EXPRESSION AND INHERITANCE OF TRAITS IN WILD MUNGBEAN (*VIGNA RADIATA* SSP. *SUBLOBATA*) X CULTIVATED MUNGBEAN (*V. RADIATA* SSP. *RADIATA*) HYBRIDS

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ABSTRACT

Mungbean (*Vigna radiata* (L.) Wilczek ssp. *radiata*) is an economically important crop in Asian countries where breeding research is being undertaken to improve varietal adaptation, yield and seed quality. The wild mungbean (ssp. *sublobata*) is a potentially useful adjunct to breeding, as the wild accessions possess traits that confer adaptation in their natural environments. The wild accession ACC 87 collected near Townsville has been identified as being perennial, a potentially useful trait for forage crop improvement. Accession ACC 1, from Mackay is very late flowering and was reported to possibly have a long juvenile (LJ) trait similar to that found in soybean. Before these and other potentially useful wild traits can be exploited, information is needed on their inheritance. Therefore, a study was conducted to examine the inheritance of traits in four hybrid cultivated X wild mungbean populations. The study examined the expression of traits in the parental plants, and the F_1, F_2, BC_{P1} and BC_{P2} progeny generations, when grown under controlled conditions in pots. The four genetic populations had been created by hybridizing using two cultivated mungbean varieties, Berken and Kiloga, with each of two wild parents, ACC 1 and ACC 87.

Several morphological traits, including lobed leaflet shape, seed testa and hilum color, and plant habit were found to be under simple (qualitative) genetic control, with the wild type generally dominant. An exception was putative resistance to powdery mildew infection in the wild accessions, which appeared to be recessive. Many other traits like phenology, nodes per plant, seed yield and biomass were under quantitative genetic control. The perenniality trait in ACC 87 appeared to be under simple genetic control, with expression of perenniality due to two dominant complementary genes. In contrast, flowering in the two ACC 1 populations appeared to be quantitatively inherited, with no evidence of a LJ trait. There were many similarities in the genetic control of both qualitative and quantitative traits among the four hybrid populations, with only small differences due to the different cultivated parents. However, larger differences were apparent between the populations involving ACC 1 and ACC 87.

Estimates of narrow sense heritability were high for many of the qualitatively inherited traits, indicating high additive genetic variance for those traits, and thus the capacity for genetic gain through selection. Transgressive segregation occurred for most of the quantitative traits in one or more of the four crosses, indicating the potential value of the wild germplasm in broadening the phenotypic range available to plant breeders. Several phenotypic and genotypic interrelations found between many of the wild traits among the four crosses. In particular, there were several significant genetic correlations among quantitative traits, indicating that selection for one of the traits should result in genetic advance in the other.

The study confirmed earlier research that Australian accessions of the wild mungbean can be considered part of the primary gene pool of the cultivated mungbean. Consequently, the wild accessions provide an additional source of triats potentially useful for mungbean improvement. The study also established that traits of possible commercial interest, perenniality and powdery mildew resistance, were qualitatively inherited and thus should be readily transferrable into cultivated varieties. While the study failied to identify the presence of a LJ trait, it suggested that the wild germplasm could be a useful source of lateness genes for breeding vegetatively vigorous forage or cover crop varieties of mungbean.

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STATEMENT OF SOURCES DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given

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CHAPTER 1: INTRODUCTION

Grain legumes play a vital role in food security as well as the sustainability of agricultural systems, particularly in developing countries. They also make a major contribution of proteins and lipids for more than two billion people worldwide (Singh and Singh 1992, Popelka *et al.* 2004, Kaviraj *et al.* 2006). Additionally, the world demand for grain legumes has been increasing because vegetarian diets have become a growing trend (Kaviraj *et al.* 2006). Grain legumes rank second behind cereals as a source of human food and animal feed and rank third for world crop production after cereals and oilseeds (Desai *et al.* 1997, Popelka *et al.* 2004). Similarly, grain legumes including soybean (*Glycine max*), mungbean (*Vigna radiata*) and peanut (*Arachis hypogea* L.) have the third highest priority for crop developments in Vietnam after rice (*Oryza sativa*) and maize (*Zea mays*) (Nguyen *et al.* 1996). Furthermore, pulses are two to three times richer in protein than cereals (Desai *et al.* 1997). Most of the proteins in legumes are generally high in lysine, but low in methionine, cysteine and / or tryptophan (Desai *et al.* 1997, Popelka *et al.* 2004). However, when they are combined with cereals, legume proteins provide a balanced diet. Furthermore, pulses supply variety of alternative food for people who have limited access to animal protein (Summerfield and Lawn 1987) or for cultural or religious reasons, cannot consume it.

The genus *Vigna* is one of the more economically important genera within the Papilionaceae, containing various vital agricultural species such as cowpea (*V. unguiculata*), mungbean (*V. radiata*), black gram (*V. mungo*), adzuki bean (*V. angularis*) and rice bean (*V. umbellata*), plus several forage, root, cover and vegetable crops. Mungbean or green gram is an important grain legume, particularly in Asia, where it is widely used for dhal, starch, flour and bean sprouts. Additionally, mungbean is also an important emerging crop for summer cropping areas in northern Australia (Lawn and Russell 1978), where it is grown in rotation with sugarcane and other crops. Moreover, mungbean is well-known as a major source of nitrogen fixation created by *Rhizobium* bacteria attached on roots. It has been estimated that worldwide annual production is 2.5-3 million tons per year (Poehlman 1991). However, the area and yield for individual grain legumes, apart from soybean (*Glycine max* L.), are not separately recorded in national agriculture statistics. Mungbean in China is grown on approximately 1 million ha and production is increasing (Tomooka *et al.* 2005). In Australia, the mungbean growing areas have increased in recent decades. The mungbean growing areas in Australia were about 41 thousand ha in 2004, but it has increased up to 72 thousand ha in 2011 (ABARES (2010) for years 2004 to 2009; Dale Reeves, AMA for years 2010 and 2011).

Mungbean belongs to the Asian subgenus Ceratotropis. Taxonomically, subgenus Ceratotropis has been divided into the following three sections: Ceratotropis, Angulares and Aconitifoliae. Section Ceratotropis also includes the South Asian cultigen black gram *V. mungo* (L.) Hepper (Tomooka *et al.* 2002). The wild mungbean, *V. radiata* ssp. *sublobata*, the putative progenitor of the cultivated mungbean, is an indigenous species throughout the moderate to higher rainfall areas of tropical and subtropical Australia (Lawn and Cottrell 1988). The species is widespread, ranging from southern Africa, across southern Asia, where it is believed to have been domesticated, eastern Asia, and the islands of Indonesia, Australia and Papua New Guinea, as far east as western Oceania (Lawn and Cottrell 1988, Tomooka *et al.* 2002). Archaebotanical finds and literary records suggest that mungbean was domesticated in India where wild mungbean is widely distributed (Smartt 1990, Tomooka *et al.* 2002). Archaeobotanical evidence points to both south-eastern India between the Godavari and Krishna rivers and western Himalayan foothills as likely places where domestication could have occurred (Fuller and Harvey 2006).

Lawn and Watkinson (2002) reported that a large collection of accessions has been assembled as part of a project to collect, describe and conserve the several indigenous/endemic *Vigna* species in Australia. Included in the collection are more than 120 accessions of the wild mungbean, primarily from mainland Australia, but also from nearby islands of Indonesia and Papua New Guinea with a few from more remote locations including India. The wild species represents a potentially useful addition to the germplasm of cultivated mungbean, by extending the range of variation available to breeders for traits of interest, notably pest resistance (e.g. Lambrides and Imrie 2000) and adaptations to the environment. Most wild traits are not useful agronomically. However, there are some traits in wild mungbean that may be useful. For example, recent reports identify several novel but potentially useful traits in the wild germplasm, including a putative 'long juvenile' photoperiodic trait (Rebetzke and Lawn 2006a), indeterminate habit, root tubers (Rebetzke and Lawn 2006b,c), and high seed protein (Lawn and Rebetzke 2006).

According to Sleeper and Poehlman (2006), one of the basic strategies in plant breeding is to search out genes that encode for useful traits from cultivated species and their close relatives, and combine these into improved varieties through hybridization, recombination and selection. It is important to understand how both useful and unwanted traits are inherited and the development of an understanding of inheritance patterns and segregation ratios is thus an important step in crop improvement. To date, there have been a number of studies on the inheritance of mungbean traits. Early studies on the topic were broadly reviewed by Fery (1980). Since then, further information on mungbean genetics has been reported in several studies (e.g. James *et al.* 1999, Khattak 2001a,b,c,

2002a,b, 2004; Sriphadet *et al*. 2007, Sukhumaporn 2009). There have been fewer studies on the inheritance of wild *vs.* cultivated mungbean traits, particularly for novel wild traits.

To sum up, *Vigna radiata* is an important grain legume that appears to possess several multi-purpose attributes that make it a potentially valuable species for improving food security in village agriculture and could contribute benefits to agricultural sustainability in the tropics. There may useful trais in wild forms of mungbean, including those that have been collected in Australia. The present research was conducted to determine the inheritance and expression of several wild physiological traits in Australian wild accessions, to provide further basic information on mungbean genetics for the developments of breeding and genetic biotechnology programs.

To that end, the current state of knowledge concerning the use of crop wild relatives is reviewed in the following chapter, with the specific objective of identifying areas where additional knowledge is needed to underpin future improvement research on mungbean. Then, in Chapter 3, two experiments designed to develop additional knowledge on the inheritance and expression of several wild physiological traits are described. The results of those experiments are respectively presented in Chapters 4 and 5. Finally, in the concluding chapter (Chapter 6), the similarities and contrasts in the results of the two experiments are discussed with reference to previous studies, and some implications for mungbean breeding are suggested.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

The concept of crop wild relatives (CWRs) was interpreted by Heywood (2005) as encompassing wild species that are more or less closely related to useful plants (i.e. food and fodder crops, forestry trees, ornamental and industrial crops and other useful species such as medicinal and aromatic plants) to which they may contribute genetic material. The relationship of CWRs to useful species is often defined in terms of their membership of the primary or secondary gene pools for that species, or their level of taxonomic affinity. The wild ancestors initially contributed the genetic characters that enabled crops and other useful plants to adapt to their environment and to provide useful products. Their wild descendants continue to contribute to modern varieties today (Kell *et al*. 2008, Maxted *et al*. 2006, 2007, 2008).

The importance and utilization of CWRs have been widely canvassed e.g. Hajjar and Hodgkin (2007), Kell *et al*. (2008), Maxted *et al*. (2006, 2007, 2008). As noted by Heywood (2007) "the genes that come from crop wild relatives make a direct contribution to increased production, food quality and human wellbeing through poverty alleviation, thus contributing to the attainment of the Millennium Development Goals". This review explores the nature and extent of wild genetic diversity and the utilization of CWRs for plant breeding and crop improvement. The main focus of the review is on the current status of CWRs, and their evaluation and use in crop breeding programs. Where practicable, emphasis is placed on mungbean and related crops.

2.2 Genetic Resources of Wild Relatives

During the last century, the natural diversity of crops received increasing consideration by botanists, agricultural scientists, and propagators, because of the potential benefits to be derived through plant exploration, taxonomy, physiology and plant breeding (Hawkes 1971). The natural diversity of crop plants, whether before, during or after their domestication, is commonly referred to as 'genetic resources', which in turn comprises the sum total of genes, gene combinations, or genotypes available for the genetic improvement of crop plants (Gepts 2006). An understanding of the nature and extent crop plant diversity is fundamental to understanding plant genetic resources. The importance of genetic diversity for current and future plant breeding was emphasized by Gepts (2006).

Collectively, the cultivated and wild genetic resources form the available gene pool of a crop. Harlan and de Wet (1971) defined three gene pool categories, based on the extent of obstacles to sexual

hybridisation and attaining fertile progenies. Their proposal was based on the practical reality that the capacity for hybrisation determines the extent that various CWRs might used to introduce new genetic diversity into a breeding population. Based on more recent developments in biotechnology, Gepts (2000) proposed that an additional gene pool category (Gene Pool IV) be added for the full potential of biodiversity to be realised.

In the cultivated legumes, Singh and Chung (2007) suggested that Gene Pool I encompasses the cultigen species, land races and weedy relatives. Crosses within this category can generally be achieved readily and the resulting progeny is usually viable and fertile. Gene Pool categories II and III comprise other species that are related, yet different from the crop of interest. With Gene Pool II species, hybrids with the crop can be attained; however, hindrances may make gene transfer difficult, or hybrid progeny may show reduced self-fertility. With Gene Pool III, which usually comprises related wild species, initial success in performing cross-hybridizations may be possible, but the hybrid seed may then die before maturity. Hence this gene pool requires special techniques, for example, *in vitro* culture of hybrid seed embryos, or the use of bridging species, to facilitate genetic transfer back to the cultigen species.

In the pulse crops, the wild progenitors, which are usually cross-fertile with the cultigen, are variously allocated varietal, sub-species or even separate species rank, depending on the taxonomist (Smartt and Hymowitz 1985). These authors propose that for consistency, the wild progenitor for each pulse species should be considered a sub-species of the cultigen. Thus for example, the wild mungbean would be *Vigna radiata* ssp. *sublobata*. This terminology is adopted in this study.

2.2.1 Centers of Diversity

A major contribution to understanding the origins of agricultural species was made by Candolle (1882). Subsequently, Vavilov (1926) introduced the concept of ‘centers of origin’ for agricultural species based on the diversity within those regions, and argued the case for conservation of plant genetic resources. Ultimately, the concept evolved into ‘centers of diversity’ (Harlan 1976). The development of these concepts was significant in two ways. Firstly, it showed that crop domestication independently happened in different areas of the world. Secondly, it demonstrated that such domestications were relatively rare events - hence the small number of primary centres of diversity (Murphy 2007). Following Vavilov’s (1926) approach, there have been significant advances in defining the origins of many species.

In the case of mungbean (*V. radiata*) and black gram (*V. mungo*), Jain and Mehra (1980) and Smartt (1985) reported that both were domesticated in the Indian subcontinent, with archaeological evidence

suggesting that their consumption there dates back at least 3500 years. The wild mungbean (*V. radiata* ssp. *sublobata*) is widely distributed from tropical Africa through southern Asia into south-east Asia, Austronesia and Oceania (Verdcourt 1970). The wild mungbean is indigenous in Australia and has long been used as a root crop by the Australian Aborigines (Lawn and Cottrell 1988).

2.2.2 Domestication

According to Pickersgill (2007) domestication is generally considered to be "the end-point of a continuum that starts with exploitation of wild plants, continues through cultivation of plants selected from the wild but not yet genetically different from wild plants, and terminates in fixation, through human selection, of morphological and hence genetic differences distinguishing a domesticate from its wild progenitor." A broadly similar definition, provided by Gepts (2004), is that domestication is "the outcome of a selection process leading to increased adaptation of plants and animals to cultivation or rearing and utilization by humans, whether as farmers or consumers." Nonetheless, domestication is a multi-stage process (Ford 1985, Harris 1989, 1996). Harris (1989) divided the process of domestication into four stages: (i) wild plant food collection (hunting and gathering), (ii) wild plant food production (the start of cultivation), (iii) systematic cultivation of wild plants and (iv) agriculture based on domesticated plants. Throughout the process, plants become more suited to human needs and more productive, but less adapted to natural survival and so more dependent on humans (Fuller 2007).

Ford-Lloyd and Jackson (1986) noted that the process of making plants more acceptable to humans has resulted in a large number of changes in phenotype and in reproductive biology. Evans (1993) argued that these changes are associated with underlying genetic changes, which arise through repeated cycles of selection and culture in different environmental conditions in different regions. These genetic changes occur not as a single event, but as part of a continuing evolutionary process. Gepts (2004) proposed that the progress of plant domestication depends on three factors and their interaction: humans, plants, and the environment. It was argued by Diamond (1997) that crop domestication is among the greatest technological advances in human history, enabling the efficient, reliable production of food on a small land area.

According to Pickersgill (2007), plants vary within and between species in their degrees of domestication, with innumerable varieties, races and cultivars of agricultural plants developed to support human and animal demand for food, fibre and building materials as well as other purposes. Zeven and de Wet (1982) estimated that a total of 2489 species from 173 families have been disseminated as domesticates. The Graminae and Leguminosae together represent almost a third of all domesticates, with 379 and 337 species respectively. The proportion of species that has been

domesticated varies greatly between families (Evans, 1993). For example, about 8.3% of the species of the Cucurbitaceae have been domesticated, but only 2.8% of the Leguminosae (Zeven and de Wet 1982).

According to Smartt (1978), grain legume crops have been found from early times in archaeological sites of both the Old and New World, and they appear to be among the earlier plants domesticated. Soybean (*Glycine max* (L.) Merr.) was domesticated in China where, according to records, it has been in cultivation for over 3000 years. Since the process of domestication takes place over time, Hymowitz (1970) suggested that for soybean this may have occurred during the Shang Dynasty (1700-1100 BC) or even earlier. Evans (1993) reported that mungbean was domesticated in Madhya Pradesh, India, 3700 BP, while cowpea (*V. unguiculata*) was domesticated in Kintampo, West Africa 3400 BP. However, different centres of diversity and origin of the cowpea have been proposed e.g., Ethiopia (Pasquet 2000), West Africa (Vaillancourt and Weeden 1992) and Eastern and Southern Africa (Baudoin 1985). No exact information exists about when and where *V. vexillata* was first domesticated, although recent research suggests that it occurred independently in Africa and Asia (Damayanti *et al.* 2010c).

2.2.3 Genetic Variability in Wild Species

There are currently ~5600 accessions of cultivated mungbean conserved by the Asian Vegetable Research and Development Center (AVRDC) in Taiwan, the main international mungbean germplasm centre (Tomooka *et al.* 2002). These accessions have been collected from throughout the Old World tropics where mungbean is widely grown, but are dominated by collections from south Asia and east Asia. The natural geographical range of the wild mungbean is even wider than that of the cultigen, and encompasses a range of climatic, edaphic and biotic conditions. It has diversified into numerous ecotypes which are of potential use for crop adaptation enhancement. Several large collections of wild mungbean accessions by Australian (Lawn and Watkinson 2002, Lawn and Rebetzke 2006) and Japanese (Vaughan *et al.* 2006, Tomooka *et al.* 2006a,b) scientists have added to the wild genetic resources of mungbean.

In Australia and nearby islands, wild mungbean has been collected from riverbanks, savannah grassland and lightly wooded areas (Lawn and Watkinson 2002, Lawn and Rebetzke 2006). The genotypic variation in a range of phenological, morphological, seed and adaptive traits within the wild mungbean has been described (Rebetzke and Lawn 2006a,b,c). In terms of morphological attributes, wild mungbean from clay soils of central Queensland, where an extended dry season occurs, were found to have well developed taproots (Lawn and Watkinson 2002). Meanwhile, a perennial tuberous form was found in the Townsville - Charters Towers region of Queensland (Rebetzke and Lawn

2006a). In contrast, wild mungbean from other habitats, such as permanently wet alluvial banks of the Sepik River of Papua New Guinea, had extensive fibrous roots (Sangiri *et al.* 2007).

Lawn and Rebetzke (2006) reported that there was very wide variation in time to flower among Australian wild mungbean accessions. Subsequent studies (Rebetzke and Lawn 2006b) indicated this variation arose because of differences in sensitivity to photoperiod and temperature among accessions, depending on their latitude of collection. In general, accessions collected from lower latitudes than the test site were more sensitive to variation in photoperiod compared with accessions that were collected at higher latitudes than the test site. One late flowering accession (ACC 1) was identified which appeared insensitive to photoperiod (Rebetzke and Lawn 2006b). These authors suggested the accession perhaps could be a source of long-juvenile genes like that used in soybean to reduce photoperiod sensitivity and broaden adaptation to latitude (Lawn and James 2011).

Hardseededness, which is a potentially useful trait for weathering damage resistance, is variable and incomplete in cultivars, but is durable and near absolute among the wild accessions. This trait may be primarily due to a single dominant gene (Lawn 1988). Large genetic variation has also been reported for pod length in wild mungbean accessions (Biswas and Bhadra 1997). Among diverse mungbean genotypes, Sharma and Gupta (1994) reported a positive correlation between pod length and yield per plant, although other authors (e.g. Chhabra *et al*. 1991, Islam *et al*. 1999) have reported multiple correlations between yield and its component traits in mungbean.

Other useful traits that may be found in wild germplasm include tolerance of adverse soils conditions, and resistance to pests and diseases. For example, Lawn (1988) reported that accessions of wild mungbean collected in West Timor, Indonesia, were more tolerant of calcareous soil conditions than introduced cultivars. James *et al.* (1999) reported resistance to bruchid seed weevils in and Australian wild mungbean accessions although subsequent studies (Lambrides and Imrie 2000) suggested this may have been partly due to small seed size.

2.3 Evaluation of Crop Wild Relatives

Evaluation of CWRs is an essential preliminary step to their utilization in plant breeding (Frankel and Soule 1981), as it allows genotypes to be compared and those with useful traits to be identified for later use. Many different methods are available for the purpose of screening, including traditional field methods and modern laboratory *in vitro* techniques. Irrespective of the potential value of a trait, it is of limited use when it occurs in a species that is distantly related and/or genetically incompatible with the species being improved. Therefore, it is important when assessing the potential utility of CWRs that early consideration be given to their taxonomic relation to the crop in question, since traits

from distantly related genotypes may operate differently in an alien genome and plasmon (Smartt 1981).

2.3.1 Taxonomic Evaluation

Taxonomy underpins many aspects of the management of genetic resources (Harlan and de Wet 1971). Firstly, it allows priorities to be developed for which sets of CWRs need to be conserved because of their close relations with crop species. Secondly, it allows clearer definition of the CWRs that should be considered for conservation, and their boundaries. Taxonomy also allows clearer communication between scientists, allowing them to exchange germplasm and to describe its properties on the basis of a shared understanding of its identity.

In broad terms, taxonomy reflects the accumulation of differences in the genome between different organisms to an extent that they are recognized as different taxa. That said, taxonomy is not an exact science, and often, different criteria and/or different procedures are sometimes used to distinguish between taxa (Hanelt 1986). While traditionally, taxonomy has been based on morphological characters, data generated using molecular techniques for taxonomic inferences has provided new insights into the phylogeny and taxonomy of many plant groups (Rao and Hodgkin 2002).

Historically, taxonomic knowledge has been empirical, and where crop breeders were not familiar with CWRs, they were often misled by inappropriate taxonomies (Li 1974). One of the major problems that arose in genetic resources work was the apparent inability to satisfactorily classify crop collections (Bedigian *et al.* 1986). For instance, teostinte, the wild progenitor of maize, has been variously assigned by taxonomists to the genus *Euchlaena*, to *Zea*, or to a race or subspecies of *Z. mays*. Confusions like this can deter the use of CWRs, especially when the crosses made with them exhibit problems of sterility and deleterious genes (Smartt 1984).

Harlan and de Wet (1971) believed that more biologically realistic taxonomic classifications would lead to a better understanding of evolutionary relationships between wild and cultivated species. Likewise, Maxted *et al.* (2006) indicated that taxonomic distance may be positively related to genetic distance, and that classical taxonomy, for practical purposes, remains a desirable means of estimating genetic relationships. In this context, the gene pool and taxon group approach to taxonomy can help reduce the confusion and misdirection of effort by crop breeders, as it provides an approximation of the relative ease of hybridization between and within the crop and the wild species (Maxted *et al.* 2006).

2.3.1.1 Mungbean taxonomy

Initial taxonomic confusion was evident within the legume tribe *Phaseolae* with many species of the genus *Phaseolus* being morphologically and cytogenetically more similar to *Vigna* and following several revisions during the period 1950-1970, many *Phaseolus* species were transferred into *Vigna* (Lawn 1995). Importantly, as a result of these revisions, the Asiatic species of *Phaseolus*, which included the mungbean (previously *P. radiatus* L. syn. *P. aureus*), black gram (previously *P. mungo* L.) and related pulses were reclassified as *Vigna* species within the sub-genus *Ceratotropis* (Verdcourt 1970). It was considered that this relocation would make the potential utility of the former *Phaseolus* species more obvious in hybridization studies (Smartt 1985).

2.3.1.2 Mungbean cytotaxonomy

According to Smartt (1980), chromosomal differences produce an effective isolating mechanism, and direct observation of the genome and its karyotype should indicate potential problems in chromosome pairing and behaviour in a cross. It has proved useful for identifying wild progenitors and polyploidy, and can contribute to understanding evolution of cultivated species (Smartt 1980). In the case of mungbean, cytogenetic and cross-hybridisation studies have been summarized (Jain and Mehra 1980, Smartt 1985, Baudoin 1985). Generally, there is substantial chromosomal homology between the species within sub-genus *Ceratotropis*, with most species exhibiting a 2n = 22 karyotype. Inter-specific hybrids can be obtained, if occasionally with some difficulties, between a wide range of pairwise combinations. The mungbean is generally cross-compatible with the wild mungbean, and to some extent, with black gram (Lawn 1995, James *et al.* 1999).

2.4 Utilization of Crop Wild Relatives

The potential value of CWRs for crop improvement was indicated by Vavilov in the early 20th century (Vavilov 1926). Probably the first documented use of CWRs occurred in the development of high-yielding, high-sugar varieties of sugar cane in the 20th century (Hajjar and Hodgkin 2007). The value of CWRs became more widely accepted in breeding programs of main crops in the 1940s and 1950s (Plucknett *et al.* 1987, Hodgkin 1992) and the wider use of wild genes in improvement of a range of crops gained prominence in the 1970s and 1980s (Hoyt 1988). The use of CWRs in plant breeding was broadly canvassed by Maxted *et al.* (2008). CWRS can provide characters of considerable use to agriculture, and modern cultivars of most main crops already inherit genes from them. Harlan (1965) noted that CWRs were instrumental in the productivity and stability of traditional agro-ecosystems, through the natural genetic exchange (gene introgression) between landrace crops and their wild, weedy relatives.

2.4.1 Crop Improvement

Hajjar and Hodgkin (2007) grouped advances in crop improvement according to four main functional categories: providing resistance to pests and/or diseases or abiotic stresses; increasing yield; providing cytoplasmic male sterility or fertility restorers for use in producing hybrids; and improving quality traits of the crop. They noted that modern breeding techniques have enabled the selection and incorporation of specific desirable traits such as resistance to pests and diseases, tolerance of drought, salinity and other abiotic stresses and ability to achieve higher yields and improved quality for all types of crops. They also noted that in the process of domestication, a crop goes through a genetic bottleneck, ending up with much less genetic variation than is available in the wild species. This genetic uniformity can make crops more vulnerable to novel biotic and abiotic stresses.

The main strategy for using wild relatives for crop improvement usually relies on the transfer of specific traits, particularly for increasing resistance to insect pests and diseases. For instance, Malik *et al.* (2003) used the wild diploid D-genome progenitor of wheat to improve resistance against wheat curl mite (*Aceria tosichella*). Other examples where genes from wild crop were used for disease resistance include late blight in potato (*Phytothora infestans*) (Pavek and Corsini 2001) and grassy stunt disease in rice (Brar and Khush 1997). Faroq *et al.* (2001) used the wild progenitors of wheat to enhance tolerance tolerance to drought while Sheehy *et al.* (2005) researched the potential for improving heat tolerance in rice using wild rice species. CWRs have also been used to improve the nutritional value of some crops, such as protein content in durum wheat (Kovacs *et al*. 1998).

With mungbean, there have been several reports on hybridisation of wild species with domesticated accessions, as a precursor to using these species for crop improvement purposes. The wild mungbean hybridizes readily with the cultivated mungbean, with normal inheritance for a range of qualitative and quantitative traits (e.g. Singh *et al.* 1983, James *et al.* 1999). A study on genetic diversity of the mungbean gene pool revealed more polymorphisms in the wild than in the cultivated mungbean accessions (Sangiri *et al.* 2007). However, for other wild relatives of mungbean within sub-genus *Ceratotropis*, such as black gram, ricebean bean (*V. umbellata*), moth bean (*V. aconitifolia*) and adzuki bean (*V. angularis*), genetic isolation barriers exist (Jain and Mehra 1980). For example, crossability in terms of pod set percentage was in the range of 0.005 – 29.63% when mungbean used as female parent was crossed with several related wild species (Bharathi *et al.* 2006). Cultivated mungbean is not cross-compatible with any of the Australian native *Vigna* species apart from wild mungbean (Lawn and Rebetzke 1991).

2.4.1.1 Yield potential and quality

Both yield and quality are frequent targets for improvement, and both traits usually the integral effects of number of additional attributes (Simmonds 1978). In the simplest terms, yield can be considered the product of two components, the accumulated biomass and the relative partitioning of biomass into economic yield (Harvest Index). Both of these components in turn depend on numerous other traits, many of which are subject to environmental factors. Attributes of quality are also often diverse, and may include improvement of chemical composition (flavour, protein, carbohydrate and vitamin content), fibre content or even colour and shape in ornamental flowers (Allard 1999).

It is in relation to environmental stresses that CWRs have most potential, through the improvement of seasonal adaptation, tolerance of adverse environmental factors, and improved pest and disease resistance (Smartt 1984). CWRs usually exhibit undesirable agronomic traits (Hajjar and Hodgkin 2007) and hence few examples of wild genes being used to improve yield in modern cultivars have been published. Indeed, according to Hajjar and Hodgkin (2007), most reports of yield improvement of crop cultivars have been achieved through the flow-on effects of other desirable characters, including biotic or abiotic stress tolerance, provided by the wild species. For example, Hajjar and Hodgkin (2007) noted that chickpea cultivar 'BG1103' yields about 40% more than competing cultivated lines, but this improvement arose because of wild genes that contributed increased drought and temperature tolerance, rather than specific direct effects on yield.

Other examples where CWRs have been used or proposed for use in crop breeding include the use wild genes from *Phaseolus* species in order to improve yields of common bean (*P. vulgaris*) cultivars (Kell *et al.* 1998), where varieties with improved yield were created from a Colombian wild bean. Additionally, Gur and Zamir (2004) reported 50% improved yield of tomato cultivars deriving from a green-fruited wild relative. Wild sorghum species e.g. *S. arundinaceum*, have been suggested as a source of genes to improve yield of cultivars (Hajjar and Hodgkin 2007). In mungbean, Lawn and Rebetzke (2006) reported finding some wild mungbean accessions with seed protein contents above that of cultivated varieties (31% vs 28%), and suggested they may provide a source of useful genes for this trait.

2.4.1.2 Pest and disease resistance

Scientists continuously try to find genes conferring resistance to key plant pests and diseases (Brar and Khush 1997) and to date, the most widespread use of CWRs has been as sources of disease resistance (Prescott-Allen 1986). Many successful examples of the utilization of CWRs in breeding new varieties of crops with wild gene resistance to pests and diseases were provided in the review by

Hajjar and Hodgkin (2007). For instance, in sunflower (*Helianthus annuus* L.), wild genes for resistance to downy mildew (*Plasmopara halstedii)*, rust (*Puccinia helianthi*) and broomrape (*Orobanche cumana*) were derived through cross-hybridisation from wild accessions and from the wild species, *H. praecox*. Examples of wild progenitors providing genes for resistance to pests or diseases given by Prescott-Allen (1986) included the development of rice (*O. sativa*) cultivars resistant to grassy stunt virus using genes from the wild species *O. nivara*, and the use of *Solanum demissum* to confer resistance to late blight (*Phytothora infestans*) in potato (*S. tuberosum*).

Examples from the crop legumes include soybean, where Riggs *et al.* (1998) reported that the wild perennial species *G. tomentella* had genes conferring resistance to cyst nematode. Similarly, in chickpea (*Cicer arietinum*), resistance to root lesion nematodes (*Pratylenchus negletus*, *P. thornei*) and *Phytophthora* root rot were found in the wild species *C. reticulatum* and *C. echinospermum* and this is being transferred into cultivars through a backcrossing program. Various *Phaseolus* CWRs were being screened for resistances to web blight, rust, white mold, bean golden yellow mosaic virus, bruchids and other seed storage insects (Singh 2001).

In mungbean, various levels of resistance to powdery mildew (*Podosphaera fusca*) have been observed in weedy landraces (Yohe and Poehlman 1972, Tickoo *et al*. 1988). Working on powdery mildew genetics of mungbean in Thailand, Khajudparn *et al.* (2007) reported that resistance appeared to be conditioned by two dominant genes whereas Kasettranan (2009), Gawande and Patil (2003) and Chaitieng *et al.* (2002) found resistance appeared to be controlled by more than one gene with both additive and dominance gene action. In India, Reddy *et al.* (1994) reported two separate genes, *Pm1* and *Pm2*, controlled resistance to powdery mildew under managed environmental conditions using field isolates of powdery mildew. In quantitative trait loci (QTL) mapping, using cultivated X wild hybrid mungbean populations, Young *et al.* (1992) found three RFLP markers linked with powdery mildew resistance. Humphry *et al.* (2003) reported that there was a major QTL conferring resistance, although they used different sources of resistance. Using QTL markers, a single locus was identified that explained up to 86% of 147 recombinant inbred individuals created from Berken x ATF3640 cross in which the resistance response to powdery mildew was evaluated. In *V. vexillata,* James and Lawn (1991) reported that resistance to powdery mildew found in a wild African line, CPI 16683, was due to a single dominant gene.

Working on resistance of mungbean to bruchid (*Callosobruchus* spp) seed weevils, Lambrides and Imrie (2000) reported that three wild mungbean accessions, the exotic accession TC1966 and two native Australian accessions, ACC 23 and ACC 41, showed zero or low levels of damage in bioassays with two bruchid species. Earlier studies by Kitamura *et al*. (1988), reported that TC1966 contained a single dominant gene controlling resistance to *C. chinensis*. Likewise, an initial study by James *et al.*

(1999) involving crosses with cultivars suggested that bruchid resistance in ACC 41 was due to a single dominant gene.

2.4.2 Adaptation to Environmental Stress

According to Evans (1993), domestication regularly led to relocation of crop plants away from their initial centre of origin, and the movement or transport away has been seen by some as an essential element of the domestication process. Burkill (1952) regarded the relocation of plants to new regions as a factor enforcing conscious sowing and planting. In new environments, plants are likely to be exposed to conditions somewhat different to where they first developed, although movement to different environments also occasionally freed crops from their usual pests and diseases. The culture of crop plants in new environmental conditions presumably exposed genetic variation that was not previously apparent at the centre of origin, a process which Darlinton (1963) and Stebbin (1950) and others regarded as an important aspect of domestication. Transport to new environments also exposed differences in adaptability between crops as well as genotypes within crops.

2.4.2.1 Weather extremes

The capacity for and extent of adaptation of a crop to weather extremes is reflected in the diversity of environments where the crop is grown, especially its distribution in new habitats (Parmesan *et al.* 2000). Crop adaptation to extreme weather conditions is an important consideration when moving plants to new regions or changing plant cultural management practices like sowing date (Evans 1993). As a consequence of past adaptive changes, modern crop genotypes that are well adapted to a specific environment may no longer retain capacity to adapt when grown in another region. However, naturally occurring CWRs may contain traits that confer adaptation to weather extremes outside the range to which modern crop cultivars are adapted.

