange between farmers in different regions. However, it seemed that intensive extension work may lead to increased seed exchange over long distances and thus may decrease the level of local adaptations. Finally, a dramatic loss of genetic diversity of BLR accessions was found with increasing storage time in *ex situ* gene banks, suggesting the presence of genetic bottlenecks associated with the rejuvenation of ageing seeds accessions. Genetic diversity levels of recently collected BLRs support the implementation of *in situ* conservation strategies.

Declaration

I, the undersigned, hereby declare that this thesis has been composed by myself.
All assistance obtained from other sources has been acknowledged in the appropriate places.

HEIKO KURT PARZIES

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Abbreviations are explained in the text when they are first mentioned. This list is provided to serve as a reference list for frequently used abbreviations in case an explanation cannot be located. Molecular formulae of chemicals are not given here but in chapter 3. Internationally recognised units of measurements and their common abbreviations have been used throughout.

AFLP	Amplified Fragment Length Polymorphism;
BAZ..	Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Braunscheweig, Germany;
BLR	Barley landrace;
CAPS	Cleavable Amplified Polymorphic Sequences;
CC	Composite Cross;
CPRO-DLO	Collections of the Centre for Genetic Resources, Wageningen, The Netherlands;
CV	Cultivar, (cultivated variety);
D	Linkage disequilibrium;
DHL	Double haploid line;
DNA	Deoxyribonucleic acid;
cpDNA	chloroplast DNA;
mtDNA	mitochondrial DNA;
rDNA	ribosomal DNA;
EMR	Effective Multiplex Ratio;
FDS	Frequency dependent selection;
GIS	Geographic Information System;
ICARDA	International Centre for Agricultural Research in the Dry Areas
IDW	Inverse distance weighted (interpolation method);
IERM	Institute of Ecology and Resource Management, University of Edinburg;
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Gernmany;
PAGE	Polyacrylamide Gel Electrophoresis;
PCR	Polymerase Chain Reaction;
RAPD	Random Amplified Polymorphic DNA;
RFLP	Restriction Fragment Length polymorphism;
SDS-PAGE	Polyacrylamid Electrophoresis using sodium dodecylsulfate;
SGE	Starch Gel Electrophoresis;
SSR	Single Sequence Repeats;
TGW	Thousand Grain Weight;
VIR	Vavilov Research Institute of Plant Industrie, St. Petersburg Russia.

Chapter 1

Introduction, background and rationale

1.1 Introduction

Barley landraces (*Hordeum vulgare* L. ssp. *vulgare*) are the evolutionary link between wild barley (*H. vulgare* ssp. *spontaneum* C. Koch) and modern barley cultivars. Until the late nineteenth century, all cultivated barley existed as highly heterogeneous landraces. Over the last 100 years the landraces have been almost wholly displaced in advanced agriculture by pure line cultivars (Nevo, 1992). However, in many parts of the world with a less advanced agriculture, landraces are still in use and are favoured by local farmers, because they often perform more stably under a range of conditions than modern cultivars (Ceccarelli, 1996a). In recognition of this possible advantage of landraces, new breeding strategies have been suggested for marginal areas of production with the aim of producing improved landraces which retain a high level of yield stability, but show a better over-all performance than traditional landraces (Ceccarelli, 1994). A considerable number of publications in the last 15 years or so have contributed valuable information about diversity of barley landrace (BLR) populations from different regions of the world using an array of genetic marker methods (e.g. Doll and Brown, 1979; Murphy and Witcombe, 1981; Brown and Munday, 1982 ; Clegg, Brown and Whitfeld, 1984; Saghai-Maroof, Soliman, Jorgensen and Allard, 1984; Damania, Jackson and Porceddu, 1985; Ceccarelli, Grando and van Leur, 1986; Holwerda, Jana and Crosby, 1986; Jana and Pietrzak, 1988; Weltzien, 1988, 1989; Van Leur, Ceccarelli and Grando, 1989; Asfaw, 1989; Kim, Lee and Chung, 1991; Konishi and Matsuura, 1991; Nevo, 1992; Konishi, Yano and Fukushima, 1993, Lashmers, Blake and Ceccarelli, 1993; Zhang, Shagai-Maroof and Kleinhofs, 1993; Baum and Johnson, 1994; Ramamoorthy, Chong and Chinappa,1994; Abay and Cahalan, 1995; Hadjichristodoulou, 1995; Jana and Bailey, 1995; Lakew, Semane, Alemayehu, Gebre, Grando, van Leur and Ceccarelli, 1997). The importance of landraces both as a means of conserving genetic diversity and as a source of breeding material is now well recognised. However, the knowledge is still very patchy and unstructured and results with a practical relevance are still relatively few. The purpose of the present study was to structure present information on genetic diversity of BLRs and gather additional data in order to increase their direct relevance for breeding strategies and conservation.

1.2. Background

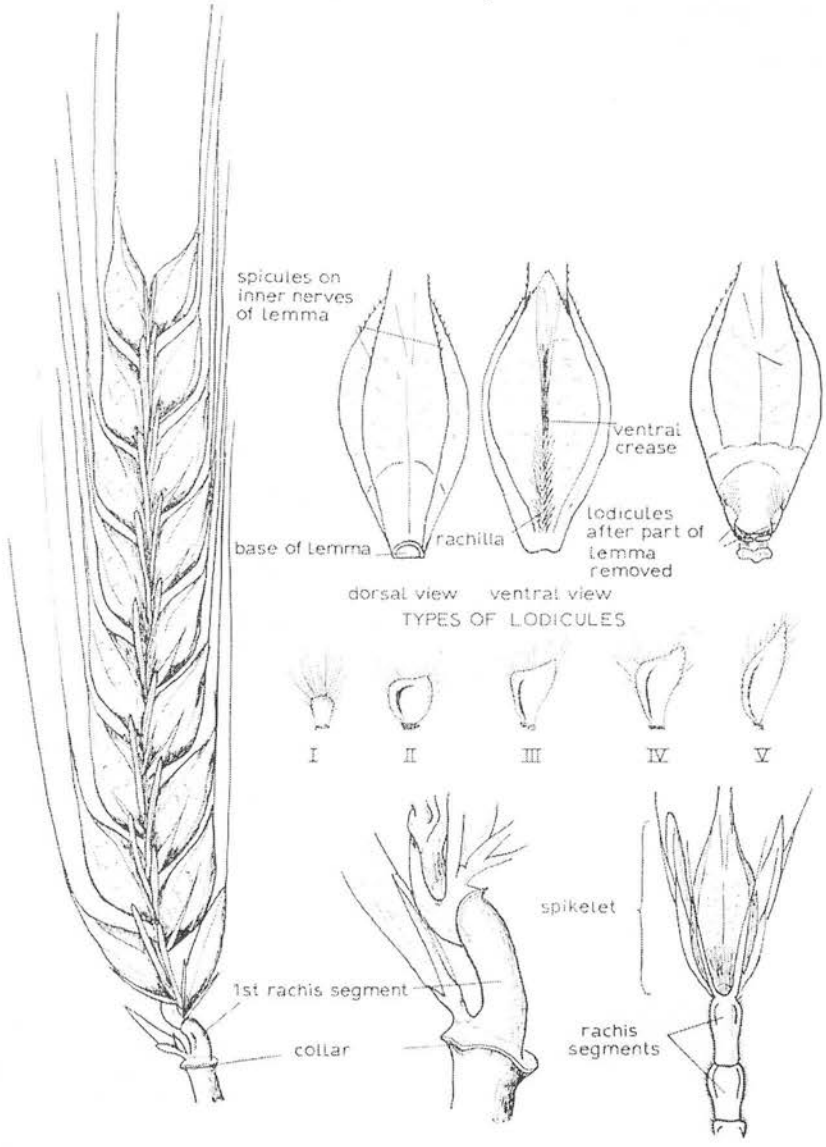
1.2.1 Barley

Barley (*H. vulgare* L.) comprises a number of features which make it a model plant species for a wide range of studies in evolutionary biology and population genetics (Brown, 1983). Barley (Plate 1.1) is a diploid member of the *Gramineae* family with a chromosome number of 2n = 14 (Franke, 1981). Outcrossing rates of cultivars of barley range from 0.5% to 10% (Sokal, 1978; Simmonds, 1979; Doll, 1987), while the outcrossing rate of wild barley has been reported as 1.6% (Brown, Zohary and Nevo, 1978). Thus, it can be regarded as a predominantly self-pollinated species. These features assist in the genetic analysis of barley, as homozygosity is the predominant state for most of its genes. Hand crosses of barley are easily performed and yield fertile offspring, so that F_2 families are easily generated (Brown, 1983), and segregation pattern can be assessed, employing codominant genetic markers (e.g. isoenzymes).

The diversity of barley and its wide adaptation to a range of extreme environments is well documented (e.g. Duke, 1982; see Section 1.2.1.2, below). Accessions of barley from all parts of the world are readily available for studies from huge collections held in *ex situ* gene banks all over the world. The availability of wheat-barley addition lines enabled extensive mapping of genes, which have significance for other, closely related members of the *Gramineae* family, as well (Brown, 1983). While wild barley is annual, both annual and biennial types of cultivated barley exist (i.e. spring and winter types) and vernalisation requirements can vary considerably. The number of grain-rows per spike of cultivated barley can be 2, 4 or 6 (Franke, 1981).

Cultivated barley (*H. vulgare* L. ssp. *vulgare*) has a single recognised wild progenitor (*H. vulgare* ssp. *spontaneum* C. Koch), and both are completely interfertile (Zohary and Hopf, 1988). Wild barley is found in a wide range of environments and is still widely distributed in the Near East, the supposed centre of domestication (Brown, 1983), where it grows frequently as a weed alongside barley fields (Nevo, 1992).

Plate 1.1 **Diagram of cultivated two-row barley (*H. vulgare* L. ssp. *vulgare*) (from Aufhammer, et al. 1968)**



The taxonomy of cultivated barley is still an ongoing debate and some authors propose the division of wild and cultivated barley into different species (Takahashi, 1955, Nevo, 1992). In the present study the classification proposed by Zohary and Hopf (1988) has been used, which considers all cultivated barleys as *H. vulgare* L. ssp. *vulgare,* and the wild progenitor as *H. vulgare* ssp. *spontaneum* C. Koch. This classification seems justified, because *H. vulgare* ssp. *spontaneum* is the only wild *Hordeum* species that is cross-compatible and fully interfertile with the cultivated

barley (Nevo, 1992), and the brittle rachis of wild barley is the only significant morphological difference to cultivated barley (Schmeil-Fitschen, 1976). However, for simplicity in the present study cultivated barley will be referred to as *H. vulgare* and the wild progenitor to as *H. spontaneum* or wild barley.

1.2.1.1 Origin, domestication and crop evolution

The supposed progenitor of cultivated barley is *H. spontaneum*, a two-row, annual barley, whose main distinguishing character from the cultivated forms is its brittle rachis (Nevo, 1992). Brittle rachis is caused by a closely linked pair of complementary loci present in the double dominant condition (Bt1, Bt2). Cultivated barley is monomorphic for the double recessive condition of the loci (bt1 and bt2) which causes a non-brittle rachis (Allard, 1992). Domestication from two-row brittle rachis types to non-brittle rachis types began about 10,000 years ago in the Near East Fertile Crescent which stretches from Israel over Lebanon and Syria, through the South of Turkey and between the rivers Euphrates and Tigris deep into Iraq and Iran. Unmistakable remains of non-brittle barley i.e. forms that could only survive under cultivation, appear in the 7th millennium BC (Zohary and Hopf, 1988) in Syria.

Barley grains in archaeological excavations were commonly found together with other cereals, grasses and legumes. In early excavations accompanying cereals were mainly emmer wheat (*Triticum turgidum* L. ssp. *dicoccum*) and einkorn wheat (*T. monococcum* L.), which both have a non-brittle rachis in common with *H. vulgare*. The progenitors of all three mentioned cereals have a brittle rachis, so that similar domestication processes are likely to have occurred. Genetic considerations suggest that the establishment of non-shattering mutants in cereals may have been accomplished in the course of a few generations (Zohary and Hopf, 1988). Legumes, which were found together with the previously mentioned cereals, were mainly lentils (*Lens culinaris* Medic.) and peas (*Pisum sativum* L.). All five above mentioned species are annual and predominantly inbreeding species, while the great majority of plant species are outbreeders. Apparently inbreeding species were better

suited to domestication than cross-pollinated species, because selfing isolates the crop reproductively from their wild progenitor and selfing enabled the maintenance of favourable types within populations (Zohary and Hopf, 1988).

Vavilov (1926) designated Nepal in the Himalayas as the centre of diversity and origin for barley, but altered his opinion after findings of great diversity in barley from Abyssinia and Eritrea (Takahashi, 1955). His theories of centres of origin have been widely criticised since (e.g. Simmonds, 1979), although in a later study Murphy and Witcombe (1981) found in large barley collections of the Himalayan region the markedly greatest variation for qualitative characters in barley landraces from Nepal. Chapman (1984) indicated that countries or regions of the greatest diversity are not necessarily those in which the crop is thought to have originated. As an example he mentioned that countries of eastern Europe are the richest for variability in barley whereas those of the Middle East and North Africa are among the poorest. Peeters (1988) claims in a study of statistical data from world-wide *ex situ* barley collections that the countries with the highest overall diversity of barley are today the USA, Turkey, Japan, USSR and China. It seems to be questionable whether different authors compare like with like when they refer to variability and there might be need for reconciliation of terminology and methodology.

However, a lot of useful traits like disease resistance and tolerance have been reported for collections of barley landraces from countries where landraces are still the predominant cultivars, for instance Ethiopia, Syria and Jordan (Lakew, et al., 1996; van Leur, Ceccarelli and Grando, 1989). In particular Ethiopia seems to be rich in barley genotypes and has long been recognised as a secondary gene centre for barley (Lakew, et al., 1996) (see Section 1.2.3.1).

According to Harlan, de Wett and Price (1972) the process of domestication of barley itself can be ascribed to two major forces, automatic selection and deliberate human selection. Simply harvesting and planting seeds caused a number of adaptations to evolve such as non-shattering and uniform maturation and favoured increase in seed

production due to harvesting, greater seed size, lower protein and higher carbohydrate content as well as the loss of germination inhibitors, loss of dormancy, due to seedling competition in the field. Deliberate human selection included selection for larger heads, larger seeds, more seeds, better seed-set, daylength sensitivity, easier threshing but also colour, flavour, texture and storage quality. The result of these selection pressures are landraces grown for different purposes or to fit different ecological niches of the agricultural system (Harlan, et al. 1972; Simmonds, 1979). Due to domestication, phenotypic diversity of *H. vulgare* has increased significantly but although the domestication of barley has led to great variation it has not produced new species (Harlan, et al., 1973).

The discussion of genetic erosion today mainly refers to the loss of diversity which was created previously by domestication and cultivation. This loss of diversity is thought to be due to increased cultivation of a few inbred line cultivars selected from a narrow gene pool (Allard, 1992). But diversity has a second dimension, which is the diversity within the population. Allard (1992) showed that there is loss of diversity within barley populations due to domestication. In extensive experiments with experimental barley populations (composite crosses) over a period of more than 50 generations the allele number of about 25 loci decreased dramatically in the progression from primitive material to the most advanced modern cultivars (Allard, 1992; Allard, et al., 1992).

1.2.1.2 Cultivation and Use

Barley is the second most important cool temperate cereal in the world after wheat (Russell, 1990) and covers about 13% of the arable agriculture world-wide (Franke, 1981). The reasons for its importance include a short growing cycle, tolerance to salinity and dry conditions as well as high yielding capacity under the cool and wet conditions of north west Europe. Barley is grown from near the equator in Ethiopia to beyond the Arctic Circle in Norway, and from below sea level in the Netherlands to the limits of cultivation at 5000m altitude in Tibet. It is cultivated where the

annual rainfall is too low or too erratic for satisfactory wheat yields (Russell, 1990). Ten million hectares in the arid regions of North Africa, Middle East and West Asia are cultivated with barley. Even under very dry conditions (200-250 mm annual rainfall) barley can produce a yield between 0.5 and 1.7 tons per ha (Wilson and Witcombe, 1984). The high adaptability of barley is demonstrated in a number of known tolerances to stress conditions such as high salt concentrations in soil, high aluminium concentrations, high and low levels of manganese, low pH, low temperatures as well as high temperatures with low humidity (Duke, 1982).

Barley is used for human consumption, for the production of beverages, for feeding livestock and for other purposes. In the human diet it is still used for bread making (mainly in dry regions of developing countries), but mostly as pot-barley and grits in soups and pulps. An important use of barley is the production of beer and spirits, but in addition non-alcoholic beverages such as malt-drinks are also produced (Franke, 1981). The main use of barley grains is for feeding purposes of livestock and in some arid regions sheep graze barley fields in the late tillering stage and at maturity. Also straw and grain are used as dry forage or for grazing (Oosterom and Acevedo, 1992). Finally, the straw is used also for other purposes like upholstery material (Hailu-Gebre and Fekadu-Alemayehu, 1991).

1.2.1.3 Plant breeding

Plant breeding of the two most important cereals for high-input agriculture (wheat and barley) of developed countries is almost entirely based on the pedigree selection method (see Chapter 6), whose final product is a homogeneous and homozygous pure-line cultivar (Jensen, 1988). Homogeneous cultivars have considerable advantages, because they allow the use of modern technology by growers, processors, packers and consumers (Marshall, 1977).

Modern wheat and barley cultivars are selected for high yield at many trial sites under high input conditions and thus show a relatively wide adaptation to high input environments, but no local adaptation (Ceccarelli, 1996a). However, the lifetime of

these cultivars is relatively short, due to continuous improvement of newly released cultivars, but also because these cultivars are particularly prone to disease and pest epidemics (Marshall, 1977). Vertical resistances, which rely on single genes, are frequently back-crossed into advanced cultivars, but may become non-effective following the evolution of the pathogens (Simmonds and Smartt, 1999). Thus, horizontal resistances (also termed field resistances), which are commonly based on multiple genes and are less effective but more stable than vertical resistances, are gradually displaced from the gene pool. Most breeding activities in modern agriculture have been aimed at plant characters such as short straw, higher resistance to lodging and higher fertiliser response which enable greater inputs to be achieved when fertilisers and herbicides are applied (Harlan, 1995).

However, the merit of modern plant breeding seems questionable, when long-term observations of barley composite cross-populations (i.e. mixtures of crosses between many cultivars) at Davis, California, are considered. In these experiments, populations have been grown for more than 60 generations without directed selection and minimised drift in a local environment in California. This mixture, which is still highly variable, showed a steady yield increase over the years, due to local adaptation, which is about 95% of the yield increase that plant breeders have achieved over the same period of time (Harlan, 1995). This last observation may suggest parallels with BLRs, which are also highly variable, locally adapted populations.

1.2.3 Barley landraces

Until the late nineteenth century, all cultivated barley existed as highly heterogeneous landraces, which were mixtures of inbred lines and hybrid segregates, the products of a low level of random crossing in earlier generations. Over the last 100 years landraces have been almost wholly displaced in advanced agriculture by pure line cultivars (Nevo, 1992).

However, in many parts of the world, landraces are still in use and are favoured by local farmers, because they often perform more predictably under a range of conditions than modern cultivars due to local adaptation (Ceccarelli, 1996b).

Ramamoorthy, Chong and Chinnappa (1994) defined BLRs concisely as "populations of *H. vulgare* that have evolved under conditions of cultivation. They depend entirely upon manual harvesting and sowing due to their tough rachis." To emphasise the importance of local adaptation, the definition can be supplemented with a statement by Harlan (1975), who pointed out that "landraces have a certain genetic integrity. They are recognisable morphologically; farmers have names for them and different landraces are understood to differ in adaptation to soil type, time of seeding, date of maturity, height, nutritive value, use and other properties. Most important, they are genetically diverse. They are balanced populations - variable, in equilibrium with both environment and pathogens, and genetically dynamic."

1.2.3.1 Agronomic characteristics of BLRs

A number of publications deal with useful traits found in BLRs, collected in different regions. The most striking cases are perhaps the finding of resistance to barley yellow dwarf virus (BYDV) (Frankel, 1977) and genotypes with a high lysine content (Hiproly) in accessions from Ethiopia (Frankel, et al. 1995).

It seems to be noteworthy that another important resistance has been found in a landrace of peas (*P. sativum* var. *abyssinicum*) from a remote area in Ethiopia. This pea landrace showed resistance against root-rot of peas (*Fusarium solani* forma *pisi*).

Not unlike barley, the main region of genetic diversity of *Pisum* are the countries of the south-eastern Mediterranean Sea and Ethiopia (Frankel, 1977). The conditions on the high plateau of Ethiopia are generally favourable for plant diseases of all sorts and local races of barley and peas seem to have responded to these conditions by developing high levels of tolerance or resistance (Harlan, 1995). Harlan (1995) stresses that resistances are not only common in BLRs from Ethiopia but that many accessions have multiple resistances and some have resistances to all races of pathogens which have been tested.

Van Leur, Ceccarelli and Grando (1989) evaluated a number of BLRs collected in Syria and Jordan for resistance to yellow rust (*Puccinia striiformis* West), powdery mildew (*Erysiphe graminis* DC.), scald (*Rhynchosporium secalis* (Oud.) J.J. Davis) and covered smut (*Ustilago hordei* (Pers.) Lagerh.). They found a large diversity in resistance for each pathogen both between populations of different collection sites as well as between head progenies within each collection site. The level of resistance varied from absolute and partial resistant types up to highly susceptible lines. No consistent association between the environmental conditions of the collection site and the level of resistance of the landrace-line could be found. Jana and Bailey (1995) found higher levels of resistance or moderate resistance in wild barley than in cultivated landraces from Jordan and Turkey against the three pathogens of net blotch (*Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*) and spot blotch (*Cochliobolus sativus*), which indicates a loss of resistance due to cultivation. Jorgensen (1992) reports that the Mlo-powdery mildew (*Erysiphe graminis hordei* Em. Marchal) resistance, that has been mutagen-introduced to barley cultivars, has been found in a barley landrace collected in Ethiopia in the 1930's. He states the importance of the Mlo-resistance which is present in some spring barley cultivars in Europe that have been grown on 700,000 ha in 1990. Testing 958 barley cultivars for resistance against barley yellow mosaic virus (BYMV) Kawada (1991) found 79 varieties with high or complete field resistance. 28 of these barley varieties were landraces. This result indicates a high potential to isolate useful genotypes from landraces for plant breeding.

Minella and Sorells (1992) tested 37 barley genotypes of diverse genetic and geographical origins for aluminium (Al) tolerance. They found 8 genotypes with a high level of Al-tolerance of which 5 were ancestral landraces.

Grando, Grillo, Cecarelli and Acevedo (1988) examined the root number and root length of different barley populations. They found that improved barley cultivars had more seedling roots (4.8-5.8) than landraces (4.6-5.0) and *H. spontaneum* the least (3.0-4.1).

However, when assessing root length they found that improved varieties had the shortest roots (61-97mm) and *H. spontaneum* (77-124mm) and barley landraces had the longest roots (92-118mm). They interpreted these results in terms of a higher efficiency of water uptake and thus a higher yield stability of BLRs and wild barley under marginal conditions.

The feeding value of straw from barley cultivars and landraces was compared by Capper, Thomson and Herbert (1988) in Ethiopia. They found out that animals performed best and had highest milk production when fed straw from the landraces rather than the improved cultivars of barley.

Weltzien and Fischbeck (1990) tested Syrian BLRs for their performance under drought stress and salinity and found that the landrace lines outyielded the best control genotypes, which were modern cultivars. In a further experiment under marginal conditions in Syria, the yield of barley breeding lines ranged from total failure to 893 kg per ha. The range for landraces in the same experiment was 486 to 1076 kg per ha which implied a higher yield stability of BLRs (Ceccarelli, Acevedo and Grando, 1991). This last result leads to the next section in which the interaction of barley genotypes and the environment is briefly reconsidered.

1.2.3.2 Genotype × environment interaction

The majority of modern barley cultivars are inbred lines, produced according to the pedigree selection method. Due to selection for high yielding genotypes in multiple trial sites, these cultivars show a good performance in a number of environments, when grown under high input conditions. But, due to their homogeneity and high level of homozygosity they do not have the capacity to adapt much further. In contrast, landraces are adapted to their cultivation environment but have retained a high level of adaptability i.e. the capacity to adapt to new environments due to their genetic variation (Ceccarelli, 1994) and higher level of heterozygosity (Einfeldt, et al., 1996). Under stress and low-input conditions barley landraces can outyield

modern cultivars due to a higher yield stability (Weltzien and Fischbeck, 1990; Ceccarelli, 1994, 1996a, 1996b; Ceccarelli, Acevedo and Grando, 1991). Ceccarelli (1994) called this phenomenon the cross-over point of a genotype ×environment interaction. He recommended that selection has to be conducted for specific adaptation, if the target environment is below this point. These results suggest that BLRs may perform more stably under a range of conditions than modern cultivars (Ceccarelli, 1996) due to population buffering (i.e. the ability to compensate for the failure of a component by a genetically different component of the same population) based on a high level of diversity (Ceccarelli, Acevedo & Grando, 1991). In contrast, Frankel, et al. (1995) explain the higher yield stability under these conditions simply with the fact that the least productive genotypes tend to have the lowest variance in yield. Experiments carried out in Syria showed that selected lines from landraces surpassed the performance of the parent landraces under adverse conditions (Ceccarelli, 1994; Weltzien and Fischbeck, 1990), which might suggest that poorer components limit the productivity of the population as a whole (Frankel, et al.,1995). However, environmental conditions seem to influence the level of diversity of barley populations (Nevo 1992). Nevo, et al. (1997) reported for wild barley significantly higher levels of genetic diversity under stressful and variable environmental conditions than under more mesic conditions in Israel.

1.2.4 Population Structure of BLRs

The genetic structure of plant populations describes the behaviour of genes in populations and the evolution of populations under natural selection. It is based on population genetic theory, whose basic ideas are fundamental to plant breeding. This is because plant breeding is a special case of evolution, in which natural selection is replaced by a mixture of natural and artificial selection (Simmonds and Smartt, 1999). The genetic structure of populations can be given in the form of different genetic indices, quantifying the genetic diversity of individual populations and differentiation between populations of one species. Population structure includes the size and demographic composition of populations, their mating system, their degree

of isolation and rate and pattern of migration in space and time (Brown, 1978) and adaptation of populations to local environments.

Genetic markers (i.e. heritable phenotypic or genotypic characters of an organism which show quantifiable variation) are used to calculate genetic indices. The characterisation of agronomic traits of a population, as given above in Section 1.2.3.1, is a way to describe the population structure. For example, the resistance against a pathogen can be a genetic marker, and the ratio of resistant and non-resistant plants in a population could be a measure of diversity of the population, for this genetic marker. However, different genetic markers have different characteristics and may produce contrasting results. For this reason genetic markers are reviewed separately in Chapter 2. A detailed account of how population structure was analysed in this study is given in Chapter 5.

The analysis of the population structure of accessions of BLRs from different regions in Syria has been conducted to address questions with relevance to plant breeding and conservation. The knowledge of the population structure of BLR enables comparisons with the population structure of wild barley and modern cultivars in order to assess the impact of domestication, cultivation and plant breeding. The knowledge of the population structure may further help to make a choice about which landraces deserve priority for conservation.

1.3 Aims and objectives of the study

In 1983 Brown wrote that despite all that has been written about landraces, there is virtually no direct evidence available in cereals on their genetic structure. A number of reports on aspects of BLRs have been published since, but comprehensive studies with relevance to plant breeding and conservation are still lacking. This is the more surprising as landraces are still the main kind of genetic plant material used in low-input agriculture under marginal conditions in developing countries – conditions on which some 1.4 billion people are dependent (Pimbert, 1994, cited by Ceccarelli,

1996a). The high value of BLRs as a still widely unexploited source of resistances for plant breeding has been emphasised in Section 1.2.3.1. Furthermore, barley is the only major cereal which has no secondary gene pool (i.e. pool of all biological species that will cross with the crop but where gene transfer is difficult) (Harlan, et al., 1973; Marshall, 1990). Consequently, *H. spontaneum* and BLRs, which comprise the primary gene pool of barley (apart from cultivars), deserve a high priority in conservation.

Characteristics of BLRs as mentioned in the preceding sections can be summarised as follows. BLRs are diverse populations of cultivated barley, which may be locally adapted. They seem to be less diverse than wild barley, but more diverse than modern barley cultivars. BLRs are able to outperform modern pure-line cultivars under marginal conditions and show useful traits for future plant breeding which be worthy of being conserved. Despite this account, a larger number of questions regarding BLRs remain unanswered.

The present study was designed to focus on a number of open questions which are stated below.

(i) How generally diverse are present day BLRs from Syria and how is diversity of BLRs maintained?

(ii) How has domestication, cultivation and breeding influenced the mating system and the population structure of barley?

(iii) Is the population structure of BLRs correlated with environmental conditions and the heterogeneity of the environment?

(v) What are the implications of the population structure of BLRs in terms of breeding strategies for yield stability in unpredictable environments?

(vi) What are the implications of the population structure of BLRs for conservation strategies?

(iv) Which genetic marker methods are suitable to investigate the population structure of BLRs and how do results of different genetic markers compare?

Chapter 2

Suitability and efficiency of different genetic markers to characterise the population structure of barley landraces

2.1 Introduction

Genetic markers are heritable phenotypic or genotypic characters of an organism which show quantifiable variation. This variation represents differences in the genetic information within or between populations of organisms such as plants and can be used to describe their genetic structure. The genetic structure of populations, or simply population structure, can be measured in terms of gene diversity, arrangement of genetic variation within individuals in each population, and the distribution of genetic diversity among populations and regions (Ennos, 1996). Variation in genetic marker loci can be detected on the DNA level (e.g. RFLP, SSR) or as primary products (e.g. isoenzymes) or secondary products (e.g. alkaloids, pigments) of genes (Ennos, 1996; Mallet, 1996).

Each genetic marker system is characterised by a unique combination of advantages and disadvantages. However, the choice of a marker system is dictated to a significant extent by the question under consideration (Birren and Lai, 1996). The ideal genetic marker to characterise the population structure of plant populations should be highly polymorphic, highly heritable i.e. unaffected by environmental variability (Bretting and Widrlechner, 1995), selectively neutral (Ennos, 1996), simply inherited (i.e. single Mendelian genes with codominant alleles) and marker loci should be well dispersed throughout the genome. Further, the marker should be amenable to scoring, using rapid, inexpensive and technically simple but reliable methods (Bretting and Widrlechner, 1995). Genetic markers should allow recognition of genetic homologies and determination of genetic distance and diversity parameters (Gepts, 1993).

Literature on genetic markers used to characterise the population structure of barley is substantial. A summary of these references is attached as Appendix 1.

The evaluation of the resources of BLRs and its wild progenitor *in situ* and *ex situ* was fuelled in the early 1980s when warnings about narrowing of the genetic base of major crop species due to modern breeding methods (Frankel, 1977, Marshall, 1977) prompted scientists to rethink strategies in genetic conservation and plant breeding. Most of the work on BLRs from the Near East and Asia (Iran, Syria, Jordan, Nepal, Bhutan, Yemen, Cyprus, Turkey, Crete (Greece), Ethiopia and Korea) was carried out using qualitative and quantitative morphological markers, mainly to assess the

agronomic value of BLRs and to find disease resistances (Murphy and Witcombe, 1981; Weltzien, 1982, 1988, 1989; Damania, et. al, 1985; Ceccarelli, et al., 1986; Van Leur, et al., 1989; Kim, et al., 1991; Abay and Cahalan, 1995; Hadjichristodoulou, 1995; Jana and Bailey, 1995; Lakew, et al., 1997). The suitability of starch gel electrophoresis (SGE) of isoenzymes and polyacrylamide gel electrophoresis (PAGE) of storage proteins was initially tested using cultivars of barley; electrophoresis of isoenzymes (Kahler and Allard, 1970; Allard, Kahler and Weir, 1972; Clegg, Allard and Kahler, 1972; Brown, 1978, 1983; Kahler, Heath-Pegliuso and Allard, 1981; Brown and Munday 1982; Jana and Pietrzak, 1988; Konishi and Matsuura, 1991; Nevo, 1992; Konishi, et al., 1993; Nevo, Appelbaum-Elkaheret, Garty and Beiles, 1997) and storage proteins (Doll and Brown, 1979; Ohms and Paradies, 1985; Asfaw, 1989; Nevo, 1992; Shewry, 1993) were consequently used for evolutionary studies and population genetic studies of wild barley (e.g. from Israel, Turkey, Jordan and Iran) and BLRs (e.g. from Turkey, Crete, Jordan Bhutan and Ethiopia). With the development of molecular methods such as restriction fragment length polymorphisms (RFLP) in the early 1980s a new era began. This time, as well as cultivars, scientists included BLRs and wild barley (e.g. from Israel, Iran, Jordan, Turkey, Syria) in their analyses to assess this method for population genetic and evolutionary studies in barley. RFLPs were used to investigate sequence length polymorphism of nuclear DNA (Zhang, et al., 1993), ribosomal DNA (rDNA) (Saghai-Maroof, et al., 1984; Saghai-Maroof, Allard and Zhang, 1990; Ramamoorthy, et al.,1994) and organelle DNA including mitochondrial DNA (mtDNA) (Holwerda, et al., 1986) and chloroplast DNA (cpDNA) (Clegg, et al., 1984; Neale, Saghai-Maroof, Allard, Zhang and Jorgensen, 1986; Holwerda, Jana and Crosby, 1986) after cleavage with restriction enzymes. With the invention of the polymerase chain reaction (PCR) in the 1980s (Mullis, Faloona, Scharf, Saiki, Horn and Ehrlich, 1986) the amplification of DNA sequences became very simple and a whole string of molecular methods were developed (e.g. Sequence Tagged PCR (ST-PCR); Cleavable Amplified Polymorphic Sequences (CAPS); Random Amplified Polymorphic DNA (RAPD); Amplified Fragment Length Polymorphism (AFLP), Single Sequence Repeats (SSR) also called microsatellites, Inter Single Sequence Repeats (ISSR), and combinations of methods

like Random Amplified Microsatellite Polymorphism (RAMP) which is a mixture of RAPD and ISSR). The use of these methods to characterise barley populations was mainly started using cultivars (*ST-PCR*: Tragoonrung, Kanazin Hayes and Blake, 1992; Ko, Weining and Henry, 1996; *CAPS*: Kanazin, Ananiev and Blake, 1993; *SSR*: Becker and Heun, 1995; Donini, Bryan and Koebner; 1998, *AFLP*: Ellis, Mcnicol, Baird, Booth, Lawrence, Thomas and Powell, 1997; *RAMP*: Sanchez de la Hoz, Davila, Loarce and Ferrer, 1996). Some methods have now been assessed for their use in barley population genetics and evolutionary studies using wild barley and BLRs. Such methods are CAPS (Lashmers, et al., 1993), PCR and sequencing of rDNA (Baum and Johnson, 1994), RAPDs (Tinker, Fortin and Mather, 1993; Gonzales and Ferrer, 1993) and SSR (Saghai Maroof, Biyashev, Yang, Zhang and Allard, 1994).

Although the ISSR method has been recommended for studies in population genetics (Wolfe and Liston, 1999) no studies on barley populations have been published so far. Thus, as part of the present study the ISSR method was evaluated for its suitability and efficiency to characterise barley landraces genetically.

The effective multiplex ratio (EMR) has been proposed as an index to measure the efficiency of genetic markers. It is the number of different genetic loci that may be simultaneously analysed per experiment (i.e. per gel lane) (Birren and Lai, 1996). The EMR was used in the present study, in which the ISSR method was tested in comparison with two different genetic markers, namely seed morphology and SGE of isoenzymes. As reference material BLRs from Syria plus wild barley and modern European barley cultivars were used. The results of this assessment are given in Table 2.1. The characteristics of the genetic marker methods are discussed further in Chapters 5, 6 and 8. The following assessment of the individual genetic markers used (Section 2.2 to 2.4) is based on published data.

2.2 Morphological traits as genetic markers

Morphological characters are phenotypic traits that can be divided generally into those that show qualitative and quantitative variation. Most traits of agronomic interest show quantitative variation (e.g. yield) and, less frequently, qualitative

morphological variation (e.g. seed colour). The main purpose of conservation of genetic resources of crop plants is to maintain a gene pool for future plant breeding activities. Breeders usually evaluate barley accessions for agronomic traits (e.g. disease resistance, yield) or nutritional quality traits (e.g. protein quantity, starch quality) rather than for population genetic indices.

2.2.1 Qualitative morphological characters

Qualitative morphological characters show discontinuous variation, i.e. they have clearly distinguishable expressions such as presence or absence of spicules on awns. Inheritance of this variation is predominantly governed by single Mendelian genes. Qualitative morphological variation, such as that used in the present study (Section 3.3.2) is widely used in cultivar verification and cultivar purity trials of seed certification schemes (SASA, 1994) because variation is easily scored and highly heritable. The variation is not selectively neutral, i.e. it may be subject to selection. The use of morphological markers in population genetic studies is limited nowadays, mainly because they are in the majority dominantly inherited and selectively adaptive. However, qualitative morphological characters in barley are extremely well studied (Nilan, 1964), are easily assessed and can be used to calculate genetic diversity indices. They have been used in the past to calculate outcrossing rates in barley (Jain and Allard, 1960). Although it is not possible to apply a measure of efficiency like EMR to qualitative characters, assessment is by far more efficient compared with the use of molecular markers (e.g. the assessment of alleles at 4 loci takes less than two hours for about 100 individuals; personal observation) and is usually unambiguous and requires no specialist equipment apart from a magnifying glass.

2.2.2 Quantitative morphological characters

Variation in quantitative characters is continuous so that classification into discrete categories is arbitrary (Mayo, 1987). Genetic variation for quantitative traits (e.g. yield, drought resistance) is controlled by alleles at multiple loci and thus called

polygenic variation. Variation for such traits is also influenced by the environment and for this and the previous reason the causes of variation are often described as multifactorial (Hartl, 1988). Selection for quantitative traits is the main concern of plant breeding but they have some relevance for population genetics as well. Statistical methods of quantitative genetics allow the calculation of indices of genetic variation such as the coefficient of genetic variation (Lawrence, 1982, Houle, 1992) and recent computer programs (e.g. Popgene, Yeh, et al., 1997) include the possibility of analysing quantitative genetic variation.

2.3 Proteins as genetic markers

The barley grain consists of from 10% to 11% of protein, of which approximately half are storage proteins and half are functional proteins such as enzymes.

2.3.1 Starch gel electrophoresis of isoenzymes

Isoenzymes are structurally different molecular forms of an enzyme system with, qualitatively, the same catalytic function (Muller-Strack, 1998). Using electrophoresis (i.e. the migration of charged molecules under the influence of an electric field within a stationary liquid held upon a porous medium such as a starch or an agarose gel) (Allaby, 1992) under native conditions (pH 7.0 to 8.5 and low temperatures) in a starch gel, isoenzyme molecules can be separated according to differences in charge and size. The method is accurate enough to detect single amino acid substitutions visualised by means of enzyme-specific stains (Brown, 1983). However, the majority of substitutions are synonymous, i.e. they do not alter amino acids. Only 1/3 of mutations of the coding regions of isoenzymes will result in an amino acid change and thus in a detectable polymorphism. This is because there are 61 base triplets (i.e. codons) that code for particular amino acid, but since there are only 20 amino acids used for making proteins, there are many codons which code for the same amino acid (Nei, 1987).

The level of polymorphism detected with different isoenzymes can be different and show different pattern of diversity. Some isoenzymes (e.g. Est 1, Idh 2, Pdg 2) are

known to be more polymorphic in cultivated barley than in wild barley, while others (e.g. Est 2, Acp 2, Adh 2, Pgm) are more polymorphic in wild barley compared with cultivated barley (Brown, 1983).

Barley was among the first plant species to be studied using SGE of isoenzymes (Nilson and Hermelin, 1966, cited by Brown, 1983), because it combines a number features suited for a model organism. Barley is a diploid, predominantly self pollinated, annual crop plant of noticeable diversity which is adapted to a wide range of environments and has a single recognised wild progenitor (*H. vulgare* ssp. *spontaneum*) (Brown, 1983). For this reason a considerable number of publications exists on methodology and characterisation of individual isoenzymes for use in a wide range of evolutionary and population genetic studies, most of which were summarised in an extensive review by Brown (1983). Hamrick and Godt (1990) reviewed the results of 653 studies in which isoenzyme diversity of a total of 449 plant species were investigated and concluded the wide range of applicability of isoenzymes for evolutionary and population genetic studies. Isoenzyme markers are biochemical markers that allow the indirect detection of genotypic differences, although they are phenotypic markers. They are predominantly codominant markers mainly governed by single Mendelian genes (Bretting and Widrlechner, 1995). Depending on the chosen combination of isoenzymes the genome can be covered sufficiently as different isoenzyme loci have been mapped on all of the 7 barley chromosomes (Brown, 1983). The combination of mentioned features make isoenzymes a genetic marker with a very high information content. SGE of isoenzymes is relatively simple to perform, does not require very expensive equipment and shows a relatively high EMR value due to the ability to assess a number of loci on a single gel for up to 20 individual plants (Table 2.1). Nevertheless, there are some reports about disadvantages of the method. Whether variation of isoenzymes is selectively adaptive or neutral is an ongoing dispute (Műller-Starck, 1998). However, it seems that isoenzymes are not particularly subject to selection but rather may be associated with quantitative trait variation (e.g. drought tolerance) due to linkage disequilibrium (Brown, 1978).

2.3.2 Electrophoresis of storage proteins

Prolamin storage proteins of barley (hordeins) account for about 35-55% of the total grain protein in barley. Hordeins are alcohol soluble and can be cleaved at their interchain disulfide bonds into polypeptide subunits of 30-100 kDa molecular weight using 2-mercaptoethanol. The additional use of the detergent sodium dodecylsulfate (SDS) for the extraction of hordeins from seeds offers the advantage that all proteins are rendered soluble, negatively charged and randomly coiled so that polypeptide subunits are separated during electrophoresis solely according to their molecular weight (Gepts, 1990). Polymorphism within these subunits can be visualised using PAGE and subsequent staining with coomassie blue. A detailed description of the method is given in Appendix 9.

Hordeins can be classified into at least 4 groups (i.e. B,C, D and γ-hordeins) of which the B and C-hordeins express the highest polymorphism within populations (Shewry, 1993; Gepts, 1990). Other authors divide the C-hordeins further into two groups, i.e. high and low-molecular C-hordeins (Ohms, p.c. 1989) (see table Appendix 9.1). Each group represents a single locus with numerous alleles. The polymorphism of hordeins is remarkable and can be 10-30 times higher than the polymorphism present in isoenzymes (Doll and Brown, 1979). Hordeins may be used in marker assisted selection as some have found to be closely linked with the Ml-a locus, coding for powdery mildew resistance (Gepts, 1990). Hordein variation is codominant and under relatively simple genetic control. This means, on the other hand, a restricted genome coverage as all hordein loci are located on chromosome 5 within a stretch of 20 cM (von Wettstein-Knowles, 1992). A further limitation of the use of hordeins as genetic markers is the relatively small number of loci and the very complex structure of alleles. Up to 20 different alleles have been reported for hordein loci (Gepts, 1990) which can consist of multiple bands. Expensive image analysis software may be required to achieve meaningful results. Furthermore it is not clear whether hordeins are selectively neutral. Some authors explain the non random distribution of hordein polypeptide pattern in European cultivars as a "hitch-hiking" effect, because of human selection for closely linked resistance genes like Ml-a (Shewry, 1993), while others explain their strong population differentiation in term of a selectively adaptive hypothesis (Nevo, 1992, Doll and Brow, 1979; Gepts, 1990).

2.4 DNA markers

The plant genome consists of three parts, namely nuclear DNA, cpDNA and mtDNA, each of which evolves at a different rate (Bretting and Widrlechner, 1995). Specific DNA extraction methods allow the exclusive extraction of either nuclear DNA, mtDNA (White, Tatum, Tegelstrom and Densmore, 1998) or cpDNA (Burg, et al., 1998) and consequently separate analysis of these genome fractions. The faster a region of DNA evolves the more likely is the occurrence of polymorphisms or, inversely, the more conserved a DNA region, the less likely it is to find polymorphisms. Organelle DNA (cpDNA and mtDNA) is generally haploid and, in most angiosperms such as barley, inherited maternally (Holwerda et al., 1986). Because of the uniparental inheritance, organelle DNA evolves clonally and thus is more conserved than nuclear DNA. Therefore organelle DNA is of more interest for taxonomic and phylogenetic studies at higher taxonomic level (species, genera, families), rather than for population genetics (Burg, Fluch and Vendramin, 1998). Whereas Petit, Demesure and Dumolin (1998) state that mtDNA evolves at a much lower rate than cpDNA, Holwerda, et al. (1986) found that in barley mtDNA seems to be equally or slightly more polymorphic than cpDNA when analysing RFLPs. This discrepancy may be explained by the technique, genes used, or by large rearrangements within evolving mtDNA, which are detected by RFLP analysis (Holwerda, et al., 1986), while the base substitution rate, which may be detected by gene sequencing, is very low.

Ribosomal DNA of plants may be found in the nucleus and in organelles. However, rDNA in organelles may be more conserved than nuclear rDNA. Internal transcribed spacers (ITS) occur between regions coding for different rDNA genes and are more variable than genes themselves (Schlőtterer, 1998). As coding regions of plant nuclear DNA are more conserved than non-coding regions (Nei, 1990), SSRs of the former seem to be more conserved than SSRs of the latter (Becker and Heun, 1995).

A high level of polymorphism is desirable to study the population structure of single species. It is therefore recommended to use molecular markers which comprise DNA of less conserved regions (Bretting and Widrlechner, 1995).

With the introduction of molecular methods like RFLP and later PCR based methods a whole array of molecular genetic marker methods have been proposed. The utilisation of PCR has made it possible to amplify DNA fragments to easily detectable quantities. Two approaches are employed to quantify differences between DNA fragments of different individuals, sequencing and detection of length variation of amplified DNA fragments. Although sequencing is certainly the more accurate approach to detect mutations, it is presently too costly to investigate large sample numbers as required for studies in population genetics, and it is also sampling a very small proportion of the genome. Thus most of the molecular marker methods described below are based on the detection of length variation of genomic or amplified DNA fragments.

2.4.1 RFLP

DNA can be cleaved into fragments using restriction enzymes. Length variation of these DNA fragments between, for example, individual plants of a population are called restriction fragment length polymorphism (RFLP) and can be detected on DNA blots using radioactively labelled DNA probes that hybridise to a single target sequence in the genome (Birren and Lai, 1996). The molecular basis of length variations are single nucleotide changes, which may cause the loss of a restriction site (i.e. the DNA sequence which can be recognised and cleaved by the restriction enzyme), or, less likely, a site gain (Gepts, 1990). Frequencies of length variants can be used to calculate genetic indices. The level of polymorphism achieved with RFLP markers has been described as higher than the diversity found with isoenzyme markers (Gepts, 1990), but RFLPs may detect a different differentiation pattern between and within populations than isoenzymes. Zhang, et al. (1993) found that RFLP analysis resolved larger amounts of between population differentiation than isoenzyme assays. This may be a result of the maternal inheritance of cpDNA in barley, as RFLPs sample the whole genome, whereas isoenzymes are restricted to nuclear DNA. RFLPs are well dispersed throughout the genome (Gepts, 1990) and always codominant (Birren and Lai, 1996). These positive characteristics are the reason that RFLPs are still used, although RFLP analysis requires large amounts of

DNA (2 – 10μg) and involves the use of radioactively labelled probes. RFLP analysis has been characterised as cumbersome and costly and therefore of limited use for studies in population genetics, where large sample sizes are required (Gepts, 1990).

2.4.2 CAPS

Similar to RFLPs, cleavable amplified polymorphic sequences (CAPS) are DNA fragments attained through the use of restriction enzymes. However, in the case of CAPS the PCR method is used to amplify restriction fragments which are subsequently separated by electrophoresis on agarose gels. During electrophoresis, DNA fragments are separated by size and short fragments migrate faster than longer ones. The resulting banding patterns can be visualised under UV light after staining with ethidium bromide.

CAPS markers show a low to medium level of polymorphism (Lashmers, et al., 1993) and are well dispersed throughout the genome. CAPS markers may be either codominant or dominant, and may be selectively adaptive when associated with coding DNA regions (Birren and Lai, 1996). Because of the low level of polymorphism the method seems to be more suitable for evolutionary studies than for studies of population genetics (Tragoonrung, et al., 1992).

2.4.3 PCR amplification of specific DNA regions

PCR amplification of coding or non-coding regions achieved with specific primers can be used to reveal variation in either length or sequence of products. Specific primers are short, single-stranded DNA fragments, usually of 20-24 bp length which are complementary to conserved annealing sites of a defined sequence of a DNA template. The primers are extended on single-stranded denatured DNA templates, by an enzyme (i.e. DNA polymerase), in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. This results in the synthesis of new DNA strands complementary to the template strands and eventually in an amplification of the original DNA fragment (Newton and Graham, 1994). Characteristics of different

DNA regions have been described above (Section 2.4). For instance, analysis of sequences from ITS regions become increasingly interesting for both phylogeny reconstruction and population differentiation (Schlőtterer, 1998) but coding regions like the α-amylase gene family (Ko, et al., 1996) or the hordein gene family in barley (Kanazin, et al., 1993) were also used and produced some polymorphic banding pattern in barley. However, the polymorphism of those amplification products is low and genetic markers associated with coding regions are not necessarily selectively neutral. Due to stringent PCR conditions a high reproducibility of results can be anticipated, but interpretation of banding patterns might be difficult due to the complexity of gene families. Consequently Ko, et al. (1996) recommend the method for evolutionary studies rather than for population genetic studies.

2.4.4 SSR

SSRs (also termed microsatellites) are of tandemly repeated units, each between one and 10 base pairs in length (e.g. ATATATAT....). They occur very frequently and are well dispersed throughout the genome of plants and can be amplified in a PCR reaction using specific primers. SSRs are highly polymorphic, codominant Mendelian markers (Becker and Heun, 1995) which are found to be selectively neutral (Saghai Maroof, et al., 1994). They can be analysed as locus/allele systems as each SSR represents a single locus, and are therefore considered as being very informative. However, the analysis of SSRs requires some special equipment in order to visualise fragment length differences of a few to one single base pair. Technical improvements like the use of low melting point agarose and sequential sample loading (Donini, et al., 1998) as well as the availability of a large number of primers for the amplification of SSRs in barley may offer great opportunities for this genetic markers in population genetics (Saghai Maroof, et al., 1994).

2.4.5 ISSR

The ISSR method is based on length variation of amplification products of genomic DNA found between SSRs. Due to the high frequency of SSRs across the genome, it is highly likely to find two SSRs with a common motif, oriented on opposing DNA strands, within an amplifiable distance (< 3000 bp) and consequently to find a high degree of polymorphic bands (Wolfe and Liston, 1999). Primers used to amplify inter-SSR sequences are SSRs (e.g. ACACACACACACACAC) which anneal to complementary SSRs of the DNA. They can be anchored at either their 5' or 3' ends, and thus include amplification of the SSR or exclude it, respectively. Anchors are short sequences (i.e. 1 to 3 bases) which prevent the SSR primer from annealing arbitrarily to SSR target sequences in the DNA and thus increase stringency. ISSR markers are highly polymorphic, selectively neutral and are well dispersed throughout the genome. They are inherited as dominant or codominant genetic markers in a Mendelian fashion, and like RAPDs, must be interpreted as diallelic with alleles designated as *band present* or *band absent* (Wolfe and Liston, 1999). However, the use of an anchored SSR primers with a sequence length of 17 to 18 base pairs and annealing temperatures of about 5°C below the melting temperature of the primer results in high stringency and subsequently a high repeatability and reproducibility of obtained banding patterns (Tsumura, Ohba and Strauss, 1996). As the ISSR method combines most advantages of RAPDs with a better reproducibility of results it has been recommended for studies in plant population genetics (Wolfe and Liston, 1999).

2.4.6 RAPD

The RAPD method is based on length polymorphism found in the amplification products of the DNA fragments between pairs of short, inverted DNA sequences scattered throughout the genome (Birren and Lai, 1996). Short non-specific primers of about ten base pairs are used in a PCR to amplify DNA fragments between annealing sites of the primer. RAPDs are among the most widely used molecular markers to date as they are relatively easily accomplished without prior knowledge of DNA sequences. They show a high level of polymorphism, are selectively neutral

and are well dispersed throughout the genome. However, RAPDs are known to have some limitations. They are mainly dominant but can show up to 5% co-dominance (Wolfe and Liston, 1999). They cannot be interpreted as locus/allele systems, and hence must be interpreted as diallelic with alleles designated as "band present" or "band absent". RAPD markers do not necessarily follow a Mendelian mode of inheritance as they might originate from uniparentally inherited organellar genomes or may be caused by artifactual, nongenetic variation (Wolfe and Liston, 1999). The reproducibility and repeatability of RAPD assays is poor (Karp, Isaak and Ingram, 1998) mainly due to unspecific amplification of the short primers and the low annealing temperatures used (Wolfe and Liston, 1999). Moreover, homology between bands can be poor; up to 50% of bands of the same length can be non-homologies, i.e. they are not the same (Gillies and Abbott, 1998).
Despite the known shortcomings of RAPD markers the method is still recommended for use in population studies of barley (Gonzalez and Ferrer, 1993).

2.4.7 AFLP

AFLPs are genetic markers that have been generated by a combination of restriction enzyme digest of DNA, ligation of specific nucleotide sequences linked to the ends of the restriction fragments, followed by two rounds of PCR amplification using labelled primers based on the linked sequences (Wolfe and Liston, 1999). AFLPs are highly polymorphic, selectively neutral and well dispersed throughout the genome. The technique is highly reproducible and standardised kits are available. However, some authors state that AFLPs are codominant markers which are inherited in a Mendelian fashion provided they are derived from nuclear DNA (Wolfe and Liston, 1999), while others question their Mendelian inheritance due to artifactual polymorphic bands resulting from incomplete digestion and indicate that they are not necessarily inherited as codominant markers (Birren and Lai, 1996). Usually AFLPs are analysed like dominant markers with alleles designated as *band present* or *band absent* (Wolfe and Liston, 1999). Although the method is potentially very informative, it involves multiple steps, requires relatively expensive material and is more laborious than other methods (Birren and Lai, 1996). Powell, et al. (1994)

conclude that AFLPs have great potential for use in barley genome research and breeding.

2.5 Comparative overview of different genetic marker methods

A characterisation of the genetic markers summarised from information given in Sections 2.1 to 2.4, is shown in Table 2.1. The table was designed with reference to Birren and Lai (1996). The effective multiplex ratio (EMR) of SGE of isoenzymes and PCR of ISSRs was calculated as an average over all tested populations from results given in detail in Chapters 5 and 6. The EMR for morphological markers is not comparable and has only been given to emphasise the efficiency of the method. It was calculated as an average over all tested populations from results given in detail in Chapters 5 and 6. The EMR for SDS-PAGE of hordeins is based on the results of a preliminary study with a set of individual plants of 10 cultivars, 10 barley landraces and 10 individuals of *H. spontaneum* from two accessions in Syria. Details are given in Appendix 9. The sources of further stated EMR values are given in the table. The assessment of costs of methods and technical requirements are excluded from Table 2.1.

Table 2.1 Characterisation of genetic markers

	Seed morphology	SGE of isoenzymes	SDS-PAGE of hordeins	RFLP	CAPS	SSR	RAPDS	ISSR	AFLP
Abundance in the genome	medium	medium/ high	low	high	high	high	high	high	high
Level of polymorphism	medium	high	very high	high	medium	high	high	high	very high
Inheritance	Mendelian	Mendelian	Mendelian	Mendelian	Mendelian for nuclear DNA	Mendelian for nuclear DNA	not always Mendelian	Mendelian	not always Mendelian
Mode of Inheritance	dominant/ codominant	mainly codominant	codominant	codominant	codominant/ dominant for organellar DNA	codominant	dominant (< 5% codominant)	dominant/ codominant	mixed
Heritability	low for QTLs	high	high	high	high	high	n.i.	high	n.i.
Selectivity	adaptive	neutral/ adaptive	neutral/ adaptive	n.i.	neutral for non-coding DNA regions	neutral	neutral	neutral	neutral
Proportion polymorphic loci (*P 0.01*)	(0.75)	0.71	0.7	0.5 ①	n.i.	1.0 ①	0.3 ①	0.4	0.24 ①
Number of loci analysed per gel (*n*)	(4 per seed)	8	3	1.0 ①	n.i.	1.0 ①	13.8 ①	12.5	55 ①
EMR (*P 0.01* x *n*) ③	(3.0)	**5.7**	**2.1**	**0.5** ①	**low** ②	**1.0** ①	**4.1** ①	**5.0**	**13.2** ①

① Powell, et al. (1994); ② Birren and Lai, (1996); ③ EMR = effective multiplex ratio (*P 0.01* x *n*) (i.e. the number of different genetic loci that may be simultaneously analysed per experiment (e.g. per gel lane), EMR is a measure of the efficiency of genetic markers); n.i. = no information available

2.6 Discussion and conclusions

It is obvious that each genetic marker has a unique profile of features and that a high EMR does not necessarily qualify a genetic marker method for a particular purpose. It has to be considered that all molecular methods require the time consuming process of DNA extraction. To visualise RFLP, SSR and AFLP markers it might be necessary to use radio labelling or polyacrylamide sequencing gels. Most molecular markers (e.g. RFLP, CAPS, RAPD, SSR, ISSR) require a number of assays using different primers or restriction enzymes to achieve a sufficient number of polymorphic marker bands.

A high information content in population genetic studies is given when a genetic marker is highly polymorphic, follows a Mendelian, codominant inheritance and is selectively neutral. This is basically true for CAPS, RFLP, SGE of isoenzymes, SDS-PAGE of hordeins and SSRs. Of these, CAPS and RFLP show a very low EMR, and the latter is time consuming and requires a relatively high technical input. As shown in a preliminary study (Appendix 9), banding patterns of SDS-PAGE of hordein alleles are too complex to be analysed as codominant markers and represent only a very limited fraction of the genome. If time and costs are not a main factor, SSRs would be the ideal marker system in population genetics, but isoenzymes are probably the best achievable compromise for their relatively high EMR and information content. However, the use of advanced statistical methods may compensate for the lack of information content of dominant markers. Of those dominant markers with a high EMR (i.e. AFLPs, ISSRs, RAPDs), ISSR shows the best repeatability and reproducibility and a clearly Mendelian inheritance.

For these reasons, ISSRs and isoenzymes were chosen to investigate the population structure of BLRs from Syria. Additionally, morphological markers were included in the present study for two reasons: (i) they were easily assessed and (ii) they are clearly adaptive and provide a good contrast to the neutral ISSR marker and the isoenzymes which are selectively equivocal.

Chapter 3

Materials and Methods

3.1 Seed samples of barley

During the course of the project a substantial number of barley samples from different sources had been accumulated, including BLRs from my own collections in farmers' fields in Syria, BLRs from gene bank collections and BLRs sampled in rejuvenation plots at gene banks and at the International Center for Agricultural Research in Dry Areas, (ICARDA), Aleppo, Syria. For comparison of the results achieved with BLRs and to investigate the effect of domestication, two populations of wild barley were collected in Syria, where wild barley grows as a weed alongside fields of cultivated barley. A number of commercial cultivars were collected in order to define boundaries between them and landraces. F_2-bulk populations from crosses between commercial cultivars and F_3-bulk populations from crosses between commercial cultivars and lines from landraces were used to evaluate the inheritance of different genetic markers and to estimate the diversity of progenies of known origin. All samples were stored at room temperature until they were planted for experimental work.

3.1.1 Barley landraces

The basis for the experimental work in the present study was formed by four barley landrace accessions from Syria that had been passed on by ICARDA to SAC in 1994 in a request to investigate the biochemical profiles of these accessions. Previous studies at ICARDA in Syria showed a high level of variability for the four accessions between and within collection sites for morphological, agronomic and developmental traits (Ceccarelli, Grando and Van Leur, 1986; Weltzien, 1989) as well as for disease resistance (Van Leur, Ceccarelli and Grando, 1989). Two each of the four accessions were white-seeded Arabi Abiad (Abiad 1 and 3, Table 3.1.1) and the others black-seeded Arabi Aswad (Aswad 1 and 2, Table 3.1.1). Arabi Abiad and Arabi Aswad are both two-rowed barley and have a mild vernalization requirement. Local populations of both landraces are grown throughout Syria. The white-seeded landrace Arabi Abiad (i.e. white Arabian) is grown in slightly more favourable environments and the black-seeded landrace Arabi Aswad (i.e. black Arabian) in harsher environments (Ceccarelli, 1989). The four samples were single head

progenies of accessions of an original collection undertaken by ICARDA in 1981 (Weltzien, 1982). The original samples consisted of 100 single ears, taken from individual plants each two steps apart. Single ears were multiplied twice at ICARDA's main experimental station Tel Hadya without any spatial isolation and kept as separate lines. Between 59 and 82 lines of these accessions survived and subsamples of each line were sent to SAC. Details of the collection sites are summarised in Table 3.1.1 and the location of accessions is shown on Plate 3.1.3. In 1997 two more accessions of Arabi Aswad (Aswad 3 and 4, Table 3.1.1) and one more accession of Arabi Abiad (Abiad 3, Table 3.1.1) were collected in farmers' fields with the assistance of ICARDA staff with the objective of covering a wider range of eco-geographical environments. Each of the collected accession consisted of 200 single ears from individual plants which were a minimum of two steps apart. The location of all accessions collected in 1997 are shown on Plate 3.1.3 and details of the collection sites are listed in Table 3.1.1.

Later in 1997 it became apparent that the Scottish Crop Research Institute (SCRI) in Dundee, Scotland was working on a similar set of accessions from ICARDA in order to investigate the diversity of accessions using a molecular marker system (microsatellites). To avoid conflicts the original outline of the present study was revised and more emphasis placed on microclimatic differences found for one single landrace (Arabi Aswad). A further collection of accessions of Arabi Aswad was undertaken *in situ* in 1998 by ICARDA staff at eight locations in the Aleppo region. The locations covered four distinct rainfall zones and two replications each were collected (Aswad 5 to 12, Table 3.1.1; Plate 3.2.2 and Plate 4.2.2 in Chapter 4). The distance between replications was 3km to 6km. Accessions consisted of 50 to 74 single ears, collected in farmers' fields from individual plants, which were at least two steps apart. The identity of all collected landraces was confirmed by ICARDA staff. Photographs of seeds samples of the two landraces Arabi Abiad and Arabi Aswad are shown on Plate 3.1.1.

Plate 3.1.1 Photograph of seed samples of black seeded Arabi Aswad (left) and white seeded Arabi Abiad (right)

3.1.2 Wild barley

In 1997 two populations of *H. spontaneum* were collected *in situ* (designated *H.spon1* and *H.spon2*, Table 3.1.1) at two locations in Syria alongside barley fields, where population size was sufficiently large to obtain samples of at least 100 individual plants. The identity of sampled plants was confirmed using a taxonomic key (Davis, 1985). An example of brittle ears of black and white seeded wild barley accessions from Syria are presented on Plate 3.1.2. The precise locations of the accessions are shown in Plate 3.1.3 and details of the collection sites are listed in Table 3.1.1.

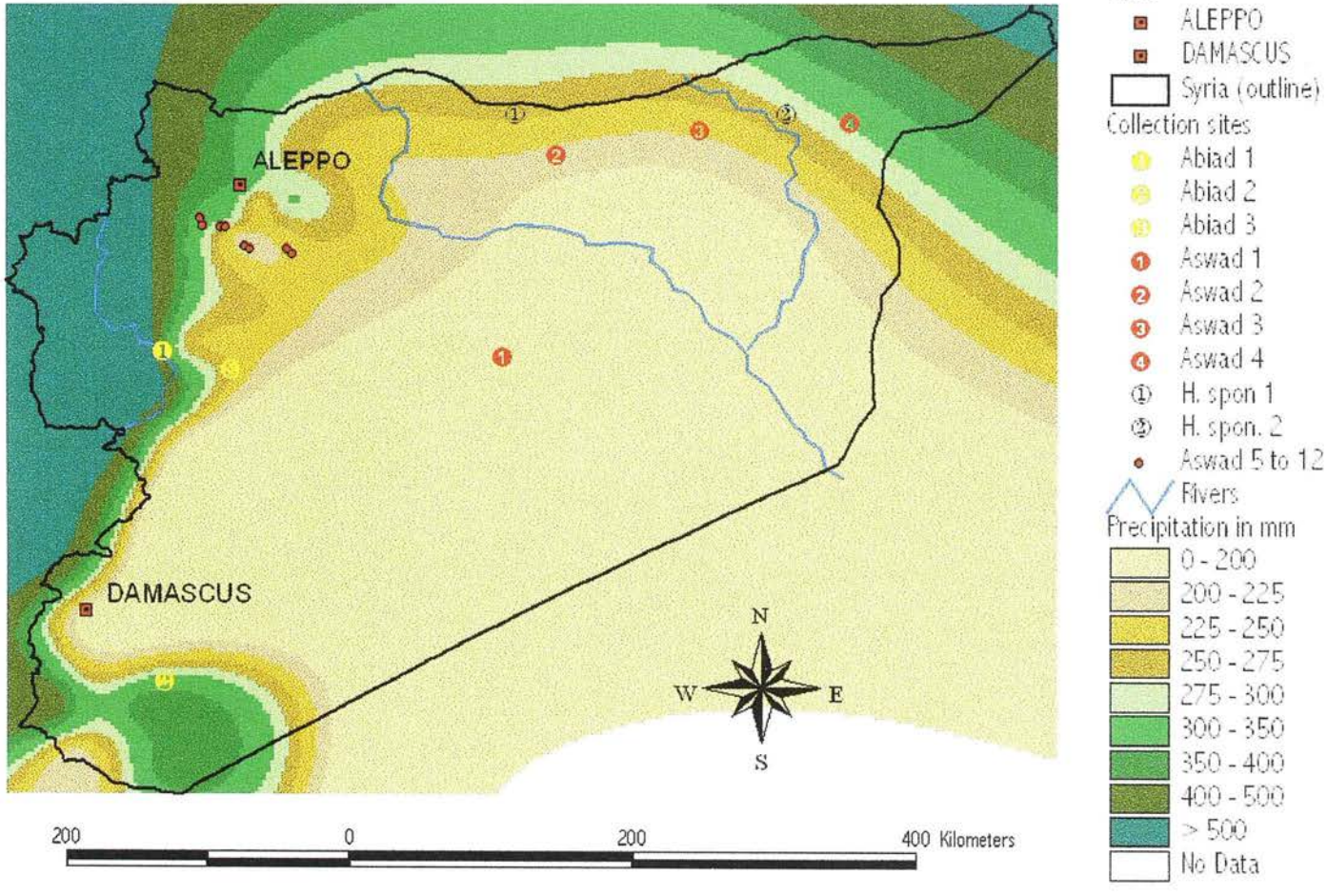
Plate 3.1.2 Example of brittle ears of black and white seeded wild barley accessions from Syria.

Table 3.1.1. Details of 12 barley landrace accessions and 2 accessions of wild barley (*H. spontaneum*) that were used in the study.

