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Gene introgression in grain legumes

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ABSTRACT

The wild species of grain legumes are valuable gene pools, particularly for resistance to biotic and abiotic stresses. These have largely remained under-utilized due to crossability barriers, but there are some examples of successful introgression of genes into the cultivated species from their wild relatives, particularly those constituting primary and secondary gene pools. In chickpea, two closely related species, *Cicer reticulatum* and *C. echinospermum*, have been used for widening genetic base of the cultigen and introgressing genes for resistance/tolerance to phytophthora root rot, cyst nematode (*Heterodera ciceri*), root-lesion nematode (*Pratylenchus* spp.), pod borer (*Helicoverpa armigera*), ascochyta blight, botrytis grey mould and low temperatures. Wild *Cajanus* species have been effectively exploited in developing cytoplasmic male sterility (CMS) systems, which made commercial hybrids possible. In addition, resistance to *Helicoverpa armigera* and sterility mosaic has been introgressed from *C. acutifolius* and *C. scarabaeoides*. The high protein content trait has been introgressed from *C. scarabaeoides*. In *Phaseolus* beans, the cultivated species of the secondary (*P. coccineus* and *P. dumosus*) and the tertiary (*P. acutifolius*) gene pools have been used for the improvement of common bean (*P. vulgaris*). The congruity back cross system and its modifications have been especially useful for tapping the tertiary gene pool. In lentil, genes for anthracnose and wilt resistance and drought tolerance have been introgressed in the cultigen from *L. lamottei*. Presence of crossability barriers has restricted greater exploitation of wild species, particularly in tertiary gene pool.

Concerted efforts are needed to overcome these crossability barriers. Cloning of desired genes from cross-incompatible wild species and their transfer through transgenic approaches may also be considered.

Introduction

Food legumes like chickpea (*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and pigeonpea (*Cajanus cajan* L.) are important sources of protein and calories in the semi-arid regions of Asia and Africa. Food legumes are usually grown with minimal inputs and with minimal or no investments on control of insect-pests and diseases. Despite being neglected, the food legumes are an integral part of cropping system in the semi-arid tropics mainly because of their ability to produce something of economic value (food or fodder) under extreme conditions and their soil ameliorative properties.

Food legumes generally have low and unstable yields compared to cereals, largely because they are grown in marginal environments and their productivity is seriously constrained by both biotic and abiotic stresses. The severity of some of the stresses like drought, heat and dry root rot, is further expected to increase due to consequences of climate change. These stresses not only limit the yield but also affect biological nitrogen fixation (Wery 1987) and quality of grain and fodder. Developing cultivars resistant/ tolerant to biotic and abiotic stresses has always been and will continue to remain a major objective in legume breeding.

Excellent progress has been made in breeding for resistance to certain diseases (e.g. fusarium wilt in chickpea) where sources with high levels of resistance were available in the germplasm of cultivated species. Developing cultivars with high level of resistance to some stresses (e.g. legume pod borer) continue to remain a challenge due to lack of adequate level of resistance available in the cultivated species. The wild species are known to have greater genetic diversity than the cultivated species. Since the wild species survive under adverse conditions in isolated geographical niches they possess genes for adaptation to these conditions, including resistance to various abiotic and biotic stresses. Thus, the wild species provide an opportunity for introgressing genes for new or diverse sources of resistance and other useful traits into the cultivated species.

The major constraints that hinder the use of wild species of food legumes in breeding include lack of evaluation data on specific traits (heat, drought and salinity), barriers to interspecific hybridization and more importantly the extensive efforts that would be required to breed out the associated undesirable traits. Exploitation of wild species, particularly those belonging to the primary and secondary gene pools, for stress response traits deserves special attention. However, traits associated with survival of the wild species, like seed shattering, hard seed coat and seed dormancy need to be eliminated during introgression of useful traits into the cultigen from wild relatives. Hawkes (1977) emphasized the need to understand crossability barriers, chemotaxonomic relationships and cytogenetic affinities between the wild species and the cultigen before attempting gene introgression.

This review provides an overview of the advances made in introgression of useful genes from wild species in major food legumes.

Gene pool of some of the food legumes

The wild relatives of food crop plants can be classified into primary, secondary and tertiary gene pools based on ease or difficulty of gene flow from the wild relative to the cultivated species. The primary, secondary and tertiary gene pools of food legumes represent potential genetic diversity that can eventually be exploited in the background of cultivated types to overcome biotic and abiotic stresses (Table 1). Reproductive isolation, embryo breakdown, hybrid sterility, and limited genetic recombination are major obstacles in the exploitation of wild species beyond those within the primary gene pool. To overcome these crossability barriers, embryo rescue technique or cloning of desired genes from cross-incompatible wild species and their transfer through transgenic approaches may be considered.

Table 1: Species in the primary, secondary and tertiary gene pools of chickpea (*Cicer* spp.), pigeonpea (*Cajanus* spp.) and lentil (*Lens* spp.)