Shannon (1997) provided several examples where genetic resistance to extreme weather conditions in CWRs was transferred into cultivated crops through hybridisation. As mentioned previously, a leading chickpea cultivar in Northern India, ‘BG1103’, contains drought and temperature tolerance derived from *C. reticulatum*, while, six barley (*Hordeum vulgare*) cultivars with drought tolerance derived from *H. spontaneum* were released for use in Syria by the International Center for Agricultural Research in Dry Areas (ICARDA). In rice, *O. rufipogon* genes have been exploited for tolerance of acidic-sulfate soils in Vietnam, and *O. longistaminata* provided genes for drought tolerance in the Philippines, allowing the spread of production to previously unusable lands (Brar 2005, Nguyen *et al.* 2003).

Extreme temperatures are occasionally a difficult climatic variable to overcome through scheduling, particularly at higher latitudes (Eagles 1979). Exposure to temperature extremes can be long-term, as often occurs in hotter habitats, or it can more acute, as a result of seasonal or daily temperature extremes. With potatoes, CWRs and land races from warmer environments tend to be less adversely affected by high temperature - as shown by Smillie *et al.* (1983) among potatoes collected at various altitudes in the Andes - and are being used to breed potatoes that are better adapted to lowland tropical conditions (Mendoza and Estrada 1979).

The wild mungbean occurs naturally over a wide geographical range encompassing a diversity of climatic, edaphic and biotic conditions (Lawn and Watkinson 2002). Genotypic variation related to environmental factors within the wild mungbean has been observed in a range of phenological, morphological, seed and adaptive traits (Lawn *et al.* 1988, 1991, 2006, Rebetzke and Lawn 2006a,b,c). The diversity of ecotypes is therefore of potential use for crop adaptation enhancement. Its tolerance to various environmental stresses such as weather damage and cool temperature has been recorded. In mungbean, resistance to weather damage is conditioned by several seed and pod traits such as hardseededness and podwall density (Immie *et al.* 1988). Incorporation of these traits or pyramiding of appropriate genes could be useful for the development of a weather resistant ideotype.

2.4.2.2 Soil factors

Salinity stress and drought stress are two abiotic constraints that limit crop performance and crop yield (Kramer *et al.* 1980, Munns 2002), and which frequently occur together. Indeed, according to Munns (2002), the initial reaction of plants to drought and to salinity stresses are comparable, and the toxic effects of the salt itself are only found later in stress development. Numerous studies have explored the physiological responses of crops to water deficits and salinity stress and traits have been identified that confer adaptation to drought-prone environments and to saline soils (e.g. in the case of drought stress response traits, see Ludlow and Muchow 1990).

Working with *Phaseolus* species, Bayuelo-Jimenez *et al.* (2002) reported large variation in salt tolerance, particularly among wild species, while in the case of soybean, James *et al.* (2008a) reported that in perennial wild *Glycine* species, levels of osmotic adjustment were higher, and lethal relative water content and epidermal conductance were both lower. All three traits were associated with enhanced plant survival of water deficit stress (James *et al.* 2008b).

In the case of mungbean, there are few detailed reports of adaptations to adverse soil conditions. Nonetheless, Lawn *et al.* (1988) reported that in West Timor, Indonesia, wild mungbean was found growing on saline degraded soils, while on calcareous soils, the wild accessions did not exhibit strong

induced chlorosis as was observed in introduced mungbean cultivars. Meanwhile, Lawn and Watkinson (2002) claimed that accessions of wild mungbean from the Central Highlands in Queensland were unusually well-adapted to heavy textured, cracking clay soils in brigalow areas.

Of particular interest was the report by Rebetzke and Lawn (2006a) of a group of perennial wild mungbean accessions which they found only in the seasonally-arid savannah grasslands in the Townsville-Charters Towers region. These accessions, typified by ACC 87, developed thickened, tuberised roots, which spread out from the main taproot in the top 15 cm of soil. While the aerial stems of these plants often died off during the dry season, after rainfall, shoots were observed to emerge from the lower stems, and / or along the thickened roots some distance from the taproot. The authors postulated that the perenniality trait conferred adaptation to the arid environment where these accessions occur, and suggested that the trait might be useful either for breeding perennial forage mungbean genotypes, or even perennial grain varieties.

2.4.2.3 Phenological adaptation

Phenology is the term used to describe the duration of the respective developmental phases, or stages of ontogeny of a plant (Evans 1993, Richards and Condon 1993). Phenology is the most important single factor determining plant adaptation to both natural and agricultural environments. Firstly, phenology determines how well the plant's total life cycle is matched to the duration where weather conditions are generally favourable to growth. Secondly, within that period, phenology determines whether sensitive stages of development or ontogeny, e.g. flowering, are likely to be exposed to occasional periods of weather extremes e.g. high or low temperature, or drought or excessive rain (Fukai 1999, Richards and Condon 1993). For example, Jearakongman *et al*. (1995) found that standing water until flowering time was essential for high rice yield in Northeast Thailand, and this favoured early-flowering varieties. Varieties which flowered after the standing water disappeared from the paddy were exposed to the late season drought which caused a large reduction in yield.

Optimum phenology thus depends partly on the length of the growing season and partly on the occurrence of unfavourable weather conditions during the growing season. The key determinant of phenology is the date of flowering, which in many plant species, is strongly influenced by temperature and photoperiod (Roberts *et al.* 1993). The timing of flowering affects the duration of vegetative growth, which in turn determines the maximum biomass that might be accumulated by a plant, assuming favourable growth conditions. Therefore, a broad understanding of the phenology required for the target environment is indispensable knowledge crop breeders (Fukai 1999).

Windauer *et al.* (2004, 2006) reported that photo-thermal conditions and moisture had considerably impact on the phenology of the oilseed species *Lesquerella*. As such, sowing date had a large effect on phenology, due to climate-related changes in photo-thermal conditions. As sowing dates were delayed from autumn to spring, the life cycles of *Lesquerella* were shorter. Similarly in kabuli chickpea, Anwar *et al.* (2003) reported substantial impacts of temperature and photoperiod on time to flowering and maturity, and consequently growth and yield of irrigated crops.

Lawn and Rebetzke (2006) and Rebetzke and Lawn (2006b) reported that the photo-thermal environment contributed to substantial variation in the phenology of Australian wild mungbean accessions. In general, warmer temperatures hastened flowering while longer days delayed flowering. The duration of the period from first flowering to maturity also appeared to be sensitive to photoperiod and temperature. Interestingly, the sensitivity of individual wild accessions differed depending on the latitude where they were first collected, indicating that each accession was adapted to the latitude where it was collected. In general, accessions collected from Papua New Guinea (lat. 4^{o}S) were more sensitive to photoperiod, and so were later flowering at Townsville (19^{o}S) and even later at Samford (27^{o}S). Conversely, accessions collected from northern New South Wales were early flowering at Samford, and even earlier at Townsville.

2.4.3 Constraints to Use of Wild Traits in Crop Breeding

There are various constraints to the use of wild traits in crop breeding for purposes of crop improvement. Firstly, and most importantly, applied breeding programs generally have important objectives to address in order to ensure the timely development of adapted, high-yielding cultivars suited to commercial production (Sleper and Poehlman 2006). Inevitably, the use of wild accessions as parents in a breeding program will introduce many undesirable wild traits that have been eliminated through the process of domestication (e.g. Carpenter and Fehr 1986). Thus while the use of wild parents may increase the overall level of genetic variability into the breeding population, genetic advance may be slowed because of the mean performance of the breeding population is lowered. Thus the use of wild parents is usually only considered for traits that are not in the normal cultivated breeding population.

Additionally, the constraints to use of wild trait in crop breeding can occur where there are crossability barriers between the cultivated and wild germplasm. In the case of mungbean, apart from the wild mungbean itself, there are a range of hybridisation barriers with its less closely related wild relatives (Ahn 1977, Chen *et al.* 1977, Chowdhury 1977). It is often difficult to identify the causes of failures to achieve interspecific hybrids in food legumes. In some instances, for example, the pollen

tubes are unable to penetrate the stigma and style (Chowdhury 1977); however, in other situations, fertilization takes place, but embryo abortion happens during embryogenesis (Honma 1956). The failure of interspecific crosses due to embryo degeneration is widespread in interspecific *Vigna* spp. hybrids (Ahn and Hartmann 1977, Chen *et al.*1977). Even where viable hybrids are achieved, they are often only partial fertile and sometimes absolutely sterile. In *V. vexillata*, for example, Damayanti *et al.* (2010b) reported genetic breakdown in hybrids between cultivated Bali types and the cultivated African var. *macrosperma*.

While associations between traits can be very useful for crop breeders, with wild traits, they may become barriers where there are tight linkages between traits of interest and other undesirable wild traits or where the value of the trait of interest is lost when introduced into another genetic background. For example, Imrie *et al.* (1991) reported that the hard seed character was difficult to breed for because of a negative association between hard-seededness and seed weight, perhaps as a consequence of a physiological relation or a pleiotropic effect. Likewise, it has been suggested that in some instances, bruchid resistance in some wild mungbean accessions may be due to the unattractiveness of small seeds to egg-laying adult weevils (Lambrides and Imrie 2000). In another example, Lawn and Rebetzke (2006) found some wild mungbean accessions had higher seed protein content than cultivated mungbean. However, there was a negative correlation between protein content and seed size, and given significant genotype x environment interaction for the trait, it may prove difficult to transfer the high protein trait into large seeded cultivars.

A major constraint in using wild types in cultivar development is linkage drag of undesirable traits and this is a common phenomenon in combining wild and cultivated legume traits for breeding. Dwivedi *et al.* (2003) indicated the linkage drag of undesirable pod traits such as poor shelling outturn and prominent reticulation and deep constriction in the pods with disease resistance which limited of success when introgressing resistance genes from wild *Arachis* species to cultivated groundnut. Additionally, linkage drag of unwanted traits such as pod dehiscence, small seed or viny twining habit is a major problem when using wild mungbean in cultivated mungbean improvement

2.5 Optimizing Use of Wild Germplasm in Plant Breeding

While there is considerable potential value in sourcing novel traits from CWRs, this value is unlikely to be easily captured by simply hybridising cultivars with CWRs. Rather, there are several necessary precursor steps that can help make the use of wild genes in a breeding program more efficient (Sleper and Poehlman 2006). Firstly, collections of CWR germplasm need to be assembled from a range of locations and the nature and extent of the diversity in those collections needs to be described; secondly, traits of interest need to be identified and described; and thirdly, gene action and heritability

need to be determined for traits of interest. These steps are considered briefly in turn, with emphasis on wild mungbean and related species.

2.5.1 Collection and Evaluation of Diversity in Wild Vigna Species

A number of studies has been reported on the diversity and genetic resources of the cultivated and wild *Vigna* species. Baudoin and Maréchal (1988) reported that the genus *Vigna* consists of eight sub-genera and seven main cultivated species, two of which are of African origin (sub-genus *Vigna)* and five of which are Asiatic (sub-genus *Ceratrotopis*). As noted earlier, Tomooka *et al.* (2002) reported there are about 5600 accessions of the cultivated mungbean conserved at the AVRDC gene bank. Many of these accessions have been systematically assessed and characterised, so that breeders can readily identify genotypes of specific interest.

Many more mungbean accessions exist in national collections. For example, Kawalkar *et al.* (1996) reported that there are more than 2000 accessions of mungbean in the National Bureau of Plant Genetic Resources collection in India. These accessions have been systematically characterized and evaluated and the data of 1532 accessions was recently documented in the form of a crop catalogue. A study on genetic diversity was also conducted using data on a set of accessions based on biased sampling towards better agronomic types (Bisht *et al*. 1998).

Based on a comparative study of mungbean collections from across south Asia, Tomooka *et al.* (1992) reported that the genetic diversity of cultivated mungbean is better-preserved in Afghanistan-Iran more than in other areas. Nevertheless, the presence of wild and weedy races of mungbean, the occurrence of archaeological remains and landrace diversity suggest that India is the most likely area of domestication (Tomooka *et al.* 2002). The conclusion that the genetic diversity in cultivated mungbean is highest in South Asia was confirmed by micro-satellite marker studies by Sangiri *et al.* (2007).

There are far fewer accessions of wild *Vigna* species in national and international gene banks, and in comparison with the cultivated species, far less research has been conducted to explore the genetic diversity within those collections. As noted vearlier, among the more significant collections that have been assembled in recent years are those by Japanese researchers in south, south-east and east Asia (e.g. Vaughan *et al*. 2006, Tomooka *et al*. 2006a,b) and by researchers in Australia and nearby islands (Lawn and Cottrell 1988, Lawn and Watkinson 2002).

The genotypic diversity of the Australian wild *Vigna* species for a range of traits of agronomic, taxonomic and adaptive significance has been reported in a series of recent papers. In addition to the more general information presented by Lawn and Cottrell (1988), Lawn *et al.* (1988), and Lawn and Watkinson (2002), variation in the endemic native species *V. lanceolata* was described by Lawn and Holland (2003). Genotypic variation in the indigenous species *V. vexillata* was described by Grant *et al.* (2003) and Damayanti *et al.* (2010a) while that in the indigenous wild mungbean was described by Lawn and Rebetzke (2006) and Rebetzke and Lawn (2006a,b,c). In general terms, the endemic species *V. lanceolata* is the most genotypically diverse of the wild *Vigna* species in Australia (Lawn and Watkinson 2002). There are at least seven different morphotypes, with genetic incompatibilities evident between some (Lawn and Holland 2003).

Interestingly, there is considerable diversity within wild mungbean in Australia, with many adaptations to specific environments suggesting the species has a long history in Australia (Lawn and Cottrell 1988). Lawn and Rebetzke (2006) reported that there is a general geographical trend whereby accessions collected from regions more remote from those areas from southern and eastern Asia where mungbean has traditionally been cultivated showed greater expression of wild-type characters. Savaranakumar *et al*. (2004) used RAPD and AFLP molecular markers to classify wild mungbean accessions on the basis of a similarity coefficient based on marker diversity. While their study had few accessions from Austronesia region, they found the accessions ranked in similarity in the order Myanmar-India-Madagascar-Indonesia-Australia/Papua New Guinea. Subsequently Sangiri *et al.* (2007), drawing on the collection assembled by Lawn and Watkinson (2002), concluded that there was close genetic relationship with high allelic diversity among accessions of wild mungbean from Australia, East Timor, and Papua New Guinea. They concluded that their findings supported the idea of Lawn and Cottrell (1988) that wild mungbean has a long history in Australasia.

2.5.2 Identifying and Describing Potentially Useful Traits in Vigna CWRs

While most wild traits are not desirable agronomically, there are some traits in wild germplasms that may be useful in crop cultivar improvement. For example, in the tuberous rooted species *V. vexillata*, Damayanti *et al.* (2010a) identified some wild types with tuber attributes comparable with or better than those of cultivated varieties that are grown for their tubers in Bali, Indonesia. These authors also reported resistance to powdery mildew disease in some wild African accessions, confirming the earlier observation by James and Lawn (1991).

Relatively few potentially useful traits have been identified in wild mungbean, reflecting the fact that grain legumes are generally under-researched crops (Summerfield and Lawn 1987) and among the legumes, mungbean has been subjected to less research than crops like soybean and peanut. The wild

trait that has attracted most interest in relation to mungbean improvement is resistance to seed weevils (Table 2.1), reflecting the fact that bruchids are a serious pest of stored mungbean seed. Resistance to yellow mosaic virus also has been found in wild mungbean. Several traits of potential values have been identified in Australian wild mungbean accessions (Table 2.1).

Table 2.1 Potentially useful traits that have been identified by various authors in wild mungbean.

Potentially useful trait	Author(s)
Bruchid resistance	Singh and Ahuja 1977, Fujii *et al.* 1989, Kitamura *et al*. 1988, Young *et al.* 1992, James *et al.* 1999, Lambrides and Imrie 2000
MYMV - mungbean yellow mosaic virus	Bisht 2005
Weather damage resistance incl. hardseededness	Lawn *et al.* 1988
Tolerance of saline or calcareous soils	Lawn and Watkinson 2002
Higher seed protein content	Lawn and Rebetzke 2006
Perenniality and tuberous roots	Rebetzke and Lawn 2006a
Late flowering trait (possible long juvenile trait)	Rebetzke and Lawn 2006b

As noted previously, perennial accessions of mungbean have been found in seasonally-arid savannah grasslands of north-eastern Australia (Lawn and Cottrell 1988). These accessions occurred only in the Townsville-Charters Towers region. A study of several of these accessions indicated that the perenniality trait was associated with the development later in the growth period of a tuberised taproot, and tuberised lateral roots, mainly in the top 10-15 cm of soil (Rebetzke and Lawn 2006a). Compared with fibrous rooted annual wild mungbean accessions, the perennial accessions produced less seed. Therefore, the perennial character may not be of immediate interest for development of commercial mungbean cultivars to be grown for seed.

Nonetheless, there has been increasing interest in the development of perennial grain crops in agriculture, in order to address perceived sustainability issues (e.g. Scheinost *et al.* 2001, Jackson and Jackson 1999). Further, it is possible that the perennial lines could be of interest for development as a forage legume adapted to the seasonally arid coastal and sub-coastal grasslands of northern Australia. The invasive spread of exotic pasture species into non-pastoral areas has led to concerns about their effect on natural ecosystems (Lonsdale 1994). The use of forage cultivated derived from native plants should be less of a concern.

As noted earlier, another potentially interesting trait was reported by Rebetzke and Lawn (2006b). They identified one wild mungbean accession, ACC 1, from Mackay in central Queensland, which was both unusually late flowering, but apparently insensitive to photoperiod. Across environments,

the time to flower ranged from 98 - 121 days, while flowering was not delayed where seedling plants were exposed to artificially extended photoperiod using incandescent lights. Those authors hypothesized that this novel response may have been due to a 'long – juvenile' (LJ) trait, similar to that found in soybean (Hartwig and Kiihl 1979). The LJ trait, which is due to a single recessive gene, has been valuable in broadening the range of adaptation of soybean in Australia (James and Lawn 2011) and an analogous LJ trait in mungbean may similarly be very useful.

2.5.4 Investigating Genetic Control of Wild Traits in Vigna

An understanding of the genetic control of a trait is an important aid to a breeder when contemplating a hybridisation and selection program to develop improved cultivars with that trait. In the cultivated *Vigna* species, there have been a large number of studies on gene action and heritability for traits of interest and many earlier studies were summarised by Fery (1980). For cultivated mungbean, information is available on the inheritance of a wide range of qualitative traits, e.g. stem twining (Sen and Ghosh 1959, Pathak and Singh 1963), leaflet size (Soehedi *et al.* 2007), leaflet shape (Yimram 2009), leaflet lobing (Sen and Gosh 1959) as well as seed testa traits (e.g. Rheenen 1965). There are also numerous reports on the heritability of the more important agronomic traits, e.g. 100 seed weight (Mak and Yap 1980, Singh and Singh 1996, Rohman 2003, Khattak 2004), seeds per pod (Malik and Singh 1983), branches per main stem (Tiwari *et al.*, 1993, Singh and Sing 1996, Khattak *et al.* 2002c, Khattak *et al.* 2004, Yimram 2009), and biomass production (Rehman *et al.* 2009),

Perhaps not surprisingly, there is less information available of the genetic control of wild traits in Vigna CWRs. In the tuberous rooted species, *V. vexillata*, James and Lawn (1991) reported on the genetic control of range of wild qualitative and quantitative traits. Damayanti *et al.* (2010c) extended this work, with research that explored the genetic control of a range of wild traits in cultivated x wild hybrid populations, including some tuber attributes. These studies involved cultivated varieties of *V. vexillata* from Bali, Indonesia, and another from Sudan, Africa, as well as wild accessions from Australia and Africa.

Numerous studies have shown that the wild mungbean is generally cross-compatible with the cultivated mungbean (Jain and Mehra 1980). This applies also to the forms of wild mungbean that occur in Australia (James *et al.* 1999), even though, as noted earlier, these forms are more 'wild' than those found closer to the centre of domestication of mungbean. The genetic control of some qualitative and quantitative wild mungbean traits in wild x cultivated hybrids has been reported in several studies (Table 2.2).

Table 2.2 Examples of studies reporting the inheritance / heritability of wild mungbean traits based on studies on cultivated x wild hybrids.

Trait	Author(s)
Qualitative	
Bruchid resistance	Singh and Ahuja 1977, Fujii *et al.* 1989, Kitamura *et al*. 1988, Young *et al.* 1992, James *et al.* 1999, Lambrides and Imrie 2000
Hardseededness	Singh *et al.* 1983, Lawn *et al.* 1988, Plhak 1989, Humphry *et al.* 2005
Leaflet lobing	James *et al*. 1999
Growth habit	Talukdar 2003, Sriphadet *et al.* 2007
Seed testa traits	James *et al*. 1999, Zubair 2004
Quantitative	
Seed yield and yield components	James *et al*. 1999, Khattak 2002c, Zubai 2004, Sriphadet *et al.* 2007, Yimram 2009, Rehim 2010
Growth and biomass	James *et al*. 1999, Sriphadet *et al.* 2007

Relative to many crops, the genetic control of wild traits in mungbean and their expression in cultivated backgrounds remain under-researched. In particular, there is no information on the perenniality trait described by Rebetzke and Lawn (2006a). James *et al.* (1999) reported that the perennial accession ACC 87 hybridised readily with the cultivated mungbean, but no information was presented on the expression of the perenniality trait in the resultant hybrids. Likewise, there is only limited information on the control of phenology, especially the very late flowering trait described by Rebetzke and Lawn (2006b).

In addition to the direct use of wild traits for improving mungbean cultivars, wild traits can provide useful genetic markers in normal crop breeding. This opportunity has become especially relevant with the development in recent years of molecular genetic markers. In mungbean as in other crops, cultivated x wild hybrid populations have proved very useful, for developing comprehensive marker maps (e.g. Menancio-Hautea *et al.* 1992, Young *et al.* 1992, Lambrides *et al.* 2000, Humphry *et al.* 2002). As noted earlier, the process of domestication often narrows the genetic base of cultivated varieties, and there may not be sufficient variability among cultivars especially for agronomic traits, to enable markers for those traits to be identified. However, because wild mungbean accessions differ from cultivars at many loci, the hybrids enable markers for many loci, throughout the genome, to be identified.

2.6 Thesis Objective

The preceding review has demonstrated that wild traits from CWRs can be potentially useful in crop improvement, but the use of CWRs in grain legume improvement, especially cultivated *Vigna* crops, remains limited. In the case of mungbean, there are several potentially useful wild traits, including the perenniality trait in ACC 87 (Rebetzke and Lawn 2006a), and perhaps the late flowering trait in ACC 1 (Rebetzke and Lawn 2006b) if it proves to be due to a LJ trait. However, for effective use to be made of wild traits, it is desirable that their genetic control and expression in cultivated backgrounds be understood.

Accordingly, the scientific objective of this thesis was to develop an understanding of the genetic control of traits that have been identified in Australian wild mungbean accessions, with particular emphasis on perenniality in ACC 87, and on the late flowering trait in ACC 1. The aim was to lay the basis for the future use of these traits, if practicable, in mungbean genetic improvement. The development of hybrid cultivated x wild mungbean populations, and the design of research experiments whereby the expression of traits in different generations could be measured and their likely genetic control could be inferred, are described in the following chapter.

CHAPTER 3: MATERIALS & METHODS

Two experiments were conducted to explore the expression and inheritance of potentially useful physiological traits from wild mungbean (*V. radiata* ssp. *sublobata*) using genetic populations created by hybridising wild mungbean genotypes known to possess the traits of interest with cultivated mungbean varieties. In addition to the target physiological traits, several other traits were recorded, in order to explore the expression and inheritance of wild and cultivated traits in mungbean. The research project comprised two related experiments that were conducted using plants grown in large pots located on benches in the field at the CSIRO Davies Laboratory, in Townsville, Queensland, Australia (19°13'S, 146°48'E; alt 100 m) (Table 3.1). In this chapter, those procedures and measurements that were common to both experiments are documented. Measurements that were specific to one experiment or the other are documented in the respective chapters reporting the results from each experiment.

Table 3.1 Experiments designed to explore the expression and inheritance of cultivated and wild traits in mungbean.

	Experiment 1	**Experiment 2**
Main objective	Phenotypic expression and inheritance of the perennial trait in mungbean	Phenotypic expression and inheritance of a very late flowering trait in mungbean
Location	CSIRO Davies Laboratory	CSIRO Davies Laboratory
Sowing date	3rd March, 2009	10th March, 2009

Both experiments used parental and segregating populations generated by hybridising an accession of wild mungbean that was known to possess the trait of interest with two different mungbean cultivars. The experiments were sown in early autumn (Table 3.1) in order to hasten flowering and minimise excessive vegetative growth by the plants, yet still provide adequate time for expression of the traits of interest. Both cultivated (Lawn 1979a) and wild mungbeans (Lawn and Rebetzke 2006) are quantitative short day plants so that flowering tends to be delayed by longer day lengths over the summer period, and occur sooner when the plants are sown in autumn. In the field, the perennial trait usually becomes evident in the winter – spring period, when plants begin to regrow as temperatures again become warmer. Meanwhile, the very late flowering trait is still expressed in late sown plants (Rebetzke and Lawn 2006a). The autumn sowing time also shortened the time when the experiment was exposed to heavy wet season rains, which can damage plants and create conditions favourable for disease.

Apart from the specific accessions used in the research, the methodology used in both experiments was broadly similar. The plants were grown in large pots, rather than in the field, because in that way,

a larger number of plants could be grown in a relatively smaller area than if the plants were planted directly into the ground. Further, greater control could be exercised on the environmental conditions that the plants experienced. For example, all plants were exposed to a uniform soil mix, and because they were grown in pots, there were minimal weed problems. All plants also experienced broadly similar growth conditions in terms of nutrient and water supply and insect pest and disease control.

3.1 Experimental Germplasm

The genetic populations used in these studies had been created in prior years using the methodology described below (RJ Lawn, personal communication 2008). Briefly, four genetic populations were established using two cultivated mungbean varieties, Berken and Kiloga, and two wild parents, ACC 1- late flowering, putative long-juvenile trait, and ACC 87- tuberous rooted, perennial trait. The characteristics of the four parental genotypes are shown in Table 3.2.

Table 3.2 Parental genotypes used to generate genetic populations to study the inheritance of wild and cultivated traits in mungbean

Varieties	Cultivated or Wild	Main Trait of Interest
Kiloga	Cultivated variety from USA	Very early maturing (68-84 d), annual (see Lawn 1979b). Erect stem, large shiny green seed.
Berken	Cultivated variety from USA	Early flowering (68-97 d), annual (see Lawn 1979a). Erect stem, large shiny green seed.
ACC 1	Wild accession from near Mackay	Prostrate vine, very late flowering (>110 d), not affected by extended photoperiod. Very small black seed. Putative long juvenile trait (see Rebetzke and Lawn 2006a).
ACC 87	Wild accession from near Townsville	Twining vine, relatively large flowers, black seed. Sensitive to photoperiod. Tuberous rooted and perennial (see Rebetzke and Lawn 2006c).

The two mungbean cultivars were chosen because they were typical cultivated varieties with a strong erect stem, large leaflets, and large green shiny seed. Kiloga was very early flowering and Berken was early flowering when grown in SE Queensland (Lawn 1979a) and both would be expected to become even earlier flowering in Townsville, because late summer days are shorter and warmer than in South East Queensland.

In contrast, the two wild mungbean accessions were fine stemmed, twining plants with smaller leaves and small, black seeds. The accession with the very late flowering or putative ‘long-juvenile’ trait, ACC 1, is a prostrate, viny plant with very small leaves (Rebetzke and Lawn 2006a). The putative ‘long-juvenile’ trait expresses as a very late flowering habit, which was shown to be unaffected when

plants were exposed to artificial long days (Rebetzke and Lawn 2006a). The other wild accession, ACC 87, is a perennial type with more robust growth habit, and larger leaflets, flowers and seeds (Rebetzke and Lawn 2006c). In the wild, the perennial forms of wild mungbean are tuberous-rooted.

In order to create hybrid populations, each cultivated parent was crossed with each wild parent to create four F_1 hybrids (Kiloga x ACC 1; Kiloga x ACC 87; Berken x ACC 1; Berken x ACC 87). In turn, each F_1 hybrid was backcrossed to each of its parents, to create two backcross populations for each initial hybrid combination. In making these backcross populations, sometimes the F_1 plant was used as the pollen donor, and sometimes as the pollen recipient, depending on whether healthy flowers were available at the time (RJ Lawn, personal communication 2008). The F_1 plants that were used to create the backcrosses were also allowed to self, so producing F_2 generation seed. Thus for each hybrid population, seed was available of the following generations: P_1 (cultivated parent), P_2 (wild parent), F_1 progeny, F_2 progeny, BC_{P1} (backcross to cultivated parent), BC_{P2} (backcross to wild parent). The F_2 and backcross populations were expected to segregate for those cultivated and wild traits, including the main traits of interest, which differed between the parents.

3.2 Cultural Details

The parental plants and their hybrids were grown under favourable conditions, in order to maximize the expression of major traits of interest. The experimental area was previously a grassy field in which 90 cm x 240 cm wire mesh benches were located. In order to control weeds and reduce the possible movement of insect pests and disease from the pasture to the experimental plants, the area was mown and the herbicide chemical Glyphosate was applied on the project area two weeks before the benches were located there. The pots in which the plants were grown were located on the benches above the soil surface. The plants were grown in round pots 210 mm diameter and 300 mm deep, each containing an equal amount of a commercial potting mix soil as the rooting medium. The pots were placed in plastic saucers and located on the benches with twelve pots per bench.

As the wild accessions are hardseeded, the seeds were scarified before sowing, by removing approximately one millimetre square of testa to assist them to imbibe water and emerge quickly. The scarified seeds were sown one per pot at 1 cm depth and then covered with moistened vermiculite. The pots were shaded by a net in the first few days after germination in order to avoid the effects of harsh weather conditions - such as high temperatures or heavy rain - during the vulnerable seedling emergence stage. The seedling plants were also dusted with a rotenone-based commercial pesticide to avoid insect damage. Shortly after emergence, the pots were moved from the field to the glasshouse, and back again 2 d later, to avoid the threat of a passing cyclone. A 90 cm bamboo stake was placed

vertically in each pot to support the young plants and in the case of the wild parents and the hybrids, to ensure that stems from adjacent plants did not intertwine.

The pots were maintained free of water stress and insects. Over time, as the plants grew, differences in plant size and water use developed. As mungbean is sensitive to waterlogging (Paisan *et al*. 1994), the plants were watered daily by hand, and the water supplied was adjusted to match the use by different plants. During periods of heavy rain, however, some influences of water logging were impossible to avoid. In order to ensure nutrient supply was adequate for good growth, 10 g of Osmocote Plus® Controlled-Release Fertilizer was carefully added to each pot before the flowering period commenced. The fertiliser was added at the same time for each population, in early morning or in the late afternoon, with care taken to make sure that the fertiliser did not come into contact with the stems, to escape burning due to the hot weather conditions. As they grew, the plants were also tied to the previously installed bamboo stake, to minimise wind damage.

As the field site was located near native grassland which provided a source of several insect pests during the experimental period, it was necessary to spray with suitable insecticides immediately after the pests appeared. Bean fly (*Ophiomyia phaseoli*), which can cause significant damage to mungbean plants, particularly at the seedling stage (Shepard *et al.* 1983), was found during most of the growing season. Therefore, when the cotyledons first emerged, the systemic insecticide Rogor® was applied to minimise bean fly damage. Thereafter, Rogor was sprayed weekly until almost all growth of new leaves and stems had finished. Other insect problems encountered included attacks during the flowering and young pod development period by the cluster caterpillar (*Spodoptera litura*) which mainly caused some minor leaf damage, and the bean pod borer (*Maruca testulalis*), which infested the inflorescences and burrowed into flowers or young pods. These latter pests were the most difficult to control, but damage was minimised by weekly sprays with the chemical Lannate®. Sprays were applied in the early morning or late afternoon until the plants almost finished producing tender pods.

The pods on the cultivated parental plants ripened over a shorter period of time than on the wild accessions, which produced several flushes of flowers and pods over an extended period. During the pod ripening period, completely ripened pods were collected by hand, to avoid loss of seeds through pod dehiscence or shattering. The pods from each pot were stored in a labelled, recycled paper envelope in a cool, dry storage shed to avoid any negative impacts on seed quality. Initially the mature pods were collected daily, but as the season progressed into late autumn and the weather conditions dried, the ripe pods were removed at less frequent intervals, until the plants stopped producing pods and the experiments were completed. The bulk harvests of pods from each pot were air-dried, and weighed, and a sub-sample taken for estimating the pod and seed weight ratio. The bulk samples were threshed by hand to separate the seeds from the pods.

3.3 Experimental Design

In each of the four hybrid combinations, a minimum number of plants was assessed for each generation as follows: 5 of each parent, 5 F_1 plants, 80 F_2 plants, and 10 plants from each of the two backcross populations, BC_{P1} and BC_{P2}. Based on Allard (1999), these sample sizes were considered large enough to enable the expression and likely inheritance / heritability of both qualitative and quantitative traits to be described, but small enough to enable a wide range of traits to be measured. Within each hybrid combination, the plants were allocated spatially across the benches in a completely randomized design, regardless of the generation. To minimise the possible confounding effects of localised spatial variation within or between benches, pots were randomly re-positioned on the benches every three weeks.

3.4 Trait Measurements

Selected traits for which variation was apparent in crosses were observed, and their modes of inheritance determined from the variation evident in the segregating generations of the test populations. In deciding which traits should be observed, reliance was placed on previous reports (James and Lawn 1991, James *et al.*1999, Rebetzke and Lawn 2006a, b, c, Zubair 2004) of the expression of traits in wild mungbean and other *Vigna* species. Traits subsequently determined to be qualitatively inherited are defined in Table 3.4. Several other traits determined to be quantitatively inherited are indicated in following sections.

Table 3.3 Putative qualitative traits observed in parental morphotypes and their progenies

Trait	Parental Morphotypes	
	Genotypes	**Score and definition**
Testa colour	Kiloga and Berken	1: Shiny green
	ACC 87 and ACC 1	2: Speckled black
Seed coat ridging	Kiloga and Berken	1: Seed coat shiny, ridging absent
	ACC 87 and ACC 1	2: Seed coat dull, ridging present
Seed coat surface	Kiloga and Berken	1: Shiny
	ACC 87 and ACC 1	2: Dull
Hilum colour	Kiloga and Berken	1: Light
	ACC 87 and ACC 1	2: Dark
Powdery mildew	Kiloga and Berken	1: Presence
	ACC 87 and ACC 1	2: Absence

3.4.1 Phenological Traits

The experimental plants were observed daily and the dates recorded for: germination, flowering (defined as the first completely open flower in each pot); first mature pod (recorded as the date that the plant had the first pod that lost chlorophyll and darkened); end of flowering (when major flowering flushes had ceased and only sporadic flowering persisted); physiological maturity (recorded when most (> 95%) of the pods had ripened). The durations of the respective phenological phases were then calculated using these dates.