Accession:	Collection Site in Syria: Region:	Location:	Longitude E	Latitude N	Altitude (meter)	Source:	Year of Collection:	No. of seed heads collected	Number of multiplication cycles
Arabi Aswad:									
Aswad 1	Palmyra		38.88	35.10	550	ICARDA	1988	58	2
Aswad 2	Raqqa		39.22	36.38	480	ICARDA	1988	82	2
Aswad 3	Hassakeh	West of Hassakeh	40.12	36.53	296	own collection	1997	100	-
Aswad 4	Quamishli	Tal Birak	41.08	36.58	n.i.	own collection	1997	200	-
Aswad 5	Aleppo	Tal Bagar	36.98	35.93	330	ICARDA	1998	74	-
Aswad 6	Aleppo	Banus	36.97	35.98	360	ICARDA	1998	65	-
Aswad 7	Aleppo	Soaibieh	37.10	35.92	330	ICARDA	1998	50	-
Aswad 8	Aleppo	Abu-Rowail	37.13	35.92	342	ICARDA	1998	54	-
Aswad 9	Aleppo	Khanasir	37.52	35.78	355	ICARDA	1998	51	-
Aswad 10	Aleppo	Im Mial	37.55	35.75	365	ICARDA	1998	80	-
Aswad 11	Aleppo	Sowaiha	37.25	35.80	303	ICARDA	1998	53	-
Aswad 12	Aleppo	Om-amood	37.28	35.78	324	ICARDA	1998	52	-
Arabi Abiad:									
Abiad 1	Hama	Hama	36.72	35.13	309	ICARDA	1988	62	2
Abiad 2	Suweida	Suweida	36.73	33.03	850	ICARDA	1988	68	2
Abiad 3	Bural Sharqui	Bural Sharqui	37.15	35.02	350	own collection	1997	200	-
H. spontaneum:									
H.spon. 1	Tal Abiad	Tal Abiad	38.95	36.63	349	own collection	1997	100	-
H.spon. 2	Hassakeh	Hassakeh	40.67	36.63	296	own collection	1997	100	-

n.i. = no information

Plate 3.1.3 Sampling sites of BLRs and *H. spontaneum* populations in Syria



3.1.3 Cultivars and breeders material

Seed samples of 20 modern two-row barley cultivars were acquired from the SAC collection, from ICARDA and from two plant breeding companies (Saatzucht Hadmersleben, Germany and Nickerson Seeds, GB). (Details in Table 3.1.2). All but one of them (Igri) were spring cultivars. Sample size ranged from 250g to about 1000g. Only 20 to 30 grains were available from the four seed samples of cultivars obtained from ICARDA (Chebec, Memosa, Hart and Puffin). These had been maintained at Tel Hadya for use in experimental crosses with lines that had been selected from BLRs. 100 single heads of each of three of these lines (Arta, Tadmor and Zambaka, Table 3.1.2) were collected from multiplication plots of 3 m^2 size at Tel Hadya. These lines had been selected for superior yield and were supposed to be homozygous.

Table 3.1.2. Details of barley cultivars and selected lines from barley landraces that were used in the study.

Name:	Description:	Source:	Country:	Breeder:	Year registered:	Sample size:
Commercial cultivars:						
KRONA	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	1990	200g
SCARLET	S 2	Saatzucht Hadmersleben	Germany	Breun	1995	200g
CAMARQUE	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	1986	200g
TRUMPF	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	1973	200g
RENATA	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	n.i.	200g
POLYGENA	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	1994	200g
IGRI	W 2	SAC	Germany	Ackermann	1976	200g
ALEXIS	S 2	SAC	Germany	Breun	1986	200g
GOLF	S 2	Nickerson Seeds	UK	Nickerson Seeds	1982	200g
CAMEO	S 2	Nickerson Seeds	UK	Nickerson Seeds	n.i.	200g
CERTINA	S 2	IPK	Germany	Saatzucht Hadmersleben	1959	20 g
CERES	S 2	IPK	Germany	Saatzucht Hadmersleben	1959	20 g
MELTAN	S 2	Saatzucht Hadmersleben	Germany	Weibull	1992	150 grains
BRENDA	S 2	Saatzucht Hadmersleben	Germany	Semundo	1995	150 grains
BARONESSE	S 2	Saatzucht Hadmersleben	Germany	Nordsaat	1989	150 grains
HANKA	S 2	Saatzucht Hadmersleben	Germany	Saatzucht Hadmersleben	n.i.	20 grains
PUFFIN	S 2	ICARDA	n.i.	n.i.	n.i.	20 grains
CHEBEC	S 2	ICARDA	n.i.	n.i.	n.i.	20 grains
HART	S 2	ICARDA	UK	PBJ Cambridge	> 1980	20 grains
MEMOSA	S 2	ICARDA	n.i.	n.i.	n.i.	20 grains
Selected lines from BLR:						
ARTA	S/W 2	ICARDA	Syria	ICARDA	n.i.	100 ears
ZAMBAKA	S/W 2	ICARDA	Syria	ICARDA	n.i.	100 ears
TADMOR	S/W 2	ICARDA	Syria	ICARDA	n.i.	100 ears

n.i. = no information, * S = spring barley; W = winter barley; S/W = intermediate; 2 = two row barley

In addition 12 F_3 bulk populations of crosses between these three selected lines (Arta, Tadmor and Zambaka) and four cultivars (Chebec, Memosa, Hart and Puffin) were also collected in multiplication plots at Tel Hadya. 100 single heads of each of the 12 crosses were collected in multiplication plots of $3m^2$ size at Tel Hadya.

F_3 bulk populations in an autogamous species like barley are produced by crossing two homozygous parent lines and growing plants from the resulting F_1 seeds in a field plot. The harvested seeds from the F_1 plants are sown in a plot in the following generation to produce F_2 seeds. The plants that are grown from these F_2 seeds form an unselected segregating population, the F_2 bulk population. The consecutive generation of plants grown from F_2 seeds form the F_3 bulk population, and so on.

A further six samples of F2 bulk populations of crosses between modern cultivars (about 150 grains each) were supplied by Saatzucht Hadmersleben, Germany. Details of all cultivars and samples of breeders material used in this study are listed in Table 3.1.3.

Table 3.1.3. Details of bulk populations that were used in the study.

Name:*	Source:	Country:	Year of collection:	No. of seed heads collected:
F_3 bulk populations:				
HART x ARTA	ICARDA	Syria	1997	100
HART x TADMOR	ICARDA	Syria	1997	100
HART x ZAMBAKA	ICARDA	Syria	1997	100
PUFFIN x ARTA	ICARDA	Syria	1997	100
PUFFIN x TADMOR	ICARDA	Syria	1997	100
PUFFIN x ZAMBAKA	ICARDA	Syria	1997	100
CHEBEC x ARTA	ICARDA	Syria	1997	100
CHEBEC x TADMOR	ICARDA	Syria	1997	100
CHEBEC x ZAMBAKA	ICARDA	Syria	1997	100
MEMOSA x ARTA	ICARDA	Syria	1997	100
MEMOSA x TADMOR	ICARDA	Syria	1997	100
MEMOSA x ZAMBAKA	ICARDA	Syria	1997	100
F_2 bulk population:				
BARONESSE x SCARLET	Saatzucht Hadmersleben	Germany	1996	150 grains

* the name refers to the original cross (mother x father)

3.1.4 Gene bank accessions

In addition to the four Arabi landraces that had been stored for more than ten years (Arabi Aswad 1 and 2; Arabi Abiad 1 and 2) at ICARDA, two further samples were obtained from the BBSRC gene bank at the John Innes Institute in Norwich, UK (Arabi Aswad 20126, Arabi Abiad 20125). These accessions were originally collected by ICARDA and had been stored at the John Innes Institute since 1988. All six populations went through two rejuvenation cycles during their time in storage. Two seed samples of the Arabi landraces collected in 1959 and stored for 40 years were supplied by the All-Union Institute of Plant Industry (VIR) gene bank located in St. Petersburg, Russia (Arabi Abiad 18881, Aswad 18882). Finally, three seed samples that had been collected in Syria in 1926 and been stored for 72 years were available, two from the VIR gene bank (*H. vulgare* 7667 and 76590) and one from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany (*H. vulgare* 7400). The latter sample had originally been collected by VIR and was a subsample of *H. vulgare* 7667. It arrived at IPK before 1977 where it was classified as *H. vulgare* convar. *distichon,* which is a previously used term for two row barley. Unfortunately there was no further information about the accessions available, but it can be assumed that the original accessions collected by VIR were large since sub-samples were tested in extensive experiments at numerous locations (Vavilov, 1957). Details of all gene bank accessions used in the present study are listed in Table 3.1.4.

Table 3.1.4. Details of 7 gene bank accessions of barley landraces that were used in the study.

Designation:	Code:	Original collection site: Region:	Longitude E	Latitude N	Altitude (meter)	Source:*	Year of Collection:	Years stored *ex situ*:	Sample size:
Arabi Aswad	Aswad 20126	Syria	-	-		BBSRC/ICARDA	< 1988	> 10	88 grains
Arabi Abiad	Abiad 20125	Syria	-	-		BBSRC/ICARDA	< 1988	> 10	95 grains
Arabi Aswad	Aswad 18882	Syria	-	-		VIR	1959	40	> 200 grains
Arabi Abiad	Abiad 18881	Damascus	36.23	33.48	729	VIR	1959	40	> 200 grains
white BLR	White H.v. 7667	Aleppo	37.22	36.18	393	VIR	1926	73	112 grains
white BLR	White H.v. 7659	Homs	36.72	34.75	451	VIR	1926	73	200 grains
white BLR	White H.v./HOR7400	Aleppo	37.22	36.18	393	IPK/VIR	1926	73	62 grains

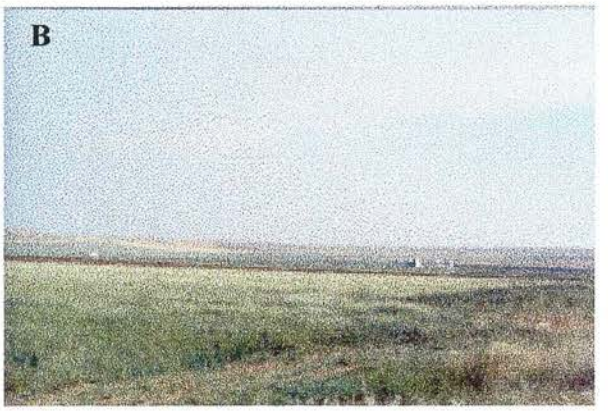
* see list of abbreviations

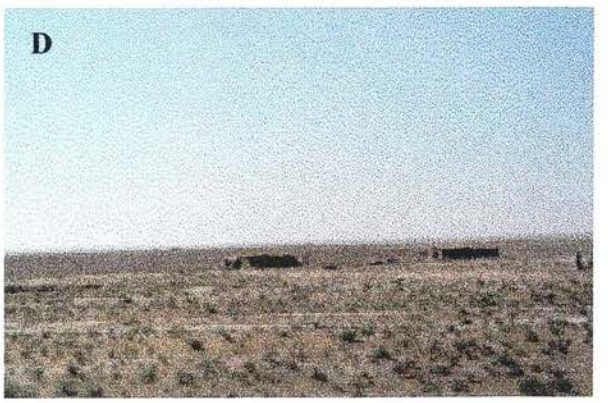
3.2 The study area

3.2.1 General overview of Syria

The Syrian Arab Republic is located in the Mediterranean region between longitude 35° and 42° and latitude 32° and 37°. It is bordered by Turkey to the north, Iraq to the east, the Mediterranean Sea and Lebanon to the west and Israel and Jordan to the south. Generally, the climate is characterised by cold and rainy winters and hot and dry summers. Rainfall distribution varies from regions with more than 1000mm of average annual rainfall to those with less than 200mm. Rain mainly falls between October and March, while the rest of the year has no considerable rainfall. Temperatures in winter can fall to –10° C in some areas for short periods while the normal range of temperatures in winter lies between 5° and 10° C. Temperatures in summer range between 25° and 30° C but may reach up to 40° C in some areas. Syria's surface area (185,000km^2) is divided eco-geographically into four main areas. (i) The small coastal area with a Mediterranean climate is plane, fertile and rich in terms of agriculture. (ii) The coastal mountain range (Plate 3.2.1 A) has a more Mediterranean climate with moderate to good, average annual rainfall (350mm to 800mm). (iii) The inland plain area (Plate 3.2.1.B) stretches along the east of the coastal mountain range and along the Turkish border. It has a semi-arid climate with low to moderate rainfall of 250mm to 350mm. In this area dryland farming of wheat, barley and legumes is still possible. Regions with more than 250mm of average annual rainfall (i; ii; iii) make up to 28% of the total surface area of Syria. (iv) The steppe (Plate 3.2.1 D) is located east of the inland plain. It makes up 55% of the total area of Syria and has a typical arid climate with very low rainfall of less than 200mm (FAO, 1996a, Brichambaut and Wallen, 1963). Agriculturally it can only be used for permanent grazing. The area between the steppe and inland plain (Plate 3.2.1.C) with 200mm to 250mm rainfall is used for agriculture but mainly barley can be grown due to its adaptability to drought. This area on the borderline between semi-arid and arid climate makes up 17% of the total surface area of Syria. Agriculture in this area is considered to be risky due to the scarcity of rainfall and frequent drought (FAO, 1996a). Yield of barley in this area will rarely exceed 500kg per ha (Anonymous, 1986) and total failure of harvest can occur in more than two out of ten years (Brichambaut and Wallen, 1963).

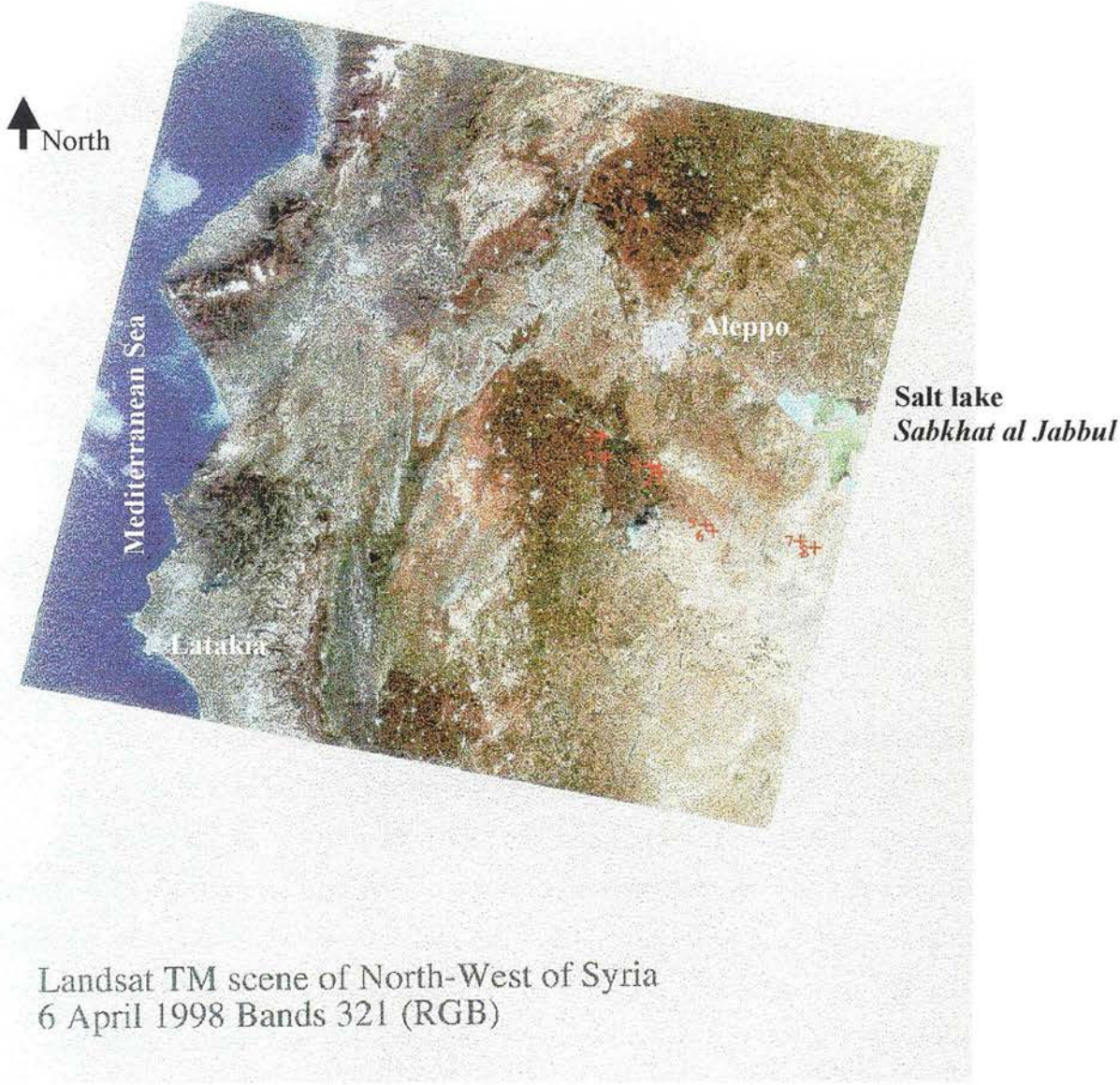
Plate 3.2.1 **A. Coastal mountain range; B. Inland plain; C. Area between steppe and inland plain, 200 – 250mm rainfall/year; D. Steppe.**





This area seemed particularly interesting for the present study as its environmental conditions are harsh and unpredictable and thus show a high level of heterogeneity. Plate 3.2.2 shows a satellite image (ICARDA, 1998) of this area from the 6th of April 1998, about 8 weeks before sampling of the landraces took place. The red crosses mark the sampling sites. From the satellite image it becomes apparent that the climatic conditions are getting gradually drier eastwards, towards the central of Syria as the colour of the surface changes from dark green over brown to yellow.

Plate 3.2.2 Satellite image of the Aleppo region of Syria, taken on the 6th of April 1998, about 8 weeks before sampling. The red crosses mark the sampling sites (x 1 = Aswad 5; x 2 = Aswad 6; x 3 = Aswad 7; x 4 = Aswad 8; x 5 = Aswad 11, x 6 = Aswad 12; x 7 = Aswad 10; x 8 = Aswad 9).



3.2.2 Characterisation of environmental conditions of the study area

Environmental conditions of the study area were characterised using the following indices: Elevation above sea level or altitude (*Alt*), average annual temperature (*T*) and average annual precipitation (*P*). The harshness of the environments was characterised using the average minimum temperature of the coldest month (T_{min}), the average maximum temperature of the warmest month (T_{max}) and percent continentality (*C*). The heterogeneity or diversity of the environmental conditions was measured in terms of relative interannual variation in rainfall (P_{cv}).

Continentality (*C*) was calculated according to Brichambaut and Wallen (1963) as

$$C = (1.3A/sin\varphi) - 36.3$$

where *C* is the continentality in per cent, *A* is the mean for the period of records of the annual range between mean maximum temperature of the hottest month (T_{max}) and the mean minimum temperature of the coldest month (T_{min}), and φ is the latitude.

The relative interannual variation in rainfall (P_{cv}) was calculated as the coefficient of variance of the average annual precipitation (*P*) over a period of *r* years. Brichambaut and Wallen (1963) calculated the interannual variability of rainfall relative to the mean ($1AV_{rel}$) as

$$1AV_{rel} = \frac{100 * \sum_{r=2}^{r=n} (P_{r-1} - P_r)}{\overline{P}(n-1)},$$

where *P* is the average annual precipitation, $\overline{P}$ is the average annual precipitation over *n* years, and the index *r-1* denotes the first year of recording.

Results of this formula (Lockwood, 1974) can take positive or negative values and change when the order of individual annual rainfall data changes. The basic concept of the formula is to divide the standard deviation by the mean of annual rainfall to achieve a value which gives the relative variability over years. For example under high rainfall conditions (e.g. $P > 1000$mm) a standard deviation (*SD*) of 100 does not have a great impact on yield but under low rainfall conditions (e.g. $P = 200$mm) a *SD*

of 100 would be devastating. In the first case the coefficient of variance would be as low as 0.1, but in the second case it would be 0.5 and would account for the higher relative variability. The coefficient of variance serves the same purpose as the $1AV_{rel}$ value but it is in contrast a unambiguous index and easier to compute. For this reason it seemed to be justified to use the coefficient of variance of the average annual precipitation (P) over a period of r years to calculate the relative interannual variation in rainfall and call it P_{cv}.

Individual annual precipitation data for 10 years (1977 – 1987) were available for 38 locations from the Syrian Ministry of Agriculture (Anonymous, 1986) and for 6 ICARDA experimental stations (10 to 19 years of observations) (ICARDA, p.c. 1999).

All meteorological data were compiled from three sources, ICARDA (p.c. 1999), FAO (1994) and the Syrian Ministry of Agriculture (Anonymous, 1986). The individual sources of data and individual values are given in Appendix 2.

Drought indices (e.g. Thorntwaite moisture index (Thorntwaite, 1948); Palmer drought severity index (Karl, 1986)) were not used in the study as their calculation requires the knowledge of the potential evapotranspiration (ETP), which was available for too few locations in the study area to carry out an interpolation with meaningful results. It was also not possible to consider differences in soil types of the sampling sites as too few data were available. Salinity did not occur in any of the collection sites. For 13 locations the average yield (1977 – 1986) for barley under rainfed conditions was available (Anonymous, 1986) and the values were used as an index for yield potential.

The spatial analysis of the computer program ArcView GIS, 3.0 (ESRI, 1996) was used to interpolate the environmental conditions for the collection sites of BLR samples and samples of *H. spontaneum* in Syria. The available meteorological data with their co-ordinates were entered into the program ArcView and were interpolated for the individual sampling sites. The procedure spline (tension, weight 0.1) was used to interpolate all data for the surface area of Syria, except for yield. The spline interpolator is a general purpose interpolation method that fits a minimum-curvature

surface through the input points. Conceptually, it is like bending a sheet of rubber to pass through the points, while minimising the total curvature of the surface. It fits a mathematical function to a specified number of nearest input points, while passing through the sample points. Only 13 data points existed for yield, therefore regularised spline (weight, 0.1) was used to interpolate these data. A lot of data points were available for the calculation of precipitation within the Aleppo region. For this reason the Inverse Distance Weighted (IDW) interpolator was used. IDW assumes that each input point has a local influence that diminishes with distance. It weights the points closer to the processing cell greater than those farther away. All points within a specified radius, can be used to determine the output value for each location.

Four maps showing the location of meteorological stations from which *(P)*, *(P_{cv})*, *(C)* and yield were interpolated as well as the sampling sites are given as Appendix 3. The co-ordinates of the sampling sites were supplied by ICARDA (Aswad 5 to Aswad 12) or estimated from the marks in the map when collection took place.

3.3 Genetic markers

3.3.1 General

The suitability and efficiency of different genetic markers was assessed in a preliminary study. Material and methods used in this study are given and discussed in a separate chapter (Chapter 2). As a result of the preliminary study three genetic markers were chosen, primarily because they cover the whole range from selectively neutral markers (inter-SSRs) over neutral to adaptive markers (isoenzymes) to clearly adaptive markers (morphology) and secondly because these three markers include co-dominant (isoenzymes) as well as dominant markers (inter-SSRs and morphology).

3.3.2 Morphological markers

Phenotype frequency was scored macroscopically and using a 1.5x stereomicroscope for four seed characters showing variation controlled by single genes (Nilan, 1964; Hockett and Nilan, 1985). The sample size ranged from 50 to 200 individuals. The characters, phenotypes and corresponding genotypes (Nilan, 1964; Hockett and Nilan, 1985) are summarised in Table 3.3.1.

Table 3.3.1 Seed morphological markers, their phenotypes and genotypes.

Seed character	Inheritance	Location on chromosome	Phenotypes (genotypes)
Lemma colour	dominant	5	white (b/b), grey (B_g/ B_g , B_g/b), brown (Bg/Bg, Bg /B_g, Bg/b), black (B/-).
Aleurone/ pericarp colour	dominant	4,5	white (bl/bl)(b/b), blue (Bl/Bl, Bl/bl, black (B/-).
spicules on lemma/awns	dominant	7	rough (r/r), smooth (R/-).
Rachilla hair length	dominant	7	short (s/s), long (S/-).

The colour of aleurone and pericarp were scored after removing the lemma of the seed. Pericarp colour and aleurone colour are controlled by two different genes (B and Bl, respectively). Because the pericarp colour may conceal a blue aleurone underneath, the two characters were scored as one locus with three alleles, black, blue and white, with black being dominant over blue, blue being dominant over white as proposed by Nilan (1964). All these characters have been used before to assess genetic diversity in barley (Brown & Munday, 1982; Allard, 1992). Allele frequencies were estimated on the assumption that all individuals are completely homozygous so that for these dominant markers, allele frequencies are equal to observed phenotype frequencies. This assumption is supported by the results of a parallel analysis of multilocus outcrossing rates in ten of the populations used in this study (Arabi Aswad 3 to Arabi Aswad 12, Table 3.1.1). The outcrossing rate obtained in this study was 1.7% (see 5.2 for details) indicating a very low expected frequency of heterozygotes at inbreeding equilibrium.

3.3.3 Isoenzymes

Variation at eight esterase (EST, E.C.3.1.1.1) loci were analysed on a mean number of 44 individuals per accession (range: 15-55). Seeds were planted individually in compost in cells of a 96-cell planting-module-tray, watered and kept for three days at 3° C to break potential dormancy. After three days the modules were placed in the glass house at a minimum temperature of 15°C and seeds germinated on average within 3 days. The germination of all recently collected landraces and cultivars was between 97% and 100%. Older material (Arabi Aswad 1 and 2, Arabi Abiad 1 and 2) and some accessions from gene banks (see 3.1.1) had a lower germination which was not assessed for individual accessions. Between 4 to 7 day old coleoptiles were cut from the plants and crushed in 40µl of a 6mM solution of the reducing agent dithiothreitol (DTT). Extracts were absorbed into filter paper wicks, which were kept frozen until use. A modified protocol for starch gel electrophoresis based on the methods of Kahler & Allard (1970), Brown (1983), Kahler *et al.* (1981) and Cheliak & Pitel (1984), was used. Gels were prepared using 11% hydrolysed potato starch in a solution of 0.01M Histidine HCl, 0.28mM EDTA, pH 7.0 adjusted with 1M Tris. The electrode buffer contained 0.125M Tris (pH 7 adjusted with 1M anhydrous citric acid). Samples were inserted in a slot of the gels and run on cooled flatbed electrophoresis units (Pharmacia Multiphor II) for 5 hours at a current of 55mA. Staining of samples was carried out at 37°C in the dark in a solution of 100mg α-Napthyl acetate, 50mg β-Naphtyl acetate (both dissolved in 1 ml of acetone) and 100mg Fast Blue RR salt in 50ml of a 0.2 M Phosphate buffer (pH 6.4 adjusted with 1M NaH_2PO_4). A detailed protocol is attached as Appendix 4.

Methods described by Kahler & Allard (1970) and Brown (1983) were used to evaluate allele frequencies at eight different loci (Est1 to Est8). The loci Est1, Est2, Est4, Est5, Est6, Est7 and Est8 are identical to the loci A to G described by Kahler & Allard (1970). Loci Est6, Est7 and Est8 showed either a single band or a null allele. Kahler and Allard (1970) found in progeny tests for these loci that the banded alleles were dominant to the null alleles. In the present study a further esterase locus was found between Est2 and Est4. The locus was called Est2b and showed two frequent alleles (single band or null allele), and a further, very rare allele (one faster band) the latter occurred only in one accession of BLR Arabi Abiad at a frequency of 0.069.

No hybrid bands were detected, however, the ratio of the two frequent alleles in F_2 progenies (i.e. *band: null allele*) was 57:0.43 and not consistent to 3:1 which would be expected for dominant inheritance at this locus. More observations are needed to clarify the inheritance of this locus. The present results (see Section 5.3.2.2) imply that locus Est2b may be located on chromosome 3, like loci Est2 and Est4, with which Est2b showed very highly significant non-random associations in all accessions of BLR Arabi Aswad. It seems unlikely that Est 2b is identical with Est3 because the latter has so far only been reported in embryo tissue, it migrates faster than Est2b in SGE, and it is located on chromosome 1 (Brown 1978; Hvid and Nielsen, 1977). A list of the eight loci and all recorded alleles is given in Table 3.3.2.

Table 3.3.2 Isoenzyme loci and observed alleles

			No. of alleles observed and (quoted)**			
Locus	Inheritance**	Location on* chromosome	Single band	Double bands	Triple and quadruple bands	Null allele
Est1	co-dominant	3	4 (4)	5 (6)	0 (0)	0 (0)
Est2	co-dominant	3	3 (3)	3 (3)	0 (0)	1 (0)
Est2b	not clear	presumably 3	2	0	0	1
Est4	co-dominant	3	2 (0)	1 (3)	0 (3)	0 (0)
Est5	co-dominant	1	2 (0)	0 (3)	0 (3)	1 (1)
Est6	dominant	3	1 (1)	0 (0)	0 (0)	1 (1)
Est7	dominant	2	1 (1)	0 (0)	0 (0)	1 (1)
Est8	dominant	n.i.	1 (1)	0 (0)	0 (0)	1 (1)

* Brown (1983); ** Kahler and Allard (1970); n.i. = no information;
Number in brackets give the number of different allele types quoted in the literature.

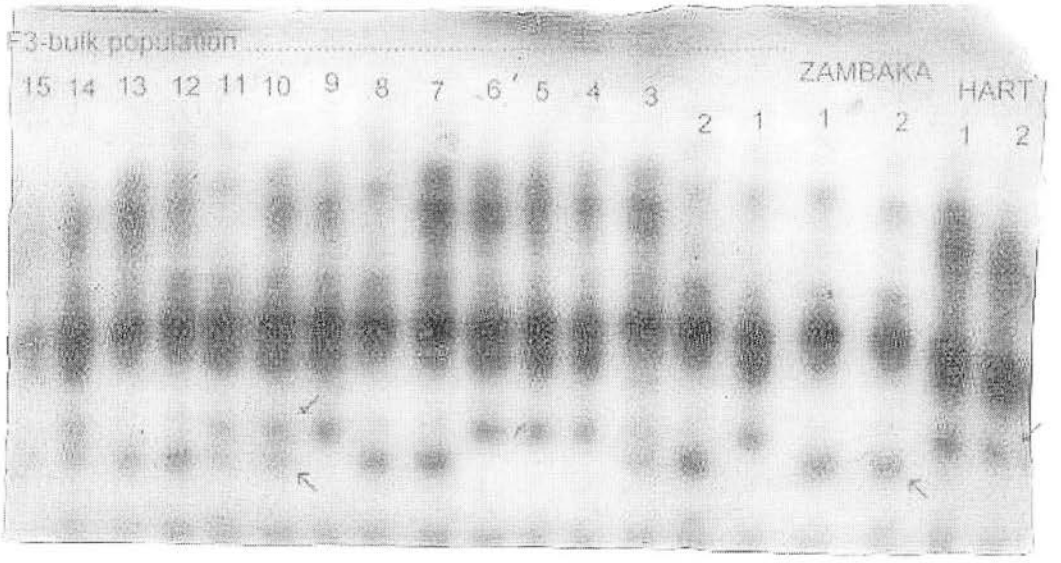
Repeatability of the method was tested by running 10 isoenzyme extracts of barley plants with different banding pattern with one week difference on different gels on two different electrophoresis units with different power supplies. Each of the 10 different leaf extracts gave identical banding patterns under both experimental conditions, apart from slight differences in the intensity of bands.

To calculate the multilocus outcrossing rate, variation was analysed at two codominant esterase loci, Est1 and Est2. Although the migration zones of these two loci overlap (bands of Est1 migrated between 0.2cm to 2.6cm, bands of Est2 migrated between 1.6cm to 3.9cm from the origin) heterozygous alleles were easily

detected as they were always double bands, so that 3 instead of the usual 2 bands appeared in the migration zone between 0.2cm to 3.9cm from the origin.

With successive scoring of gels assessment of banding pattern became clearer and easier. An example for the co-dominant locus Est2 is given in Plate 3.3.5.

Plate 3.3.5 Starch gel electrophoresis of esterases in barley coleoptiles. Example of co-dominance of Est2 in a F_3 bulk population between cv Hart and Zambaka. The heterozygous allele (double bands) in the segregating F_3 population is marked with two arrows, the two single bands in the homozygous parent lines are marked with individual arrows.



Per population 50 families (i.e. progenies of a single spike of a single plant) were analysed following the above-mentioned procedure, using a quantity of 6 individuals per family. Expressed alleles at Est1 and Est2 were scored and results were computed using the computer program MLT (Ritland, 1990). The data of ten populations of Arabi Aswad (Aswad 3 to Aswad 12, Table 3.1.1) were analysed and MLT calculated the multilocus outcrossing rate (t_m) as well as Wright's fixation index (F_{IS}).

3.3.4 Inter-SSR markers

20 inter-SSR primers (17 dinucleotide and 3 trinucleotide) were screened for polymorphism in barley populations of (Table 3.3.3).

Table 3.3.3 Characterisation and assessment of 20 inter-SSR primers.

Primer code:	Primer ① sequences:	Number of base pairs	Annealing temp.(°C) ②	Amplification	Number of polymorphic bands
	dinucleotide motifs:				
808	5'-$[GA]_8$-C-3'	17	45	good	11
810	5'-$[GA]_8$-T-3'	17	45	very good	5-6
812	5'-$[GA]_8$-A-3'	17	45	very good	14-16
814	5'-$[CT]_8$-A-3'	17	45	poor	> 2
818	5'-$[CA]_8$-G-3'	17	45	good	③
820	5'-$[GT]_8$-C-3'	17	47	very good	3
827	5'-$[AC]_8$-G-3'	17	47	good	> 5
828	5'-$[TG]_8$–A-3'	17	45	poor	③
830	5'-$[TG]_8$–G-3'	17	47	good	③
838	5'-$[TA]_8$–RC-3'	18	33	no amplification	--
840	5'-$[GA]_8$-YT-3'	18	48	good	③
841	5'-$[GA]_8$-YC-3'	18	50	very good	1
848	5'-$[CA]_8$-RG-3'	18	51	poor	1-2
850	5'-$[GT]_8$-YC-3'	18	50	very good	3
857	5'-$[AC]_8$-YG-3'	18	50	good	14
858	5'-$[TG]_8$-RT-3'	18	48	poor	1-2
888	5'-BDB-$[CA]_7$-3'	17	52	no amplification	--
	trinucleotide motifs:				
861	5'-$[ACC]_6$-3'	18	55	no amplification	--
1204	5'-BDV-$[CAG]_5$ - 3'	18	44	good	③
1425	5'-BDB-$[GAT]_5$ - 3'	18	53	good	> 8

① R=purine (A+G); Y=pyrimidine(C+T); N= any nucleotide; B= not A; D= not C; H= not G; V= not T; ② Annealing temp. = 5°C less than melting temperature; ③ All bands were monomorphic.

Primers originated from the University of British Columbia Biotechnology Laboratory and were obtained from IERM, University of Edinburgh. The objective was to find a primer combination which gave at least 20 reproducible polymorphic bands. Only two primers were needed to meet this target, and primer 808 with 11 polymorphic bands and primer 857 with 14 polymorphic bands (Table 3.3.3) were finally chosen because they showed the best repeatability and reproducibility of the achieved banding pattern. Repeatability was tested by amplifying DNA-extracts of four different barley accessions (1x cultivar, 2x BLRs and 1x *H. spontaneum,*

respectively) on two different thermocyclers (Biometra, Techne) and assessing the banding pattern of amplification products on agarose gels. Reproducibility was tested by amplifying two sets of DNA extracts of these four different barley accessions in two laboratories on the campus (at SAC, Department of Biotechnology and IERM, University of Edinburgh, Darwin Building) and assessing the banding pattern of amplification products on agarose gels. The whole procedure was repeated for both primers (808 and 857) which showed repeatable and reproducible banding patterns for the different four individual barley plants, apart from minor differences in the intensity of bands. DNA was extracted from second leaves of 10 to 20 day old barley plants. The mean number of individuals tested per accession was 44 (range: 15-50). Only leaf parts which were still covered by the first leaf were used in order to exclude fungal infection or insect colonisation. Extraction was carried out according to a modification of the method described by Doyle and Doyle (1986) and Khan (1997) (for details, used equipment and sources of chemicals see protocol II, Appendix 5). A leaf fragment of about 2cm length was crushed in a sterile mortar in liquid nitrogen, dissolved in 1 ml extraction buffer and poured into a sterile 2ml Eppendorf tube. The extraction buffer (see Table 3.3.4 and Appendix 5) contained the cationic detergent CTAB to solubilize the plant membranes and form a complex with DNA, as well as EDTA which chelates cations and thereby inhibits metal dependant enzymes such as DNase (Milligan, 1998). Sodium chloride was added to stabilise the nucleic acids in an isotonic medium, Trizma base to maintain the pH of the buffer and PVP-40 to decreases the effect of polyphenols, quinons and tannins, and finally the reducing agent DTT to protect the DNA against disulfides, peroxidases and polyphenoloxidases (Milligan, 1998).

Table 3.3.4 Components of the DNA-Extraction Buffer:

Concentration/Component
2% CTAB (Hexadecyltrimethylammoniumbromide)
100 mM TRIZMA Base
1.4 M Sodium Chloride
20 mM EDTA
3% PVP-40 (Polyvinylpyrolidone)
Adjust to pH 8.0 with 1M HCl
Autoclave
Add 0.1% DTT (DL-Dithiothreitol)

After 30 min incubation at 65°C, 750µl of a 24:1 Chloroform/IAA solution was added and the sample was mixed well and spun for 10min at 10,000 rpm. The supernatant was transferred into a sterile 2ml Eppendorf tube and 500µl ice cold Isopropanol was added so that precipitation was visible. The sample was mixed well and kept in the refrigerator for 15min at 4°C. After spinning for another 10min at 10,000 rpm a pellet was visible on the bottom of the Eppendorf tube. The supernatant was discarded and the pellet was washed with 500µl of a 70% ethanol solution. The ethanol was discarded and the pellet was dried in a laminar flow cabinet overnight by inverting the Eppendorf tube on a layer of sterile tissue. Next day the pellet was resuspended in 200µl of 1xTE-buffer and mixed gently 50 times. To degrade any RNA present in the sample 1µl of RNase was added and the sample was incubated for 1h at 37°C. Samples of one population were kept in the refrigerator at 4°C until the DNA concentration was determined. To confirm the presence of DNA in the extracts, 2µl of samples were mixed with 2µl of a sucrose based loading buffer and loaded on a 1.4% Agarose gel (prepared with 1x TBE buffer). One sample well was loaded with 2µl of distilled water plus 2µl of loading buffer (control), a further sample well was loaded with 2µl of a low DNA mass ladder to calculate the DNA concentration of the sample. Table 3.3.5 gives the DNA concentration of the bands of the used low DNA mass ladder in ng DNA per µl as well as the DNA fragment size of each band in bp.

Table 3.3.5 Characterisation of the DNA mass ladder used to calculate the concentration of extracted DNA

Fragment size	Amount of DNA in each band per 1µl
2000 bp	50 ng
1200 bp	30 ng
800 bp	20 ng
400 bp	10 ng
200 bp	5 ng
100 bp	2.5 ng

The gel was submerged in 0.5x TBE buffer in a electrophoresis unit and run at 6 Volt per cm gel length for 1h. After 1h the gel was stained in a 1% ethidium bromide solution for 30 minutes and the image of the gel was captured with a video capture system (Flowgen, IS1000) under UV light.

The DNA concentration of the DNA extracts was calculated using a densitometer analysis of the gel image. A calibration curve was drawn by plotting the peak area of bands with known DNA concentration of the low DNA mass ladder against the analogous DNA concentration. The DNA concentration of the sample DNA for which the peak area had been analysed with the densitometer was estimated from the graph.

To confirm the accuracy of this method the DNA concentrations of sample extracts were additionally determined using a spectrophotometer (260nm and 280nm of the UV spectrum). The spectrophotometer was programmed so that the reading of 1 at 260nm wavelength corresponded to a DNA concentration of 50µg/ml. The ratio of the reading at 260/280nm was used to assess the purity of the DNA. A high purity was presumed for values between 1.8 and 2.0, a value < 1.8 implied the possibility of presence of polysaccharides. After about 200 parallel measurements it was decided to rely on the calculation of DNA concentration using the DNA mass ladder as the obtained values were sufficiently accurate compared with the results obtained using the spectrophotometer. No significant impurities were detected in the samples. A concentration of 10ng/µl of DNA was achieved for later PCR amplification and samples were diluted accordingly using sterile deionised water (SDW).

For PCR amplification in a thermocycler a 25µl reaction mix was prepared in DNase-free 200µl flat-cap micro tubes using 1.5µl of DNA extracts (i.e. DNA template) (10ng DNA/µl), 9.87µl of SDW, 2.5µl of 10x PCR buffer, 4µl of dNTP-mix (0.2mM, for each of dATP, dCTP, dGTP and dTTP), 2.5µl of 25mM $MgCl_2$, 3µl of a 5µM primer solution, 0.13µl Amplitaq® (10 U/µl). The final concentration of this mixture was 0.6ng/µl DNA template; 1x PCR buffer; 32µM of each of dATP, dCTP, dGTP and dTTP; 2.5mM $MgCl_2$; 0.6µM primer , 0.05U of Amplitaq®.
PCR of the reaction mix was carried out in a Biometra thermocycler with heated lid. The program of the thermocycler is given in Table 3.3.6.

Table 3.3.6 PCR program for amplification using Inter-SSR primer

	Condition	No. of cycles
Hot start	94°C for 1.5 min	
Denaturation of DNA	94°C for 1.0 min	repeated for
Annealing of primers	50°C for 2.0 min	30 cycles
Extension	72°C for 1.0 min	
Final extension	72°C for 5.0 min	

Although the two primers 808 and 857 had different annealing temperatures (45°C and 50°C respectively) the same annealing temperature was used for both of them in the PCR process because the obtained amplification products showed good results on Agarose gels. Amplified samples were stored at 4°C. To visualise the amplification products 8µl of the amplified samples were mixed with 4µl of a loading buffer and loaded on a 2% Agarose gel (prepared with 1x TBE buffer). One sample well was loaded with 8µl control (amplified reaction mix without template) plus 4µl of loading buffer and another sample well was loaded with 4µl of a 100 bp DNA ladder to enable the estimation of fragment size of the amplification products. The gel was submerged in 0.5x TBE buffer in a electrophoresis unit (Flowgen) and run at 6 Volt per cm gel length for 2h. After 2h the gel was stained in a 1% ethidium bromide solution for 30 minutes and the image of the gel was captured with a video capture system (Flowgen, IS1000) under UV light.

11 polymorphic bands were identified in banding patterns of amplification products using primer 808 and 14 bands using primer 857. Bands of primer 808 represented DNA fragments of 770, 750, 640, 600, 490, 475, 415, 370, 330, 320 and 300 base pairs and bands of primer 857 represented DNA fragments of 880, 830, 790, 700, 640, 550, 460, 440, 420, 340, 320, 270, 240, and 220 base pairs. An example of banding patterns of individuals of accession Arabi Aswad 1 is given for both primers 808 and 857 in Plate 3.3.6 and 3.3.7, respectively.

Plate 3.3.6 Banding pattern of individuals of BLR accession Arabi Aswad 1 achieved with primer 808 (Inverse image, bp = base pairs, Variable bands were marked with arrows.).

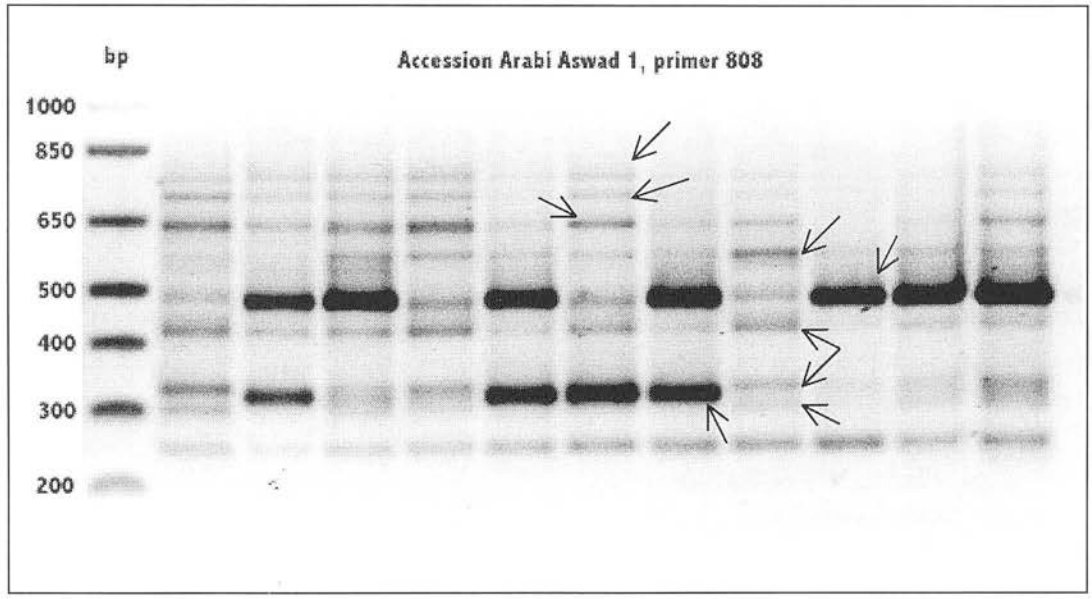
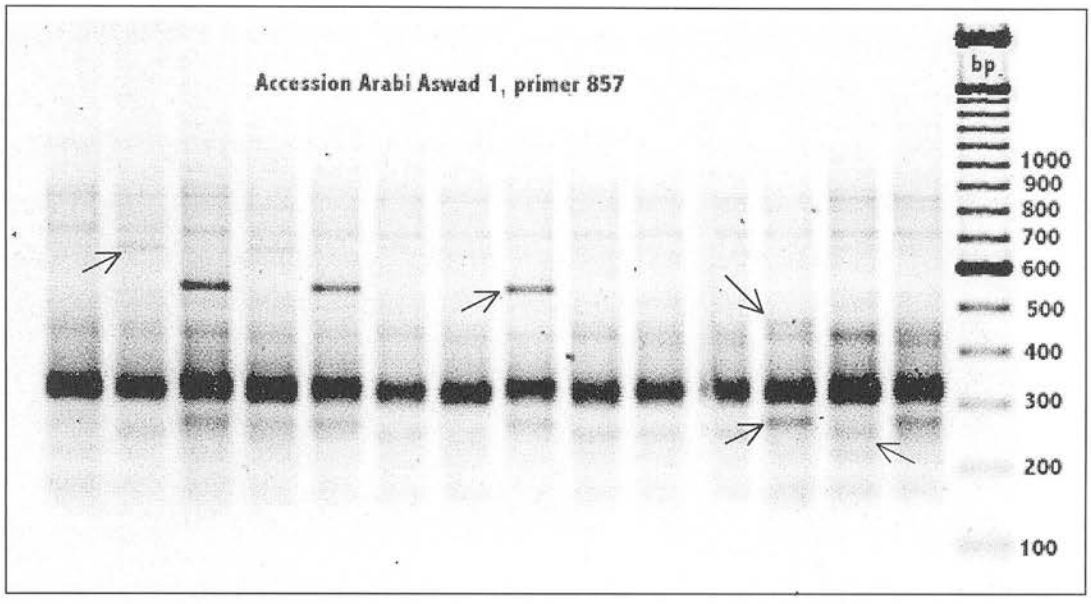


Plate 3.3.7 Banding pattern of individuals of BLR accession Arabi Aswad 1 achieved with primer 857 (Inverse image, bp = base pairs, Variable bands were marked with arrows.).



Presence and absence of bands was assessed visually and entered as '1' (present) and '0' (absent), or if uncertain as '.' (missing value) into data files to be analysed with the computer programme Popgene (Yeh, et al., 1997). Allele frequencies were estimated on the assumption that all individuals are completely homozygous so that for these dominant markers, allele frequencies are equal to observed phenotype frequencies, and that no outcrossing occurs.

3.4 Glasshouse experiment

A glasshouse experiment was carried out in 1999 to investigate the performance of 12 BLR accessions from different eco-geographical environments in Syria when grown under identical conditions in the glasshouse. From 10 accessions of BLRs the thousand grain weight (TGW) and the number of seeds per ear were assessed after collection in the field from about twenty individual plants per population. The original idea was to design an experiment that enables the calculation of heritability and the coefficient of genetic variation (Lawrence, 1982; Houle, 1992; Ennos, 1996). However, by mistake those plants grown for the glass house experiment were

progenies of different lines so that heritability and the coefficient of genetic variation could not be calculated and obtained results could only be interpreted on a population basis. However, 20 plants per population were grown in an randomised pot experiment in a glasshouse and TGW and number of seeds per ear were scored. Additionally the number of ears per plant, total yield per plant (in g) and the total number of seeds per plant were assessed.

Seeds were planted on the 12 of February 1999 individually in compost in cells of 96-cell planting-module-tray, watered and kept for three days at 3° C to break potential dormancy. After three days the modules were placed in the glass house at a minimum temperature of 15°C and seeds germinated on average within 3 days. On the 3rd of March 1999 twenty random plants per population were transplanted into multipurpose compost in pots of 18cm diameter and randomised within the glasshouse at Bush, the SAC experimental station. The glasshouse was not heated, to expose plants to low temperatures overnight in order to meet their mild vernalization requirements. Plants were watered regularly and treated twice (late April and mid June 1999) against mildew with the fungicide Tilt Turbo (active ingredient: Propiconazole: Tridemorph, 0.25:0.5g/l) and on the same dates against aphids using the insecticide Pirimor (active ingredient: Pirimicarb, 0.5g/l). In late April it was discovered that some labels were written with a non-light-resistant marker. Some labels were not readable any more so that about 10% of all plants had to be discarded. However, the number of replications for all populations was sufficient to analyse results statistically. From mid July onwards plants were no longer watered, and harvested individually on the 3rd and 4th of August. Ears per plant were counted and threshed using a single ear thresher (Wintersteiger). All seeds per plant were weighed and TGW was estimated from weighing 100 grains in three replications. The total number of seeds per plant was calculated as total yield per plant/TGW x 1000.

3.5. Statistical Analysis

Descriptive statistics, ANOVA and regressions as well as all graphs and figures were produced using the programmes MS-Excel 97 or Minitab 11.1. Genetic indices were calculated using Popgene (Yeh, 1997) and FSTAT (Goudet, 1999), or manually using formulas given by Hartl (1988), Nei (1972, 1973, 1987), Falconer and Mackay (1996), Weir (1996) and Kremer, Petit and Pons (1998). Morphological, isoenzyme genetic markers and ISSR markers were initially analysed separately since they may differ in important characteristics such as mutation rate and degree of selective neutrality (Bataillon et al., 1996).

Throughout the study genetic diversity was quantified in terms of mean proportion of polymorphic loci using the 1% criterion (*P(0.01)*), mean number of alleles per locus (*A*) and average gene diversity (*H*). Gene diversity (*H*) is defined as the probability that two variants taken at random in the population are different (Nei, 1987) and calculated as :

$$H = 1 - \sum x_i^2$$

where x_i is the population frequency of the *i*th allele at a locus. The average gene diversity (H_S) was calculated as the average of sub-populations (i.e. accessions) and the total gene diversity (H_T) denotes the gene diversity of the total population under consideration (i.e. all accessions combined) (Hartl, 1988). (*H*) of dominant markers can only take values between 0 and 0.5, while (*H*) calculated for co-dominant markers can take values between 0 and 1.

To enable analogous comparisons between co-dominant and dominant markers, Shannon's information index (*S*) was calculated as:

$$S = - \sum x_i \, ln(x_i),$$

where x_i is again the population frequency of the *i*th allele at a locus. (*S*) can take a maximum value of $ln(x_i)$ (Kremer, et al., 1998).

The observed heterozygosity (H_{et}) was given as the frequency of heterozygotes, based on direct counts (f_{ij}) with alleles i,j within a population, which was calculated according to Mallet (1996) as

$$H_{et} = \frac{1}{N} \sum f_{ij} \ .$$

The occurrence of alleles of different loci was classified according to Brown (1978) as widespread (occurrence in more than two regions), sporadic (occurrence in 2 regions) or localised (occurrence in only one region). Alleles were further classified as common, when they occurred at least in one population at a frequency of >10% and as rare, when they never occurred at a frequency >10 %.

Population differentiation was given as F_{ST} or G_{ST} (i.e. multiallelic F_{ST}) which was calculated according to Hartl (1988) as:

$$F_{ST} = (H_T - H_S)/H_T.$$

Population differentiation of dominant markers was expressed as theta (θ), as employed in the FSTAT programme (Goudet, 1999).

Genetic identity (I) and genetic distance (D) were measured according to Nei (1972) and as employed in the programme Popgene (Yeh, 1997). The calculation of genetic identity follows the formula:

$$I = \frac{J_{XY}}{\sqrt{J_X J_Y}}$$

with $J_X = \sum x_i^2$, $J_Y = \sum y_i^2$ and $J_{XY} = \sum x_i y_i$, where x_i and y_i are the frequency of the ith allele in population X and Y, respectively. Genetic distance is defined as:

$$D = - ln(I).$$

Gene flow was given as (Nm) which was calculated by the programme Popgene as:

$$Nm = 0.25\ (1 - F_{ST}) / F_{ST}$$

Linkage disequilibrium was measured in terms of the number of significant disequilibria ($p < 0.05$) between alleles of loci per population (Weir, 1979), as calculated by the programme Popgene.

Outcrossing was expressed in terms of the multilocus outcrossing rate (t_m) and the inbreeding coefficient F_{IS} as employed by the programme MLT (Ritland, 1990). The fixation rate at inbreeding equilibrium ($\hat{F}_{IS}$) is given by $S/2\text{-}S$, where the rate of selfing (S) is calculated as $S = 1 - t_m$ (Ennos, 1994).

The calculation of effective population size (N_e) was conducted under the following proposition (Crow and Kimura, 1970): in an isolated population of effective size N_e, gene diversity is expected to decline every generation as a consequence of genetic drift. Given an initial gene diversity H_o, after t generations the remaining value of gene diversity H_t is given by:

$$H_t = H_o(1 - 1/2N_e)^t,$$

$$\text{then } log(H_t) = log\ (H_o) + t.log\ (1\text{-}1/2N_e).$$

The slope of the regression of $\log(H_t)$ on t provides an estimate of $\log(1 - 1/2N_e)$ from which N_e can be calculated. Conducting this calculation it has to be considered that in an inbreeding species like barley the effective population size $N_e = 1/2N$, where N is the actual population size (Crow and Kimura, 1970).

Multilocus combinations (M) were calculated as the number of different allele combinations for the number of loci used of each genetic marker (Brown, Feldmann and Nevo, 1980; Zhang, Saghai-Maroof & Kleinhofs, 1993).

Significance levels were symbolised using asterisks. Significant differences ($p \leq 0.05$) where symbolised with '*', highly significant differences ($p \leq 0.01$) with '**' and very highly significant differences ($p \leq 0.001$) with '***'. No significant differences ($p > 0.05$) were symbolised with 'ns'.

A list of all used indices is given in Table 3.5.1.

Table 3.5.1 List of genetic indices used.

Index	Symbol	Reference
Mean proportion of polymorphic loci (1% criterion)	$P\ (0.01)$	Nei (1987)
Mean number of alleles per locus	A	Nei (1987)
Average gene diversity	H_S	Nei (1987)
Total gene diversity	H_T	Hartl (1988)
Observed heterozygosity	H_{et}	Mallet (1996)
Shannon's information index	S	Kremer, et al. (1998)
Multilocus combinations	M	Zhang, et al. (1993)
Population differentiation, Fixation index	F_{ST} or G_{ST}	Hartl (1988)
Population differentiation, theta	θ	Goudet (1999)
Genetic identity	I	Nei (1972)
Genetic distance	D	Nei (1972
Gene flow or isolation by distance	N_m	Hartl (1988)
Multilocus outcrossing rate	t_m	Ritland (1990)
Fixation rate at inbreeding equilibrium	$\hat{F}_{IS}$	Ennos (1994)
Inbreeding coefficient	F_{IS}	Hartl (1988)
Effective population size	N_e	Crow and Kimura (1970)

Chapter 4

Characterisation of environmental conditions of the study area

4.1 Introduction

The variation of environmental conditions has a spatial and a temporal dimension (Falconer, 1989), just like the genetic structure of populations (Brown, 1978). This analogy is not surprising, as plant populations tend to adapt to environments. Furthermore, environmental conditions can be heterogeneous over space and time and it seems as if plant populations adapt to these conditions with increased genetic heterogeneity. Stable polymorphism can arise in a population if one allele is advantageous in one environment and another in a different environment without heterozygotes necessarily being on average superior (Falconer, 1989). Nevo, et al. (1997) have shown that in a microsite in Israel (Mt. Carmel), which stretches over a distance of only about 400 m, extremely different environmental conditions are found (Nevo, 1995, 1997; Nevo, et al. 1997). On the south facing, xeric slope of the valley, African and Asian xeric tropical biota are predominant, whereas on the mesic, north-facing slope of the valley biota of temperate Europe are prevalent. Furthermore, diversity within populations of most species of all organisms tested displayed a higher diversity under xeric than under mesic environmental conditions. One of them was wild barley (Nevo, et al. 1997). Nevo et al. (1997) concluded, that higher genetic diversity found in plant populations, such as wild barley, was caused by the higher environmental heterogeneity found in the xeric part of the microsite. However, the climatic indices that they had considered (solar radiation, temperature and drought) are suited to describe climatic stress conditions, but not the heterogeneity of an environment.

Measures of environmental heterogeneity are not readily available and the designation of parameters as measures of environmental heterogeneity seems rather arbitrary. In the spatial dimension heterogeneity could, for example, be measured in terms of soil differences, occurrence of salinity and water potential of soils (FAO, 1996). But even if detailed soil maps existed (Ryan, 1997), there seem to be no standardised ways to describe the variability of soils in a given area. The description of temporal environmental heterogeneity seems to be somewhat easier, if long term observations are available. Climatic data such as precipitation and temperature may be used to calculate indices that describe fluctuations over time. Brichambaut and Wallen (1963) carried out a study of agroclimatology in semi-arid and arid zones of the Near East with the purpose of defining limits to dryland farming. They used

simple measures such as temperature and precipitation and combinations of those two to describe the environmental conditions, but had problems to describe the water balance sufficiently because too few measurements were available to calculate evapotranspiration for the whole region. They proposed using the interannual variation in rainfall as a measure of heterogeneity. The significance of rainfall variability in semi-arid and arid regions has been well recognised (Gommes and Petrassi, 1996). For Syria, precipitation data for a minimum period of ten successive years were available for 44 locations, so that a reasonably sufficient interpolation of data was possible. Further climatic data were collected (coldest winter temperature, hottest summer temperature and percent continentality), which are, however, not parameters of environmental heterogeneity, but descriptors of environmental harshness. Details are given in Chapter 3, Section 3.2.2.

The importance of environmental characterisation for understanding genotype × environment interactions has been highlighted by Wade, et al. (1996), who propose the use of geographical information systems (GIS). The analysis of spatial data has developed considerably with the introduction of computer-based GIS. Programmes such as ArcView's spatial analyst allow the integration and combination of data which have a spatial component (ESRI, 1996). In the present study ArcView was used to characterise the average environmental conditions, harshness and heterogeneity of the target area (i.e. Syria). Available data from different sources (Appendix 2) were collected and interpolated for the target area. As coordinates of the collection sites of BLRs and *H. spontaneum* accessions were known, it was possible to estimate environmental conditions, harshness and heterogeneity for each sampling site. The results of this spatial analysis are given below for the target area and individual sampling sites of accessions used in the present study.

The main purpose of the spatial analysis was to correlate estimated environmental data of the sampling sites with genetic indices calculated for the accessions collected at these sites, using genetic markers. Results of this investigation are given in Chapter 5.

4.2 Characterisation of the target area

Environmental conditions of the study area were characterised as described in detail in Section 3.2.2, using the following indices: Altitude (Alt), average annual temperature (T) and average annual precipitation (P). The harshness of the environments was characterised using the average minimum temperature of the coldest month (T_{min}), the average maximum temperature of the warmest month (T_{max}) and percent continentality (C). The heterogeneity or diversity of the environmental conditions was measured in terms of relative interannual variation in rainfall (P_{cv}). Too few data were available to include drought indices or to consider differences in soil types of the sampling sites. It was further planned to include information about the 10 year average yield achieved for barley under rainfed conditions in Syria as an index for regional yield potential. However, data of only 13 locations were available, so that a meaningful interpolation for the whole target area was not possible. Nevertheless, results (Plate 4.2.8) have been given to demonstrate the potential of such analysis. Results of the spatial analysis are given in form of maps created with the programme ArcView (Plates 4.2.1 to 4.2.8).

Plate 4.2.1 Average annual precipitation (P) in Syria. Surface interpolated from data from 55 meteorological stations in Syria.

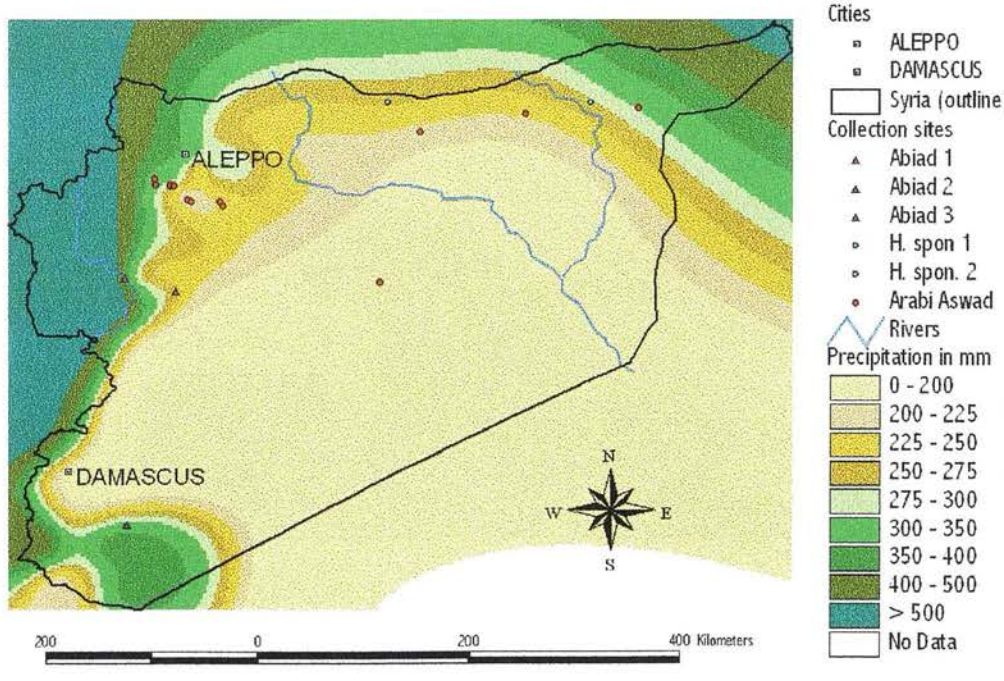


Plate 4.2.2 Average annual precipitation (P) in the Aleppo region. Surface interpolated from local meteorological stations (Aleppo region) using method IDW with a fixed radius of 22km. Interannual variation of rainfall (P_{cv}) is given as light green contour lines.

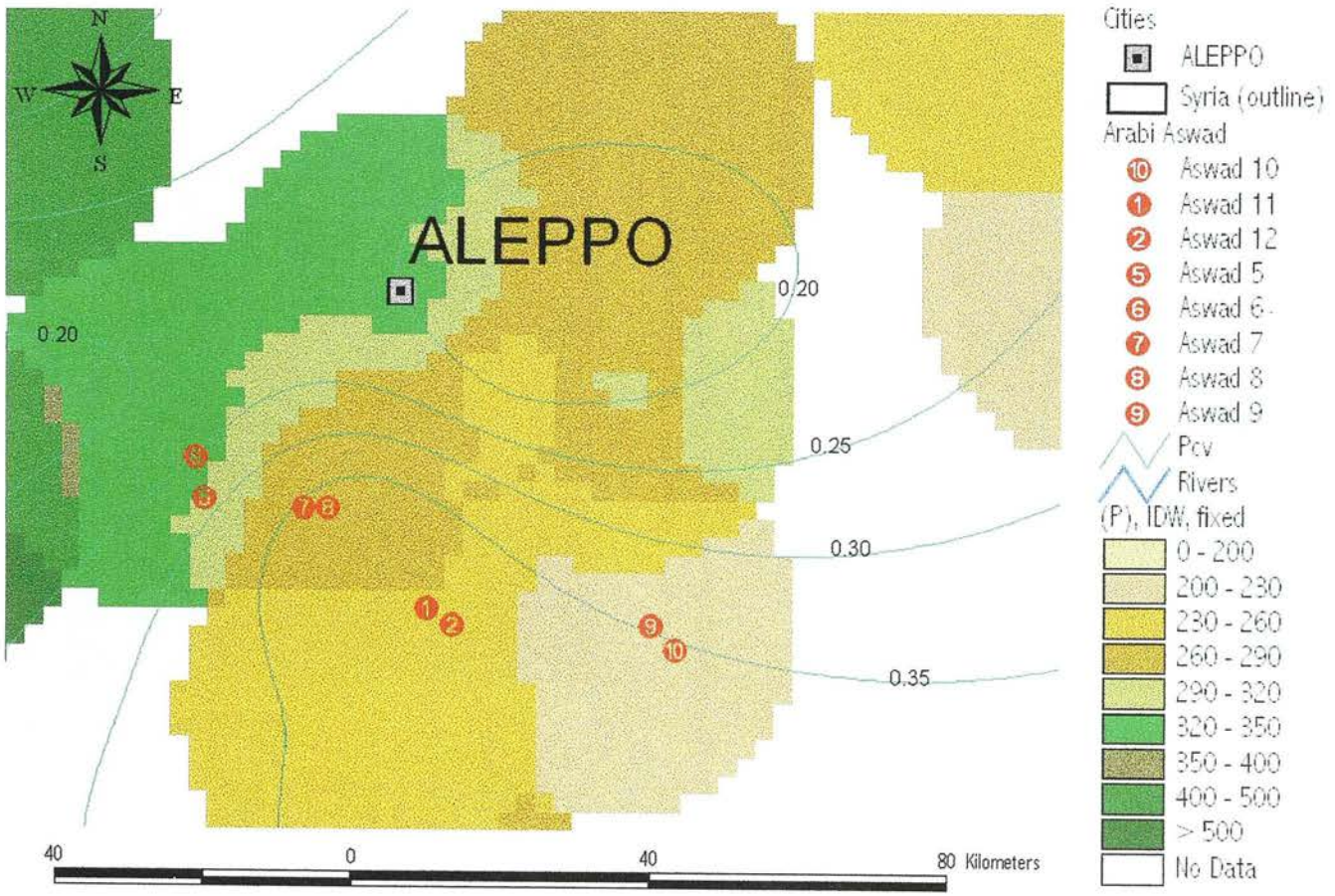


Plate 4.2.3 Interannual variation in rainfall (P_{cv}) in Syria. Surface interpolated from data from 45 meteorological stations in Syria.