Legume species	Primary gene pool	Secondary gene pool	Tertiary gene pool
Chickpea	*Cicer reticulatum*	*C. echinospermum*	*C. bijugum* *C. pinnatifidum* *C. judaicum* *C. yamashitae* *C. chorassanicum* *C. cuneatum*
Pigeonpea	*Cajanus cajan*	*C. acutifolius* *C. albicans* *C. cajanifolius* *C. lanceolatus* *C. latisepalus* *C. lineatus* *C. reticulates* *C. scarabaeoides* *C. sericeus* *C. trinervius* *Flemingia* spp.	*C. goensis* *C. heynei* *C. kerstingii* *C. mollis* *C. platycarpus* *C. rugosus* *C. volubilis* *Rhynchosia* spp. *Dunbaria* spp. *Eriosema* spp.
Lentil	*Lens culinaris* ssp. *culinaris* *L. culinaris* ssp. *orientalis* *L. culinaris* ssp. *odemensis*	*L. nigricans* ssp. *nigricans* *L. nigricans* ssp. *ervoides*	

Gene introgression from wild species

Chickpea

Wild species of *Cicer* include 8 annual and 34 wild perennial species. The low level of molecular diversity observed in the cultivated chickpea (*Cicer arietinum* L.) is a major concern to plant breeders. The wild species offer opportunities for enhancing genetic variability and introgressing desired traits, particularly resistance to stresses, in the cultigen. Resistance to some of biotic stresses, such as seed beetle and cyst nematode, was found only in the wild species. Most studies on wild *Cicer* species have been confined to annual species because of crossability barriers and difficulties associated with propagation of perennial species. Some of the important traits associated with wild species are listed in Table 2.

Table 2: Some important traits present in wild *Cicer* species

Trait	*Cicer* species	Reference
Resistance to fusarium wilt	*judaicum*	Kaiser et al. 1994; Infantino et al. 1996
	bijugum	Kaiser et al. 1994; Haware et al. 1992; Infantino et al. 1996
	echinospermum	Haware et al. 1992; Infantino et al. 1996
	canariense chorassanicum cuneatum pinnatifidum	Kaiser et al. 1994
	reticulatum	Infantino et al. 1996
Resistance to phytophthora root rot	*echinospermum*	Knights et al. 2008
Resistance to soil borne diseases	*bijugum cuneatum judaicum pinnatifidum*	Reddy et al. 1991
Resistance to botrytis gray mold	*bijugum*	Haware et al. 1992
Resistance to ascochyta blight	*bijugum*	Haware et al. 1992; Singh and Reddy 1993; Collard et al. 2001
	judaicum	Singh et al. 1981; Haware et al. 1992
	pinnatifidum	Singh and Reddy 1993
	echinospermum	Collard et al. 2003
	montbretii	Singh et al. 1981
Resistance to cyst nematode	*bijugum pinnatifidum reticulatum*	Greco and Di Vito 1993; Di Vito et al. 1996
Resistance to leaf miner	all wild annual species	Singh and Weigand 1994
Tolerance to cold	*bijugum judaicum echinospermum pinnatifidum reticulatum*	Singh et al. 1990; Berger et al. 2005
High seed protein	*bijugum reticulatum*	Singh and Pundir 1991
Multiple seeds	*cuneatum montbretii*	van der Maesen 1987; Robertson et al.1995

C. reticulatum and *C. echinospermum* are the two wild *Cicer* species that are cross-compatible with the cultivated species and have been used in chickpea improvement. These species have narrow ecogeographic adaptation and may therefore provide limited array of adaptive alleles. Use of more distantly related species, those in tertiary gene pool, may offer more allelic diversity (Badami et al. 1997). Due to limited cross compatibility, reduced chromosome pairing and differential seed set in the segregating populations, only a fraction of all the possible gametic/zygotic combinations can be recovered by the breeder.

Resistance to ascochyta blight

Collard et al. (2001) identified a number of resistant wild *Cicer* accessions in controlled environment, particularly those belonging to *C. bijugum*. Quantitative trait loci (QTL) associated with seedling and stem resistance were detected in the F_2 population of a cross between a resistant accession of *C. echinospermum* and a susceptible accession of cultivated chickpea (Collard et al. 2003). Several QTL were clustered in LG 4, but there is also evidence for QTL on other linkage groups (Santra et al. 2000). Crosses between *C. echinospermum* accession L204 and susceptible chickpea parents have produced at least one line (90102-5Q-1103) with high level of resistance to ascochyta blight in field experiments in Australia (Ted Knights, unpublished data).

Resistance to phytophthora root rot

Phytophthora root rot (*Phytophthora medicaginis*) is a major disease of chickpea in north-eastern Australia. Host resistance is the only practicable management option, however only limited resistance has been observed within the chickpea germplasm (Brinsmead et al. 1985). Extensive screening of wild annual *Cicer* species revealed existence of higher resistance in some *C. echinospermum* accessions. Further it was demonstrated that this resistance could be recovered fully in interspecific lines (Knights et al. 2008). Subsequently some of the resistant interspecific lines were backcrossed to chickpea parents, showing that the *C. echinospermum* resistance could be combined with the domesticated features of the cultigen (Ted Knights, unpublished data). Progeny lines obtained from a cross between *C. echinospermum* x *C. arietinum* were tested in a field nursery at Tamworth, NSW, Australia. The most resistant

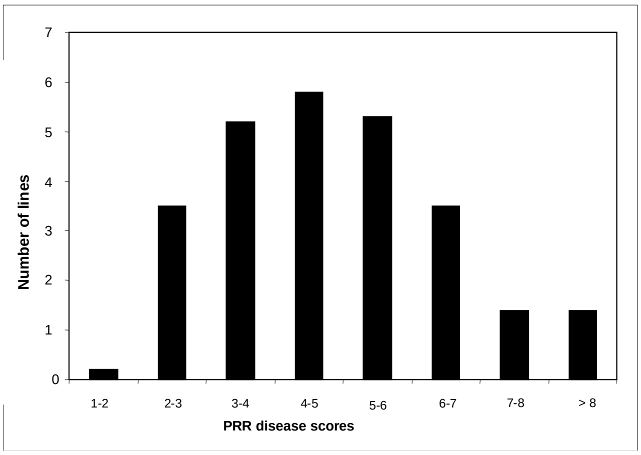

Fig. 1: Distribution of Phytophthora root rot (PRR) disease scores on 1 to 9 scale (1= highly resistant; 9=highly susceptible) for advanced lines derived from *C. echinospermum* x *C. arietinum* cross in a field phytophthora nursery.

first-backcross lines had disease scores <2 (where 1=no disease; 9=all plants dead) compared to Yorker and Jimbour, locally adapted, resistant cultivars having a score of 6.6 and 7.8, respectively (Figure 1).