3.4.2 Morphological Traits

Vegetative traits recorded included stem thickness, defined as the stem diameter (mm) below the fourth, fully-expanded trifoliolate leaf; leaflet length (mm) and width (mm), from which leaf length to width ratio was calculated, were measured on the terminal leaflet of the fourth fully expanded trifoliolate leaf; and leaflet lobing score, rated from 0 (leaves entire) to 3 (deeply scalloped leaflets with pronounced basal lobes) was recorded. At flowering, the width of the floral standard (mm) was observed on the first three completely opened flowers. Other traits were plant growth habit, recorded as a rating from 0 (erect) to 4 (prostrate) observed eight weeks after germination; and twining score, observed as a rating from 0 (absence) to 2 (strongly twining) on the wooden stakes in the experiments. Disease reaction to powdery mildew was also recorded as 0 (no disease symptoms) and 1 (appearance of disease symptoms). Pod dehiscence was also observed as 0 (none pod shattering) to 2 (strong pod shattering).

At harvest, a number of morphological traits were observed, including the numbers of main (primary) branches per plant; the number of nodes on the primary stem and on five primary branches; the number of seeds per pod as recorded on the first ten harvested pods; pod size, measured as the length (mm) and width (mm) of the first five harvested pods; and the number of pods per peduncle and peduncle length (mm) as observed on five randomly selected peduncles during the first flush of pods. Other traits of interest included seed size, defined as the weight of 100 seeds from the first flush of pods; and hardseededness, measured by calculating the percentage seeds that germinated after exposing 100 seed samples to conditions favourable to germination for a period of 72 hours, using seeds from the first-harvested mature pods from each plant.

3.4.3 Agronomic Traits

Total standing dry matter (g/plant) was estimated for each population after plant maturity. The standing biomass was removed and dried in a fan forced dehydrator at 60^0C for 72 hours. Pod biomass

and seed yield per plant in each population were calculated using the cumulative weekly harvests of pod and seeds. Total aboveground dry matter production (TDM) was then calculated as the sum of these several components. Harvest index (HI) was measured as the ratio of seed yield to TDM.

3.5 Data Analysis

The primary aim was to obtain statistical information on genotypic variation in the respective populations for each of the traits of interest. Initially, frequency distributions for each trait were used to separate qualitative traits with discrete distributions (i.e. those apparently under simple genetic control) from quantitative traits with continuous distributions (i.e. those apparently under multi-gene control). Additionally, a linkage analysis of variance was conducted to detect when variation for a qualitatively inherited trait conditioned a significant difference in other qualitatively or quantitatively inherited traits. Finally, comparisons between populations were made using analysis of variance. The above-mentioned analyses are discussed in more detail in the following sections.

3.5.1 Qualitative Trait Analyses

For traits that appeared to be qualitatively inherited (i.e., there was discontinuous variation in the segregating populations), the standard Chi -square (χ^2) tests were used to test hypotheses related to categorical data such as would be collected from inheritance studies. The Chi -square tests were evaluated to test the goodness of fit of the observed data to a model assuming the simplest case of single gene control. The segregation ratios observed in the three segregating generations (F_2, BC_{P1}, and BC_{p2}), were compared with expectations from the model for single gene control. If the Chi-squared probability was <0.90, more complex digenic models of control were also evaluated (Acquaah 2008).

$$\chi^2 = \sum[(f_o - f_e)^2 / f_e]$$

Where f_o = Observed sample frequency and f_e = Expected frequency based on Mendelian ratios (≡ the null hypothesis (Ho)), the hypothesis to be disproved. Subsequently, a chi-square analysis was used to test the goodness of fit of the data from the segregating progeny generations (F_2; BC_1 and BC_2) as a whole to the putative genetic model, based on the method of Flanders and Khoury (1996) and as applied by James *et al.* (1999) and Damayanti *et al.* (2010c).

3.5.2 Quantitative Trait Analyses

For putative quantitative traits (i.e. those exhibiting continuous variation), several analyses were undertaken to explore the expression of the traits in the various generation, and assess their heritability.

3.5.2.1 Trait expression in different generations

For each trait, the analysis of variance was applied to test for differences in mean expression of traits in the parental and progeny generations. The SPSS 17.0 Graduate Student Version software (SPSS, Inc,) was used for all of these data analyses.

3.5.2.2 Estimates of environmental and genetic variances and heritability

For each trait, the values for individual plants were used to estimate variances for the parental and progeny generations. In turn, these variance estimates were used to calculate estimates of environmental, phenotypic, additive genetic, dominance genetic and total genetic variances for each trait. These variances estimates were then used to estimate broad sense heritabilities and narrow sense heritabilities using the variance ratios method (Acquaah 2007). These entities are defined as per the following formulas (Acquaah 2007):

$$s^2 = \sum(Y_i - \bar{Y})^2 / (n-1)$$

$$Co(X,Y) = \frac{\sum(Xi - \dot{X})(Yk - \bar{Y})}{(n-1)}$$

$$V_E = (V_{P1} + V_{P2} + V_{F1})/3$$

$$V_D = [(V_{B1} + V_{B2}) - V_{F2} - V_E$$

$$V_A = 2V_{F2} - (V_{B1} + V_{B2})$$

$$V_G = V_A + V_D$$

$$V_P = V_A + V_D + V_E$$

$$h^2_b = V_G / V_P$$

$$h^2_n = V_A / V_P$$

where:

s^2: Variance of trait

Co(X,Y): Covariance between the two traits, Y_i and $\bar{Y}$ are value of an individual observation and mean value of observations

n: the number of observations

V_{P1}; V_{P2}; V_{F1}; V_{BCP1}; V_{BCP2}; V_{F2}: Parents, F_1 progeny, Backcross parents and F_2 progeny variances

V_E; V_A ; V_D; V_G; V_P: Environmental, additive, dominance, genetic and total phenotypic variances

h^2_b: Broad sense heritability

h^2_n: Narrow sense heritability

The standard errors of estimates of heritability were calculated by method of Acquaah (2007).

3.5.3 Associations between traits

Phenotypic correlations: Simple linear correlation was applied to show how much multiple characteristics of the units of a population associate. If there is no association, covariance will be zero or close to zero. The magnitude of covariance is often related to the size of the variables themselves, and also depends on the scale of measurement. The simple linear correlation measures the linear relationship between two variables. The correlation coefficient formula is given by Acquaah (2007):

$$r_P = [N*\sum(X*Y)- (\sum X)*(\sum Y)]/ \sqrt{[N*\sum X^2 – (\sum X)^2][N*\sum Y^2 – (\sum Y)^2]}$$

where N = population, X, Y = Measured values of the traits X and Y, respectively.

Genotypic correlations: Genetic correlation is the proportion of variance that two traits share due to genetic causes. The genetic correlation of traits is independent of their heritability: i.e., two traits can have a very high genetic correlation even when the heritability of each is low and vice versa. The genetic correlation estimates how much of the genetic influence on two traits is common to both. To the extent that it is above zero, this suggests that the two traits are linked or influenced by common genes. The genotypic correlation coefficient formula is given by Falconer (2000):

$$r_G = \text{Covariance } (X,Y)/ \sqrt{(s^2_X * s^2_Y)}$$

where s^2_X and s^2_Y are variances of two traits x and y.

Linkage between qualitative traits: To evaluate the extent of linkage between qualitative traits, an analysis was conducted to detect when variation for qualitatively inherited traits conditioned a significant difference in other qualitatively inherited traits. The joint segregation ratios of qualitative trait pairs observed in the F_2 generation were compared to the expected ratio of normal distribution of independent assortment (9:3:3:1) (Allard, 1999).

CHAPTER 4: PERENNIALITY & OTHER WILD TRAITS FROM ACC 87

4.1 Introduction

As outlined in Chapter 2, there have been a number of studies on the inheritance of mungbean traits. Early studies on the topic were broadly reviewed by Fery (1980). Since then, further information on mungbean genetics has been reported in several studies (e.g. James *et al.* 1999, Khattak 2002a,b,c, 2004, Sriphadet *et al*. 2007, Sukhumaporn 2009). There have been fewer studies on the inheritance of wild *vs.* cultivated mungbean traits, particularly for novel wild traits. Generally, the wild species represents a potentially useful additional source of genetic diversity for cultivated mungbean. While most wild traits are not desirable agronomically, there are some traits in wild mungbean that may be useful (e.g. perenniality, hardseededness, pest resistance). It is essential to understand how both useful and unwanted traits are inherited. The development of an understanding of inheritance pattern and segregation ratios is thus an important step in crop improvement.

As discussed previously, wild mungbean accessions have been identified from north-eastern Australia that, in contrast with the short duration, annual life cycle of cultivated mungbean, exhibit a perennial growth habit (Rebetzke and Lawn 2006c). The perennial plants, which are found in seasonally arid savannah grassland, regenerate from tuberised roots. The inheritance of the perennial trait has not yet been established. The aim of the experiment reported in this chapter was therefore to document the expression and inheritance of perenniality in cultivated x wild mungbean hybrid populations. In addition, observations were made on several other wild *vs.* cultivated mungbean traits of potential agronomic or adaptive significance.

4.2 Materials & Methods

The broad details of the genetic populations, the plant cultural procedures, experimental designs, the traits observed, and the analytical approaches used, were outlined previously in Chapter 3. Of particular interest with the two hybrid populations involving the wild accession ACC 87 was expression of the so-called perenniality trait, which had been observed in ACC 87. Previous observations on this trait (Rebetzke and Lawn 2006c) had suggested that expression of the trait was most evident by i. the development of tuberised taproots and main root laterals and ii. the survival of the main taproot and lateral roots over the winter period. In some instances, especially in ACC 87, shoot initials were observed on the tuberised taproots and lateral roots in the following springtime.

4.2.1 Germplasm & Experimental Design

The numbers of plants that were grown in each generation of the two cultivated x wild populations involving the perennial wild accession ACC 87 are shown in Table 4.1.

Table 4.1 The numbers of individual plants sown for each generation in each hybrid combination involving the wild perennial accession ACC 87.

	Cultivated parent	
Generation	**Kiloga**	**Berken**
Cultivated parent (P_1)	5	5
Wild parent (P_2)	5	5
F_1 Progeny	5	5
F_2 Progeny	84	84
BC_{p1} Progeny	13	12
BC_{p2} Progeny	13	13

4.2.2 Cultural Details

The plants were germinated on 3rd March i.e. late summer, and allowed to grow, flower and set seed during the autumn to mid-winter period (i.e. over the period March-July). The plant shoots were harvested as they matured and died over the latter part of this period. According to Rebetzke and Lawn (2006c), whereas the roots of normal annual mungbean plants die when the shoot dies, the roots of the perennial forms in the field usually thicken, and remain alive. However, tuber development in the wild perennial accessions did not become apparent until plants had been grown for an extended period of at least 120 days (Rebetzke and Lawn 2006c). Indeed, those authors suggested that expression of the trait was maximised where plants were allowed to over-winter.

Therefore, to maximise the opportunity for the expression of the trait in this study, the roots of the plants in the ACC 87 hybrid populations were allowed to remain undisturbed over the autumn-winter period unless it became clear that the stems and roots had died. Where the roots had clearly died, plants were removed from the soil to establish whether there was any evidence of tuberisation. Those plants that did not die were examined in the spring (September) for evidence of tuberisation. During the over-winter period, the pots were watered very sparingly to minimise rotting of the roots. The plant pots were left standing on the wire mesh benches in the outside field environment, because it was expected that the warm humid environment in a glasshouse or shade house may promote root rot.

4.2.3 Data Collection & Statistical Analysis

The variation apparent of the traits of interest in each population was recorded, and their modes of inheritance determined from the variation evident in the segregating generations of the test populations. Traits determined to be qualitatively or quantitatively inherited are defined in following sections. For many traits, the F_1 hybrid was similar to one or the other parent, suggesting dominant gene action. In others, the F_1 hybrid was intermediate suggesting additive response. The methods of observing and statistically analysing data were as described in Chapter 3, using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc,).

4.2.3.1 Perenniality

A quantitative score for the perennial trait was developed based in part on the observations of Rebetzke and Lawn (2006c) and in part on observation of the range of responses observed in this study. After the aerial stems had died back and were harvested, individual plants were marked and then examined at two-weekly intervals to establish whether there was evidence that (i) the stem base and taproot remained alive after the aerial stems had died, (ii) there was any thickening of the main taproot or main side lateral roots (thickened lateral roots were defined as > 2 mm in diameter more than 10 mm away from the main stem) (iii) new shoots were emerging on the basal nodes of aboveground stems after the harvest of the mature shoots roots and (iv) whether any shoots were emerging from thickened tap or lateral roots. Plants were marked and examined every two weeks for the appearance of new shoots emerging on the aboveground stem after the mature shoots were harvested. By combining these observations, the roots were visually scored from zero (roots fibrous with no evidence of tuberisation) to four (completely tuberous or perennial with shoots emerging above ground from the roots).

Some plants, including the cultivated parents, died before harvest of the dead aerial shoots. Most of these plants were found to have fibrous roots and exhibited no evidence of perenniality. However, in a few cases, plants which died after harvest showed some evidence of tuberisation. Some of these plants showed evidence of infection by charcoal rot disease (*Macrophina phaseoli*) after the dead aerial shoots had been cut back. Some roots may also have rotted out due to transient water-logging, because it was difficult to match water supply to plant need in winter when conditions were cool and after the shoots had died back. Whenever it was evident that roots had died, the roots were then quickly recovered and examined to establish if there was evidence of root thickening. Where there was evidence of tuberisation, the perenniality score was usually a 1 or a 2.

4.2.3.2 Putative qualitative traits

Trait expression was observed in the parents and the F_1 generation, and the segregation ratios observed in the three segregating generations (F_2, BC_{P1} and BC_{P2}). As previously discussed in section 4 of Chapter 3, the goodness of fit of a model assuming the simplest case of single gene control was tested using the Chi-square (χ^2) test, in which the observed segregation ratios in the segregating generations were compared with expectation from the model for single gene control. If the Chi-squared probability was <0.90, digenic control was also explored. Comparisons were made separately for each segregating generation and then in combination. Where the mode of inheritance appeared to be the same in different crosses, a combined χ^2 test was undertaken using all the data. The goodness of fit was tested using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc,).

4.2.3.3 Putative quantitative traits

For putative quantitative traits (i.e. those exhibiting continuous variation), broad sense and narrow heritability were calculated by the variance ratios method (Acquaah 2007, Allard 1999). The standard errors were calculated by method of Acquaah (2007). The variance of each component in the variance ratios method was calculated using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc,).

4.3 Results & Discussion

4.3.1 Perenniality

The degree of expression of perenniality observed in this study varied considerably between plants. Examples of the different levels of expression of the trait and the scoring system used for the trait are shown in Figure 4.1. The numbers of plants observed at the various levels of expression, in each generation of the two hybrid populations involving ACC 87, are shown in Table 4.2. Consistent with previous experience (Rebetzke and Lawn 2006c), the parental plants exhibited extremes of response, with Kiloga and Berken having fibrous roots and a typical annual growth cycle, while ACC 87 exhibited the perennial habit (Table 4.2), with the tuberised roots remaining alive and shoots emerging from the roots after the winter. In both hybrid populations, the F_1 plants showed reasonably strong expression of the trait, with tuberised roots giving rise to adventitious shoots after the winter. The fact that the F_1 plants exhibited root tuberisation and formation of adventitious shoots suggested a degree of dominant gene action. However, the level of expression of the trait in the F_1 was not as strong as in ACC 87, suggesting the trait may be multi-genic.

Score zero Roots fibrous Annual habit	Score 1 Roots tuberised (> 2 mm diameter) and living over winter	Score 2 Roots tuberised, stem base alive, shoots emerging from basal stem nodes after winter	Score 3 Roots tuberised Small adventitious shoots from roots below-ground	Score 4 Roots tuberised Emergent vigorous adventitious shoots from roots after winter

Figure 4.1 Representative examples illustrating the degree of expression of the perenniality trait, for each of the five scoring levels used in the study. The cultivated parent plants scored zero (i.e. annual, fibrous rooted), while ACC 87 scored 4 (i.e. perennial, roots tuberised, with strong shoots emerging from the soil after winter) Arrows indicate tuberous roots and/or adventitious shoots.

In the F_2 generation, the pattern of response in perenniality was broadly similar in both populations (Table 4.2). There was a range of response types observed in the F_2, spanning the complete range from the cultivated to the wild parental types. However, in contrast to the F_1 generation, nearly half the F_2 plants were fibrous rooted and exhibited no evidence of perenniality. Conversely, in just over half the F_2 plants in both populations, there was evidence of some level of perenniality, although only a few exhibited the trait as strongly as in the wild parent.

Table 4.2 Observed frequencies of different levels of expression of the perennial trait in the parent and hybrid generations for two populations, Kiloga x ACC 87 and Berken x ACC 87.

Generation	Rating					Perenniality concluded	
	0	1	2	3	4	No	Yes
Kiloga (P1)	5	0	0	0	0	5	0
ACC 87 (P2)	0	0	0	0	5	0	5
F1	0	0	0	5	0	0	5
F2	41	6	21	5	11	41	43
BCP1	11	2	0	0	0	11	2
BCP2	0	0	1	5	7	0	13
Berken (P1)	5	0	0	0	0	5	0
ACC 87 (P2)	0	0	0	0	5	0	5
F1	0	0	0	5	0	0	5
F2	38	10	17	12	7	38	46
BCP1	9	3	0	0	0	9	3
BCP2	0	0	2	2	9	0	13

Among the backcrosses to the cultivated parents (BC_{P1}), most plants were fibrous rooted like the cultivated parent, with only a small number (15-25%) showing any evidence of tuberisation. In the BC_{P1} generation, no plants were recovered that showed evidence of regeneration of shoots from either the roots or from basal stem nodes. The failure to recover any plants expressing a level of response close the wild parent again suggested that the trait may be conditioned by several genes. Among the

backcrosses to the wild parent, most plants exhibited stronger evidence of perenniality, with scores of 3 or 4, with only one and two BC_{P2} plants scored at 2 for Kiloga and Berken, respectively (Table 4.2).

Because of the range of response types observed in the F_2 and backcross populations (Table 4.2), the data were aggregated across the different perenniality rating scores to create two contrasting groups: fibrous rooted types that showed no evidence of tuberisation and types that showed evidence of root tuberisation, but did not necessarily produce either basal stem shoots or adventitious shoots from the roots after winter. In neither cross was the segregation ratio of the pooled F_2 data consistent with a single dominant gene ($P < 0.01$) based on the χ^2 test (data not shown). The number of F_2 individuals with no evidence of the perennial trait was much greater than would be expected with single gene action. Because a more complex mode of inheritance for the perennial trait was indicated, a two-gene model was then tested.

The simplest model that provided an acceptable fit to the data was two dominant genes with complementary action, that is, two genes which each need to be present as the dominant allele to enable expression of the perenniality trait (Table 4.3). With this model, it would be anticipated that in addition to the wild parent, expression of perenniality would be apparent in the F_1 plants and also all of the BC_{P2} plants, in just over half (56%) of the F_2 plants, but in only 25% of the BC_{P1} plants. In both crosses, the anticipated phenotypes were observed, and in each case, the ratios between the two contrasting phenotypes were broadly consistent with those expected (Table 4.3). The goodness of fit for the individual generations generally was not as strong in the Kiloga x ACC 87 cross as in the Berken x ACC 87 cross. However, it was still concluded that the presence or absence of the expression of perenniality was largely conditioned by two complementary genes.

There are several possible environmental or genetic reasons why the degree of expression of the perenniality trait may have varied in the segregating progeny (Table 4.2). Because the experimental plants were grown in pots, the development of the root system was inevitably restricted, and it is possible that the expression of the perenniality trait may have been limited in some plants. However, the fact that the perennial trait was expressed in ACC 87 suggested that the pot culture system used for the study was adequate for expression of the trait. Another possibility is that some of the tuberous rooted plants that were scored as 1, 2 or 3 after winter may have received a higher score at a later time. That is, it is possible that some tuberous rooted plants that had not developed basal shoots or adventitious shoots on the roots when the harvest was conducted (September 7) may have done so if the plants had been left to overwinter for longer. Related to this is the likelihood that some of the plants that died prematurely during the winter because of disease and/or waterlogging, but showed evidence of tuberous roots and scored 1 or 2 at that time, may have scored higher if they had survived.

Table 4.3 Models used to test the hypothesis that the segregation ratios for the perennial trait in the two crosses, Kiloga x ACC 87 and Berken x ACC 87, were consistent with the action of two complementary dominant genes.

Cross & Generation	Expected segregation ratio (no : yes)	Observed (Perenniality rating)					χ^2	df	Probability
		0	**1**	**2**	**3**	**4**			
Kiloga x ACC 87									
	F_2	41	6	21	5	11			
P_1	All no	5	-	-	-	-			
P_2	All yes	-	-	-	-	5			
F_1	All yes	-		-	5	-			
F_2	7:9	41			43		0.874	1	0.350
BC_{P1}	3:1	11			2		0.641	1	0.423
BC_{P2}	All yes	0			13		0.000	1	1.000
	Combined fit of segregation data to model						1.515	4	0.824
Berken x ACC 87									
	F_2	38	10	17	12	7			
P_1	All no	5	-	-	-	-			
P_2	All yes	-	-	-	-	5			
F_1	All yes	-		-	5	-			
F_2	7:9	38			46		0.076	1	0.783
BC_{P1}	3:1	9			3		0.000	1	1.000
BC_{P2}	All yes	0			13		0.000	1	1.000
	Combined fit of segregation data to model						0.076	4	0.999

Possible genetic reasons for the different levels of expression of perenniality include the fact that, assuming the di-genic model illustrated in Table 4.3, the F_2 and BC_{P2} progeny exhibiting the trait would be those genotypes that were either homozygous dominant or heterozygous at each locus. Thus four possible genotypes, AABB, AABb, AaBB and AaBb would exhibit the trait and that the degree of expression would vary to the extent there were any additive or partial dominance effects at either or both loci. Finally, it is possible that in addition to the two major loci suggested by the aggregate data (Table 4.3), there may be additional minor genes that affect the expression of the perenniality trait. Prior to this study, there was no information available on the inheritance of the perennial trait in mungbean, and little information for *Vigna* species generally. In *Vigna vexillata*, many of the root characters, such as tuber dry weight, and tuber harvest index, which are related to the expression of perenniality of this species, appeared to be quantitatively inherited (James *et al*. 1991, Karuniawan *et al*. 2006, Damayanti *et al*. 2010c). Damayanti (2010 a,b,c) suggested that tuber form in *V. vexillata* may be qualitatively inherited, but did not give any possible models of inheritance for this trait.

4.3.2 Qualitatively Inherited Traits

4.3.2.1 Morphological traits

The numbers of plants in each phenotypic category in the parental and the hybrid generations for each of the four putative qualitative morphological traits evaluated in this study (namely leaflet lobing, twining habit, growth habit and pod dehiscence) are shown in Table 4.4, for the both the Kiloga x ACC 87 and Berken x ACC 87 crosses. Also shown in Table 4.4 are the aggregate χ^2 values for the most appropriate model of gene action based on the frequency distributions of the different phenotypes in each generation. The various phenotypic categories used to score the main qualitative traits of interest were as described in Chapter 3. For none of the traits was there any evidence of variation among plants within either of the cultivated or the wild parental lines. This uniformity within the parental genotypes reflected the fact that mungbean is an inbreeding plant, so that cultivars generally remain homozygous and 'true-to-type' over successive generations. Mungbean flowers are visited by a range of nectar-feeding insects (mainly ants, bees and wasps) and there can occasionally be a small level of chance out-crossing. However, the data in Tables 4.4 indicated no evidence of that having occurred within either the wild or cultivated parents.

Leaflet lobing. The two cultivars showed no evidence of leaflet lobing, whereas the wild parent ACC 87 exhibited leaflet lobing, consistent with the descriptive name of '*sublobata*' for the wild type. In both crosses, the F_1 plants exhibited some lobing, but less than in the wild type (Table 4.4a). This suggested a degree of dominance for the wild type trait, but that its expression was either additive (and therefore less strongly expressed in the heterozygous state) or perhaps modified by other genes. In the F_2 generation, a range of expression of lobing was observed, with about three quarters of the plants showing some lobing (Table 4.4a). In the backcrosses to the cultivated parents (BC_{P1}), on average about half the plants exhibited some lobing in each cross, while in the backcross to the wild parent (BC_{P2}), the leaves of all the plants were lobed. These distributions were consistent with a single dominant gene conditioning the presence of lobing.

Collectively, in both crosses, when the two higher lobing scores (scores 2 and 3) were combined into a single group, the F_2 segregation ratio was consistent with a 1: 2: 1 none: weak: lobed distribution, the BC_{P1} distribution was consistent with 1: 1: 0 while the BC_{P2} distribution was 0: 1: 1. Thus, collectively, the data in both crosses was consistent with leaflet lobing in these crosses being conditioned by a single dominant gene, with additive gene action contributing to stronger lobing in plants homozygous dominant for the trait (data not shown). However, to confirm this point, it would be necessary to test the segregation patterns of the different lobed classes in the F_3 generation.

Twining habit. The cultivated parents showed no evidence of twining, while ACC 87 was strongly twining and in both crosses, the F_1 was weakly twining (Table 4.4b). About three quarters of the F_2 generation showed some twining, the backcrosses to the cultivated parents segregated and the backcrosses to the wild parent were all twining. These patterns were consistent with twining being conditioned by a single dominant gene. Collectively, in both crosses, the proportions of weakly twining plants in the F_1, F_2, BC_{P1} and BC_{P2} generations suggested that these may be heterozygous plants, and so were consistent with twining in both crosses being conditioned by additive gene action. Again, to confirm this point, it would be necessary to test the segregation patterns of the weakly and strongly twining classes in the F_3 generation.

Table 4.4 Phenotypic scores for putative qualitative morphological traits, the likely model of inheritance, and Chi-square tests for observed ratios, for two cultivated x wild mungbean crosses.

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories				χ^2	df	Probability
(a) Leaflet lobing – presence dominant over absence; additive gene action?								
Kiloga x ACC 87		**0[A]**	**1[B]**	**2[B]**	**3[B]**			
P_1	All none	5	0	0	0			
P_2	All strong	0	0	0	5			
F_1	All medium	0	0	5	0			
F_2	1:3	24	34	16	10	0.572	1	0.450
BC_{P1}	1:1	5	8	0	0	0.692	1	0.405
BC_{P2}	All lobed (0:n)	0	6	2	5	0.000	1	1.000
	Combined fit of segregation data to model					1.264	4	0.867
Berken x ACC 87		**0[A]**	**1[B]**	**2[B]**	**3[B]**			
P_1	All none	5	0	0	0			
P_2	All strong	0	0	0	5			
F_1	All medium	0	0	5	0			
F_2	1:3	19	39	11	15	0.254	1	0.614
BC_{P1}	1:1	8	4	0	0	1.333	1	0.248
BC_{P2}	All lobed (0:n)	0	4	4	5	0.000	1	1.000
	Combined fit of segregation data to model					1.587	4	0.811
[A]category 0 = no lobing; [B] category1, 2 & 3 = lobing present								
(b) Twining habit – presence dominant over absence; additive gene action?								
Kiloga x ACC 87		**0[C]**	**1[D]**	**2[D]**				
P_1	All none	5	0	0				
P_2	All strong	0	0	5				
F_1	All medium	0	5	0				
F_2	1:3	24	37	23		0.571	1	0.450
BC_{P1}	1:1	8	5	0		0.693	1	0.405
BC_{P2}	All twining (0:n)	0	6	7		0.000	1	1.000
	Combined fit of segregation data to model					1.264	4	0.867
Berken x ACC 87		**0[C]**	**1[D]**	**2[D]**				
P_1	All none	5	0	0				
P_2	All strong	0	0	5				
F_1	All medium	0	5	0				
F_2	1:3	20	39	25		0.063	1	0.996
BC_{P1}	1:1	9	3	0		3.000	1	0.083
BC_{P2}	All twining (0:n)	0	5	8		0.000	1	1.000
	Combined fit of segregation data to model					3.063	4	0.547
[C] category 0 = non twining; [D] category 1 & 2 = twining present								

Table 4.4 continued …

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories					χ^2	df	Probability
(c) Growth habit – digenic, prostrate / spreading partially dominant over erect									
Kiloga x ACC 87		**0**	**1**	**2**	**3[E]**	**4[E]**			
P_1	Erect	5	0	0	0	0			
P_2	Prostrate spreading	0	0	0	0	5			
F_1	Prostrate spreading	0	0	0	5	0			
F_2	1:3:3:9	6	16	13	23	26	0.656	3	0.884
BC_{P1}	1:1:1:1	4	4	3	2	0	0.846	3	0.838
BC_{P2}	0:0:0:n	0	0	0	2	11	0.000	1	1.000
	Combined fit of segregation data to model						1.502	8	0.993
Berken x ACC 87		**0**	**1**	**2**	**3[E]**	**4[E]**			
P_1	Erect	5	0	0	0	0			
P_2	Prostrate spreading	0	0	0	0	5			
F_1	Prostrate spreading	0	0	0	5	0			
F_2	1:3:3:9	2	16	14	25	27	2.688	3	0.442
BC_{P1}	1:1:1:1	1	4	6	1	0	6.000	3	0.112
BC_{P2}	0:0:0:n	0	0	0	0	13	0.000	1	1.000
	Combined fit of segregation data to model						8.688	8	0.369
[E] Categories 3 & 4 combined as strongly prostrate and spreading									
(d) Pod dehiscence — presence dominant over absence; additive gene action?									
Kiloga x ACC 87		**0[F]**		**1[G]**		**2[G]**			
P_1	Non-dehiscent	5		0		0			
P_2	Present (strong)	0		0		5			
F_1	Present (intermed.)	0		5		0			
F_2	1:3	21		42		21	0.000	1	1.000
BC_{P1}	1:1	8		5		0	0.692	1	0.405
BC_{P2}	0:n	0		3		10	0.000	1	1.000
	Combined fit of segregation data to model						0.692	4	0.952
Berken x ACC 87		**0[F]**		**1[G]**		**2[G]**			
P_1	Non-dehiscent	5		0		0			
P_2	Present (strong)	0		0		5			
F_1	Present (intermed.)	0		5		0			
F_2	1:3	21		40		23	0.000	1	1.000
BC_{P1}	1:1	7		5		0	0.333	1	0.564
BC_{P2}	0:n	0		4		9	0.000	1	1.000
	Combined fit of segregation data to model						0.333	4	0.988
[F] category 0 = non-dehiscent; [G] categories 1 & 2 = dehiscence present									

Score 0
No twining present

Score 1
Intermediate twining present

Score 2
Strongly twining

Figure 4.2 Representative examples illustrating the degree of expression of the twining habit trait, for each of the three scoring levels used in the study. The cultivated parent plants scored zero (i.e. non - twining habit), while ACC 87 scored 4 (i.e. strongly twining habit).

Growth habit. Representative growth habit phenotypes observed in both crosses are illustrated in Figure 4.4. Based on a rating score of 0 (erect habit) to 4 (prostrate habit), the two parental cultivars scored 0 compared with 4 for ACC 87, while the F_1 plants were all spreading to a large degree, but not as prostrate as the wild parent (Table 4.4c). In the F_2 and BC_{P1} generations, only a small number of erect plants similar to the cultivated parental type were recovered. In contrast, more than half of the F_2 plants in both crosses were scored in the two highest (3 and 4) categories. In the BC_{P2} generation, all of the plants were prostrate and scored in the two highest categories. The range of F_2 phenotypes suggested at least two genes controlling this trait, with prostrate spreading habit dominant to erect habit. When the two high score categories (3 and 4) were combined into a 'prostrate and spreading' group, the F_2 distribution in both crosses was consistent with a segregation ratio of 9: 3: 3: 1 digenic model of inheritance for growth habit, with the goodness-of-fit stronger in the Kiloga cross than in the Berken cross (Table 4.4c). In this model, both genes exhibit partial dominance for the prostrate, spreading habit.

Score zero Strongly erect habit (cultivated parent)	Score 1 Partly erect habit (hybrid progeny)	Score 2 Intermediate habit (hybrid progeny)	Score 3 Partly prostrate habit (hybrid progeny)	Score 4 Strongly prostrate habit (wild parent)

Figure 4.3 Representative examples illustrating the degree of expression of the growth habit trait, for each of the five scoring levels used in the study. The cultivated parent plants scored zero (i.e. erect habit), while ACC 87 scored 4 (i.e. prostrate spreading habit).

Pod dehiscence. The pods of the cultivated varieties were both non-dehiscent while ACC 87 was strongly dehiscent and the F_1 was weakly dehiscent (Table 4.4d). In the F_2 generation, three quarters of the plants in both crosses were dehiscent, while in the backcrosses to the cultivated parents, just over half the progeny were non-dehiscent. Collectively, when ignoring type of pod shattering, these data were consistent with pod dehiscence in both crosses being conditioned by a single dominant gene model (Table 4.4d). However, there were differences in the degree of shattering, in that both strongly dehiscent and weakly dehiscent progeny were recovered (Table 4.4d). In both crosses, the distributions in the F_2 were consistent with a 1: 2 : 1 non : weak : strong ratio, while the BC_{P1} ratio was consistent with 1: 1 non: weak. These patterns suggested additive gene action, whereby the heterozygous progeny plants expressed weak rather than strong dehiscence. However, the goodness of fit to this model (data not shown) was not as strong as for the simpler model illustrated in Table 4.4d.

4.3.2.2 Visual seed characters

Visual appearance of seed is of major commercial importance in mungbean. The heritability of traits contributing to the appearance, and the presence of linkages with any undesirable traits are therefore of some importance to establishing how easily advantage might be taken of wild germplasm in mungbean breeding. The observations on phenotypic ratios for testa colour, seed-coat ridging, seed-coat surface, seed-coat ridging colour and testa mottling, and the probable models for inheritance based on these observations, are shown in Table 4.5.

Testa colour. In both crosses, the wild type trait was speckled black while the cultivated phenotype was uniform green (Table 4.5a). The F_1 was also speckled, although the number of speckled F_2 plants in both crosses was much higher than expected for a single gene. All of the BC_{P2} plants showed the speckled testa, as did just a half of BC_{P1} progenies. Together, the simplest most likely inheritance model for seed testa colour character was control by two genes with dominant and recessive epistasis (a dominant gene for the speckled wild type, moderated by a recessive suppressor gene inherited from the cultivated parent green testa). In both crosses, there were differences in expression of the mottling trait in that in the F_1 and BC_{P1} progeny, the mottling was light. However, in the F_2 and BC_{P2} progeny, there were both lightly and densely mottled progeny, with the latter representing more than half the numbers. The differences in mottling density suggest either partial dominance or a multigenic effect for the trait.

Seed-coat surface texture layer. In both crosses, the wild type trait had a ridged surface texture layer while the cultivated phenotype was smooth (Table 4.5b). The F_1 and BC_{P2} progeny were similar to the wild type, while the F_2 generation in both crosses segregated for both traits. While the goodness-of-fit in the F_2 generation, especially in the Berken cross, was not very strong (Table 4.5b), the simplest inheritance model for both crosses was a single dominant gene for presence of the ridged texture layer.

Seed-coat surface. The dull seed coat shows a fine network of ridges, which in transverse section gives the outer epidermis surface an undulated or dentate appearance. The shiny seed coat has a smooth surface. The cultivated parents had shiny seed coat, whereas the wild parent and the F_1 progeny in both crosses exhibited dull seed coat, suggesting dominant gene action conditioning the dull trait (Table 4.5c). In both crosses, there was segregation in the F_2 and BC_{P1} generations, but not in the BC_{P2}, which were all dull. The F_2 plants in the Kiloga x ACC 87 cross segregated for dull: shiny in a reasonable fit to a 3:1 ratio, and near 1:1 BC_{P1} ratio, supporting this model. In contrast, the F_2

generation of the Berken x ACC 87 cross fitted better to a model for two dominant genes with complementary action (9:7 ratio expected). The near 1:3 BC_{P1} ratio also supported this digenic model.