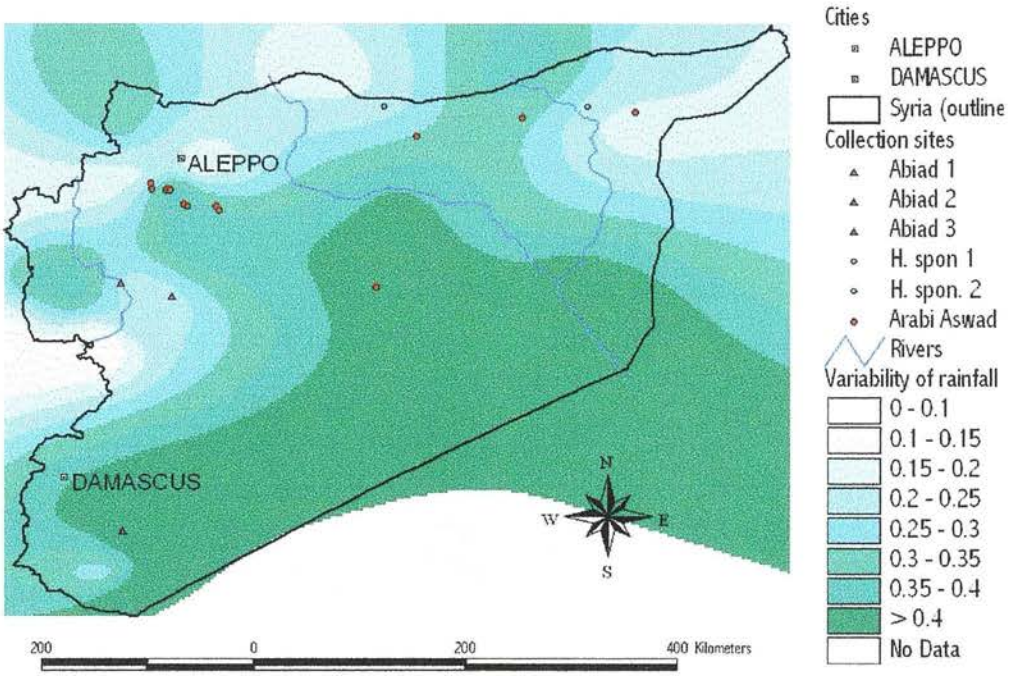


Plate 4.2.4 Average annual temperature (T) in Syria. Surface interpolated from data from 34 meteorological stations in Syria.

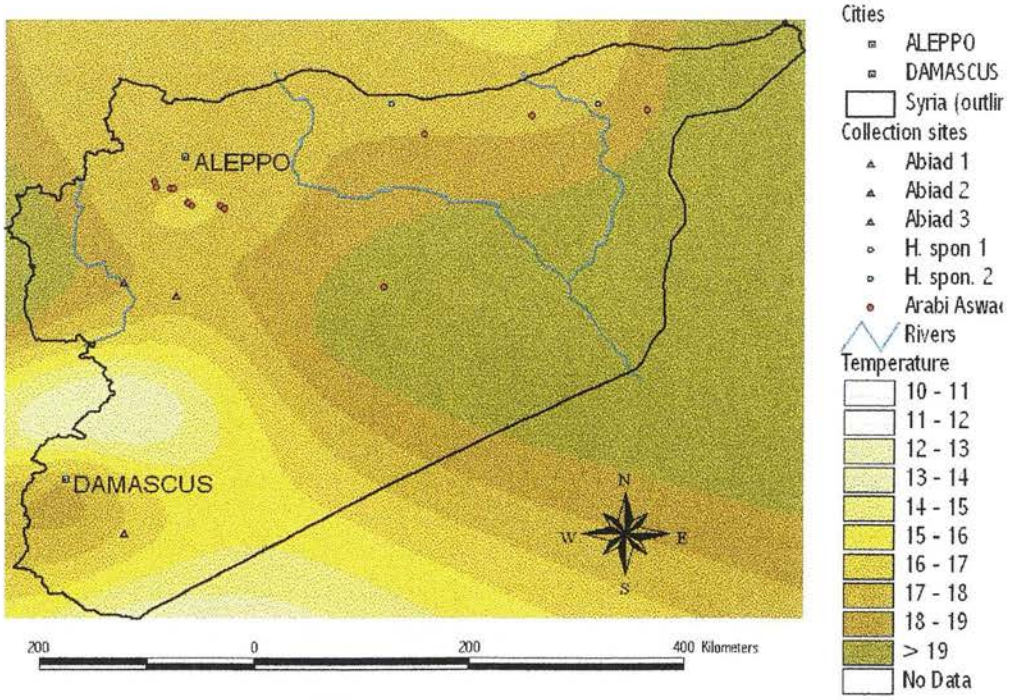


Plate 4.2.5 Average minimum temperature of the coldest month(T_{min}) in Syria. Surface interpolated from data from 34 meteorological stations in Syria.

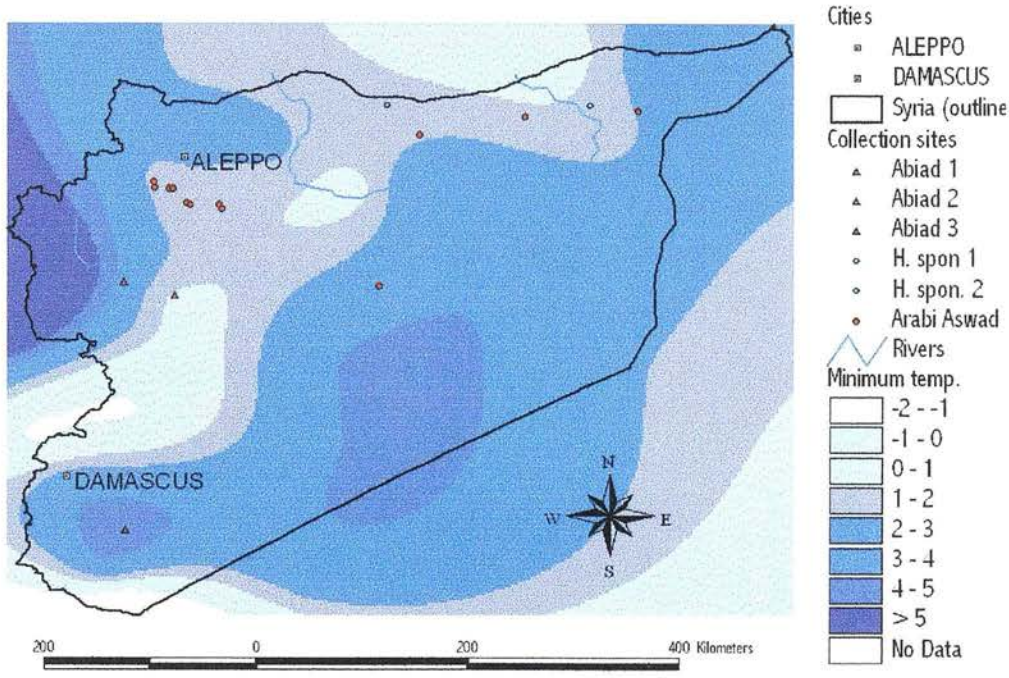


Plate 4.2.6 Average maximum temperature of the hottest month(T_{max}) in Syria. Surface interpolated from data from 34 meteorological stations in Syria.

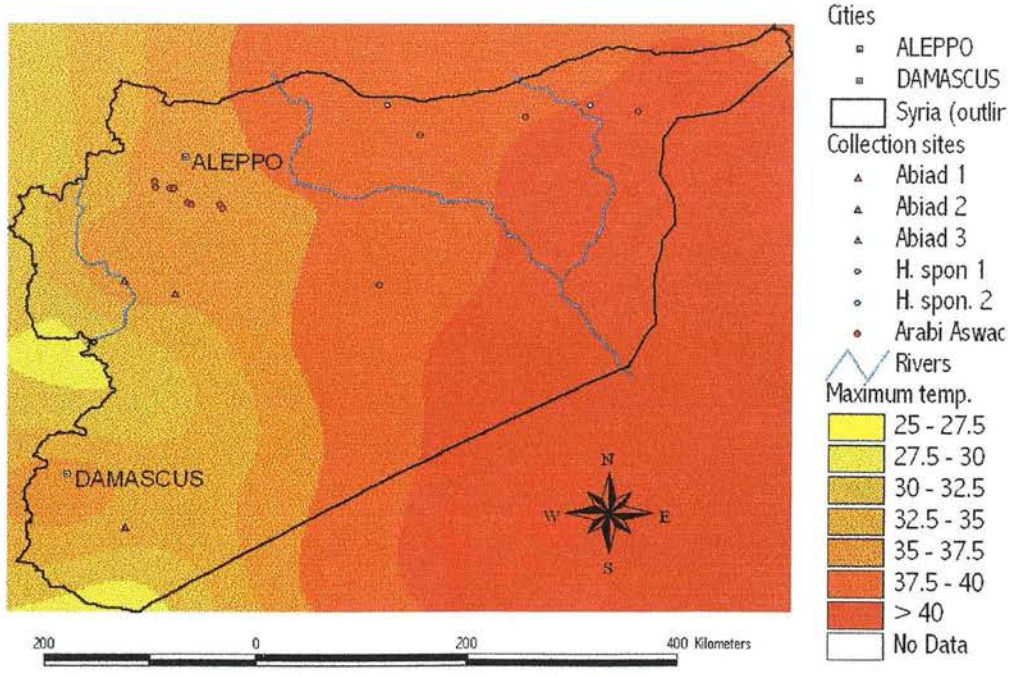


Plate 4.2.7 Continentality in % (C) in Syria. Surface interpolated from data from 34 meteorological stations in Syria.

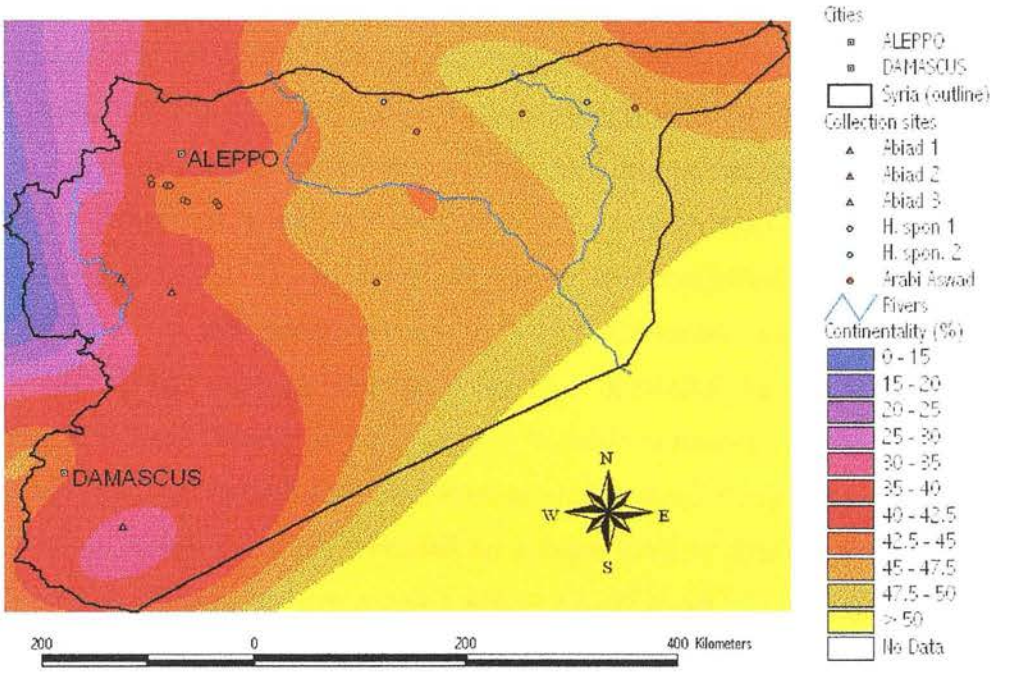


Plate 4.2.8 10 year average yield achieved for barley without irrigation in Syria. Surface interpolated from data from 13 experimental stations in Syria.

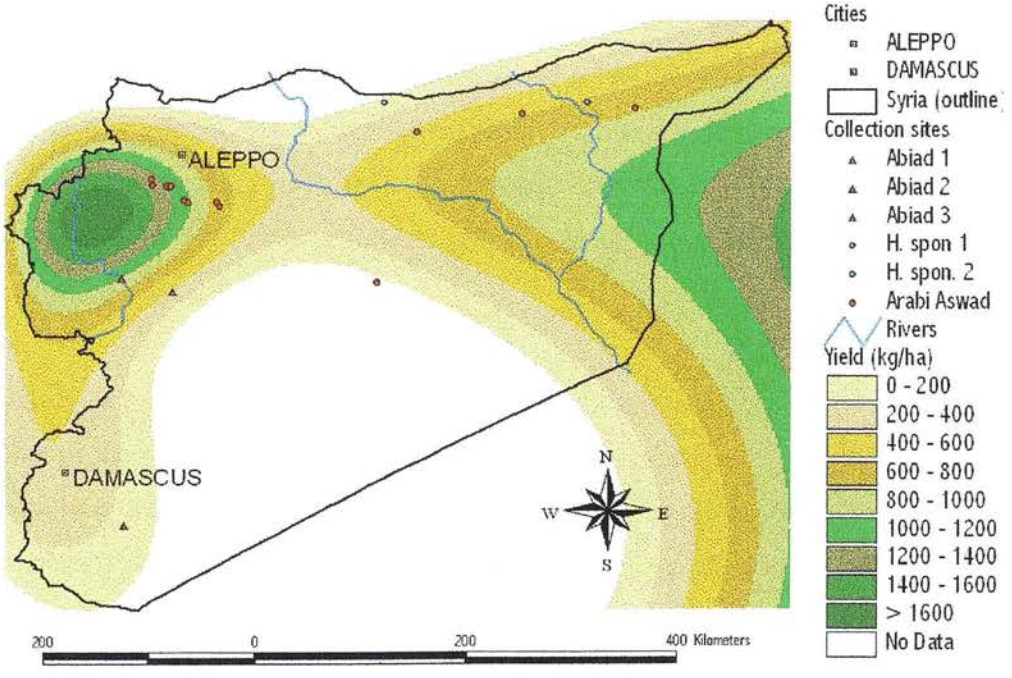


Plate 4.2.1 shows a very rapid decrease of average annual rainfall from north-west to south-east towards the arid central steppe of Syria. It was possible to interpolate the average annual rainfall in the Aleppo region with more precision as a lot of meteorological data were available from this region. For this reason a different method of interpolation (Inverse distance weighted, IDW) was used. Results given on Plate 4.2.2 show that accessions of Arabi Aswad originated from four distinct zones of average rainfall. Contour lines of the interannual variation in rainfall were overlaid and show that the collection sites of four accessions (Aswad 7, 8, 9 and 10) had a similar interannual variation in rainfall. Collection sites of accessions Aswad 11 and 12 had a higher interannual variation in rainfall, but higher average rainfall than collection sites of Aswad 9 and 10. Accessions Aswad 5 and 6 were collected in zones of higher rainfall and lower interannual variation of rainfall.

The interannual variation in rainfall on a larger scale in Syria (Plate 4.2.3) increases in wider steps in the same geographical direction as the average annual rainfall decreases.

Average maximum temperature (Plate 4.2.6) shows a trend of increasing temperatures from west to east. This trend is also seen in Plate 4.2.7 which shows increasing continentality from east to west. Continentality is based on the range between the lowest and the hottest temperatures. However, the average lowest temperature of the coldest month (Plate 4.2.5) shows a different pattern. The regions with the coldest temperatures stretch from south-west to north-east, while regions closer towards the Mediterranean Sea and the inland steppe have milder winters. The average temperature in Plate 4.2.4 shows a similar pattern, though less pronounced. A corridor of milder temperatures stretches from the mountains north of Damascus north-eastwards.

Plate 4.2.8 shows the potential yield of barley without irrigation, which is highest east of the coastal mountain range and decreases rapidly towards the inland or plain area and the inland steppe. It increases along the Turkish border towards the north eastern part of Syria, where higher average rainfall occurs (Plate 4.2.1).

4.3 Characterisation of the sampling sites of BLRs and *H. spontaneum* accessions

Interpolated data of environmental conditions of the target area as shown in form of maps (Plate 4.2.1 to 4.2.7) were used to estimate the environmental conditions of the sampling sites of accessions of BLRs and *H. spontaneum*. This was done within the programme ArcView. With a simple procedure, interpolated values were available for each location of a surface map. Results are summarised in Table 4.1.

Table 4.1 Environmental conditions of sampling sites calculated using ArcView spatial analyst, interpolated data from meteorological stations in Syria.

Code	Region	Location	Longitude (E)	Latitude (N)	Altitude (m)*	P (mm/year)	P_{cv}	T (°C)	T_{min} (°C)	T_{max} (°C)	Continentality (%)	Potential yield (kg/ha)**
Aswad 1	Palmyra		38.88	35.1	550	153	0.434	19.5	3.1	39.0	45.4	n.i.
Aswad 2	Raqqa		39.22	36.38	480	218	0.307	18.0	1.9	39.4	46.5	431.0
Aswad 3	Hassakeh	West of	40.12	36.53	296	229	0.318	17.8	1.3	39.9	48.4	603.0
Aswad 4	Quamishli	Tal Birak	41.08	36.58		305	0.195	18.8	2.1	40.5	47.8	765.0
Aswad 5	Aleppo	Tal Bagar	36.98	35.93	330	313 ①	0.280	17.3	1.8	37.0	42.1	1328.0
Aswad 6	Aleppo	Banus	36.97	35.98	360	336 ①	0.253	17.5	1.8	37.1	41.8	1259.0
Aswad 7	Aleppo	Soaibieh	37.10	35.92	330	269 ①	0.358	17.2	1.5	37.2	43.4	1175.0
Aswad 8	Aleppo	Abu-Rowail	37.13	35.92	342	265 ①	0.369	17.2	1.4	37.1	43.5	1148.0
Aswad 9	Aleppo	Khanasir 1	37.52	35.78	355	227 ①	0.354	17.1	1.2	37.2	44.1	729.0
Aswad 10	Aleppo	Im Mial	37.55	35.75	365	227 ①	0.356	17.2	1.2	37.2	44.2	702.0
Aswad 11	Aleppo	Sowaiha	37.25	35.8	303	257 ①	0.384	16.8	1.3	37.3	44.5	1037.0
Aswad 12	Aleppo	Om-amood	37.28	35.78	324	253 ①	0.382	16.8	1.3	37.3	44.5	1009.0
H. spont.1	Tal Abiad	Tal Abiad	38.95	36.63	349	255	0.229	17.7	1.4	39.3	46.8	n.i.
H. spont 2	Hassakeh	Hassakeh	40.67	36.63	296	274	0.240	18.0	1.3	40.2	48.8	n.i.
Abiad 1	Hama	Hama	36.72	35.13	309	394	0.248	18.1	3.3	37.1	40.1	944.0
Abiad 2	Suweida	Suweida	36.73	33.03	850	323	0.442	16.9	4.7	33.7	33.4	127.0
Abiad 3	Bural Sharqui		37.15	35.02	350	254	0.227	17.4	0.9	35.6	42.2	349.0

n.i. = no information available; P = Average annual rainfall; P_{cv} = Relative interannual variation in rainfall;
T_{min} = Average minimum temperature of the coldest month; T_{max} = Average maximum temperature of the warmest month;
* = Altitude values based on ICARDA and FAO (1994); ** = Potential yield denotes the average yield for barley without irrigation;
① = different method (fixed radius, IDW) to interpolate rain.

Altitudes of the sampling sites as given in Table 4.1, are based on ICARDA observations or were estimated from a geographic map (scale 1 : 1 000 000) of Syria, which had a higher accuracy than could be achieved using interpolation of available data points. The average annual precipitation in Syria was measured using the spline method (see 3.2.2) of the programme ArcView as shown in Plate 4.2.1. A different method (IDW) has been used to interpolate average annual precipitation for the Aleppo region because the use of method IDW is expected to give more accurate results when a high number of data points is available within a small region (see 3.2.2 for details). A correlation of genetic indices with environmental indices for the sampling sites of accessions of BLRs and populations of *H. spontaneum* (Table 4.1) is described in Chapter 5.

4.4 Discussion and conclusions

Plates 4.2.1. to 4.2.8 illustrate the potential of GIS to analyse data with a spatial dimension. However, the significance of the above maps lies more accurately in the underlying, interpolated data, which can be used to give estimates of the corresponding index for each location in the interpolated surface area (i.e. coloured area within the outline). However, the accuracy of results of interpolations depend greatly on the number of available measurements.

In a study of the agroclimatology of the Near East (Brichambaut and Wallen, 1963) contour maps based on manual interpolation were given for average precipitation, relative interannual variation rainfall, average minimum temperature of the coldest month and continentality in percent. Rainfall data were based on 80, and temperature data on 40 stations for a region that included the south of Turkey, Syria, Iraq, Iran, Jordan, Lebanon, Israel, the Sinai peninsula, the North of Saudi Arabia and Kuwait and stretches from 35°E to 62° E and from 26°N to 38°N. In the present study rainfall data were based on 54 and temperature data on 34 stations in Syria alone, and are expected to be more detailed. However, surface maps of precipitation (Plate 4.2.1) and interannual variation of rainfall (Plate 4.2.3) are fairly similar to those achieved by Brichambaut and Wallen (1963). The surface map of minimum winter

temperatures (Plate 4.2.5) seems much more precise than the published map. The surface map showing percent continentality (4.2.7) shows a much slower increase of continentality from west to east compared with Brichambaut and Wallen (1963) map, which is only based on 9 stations in Syria. These observations may imply that interpolated data of the present study have a fairly high accuracy.

The surface maps of average precipitation and interannual variation in rainfall (Plates 4.2.1 and 4.2.3) illustrate increasing harshness of environmental conditions from north-west to south-east. Average maximum temperature and continentality (Plates 4.2.6 and 4.2.7) showed increasing values from west to east, or from the coastal area land inwards. The average temperature and the average minimum winter temperature show a different pattern. A corridor of colder temperatures stretches from the mountains north of Damascus north-eastwards. The milder average temperatures in this corridor are mainly caused by the lower winter temperatures, because the average maximum summer temperatures increase clearly from west to the east (Plate 4.2.6).

The fluctuation (cyclicity) of farm crop yield was studied by Novogrudskii (1991), who based his observations on wheat and barley in Russia. In this study it was attempted to use barley yield under rainfed conditions as an indicator for environmental variability (Plate 4.2.8). Unfortunately data for too few locations were available, to achieve meaningful results. It would have been further interesting to investigate long term fluctuations in yield, if annual data for a long period were available. Interpolation of the coefficient of variance of barley yield under rainfed conditions could have resulted in a useful additional map. Maps of average barley yield and interannual variation of barley yield under rainfed conditions would be suited to describe regions in Syria in terms of spatial and temporal ecological variability.

It was possible to estimate environmental conditions of all sampling sites of BLRs and *H. spontaneum* populations from interpolated surface maps presented in this chapter (Plate 4.2.1 to 4.2.8). Data were used in the following chapter to investigate correlations between environmental and genetic indices.

Chapter 5

Results of experimental investigations of the population structure of barley landraces from different eco-geographical environments in Syria

5.1 Introduction

BLRs are the evolutionary link between wild barley and modern barley cultivars. However, relatively little is known about their population structure. For instance no specific figure of the outcrossing rate of BLRs was found in the literature. A better knowledge of the population structure of BLRs may have consequences for plant breeding particularly for yield stability in unpredictable environments as well as for conservation strategies.

The results of previous studies suggest, for example, that BLRs may perform more stably under a range of conditions than modern cultivars (Ceccarelli, 1996a) due to population buffering (i.e. the ability to compensate for the failure of a component by a genetically different component of the same population) based on a high level of diversity (Ceccarelli, Acevedo & Grando, 1991). Furthermore, the level of diversity of barley populations seems to be influenced by environmental conditions (Nevo 1992). Nevo et al. (1997) reported for wild barley significantly higher levels of gene diversity under stressful and variable environmental conditions than under more mesic conditions in Israel.

The results of the following chapter may help to answer questions about the mating system of BLRs and the influence of environmental conditions and heterogeneity of the environment on the population structure of BLRs. They may further illuminate the impact of human activities due to cultivation on BLRs and the importance of seed exchange between farmers.

5.1.1 Population structure

Methods of population genetics have been employed to analyse the genetic structure of BLRs, i.e. the impact of evolutionary forces such as mutation, migration, genetic drift and selection on allele frequencies, heterozygosity, the correlation of alleles between loci and the differentiation of populations. The expression of these forces is influenced significantly by the mating system and the effective size of the population in question.

Population genetic theory is usually concerned with natural populations. In the present study principles of population genetics were applied to a crop plant such as barley. This made it necessary in addition to evolutionary forces mentioned above, to consider the influence of human activities associated with domestication and cultivation of barley, such as deliberate selection and migration through seed exchange between farmers.

The knowledge of the mating system of a population is important as it determines the organisation of genes into genotypes. Inbreeding increases homozygosity and the occurrence of linkage disequilibria in a population. However, the mating system does not change allele frequencies by itself (Hartl, 1988).

The ultimate source of genetic variation is mutation (Hartl, 1988), however, in the present study the effect of mutation was neglected because effects on change in gene frequencies are likely to be insignificant (Nei, 1987).

Migration is the exchange of genetic information between populations through gene flow by vectors such as pollen and seeds. In a predominantly inbreeding species such as barley, the impact of the migration of pollen between local populations is expected to be small. Natural dispersal of seeds in cultivated barley is non-existent, because of a tough rachis, which prevents shattering of seeds so that reproduction depends entirely on cultivation. Consequently, gene flow found between local populations of cultivated barley may be primarily attributed to seed exchange between farmers.

Genetic drift is the random change of gene frequencies. Although drift occurs in all populations, its effect is more pronounced in very small isolated populations, in which it gives rise to the random fixation of alternative alleles. Consequently, the variation which was originally present in the initial population, appears as variation between isolated local populations (Falconer, 1989; Allaby, 1992) i.e. the diversity within populations decreases while diversity between populations increases.

Selection is a process that results from different rates of reproduction of individuals within a population. Those individuals which produce more viable seeds under a particular set of conditions (i.e. have a higher fitness), will contribute more offspring to the next generation than those individuals which produce less seeds. Natural selection acts on phenotypes. However, if the differences of fitness are in any way associated with the presence or absence of a particular gene in the individual's genotype, then selection operates on the gene. Consequently, the frequency of the gene of a fitter plant will increase in the next generation (Falconer, 1989). Thus, local populations are able to become more adapted to a particular environment under natural conditions. Human activity can lead to selection in populations. Harlan, et al., (1972) distinguish between automatic and deliberate human selection. Automatic selection is caused by the domestication process and cultivation of a plants and favours traits such as uniform maturation, increase in seed production due to harvesting, greater seed size and loss of dormancy due to seedling competition in the field. If man deliberately selects a population for a preferred trait (e.g. seed colour), the proportion of individuals with this trait will increase and the diversity of the population will decrease. Whereby traits for which man select are almost generally phenotypes. The presence of heterozygote advantage (i.e. the heterozygote state at a certain locus has a greater fitness than the homozygote) acts as well as a selective force and is an explanation for maintenance of polymorphism (Falconer, 1989). A further kind of selection, termed frequency-dependent selection, has been extensively demonstrated in cultivated plants grown in heterogeneous environments. Under heterogeneous conditions a genotype may have high fitness when it is rare, because the sub-environments in which it is favoured are relatively abundant. But when this genotype is common, its fitness may be low, because its favourable sub-environments are saturated (Ayala, 1982) and consequently it will be selected against (Falconer, 1989). This process may maintain a high level of polymorphism within a population.

Selection favouring one combination of alleles over another can produce and maintain linkage disequilibrium (Falconer, 1989). Permanent linkage disequilibrium, i.e. the genetic coadaptation of alleles, may result from natural selection if some

genetic combinations result in a higher fitness than other combinations. The less closely linked two loci are, the greater the strength of natural selection required to maintain linkage disequilibrium (Ayala, 1982).

BLRs are subject to natural selection, but, additionally are likely to be affected by selection due to human activities. Deliberate selection by man seems to play a minor role in BLRs, as resource-poor farmers seem to favour diverse populations (Ceccarelli, 1996a). However, automatic selection, as explained above, will certainly influence the population structure of BLRs. Heterozygote advantage, if present, and frequency dependent selection may as well be acting on BLRs and will be considered in this study. Furthermore, the influence of the heterogeneity of the environment (Falconer, 1989; Nevo, et al., 1997) may have an influence on the population structure of BLRs.

The analysis of the population structure using different genetic markers will produce different results. One of the main reasons for this is the different selectivity of markers, which can be either neutral or adaptive.

Most polymorphisms observed at the molecular level are selectively neutral. Mutation that are selectively neutral produce such small effects on the ability of their carriers to survive and reproduce that they are completely equivalent in terms of natural selection. The ultimate fate of neutral alleles in a population is determined largely by the process of genetic drift (Kimura, 1968, cited by Hartl, 1988).

Selectively adaptive markers are subject to selection themselves or are associated with genes which are subject to selection. With selection the gene frequency of a favoured gene within a population will increase and the diversity will decrease. Thus the proportion of diversity found between populations will increase. Random genetic drift may cause additional decrease of diversity within populations, in particular when the effective population size is small. In contrast, migration may lead to an increase of diversity within populations.

5.2 Mating system of BLRs

BLR populations are thought to be structured as highly heterogeneous mixtures of inbred lines and hybrid segregates which are maintained by a low level of random crossing in each generation (Nevo, 1992). While the outcrossing rate of wild barley (Brown, et al., 1978) and modern cultivars (Doll, 1987) has been well studied, no particular information on the outcrossing rate of BLRs is available. The knowledge of the mating system of BLRs has direct implications for the application of principles of population genetics (Brown, 1990) such as the Hardy-Weinberg principle and for the way in which genetic indices such as the effective population size, linkage disequilibrium and heterozygosity are calculated. Furthermore, the investigation of the mating system of BLRs had three objectives. First, to investigate if domestication has led to a change of the outcrossing rate. Second, to investigate if the level of heterozygosity found can be explained with the present level of outcrossing, and third, to investigate the influence of environmental conditions on the outcrossing rate of BLRs.

Samples of 10 populations of the black seeded BLR Arabi Aswad (Table 3.1.1) which is still cultivated throughout Syria, were collected in 1997 and 1998 under the supervision of ICARDA. Spikes of fifty individual plants from at least two metre distance were collected per population and 6 seeds per spike (i.e. family) were used for electrophoresis. Thus a total of about 300 seeds per population were grown in soil. Starch gel electrophoresis was carried out on extracts of four to ten day old coleoptiles (Kahler & Allard, 1970). Allele frequencies at two different codominant isoenzyme loci (*Est1, Est2*, E.C.3.1.1.1) were analysed and multilocus outcrossing rate (t_m) and fixation index (F_{IS}) were calculated using the programme MLT (Ritland, 1990). Climatic data (FAO, 1994; Anonymous, 1986) were interpolated for the sampling sites using the computer programme ArcView GIS 3.0, spatial analyst. For details see Section 3.3.3.

5.2.1 Results of the analysis of the outcrossing rate of BLRs

The multilocus outcrossing rate (t_m) of 10 populations of the black seeded barley landrace Arabi Aswad from different regions in Syria was on average 1.7%.

The 95 % confidence interval for t_m ranged from 0.011 to 0.023, so that accessions Aswad 3 and Aswad 7 showed significantly higher outcrossing rates and accession Aswad 4 a significantly lower outcrossing rate. The analysis of variance, however, showed no significant differences between regions or years of collection. The fixation index (F_{IS}) for these populations was on average 0.866. The results are given in detail in Table 5.2.1.

Table 5.2.1 Multilocus outcrossing rate (t_m) and Fixation index (F_{IS}) of 10 populations of BLR Arabi Aswad from Syria. Variances (bootstrap method) are given in parenthesis.

Accession code	Region/ Year of collection	Location	Number of plants	Multilocus outcrossing rate (t_m)	Fixation index (F_{IS})
Aswad 3	Hassakeh/ 1997	West of Hassakeh	300	**0.025** (0.013)	**0.909** (0.033)
Aswad 4	Quamishli/ 1997	Tal Birak	297	**0.002** (0.006)	**0.939** (0.031)
Aswad 5	Aleppo/ 1998	Tal Bagar	300	**0.012** (0.015)	**0.917** (0.051)
Aswad 6	Aleppo/ 1998	Banus	299	**0.012** (0.006)	**0.823** (0.079)
Aswad 7	Aleppo/ 1998	Soaibieh	302	**0.030** (0.020)	**0.963** (0.036)
Aswad 8	Aleppo/ 1998	Abu-Rowail	300	**0.013** (0.008)	**0.861** (0.066)
Aswad 9	Aleppo/ 1998	Khanasir	300	**0.023** (0.015)	**0.876** (0.053)
Aswad 10	Aleppo/ 1998	Im Mial	299	**0.017** (0.009)	**0.835** (0.063)
Aswad 11	Aleppo/ 1998	Sowaiha	300	**0.023** (0.021)	**0.845** (0.052)
Aswad 12	Aleppo/ 1998	Om-amood	301	**0.015** (0.016)	**0.696** (0.061)
Average {+/- SE}			300	**0.0172** {+/- 0.0025}	**0.866** {+/- 0.0239}

A two sample t-test between the present results (Table 5.2.1) and results reported by Brown, Zohary & Nevo (1978) for wild barley showed that the mean outcrossing rate of 1.72 % found for BLRs was not significantly different from the mean outcrossing rate of 1.6 % found for wild barely.

5.2.2 Results of the analysis of fixation at inbreeding equilibrium

The heterozygosity of a highly inbreeding plant population such as barley may be considerably higher than anticipated from the mating system. Exceeding levels of heterozygosity can be quantified as the difference between the expected fixation index at inbreeding equilibrium ($\hat{F}_{IS}$) and the calculated fixation index (F_{IS}).

The expected fixation index at inbreeding equilibrium ($\hat{F}_{IS}$) was calculated from the rate of selfing (S) as $S/2$-S, where ($S = 1 - t_m$) (see Section 3.5) and gave an average of ($\hat{F}_{IS}$) of 0.966 (range: 0.942 to 0.996) over all 10 populations tested. All but one accession (Aswad 7) showed positive differences i.e. a fraction of heterozygosity which cannot be explained by the outcrossing rate alone. A Mann-Whitney test showed that the average F_{IS} of 0.866 (Table 5.2.1) was very highly significantly lower ($P = 0.0003$) than the calculated fixation at inbreeding equilibrium ($\hat{F}_{IS}$).

5.2.3 Influence of environmental conditions on the outcrossing rate of BLRs

Diversity levels of populations of wild barley may be increased by the severity and heterogeneity of environmental conditions (Nevo, et al., 1997). One reason for increased diversity levels could be a higher outcrossing rate caused by environmental conditions such as drought or heat.

To investigate this question, outcrossing rates and fixation indices of single accessions (Table 5.2.1) were correlated with interpolated environmental data for the collection sites of the corresponding accessions (Table 4.1). Outcrossing rate showed a significant positive correlation ($P = 0.031$) with relative interannual variation in rainfall (P_{cv}) and a significant negative correlation ($P = 0.024$) with the average minimum temperature of the coldest month (T_{min}). Non-linear regression curves for both correlations gave a better fit than the linear regression and are shown in Figure 5.2.1 and Figure 5.2.2, respectively. The regression function and R^2 values are given within the figures.

Figure 5.2.1 Influence of relative interannual variation in rainfall (P_{cv}) on outcrossing rate (t_m) of BLRs.

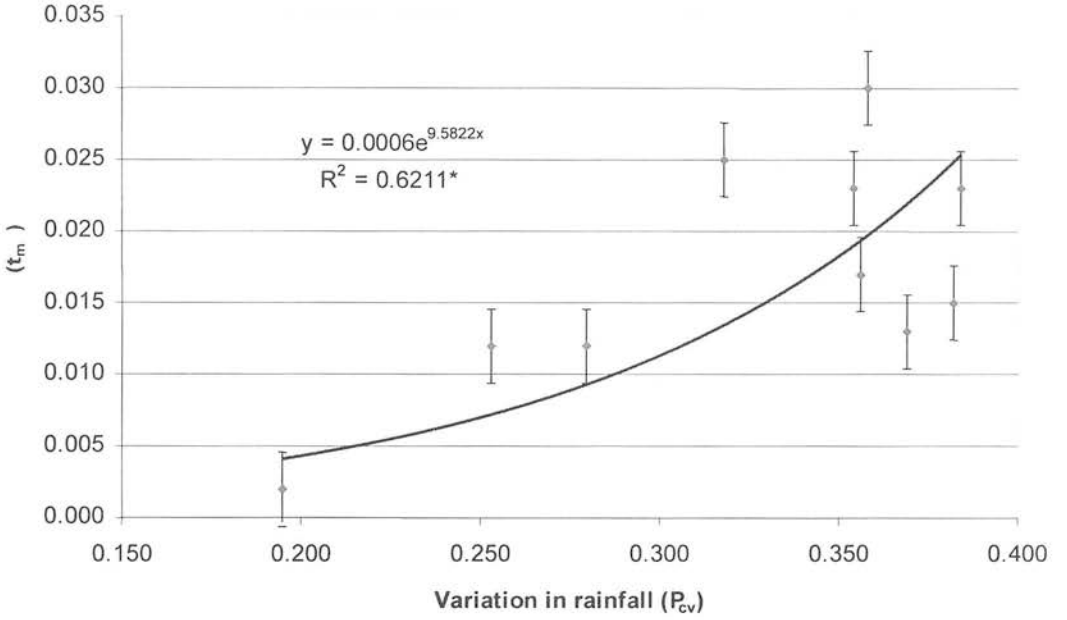
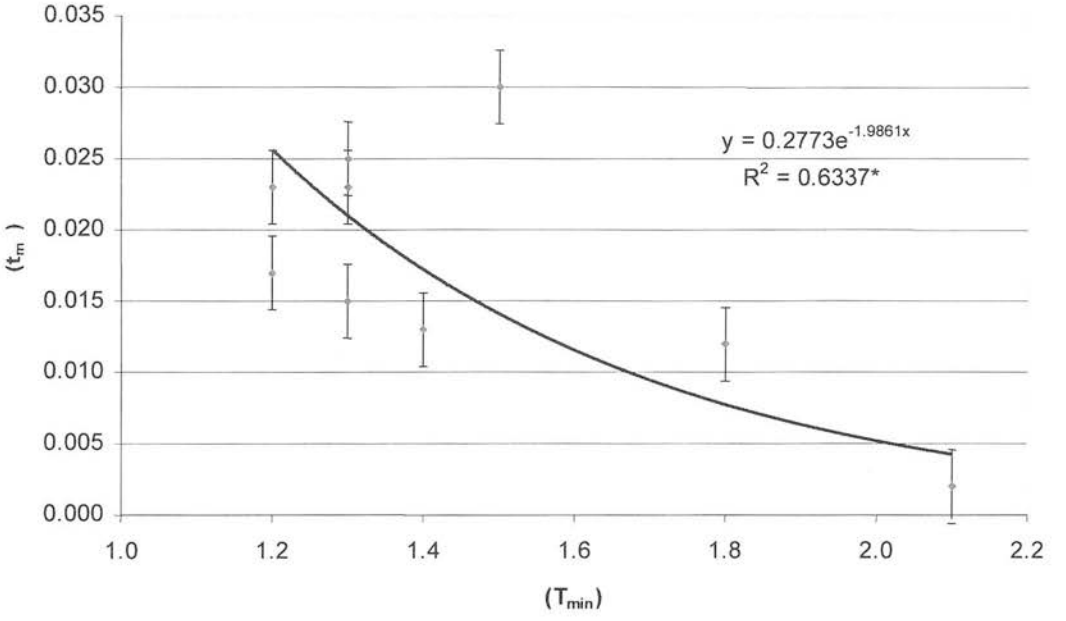


Figure 5.2.2 Influence of average minimum temperature of the coldest month (T_{min}) on outcrossing rate (t_m) of BLRs.



5.2.4 Discussion

The results given in this section showed that BLRs can be considered as inbreeding populations in which a very small level of random outcrossing of about 1.7% occurs. An increasing rate of selfing may be expected under domestication (Jarne & Charlesworth, 1993), however, outcrossing in barley may not have been affected by domestication as the outcrossing rate found for BLRs is not significantly different to the outcrossing rate of 1.6 % reported by Brown, Zohary & Nevo (1978) for wild barley. Outcrossing rates reported for modern cultivars ranged from 0.5% to 10% (Sokal, 1978; Simmonds, 1979; Doll, 1987) and disagrees as well with the theory of increasing selfing rates through domestication, for barley.

Expected fixation at inbreeding equilibrium was significantly higher (about 10%) than Wright's fixation index. This excess level of heterozygosity may be explained either by the presence of heterozygote advantage (also termed overdominance, i.e. the heterozygote state at a certain locus has a greater fitness than the homozygote) (Falconer, 1989), or an interannual fluctuation of outcrossing rates (Brown and Albrecht, 1980). However, in the present study outcrossing rates from two successive years (1997, 1998) did not show significantly different results. Therefore it seems more likely that overdominance may have caused the higher proportion of heterozygosity, which may partly explain the high level of diversity sustained in BLR populations as shown in Tables 5.3.1 to 5.3.3. An alternative explanation to overdominance for the phenomenon of an excess level of heterozygosity may well be, that heterozygotes cover up deleterious mutations rather than having *per se* a higher fitness (Ennos, 1983; Nei, 1987).

A highly significant correlation between the outcrossing rate of BLRs and interannual variation in rainfall (P_{cv}) was observed (Figure 5.2.1). Interannual variation in rainfall can be seen as a measure of the heterogeneity of rainfall and thus includes the effect of droughts. It is known that water stress during pollen mother cell meiosis and, independently, high temperature can cause pollen (male) sterility in wheat, while the female fertility is not affected. In addition a reduced number of grains per spike is reported for the same climatic conditions (Aspinall, 1985).

Chemically-induced male sterility is used in wheat hybrid breeding – male-sterile flowers tend to open up and allow cross pollination. The very similar flower biology of wheat and barley may indicate a similar mechanism in barley where water stress during pollen mother cell meiosis would induce male sterility and favour cross pollination. This mechanism may explain a higher rate of outcrossing under dry and unpredictable rainfall conditions as found for BLRs. However, the result is contrary to findings of Nevo (1992) who found significantly higher outcrossing rates in populations of *H. spontaneum* under mesic conditions rather than under xeric conditions in Israel, which on the other hand seems to be at odds with findings of higher diversity levels of *H. spontaneum* under more xeric conditions at 'evolution canyon' in Israel (Nevo, et al., 1997).

Outcrossing rate also showed a highly significant negative correlation with average minimum temperature of the coldest month (T_{min}) (Figure 5.2.2). This result is in accordance with Doll (1978) and Giles (1989) who found significantly higher outcrossing rates for autumn-sown barley than for spring-sown barley, the latter being apparently less exposed to colder temperatures. However, it seems noteworthy that the two climatic indices ($P_{cv;}$ T_{min}) were closely correlated themselves (R^2 = 0.82) so that correlations with the multilocus outcrossing rate have to be interpreted with caution. Originally, self-pollination is believed to have evolved later than cross-pollination, predominantly in annual species in order to ensure seed set in the absence of appropriate mating partners and under warm and dry conditions with fluctuating rainfall (Stebbins, 1950; Cronquist, 1968; Jarne & Charlesworth, 1993). However, as mentioned earlier, the same climatic conditions seem to favour male sterility and subsequently cross-pollination in wheat (Aspinall, 1985).

Generally, the results may indicate that the same mechanism (i.e. self-pollination with a low level of random outcrossing) that guarantees a high seed set but sustains a high level of diversity in wild barley, has been maintained in BLRs. The presence of overdominance may furthermore sustain a higher level of heterozygosity than anticipated from the observed outcrossing rate. The level of outcrossing seems to be positively influenced by the heterogeneity and severity of the environment.

5.3 Genetic structure of BLR populations an *H. spontaneum* from different regions in Syria

The investigation of the genetic structure of BLRs and *H. spontaneum* was based on allele frequencies at numerous loci of three genetic markers, as described in Section 3.3.2 to 3.3.4. Morphological, isoenzyme and ISSR markers were analysed separately since they may differ in important characteristics such as mutation rate and degree of selective neutrality (Bataillon *et al.*, 1996). The characterisation of the population structure was confined to the measurement of diversity, differentiation between populations and isolation by distance.

As mentioned previously, Nevo et al. (1997) reported for wild barley significantly higher diversity levels under stressful and variable environmental conditions than under more stable and moderate conditions in Israel. One purpose of the present study was to investigate whether the same is true for BLRs from different eco-geographical environments in Syria and to investigate further the influence of environmental conditions on the population structure of BLRs.

5.3.1 Diversity of BLR populations and *H. spontaneum* from Syria

Generally, the diversity of BLRs is believed to be intermediate (Brown and Munday, 1982) to high (Jana and Pietrzak, 1988) compared with other plant species. According to the majority of published data the diversity of BLRs is expected to be lower than the diversity of wild barley due to genetic bottle-necks during domestication, when morphological, isoenzyme and molecular markers are considered (e.g. Allard, 1992; Brown and Munday, 1982; Nevo, 1992, Saghai-Maroof, et al., 1994). However, some authors reported about equal or higher diversity of BLRs compared with wild barley when organellar DNA marker loci (Holwerda, et al., 1986) or ribosomal DNA marker loci (Ramamoorthy et al., 1994) were used. Three different genetic marker types were used in this study to address this question. Main consideration was given to the environmental influence on diversity levels of BLRs.

5.3.1.1 General overview of results

Average results of all accessions are given in Tables 5.3.1 to 5.3.3. A summary of results is given in Table 5.3.4.

Morphological marker loci

Table 5.3.1 Genetic indices calculated from allele frequencies of 4 seed-morphological marker loci of accessions of BLR and *H. spontaneum* from Syria (standard errors in parentheses).

Code	Region	Location	Longitude (E)	Latitude (N)	Altitude (m)	n	Morphological markers: H	A	P (0.01)	S	M
Aswad 1	Palmyra		38.88	35.10	153	58	**0.2537** (+/-0.0361)	**2.3** (+/-0.1)	**0.8**	**0.4374** (+/-0.0599)	**12**
Aswad 2	Raqqa		39.22	36.38	218	82	**0.3326** (+/-0.0367)	**2.5** (+/-0.1)	**0.8**	**0.5840** (+/-0.0627)	**13**
Aswad 3	Hassakeh	West of	40.12	36.53	229	197	**0.3150** (+/-0.0257)	**2.5** (+/-0.1)	**0.8**	**0.5455** (+/-0.0456)	**5**
Aswad 4	Quamishli	Tal Birak	41.08	36.58	305	200	**0.3271** (+/-0.0309)	**2.8** (+/-0.1)	**1.0**	**0.5718** (+/-0.0363)	**9**
Aswad 5	Aleppo	Tal Bagar	36.98	35.93	317	74	**0.1770** (+/-0.0264)	**2.3** (+/-0.1)	**1.0**	**0.2931** (+/-0.0371)	**9**
Aswad 6	Aleppo	Banus	36.97	35.98	328	65	**0.2157** (+/-0.0294)	**2.0** (+/-0.1)	**0.8**	**0.3684** (+/-0.0486)	**11**
Aswad 7	Aleppo	Soaibieh	37.10	35.92	273	50	**0.1918** (+/-0.0330)	**2.3** (+/-0.1)	**1.0**	**0.3385** (+/-0.0498)	**16**
Aswad 8	Aleppo	Abu-Rowail	37.13	35.92	265	54	**0.2172** (+/-0.0365)	**2.0** (+/-0.1)	**0.8**	**0.3773** (+/-0.0599)	**11**
Aswad 9	Aleppo	Khanasir 1	37.52	35.78	231	51	**0.2186** (+/-0.0381)	**2.5** (+/-0.2)	**0.8**	**0.4080** (+/-0.0655)	**14**
Aswad 10	Aleppo	Im Mial	37.55	35.75	232	80	**0.2413** (+/-0.0312)	**2.8** (+/-0.1)	**1.0**	**0.4426** (+/-0.0518)	**11**
Aswad 11	Aleppo	Sowaiha	37.25	35.80	219	53	**0.2025** (+/-0.0364)	**2.3** (+/-0.2)	**0.8**	**0.3828** (+/-0.0663)	**15**
Aswad 12	Aleppo	Om-amood	37.28	35.78	220	52	**0.1855** (+/-0.0354)	**2.0** (+/-0.1)	**0.8**	**0.3204** (+/-0.0554)	**11**
Abiad 1	Hama		36.72	35.13	394	62	**0.0905** (+/-0.0078)	**2.3** (+/-0.1)	**1.0**	**0.1919** (+/-0.0130)	**10**
Abiad 2	Suweida		36.73	33.03	323	68	**0.2079** (+/-0.0288)	**2.3** (+/-0.1)	**1.0**	**0.3648** (+/-0.0439)	**10**
Abiad 3	Bural Sharqui		37.15	35.02	254	200	**0.2429** (+/-0.0175)	**2.0** (+/-0.0)	**1.0**	**0.3615** (+/-0.0236)	**3**
H. spont. I	Tal Abiad		38.95	36.63	255	100	**0.1815** (+/-0.0214)	**2.0** (+/-0.1)	**0.5**	**0.3246** (+/-0.0378)	**6**
H. spont II	Hassakeh		40.67	36.63	274	100	**0.2538** (+/-0.0294)	**2.0** (+/-0.1)	**0.5**	**0.4372** (+/-0.0506)	**9**

n = number of individuals tested; H = Average gene diversity; A = Mean number of alleles per locus;
P 0.01= Proportion of polymorphic loci; M = Number of multilocus combinations per100 individuals;
S = Shannon's information index.

Isoenzyme marker loci

Table 5.3.2 Genetic indices calculated from allele frequencies of 8 isoenzyme marker loci of accessions of BLR and *H. spontaneum* from Syria (standard errors in parentheses).

Code	Region	Location	Longitude (E)	Latitude (N)	Altitude (m)	n	Isoenzyme markers: H	A	P (0.01)	S	M
Aswad 1	Palmyra		38.88	35.10	153	39	**0.3166** (+/-0.0433)	**2.1** (+/-0.2)	**0.75**	**0.5031** (+/-0.0706)	**64**
Aswad 2	Raqqa		39.22	36.38	218	42	**0.2917** (+/-0.0377)	**2.0** (+/-0.1)	**0.75**	**0.4741** (+/-0.0651)	**57**
Aswad 3	Hassakeh	West of	40.12	36.53	229	50	**0.2926** (+/-0.0314)	**2.0** (+/-0.1)	**0.88**	**0.4442** (+/-0.0443)	**40**
Aswad 4	Quamishli	Tal Birak	41.08	36.58	305	50	**0.2635** (+/-0.0.05)	**2.0** (+/-0.1)	**0.88**	**0.4033** (+/-0.0417)	**30**
Aswad 5	Aleppo	Tal Bagar	36.98	35.93	317	50	**0.2744** (+/-0.0288)	**1.9** (+/-0.1)	**0.88**	**0.4136** (+/-0.0379)	**28**
Aswad 6	Aleppo	Banus	36.97	35.98	328	50	**0.2765** (+/-0.0409)	**1.9** (+/-0.1)	**0.63**	**0.4200** (+/-0.0623)	**40**
Aswad 7	Aleppo	Soaibieh	37.10	35.92	273	50	**0.2914** (+/-0.0289)	**2.0** (+/-0.1)	**0.88**	**0.4443** (+/-0.0389)	**32**
Aswad 8	Aleppo	Abu-Rowail	37.13	35.92	265	50	**0.3043** (+/-0.0356)	**1.9** (+/-0.1)	**0.75**	**0.4642** (+/-0.0532)	**46**
Aswad 9	Aleppo	Khanasir 1	37.52	35.78	231	50	**0.2687** (+/-0.3530)	**2.0** (+/-0.1)	**0.75**	**0.4142** (+/-0.0529)	**36**
Aswad 10	Aleppo	Im Mial	37.55	35.75	232	50	**0.3309** (+/-0.0310)	**2.0** (+/-0.1)	**0.88**	**0.5091** (+/-0.0459)	**50**
Aswad 11	Aleppo	Sowaiha	37.25	35.80	219	50	**0.2624** (+/-0.0329)	**1.6** (+/-0.1)	**0.63**	**0.3738** (+/-0.0465)	**28**
Aswad 12	Aleppo	Om-amood	37.28	35.78	220	50	**0.3138** (+/-0.0362)	**2.0** (+/-0.1)	**0.75**	**0.4803** (+/-0.0546)	**54**
Abiad 1	Hama		36.72	35.13	394	29	**0.3480** (+/-0.0383)	**2.1** (+/-0.1)	**0.88**	**0.5465** (+/-0.0588)	**69**
Abiad 2	Suweida		36.73	33.03	323	17	**0.2459** (+/-0.0533)	**2.0** (+/-0.2)	**0.75**	**0.4150** (+/-0.0927)	**47**
Abiad 3	Bural Sharqui		37.15	35.02	254	55	**0.2825** (+/-0.0292)	**2.0** (+/-0.1)	**0.88**	**0.4366** (+/-0.0409)	**44**
H. spont. I	Tal Abiad		38.95	36.63	255	48	**0.3637** (+/-0.0285)	**2.1** (+/-0.1)	**0.88**	**0.5612** (+/-0.0463)	**50**
H. spont II	Hassakeh		40.67	36.63	274	40	**0.4423** (+/-0.0066)	**2.1** (+/-0.1)	**1.00**	**0.6391** (+/-0.0054)	**50**

n = number of individuals tested; H = Average gene diversity; A = Mean number of alleles per locus;
P 0.01= Proportion of polymorphic loci; M = Number of multilocus combinations per100 individuals;
S = Shannon's information index.

ISSR marker loci

Table 5.3.3 Genetic indices calculated from allele frequencies of 25 ISSR marker loci of accessions of BLR and *H. spontaneum* from Syria (standard errors in parentheses).

Code	Region	Location	Longitude (E)	Latitude (N)	Altitude (m)	n	ISSR markers: H	A	P (0.01)	S	M
Aswad 1	Palmyra		38.88	35.10	153	39	**0.1895** (+/-0.0307)	**1.6** (+/-0.1)	**0.48**	**0.2892** (+/-0.0445)	**74**
Aswad 2	Raqqa		39.22	36.38	218	48	**0.3386** (+/-0.0254)	**1.9** (+/-0.0)	**0.88**	**0.4952** (+/-0.0346)	**95**
Aswad 3	Hassakeh	West of	40.12	36.53	229	50	**0.1673** (+/-0.0263)	**1.6** (+/-0.1)	**0.56**	**0.2576** (+/-0.0383)	**71**
Aswad 4	Quamishli	Tal Birak	41.08	36.58	305	48	**0.2242** (+/-0.0289)	**1.7** (+/-0.1)	**0.56**	**0.3379** (+/-0.0409)	**46**
Aswad 5	Aleppo	Tal Bagar	36.98	35.93	317	48	**0.1416** (+/-0.0276)	**1.4** (+/-0.1)	**0.36**	**0.2135** (+/-0.0403)	**48**
Aswad 6	Aleppo	Banus	36.97	35.98	328	48	**0.1629** (+/-0.0268)	**1.5** (+/-0.1)	**0.4**	**0.2501** (+/-0.0394)	**51**
Aswad 7	Aleppo	Soaibieh	37.10	35.92	273	48	**0.1276** (+/-0.0275)	**1.4** (+/-0.1)	**0.32**	**0.1905** (+/-0.0400)	**57**
Aswad 8	Aleppo	Abu-Rowail	37.13	35.92	265	48	**0.1325** (+/-0.0255)	**1.4** (+/-0.1)	**0.36**	**0.2034** (+/-0.0382)	**60**
Aswad 9	Aleppo	Khanasir 1	37.52	35.78	231	48	**0.2040** (+/-0.0304)	**1.6** (+/-0.1)	**0.4**	**0.3039** (+/-0.0431)	**62**
Aswad 10	Aleppo	Im Mial	37.55	35.75	232	48	**0.1556** (+/-0.0219)	**1.7** (+/-0.1)	**0.52**	**0.2544** (+/-0.0331)	**56**
Aswad 11	Aleppo	Sowaiha	37.25	35.80	219	48	**0.0947** (+/-0.0215)	**1.4** (+/-0.1)	**0.36**	**0.1543** (+/-0.0321)	**39**
Aswad 12	Aleppo	Om-amood	37.28	35.78	220	48	**0.1261** (+/-0.0269)	**1.4** (+/-0.1)	**0.36**	**0.1919** (+/-0.0388)	**57**
Abiad 3	Bural Sharqui		37.15	35.02	254	48	**0.1266** (+/-0.0258)	**1.5** (+/-0.1)	**0.4**	**0.1956** (+/-0.0377)	**72**
H. spont. I	Tal Abiad		38.95	36.63	255	48	**0.1996** (+/-0.0316)	**1.6** (+/-0.1)	**0.5**	**0.2946** (+/-0.0443)	**48**
H. spont II	Hassakeh		40.67	36.63	274	50	**0.1375** (+/-0.0290)	**1.3** (+/-0.1)	**0.24**	**0.2001** (+/-0.0417)	**50**

n = number of individuals tested; H = Average gene diversity; A = Mean number of alleles per locus;
P 0.01= Proportion of polymorphic loci; M = Number of multilocus combinations per100 individuals;
S = Shannon's information index.

Summary

A summary of means of diversity indices for three groups of populations (BLR Arabi Aswad, BLR Arabi Abiad and *H. spontaneum*) is given separately for all three genetic markers in Table 5.3.4.

Table 5.3.4 Mean diversity indices of BLRs and populations of *H. spontaneum* for three different genetic markers (standard errors in parenthesis).

	n	H	A	P (0.01)	S	M
Morphological marker loci:						
		ns	ns	*	ns	ns
Arabi Aswad	12	**0.2398** (+/-0.0161)	**2.34** (+/-0.08)	**0.83** a (+/-0.04)	**0.4225** (+/-0.0282)	**12** (+/-1)
Arabi Abiad	3	**0.1804** (+/-0.0461)	**2.17** (+/-0.08)	**1.00** a (+/-0.00)	**0.3061** (+/-0.0571)	**7** (+/-2)
{all BLRs	15}	**0.2280** (+/-0.0162)	**2.30** (+/-0.07)	**0.87** (+/-0.03)	**0.3992** (+/-0.0274)	**11** (+/-1)
H. spontaneum	2	**0.2177** (+/-0.0362)	**2.00** (+/-0.00)	**0.50** b (+/-0.00)	**0.3809** (+/-0.0563)	**8** (+/-2)
Isoenzyme marker loci:						
		***	*	ns	**	ns
Arabi Aswad	12	**0.2906** a (+/-0.0065)	**1.95** a (+/-0.03)	**0.78** (+/-0.03)	**0.4454** a (+/-0.0121)	**42** (+/-4)
Arabi Abiad	3	**0.2921** a (+/-0.0299)	**2.04** a (+/-0.04)	**0.83** (+/-0.04)	**0.4660** a (+/-0.0407)	**53** (+/-8)
{all BLRs	15}	**0.2909** (+/-0.0072)	**1.97** (+/-0.03)	**0.79** (+/-0.02)	**0.4495** (+/-0.0120)	**44** (+/-3)
H. spontaneum	2	**0.4030** b (+/-0.0393)	**2.13** b (+/-0.00)	**0.94** (+/-0.06)	**0.6002** b (+/-0.0390)	**50** (+/-0)
ISSR marker loci:						
		ns	ns	ns	ns	ns
Arabi Aswad	12	**0.1721** (+/-0.0184)	**1.55** (+/-0.05)	**0.46** (+/-0.04)	**0.2618** (+/-0.0262)	**60** (+/-4)
Arabi Abiad	1	**0.1266** (+/-0.0000)	**1.50** (+/-0.00)	**0.40** (+/-0.00)	**0.1956** (+/-0.0000)	**72** (+/-0)
{all BLRs	13}	**0.1686** (+/-0.0173)	**1.55** (+/-0.04)	**0.46** (+/-0.04)	**0.2567** (+/-0.0246)	**61** (+/-4)
H. spontaneum	2	**0.1686** (+/-0.0311)	**1.45** (+/-0.15)	**0.36** (+/-0.12)	**0.2474** (+/-0.0473)	**49** (+/-1)

n = number of accessions tested; H = Average gene diversity; A = Number of polymorphic alleles; P (0.01) = Proportion of polymorphic loci; M = Number of multilocus combinations per 100 individuals; S = Shannon's information index

A statistical analysis of each genetic index per molecular marker group was carried out between results of three groups of populations, namely BLR Arabi Aswad, BLR Arabi Abiad and *H. spontaneum*. Genetic indices that showed significant differences between groups were marked with blue asterisks. Small blue letters were used to indicate significances between different groups. The statistical analysis of the results was carried out using the ANOVA procedure for genetic indices which were predominantly normally distributed such as *H, S* and *M.* Non-normally distributed indices (*A, P(0.01)*) were analysed with the non-parametric Mood-Median test. Details of the statistical analysis are given in Appendix 7.

The results show a significantly lower proportion of polymorphic loci (*P (0.01)*) for the *H. spontaneum* populations compared with BLRs considering the morphological markers. The isoenzyme markers produced significantly higher diversity indices (*H, A* and *S*) for the *H. spontaneum* populations than for BLRs. This result was highly significant for Shannon's information index (*S*) and very highly significant for the average gene diversity (*H*). No significant differences between BLRs and *H.spontaneum* populations were detected when results of the molecular ISSR markers were analysed.

5.3.1.2 Regional variation of diversity levels of BLRs

ANOVA

Only accessions of BLR Arabi Aswad were considered to investigate differences in the diversity levels between populations from different regions, in order to rule out differences caused by dealing with different landraces or *H. spontaneum* populations. Again, the statistical analysis of the results was carried out using the ANOVA procedure for genetic indices which were predominantly normally distributed such as *H, S* and *M.* Non-normally distributed indices (*A, P(0.01)*) could not be analysed using non-parametric tests, because too many observations had no replication. Results are given in Table 5.3.5, details of the statistical analysis are given in Appendix 7, part 2.

Table 5.3.5 Mean diversity indices of BLRs accessions of Arabi Aswad from five distinct regions in Northern Syria. Results are given for three different genetic markers.

Region	n	H	A	P (0.01)	S	M
Morphological marker loci:						
		***			**	ns
Palmyra	1	0.2537 ab	2.3	0.8	0.4374 ab	12
Raqqa	1	0.3326 b	2.5	0.8	0.5840 b	13
Hassakeh	1	0.3150 b	2.5	0.8	0.5455 b	5
Quamishli	1	0.3271 b	2.8	1.0	0.5718 b	9
Aleppo	8	0.2062 a	2.3	0.9	0.3664 a	12
Isoenzyme marker loci:						
		ns			ns	ns
Palmyra	1	0.3166	2.1	0.8	0.5031	64
Raqqa	1	0.2917	2.0	0.8	0.4741	57
Hassakeh	1	0.2926	2.0	0.9	0.4442	40
Quamishli	1	0.2635	2.0	0.9	0.4033	30
Aleppo	8	0.2903	1.9	0.8	0.4399	39
ISSR marker loci:						
		**			**	**
Palmyra	1	0.1895 ab	1.6	0.5	0.2892 ab	74 ab
Raqqa	1	0.3386 b	1.9	0.9	0.4952 b	95 b
Hassakeh	1	0.1673 ab	1.6	0.6	0.2576 ab	71 ab
Quamishli	1	0.2242 ab	1.7	0.6	0.3379 ab	46 a
Aleppo	8	0.1431 a	1.5	0.4	0.2203 a	53 a

n = number of accessions; H = Average gene diversity; A = Mean number of alleles per locus; P (0.01) = Proportion of polymorphic loci; M = Number of multilocus combinations per 100 individuals; S = Shannon's information index

No standard errors were given in Table 5.3.5, as results related mainly to one region only. However, ANOVA procedures produced significant differences between regions for some genetic indices, which were marked with blue asterisks. Small blue letters were used to indicate significant differences between regions.

Collection sites of BLR Arabi Aswad were separated into 5 major regions (i.e. Palmyra, Raqqa, Hassakeh, Quamishli and Aleppo) in which genetic indices (*H* and *S*) of morphological and ISSR markers showed consistently that accessions of the Aleppo region had the highly significantly lowest diversity indices. Considering the morphological markers, accessions from Raqqa, Hassakeh and Quamishli showed highly significantly higher H and S values compared with accessions from the Aleppo region, while results of the ISSR-markers showed highly significantly higher diversity indices (*H, S*) for the accession from the Raqqa region. The number of

multilocus combinations (*M*) of the ISSR markers showed equally low values for accessions from Quamishli and the Aleppo region and the highly significantly highest value for the accession of the Raqqa region.

Furthermore, diversity indices of 8 accessions from the Aleppo region were analysed on a regional scale. Two accessions from close locations (see Section 3.1.1 and Plate 3.2.5) were used as replicates for four distinct rainfall zones of the Aleppo region. Zones were marked A (> 300 mm rainfall), B (260-300 mm rainfall), C (230-260 mm rainfall) and D (200 – 230 mm rainfall). Results are given in Table 5.3.6. Details of the statistical analysis are given in Appendix 7, part 3.

Table 5.3.6 Mean diversity indices of BLRs accessions of Arabi Aswad from 4 zones of the Aleppo region (see text for details). Results are given for three different genetic markers.

Aleppo region	n	H	A	P (0.01)	S	M
Morphological marker loci:						
		ns			ns	ns
Zone A	2	0.1964	2.2	0.9	0.3308	10
Zone B	2	0.2045	2.2	0.9	0.3579	14
Zone C	2	0.1940	2.2	0.9	0.3516	13
Zone D	2	0.2299	2.7	0.9	0.4253	13
Isoenzyme marker loci:						
		ns			ns	ns
Zone A	2	0.2755	1.9	0.8	0.4168	34
Zone B	2	0.2979	2.0	0.8	0.4542	39
Zone C	2	0.2881	1.8	0.7	0.4271	41
Zone D	2	0.2998	2.0	0.8	0.4617	43
ISSR marker loci:						
		ns			ns	ns
Zone A	2	0.1523	1.5	0.38	0.2318	49
Zone B	2	0.1301	1.4	0.34	0.1970	58
Zone C	2	0.1104	1.4	0.36	0.1731	48
Zone D	2	0.1798	1.7	0.46	0.2792	59

n = number of accessions; H = Average gene diversity; A = Number of polymorphic alleles; P (0.01) = Proportion of polymorphic loci; M = Number of multilocus combinations per 100 individuals; S = Shannon's information index;

No standard errors were given in order not to complicate the table. None of the normally distributed genetic indices (*H, S* and *M*) showed significantly different diversity level for any of the four rainfall zones in the Aleppo region, consistently for all three genetic markers. However, it is apparent from Table 5.3.6 that all diversity indices based on accessions from zone D (i.e. the zone with the lowest rainfall) show the highest diversity indices. To investigate this phenomenon further, a two–way-ANOVA was conducted, analysing the influence of the factors *zones* (i.e. 4 zones) and *markers* (i.e. 3 genetic markers) on the diversity indices *H, S* and *M*. For all three cases (i.e. diversity indices) very highly significantly differences between genetic markers were found, but only the Shannon Index showed significant differences between accessions from different rainfall zones. Accessions from the zone with the lowest rainfall (i.e. 200 – 230 mm) showed significantly higher Shannon indices in comparison with accessions from all other zones. Details of the analysis are given as Appendix 7, part 4.