Resistance to root-lesion nematode

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) are significant pathogens of annual crops in Australia. Chickpea is a susceptible host and its role as a rotational crop in farming systems is compromised by the susceptibility of other species (particularly wheat) that precede and follow it in the cropping sequence. Genotypic differences in host reaction to both nematode species is known to exist between cultivars (J Thompson, unpublished data), but economically significant increases in the population of nematodes will still occur under cultivation of the least susceptible genotypes. Improved resistance, as measured by low nematode populations in the roots/soil under controlled inoculation is apparent within both *C. reticulatum* and *C. echinospermum*. In some greenhouse experiments nematode populations for interspecific lines were less than 1% for a range of chickpea cultivars (J Thompson, unpublished data). Much of this resistance has been recovered in backcross derivatives, and second backcross lines are now undergoing field assessment.

Chilling tolerance

In Australia, and potentially in other environments where chickpeas are autumn/ winter sown, floral abortion due to low spring temperatures can adversely affect yield. Berger et al. (2005) showed that some annual wild *Cicer* species (including *C. reticulatum* and *C. echinospermum*) were almost insensitive to the temperature range experienced during flowering at a strategically located cool site; interspecific hybrids, despite being selected for an unrelated trait (root-lesion nematode resistance) also showed a significant increase in chilling tolerance as reflected in a shorter interval from anthesis to pod appearance.

Resistance to pod borer

There has been limited progress in breeding for pod borer (*Helicoverpa armigera)* resistance in chickpea due to unavailability of high level of resistance in the cultivated species. Screening of 32 accessions of annual *Cicer* species under greenhouse conditions indicated that some accessions of *C. bijugum, C. cuneatum*, *C. judaicum, C. pinnatifidum* and *C. reticulatum* have higher levels of resistance than the best sources of resistance available in the cultivated species. Larval weights on many accessions of these wild *Cicer* species were much lower than those on the cultivated species, indicating the existence of antibiosis mechanism of resistance in the wild species (Sharma et al. 2005). One of these species, *C. reticulatum* can be easily crossed with the cultigen and thus *C. arietinum* ⁰*C. reticulatum* crosses are being used to enhance level of resistance by combing different mechanisms of resistance available in the two species.

Recombinant inbred lines (RILs) derived from ICC 4958 (*Cicer arietinum*) x PI 489777 (*Cicer reticulatum*) were evaluated for resistance to pod borer using detached leaf assay in the laboratory and under natural infestation in the field (ICRISAT 2008). The results indicated considerable variation in resistance to pod borer damage, growth and survival of the larvae. Several RILs (nos. 2, 13, 16, 17, 31, 40, 60, 65, 72, 81, 92, 95 and 123) showed low leaf feeding and low larval weight gain at the vegetative and/or the flowering stages. These lines can be exploited in chickpea breeding programs.

Linkage drag

C. arietinum and *C.echinospermum* exhibit resistance to phytopthora root rot and root-lesion nematode. These have been systematically used in Australian chickpea breeding program. Interspecific lines, particularly those involving *C. echinospermum*, were well represented amongst elite lines tested in pre-release trials. A comparison of interspecific *C. arietinum* x *C. echinospermum* with *C. arietinum* desi lines did not show any significant difference in predicted yield, averaged over 39 trials during the period 1998-2001 (Knights et al. 2002). However, significant differences in seed quality were observed. Further, cooking time was faster whereas, dhal yield and water absorption were reduced.

Successful utilization of *C. reticulatum* for increasing yield (up to 39%) was reported by Singh and Ocampo (1997). Yadav et al. (2002) developed a cultivar BG 1103 by using *C. reticulatum* in the backcross breeding.

Pigeonpea

It is often said that pigeonpea has reached its performance plateau (Saxena 2008). Although ample morphological diversity is exhibited by pigeonpea as a crop the same is not true at the molecular level (Yang et al. 2006). A range of diseases and insect pests attack pigeonpea and sources of resistance for some of the stresses are lacking in cultivated germplasm. The crop has a rich source of variability in the form of wild species, which have played a major role in the introduction of genes for disease resistance, quality traits (high protein content), identification and diversification of cytoplasmic base of CMS system.

The genebank at ICRISAT conserves 555 accessions of wild relatives of pigeonpea representing six genera and 57 species. Five unique CMS systems have been developed in pigeonpea by utilizing wild relatives. Amongst these, the A_4 cytoplasm from *C. cajanifolius* is currently being used to exploit heterosis in pigeonpea (Saxena et al. 2005). The system is stable across environments with very good fertility restoration.

Crosses between *C. cajan* and *C. acutifolius* gave rise to A_5 CMS system (Mallikarjuna and Saxena 2005). The A_5 system is in developmental stages and will be available commercially in the near future. Open flower segregants were produced from crosses between *C. platycarpus,* a wild relative of tertiary gene pool and cultivated pigeonpea (Mallikarjuna et al. 2006). Some of the progenies were completely male sterile with white anthers. In the semi-fertile progeny, pollen shedding was not observed as the anthers had a thick cell wall. Self-pollination did not set seeds but seed set was observed when pollinated with a range of other cultivars. This may be another source of CMS in pigeonpea (N Mallikarjuna, unpublished data).