Surface texture layer and hilum colour. When present, the surface texture layer was either dark or light and this pigmentation extended to the hilum. In both crosses, the wild type trait was dark while the cultivated phenotype was light (Table 4.5d). The F_1 was similar to the wild type, while the F_2 generation segregated for both phenotypes in a moderate fit to a 3:1 ratio. In both crosses, almost all of the BC_{P2} plants were dark, whereas about half the BC_{P1} plants were light. The simplest most likely inheritance model was a single dominant gene for dark pigmentation.

Table 4.5 Phenotypic scores for putative qualitative seed appearance traits, the likely model of inheritance, and Chi-square tests for observed ratios, for two cultivated x wild mungbean crosses.

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories			χ^2	df	Probability
(a) Testa colour – green colour conditioned by two genes in recessive-dominance epistasis							
Kiloga x ACC 87		Green	Speckled black				
			Light	Dense			
P_1	Green	5	-	-			
P_2	Speckled black	0	0	5			
F_1	Speckled black	0	5	0			
F_2	3:13	14	30	40	0.239	1	0.625
BC_{P1}	1:1	8	5	0	0.692	1	0.405
BC_{P2}	All sp. black (0:n)	0	4	9	0.000	1	1.000
	Combined fit of segregation data to model				0.931	4	0.920
Berken x ACC 87		Green	Speckled black				
			Light	Dense			
P_1	Green	5	-	-			
P_2	Speckled black	0	0	5			
F_1	Speckled black	0	5	0			
F_2	3:13	16	31	37	0.005	1	0.944
BC_{P1}	1:1	8	4	0	1.333	1	0.248
BC_{P2}	All sp. black (0:n)	0	6	7	0.000	1	1.000
	Combined fit of segregation data to model				1.338	4	0.855
(b) Surface texture layer – single gene, presence dominant over absence							
Kiloga x ACC 87		Absent	Present				
P_1	Absent	5	0				
P_2	Present	0	5				
F_1	Present	0	5				
F_2	1:3	25	59		1.016	1	0.313
BC_{P1}	1:1	7	6		0.077	1	0.781
BC_{P2}	All present (0:n)	0	13		0	1	1.000
	Combined fit of segregation data to model				1.093	4	0.895
Berken x ACC 87		Absent	Present				
P_1	Absent	5	0				
P_2	Present	0	5				
F_1	Present	0	5				
F_2	1:3	26	58		1.587	1	0.208
BC_{P1}	1:1	8	4		1.333	1	0.248
BC_{P2}	All present (0:n)	0	13		0	1	1.000
	Combined fit of segregation data to model				2.920	4	0.571

Table 4.5 continued …

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories		χ^2	df	Probability
(c) Seed-coat surface – dull dominant over shiny						
Kiloga x ACC 87		Shiny	Dull			
P_1	Shiny	5	-			
P_2	Dull	-	5			
F_1	Dull	-	5			
F_2	1:3	25	59	1.061	1	0.303
BC_{P1}	1:1	8	5	0.692	1	0.405
BC_{P2}	All dull (0:n)	0	13	0.000	1	1.000
	Combined fit of segregation data to model			1.753	4	0.781
Berken x ACC 87		Shiny	Dull			
P_1	Shiny	5	-			
P_2	Dull	-	5			
F_1	Dull	-	5			
F_2	7:9	34	50	0.366	1	0.562
BC_{P1}	3:1	10	2	0.444	1	0.505
BC_{P2}	All dull (0:n)	0	13	0.000	1	1.000
	Combined fit of segregation data to model			0.810	4	0.937
(d) Texture layer / hilum colour – dark dominant over light						
Kiloga x ACC 87		Light	Dark			
P_1	Light	5	0			
P_2	Dark	0	5			
F_1	Dark	0	5			
F_2	1:3	26	58	1.587	1	0.208
BC_{P1}	1:1	6	7	0.077	1	0.781
BC_{P2}	All dark (0:n)	0	13	0.000	1	1.000
	Combined fit of segregation data to model			1.644	4	0.801
Berken x ACC 87		Light	Dark			
P_1	Light	5	0			
P_2	Dark	0	5			
F_1	Dark	0	5			
F_2	1:3	24	60	1.097	1	0.450
BC_{P1}	1:1	6	6	0.000	1	1.000
BC_{P2}	All dark (0:n)	0	13	0.000	1	1.000
	Combined fit of segregation data to model			1.097	4	0.895

Apparent resistance to powdery mildew disease. Powdery mildew infection on the leaves occurred in the cultivated parents in both crosses but not on ACC 87, suggesting that ACC 87 was not as susceptible as the cultivated parents. The F_1 and the BC_{P1} progeny were all similar to the cultivated type, indicating that putative susceptibility was dominant (Table 4.6). Meanwhile, the F_2 and the BC_{P2} populations of both crosses contained plants with and without the disease. In the F_2 generation, the data for Berken x ACC 87 provided a reasonable fit to a single recessive gene (χ^2= 0.254, P = 0.614). The backcross data supported this model. However, a single recessive gene model did not fit the Kiloga x ACC 87 cross in the F_2 generation, because there were too few plants without infection (χ^2 = 5.143, P = 0.023). Instead, the distribution in the Kiloga x ACC 87 cross was closer to a digenic 13:3 presence: absence ratio, consistent with the absence of powdery mildew disease infection in this cross being conditioned by two genes with dominant and recessive epistasis. Indeed, the same model could

be applied to the Berken x ACC 87 data, albeit with a slightly lower probability than the single recessive gene model. As all the plants were randomly distributed on the benches, and as the infected plants had many affected leaves, there is no chance that the non-infected plants could have simply 'escaped' exposure to spores.

Table 4.6 Phenotypic scores for putative resistance to powdery mildew, the likely mode of inheritance, and Chi-square tests for observed ratios, for the two cultivated x wild mungbean crosses Kiloga x ACC 87 and Berken x ACC 87.

Cross & Generation	Expected segregation ratio	Observed presence or absence of powdery mildew disease		χ^2	df	Probability
		Presence	Absence			
Kiloga x ACC 87						
Powdery mildew infection – absence conditioned by two genes in recessive-dominance epistasis						
P_1	Presence	5	0			
P_2	Absence	0	5			
F_1	Presence	5	0			
F_2	13:3	72	12	1.099	1	0.294
BC_{P1}	All with (n:0)	13	0	0.000	1	1.000
BC_{P2}	1:1	8	5	0.692	1	0.405
	Combined fit of segregation data to model			1.791	4	0.774
Berken x ACC 87						
Powdery mildew infection – absence conditioned by a single recessive gene						
		Presence	**Absence**			
P_1	Presence	5	0			
P_2	Absence	0	5			
F_1	Presence	5	0			
F_2	3:1	65	19	0.254	1	0.614
BC_{P1}	All with (n:0)	12	0	0.000	1	1.000
BC_{P2}	1:1	9	4	1.923	1	0.166
	Combined fit of segregation data to model			2.177	4	0.703

4.3.3 Quantitatively Inherited Traits

4.3.3.1 Phenological traits

Means for phenological traits for the parents and the F_1, F_2, BC_{P1} and BC_{P2} generations are shown in Table 4.7. In both crosses, analysis of variance revealed that there were significant differences between the cultivated and wild parents in times to flowering, the duration of flowering, and the total life cycle. There were no differences ($P > 0.05$) between the cultivated and wild parents in the time for pod growth. The two cultivars flowered in less than five weeks after sowing and matured their first pods about 19 d later. Both Kiloga and Berken are early flowering varieties in the subtropics (Lawn 1979a), so it was expected that they would also be early flowering under the shorter days of the tropics. In contrast, ACC 87 did not flower until just over six weeks after sowing, but like the cultivars, matured its first pods about 19 d later. However, maturity of the last flush of pods on ACC 87 did not occur until more than five months after sowing, much later than in either of the two

cultivars (Table 4.7). This longer growth cycle of the wild accession was consistent with the previous observations of Rebetzke and Lawn (2006 a,b and c), and reflected the fact that growth of the wild accession was strongly indeterminate, with successive flushes of flowers and pods being produced, until mid-winter, when cool temperatures finally slowed growth and the plant shoots died back. While the two cultivars flowered sooner after flowering than ACC 87, their life cycle was two to four weeks longer than mid-summer sowings of these cultivars in south-east Queensland (e.g. see Lawn 1979a). The longer life cycle in the tropics may have been due to the fact that after March 21, these cultivars were exposed to longer days and warmer temperatures than are summer sowings after that date in the subtropics. Warm temperatures and long photoperiods are known to promote continued vegetative growth and successive flushed of flowering and podding in mungbean (Lawn 1979b).

The time to flowering of the F_1 hybrids in both crosses were intermediate between the two parents while the time to first mature pod was not significantly different from the parents (Table 4.7). However, the growth duration of the F_1 hybrid in both crosses was comparable with the wild parent, reflecting the fact that the F_1 hybrids were strongly indeterminate like the wild parent. The mean time to flowering of the F_2 population was again intermediate between the two parents, although a little later than the F_1. In both crosses, there was evidence of transgressive segregation, with some F_2 individuals recovered that were either earlier flowering than the cultivated parent, or later flowering than ACC 87 (Table 4.7, Figure 4.4). In the Berken x ACC 87 cross, some F_2 individuals were recovered that were much later flowering than ACC 87 (Figure 4.4). These transgressive phenotypes could be produced when alleles at multiple loci that originated in the wild parental populations recombined in the hybrids. According to Bell (2005), diverse traits may exhibit transgressive segregation, and they could contribute to ecological divergence and reproductive isolation between hybrids and parental species. The appearance of transgressive segregation in hybrids could be useful in exploring hybrid zone dynamics or hybrid speciation (Rieseberg *et al.* 1999). Transgressive segregation was a common occurrence in crosses between domesticated lines and wild populations.

In terms of the duration of the main flush of flowering, the F_1 plants were closer to the cultivated parents and not significantly different from them (Table 4.7). As with time to flowering, the range for this trait in the F_2 populations was very wide in both crosses. The BC_{P2} plants of the Kiloga cross were not significantly different from the wild parents, but in the Berken cross, the duration of flowering trait in the BC_{P2} was closer to Berken. The overall growth duration of the cultivated parents was significantly shorter than that of the wild types. In both crosses, the F_1, F_2, and BC_{P2} population means for growth duration were comparable with the wild parents, whereas in the BC_{P1}, growth duration was closer to the cultivated parents. The additive genetic components for the phenology traits were much greater in magnitude than the corresponding dominance genetic components and

environmental variance (data not shown), reflecting the presence of additive gene action, or additive-dominance modes of inheritance for this trait (Brown *et al.* 2008, Rehman *et al.* 2009).

Table 4.7 Phenological trait means for the parental, F_1, F_2, BC_{P1} and BC_{P2} generations, the range for the F_2 generation, and broad and narrow sense heritability estimates, for two cultivated x wild mungbean crosses (a) Kiloga x ACC 87 (b) Berken x ACC 87. Means followed by the same letters are not significantly different ($P > 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad (h^2_b)	Narrow (h^2_n)
(a) Kiloga x ACC 87									
Time to flowering (d)	31.1a	37.8b	43.8c	30-48	38.4b	36.2b	41.5c	0.91±0.01	0.53±0.24
Time for pod growth (d)	19.0ab	16.0a	18.8ab	14-29	17.7a	17.8a	19.6b	0.92±<0.01	0.00
Duration of flowering (d)	38.4a	37.4a	56.6b	21-60	42.3a	36.3a	44.2a	0.92±0.00	0.81±0.23
Growth duration (d)	112a	166c	171c	87-170	144bc	123ab	154bc	0.93±0.01	0.96±0.15
(b) Berken x ACC 87									
Time to flowering (d)	33.5a	38.2ab	45.6c	31-82	41.3bc	35.2ab	40.8bc	0.97±0.01	1.00[A]±0.001
Time for pod growth (d)	18.7ab	16.4a	18.7ab	16-29	18.1ab	18.2ab	19.0b	0.70±0.08	0.00
Duration of flowering (d)	45.8ab	37.2a	62.3c	29-98	42.1ab	38.3a	51.7b	0.75±0.06	1.00[A]±0.01
Growth duration (d)	132ab	165bc	174c	92-188	157bc	128a	156bc	0.90±0.02	1.00[A]±0.03

[A] indicates where the estimate of heritability exceeded 1

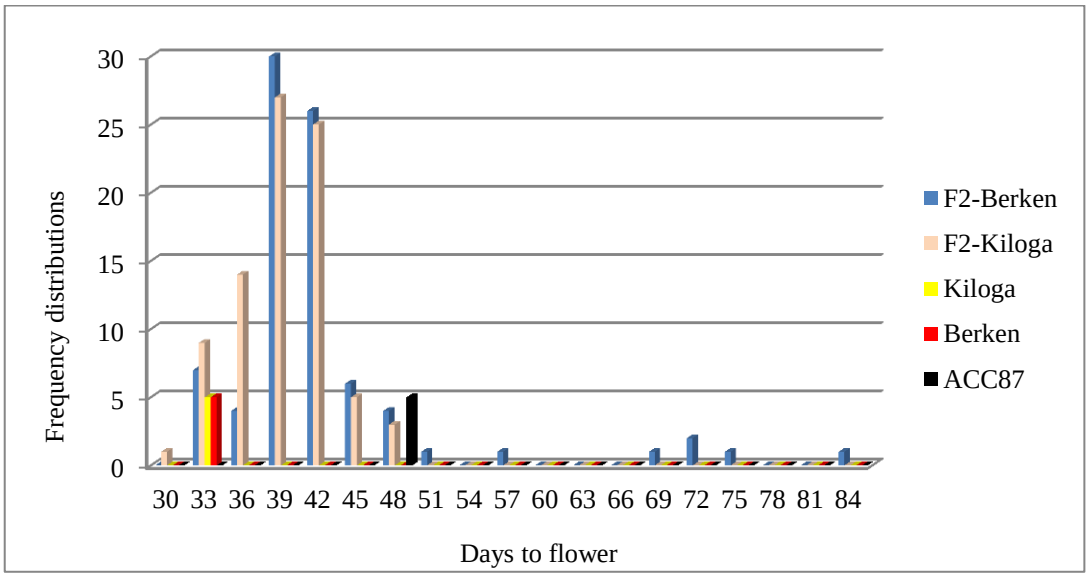


Figure 4.4 Time to flowering (d) for the parents and F_2 generations for two cultivated x wild mungbean crosses Kiloga x ACC 87 and Berken x ACC 87

Based on the heritability classification of Acquaah (2007), broad sense heritability estimates for all four phenological traits appeared generally to be high in both crosses (Table 4.7). However, narrow sense heritability values for those traits were variable. In both crosses, the values of narrow sense

heritability for the duration of pod growth were not significantly different from zero, presumably reflecting the fact that the duration of pod growth exhibited in both the wild and cultivated parents was not significantly different. Narrow sense heritability for the remaining phenological traits in both crosses was moderate to high, suggesting a high level of additive genetic variance, and thus a strong prospect of making genetic gains through selection for these traits. In the Berken x ACC 87 cross, some narrow sense heritability estimates actually exceeded 1, which is not possible, but which nonetheless meant that the traits were highly heritable.

4.3.3.2 Morphological traits

The analysis of variance revealed that there were large differences between the cultivated and wild parents, and consequently in the F_1, F_2, BC_{P1} and BC_{P2} generations, for all of the morphological traits observed (Table 4.8). Most of the size related traits, such as leaflet length and width, seed weight, pod length and width, stem thickness and width of the floral standard, were all much greater in magnitude in the cultivated parents than in ACC 87.However, some traits such as number of branches, nodes per main stem and level of hardseededness were much higher in ACC 87. The expression of the individual traits in the different generations, and the estimates of their heritability are discussed in turn below.

Stem diameter. In both crosses, the stem thickness of the F_1 hybrid was closer to the cultivated parent than to the wild type, suggesting some level of dominance for the trait (Table 4.8). However, the F_2 populations of both crosses were close to the mid-parent value. The BC plants in both crosses were close to, and not statistically different from the respective backcross parent. The broad sense heritability values were moderate in both crosses (0.66 and 0.57, respectively), but the narrow sense heritability in the Berken cross was only 0.41 and given the high standard error, was not statistically greater than zero.

Floral standard. While ACC 87 and other perennial accessions have a relatively wider floral standard than most wild mungbean accessions (Lawn and Rebetzke 2006c), the floral standard was still smaller than in the two cultivars (Table 4.8). In both crosses, the width of the floral standard of both the F_1 and F_2 generations was closer to the cultivated than the wild parent, and only for the BC_{P2} population was mean floral standard width closer to the wild type. Both broad and narrow sense heritability of standard width was moderate in the Kiloga x ACC 87 cross, and high in the Berken x ACC 87 cross.

Table 4.8 Morphological trait means for the parental, F_1, and F_2BC_{P1} and BC_{P2} populations, the range for the F_2 generation, and broad and narrow sense heritability estimates, for two cultivated x wild mungbean crosses (a) Kiloga x ACC 87 (b) Berken x ACC 87.Means followed by the same letters are not significantly different ($P < 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad (h^2_b)	Narrow (h^2_n)
(a) Kiloga x ACC 87									
Stem diameter (mm)	8.7c	8.4c	5.3a	4.5-11.7	7.0b	9.7c	5.7a	0.66±0.01	1.00[A]±0.09
Floral standard (mm)	17.4b	17.9b	14.9a	14.2-19.8	17.5b	18.1b	15.2a	0.59±0.04	0.52±0.26
Leaflet length (mm)	123bc	120bc	89a	64-153	112b	128c	88a	0.70±0.03	0.00
Leaflet width (mm)	100bc	101bc	58a	61-129	94b	110c	67a	0.75±0.03	0.84±0.15
Leaflet width-length ratio	0.81bc	0.84bc	0.66a	0.51-1.14	0.84bc	0.87c	0.75b	0.79±1.44	1.00[A]±0.07
Branches per main stem	4.3a	7.4b	8.4b	3-13	7.5b	5.5a	8.0b	0.80±0.01	0.00
Nodes per main stem	8.0a	10.6bcd	12.1d	3-15	9.7abc	9.1ab	11.1cd	0.86±0.01	0.95±0.13
Nodes per branch	3.7a	6.4b	9.1c	2.8-8.0	5.6b	4.0a	8.4c	0.77±0.03	0.39±0.38
Pods per peduncle	4.6a	5.2ab	6.1c	3.0-7.4	4.9ab	4.6a	5.5bc	0.67±0.09	0.00
Peduncle length (mm)	78a	119c	169d	51-137	90ab	103bc	117c	0.53±0.18	0.58±0.27
Pod length (mm)	113d	84bc	62a	50-120	74b	95c	74b	0.61±0.18	0.87±0.15
Pod width (mm)	6.0c	4.9b	4.0a	3.6-6.8	4.7b	5.9c	4.5ab	0.63±0.13	0.44±0.34
Seeds per pod	11.9c	7.9a	9.5b	5.8-14.5	11.4c	12.5c	9.4b	0.79±0.01	1.00[A]±0.01
Seed size (g /100)	6.65c	5.54b	3.99a	0.39-7.40	5.17b	7.06c	4.98b	0.82±0.09	0.70±0.25
Hard seed	0.01a	0.10a	1.00c	0.12-0.98	0.41b	0.13a	0.83bc	0.99±<0.01	0.75±0.24
(b) Berken x ACC 87									
Stem diameter (mm)	9.4c	7.2b	4.9a	5.0-10.8	6.7b	9.5c	5.6a	0.57±0.07	0.00
Floral standard (mm)	18.7d	17.7cd	15.3a	13.5-19.0	17.2c	18.4d	16.2b	0.81±002	0.87±0.14
Leaflet length (mm)	129d	122cd	87a	68-164	110bc	120cd	97ab	0.68±0.05	0.36±0.30
Leaflet width (mm)	104c	100c	65a	62-125	88b	110c	65a	065±007	0.00
Leaflet width-length ratio	0.79bc	0.82bc	0.75ab	0.54-1.45	0.80bc	0.86bc	0.67a	0.71±0.0	1[A]±0.14
Branches per main stem	3.9a	6.8b	9.0c	3.0-16.0	7.8bc	6.3b	8.1bc	0.95±0.01	0.89±0.10
Nodes per main stem	8.7a	12cd	12.7d	6.0-16.0	10.6bc	9.8ab	11.9cd	0.89±002	0.92±0.15
Nodes per branch	4.02a	6.80bc	9.79d	2.5-12.4	5.99b	4.15a	7.98c	0.82±0.03	0.82±0.18
Pods per peduncle	4.6a	5. 0ab	6.1c	4.2-7.2	5.4bc	4.8ab	5.1ab	0.82±0.06	0.00
Peduncle length (mm)	107a	99a	178b	61-179	111a	116a	116a	0.84±0.03	0.39±0.36
Pod length (mm)	109d	74b	59.9a	55-139	92.3c	102.8d	69.1b	0.69±0.16	0.83±0.18
Pod width (mm)	6.4d	4.9c	3.9a	4-.0-7.6	4.7bc	6.3d	4.4b	0.55±0.09	0.80±0.23
Seeds per pod	12.2b	8.1a	9.1a	5.7-14.5	11.9b	12.5b	11.7b	0.88±0.02	0.94±0.13
Seed size (g /100)	7.14e	5.90cd	3.87a	4.00-8.80	5.44bc	6.72de	4.87b	0.88±0.04	0.59±0.37
Hard seed	0.01a	0.11a	1.00c	0.04-0.95	0.45b	0.12a	0.78bc	0.97±<0.01	0.96±0.14

[A] indicates where the estimate of heritability exceeded 1

Leaflet size and shape. In both crosses, the leaflet length and width, and the leaflet length: width ratio of the F_1 progeny were all closer to the cultivated parents, whereas in the F_2 generation, the mean was closer to the mid-parent value (Table 4.8). Among the backcross progeny, the means were close to the respective backcross parents, and not significantly different from them. The narrow sense heritability of the leaflet length and width characters in both crosses was low, excepted for leaflet width in the Kiloga cross which was relatively high (0.84). Narrow sense heritability of the leaflet length: width ratio was very high in both crosses.

Number of branches on main stem. In the Kiloga x ACC 87 cross, the means of the F_1, F_2, and BC_{P2} plants were closer to the wild parent, whereas the BC_{P1} population was similar to the cultivar (Table

4.8). In the Berken x ACC 87 cross, the means of the F_1 and BC_{P1} populations were intermediate between the parents, while the F_2 and BC_{P2} progeny means were closer to the wild type. Narrow sense heritability was very low in the Kiloga cross, but was very high in the Berken cross.

Number of nodes per main stem and nodes per branch. In both crosses, the number of nodes per main stem and per branch was significantly greater in the wild parent than in the two cultivars (Table 4.8). In both crosses, the number of nodes per stem in the F_1 plants was closer to the wild parent, suggesting perhaps some dominant gene action. In the F_2 generation in both crosses, while there was transgressive segregation beyond both parental values, the means were closer to the mid-parent values. With the number of nodes per branch, the F_1 progenies in both crosses were close to the mid-parent values. Except for the narrow sense heritability of nodes/branch in the Kiloga cross, both broad and narrow sense heritability for both node traits was high.

Number of pods per peduncle. In both crosses, the number of pods per peduncle of ACC 87 was significantly greater than in the cultivated parents (Table 4.8). The F_1 hybrids were closer to the cultivated parents than to ACC 87. There was some transgressive segregation in the F_2 generation, with the mean closer to the mid-parent value. Narrow sense heritability was generally considerably smaller than broad sense heritability in both crosses, suggesting a significant non-additive contribution to total genetic variance. This non-additive component could consist of dominance, epistatic (Lynch and Walsh, 1998); however, the present results can not distinguish among these possibilities.

Peduncle length. There were large differences between parental lines and their offspring with the cultivated parents exhibiting considerably shorter peduncles than the wild parent (Table 4.8). In the Kiloga cross, the F_1 plants were intermediate between and significantly different from the parents, whereas in the Berken cross, the F_1, F_2, BC_{P1} and BC_{P2} generation means were all similar to Berken, suggesting dominant gene action. Both broad and narrow sense heritabilities for the trait in the Kiloga cross were moderate (0.53 and 0.58, respectively). However, the narrow sense heritability in the Berken cross was only 0.39, much lower than the broad sense heritability (0.83), which again may reflect a significant non-additive genetic contribution to total genetic variance.

Pod size. In both crosses, pod size in the wild parent was generally smaller than that of the cultivated varieties in terms of both length and width (Table 4.8). This was consistent with the previous observations of James *et al*. (1999) and Lawn and Rebetzke (2006). In both crosses, the F_1 hybrid was intermediate between and significantly different from both parents for pod width and pod length. Generally, both narrow and broad sense heritabilities were moderate to high (more than 0.65), the exception being the narrow sense heritability of pod width in the Kiloga cross (just 0.44).

Number of seeds per pod. In both crosses, the number of seeds per pod was significantly greater in the cultivated parents than in ACC 87 (Table 4.8). Interestingly, the F_1 hybrid tended to have fewer seeds per pod than either parent. However, the F_2 and BC_{P1} generation means were close to the cultivated parent. Nonetheless, there was considerable transgressive segregation for seeds per pod in the F_2 generation (Figure 4.5). In both crosses, there were several segregants above and several below the respective parental values. Broad sense heritability was high and narrow sense heritability was very high in both crosses (Table 4.8).

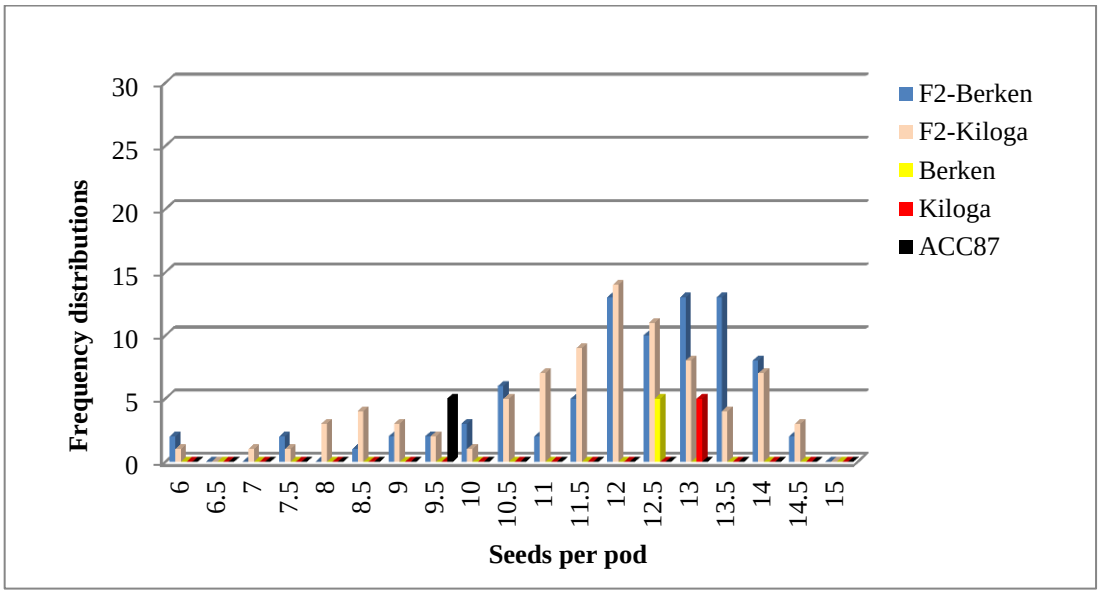


Figure 4.5 Number of seeds per pod for the parents and F_2 generations for two cultivated x wild mungbean crosses Kiloga x ACC 87 and Berken x ACC 87

Seed size or 100 seed weight. The 100 seed weight of ACC 87 was around 3.9 g (Table 4.8), which is at the upper end of the range for wild Australian accessions (Rebetzke and Lawn 2006a). In contrast, seed size of the two cultivars was much larger. In both crosses, the seed size of the F_1 hybrid was intermediate between and significantly different from both the parents. Likewise, the mean of the F_2 generation was close to the mid-parent value, although in both crosses, a few very large seeded segregants were recovered. Broad sense heritability estimates for seed size were high, and narrow sense estimates were moderate to high, suggesting considerable additive gene action for this trait.

Hardseededness. In both crosses, the wild parent showed extremely strong hardseededness (Figure 4.6a), while the cultivated lines were almost completely soft seeded (Figure 4.6c). This finding was consistent with previous observations. For example Lawn *et al.* (1988) and James *et al.* (1999) reported 100% hard seeds in wild mungbean. While the F_1 hybrid showed only about 10% hard seed (Figure 4.6b, Table 4.8), there was considerable variation in the F_2 generation, from almost completely soft seed to almost completely hard seed (Table 4.8). There was no apparent relation with

seed testa colour (Figures 4.6d-h). The mean of the F_2 progenies was about 40% hard seed in both crosses (Table 4.8). In the BC_{P1} generation, the mean level of hard seed was comparable with the F_1, whereas in the BC_{P2} plants, a higher level of hard seed (average c. 80%) was exhibited. Both broad and narrow sense heritabilities for hardseededness were high to very high.

Figure 4.6a:
ACC 87 had speckled black testa and hard seed

Figure 4.6b:
Seed of F_1 plants had speckled black testa and mostly soft seed

Figure 4.6c:
Cultivated type (Berken) had green testa and mostly soft seed

Figure 4.6d
Seed of an F_2 plant with green testa and hard seed

Figure 4.6e
Seed of an F_2 plant with green testa and soft seed

Figure 4.6f
Seed of an F_2 plant with speckled black testa and mostly hard seed

Figure 4.6g
Seed of an F_2 plant with speckled black testa and mostly soft seed

Figure 4.6h
Seed of an F_2 plant with speckled black testa and no hard seed

Figure 4.6 Representative examples of different levels of hardseededness, as illustrated by the numbers of seed that germinated after 48 hours under warm, moist conditions in petri dishes: (a) ACC 87, (b) F_1 progeny, (c) cultivated type (Berken), (d-h) F_2 progenies with different seed testa colours and different levels of hard seed.

4.3.3.3 Agronomic traits

Analysis of variance showed that there were significant differences ($P< 0.05$) between the parental means, and among the F_1, F_2, BC_{P1} and BC_{P2} generation means, for most of the agronomic traits observed, the main exception being vegetative biomass (Table 4.9). Generally, vegetative biomass, total pod weight and total dry matter were greater in the wild accession than in the cultivars, whereas seed yield, harvest index and seed: pod weight ratio were each generally smaller in the wild accession. The estimates of broad sense heritability for all the agronomic traits were moderate to high in both crosses. However, the estimates of narrow sense heritability ranged from very low to very high values, depending on the trait.

Table 4.9 Agronomic trait means for the parental, F_1, and F_2, BC_{P1} and BC_{P2} populations, the range for the F_2 generation, and broad and narrow sense heritability estimates, for two crosses (a) Kiloga x ACC 87 (b) Berken x ACC 87. Means followed by the same letters are not significantly different ($P < 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad (h^2_b)	Narrow (h^2_n)
(a) Kiloga x ACC 87									
Vegetative biomass (g)	25.1a	31.1b	34.8bc	13-36	26.0a	26.8a	35.7c	0.68±<0.01	0.80±0.18
Pod weight (g/plant)	70.1a	94.6bc	85.1abc	42-175	79.5ab	79.1ab	100.7c	0.89±<0.01	0.61±0.22
Seed yield (g/plant)	49.5bcd	56.7d	38.4a	30-71	46.3abc	55cd	41.1ab	0.85±<0.05	0.44±0.38
Seed: pod weight ratio	0.70c	0.56b	0.45a	0.22-0.80	0.60b	0.71c	0.44a	0.90±<0.01	0.00
Total dry matter (g)	97a	130c	118bc	70-210	105ab	105ab	130c	0.91±<0.01	0.55±0.24
Harvest index	0.49cd	0.43b	0.32a	0.18-0.62	0.44bc	0.53d	0.32a	0.75±0.14	1.00[A]±0.17
(b) Berken x ACC 87									
Vegetative biomass (g)	25.4a	31.1abc	36.4c	18-69	28.6ab	28.5ab	34.2bc	0.94±0.01	1.00[A]±0.02
Pod weight (g/plant)	80.3a	101.8bc	100.6bc	20-140	76.5a	87.8ab	114.3c	0.69±0.06	0.54±0.26
Seed yield (g/plant)	51.4ab	57.4b	40.2a	12-76	44.3a	58.8b	49.9ab	0.75±0.07	0.00
Seed: pod weight ratio	0.63cd	0.57b	0.45a	0.42-0.72	0.59bc	0.67d	0.44a	0.69±0.07	0.00
Total dry matter (g)	105a	132bc	135bc	43-188	105a	115ab	148c	0.81±0.03	0.90±0.15
Harvest index	0.49c	0.43b	0.32a	0.29-0.49	0.42b	0.51c	0.34a	0.81±0.04	0.93±0.21

[A] indicates where the estimate of heritability exceeded 1

Vegetative biomass. The differences in mean vegetative biomass between the various generations in the Kiloga cross were generally small (Table 4.9). The F_1 and BC_{P2} means were close to the wild parent, while the F_2 and BC_{P1} means were close the cultivated parent. In the Berken cross, while vegetative biomass was greater in the wild parent, mean biomass for the F_1, F_2 and BC_{P1} generations was intermediate between the parents and different from neither. Broad and narrow sense heritabilities for both crosses were moderate to very high.

Pod weight. As with vegetative biomass, the difference in total pod weight between the different generations of the Kiloga cross were generally small and not significant (Table 4.9). The exception was the BC_{P2} generation, where mean pod weight was greater than both the cultivated parent, and the F_2 and BC_{P1} means. In the Berken cross, the difference in total pod weight between the parents was statistically significant. The F_2 and BC_{P2} means were around the mid-parent value, while the F_1 and BC_{P2} means were around the wild parent value. Broad sense heritabilities were moderate to high while narrow sense heritabilities in both crosses were moderate (0.61 and 0.54 respectively).

Seed yield. Notwithstanding the very long growth duration of ACC 87, in both crosses, seed yield per plant tended to be higher in the cultivated parent than in ACC 87, but was statistically so only in the Kiloga cross (Table 4.9). In both crosses, mean seed yields of the F_1 and BC_{P1} populations were not statistically greater than for the cultivated parent, but in the F_2 generations, there were some segregants with higher yields than the cultivated parents. Broad sense hertitabilities for seed yield in both crosses were moderately high (0.85 and 0.75, respectively), but narrow sense heritabilities in both crosses were low.

Seed-pod weight ratio. The proportion of total pod weight that was seed was significantly greater in the cultivated parents than in ACC 87 (Table 4.9). This finding was consistent with the study by Bushby and Lawn (1992) who found that wild mungbean invested relatively more dry matter in pod walls than cultivated mungbean. The F_1 hybrid plants were intermediate between the parents and significantly different from both. The F_2 generation mean was closer to the cultivated parent, while the means for the backcross plants were each closer to the respective backcross parent. While broad sense heritability for this trait was moderate to high, narrow sense heritability was zero in both crosses, implying non-additive gene action for the trait.