Application of GIS ArcView

Average gene diversity (*H*) of BLR Arabi Aswad as given in Tables 5.3.1 to 5.3.3 were further analysed for all three genetic markers using the spatial analyst of the GIS-programme ArcView (see Section 3.2.2). However, the sampling regime was not suitable to allow a meaningful interpolation using the programme ArcView, so that results were not analysed further. For completeness results of the interpolation are given as Appendix 10.

Principal component analysis

A Principal component analysis (PCA) was carried out to show whether accessions were clustering together when all genetic indices of the three genetic markers were considered (as given in Tables 5.3.1 to 5.3.3). Only accessions of BLR Arabi Aswad and the two *H. spontaneum* accessions were included in the PCA, because data were incomplete for accessions of Arabi Abiad. The first component of the PCA explained 41% of the variation, the second component another 28% and the third component another 12%. Thus considering the first three components of the PCA (Figure 5.3.1

and 5.3.2) 81% of the total variation of five genetic indices, of three genetic markers each was explained.

Figure 5.3.1 The first and second component of a principal component analysis of 5 genetic indices (*H, A, P 0.01, S, M*) of three genetic markers (Morphological, isoenzyme and ISSR markers) of accessions from different regions of BLR Arabi Aswad and *H. spontaneum*.

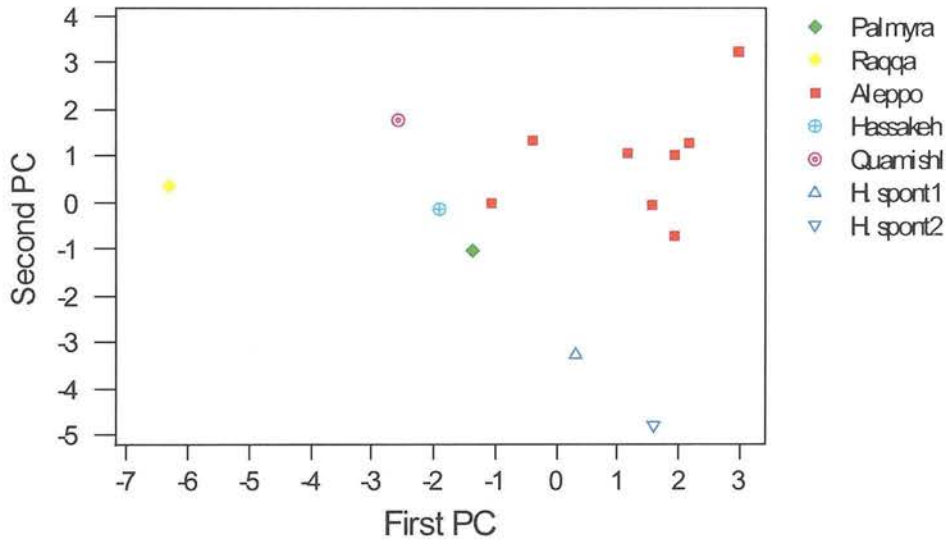
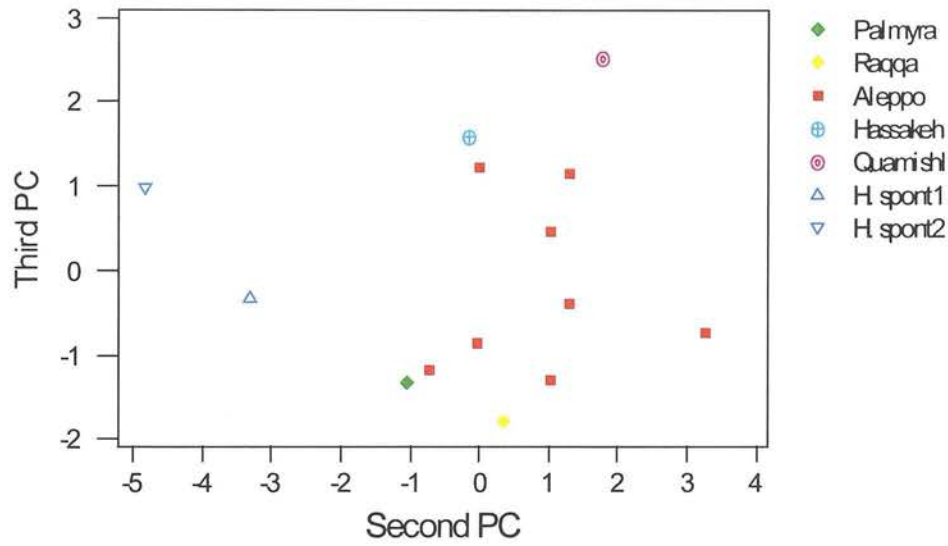


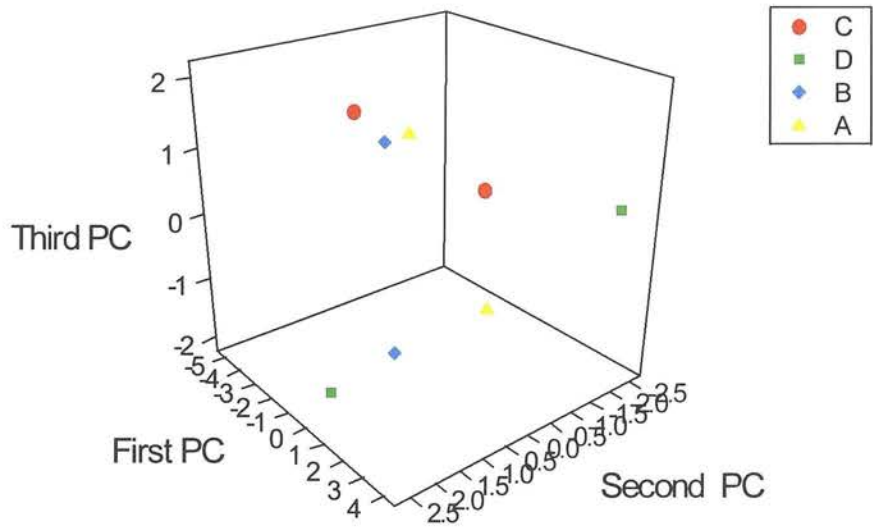
Figure 5.3.2 The second and third component of a principal component analysis of 5 genetic indices (*H, A, P 0.01, S, M*) of three genetic markers (Morphological, isoenzyme and ISSR markers) of accessions from different regions of BLR Arabi Aswad and *H. spontaneum*.



The first three principal components show a clear grouping of BLR Arabi Aswad from the Aleppo region and the two *H. spontaneum* accessions. BLR Arabi Aswad from Raqqa, which showed the highest diversity over all genetic indices and markers takes an outlying position considering the first and second principal component.

A further PCA was carried out to investigate the association of BLR accessions of Arabi Aswad from the Aleppo region. The letters used in the figure denote zones with different average annual rainfall (i.e. A > 300 mm, B = 260 - 300 mm, C = 230 - 260 mm and D = 200 – 230 mm) from which two accessions each were collected. The distance between collection sites per zone were on average about 4.5 km, the distance between zones on average 30 km.

Figure 5.3.3 Three components of a principal component analysis of 5 genetic indices (*H, A, P 0.01, S, M*) of three genetic markers (Morphological, isoenzyme and ISSR markers) of accessions of BLR Arabi Aswad two each from different rainfall zones from the Aleppo region.



A total of 78% of the total variation was explained considering the first three components of the PCA of the correlation matrix. However, no grouping of accessions of BLR Arabi Aswad from identical zones of the Aleppo region was observed.

5.3.1.3 Correlation between genetic indices and environmental conditions of BLR populations from different eco-geographical environments

On a macro-geographic scale Nevo (1992) found that a substantial portion of allozyme diversity of wild barley populations is significantly correlated with environmental conditions, namely mean annual rainfall, humidity and August temperatures. It appeared that allozyme diversity was significantly associated with an increase of mean annual rainfall. On a micro-geographic scale aridity stress seems to be associated with increased allozyme diversity (Nevo, 1992; Nevo et al., 1997).

In the present study the diversity of accessions of BLR Arabi Aswad was investigated on a macro-geographic scale using 12 accessions of BLR Arabi Aswad from five regions of Northern Syria, and on a smaller scale (i.e. 65 km diameter) using eight accessions of BLR Arabi Aswad from the Aleppo region. Three genetic markers (morphological-, isoenzyme- and ISSR-markers) were used to quantify genetic diversity. Environmental data were interpolated for the sampling sites as described in Chapter 4. Three kinds of environmental indices were considered, namely general environmental indices (i.e. altitude (*Alt*), average annual rainfall (*P*), average annual temperature (*T*)), environmental stress indices (i.e. average minimum temperature of the coldest month (T_{min}), average maximum temperature of the hottest month (T_{max}) and percent continentality (*C*)) and one index to describe environmental heterogeneity (i.e. relative interannual variation in rainfall (P_{CV})). Further details are given in Section 3.2.2. The results of the interpolation of environmental data are summarised in Table 4.1, Chapter 4. These results were correlated with the genetic indices (*H, A, P 0.01, S* and *M*) achieved for all three genetic markers. Pearson's correlation coefficient were calculated to find significant associations between environmental and genetic indices. A linear regression was calculated for all comparisons with Pearson's correlation coefficients greater than 0.5 or less than -0.5. Results are given for all 12 Arabi Aswad accessions and the 8 accessions from the Aleppo region in Tables 5.3.7 and 5.3.8, respectively.

Table 5.3.7 Pearson's correlation coefficient (> 0.5 or < -0.5) for comparisons between environmental and genetic indices of 12 BLR accessions from northern Syria.

	Alt.	P	P_{cv}	T	T_{min}	T_{max}	C
Morphology:							
H				**0.63** *		**0.92** ***	**0.86** ***
A						0.53 ns	0.57 ns
P (0.01)							
S				0.57 ns		**0.89** ***	**0.87** ***
M							
Isoenzymes:							
H		-0.55 ns	0.56 ns				
A	0.50 ns			0.53 ns			
P (0.01)							
S	0.56 ns	**-0.61** *					
M	**0.76** **	**-0.69** *					
ISSR:							
H	**0.65** *			0.51 ns		**0.61** *	
A	0.59 ns			0.51 ns		**0.66** *	**0.60** *
P (0.01)	0.54 ns					**0.70** *	**0.64** *
S	**0.66** *			0.52 ns		**0.62** *	
M	**0.67** *	**-0.64** *					

Alt.= Altitude; P = average annual rainfall; P_{cv} = relative interannual variation in rainfall; T = average temperature; T_{min} = average minimum temperature of the coldest month; T_{max} = average maximum temperature of the hottest month; C = continentality in %.

Considering the northern part of Syria, the coefficients in Table 5.3.7 show a positive correlation between altitude of the collection site and genetic indices of BLR accessions of Arabi Aswad. The correlation coefficients predict a significantly higher number of multilocus combinations for both the isoenzyme and the ISSR marker loci, as well as higher average gene diversity and higher Shannon's indices for the ISSR marker loci, with increasing altitude. The range of the altitude of collection sites was 296 to 550 m. The significant correlation between average annual rainfall and the genetic indices *S* and *M* of the isoenzyme markers and *M* of the ISSR markers predict significantly higher diversity indices at lower levels of average annual rainfall. Further positive correlations were found between temperature indices and diversity indices of the morphological and the ISSR marker loci. These correlation coefficients predict significantly higher diversity (*H*, morphological marker loci) with higher average temperature and very highly significantly higher diversity indices (i.e. *H, S* of morphological marker loci) with increasing average maximum temperature (T_{max}) and continentality (*C*).

Average maximum temperature and continentality were significantly positively correlated with diversity indices achieved with the ISSR markers. *H, A, P (0.01)* and S were significantly higher at higher average maximum temperatures of the sampling sites, and *A* and *P(0.01)* were significantly higher with increasing continentality.

A multiple regression combining all environmental indices (see Table 5.3.7, columns) gave significant correlation coefficients for the following genetic indices of morphological marker loci: *H**, *S** and of ISSR marker loci: *A**, *P (0.01)** and *S**. These results agree in principle with results given Table 5.3.7 and are caused mainly by the high correlation coefficients of altitude, continentality and maximum temperature. A multiple regression combining altitude, average annual rainfall and average annual temperature gave no significant correlation with any one of the genetic indices of the three different markers.

On a smaller geographical scale, only the altitude of the collection site showed significant correlation coefficients with diversity indices of the morphological and the ISSR marker loci, suggesting higher diversity at higher altitudes. However, the range of the altitude of these collection sites was only 303 to 365 m. Results are given in Table 5.3.8. No further significant associations between environmental and genetic indices were found within the Aleppo region.

Table 5.3.8 Pearson's correlation coefficient (> 0.5 or < -0.5) for comparisons between environmental and genetic indices of 8 BLR accessions from the Aleppo region in Syria.

	Alt.	P	P_{cv}	T	T_{min}	T_{max}	C
Morphology:							
H	**0.71** *						
A		-0.57 ns					
P (0.01)							
S	0.53 ns	-0.59 ns			0.63 ns		
M			0.54 ns			0.63 ns	
Isoenzymes:							
H							
A	0.64 ns						
P (0.01)							
S							
M							
ISSR:							
H	**0.81** *						
A	**0.78** *						
P (0.01)	0.69 ns						
S	**0.86** *						
M	0.60 ns						

Alt.= Altitude; P = average annual rainfall; P_{cv} = relative interannual variation in rainfall; T = average temperature; T_{min} = average minimum temperature of the coldest month; T_{max} = average maximum temperature of the hottest month; C = continentality in %.

5.3.2 Differentiation of BLR populations and *H. spontaneum* from Syria

The comparison of populations which are components of a meta-population (i.e. the entirety of populations under consideration) does not only concern diversity levels. It can also include the way in which diversity is partitioned within and between populations and similarities between populations expressed in form of genetic identity and genetic distance.

5.3.2.1 Partitioning of diversity within and between populations

The total diversity (H_T) of populations can be divided into diversity within populations and diversity found between populations. The Fixation index (F_{ST}) gives the relative amount of the total diversity which is found between populations and can be expressed as a percentage. The average gene diversity (H_S), when expressed as a percentage of H_T, gives the percentage of the total diversity which is found within populations. For example, modern barley cultivars are genetically pure lines, i.e. the diversity within cultivar populations is zero. A comparison between cultivars from different breeding programmes will show some genetic variation between them. In this case the total diversity of the populations under consideration (i.e. a certain number of cultivars) lies *between* the populations and the fixation index would be $F_{ST} = 1$.

It can be expected for an inbreeding species like barley, that evolutionary forces such as genetic drift and selection will decrease the within-population diversity and increase the diversity between populations (Baur and Schmid, 1996).

The following table (5.3.9) gives the results of the analysis of the partitioning of diversity in a hierarchical way, i.e. from the species to the subspecies to the population level. The results of three genetic markers were considered. No standard errors were given in order not to complicate the table. Percentages were given next to H_S and F_{ST} values to simplify their interpretation.

Table 5.3.9 Partitioning of diversity within and between barley populations in a hierarchical order (i.e. species to subspecies to population level). Results are given for three different genetic markers.

	n	Total diversity H_T	Diversity within populations H_S [%]	Diversity between populations F_{ST} [%]
Morphology:				
H. vulgare	17	**0.4318**	**0.2267** [52.5%]	**0.475** [47.5%]
H. v. ssp. vulgare (=BLRs)	15	**0.3891**	**0.2280** [58.6%]	**0.414** [41.4%]
H. v. ssp. spontaneum (= wild barley)	2	**0.2223**	**0.2176** [97.9%]	**0.021** [2.1%]
BLR Arabi Aswad	12	**0.3054**	**0.2398** [78.5%]	**0.215** [21.5%]
BLR Arabi Abiad	3	**0.2463**	**0.1804** [73.3%]	**0.267** [26.7%]
Isoenzymes:				
H. vulgare	17	**0.3709**	**0.3041** [82.0%]	**0.180** [18.0%]
H. v. ssp. vulgare (=BLRs)	15	**0.3449**	**0.2909** [84.3%]	**0.157** [15.7%]
H. v. ssp. spontaneum (= wild barley)	2	**0.4800**	**0.4030** [84.0%]	**0.160** [16.0%]
BLR Arabi Aswad	12	**0.3397**	**0.2906** [85.5%]	**0.145** [14.5%]
BLR Arabi Abiad	3	**0.3285**	**0.2921** [88.9%]	**0.111** [11.1%]
ISSR:				
H. vulgare	15	**0.3185**	**0.1686** [52.9%]	**0.471** [47.1%]
H. v. ssp. vulgare (=BLRs)	13	**0.3143**	**0.1686** [53.6%]	**0.464** [46.4%]
H. v. ssp. spontaneum (= wild barley)	2	**0.2216**	**0.1686** [76.1%]	**0.239** [23.9%]
Arabi Aswad	12	**0.3154**	**0.1721** [54.6%]	**0.454** [45.4%]
Arabi Abiad	1		**0.1266**	

n = Number of accessions

The results in Table 5.3.9 show lower levels of F_{ST} values with the isoenzyme markers compared with F_{ST} values achieved with both the isoenzyme and the ISSR markers. However, in general the data in Table 5.3.9 show that most of the diversity was found *within* populations for both BLRs and *H. spontaneum* populations. The diversity within populations of BLRs ranged from 55% to 89% and between 76% to 98% within *H. spontaneum* populations. The results achieved with the isoenzyme markers showed very little difference between F_{ST} values of BLRs and *H. spontaneum* populations. In contrast, the F_{ST} values of *H. spontaneum* populations achieved with the morphological markers was very low (i.e. 2.1%) and had a proportion of only 5% of the F_{ST} values of the BLRs (i.e. 41%). F_{ST} values of *H. spontaneum* populations based on the ISSR markers were as well lower, as those obtained for BLRs. However, F_{ST} values of *H. spontaneum* populations were about 50% of the F_{ST} values of BLRs.

5.3.2.2 Linkage disequilibrium

When alleles of certain genes appear in non-random association they are said to be in linkage disequilibrium (LD) (Nei, 1987). For example in individuals of BLR population Arabi Abiad 3, the allele *short rachilla hair* occurred always jointly with the allele *rough awn*, and the allele *long rachilla hair* with the allele *smooth awn*. The two alleles were non-randomly associated and a significance test showed that they were in linkage disequilibrium ($p < 0.01$). LD appears much more frequently in inbreeding species than in outbreeding species, because with random mating there is a higher likelihood that linked genes are separated through crossing over (Hartl, 1988). LD is of some importance for selection, because if the allele of one gene is subject to selection, the allele of a linked gene will automatically be selected as well. Therefore LD may be a measure of the intensity of natural selection and thus the level of adaptation (Ayala, 1982).

In the present study LD was analysed using the programme Popgene (Yeh, et al., 1997) and given as number of significant LDs at $p < 0.05$ for all accessions of BLRs Arabi Aswad, Arabi Abiad and *H. spontaneum*, for all tested morphological and isoenzyme marker loci (Weir, 1979). Results are given in Table 5.4.1.

Table 5.4.1 Observed number and kind of LD in populations of BLRs and *H. spontaneum* from Syria.

		Morphological markers:		Isoenzyme markers:	
Code	Region	LDs	Linked loci*	LDs	Linked Esterase loci
Aswad 1	Palmyra	1	②+③	4	2+2b
Aswad 2	Raqqa	4	②+③	4	2+2b
Aswad 3	Hassakeh	10	②+③, *①+③*	8	2+2b, *5+7*
Aswad 4	Quamishli	4	②+③	4	2+2b
Aswad 5	Aleppo	0		4	2+2b
Aswad 6	Aleppo	0		4	2+2b
Aswad 7	Aleppo	4	*③+④*	4	2+2b
Aswad 8	Aleppo	0		4	2+2b
Aswad 9	Aleppo	3	②+③	8	2+2b, 6+8
Aswad 10	Aleppo	2	②+③	8	2+2b, 6+8
Aswad 11	Aleppo	2	②+③	4	2+2b
Aswad 12	Aleppo	0		4	2+2b
Abiad 1	Hama	0		6	2+2b, *2b+5*
Abiad 2	Suweida	2	②+③	4	6+8
Abiad 3	Bural Sharqui	4	①+④	0	
H. spont. I	Tal Abiad	1	②+③	8	*4+5, 6+7*
H. spont II	Hassakeh	4	②+③	28	2+2b, 2+4, *2+5*, 2b+4, *2b+5*, *4+5*, 7+8

* ① = Rachilla hair length, ② = Lemma colour, ③ = Seed colour, ④ = Spicules on lemma and awn.

Association between alleles of loci which are located on different chromosomes are given in blue italics. Results in Table 5.4.1 show regional differences of the number of occurring of LDs of morphological marker loci within BLRs and populations of *H.spontaneum*. The majority of LDs between alleles of morphological marker loci occurred between lemma colour and seed colour. Associations between alleles of the loci rachilla hair length and seed colour as well as seed colour and spicules on lemma and awns occurred only once each in accessions of BLR Arabi Aswad. Only in one accession of BLR Arabi Abiad an association between rachilla hair length and spicules on lemma and awns was observed.

Associations between isoenzyme loci were similar for most accessions of BLR Arabi Aswad, which all showed a LD between alleles of the Est 2 and Est 2b loci. A different spectrum of LDs were observed in accessions of BLR Arabi Abiad. Accessions of *H. spontaneum* showed a different spectrum of LDs compared with

BLRs. The by far highest number of LDs was observed in accession *H. spont.* 2, from Hassakeh.

5.3.2.3 Gene flow

Gene flow is the movement of genes between geographically distinct populations through migration of pollen or seeds (Allaby, 1992). Gene flow between geographically distant populations is restricted. Populations at greater distance are more genetically differentiated by genetic drift, than those that are close geographically. This phenomenon is called isolation by distance.

In the case of a self-pollinating crop species such as barley, gene flow can be expected to be associated mainly with seed exchange between farmers, unanticipated admixtures and to a small extent outcrossing with foreign pollen. Slatkin (1993) demonstrated using simulations that gene flow depends on the geographic distance between populations such that the likelihood of exchange of genes between populations decreases linearly for log-transformed variables of both inferred gene flow (*Nm*) and geographic distance (*km*).

Gene flow was measured using the index *Nm* (where *N* is the population size and *m* is the rate of migration, Hamrick and Godt, 1990), which is directly derived from F_{ST} (for details see Section 3.5). *Nm* was calculated between 12 accessions of BLR Arabi Aswad and two populations of *H. spontaneum*, from allele frequencies at 8 isoenzyme marker loci (Est1 to Est8) as described in Section 3.5. Geographic distance was calculated from the co-ordinates of the collection sites. Results are given in Table 5.4.2.

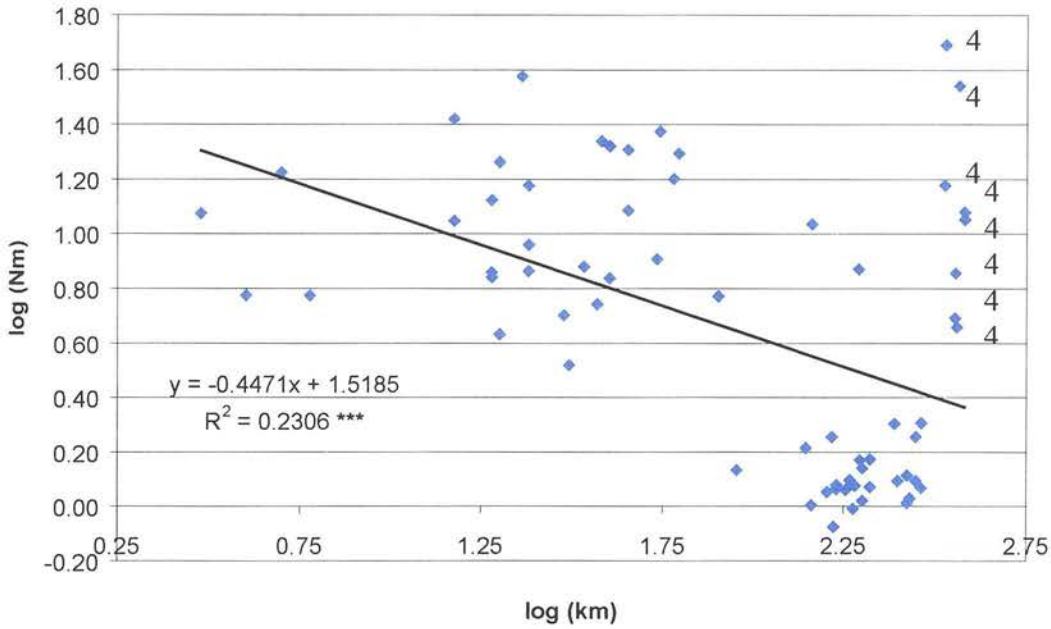
Table 5.4.2 Gene flow (Nm) given above the diagonal and geographic distance in km given below the diagonal, between populations of BLR Arabi Aswad from 12 collection sites and *H. spontaneum* from two collection sites in Syria.

Region: Location:	Aswad 1 Palmyra	Aswad 2 Raqqa	Aswad 3 West of Hassakeh	Aswad 4 Quamishli Tal Birak	Aswad 5 Aleppo Tal Bagar	Aswad 6 Aleppo Banus	Aswad 7 Aleppo Soaibieh	Aswad 8 Aleppo Abu-Rowail	Aswad 9 Aleppo Khanasir 1	Aswad 10 Aleppo Im Mial	Aswad 11 Aleppo Sowaiha	Aswad 12 Aleppo Om-amood	H.spont.1 Tal Abiad	H. spont. 2 Hassakeh
Aswad 1	-	10.86	7.44	1.03	1.38	1.05	1.25	1.15	1.01	1.64	0.84	1.13	1.47	1.66
Aswad 2	145	-	5.92	1.20	1.49	1.18	1.48	1.19	1.16	1.80	0.98	1.18	1.08	1.94
Aswad 3	195	80	-	1.36	2.03	1.17	1.80	1.24	1.24	2.01	1.07	1.30	1.31	2.34
Aswad 4	265	170	90	-	12.02	11.30	34.80	4.56	49.06	15.09	4.92	7.19	1.07	1.52
Aswad 5	200	210	290	380	-	5.97	26.40	4.30	8.09	15.91	3.30	5.53	1.38	1.42
Aswad 6	200	210	290	380	4	-	11.18	18.35	23.70	19.72	7.59	21.82	1.26	1.29
Aswad 7	185	197	280	367	15	15	-	5.95	20.93	20.30	6.95	9.14	1.24	1.67
Aswad 8	180	191	280	362	20	20	6	-	6.90	12.19	7.24	37.69	1.79	1.27
Aswad 9	145	170	250	337	54	55	40	40	-	16.73	7.31	13.30	1.09	1.48
Aswad 10	140	165	245	335	60	62	45	45	5	-	5.04	15.04	1.88	1.76
Aswad 11	167	189	270	358	31	34	19	19	24	30	-	11.89	0.96	1.39
Aswad 12	160	184	265	359	37	38	24	23	19	24	3	-	1.57	1.48
H. spont. 1	170	39	105	195	190	190	180	180	160	156	176	174	-	1.26
H. spont. 2	220	131	52	44	340	340	325	320	295	290	315	310	160	-

The figures in Table 5.4.2 show that gene flow between the two *H. spontaneum* accessions and BLRs was low (*Nm* range: 0.96 to 2.34). The highest gene flow of the *H. spontaneum* accessions (*Nm* = 2.34) was found between BLR Arabi Aswad 3 from the Hassakeh region and *H. spontaneum* 1 which is located in the same region in about 55 km distance in the main wind direction (i.e. westerly).

To illustrate the association of gene flow and geographic distance of accessions of BLR Arabi Aswad given in Table 5.4.2, the log of *Nm* values was plotted against the log of the geographic distance between populations and is shown in Figure 5.3.4.

Figure 5.3.4 Gene flow (log (*Nm*)) vs geographic distance (log(*km*)) of 12 populations of BLR Arabi Aswad from Syria.

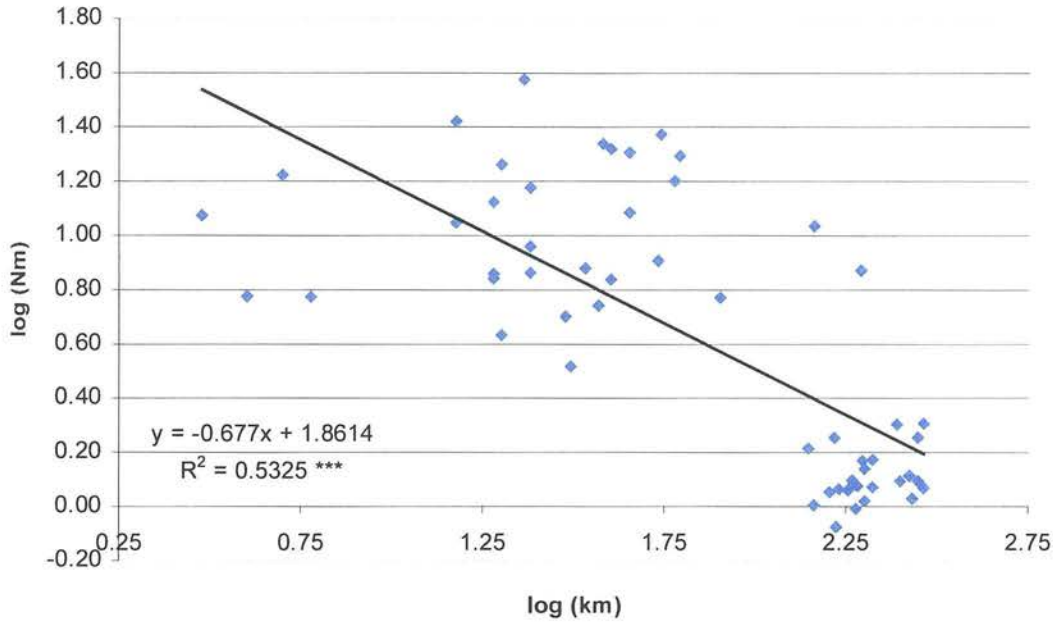


Following Slatkin (1993) a log-transformation of gene flow (*Nm*) and geographic distance (*km*) was conducted to look for evidence of isolation by distance. The regression function and R^2 values are given within the figure.

The trend line in Figure 5.3.4 shows a low R^2 value, which was still very highly significant. This poor correlation (Pearson's correlation coefficient of – 0.48) was mainly caused by comparisons between BLR Arabi Aswad 4 from Quamishli, Tal Birak (marked *4* in Figure 5.3.4) and those BLRs from the Aleppo region. The gene flow between the accession Arabi Aswad 4 and geographically distant accessions

from the Aleppo region (about 350 km distance) was very high (see Table 5.4.2). Excluding the gene flow data of the accession Aswad 4 from the sampling site Quamishli, Tal Birak, gave a different picture which is shown in Figure 5.3.5.

Figure 5.3.5 Gene flow (log (*Nm*)) vs geographic distance (log(*km*)) of 11 populations of BLR Arabi Aswad from Syria, excluding the collection site of Arabi Aswad 4 from Quamishli, Tal Birak.



The trend line in Figure 5.3.5 shows a very much higher R^2 value than the one in Figure 5.3.4. The trend line in Figure 5.3.5 describes the relationship between gene flow and geographic distance as expected, i.e. increasing exchange of genes between populations grown in close proximity, and little gene flow between geographically distant populations.

5.3.2.4 Genetic distance

Genetic identity (*I*) quantifies the similarity and genetic distance (*D*) the distance between two populations on a scale of 0 to 1. Results of BLR Arabi Abiad were excluded from the present sections of this chapter because only three accessions of Arabi Abiad were available which originated from the middle and south of Syria, while all accessions of Arabi Aswad and *H. spontaneum* originated from the northern part of the country. Genetic identity and genetic distance were calculated separately for the three genetic markers used. All results are listed in Tables 5.4.3 to 5.4.5 in Appendix 8. Results were summarised in dendrograms which were produced from genetic distances (*D*) given in Tables 5.4.3 to 5.4.5, using the Unweight Pair Group Method with Arithmetic Averages (UPGMA) as employed by the programme Popgene (Yeh, et al., 1997). Dendrograms are given in the following Figures 5.3.6 to 5.3.8, and branch lengths of dendrograms are given in the corresponding Tables 5.4.6 to 5.4.8 as Appendix 9.

Figure 5.3.6 Dendrogram of Arabi Aswad populations and *Hordeum spontaneum* from Syria, based on Nei's Genetic distance (Nei, 1972) of morphological markers (4 loci) (Method = UPGMA).

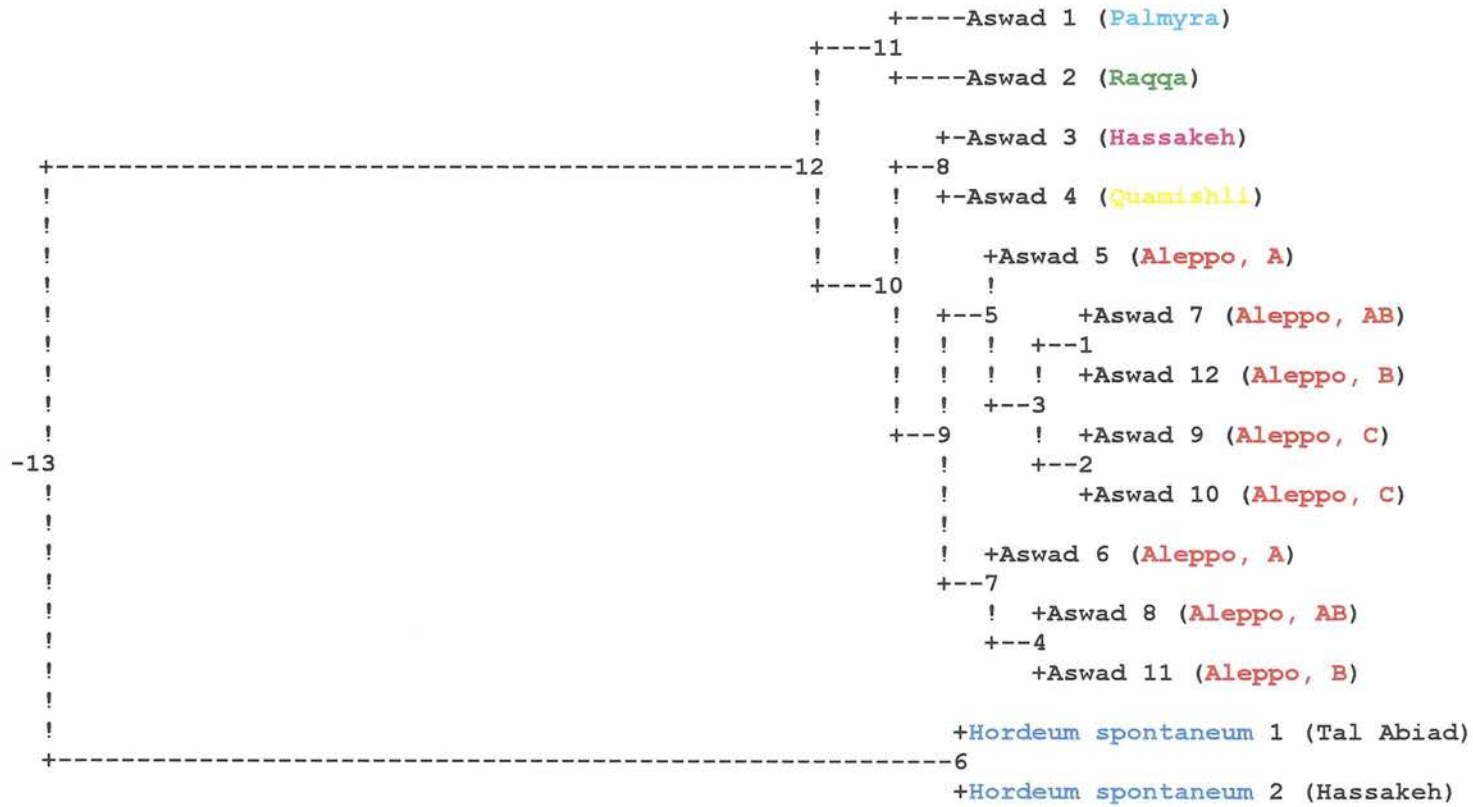


Figure 5.3.7 Dendrogram of Arabi Aswad populations and *Hordeum spontaneum* from Syria, based on Nei's Genetic distance (Nei, 1972) of isoenzyme markers (8 loci) (Method = UPGMA).

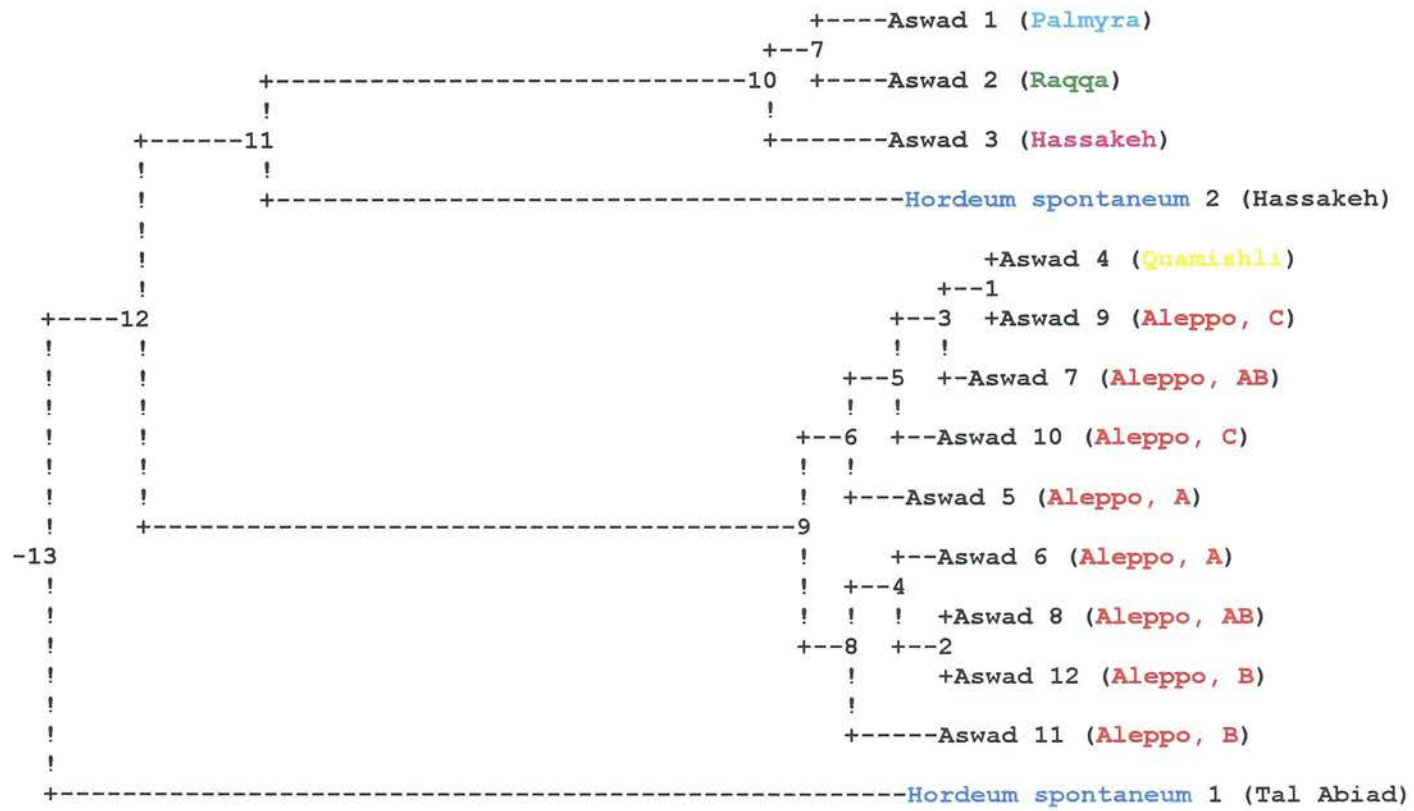
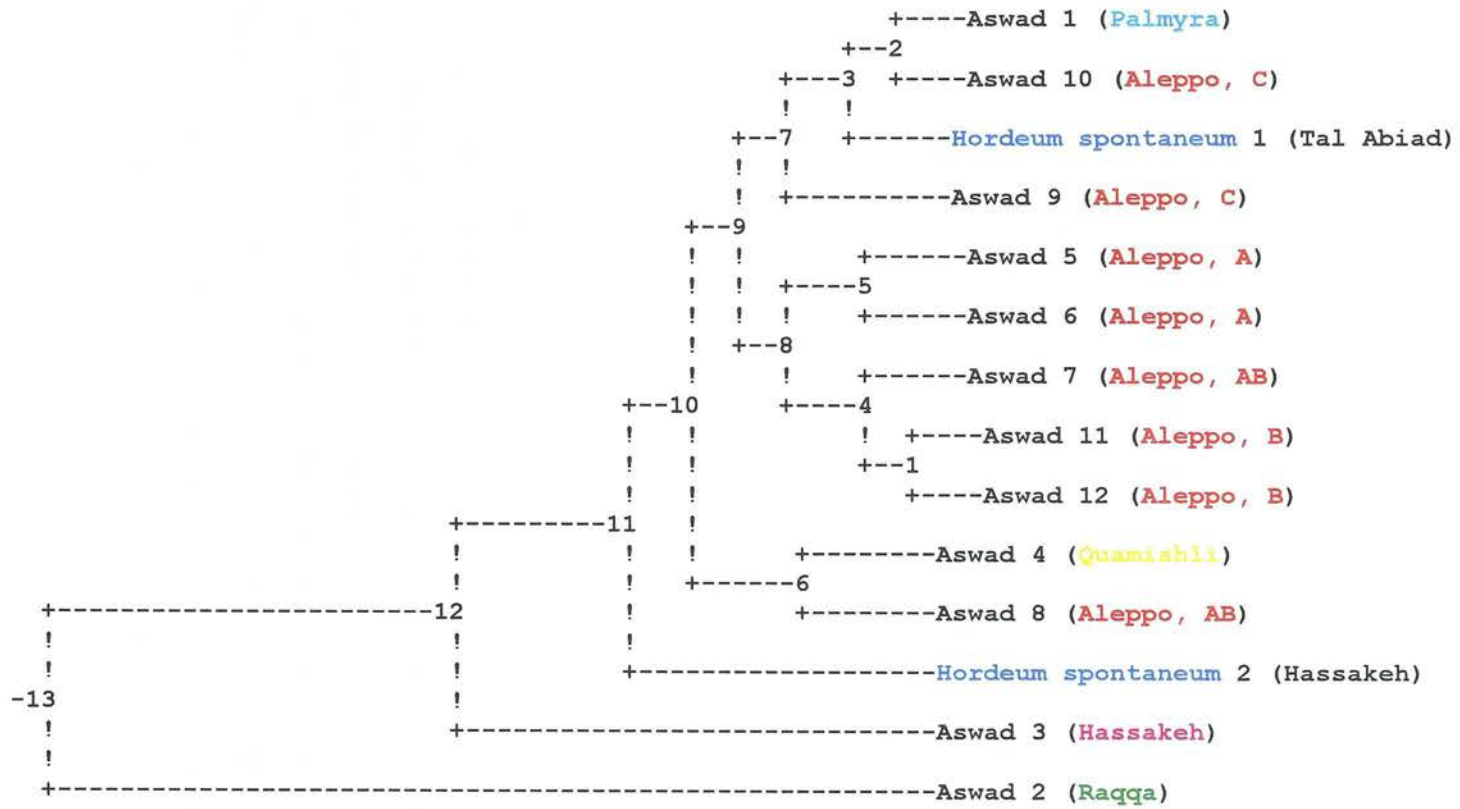


Figure 5.3.8 Dendrogram of Arabi Aswad populations and *Hordeum spontaneum* from Syria, based on Nei's Genetic distance (Nei, 1972) of ISSR markers (25 loci) (Method = UPGMA).



The three dendrograms (Figure 5.3.6 to 5.3.8) give different images of the relatedness of accessions of BLRs and *H. spontaneum*. The dendrogram produced of genetic distances based on morphological marker loci (Figure 5.3.6) shows a clear grouping of the two *H. spontaneum* accessions, the 8 accessions of Arabi Aswad from the Aleppo region and the four further accessions of BLR Arabi Aswad.

Figure 5.3.7 which is based on genetic distances obtained from isoenzyme marker loci shows a slightly different picture. The eight accessions of BLR Arabi Aswad from the Aleppo region are still grouping together and so do the accessions from Palmyra, Raqqa and Hassakeh. However, the *H. spontaneum* accession from Hassakeh is located closer to the BLR accession from the same region than it is to the second accession of *H. spontaneum.*

The dendrogram produced from genetic distances based on results of the molecular ISSR markers (Figure 5.3.8) shows all BLR accessions intermingled with each other and with the *H. spontaneum* accessions. Five accessions from the Aleppo region are grouped closely together, while the remaining three accessions from that region are positioned in close vicinity of the main group.

5.3.3 Discussion

Diversity of BLR populations and H. spontaneum from different regions in Syria

Average gene diversity achieved with morphological markers (on average $H = 0.23$) and isoenzyme markers (on average $H = 0.29$) for BLR accessions collected in farmers' fields in Syria were within the range of values found for H in the literature for corresponding marker types used in BLRs. In comparison with the present study Brown and Munday (1982) reported for BLR from Iran a slightly lower average gene diversity ($H = 0.16$) using a similar set of seed morphological markers, but a much lower average gene diversity calculated from isoenzyme marker loci ($H = 0.08$). However, other authors (Jana and Pietrzak, 1988) reported H values for BLRs from Jordan and Turkey of about 0.55 and for wild barley from the same region of about 0.66. The fairly high diversity indices achieved in the present study may partly be explained with the fact that all marker loci used proved to be polymorphic in at least

one of the populations tested. However, the presence of heterozygote advantage, as shown in Section 5.2.4, may have positively influenced diversity indices as well.

The diversity level of BLR accessions was similar to the diversity found in two accessions of *H. spontaneum* when morphological and isoenzyme markers were considered. Allele frequencies of isoenzyme marker loci gave significantly higher diversity indices for *H. spontaneum* than for BLRs. The latter result is in accordance with results by Nevo (1992), Allard (1988, 1992) and Brown and Munday (1982), who explain the difference with the progressive loss of variation with increasing selection under domestication (Brown and Munday, 1982), i.e. genetic drift created bottlenecks during the process of domestication through which a loss of diversity may have occurred. The diversity indices of the present study achieved with the morphological markers do not show significant differences between BLRs and *H. spontaneum* populations. This might be due to the fact that only four loci were analysed, of which two were seed and lemma colour variants and populations analysed included black and white BLRs with a high diversity for these traits.

Diversity indices calculated from the ISSR markers did not show a difference between wild and cultivated barley either. In this case a large number of loci (i.e. 25) was analysed. Reports about the diversity of BLRs in comparison with its wild progenitor achieved with molecular markers give contrasting results. While some authors demonstrate a higher diversity of wild barley compared with barley landraces for SSR markers (Saghai-Maroof, et al., 1994), rDNA markers (Saghai-Maroof, et al., 1990) and cpDNA markers (Clegg, et al., 1984), others have shown the opposite result for rDNA markers (Ramamoorthy et al., 1994) or equal diversity of BLRs and wild barley for organellar DNA marker loci (i.e. mtDNA and cpDNA) (Holwerda, et al., 1986). These contrasting results imply that the effect of domestication on molecular markers is not clear. It seems that in particular organellar DNA, which is inherited maternally and supposed to be much more conserved than nuclear DNA (Petit, et al., 1998) has not been affected by domestication in the same way as, for instance, isoenzymes. ISSR markers, as used in the present study, are molecular markers which may include a proportion of organellar DNA loci, which may explain,

at least partly, the similar results for BLRs and wild barley achieved with ISSR markers. However, molecular markers, such as ISSRs, are expected to recognise much more mutations compared with isoenzyme markers, because isoenzymes will only result in different alleles when the protein structure of the enzyme has been changed. This may only happen in about 1/3 of all mutation events of bases of the coding regions for isoenzymes. Only when amino acid changes happen the structure of the enzyme may be changed subsequently (Nei, 1987). Therefore isoenzyme markers should produce only about 33% of the diversity achieved with molecular markers. However, molecular markers may also only detect a proportion of the DNA differences that are present, and the quantification of undetected differences is not easy.

Molecular markers cover the whole genome including coding and non-coding regions. The inclusion of non-coding regions may cause higher diversity indices, as non-coding regions are not subject to purifying selection, because mutations are not expressed and thus not selected against. However, the level of diversity indices achieved using isoenzyme marker loci may be positively influenced because they are a selection of potentially polymorphic coding regions of the nuclear genome, which may even be associated with genes which are subject to selection.

As a consequence, a direct comparison between genetic indices achieved with different genetic marker methods is not possible, results are relative rather than absolute (Ennos, 1996). Average gene diversity values depend on the number of polymorphic loci that have been used to calculate them, i.e. every additional monomorphic locus included in a study will decrease the over-all level of *H*. The mean number of alleles per locus (*A*) of different markers depends on the method and is for molecular markers with dominant loci (e.g. ISSR, RAPD) limited to a maximum of 2 (i.e. presence or absence of a band), while for isoenzymes it may take values up to 15 alleles per individual loci in barley (Brown, 1983). The proportion of polymorphic loci (*P 0.01*) depends on the number of polymorphic loci included in the study and thus is a rather ambiguous genetic index.

A comparison of diversity indices of BLR Arabi Aswad from different locations was carried out on a large scale (Northern Syria) and on a smaller scale (Aleppo region). Out of 5 distinct regions of Northern Syria, accessions from the Aleppo region showed the significantly lowest diversities when morphological and ISSR markers were considered, and the accession from the Raqqa region the significantly highest diversity indices. This result may be explained mainly with environmental differences between regions, as will be discussed below. However, the close vicinity of the ICARDA research stations may also have an influence on the intensity of farming practices and thus the selection pressure on BLRs.

On a smaller scale, diversity indices of 8 accessions from 4 distinct rainfall zones in the Aleppo region were analysed. No significant differences between accessions from different rainfall zones were detected, although accessions from zone D (lowest rainfall zone with 200 –230 mm rainfall) showed the highest values for all genetic indices for all three genetic markers. A two-way analysis of variance, including results from all three different genetic markers showed, as expected, significant differences between genetic markers, but also significantly higher Shannon indices for the zone with the lowest rainfall (200 – 230 mm). This result may indicate that water stress could be a factor causing increased diversity levels in BLRs.

A PCA was carried out to investigate whether the combination of all genetic indices over all genetic markers can be used to explain spatial population structuring. It was in fact possible to demonstrate a clustering of local accessions of BLR Arabi Aswad from Aleppo, and of accessions of *H. spontaneum*. No grouping was found for accessions from different zones within the Aleppo region. This lack of differentiation between accessions from one region may be explained with the low genetic distance and high gene flow between accessions (Table 5.4.2).

Correlation between genetic indices and environmental conditions of BLR populations from different eco-geographical environments

The comparison of environmental and genetic indices was carried out on a larger scale (i.e. North Syria) and on a smaller scale (i.e. Aleppo region). On the larger

scale significant correlations were observed for results achieved with isoenzyme and ISSR markers. Diversity indices showed a significantly positive correlation with increasing altitude, i.e. at higher elevations higher diversity indices were found. This result corroborates results by Nevo et al. (1997) who found significantly higher diversity indices for wild barley at highest, south-facing collection points of a microsite in Israel. No significant influence of interannual variation of rainfall on diversity could be demonstrated. However, average annual rainfall was significantly negatively correlated with the diversity indices *S* and *M* for isoenzyme marker loci and *M* for ISSR marker loci. Thus diversity was higher at lower rainfall levels. These results corroborate findings by Nevo (1992) for *H. spontaneum* accessions from Israel using isoenzyme markers.

The results showed further that high temperatures (T_{max}) and a high percentage of continentality (*C*) may cause an increase of diversity indices considering morphological and ISSR marker loci. Considering *C* and T_{max} as measures of environmental stress the data seem to indicate that it is mainly high temperature stress which is associated with an increase of diversity within BLRs. Taking the relative interannual variation in rainfall as the only true index of heterogeneity of the environment in this study, it seems that heterogeneity of environment did not influence the diversity within BLRs significantly. However, it seemed reasonable to investigate the influence of a combination of climatic factors on the diversity of BLRs as proposed by Nevo (1992). A multiple regression of all combined environmental conditions, as proposed by Epperson (1990), gave significant correlations with morphological and ISSR marker loci as shown in Section 5.3.1.3. These findings may indicate that diversity of BLRs are influenced by local environmental conditions. However, environmental conditions may interact with the genotype in a much more complex way than measurable by individual indices such as temperature or derived indices such as continentality or interannual variation in rainfall.

On a smaller geographic scale, within a radius of 65 km in the Aleppo region, no significant influence of precipitation, temperature or the variation of both could be

demonstrated. Correlation coefficients of Table 5.3.8 indicate that only altitude had a significant influence on the diversity of BLRs. This result is unexpected as the range in altitude of the region under consideration is only 296 to 365 m. The variation of environmental indices within the restricted region seems to be too small to influence the diversity of BLRs significantly. As expected (Baur and Schmid, 1996), a high level of gene flow between geographically close accessions, as shown in Figure 5.3.4 in Section 5.3.2.3, seems to blur differences in the diversity levels due to environmental influences. In contrast, Nevo (1997, 1992) found remarkable non-random differences in allozyme and hordein diversities of *H. spontaneum* populations from different collection points of a microsite in Israel, despite considerable gene flow. He mainly investigated the influence of intensity of sunshine (north and south facing slopes) and different soil conditions in a microsite that he coined *Evolution Canyon.*

To investigate possible interactions of different environmental indices a multiple regression combining altitude, average annual rainfall and average annual temperature was conducted. However, no significant correlation with any one of the genetic indices of the three different genetic markers could be found.

Differentiation of BLR populations and H. spontaneum from Syria

Genetic drift and selection can cause a decrease of diversity within populations and an increase of differentiation between populations measured in terms of higher F_{ST} values (Baur and Schmid, 1996). Drift and increasing selection under domestication (Brown and Munday, 1982) may have reduced diversity within populations and increased diversity between populations.

To test this hypothesis F_{ST} values were analysed in a hierarchical way, i.e. from the species level (*H. vulgare*), to the subspecies level (*H. vulgare* ssp. *vulgare* and ssp. *spontaneum*), to the population level (BLRs) for three different genetic markers.

The morphological markers showed for the combination of all populations of *H.vulgare* a partitioning of about 50%/50% within and between populations. Considering the two subspecies *vulgare* and *spontaneum* (i.e. cultivated and wild

barley, respectively) about 98% of the diversity was found within and only about 2% between populations of wild barley, while BLRs showed a partitioning of 59%/41% within/between populations. This result may be explained with the assumption that if human selection has occurred, it will have occurred on morphological characters and thus decreased the diversity within populations.
The results achieved with the ISSR markers gave a similar picture to the morphological markers, though F_{ST} values of *H. spontaneum* were only 50% of the F_{ST} values found for BLRs.

Considering the isoenzyme markers, differentiation between population was not much different for BLR populations and populations of *H. spontaneum*. Only about 15% of diversity was found between populations, which is much lower than expected from findings by Hamrick and Godt (1990), who reported G_{ST} values calculated from allozyme marker loci of 51% on average for inbreeding species.

In studies similar to the present, G_{ST} values between BLR populations ranged from 34% to 57% (Jana and Pietrzak, 1988; Brown and Munday, 1982), and the G_{ST} value found between populations of wild barley from Israel was about 46% (Nevo, 1992). However, in a different study Zhang, et al. (1993) compared diversity results achieved with a molecular markers (RFLP) and an isoenzyme markers between populations of *H. spontaneum* from Israel. They found that differentiation between populations was much lower for the isoenzyme markers (29.6%) than for the molecular markers (47.6%). This result, which is somewhat closer to the result of the present study, illustrates again the complexities associated with the use of different genetic markers and of diversity studies in general. Nevertheless, the analyses of isolation by distance, measured as *Nm*, which is directly derived from F_{ST} values, gave a very clear picture.

Linkage disequilibrium

Linkage disequilibria i.e. the non-random association of alleles, occurs in inbreeding populations due to a lack of recombination and can be seen as a measure of population differentiation.

The number of LDs of morphological marker loci showed regional differences. Arabi Aswad accessions from Hassakeh showed a higher number of LDs than all other accessions. BLR Arabi Abiad 3 from Bural Sharqui showed an association between *long rachilla hairs* and the *absence of spicules on lemma and awn*, and *vice versa*. Long rachilla *hair* and *no spicules on lemma and awn* had a frequency of about 35% within the accession Arabi Abiad 3, but were very rare alleles (on average 2.5% and 2.7%, respectively) in any other population of the BLRs or *H. spontaneum*. These alleles are, however, the predominant alleles in modern barley cultivars. It does not seem unlikely, that the accession of BLR Arabi Abiad 3 was in fact a mixture or a segregating population, as the farmer of the collection site was also managing an experimental field of the ICARDA project of *Participatory Plant Breeding* and thus had access to a wide spectrum of barley material. This inference may be corroborated by the observation that Arabi Abiad 3 was the only accession which showed no LD between alleles of isoenzyme marker loci. All other BLR accessions showed between 4 and 8 LDs as expected for predominantly inbreeding species (Hartl, 1988).

Occurrence of LDs of isoenzyme marker loci made it possible to discriminate between BLRs and populations of *H. spontaneum*. BLR accessions displayed a different pattern and low number of LDs, while *H. spontaneum* population showed on average higher number of LDs and the presence of LDs between alleles located on different loci. In particular the *H. spontaneum* population from Hassakeh showed a very high number of 28 LDs. This observation is in accordance with observations by Nevo (1992) who found more LDs in wild barley than in a composite cross of cultivated barley and reported regionally localised and geographically variable pattern of LDs. A combination of LDs found for morphological and isoenzyme marker loci shows that among BLRs the accession of Arabi Aswad from Hassakeh had the highest number of LDs. The population *of H. spontaneum* from the same region showed the by far highest number of LDs which included the most frequent LDs found in the BLR from this region. The BLR and *H. spontaneum* accessions from Hassakeh showed LDs between alleles of loci which are located on different chromosomes. Maintenance of LD's between distant linked loci, such as those on different chromosomes, requires a greater strength of natural selection (Ayala, 1982).

Thus, results may indicate that accessions from the Hassakeh region showed a higher degree of local adaptation, as natural selection seems to have been more intense than in other regions (e.g. Aleppo). The same seems to be true for both *H. spontaneum* populations, for which a higher number of LDs between distant loci was found.

Kahler and Allard (1970) reported tight linkage between loci Est 1/Est 2, Est 1/ Est 4 and Est 2/Est 4. The locus Est 2b, which showed LD with Est 2 and Est 4 in the present study was not included in Kahler and Allards (1970) study. However, the present results may imply that locus Est 2b of the present study is also located on chromosome 3, like loci Est 2 and Est 4, with which it showed a very highly significant non-random association.

Gene flow

A high level of gene flow is expected between natural populations of geographically close distance (Baur and Schmid, 1996). According to Epperson (1990) the exchange of genes between populations will decrease exponentially with distance. However, Slatkin (1993) proposed a linear relationship for a double log plot of log(*Nm*) against the log of geographic distance (*km*) to characterise isolation by distance. If the same situation was found in populations of BLRs, it could be assumed that seed exchange between farmers mainly happens over short distances.

Only populations of BLR Arabi Aswad were considered. A log transformation of values was conducted and log (*Nm*) was plotted against log (*km*). The trend-line (Figure 5.3.4) showed a significant regression line between gene flow and geographic distance, although its R^2 value was low. This poor correlation (Pearson's correlation coefficient of – 0.48) was mainly caused by comparisons between BLR Arabi Aswad 4 from Quamishli, Tal Birak and those BLRs from the Aleppo region. The gene flow between the accession Aswad 4 and geographically distant accessions from the Aleppo region (about 350 km distance) was very high (see Table 5.4.2). During sampling at the location together with a member of ICARDA staff, it became apparent, that the farmer of the sampled field was a close friend of the ICARDA extension officer. Based on this observation it cannot be excluded that seed exchange

between the ICARDA extension officer and his farmer friend may have occurred. Excluding the gene flow data of the accession of the sampling site Quamishli, Tal Birak (Aswad 4) gave a different picture which is shown in Figure 5.3.5.

The trend line in Figure 5.3.5 shows a much higher R^2 value than the one in Figure 5.3.4. The trend line in Figure 5.3.5 describes the relationship between gene flow and geographic distance as expected, i.e. increasing exchange of genes between populations grown in close proximity, and little gene flow between geographically distant populations. Thus it seems not unlikely that seed exchange of BLR material from the Aleppo region, where ICARDA is located, and the farmer in the Quamishli region may have occurred. The higher R^2, based on a correlation coefficient of -0.73, may confirm the general suggestion that the exchange of seeds between farmers is mainly limited to local areas and that seed exchange over long distances is the exception which may, however, have been facilitated by extension activities. These results confirm observations by Weltzien (1989), who revealed that farmers in Syria are very concerned about seed quality of their BLRs. But when their own supplies are insufficient they tend to buy seeds from those neighbours who are well recognised for their farming skills. It is also known, that the barley growing area in Syria has been extended significantly since the introduction of mechanisation, but seeds brought into the new areas did usually originate from geographically close sources (Van Leur, et al., 1989).

Genetic distance between BLR populations from different regions in Syria

The dendrogram produced of genetic distances based on morphological marker loci (Figure 5.3.6) shows a clear grouping of the two *H. spontaneum* accessions, the eight accessions of Arabi Aswad from the Aleppo region and accessions from the four further regions. This configuration is similar to the one which was obtained with PCA of all genetic indices when all three genetic markers were combined (Figure 5.3.1). Figure 5.3.7 which is based on genetic distances obtained from isoenzyme marker loci shows a slightly different picture. The eight accessions of BLR Arabi Aswad from the Aleppo region are still grouping together and so do the accessions from Palmyra, Raqqa and Hassakeh. However, the *H. spontaneum* accession from

Hassakeh is located closer to the BLR accession from the same region than it is to the second accession of *H. spontaneum*. This configuration may indicate a strong local effect on the arrangement of isoenzymes, which seems more significant than the subspecies boundary. In the third dendrogram (Figure 5.3.8) which is based on the molecular ISSR markers, subspecies boundaries seem to be even more dissolved. *H. spontaneum* accessions are amalgamated with BLR accessions, although like for the isoenzyme markers, the *H. spontaneum* accession from Hassakeh is located closely to the BLR accession from the same region. Five of the Arabi Aswad accessions from the Aleppo region form a closely associated group, but the remaining three accessions from this region are not positioned very far away from this group in the dendrogram. Thus it seems that the molecular markers may still detect regional similarities between populations, a result which is supported by the correlations observed between environmental conditions and genetic indices achieved with the ISSR markers. The results may partly reflect successive neutrality from the isoenzyme towards the molecular ISSR markers, and infer the close relationship between wild and cultivated barley.

5.4 Conclusions

The results of the present study have shown that the outcrossing rate of BLRs from Syria was 1.7% and therefore not significantly different from the outcrossing rate of wild barley, *H. spontaneum*. This results may imply that domestication had no effect on the outcrossing rate of barley. The results suggest further that a high variation in interannual rainfall as well as low winter temperatures may lead to increased outcrossing rates.

An excess level of heterozygosity was observed which may be explained with the presence of heterozygote advantage, and thus may partly explain the maintenance of diversity within populations of BLRs.

BLRs form Syria showed a high level of diversity which was regionally different. Wild barley showed a significantly higher diversity compared with BLRs

considering results achieved with the isoenzyme marker. Within the Aleppo region significantly higher diversity indices were observed in the zone with the lowest rainfall.

Different genetic markers produced different diversity levels. This result confirms the expectation that results from different genetic markers are relative rather than absolute (Ennos, 1996).

Genetic indices showed significant correlations with environmental indices. Diversity indices showed significantly higher values at higher altitudes, at higher maximum temperatures of the hottest month, at a higher percentage of continentality and at lower levels of average annual rainfall. However, there was no significant correlation between the outcrossing rate and any one of the calculated diversity indices, therefore it could not be shown that higher diversity was caused by a higher outcrossing rate. Outcrossing rate and diversity seem to be influenced by different environmental factors.

The partitioning of diversity of BLRs and populations of wild barley showed that most of the diversity was found within populations and less between populations. Wild barley populations showed up to 98% diversity within populations compared with BLRs which showed up to 84%. Different ratios of partitioning within and between populations were achieved with different genetic markers. Results achieved with the isoenzyme markers gave the lowest diversity values between populations. These results highlighted again the complexities associated with genetic marker types, in particular relating to the neutrality and adaptiveness of markers.

The analysis of the occurrence of linkage disequilibria (LDs) may indicate differences in the intensity of local adaptation between BLRs and a higher level of local adaptation of wild barley.

Gene flow between accessions of BLR Arabi Aswad was low between distant and high between close accessions, as expected for natural populations (Baur and

Schmid, 1996). The results confirm Slatkin's (1993) model of isolation by distance which proposes a linear decrease in a double log plot of gene flow on geographic distance. Thus the exchange of seeds between farmers over long distances seemed unlikely. The analysis of results may have been able to reveal a potential case of seed exchange over long distance. The influence of extension work on increased seed exchange over long distance has been discussed.

Finally, the analysis of genetic distance between populations of BLR Arabi Aswad and wild barley showed regional clustering of populations for neutral genetic markers such as isoenzymes and ISSR markers, and clustering of wild and cultivated barley as well as regional clustering for the adaptive morphological markers.

Chapter 6

Results of experimental investigations of the population structure of barley landraces with relevance to plant breeding for marginal conditions

6.1 Introduction

Breeding of barley for low-input agriculture and marginal conditions may require approaches different from the accepted pure-line breeding methodology. Modern pure-line cultivars can be outperformed by local landraces under extreme and unpredictable environmental conditions (Ceccarelli, 1994, 1996a). The same seems to be true for landraces of durum wheat (Elings, 1993). The reasons for this advantage may rest on characteristics of the population structure of landraces such as diversity and local adaptation (Ceccarelli, 1994, 1996a). Ceccarelli (1996a) proposed for plant breeding for low-input agriculture of marginal environments to select in the target area and to use locally adapted germplasm. To investigate how diversity created in a modern breeding programme compares to the diversity found in local germplasm such as BLRs, the diversity of the latter was compared with the diversity of F_2 and F_3-bulk populations of crosses between closely and distantly related parent-lines of barley. It was further intended to confirm that modern cultivars are homozygous and homogeneous lines as anticipated from their breeding method, and to investigate the diversity found among modern barley cultivars from Northern Europe, which predominantly build the gene pool of most breeding programmes in this region (Simmonds and Smartt, 1999).