High protein line ICPL 87162 was developed from a cross between *C. cajan* x *C. scarabaeoides* (Reddy et al. 1997). Dhal protein content of ICPL 87162 ranged from 30-34% compared to 23% in the control cultivar. More recently crosses between cultivated pigeonpea and *C. acutifolius* yielded progeny with high seed weight of 18 gm/100 seed compared to 8.0 gm/100 seed in cultivar ICPL 85010 (N Mallikarjuna, unpublished data). The relationship between seed weight and protein content is yet to be determined.

C. scarabaeoides, *C. acutifolious*, *C. sericeus*, and *C. albicans* are some of the wild *Cajanus* species showing resistance to pod borer, *Helicoverpa armigera* (Sujana et al. 2008). *C. acutifolius*, a native of Australia, can be crossed with cultivated pigeonpea in a one way cross combination (using *C. acutifolius* as the male parent). But, *in vitro* interventions are necessary to obtain hybrid plants (Mallikarjuna and Saxena 2002). Advanced breeding lines from the cross involving *C. acutifolius* as the pollen parent have shown resistance to pod borer damage, variation for seed color and high seed weight (Mallikarjuna et al. 2007). Some of the lines showed high level of resistance to pod borer, pod fly and bruchids under unprotected field conditions. *C. scarabaeoides* (ICPW 94), which is resistant to all the isolates of sterility mosaic disease (SMD), was used in the crossing program and most of the progenies were resistant or moderately resistant to SMD. The resistant plants flowered and set seeds. The susceptible plants had mosaic disease symptoms with crinkled leaves and did not flower (N Mallikarjuna unpublished data).

Wide crosses with distantly related species will give rise to novel variations not observed in the parents used in the crossing program (Hoisington et al. 1999). *C. platycarpus* was crossed (hormone aided cross pollination) with cultivated pigeonpea and aborting hybrid embryos obtained from the cross were rescued. Many peculiarities were noticed when the cross *C. platycarpus* x *C. cajan* was advanced to BC_4 generation. Some of the lines (F_1BC_4-A8-4 and F_1BC4-A14-6) were partially male sterile with pollen fertility <30% and had non-dehiscent anthers. Non-dehiscent anthers coupled with high pollen sterility are desirable traits of a CMS source. Three lines F_1BC4-A10-7, F_1BC4-A17-8 and F_1BC4-A14-6 had higher 100 seed weight than both the parents and these lines can potentially be a good source of large seed size. Protein content in all the hybrid lines was more than that in *C. platycarpus*, and F1BC4-A4 and F1BC4-A19-14 showed marginally higher protein content than the cultivated parent. All the lines were screened for *Helicoverpa armigera* (pod borer), *Melanagromyza obtusa* (pod fly) and *Callosobruchus chinensis* (bruchids) under unprotected field conditions. Damage due to *H. armigera*, in the wild parent *C. platycarpus* and cultivated parent ICPL 87 was <1% and ~ 69 % respectively. Damage in F_1BC4-A derived lines ranged from 2 to 37 % with majority of them showing <15% damage. Significant differences were observed between the lines for pod borer/bruchid resistance and 100 seed weight. The results show that there is a good scope to transfer *H. armigera* resistance from *C. platycarpus* into the cultivated species.

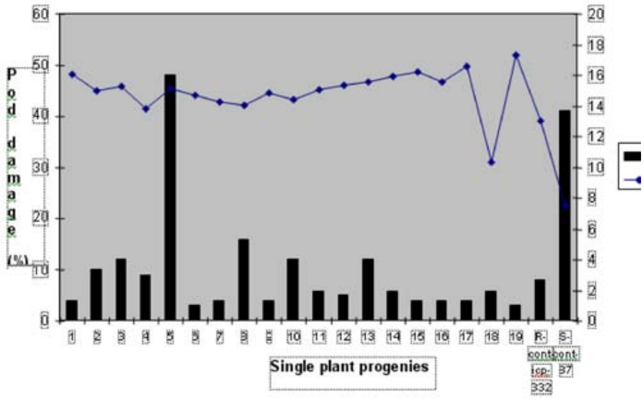

Fig. 2: *Helicoverpa armigera* pod damage and 100 seed wt in *C. acutifolius* line 7018-40-26-6

Screening thousands of germplasm lines for phytopthora blight, especially for the most virulent race P_3, has failed to identify resistant lines. But, screening of wild species has identified *C. platycarpus* showing resistance to all the isolates of phytopthora blight fungi. Although *C. platycarpus* belongs to tertiary gene pool, it has been successfully crossed and progenies generated (Mallikarjuna et al. 2006). Some progenies showed resistance to phytopthora blight under greenhouse screening, suggesting that it is possible to transfer resistance to phytopthora blight from *C. platycarpus* (Mallikarjuna et al 2007; Saxena et al. 2005).