Total dry matter (TDM). In both crosses, the mean TDM in the cultivated parents was generally smaller than for ACC 87, presumably reflecting their much shorter growth duration (Table 4.9). In the F_1 hybrid plants, mean TDM was closer to that of the wild parent, while in the F_2 generation, mean TDM was closer to the cultivated type. In the backcross populations, mean TDM was close to the respective backcross parent. In both crosses, broad sense heritability for TDM was high whereas narrow sense heritability was high in the Berken cross but only moderate in the Kiloga cross.

Harvest index (HI). In both crosses, HI of the wild parent was significantly smaller than in the cultivated parents (Table 4.9). In both crosses, the F_1 plants were intermediate between and significantly different from the parents. Similarly, the F_2 generation means were intermediate between the parents. In the backcross generations, means were close to the respective backcross parent. Both broad and narrow sense heritabilities for HI were high to very high. The high narrow sense heritability of HI in these crosses, suggests predominantly additive gene action, in which case, breeding progress to change HI should be rapid.

4.3.4 Associations between traits

4.3.4.1 Phenotypic correlations between quantitative traits in F_2 plants

There were a large number of statistically significant phenotypic correlations between pairs of quantitative traits that were observed in the F_2 plants in both crosses. The pairwise interrelations between a subset of key traits are summarised in Table 4.10 while the interrelations between the full set of traits are listed in Appendix I.

In the Kiloga x ACC 87 cross, there were few interrelations between the phenological traits, time to flowering and time to maturity, and other key morphological or agronomic traits (Table 4.11a). There was a weak relation (r = 0.22*) between crop duration and total biomass per plant. In contrast, in the Berken x ACC 87 F_2 populations, there was a significant correlation between time to flowering and time to maturity (r = 0.22*) and both traits were positively related to number of stem nodes (r = 0.38** and r = 0.39** respectively), total biomass (r = 0.51** and r = 0.30** respectively), and seed yield per plant (r = 0.26* and r = 0.20** respectively), indicating that later flowering plants grew longer, had more stem nodes, produced greater biomass and more seed yield. There was a significant negative correlation (r = 0.38**) between time to flowering and HI, indicating that earlier flowering plants produced a higher proportion of seed per unit biomass than the later flowering plants. The cultivated parents were both earlier flowering and had higher HI than the wild parents (Table 4.10). Lawn and Rebetzke (2006) reported a positive correlation between time to flowering and the total growth cycle among wild mungbean accessions. Makeen *et al.* (2007) reported a positive correlation between longer growth cycle and total biomass while Arshad *et al.* (2009) reported a correlation between days to flowering and seed yield per plant.

Among the morphological traits, in the Kiloga x ACC 87 population, the number of seeds per pod was negatively correlated (r = -0.33**) with seed size (Table 4.11a). This relation was of interest because the cultivated parents had fewer but larger seed whereas the wild parent had more but smaller seed (Table 4.10). However, this relation was not evident in the Berken x ACC 87 cross (Table 4.11b). In both crosses, pod width was strongly positively correlated with pod length (r = 0.73** and r = 0.58** respectively). In the Berken x ACC 87 cross, the number of stem nodes was positively correlated with both seed yield (r = 0.51**) and total biomass (r = 0.59**), but these relations were not evident in the Kiloga x ACC 87 cross. The reasons for this are unknown, but could be due to eother genetic or environmental factors. Among the main agronomic traits (Table 4.11), seed yield per plant in both the Kiloga x ACC 87 and Berken x ACC 87 crosses was positively correlated with total biomass per plant (r = 0.62** and 0.88**, respectively) indicating that those plants which produced more biomass also produced more seed.

Table 4.10 Pairwise phenotypic correlations among a subset of quantitative traits observed in the F_2 generation plants from two cultivated x wild mungbean crosses (a) Kiloga x ACC 87 and (b) Berken x ACC 87. Entries are the linear correlation co-efficients (*r*) between the respective trait pairs.

(a) Kiloga x ACC 87

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	-.07	1								
SP	-.10	.07	1							
PL	.09	.09	-.03							
PW	.10	.10	.03	.73**	1					
No	.12	.01	-.12	-.12	.03	1				
Ye	-.02	.08	.07	-.17	-.18	.03	1			
SeS	-.09	-.02	-.33**	-.06	-.03	-.02	.21*	1		
TM	-.08	.22*	.03	-.11	-.11	.03	.62**	.08	1	
HI	.03	-.16	.10	-.11	-.12	.01	.53**	.15	-.31**	1

(b) Berken x ACC 87

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	.22*	1								
SP	-.33**	.13	1							
PL	-.22*	.00	.52**	1						
PW	.00	-.11	-.12	.58**	1					
No	.38**	.39**	.07	.07	-.10	1				
Ye	.26**	.20*	-.01	-.10	-.09	.51**	1			
SeS	.02	.12	.08	.07	-.07	.14	.10	1		
TM	.51**	.30**	-.12	-.24*	-.17	.59**	.88**	.13	1	
HI	-.38**	-.17	.18	.25*	.16	-.08	.38**	-.01	-.10	1

*, ** indicates significant correlation at $P < 0.05$, $P < 0.01$ respectively

Flo = flowering date; Ma = physiological maturity; SP = seeds per pod; PL= pod length; PW = pod width; No = Nodes per main stem; Ye = seed yield; SeS = 100 seeds weight; TM = total biomass; HI = harvest index.

There was also a positive correlation in both crosses between seed yield and HI (r = 0.53** and r = 0.38**, respectively). Interestingly, in the Kiloga x ACC 87 cross, there was a negative relation (r = -0.31**) between HI and total dry biomass (Table 4.11a), reflecting the fact that seed yield and vegetative biomass are alternative sinks for dry matter. Indeed, in both crosses, there was a negative

relation between HI and dry vegetative biomass per plant (r = -0.35** and r = -0.50** respectively – see Appendix I).

4.3.4.2 Genetic correlations among quantitative traits

Estimated genetic correlations between a subset of key quantitative traits for the two crosses are shown in Table 4.11. Genetic correlations provide information on the extent that genes affecting one trait also affect other traits. Estimates of genetic correlation can be used to choose the breeding system to be adopted, to decide the method of selection, to predict direct and correlated response to selection, and for estimating genetic gains (Falconer and Mackay 2000, Javed *et al.* 2004). If there is strong genetic correlation between the two traits, selection for one trait should result in an improvement/ deterioration for the other trait as a correlated response (Javed *et al.* 2004).

There were several significant genetic correlations ($P < 0.05$) between pairs of quantitative traits, which suggested underlying associations in the additive gene action for the respective traits. Among the phenological traits observed, the time to flowering showed significant positive genetic correlation with physiological maturity, but the relation appeared to be stronger in Kiloga cross (r_G = 0.70**) than in Berken (r_G = 0.22*) (Table 4.11), suggesting that genes controlling flowering also considerably affected the maturity trait. There were no other significant genetic correlations between time to flowering and other traits in the Kiloga cross, but there were genetic associations between this trait and number of stem nodes, seed yield, dry biomass and HI in the Berken cross. In terms of morphological traits there were both similarities and differences in the genetic correlations between traits in the two crosses (Table 4.11). Seeds per pod was associated positively with pod length in both crosses, but more strongly in Berken cross (r_G = 0.51**) than in Kiloga population (r_G = 0.20*). While seeds per pod showed a negative genetic correlation with seed size in Kiloga cross (r_G = 0.38**), there was no relation in Berken cross. The pod length showed positive genetic correlation with pod width in the two crosses.

Among the agronomic characters in both crosses, there were generally highly significant positive genetic correlations between key pairs of agronomic traits (Table 4.11). Genes controlling for seed yield per plant involving ACC 87 were highly positively associated with total dry biomass per plant (r_G = 0.61** and 0.87**, respectively). Similarly, there were also highly positive correlations in both crosses between seed yield and HI (r_G = 0.52** and r_G = 0.38**, respectively). Interestingly, in both crosses, the total dry biomass was negatively linked with the HI, but the relation was significant only in Kiloga cross (Table 4.11).

Table 4.11 Pairwise genetic correlations among a subset of quantitative traits observed in two cultivated x wild mungbean crosses (a) Kiloga x ACC 87 and (b) Berken x ACC 87. Entries are the genetic correlation co-efficients (r_G) between the respective trait pairs.

(a) Kiloga x ACC 87

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	0.70**	1								
SP	-0.10	0.05	1							
PL	0.09	0.09	0.20*	1						
PW	0.10	0.10	0.03	0.72*	1					
No	0.12	0.01	-0.11	-0.11	0.03	1				
Ye	-0.02	0.07	0.08	-0.17	-0.17	0.03	1			
SeS	-0.09	-0.02	-0.32**	-0.06	-0.03	-0.02	0.21	1		
TM	0.07	0.22*	0.03	-0.11	-0.11	0.03	0.61**	0.08	1	
HI	0.02	-0.16	0.10	-0.11	-0.13	0.00	0.52**	0.14	-0.31**	1.00

(b) Berken x ACC 87

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	0.22*	1								
SP	-0.32**	0.12	1							
PL	-0.22*	0.00	0.51**	1						
PW	0.00	-0.11	-0.12	0.58**	1					
No	0.38**	0.38**	0.07	0.07	-0.10	1				
Ye	0.26*	0.20*	-0.01	-0.10	-0.09	0.50**	1			
SeS	0.02	0.12	0.08	0.07	-0.07	0.14	0.10	1		
TM	0.50**	0.30**	-0.12	-0.24*	-0.17	0.59**	0.87**	0.13	1	
HI	-0.38**	-0.15	0.19*	0.26*	0.15	-0.06	0.38**	0.01	-0.09	1

*, ** indicates significant linkage at $P < 0.05$, $P < 0.01$ respectively

Flo = flowering date; Ma = physiological maturity; SP = seeds per pod; PL = pod length; PW = pod width; No = Nodes per main stem; Ye = seed yield; SeS = 100 seeds weight; TM = total biomass; HI = harvest index.

4.3.4.3 Linkage between qualitative traits

Analysis of linkage between qualitative traits, which were previously found to be conditioned by a single dominant gene, was accomplished by using Chi-square of goodness of fit. The joint segregation

ratios of trait pairs, which were observed in F_2 generation, were compared to the expected distribution based on independent assortment (9:3:3:1). Where the deviation was statistically significant ($P < 0.05$), it suggested that there was an apparent linkage between those two traits (Allard 1999, Acquaah 2007, Brown 2008). Generally, the linkage between the pairs of qualitative traits observed appeared to be different between the two crosses involving ACC 87 (Table 4.12).

Table 4.12 Chi-square values for qualitative trait pairs observed in K x ACC 87 and B x ACC 87 hybrid populations used to detect genetic linkage among those traits.

(a) Kiloga x ACC 87

	Leaflet lobing	**Twining habit**	**Dehiscent pod**	**Texture layer**	**Testa lustre**	**Hilum colour**
Leaflet lobing	-					
Twining habit	1.164	-				
Dehiscent pod	1.926	0.910	-			
Texture layer	1.926	4.635	1.206	-		
Testa lustre	1.926	2.942	1.037	2.053	-	
Hilum colour	3.196	6.921+	2.349	9.460*	27.069***	-

(b) Berken x ACC 87

	Leaflet lobing	**Twining habit**	**Dehiscent pod**	**Texture layer**	**Hilum colour**	**Powdery mildew**
Leaflet lobing	-					
Twining habit	0.402	-				
Dehiscent pod	1.968	1.418	-			
Texture layer	2.180	5.228	8.444*	-		
Hilum colour	19.873***	3.196	0.571	13.354**	-	
Powdery mildew	0.698	0.402	1.291	2.180	0.910	-

+,*, **, *** indicates observed digenic ratios diverge significantly from independent assortment at 0.10 > P < 0.05, 0.01, 0.001 respectively

In the Kiloga population, there was no association between the morphological traits and seed characters ($P < 0.05$), although twining was weakly linked with hilum colour (Table 4.12a). The only seed traits that were linked were hilum colour and texture layer, and hilum colour and testa lustre. In the Berken cross, however, leaflet lobing was highly linked with hilum colour ($P<0.001$) (Table 4.12b). Additionally, there was also an association between pod dehiscence and texture layer ($P<0.05$). Similar to the Kiloga cross, the texture layer trait was strongly linked with the hilum colour character ($P<0.01$).

Genetic linkage is a term which describes the tendency of certain loci or alleles to be inherited together. Genetic loci on the same chromosome are physically close to one another and tend to stay together during meiosis, and are thus genetically linked (Allard 1999). For these instances where the deviation was statistically significant, it suggested that there was an apparent linkage between those two traits (Allard 1999, Acquaah 2007 and Brown 2008).

CHAPTER 5: LATE FLOWERING & OTHER WILD TRAITS FROM ACC 1

5.1 Introduction

According to Sleper and Poehlman (2006), one of the basis strategies in plant breeding is to search out genes that encode for useful traits from cultivated species and their close relatives, and combine these into improved varieties through hybridization, recombination and selection. It is important to understand how both useful and unwanted traits are inherited and the development of an understanding of inheritance pattern and segregation ratios is thus an important step in crop improvement. The late flowering varieties of mungbean are very important in some situations, for example, in areas that have only two crop seasons per year like in the north of Vietnam. Late flowering may extend the crop duration and so increase yield potential. Alternatively, in monsoonal tropical regions, late flowering can be useful to delay pod maturity until after the rains have ceased, to avoid weather damage to seeds (Yeates *et al.* 2000).

The aim of the research reported in this chapter was to document the inheritance of selected wild *vs.* cultivated mungbean traits, especially a very late flowering trait that had previously been identified in the wild accession ACC 1. Generally, wild mungbeans are photoperiod-sensitive, quantitative short day plants, so that they flower more quickly under shorter days (Rebetzke and Lawn 2006a). As such, they are later flowering under the longer days in mid-summer, and earlier flowering under the shorter days of autumn. However, accession ACC 1 was found which is late flowering under short days, but does not appear to be photoperiod sensitive. It was suggested that this accession may possess a so-called long-juvenile trait. The putative 'long-juvenile' trait expresses as a very late flowering habit, which has been shown to be unaffected when plants are exposed to artificial long days (Rebetzke and Lawn 2006a).

5.2 Materials & Methods

The broad details of the genetic populations, the plant cultural procedures, experimental design, and the traits observed, and the analytical approaches used, were outlined previously in Chapter 3.

5.2.1 Germplasm & Experimental Design

The number of plants that were grown in each generation of the two cultivated x wild populations involving the perennial wild accession ACC 1 are shown in Table 5.1.

Table 5.1 The number of individual plants sown for each generation in each hybrid combination involving the wild perennial accession ACC 1

	Cultivated parent	
Generation	**Kiloga**	**Berken**
Cultivated parent (P_1)	5	5
Wild parent (P_2)	5	5
F_1 Progeny	2	6
F_2 Progeny	86	82
BC_{P1} Progeny	13	11
BC_{P2} Progeny	12	12

5.2.2 Cultural Details

The plants were germinated on 10th March i.e. late summer, so that the plants experienced short photoperiods (~12 hrs and shortening) from soon after emergence. Under short day conditions, the cultivated parents Berken and Kiloga were expected to flower quickly (around 35 days or less) whereas ACC 1 were expected to take longer to flower (> 60 days) based on previous experience (RJ Lawn, personal communication 2008). The plants were grown under favourable conditions to enhance expression of the late-flowering trait in ACC 1. The plants were allowed to set pods and seeds during the autumn to mid-winter period (i.e. over the period March-July). They were kept until they matured and died. The plant shoots then were harvested by cutting the stems at ground level.

5.2.3 Data Collection & Statistical Analysis

The expression of the traits of interest was recorded from each of the plants in each population, and the mode of inheritance inferred from the variations in phenotype evident in the segregating generations of the test populations. The traits determined to be qualitatively or quantitatively inherited are defined in following sections. For many traits, the F_1 hybrid was similar to one or the other parent, suggesting dominant gene action. In others, the F_1 hybrid was intermediate, suggesting additive gene action. The methods of statistically analysing the data were broadly as described in Chapter 3, using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc.).

5.2.3.1 Late flowering trait

The date of appearance of the first flowers (date of flowering) was recorded individually for each plant in each generation. The time to flowering data of the F_2 plants were then evaluated using frequency distributions in order to test whether the late flowering trait was qualitatively inherited or not. Qualitative inheritance would be suggested if the frequency distributions showed evidence of discontinuities, while quantitative inheritance would be suggested by a continuous distribution.

5.2.3.2 Putative qualitative traits

All of the putative qualitative traits were observed based on the methods described previously in Chapter 3, excepting for the leaflet lobing and pod dehiscence characters. Observation showed that there were differences between ACC 1 and ACC 87 in the extent of both leaflet lobing and pod shattering. The expression of these two traits was stronger in ACC 1 than in ACC 87, and there was a wider range of expression of these two traits in hybrid offspring of ACC 1 populations. Therefore, in the ACC 1 x Kiloga and ACC 1 x Berken populations, one more score category was added to describe the expression in each trait. This meant that there were 5 scoring categories of leaflet lobing (from 0 to 4) and 4 scoring categories of pod dehiscence (from 0 to 3), in which the added score 4 of leaflet lobing and the added score 3 of pod shattering showed the higher expression of ACC 1 compared with ACC 87.

As previously discussed (Section 4, Chapter 3), the Chi-square goodness-of-fit of a model assuming the simplest case of single gene control was considered first, and the segregation ratios observed in the four segregating generations (F_1, F_2, BC_{P1} and BC_{P2}) were compared with expectations from the model for single gene control. If the Chi-squared probability was < 0.90, more complex models were also considered. The goodness of fit was tested using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc.).

5.2.3.3 Putative quantitative traits

For putative quantitative traits (i.e. those exhibiting continuous variation), broad sense and narrow sense heritability were calculated by the variance ratios method (Acquaah 2007, Allard 1999) as outlined in Chapter 3, while the standard errors were calculated by method of Acquaah (2007). The variance of each component in the variance ratios method was calculated using GENSTAT IV and SPSS 17.0 Graduate Student Version software (SPSS, Inc.).

5.3 Results & Discussion

5.3.1 Late Flowering Trait

There were large differences between the means for days to flowering in each generation of the two hybrid populations involving ACC 1 (Table 5.2). Consistent with previous experience, the parental plants exhibited extremes of response, with the cultivars Kiloga and Berken being early flowering (< 5 weeks), and the wild accession ACC 1 being very late flowering (~ 11 weeks) (Table 5.2). As in the crosses involving ACC 87 (Chapter 4) and also in southern Queensland (Lawn 1979a), Kiloga was slightly earlier than Berken. In both crosses, all the F_1 plants exhibited early flowering (closer to the cultivated parents), suggesting a degree of dominant gene action for the trait. However, given that the earliness of the F_1 was not as strong as in Berken and Kiloga, it was clear there was not complete dominance for earliness. In the BC_{P1}, plants were very early or early flowering, and the BC_{P1} means in both crosses were not significantly different from the cultivated parents (Table 5.2). However, the mean for the BC_{P2} generation was much closer to, although still significantly earlier than, the wild parent. Together these responses suggested both additive and dominance effects for this trait.

Table 5.2 Mean times to flowering (d) for the parental, F_1, F_2, BC_{P1} and BC_{P2} populations, for two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 (b) Berken x ACC 1. Means followed by the same letters are not significantly different ($P < 0.05$) based on the analysis of variance.

Traits	Mean square test of between generation	P_1	F_1	P_2	F_2	BC_{P1}	BC_{P2}
(a) Kiloga x ACC 1							
Time to flowering (d)	2780.934**	33.2a	37.5a	87.6c	41.3a	36.6a	58.2b
(b) Berken x ACC 1							
Time to flowering (d)	2673.028**	34.6a	36.5ab	88.2d	43.1b	34.3a	56.2c

** Significant differences at $P < 0.01$

Mean times to flowering of the F_2 generation were slightly later than for the F_1 but still closer to the cultivated parent than to the wild parent (Table 5.2). When the days to flowering for individual F_2 plants were grouped into 3-day intervals, the resulting frequency distributions indicated the variation in time to flowering was essentially continuous (Figure 5.1). The distributions were strongly skewed, with more than half the F_2 plants flowering less than 10 d after the cultivated parent. In neither cross were F_2 plants recovered that flowered as late as ACC 1. In the Kiloga cross, the latest F_2 plant flowered > 3 weeks before ACC 1. In the Berken cross, only three late flowering plants were recovered, and then the latest still flowered > 10 d before ACC 1.

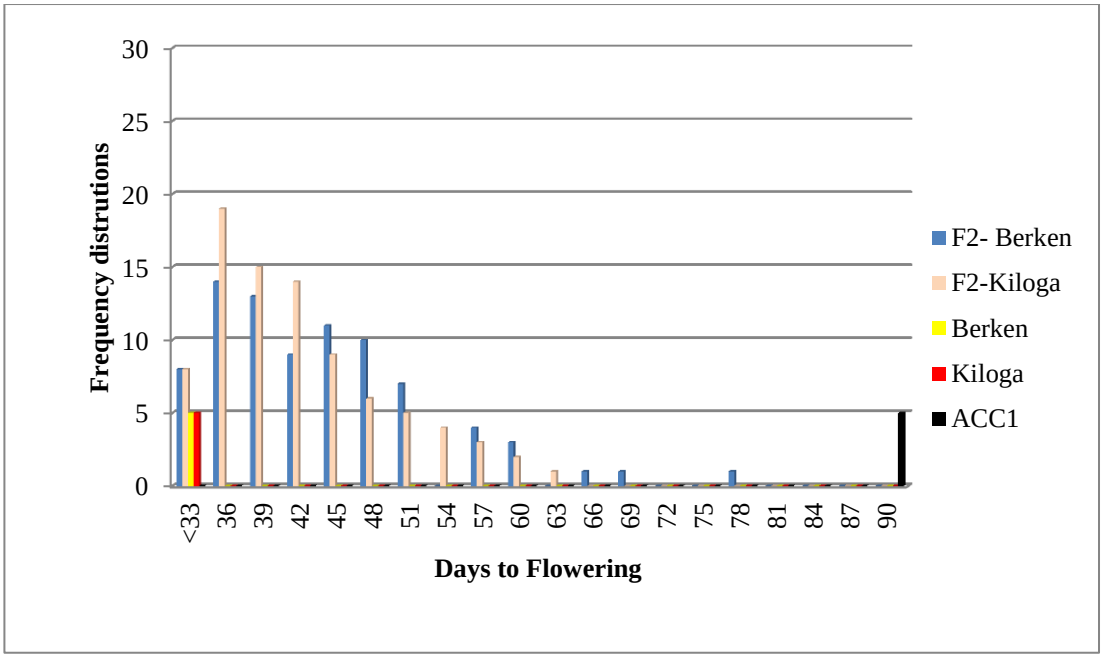


Figure 5.1 Variation in flowering date for parents and F_2 progenies for two cultivated x wild mungbean crosses, Kiloga x ACC 1 (red bars) Berken x ACC 1 (blue bars)

Only in the Kiloga population was there any indication of discontinuity, with no individual plants flowering during the 51-54 d and 60-63 periods. However, taken together, the data from the two crosses involving ACC 1 suggested that the flowering trait was quantitatively inherited. Analysis of the variance structures of the different generations in the two crosses suggested an additive-dominance model of inheritance for the flowering trait (Table 5.3).

Table 5.3 Components of genetic variation for the flowering trait in two cultivated x wild mungbean crosses, Kiloga x ACC 1 and Berken x ACC 1

Components of variation	Kiloga x ACC 1	Berken x ACC 1
V_E	2.44	3.92
V_A	91.34	151.38
V_D	32.18	8.13
A	-5.61±15.68^{ns}	-9.06±11.84^{ns}
B	3.95±6.37^{ns}	-1.18±7.63^{ns}
C	-30.55±15.23*	-18.82±18.70^{ns}
V_D/V_A	0.35	0.05
h^2_b	0.96±0.02	0.95±0.01
h^2_n	0.81±0.23	0.93±0.16

A, B, C are individual scaling tests (Brown, 2008), V_E = Environment variance, V_A = Additive effects, V_D = Dominance effects, V_D/V_A = Dominance ratio, h^2_b = Broad sense heritability, H^2_n = Narrow sense heritability, ns = not significant, * = significant at *P<0.05*

In both crosses, the estimates of the A, B, C scaling test (Brown 2008) were not significant in either cross, excepted for C scaling test in Kiloga cross. According to Brown (2008), at least one of A, B or C scaling test is not significantly different from zero, it still suggests an additive-dominance model of inheritance. Consistent with this, the additive genetic variance components (V_A) were greater in magnitude than the corresponding dominance genetic variance components (V_D) and environmental variances (V_E). Consequently, the degree of dominance (V_D/V_A) was relatively small in both crosses. Taken together, these data suggested strong additive gene effects on time to flowering in both crosses. Both broad and narrow sense heritabilities were high in both crosses (Table 5.3). The high narrow sense heritabilities (0.81 and 0.93 in the Kiloga and Berken crosses, respectively), reflected the high degree of additive gene action for the trait in these crosses.

5.3.2 Qualitatively Inherited Traits

The expression of each of the putative qualitative traits in the various hybrid generations is discussed individually below for the two crosses involving ACC 1 as the wild parent, together with most likely model of inheritance based on the observed data. The various phenotypic categories used to score the main qualitative traits of interest were broadly as described in Chapters 3 and 4, with minor modifications where trait expression in ACC 1 differed from that observed in ACC 87. For none of the traits was there any evidence of variation among plants within the cultivated and wild parental lines used in this study. This homogeny reflected the fact that mungbean is an inbreeding plant, so that accessions generally remain homozygous and 'true-to-type' over successive generations. While mungbean flowers are visited by a range of nectar-feeding insects (mainly ants, bees and wasps) and there can occasionally be a small level of chance out-crossing, the data in Tables 5.4 and 5.5 indicated no evidence of that having occurred.

5.3.2.1 Morphological traits

Leaflet lobing. As previously observed with the ACC 87 crosses (Chapter 4), the two cultivars Berken and Kiloga showed no evidence of leaflet lobing (Table 5.4a). However, the wild parent ACC 1 displayed even stronger leaflet lobing than ACC 87. In both crosses, the F_1 plants exhibited lobing, but less than in the wild parent. Additionally, in the F_2 plants, the complete range of expression of lobing, from absence to very strong expression was observed. These data suggested a degree of dominance for the wild type trait. However, its expression was either additive, and therefore less strongly expressed in the heterozygous state, or perhaps modified by other genes. For the backcrosses to the cultivated parents (BC_{P1}), less than half the plants exhibited slight lobing, while in the backcross to the wild parent (BC_{P2}), the leaves were moderately to very strongly lobed (Table 5.4a).

In both crosses, the data for presence or absence of leaflet lobing fitted very well to a single dominant gene conferring the presence of lobing (Table 5.4a). However, again, as for the ACC 87 crosses, there was also evidence of additive gene action. In both the Berken x ACC 1 and Kiloga x ACC 1 crosses, when the two lower scores for lobing and the two higher scores for lobing were each combined to create three lobing classes (none, 'intermediate' and 'strong'), the F_2 segregation ratios were consistent with a 1: 2: 1 absent: intermediate: strong distribution. Moreover, the BC_{P1} distribution was consistent with 1:1 absent: intermediate, while the BC_{P2} distribution was consistent with 1:1 intermediate: strong distribution.

Table 5.4 Phenotypic scores for putative qualitative morphological traits, the likely model of inheritance, and Chi-square tests for observed ratios, for two cultivated x wild mungbean crosses.

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories					χ^2	df	Probability
(a) Leaflet lobing - presence dominant over the absence; additive gene action?									
Kiloga x ACC 1		**0[A]**	**1[B]**	**2[B]**	**3[B]**	**4[B]**			
P_1	None	5	-	-	-	-			
P_2	Very strong	-	-	-	-	5			
F_1	Moderate	-	-	2	-	-			
F_2	1:3	24	16	25	14	7	0.388	1	0.533
BC_{P1}	1:1	7	0	6	-	-	0.077	1	0.782
BC_{P2}	All lobed (0:n)	-	-	4	2	6	0.000	1	1.000
	Combined fit of segregation data to model						0.465	4	0.977
Berken x ACC 1		**0[A]**	**1[B]**	**2[B]**	**3[B]**	**4[B]**			
P_1	None	5	-	-	-	-			
P_2	Very strong	-	-	-		5			
F_1	Moderate	-	-	6	-	-			
F_2	1:3	23	28	13	15	3	0.407	1	0.523
BC_{P1}	1:1	7	2	2	-	-	0.818	1	0.366
BC_{P2}	All lobed (0:n)	-	-	5	1	6	0.000	1	1.000
	Combined fit of segregation data to model						1.225	4	0.874
[A] category 0 = no lobing ; [B] categories 1, 2, 3 & 4 = lobing present									
(b) Twining habit – digenic, presence dominant over absence, with suppressor gene action									
Kiloga x ACC 1		**0[C]**	**1[D]**	**2[D]**					
P_1	None	5	-	-					
P_2	Strong	-	-	5					
F_1	Strong	-	-	2					
F_2	3:13	18	33	35			0.268	1	0.605
BC_{P1}	1:1	8	5	-			0.692	1	0.405
BC_{P2}	All twining (0:n)	-	5	8			0.000	1	1.000
	Combined fit of segregation data to model						0.961	4	0.916
Berken x ACC 1		**0[C]**	**1[D]**	**2[D]**					
P_1	None	5	-	-					
P_2	Strong	-	-	5					
F_1	Strong	-	-	6					
F_2	3:13	13	28	41			0.452	1	0.501
BC_{P1}	1:1	7	3	1			0.818	1	0.366
BC_{P2}	All twining (0:n)	-	3	9			0.000	1	1.000
	Combined fit of segregation data to model						1.270	4	0.866
[C] category 0 = non-twining; [D] categories 1 & 2 = twining present									

Table 5.4 continued …

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories					χ^2	df	Probability
(c) Growth habit – Prostrate dominant over erect; digenic; additive effects?									
Kiloga x ACC 1		**0**	**1 [E]**	**2 [E]**	**3 [E]**	**4 [E]**			
P_1	Erect	5	-	-	-	-			
P_2	Prostrate	-	-	-	-	5			
F_1	Prostrate	-	-	-	2	-			
F_2	1:15	3	7	6	40	30	1.119	1	0.290
BC_{P1}	1:3	2	2	2	7	-	0.641	1	0.423
BC_{P2}	All prostrate (0:n)	-	-	-	-	12	0.000	1	1.000
	Combined fit of segregation data to model						1.760	4	0.780
Berken x ACC 1		**0**	**1 [E]**	**2 [E]**	**3 [E]**	**4 [E]**			
P_1	Erect	5	-	-	-	-			
P_2	Prostrate	-	-	-	-	5			
F_1	Prostrate	-	-	-	6	-			
F_2	1:15	6	4	12	35	25	0.159	1	0.690
BC_{P1}	1:3	2	1	3	5	-	0.273	1	0.601
BC_{P2}	All prostrate (0:n)	-	-	-	3	9	0.000	1	1.000
	Combined fit of segregation data to model						0.432	4	0.980
[E] Categories 1 - 4 combined into a spreading-prostrate grouping									
(d) Pod dehiscence – two dominant genes with additive gene action									
Kiloga x ACC 1		**0 [E]**		**1 [F]**	**2 [G]**	**3 [G]**			
P_1	Non-dehiscent	5		-	-				
P_2	Very strong	-		-		5			
F_1	Strong	-		-	2	-			
F_2	1:6:9	5		32	28	21	0.036	2	0.982
BC_{P1}	1:2:1	5		6	2	-	1.462	2	0.482
BC_{P2}	All strong (0:0:n)	-		-	4	8	0.000	2	1.000
	Combined fit of segregation data to model						1.498	6	0.960
Berken x ACC 1		**0 [E]**		**1 [F]**	**2 [G]**	**3 [G]**			
P_1	Non-dehiscent	5		-	-				
P_2	Very strong	-		-		5			
F_1	Strong	-		-	6	-			
F_2	1:6:9	7		35	20	20	2.087	2	0.353
BC_{P1}	1:2:1	3		6	2	-	0.273	2	0.872
BC_{P2}	All strong (0:0:n)	-		3	2	7	0.750	2	0.687
	Combined fit of segregation data to model						3.109	6	0.795
[E] non-dehiscent; [F] intermediate; [G] categories 2 & 3 = strongly dehiscent									

Thus, the observed data in both crosses (Table 5.4a) could be interpreted to suggest that not only was leaflet lobing in these crosses conditioned by a single dominant gene, but there was additive gene action contributing to less strong lobing in plants heterozygous for the trait. These observations were consistent with the two crosses of ACC 87 with Berken and Kiloga discussed in Chapter 4.

Twining. Again, the cultivated parents showed no evidence of twining, whereas ACC 1 and the F_1 were both strongly twining (Table 5.4b). In the F_2 and BC_{P1} generations, non-twining and twining phenotypes were observed, while in the BC_{P2} generation, intermediate and strongly twining phenotypes were observed. The data were consistent with a single dominant gene conferring the presence of twining, as was found for the ACC 87 crosses (Chapter 4). However, the fit to this simple model was not strong, especially in the F_2 generation where too few plants did not show any evidence

of twining trait, particularly in the Berken x ACC 1 cross. The data were inconsistent with the more complex additive gene action model. Given the poor fit to the single gene model, digenic models were also evaluated. A digenic model involving two genes in recessive-dominance epistasis (a dominant gene for the wild type twining trait, moderated by a recessive suppressor gene inherited from the cultivated parent) provided a better model of inheritance for the trait in these two crosses (Table 5.4b).

Growth habit. Based on a rating score of 0 (erect habit) to 4 (prostrate habit), the two parental cultivars scored 0 compared with 4 for ACC 1, while the F_1 plants were all spreading to a large degree, but not as prostrate as the wild parent (Table 5.4c). As in the ACC 87 crosses, in the F_2 and BC_{P1} generations, only a very small number of erect plants similar to the cultivated parental type were recovered. However, in contrast to the ACC 87 crosses, almost all of the F_2 plants in both crosses were scored in the two highest categories (3 and 4). The range of F_2 phenotypes suggested at least two genes controlling this trait, with prostrate spreading habit dominant to erect habit. However, when the two highest score categories (3 and 4) were combined to create the score categories applied in ACC 87 crosses, the data collected from ACC 1 populations did not fit that digenic model of inheritance ($P < 0.01$, data not shown). The best fitting model was for two dominant genes with the variation within the non-erect class perhaps being the result of partial dominance or additive gene effects. The fit to this model was more satisfactory for the Berken x ACC 1 cross (Table 5.4c).

Pod dehiscence. The pods of the cultivated varieties were both non-dehiscent (0) while the wild-type and the F_1 were respectively very strongly (3) and strongly (2) dehiscent (Table 5.4d). In the F_2 generation, a very small number of individual plants showed the non-dehiscence character similar to the cultivated parental type, while about 40 per cent were intermediate (1). More than half of the F_2 progenies in both crosses were scored in the two highest categories (2 and 3). While the BC_{P1} generations of the two crosses segregated into three categories (non-, intermediate and strong dehiscence), all but three BC_{P2} plants exhibited strong or very strong shattering, suggesting two dominant genes in action for this trait. The F_2 distributions of the two crosses were consistent with a 1: 6: 9 non- : intermediate: strong ratio, although the fit was not as strong in the Berken cross (Table 5.4d). The BC_{P1} ratios were consistent with 1: 2: 1 non- : intermediate: strong and the BC_{P2} ratios were consistent with 0: 0: n non- : intermediate: strong although again the fit was not as strong in the Berken cross. Overall, the data were consistent with pod dehiscence in both ACC 1 crosses being conditioned by two dominant genes, with additive gene action, so that plants with only one of the genes as the dominant allele were intermediate rather than strongly dehiscent (Table 5.4d).