Modern cultivars are predominantly based on single plants, selected from F_3 or F_4-bulk populations. Bulk populations in an inbreeding species such as barley are produced by crossing two homozygous parent lines and growing plants from resulting F_1 seeds (i.e. first filial generation). All harvested seeds of these plants (i.e. bulk) are then grown for one or two more consecutive generations without selection, and are subsequently called F_2 and F_3-bulk population, respectively. Progeny of selected plants of F_3 or F_4-bulk populations are inbred for about four more generations (Allard, 1960, Jensen, 1988). At this point (F_7 to F_8) the obtained lines are expected to be nearly homozygous (Simmonds and Smartt, 1999). Selected lines are tested in multiple trials at different locations under high input conditions (i.e. conditions which should reflect current agricultural practice). Those lines whose performance is superior at all trial sites are selected, included in official trials and may subsequently be registered as new cultivars (Jensen, 1988). This breeding

method is called the pedigree selection method and the resulting cultivars are homozygous and homogeneous and usually show wide adaptation (i.e. they may perform equally well throughout a country, or even in different countries) when grown under similar high input conditions. In contrast, BLRs are heterogeneous populations with a fairly high level of homozygosity due to a very low outcrossing rate of about 1.7% (see Chapter 5). BLRs are expected to be adapted to local conditions (Ceccarelli, 1996), and results of the present study seem to corroborate this (Section 5.3.1.2) this. BLRs have been shown to outperform modern cultivars under low input conditions in Syria due to their local adaptation and population buffering (i.e. the ability to compensate for the failure of a component by a genetically different component of the same population) based on a high level of diversity (Ceccarelli, Acevedo and Grando, 1991). These properties of BLRs have fuelled the idea of using local adaptation and population diversity as alternative breeding strategies for marginal environments and low input agriculture (Ceccarelli, 1994, 1996). A better understanding of the population structure of BLRs in comparison with modern cultivars and defined breeding populations is highly desirable for developing alternative breeding strategies which exploit advantageous characteristics of BLRs. To explore this further the following comparisons of diversity and differentiation of BLRs were made with (i) an assembled population of up to 20 modern two row barley cultivars (Table 3.1.2), (ii) a single modern two-row spring barley cultivar (i.e. Baronesse), (iii) one F_2-bulk population of the initial cross of two similar modern cultivars (Baronesse × Scarlet) and (iv) one F3-bulk population of the initial cross of a modern two-row barley cultivar and a selected line from BLRs Arabi Aswad (Hart × Zambaka).

6.2 Diversity of BLRs compared with wild barely and defined barley breeding populations

Diversity indices of BLRs and populations of *H. spontaneum* as given in Chapter 5 were compared with diversity indices obtained for barley breeding populations. One modern cultivar (Baronesse) was used as an example to show whether it complied with the expectation of total homogeneity and homozygosity. A population of up to

20 modern two row barley cultivars, of which 19 were spring types, was assembled in order to analyse the genetic diversity between them. Allele differences between homozygous parent lines of F_2 and F_3-bulk populations were known for three genetic markers. Thus it was possible to estimate the theoretical diversity of the F_2 and F_3 generations and conduct a comparison with observed diversity indices. Subsequently it was possible to estimate the accuracy of the genetic markers used and to relate diversity indices of F_2 and F_3-bulk populations to those of BLRs and *H. spontaneum* populations. The relative allele differences between parents of the F_2 and F_3-bulk populations are given in Table 6.2.1.

Table 6.2.1 Allele differences between parents of two bulk populations

	F_2-bulk population (Baronesse x Scarlet)	**F3-bulk population (Hart x Zambaka)**
Allele differences between parents for 4 morphological marker loci (%)	0/4 (0%)	3/4 (75%)
Allele differences between parents for 8 isoenzyme marker loci (%)	2/8 (25%)	4/8 (50%)
Allele differences between parents for 25 ISSR marker loci (%)	not analysed	5/25 (20%)

Considering the allele differences between parent lines, it was possible to calculate the theoretical average gene diversity (H) and Shanon's Information index (S) based on results of dominant and codominant molecular marker loci with two alleles, for segregating, inbreeding populations in the absence of linkage, fitness differences between alleles, mutation, selection and drift. Under these assumptions, the average gene diversity (H) and Shanon's information index (S) of F_2 and following generations of a cross between parent lines different at all alleles, would be $H = 0.5$ and $S = 0.6931$, respectively, when calculated from allele frequencies of a codominant genetic marker. If only a percentage of alleles between homozygous

parent lines are different (e.g. 50%), then H and S will also only take values of the according percentage. The assumption of completely homozygous parents justified the consideration of only two alleles per locus, because even if multiple alleles were possible at a certain locus, the genotype of the heterozygous offspring (i.e. F_1) of two homozygous parents and subsequent inbred generations would show a maximum of two alleles. Mean diversity indices of *H. spontaneum*, BLRs and breeding populations for three different genetic markers are shown in Table 6.2.2. Expected values for average gene diversity (H) and Shanon's information index (S) following the above assumptions were given in square brackets, where applicable. Standard errors were not shown, in order to avoid confusion in the table, but have been partly given elsewhere (Chapter 5).

Table 6.2.2 Mean diversity indices of *H. spontaneum*, BLRs and breeding populations for three different genetic markers

	n	H	A	P (0.01)	S	M
Morphological marker:		***				
H. spontaneum	2	**0.2177** ab	**2.00**	**0.50**	**0.3809**	**8**
all BLRs	15	**0.2280** ab	**2.30**	**0.87**	**0.3992**	**11**
F3-bulk (100)* [expected ①]	1	**0.4475** a [0.3516]	**2.50**	**1.00**	**0.7210** [0.49617]	**19**
F2-bulk (54)* [expected ②]	1	**0** b [0]	**1.00**	**0**	**0** [0]	**1**
CVs (20)*	1	**0.1325** ab	**1.75**	**0.75**	**0.2366**	**25**
CV Baronesse (100)*	1	**0** b	**1.00**	**0**	**0**	**1**
Isoenzyme marker:		***				
H. spontaneum	2	**0.4030** a	**2.13**	**0.94**	**0.6002**	**50**
all BLRs	15	**0.2909** b	**1.97**	**0.79**	**0.4495**	**44**
F3-bulk (15)* [expected ③]	1	**0.2289** bc [0.2500]	**1.50**	**0.50**	**0.3248** [0.34655]	**53**
F2-bulk (50)* [expected ④]	1	**0.1335** cd [0.1250]	**1.40**	**0.25**	**0.1638** [0.1733]	**38**
CVs (16)*	1	**0.1309** cd	**1.50**	**0.50**	**0.2148**	**38**
CV Baronesse (50)*	1	**0** d	**1.00**	**0**	**0**	**2**
ISSR marker:		ns				
H. spontaneum	2	**0.1686**	**1.45**	**0.36**	**0.2474**	**49**
all BLRs	13	**0.1686**	**1.55**	**0.46**	**0.2567**	**61**
F3-bulk (15)* [expected ⑤]	1	**0.0396** [0.09376]	**1.10**	**0.10**	**0.0570** [0.1323]	**42**
CVs (18)*	1	**0.1638**	**1.50**	**0.36**	**0.3050**	**71**
CV Baronesse (39)*	1	**0**	**1.00**	**0**	**0**	**2**

n = Number of populations tested; H = Average gene diversity; H_{et} = Mean proportion of observed heterozygous loci; A = Number of polymorphic alleles; P (0.01) = Proportion of polymorphic loci; M = Number of multilocus combinations per 100 individuals; S = Shanon's information index;
* Number of individuals tested per population; Expected values in brackets [...]; *abc* = small blue letters next to the mean show significant differences of pairwise comparison of means, those means which have a letter in common are not significantly different (Scheffe's procedure, P = 0.05);
① 75 % different alleles between parents, dominant loci; ② 0 % different alleles between parents, dominant loci; ③ 50% different alleles between parents, codominant loci; ④ 25% different alleles between parents, codominant loci; ⑤ 20% different alleles between parents, dominant loci.

An ANOVA procedure was carried out for average gene diversity (*H*) per molecular marker group, between results of populations, namely *H. spontaneum,* BLRs, F_3 and F_2-bulk populations, the assemblage of cultivars and cultivar Baronesse. Where average gene diversity showed significant differences between groups these were marked with blue asterisks. Small blue letters were used to indicate significances between different groups, obtained with Scheffe's Test ($p = 0.05$). Details of the statistical analysis are given in Appendix 7.

Results in Table 6.2.2 show for all three genetic markers, no diversity within the modern two-row spring barley cultivar *Baronesse*. Diversity indices for the assemblage of 16 to 20 modern barley cultivars were almost equally low for all three genetic markers. *H. spontaneum* populations and BLRs showed the highest diversity indices, with one exception. The F_3-bulk population had the highest diversity indices for the morphological marker loci. Indices were even higher than anticipated for a F_3-generation of parent lines with 75% different alleles.

The F_2-bulk population showed no diversity for the morphological marker loci, which was in accordance with the expected values, because parent lines had identical alleles at all loci. The level of diversity indices of F_2 and F_3-bulk populations calculated from frequencies at isoenzyme marker loci were almost as high as expected from the number of allele differences between parents. The diversity of the F_3-bulk population for the isoenzyme marker had a similar level to the BLRs, while diversity level of the F_2-bulk population was close to the diversity level of the assemblage of modern barley cultivars. The latter included the parent lines of the F_2-bulk population. Diversity indices based on allele frequencies of the ISSR marker loci showed almost identical diversity indices for *H. spontaneum* populations, BLRs and the assemblage of modern barley cultivars. In contrast, diversity indices of the F_3-bulk population were very low, even lower than the expected value, calculated for the allele difference between parent lines of 20%.

In addition to diversity indices, observed heterozygosity was calculated using the programme Popgene (Yeh, et al., 1997) based on allele frequencies of isoenzyme marker loci. The heterozygosity index (H_{et}) gives the average frequency of

heterozygous loci, based on direct counts (Mallet, 1996). In Table 6.2.3 means of the observed heterozygosity over all 8 esterase loci are given for different groups of populations. Furthermore, observed heterozygosity is given for one individual codominant esterase loci, namely Est 2, which accounted for most of the heterozygosity found. Heterozygosity in an F_1-population for a segregating codominant locus in an inbreeding population is 1 (i.e. 100%). Theoretically, the level of heterozygosity is halved in every further generation of selfing (Simmonds and Smartt, 1999). Corresponding theoretical values have been added to the table for the F_2 and F_3-bulk populations in order to allow a comparison, which was not possible with statistical test because the numbers of variables were too low.

Table 6.2.3 Observed heterozygosity of *H. spontaneum* populations, BLRs and barley breeding populations achieved with isoenzyme marker.

	n	Observed heterozygous loci	Observed ① average H_{et}	Observed ② 1-locus H_{et}	Theoretical 1-locus H_{et} ③
Isoenzyme marker:					
H. spontaneum	2	**3**	**0.0227** (+/- 0.0167)	**0.1364**	
all BLRs	15	**2**	**0.0167** (+/- 0.0122)	**0.0381**	
F_3-bulk (15)*	1	**1**	**0.0333** (+/- 0.0333)	**0.2667**	**0.25**
F_2-bulk (50)*	1	**1**	**0.0575** (+/- 0.0575)	**0.4600**	**0.5**
CVs (16)*	1	**0**	**0**	**0**	
CV Baronesse (50)*	1	**0**	**0**	**0**	

Standard errors in parenthesis; * Number of individuals tested per population;
① = Observed heterozygosity as average of 8 Est loci;
② = Observed heterozygosity at one codominant locus (Est 2);
③ = Theoretical heterozygosity for one codominant locus.

Considering only one locus, results in Table 6.2.3 showed that heterozygosity of the F_2 and F_3-bulk populations were almost as high as theoretically expected. For locus Est 2, *H. spontaneum* populations showed about 1/2 and BLRs about 1/8 of the observed heterozygosity found in the F_3-bulk populations.

When the average of all 8 analysed Est loci was considered, modern barley cultivars showed no heterozygosity, while F_2 and F_3-bulk populations were as heterozygous as expected. *H. spontaneum* had a lower heterozygosity than F_2 and F_3-bulk populations, but was slightly more heterozygous compared to BLRs.

6.3 Differentiation of BLRs compared with wild barley and modern cultivars

Selection pressure may increase the differentiation between populations and decrease the diversity within populations (Baur and Schmid, 1996). Thus, plant breeding may influence the partitioning of barley populations substantially. Modern barley cultivars are supposed to be genetically pure lines, i.e. the diversity within cultivar populations is expected to be zero (Simmonds and Smartt, 1999). However, different modern barley cultivars are expected to show genetic variation between them (Allard, et al., 1970; Ellis, et al., 1997). To identify the position of BLRs in relation to wild barley and modern cultivars, differentiation was compared between these groups. Results are given in Table 6.3.1. The table includes some results given earlier in Table 5.3.9.

Table 6.3.1 Partitioning of diversity within and between barley populations of wild barley populations, BLRs and modern cultivars. Results are given for three different genetic markers.

	n	Total diversity H_T	Diversity within populations H_S [%]	Diversity between populations F_{ST} [%]
Morphology:				
Wild barley	2	**0.2223**	**0.2176** [97.9%]	**0.021** [2.1%]
BLRs	15	**0.3891**	**0.2280** [58.6%]	**0.414** [41.4%]
Modern Cultivars (20)*	1	**0.1325**	**0** ①	**1.0** [100%]
Isoenzymes:				
Wild barley	2	**0.4800**	**0.4030** [84.0%]	**0.160** [16.0%]
BLRs	15	**0.3449**	**0.2909** [84.3%]	**0.157** [15.7%]
Modern Cultivars (16)*	1	**0.1309**	**0** ①	**1.0** [100%]
ISSR:				
Wild barley	2	**0.2216**	**0.1686** [76.1%]	**0.239** [23.9%]
BLRs	13	**0.3143**	**0.1686** [53.6%]	**0.464** [46.4%]
Modern Cultivars (18)*	1	**0.1638**	**0** ①	**1.0** [100%]

n = Number of accessions; * Number of different CVs included in analysis;
① = Diversity within cultivars, calculated using cultivar Baronesse as an example

The last column of Table 6.3.1 shows that diversity between populations (F_{ST}) increases from wild barley over BLRs to modern cultivars, when the morphological and ISSR marker are considered. Results based on isoenzyme marker loci show the same trend, although the difference of F_{ST} values between wild barley and BLRs was almost zero. Total diversity of modern cultivars showed the consistently lowest values for all three genetic markers in comparison with wild barley and BLRs. The diversity within cultivars was measured using cultivar Baronesse as an example and was zero for all three genetic markers under consideration. Differences of H_T between wild barley and BLRs were analysed and discussed earlier (Section 5.3.3).

6.4 Analysis of the interaction between genotype and environment for quantitative traits

Local populations of BLR Arabi Aswad are grown throughout Syria. However, they are particularly preferred in drier regions. The performance of local accessions of the same BLR differs significantly (Ceccarelli, 1996), and might be influenced by environmental conditions through local adaptation and by human selection.

Samples of different accessions of BLR Arabi Aswad from different collection sites in Syria were assessed for quantitative traits such as number of grains per seed head and thousand kernel weight (TGW), as described in Section 3.4. To investigate the influence of environmental conditions of the sampling site, the same accessions were grown under controlled conditions in a glasshouse together with two further accessions of the same landrace. Average values per trait and accession and significant differences for each trait between accessions found in an ANOVA procedure are given in Table 6.4.1.

Table 6.4.1 Variation of quantitative traits *in situ* and under controlled conditions in the glasshouse of BLR Arabi Aswad from different collection sites in Syria (standard deviation in parentheses)

			In situ (Syria):		Glasshouse (Edinburgh):				
Code	Region	Average rainfall	Seeds/ear	TGW (g)	Seeds/ear	TGW (g)	Ears/plant	Seeds/plant	Total yield/plant (g)
			*** ①	*** ①	ns ①	ns ①	ns ①	ns ①	ns ①
Aswad 1	Palmyra	153			**13.6** (+/- 2.5)	**38.6** (+/- 8.2)	**27.0**	**390**	**15.7**
Aswad 2	Raqqa	218			**16.1** (+/- 0.8)	**40.4** (+/- 1.7)	**33.3**	**499**	**21.0**
Aswad 3	Hassakeh	229	**13.1** *bc* (+/- 2.0)	**34.0** *cd* (+/- 4.1)	**14.6** (+/- 0.9)	**41.0** (+/- 2.8)	**27.9**	**415**	**17.9**
Aswad 4	Quamishli	305	**16.3** *ac* (+/- 3.5)	**38.3** *bcd* (+/- 4.8)	**16.6** (+/- 0.7)	**42.0** (+/- 1.4)	**26.4**	**451**	**18.5**
Aswad 5	Aleppo	317	**19.5** *a* (+/- 3.1)	**50.7** *a* (+/- 5.3)	**12.7** (+/- 1.2)	**42.7** (+/- 1.9)	**29.2**	**353**	**15.1**
Aswad 6	Aleppo	328	**19.0** *a* (+/- 2.9)	**41.5** *b* (+/- 6.8)	**16.6** (+/- 1.2)	**43.8** (+/- 1.6)	**27.2**	**435**	**18.9**
Aswad 7	Aleppo	273	**11.9** *b* (+/- 1.7)	**37.3** *bcd* (+/- 5.1)	**14.9** (+/- 1.1)	**43.0** (+/- 1.9)	**29.5**	**416**	**17.7**
Aswad 8	Aleppo	265	**16.8** *ac* (+/- 3.4)	**39.0** *bcd* (+/- 6.7)	**15.2** (+/- 0.6)	**41.7** (+/- 0.9)	**32.5**	**478**	**20.2**
Aswad 9	Aleppo	231	**11.0** *b* (+/- 2.0)	**33.0** *cd* (+/- 5.5)	**15.2** (+/- 0.7)	**41.8** (+/- 1.2)	**28.4**	**408**	**17.3**
Aswad 10	Aleppo	232	**12.8** *bc* (+/- 4.1)	**38.2** *bcd* (+/- 6.7)	**15.8** (+/- 0.8)	**40.3** (+/- 1.3)	**32.0**	**474**	**19.1**
Aswad 11	Aleppo	219	**14.6** *bc* (+/- 2.7)	**40.0** *bc* (+/- 4.4)	**15.9** (+/- 0.7)	**44.0** (+/- 1.4)	**29.7**	**463**	**19.7**
Aswad 12	Aleppo	220	**13.6** *bc* (+/- 1.7)	**32.2** *d* (+/- 6.6)	**15.2** (+/- 0.6)	**44.0** (+/- 1.5)	**28.1**	**419**	**18.2**

① = significance level for ANOVA of this trait among accessions; *abc* = small blue letters next to the mean show significant differences of pairwise comparison of means, those means which have a letter in common are not significantly different (Scheffe's procedure, P = 0.05); TGW = Thousand grain weight

Results in Table 6.4.1 illustrate that BLR accessions from different eco-geographical environments when grown *in situ* in Syria showed both significantly different number of seeds per ear and TGW. When all accessions were grown under identical conditions in the glasshouse, there were no significant differences for any one of the five traits analysed. ANOVA tables of results are given in Table 6.4.2.

Table 6.4.2 ANOVA tables for response variable: Seeds/ear and TGW

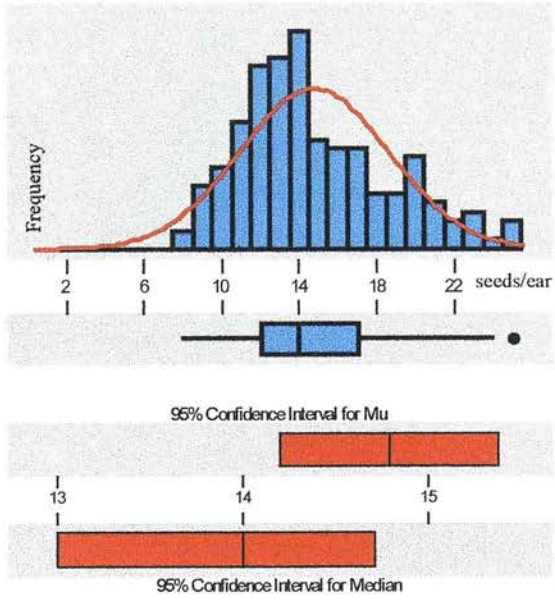
Variable	Source	DF	Sum-Squares	Mean-Square	F-Ratio	Prob>F
Response variable: Seed/ear, *in situ* Syria						
Accessions	A	9	1224.41	136.05	16.98	0.0000 ***
	Error	159	1273.92	8.01		
	Total	168	2498.33			
Response variable: TGW, *in situ* Syria						
Accessions	A	9	5042.91	560.323	17.31	0.0000 ***
	Error	190	6151.94	32.38		
	Total	199	1194.84			
Response variable: Seeds/ear, glasshouse (Scotland)						
Accessions	A	9	170.78	18.98	1.43	ns
	Error	169	2241.44	13.26		
	Total	178	2412.22			
Response variable: TGW, glasshouse (Scotland)						
Accessions	A	9	105.89	11.77	0.27	ns
	Error	169	7364.28	43.58		
	Total	178	7470.17			

The results of a Scheffe's procedure, which is a stringent significance test for the comparisons of means, are given as small blue letters next to the means in Table 6.4.1. Means which have at least one letter in common are not significantly different at a level of $p = 0.05$.

Descriptive statistics and histograms for the two tested quantitative traits *in situ* and under controlled conditions are given in Figure 6.4.1 to 6.4.4, to illustrate the range of variation found.

Figure 6.4.1 Descriptive statistics and frequency distribution of *number of seeds per ear*, of accessions of BLR Arabi Aswad *in situ* in Syria.

Descriptive Statistics



Variable: seeds/ea
Group: 1

Anderson-Darling Normality Test

A-Squared:	2.879
P-Value:	0.000
Mean	14.7870
StDev	3.8563
Variance	14.8710
Skewness	0.663272
Kurtosis	-1.9E-01
N	169
Minimum	8.0000
1st Quartile	12.0000
Median	14.0000
3rd Quartile	17.0000
Maximum	25.0000

95% Confidence Interval for Mu

14.2014	15.3726

95% Confidence Interval for Sigma

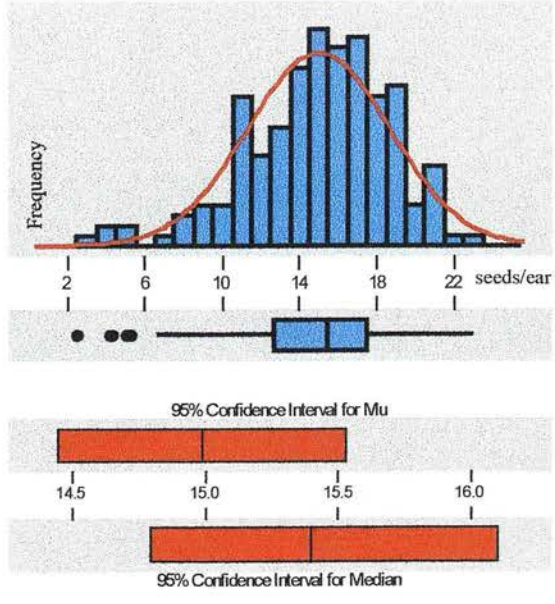
3.4843	4.3178

95% Confidence Interval for Median

13.0000	14.7104

Figure 6.4.2 Descriptive statistics and frequency distribution of *number of seeds per ear*, of accessions of BLR Arabi Aswad under controlled conditions in a glasshouse in Scotland.

Descriptive Statistics



Variable: seeds/ea
Group: 2

Anderson-Darling Normality Test

A-Squared:	0.973
P-Value:	0.014
Mean	14.9899
StDev	3.6813
Variance	13.5518
Skewness	-6.4E-01
Kurtosis	0.609267
N	179
Minimum	2.5000
1st Quartile	12.7000
Median	15.4000
3rd Quartile	17.5000
Maximum	22.9000

95% Confidence Interval for Mu

14.4470	15.5329

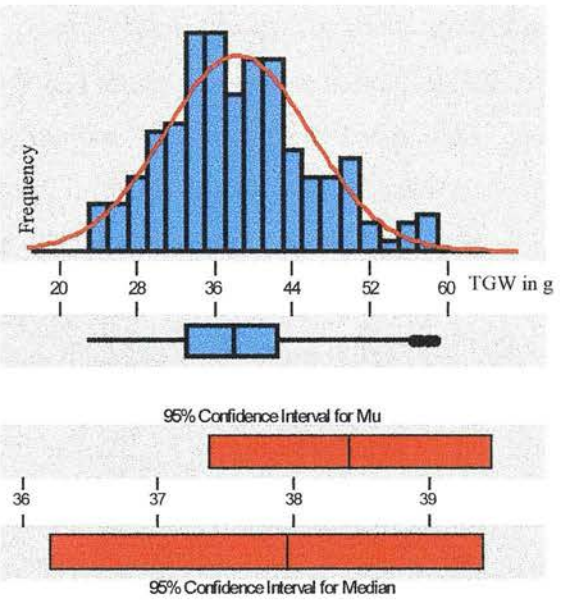
95% Confidence Interval for Sigma

3.3354	4.1079

95% Confidence Interval for Median

14.7903	16.1000

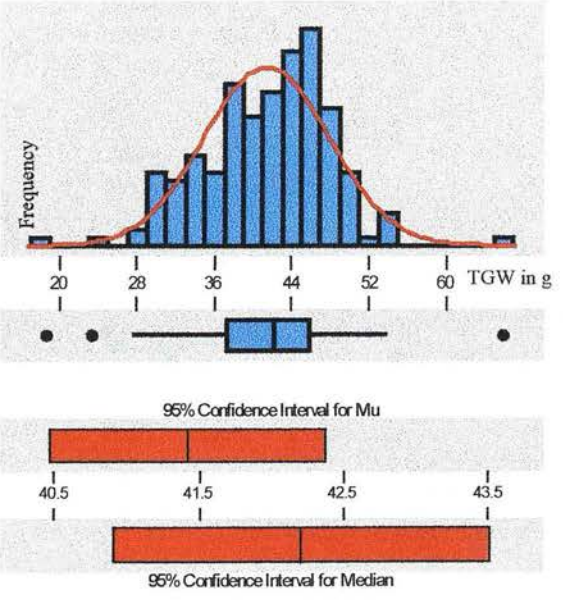
Figure 6.4.3 Descriptive statistics and frequency distribution of *TGW*, of accessions of BLR Arabi Aswad *in situ* in Syria.



Variable: TGW in g
Group: 1

Anderson-Darling Normality Test	
A-Squared:	0.774
P-Value:	0.044
Mean	38.4155
StDev	7.5004
Variance	56.2555
Skewness	0.394499
Kurtosis	-1.3E-01
N	200
Minimum	23.0000
1st Quartile	33.1250
Median	37.9500
3rd Quartile	42.4500
Maximum	58.6000
95% Confidence Interval for Mu	
37.3697	39.4613
95% Confidence Interval for Sigma	
6.8303	8.3173
95% Confidence Interval for Median	
36.2000	39.4000

Figure 6.4.4 Descriptive statistics and frequency distribution of *TGW*, of accessions of BLR Arabi Aswad under controlled conditions in Scotland.



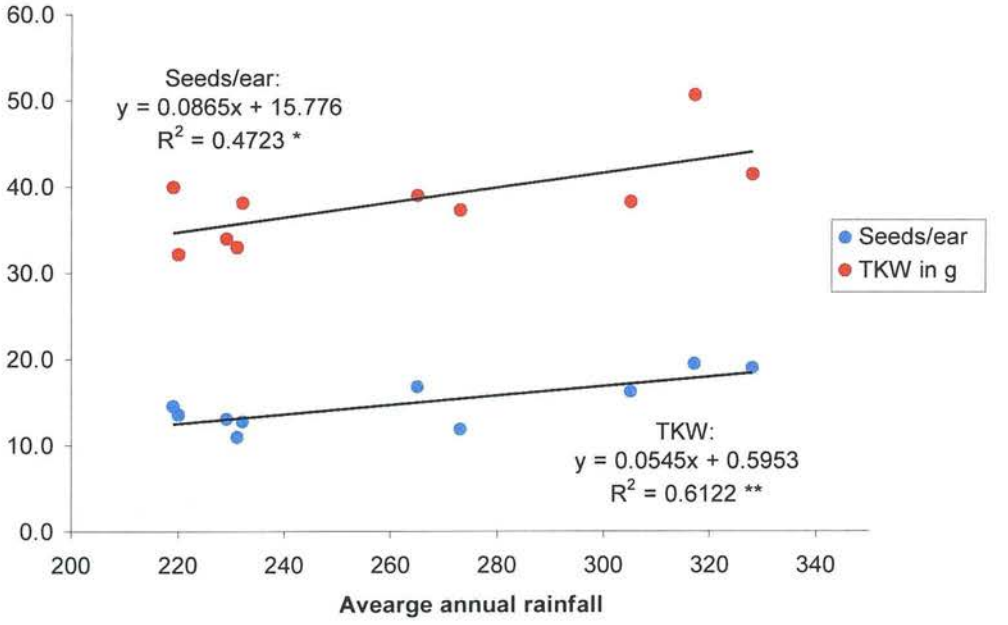
Variable: TGW in g
Group: 2

Anderson-Darling Normality Test	
A-Squared:	1.129
P-Value:	0.006
Mean	41.4218
StDev	6.4782
Variance	41.9672
Skewness	-2.6E-01
Kurtosis	0.871608
N	179
Minimum	18.6000
1st Quartile	37.3000
Median	42.2000
3rd Quartile	45.8000
Maximum	65.8000
95% Confidence Interval for Mu	
40.4663	42.3773
95% Confidence Interval for Sigma	
5.8695	7.2289
95% Confidence Interval for Median	
40.9000	43.5097

Significantly higher TGW ($p = 0.001$) and a non-significantly, higher mean number of seeds per ear were produced under controlled conditions. However, standard deviation and variance of both quantitative traits (Figure 6.4.1 to 6.4.4) were greater, though not significantly, *in situ* than under controlled conditions. In contrast, the range of individual values for both quantitative traits was slightly wider (not significantly) under glasshouse conditions than *in situ*. This was mainly caused by a higher number of individuals with very poor performance under glasshouse conditions in Scotland, as can be seen from the histograms. All four histograms showed that data were not normally distributed, as the *p*-value for the Anderson-Darling test were < 0.05. However, when results from both environments were combined, they showed normality for both number of seeds per ear and TGW (result not shown).

A regression between average annual rainfall and both average number of seeds per ear and TGW of accessions collected *in situ* in Syria was conducted and results are shown in Figure 6.4.5.

Figure 6.4.5 Influence of average annual rainfall on number of seeds per ear and TGW in BLR accessions of Arabi Aswad collected in Syria



With increasing average annual rainfall the average number of seeds increased highly significantly and TGW increased significantly. There was no significant correlation between average annual rainfall of the collection site and both average number of seeds per ear and TGW when accessions were grown under identical conditions in the glasshouse.

6.5 Discussion

Diversity

No diversity was observed within the modern two-row spring barley cultivar Baronesse, considering morphological, isoenzyme and ISSR markers, as was expected from the cultivar type. The diversity of the cultivar was analysed as an example to confirm the assumption that pure-line cultivars are homozygous and homogenous.

The assemblage of 16 to 20 modern barley cultivars showed almost equally low diversity indices for all three genetic markers. Average gene diversity of cultivars, based on the morphological marker, was about 60% of that of *H. spontaneum* populations and BLRs. Based on isoenzyme marker loci, average gene diversity of cultivars was about 45% of the *H* value observed for BLRs and about 32% of the *H* value observed for wild barley. However, considering the molecular ISSR marker, almost no differences in average gene diversity were found between cultivars, BLRs and wild barley. The latter result may cast doubt on the argument that cultivars were less diverse because they had a narrow genetic base, originating predominantly from Northern Europe. It rather seems that the characteristics of the employed genetic marker has a major impact on achieved diversity levels of populations. While the morphological and the isoenzyme marker may show the impact of domestication and selection through plant breeding on diversity indices of barley populations, the ISSR marker seems to produce diversity indices which seem to be unaffected by evolutionary forces of the magnitude that were present during domestication, cultivation and plant breeding.

The F_2-bulk population showed no diversity for the morphological marker loci, which was expected, as parent lines had identical alleles at all loci. The level of diversity for isoenzyme marker loci was also as high as expected from the number of allele differences between parents.

The F_3-bulk population had the highest diversity indices for the morphological marker loci. Indices were even higher than anticipated for a F_3-generation of parent lines with 75% different alleles. However, the parent lines of the segregating F_3-bulk population were a modern cultivar with white lemma and seed colour and a selected line from a black seeded landrace. The selected line (Zambaka) was not a pure line and had itself an average gene diversity of 0.1128 for morphological marker loci (results not shown). The diversity for isoenzyme marker loci of the F_3-bulk population was almost as expected from allele differences between parent lines. Diversity indices based on ISSR marker loci for the F_3-bulk population were very low, and only about half as high as expected for a F_3-popualtion of parent lines with 20% allele differences. Two of the four loci for which parent lines had different alleles, did not segregate in the F_3-bulk population. One explanation for this may be that ISSRs can include loci of maternally inherited DNA such as mtDNA and cpDNA. These organellar DNA loci will not show Mendelian inheritance and are expected to show the maternal allele in all segregating filial generations, as was the case for the two ISSR-loci 857-640 and 857-550.

Results above may indicate a high predictability of genetic indices achieved with isoenzyme markers due to a clearly Mendelian inheritance. This comprehensibility of the isoenzyme marker enables clear quantification of the impact of evolutionary forces such as migration. The high predictability of heterozygosity based on direct counts, using isoenzyme marker loci, may further corroborate the previous assumption. Based on a single codominant locus with two different alleles between parents of a segregating inbred population, the heterozygosity found in BLRs and in *H. spontaneum* populations was of a magnitude expected for a F_6- and a F_4-generation, respectively.

However, BLRs and *H. spontaneum* accessions showed almost the same level of diversity for the morphological and the ISSR marker, while results based on the isoenzyme marker showed significantly higher diversity for *H. spontaneum* accessions. The latter result is in accordance with findings by Brown and Munday (1982) and has been discussed earlier in Chapter 5.

Differentiation

Results given in Table 6.3.1 can be seen as an adjunct to Table 5.3.9 in Chapter 5, in which partitioning between wild barley and barley landraces was analysed in a hierarchical order. The diversity found in cultivars lies wholly between populations, because no diversity is found within cultivars, when morphological, isoenzyme and ISSR marker are considered. A trend of increasing F_{ST} values (i.e. decreasing diversity within populations) was observed for data based on the morphological and the ISSR marker. The trend was also present, though less clear, for the isoenzyme marker, which gave similar F_{ST} values for BLRs and *H. spontaneum* populations. However, increasing F_{ST} values may indicate the presence of genetic drift and selection (except selection caused by heterozygote advantage, which may cause decreasing F_{ST}-values) (Weir, 1996).

The results confirmed the expectations that within modern pure-line cultivars no diversity can be found. On the other hand, it points again to the characteristics of employed genetic markers, as discussed in Chapter 5, Section 5.3.3. It can not be excluded that other genetic marker methods which cover a larger part of the genome or analyse faster evolving DNA fractions such as AFLP or SSR, respectively, would detect polymorphism and thus diversity even within pure-line cultivars. Even SDS-PAGE of storage proteins is able to reveal some diversity within modern barley cultivars. Purity checks by the German Bundes Sorten Amt (i.e. German variety registration authority, BSA) do frequently reveal impurities and segregates in seed lots of modern commercial cultivars of barley (Ohms, BSA, 1995, p.c.). Thus, it may be expected that under cultivation in the absence of any deliberate purifying selection even modern cultivars would finally become populations with a certain level of

diversity again. However, intensive maintenance breeding in seed companies is aimed at detecting and eliminating such unwanted impurities.

Genotype × environment interaction

A number of accessions of BLR Arabi Aswad were grown under identical conditions in a controlled environment in a glasshouse and quantitative traits (seeds per ear, TGW) were compared with those scored in the same accessions grown at the original collection site *in situ* in Syria. Unfortunately, not exactly the same families per accession were grown in the glasshouse as were analysed at the original collection site, so that the calculation of heritability on a family basis was not possible. However, the results showed clearly that individual accessions performed significantly differently depending on the environmental conditions of the collection site, such as average annual rainfall. When grown under identical conditions there was no significant difference between the performance of individual accessions. The results do not allow conclusions about local adaptation of BLRs, since it would be necessary to conduct yield experiments in the appropriate environment using a reciprocal transplant approach to analyse local adaptation and estimate the heritability of variation (Ennos, 1996).

Histograms (Figure 6.4.1 to 6.4.4) of the quantitative traits seeds/ear and TGW in both environments illustrate a remarkable variation of quantitative traits within accessions of Arabi Aswad, however, the source of this variation could not be identified using the experimental approach of this study. The high level of variation found in BLRs *per se* may bring about plant breeders to select high yielding lines for pure-line breeding strategies. It has in fact been shown that selection of well performing lines from landraces resulted in significant yield increase over local landraces in Syria (Ceccarelli, 1996a). However, this strategy can be seen as a conventional approach which does not account for marginal and unpredictable conditions. The advantageous performance of BLRs under adverse conditions seem to have other reasons than a proportion of lines performing well under certain conditions.

The role of heterozygosity and heterogeneity to improve performance of barley under drought stress has been investigated in experiments in Syria (Mayer, Gland, Ceccarelli and Geiger, 1995; Einfeldt, et al., 1996). Double haploid lines (DHL) were produced from locally adapted barley material and crossed with each other. Yield of DHL in pure stand (i.e. homogeneous and homozygous) and mixed stand (i.e. heterogeneous and homozygous) and F_2-bulk populations (i.e. heterozygous) from crosses between DHLs was analysed in four drought environments in Syria. Einfeldt, et al. (1996) concluded that heterozygosity (as found in F_2-bulk populations) was more important for grain yield under drought conditions than the effect of heterogeneity (as found in mixed stand of DHLs). However, their conclusions seem questionable, as F_2-bulk populations are not only heterozygous but also heterogeneous at the same time. Thus their superior performance under drought stress may be caused by a combined effect of heterozygosity and heterogeneity rather than by heterozygosity alone.

In a similar experiment (Mayer, et al., 1995) DHLs proved to show a much higher genotype variance than F_2-bulk populations and the predictability of their yield was reduced consequently. Mayer, et al. (1995) concluded that the high genetic variation of homogeneous and homozygous DHLs (and pure lines in general) under adverse and fluctuating climatic conditions are the main reason why heterogeneous BLRs are favoured in this region.

A higher proportion of plants with a low number of seeds per ear and a low TGW were observed in the glasshouse in Scotland. If the number of seeds per ear is understood as a measure of fitness, it seems that a number of genotypes had lower fitness under glasshouse conditions in Scotland than *in situ* in Syria. This was expected because accessions of BLRs are supposed to be adapted to local environments in Syria (Ceccarelli, 1996a), and growing them under glasshouse conditions in Scotland may have covered up possible differences which might become apparent under drought conditions in Syria.

Phenotypes with low performance may be adapted to particular environmental conditions and may show a higher fitness when these particular conditions are

present. Climatic conditions, in particular in semiarid and arid regions, are subject to inter-annual fluctuations. The occurrence of droughts seems to follow certain patterns which may be regionally different (Gommes, 1996). Novogrudskii (1991) proposed a model which predicts a 12 year cyclicity of farm crop yield, based on observations on wheat and barley in Russia. In Syria extreme drought, which can cause near failure of harvest, can occur in more than two years out of ten (Brichambaut and Wallen, 1963). Certain genotypes may have a superior fitness under these extreme conditions and would contribute an increased number of offspring to the next generation, but would contribute continuously less offspring over following seasons with sufficient precipitation. The proportion of this genotype in the population has to be large enough to survive for several seasons without its individually favourable conditions. It seems that adaptation to stress conditions is rather complex and combinations of different stress factors such as heat, drought and salinity may cause complex patterns of adaptation in individual genotypes and populations (Ceccarelli, 1984, 1991, 1994). Thus, a large number of experiments in different controlled environments would be necessary to investigate the composition of BLRs regarding adaptation to different stress factors. Genotypes tolerant to certain stress factors could be identified and used to compose artificial landraces.

Another strategy is selection of BLRs *in situ* in order to exploit local adaptation (Simmonds, 1991; Ceccarelli, 1996a). However, even if selection is carried out in the target environment on adapted germplasm, it will reduce the diversity of landrace populations, which components seem to be in a very complex balance. Phenotypic plasticity (i.e., adaptation to variation in the physical environment) itself seems to be a heritable trait that evolves under natural selection in diverse environments and can be manipulated by artificial selection (Bell and Lechowicz, 1994). A further complication of selection under stress conditions may be a lower heritability as a consequence of the higher environmental component of the phenotypic variance (Blum, 1985, cited by Ceccarelli, 1989). Ceccarelli (1989) found no clear trend of heritability for grain yield of barley in contrasting environments. The concept of narrow sense heritability has been criticised by Houle (1992) who proposed the additive genetic coefficient of variation to measure the ability to respond to selection.

While fitness related traits usually show a low narrow sense heritability, they have a higher additive genetic and nongenetic variability by the coefficient of variation criterion than characters under weak selection.

A further approach to plant breeding of barley for low-input agriculture under marginal conditions may be the use of long term bulk populations (or composites) (Simmonds 1993; Simmonds and Smartt, 1999), which could be produced from selected lines of locally adapted germplasm. As a result of the experimental work of barley composite crosses (Allard, 1992), it can be expected that natural selection for fertility may produce long-continued yield improvements in bulk populations (Simmonds and Smartt, 1999).

It seems reasonable to follow different approaches at the same time. However, new material should be tested and compared with local BLRs in local trials, with farmers' participation for many seasons in order to take local requirements and influences of inter-annual environmental fluctuations into consideration and conserve the basic germplasm (i.e. BLRs) at the same time *in situ.*

Chapter 7

Results of experimental investigations of the population structure of barley landraces with relevance to conservation strategies

7.1 Introduction

The global extension of modern cultivars of crop plants has prompted widespread concern about genetic erosion, i.e. the loss of genetic diversity of genetic resources for future plant breeding activities (Simmonds, 1962; Harlan, 1975; Frankel, 1977, Marshall, 1990, Dempsey, 1992). The immediate answer to the problem was the creation of extensive *ex situ* collections of accessions in form of dried seeds samples in cold stores and freezers, i.e. gene banks. Until recently the number of accessions of barley alone, held in gene banks around the world exceeded 100,000 (www-sites of gene banks, 1999). The huge number of amassed accessions raised a number of questions about the reasoning for such a venture (Hamilton, 1994). The main criticism included the huge costs, a high possible number of duplicates and the possible loss of diversity within accessions. The current approach to solve these problems is the subdivision of existing collections into a core collection, which would include with minimum redundancy the genetic diversity of a crop species, and a reserve collection that would include all other material (Frankel, 1984, cited by Marshall, 1990). The creation of a core collection is about to become reality for barley (Van Hintum, et al., 1995, Knupffer and Van Hintum, 1995) and may result in a reduced, manageable number of accessions without duplications at gene banks involved.

However, as early as in the 1960's there were a number of critical scientists who questioned the concept of *ex situ* conservation *per se*. For instance, Simmonds (1962) branded gene banks as 'museum collections' and proposed *in situ* conservation in the form of 'mass reservoirs', i.e. locally adapted broadly based populations. The main arguments against the sole reliance on *ex situ* conservation strategies derives from the worry that gene banks are static stores, while pathogens continue to evolve. *In situ* conservation, in contrast, would enable plants to co-evolve with pathogens and be able to develop resistances (Dempsey, 1992). Furthermore, the possible loss of genetic diversity within accessions in gene banks due to common gene bank practices has been mentioned as another argument against *ex situ* conservation (Frankel, 1977). In the meantime several *in situ* strategies have been proposed, one being the *in situ* conservation of crop plants in farmers' fields (Dempsey, 1992).

However, whatever conservation strategy is followed, only a small sample of the existing genetic diversity can be conserved at reasonable expense. The knowledge of the population structure of potential candidates, based on genetic markers, may help decide which accessions to include in a conservation project (Marshall and Brown, 1975). The use of molecular markers may also be of value to assess existing collections and identify duplicates, and in addition, identify possible loss of genetic diversity in *ex situ* collections. The objective of the two following sections was (i) to evaluate the collected accessions of BLRs from Syria for potential inclusion in a conservation programme and (ii) to investigate potential loss of genetic diversity within BLR accessions held in *ex situ* gene banks.

7.2 Genetic resource evaluation of BLRs from Syria

Managers and users of conservation programmes decide which accessions will be included in their programmes. The knowledge of the population structure of accessions, as given in Chapter 5 for BLRs in Syria, can be seen as the base for decisions which accessions to include in conservation programmes *in* or *ex situ*. Brown (1978) argues that alleles which deserve priority in sampling procedures for conservation are those which have a restricted or localised occurrence, but high frequencies (> 10%). He further recommends prioritising accessions with a higher average gene diversity.

The present study was not primarily designed as a genetic resource evaluation, as only a limited number of sites were sampled. However, principles for sampling as outlined by Brown (1978) were mainly followed. These principles advise (i) to collect 50-100 random individuals per site, (ii) to sample as many sites as possible within the time available, and (iii) to ensure that sampling sites represent as broad a range of environments as possible, within a target area.

The following results show the distribution of average gene diversity found within accessions of BLRs and the occurrence of alleles.

7.2.1 Results of the distribution of average gene diversity

Individual results of average gene diversity are given in Tables 5.3.1 to 5.3.3 for three genetic markers. Histograms of these results are given in Figures 7.1 to 7.3.

Figure 7.1 Histogram of average gene diversity (H) of 12 accessions of BLR Arabi Aswad, based on 4 morphological marker loci.

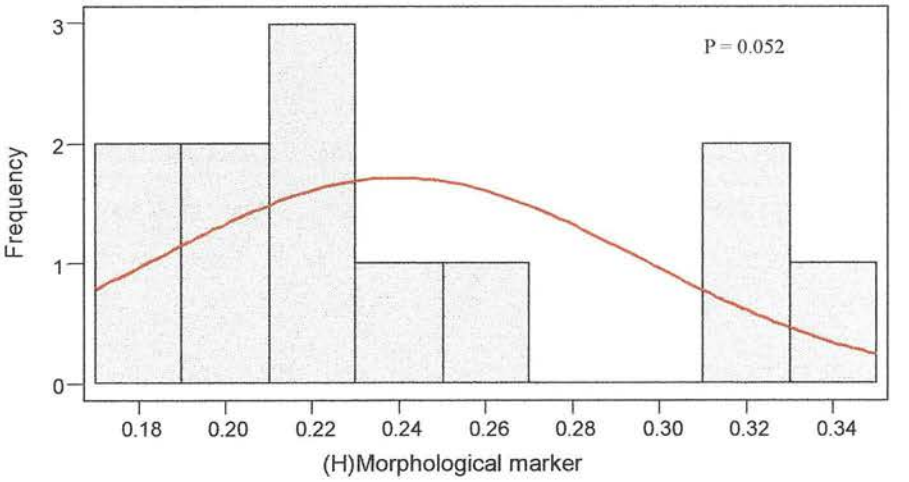


Figure 7.2 Histogram of average gene diversity (H) of 12 accessions of BLR Arabi Aswad, based on 8 isoenzyme marker loci.

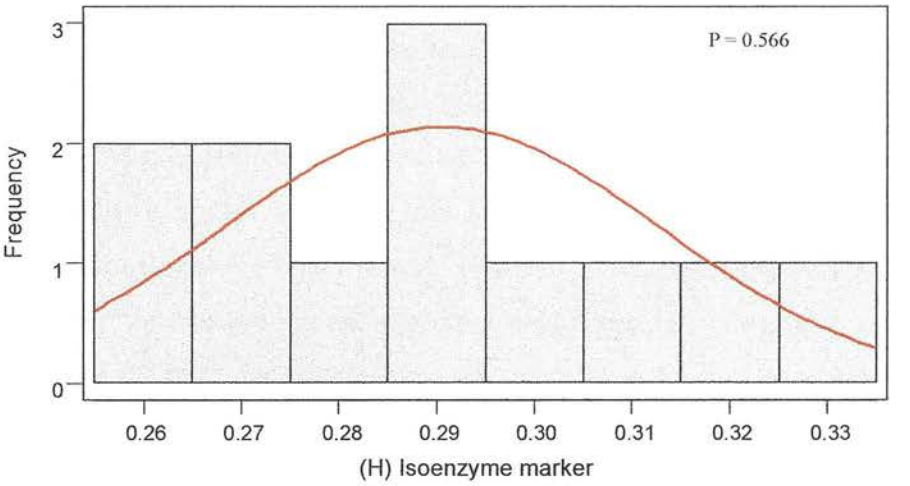
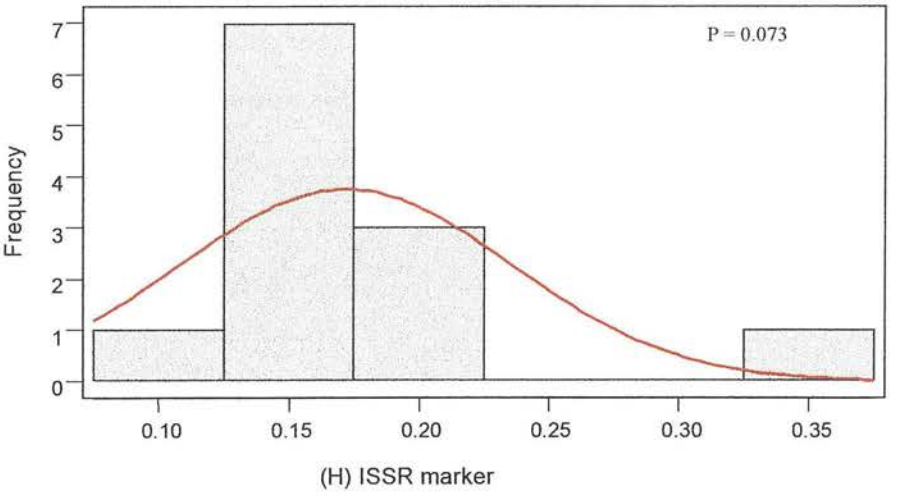


Figure 7.3 Histogram of average gene diversity (H) of 12 accessions of BLR Arabi Aswad, based on 25 ISSR marker loci.



All three histograms (Figure 7.1 to 7.3) showed a normal distribution according to the Anderson-Darling Normality Test. The significance level of this test (*P*) refers to the null hypothesis that the sample is normally distributed. Values of $P > 0.05$ indicate normality.

Results based on the morphological and ISSR marker showed a *P* value just over 0.05, while results based on isoenzyme markers were clearly normally distributed.

Both the morphological marker and the ISSR marker showed a number of accessions with low to moderate gene diversity, followed by a gap and a few number of accessions with a higher diversity. The highest gene diversity was found in the accessions from Raqqa for both markers, followed by accessions from Hassakeh and Quamishli for the morphological marker. Considering the isoenzyme marker the distribution of average gene diversities was continuous over a range from 0.26 to 0.33. Accessions from Aleppo (Im Mial and Om-amood) and Palmyra showed the highest average gene diversities.

7.2.2 Results of the occurrence and frequency of alleles

The occurrence of alleles of three different kinds of genetic markers were assessed. According to Brown (1978) alleles were categorised as common when they occurred at least in one sample with a frequency > 10%. Alleles that never occurred with a frequency > 10% were classified as rare. Alleles were considered widespread, sporadic and localised when they occurred in more than two locations, two locations or in only one location, respectively. Results are summarised in Table 7.1.

Table 7.1 Occurrence of alleles of three genetic markers in BLRs and populations of *H. spontaneum* in Syria.

	Number of alleles	Common			Rare	
	Total	Widespread	Sporadic	Local	Widespread	Local
Morphological marker (4 loci):						
Arabi Aswad (12)	**11**	**9**			**2**	
Arabi Abiad (3)	**11**	**8**		**3**		
H. spontaneum (2)	**8**	**8**				
Isoenzyme marker (8 loci):						
Arabi Aswad (12)	**20**	**17**	**1**			**2**
Arabi Abiad (3)	**19**	**16**				**3**
H. spontaneum (2)	**20**	**14**		**5**		**1**
ISSR-marker (25 loci):						
Arabi Aswad (12)	**49**	**49**				
Arabi Abiad (1)	**35**	**33**				**2**
H. spontaneum (2)	**38**	**37**				**1**

Number of populations in parenthesis

Morphological marker loci

Eleven alleles were found using 4 seed-morphological marker loci in populations of BLRs Arabi Aswad and Arabi Abiad. In Arabi Aswad nine alleles were common and widespread and two rare but widespread. Within populations of BLR Arabi Abiad 8 alleles were common and widespread and 3 were common but occurred only locally. These 3 alleles were black and dark lemma colour and black pericarp, which were the predominant alleles at these loci in Arabi Aswad. Three alleles occurred only in populations of the BLRs but not in *H. spontaneum* populations. *H. spontaneum* populations showed exclusively long hair rachillas (S/-), had never absolutely black lemma colour (B/-) and had always spicules present on lemma and awns (r/r). The eight alleles of the *H. spontaneum* populations were all common and widespread.

Isoenzyme marker loci

Allele frequencies at 8 esterase loci were resolved and resulted in a total of 20 different alleles within populations of BLR Arabi Aswad. 17 of these 20 alleles were common and wide spread, one was common but occurred only sporadically (Est 1, D). Two of all alleles were rare and occurred only locally (Est 2, C; Est 5, B). One of

these (Est 5, allele B) was exclusively found in BLR Arabi Aswad. 19 alleles occurred in populations of BLR Arabi Abiad, of which 16 were common and widespread and three rare and occurred only locally (Est 1, D; Est 2, C; Est 2b, B). One of these rare alleles (Est 2b, allele B) was unique to populations of BLR Arabi Abiad. *H. spontaneum* populations showed a total of 20 different alleles of which 14 were common and widespread, 5 common and localised and one allele was rare and occurred only locally (Est 1, D). The common, widespread allele B of the locus Est 4 was unique to *H. spontaneum* , i.e. did not occur in any of the BLR populations.

ISSR marker loci

Only one of the three populations of Arabi Abiad were analysed using the ISSR marker. For this reason all comparison between data achieved with the ISSR marker are restricted to the number of accessions given in Table 5.3.3. At 25 ISSR marker loci all 50 possible alleles were found in populations of the BLRs and all of them were common and widespread. In the two *H. spontaneum* populations only 38 alleles were assessed due to missing data for 6 loci. One of the 38 alleles scored was rare and occurred only in one of the two accessions, the remainder were common and widespread.

7.2.3 Discussion

The analysis of the population structure using genetic markers made it possible to discriminate between accessions according to their value for conservation purposes.

The histograms of average gene diversity (*H*) distinguishes some accessions with a higher level of *H* from the great majority of accessions with moderate to low *H* values. However, analysis of the genetic diversity does not provide sufficient information to select accessions for conservation. The differentiation of accessions, as given in Chapter 5, may offer very valuable information. For instance, the analysis of genetic distance between accessions in form of dendrograms (Figure 5.5.1 to 5.5.3) shows that accessions from the Aleppo region are quite similar, when all three genetic markers are considered. This implies that it might be sufficient to conserve

only one of these accessions in order to capture most of the allelic diversity from this region.

The analysis of allele frequencies per accession and their regional rate of recurrence may offer possibilities to select certain accessions for conservation. Results given in Table 7.1 are based on allele frequencies analysed for all accessions over all loci of the three genetic markers used. Brown (1978) argues that alleles which deserve priority in sampling procedures for conservation are those which have a restricted or localised occurrence, but high frequencies. For morphological marker loci localised common alleles (LCA) occurred exclusively in accessions of BLR Arabi Abiad. These loci were variants of black lemma and black seed colour and would be captured with the conservation of one accessions of the black seeded BLR Arabi Aswad. Five alleles of isoenzyme marker loci were LCAs in *H. spontaneum* accessions, but only one of them was unique to *H. spontaneum*. The reason for the local occurrence of alleles in *H. spontaneum* accessions was clearly because only two accessions were analysed. Combining all BLR and *H. spontaneum* accessions only one isoenzyme allele would be localised and common. No LCA was found for ISSR marker loci. Therefore it seems worthwhile to take the occurrence of rare alleles into consideration for the selection of accessions for conservation. Two rare morphological alleles occurred in Arabi Aswad (i.e. long rachilla hair and absence of spicules on lemma and awns), which were common in BLR Arabi Abiad and which are the predominant alleles for this locus in modern cultivars. Six rare isoenzyme alleles and three rare ISSR alleles occurred considering all accessions of BLRs and *H. spontaneum.*

The analysis of allele frequencies showed that it was possible to capture all morphological diversity present, including LCAs and rare alleles, by conserving one single accession of BLR Arabi Aswad (i.e. Aswad 3, Hassakeh). The conservation of both *H. spontaneum* accessions and BLR accession Arabi Aswad 3 from Hassakeh would be sufficient to capture all isoenzyme alleles that appeared in the present study. BLR accession Arabi Aswad 2 from Raqqa showed all but two of the 49 ISSR alleles found in the study, the remaining two alleles were present in Arabi Aswad 3 from Hassakeh.

To capture the entire genetic diversity found for three genetic markers in all 17 BLRs and *H. spontaneum* accessions it would be sufficient to conserve the two *H. spontaneum* accessions and BLR Arabi Aswad 2 and 3, from Raqqa and Hassakeh, respectively. However, this approach would disregard the importance of locally adapted variability for plant breeding, which has been emphasised by Simmonds (1962). Thus it would be advisable to conserve at least one BLR accession per region in order to supply breeders with a continually replenished store of locally adapted variability. Continuity in local adaptation could only be achieved by means of an *in situ* conservation approach.

7.3 Results of experimental investigations of the diversity of BLRs which have been stored in gene banks for different periods of time

Large numbers of crop plant accessions from all over the world have been amassed in gene banks to secure a gene pool for future breeding programmes. A large proportion of the material in these gene banks comprises accessions of traditional landraces of cultivated species. For instance, approximately one half of the existing accessions of *H. vulgare* in the BBSRC collection are landraces (BBSRC, 1999). However, about 50% to 60% of genetic variation of initial collections of BLRs are expected to reside within accessions, the remainder between accessions (Brown and Munday, 1982; Jana and Pietrzak, 1988).

Great emphasis has been placed on devising sampling strategies to ensure that accessions entering gene banks from the wild contain a high proportion of the 'within population' genetic variation that exists in traditional landraces (Brown & Briggs, 1991). Much less attention has been paid, however, to ensuring the maintenance of this genetic diversity throughout the lifetime of the gene bank (Sackville Hamilton & Chorlton, 1997). It has been common practice in gene banks to retain seed of many crops in airtight containers in cold stores (Clark *et al.*, 1997). In these circumstances, frequent rejuvenation of seed is needed to maintain high seed viability. To accomplish this in a species like barley, samples are taken from the stored accessions

and grown in plots that rarely exceed $3m^2$ in area. Rejuvenated seed is harvested from these plots for the next period of storage.

If the number of parents contributing to the rejuvenated seed is low i.e. the effective population size N_e, of rejuvenation populations is small, genetic diversity of landraces could be lost rapidly with each rejuvenation cycle (Frankel, 1977; Marshall, 1990; Hamilton 1994; Brown *et al.*, 1997), and accumulation of plants homozygous for deleterious mutations could occur (Bataillon *et al.*, 1996; Schoen *et al.*, 1998). Computer simulations suggest that effective population sizes of $N_e = 100$ (equivalent to 200 equally contributing parents in a self-fertilising species) in the rejuvenation population are needed to avoid these deleterious effects. It is possible that such population sizes have not been achieved in practice, and that the problem of genetic erosion from accessions of landraces in gene banks is a real one. However there are no empirical data presently available to determine whether loss of variation has actually taken place within gene bank collections as a consequence of repeated rounds of rejuvenation.

One purpose of the present study was to establish whether there is any evidence to support the prediction of loss of genetic variation from landrace populations that have been maintained *ex situ* within gene banks. Accessions of barley landraces from Syria were collected from *in situ* populations in farmers' fields, and from a variety of *ex situ* gene banks where they had been held for different periods since the initial collection. These periods ranged from 10 to 40 and 72 years (see Table 3.1.4, Chapter 3).

Four seed morphological and eight isoenzyme markers were analysed to determine whether a significant reduction in genetic diversity could be detected in populations that had been maintained in gene banks, and whether this reduction in diversity was related to the number of generations spent in the gene bank collection. Making a number of assumptions about the origins of gene bank population and their rate of rejuvenation in gene banks, the observed changes in genetic diversity were used to infer the genetically effective sizes of gene bank accessions during their time in storage.

7.3.1 Diversity levels within accessions of BLRs

Table 7.2 summarises the mean genetic diversity indices within accessions calculated from morphological and isoenzyme markers for groups of barley landrace accessions from Syria with identical storage periods. The individual results for all accessions are listed in Tables 7.3 and 7.4, and were plotted against time in storage in Figures 7.4 and 7.5.

Table 7.2 Diversity indices measuring mean genetic variation within 22 accessions of barley landraces stored in gene banks for identical time periods. *H*, gene diversity; *A*, mean alleles per locus; *P*(0.01), percentage polymorphic loci using the 1% criterion. Standard deviations are in parentheses. Values calculated from morphological markers and isoenzyme markers are shown separately.

Accessions with a storage period of:	Number of accessions	Morphological markers:			Isoenzyme markers:		
		H	*A*	P (0.01)	*H*	*A*	P(0.01)
0 years	11	**0.2327** (+/- 0.0544)	**2.3** (+/- 0.3)	**0.89** (+/-0.10)	**0.2874** (+/- 0.0219)	**1.9** (+/- 0.1)	**0.80** (+/- 0.10)
10 years	2	**0.1783** (+/- 0.067)	**1.8** (+/- 0.3)	**0.60** (+/- 0.10)	**0.2053** (+/- 0.0762)	**1.7** (+/- 0.21)	**0.57** (+/- 0.09)
40 years	2	**0.1795** (+/- 0.0292)	**1.8** (+/- 0.0)	**0.65** (+/- 0.21)	**0.06505** (+/-0.0712)	**1.5** (+/- 0.07)	**0.44** (+/- 0.08)
72 years	3	**0.0308** (+/- 0.0534)	**1.2** (+/- 0.3)	**0.17** (+/- 0.29)	**0.1015** (+/- 0.1085)	**1.5** (+/- 0.1)	**0.46** (+/- 0.14)
all accessions	18	**0.1871** (+/- 0.0898)	**2.0** (+/- 0.5)	**0.71** (+/- 0.31)	**0.2226** (+/- 0.1028)	**1.8** (+/- 0.2)	**0.68** (+/- 0.19)

Table 7.3 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at four loci affecting seed morphology. *H* gene diversity, *A* mean alleles per locus, *P*(0.01) percentage polymorphic loci using 1% criterion.

Landrace Code:	Collection year	Years stored	Number of individuals (n)	Morphological diversity				
				H	Sd(*H*)	*A*	Sd(*A*)	*P*(0.01)
Aswad 5	1998	0	74	0.1771	(+/-0.2266)	2.3	(+/-0.5)	1.0
Aswad 6	1998	0	65	0.2157	(+/-0.2374)	2.0	(+/-0.8)	0.8
Aswad 7	1998	0	50	0.1918	(+/-0.2330)	2.3	(+/-0.5)	1.0
Aswad 8	1998	0	54	0.2172	(+/-0.2680)	2.0	(+/-0.8)	0.8
Aswad 9	1998	0	51	0.2186	(+/-0.2722)	2.5	(+/-1.3)	0.8
Aswad 10	1998	0	80	0.2413	(+/-0.2787)	2.8	(+/-1.0)	1.0
Aswad 11	1998	0	52	0.2025	(+/-0.2650)	2.3	(+/-1.3)	0.8
Aswad 12	1998	0	53	0.1855	(+/-0.2553)	2.0	(+/-0.8)	0.8
Aswad 3	1997	0	197	0.3469	(+/-0.3297)	2.8	(+/-1.0)	1.0
Aswad 4	1997	0	200	0.3207	(+/-0.2988)	2.5	(+/-1.3)	0.8
Abiad 3	1997	0	200	0.2422	(+/-0.2457)	2.0	(+/-0.0)	1.0
Aswad 20126	< 1988	10	88	0.2259	(+/-0.2175)	2.0	(+/-1.0)	0.7
Abiad 20125	< 1988	10	95	0.1308	(+/-0.2420)	1.5	(+/-0.6)	0.5
black BLR 18882	1959	40	100	0.1588	(+/-0.2150)	1.8	(+/-1.0)	0.5
Abiad 18881	1959	40	100	0.2002	(+/-0.1840)	1.8	(+/-0.5)	0.8
white BLR 7667	1926	72	112	0.0000	(+/-0.0000)	1.0	(+/-0.0)	0.0
white BLR 7659	1926	72	100	0.0925	(+/-0.1082)	1.5	(+/-0.6)	0.5
white BLR HOR 7400	1926	72	62	0.0000	(+/-0.0000)	1.0	(+/-0.0)	0.0

Table 7.4 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at eight enzyme loci. *H* gene diversity, *A* mean alleles per locus, *P*(0.01) percentage polymorphic loci using 1% criterion.

Landrace Code:	Collection year	Years stored	Number of individuals (n)	Isoenzyme diversity: *H*	Sd(*H*)	*A*	Sd(*A*)	*P*(0.01)
Aswad 5	1998	0	50	0.2744	(+/-0.2033)	1.9	(+/-0.4)	0.9
Aswad 6	1998	0	50	0.2765	(+/-0.2895)	1.9	(+/-0.8)	0.6
Aswad 7	1998	0	50	0.2914	(+/-0.2043)	2.0	(+/-0.5)	0.9
Aswad 8	1998	0	50	0.3043	(+/-0.2515)	1.9	(+/-0.6)	0.8
Aswad 9	1998	0	50	0.2687	(+/-0.2498)	2.0	(+/-0.9)	0.8
Aswad 10	1998	0	50	0.3309	(+/-0.2193)	2.0	(+/-0.5)	0.9
Aswad 11	1998	0	50	0.2624	(+/-0.2325)	1.6	(+/-0.5)	0.6
Aswad 12	1998	0	50	0.3138	(+/-0.2560)	2.0	(+/-0.8)	0.8
Aswad 3	1997	0	50	0.2926	(+/-0.2217)	2.0	(+/-0.5)	0.9
Aswad 4	1997	0	50	0.2635	(+/-0.2159)	2.0	(+/-0.5)	0.9
Abiad 3	1997	0	55	0.2825	(+/-0.2165)	2.0	(+/-0.5)	0.9
Aswad 20126	< 1988	10	50	0.2592	(+/-0.2431)	1.8	(+/-0.7)	0.6
Abiad 20125	< 1988	10	50	0.1514	(+/-0.2176)	1.5	(+/-0.5)	0.5
black BLR 18882	1959	40	50	0.1154	(+/-0.1696)	1.5	(+/-0.5)	0.5
Abiad 18881	1959	40	50	0.0147	(+/-0.0203)	1.4	(+/-0.5)	0.4
white BLR 7667	1926	72	50	0.0469	(+/-0.0434)	1.4	(+/-0.8)	0.4
white BLR 7659	1926	72	44	0.0312	(+/-0.0737)	1.5	(+/-0.5)	0.4
white BLR HOR 7400	1926	72	35	0.2265	(+/-0.2122)	1.6	(+/-0.5)	0.4

Figure 7.4 Relationship between genetic diversity indices, measured using four morphological markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((*H*), Fig. 7.4 A.); mean alleles per locus ((*A*), Fig 7.4 B); and percentage polymorphic loci using 1% criterion ((*P* (0.01)), Fig. 7.4 C).