Phaseolus beans

The genus *Phaseolus* is remarkably diverse, with five cultigens (*vulgaris, coccineus, dumosus, acutifolius,* and *lunatus*) and another 80 or so fully wild species (Freytag and Debouck 2002). Among these, there is a group of tall, short living perennial lianas with fibrous roots, epigeal germination, multi-flowered inflorescences, and medium sized multi-seeded pods. Convergent information from interspecific hybridizations and maternally inherited molecular marker studies (Schmit et al. 1993; Delgado Salinas et al. 2006) has shown that these different species (Table 3) form a natural group referred to as section *Phaseoli* within the genus (Freytag and Debouck 2002). Although *P. vulgaris* is derived from the same ancestral stock, it appears to have adapted to a new environment (open

Table 3: Species of the Phaseoli section of genus *Phaseolus*

Species	Geographic range	References
Phaseolus vulgaris L.	NW Mexico to NW Argentina	Toro et al. 1990
Phaseolus dumosus Macfady.	Western Guatemala	Schmit and Debouck 1991; Freytag and Debouck 2002
Phaseolus albescens McVaugh ex Ramírez (3) & Delgado	Western Mexico	Freytag and Debouck 2002; Ramírez and Delgado Salinas1999
Phaseolus costaricensis Freytag & Debouck	Central Costa Rica and W Panama	Araya Villalobos et al. 2001; Freytag and Debouck 2002

and drier) and has become an annual species (Freytag and Debouck 2002; Gentry 1969).

Muñoz et al. (2006) do not consider the inclusion of *P. acutifolius* nor *P. parvifolius* into the *vulgaris* group [contrast with Delgado Salinas et al. (2006)] due to their little compatibility and recombination in interspecific crosses. Although, these two taxa could have been part of this phylum very early on. As discussed elsewhere (Freytag and Debouck 2002), *P. coccineus* is also very close to the *Phaseoli* section, another case of early separation. *P. glabellus,* another wild species has no affinity with either of them (also confirmed by Delgado Salinas et al. 2006). Surprisingly, *P. persistentus* was recently shown to belong to the *Phaseoli* (Delgado Salinas et al. 2006, Delgado Salinas and Carr 2007). This is unexpected because *P. persistentus* is quite dissimilar morphologically from the tall lianas having large showy inflorescences that characterize the other members of *Phaseoli*. This result needs further confirmation, but it might be indicative of other members of this section yet to be identified.

The cultivated species of the secondary (*P. coccineus* and *P. dumosus*) or tertiary (*P. acutifolius*) gene pool have mostly been used in the breeding program in spite of wide diversity of common bean.This knowledge can be utilized in using non-domesticated species in different gene pools (*P. costaricensis* and *P. albescens*; and *P. parvifolius*, respectively) that have comparable physiological and morphological traits.

The growth characteristics of the *Phaseoli* are evidently an adaptation to the environment in which they have evolved. The tall lianas permit competition with the surrounding vegetation that ranges from weeds/bushes to tall trees. For instance, where *Phaseolus dumosus* exists as an escaped domesticate, it can grow to several meters in height through and above the competing perennial stands. Until it is well established, its survival depends more on vigorous vegetative growth than seed production. As a result, partitioning of photosynthates is increased towards vegetative parts, and seed production is scant with low harvest index. This trait is still expressed in a cultivated environment, and yields are normally low. Poor partitioning to grain and low harvest index are consistent problems with *Phaseolus* species expressing this pattern of growth, and also in the interspecific progeny derived from them in crosses with *P. vulgaris*.

Recent experience with interspecific crosses between common bean and *P. coccineus* may shed light on strategies to employ these species for the improvement of common bean. Selection of drought resistance within common bean germplasm has produced lines with improved yield under both drought stress and in favorable conditions (Beebe et al. 2008). This improved yield performance across different environments was hypothesized to be due to improved partitioning to grain. One such drought resistant line, SER 16, was crossed with G35346, an aluminum tolerant accession of *P. coccineus*, and the F_1 was backcrossed to SER 16. The agronomic quality of the backcross progenies was substantially better than that obtained in other *vulgaris* - *coccineus* crosses, to the extent that yield was improved in relation to the drought resistant common bean parent, even under moderate intermittent drought (Table 4). We believe that the improved partitioning observed in SER 16 is a key to recovering superior progeny in these crosses, and this experience could be extended to crosses with other species of similar physiology.

Table 4: Yield of interspecific progeny of the cross SER 16 x (SER 16 x G35346) under intermittent drought condition

Entries	Yield kg ha^{-1}	Maturity (days)	Yield kg $ha^{-1}d^{-1}$
Interspecific lines			
ALB 205	3199	68	47
ALB 167	3174	69	46
ALB 213	3029	67	45
Drought tolerant checks			
SER 16	2520	63	40
BAT 477	2165	68	32

The tepary bean (*Phaseolus acutifolius*) possesses multiple traits that are important for common bean breeding. Furthermore, this species is suitable for the production of transgenic plants through *Agrobacterium* transformation. However, crosses with the tepary bean, or its close relative, *P. parvifolius*, presents multiple problems. Tepary bean is characterized by good partitioning of photosynthates to grain (thus its problems are not those of the *Phaseoli* group cited above), but its phylogenetic distance from *P. vulgaris* gives rise to different postzygotic incompatibility barriers such as embryo abortion, presence of dominant lethal alleles, self or complete sterility of the resulting hybrids and incongruity of homeologous chromosomes. Through the use of embryo rescue and facilitator genotypes of both species, the production of viable hybrids and the transfer of resistance to bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) have been possible. However, the production of fertile progeny with other genotypes of tepary bean that carry resistance to drought, leafhopper (*Empoasca kraemeri* R. & M.) or the bean weevil (*Acanthoscelides obtectus* Say) has not been possible using the above mentioned strategies.