5.3.2.2 Visual seed characters

Testa colour. Again, the wild type trait was speckled black, while the cultivated phenotype was uniform green in both crosses (Figure 5.2). The F_1 was similar to the wild type, while the F_2 population segregated for both parental forms. However, the number of F_2 plants that exhibited the uniform green testa was much smaller than expected for a single dominant gene (Table 5.5a). All of the BC_{P2} progenies segregated for the speckled black testa, and about a quarter of the BC_{P1} individual plants showed the uniform green testa. While the fit to the F_2 data was not strong, the simplest most likely inheritance model was two dominant genes with duplicate action for black speckling.

Green testa (Kiloga) | Green testa (Berken) | Speckled black (F_2) | Speckled black (F_2) | Speckled black (ACC 1)

Figure 5.2 Representative examples illustrating the expression of the seed testa colour trait.

As for the ACC 87 crosses, there were differences in expression of the black speckle trait in the F_2 and BC_{P1} generations, with some light and others dense. Again, it is possible that the differences in degree of speckling reflect additive gene effects, with homozygous dominant individuals expressing darker speckling than heterozygotes.

Seed-coat texture layer. The wild type trait had a ridged surface texture layer while the cultivated phenotype was smooth (Table 5.7). The F_1 and BC_{P2} progenies were similar to the wild type, while the F_2 generation in both crosses segregated for both traits. Interestingly, just over a half of the plants in the Kiloga F_2 hybrid population showed the texture layer trait, whereas in the Berken cross, more than four fifths of the F_2 progeny exhibited a texture layer on the seed coat. For the BC_{P1} population, both crosses segregated for absence and presence of the texture layer, but there were fewer plants with texture layer present.

Together, the simplest model that provided an acceptable fit in the Kiloga cross was two dominant genes with complementary action, that is, two genes which each need to be present as the dominant allele to enable expression of the texture layer trait. However, the distribution in the Berken x ACC 1 cross was closer to a digenic 13:3 presence: absence ratio, consistent with the presence of texture layer on seed-coat in this cross being conditioned by two genes with dominant and recessive epistasis.

Table 5.5 Phenotypic scores for putative qualitative seed appearance traits, the likely model of inheritance, and Chi-square tests for observed ratios, for two cultivated x wild mungbean crosses.

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories			χ^2	df	Probability
(a) Testa colour-speckled black colour conditioned by two dominant genes (additive gene action?)							
Kiloga x ACC 1		Green	Speckled				
			Light	Dark			
P_1	All green	5	-	-			
P_2	All speckled	-	-	5			
F_1	All speckled	-	2	-			
F_2	1:15	7	32	47	0.524	1	0.470
BC_{P1}	1:3	3	6	4	0.026	1	0.872
BC_{P2}	All speckled (0:n)	-	-	11	0.000	1	1.000
	Combined fit of segregation data to model				0.550	4	0.968
Berken x ACC 1		Green	Speckled				
			Light	Dark			
P_1	All green	5	-	-			
P_2	All speckled	-	-	6			
F_1	All speckled	-	5	-			
F_2	1:15	7	35	40	0.732	1	0.392
BC_{P1}	1:3	3	6	2	0.030	1	0.862
BC_{P2}	All speckled (0:n)	-	-	12	0.000	1	1.000
	Combined fit of segregation data to model				0.762	4	0.943
(b) Surface texture layer- presence controlled by two dominant genes with complementary gene action in Kiloga x ACC 1 cross, but with dominance and recessive epistasis in Berken x ACC 1 cross							
Kiloga x ACC 1		Absent	Present				
P_1	Absent	5	-				
P_2	Present	-	2				
F_1	Present	-	5				
F_2	7:9	34	52		0.621	1	0.431
BC_{P1}	3:1	10	3		0.026	1	0.872
BC_{P2}	All present (0:n)	-	12		0.000	1	1.000
	Combined fit of segregation data to model				0.647	4	0.956
Berken x ACC 1		Absent	Present				
P_1	Absent	5	-				
P_2	Present	-	6				
F_1	Present	-	5				
F_2	3:13	14	68		0.151	1	0.698
BC_{P1}	1:1	7	4		0.818	1	0.366
BC_{P2}	All present (0:n)	-	12		0.000	1	1.000
	Combined fit of segregation data to model				0.969	4	0.914

Table 5.5 continued …

Cross & Generation	Expected segregation ratio	Observed distribution for rating categories		χ^2	df	Probability
(c) Seed-coat surface - dull seed-coat controlled by two dominant genes with complementary action						
Kiloga x ACC 1		Shiny	Dull			
P_1	Shiny	5	-			
F_1	Dull	-	2			
P_2	Dull	-	5			
F_2	7:9	37	49	0.018	1	0.893
BC_{P1}	3:1	11	2	0.641	1	0.423
BC_{P2}	All dull (0:n)	-	12	0.000	1	1.000
	Combined fit of segregation data to model			0.659	4	0.956
Berken x ACC 1		Shiny	Dull			
P_1	Shiny	5	-			
F_1	Dull	-	6			
P_2	Dull	-	5			
F_2	7:9	38	44	0.224	1	0.636
BC_{P1}	3:1	8	3	0.030	1	0.825
BC_{P2}	All dull (0:n)	-	12	0.000	1	1.000
	Combined fit of segregation data to model			0.254	4	0.993
(d) Texture layer / hilum colour – single gene, dark dominant over light						
Kiloga x ACC 1		Light	Dark			
P_1	Light	5	-			
P_2	Dark	-	5			
F_1	Dark	-	2			
F_2	1:3	20	66	0.140	1	0.708
BC_{P1}	1:1	7	6	0.077	1	0.782
BC_{P2}	All dark (0:n)	0	12	0.000	1	1.000
	Combined fit of segregation data to model			0.217	4	0.995
Berken x ACC 1		Light	Dark			
P_1	Light	5	-			
P_2	Dark	-	5			
F_1	Dark	-	6			
F_2	1:3	19	63	0.146	1	0.702
BC_{P1}	1:1	5	6	0.091	1	0.763
BC_{P2}	All dark (0:n)	0	12	0.000	1	1.000
	Combined fit of segregation data to model			0.237	4	0.994

Seed-coat surface. As mentioned in Chapter 4, the dull seed coat shows a fine network of ridges, which in transverse section gives the outer epidermis surface an undulated or dentate appearance. The shiny seed coat has a smooth surface. The cultivated parents had shiny seed coat, whereas the wild parent and the F_1 progeny in both crosses exhibited dull seed coat, suggesting dominant gene action giving the dull trait (Table 5.7c). In both hybrid populations, there was segregation in the F_2 and BC_{P1}

generations, but not in the BC_{P2}, which were all dull. The F_2 plants in both crosses segregated for dull: shiny in a reasonable fit to a 9:7 ratio, and a near 1:3 BC_{P1} ratio supported this model.

Green with texture layer (F_2) | Green with no layer (Kiloga) | Speckled black with texture layer (ACC 1) | Speckled black with no layer (F_2)

Figure 5.3 Representative examples illustrating the expression of the seed-coat surface texture layer. The cultivated parents had green testa with no layer, while ACC 1 was speckled black with a surface texture layer.

Texture layer / hilum colour. In both crosses, the wild type trait was dark while the cultivated phenotype was light (Table 5.4d). The F_1 was similar to the wild type, while the F_2 population segregated for both phenotypes (Figure 5.4), with about three-quarters exhibiting dark colour. Additionally, while all of the BC_{P2} plants exhibited the dark colour, just about half of the BC_{P1} plants showed the light colour. Together, the data suggested the simplest most likely inheritance model was a single dominant gene for dark texture layer / hilum colour.

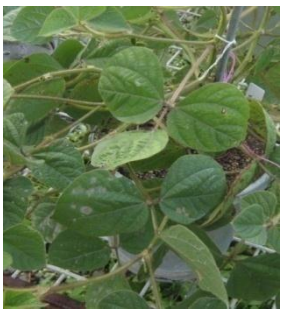
Green testa with dark texture layer | Green testa with light texture layer | Speckled black with dark texture layer | Speckled black with light texture layer

Figure 5.4 Representative examples illustrating the expression of the seed-coat texture layer / hilum colour.

Apparent resistance to powdery mildew disease. Powdery mildew infection on the leaves occurred in the cultivated parents in both crosses but not on ACC 1, suggesting that ACC 1 may not have been as susceptible as the cultivated parents (Figure 5.5). The F_1 and the BC_{P1} progeny were all similar to the

cultivated type, indicating that putative susceptibility was dominant (Table 5.6). The F_2 populations of both crosses contained plants with and without the disease.

Cultivated parent with dense powdery mildew infection on leaves.



Hybrid progeny with light powdery mildew infection on leaves.

Hybrid progeny with complete absence of infection.

Wild parent with complete absence of infection.

Figure 5.5 Representative examples illustrating the expression of the powdery mildew infection in the study.

Table 5.6 Models used to test the hypothesis that the segregation ratios for apparent powdery mildew resistance were consistent with the action of a single dominant gene in the two crosses, Kiloga x ACC 1 and Berken x ACC 1.

Cross & Generation	Expected segregation ratio	Observed occurrence of Powdery Mildew disease		χ^2	df	Probability
Powdery mildew infection-absence controlled by a single recessive gene?						
Kiloga x ACC 1						
		Present	Absent			
P_1	Present	5	-			
P_2	Absence	-	5			
F_1	Present	2	-			
F_2	3:1	66	20	0.140	1	0.708
BC_{P1}	1:0	13	0	0.000	1	1.000
BC_{P2}	1:1	9	3	3.000	1	0.083
	Combined fit of segregation data to model			3.140	4	0.534
Berken x ACC 1						
		Presence	Absence			
P_1	Present	5	-			
P_2	Absence	-	5			
F_1	Present	6	-			
F_2	3:1	67	15	1.967	1	0.160
BC_{P1}	1:0	11	0	0.000	1	1.000
BC_{P2}	1:1	7	5	0.333	1	0.564
	Combined fit of segregation data to model			2.301	4	0.681

The data of the F_2 generation for the Kiloga x ACC 1 cross provided a reasonable fit to a single recessive gene (Table 5.6). However, while the BC_{P1} data completely supported this model, the number of BC_{P2} plants with presence of the disease was higher than expected, providing only weak

support for the model for the F_2 segregation. Nonetheless, the simplest model of inheritance was a single recessive gene controlling the absence of disease. However, the fit to this simple model was very weak for the Berken x ACC 1 cross in the F_2 generation, because there were too few plants without infection. For this second cross, the alternative model of recessive-dominance epistasis (13:3), as was indicated for the Kiloga x ACC 87 cross (Chapter 4), provided a better fit to the F_2 data (χ^2 = 0.011, P = 0.740).

5.3.3 Quantitatively Inherited Traits

5.3.3.1 Phenological traits

In both crosses, analysis of variance revealed that, in addition to time to flowering (Table 5.2), there were significant differences between the cultivated and wild parents in the duration of flowering and the total growth duration (Table 5.7). There were no differences ($P > 0.05$) between the cultivated and wild parents in the duration of pod growth. Maturity of the last flush of pods on ACC 1 did not occur until more than six months after sowing, much later than in either of the two cultivars. Because of its indeterminate growth and flowering habit, it was difficult to accurately determine the exact dates on which flowering ceased in the ACC 1 plants and also many of the F_2, and BC_{P2} hybrid progeny plants. Plants still alive at the end of the experiment were harvested on the same date to measure biomass and as a consequence, total growth duration of these plants was artificially truncated.

The duration of flowering in ACC 1 was > 30 days longer than for Kiloga, while the total life cycle was > 60 days longer. The duration of flowering and the total cycle of the F_1 progeny were both intermediate to the parental lines. In the F_2 and BC_{P2} generations, means for total growth duration were closer to the wild parent. In the Berken cross (Table 5.9b), the duration of pod growth of Berken and the F_1, F_2 and backcross generations was marginally but significantly shorter than in the wild parent. The mean duration of flowering of the F_1, F_2 and BC_{P2} progeny was intermediate between the parents whereas the BC_{P1} mean was close to Berken. Mean life cycle duration of the F_1 and BC_{P1} progeny were close to Berken, whereas the other progeny were closer to ACC 1.

Generally, in both crosses, broad sense heritability estimates for most phenological traits was higher than that of narrow sense heritability (Table 5.9). In the Kiloga hybrid population, broad sense heritability for the three phenological traits was very high, whereas with the exception of the duration of flowering, narrow sense heritability was low to moderate. In the Berken cross, broad sense heritability varied from moderate to very high while narrow sense heritability for the three traits was moderate.

Table 5.7 Phenological trait means for the parental, F_1, F_2, BC_{P1} and BC_{P2} generations, the range for the

F_2 generation, and broad and narrow sense heritability estimates, for two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 (b) Berken x ACC 1. Means followed by the same letters are not significantly different ($P > 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad ($h^2_{b)}$	Narrow (h^2_n)
(a) Kiloga x ACC 1									
Time for pod growth (d)	19.4a	19.5a	20.4a	15-24	19.8a	20.1a	18.7a	0.92±0.47	0.47±0.36
Duration of flowering (d)	55.8a	63ab	86.4c	43-106	65.7ab	60.6ab	71.0b	0.84±0.07	0.70±0.23
Growth duration (d)	118a	143b	181c	142-181	171c	153b	173c	0.90±0.01	0.44±0.39
(b) Berken x ACC 1									
Time for pod growth (d)	17.8a	18.2a	21.2 b	15-22	18.2a	16.8a	18.3a	0.56±0.11	0.47±0.41
Duration of flowering (d)	54.6a	68.2b	85.2c	36-79	63.8b	56.2a	69.5b	0.57±0.14	0.61±0.24
Growth duration (d)	139a	148a	181b	138-181	172b	147a	173b	0.95±0.02	0.56±0.34

5.3.3.2 Morphological traits

The analysis of variance revealed that there were large differences between the cultivated parents and ACC 1, and consequently in the F_1, F_2, BC_{P1} and BC_{P2} generations, for most of the morphological traits observed (Table 5.10). Most of the size-related traits, such as leaflet length and width, seed weight, pod length and width, stem thickness and width of the floral standard, were all much greater in the cultivated parents than in ACC 1. However, some traits such as number of branches, nodes per main stem and level of hard seed were much higher in ACC 1. The expression of the individual traits in the different generations, and the estimates of their heritability are discussed below.

In theory, broad sense heritability should always be greater than narrow sense heritability because the former includes dominance as well as additive genetic effects. In most cases, when standard errors were taken into account, the narrow sense heritabilities were smaller than the broad sense estimates. The fact that a few estimates of narrow sense heritability were greater than broad sense heritability, even after taking account of the standard errors of estimates, reflects the fact that the arithmetical estimates of the component variances were subject to greater error than that estimated statistically. This was arithmetically most likely where the dominance effect was small relative to additive.

Stem diameter. Expression of this trait in the F_1 hybrid progenies was closer to the cultivated than to the wild parent (Table 5.10), suggesting some level of dominance for the trait. The estimates of broad

and narrow sense heritability for stem thickness were both high in the Kiloga hybrid population (Table 5.10a) but very low in the Berken cross (Table 5.10b).

Floral standard. In both crosses, the mean width of the floral standard of the F_1 and subsequent progeny generations was intermediate between the parents and significantly different from them (Table 5.10). Broad and narrow sense heritability estimates ranged from moderate (Berken cross) to very high (Kiloga cross).

Leaflet size and shape. In general, the mean F_1 values for leaflet size and shape were closer to the cultivated parent than to ACC 1 (Table 5.10), with the possible exception of leaflet width in the Kiloga cross. This suggested a level of dominance for this trait. Heritability estimates for leaflet length and width were generally in the range of moderate to very high. In contrast, while broad sense heritability for leaflet shape, as indicated by length: width ratio, was moderate in both crosses, narrow sense heritability was zero.

Number of main stem branches. The F_1 progeny was much closer to the wild parent than the cultivated lines (Table 5.10), suggesting a level of dominance for the trait. While broad sense heritability of the trait in both populations was moderately high (79% and 70%, respectively), narrow sense heritability in both crosses was zero, being smaller than the (relatively high) standard errors of estimate.

Number of main stem nodes. The F_1 progeny means in both crosses were intermediate between the two parents and differed significantly from them (Table 5.10), consistent with additive gene action. There was very wide variation in the F_2 generation, while in the respective backcross generations, mean values reverted toward the respective parents. Generally, both broad and narrow sense heritability for the trait in the two crosses was moderate to high (Table 5.10).

Number of pods per peduncle. In both hybrid populations, analysis of variance showed that there were no significant differences in the mean number of pods per peduncle between the two parents, the F_1, F_2, BC_{P1} and BC_{P2} progenies (Table 5.10). In both crosses, however, there was transgressive segregation in the F_2 generation, with some individual values considerably lower, and others higher than the parental plants. Broad sense heritability for the trait in the two crosses appeared to be high (0.89 and 0.80, respectively). However, the narrow sense heritability in both crosses was very low, and given higher standard error, was not statistically greater than zero.

Peduncle length. In the Kiloga cross, while peduncle length appeared somewhat longer in Kiloga, analysis of variance showed there were no significant differences in the trait between the two parents and the F_1, F_2, BC_{P1} and BC_{P2} generations (Table 5.10a).

Table 5.8 Morphological trait means for the parental, F_1, F_2, BC_{P1} and BC_{P2} populations, the range for the F_2 generation, and broad and narrow sense heritability estimates, for two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 (b) Berken x ACC 1. Means followed by the same letters are not significantly different ($P < 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad (H_b)	Narrow (h_n)
(a) Kiloga x ACC 1									
Stem diameter (mm)	11.8d	8.9c	4.3a	5.5-12.9	8.2bc	8.7c	6.6b	0.70±0.15	1^A±0.11
Floral standard (mm)	17.5c	15.7b	13.5a	12.9-18.1	15.5b	15.4b	14.8ab	0.91±0.03	1^A±0.11
Leaflet length (mm)	141b	129b	83a	58-165	120b	126b	86a	0.85±0.06	0.54±0.28
Leaflet width (mm)	113c	89b	51a	57-129	91b	101c	62a	0.85±0.07	0.66±0.23
Leaflet width:length ratio	0.81b	0.69ab	0.61a	0.55-0.99	0.76ab	0.81b	0.72ab	0.69±0.13	0.27±0.38
Branches per main stem	4.8a	8.0bc	8.6c	4-9	6.0ab	6.3abc	5.8ab	0.79±0.04	0.31±0.38
Nodes per main stem	9.4a	13.5b	17.2c	5-18	11.6ab	10.2a	13.6b	0.70±0.04	0.70±0.31
Pods per peduncle	4.6a	5.2a	4.1a	2.2-6.6	4.2a	4.2a	4.8a	0.89±0.04	0.14±0.45
Peduncle length (mm)	117a	73a	82a	22-207	94a	114a	104a	0.89±0.04	0.52±0.28
Pod length (mm)	108b	63a	69a	52-117	71a	78a	74a	0.88±0.05	0.49±0.27
Pod width (mm)	6.3b	4.7a	4.1a	3.6-6.6	4.8a	5.0a	4.8a	0.88±0.04	0.66±0.33
Seeds per pod	13.2d	8.1ab	9.8bc	5.7-13.9	11.1c	11.7c	7.2a	0.90±0.03	0.43±0.41
Seed size (g /100 seed)	6.4c	3.4b	1.0	1.2-6.1	3.0	3.8b	3.1b	0.81±0.04	0.98±0.13
Hard seed (%)	0.02a	0.13a	1.00c	0.02-0.97	0.41b	0.07a	0.86c	0.99± 0^B	1^A±0.03
(b) Berken x ACC 1									
Stem diameter (mm)	9.2cd	8.2bc	4.6a	5.1-10.4	7.4b	10.4d	5.2a	0.20±0.10	0.36±0.42
Floral standard (mm)	18.1c	15.6b	13.6a	11.5-18.1	14.9ab	15.7b	14.6ab	0.77±.03	0.61±0.24
Leaflet length (mm)	129cd	118bc	87a	87-203	121c	142d	103ab	0.60±0.14	0.94±0.15
Leaflet width (mm)	100cd	87c	51a	58-137	89c	110d	70b	0.79±0.13	0.93±0.16
Leaflet width-length ratio	0.77d	0.74cd	0.59a	0.60-0.95	0.74cd	0.77d	0.68bc	0.56±0.17	0.19±0.48
Branches per main stem	4.7a	7.8c	8.2c	4.0-11.0	6.8bc	5.4ab	6.8bc	0.70±0.07	0.30±0.35
Nodes per main stem	8.3a	13.2c	16.8d	4.0-18.0	11.1abc	9.5ab	12.0cd	0.70±0.03	0.95±0.13
Pods per peduncle	4.3a	4.2a	3.9a	2.4-7.2	4.4a	4.8a	4.7a	0.80±0.07	0.08±0.54
Peduncle length (cm)	13.3c	10.4abc	8.5ab	3.8-18.9	9.4ab	11.6bc	7.1a	0.77±0.05	0.19±0.66
Pod length (mm)	111c	71ab	71ab	46-92	68ab	78b	67a	0.59±0.09	0.13±0.42
Pod width (mm)	6.7c	4.9ab	4.0a	2.9-7.2	4.4ab	5.6b	4.6ab	0.81±0.03	0.67±0.28
Seeds per pod	12.3c	9.1ab	9.2ab	4.8-13.8	10.6bc	10.5bc	7.3a	0.67±0.06	0.53±0.36
Seed size (g /100 seed)	6.8d	3.7bc	1.0a	1.4-6.1	3.3bc	4.3c	3.1b	0.27±0.04	0.74±0.22
Hard seed (%)	0.02a	0.20a	1.00c	0.02-0.96	0.47b	0.18a	0.90c	0.98± 0^B	0.81±0.25

1^A indicates the estimate of heritability exceeded 1; 0^B indicates the value of the standard error was < 0.01

However, there was transgressive segregation in the F_2 generation, with some individuals either considerably above or below the parental values. Broad sense heritability in this cross was high and narrow sense heritability moderate (Table 5.10a). In contrast, peduncle length in Berken was significantly longer than in ACC 1 (Table 5.10b). Again, there was transgressive segregation in the F_2 generation. However, in this cross, broad sense heritability was only moderately high while narrow sense heritability was not statistically greater than zero.

Pod size. In both crosses, the mean values for the F_1 hybrid and F_2 plants were much closer to the wild than cultivated parents for pod width and pod length (Table 5.10). In the Kiloga cross, the broad sense heritability for pod length and width was high (0.88), while the narrow sense heritability was moderate (0.49 and 0.66, respectively). In the Berken hybrid population, broad sense heritability for these two traits was moderate to high, but narrow sense heritability was not significantly greater than zero.

Number of seeds per pod. In both crosses, the number of seeds per pod was significantly greater in the cultivated parents (12.3-13.2) than in ACC 1 (9.2-9.8) (Table 5.10). Interestingly, the F_1 hybrid tended to have fewer seeds per pod than either parent. However, the F_2 and BC_{P1} generation means were much closer to the cultivated parent than wild species. There was considerable transgressive segregation for seeds per pod in the F_2 generation, with some segregants above and some below the respective parental values. Inspection of the F_2 and backcross progeny distributions for seeds per pod indicated discrete progeny groupings. In general, the groupings were consistent in the two crosses. In the F_2 population (Figure 5.6a), about a quarter of the plants had between 6.0-9.0 seeds per pod (mean ~ 7.5) i.e. fewer than ACC 1. The other three quarters had 9.5-14.5 seeds per pod, with a mean of c. 12.0-12.5 (i.e. similar to the cultivated parents). In the BC_{P1} generation, there were two very discrete groups, with about half the individuals with 7.5-9.5 seeds per pod and half with 12.5-14.5. In the BC_{P2} generation, there was only one grouping, with 6.0-9.5 seeds per pod (Figure 5.6c). A plausible qualitative inheritance model for these distinctive patterns was not apparent.

Assuming quantitative inheritance, broad sense heritability was generally higher in magnitude than narrow sense heritability for the trait in both crosses. In the Kiloga cross, while broad sense heritability was very high (0.90), the narrow sense heritability was low (0.43). However, narrow sense heritability for the trait in the Berken cross was medium, at 0.53.

Seed size or 100 seed weight. The 100 seed weight of ACC 1 was just around 1.0 g (Table 5.10), which is at the lower end of the range for wild Australian accessions (Rebetzke and Lawn 2006a). In contrast, seed size of the two cultivars was significantly higher. In both crosses, the seed size of the F_1 hybrid was intermediate between and significantly different from both the parents. While the F_2 frequency distribution was somewhat skewed toward the wild parent (Figure 5.7a), the F_2 mean was still fairly close to the mid-parent value. The estimate of broad sense heritability for seed size in the Kiloga cross was high (0.81), but in Berken cross was very low (0.27). However, narrow sense heritability for the trait in both crosses was very high and high (0.98 and 0.74, respectively), suggesting considerable additive gene action for this trait.

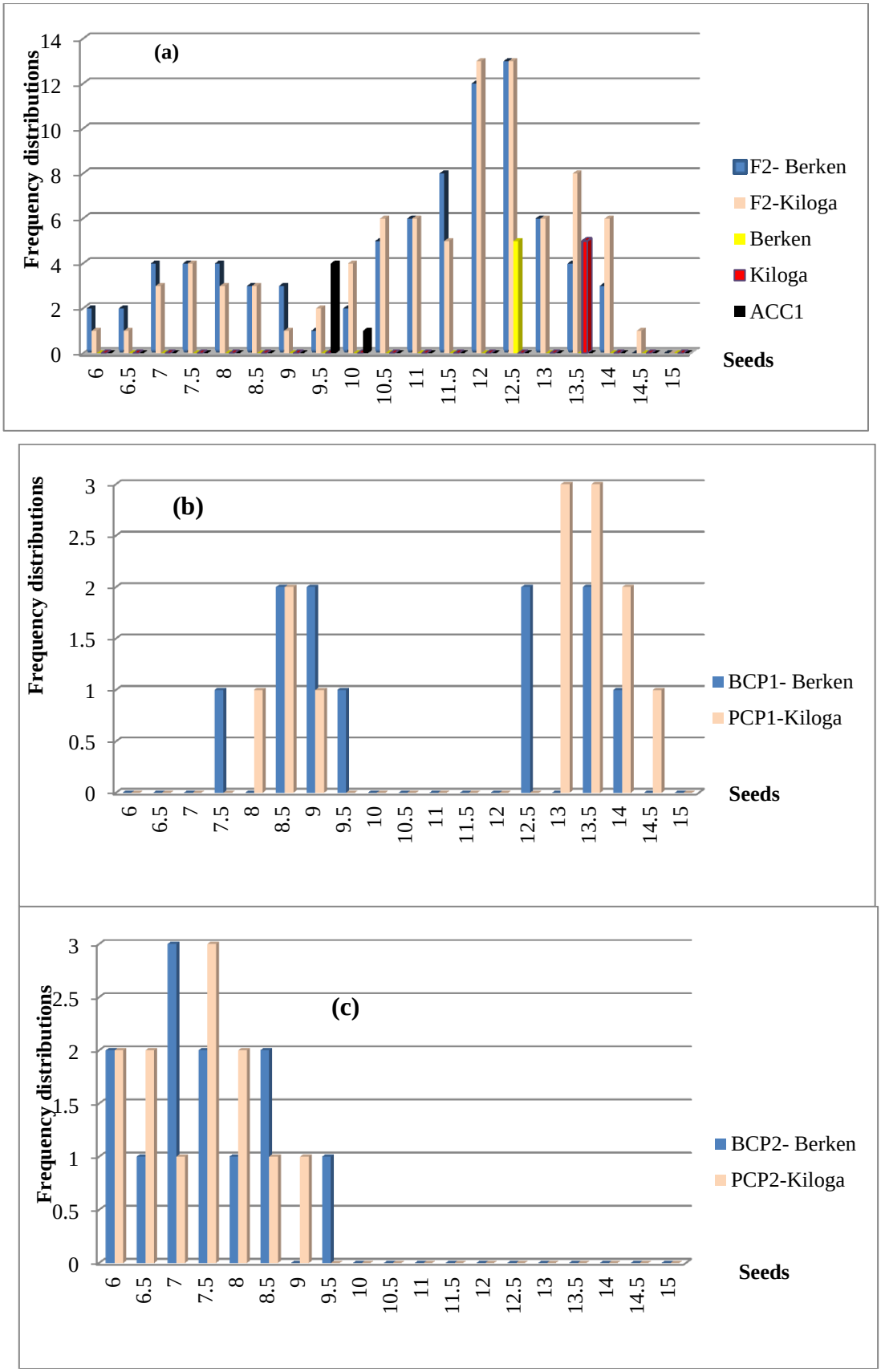


Figure 5.6 Frequency distributions for seeds per pod within the F_2 and backcross progenies from the Kiloga x ACC 1 and Berken x ACC 1 crosses (a) F_2 generation, (b) backcross to cultivated varieties Kiloga and Berken, (c) backcross to wild parent ACC 1

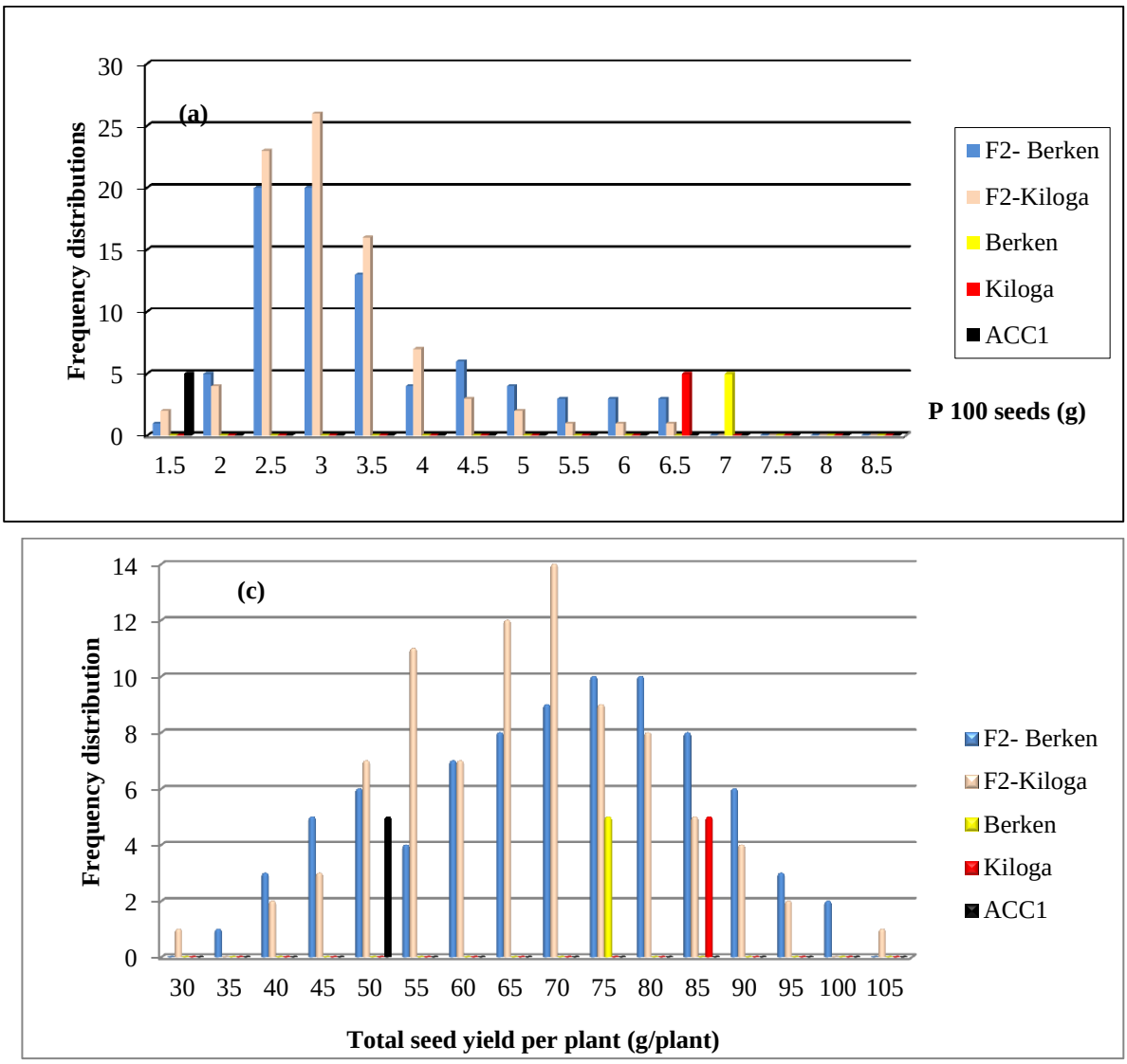


Figure 5.7 Frequency distributions for (a) 100 seed weight (g) and (b) total seed yield (g/per plant) of 86 and 82 F_2 and parental plants respectively, from the Kiloga x ACC 1 and the Berken x ACC 1 crosses

Hardseededness. In both crosses, the wild parent showed extremely strong hardseededness, while the cultivated lines were almost completely soft seeded (Table 5.10). In the Kiloga cross, while the F_1 hybrid showed 13% hard seed, the mean of the F_2 progenies was about 41% hard seed, which was significantly different from both the cultivated and wild parents. Additionally, in the BC_{P1} generation, the mean level of hard seed was comparable with the F_1, whereas in the BC_{P2} plants, a higher level of hard seed was exhibited in the two crosses (86 and 90%, respectively). Broad sense heritability for hardseededness was very high in both crosses (99% and 98%, respectively). The estimate of narrow sense heritability exceeded 100%, which was not possible, but nonetheless meant that the trait was highly heritable

5.3.3.3 Agronomic traits

Analysis of variance showed that there were significant differences ($P < 0.05$) between the parental means, and among the F_1, F_2, BC_{P1} and BC_{P2} generation means, for most of the agronomic traits observed, the main exception being pod weight per plant (Table 5.11). Generally, vegetative biomass and total dry matter were greater in the wild accession than in the cultivars, whereas seed yield, harvest index and seed: pod weight ratio were each generally smaller in the wild accession. The estimates of both broad and narrow sense heritabilities for agronomic traits varied considerably, depending on the trait and cross.

Table 5.9 Agronomic trait means for the parental, F_1, and F_2 BC_{P1} and BC_{P2} populations, the range for the F_2 generation, and broad and narrow sense heritability estimates, for two crosses (a) Kiloga x ACC 1 (b) Berken x ACC 1. Means followed by the same letters are not significantly different ($P < 0.05$).