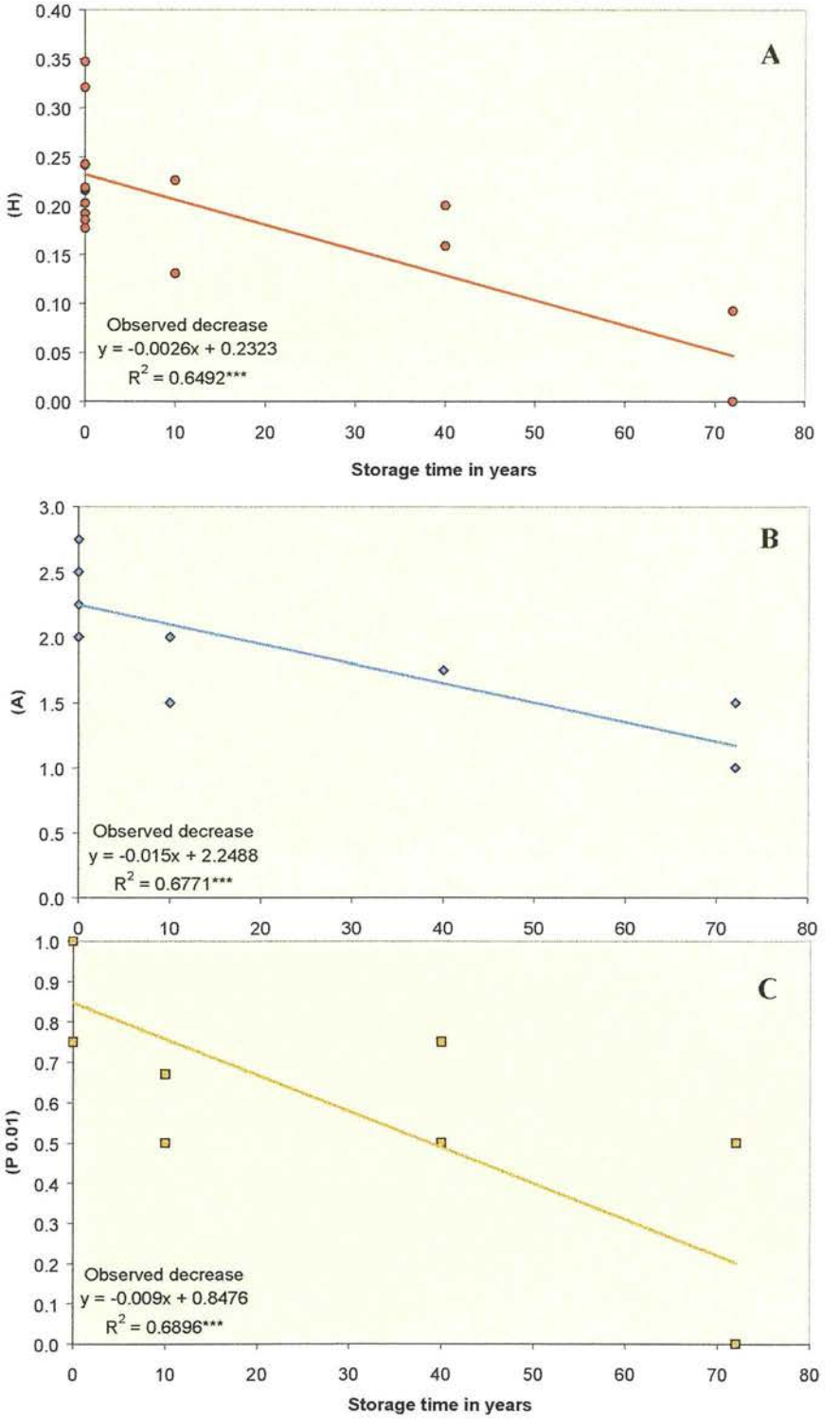
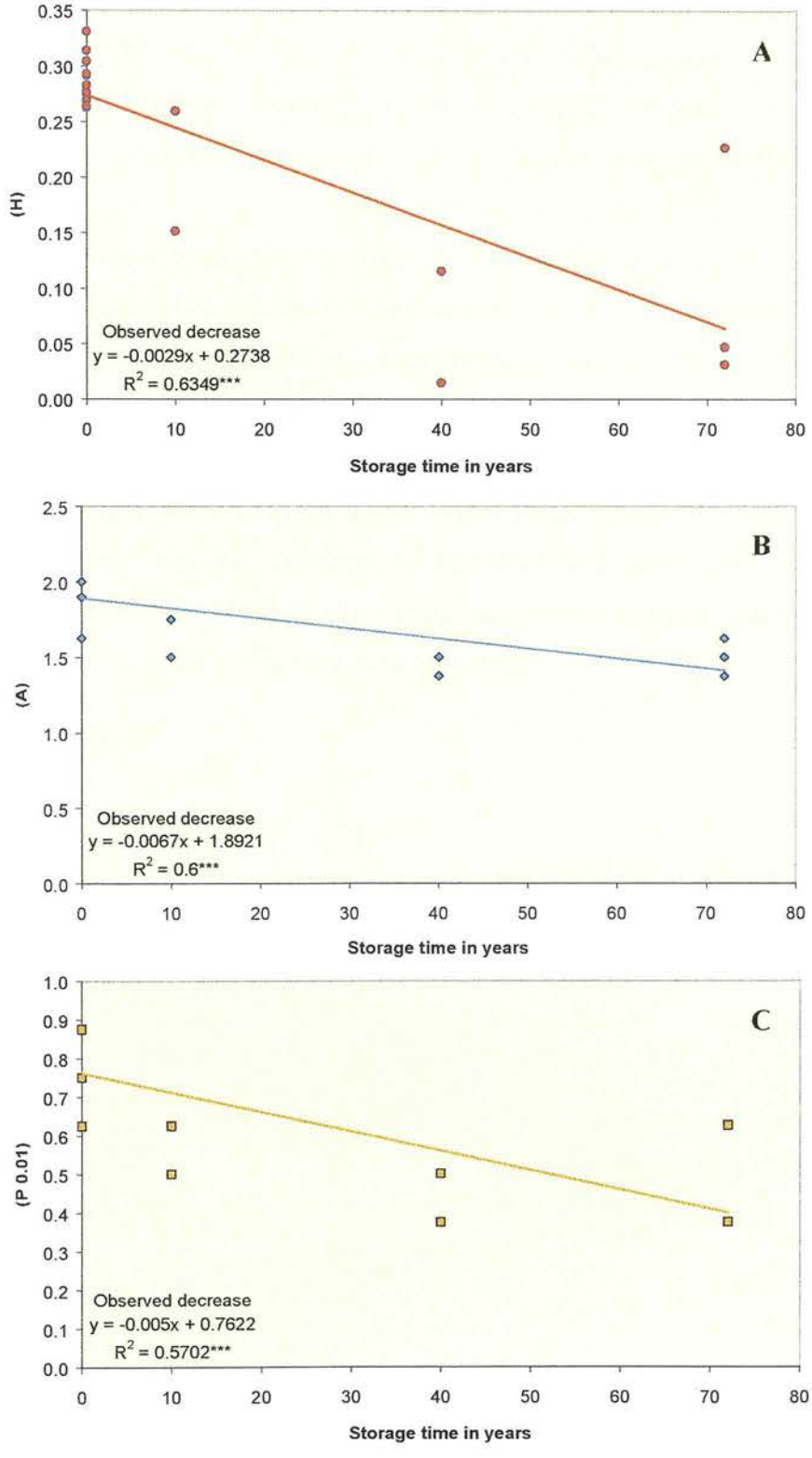


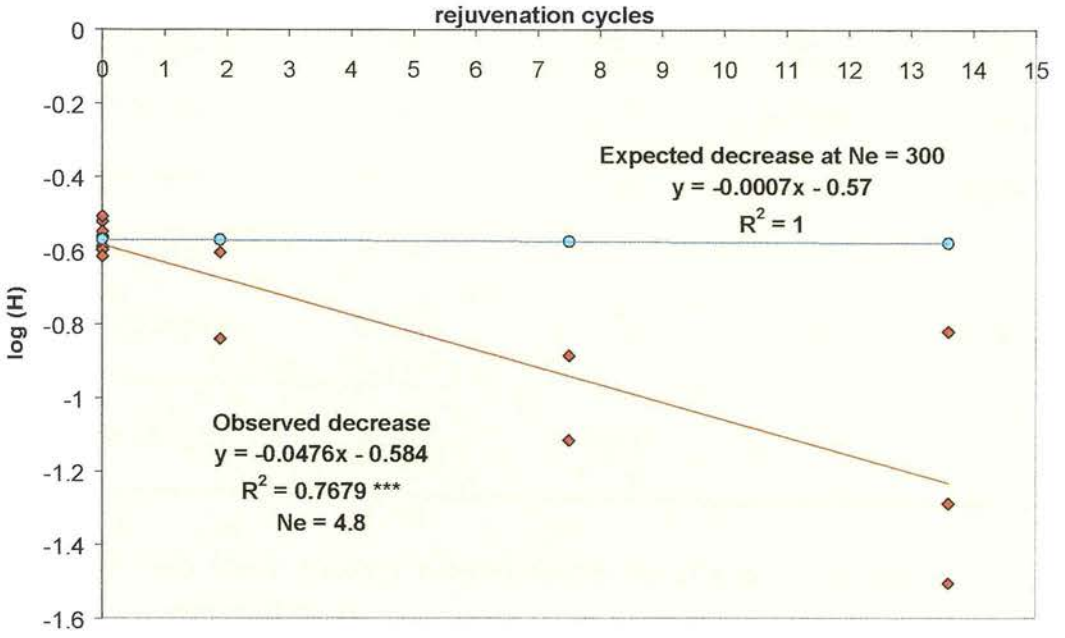
Figure 7.5 Relationship between genetic diversity indices, measured using eight isoenzyme markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((*H*), Fig.7.5 A.); mean alleles per locus ((*A*), Fig. 7.5 B); and percentage polymorphic loci using 1% criterion ((*P* (0.01)), Fig. 7.5 C).



A decrease of genetic diversity with increase in storage period was observed for all three indices of diversity and for both marker types. Average maximum declines in diversity indices after 72 years storage were 62% for $P(0.01)$, 37% for A and 78% for H. For both the morphological and the isoenzyme markers, all three genetic diversity indices showed significant negative regressions ($P < 0.001$) on storage time (Figs. 7.4 and 7.5). The only exceptional result was a slight increase in average gene diversity (H) of isoenzyme markers from the 40 to the 72 year accessions. This result was caused by the high H value of one individual accession (HOR 7400) (Table 7.4).

Comprehensive documentation of rejuvenation practices was available from the IPK gene bank, Gatersleben, Germany. An analysis of 20 different files, monitoring accessions collected from 11 to 51 years ago, showed that on average one rejuvenation cycle took place for every 5.3 years of storage time. This accords with the 5 year rejuvenation cycle for the 10 year old material obtained from the BBSRC gene bank (Arabi Aswad 20126, Arabi Abiad 20125, Table 7.3 and 7.4). These accessions were originally collected by ICARDA and have been stored at the BBSRC gene bank since 1988. It was known that these accessions have been through two rejuvenation cycles during their time in storage.

Figure 7.5 Regression of log (gene diversity) (log(H)) on number of cycles of rejuvenation experienced by accessions of barley landraces in gene banks. The slope of the line is an estimate of log (1-1/2N_e) where N_e is the effective size of rejuvenation populations.



Assuming a mean time between rejuvenation of 5.3 years, gene diversity (H) within accessions decreased on average by approximately 10% with every cycle of rejuvenation for both sets of markers. From the regression of $log(H_t)$ on t, the genetically effective size of *ex situ* populations during their time in storage, estimated from the rate of decline of gene diversity, was $N_e = 4.8$ (see Figure7.5).

7.3.2 Total diversity and its distribution among accessions of BLRs

If genetic drift occurs independently in accessions over cycles of rejuvenation, an increase in genetic divergence between them is expected. To test for this effect all accessions held for the same length of time in gene banks were grouped and treated as a single subdivided population. Values of total genetic diversity H_T, for accessions in each age class (0, 10, 40 and 72 years), and of F_{ST} measuring the extent of genetic divergence among accessions in these age classes were estimated separately for morphological and isoenzyme markers and given in Table 7.5.

Table 7.5 Total genetic variation H_T of accessions held for particular time period, and genetic differentiation F_{ST} found among accessions. Results are shown separately for morphological and isoenzyme markers.

Accessions with a storage period of:	Morphological markers:		Isoenzyme markers:	
	H_T	F_{ST}	H_T	F_{ST}
0 years	0.3197	0.2722	0.3209	0.0929
10 years	0.2967	0.3990	0.2502	0.1795
40 years	0.2661	0.3254	0.2302	0.8477
72 years	0.1242	0.7518	0.1127	0.2832
All accessions	0.3446	0.4666	0.3545	0.3721

Total diversity levels H_T for combined accessions of a particular age held in gene banks show a decline with storage time (Table 7.5). Over 72 years storage H_T declined from 0.32 to 0.12 when measured with morphological markers and from 0.32 to 0.11 when isoenzyme markers were used. Measures of the proportion of genetic variation found among accessions relative to total genetic variation, F_{ST}, showed an increase in value with time of storage. Thus total genetic variation was lower in older samples, and a greater proportion of this variation was distributed between rather than within samples. One exception to this trend was the group of accessions with a 72-year storage period scored for isoenzyme markers. The low F_{ST} value of this group is caused by a high H value in one of the three accessions (HOR 7400, Table 7.4).

7.3.5 Discussion

Results of this section show that gene diversity within landrace accessions of barley in Syria is related to the time of collection of the accession. Genetic diversity is lower in early accessions than in accessions made more recently. An additional result is that a greater proportion of the total genetic variation is found between rather than within landrace accessions in older collections than in recent collections. These results are consistently found using both morphological and isoenzyme genetic markers.

A possible explanation for both these results is that the differences in genetic diversity and structure are due to genetic drift occurring during the rejuvenation cycles through which the *ex situ* collections have passed during their time in the gene bank. For this to be a convincing explanation a number of conditions must be met. The first is that the initial samples used to found the early gene bank accessions were large enough to include a high proportion of the genetic diversity present in the contemporary landrace populations. The second is that there has been no systematic change in the gene diversity of the *in situ* landrace populations over time.

It can be anticipated that 10 year old samples (Arabi Aswad 20126, Arabi Abiad 20125) which originated from ICARDA, were based on at least 50 to 100 seed heads (Weltzien, 1989), and thus would have sampled a similar proportion of the genetic diversity available in landraces as present day collections (Marshall, 1990). Unfortunately details of the sampling procedures used to obtain the oldest accessions used in this study are not available. However the fact that such early collections were large enough to provide the material for extensive field trials demonstrating genetic heterogeneity within landraces suggests that they were not genetically depauperate at the time when they entered the gene banks (Vavilov, 1957). It therefore seems reasonable to conclude that observed differences in gene diversity among collections held for different lengths of time are unlikely to be accounted for by differences in initial sampling procedures.

The second assumption, that there has been no systematic change in the genetic diversity of *in situ* populations of barley landraces in Syria over the last 72 years, is supported by results of this study (Section 5.3.2.2) and extensive fieldwork at ICARDA. Both sources indicate restricted exchange of barley landrace germplasm

among climatic regions within Syria, and the minimal influence of modern barley cultivars on barley landraces in this region (Weltzien & Fischbeck, 1990; Ceccarelli, 1996a). The continuity and stability of landrace cultivation in the area means that gene diversity levels in present day barley landraces are likely to be very similar to gene diversity levels in landrace populations grown throughout the last 72 years. If any changes have taken place over this time they are likely to have led to a reduction rather than an increase in gene diversity levels in contemporary landrace populations.

Comparison of gene diversity levels in accessions held for different lengths of time show decreasing levels of genetic diversity with increasing length of storage, and this is consistent over diversity indices and genetic markers. If it is accepted that these differences were not caused by differences in initial sampling procedures or by a systematic increase in gene diversity over time within *in situ* populations, the observed decline of genetic diversity is most easily explained by the effects of genetic drift in rejuvenation populations used to maintain the accessions in the gene banks. The rate of decline of genetic diversity is consistent with a model of genetic drift in rejuvenation populations with an effective population size N_e = 4.8. In a predominantly inbreeding species where N_e is half the number of observed reproductive individuals, this is equivalent to a population of 9.6 unrelated adults contributing equally to the seed produced.

Such a low number of parents contributing to rejuvenated seed appears at odds with what is known of rejuvenation procedures in gene banks. Here accessions are grown on $3m^2$ field plots likely to accommodate 600 plants, and if all individuals contributed equally, effective population size would be N_e = 300. The expected decline in gene diversity every cycle of rejuvenation is negligible when the effective population size is as large as 300 (Figure 7.5).

There are a number of factors that could account for this discrepancy between anticipated and inferred effective population size in the rejuvenation populations. Restricted sampling of the accession from the gene bank to establish the rejuvenation plot, and limited sampling of the seed produced from this plot could both contribute to a low value of N_e. In addition the differences in environmental conditions between the original site of collection and the rejuvenation plots may be so large that only a

limited set of genotypes are able successfully to grow and produce seed. A large variance in the reproductive contribution of individuals will significantly reduce the effective size of a population (Lande & Barrowclough, 1987).

It should also be remembered that the value of N_e calculated relates to the genetic behaviour of accessions over a number of generations. The value of N_e in these circumstances is governed by the value of the smallest effective population size found over the time period in question (Lande & Barrowclough, 1987). Thus the low value of N_e could be accounted for by a single bottleneck event in the gene bank, rather than a chronically low effective population size at each round of rejuvenation.

If these estimated values of N_e in rejuvenation populations are reasonable, problems are likely to arise in gene bank collections not only as a consequence of loss of genetic variation, but also through fixation of deleterious mutations that are much more difficult to monitor (Schoen *et al.*, 1998). In this respect it is interesting to note that during growth of material for electrophoresis, chlorophyll deficient mutants were observed in accessions that had been stored for 10 years (Aswad 20126) and 72 years (white H.v. 7667) at frequencies of 1.1% and 3.8% respectively, but not in any recent accessions. Computer simulations by Schoen *et al.* (1998) suggest that accumulation of plants homozygous for deleterious mutations will accompany recurrent rejuvenation of germplasm when effective population size is less than 75.

Another effect that is expected in gene banks, if genetic drift is occurring in rejuvenation populations, is that genetic differentiation among accessions with identical storage times should increase with time of storage. A trend of increasing values of F_{ST} with storage time is indeed seen for the barley landraces and this is clearest for the morphological markers. These show a greater than 2 fold increase in F_{ST} after 72 years in storage (Table 7.5). This change cannot be attributed to collection of a less diverse range of populations in more recent years since the present day collections cover the wide range of barley landrace sites within northern Syria.

7.4 Conclusions

One problem that all conservation strategies have in common is that only a limited number of accessions can be conserved at reasonable expense.

The analysis of the population structure of BLRs employing genetic markers has proved useful to assess the value of individual accessions for inclusion in conservation programmes. In particular the analysis of allele frequencies of individual accessions enables the selection of accessions which possess most of the allelic diversity present. If local adaptation is to be included in a conservation programme, population differentiation may be a tool to identify accessions that represent the diversity found within a defined region. However, all endeavours to sample and identify a maximum of genetic diversity within collected accessions may be in vain, if practical conservation strategies are unable to preserve it.

The results of experimental investigations of the diversity of BLRs which have been stored in gene banks for different periods of time may indicate that genetic erosion i.e. the loss of genetic diversity is occurring in gene bank accessions. The genetic variation originally present in the accessions collected at a particular time has not only declined overall (shown by a decrease in H_T, Table 7.5) but has become rearranged such that in older collections an increased proportion of the variation is distributed between accessions while less is found within accessions.

Simmonds (1962) has referred to gene banks as "museum collections" because they contain a selection of ill-adapted genotypes (from the point of view of high input agriculture) which either cannot be preserved at all in the wild or can be preserved only with great difficulty and uncertainty. The present results suggest that the reality is even worse, because genetic drift during rejuvenation may lead to erosion of the original genetic diversity of seed accessions lodged in gene banks. The present study is only of a preliminary nature, relies on assumptions about early sampling procedures and continuity of landrace populations over time, and deals only with one crop species. However, it implies the possibility that other related inbreeding crop species such as wheat and rice are subject to similar influences. Thus more detailed empirical studies of genetic changes occurring during rejuvenation of gene bank accessions are justified. If genetic erosion proves to be a widespread phenomenon,

changes in management are clearly required to prevent its undesirable effects and enable gene banks to serve as sustained reservoirs of crop genetic resources.

A rapid improvement of *ex situ* storage conditions and facilities (i.e. deep freezing of samples) would facilitate maintenance of collections with fewer rejuvenation cycles. The use of larger rejuvenation plots and greater emphasis on adequate sample sizes for establishing and collecting from rejuvenation plots may also be important. To make the best use of old collections, in which genetic variation may now be found predominantly between rather than within accessions, it may be advisable to combine duplicate collections from a variety of different gene banks. Once a genetically variable collection had been amassed, this could be conserved *ex situ* with more modern techniques involving deep-freezing, possibly complemented by *in situ* conservation approaches.

The consistently high diversity indices of all recently collected BLR accessions found in this study are a clear justification for the use of *in situ* conservation strategies. Successful examples of *in situ* conservation of landraces in farmers fields' have been shown for maize (*Zea mays* L.) in southern Mexico, wheat (*Triticum* spp.) in western Turkey and Potatoes (*Solanum* spp.) in the Andes of Peru (Brush, 1995). However, given the inherent weaknesses of both systems it seems appropriate to promote crop conservation through a combination of *in* and *ex situ* conservation practices rather than favouring a single strategy.

Chapter 8

General conclusions

8.1 Introduction

The investigation of the population structure of barley landraces from different eco-geographical environments in Syria has been opened with an account of barley, and in particular of BLRs (Chapter 1). A review and evaluation of genetic markers was conducted in order to select suitable and efficient genetic markers to be used in the study (Chapter 2). Material and methods (Chapter 3) were presented referring to all following result chapters. The analysis of the environmental conditions in Syria using GIS (Chapter 4) preceded the main chapter (Chapter 5) in which the population structure of BLRs was analysed. Consequences of the population structure of BLRs for plant breeding of barley in low input agriculture under marginal conditions (Chapter 6) and for conservation strategies (Chapter 7) were analysed in the last two result chapters. Results were discussed at the end of each chapter and will be discussed jointly in the following chapter in order to answer the questions raised at the beginning of the study.

8.2 Diversity of BLRs

The study has shown that present day BLRs, as still predominantly grown throughout Syria, show a high level of diversity and are geographically variable. This was true for results achieved with three different genetic markers (morphological, isoenzymes and molecular ISSR markers), of which the ISSR marker produced the lowest diversity indices. Achieved results agree with results of other studies using morphological and isoenzyme markers (Brown and Munday, 1982; Jana and Pietrzak, 1988; Nevo, 1992; Konishi, et al., 1993). Other studies, based on specific molecular markers such as rDNA (Saghai-Maroof, et al., 1984), cpDNA (Holwerda, et al., 1986; Neale, et al., 1986) and mtDNA (Neale, et al., 1986) found, in agreement with the present study, a lower level of diversity compared with isoenzymes. This was expected in particular for the organelle markers where mutation rates are low compared with nuclear markers. The above studies also reported local adaptation and geographical variation of BLRs. Results for molecular fingerprint methods (e.g. ISSR, RAPD, AFLP) are not available for BLRs, but in barley cultivars these methods found high levels of polymorphism (Tinker, et al., 1993; Ellis, et al., 1997).

Different forces have been considered to explain how the high diversity level of BLRs has been maintained over many generations of cultivation.

One theory presented in the present study was that the outcrossing rate of BLRs may be higher than expected for barley, due to harsh and heterogeneous environmental conditions in Syria. Although it could be shown that low winter temperatures and variation in rainfall over years may increase the outcrossing rate of BLRs, the overall level of outcrossing of BLRs of 1.7% was not higher than the rate reported for wild barley of 1.6% (Brown, Zohary & Nevo, 1978) and was near the bottom end of the range of outcrossing rates found in modern cultivars (0.5% to 10%) (Sokal, 1978; Simmonds, 1979; Doll, 1987). Thus, it seems that the outcrossing rate alone cannot explain the high level of diversity found in BLRs.

Heterozygote advantage is known to maintain polymorphism in plant populations (Falconer, 1989). Results of the present study showed the presence of heterozygote advantage in BLRs from Syria, since expected fixation at inbreeding equilibrium was significantly higher (about 10%) than Wright's fixation index. Therefore it seems likely that heterozygote advantage may be one reason for the maintenance of diversity of BLRs. Results of the present study are in accordance with results by Jain and Allard (1960) who observed constant heterozygote advantage over 18 generations in a barley composite cross. However, heterozygote advantage in inbreeding species has to be interpreted with great care, since it can not be excluded that heterozygotes may be at selective advantage just because they cover up deleterious mutations (Ennos, 1983).

Nevo (1997) found that heterogeneity and harshness of the environment are associated with increased genetic diversity of a number of organisms such as landsnail (*Buliminus labrosus*), beetles (*Carabus hemprichi* and *Oxythyrea noemi*), lichen (*Caloplaca aurantia*) and wild barley (*H. spontaneum*). The results of the present study corroborate these findings for Northern Syria. Hot summer temperatures, high percentage of continentality, low average annual rainfall and high altitude all showed significant correlations with genetic diversity indices. However,

the only indisputable index to measure environmental heterogeneity in the present study was *relative interannual variation in rainfall*, which was not significantly correlated with any genetic diversity index. Lacking sufficient data of environmental heterogeneity indices, it seems only justified to link increased genetic diversity to the harshness of the environment. Further studies are required to characterise environmental conditions in terms of heterogeneity in time and space, before comparisons between genetic and environmental heterogeneity are justified.
On a smaller scale in the Aleppo region (65km diameter) only altitude had positive significant influence on genetic indices. A trend was found, though not significant, towards higher diversity indices for the zone with the lowest average annual rainfall (200 to 230mm). Thus, environmental harshness seems to maintain a high level of genetic diversity of BLR populations, as anticipated from findings for wild barley.

One explanation for increased diversity under heterogeneous environmental conditions is frequency-dependent selection (FDS). FDS is known to maintain genetic diversity of plant populations in heterogeneous environments (Ayala, 1982; Falconer, 1989). The experimental design of the present study did not allow the analysis of data for the presence of FDS.

However, the temporal environmental heterogeneity of the study area and the presence of rare genotypes may allow some speculations about a possible role of temporal variation in selection pressure (Perry and Lotan, 1979) as a force to maintain diversity in BLRs from Syria. The zone between the steppe and inland plain of Syria, with a precipitation of 200mm to 250mm can be seen as an environment highly heterogeneous in time, since near failure of harvest can occur in more than two out of ten years (Brichambaut and Wallen, 1963). Assuming that a rare genotype of a BLR population may show a higher fitness under extreme drought conditions and will thus contribute relatively more offspring to the next generation than other genotypes, its proportion will increase in the following generation. Assuming relaxed environmental conditions in the following generation, selection will work against the genotype which showed higher fitness under extreme drought, and will reduce its proportion over several generations until another extreme drought occurs. It could be

expected that this process would maintain a high level of polymorphism within the population. However, the role of temporal selection as a force to maintain polymorphism has been questioned (Ennos, 1983). To prove the validity of this hypothesis it would be necessary to test rare lines of BLRs with low fitness (i.e. low number of offspring) when grown under relaxed conditions in the heterogeneous environment. These lines should be tested under different controlled environmental conditions, to uncover conditions of their assumed adaptation.

The design of the present study allowed demonstration that diversity of BLRs seems to be based on a low outcrossing rate. Part of the heterozygosity found in BLRs can not be explained by the outcrossing rate alone and may be maintained by heterozygote advantage and the severity of environmental conditions. It seems likely that further forces such as frequency-dependent selection may influence the diversity level of BLRs.

8.3 Effects of domestication and cultivation of barley

An increasing rate of selfing may be expected under domestication (Jarne & Charlesworth, 1993). However, outcrossing in barley may not have been affected by domestication as the outcrossing rate found for BLRs is not significantly different to the outcrossing rate of wild barley of 1.6 %, as reported by Brown, Zohary & Nevo (1978). Outcrossing rates reported for modern cultivars ranged from 0.5% to 10% (Sokal, 1978; Simmonds, 1979; Doll, 1987) and disagree as well with the theory of increasing selfing rates through domestication, for barley.

The diversity of cultivated barley is expected to be lower than the diversity of wild barley, due to genetic bottle-necks during domestication (Brown and Monday, 1982; Allard, 1992; Nevo, 1992, Saghai-Maroof, et al., 1994). The results of the present study corroborate this expectation in principle, although not equally for all genetic markers used. Results based on the molecular ISSR markers gave strikingly similar diversity levels for wild barley, BLRs and modern cultivars. These results may indicate that selection due to domestication has mainly involved morphological

characters and those genetic markers which might be associated with characters under strong selection, such as some isoenzymes (Brown, 1978). The average gene diversity based on neutral molecular markers seems to be almost unaffected by the domestication process. However, the partitioning of diversity shows clearly that differentiation between populations increases with intensity of selection, i.e. diversity of progressively selected material is found between populations and less within populations. The limits are completely homogeneous and homozygous pure-line cultivars, which harbour no diversity at all. An assemblage of modern cultivars showed a considerable level of total gene diversity based on neutral markers which was, however, lower than the total diversity found in BLRs.

A higher number of linkage disequilibria, in particular of distant loci, may indicate a higher degree of local adaptation of *H. spontaneum* populations compared with BLRs. This result was expected, since *H. spontaneum* populations are not directly influenced by the activity of man, and their population structure is thus mainly shaped by natural selection.

Gene flow between collection sites of BLR accessions can be predominantly attributed to seed exchange between farmers, since propagation of seeds of cultivated barley depends entirely on human activity, and pollen flow is unlikely to have a great effect due to the very low outcrossing rate. Consequently, gene flow between populations of BLRs may be primarily attributed to seed exchange between farmers. The analysis of gene flow between populations in the present study may imply that exchange of seeds between farmers occurs on a local basis, but is unlikely to occur over longer distances. However, the results were able to demonstrate that agricultural developments, such as systematic extension work, may increase seed exchange even over long distances and thus may influence the degree of local adaptation of BLRs.

In conclusion, results of the present study corroborate observations that early domestication has lead to increased morphological variation in barley (e.g. two-row/six-row, naked/hulled, hooded/awned types) (Harlan, et al., 1973), but domestication seems to have diminished genetic diversity, as measured with

isoenzyme markers, due to genetic bottlenecks. The effect of domestication seems less clear, when neutral molecular DNA marker loci are considered. These are more frequent and randomly dispersed throughout the genome and thus less likely to be effected by genetic bottlenecks (Nei, 1987).

A clear loss of average gene diversity of barley, however, can be attributed to modern pure-line breeding because there is no diversity within pure lines, as could be shown for morphological, isoenzyme and molecular ISSR markers. Thus, the average gene diversity of modern cultivars is zero, while both BLRs and wild barley showed high levels of average gene diversity for all three genetic markers used.

8.4 Environmental impact

The characterisation of environmental conditions is in itself a complex endeavour and accuracy depends on the availability of environmental measurements for as many locations and seasons as possible. GIS seems to offer great possibilities to increase the accuracy to analyse measurements considerably, employing interpolation procedures. However, the availability of average climatic data and extremes (e.g. hottest summer temperature) might not be sufficient to describe the expected complex interactions with genotypes. A characterisation of the heterogeneity of environmental conditions over time is possible if long-term observations are available, so that coefficients of variance may be calculated. Heterogeneity over space is affected by many parameters (e.g. soil differences, availability of water and nutrients, salinity, climatic conditions), so that classification seems difficult. It may be more practical to use the average and long-term variation of local yields (e.g. coefficient of variation) of crop plants (e.g. lentils) under rainfed conditions in Syria in common agricultural systems as indicators for the effect of all combined environmental conditions. Nevertheless, accuracy of this approach depends again on the number of available observations.

The outcrossing rate of BLRs seems to be positively influenced by environmental conditions such as low winter temperatures and high interannual variation in rainfall.

Other climatic factors, however, seem to be associated with the genetic diversity of BLR populations.

Nevo, (1988) observed a general pattern of increasing genetic diversity towards xeric and ecologically heterogeneous environments of many unrelated taxa such as snails, lichen and wild barley. He explains the increase in observed heterozygosity and gene diversity with the niche-width variation hypothesis (Van Valen, 1965). The hypothesis predicts a positive correlation between genetic and ecological heterogeneities, i.e. species are more polymorphic if they live in broader climatic, ecological, or biotic spectra. Diversity indices of BLRs in the present study were higher in regions with high summer temperatures, a high percentage of continentality, low average annual rainfall and at higher elevations. These observations seem to corroborate Nevo's (1988) observations and may imply that the niche-width variation hypothesis (Van Valen, 1965) might hold for BLRs as well.

However, the results of the present study may indicate that outcrossing is not directly influencing diversity levels of BLRs. Outcrossing and diversity seem to be affected by different environmental factors. Furthermore, it seems that extreme conditions such as high summer temperatures or a high percentage of continentality increase diversity levels in BLRs, but no dependency of diversity on the heterogeneity of the environment could be found. The latter result may be rather caused by the lack of parameters defining environmental heterogeneity. Spatial heterogeneity of environments (e.g. soil differences, salinity) may cause seeds of annual species to be confronted with a different set of environmental conditions every generation. This situation may promote the occurrence of frequency-dependent selection (Ayala, 1982) and may partly explain the fact that phenotypic plasticity itself can be a heritable trait (Bell and Lechowicz, 1994).

Yield of BLRs *in situ* was positively influenced by increasing amount of average annual rainfall, as expected, because moisture availability is known as the predominant growth limiting factor (Ceccarelli, 1984). Accessions of BLR Arabi Aswad showed no significant differences for yield, when grown under identical controlled environmental conditions in a glasshouse. However, the experimental

design was not suited to calculate heritability of the variation of yield. Thus it was not possible to attribute yield differences observed between accessions *in situ,* to environmental conditions or genetic differences between accessions. The interaction between genotypes and environments may best be analysed by using variation of quantitative traits and calculating heritability and the coefficient of genetic variation in different environments using progenies of individual plants.

8.5 Consequences for plant breeding

The experimental results confirmed that modern pure-line cultivars of barley are homogeneous and homozygous and possess no gene diversity. Thus, all possible diversity of modern barley cultivars may be found between cultivars. To produce F_3-bulk populations with the same diversity level as BLRs, parent lines had to possess at least 50% allele differences. Observed heterozygosity of F_2 and F_3–bulk populations was 50% and 25% respectively as expected for an inbreeding species (Simmonds and Smartt, 1999). *H. spontaneum* populations and BLRs had a level of heterozygosity of a magnitude expected for a F_4- and a F_6-generation of a bulk population, respectively.

The high level of diversity and the wide range of performance of quantitative traits found in BLRs may indicate a high expected response to selection. Local adaptation and geographical variation of BLRs seem likely, since genetic indices were able to cluster accessions of BLRs into distinct regional groups, and linkage disequilibria revealed locally different intensities of natural selection. Thus BLRs *in situ* may be a useful source of selection for low-input agriculture under marginal conditions, as proposed by Ceccarelli (1991). BLRs may further offer the possibility to supply lines to be tested for their suitability as components for artificial landraces.

As a result of his research, Ceccarelli (1989, 1994, 1996a) proposed to select in the target area for specific adaptation, if the target environment is below the cross-over point of a genotype × environment interaction. It has been shown that heritability of quantitative traits may be low, if the environmental component of the phenotypic

variance is high (Blum, 1985, cited by Ceccarelli, 1989), as can be expected from the definition of narrow sense heritability. Thus it seems that heritability may be the wrong parameter to predict the response to selection. Houle (1991) proposed to use the additive genetic coefficient of variation to predict the ability of quantitative traits to respond to selection, which is a parameter that further allows inferences about the forces that maintain genetic variability.

In conclusion, it seems that long-term fluctuations have to be taken into account when considering future breeding strategies for marginal environmental conditions in low-input agriculture. Apart from the use of locally adapted BLRs for selection, other strategies should be considered such as long-term bulk populations and artificial landraces. A strong *in situ* conservation approach for BLRs seems imperative in order to identify the long-term breeding success of employed strategies.

8.6 Consequences for conservation

Two findings of the present study have direct implications for conservation strategies of inbreeding crop species such as barley.

First, present day BLRs, which are still predominantly grown throughout Syria and other developing countries such as Ethiopia or Nepal, show a high level of diversity. If cultivation of BLRs is seen as a form of *in situ* conservation in farmers' fields, it can be considered as a very successful conservation strategy, because both diversity and local adaptation seem to have been conserved well, and allow the possible co-evolution of BLRs and local pathogens. This example may illustrate the practicability and benefit of *in situ* conservation strategies. This and previous studies (Weltzien, 1989) have identified regions in Syria in which BLRs of similar population structure have evolved. If per region at least one farmer could be subsidised in order to continue to grow his local landrace, most requirements of modern conservation would be fulfilled at reasonable expense. Successful examples

of *in situ* conservation of landraces in farmers' fields have been reported by Brush (1995) for maize, potatoes and wheat.

Second, it could be shown that within BLR accessions held in *ex situ* gene banks genetic diversity has decreased significantly with increasing storage periods. The reason for this dramatic decrease seems to be associated with common practices in gene banks which require frequent rejuvenation of seed samples held in air tight containers in cold stores, to maintain viability of seeds (Clark, et al., 1997). For BLRs these rejuvenation cycles in small seed plots of about $3m^2$ were likely to occur every 5 years. It was estimated from results of two genetic markers that about 10% of diversity was lost with every rejuvenation cycle and that the number of non-related parent plants contributing seeds to the following generation was just 10, over a period of about 14 generations or about 72 years.

This result was most alarming considering that (i) most of the diversity (50% to 60%) of initial collections of BLRs lies within populations (Brown and Munday, 1982, Jana and Pietrzak, 1988) and the remainder between populations and (ii) most *ex situ* gene banks still have no, or limited deep freeze facilities and will still practice frequent rejuvenation of seed samples, (iii) conditions of conservation of related inbreeding crop species such as rice (*Oryza* spp.), wheat (*Triticum* spp.) and oats (*Avena* spp.) are comparable to those described for barley in the present study.

In conclusion, common *ex situ* conservation in gene banks may only be suitable to conserve the diversity between accessions, while the diversity within initial collection of early accessions may have been lost or is about to erode. *In situ* conservation of BLRs in farmers' field has been shown in the present study to be able to conserve locally adapted diversity. At the same time plant populations are able to co-evolve with pathogens in the field. However, e*x situ* conservation under improved conditions (optimised sampling, deep freezing, large samples) may be useful to ensure long-term conservation of germplasm. More information is required to investigate a possible loss of diversity within accessions of barley and other major cereal species, due to common *ex situ* gene bank practices.

8.7 Genetic markers

Throughout the present study results were obtained in parallel with up to three different genetic markers, namely morphological, isoenzymes and ISSR markers. The large amount of different experimental results achieved with these genetic markers for different populations of barley in conjunction with published results made it possible to assess the efficiency and suitability of individual genetic markers for different purposes.

Efficiency of different markers can be assessed using the effective multiplex ratio (EMR), i.e. the number of different genetic loci that may be simultaneously analysed per experiment. The highest EMR was found for AFLPs, a molecular fingerprint method. However, the second highest EMR out of nine different genetic marker types was found for starch gel electrophoresis (SGE) of isoenzymes, a method that was supposed doomed when PCR based molecular marker methods were proposed in the 1980s. However, the high information content as a genetic marker in population genetics and the relatively high efficiency and low costs of SGE have revived the method in recent years. If efficiency and costs are not the most important criteria, then it appears that the SSR method combines the most positive characteristics of all genetic markers, for studies in population genetics.

Different genetic markers have different characteristics such as selective neutrality or adaptiveness and can be based on polymorphisms of DNA or gene products (e.g. proteins, morphological traits). The degree of detectable polymorphism depends on the rate at which genetic marker loci evolve. An example from the present study may illustrate this. Domestication is supposed to have lead to a decrease of diversity in barley as a consequence of genetic bottlenecks, i.e. only a fraction of wild barley plants have been used to found lines for cultivation (Brown and Munday, 1982). It seems likely that only a part of the diversity present in wild barley has been carried over into the cultivated barley gene pool. However, a wide range of morphological variation has been created from this restricted gene pool (Harlan, et al., 1973). Considering morphological markers, which are known to be selectively adaptive, diversity of barley has increased as an effect of domestication, as BLRs show higher

average and total gene diversity in comparison with wild barley populations in the present study. Considering isoenzymes, average and total gene diversity was found to be lower in BLRs than in wild barley. Thus, domestication seem to have decreased the isoenzyme diversity as a consequence of genetic drift (i.e. genetic bottlenecks). Considering molecular marker loci such as ISSRs, the level of average gene diversity has not been changed by domestication. Both isoenzymes and molecular markers are considered to be neutral markers, i.e. they do not directly respond to selection. However, isoenzymes represent only a very limited number of highly polymorphic loci. If a few alleles of these loci are lost during the process of domestication, following generations will lack this allele and gene diversity of these populations will be lower compared with wild barley. It is unlikely that the allele would be regained by mutations in the cultivated gene pool. A lot of mutations would be necessary to change the amino acid sequence of the isoenzyme (only 1/3 of all base mutations are amino acid-changing mutations; Nei, 1987) and in addition, the exact amino acid sequence of the lost isoenzyme may require several amino acid changes. In contrast, single base substitutions within the DNA may result in polymorphisms detectable by molecular marker techniques. Thus, even if a number of alleles of a molecular marker loci had been lost during domestication, new mutations may directly result in new polymorphism and increase the level of detectable genetic diversity. Considering the molecular ISSR marker in the present study, no differences in average gene diversity were found between wild barley, BLRs and modern cultivars. In fact the total gene diversity of BLRs was higher than the total gene diversity in wild barley, presumably because more populations (13) of BLRs than wild barley (2) were screened, so that there was a higher chance of finding mutations in BLRs. This example may illustrate that the results of different genetic markers are relative rather than absolute (Ennos, 1996) and a direct comparison of diversity levels achieved with different markers is not possible. Even different molecular markers will produce different diversity levels, as some of them (e.g. RAPD, ISSR) may include a fraction of loci which are maternally inherited (mtDNA, cpDNA) and evolve at a different rate to nuclear DNA. It has further to be considered that different fractions of the DNA evolve at different rates (non-coding DNA/coding DNA) and so are likely to produce different degrees of polymorphism.

The choice of a genetic marker depends greatly on the purpose of the study. The plant breeder, who ultimately utilises crop genetic resources, is interested in phenotypic characters such as pest and disease resistances, tolerances to environmental factors (e.g. drought, salinity) and nutritional qualities. Studies with relevance to plant breeding should include the analysis of quantitative traits and should be designed to enable the analysis of heritability of these traits. The coefficient of genetic variation calculated from quantitative traits allows both the comparison of genetic variation among characters and populations (Ennos, 1996) and analysis of the ability of a population to respond to selection (Houle, 1991). For studies with relevance to conservation, however, it may be more important to analyse how much of the present diversity has been captured within a given set of samples. Molecular markers and in particular DNA fingerprint methods (RAPDs, ISSRs, AFLPs) may be sufficient to allow a comparison of diversities within a given set of samples but may not allow comparisons with other studies. A comprehensive study of the population structure including the analysis of the mating system and isolation by distance would require the use of neutral, codominant markers of known mode of inheritance such as SSRs or isoenzymes. The additional analysis of the variation of quantitative traits may be useful to estimate the response to selection and quantitative variation.

It can be concluded that a comparison of results achieved with different genetic markers is not possible. The individual characteristics of the genetic marker used have to be considered when results are analysed. It may be advisable to include internal standards, i.e. populations of known diversity into investigations in order to be able to compare achieved and expected results. For population genetic studies neutral, codominant genetic markers with unambiguous inheritance (e.g. SSRs, isoenzymes) are preferable to dominant markers which may include uniparental inherited loci (e.g. ISSRs, RAPDs).

8.8 Future studies

The present study was able to demonstrate a high level of genetic diversity and a low outcrossing rate of present day BLRs and illuminate the influence of environmental conditions and of human activity on their population structure. It was further possible to show a dramatic decrease of genetic diversity of BLRs depending on the period of time they were kept in *ex situ* gene banks and demonstrate the relevance of individual properties of different genetic markers. Analysis and discussion of individual results raised a number of questions which may provide the basis for further studies.

The results of the present study showed a dramatic decrease of genetic diversity of accessions of BLRs with time held in *ex situ* gene banks. It seems imperative to investigate this preliminary trend further as it may have significant relevance for a substantial number of accessions of barley held in *ex situ* collections around the world, and furthermore, for other related inbreeding crop species such as wheat, rice and oats. Two kinds of studies seem appropriate. First, similarly to the present study, to collect landraces (e.g. barley, wheat, rice) *in situ* and conduct exhaustive searches in gene banks for well documented accessions collected in the same region and held in *ex situ* gene banks for different periods of time. Diversity of these accessions can be analysed using genetic markers and changes in the obtained diversity levels can be related to the period of storage. Second, the common practice of a number of *ex situ* gene banks (in developed and developing countries) could be assessed in terms of storage facilities, sample sizes, requirements for rejuvenation cycles as well as conditions (plot sizes) and frequency of rejuvenation cycles for a number of crop species (e.g. barley, wheat, rice). The results of this evaluation could be used to design experimental conditions (sample size, plot size, frequency of rejuvenation) in which landraces of known genetic diversity are grown for several generations. Changes in the level of genetic diversity with rejuvenation cycles can be analysed using genetic markers and compared with the expected loss of diversity due to genetic drift.

The high level of genetic diversity found in present day collections of BLRs in farmers' fields may imply the value of *in situ* conservation strategies. It may be

useful to initiate a pilot project to maintain BLRs on subsidised farms in centres of known diversity. In this project the possibility of a continuous cultivation of local barley landraces on farms in distinct regions of a few countries (e.g. Syria, Ethiopia and Nepal) could be assessed, planned, organised, implemented and managed for several years. The practicability and financial requirements of a long-term on farm (i.e. *in situ*) conservation project may be compared with common *ex situ* conservation practices.

The use of long-term bulk populations (composite crosses) has been recommended as an alternative strategy for plant breeding. Locally maintained composite crosses are able to serve two purposes in plant breeding and conservation. The value of composite crosses for plant breeding is not immediately present but it can be expected that natural selection for fertility may produce long-continued yield improvements after a number of generations (Allard, et al. 1992). Initiation of composite cross breeding programmes in marginal regions with a low-input agriculture seems to be a promising alternative to pure-line breeding strategies under these conditions.

Finally it seems important to gain more data to describe spatial and temporal variation of environmental conditions in order to investigate the ecological relevance of the population structure of crop plants grown under marginal conditions and to use this knowledge for breeding strategies in low-input agriculture. GIS may be a useful tool to characterise spatial heterogeneity of environments but also to create surface maps that show long-term fluctuations, i.e. the predictability of environmental conditions within a region. Yield and long-term variation of yield of indicator crops grown under rainfed conditions may be useful indices to define agro-ecological zones.

All of the above suggestions have one issue in common, namely the temporal dimension. It seems that the temporal component is immensely important but has possibly not been given adequate attention where results of ecology and population genetics are applied to plant breeding and conservation.

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Table appendix 1 Reference list of evolutionary or population genetic studies of barley populations

Genetic marker/ Reference	Type of population	Country of origin	Observed level of polymorphism
Seed morphology:			
Jain and Allard, 1960	CVs (CC)	World	large
Brown and Mundy, 1982	BLRs, (H.s.,CVs)	Iran	large
Konishi, et al., 1993	BLRs	Bhutan	medium
Plant morphology, QTL (i.e. agronomic traits, disease resistance):			
Ceccarelli, et al., 1986	BLRs	Syria/Jordan	large
Damania, et al., 1985	BLRs	Nepal, Yemen	medium
Weltzien, 1988; 1989	BLRs	Syria/Jordan	large
Hadjichristodoulou, 1995	BLRs, (H.s.)	Cyprus and others	
Abay and Cahalan, 1995	BLRs	Tigray (N-Ethiopia)	large
Murphy and Witcombe, 1981	BLRs	Himalaya (gene bank)	large
van Leur, et al., 1989	BLRs	Syria, Jordan	large
Jana and Bailey, 1995	BLRs, (H.s.)	Turkey, Jordan	large
Lakew, et al., 1997	BLRs	Ethiopia	large
Kim, et al., 1991	BLRs	Korea	large
SGE of isoenzymes:			
Kahler and Allard, 1970	CVs (CC)	World	large
Allard, et al., 1972	CVs (CC)	World	large
Kahler, et al., 1981	General		large
Clegg, et al., 1972	CVs (CC)	World	
Nevo, 1992	H.s. (BLRs)	Israel, Turkey, Iran	medium
Nevo, et al. 1997	H.s.	Israel	medium
Jana and Pietrzak, 1988	BLRs, H.s.	Turkey, Crete, Jordan	large
Brown, 1978, 1983	General	General	large
Brown and Mundy 1982	BLRs, (H.s.,CVs)	Iran	large
Konishi and Matsuura, 1991	BLRs	Himalaya	large
Konishi, et al., 1993	BLRs	Bhutan	large
PAGE of storage proteins:			
Shewry, 1993	CVs		very large
Doll and Brown, 1979	CVs, (H.s.,BLRs)	World	very large
Ohms and Paradies, 1985	CVs		
Nevo, 1992	H.s. (BLRs)	Israel, Turkey, Iran	very large
Asfaw, 1989	BLRs	Ethiopia	very large

Table appendix 1 Reference list of evolutionary or population genetic studies of barley populations (continued)

Genetic marker/ Reference	Type of population	Country of origin	Observed level of polymorphism
RFLP of rDNA:			
Saghai-Maroof, et al., 1984	H.s., BLRs, CVs		
Saghai-Maroof, et al., 1990	H.s.	Israel, Iran	substantial
Ramamoorthy, et al.,1994	BLRs, (H.s.)	Jordan, Turkey, Crete	large
PCR of rDNA:			
Baum and Johnson, 1994	BLRs, CVs	gene bank	low
RFLP of cpDNA:			
Clegg, et al., 1984	H.s., BLRs, CVs	Israel, Iran	moderate
Neale, et al., 1986	H.s.	Israel, Iran	low
Holwerda, et al., 1986	H.s., BLRs	Near East	little
RFLP of mtDNA:			
Holwerda, et al., 1986	H.s., BLRs	Near East	little
RFLP of nuclear DNA:			
Zhang, et al.,1993	H.s., BLRs	Israel, Iran	large
RAPD:			
Gonzalez, Ferrer, 1993	H. species		very large
Tinker, et al., 1993	H.v., CVs, lines		very large
PCR, using specific primer:			
Ko, et al., 1996	CVs		medium
CAPS of nuclear DNA:			
Tragoonrung, et al., 1992	CVs		relatively low
Lashermers, et al. , 1993	H.s., BLRs, CVs	Syria, Israel	relatively low
Kanazin, et al., 1993	CVs		
SSR:			
Donini, et al., 1998	CVs		relatively low
Becker and Heun, 1995	CVs		medium
Saghai-Maroof, et al., 1994	H.s./H.v.	Israel/world	very large
AFLP:			
Ellis, et al., 1997	CVs	UK	very large

Abreviations used:
AFLP = Amplified Fragment Length Polymorphism;
CAPS = Cleavable Amplified Polymorphic Sequences;
EMR = Effective Multiplex Ratio
PAGE = Polyacrylamid Electrophoresis;
PCR = Polymerase Chain Reaction;
RAPD = Random Amplified Polymorphic DNA;
RFLP = Restriction Fragment Length polymorphism;
SDS-PAGE = Polyacrylamid Electrophoresis using sodium dedocylsulfate (SDS)
SGE = Starch Gel Electrophoresis;
SSR = Single Sequence Repeats;
H.s. = Hordeum vulgare ssp. spontaneum
H.v. = Hordeum vulgare ssp. vulgare, cultivated barley
BLRs = Barley Landrces
CVs = Cultivars, varieties
CC = Composite Cross

Table Appendix 2 **Sources of climatic data**

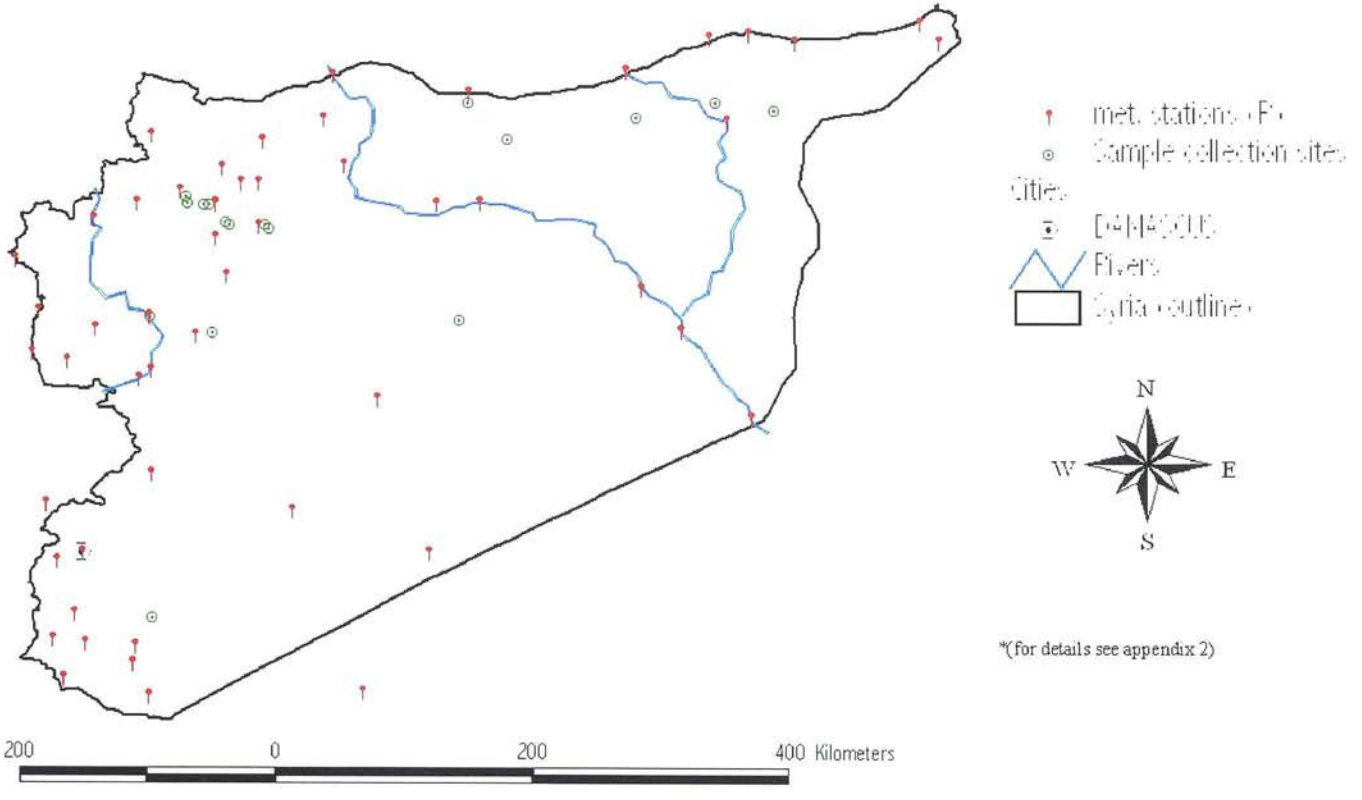
Source:	Station name:	lon (E)	lat (N)	Altitude (m)	P (mm)	Cv of P	Avg. Temp	Min. Temp.	Max. Temp.	Cont. (%)
FAO/SMA	DAMASCUS	36.23	33.48	729	193.0	0.389	17.7	2.6	36.1	43.04
FAO/SMA	PALMYRA	38.30	34.55	404	122.1	0.384	18.8	3.5	38.2	43.69
FAO/SMA	DEIR-EZZOR	40.15	35.32	212	140.1	0.401	19.9	3.6	40.2	46.26
FAO/SMA	LATTAKIA	35.77	35.53	7	781.9	0.217	20.1	8.7	30.5	12.72
FAO/SMA	HAMA	36.71	35.13	309	396.6	0.248	18.1	3.5	37.1	39.70
FAO/SMA	ALEPPO	37.22	36.18	393	329.6	0.200	17.4	2.2	36.5	39.36
FAO/SMA	AL BAB	37.50	36.37	470	271.5	0.202	17.7			
FAO/SMA	KAMISHLI	41.22	37.05	455	402.8	0.238	18.9	3.0	40.2	44.00
FAO/SMA	TEL ABIAD	38.95	36.70	349	262.9	0.219	17.7	1.3	39.3	46.91
FAO/SMA	HASSAKEH	40.75	36.50	296	261.0	0.206	18.3	1.5	40.4	49.12
FAO/SMA	RAQQA	39.02	35.93	246	186.2	0.398	18.6	2.4	38.9	45.29
ICARDA	Tel Hadya	36.93	36.02	284	347.3	0.216	17.6	1.9	37.0	41.31
ICARDA	Breda	37.17	35.93	300	258.4	0.371	17.1	1.4	37.1	43.50
ICARDA	Jindiress	36.73	36.40	210	469.8	0.305	17.2	2.3	34.6	34.73
ICARDA	Kfardane	36.05	34.02	1080						
ICARDA	Ghrerife	37.25	35.42	320	263.6	0.382	17.7	1.7	36.6	41.83
ICARDA	Boueidar	37.17	35.68	268	247.0	0.357	16.7	1.5	37.4	44.25
ICARDA	Terbol	35.98	33.82	890	567.5	0.276	14.0	-1.1	33.0	44.04
ICARDA	Maragha	37.67	35.55	370						
FAO/SMA	Al Mayadeen	40.43	35.03	197	155.4	0.515				
FAO/SMA	Al Boukamal	40.92	34.42	182	134.6	0.484	20.2	2.3	40.5	51.94
FAO/SMA	Salkhad	36.70	32.48	1447	364.7	0.467	14.4	-1.8	29.2	39.14
FAO/SMA	Al Nabek	36.72	34.03	1333	103.8	0.292	12.7	-1.1	30.6	37.36
FAO/SMA	Homs	36.72	34.75	451	412.5	0.221	16.9	2.3	32.8	33.79
FAO/SMA	Idleb	36.62	35.93	451	519.7	0.183	17.5	3.6	33.6	30.76
FAO/SMA	Tartous	35.88	34.88	5	855	0.187	19.4	8.7	30.9	14.61
FAO/SMA	Sfeereh	37.35	36.07	340	245.1	0.194				
FAO/SMA	Manbej	37.93	36.52	460	243.3	0.232				
FAO/SMA	Izra	36.25	32.85	575	315.1	0.341	17.2	2.9	33.5	37.72
FAO/SMA	Sweida	36.58	32.70	997	339	0.288	15.9	3.1	31.2	31.84
FAO/SMA	Shaba	36.60	32.83	1250	383.9	0.367				

Table Appendix 2 continued

Source:	Station name:	lon (E)	lat (N)	Altitude (m)	P (mm)	Cv of P	Avg. Temp	Min. Temp.	Max. Temp.	Cont. (%)
FAO/SMA	Dara	36.10	32.60	543	232.3	0.335				
FAO/SMA	Al-Salamiyeh	37.03	35.00	480	282.9	0.21	17.4	1.2	35.6	41.67
FAO/SMA	Missiaf	36.33	35.05	530	1220.8	0.376				
FAO/SMA	Derbassieh	40.63	37.08	430	339.8	0.317	17.4	0.9	39.4	46.77
FAO/SMA	Ras El-Eyn	40.05	36.85		269.6	0.369				
FAO/SMA	Banias	35.93	35.17	30	717.7	0.356				
FAO/SMA	Safita	36.13	34.82	359	1105.2	0.227	18.2	6.1	30.1	18.80
FAO/SMA	Al Sanameine	36.18	33.05	750	286.7	0.391	16.8			
FAO/SMA	Nawa	36.02	32.87	563	448.3	0.357				
FAO/SMA	Quateneh lake	36.63	34.70	500	423.2	0.141				
FAO/SMA	Jarablus	38.00	36.82	351	281.4	0.192	17.7	1.7	38.7	44.58
FAO/SMA	Katana	36.05	33.42	875	248.7	0.373				
FAO/SMA	Jisir	36.32	35.82	200	744.2	0.185	18.4	4.7	34	29.31
FAO/SMA	Al Malkiyeh	42.1	37.18		520.5	0.201				
FAO/SMA	Amouda	40.9	37.11		391.5	0.252				
FAO	Jabal-Ettauf	38.66	33.48	708	107					
FAO	Irwaished	38.20	32.50	608	84			2.1	37.4	49.58
FAO	Bailaneh	38.72	35.92	268	209			0.8	38	46.88
FAO	El Khafseh	38.07	36.20	337	231			2	38	43.09
FAO	Bar Dah	37.17	35.92	300	272					
FAO	Jabboul	37.48	36.07	330	292					
FAO	Sferah	37.35	36.07	340	287					
FAO	Khanasser	37.48	35.77	350	227					
FAO	Quarachock	42.23	37.05	475	507			2.7	39.9	44.00
FAO	Sabe Abar	37.70	33.78	825	97			2.1	34.3	39.60

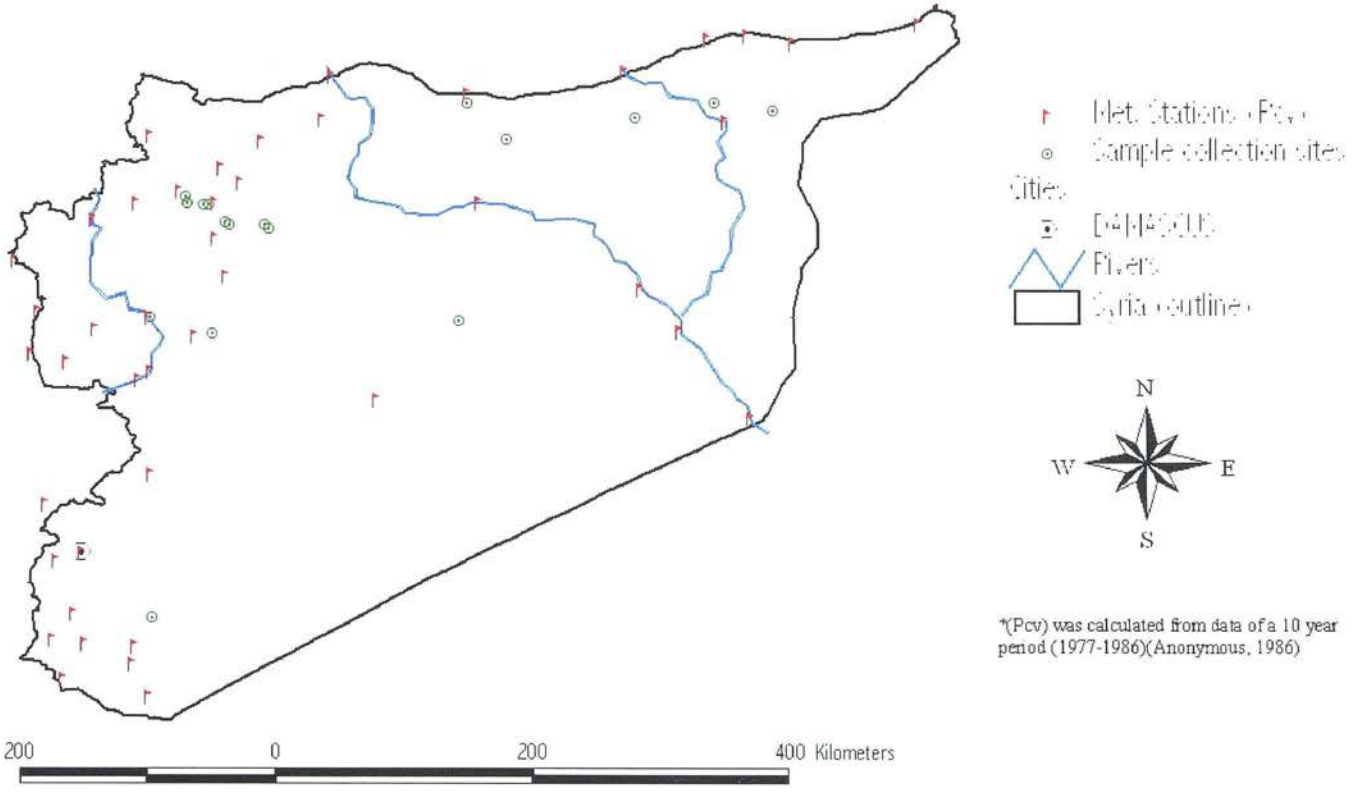
FAO (FAO, 1994)
SMA = Syrian Ministry of Agriculture (Anonymous, 1986)
ICARDA (p.c. 1999; Ryan 1997)
P = Average annual precipitation
Cont. = Continentality

55 meteorological stations in Syria,
for which average annual precipitation (P) data were available *

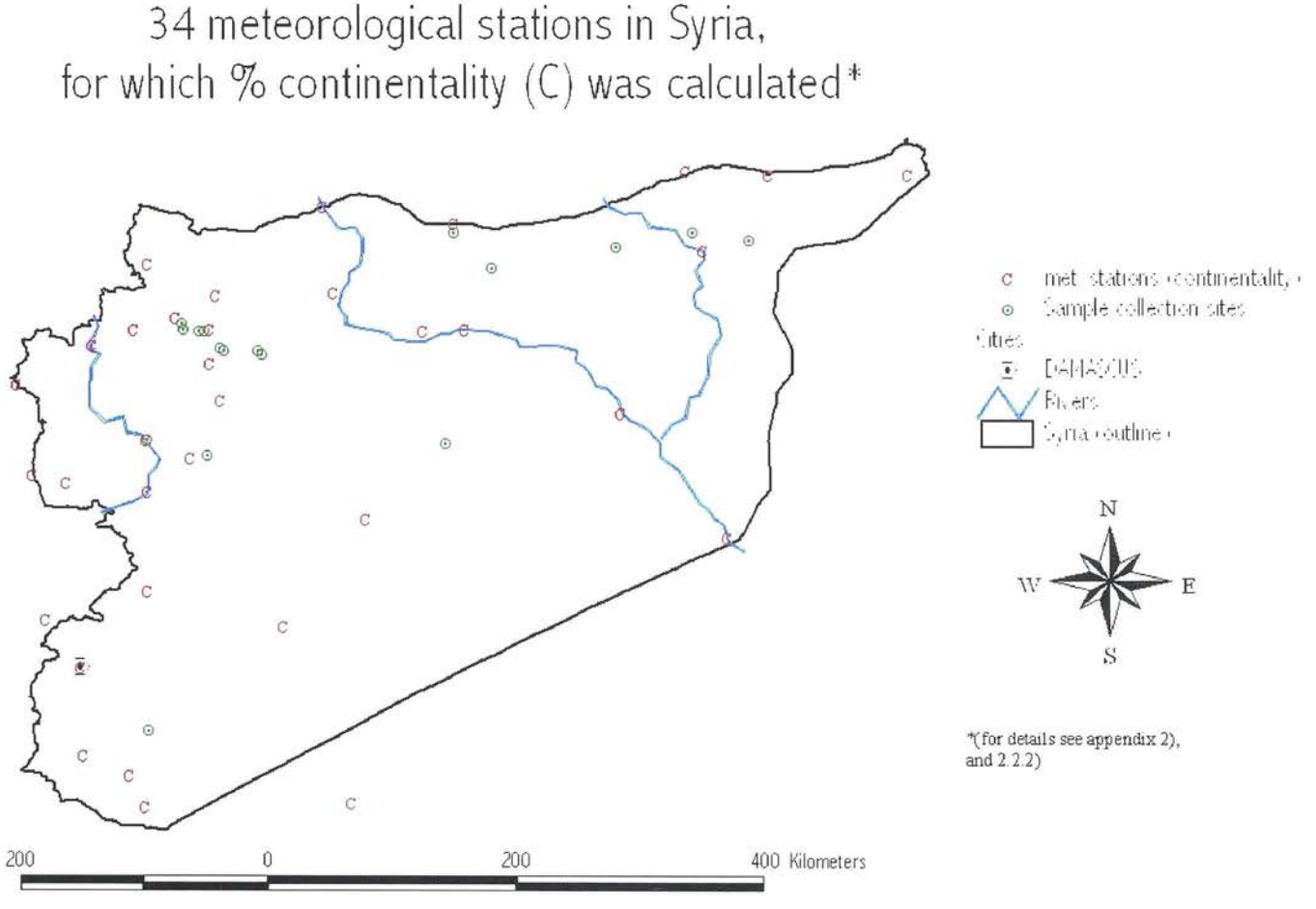


*(for details see appendix 2)

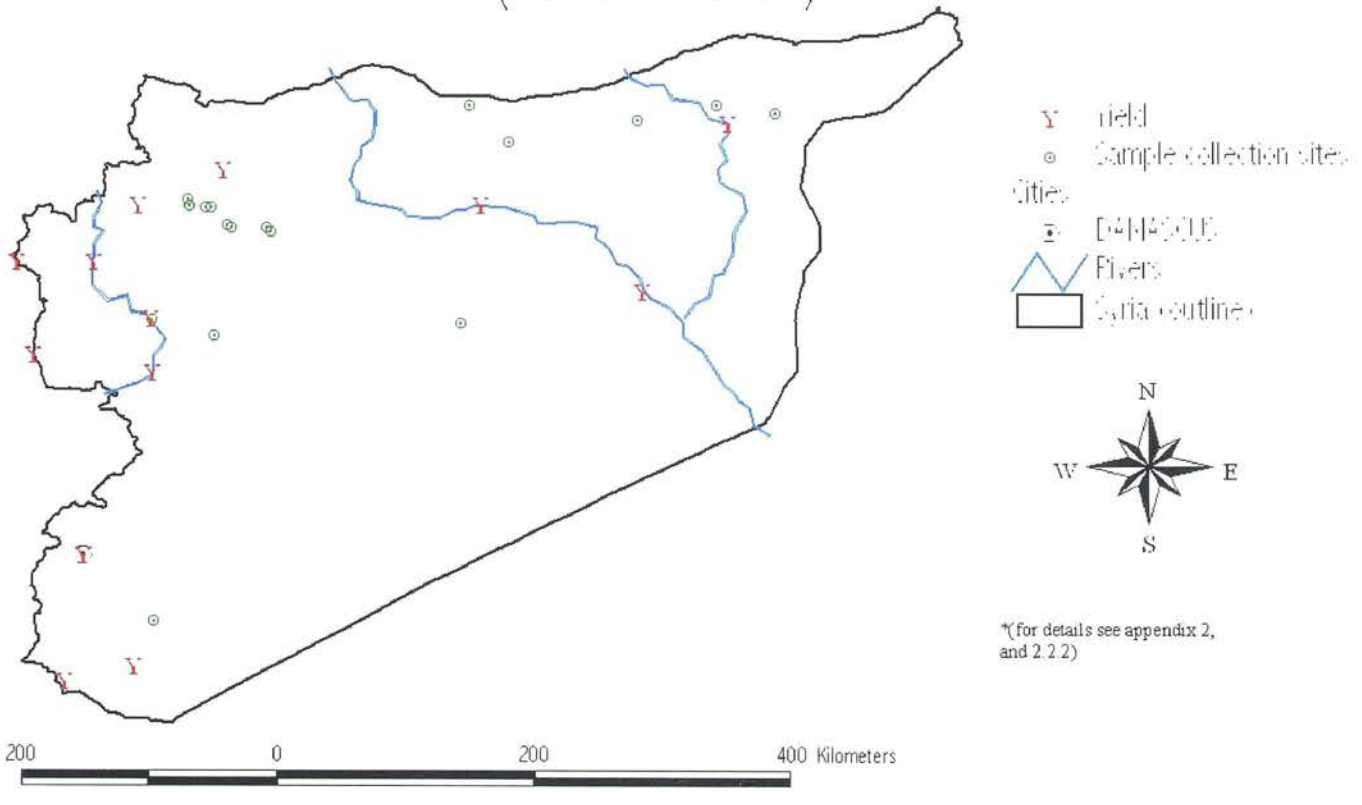
44 meteorological stations in Syria, for which interannual variation in rainfall (Pcv) was calculated*



*(Pcv) was calculated from data of a 10 year period (1977-1986)(Anonymous, 1986)


34 meteorological stations in Syria,
for which % continentality (C) was calculated*
met. stations (continentality)
Sample collection sites
Cities
DAMASCUS
Rivers
Syria (outline)
N
W
E
S
200
0
200
400 Kilometers
*(for details see appendix 2), and 2.2.2)

13 locations in Syria,
for which average barley yield under rainfed conditions was available
(for 1977-1986) *



*(for details see appendix 2, and 2.2.2)

Protocol I
Starch Gel Electrophoresis of Esterases in Barley

Esterases (EST, E.C.3.1.1.1)

1. Extraction of Enzymes

1.1 Cut barley plumules 4 to 10 days after germination and grind a 5 cm segment in 40µl of cold DTT solution (6mM) with the help of a 1000µl pipette tip;

1.2 Absorb plant extracts in filter paper wicks (5mm x 20mm ,Whatman no. 3) and place single wicks on the inner wall of an Eppendorf tube. Keep samples cold (on ice) at all times and freeze them until use (defrosted samples loose their enzyme activity quickly!). Take out a single wick for use and keep the remaining wicks frozen.