To overcome this problem, a unique breeding system called congruity backcrossing was suggested to improve chromosome pairing and recombination (Haghighi and Ascher 1988). In this system the F1 (normally employing *P. vulgaris* as female and source of cytoplasm) is immediately backcrossed to common bean and this backcross F_1 is in turn pollinated by tepary bean again. This is continued for several generations, alternating between the two parents, in the hope of obtaining inter-chromosomal recombinations that will improve chromosome pairing and fertility. A variant of this system employs a parallel crossing program to facilitate maintaining chromosomes of this species, and after several cycles, progeny from the parallel schemes are intercrossed. This intensive method has resulted in families with improved fertility and intermediate morphology, although more important economic traits have not yet been transferred to common bean.

Lentil

Seven taxa are currently recognized in the genus *Lens*. These include the cultivated lentil (*Lens culinaris* subsp. *culinaris* Medik), its wild progenitors (*L. culinaris* Medik. subsp. *orientalis* (Boiss.) Ponert, *L. odemensis* Ladiz., *L ervoides* (Brign.) Grande, *L. nigricans* (Bieb.) Godr. [Ladizinsky, 1993]) and two recent species, *L. tomentosus* Ladiz. and *L. lamottei* Czefr. The wild relatives of lentil are distributed around the Mediterranean basin and further East to Central Asia. The wild *Lens* species and the cultigen, *L. culinaris* are all diploid (2n = 14) and are predominantly self pollinators.

All the wild *Lens* species are considered to be crossable to the cultigen (Ladizinsky et al. 1988), except in few cases where the hybrids are difficult to obtain. *L. culinaris* subsp. *orientalis* is readily crossable with the domesticated lentil, although the fertility of the hybrids depends on the chromosome arrangement of the wild parent (Ladizinsky 1979; Ladizinsky et al. 1984). Crosses between *L. odemensis* and *L. culinaris* subsp. *orientalis* yield partially fertile F_1 hybrids (Ladizinsky et al. 1984). Poor seed set has been observed in F_1 hybrids of *L. odemensis* x *L. culinaris* subsp *culinaris* cross (Goshen et al. 1982). Pod abortion takes place in the crosses involving cultivated lentil and *L. ervoides* or *L. nigricans*. Hybrids between *L. ervoides* and *L. nigricans* produce pods with seeds but at low frequency. Ahmad et al. (1995) reported that use of GA3 hormone produced viable hybrids in crosses of cultivated lentil with *L. culinaris* subsp. *orientalis*, *L. odemensis*, *L. ervoides* and *L. nigricans*. Thus, three crossability groups exist in *Lens*: (1) *L. culinaris* and *L. odemensis*, (2) *L. ervoides*, *L. nigricans* and *L. lamottei*, and (3) *L. tomentosus*.

Wild lentils possessing substantial genetic diversity (Muehlbauer 1993; Ferguson and Robertson 1996) are potentially important source of genetic variation for improvement of cultivated lentil (Table 5). Their restricted use in breeding programmes is primarily due to lack of evaluation data for characters of economic importance. A portion of wild *Lens* collection has been evaluated at ICARDA for selected agronomic traits, biotic and abiotic stresses. Bayaa et al. (1991) systematically screened the ICARDA wild lentil collection for resistance to fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* and for resistance to ascochyta blight caused by *Ascochyta fabae* f. sp. *lentis*. Resistance to fusarium wilt was identified in 3 of 109 accessions of *L. culinaris* ssp. *orientalis,* 3 of 30 accessions of *L. nigricans* ssp. *nigricans*, and 2 of 63 accessions of *L. nigricans* ssp. *ervoides*. Resistance to ascochyta blight was identified in 24 of 86 accessions of *L. culinaris* ssp. *orientalis*, 12 of 35 accessions of *L. culinaris* ssp *odemensis*, 3 of 35 *accessions* of *L. nigricans* ssp. *nigricans* and 39 of 89 accessions of *L. nigricans* ssp. *ervoides*. One accession of *L. nigricans* ssp. *ervoides*, ILWL 138, had combined resistance to both the diseases.

Table 5: Some useful traits in wild *Lens* species

Trait	*Lens* species	Reference
Early flowering and maturity	*L. culinaris* subsp *orientalis*	ICARDA 1991
Pods/plant, Seeds/plant, straw yield and biomass	*L. culinaris* subsp *orientalis*, *L. nigricans* and *L. lamottei*	Ferguson and Robertson 1999
100-seed weight	*L. lamottei*	Ferguson and Robertson1999
Resistance to drought	*L. odemensis*, *L. ervoides*	Hamdi and Erskine1996
Winter hardiness	*L. culinaris* subsp. *orientalis*	Hamdi et al. 1996
Resistance to vascular wilt	*L. culinaris* subsp. *Orientalis,* *L. ervoides*	Bayaa et al. 1995
Ascochyta blight	All wild taxa	Bayaa et al. 1995
Resistance to anthracnose	*L. ervoides*	Fiala et al. 2009

Resistance to anthracnose (caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore) races Ct1/Ct0 has been successfully transferred from *L. ervoides* to cultivated lentil (Fiala et al., 2009). The studies on genetics of introgressed resistance of interspecific hybrid ($F_{7:8}$) inbred line population identified 1-2 recessive genes controlling both the races of anthracnose (Ct1/Ct0).

Concluding remarks

The cultivated species of several important legumes, such as chickpea and pigeonpea, show limited variability at the molecular level, despite the fact that high level of variability exists for morphological traits. The vast variability seen for morphological traits may be reflection of the expression of limited number of mutant loci. As the genetic variability is a perquisite for progress of any breeding program, efforts should be made to widen the genetic base of the cultigen by exploiting wild species. The wild species also offer opportunities of bringing novel alleles for important traits, particularly resistance to abiotic and biotic stresses. There has been limited success in exploiting the species of tertiary gene pool. Concentrated efforts are needed to introgress desired genes from wild species, including cloning of genes and introducing these genes through transgenic approaches.