Traits	P_1	F_1	P_2	F_2		BC_{P1}	BC_{P2}	Heritability	
				Range	Mean			Broad ($h_{b)}$	Narrow (h_n)
(a) Kiloga x ACC 1									
Vegetative biomass (g)	29.7a	52.0c	93.6d	26-87	46.5bc	35.7ab	85.5d	0.85±0.05	0.44±0.37
Pod weight (g/plant)	124a	173b	129a	26-166	123a	142a	137a	0.77±0.10	0.70±0.20
Seed yield (g/plant)	82.2c	78.9bc	49.8a	17-102	64.5ab	80.9c	61.8a	0.90±0.04	0.71±0.27
Seed: pod weight ratio	0.66d	0.46b	0.39a	0.39-0.67	0.52c	0.57c	0.45b	0.64±0.23	0.35±0.33
Total dry matter (g)	154a	225b	222b	60-229	170a	178a	222b	0.76±0.15	0.49±0.28
Harvest index	0.52e	0.35bc	0.20a	0.25-0.49	0.38c	0.45d	0.29b	0.79±0.12	0.78±0.21
(b) Berken x ACC 1									
Vegetative biomass (g)	25.6a	47.7b	82.8c	3-111	41.2b	35.6ab	85.4c	0.84±0.09	0.53±0.41
Pod weight (g/plant)	110a	114b	105a	61-171	122ab	139b	102a	0.41±0.08	0.58±0.28
Seed yield (g/plant)	73.4b	78.2b	45.0a	33-100	67.3b	74.7b	47.5a	70±0.03	0.91±0.15
Seed: pod weight ratio	0.66c	0.55b	0.43a	0.42-0.07	0.55b	0.54b	0.46a	48±0.36	0.41±0.30
Total dry matter (g)	135a	192b	188b	93-240	163ab	174b	188b	0.62±0.05	0.82±0.17
Harvest index	0.45b	0.41b	0.24a	0.26-0.53	0.41b	0.43b	0.25a	0.57±0.19	0.50±0.29

Vegetative biomass. In both crosses, the F_1 progenies intermediate between the two parents and differed from them (Table 5.11). While the F_2 generation showed a wide range of variation with some segregants above and some below the respective parental values, the mean values were still intermediate between the two parents and differed significantly from each. The BC_{P1} plants exhibited vegetative biomass that was close to the cultivated parents, while in BC_{P2} population, the trait was comparable with the wild parent. Broad sense heritability was high in both crosses; however, the narrow sense heritability was medium (0.47 and 0.53, respectively).

Pod weight. The F_1 progenies had considerably higher pod weight than the parents (Table 5.11), perhaps suggesting some hybrid vigour for this trait. There was considerable transgressive segregation for pod weight in the F_2 generation, with some segregants above and some below the respective parental lines. However, there were no significant differences between the parents and the means of the F_2, BC_{P1} and BC_{P2} generations in both crosses, except for the BC_{P1} plants of the Berken x ACC 1 cross. Both broad and narrow sense heritabilities for the trait in the Kiloga cross were moderate, at 77% and 70% respectively. In the Berken cross, about 60% of the total phenotypic variation in pod weight was heritable.

Seed yield. In both crosses, the F_1 progeny were close to and not significantly different from the cultivated parent (Table 5.11). The variation in the F_2 generation in both crosses was essentially continuous (Figure 5.7b) but with considerable transgressive segregation, with some segregants above and some below the respective parents. While the BC_{P1} populations were close to the cultivars in both crosses, the BC_{P2} means were comparable with the wild parent. Both broad and narrow sense heritabilities in both hybrid populations were moderate to high. The narrow sense heritabilities were 0.71 and 0.91 for both crosses, respectively, which is unusually high for a trait such as seed yield.

Seed-pod weight ratio. The proportion of total pod weight that was seed was significantly greater in the cultivated parents than in ACC 1 (Table 5.11). This finding was consistent with the study by Bushby and Lawn (1992) who found that wild mungbean invested more dry matter in pod walls than cultivated mungbean. Among the F_1 hybrid plants, F_2, BC_{P1} and BC_{P2} generations, the mean values were intermediate between the parents and significantly different from both, excepting for the BC_{P2} of the Berken cross. Heritabilities in the broad and narrow sense ranged from low to moderate, depending on the cross.

Total dry matter (TDM). In both crosses, the mean TDM of the cultivated parents was generally smaller than in ACC 1, presumably reflecting their much shorter growth duration (Table 5.11). In the F_1 hybrid plants, mean TDM was similar to that of the wild parent. In the Kiloga cross, while the mean TDMs of the F_2 and BC_{P1} generations were statistically similar to the cultivated parent, the BC_{P2} generation mean was close to the wild parent. However, in the Berken cross, the F_2 generation showed a wide range of variation for TDM, while for the backcross populations, mean TDM was comparable with the wild parent. In both crosses, broad sense heritability for TDM was moderate whereas narrow sense heritability was high in the Berken cross but only medium in the Kiloga cross.

Harvest index (HI). In the Kiloga cross, the F_1 plants were intermediate between and significantly different from the parents (Table 5.11). Similarly, the F_2 generation mean was intermediate between

the cultivated and wild parents. However, in the Berken cross, there were no significant differences in HI between the cultivated parent, and the F_1 and F_2 progeny means. In the backcross generations of the two crosses, means were close to the respective backcross parent. Both broad and narrow sense heritabilities for HI were moderately high in the Kiloga cross, but only medium in the Berken cross.

5.3.4 Associations between Traits

5.3.4.1 Phenotypic correlations between key quantitative traits

There was a large number of statistically significant phenotypic correlations between pairs of quantitative traits observed in the F_2 plants in both crosses. The pairwise interrelations between a subset of key traits are summarised in Table 5.10 while the interrelations between the full set of traits are listed in Appendix II. In both the Kiloga x ACC 1 and Berken x ACC 1 crosses, there were significant positive associations between the phenological traits, time to flowering and time to physiological maturity (r = 0.41** and 0.47**, respectively) (Table 5.10). Similarly, there were close relations between these phenological traits and number of nodes per main stem in both crosses. However, there were negative correlations between both traits and seed yield, pod length and seed size for both crosses. In contrast, there were significant positive relations between these two phenological traits and total dry biomass. All these relations indicated that later flowering F_2 plants grew longer, had more stem nodes, and produced greater biomass; but gave smaller pod size and seed size, and also smaller total seed yield per plant. There was a significant negative correlation (r = -0.64** and -0.59**, respectively) between time to flowering and HI, indicating that earlier flowering plants produced a higher proportion of seed per unit biomass than the later flowering plants. The cultivated parents were both earlier flowering and had higher HI than the wild parents (Table 5.10)

Among the morphological traits, in both crosses, the number of seeds per pod was positively correlated with pod length (r = 0.52** and 0.49** for the two crosses, respectively) and also positively associated with total seed yield per plant (r = 0.37** and 0.45**) (Table 5.10).These correlations are of interest, because the cultivated parents had more seeds per pod, greater pod size and seed yield which contrasted with the wild parent (Table 5.10). In the Kiloga cross, there was a weak positive correlation (r = 0.22*) between seeds per pod and seed size. Perhaps not surprisingly, pod width was positively correlated with seed size in both crosses (r = 0.35** and 0.27** respectively). While number of nodes per main stem was negatively correlated with seed yield, HI and seed size for both crosses, there was a positive correlation between nodes per main stem and TDM (r = 0.35** and 0.19*, respectively).

Table 5.10 Pairwise phenotypic correlations among a subset of quantitative traits observed in the F2 generation plants from two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 and (b) Berken x ACC 1. Entries are the linear correlation co-efficients (*r*) between the respective trait pairs.

(a) Kiloga x ACC 1

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	0.41**	1								
SP	-0.34**	-0.36**	1							
PL	-0.20*	-0.51**	0.52**	1						
PW	-0.13	-0.26**	0.02	0.26**	1					
No	0.50**	0.30**	-0.26**	-0.12	-0.07	1				
Ye	-0.32**	-0.49**	0.37**	0.32**	0.17	-0.27**	1			
SeS	-0.44**	-0.56**	0.22*	0.50**	0.35**	-0.42**	0.42**	1		
TM	0.44**	0.70**	-0.27**	-0.06	-0.11	0.35**	0.51**	-0.14	1	
HI	-0.64**	-0.56**	0.50**	0.35**	0.25**	-0.53**	0.71**	0.57**	-0.25*	1

(b) Berken x ACC 1

	Flo	**Ma**	**SP**	**PL**	**PW**	**No**	**Ye**	**SeS**	**TM**	**HI**
Flo	1									
Ma	0.47**	1								
SP	-0.24**	-0.22*	1							
PL	-0.19*	-0.44**	0.49**	1						
PW	-0.19*	-0.24**	-0.16	0.10	1					
No	0.42**	0.25**	-0.11	-0.22*	-0.06	1				
Ye	-0.47**	-0.28**	0.45**	0.19*	0.08	-0.10	1			
SeS	-0.28**	-0.27**	0.10	0.30**	0.27**	-0.42**	0.16	1		
TM	0.20*	0.40**	0.16	0.04	-0.04	0.19*	0.59**	-0.15	1	
HI	-0.59**	-0.31**	0.40**	0.22*	-0.05	-0.32**	0.67**	0.36**	-0.19*	1

*, ** indicates significant linkage at $P < 0.05$, $P < 0.01$ respectively

Flo=flowering date; Ma =physiological maturity; SP = seeds per pod; PL=pod length; PW= pod width; No=Nodes per main stem; Ye= seed yield; SeS= 100 seeds weight; TM= total biomass; HI=harvest index.

Among the main agronomic traits (Table 5.10), seed yield per plant in both the Kiloga x ACC 1 and Berken x ACC 1 crosses was positively correlated with total biomass per plant (r = 0.51** and 0.59**, respectively) indicating that those plants which produced more biomass also produced more seed. Similarly, there was also a positive correlation in both crosses between seed yield and HI (r = 0.71** and r = 0.67**, respectively). Interestingly, in both crosses, while the total seed yield per plant

was positively correlated with seed size, there were negative relations (r = -0.25** and -0.19* for the two crosses, respectively) between HI and total dry biomass (Table 5.12), reflecting the fact that seed yield and vegetative biomass are alternative sinks for dry matter.

5.3.4.2 Genetic correlations among quantitative traits

There were several significant genetic correlations ($P < 0.05$) between pairs of quantitative traits (Table 5.11), which suggested underlying associations in the additive gene action for the respective traits. While the phenological traits had negative genetic correlations with almost all of the morphological and agronomic traits collected, there were significantly positive genetic correlations between time to flowering and time to physiological maturity (r = 0.40** each), suggesting that genes controlling flowering considerably affected the maturity trait. The trait of time to maturity in the two hybrid populations exhibited positive genetic associations with nodes per main stem and TDM characters. However, physiological maturity shared negative but weak genetic correlations with seed yield, pod size and seed size in both crosses. The HI trait exhibited highly negative genetic correlation with time to maturity in Kiloga cross (r = -0.44**), but just only -0.19* for Berken cross.

In both crosses, while seeds per pod showed highly positive genetic correlation with pod length (r = 0.54** and 0.62**, respectively) and seed yield (r = 0.36** and 0.39**, respectively) (Table 5.11), it shared negative genetic associations with pod width and seed size. Interestingly, in both crosses, while pod length exhibited positive correlation with seed yield (r =0.23* and 0.30** for two crosses, respectively), there were negative genetic associations of pod width with seed yield. There were negative correlations of nodes per main tem with all of agronomic traits observed, excepting for TDM with very weak associations in both crosses.

Among the agronomic characters in both crosses, there were generally highly significant positive genetic correlations between key pairs of agronomic traits (Table 5.11). Genes controlling for seed yield per plant were highly positively associated with total dry biomass matter per plant (r = 0.77** and 0.83**, respectively in the two crosses. Similarly, there were also highly positive correlations in both crosses between seed yield and HI (r = 0.78** and r = 0.57**, respectively). Interestingly, in both crosses, the total seed yield per plant was positively correlated with seed size, but was only just significant in Kiloga cross (r =0.20*).

Table 5.11 Pairwise genetic correlations among a subset of quantitative traits observed in the F_2 generation plants from two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 and (b) Berken x ACC1

(a) Kiloga x ACC 1

	Flo	Ma	SP	PL	PW	No	Ye	SeS	TM	HI
Flo	1									
Ma	0.40**	1								
SP	-0.17	-0.29**	1							
PL	-0.21*	-0.28**	0.54**	1						
PW	-0.20*	-0.02	-0.12	0.10	1					
No	0.17	0.18	-0.04	0.00	0.11	1				
Ye	-0.12	-0.31**	0.36**	0.23*	-0.02	-0.06	1			
SeS	-0.18	-0.14	-0.01	0.26*	0.11	-0.21*	0.20*	1		
TM	0.04	0.05	0.10	0.04	-0.07	0.11	0.77**	0.08	1	
HI	-0.25*	-0.44**	0.42**	0.30**	0.10	-0.21*	0.78**	0.24*	0.24*	1

(b) Berken x ACC 1

	Flo	Ma	SP	PL	PW	No	Ye	SeS	TM	HI
Flo	1									
Ma	0.40**	1								
SP	-0.07	-0.28**	1							
PL	-0.06	-0.24*	0.62**	1						
PW	-0.04	-0.11	-0.31**	-0.32**	1					
No	0.19*	0.16	0.04	-0.08	0.09	1				
Ye	-0.31**	-0.23*	0.39**	0.30**	-0.13	-0.01	1			
SeS	-0.22*	-0.21*	-0.02	-0.12	-0.04	-0.24*	0.16	1		
TM	0.32**	0.15	0.36**	0.28**	-0.02	0.06	0.83**	0.08	1	
HI	-0.13	-0.19*	0.22*	0.15	-0.19*	-0.15	0.57**	0.18	0.05	1

*, ** indicates significant linkage at $P < 0.05$, $P < 0.01$ respectively

Flo=flowering date; Ma =physiological maturity; SP = seeds per pod; PL=pod length; PW= pod width; No=Nodes per main stem; Ye= seed yield; SeS= 100 seeds weight; TM= total biomass; HI=harvest index.

5.3.4.3 Linkage between qualitative traits

Similar to the ACC 87 crosses, the linkages between the pairs of qualitative traits for the two crosses involving ACC 1 populations were estimated and are shown in Table 5.12. Generally, the linkages between the pairs of qualitative traits observed appeared to be almost similar between the two crosses involving ACC 1. In both crosses, the linkage was strongly detected between leaflet lobing and hilum colour (Table 5.12). While the leaflet lobing was linked with powdery mildew in Kiloga cross, this was not evident for the Berken population.

Table 5.12 Chi-square values were calculated from some qualitative traits observed in K x ACC 1 and B x ACC 1 hybrid populations to detect genetic linkage among those traits.

a) Kiloga x ACC 1

	Leaflet lobing	**Hilum colour**	**Powdery mildew**
Leaflet lobing	1		
Hilum colour	29.597***	1	
Powdery mildew	10.083*	6.610^{+}	1

b) Berken x ACC 1

	Leaflet lobing	**Hilum colour**
Leaflet lobing	1	
Hilum colour	20.721***	1

$^{+}$,*, **, *** indicates observed digenic ratios diverge significantly from independent assortment at 0.10 > P < 0.05, 0.01, 0.001 respectively

CHAPTER 6: GENERAL CONCLUSIONS AND IMPLICATIONS

In this concluding chapter, the similarieties and contrasts between the expression and genetic control of cultivated and wild traits in the four different hybrid populations are discussed, and compared with previous reports where these are available. Finally, some of the implications of the findings, especially for mungbean improvement, are suggested.

Generally, there were many similarities in the expression and genetic control of both qualitative and quantitative traits among the four hybrid populations, with only small differences due to the different cultivated parents. However, larger differences were apparent between the populations involving the wild parents ACC 1 and ACC 87. Some quantitative traits showed very high values of narrow sense heritability in one cross whereas in the others they were medium or low, suggesting significant differences occurred between the hybrid populations in the range of genetic variation for those traits in F_2 and backcross generations. High values of narrow sense heritability for traits of interest would be helpful for the breeder who looking for the genetic movement. The present study also showed many similarities and some differences in the associations between traits in the four populations.

6.1 Parental Genotypes

6.1.1 Cultivated Parents

The two cultivated parental accessions Berken and Kiloga were broadly similar for a range of traits. They were typical cultivated varieties with a strong erect stem, large leaflets, and large green shiny seed. They were very early or early flowering when grown in south east Queensland where the length of the pre-flowering period varied by less than 14 days over sowing date (Lawn 1979a). As expected, they were even more early flowering when grown in Townsville where late summer days are shorter and warmer than in south east Queensland. While most qualitative traits in Kiloga and Berken were the same, there were some slight differences in the mean values of the quantitative traits in these two parental cultivars over the four crosses.

6.1.2 Wild Parents

The two wild mungbean accessions were fine stemmed, twining plants with smaller leaves than the cultivars, lobed leaflets and smaller, black seeds. The accession with the very late flowering trait, ACC 1, was a strongly prostrate, viny plant with quite small leaves that were more deeply

lobed than in ACC 87. Seed size was also smaller in ACC 1 than ACC 87. The other wild accession, ACC 87, was a perennial type with more robust, twining growth habit, and larger leaflets, flowers and pods. ACC 87 was also tuberous-rooted, with adventitious shoots emerging from the roots when warmer temperatures returned in the spring. Thus, while ACC 1 and ACC 87 shared a number of wild traits, there was also greater divergence between the two wild parents than was apparent between the two cultivated accessions. These observations reflect those of Lawn and Rebetzke (2006) and Rebetzke and Lawn (2006c) who observed that among Australian wild accessions, fine-stemmed types like ACC 1 exhibited fewer cultivated traits than more robust perennial types like ACC 87.

6.2 Expression and Inheritance of Novel Traits

6.2.1 Perenniality

In both crosses, the simplest model of inheritance was that the perennial trait from ACC 87 was conditioned by two dominant genes with complementary gene action (Chapter 4). The fit to this suggested model of inheritance for the trait was much stronger in Berken x ACC 87 cross than in Kiloga x ACC 87 population. The data nonetheless suggested that perenniality in wild mungbean is a qualitatively inherited trait. As such, it should prove relatively simple for breeders to transfer the trait across into a cultivated mungbean background. Prior to this study, there was no information available on the inheritance of the perennial trait in mungbean, and little information for *Vigna* species generally. In *V. vexillata*, many of the root characters, such as tuber dry weight, and tuber harvest index, which are related to the expression of perenniality of this species, appeared to be quantitatively inherited (James and Lawn 1991, Karuniawan *et al*. 2006, Damayanti 2010 a,b,c). Damayanti (2010c) suggested that tuber form in *V. vexillata* may be qualitatively inherited, but did not give any possible models of inheritance. While the present study indicated that perenniality is conditioned by as few as two complementary genes, it is likely that additional genes affect tuber attributes such as root diameter and length. Genotypic variation has been reported for these attributes (Rebetzke and Lawn 2006c).

From an adaptation viewpoint, it is perhaps not surprising that perenniality is controlled by relatively few genes. While perenniality and tuberous roots are relatively common traits in the genus *Vigna*, it is not common in mungbean. Indeed, whereas wild mungbean has a wide natural range, that includes Madagascar, and eastern and northern Africa (Verdcourt 1970), southern and south-east Asia, Indonesia and Papua New Guinea (Sangiri *et al.* 2007) and tropical Australia (Lawn and Cottrell 1988, Lawn and Watkinson 2002), the perenniality trait is unique to a relatively small region of eastern Australia around Townsville – Charters Towers (Rebetzke and

Lawn 2006c). It appears to confer adaptation in seasonally-arid, lightly-wooded, speargrass dominant ecosystems where annual fire is common, usually late in the dry season.

6.2.2 Late Flowering

The ACC 1 plants had very late flowering, consistent with previous findings of Lawn and Rebetzke (2006) and Rebetzke and Lawn (2006a). However, the inheritance of late flowering observed in ACC 1 crosses appeared to be complex. Observation of the F_1 plants suggested some level of dominance for the early flowering trait, while no F_2 or backcross plants were recovered that were as late flowering as ACC 1. Indeed, the data for the crosses involving ACC 1 appeared to be remarkably similar to those for the crosses involving ACC 87 (Table 6.1).

Table 6.1 Mean times to flowering (d) for the parental, F_1, F_2, BC_{P1} and BC_{P2} populations, for four cultivated x wild mungbean crosses. Means followed by the same letters are not significantly different ($P < 0.05$) based on the analysis of variance.

Cross	P_1	F_1	P_2	F_2 Range	Mean	BC_{P1}	BC_{P2}	Heritability	
								Broad (h^2_b)	Narrow (h^2_n)
Kiloga x ACC 87	31.1a	37.8b	43.8c	30-48	38.4b	36.2b	41.5c	0.91±0.01	0.53±0.24
Berken x ACC 87	33.5a	38.2ab	45.6c	31-82	41.3bc	35.2ab	40.8bc	0.97±0.01	1.00[A]±0.001
Kiloga x ACC 1	33.2a	37.5a	87.6c	31-63	41.3a	36.6a	58.2b	0.96±0.02	0.81±0.23
Berken x ACC 1	34.6a	36.5ab	88.2d	32-77	43.1b	34.3a	56.2c	0.95±0.01	0.93±0.16

The F_1 means for time to flowering were very similar for all four crosses (Table 6.1), as were the BC_{P1} means, while in the F_2 generation, the respective means for the ACC 1 crosses were only marginally later than those for the ACC 87 crosses. In general, with both wild parents, more late flowering plants were recovered with Berken rather than Kiloga as the cultivated parent (*cf.* Figures 4.5 and 5.1). More surprisingly, there were as many very late flowering F_2 plants recovered from the Berken x ACC 87 cross (including the latest flowering F_2 plant of all) than from the Berken x ACC 1 cross. That said, most of the F_2 plants in the ACC 87 crosses were in the 33 – 45 d range (Figure 4.5), whereas the ACC 1 crosses included many more progeny in the 45 – 60 d range (Figure 5.1). The BC_2 means were clearly later for the ACC 1 crosses than the ACC 87 crosses (Table 6.1), perhaps indicating the accumulation of more genes for lateness. Consistent with that interpretation, both narrow and broad sense heritability exhibited in ACC 1 crosses were very high, suggesting that the trait was highly heritable in nature and most of the total phenotypic variance was due to additive genetic variance.

The fact that the ACC 87 cross produced a small number of very late flowering genotypes indicates that there are also lateness genes in that accession, but that they are likely masked by

other genes for earliness. In contrast, the extreme lateness of ACC 1 suggests that it lacks genes for earliness, but when it was crossed with the earlier cultivated genotypes, mainly early flowering progeny were initially recovered. ACC 1 was originally collected from a swampy, coastal location near Mackay, where water is often available for long periods outside the normal wet season. It is possible that this situation favours a later flowering phenotype than more inland locations where dry conditions can abruptly shorten the active growth period.

There is little information on genetics of flowering in mungbean, particularly the late flowering character. Rehman (2010) reported both additive and non-additive gene action controlling the earliness of mungbean. Tah (2009) reported additive and dominance gene action for days to first flower and additive effects were reported for days to 50% flowering by Malik and Singh (1983) and Chhabra and Singh (1988). However, Malarvizhi (2000) found non-additive genetic control for this parameter in cowpea. Sadiq *et al.* (2000) and Vikas *et al.* (1999) reported high heritability for days to flowering in mungbean while Poehlman (1991) reported high broad sense heritability for both days to flowering and maturity.

6.3 Expression and Inheritance of Qualitative Traits

With the exception of apparent resistance to powdery mildew, the wild qualitative traits observed in the study were invariably dominant over the cultivated phenotype (Table 6.2). Among the four hybrid populations, there were only small differences observed in the best-fitting models of inheritance due to the two different cultivated parents. Larger differences were found between the populations depending on whether ACC 1 or ACC 87 was the wild parent, supporting the earlier observation that there was greater genetic divergence between the wild parents than between the cultivated parents. For example, more qualitative traits appeared to be under monogenic control in the crosses involving ACC 87 whereas dignic control was more common with the ACC 1 crosses.

6.3.1 Morphological Traits

Leaflet lobing. In all four crosses, the data supported the conclusion that the presence of the wild leaflet lobing trait was due to a single dominant gene (Table 6.2a). However, given that the degree of lobing was greater in ACC 1 than ACC 87, and in all four crosses, the F_1 progeny were less strongly lobed than the respective wild parent, and differences in the extent of lobing were also observed in the F_2 and backcross generations, the level of expression of the trait was either under additive gene action and/or other genes were involved. Single dominant gene control was consistent with Singh and Mehta (1953), who reported that lobed leaflet was dominant over entire leaflet and was conditioned by a single gene, which they symbolized E. Similarly, working with

Australian wild mungbean, James *et al*. (1999) also reported lobing was controlled by a single dominant gene. The fact that all the F_1 plants had intermediate lobing suggested that the heterozygous plants have intermediately lobed leaflets. This suggestion was consistent with a finding Sen and Ghosh (1959) who also reported that heterozygous plants have intermediately lobed leaflets.

Stem twining. In the two ACC 87 crosses, stem twining appeared to under the control of a single dominant gene (Table 6.2a) whereas in the ACC 1 crosses, there was a better fit to two genes with recessive-dominance epistasis (a recessive suppressor gene inherited from the cultivated parent). Again, the occurrence of different levels of expression in the segregating progeny suggested either additive gene effects (supported by the intermediate response of the F_1 plants in the ACC 87 crosses), or the action of additional genes. Sen and Ghosh (1959) and Khathak (1999) both concluded that the twining habit was dominant over non-twining habit in mungbean and was conditioned by a single dominant gene, whereas Pathak and Singh (1963) suggested that twining was governed by a single recessive gene. A single gene also controls the twining habit in common bean (Koinange *et al.* 1996).

Prostrate or spreading stems. In all four crosses, the data suggested that the wild trait was conditioned by the presence of two dominant genes with duplicate action (Table 6.2a). In both crosses, there were differences in the degree of expression of the wild trait in the progeny, and in the ACC 87 crosses, there was clear evidence of partial dominance. Talukdar (2003) found that when the wild mungbean that occurs in India was crossed with the cultigens, the wild growth habit was conditioned by a single dominant gene, with the spreading habit in the wild types was dominant over the erect habit of the cultivated lines. Pathak and Singh (1963) also reported that semi-spreading growth habit in mungbean was conditioned by a single dominant gene whereas Fery (1980), Khattak (1999) and Sukhumaporn (2009) concluded that other genes also contributed to variable expression of the trait.

Pod dehiscence. Pod dehiscence in the ACC 87 populations appeared to be controlled by a single recessive gene with evidence of additive gene action (Table 6.2a), whereas the responses in the ACC 1 populations were consistent with two dominant genes with additive gene action (the non-dehiscent pods from cultivated parents was recessive). Consistent with this, fewer non-dehiscent progeny were recovered in the ACC 1 crosses than from the ACC 87 crosses. The ACC 87 response was consistent with Verma and Krishi (1969) who reported that pod dehiscence in mungbean was dominant to non-shattering and was probably conditioned by a single gene pair. Likewise in *V. vexillata*, Damayanti *et al.* (2010c) reported pod dehiscence to be controlled by a single dominant gene based on crosses between cultivated and wild accessions. However, Singh

et al. (1975) reported while shattering was dominant in F_1 plants of crosses between *Vigna mungo* (non-dehiscent) and *Vigna radiata* (dehiscent), the non-shattering characteristic could not be recovered in the F_2. They concluded that resistance to shattering in those crosses was quantitatively inherited. Likewise, after rating pod dehiscence in cultivated x wild mungbean progeny from zero (nil shattering) to five (complete shattering), James *et al.* (1999) classified pod dehiscence as a quantitative trait with moderate heritability.

6.3.2 Seed Traits

Testa colour. In all four crosses, black speckling of the testa appeared to be under the control of two genes. In the ACC 87 populations, the trait was suggested to be conditioned by two genes with dominance-recessive epistasis (Table 6.2b). However, in the ACC 1 crosses, the responses were consistent with two dominant genes with duplicate gene action. In all four crosses, there were clear differences in the intensity of the mottling due to the testa speckling, with slightly more than half the F_2 progeny exhibiting darker intensity than the remainder. These differences in intensity may have been due either to additive gene action (supported by the fact that the heterozygous F_1 plants were always lighter) or additional genes. Rheenen (1965) and Sen and Jana (1963) both reported that 'mottled' seed coat in mungbean was monofactorially dominant over the non-mottled one. In black gram, Arshad *et al.* (2005) and Muhammad *et al.* (2005) reported that the speckled black seed coat colour of black gram was controlled by a single dominant gene. In *V. vexillata*, Damayanti *et al.* (2010c) also reported speckled black testa colour to be conditioned by a single dominant gene, based on crosses between cultivated and wild accessions.

Ridged surface texture layer. In the ACC 87 crosses, the presence of a ridged surface texture layer was conditioned by a single dominant gene (Table 6.2b). This model was consistent with some previous studies e.g. Watt (1977), Lawn *et al.* (1988), and Poehlman (1991). However, two genes appeared to be involved in the ACC 1 crosses (Table 6.2b). In the Kiloga x ACC 1 cross, the data were consistent with the texture layer being conditioned by two complementary dominant genes, while in the ACC 1 x Berken cross, the best model was two genes in recessive-dominance epistasis (the dominant trait from ACC 1 and a recessive suppressor gene inherited from the cultivated parent). Sen and Ghosh (1959) identified a second dominant gene which is sometimes present and affects the presence of the texture layer on seed coat. James *et al.* (1999) reported two modes of inheritance for this trait, one consistent with a single dominant gene and the other one a more complex mode of inheritance. In cowpea, Yilwa (2001) reported the seed coat texture layer

was controlled by a single dominant gene, whereas Mashi (2006) found two dominant genes conditioning the seed coat texture layer in cowpea.

Testa lustre. In the Kiloga x ACC 87 cross, the presence or absence of a dull seed coat appeared to be due to a single dominant gene (Table 6.2b). However, in the other three crosses, the trait appeared to be conditioned by two dominant genes with complementary gene action. In terms of seed-coat surface, while the dull seed-coat was controlled by a single dominant gene in ACC 87 populations, two dominant genes with complementary gene action were found to condition the dull seed-coat in ACC 1 crosses. There has been very little information on the inheritance of seed-coat surface of mungbean reported. In looking at the inheritance of the wild and cultivated mungbean traits, Rheenen (1965) found that the dull seed-coat trait was conditioned by a single dominant gene. However, Zubair (2004) classified seed-coat surface as a qualitative trait, but did not mention any possible models of the inheritance for this trait.

Surface texture layer / hilum pigmentation. The presence of dark pigmentation in the surface texture layer and the hilum appeared to be due to a single dominant gene in all the four populations (Table 6.2b). Also working on the inheritance of wild and cultivated traits in mungbean, James *et al.* (1999) reported two modes of inheritance for hilum colour trait, one consistent with a single dominant gene and the other one a more complex mode of inheritance. In cowpea, Drabo *et al.* (1984) reported that the hilum colour appeared to be conditioned by three dominant genes.

6.3.3 Susceptibility to Powdery Mildew Disease

In two of the four crosses, the presence of powdery mildew on leaves was suggested to be conditioned by a single dominant gene while in the other two crosses, by two genes in dominance-recessive epistasis (a dominant gene for the presence from the cultivated parents, moderated by a recessive suppressor gene inherited from the wild parent) (Table 6.2c). In the literature, both qualitative and quantitative genetic control of powdery mildew resistance have been reported. In varieties of mungbean in Thailand, Khajudparn (2007) reported that the trait appeared to be conditioned by two dominant genes whereas Kasettranan (2009), Gawande (2003) and Chaitieng (2002) reported that the trait is governed by more than one gene with both additive and dominance gene actions. Under controlled environmental conditions using field isolates of powdery mildew, two independent dominant genes *Pm1* and *Pm2* have been identified in the resistance to powdery mildew of mungbean in India (Reddy *et al.* 1994). Yohe and Poehlman (1972) and Tickoo *et al.* (1988) reported high variation in reaction to the disease in mungbean landraces. AVRDC (1979) reported monogenic dominant resistance in *ML3* and *ML5*. Using

quantitative trait loci (QTL) mapping, Young *et al.* (1993) identified three RFLP loci associated with powdery mildew resistance in mungbean. Humphry *et al.* (2003) reported that there was a major QTL conferring resistance, although they used different sources of resistance. Using QTL markers, a single locus was identified that explained up to 86% of the differences among 147 recombinant inbred individuals derived from a Berken x ATF3640 cross.

6.3.4 Interpretation of 'Best-Fit' Inheritance Models

The nature of the process whereby models are fitted to progeny segregation ratios, combined with the limited numbers of plants that could be evaluated in each generation of each cross, means that some caution is required in interpreting the models of inheritance proposed above. In the present study, greatest reliance was placed on the segregation ratios in the F_2 generation, where the numbers of segregating plants were largest. Even so, relatively small variations in the number of plants scored in a particular phenotype could have altered the apparent 'best-fit' model. For example, too few homozygous recessive F_2 plants could result in a 3: 1 dominant: recessive ratio being misclassified as a digenic 13: 3 segregation ratio. Conversely, too many homozygous recessive F_2 plants could cause a digenic 13: 3 segregation ratio to appear to be a monogenic 3:1 ratio. In both of these situations, the backcross generations would be of limited value in determining the actual model, because the expected backcross ratios are the same in both cases.

Possible reasons why the numbers of plants with a particular phenotype might differ enough for the incorrect model to be chosen include simple chance, as well as genetic and even environmental factors. For example, using a different Australian wild mungbean accession, Lambrides *et al.* (2004) reported that the F_2 segregation in crosses between cultivated and wild mungbean accessions was an excellent fit to a two-gene model that included both dominant and recessive epistasis (13:3 ratio expected) (a dominant gene for the wild type speckled black trait). This finding was consistent with the two present crosses involving ACC 87. However, on the basis of molecular marker data, Lambrides *et al.* (2004) ultimately rejected the digenic model in favour of a single dominant gene. They attributed the misleading ratios to 'segregation distortion', possibly due to gametic and/or zygotic selection.

In wide crosses as used in this study, genetic factors are possibly more likely causes of segregation distortion. However, as indicated in Chapter 4, there were possible environmental as well as genetic factors that may have influenced the measurement of the perenniality trait. Likewise, the expression of susceptibility to powdery mildew infection requires that the environmental conditions around each plant are suitable for development of the disease if the plant is susceptible. Given the high level of infection of susceptible plants in the study, it is likely

that the inoculum pressure was high enough to ensure no susceptible plants remained disease free. Conversely, however, the disease pressure may have been so high that some infection occurred even on plants that might ordinarily not develop the disease, in which case, a 3:1 ratio might appear to be 13:3.

A traditional strategy to evaluate putative models of inheritance is the evaluation of segregation ratios in the progeny of selected hybrid plants through further generations using pedigree breeding methods. An alternative recent strategy is the use of molecular markers to 'tag' loci associated with particular phenotypes, as was used by Lambrides *et al.* (2004). Both strategies were beyond the scope of the present study. However, the F_2 plants from the present study have subsequently been used to develop F_5 recombinant inbred lines which have been phenotyped for the traits observed in the present study (Hang Vu, pers. comm. 2010). It is planned to use micro-array DNA molecular markers (Vu *et al.* 2012) to match genetic and phenotypic data. These additional studies are expected to confirm or clarify the results of the present study.