2. Preparation of Gels

2.1 Prepare a 11% solution of hydrolysed potato starch using gel buffer solution (6.1). For a gel of about 15 cm x 18 cm use 250ml.

2.2 Homogenise starch and buffer carefully in a 0.5l Buchner flask and heat with continuous swirling on a magnetic stirrer hotplate or a bunsen burner. Stop heating when solution starts to gelatinze (before solution starts boiling);

2.3 Deaerate the solution with a tap aspirator;

2.4 Pour a 0.6cm thick gel in a plexiglas frame;

2.5 Allow the gel to cool over night at room temperature or for 1 hour at 4°.

3. Electrophoresis

3.1 Place the gel on in a horizontal electrophoresis unit on a cooled ceramic plate (2° C) (e.g. LKB-Multiphor II);

3.2 Prepare a slit app. 5cm from the cathodal end, place the frozen wicks in the slit of the gel, leave sufficient space between each of them;

3.3 Add one wick absorbed in 0.2% bromophenol blue solution as a tracking dye, remove excess extract with absorbent paper,

3.4 Push gel parts tightly together;

3.5 Fill electrode buffer (6.2) into buffer tanks and place drenched paper towels on the ends of the gel, so that they build a bridge between gel and electrode buffer tank.

4. Running conditions

Run gel at a current of 55mA (power pack: 2197, LKB) for approximately 5 hours, remove filter paper wicks after 30 minutes to avoid further flow of isoenzymes from filter paper wick into gel.

5. Staining of Esterases (EST)

5.1 Remove gel from electrophoresis unit and incubate gel for 1 h. 30 min. at 35° C in the dark in a solution of:

- 50ml of a 0.2M Phosphate buffer, pH 6.4 with 1M NaH_2PO_4,

- α-Naphtyl acetate 100mg
- β-Naphtyl acetate 50mg
  (dissolve both in 1ml of acetone),

- 100mg Fast Blue RR salt.

5.2 Shake the solution until the Fast Blue RR salt is dissolved.

5.3 If the gel is thick enough (>5mm) it is possible to slice it into 2 or 3 slices and stain each slice for a different isoenzyme system.

5.4 After staining remove staining solution and pour 100ml fixation buffer (6.3) over the gels. Keep gels over night in fixation buffer. To preserve gels, put them with the help of a plastic scoop into a plastic bag and seal the bag.

6. Stock solutions

6.1 Gel buffer: 0.05M Histidine Hcl, 1.4mM EDTA,
pH 7.0 with 1M Tris,
dilute stock 4:1 before use

6.2 Electrode buffer: 0.125M Tris,
pH 7 with 1M Citric acid (anhydrous)

6.3 Fixation buffer: 400ml Methanol
100ml Acetic acid
1000ml H_2O dist

7. Chemicals

Chemical:	Source:
α-Naphtyl acetate	Sigma, N-8505
β-Naphtyl acetate	Sigma, N-6875
Acetic acid, glacial	Sigma, A-6283
Acetone	Sigma, A-4206
Bromophenol Blue	Sigma, B-6131
Citric acid (anhydrous)	Sigma, C-0759
DTT (DL-Dithiothreitol)	Sigma, D-0632
EDTA (Ethylen diaminetetraacetic acid)	Amresco, 0105
Fast Blue RR salt	Sigma, F-0500
L Histidine HCl,	Sigma, H-8125
Methanol	Sigma, M-3641
Sodium Phosphate (NaH_2PO_4) monobasic, anhydrous	Sigma, S-0751
Phosphate buffer (pH 6.6)	Sigma, P-8165
Starch, potato, hydrolysed	Sigma, S-4501
TRIZMA Base ((tris[hidroxymethyl]amino methane)	Sigma, T-1503

(method modified after methods described by Cheliak and Pitel (1984), Brown (1983); Kahler and Allard (1970); Kahler, Heath-Peglusio and Allard (1981); Glaszmann, De los Reyes and Kush (1988))

Protocol II
PCR analysis of barley using 5' anchored SSR primer

1. DNA Extraction of barley leaves

1.01 Grind approximately 0.15g of fresh leaf material in liquid nitrogen using pestle and mortar (autoclaved!), use youngest leaves, grind thoroughly to destroy cell walls;

1.02 Add 1 ml of DNA-extraction buffer, mix well (if frozen, place on hotplate at 65° C for a few minutes to defrost) and pour into 2ml Eppendorf test tube;

DNA-Extraction Buffer:
2% CTAB (Hexadecyltrimethylammoniumbromide)
100mM TRIZMA Base
1.4M Sodium Chloride
20mM EDTA
3% PVP-40 (Polyvinylpyrolidone)
Adjust to pH 8.0 with 1M HCl
Autoclave
Add 0.1% DTT (DL-Dithiothreitol)

1.03 Incubate samples for 30min. On a hotplate at 65° C;

1.04 Add 750µl of Chloroform/IAA (24:1) and mix well;

1.05 Centrifuge for 5min in lab centrifuge (10min at 10000rpm);

1.06 Transfer supernatant (aqueous layer) into clean Eppendorf test tube;

1.07 Add 500µl of ice cold Isopropanol, mix and stand at room temperature for 15 minutes. If there is no precipitation, incubate at 0°-5°C for 30min;

1.08 Pellet DNA by centrifugation for 10min (10000rpm), discard supernatant and wash with 500µl wash buffer;

1.09 Dry pellets in a laminar flow cabinet by inverting the Eppendorf tubes on sterile tissue over night;

1.10 Resuspend DNA-pellet in 200µl of 1x TE-buffer and mix gently 50 times;

1.11 add 1µl of RNase to the DNA solution and incubate for 15min. at 65° C or 1 hour at 37°C;

1.12 Store samples in fridge until dilution and measurement of concentration.

2. Preparation of Agerose gels

2.01 Dissolve Agerose in 1x TBE buffer (e.g. 1.4g in 100ml of TBE buffer for a 1.4% gel);

2.02 Microwave until bubbles appear, mix well until all agerose is dissolved;

2.03 Allow solution to cool down to 60°C, pour into gel casting plates and apply a comb for wells;

2.04 Leave to set for 30 minutes before use.

3. Running gels to check and quantify DNA

3.01 Prepare a 1.4% gel;

3.02 Submerge gel in 0.5x TBE buffer in electrophoresis unit (e.g. Flowgen, Biorad), so that it is covered 3mm with buffer solution;

3.03 Apply a mixture of 2µl DNA sample and 2µl loading buffer to the wells;

3.04 Mix 2µl of a low DNA mass ladder (GibcoBRL, 10068-013) with 2µl of loading buffer and apply to the middle well of the gel, apply 2µl distilled water mixed with 2µl loading buffer to another well as a control;

3.05 Run gel at 5 to 8 Volt per cm gel length for about 1h (power pack: BioRad 200/2.0).

3.06 Stain gel in a 1% ethidium bromide solution for 30 minutes.

4. Quantification of DNA

4.01 Scan gel under UV light using a video capture system (Flowgen, IS1000);

4.02 Analyse band intensity with the densitometer of the image analysis system (Flowgen, IS500, ID-multi);

4.03 Draw a calibration curve by plotting the peak area of bands with known DNA concentration of the low DNA mass ladder (GibcoBRL, 10068-013) against the analogous DNA concentration;

Table, appendix 5.1 DNA mass ladder (GibcoBRL, 10068-013)

Fragment size	Amount of DNA in each band per 1µl
2000 bp	50 ng
1200 bp	30 ng
800 bp	20 ng
400 bp	10 ng
200 bp	5 ng
100 bp	2.5 ng

4.04 Calculate the peak area of samples and read the corresponding DNA concentration of samples from the graph;

4.05 To confirm results use a spectrophotometer. Determine the DNA concentration of the DNA extract at 260nm wavelength (a reading of 1 equals 50µg/ml DNA);

4.06 Check purity of DNA extract: a ratio of the 260/280nm reading of 1.8 to 2.0 means high purity, values , 1.8 could imply presence of polysaccharides;

4.07 Dilute samples to obtain DNA templates with 10ng DNA per µl.

5. PCR amplification of DNA templates

5.01 prepare 25 µl reaction mix in DNase free 200µl flat-cap micro tubes, work in a laminar flow cabinet:

Table, appendix 5.2 PCR reaction mix for inter-SSR primer 808 and 857

Component	Amount for 1 sample	Final concentration
Sterilised distilled water (SDW)	9.87µl	
10x PCR buffer	2.5µl	1x
dNTP-mix (0.2mM, for each of dATP, dCTP, dGTP and dTTP)	4 µl	32µM of each of the 4 dNTPs
Amplitaq® (10 U/µl)	0.13µl	0.05U
$MgCl_2$ (25mM)	2.5µl	2.5mM
primer solution, 5 µM	3µl	0.6µM
DNA template (10 ng DNA/µl)	1.5µl	0.6ng/µl
Total	25µl	

5.02 Place samples immediately in a thermocycler (Biometra TrioThermoblock with heated lid) and use the following conditions for the PCR:

Table appendix 5.3 PCR program for amplification using Inter-SSR primer

	Condition	No. of cycles
Hot start	94°C for 1.5 min	
Denaturation of DNA Annealing of primers Extension	94°C for 1.0 min 50°C for 2.0 min 72°C for 1.0 min	repeated for 30 cycles
Final extension	72°C for 5.0 min	

5.03 Soon after amplification store samples at 4°C.

6. Running gels to visualise PCR amplification products

6.01 Prepare a 2.0% gel using 1x TBE buffer (see2);

6.02 Submerge gel in 0.5x TBE buffer in electrophoresis unit (e.g. Flowgen, Biorad), so that it is covered 3mm with buffer solution;

6.03 Apply a mixture of 8µl PCR sample and 4µl loading buffer to the wells;

6.04 Mix 4µl of a 100bp DNA ladder (GibcoBRL, 15628-019) with 2µl of loading buffer and apply to the middle well of the gel, apply 8µl control (amplified reaction mix without template) mixed with 2µl loading buffer to another well as a control;

6.05 Run gel at 5 to 8 Volt per cm gel length for about 2h (power pack: BioRad 200/2.0).

6.06 Stain gel in a 1% ethidium bromide solution for 30 minutes;

6.07 Scan gel under UV light using a video capture system (Flowgen, IS1000);

6.08 Analyse band intensity with the image analysis system (Flowgen, IS500), use the tool 'molecular weight' (linear) but insert bp for the standard at the position of bands of the 100 bp DNA ladder and calculate the fragment size (query) in bp of the amplification products (bands);

6.09 Note results in a table where 1 is presence and 0 absence of band, uncertain bands are marked as missing.

7. Chemicals

Chemical:	Source:
Agarose	Amresco, 0710
Amplitaq® DNA Polymerase (10U/µl)	PE, F0717
Bromophenol Blue	Sigma, B-6131
CTAB (Hexadecyltrimethylammonium bromide)	Sigma, H-6269
dNTP-mix (5mM, for each of dATP, dCTP, dGTP and dTTP)	Bio Gene Ltd., 300-113
DNA mass ladder (low)	GibcoBRL, 10068-013
DNA ladder (100bp)	GibcoBRL, 15628-019
DTT (DL-Dithiothreitol)	Sigma, D-0632
EDTA (Ethylen diaminetetraacetic acid)	Amresco, 0105
Ethanol	Amresco, E 193
Ethidium Bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide)	Sigma, E-8751
HCl (1M)	Sigma, H-7020
Chloroform/Isoamyl alcohol (IAA) 24:1	Sigma, C-0549
Isopropanol	Sigma, 405-7
Magnesium Chloride ($MgCl_2$) (25mM)	PE, H0994
PCR buffer (10x), Stoffel buffer	PE, H1240
Primer solution, 5µM	GibcoBRC (custom made)
PVP-40 (Polyvinylpyrolidone)	Sigma, PVP-40
Rnase, DNase-free, from bovine pancreas	Boehringer, 1119 915
Sodium Chloride (NaCl)	Sigma, S-3014
Sucrose	Sigma, S-7903
TBE-Buffer (Tris-borate-EDTA buffer) (5x)	Sigma, T-4415
TE-Buffer (Tris-EDTA buffer) (100x)	Sigma, T-9285
TRIZMA Base (tris[hidroxymethyl]amino methane)	Sigma, T-8524

(method modified after methods described by Doyle and Doyle (1987); Khan (1997) and Zietkiewicz, Rafalski and Labuda (1993))

Assessment of SDS-PAGE of storage proteins (hordeins) to characterise barley populations (Preliminary study)

1. Introduction

A preliminary study was carried out to investigate the suitability of SDS-PAGE of barley storage proteins (hordeins) to characterise barley populations using seeds as basic material. A set of individual plants of 10 cultivars (Krona, Scarlett, Camarque, Trumpf, Renata, Polygenea, Igri, Alexis, Golf, Cameo), 10 barley landraces (Arabi Abiad 3, Arabi Aswad 3, Afghanistan 8, China 29, Ethiopia 44, Iran 62, Pakistan 07, Libya 86, Tunisia 110, Turkey 123) and 10 individuals of *H. spontaneum* from two accessions in Syria (H. spon 1 and H. spon. 2) were used to evaluate the method. Cultivars originated from SAC collections or Saatzucht Hadmersleben, Germany. All landraces originated from ICARDA, Aleppo and *H. spontaneum* from own collections in Syria in 1997. The 10 *H. spontaneum* plants were 4 and 6 plants from accession H. spon 1 and 2, respectively, and thus represent the diversity within rather than between accessions.

2. Protocol SDS-PAGE of storage proteins (hordeins) (Protocol III)

2.1. Extraction of Hordeins

2.1.1 Crush one quarter of a grain without embryo with a pair of pliers, then place in a 1.5ml Eppendorf test tube with lid;

2.1.2 Incubate over night in 0.375ml extraction buffer (15g Urea, 16ml 2-Chlorethanol, 1ml ß-Mercaptoethanol, 2g SDS, make up to 100ml with distilled water and add a few crystals of Bromophenol Blue as a marker);

2.1.3 Centrifuge for 10 minutes at 10,000 rpm;

2.2. Electrophoresis

2.2.1 Place precast gels (Pharamacia ExcelGel gradient 8-18 or homogenous 12.5%) on a LKB-Multiphor II (or similar) horizontal electrophoresis unit, and use precast buffer-strips for SDS-electrophoresis (Pharamacia), separation direction:'-' to '+';

2.2.2 Fill wells of the gel or of rubber strips with 10µl of the supernatant from the Eppendorf test tubes, fill one well with distilled water as a control and another well with with a molecular weight marker (Pharmacia, LMW Marker kit, 14.4 to 94 kDa);

2.2.3 Running conditions:
Electrophoresis Power supply: Pharamacia EPS 3500XL or similar,
Gel prefocussed at 600V, 30mA and 30 Watt for 20 minutes,
Separation at 600V, 50mA and 30 Watt for about 1 hours, until the Bromophenol Blue marker has approached
the electrode buffer strip (about 1cm).;

2.3 Staining of Hordeins

2.3.1 Remove gel from the unit, mark it (cuts) and fix it for about 30 minutes (fixation solution: 400ml Ethanol, 100ml Acetic Acid, make up to 1000ml with distilled water);

2.3.2 Stain proteins in staining solution for 10 minutes and up to 1 day, then wash gel twice in distilled water; (staining solution: dissolve 1 tablet PhastGel Blue R per 400ml destaining solution (see next step), heat to 60°C, filter through filter paper (Whatman No. 1) and cool down to room temperature)

2.3.3 Destain background of gels until background is clear (destaining solution: (250ml Ethanol, 80ml Acetic Acid, make up to 1000ml with distilled water);

2.3.4 Preserve gels for several weeks in preserving solution (i.e. 25ml of Glycerol (87% w/v), make up to 250ml with destaining solution), or cover the gel with a layer of cellophane that has been imbibed in preserving solution and dry at room temperature on a glass plate or using a suitable gel drier.

Table appendix 6.1 Chemicals

Chemical:	Source:
2-Chlorethanol	Fisons, C/4880/PB08
Acetic acid, glacial	Sigma, A-6283
Bromophenol Blue	Sigma, B-8026
Ethanol	local supplier
Glycerol (87% w/v)	Sigma, G-8773
ß-Mercaptoethanol,	Sigma, M-7154
LMW Molecular marker kit, bands: 14.4, 20.1, 30, 43, 67 and 94kDa)	Pharmacia 17-0446-01
PhastGel Blue R	Pharmacia 17-0518-01
precast gel, Pharamacia ExcelGel gradient 8-18	Pharmacia 80-1255-53
precast gel, Pharamacia homogenous, 12.5%	Pharmacia 80-1261-01
SDS (Sodium dedocylsulfate)	Sigma, L-3771
Urea	Sigma, U-6504

3. Evaluation of Hordeins

The use of a video-capture and image-analysis-system to evaluate the molecular weight of present bands is recommended, but comparison with a molecular-weight-marker on the gel or a reference cultivar with known banding pattern enables the determination of bands macroscopically. According to Kreis & Shewry (1992) there are four groups of polypeptides in the hordeins of which the B- and γ-hordeins are not clearly distinguishable using SDS-PAGE. Ohms (p.c. 1989) divided the C-hordeins further into high molecular weight C-hordeins (C-hmw) and those with low molecular weight (C-lmw) and treated them as single loci. However, banding pattern within the below given molecular weight ranges can be evaluated as loci/allele structures. Table appendix 6.2 shows the hordein loci that are distinguishable using this protocol.

Table appendix 6.2 Different hordein groups in barley

Hordein	Chromosome (locus)	Molecular weight (in kDa)**
D*	5 (Hor3)	105 to 82
C-hmw or F**	5 (Hor1)**	71 to 59
C-lmw**	5 (Hor1)**	56 to 48
B* and γ 1,2,3	5 (Hor2) and (Hor5)	47 to 30

(according to *Kreis & Shewry, 1992, and **Ohms, p.c. 1989)

Figure appendix 6.1 Frequent bands of different hordeins in barley, SDS Electrophoresis

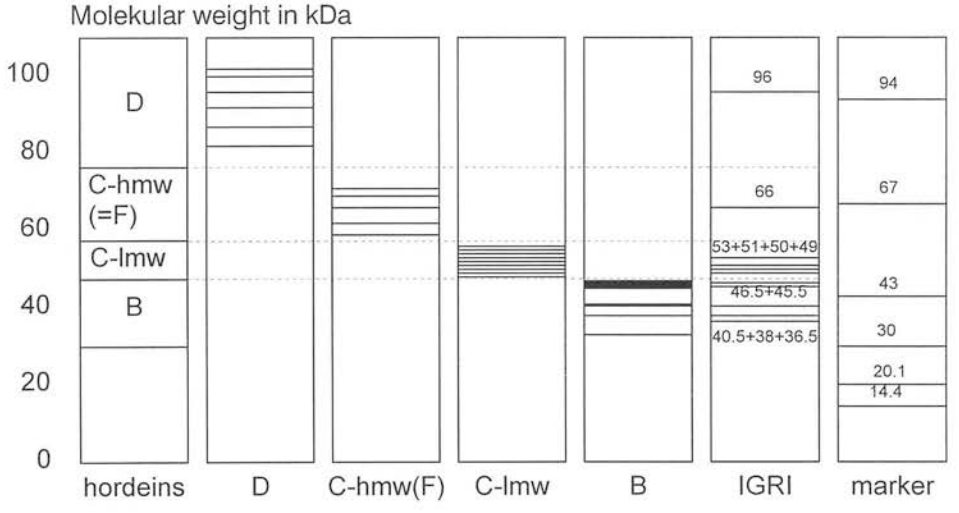


Figure Appendix 6.1 illustrates the different hordein groups, that are listed in Table appendix 6.2. Frequent hordein bands are shown for each group. All hordein bands are given for the cultivar IGRI, which is used as reference cultivar.

(Method modified after Ohms and Paradies, 1985)

4. Results and discussion

Three loci (B, C, and D-hordeins) were evaluated over all barley populations.

Table appendix 6.3 Diversity indices achieved over all barley populations

	Maximal number of bands observed per allele	Gene diversity (H)	Number of alleles per locus (A)	Proportion of polymorphic loci (P (0.01))
D-hordeins	2	0.6977	5	0.9
C-hordeins	6	0.9600	25	0.9
B-hordeins	6	0.9600	27	0.2
Average		0.8725	19	0.7

Table appendix 6.4 Diversity indices achieved for individual barley populations

	Average gene diversity (H)	Observed number of alleles per locus (A)	Proportion of polymorphic loci (P 0.01)
10 cultivars	0.5930	6.7	0.7
10 BLRs	0.7530	7.6	1.0
10 H.s.	0.5733	5.7	0.7
Average	0.6397	6.7	0.8

The diversity indices in table 6.3 are very high and signify the high complexity of alleles with up to 6 bands. Due to the complexity of banding pattern it is almost impossible to score the marker as co-dominant marker and thus diminish the information content of the method.
Cultivars and *H. spontaneum* showed a lower diversity than BLRs, though not significantly different. The lower diversity of H. spontaneum might be explained with the fact that the 10 plants scored came from only two different accessions.

Part 1 Statistical analysis of genetic indices given in table 5.3.4. ANOVA was carried out for predominantly normally distributed genetic indices H, S and M; the non-parametric Mood-Median test was carried out for non-normally distributed indices A and P (0.01). Tested level were: BLR Arabi Aswad (1), Arabi Abiad (2) and *H. spontaneum* populations (3).

One-Way Analysis of Variance

```
Analysis of Variance for H mo
Source     DF        SS        MS        F        P
BLR ssp     2   0.00866   0.00433     1.22    0.324
Error      14   0.04957   0.00354
Total      16   0.05823

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev ---+---------+---------+---------+---
1          12   0.23983   0.05577                    (-----*-----)
2           3   0.18043   0.07983   (-----------*-----------)
3           2   0.21765   0.05112      (--------------*--------------)
                                  ---+---------+---------+---------+---
Pooled StDev =  0.05950            0.120     0.180     0.240     0.300
```

One-Way Analysis of Variance

```
Analysis of Variance for S mo
Source     DF        SS        MS        F        P
BLR ssp     2   0.03312   0.01656     1.77    0.206
Error      14   0.13100   0.00936
Total      16   0.16412

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev --+---------+---------+---------+----
1          12   0.42248   0.09775                    (-----*-----)
2           3   0.30607   0.09889   (-----------*-----------)
3           2   0.38090   0.07962      (--------------*--------------)
                                  --+---------+---------+---------+----
Pooled StDev =  0.09673           0.20      0.30      0.40      0.50
```

One-Way Analysis of Variance

```
Analysis of Variance for M mo
Source     DF        SS        MS        F        P
BLR ssp     2  0.005847  0.002923     2.96    0.085
Error      14  0.013834  0.000988
Total      16  0.019680

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev -+---------+---------+---------+-----
1          12   0.11550   0.02963                         (------*-----)
2           3   0.07467   0.04315     (------------*------------)
3           2   0.07500   0.02121  (---------------*---------------)
                                  -+---------+---------+---------+-----
Pooled StDev =  0.03143          0.030     0.060     0.090     0.120
```

One-Way Analysis of Variance

```
Analysis of Variance for H iso
Source     DF        SS        MS        F        P
BLR ssp     2  0.022190  0.011095    11.14    0.001
Error      14  0.013943  0.000996
Total      16  0.036133

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1          12   0.29057   0.02237     (--*---)
2           3   0.29213   0.05173  (------*-----)
3           2   0.40300   0.05558                  (-------*-------)
                                    --------+---------+---------+--------
Pooled StDev =  0.03156           0.300     0.360     0.420
```

One-Way Analysis of Variance

```
Analysis of Variance for S iso
Source     DF        SS        MS        F        P
BLR ssp     2   0.04108   0.02054     8.91    0.003
Error      14   0.03229   0.00231
Total      16   0.07338

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          12   0.44535   0.04190    (---*--)
2           3   0.46603   0.07052  (------*-------)
3           2   0.60015   0.05508                (--------*--------)
                                    ----------+---------+---------+------
Pooled StDev =  0.04803           0.480     0.560     0.640
```

One-Way Analysis of Variance

```
Analysis of Variance for M iso
Source     DF        SS        MS        F        P
BLR ssp     2    0.0353    0.0176     1.24    0.320
Error      14    0.1996    0.0143
Total      16    0.2349

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+-------
1          12    0.4210    0.1214     (------*------)
2           3    0.5320    0.1371        (--------------*--------------)
3           2    0.5000    0.0000   (-----------------*-----------------)
                                    ---------+---------+---------+-------
Pooled StDev =  0.1194            0.40      0.50      0.60
```

One-Way Analysis of Variance

```
Analysis of Variance for H issr
Source     DF        SS        MS        F        P
BLR ssp     2   0.00191   0.00095     0.25    0.786
Error      12   0.04665   0.00389
Total      14   0.04856

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  --+---------+---------+---------+----
1          12   0.17205   0.06376                    (----*---)
2           1   0.12660   0.00000  (----------------*----------------)
3           2   0.16855   0.04391            (-----------*-----------)
                                    --+---------+---------+---------+----
Pooled StDev =  0.06235           0.000     0.080     0.160     0.240
```

One-Way Analysis of Variance

```
Analysis of Variance for S issr
Source     DF        SS        MS        F        P
BLR ssp     2   0.00420   0.00210     0.27    0.771
Error      12   0.09473   0.00789
Total      14   0.09893

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          12   0.26183   0.09059                  (----*---)
2           1   0.19560   0.00000  (---------------*---------------)
3           2   0.24735   0.06682          (-----------*----------)
                                  ----------+---------+---------+------
Pooled StDev =  0.08885                  0.12      0.24      0.36
```

One-Way Analysis of Variance

```
Analysis of Variance for M issr
Source     DF        SS        MS        F        P
BLR ssp     2    0.0378    0.0189     0.91    0.427
Error      12    0.2482    0.0207
Total      14    0.2860

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          12    0.5961    0.1501           (---*--)
2           1    0.7210    0.0000       (------------*-----------)
3           2    0.4895    0.0148   (--------*-------)
                                  ----------+---------+---------+------
Pooled StDev =   0.1438                  0.50      0.75      1.00
```

Mood Median Test

```
Mood median test for A mo

Chi-Square = 2.95   DF = 2   P = 0.229

                                        Individual 95.0% CIs
BLR ssp    N<=    N>   Median    Q3-Q1  -------+---------+---------+---------
1            7     5    2.300    0.425        (-------------+-------------)
2            3     0    2.300    0.300  (-------------------+
3            2     0    2.000    0.000  +
                                        -------+---------+---------+---------
                                            2.10      2.25      2.40
Overall median = 2.300
* NOTE * Levels with < 6 observations have confidence < 95.0%
```

Mood Median Test

```
Mood median test for P mo

Chi-Square = 5.99   DF = 2   P = 0.050

                                        Individual 95.0% CIs
BLR ssp    N<=    N>   Median    Q3-Q1  -------+---------+---------+---------
1            8     4    0.800    0.200                        +-------------)
2            0     3    1.000    0.000                                      +
3            2     0    0.500    0.000  +
                                        -------+---------+---------+---------
                                            0.60      0.75      0.90
Overall median = 0.800
* NOTE * Levels with < 6 observations have confidence < 95.0%
```

Mood Median Test
Mood median test for A iso

Chi-Square = 8.20 DF = 2 P = 0.017

BLR ssp	N<=	N>	Median	Q3-Q1	Individual 95.0% CIs
1	11	1	2.000	0.100	(--------------+
2	2	1	2.000	0.100	+-------------)
3	0	2	2.125	0.000	+
					1.960 2.030 2.100

Overall median = 2.000
* NOTE * Levels with < 6 observations have confidence < 95.0%

Mood Median Test
Mood median test for P iso

Chi-Square = 2.62 DF = 2 P = 0.270

BLR ssp	N<	N>=	Median	Q3-Q1	Individual 95.0% CIs
1	7	5	0.750	0.130	+------------------)
2	1	2	0.880	0.130	(------------------+
3	0	2	0.940	0.120	(-------+--------)
					0.770 0.840 0.910 0.980

Overall median = 0.880
* NOTE * Levels with < 6 observations have confidence < 95.0%

Mood Median Test
Mood median test for A issr

Chi-Square = 0.00 DF = 1 P = 1.000

BLR ssp	N<=	N>	Median	Q3-Q1	Individual 95.0% CIs
1	6	6	1.550	0.275	(-----------+---------)
2	1	0	1.500	Not used	
3	1	1	1.450	0.300	(------------+-----------)
					1.32 1.44 1.56 1.68

Overall median = 1.500
* NOTE * Levels with < 6 observations have confidence < 95.0%

Mood Median Test
Mood median test for P issr

Chi-Square = 0.05 DF = 1 P = 0.825

BLR ssp	N<=	N>	Median	Q3-Q1	Individual 95.0% CIs
1	7	5	0.400	0.190	(---+--------------)
2	1	0	0.400	Not used	
3	1	1	0.370	0.260	(------------+------------)
					0.30 0.40 0.50 0.60

Overall median = 0.400
* NOTE * Levels with < 6 observations have confidence < 95.0%

Part 2 Statistical analysis of genetic indices given in section 5.3.1.2. ANOVA was carried out for predominantly normally distributed genetic indices H, S and M; non-normally distributed indices A and P (0.01) could not be tested with non-parametric tests, because data had not enough replications. However, ANOVA was carried out in order to see trends and calculate means and standard deviations. Regions tested were: Palmyra (1), Raqqa (2), Hassakeh (3), Quamishli (4) and Aleppo (5)

One-Way Analysis of Variance

Analysis of Variance for H mo

Source	DF	SS	MS	F	P
Region	4	0.031113	0.007778	17.57	0.001
Error	7	0.003099	0.000443		
Total	11	0.034212			

Level	N	Mean	StDev
1	1	0.25370	0.00000
2	1	0.33260	0.00000
3	1	0.31500	0.00000
4	1	0.32710	0.00000
5	8	0.20620	0.02104

Pooled StDev = 0.02104

Individual 95% CIs For Mean Based on Pooled StDev

```
(-------*--------)
                (-------*--------)
             (--------*-------)
               (--------*-------)
(--*--)
---------+---------+---------+-------
       0.240     0.300     0.360
```

One-Way Analysis of Variance

Analysis of Variance for S mo

Source	DF	SS	MS	F	P
Region	4	0.08891	0.02223	9.61	0.006
Error	7	0.01620	0.00231		
Total	11	0.10511			

Level	N	Mean	StDev
1	1	0.43740	0.00000
2	1	0.58400	0.00000
3	1	0.54550	0.00000
4	1	0.57180	0.00000
5	8	0.36639	0.04810

Pooled StDev = 0.04810

Individual 95% CIs For Mean Based on Pooled StDev

```
 (--------*---------)
             (---------*--------)
           (--------*---------)
             (---------*--------)
 (---*--)
----+---------+---------+---------+--
  0.36      0.48      0.60      0.72
```

One-Way Analysis of Variance

Analysis of Variance for M mo

Source	DF	SS	MS	F	P
Region	4	0.005728	0.001432	2.55	0.132
Error	7	0.003931	0.000562		
Total	11	0.009659			

Level	N	Mean	StDev
1	1	0.12100	0.00000
2	1	0.13400	0.00000
3	1	0.05100	0.00000
4	1	0.09000	0.00000
5	8	0.12375	0.02370

Pooled StDev = 0.02370

Individual 95% CIs For Mean Based on Pooled StDev

```
             (--------*---------)
               (--------*---------)
(---------*--------)
       (--------*--------)
                    (---*--)
-+---------+---------+---------+-----
0.000     0.060     0.120     0.180
```

One-Way Analysis of Variance

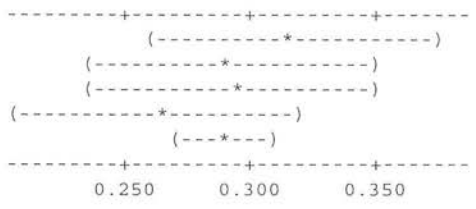
Analysis of Variance for H iso

Source	DF	SS	MS	F	P
Region	4	0.001416	0.000354	0.61	0.671
Error	7	0.004086	0.000584		
Total	11	0.005502			

Level	N	Mean	StDev
1	1	0.31660	0.00000
2	1	0.29170	0.00000
3	1	0.29260	0.00000
4	1	0.26350	0.00000
5	8	0.29030	0.02416

Pooled StDev = 0.02416

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

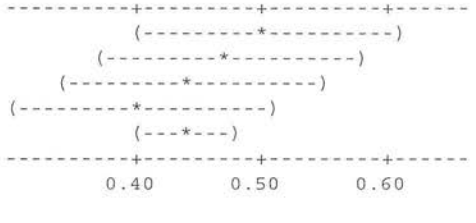
Analysis of Variance for S iso

Source	DF	SS	MS	F	P
Region	4	0.00617	0.00154	0.82	0.551
Error	7	0.01315	0.00188		
Total	11	0.01931			

Level	N	Mean	StDev
1	1	0.50310	0.00000
2	1	0.47410	0.00000
3	1	0.44420	0.00000
4	1	0.40330	0.00000
5	8	0.43994	0.04334

Pooled StDev = 0.04334

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

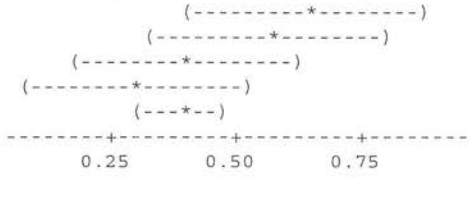
Analysis of Variance for M iso

Source	DF	SS	MS	F	P
Region	4	0.09248	0.02312	2.33	0.155
Error	7	0.06955	0.00994		
Total	11	0.16203			

Level	N	Mean	StDev
1	1	0.64100	0.00000
2	1	0.57100	0.00000
3	1	0.40000	0.00000
4	1	0.30000	0.00000
5	8	0.39250	0.09968

Pooled StDev = 0.09968

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

Analysis of Variance for H issr

Source	DF	SS	MS	F	P
Region	4	0.03748	0.00937	9.05	0.007
Error	7	0.00724	0.00103		
Total	11	0.04472			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	1	0.18950	0.00000	(-------*-------)
2	1	0.33860	0.00000	(-------*------)
3	1	0.16730	0.00000	(-------*------)
4	1	0.22420	0.00000	(------*-------)
5	8	0.14313	0.03217	(-*--)

Pooled StDev = 0.03217 0.10 0.20 0.30 0.40

One-Way Analysis of Variance

Analysis of Variance for S issr

Source	DF	SS	MS	F	P
Region	4	0.07485	0.01871	8.49	0.008
Error	7	0.01542	0.00220		
Total	11	0.09027			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	1	0.28920	0.00000	(------*-------)
2	1	0.49520	0.00000	(------*------)
3	1	0.25760	0.00000	(------*-------)
4	1	0.33790	0.00000	(-------*------)
5	8	0.22025	0.04694	(--*-)

Pooled StDev = 0.04694 0.15 0.30 0.45 0.60

One-Way Analysis of Variance

Analysis of Variance for M issr

Source	DF	SS	MS	F	P
Region	4	0.21002	0.05251	9.68	0.006
Error	7	0.03797	0.00542		
Total	11	0.24799			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	1	0.7420	0.0000	(------*------)
2	1	0.9530	0.0000	(------*------)
3	1	0.7140	0.0000	(------*------)
4	1	0.4630	0.0000	(------*-----)
5	8	0.5351	0.0736	(-*--)

Pooled StDev = 0.0736 0.50 0.75 1.00

One-Way Analysis of Variance

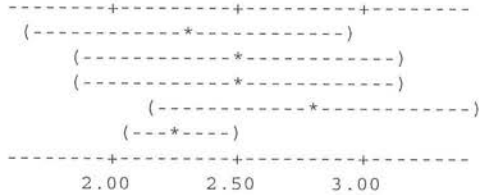
Analysis of Variance for A mo

Source	DF	SS	MS	F	P
Region	4	0.2942	0.0735	0.93	0.499
Error	7	0.5550	0.0793		
Total	11	0.8492			

Level	N	Mean	StDev
1	1	2.3000	0.0000
2	1	2.5000	0.0000
3	1	2.5000	0.0000
4	1	2.8000	0.0000
5	8	2.2750	0.2816

Pooled StDev = 0.2816

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

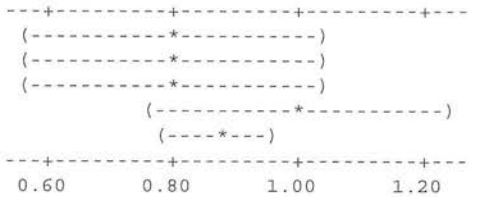
Analysis of Variance for P mo

Source	DF	SS	MS	F	P
Region	4	0.0317	0.0079	0.74	0.594
Error	7	0.0750	0.0107		
Total	11	0.1067			

Level	N	Mean	StDev
1	1	0.8000	0.0000
2	1	0.8000	0.0000
3	1	0.8000	0.0000
4	1	1.0000	0.0000
5	8	0.8750	0.1035

Pooled StDev = 0.1035

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

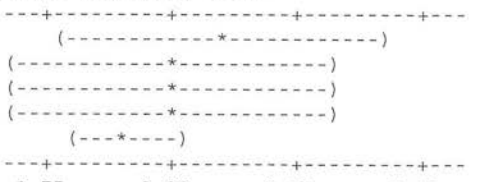
Analysis of Variance for A iso

Source	DF	SS	MS	F	P
Region	4	0.0413	0.0103	0.56	0.699
Error	7	0.1287	0.0184		
Total	11	0.1700			

Level	N	Mean	StDev
1	1	2.1000	0.0000
2	1	2.0000	0.0000
3	1	2.0000	0.0000
4	1	2.0000	0.0000
5	8	1.9125	0.1356

Pooled StDev = 0.1356

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

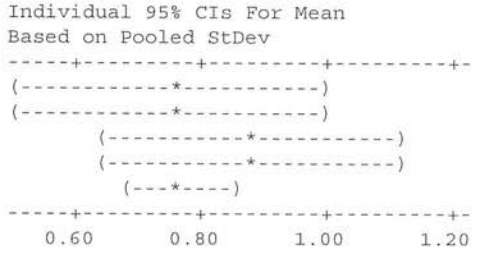
Analysis of Variance for P iso

Source	DF	SS	MS	F	P
Region	4	0.0226	0.0057	0.52	0.728
Error	7	0.0767	0.0110		
Total	11	0.0993			

Level	N	Mean	StDev
1	1	0.7500	0.0000
2	1	0.7500	0.0000
3	1	0.8800	0.0000
4	1	0.8800	0.0000
5	8	0.7688	0.1047

Pooled StDev = 0.1047

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

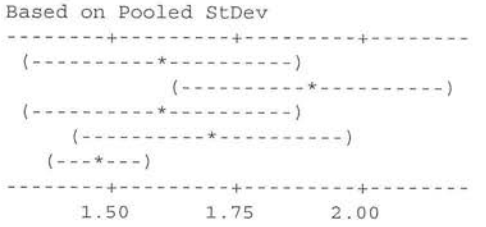
Analysis of Variance for A issr

Source	DF	SS	MS	F	P
Region	4	0.1950	0.0488	3.59	0.067
Error	7	0.0950	0.0136		
Total	11	0.2900			

Level	N	Mean	StDev
1	1	1.6000	0.0000
2	1	1.9000	0.0000
3	1	1.6000	0.0000
4	1	1.7000	0.0000
5	8	1.4750	0.1165

Pooled StDev = 0.1165

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

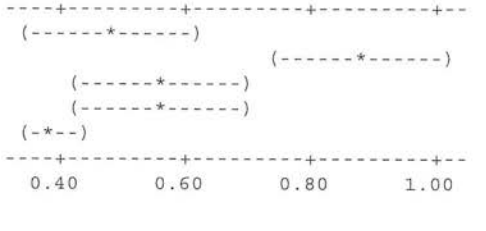
Analysis of Variance for P issr

Source	DF	SS	MS	F	P
Region	4	0.24167	0.06042	16.65	0.001
Error	7	0.02540	0.00363		
Total	11	0.26707			

Level	N	Mean	StDev
1	1	0.4800	0.0000
2	1	0.8800	0.0000
3	1	0.5600	0.0000
4	1	0.5600	0.0000
5	8	0.3850	0.0602

Pooled StDev = 0.0602

Individual 95% CIs For Mean Based on Pooled StDev



Part 3 Statistical analysis of genetic indices given in section 5.3.1.2 (Aleppo region). ANOVA was carried out for predominantly normally distributed genetic indices H, S and M; non-normally distributed indices A and P (0.01) could not be tested with non-parametric tests, because data had not enough replications. However, ANOVA was carried out in order to see trends and calculate means and standard deviations. Locations of the Aleppo region had the following rainfall in mm: > 300 mm (1), 260-300 mm (2), 230 - 260 mm (4), 200-230 mm (3).

One-Way Analysis of Variance

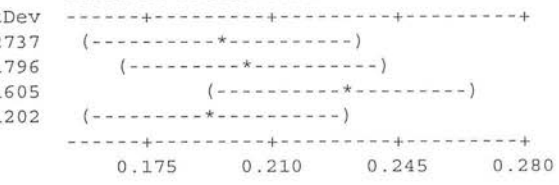
Analysis of Variance for H mo

Source	DF	SS	MS	F	P
Location	3	0.001626	0.000542	1.47	0.349
Error	4	0.001474	0.000368		
Total	7	0.003099			

Level	N	Mean	StDev
1	2	0.19635	0.02737
2	2	0.20450	0.01796
3	2	0.22995	0.01605
4	2	0.19400	0.01202

Pooled StDev = 0.01919

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

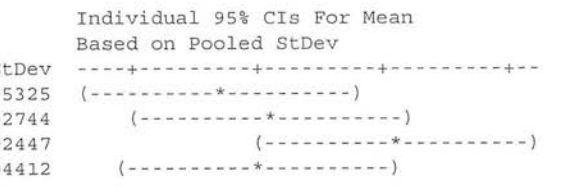
Analysis of Variance for S mo

Source	DF	SS	MS	F	P
Location	3	0.01006	0.00335	2.19	0.232
Error	4	0.00613	0.00153		
Total	7	0.01620			

Level	N	Mean	StDev
1	2	0.33075	0.05325
2	2	0.35790	0.02744
3	2	0.42530	0.02447
4	2	0.35160	0.04412

Pooled StDev = 0.03916

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

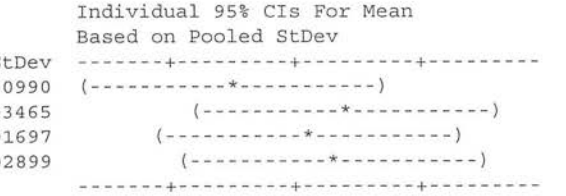
Analysis of Variance for M mo

Source	DF	SS	MS	F	P
Location	3	0.001504	0.000501	0.83	0.544
Error	4	0.002427	0.000607		
Total	7	0.003931			

Level	N	Mean	StDev
1	2	0.10100	0.00990
2	2	0.13550	0.03465
3	2	0.12500	0.01697
4	2	0.13350	0.02899

Pooled StDev = 0.02463

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

Analysis of Variance for H iso

Source	DF	SS	MS	F	P
Location	3	0.000745	0.000248	0.30	0.827
Error	4	0.003341	0.000835		
Total	7	0.004086			

Level	N	Mean	StDev
1	2	0.27545	0.00148
2	2	0.29785	0.00912
3	2	0.29980	0.04398
4	2	0.28810	0.03635

Pooled StDev = 0.02890

Individual 95% CIs For Mean Based on Pooled StDev

```
------+---------+---------+---------+
 (-------------*-------------)
       (-------------*-------------)
        (-------------*-------------)
     (-------------*-------------)
------+---------+---------+---------+
    0.240     0.280     0.320     0.360
```

One-Way Analysis of Variance

Analysis of Variance for S iso

Source	DF	SS	MS	F	P
Location	3	0.00276	0.00092	0.35	0.790
Error	4	0.01039	0.00260		
Total	7	0.01315			

Level	N	Mean	StDev
1	2	0.41680	0.00453
2	2	0.45425	0.01407
3	2	0.46165	0.06710
4	2	0.42705	0.07531

Pooled StDev = 0.05097

Individual 95% CIs For Mean Based on Pooled StDev

```
-----+---------+---------+---------+-
(--------------*-------------)
      (-------------*-------------)
       (-------------*-------------)
  (-------------*-------------)
-----+---------+---------+---------+-
   0.350     0.420     0.490     0.560
```

One-Way Analysis of Variance

Analysis of Variance for M iso

Source	DF	SS	MS	F	P
Location	3	0.0090	0.0030	0.20	0.894
Error	4	0.0606	0.0151		
Total	7	0.0695			

Level	N	Mean	StDev
1	2	0.3400	0.0849
2	2	0.3900	0.0990
3	2	0.4300	0.0990
4	2	0.4100	0.1838

Pooled StDev = 0.1231

Individual 95% CIs For Mean Based on Pooled StDev

```
----+---------+---------+---------+--
(--------------*--------------)
   (--------------*--------------)
      (--------------*--------------)
     (--------------*--------------)
----+---------+---------+---------+--
  0.16      0.32      0.48      0.64
```

One-Way Analysis of Variance

Analysis of Variance for H issr

Source	DF	SS	MS	F	P
Location	3	0.005340	0.001780	3.74	0.117
Error	4	0.001903	0.000476		
Total	7	0.007244			

Level	N	Mean	StDev
1	2	0.15225	0.01506
2	2	0.13005	0.00346
3	2	0.17980	0.03422
4	2	0.11040	0.02220

Pooled StDev = 0.02181

Individual 95% CIs For Mean Based on Pooled StDev

```
-------+---------+---------+---------
         (-------*--------)
     (--------*--------)
               (--------*--------)
  (-------*--------)
-------+---------+---------+---------
     0.100     0.150     0.200
```

One-Way Analysis of Variance

Analysis of Variance for S issr

Source	DF	SS	MS	F	P
Location	3	0.012737	0.004246	6.33	0.053
Error	4	0.002685	0.000671		
Total	7	0.015422			

Level	N	Mean	StDev
1	2	0.23180	0.02588
2	2	0.19695	0.00912
3	2	0.27915	0.03500
4	2	0.17310	0.02659

Pooled StDev = 0.02591

Individual 95% CIs For Mean Based on Pooled StDev

```
---------+---------+---------+------
          (--------*-------)
     (--------*-------)
                    (--------*-------)
(--------*-------)
---------+---------+---------+------
        0.180     0.240     0.300
```

One-Way Analysis of Variance

Analysis of Variance for M issr

Source	DF	SS	MS	F	P
Location	3	0.01993	0.00664	1.47	0.349
Error	4	0.01804	0.00451		
Total	7	0.03797			

Level	N	Mean	StDev
1	2	0.49300	0.02546
2	2	0.58400	0.02263
3	2	0.58550	0.04172
4	2	0.47800	0.12304

Pooled StDev = 0.06715

Individual 95% CIs For Mean Based on Pooled StDev

```
--+---------+---------+---------+----
  (----------*----------)
           (----------*----------)
           (----------*----------)
 (----------*----------)
--+---------+---------+---------+----
0.36      0.48      0.60      0.72
```

Mood Median Test

* ERROR * Too few observations > median

One-Way Analysis of Variance

Analysis of Variance for A mo

Source	DF	SS	MS	F	P
Location	3	0.3750	0.1250	2.78	0.174
Error	4	0.1800	0.0450		
Total	7	0.5550			

Level	N	Mean	StDev
1	2	2.1500	0.2121
2	2	2.1500	0.2121
3	2	2.6500	0.2121
4	2	2.1500	0.2121

Pooled StDev = 0.2121

Individual 95% CIs For Mean Based on Pooled StDev

```
-------+---------+---------+---------
(----------*---------)
(----------*---------)
             (---------*----------)
(----------*---------)
-------+---------+---------+---------
     2.00      2.40      2.80
```

One-Way Analysis of Variance

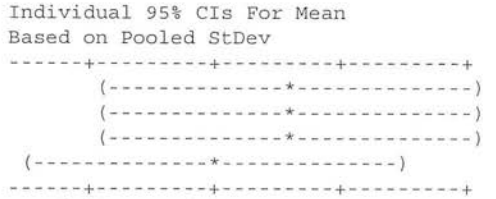
Analysis of Variance for P mo

Source	DF	SS	MS	F	P
Location	3	0.0150	0.0050	0.33	0.803
Error	4	0.0600	0.0150		
Total	7	0.0750			

Level	N	Mean	StDev
1	2	0.9000	0.1414
2	2	0.9000	0.1414
3	2	0.9000	0.1414
4	2	0.8000	0.0000

Pooled StDev = 0.1225

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

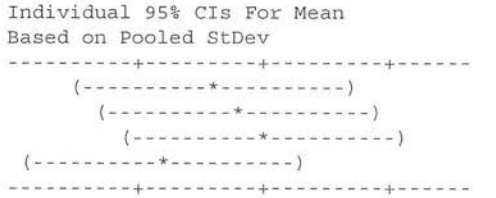
Analysis of Variance for A iso

Source	DF	SS	MS	F	P
Location	3	0.0437	0.0146	0.69	0.606
Error	4	0.0850	0.0212		
Total	7	0.1287			

Level	N	Mean	StDev
1	2	1.9000	0.0000
2	2	1.9500	0.0707
3	2	2.0000	0.0000
4	2	1.8000	0.2828

Pooled StDev = 0.1458

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

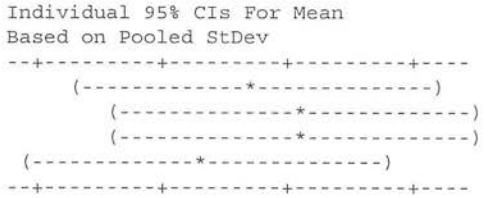
Analysis of Variance for P iso

Source	DF	SS	MS	F	P
Location	3	0.0213	0.0071	0.51	0.694
Error	4	0.0553	0.0138		
Total	7	0.0767			

Level	N	Mean	StDev
1	2	0.7550	0.1768
2	2	0.8150	0.0919
3	2	0.8150	0.0919
4	2	0.6900	0.0849

Pooled StDev = 0.1176

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

Analysis of Variance for A issr

Source	DF	SS	MS	F	P
Location	3	0.08500	0.02833	11.33	0.020
Error	4	0.01000	0.00250		
Total	7	0.09500			

Level	N	Mean	StDev
1	2	1.4500	0.0707
2	2	1.4000	0.0000
3	2	1.6500	0.0707
4	2	1.4000	0.0000

Pooled StDev = 0.0500

Individual 95% CIs For Mean Based on Pooled StDev

```
----+---------+---------+---------+--
    (------*-----)
 (-----*------)
                  (------*------)
 (-----*------)
----+---------+---------+---------+--
  1.35      1.50      1.65      1.80
```

One-Way Analysis of Variance

Analysis of Variance for P issr

Source	DF	SS	MS	F	P
C3	3	0.01660	0.00553	2.52	0.197
Error	4	0.00880	0.00220		
Total	7	0.02540			

Level	N	Mean	StDev
1	2	0.38000	0.02828
2	2	0.34000	0.02828
3	2	0.46000	0.08485
4	2	0.36000	0.00000

Pooled StDev = 0.04690

Individual 95% CIs For Mean Based on Pooled StDev

```
------+---------+---------+---------+
      (--------*--------)
 (--------*--------)
           (--------*--------)
   (--------*--------)
------+---------+---------+---------+
    0.30      0.40      0.50      0.60
```

Part 4 Statistical analysis of genetic indices given in section 5.3.1.2 (Aleppo region). Two-way-ANOVA was carried out for predominantly normally distributed genetic indices H, S and M. Factores tested were A (3 genetic markers) and B (zones) Locations of the Aleppo region had the following rainfall in mm: > 300 mm (1), 260-300 mm (2), 230 - 260 mm (3), 200-230 mm (4).

ANOVA Table for Response Variable: H

Variable	Source	DF	Sum-Squares	Mean-Square	F-Ratio	Prob>F	Error Term
[METHODE]	A	2	8.723E-02	4.361E-02	77.91	0.0000	S(AB)
[ZONE]	B	3	4.936E-03	1.645E-03	2.94	0.0763	S(AB)
	AB	6	2.774E-03	4.624E-04	0.83	0.5714	S(AB)
	S(AB)	12	6.717E-03	5.597E-04			
	TOTAL(Adj)	23	.1016601				

ANOVA Table for Response Variable: S

Variable	Source	DF	Sum-Squares	Mean-Square	F-Ratio	Prob>F	Error Term
[METHODE]	A	2	.2000757	.1000378	62.49	0.0000	S(AB)
[ZONE]	B	3	1.840E-02	6.133E-03	3.83	0.0390	S(AB)
	AB	6	7.154E-03	1.192E-03	0.74	0.6247	S(AB)
	S(AB)	12	1.921E-02	1.600E-03			
	TOTAL(Adj)	23	.244842				

Fisher's LSD Comparison Report
Response Variable: S Factor(B,ZONE) Error Term: S(AB)
Summary Results Ó= .05

Code(Level)	Mean	Level Codes ABCD
A(3)	.31725	...S
B(1)	.32645	...S
C(2)	.3363667	...S
D(4)	.3887	SSS.

ANOVA Table for Response Variable: M

Variable	Source	DF	Sum-Squares	Mean-Square	F-Ratio	Prob>F	Error Term
[METHODE]	A	2	.6981276	.3490638	51.67	0.0000	S(AB)
[ZONE]	B	3	1.732E-02	5.775E-03	0.85	0.4906	S(AB)
	AB	6	1.305E-02	2.175E-03	0.32	0.9129	S(AB)
	S(AB)	12	.0810655	6.755E-03			
	TOTAL(Adj)	23	.809576				

Part 5 Statistical analysis of genetic indices given in table 6.2.2. ANOVA was carried out for predominantly normally distributed genetic indices H and S; Scheffe's test (p = 0.05) was carried out to identify significances between populations. Tested level were: BLRs (1), *H. spontaneum* populations (2), F_3 population (3), F_2 population (4), Mix of cultivars (5) and cultivar Baronesse (6).

```
GLM ANOVA                    (Analysis of Variance Report)

Morphology:

ANOVA Table for Response Variable: MOH
Source        DF   Sum-Squares  Mean Square  F-Ratio  Prob>F
A (SORTPOP )  5     .1555356     3.110E-02     8.04    0.0007
ERROR         15    5.803E-02    3.869E-03
TOTAL(Adj)    20    .213574

Scheffe's Procedure
Response Variable: MOH   Factor(A,SORTPOP)
Summary Results Ó= .05       Level Codes
Code(Level)     Mean         ABCDEF
A(6)          -5.626148E-09  .....S
B(4)          -1.526409E-09  .....S
C(5)           .1325         ......
D(2)           .21765        ......
E(1)           .2279533      ......
F(3)           .4475         SS....

ANOVA Table for Response Variable: MOS
Source        DF   Sum-Squares  Mean Square  F-Ratio  Prob>F
A (SORTPOP )  5     .4276372     8.552E-02     7.85    0.0008
ERROR         15    .1635314     1.090E-02
TOTAL(Adj)    20    .5911686

Scheffe's Procedure
Response Variable: MOS   Factor(A,SORTPOP)
Summary Results Ó= .05       Level Codes
Code(Level)     Mean         ABCDEF
A(6)          -3.38157E-09   .....S
B(4)           7.518133E-09  .....S
C(5)           .2366         ......
D(2)           .3809         ......
E(1)           .3992         ......
F(3)           .721          SS....

Isoenzymes:
ANOVA Table for Response Variable: ISOH
Source        DF   Sum-Squares  Mean Square  F-Ratio  Prob>F
A (SORTPOP )  5     .1544852     3.089E-02    33.23    0.0000
ERROR         15    1.394E-02    9.299E-04
TOTAL(Adj)    20    .1684338

Scheffe's Procedure
Response Variable: ISOH   Factor(A,SORTPOP)
Summary Results Ó= .05       Level Codes
Code(Level)     Mean         ABCDEF
A(6)           3.422101E-09  ...SSS
B(5)           .1309         ....SS
C(4)           .1335         ....SS
D(3)           .2289         S....S
E(1)           .29088        SSS..S
F(2)           .403          SSSSS.
```

ANOVA Table for Response Variable: ISOS

Source	DF	Sum-Squares	Mean Square	F-Ratio	Prob>F
A (SORTPOP)	5	.3697168	7.394E-02	33.29	0.0000
ERROR	15	3.332E-02	2.221E-03		
TOTAL(Adj)	20	.4030369			

Scheffe's Procedure
Response Variable: ISOS Factor(A,SORTPOP)
Summary Results Ó= .05 Level Codes

Code(Level)	Mean	ABCDEF
A(6)	1.074659E-08	...SSS
B(4)	.1638	....SS
C(5)	.2148	....SS
D(3)	.3248	S....S
E(1)	.4494867	SSS..S
F(2)	.60015	SSSSS.