There has been a rapid progress in development of molecular markers and molecular maps in many legumes. We are now better prepared for marker-assisted introgression of traits, using both foreground and background selection. This will greatly facilitate introgression of genes from the wild species.

References

Ahmad M, Fautrier AG, McNeil DI, Burritt DJ and Hill GD. 1995. Attempts to overcome post-fertilization barrier in interspecific crosses of the genus *Lens*. Plant Breeding **114**: 558-560.

Araya Villalobos R, González Ugalde WG, Camacho Chacón F, Sánchez Trejos P and Debouck DG. 2001. Observations on the geographic distribution, ecology and conservation status of several *Phaseolus* bean species in Costa Rica. Genet. Resources & Crop Evol. **48**: 221-232.

Badami PS, Mallikarjuna N and Scoles GJ. 1997. Interspecific hybridization between *Cicer arietinum* and *C. pinnatifidum*. Plant Breeding **116**: 393-395.

Bayaa B, Erskine W and Hamdi A. 1991. Screening wild lentil for resistance to vascular wilt and Ascochyta blight diseases. Presented at Fourth Arab Congress of Plant Protection, Cairo, December 1991.

Bayaa B, Erskine W and Hamdi A. 1995. Evaluation of a wild lentil collection for resistance to vascular wilt. Genetic Resources and Crop Evolution **42**: 231-235.

Beebe SE, Rao IM, Cajiao C and Grajales M. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Science **48**: 582-592.

Berger JD, Buck R, Henzell JM and Turner NC. 2005. Evolution in the genus *Cicer* – vernalization response and low temperature pod set in chickpea (*C. arietinum* L.) and its annual wild relatives. Australian Journal of Agricultural Research **56**: 1191-1200.

Brinsmead RB, Rettke ML, Irwin JAG and Langdon PW. 1985. Resistance in chickpea to *Phytophthora megasperm*a f. sp. *medicaginis*. Plant Disease **69**: 505-506.

Collard BCY, Ades PK, Pang ECK, Brouwer JB and Taylor PWJ. 2001 Prospecting for sources of resistance to ascochyta blight in wild *Cicer* species. Australasian Plant Pathology **30**: 271-276.

Collard BCY, Pang ECK, Ades PK and Taylor PWJ 2003. Preliminary investigation of QTLs associated with seedling resistance to ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. Theoretical and Applied Genetics **107**: 719-729.

Delgado Salinas A, Bibler R and Lavin M. 2006. Phylogeny of the genus *Phaseolus* (Leguminosae): a recent diversification in an ancient landscape. Systematic Botany **31** (4): 779-791.

Delgado Salinas A and Carr WR. 2007. *Phaseolus texensis* (Leguminosae: Phaseolinae): a new species from the Edwards Plateau of Central Texas. Lundellia **10**: 11-17.

Di Vito M, Singh KB, Greco N and Saxena MC. 1996. Sources of resistance to cyst nematode in cultivated and wild *Cicer* species. Genetic Resources and Crop Evolution **43**: 103-107.

Ferguson ME and Robertson LD. 1996. Genetic diversity and taxonomic relationships within the genus *Lens* as revealed by allozyme polymorphism. Euphytica **91**: 163-172.

Ferguson ME and Robertson LD. 1999. Morphological and phenological variation in the wild relatives of lentil. Genetic Resources and Crop Evolution **46**: 3-12.

Fiala JV, Tullu A, Banniza S, Seguin-Swartz G and Vandenberg V. 2009. Interspecies transfer of resistance to Anthracnose in lentil (*Lens culinaris* Medic.). Crop Science **49**: 825-830.

Freytag GF and Debouck DG. 2002. Taxonomy, distribution and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. SIDA Botanical Miscellany **23**: 1-300.

Gentry HS. 1969. Origin of the common bean, *Phaseolus vulgaris* L. Economic Botany **23**: 55-69.

Goshen D, Ladizinsky G and Muehlbauer FJ. 1982. Restoration of meiotic regularity and fertility among derivatives of *Lens culinaris* x *L. nigricans* hybrids. Euphytica **31**: 795-799.

Greco N and Di Vito M. 1993. Selection for nematode resistance in cool season food legumes. In: Breeding for stress tolerance in cool season food legumes (Eds., KB Singh and MC Saxena). John Wiley and Sons, Chichester, UK. Pp 157-166.

Haghighi KR and Ascher PD. 1988. Fertile intermediate hybrids between *Phaseolus vulgaris* and *P. acutifolius* from congruity backcrossing. Sex and Plant Reproduction **1**: 51-58.

Hamdi A and Erskine W 1996. Reaction of wild species of the genus Lens to drought. Euphytica **91**: 173-179.

Hamdi A, Kusmenoglu I and Erskine W. 1996. Sources of winter hardiness in wild lentil. Genetic Resources and Crop Evolution **43**: 63-67.

Haware MP, Narayan Rao J and Pundir RPS. 1992. Evaluation of wild Cicer species for resistance to four chickpea diseases. International Chickpea Newsletter **27**: 16-18.

Hawkes JG. 1977. The importance of wild germplasm in Plant Breeding. *Euphytica* **26**: 615-621.

Hoisington D, Khairallah M, Reeves T, Ribaut JM, Skovmand B, Taba S and Warburton M. 1999. Plant genetic resources: What can they contribute toward increased crop productivity? Proceedings of National Academy of Sciences, USA **96**: 5937-5943.

ICARDA 1991. Food Legume Improvement Program Annual Report for 1991. International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.