6.4 Expression and Inheritance of Quantitative Traits

In general, the mean values of the quantitative traits observed in the two cultivated parents were broadly similar between the four crosses. As would be expected, there were generally large differences in the mean values of most quantitative characters observed between the cultivated and wild parents. The differences between the two wild parents were also much greater than between the two cultivars. As a consequence of the large differences between the cultivated and wild parents, generally there were also large differences between and within hybrid progeny generations. For most quantitative traits, there were consistencies in response between the different crosses, indicating broad similarities in genetic control. Nevertheless, for several traits, there were clear differences between crosses in the patterns of variation in the hybrid progeny and estimates of heritability. Not surprisingly in view of the differences between the parents, the greatest differences in trait expression in hybrid progeny and heritability were observed between crosses involving the two wild parents. There were some growth differences in Kiloga between the two sets of crosses, with the later sown plants in the ACC 1 crosses, showing more vigour. Presumably, these effects were due to the different environments that were experienced at the different planting dates of the two sets of crosses (Chapter 3). These differences were small compared with those between the wild parents, and between the cultivated and wild parents.

6.4.1 Phenological Traits other than Flowering

There were only very small differences between the four parents and their progeny in the mean duration of individual pod growth, which was different to the finding by Damayanti (2010) that

larger seed size in *V. vexillata* was associated with a longer duration of pod growth. In terms of the duration of flowering and the duration of the growth cycle, the F_1, F_2 and BC_{P2} progeny all tended to exhibit extended periods of flowering and growth like the wild parents. Even so, there were some differences between the wild parents and so their progenies in the duration of flowering. The ACC 1 parents and progeny flowered for a longer time than the ACC 87 plants and progenies, despite the fact the ACC 1 crosses were planted later (Chapter 3). The difference may reflect the fact that ACC 87 is perennial, and the developing tubers represent an alternative sink to flowers and pods (Rebetzke and Lawn 2006c). Narrow sense heritabilities for duration of flowering were high in the ACC 87 crosses, and moderate-high in the ACC 1 crosses, indicating that the wild germplasm may provide a useful source of indeterminateness as is found in black gram.

Rehman (2010) concluded that the gene action controlling earliness of maturity in mungbean was complex. Both additive and non-additive components were identified and seasonal as well as environmental factors were also found to be significant. Malik and Singh (1983) and Khattak *et al.* (2001a,b) reported both additive and dominance components for days to maturity while Naidu and Satyanarayana (1993) reported the incidence of additive gene effects only. Khattak *et al.* (2002a, b) also reported the influence of seasonal effects on gene action in mungbean. In the present study, while the narrow sense heritability of growth duration was moderate (ACC 1 crosses) to very high (ACC 87 crosses), the data need to be interpreted with caution in light of the fact that in all four crosses, the final harvest of the latest-maturing plants necessarily occurred before all plants had died.

6.4.2 Key Morphological and Agronomic Traits

In the present study, the broad differences between the cultivated and wild morphological traits were generally consistent with what would be anticipated based on other studies (e.g. Donald and Hamblin 1983, Damayanti et al. 2010a,b,c). In general, the wild accessions were thinner stemmed and less robust plants, with smaller leaves, pods, and seeds than the two cultivars. Depending on the cross, the narrow sense heritability of these several traits of domestication ranged from negligible to very high. While morphological traits like stem thickness are recognised as traits that differentiate between domesticated and wild genotypes (Donald and Hamblin 1983), there is little information in the literature on the heritability of the trait in mungbean. In *V. vexillata*, Damayanti *et al.* (2010) found that both broad and narrow sense heritability for the stem thickness character were moderate. In the present studies, narrow sense heritability for stem thickness was high in the crosses involving Kiloga, but low or negligible for those involving Berken.

Similarly, while floral standard width is usually recognised as a trait that has increased with domestication (Lawn and Rebetzke, 2006), there are no reports of its inheritance in the literature. While it is usually considered a trait of taxonomic rather than agronomic interest, flower size could become more important if attempts are made to develop hybrid varieties of mungbean, since it may affect attractiveness to cross-pollinating insects. In the present study, the mean floral standard width in the two wild parents was smaller than that of Berken and Kiloga and was higher in ACC 87 than ACC 1, consistent with finding of Lawn and Rebetzke (2006). Narrow sense heritability for the trait was moderate to very high, depending on the cross.

While both wild accessions had smaller leaflet size than the two cultivars, ACC 1 had smaller leaves than ACC 87. Leaf shape as shown by width: length ratio was similar and narrower in the two wild types. As for other traits, narrow sense heritability for leaflet size and shape attributes varied with trait and cross. In cultivated mungbean, Dwiwedi and Sing (1985) reported that a narrow leaflet character appeared to be controlled by two recessive genes which they symbolized by nl_1 and nl_2. Soehedi *et al.* (2007) reported that large vs small leaflet size in mungbean was conditioned by a single locus 's'. Yimram (2009) reported moderate heritability for leaflet length and leaflet width in cultivated mungbean. In *V. vexillata*, James and Lawn (1991) and Damayanti (2010) found that leaflet shape and leaflet width were both conditioned by single genes.

The two wild accessions generally had more branches with more nodes than the cultivated varieties, attributes generally shared by the progeny generations. However, narrow sense heritability for these traits was, as for other morphological traits, variable depending on the cross. In looking at the inheritance of wild x cultivated mungbean traits, Yimram (2009) reported very low narrow sense heritability for the branches per major stem. Both additive and dominance gene action for branches per main stem was reported by Tiwari *et al.*(1993) and Khattak *et al.* (2004). Singh and Singh (1996) reported three models of inheritance for branches/plant of mungbean including additive genes and/or dominance interaction. In all four crosses, the number of main stem nodes was much higher in the wild parents, and in all progeny generations except BC_{P1}. Narrow sense heritability for the number of main stem nodes was very high among all four crosses. Similarly, Khattak (2001a) reported high broad and narrow sense heritabilities for the number of main stem nodes in mungbean, indicating that the greater proportion of heritable variation of the trait was of additive nature. Given that the extent of nodal development is related to phenological development, it is likely that there are confounding effects of phenology on the extent of stem and branch development.

In terms of pods per peduncle, there were no differences between the cultivated parents and ACC 87, and only small differences between them and ACC 1. The narrow sense heritability for this

trait was not significantly greater than zero due to higher standard errors, suggesting that little variability in the population was due to heritable genetic differences. In contrast, working on genetic variability in mungbean, Markeen (2007) and Rahim (2010) reported high broad sense heritability for the number of pods per cluster (c. 75%). However, moderate narrow sense heritability (0.58) for the trait in mungbean was published by Sukhumaporn (2009). Singh and Singh (1996) reported that the number of pods per cluster was under control of additive, dominance, digenic interaction in three mungbean crosses.

For pod size, there were slight differences in pod size between the two cultivated parents whereas the two wild parents were considerably smaller. The narrow sense heritability for the components of pod sizes varied between crosses and ranged from low to moderate. In looking at the inheritance of wild x cultivated mungbean traits, Sukhumaporn (2009) reported very high narrow sense heritabilities for pod length and pod width (0.93 each) whereas Yimram (2009) reported only moderate broad sense heritability for those traits. Both Khattak (2002a) and Zubai (2004) concluded that the additive genetic variance component was significant for pod length in mungbean, whereas Rahim (2010) reported very low broad sense heritability. Lawn and Rebetzke (2006) found very high broad sense heritability for this trait (0.93).

While both wild parents had similar seeds per pod, and both had fewer than the cultivars, there were differences in the values of narrow sense heritability between the respective crosses of ACC 1 and ACC 87. Heritability estimates were higher in the ACC 87 than in ACC 1 crosses. While there was clear evidence of discontinuous variation in the segregating generations in the ACC 1 crosses, there was no obvious simple model of qualitative inheritance. Presumably because it is an important commercial trait, there is extensive published information on the inheritance of seeds per pod in mungbean. In early studies, additive and dominant gene action was reported by (Singh and Jain 1971) while Murty *et al.* (1976) reported dominance x dominance gene action for this trait. Additionally, Malik and Singh (1983) reported that both additive and dominant gene action was involved in the expression of seeds per pod in mungbean. In later studies, Singh and Singh (1996) reported additive and dominance digenic interaction in three mungbean crosses while Khattak (2002a) reported significant additive genetic variance for the trait. Medium broad and narrow sense heritabilities for seeds per pod were reported by Makeen (2007) and Yimram (2009), while Shukhumaporn (2009) reported moderately high narrow sense heritability.

Among the parents, seed size ranked in the order Berken > Kiloga >> ACC 87 >> ACC 1. In the ACC 87 crosses, narrow sense heritability for seed size was moderate to high, while within the ACC 1 crosses, it was high to very high. Working on wild x cultivated mungbean crosses, moderately high narrow sense heritability for 100 seeds weight was reported by Fery (1980),

James *et al.* (1999), Khattak *et al.* (2002), Makeen *et al.* (2007) and Sukhumaporn (2009). Similarly, very high broad sense heritability (0.92) was reported by Yimram (2009). Singh and Singh (1996) reported that 100 seed weight trait in three mungbean crosses was under the control of additive genes and/or dominance type interaction. However, Rohman (2003) reported that 100 seeds weight was governed to a greater extent by additive gene effects. Mak and Yap (1980) reported additive genetic variation was higher than dominant variation in governing 100 seeds weight. Khattak (2004) reported complex inheritance for 100 seeds weight.

Both wild parents showed extremely strong hardseededness, while the cultivated lines were almost completely soft seeded. This finding was consistent with previous observations (Williams 1989, Lawn *et al.* 1988, James *et al.* 1999). Transient hardseededness is common in some mungbean cultivars, with levels usually in the range of 0-70% (Lawn and Rebetzke, 2006). In the present study, narrow sense heritability for level of hard seed was high to very high. In the literature, both qualitative and quantitative inheritance of hard seeds has been reported in mungbean. Singh *et al.* (1983) reported that the F_2 segregation of a cross between ssp. *radiata* with ssp. *sublobata* suggested a ratio of 3 hard to 1 normal seed, indicating that this trait is conditioned by a single dominant gene. Likewise, Plhak (1989) identified one major QTL associated with hardseededness in mungbean. However, Humphry *et al.* (2005) analysed an RIL population derived from a cross between a completely soft seeded cultivar and a hard seeded wild mungbean and found four QTLs associated with hardseededness. Sukhumaporn (2009) reported that the narrow sense hertitabilities for germination at three days and seven days were very high (0.99 and 0.95 respectively). Broad sense heritabilities of 99% and 74% were reported by James *et al.* (1999) in two crosses between wild and cultivated mungbean.

TDM was generally greater in the two wild parents, reflecting their generally longer growth duration. Among the four crosses, narrow sense heritability for TDM was moderate to very high. Consistent with this, Rehman *et al.* (2009) reported predominantly additive genetic effects on TDM of mungbean. In *V. vexillata*, Damayanti *et al*. (2010c) reported low narrow sense heritability for dry above ground biomass, while James and Lawn (1991) reported very low and moderately high narrow sense heritabilities in *V. vexillata* depending on the cross. Dijee *et al.* (2000) found that gene action was predominantly non-additive for dry matter production in cowpea. In contrast to TDM, seed yield was generally greater in the cultivated parents. Narrow sense heritability for yield was low in the ACC 87 crosses, but moderate to high in the ACC 1 crosses. There are numerous reports on heritability of seed yield in mungbean in the literature, with very diverse findings. Joseph and Santhoshkumar (2000) reported additive genetic effects for yield per plant, while Loganathan *et al.* (2000) reported over-dominance for this trait in green gram. While a narrow sense heritability of 87% for grain yield in mungbean was reported by

Sukhumaporn *et al.*, (2009), very low broad sense heritability (0.22) was reported by Yimram (2009). In cowpea, Dijee *et al.* (2000) reported that gene action was predominantly non-additive for seed yield.

Seed: pod weight ratio was smaller in both wild accessions than in the cultivated varieties. In the ACC 87 crosses, narrow sense heritability for the trait was zero, and in the ACC 1 crosses, it was low. Likewise, HI was lower in the wild parents, especially ACC 1. Narrow sense heritability of HI was very high in the ACC 87 crosses, and moderate to high in the ACC 1 crosses.

6.5 Implications for Mungbean Genetic Improvement

The present study examined the inheritance and expression of cultivated and wild traits in hybrid populations between two mungbean cultivars, Kiloga, and two Australian wild accessions, ACC 1 and ACC 87. The appearance of most cultivated traits was broadly similar between Kiloga and Berken plants. They both exhibited early flowering, strong erect stem, large leaflets, and large green shiny seed and normal fibrous roots. In strong contrast, the two wild mungbean accessions, ACC 1 and ACC 87, were fine stemmed, twining plants with smaller leaves and small, black seeds. Nonetheless, there were differences in mean or score observed for some traits present in ACC 1 and ACC 87. For instance, the mean of time to flowering in ACC 87 was much shorter than that of ACC 1. While root tuberisation and perenniality expressed in ACC 87 plants, there was no evidence of perenniality in ACC 1 plants. Despite these very large phenotypic differences between the cultivated and wild parents, hybrid progeny of the several different generations that were studied were readily obtained.

Additionally, there was apparently normal expression of both cultivated and wild traits, whether they were qualitative or quantitative characters, in the hybrid progenies. These studies thus provided more evidence that the wild mungbean accessions are clearly part of the primary gene pool for the cultivated mungbean. Consequently, exploitation of wild traits of interest for mungbean improvement purposes is quite feasible. In general, the best fitting models of inheritance appeared to be only slightly different between the two cultivated parents. However, there were larger differences observed in the best fitting models of inheritance for qualitative traits, and in the estimates of heritability for quantitative traits, between the hybrid populations depending on whether ACC 1 or ACC 87 was the wild parent. Interestingly, despite the fact that ACC 87 was a perennial accession, it shared more traits in common with the cultivated parents than ACC 1. For example, for several of the qualitative traits, the cultivars appeared to be separated from ACC 87 by just one gene, but by two from ACC 1. This indicates that genetic divergence appears to be greater between the wild parents than between the cultivated parents.

Consequently, in a breeding program it would be likely that the choice of the wild accession used in crosses may influence the inheritance / heritability of any wild traits of interest more than the choice of cultivar.

6.5.1 Potentially Useful Traits

Generally, there was little information on the inheritance of perenniality in *Vigna* species, and no information available for mungbean, before this study. In *Vigna vexillata*, James and Lawn (1991), Karuniawan *et al*. (2006) and Damayanti (2010) grouped some of the root characters, such as tuber dry weight, and tuber harvest index, which are related to the expression of perenniality of this species, into the quantitative trait category. However, in the present study, the perennial character as observed in ACC 87 was suggested to be controlled by two dominant genes with complementary action. Based on this result, it can be concluded that the perenniality trait should be easily transferred into cultivated mungbean. Perenniality is a potentially desirable wild trait as it contributes to the possibility of creating a perennial mungbean crop or persistent forage cultivars.

The two wild parents may also provide a novel source of resistance to powdery mildew. While there was different expression of presence of powdery mildew in hybrid progeny among the four crosses, the observations suggested that the apparent resistance to powdery mildew may be conditioned by a single recessive gene, or perhaps two genes. However, the resilience of the trait needs to be re-checked in different environments and over years, and using known disease isolates. If it proves resilient, it would then be worthwhile to utilize molecular marker technology to reliably establish the underlying genetic control of the trait. Powdery mildew can be an important disease in grain legume crops. In soybean, for example, Graud (2006) reported that an epidemic of powdery mildew disease (*Microsphaera diffusa* Cook & Peck) occurs every 10-15 years in Wisconsin, United States. Additionally, the powdery mildew disease decreased seed yield of soybean by up to 35% in Japan, Canada and United states (Dunleavy 1978, Phillip 1984, Graud 2006). In Vietnam, over the last few years, the presence of this disease on both mungbean and soybean has been identified as an urgent issue. The Vietnamese government has invested heavily in creating and breeding mungbean and soybean varieties resistant to powdery mildew (Tran and Nguyen 2011).

In some crops, characters such as indeterminateness, stem growth habit and twining are considered to be useful or potentially useful traits. For instance, most soybean cultivars in the northern temperate regions of the USA are indeterminate varieties, in which new stem nodes continue to grow after flowering has commenced (e.g. Hicks *et al.* 1969). It is possible that

indeterminateness might be a useful trait in mungbean, as Lawn and Russell (1978) showed this trait contributed to the greater responsiveness of black gram to environment compared with mungbean. However, this is unlikely to be the case in Vietnam, where short or medium growth duration cultivars are best adapted to the local agricultural system (Tran *et al.* 2000). Cultivated varieties of the mat bean, *V. aconitifolia* have prostrate plant habit, which might be useful if mungbean were to be grown as a nitrogen-fixing cover crop in the interrow space between a row crop like sugarcane. Meanwhile, vegetable varieties of other *Vigna* crops, including the long bean, *V. unguiculata* ssp. *sesquipedalis* and the rice bean, *V. umbellata*, have twining stem habit. The twining habit allows these crops to be successfully intercropped with cereal species like maize, as they climb up over the mature maize plants. In mechanised systems, it is conceivable that such traits might be useful in mungbean cultivars developed for use as forages or cover crops, or perhaps even novel future mixed cropping systems focussed on sustainability.

Hard-seededness has been identified as a possible trait in mungbean breeding programs where the aim is to develop weather-resistance (Williams 1989, lmrie *et al*. 1991). Transient hardseededness is common in some mungbean cultivars, with levels usually in the range of 0-70% (Lawn and Rebetzke 2006). The two wild parents, ACC 87 and ACC 1, presented complete hardseededness, whereas the two cultivars were soft-seeded. There was strong expression of this trait in hybrid progenies among the four crosses. Therefore, it can be concluded that the hardseededness trait can be transferred into cultivated mungbean to create weather-tolerant varieties. However, both qualitative and quantitative inheritance of hard seeds has been reported in mungbean. Singh *et al.* (1983) reported that the F_2 segregation of a cross between ssp. *radiata* with ssp. *sublobata* suggested a ratio of 3 hard to 1 normal seed, indicating that this trait is conditioned by a single dominant gene. However, Humphry *et al.* (2005) analysed an RIL population derived from a cross between a completely soft seeded cultivar and a hard seeded wild mungbean and found four QTLs associated with hardseededness.

6.5.2 Undesirable or Deleterious Traits

Generally, most wild mungbean traits are not agronomically useful. In the present study, most of the wild qualitative traits appeared to be dominant over the cultivated traits. This suggested that the cultivated traits may therefore have arisen through mutations that broke the biosynthetic pathways present in their wild relatives. The cultivated traits presumably persisted because they were favoured during domestication. There were several associations between wild and cultivated traits among the four crosses. Some of these were phenotypic associations which may or may not reflect underlying physiological processes. In other instances, there were genetic correlations between wild traits. In a breeding program, these various linkages might either enhance or retard

progress, depending on the nature of the traits and the linkages. Lawn and Rebetzke (2006) reported that wild accessions collected from subtropical eastern Australia contained fewer traits associated with cultivated mungbean, whereas with accessions collected in north Western Australia, and eastern Indonesia, the frequency of 'cultivated' traits increased.

The dehiscence of pods (shattering) prior to harvest is generally an undesirable trait of mungbean and soybean. Pod dehiscence is relatively uncommon in cultivated varieties, but it is often observed when wild species are used as parents to introgress useful genes or to develop genetically diverse breeding populations (Bailey *et al.* 1996). In the present studies, the pod dehiscence appeared to be controlled by a single dominant gene among the four crosses. However, the pod shattering in F_1 plants was not as strong as in the wild plants, suggesting additive gene action for the trait. Singh *et al.* (1975) reported while shattering was dominant in F_1 plants of crosses between *V. mungo* (non-dehiscent) and *V. radiata* (dehiscent), the non-shattering characteristic could not be recovered in the F_2. They concluded that resistance to shattering in those crosses was quantitatively inherited.

For the seed characters, the two wild varieties presented small black seeds while the two cultigens presented large green shiny seeds, which is good appearance for commercial purposes. In most cases, the wild visual seed characters are considered unwanted traits. In the present study, there was strong expression of wild seed traits in the hybrid progeny in the four crosses, particular in F_1 plants and backcrosses to wild plants. It suggested that the unwanted wild visual seed traits were dominant over the domesticated traits.

For phenological traits, the cultivated phenotypes were early flowering and of shorter growth duration than the wild accessions. There was strong expression of wild phenological traits in the hybrid progeny, particularly for the duration over which flowers and pods were produced, as well as for the duration of growth cycle. In most cases, these wild traits would be undesirable in a mungbean breeding program. For example, in Vietnam, the mungbean crop is usually grown in rotating or intercropping areas. If the mungbean varieties have long growth duration, it is hard to fit into those agricultural systems. Most Vietnamese scientists therefore would target mungbean varieties with short or medium growth duration in order to fit them to rotating or intercropping areas. In particular, they would target varieties with just two or three times of harvesting mature pods to reduce labour costs.

Reflecting the effects of domestication, the mean values for seed yield, and for components such as seeds per pod and 100 seed weight, were greater in the cultivated phenotypes than in the wild accessions. There was wide variation for those traits in the hybrid progeny and there were some

phenotypic and genotypic associations between those traits. The mean value of 100 seed weight was much smaller in the wild accessions than in the cultivars and small seed size seemed to be strongly expressed in the hybrid progeny in all four crosses. This trait is also usually considered to be an unwanted character for commercial purposes. However, in Japan, mungbean varieties with about 3.5 g per 100 seeds are considered to be good for making mungbean sprouts (Noriyuki Aoki, personal communication, 2011).

6.6 Concluding Statement

The present studies showed two Australian wild mungbean accessions to be part of the primary gene pool of cultivated mungbean, and as such, a potential source of useful genetic variation for mungbean breeding. In conventional plant breeding programs, most of the wild traits observed in these studies would ordinarily be considered undesirable. However, some traits are potentially useful, especially those that improve environmental adaptation. In the present study, possible useful traits included the tuberous rooted perenniality trait from ACC 87 and resistance to powdery mildew, both of which seemed to be relatively simply inherited. There was no evidence that the late flowering of ACC 1 was due to the action of a long juvenile trait analogous to that found in soybean. Rather, late flowering in ACC 1 seemed to be due to the combined action of several additive lateness genes. Given the interrelations observed between many of the wild traits, it is important that their modes of expression and inheritance are understood, so that the useful wild traits can be more easily manipulated in the breeding program. The present studies have assisted in that task for a range of wild traits. Only two wild accessions were evaluated in these studies, and it is certain that other potentially useful traits are likely to exist in accessions adapted to different regions.

CHAPTER 7: BIBLIOGRAPHY

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Appendix I: Pairwise phenotypic correlations among a subset of quantitative traits observed in the F_2 generation plants from two cultivated x wild mungbean crosses (a) Kiloga x ACC 87 and (b) Berken x ACC 87. Entries are the linear correlation co-efficients (*r*) between the respective trait pairs.

a) Kiloga x ACC87

	Fl	**PG**	**DoFl**	**Mat**	**LW**	**LL**	**Ste**	**FlS**	**SP**	**PL**	**PW**	**PeL**	**Ppe**	**No**	**Bra**	**NoB**	**PW**	**Ye**	**SeS**	**Har**	**Bio**	**TM**	**HI**
Fl	1																						
PG	-.55**	1																					
DoFl	-.46**	.42**	1																				
Mat	-.07	.11	.57**	1																			
LW	.05	-.09	-.01	-.10	1																		
LL	.00	-.08	-.03	-.06	.56**	1																	
Se	.11	-.05	-.11	-.34**	.26**	.22*	1																
FlS	-.05	-.11	.12	.02	.07	.13	.06	1															
SP	-.10	-.01	-.01	.07	-.08	-.01	-.20*	-.02	1														
PL	.09	-.01	-.06	.09	.12	-.01	.04	-.14	-.03	1													
PW	.10	-.17	0.10	.10	.10	-.01	.03	-.09	.03	.73**	1												
PeL	-.10	.09	.10	.18	.19*	.22*	-.05	.00	.02	-.07	-.10	1											
Ppe	.11	-.10	.02	-.07	.14	.25**	.26**	.18	-.33**	.09	.11	.04	1										
No	.12	-.09	-.01	.01	.18*	.15	-.01	.04	-.12	-.12	.03	.03	-.014	1									
Bra	.04	-.01	.01	.22*	-.27**	-.07	-.22*	-.09	.16	-.14	-.10	-.14	-.10	-.01	1								
NoB	.21*	-.04	.09	.03	.00	.10	-.06	.15	-.31**	-.14	-.19*	.07	.13	.32**	.24**	1							
PW	-.09	-.15	.11	.23*	.13	.15	-.13	.06	.01	-.12	-.11	.04	.01	.02	.49**	.18	1						
Ye	-.02	-.11	-.01	.08	.25**	.26**	-.09	-.02	.07	-.17	-.18	.02	.131	.03	.46**	.30**	.64**	1					
SeS	-.09	-.01	.02	-.02	.26**	.11	.22*	.20*	-.33**	-.06	-.03	-.06	.15	-.02	-.14	-.09	.12	.21*	1				
Har	-.03	.03	.13	.10	-.12	-.12	-.04	.07	.29**	-.08	-.09	.03	-.06	.06	.036	-.05	-.17	-.04	-.09	1			
Bio	.05	.02	.07	.03	.03	.01	.06	.12	.14	.04	-.02	-.03	-.02	.04	.27**	.16	.15	.04	-.17	-.06	1		
TM	-.08	-.14	.12	.22*	.13	.14	-.12	.08	.03	-.11	-.11	.04	.01	.03	.51**	.20*	.99**	.62**	.08	-.17	.32**	1	
HI	.03	.07	-.18	-.16	.13	.11	-.03	-.12	.10	-.11	-.12	.01	.08	.01	.02	.09	-.26**	.53**	.15	.10	-.35**	-.31**	1

b) Berken x ACC87

	Fl	PG	DoFl	Mat	LW	LL	Ste	FlS	SP	PL	PW	PeL	Ppe	No	Bra	NoB	PW	Ye	SeS	Har	Bio	TM	HI
Fl	1																						
PG	.47**	1																					
DoFl	.53**	.32**	1																				
Mat	.22*	.17	.28*	1																			
LW	-.19	-.02	-.25*	.09	1																		
LL	-.09	-.07	-.15	.27*	.61**	1																	
Se	.22*	.03	.05	.11	.35**	.25*	1																
FlS	-.35**	-.23*	-.34**	-.13	.38**	.18	-.14	1															
SP	-.33**	-.35**	-.20	.13	.04	.08	.09	.02	1														
PL	-.22*	-.11	-.23*	.00	.23*	.16	.13	.25*	.52**	1													
PW	.00	.10	-.07	-.11	.28**	.11	.01	.24*	-.12	.58**	1												
PeL	-.12	-.13	-.08	.14	.07	.21	.13	-.08	.07	.15	-.09	1											
Ppe	-.14	-.07	-.21	.12	.14	.14	.01	.13	-.01	.08	-.04	.33**	1										
No	.38**	.13	.38**	.39**	-.02	.25*	.18	-.15	.07	.07	-.10	.04	-.06	1									
Bra	.36**	.26*	.11	.19	-.16	.05	.10	-.30**	-.05	-.08	-.22*	.02	-.17	.55**	1								
NoB	.73**	.36**	.56**	.32**	-.09	-.00	.30**	-.40**	-.15	-.17	-.13	.06	-.10	.57**	.47**	1							
PW	.32**	.10	.42**	.21	-.16	-.05	-.06	-.25*	-.08	-.20	-.14	.11	-.11	.50**	.40**	.54**	1						
Ye	.26*	.06	.34**	.20	-.08	-.01	.03	-.20	-.01	-.10	-.09	.17	-.07	.51**	.39**	.56**	.93**	1					
SeS	.02	-.13	.19	.12	.11	.05	.22*	-.05	.08	.07	-.07	.07	.06	.14	-.07	.12	.10	.10	1				
Har	-.18	-.13	-.02	-.20	-.14	-.15	-.09	-.06	.11	-.04	-.18	.07	-.00	-.19	-.17	-.08	.03	.03	.01	1			
Bio	.73**	.38**	.60**	.39**	-.06	.08	.32**	-.25*	-.16	-.23*	-.16	-.05	-.09	.57**	.41**	.77**	.46**	.41**	.14	-.14	1		
TM	.51**	.21	.55**	.30**	-.15	-.01	.06	-.29**	-.12	-.24*	-.17	.07	-.12	.59**	.46**	.69**	.95**	.88**	.13	-.03	.72**	1	
HI	-.38**	-.33**	-.31**	-.17	.12	-.03	-.02	.13	.18	.25*	.16	.24*	.05	-.08	-.11	-.24*	.10	.38**	-.00	.09	-.50**	-.10	1

*, ** indicates significant linkage at $P < 0.05$, $P < 0.01$ respectively

Flo=flowering date; PG = duration of pod growth; DoFl = duration of flowering; Ma =physiological maturity; LW = leaflet width; LL = Leaflet length; Ste=stem thickness; FlS = floral standard; SP = seeds per pod; PL=pod length; PW= pod width; PeL=peduncle length; No=Nodes per main stem; Bra = number of brances per main stem; PW = dry pod weight, Ye= seed yield; SeS= 100 seeds weight; Har = hardseededness; Bio = biomass;TM= total biomass; HI=harvest index.

Appendix II: Pairwise phenotypic correlations among a subset of quantitative traits observed in the F_2 generation plants from two cultivated x wild mungbean crosses (a) Kiloga x ACC 1 and (b) Berken x ACC 1. Entries are the linear correlation co-efficients (*r*) between the respective trait pairs.

a) Kiloga x ACC1

	Fl	**PG**	**DoFl**	**Mat**	**LW**	**LL**	**Ste**	**FlS**	**SP**	**PL**	**PW**	**PeL**	**Ppe**	**No**	**Bra**	**PW**	**Ye**	**SeS**	**Har**	**Bio**	**TM**	**HI**
Fl	1																					
PG	.08	1																				
DoFl	.17	.66**	1																			
Mat	.40**	.24*	.58**	1																		
LW	-.38**	-.24*	-.29**	-.35**	1																	
LL	-.28**	-.16	-.16	-.15	.78**	1																
Se	-.10	.07	-.09	-.19	.23*	.25*	1															
FlS	-.52**	-.35**	-.38**	-.31**	.25*	.06	.03	1														
SP	-.17	-.03	-.24*	-.30**	-.10	-.15	.06	.23*	1													
PL	-.21	-.11	-.16	-.29**	.12	.11	.19	.20	.54**	1												
PW	.20	-.08	-.04	-.02	-.03	-.02	-.14	-.20	-.12	.10	1											
PeL	-.56**	-.36**	-.35**	-.33**	.31**	.27*	-.11	.40**	.22*	.17	-.125	1										
Ppe	-.13	-.04	.05	.02	.08	.18	.06	-.01	-.28**	-.23*	-.03	.06	1									
No	.18	.34**	.27*	.18	-.10	-.20	.15	-.26*	-.04	.00	.11	-.44**	.04	1								
Bra	.10	.18	.29**	.28**	-.25*	-.26*	-.14	-.24*	-.09	-.14	.07	-.17	-.02	.27*	1							
PW	-.17	-.32**	-.20	-.26*	.12	.08	.10	.07	.24*	.13	-.09	.12	.15	-.08	-.06	1						
Ye	-.13	-.19	-.19	-.32**	.05	.01	.09	.01	.36**	.23*	.02	.06	.02	-.06	-.01	.90**	1					
SeS	-.18	-.13	-.09	-.14	.12	.10	.15	.05	-.01	.27*	.11	.12	-.10	-.22*	.01	.18	.20	1				
Har	-.03	.09	-.18	-.07	-.05	-.01	.17	.01	.03	-.08	.04	-.05	.04	.12	-.14	.03	-.01	-.05	1			
Bio	.40**	.33**	.44**	.38**	-.19	-.08	-.14	-.51**	-.23*	-.16	.02	-.36**	-.11	.38**	.13	-.06	-.08	-.18	-.08	1		
TM	.04	-.13	.04	-.05	.02	.03	.02	-.18	.10	.04	-.07	-.07	.08	.11	.01	.88**	.78**	.08	-.02	.43**	1	
HI	-.31**	-.23*	-.25*	-.39**	.14	.09	.17	.19	.35**	.22*	.02	.20	.01	-.21	.06	.49**	.68**	.26*	-.11	-.46**	.22*	1

b) Berken x ACC1

	Fl	PG	DoFl	Mat	LW	LL	Ste	FlS	SP	PL	PW	PeL	Ppe	No	Bra	PW	Ye	SeS	Har	Bio	TM	HI
Fl	1																					
PG	.21	1																				
DoFl	-.01	.08	1																			
Mat	.40**	.17	.67**	1																		
LW	-.24*	-.01	.08	-.03	1																	
LL	-.17	-.01	.16	.07	.82**	1																
Se	-.06	.08	.18	.08	.16	.12	1															
FlS	-.40**	.02	-.15	-.28**	.19	.15	.01	1														
SP	-.07	-.14	-.26*	-.29**	-.03	-.07	.10	-.03	1													
PL	-.06	.02	-.11	-.24*	.11	.09	.20	.06	.63**	1												
PW	-.04	.29**	.16	.11	.12	.21	-.13	.14	-.32**	-.32**	1											
PeL	-.56**	-.07	.07	-.25*	.40**	.31**	.09	.27*	.15	.09	.17	1										
Ppe	-.05	.28*	.16	.11	.12	.21	-.13	.14	-.32**	-.32**	.10**	.17	1									
No	.19	.01	.15	.16	.04	.08	.10	-.14	.04	-.08	.09	-.11	.09	1								
Bra	.05	-.19	.14	.12	.05	.00	.12	-.09	.16	.01	-.14	-.05	-.14	.46**	1							
PW	-.36**	-.30**	-.05	-.21	.13	.01	.19	-.02	.42**	.30**	-.11	.31**	-.11	.01	.18	1						
Ye	-.32**	-.26*	-.10	-.23*	.10	-.05	.19	.04	.40**	.30**	-.13	.25*	-.12	-.01	.15	.94**	1					
SeS	.22*	.11	.06	.21	-.29**	-.28*	.03	-.10	-.02	-.12	-.04	-.14	-.04	-.24*	-.12	.13	.16	1				
Har	.07	-.04	-.00	.05	-.09	-.07	-.20	.09	-.15	-.19	-.05	-.08	-.05	-.05	.02	-.00	-.00	.05	1			
Bio	-.09	.13	-.02	.02	-.01	.02	.13	.07	.09	.10	.15	.05	.16	.12	.15	.26*	.24*	-.05	-.02	1		
TM	-.32**	-.18	-.04	-.15	.09	.02	.21	.02	.36**	.28*	-.02	.26*	-.01	.06	.21	.90**	.84**	.08	-.01	.66**	1	
HI	-.13	-.23*	-.11	-.19	.06	-.08	.05	.10	.22*	.15	-.20	.08	-.20	-.15	-.08	.38**	.57**	.19	-.02	-.51**	.06	1

*, ** indicates significant linkage at $P < 0.05$, $P < 0.01$ respectively

Flo=flowering date; PG = duration of pod growth; DoFl = duration of flowering; Ma =physiological maturity; LW = leaflet width; LL = Leaflet length; Ste=stem thickness; FlS = floral standard; SP = seeds per pod; PL=pod length; PW= pod width; PeL=peduncle length; No=Nodes per main stem; Bra = number of brances per main stem; PW = dry pod weight, Ye= seed yield; SeS= 100 seeds weight; Har = hardseededness; Bio = biomass;TM= total biomass; HI=harvest index.