ISSR:

ANOVA Table for Response Variable: ISSRH

Source	DF	Sum-Squares	Mean Square	F-Ratio	Prob>F
A (SORTPOP)	4	3.998E-02	9.996E-03	2.68	0.0793
ERROR	13	4.855E-02	3.735E-03		
TOTAL(Adj)	17	8.854E-02			

ANOVA Table for Response Variable: ISSRS

Source	DF	Sum-Squares	Mean Square	F-Ratio	Prob>F
A (SORTPOP)	4	9.818E-02	2.454E-02	3.23	0.0478
ERROR	13	9.878E-02	7.598E-03		
TOTAL(Adj)	17	.1969639			

Table 5.4.3 Nei's genetic identity (I, above diagonal) and genetic distance (D, below diagonal) between populations of Arabi Aswad and *H. spontaneum* using morphological markers (4 loci)

	Aswad 1	Aswad 2	Aswad 3	Aswad 4	Aswad 5	Aswad 6	Aswad 7	Aswad 8	Aswad 9	Aswad 10	Aswad 11	Aswad 12	H.spont. 1	H. spont. 2
	Palmyra	Raqqa	Hassakeh	Quamishli	Aleppo (A)	Aleppo (A)	Aleppo (B)	Aleppo (B)	Aleppo (D)	Aleppo (D)	Aleppo (C)	Aleppo (C)	Tal Abiad	Hassakeh
Aswad 1	****	0.9363	0.8739	0.8494	0.8278	0.8815	0.8639	0.8529	0.8556	0.8684	0.8662	0.8579	0.4094	0.4643
Aswad 2	0.0659	****	0.9580	0.9148	0.8494	0.9114	0.8841	0.8685	0.8785	0.8983	0.8820	0.8806	0.5348	0.5561
Aswad 3	0.1348	0.0429	****	0.9786	0.9247	0.9597	0.9440	0.9425	0.9485	0.9633	0.9494	0.9465	0.5261	0.5613
Aswad 4	0.1633	0.0891	0.0217	****	0.8915	0.9638	0.9222	0.9357	0.9289	0.9422	0.9549	0.9229	0.4899	0.5324
Aswad 5	0.1890	0.1632	0.0782	0.1148	****	0.9439	0.9914	0.9816	0.9936	0.9891	0.9611	0.9944	0.3541	0.4298
Aswad 6	0.1261	0.0928	0.0411	0.0369	0.0577	****	0.9770	0.9832	0.9645	0.9728	0.9935	0.9730	0.3891	0.4517
Aswad 7	0.1463	0.1231	0.0577	0.0810	0.0087	0.0232	****	0.9941	0.9941	0.9932	0.9853	0.9993	0.3586	0.4322
Aswad 8	0.1591	0.1410	0.0592	0.0664	0.0185	0.0169	0.0060	****	0.9857	0.9874	0.9947	0.9934	0.3957	0.4728
Aswad 9	0.1559	0.1296	0.0528	0.0737	0.0065	0.0361	0.0059	0.0144	****	0.9976	0.9745	0.9963	0.3451	0.4156
Aswad 10	0.1411	0.1072	0.0374	0.0595	0.0110	0.0276	0.0068	0.0127	0.0024	****	0.9781	0.9957	0.3773	0.4422
Aswad 11	0.1436	0.1256	0.0519	0.0462	0.0396	0.0065	0.0148	0.0054	0.0258	0.0221	****	0.9828	0.3971	0.4711
Aswad 12	0.1533	0.1271	0.0550	0.0802	0.0056	0.0274	0.0007	0.0066	0.0037	0.0043	0.0174	****	0.3621	0.4348
H. spont. 1	0.8930	0.6258	0.6423	0.7136	1.0382	0.9440	1.0254	0.9272	1.0638	0.9748	0.9237	1.0157	****	0.9891
H. spont. 2	0.7672	0.5868	0.5775	0.6304	0.8443	0.7947	0.8389	0.7490	0.8779	0.8159	0.7527	0.8329	0.0109	****

Table 5.4.4 Nei's genetic identity (I, above diagonal) and genetic distance (D, below diagonal) between populations of Arabi Aswad and *H. spontaneum* using isoenzyme markers (8 loci)

	Aswad 1	Aswad 2	Aswad 3	Aswad 4	Aswad 5	Aswad 6	Aswad 7	Aswad 8	Aswad 9	Aswad 10	Aswad 11	Aswad 12	H.spont. 1	H. spont. 2
	Palmyra	Raqqa	Hassakeh	Quamishli	Aleppo (A)	Aleppo (A)	Aleppo (B)	Aleppo (B)	Aleppo (D)	Aleppo (D)	Aleppo (C)	Aleppo (C)	Tal Abiad	Hassakeh
Aswad 1	****	0.9800	0.9627	0.7974	0.8481	0.7953	0.8228	0.8044	0.7918	0.8543	0.7550	0.7952	0.8225	0.8205
Aswad 2	0.0202	****	0.9720	0.8381	0.8677	0.8300	0.8593	0.8213	0.8299	0.8744	0.8025	0.8150	0.7707	0.8564
Aswad 3	0.0380	0.0284	****	0.8588	0.9027	0.8306	0.8854	0.8290	0.8429	0.8879	0.8214	0.8331	0.8148	0.8790
Aswad 4	0.2263	0.1766	0.1522	****	0.9847	0.9837	0.9947	0.9569	0.9963	0.9871	0.9637	0.9724	0.7897	0.8237
Aswad 5	0.1647	0.1420	0.1024	0.0154	****	0.9682	0.9926	0.9529	0.9770	0.9872	0.9445	0.9627	0.8328	0.8088
Aswad 6	0.2290	0.1863	0.1856	0.0165	0.0324	****	0.9823	0.9890	0.9921	0.9897	0.9757	0.9908	0.8149	0.7818
Aswad 7	0.1950	0.1517	0.1217	0.0053	0.0074	0.0178	****	0.9644	0.9908	0.9893	0.9727	0.9764	0.8054	0.8293
Aswad 8	0.2177	0.1969	0.1875	0.0441	0.0483	0.0110	0.0363	****	0.9712	0.9811	0.9731	0.9941	0.8611	0.7669
Aswad 9	0.2334	0.1864	0.1709	0.0037	0.0233	0.0079	0.0092	0.0292	****	0.9882	0.9753	0.9850	0.7899	0.8149
Aswad 10	0.1574	0.1342	0.1189	0.0130	0.0129	0.0104	0.0108	0.0191	0.0119	****	0.9593	0.9843	0.8596	0.8226
Aswad 11	0.2810	0.2201	0.1968	0.0370	0.0571	0.0245	0.0277	0.0273	0.0250	0.0415	****	0.9836	0.7656	0.8071
Aswad 12	0.2291	0.2046	0.1827	0.0280	0.0380	0.0093	0.0239	0.0059	0.0151	0.0159	0.0165	****	0.8381	0.7947
H. spont. 1	0.1954	0.2605	0.2048	0.2361	0.1830	0.2046	0.2164	0.1496	0.2358	0.1513	0.2672	0.1767	****	0.7294
H. spont. 2	0.1979	0.1550	0.1290	0.1939	0.2122	0.2462	0.1872	0.2653	0.2047	0.1953	0.2143	0.2298	0.3155	****

Table 5.4.5 Nei's genetic identity (I, above diagonal) and genetic distance (D, below diagonal) between populations of Arabi Aswad and *H. spontaneum* using ISSR markers (25 loci)

	Aswad 1	Aswad 2	Aswad 3	Aswad 4	Aswad 5	Aswad 6	Aswad 7	Aswad 8	Aswad 9	Aswad 10	Aswad 11	Aswad 12	H.spont. 1	H. spont. 2
	Palmyra	Raqqa	Hassakeh	Quamishli	Aleppo (A)	Aleppo (A)	Aleppo (B)	Aleppo (B)	Aleppo (D)	Aleppo (D)	Aleppo (C)	Aleppo (C)	Tal Abiad	Hassakeh
Aswad 1	****	0.6950	0.8249	0.9280	0.9023	0.9245	0.8965	0.9095	0.9356	0.9705	0.9408	0.9348	0.9601	0.8646
Aswad 2	0.3638	****	0.7433	0.6805	0.7086	0.7200	0.7451	0.6644	0.7087	0.6887	0.6913	0.6998	0.7052	0.7414
Aswad 3	0.1925	0.2967	****	0.7838	0.8532	0.9038	0.8429	0.8469	0.7631	0.8396	0.8487	0.8382	0.7674	0.8400
Aswad 4	0.0747	0.3850	0.2436	****	0.8845	0.8816	0.8965	0.9481	0.9048	0.9167	0.8832	0.8936	0.9205	0.8600
Aswad 5	0.1028	0.3445	0.1588	0.1228	****	0.9575	0.9195	0.9121	0.8533	0.9338	0.9380	0.9109	0.9257	0.9036
Aswad 6	0.0785	0.3285	0.1011	0.1260	0.0434	****	0.9150	0.9130	0.8929	0.9598	0.9378	0.9377	0.9078	0.9402
Aswad 7	0.1092	0.2942	0.1709	0.1093	0.0840	0.0888	****	0.9461	0.8974	0.9248	0.9617	0.9538	0.9085	0.9048
Aswad 8	0.0949	0.4089	0.1662	0.0533	0.0920	0.0910	0.0554	****	0.8897	0.9183	0.9469	0.9501	0.8841	0.8747
Aswad 9	0.0665	0.3443	0.2704	0.1000	0.1587	0.1133	0.1082	0.1169	****	0.9380	0.8941	0.9473	0.9326	0.8531
Aswad 10	0.0300	0.3729	0.1748	0.0870	0.0684	0.0410	0.0782	0.0852	0.0640	****	0.9531	0.9439	0.9554	0.8839
Aswad 11	0.0611	0.3692	0.1641	0.1242	0.0640	0.0642	0.0390	0.0545	0.1119	0.0480	****	0.9715	0.9330	0.8956
Aswad 12	0.0674	0.3570	0.1765	0.1125	0.0933	0.0643	0.0473	0.0512	0.0541	0.0578	0.0289	****	0.9280	0.8910
H. spont. 1	0.0407	0.3493	0.2647	0.0829	0.0772	0.0967	0.0960	0.1231	0.0698	0.0456	0.0693	0.0747	****	0.8847
H. spont. 2	0.1455	0.2992	0.1744	0.1508	0.1014	0.0617	0.1000	0.1339	0.1588	0.1234	0.1102	0.1154	0.1225	****

Table 5.4.6 Branch length for dendrogram in figure 5.3.6

Between	And	Length
13	12	34.73058
12	11	3.34215
11	pop1	3.29351
11	pop2	3.29351
12	10	3.53462
10	8	2.01787
8	pop3	1.08318
8	pop4	1.08318
10	9	1.93407
9	5	0.77064
5	pop5	0.39633
5	3	0.13688
3	1	0.22237
1	pop7	0.03708
1	pop12	0.03708
3	2	0.13792
2	pop9	0.12153
2	pop10	0.12153
9	7	0.58135
7	pop6	0.58562
7	4	0.31771
4	pop8	0.26790
4	pop11	0.26790
13	6	40.82017
6	pop16	0.54608
6	pop17	0.54608

Table 5.4.7 Branch lengths for dendrogram in figure 5.3.7

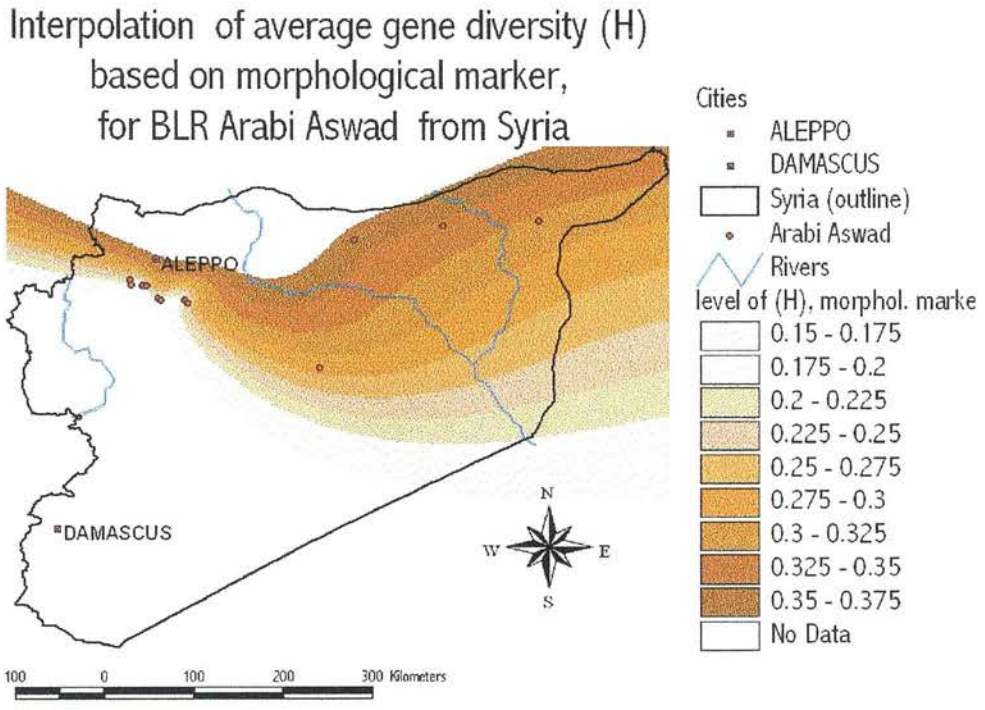
Between	And	Length
13	12	1.17352
12	11	1.55247
11	10	6.37241
10	7	0.64822
7	pop1	1.01050
7	pop2	1.01050
10	pop3	1.65872
11	pop14	8.03113
12	9	8.15591
9	6	0.68975
6	5	0.14398
5	3	0.23004
3	1	0.17907
1	pop4	0.18484
1	pop9	0.18484
3	pop7	0.36391
5	pop10	0.59395
6	pop5	0.73793
9	8	0.28896
8	4	0.63094
4	pop6	0.50779
4	2	0.21137
2	pop8	0.29642
2	pop12	0.29642
8	pop11	1.13872
13	pop13	10.75712

Table 5.4.8 Branch lengths for dendrogram in figure 5.3.8

Between	And	Length
13	12	7.95136
12	11	3.39111
11	10	1.28507
10	9	0.55030
9	7	0.84289
7	3	1.18116
3	2	0.65788
2	pop1	1.49973
2	pop10	1.49973
3	pop13	2.15761
7	pop9	3.33877
9	8	0.36022
8	5	1.65248
5	pop5	2.16896
5	pop6	2.16896
8	4	1.66287
4	pop7	2.15857
4	1	0.71131
1	pop11	1.44726
1	pop12	1.44726
10	6	2.06908
6	pop4	2.66288
6	pop8	2.66288
11	pop14	6.01703
12	pop3	9.40814
13	pop2	17.35950

Figure A 16.1 Interpolation of average gene diversity (H) based on morphological marker. A in Northern Syria, B in the Aleppo region

A



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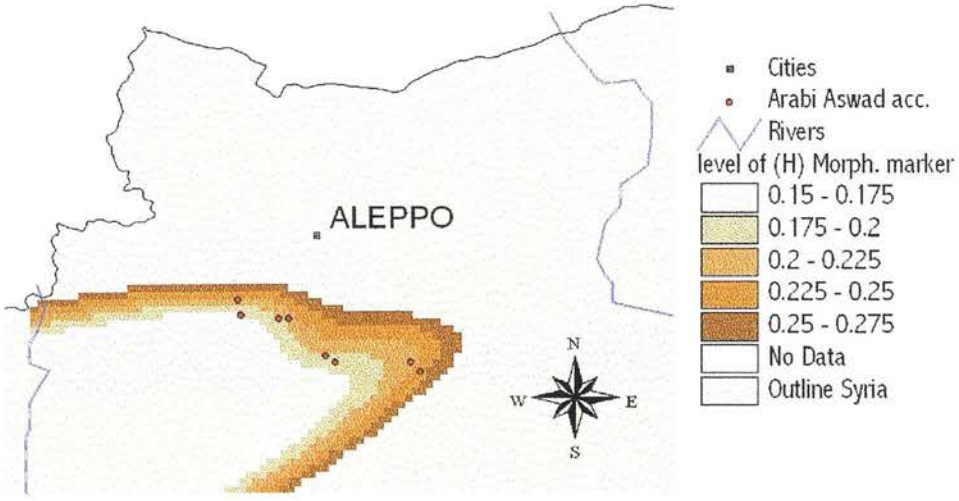
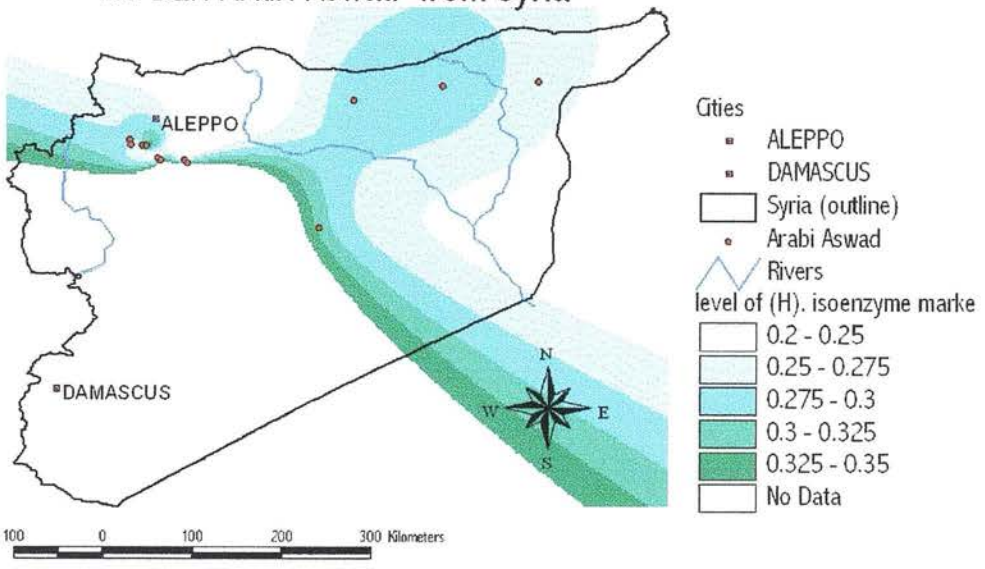


Figure A 16.2 Interpolation of average gene diversity (H) based on isoenzyme markers. A in Northern Syria, B in the Aleppo region

A



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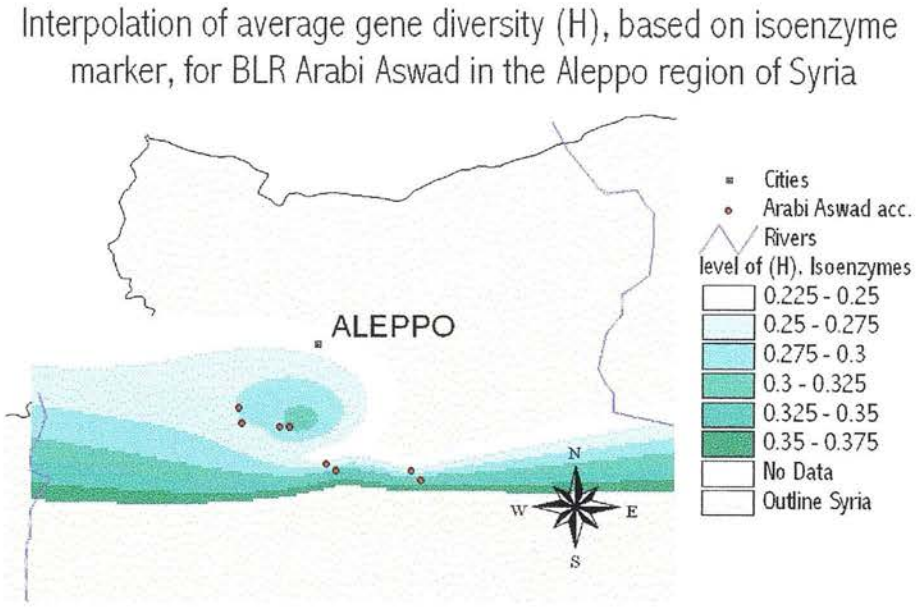
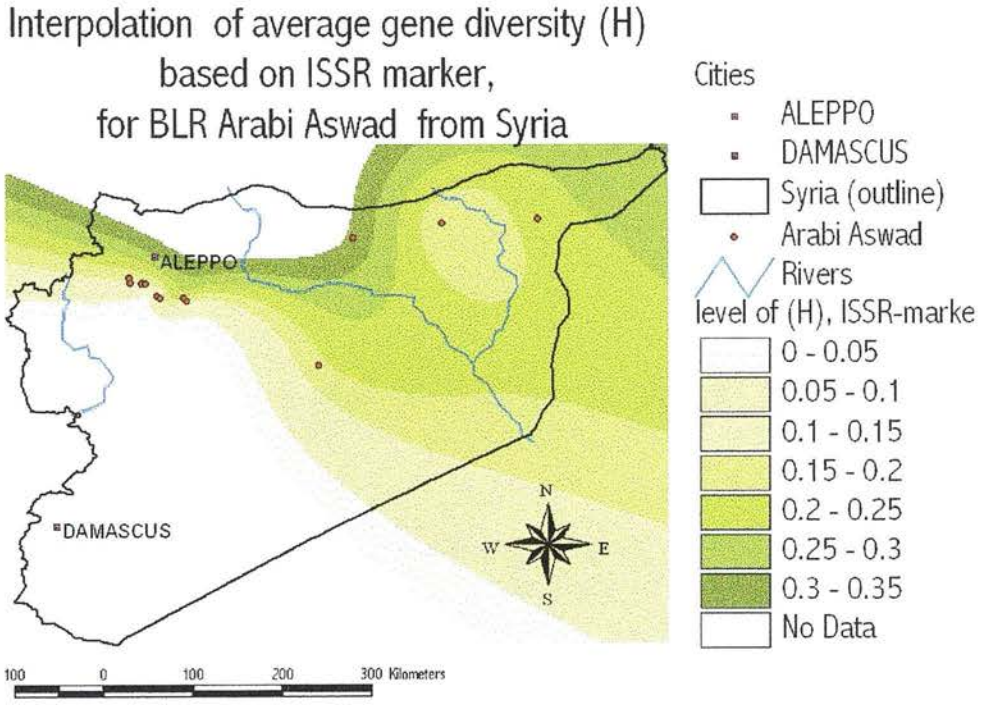
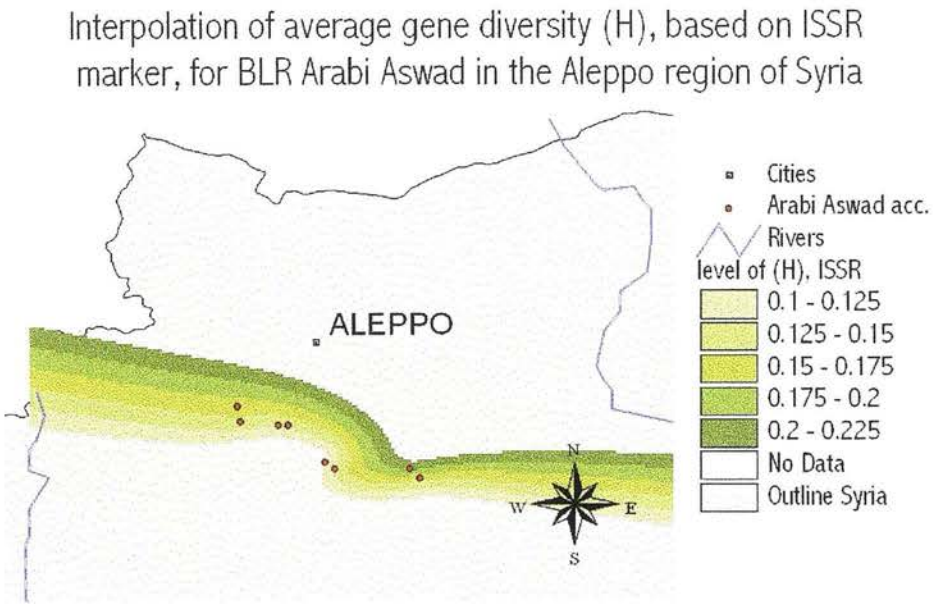


Figure A 16.3 Interpolation of average gene diversity (H) based on ISSR marker. A in Northern Syria, B in the Aleppo region

A



B



Short communication

Outcrossing Rates of Barley Landraces from Syria

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Abstract

Diversity levels in populations of barley landraces may be influenced by varying levels of natural outcrossing due to environmental conditions. Outcrossing was studied in 10 accessions of the barley landrace Arabi Aswad from different environments in Syria. Electrophoretic variation at two codominant isoenzymes (*Est1* and *Est2*) in six seeds of 50 families per population were analysed and multilocus outcrossing rates calculated. Results were correlated with interpolated environmental conditions. Outcrossing was, on average, 1.7% and not significantly different from the outcrossing rate of wild barley. A significant increase of outcrossing was observed with increasing interannual variation in rainfall and decreasing minimum temperatures of the coldest month.

Key words: *Hordeum vulgare* ssp. *vulgare* - barley landrace – outcrossing - electrophoresis - environmental heterogeneity - isoenzymes

Barley landraces (*Hordeum vulgare* L ssp. *vulgare*) are the evolutionary link between wild barley (*Hordeum vulgare* ssp. *spontaneum* C. Koch) and modern barley cultivars. Until the late nineteenth century, all cultivated barley existed as highly heterogeneous landraces. Over the last 100 years the landraces have been almost wholly displaced in advanced agriculture by pure line cultivars (Nevo, 1992). However, in many parts of the world with a less developed agriculture, landraces are still in use and are favoured by local farmers, because they often perform more stably over a range of conditions than modern cultivars (Ceccarelli, 1996). This advantage of barley landraces may be

explained by their buffering ability wherein better adapted components of the population compensate for the losses of less adapted lines under adverse conditions. Barley landrace populations are thought to be structured as highly heterogeneous mixtures of inbred lines and hybrid segregates which are maintained by a low level of random crossing in each generation (Nevo, 1992). While the outcrossing rate of wild barley (Brown, et al., 1978) and modern cultivars (Doll, 1987) has been well studied, no particular information on the outcrossing rate of barley landraces is available. Samples of 10 populations of the black seeded barley landrace Arabi Aswad (table 1) which is still cultivated throughout Syria, were collected in 1997 and 1998 under the supervision of the International Center for Agricultural Research in Dry Areas, (ICARDA), Aleppo, Syria. Spikes of fifty individual plants from at least two metre distance were collected per population and 6 seeds per spike (i.e. family) were used for electrophoresis. Thus a total of 300 seeds per population were grown in soil and starch gel electrophoresis was carried out on extracts of four to ten day old coleoptiles (Kahler and Allard, 1970). Allele frequencies at two different codominant isoenzyme loci (*Est1, Est2*, E.C.3.1.1.1) were analysed and multilocus outcrossing rate (t_m) and fixation index (F_{IS}) were calculated using the programme MLT (Ritland, 1990). Climatic data (FAO, 1994; Anonymous, 1986) were interpolated for the sampling sites using the computer programme ArcView GIS 3.0, spatial analyst.

The multilocus outcrossing rate (i.e. the proportion of offspring produced by outcrossing) of barley landrace Arabi Aswad was on average 1.7% (table 1). Domestication may not have influenced the outcrossing rate of barley significantly as this result is not significantly different from the outcrossing rate reported by Brown, Zohary and Nevo (1978) for wild barley.

The inbreeding coefficient for the adult plants (F_{IS}), i.e. the probability that two alleles of a gene are identical by descent (Hartl, 1988), was on average 0.866 for the populations analysed (Tab. 1). Fixation at inbreeding equilibrium (F_i) was calculated from the rate of selfing as $F_i = S/(2-S)$, where ($S = 1 - t_m$), and gave an average value of F_i of 0.966 (+/- 0.005). A Mann-Whitney test showed that the average F_{IS} of 0.866 was very highly significantly lower (P = 0.0003) than the calculated fixation at inbreeding equilibrium (F_i). This may indicate the presence of multilocus heterozygote advantage, or the advantage of multiply heterozygous individuals produced by outcrossing.

Outcrossing rates were correlated with interpolated environmental data for the collection sites of the corresponding accessions and showed a highly significant positive correlation (P = 0.03) with relative interannual variation in rainfall given as the coefficient of variation of 10 year rainfall data (P_{CV}) (Fig. 1A). This finding may imply that the outcrossing rate is higher in unpredictable environments than in more stable environments. Outcrossing rate also showed a highly significant negative correlation (P = 0.02) with average minimum temperature of the coldest month (T_{min}) (Fig. 1B). The latter result is in accordance with Doll (1978) who found significantly higher outcrossing rates for autumn-sown barley than for spring-sown barley, the latter being apparently less exposed to colder temperatures. The results may indicate that natural outcrossing plays an active role in maintaining diversity within populations of barley landraces, while heterogeneity and severity of the environment seem to influence the level of outcrossing.

Acknowledgements

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Caption for figure 1

Figure 1. A. Relationship between relative annual variation in rainfall (P_{CV}), measured as the coefficient of variance of 10 year rainfall data, and multilocus outcrossing rate (t_m) as given in table 1, of 10 barley landrace populations from Syria. B. Relationship between the average minimum temperature of the coldest month (T_{min}), and multilocus outcrossing rate (t_m) as given in table 1, of 10 barley landrace populations from Syria. Accession numbers of Arabi Aswad are given next to the data points.

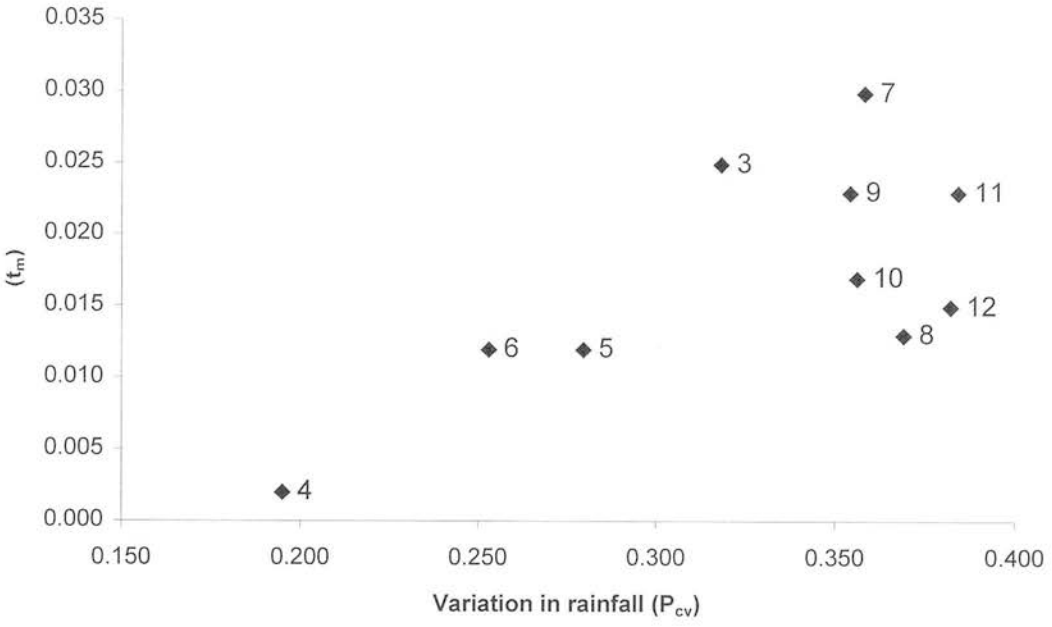
Table 1 Multilocus outcrossing rate (t_m) and Fixation index (F_{IS}) of 10 populations of barley landrace Arabi Aswad from Syria with 50 families each. Allele frequencies of adults are given for two esterase loci.

Table 1

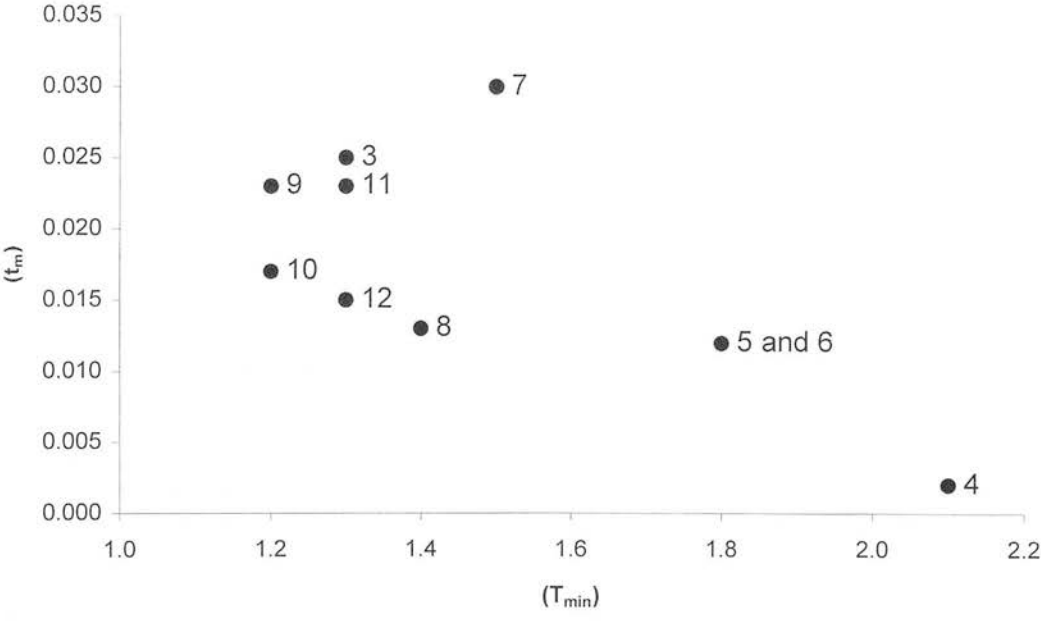
Accession code	Region	Location	Allele Frequencies				Multilocus outcrossing rate (t_m)	Fixation index (F_{IS})
			locus *Est1*		locus *Est2*			
			allele	Freq.	allele	Freq.		
Aswad 3	Hassakeh	West of Hassakeh	1	0.07	1	0.184	**0.025**	**0.909**
			2	0.115	2	0.816		
			3	0.815	3	0		
Aswad 4	Quamishli	Tal Birak	1	0.114	1	0.488	**0.002**	**0.939**
			2	0.868	2	0.512		
			3	0.018	3	0		
Aswad 5	Aleppo	Tal Bagar	1	0.42	1	0.648	**0.012**	**0.917**
			2	0.07	2	0.352		
			3	0.51	3	0		
Aswad 6	Aleppo	Banus	1	0.081	1	0.813	**0.012**	**0.823**
			2	0.875	2	0.183		
			3	0.044	3	0.004		
Aswad 7	Aleppo	Soaibieh	1	0.319	1	0.194	**0.030**	**0.963**
			2	0.681	2	0.612		
			3	0	3	0.194		
Aswad 8	Aleppo	Abu-Rowail	1	0.322	1	0.918	**0.013**	**0.861**
			2	0.076	2	0.082		
			3	0.602	3	0		
Aswad 9	Aleppo	Khanasir	1	0.146	1	0.614	**0.023**	**0.876**
			2	0.315	2	0.386		
			3	0.539	3	0		
Aswad 10	Aleppo	Im Mial	1	0.532	1	0.095	**0.017**	**0.835**
			2	0.055	2	0.905		
			3	0.413	3	0		
Aswad 11	Aleppo	Sowaiha	1	0.879	1	0.892	**0.023**	**0.845**
			2	0.121	2	0.104		
			3	0	3	0.004		
Aswad 12	Aleppo	Om-amood	1	0.076	1	0.094	**0.015**	**0.696**
			2	0.90	2	0.902		
			3	0.024	3	0.004		
Average							**0.0172**	**0.866**
Std. dev.							+/- 0.0082	+/- 0.0756

Figure 1

A


0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
(tm)
7
3
9
11
10
12
8
6
5
4
0.150
0.200
0.250
0.300
0.350
0.400
Variation in rainfall (Pcv)

B


0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
(tm)
7
3
9
11
10
12
8
5 and 6
4
1.0
1.2
1.4
1.6
1.8
2.0
2.2
(Tmin)

Heredity **84** (2000) 476–486 *Received 20 August 1999, accepted 18 January 2000*

Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare*) conserved for different lengths of time in *ex situ* gene banks

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Large numbers of crop plant accessions from all over the world have been amassed in gene banks to secure a gene pool for future breeding programmes. Maintenance of accessions held as seed samples in cold stores involves frequent rejuvenation cycles to ensure the viability of seeds. The practice of rejuvenation by multiplication of a sample of each accession in small field plots has the potential to create population bottlenecks, leading to loss of genetic diversity and changes in gene frequencies every rejuvenation cycle. In order to determine whether these undesirable effects occur, genetic diversity levels were assessed for morphological and isozyme markers within gene bank accessions of two barley landraces from Syria that had been stored for 10, 40 and 72 years. These were compared with genetic diversity levels for the same markers in barley landraces collected recently at locations in Syria where they are still under cultivation. Average gene diversity (H), alleles per locus (A) and percentage polymorphic loci ($P(0.01)$) all showed very significant declines with length of time in storage, and genetic differentiation F_{ST} among accessions increased over time. If the observed differences in genetic diversity are caused by genetic drift in gene bank accessions rejuvenated every 5.3 years, it was estimated that the effective population size N_e of rejuvenation populations over their period in storage was only 4.7. Implications for gene bank management are discussed.

Keywords: barley landrace, gene bank, genetic conservation, genetic erosion, genetic markers, isozymes.

Introduction

Much of the effort expended in conservation of crop genetic resources has involved the establishment of *ex situ* gene banks. A large proportion of the material in these gene banks comprises accessions of traditional landraces of cultivated species. For instance in barley, *Hordeum vulgare* ssp. *vulgare* approximately one-half of the existing accessions in the BBSRC collection take the form of landraces (BBSRC, 1999). Landraces of inbreeding crops such as barley are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of outcrossing (Nevo, 1992). In the initial collections of landraces held in gene banks at least 50–60% of the total genetic variation captured resides within the landraces, the remainder being accounted for by differences between landraces (Brown & Munday, 1982; Jana & Pietrzak, 1988).

A great deal of thought has been put into devising sampling strategies to ensure that the accessions entering gene banks from the wild contain a high proportion of the 'within population' genetic variation that exists in traditional landraces (Brown & Briggs, 1991). Much less attention has been paid, however, to ensuring the maintenance of this genetic diversity throughout the lifetime of the gene bank (Sackville Hamilton & Chorlton, 1997). It has been common practice in gene banks to retain seed of many crops in airtight containers in cold stores (Clark *et al.*, 1997). In these circumstances, frequent rejuvenation of seed is needed to maintain high seed viability. To accomplish this in a species like barley, samples are taken from the stored accessions and grown in plots that rarely exceed 3 m^2 in area. Rejuvenated seed is harvested from these plots for the next period of storage.

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If the number of parents contributing to the rejuvenated seed is low, i.e. the effective population size, N_e, of rejuvenation populations is small, genetic diversity could be lost rapidly each rejuvenation cycle from each landrace (Frankel, 1977; Marshall, 1990; Hamilton, 1994; Brown *et al.*, 1997), and an accumulation of plants homozygous for deleterious mutations could occur (Bataillon *et al.*, 1996; Schoen *et al.*, 1998). Computer simulations suggest that effective population sizes of $N_e = 100$ (equivalent to 200 equally contributing parents in a self-fertilizing species) in the rejuvenation population are needed to avoid these deleterious effects. It is possible that such population sizes have not been achieved in practice, and that the problem of genetic erosion from accessions of landraces in gene banks is a real one. However there are no empirical data presently available to determine whether loss of variation has actually taken place within gene bank collections as a consequence of repeated rounds of rejuvenation.

The purpose of the present, preliminary study was to establish whether there is any evidence to support the prediction of loss of genetic variation from landrace populations that have been maintained *ex situ* within gene banks. Accessions of barley landraces from Syria were obtained from current *in situ* populations, and from a variety of *ex situ* gene banks where they had been held for different periods since initial collection. These periods ranged from 10 to 72 years.

Four seed morphological and eight isozyme markers were analysed to determine whether a significant reduction in genetic diversity could be detected in populations that had been maintained in gene banks, and whether this reduction in diversity was related to the number of generations spent in the gene bank collection. Making a number of assumptions about the origins of gene bank populations and their rate of rejuvenation in gene banks, the observed changes in genetic diversity were used to infer the genetically effective sizes of gene bank accessions during their time in storage.

Materials and methods

Sample material

All material used was of the two barley landraces, Arabi Aswad (black seeded) and Arabi Abiad (white seeded) from Syria. Barley production in Syria is entirely based on these two land races (Ceccarelli, 1996). Arabi Abiad is typically grown in more favourable environments than is Arabi Aswad. Extant populations were sampled *in situ* with the assistance of the International Centre for Agriculture in Dry Areas (ICARDA), Aleppo, Syria (Table 1). At least 100 seed heads were collected randomly from each of 11 populations located in different rainfall zones in the vicinity of Aleppo and elsewhere in northern Syria. Single seeds were taken from each seed head for analysis.

An exhaustive search of existing gene bank accessions of barley landraces derived from the same areas of Syria yielded a total of 11 accessions, ranging in storage time from 10 to 72 years, that were suitable for this study. Six samples of seed of the two Arabi landraces stored for 10 years were available (Table 1). Four were from ICARDA, Aleppo, and two from the BBSRC gene bank at the John Innes Institute in Norwich, UK, but originally collected by ICARDA. It is known that this material was derived from field collections of seed heads from at least 50 parents in each population. The populations have been through two rejuvenation cycles during their time in storage.

Two seed samples of the Arabi landraces stored for 40 years were supplied by the All-Union Institute of Plant Industry (VIR) gene bank located in St. Petersburg, Russia. Finally three seed samples that had been stored for 72 years were available, two from the VIR gene bank and one from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany (Table 1). This sample had originally been collected by VIR. It can be assumed that the original accessions collected by VIR were large because subsamples were tested in extensive experiments at numerous locations (Vavilov, 1957).

Comprehensive documentation of rejuvenation practices was available from the IPK gene bank. An analysis of 20 different files, monitoring accessions collected from 11 to 51 years ago, showed that on average one rejuvenation cycle took place for every 5.3 years of storage time. This accords with the 5 years rejuvenation cycle for the 10-year-old material in the ICARDA gene bank.

Assessment of genetic diversity

Morphological markers Phenotype frequency was scored for four seed characters showing variation controlled by single genes (Nilan, 1964; Hockett & Nilan, 1985). Sample size ranged from 50 to 200 individuals (Table 3). The characters, phenotypes and corresponding genotypes were:

lemma colour — white (b/b), grey (B_g/B_g, B_g/b), brown (Bg/Bg, Bg/B_g, Bg/b) or black($B/$-);

aleurone/pericarp colour — white (bl/bl), blue (Bl/Bl, Bl/bl), or black ($B/$-);

awns — rough (r/r) or smooth ($R/$-);

rachilla hairs — short (s/s) or long ($S/$-).

These simply inherited morphological characters have been used before to assess genetic diversity in barley and for testing the distinctiveness and uniformity of

Table 1 Details of 22 barley landrace accessions used in the study

Landrace	Collection site in Syria at	Location in Syria: longitude E/latitude N	Code	Source	Year of collection	No. of seed heads collected	Years stored
Arabi Aswad	Hassakeh	40.07/36.32	Aswad 3	Own collection	1997	100	0
Arabi Aswad	Quamishli	41.05/36.35	Aswad 4	Own collection	1997	200	0
Arabi Aswad	Aleppo, Zone A†	36.59/35.56	Aswad 5	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A	36.58/35.59	Aswad 6	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A-B	37.06/35.55	Aswad 7	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone A-B	37.08/35.55	Aswad 8	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone C	37.31/35.47	Aswad 9	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone C	37.33/35.45	Aswad 10	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone B	37.15/35.48	Aswad 11	Own collection	1998	100	0
Arabi Aswad	Aleppo, Zone B	37.17/35.47	Aswad 12	Own collection	1998	100	0
Arabi Abiad	Bural Sharqui	37.09/35.01	Abiad 3	Own collection	1997	200	0
Arabi Aswad	Palmyra	38.53/35.06	Aswad 1	ICARDA	1988	58	10
Arabi Aswad	Raqqa	39.13/36.23	Aswad 2	ICARDA	1988	82	10
Arabi Aswad	Syria	—	Aswad 20126	BBSRC/ICARDA	<1988		10
Arabi Abiad	Hama	36.43/35.08	Abiad 1	ICARDA	1988	62	10
Arabi Abiad	Suweida	36.44/33.02	Abiad 2	ICARDA	1988	68	10
Arabi Abiad	Syria	—	Abiad 20125	BBSRC/ICARDA	<1988		10
Arabi Aswad	Syria	—	H. vs. 18882	VIR	1959		40
Arabi Abiad	Damascus	36.31/33.25	Abiad 18881	VIR	1959		40
white BLR	Aleppo	37.13/36.11	White H.v. 7667	VIR	1926		72
white BLR	Homs	36.43/34.43	White H.v. 7659	VIR	1926		72
white BLR	Aleppo	37.13/36.11	White H.v.HOR7400	IPK/VIR	1926		72

† Zones refer to annual rainfall. Zone A, >350 mm; Zone B, 250–350 mm; Zone C, 200–250 mm.

cultivars (Brown & Munday, 1982; Allard, 1992). Allele frequencies were estimated on the assumption that all individuals were completely homozygous so that for these dominant markers, allele frequencies are equal to observed phenotype frequencies. This assumption is supported by the results of a parallel analysis of multilocus outcrossing rates in 10 of the populations used in this study (Arabi Aswad 3 to Arabi Aswad 12, Table 1) (Parzies, unpubl. data). Outcrossing rate was 1.7% and was not significantly different from the value of 1.6% found in wild barley, *H. vulgare* ssp. *spontaneum* (Brown *et al.*, 1978), indicating a very low expected frequency of heterozygotes at inbreeding equilibrium.

Isozyme markers Variation at eight esterase (EST, E.C.3.1.1.1) loci was analysed in samples from the same 22 accessions scored for morphological markers (Table 1). The mean number of individuals scored per accession was 46 (range 17–55, Table 2). A modified protocol for starch gel electrophoresis based on the methods of Kahler & Allard (1970), Brown (1983), Kahler *et al.* (1981) and Cheliak & Pitel (1984), was used. Four to 10 day old coleoptiles were crushed in 40 μL of a 6-mM DTT solution and extracts absorbed into filter paper wicks, which were kept frozen until use. Gels were prepared using 11% hydrolysed potato starch in a solution of 0.01 M histidine HCl, 0.28 mM EDTA, pH 7.0 adjusted with 1 M Tris. The electrode buffer contained 0.125 M Tris (pH 7.0 adjusted with 1 M anhydrous citric acid). Samples were inserted into a slot of the gels and run on cooled flatbed electrophoresis units for 5 h at a current of 55 mA. Staining of samples was carried out at 37°C in the dark in a solution of 100 mg α-naphthyl acetate, 50 mg β-naphthyl acetate (both dissolved in 1 mL of acetone) and 100 mg Fast Blue RR salt in 50 mL of a 0.2-M phosphate buffer (pH 6.4 adjusted with 1 M NaH_2PO_4).

Methods described by Kahler & Allard (1970) and Brown (1983) were used to evaluate allele frequencies at eight different loci (*Est1* to *Est8*). The loci *Est1*, *Est2*, *Est4*, *Est5*, *Est6*, *Est7* and *Est8* are identical with the loci *A* to *G* described by Kahler & Allard (1970). A further locus between *Est2* and *Est4* with two alleles (single band or null allele) was found. This may be identical with *Est3* which has so far only been reported in embryo tissue (Brown, 1983).

Data analysis

Genetic diversity within accessions was quantified in terms of mean proportion of polymorphic loci using the 1% criterion ($P(0.01)$), mean number of alleles per

Table 2 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at eight enzyme loci

Landrace code	Collection year	Years stored	Number of individuals (n)	Isoenzyme diversity				
				H	SD(H)	A	SD(A)	$P(0.01)$
Aswad 5	1998	0	50	0.2744	(±0.2033)	1.9	(±0.4)	0.88
Aswad 6	1998	0	50	0.2765	(±0.2895)	1.9	(±0.8)	0.63
Aswad 7	1998	0	50	0.2914	(±0.2043)	2.0	(±0.5)	0.88
Aswad 8	1998	0	50	0.3043	(±0.2515)	1.9	(±0.6)	0.75
Aswad 9	1998	0	50	0.2687	(±0.2498)	2.0	(±0.9)	0.75
Aswad 10	1998	0	50	0.3309	(±0.2193)	2.0	(±0.5)	0.88
Aswad 11	1998	0	50	0.2624	(±0.2325)	1.6	(±0.5)	0.63
Aswad 12	1998	0	50	0.3138	(±0.2560)	2.0	(±0.8)	0.75
Aswad 3	1997	0	50	0.2926	(±0.2217)	2.0	(±0.5)	0.88
Aswad 4	1997	0	50	0.2635	(±0.2159)	2.0	(±0.5)	0.88
Abiad 3	1997	0	55	0.2825	(±0.2165)	2.0	(±0.5)	0.88
Aswad 1	<1988	10	39	0.3166	(±0.2704)	2.1	(±1.0)	0.75
Aswad 2	<1988	10	42	0.2917	(±0.2444)	2.0	(±0.9)	0.75
Aswad 20126	<1988	10	50	0.2592	(±0.2431)	1.8	(±0.7)	0.63
Abiad 1	<1988	10	29	0.3480	(±0.2061)	2.1	(±0.6)	0.88
Abiad 2	<1988	10	17	0.2459	(±0.2197)	2.0	(±0.9)	0.75
Abiad 20125	<1988	10	50	0.1514	(±0.2176)	1.5	(±0.5)	0.50
Black BLR 18882	1959	40	50	0.1154	(±0.1696)	1.5	(±0.5)	0.50
Abiad 18881	1959	40	50	0.0147	(±0.0203)	1.4	(±0.5)	0.38
White BLR 7667	1926	72	50	0.0469	(±0.0434)	1.4	(±0.8)	0.38
White BLR 7659	1926	72	44	0.0312	(±0.0737)	1.5	(±0.5)	0.38

H, gene diversity; A, mean alleles per locus; $P(0.01)$ percentage polymorphic loci using 1% criterion.

locus (A) and average gene diversity (H) using the POPGENE programme (Yeh *et al.*, 1997). Morphological and isozyme genetic markers were initially analysed separately since they may differ in important characteristics such as mutation rate and degree of selective neutrality (Bataillon *et al.*, 1996).

In an isolated population of effective size N_e, gene diversity is expected to decline every generation as a consequence of genetic drift. Let the initial gene diversity be H_o. After t generations the remaining value of gene diversity H_t is given by:

$$H_t = H_o(1 - 1/2N_e)^t.$$

Then $\log(H_t) = \log(H_o) + t \cdot \log(1 - 1/2N_e)$.

In order to estimate the genetically effective size of gene bank populations during their time in storage that is compatible with the observed decline in gene diversity by genetic drift, a regression of $\log(H_t)$ on t was calculated. Because observed rates of decline for morphological and isozyme markers were very similar (Fig. 1a, and 1b) H_t for this analysis was calculated using all 12 loci. t is the number of cycles of regeneration passed through by accessions during their time in the gene bank, assuming a rejuvenation cycle time of 5.3 years. The slope of these regressions provides an estimate of $\log(1 - 1/2N_e)$ from which N_e can be calculated.

If genetic drift occurs independently in accessions over cycles of rejuvenation, an increase in genetic divergence between them is expected. To test for this effect all accessions held for the same length of time in gene banks were grouped and treated as a single subdivided population. Values of total genetic diversity H_T, for accessions in each age class (0, 10, 40 and 72 years), and of F_{ST} measuring the extent of genetic divergence among accessions in these age classes were estimated separately for morphological and isozyme markers using the POPGENE and FSTAT (Goudet, 1999) programs.

Results

Diversity levels within accessions

Table 4 summarizes the mean genetic diversity indices within accessions calculated from morphological and isozyme markers for groups of barley landrace accessions from Syria with identical storage periods. The individual results for all accessions are listed in Tables 2 and 3 and plotted against time in storage in Figs 1 and 2.

A decrease of genetic diversity with increase in storage period was observed for all three indices of diversity and for both marker types. Average maximum declines in diversity indices after 72 years storage were 60% for $P(0.01)$, 35% for A and 75% for H. For both the morphological and the isozyme markers, all three genetic diversity indices showed significant negative regressions ($P < 0.001$) on storage time (Figs 1 and 2). The only exceptional result was a slight increase in average gene diversity (H) of isoenzyme markers from the 40 to the 72 years accessions. This result was caused by the high H-value of one individual accession (HOR 7400) (Table 2).

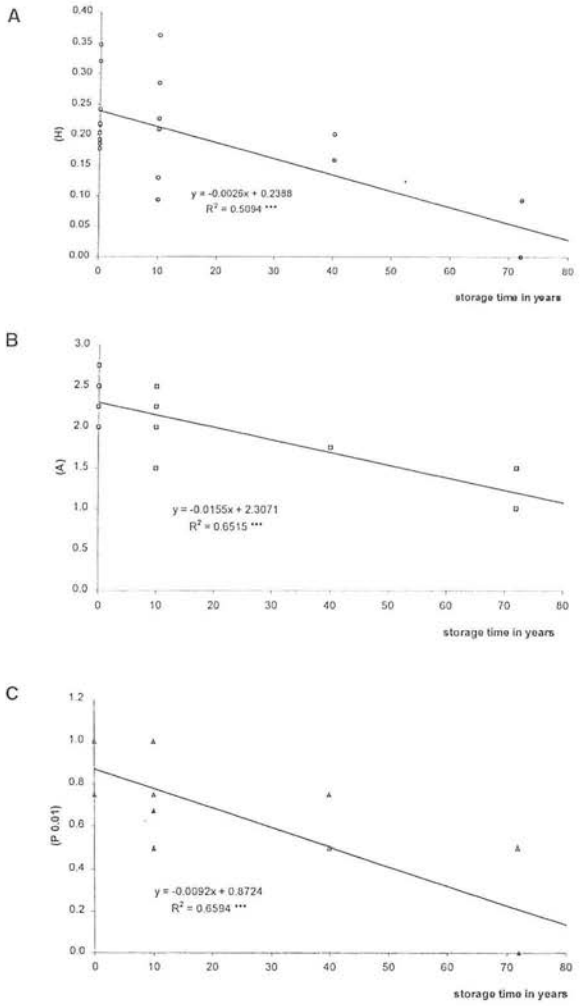

Fig. 1 Relationship between genetic diversity indices, measured using four morphological markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((H), Fig. 1 A); mean alleles per locus (A), Fig. 1(B); and percentage polymorphic loci using 1% criterion ($(P(0.01))$, Fig. 1(C).

Table 3 Genetic diversity indices for 22 individual accessions of barley landraces from Syria calculated from variation at four loci affecting seed morphology

Landrace code	Collection year	Years stored	Number of individuals (n)	Morphological diversity				
				H	SD(H)	A	SD(A)	P(0.01)
Aswad 5	1998	0	74	0.1771	(±0.2266)	2.3	(±0.5)	1.0
Aswad 6	1998	0	65	0.2157	(±0.2374)	2.0	(±0.8)	0.8
Aswad 7	1998	0	50	0.1918	(±0.2330)	2.3	(±0.5)	1.0
Aswad 8	1998	0	54	0.2172	(±0.2680)	2.0	(±0.8)	0.8
Aswad 9	1998	0	51	0.2186	(±0.2722)	2.5	(±1.3)	0.8
Aswad 10	1998	0	80	0.2413	(±0.2787)	2.8	(±1.0)	1.0
Aswad 11	1998	0	52	0.2025	(±0.2650)	2.3	(±1.3)	0.8
Aswad 12	1998	0	53	0.1855	(±0.2553)	2.0	(±0.8)	0.8
Aswad 3	1997	0	197	0.3469	(±0.3297)	2.8	(±1.0)	1.0
Aswad 4	1997	0	200	0.3207	(±0.2988)	2.5	(±1.3)	0.8
Abiad 3	1997	0	200	0.2422	(±0.2457)	2.0	(±0.0)	1.0
Aswad 1	<1988	10	58	0.2846	(±0.3096)	2.5	(±1.3)	0.8
Aswad 2	<1988	10	82	0.3630	(±0.3667)	2.5	(±1.3)	0.8
Aswad 20126	<1988	10	88	0.2259	(±0.2175)	2.0	(±1.0)	0.7
Abiad 1	<1988	10	62	0.0934	(±0.0624)	2.3	(±0.5)	1.0
Abiad 2	<1988	10	68	0.2086	(±0.2357)	2.3	(±0.5)	1.0
Abiad 20125	<1988	10	95	0.1308	(±0.2420)	1.5	(±0.6)	0.5
Black BLR 18882	1959	40	100	0.1588	(±0.2150)	1.8	(±1.0)	0.5
Abiad 18881	1959	40	100	0.2002	(±0.1840)	1.8	(±0.5)	0.8
White BLR 7667	1926	72	112	0.0000	(±0.0000)	1.0	(±0.0)	0.0
White BLR 7659	1926	72	100	0.0925	(±0.1082)	1.5	(±0.6)	0.5

H, gene diversity; A, mean alleles per locus; P(0.01) percentage polymorphic loci using 1% criterion.

Assuming a mean time between rejuvenation of 5.3 years, gene diversity (H) within accessions decreased on average by approximately 11% with every cycle of rejuvenation for both sets of markers. From the regression of $\log(H_t)$ on t, the genetically effective size of *ex situ* populations during their time in storage, estimated from the rate of decline of gene diversity, was $N_e = 4.7$ (Fig. 3).

Total diversity and its distribution among accessions

Total diversity levels (H_T) for combined accessions of a particular age held in gene banks show a decline with storage time (Table 5). Over 72 years storage H_T declined from 0.32 ± 0.08 to 0.12 ± 0.12 when measured with morphological markers and from 0.32 ± 0.07 to 0.11 ± 0.11 when isozyme markers were used. Measures of the proportion of genetic variation found among accessions relative to total genetic variation, F_{ST}, showed an increase in value with time of storage (Table 5). Thus total genetic variation was lower in older samples, and a greater proportion of this variation was distributed between rather than within samples. One exception to this trend was the group of accessions with a 72-year storage period scored for isoenzyme markers. The low F_{ST} value of this group is caused by a high H-value in one of the three accessions (HOR 7400, Table 2).

Discussion

The major finding of this study is that gene diversity within landrace accessions of barley in Syria is related to the time of collection of the accession. Genetic diversity is lower in early accessions than in accessions made more recently. An additional result is that a greater proportion of the total genetic variation is found between rather than within landrace accessions in older collections than in recent collections. These results are consistently found using both morphological and isozyme genetic markers.

A possible explanation for both these results is that the differences in genetic diversity and structure are due to genetic drift occurring during the rejuvenation cycles through which the *ex situ* collections have passed during their time in the gene bank. For this to be a convincing explanation a number of conditions must be met. The first is that the initial samples used to found the early gene bank accessions were large enough to include a high proportion of the genetic diversity present in the contemporary landrace populations. The second is that

Table 4 Diversity indices measuring mean genetic variation within 22 accessions of barley landraces stored in gene banks for identical time periods

Accessions with storage period of	Number of accessions	Morphological markers			Isozyme markers		
		H	*A*	*P*(0.01)	*H*	*A*	*P*(0.01)
0 years	11	0.2327 (±0.0544)	2.3 (±0.3)	0.89 (±0.10)	0.2874 (±0.0219)	1.9 (±0.1)	0.80 (±0.10)
10 years	6	0.2177 (±0.0988)	2.2 (±0.4)	0.80 (±0.19)	0.2688 (±0.0685)	1.9 (±0.23)	0.71 (±0.13)
40 years	2	0.1795 (±0.0292)	1.8 (±0.0)	0.65 (±0.21)	0.06505 (±0.0712)	1.5 (±0.07)	0.44 (±0.08)
72 years	3	0.0308 (±0.0534)	1.2 (±0.3)	0.17 (±0.29)	0.1015 (±0.1085)	1.5 (±0.1)	0.46 (±0.14)
All accessions	22	0.1962 (±0.0938)	1.6 (±0.9)	0.75 (±0.29)	0.2368 (±0.0988)	1.8 (±0.2)	0.70 (±0.18)

H, gene diversity; *A*, mean alleles per locus; *P*(0.01), percentage polymorphic loci using the 1% criterion. Standard errors are in parentheses. Values calculated from morphological markers and isozyme markers are shown separately.

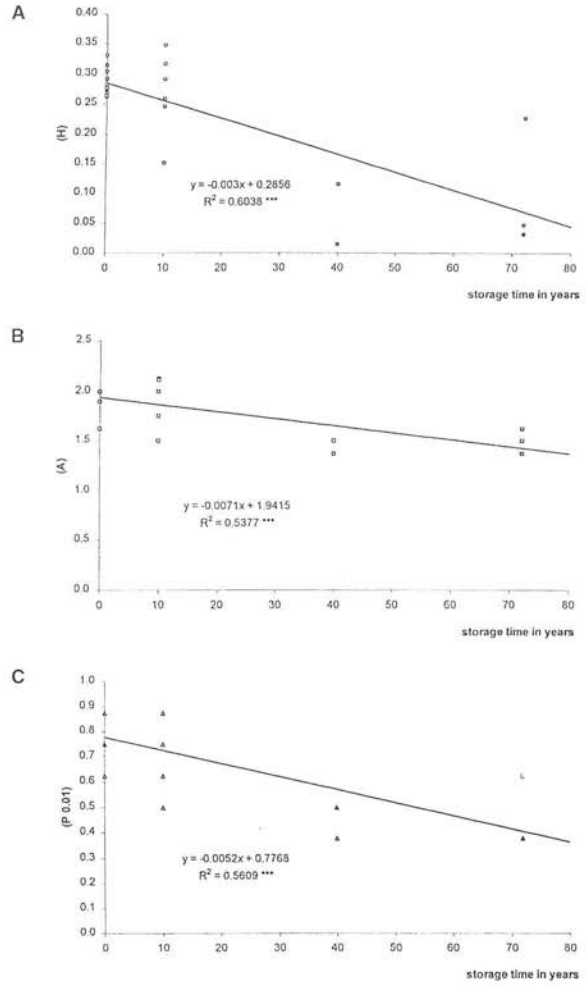

Fig. 2 Relationship between genetic diversity indices, measured using eight isozyme markers, and length of time for which barley landrace accessions have been held in gene banks. Indices of genetic diversity are gene diversity ((*H*), Fig. 2A); mean alleles per locus (*A*), Fig. 2(B); and percentage polymorphic loci using 1% criterion ((*P*(0.01)), Fig. 2(C).

there has been no systematic change in the gene diversity of the *in situ* landrace populations over time.

In the present study it is certain that the 10-year-old collections used were based on at least 50 seed heads and would have sampled a similar proportion of the genetic diversity available in landraces as present day collections (Marshall, 1990). Unfortunately details of the sampling procedures used to obtain the oldest accessions used in this study are not available. However the fact that such early collections were large enough to provide the material for extensive field trials demonstrating genetic heterogeneity within landraces,

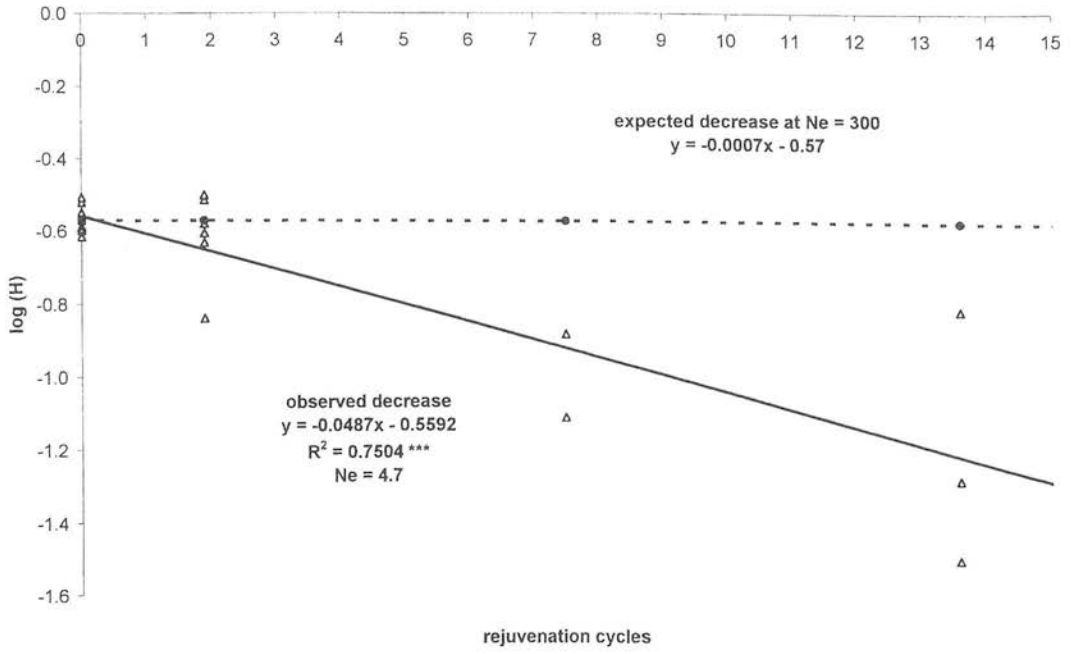

Fig. 3 Regression of log (gene diversity) (log(H)) on number of cycles of rejuvenation experienced by accessions of barley landraces in gene banks. The slope of the line is an estimate of log $(1 - 1/2N_e)$ where N_e is the effective size of rejuvenation populations.

Table 5 Total genetic variation H_T within collections of accessions held for particular time period, and genetic differentiation F_{ST} found among accessions within these collections. Results are shown separately for morphological and isozyme markers. Standard deviations are in parentheses

Accessions with storage period of	Morphological markers		Isoenzyme markers	
	(H_T)	(F_{ST})	(H_T)	(F_{ST})
0 years	0.3197 (±0.0823)	0.2722 (±0.0513)	0.3209 (±0.0670)	0.0929 (±0.042)
10 years	0.3204 (±0.1328)	0.3205 (±0.1258)	0.3266 (±0.1053)	0.2110 (±0.051)
40 years	0.2661 (±0.1850)	0.3254 (±0.0739)	0.2302 (±0.1856)	0.8477 (±0.078)
72 years	0.1242 (±0.1157)	0.7518 (±0.2482)	0.1127 (±0.0610)	0.2832 (±0.113)
All accessions	0.3549 (±0.0678)	0.4472 (±0.0564)	0.3594 (±0.0496)	0.3461 (±0.079)

suggests that they were not genetically depauperate at the time when they entered the gene banks (Vavilov, 1957). It therefore seems reasonable to conclude that observed differences in gene diversity among collections held for different lengths of time are unlikely to be accounted for by differences in initial sampling procedures.

The second assumption, that there has been no systematic change in the genetic diversity of *in situ* populations of barley landraces in Syria over the last 72 years, is supported by extensive fieldwork at ICARDA. This indicates the restricted exchange of barley landrace germplasm among climatic regions within Syria, and the minimal influence of modern barley cultivars on barley landraces in this region (Weltzien & Fischbeck, 1990; Ceccarelli, 1996). The continuity and stability of landrace cultivation in the area means that gene diversity levels in present day barley landraces are likely to be very similar to gene diversity levels in landrace populations grown throughout the last 72 years. If any changes have taken place over this time they are likely to have led to a reduction rather than an increase in gene diversity levels in contemporary landrace populations.

Comparison of gene diversity levels in accessions held for different lengths of time show decreasing levels of

genetic diversity with increasing length of storage, and this is consistent over diversity indices and genetic markers. If it is accepted that these differences were not caused by differences in initial sampling procedures or by a systematic increase in gene diversity over time within *in situ* populations, the observed decline of genetic diversity is most easily explained by the effects of genetic drift in rejuvenation populations used to maintain the accessions in the gene banks. The rate of decline of genetic diversity is consistent with a model of genetic drift in rejuvenation populations with an effective population size $N_e = 4.7$. In a predominantly inbreeding species where N_e is half the number of observed reproductive individuals, this is equivalent to a population of 9.4 unrelated adults contributing equally to the seed produced.

Such a low number of parents contributing to rejuvenated seed appears at odds with what is known of rejuvenation procedures in gene banks. Here accessions are grown on 3 m^2 field plots likely to accommodate 600 plants, and if all individuals contributed equally, effective population size would be $N_e = 300$. The expected decline in gene diversity every cycle of rejuvenation is negligible when the effective population size is as large as 300 (Fig. 3).

There are a number of factors that could account for this discrepancy between anticipated and inferred effective population size in the rejuvenation populations. Restricted sampling of the accession from the gene bank to establish the rejuvenation plot, and limited sampling of the seed produced from this plot could both contribute to a low value of N_e. In addition the differences in environmental conditions between the original site of collection and the rejuvenation plots may be so large that only a limited set of genotypes are able successfully to grow and produce seed. A large variance in the reproductive contribution of individuals will significantly reduce the effective size of a population (Lande & Barrowclough, 1987).

It should also be remembered that the value of N_e calculated relates to the genetic behaviour of accessions over a number of generations. The value of N_e in these circumstances is governed by the value of the smallest effective population size found over the time period in question (Lande & Barrowclough, 1987). Thus the low value of N_e could be accounted for by a single bottleneck event in the gene bank, rather than a chronically low effective population size at each round of rejuvenation.

If these estimated values of N_e in rejuvenation populations are reasonable, problems are likely to arise in gene bank collections not only as a consequence of loss of genetic variation, but also through fixation of deleterious mutations that are much more difficult to monitor (Schoen *et al.*, 1998). In this respect it is interesting to note that during growth of material for electrophoresis, chlorophyll deficient mutants were observed in accessions that had been stored for 10 years (Aswad 20126) and 72 years (white H.v. 7667) at frequencies of 1.1% and 3.8%, respectively, but not in any recent accessions. Computer simulations by Schoen *et al.* (1998) suggest that accumulation of plants homozygous for deleterious mutations will accompany recurrent rejuvenation of germplasm when effective population size is less than 75.

Another effect that is expected in gene banks, if genetic drift is occurring in rejuvenation populations, is that genetic differentiation among accessions with identical storage times should increase with time of storage. A trend of increasing values of F_{ST} with storage time is indeed seen for the barley landraces and this is clearest for the morphological markers. These show a greater than twofold increase in F_{ST} after 72 years in storage (Table 5). This change cannot be attributed to collection of a less diverse range of populations in more recent years because the present day collections cover the wide range of barley landrace sites within northern Syria.

The results are, however, as anticipated if genetic drift is occurring in gene bank accessions. The genetic variation originally present in the accessions collected at a particular time has not only declined overall (shown by a decrease in H_T, Table 5) but has become rearranged such that in older collections an increased proportion of the variation is distributed between accessions while less is found within accessions.

Simmonds (1962) has referred to gene banks as 'museum collections' because they contain a selection of ill-adapted genotypes (from the point of view of high input agriculture) which either cannot be preserved at all in the wild or can be preserved only with great difficulty and uncertainty. The present results suggest that gene banks face further problems because genetic drift during rejuvenation may lead to erosion of the original genetic diversity of seed accessions lodged in gene banks. The present study is only of a preliminary nature, relies on assumptions about early sampling procedures and continuity of landrace populations over time, and deals only with one crop species. However it suggests that more detailed empirical studies of genetic changes occurring during rejuvenation of gene bank accessions are justified. If genetic erosion proves to be a widespread phenomenon, changes in management are clearly required to obviate its undesirable effects and enable gene banks to serve as sustained reservoirs of crop genetic resources.

A rapid improvement of *ex situ* storage conditions and facilities (i.e. deep freezing of samples) would

facilitate maintenance of collections with fewer rejuvenation cycles. The use of larger rejuvenation plots and greater emphasis on adequate sample sizes for establishing and collecting from rejuvenation plots may also be important. To make the best use of old collections, in which genetic variation may now be found predominantly between rather than within accessions, it may be advisable to combine duplicate collections from a variety of different gene banks. Once a genetically variable collection had been amassed, this could be conserved *ex situ* with more modern techniques involving deep-freezing, possibly augmented by *in situ* conservation approaches.

The consistently high diversity indices of all recently collected barley landrace accessions found in this study is a clear justification for the use of *in situ* conservation strategies. However, given the inherent weaknesses of both systems it seems appropriate to promote crop conservation through a combination of *in* and *ex situ* conservation practices rather than favouring a single strategy.

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