ICRISAT 2008. Crop Improvement Archival Report 2008. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India.

Infantino A, Porta-Puglia A and Singh KB. 1996. Screening wild *Cicer* species for resistance to fusarium wilt. Plant Disease **80**: 42-44.

Kaiser WJ, Alcala-Jimenez AR, Hervas-Vargas A, Trapero-Casas JL and Jimenez-Diaz RM. 1994. Screening of wild cicer species for resistance to races 0 and 5 of *Fusarium oxysporum f. sp. ciceris*. Plant Disease **78**: 962-967.

Knights EJ, Brinsmead RB, Fordyce M, Wood JA, Kelly and Harden S. 2002. Use of the wild relative *Cicer echinospermum* in chickpea improvement. In: 'Plant Breeding for the 11th Millenium' (Ed. JA McComb). Proceedings of the 12th Australasian Plant Breeding Conference, Perth, Western Australia, 15-20 September, 2002. Pp 150-154.

Knights EJ, Southwell RJ, Schwinghamer MW and Harden S. 2008. Resistance to *Phytophthora medicaginis* Hansen and Maxwell in wild *Cicer* species and its use in breeding root rot resistant chickpea (*Cicer arietinum* L.). Australian Journal of Agricultural Research **59**: 383-387.

Ladizinsky G. 1979. The origin of lentil and its wild genepool. Euphytica **28**: 179-187.

Ladizinsky G, Braun D, Goshen D and Muehlbauer F. 1984. The biological species of genus *Lens*. Bot. Gaz. **145**: 253-261.

Ladizinsky G, Pickersgill B and Yamamato K. 1988. Exploitation of wild relatives of the food legumes. In: World Crops: Cool Season Food Legumes (Ed. RJ Summerfield). Kluwer Publications, The Netherlands.

Ladizinsky G. 1993. Wild lentils. Critical Reviews in Plant Sciences **12**: 169-184.

Mallikarjuna N and Saxena KB. 2002. Production of hybrids between *Cajanus acutifolius* and *C. cajan*. Euphytica **124**: 107-110.

Mallikarjuna N and Saxena KB. 2005. A new cytoplasmic male-sterility system derived from cultivated pigeonpea cytoplasm. Euphytica **142**: 143-148.

Mallikarjuna N, Jadhav D and Reddy P. 2006. Introgression of *Cajanus platycarpus* genome into cultivated pigeonpea, *C. cajan*. Euphytica **149**: 161-167.

Mallikarjuna N, Jadhav D, Reddy MV and Dutta-Tawar U. 2007. Introgression of Phytophthora blight disease resistance from *Cajanus platycarpus* into short duration pigeonpeas. Indian Journal of Genetics and Plant Breeding **65**: 261-264.

Muehlbauer FJ. 1993. Use of wild species as a source of resistance in cool-season food legume crops. In: Breeding for stress tolerance in cool-season food legumes (Eds., KB Singh and MC Saxena). John Wiley and Sons, Chichester, UK. Pp 359-372.

Muñoz LC, Duque MC, Debouck DG and Blair MW. 2006. Taxonomy of Tepary Bean and Wild Relatives as Determined by Amplified Fragment Length Polymorphism (AFLP) Markers. Crop Science **46**:1744–1754.

Ramírez Delgadillo R and Delgado Salinas A. 1999. A new species of *Phaseolus* (Fabaceae) from West-Central Mexico. SIDA **18**: 637-646.

Reddy MV, Raju TN and Pundir RPS. 1991. Evaluation of wild *Cicer* accessions to wilt and root rots. Indian Phytopathology **44**: 388-391.

Reddy LJ, Saxena KB, Jain KC, Singh, Green JM, Sharma D, Faris DG, Rao AN, Kumar RV and Nene YL. 1997. Registration of high protein elite germplasm ICPL 87162. Crop Science **37**: 94.

Robertson LD, Singh KB and Ocampo B. 1995. A catalog of annual wild *Cicer* species. ICARDA, Aleppo, Syria. Pp 171.

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ and Muehlbauer FJ (2000). Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Science **40**: 1606-1612.

Saxena KB, Kumar RV, Dalvi VA, Mallikarjuna N, Gowda CLL, Singh BB, Tikka SBS, Wanjari KB, Pandet LB, Paralkar LM, Patel MK, Shiying B and Xuxiao Z. 2005. Hybrid breeding in grain Legumes-A Success Story of Pigeonpea. In: International Food Legume Research Conference (Ed. Khairwal). New Delhi, India.

Saxena KB. 2008. Genetic improvement of pigeonpea–A review. Tropical Plant Biology **1**: 159-178.

Schmit V and Debouck DG. 1991. Observations on the origin of *Phaseolus polyanthus* Greenman. Economic Botany **45**: 345-364.

Schmit V, du Jardin P, Baudoin JP and Debouck DG. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P._coccineus*. Theoretical and Applied Genetics **87**: 506-516.

Sharma HC, Pampapathy G, Lanka SK and Ridsdill-Smith TJ. 2005. Antibiosis mechanism of resistance to pod borer, *Helicoverpa armigera* in wild relatives of chickpea. Euphytica **142**: 107-117.

Singh KB, Hawtin GC, Nene YL and Reddy MV. 1981. Resistance in chickpea to *Ascochyta rabiei*. Plant Disease **65**: 586-587.

Singh KB, Malhotra RS and Saxena MC. 1990. Sources for tolerance to cold in *Cicer* species. Crop Science **30**: 1136-1138.

Singh KB and Reddy MV. 1993. Sources of resistance to ascochyta blight in wild *Cicer* species. Netherland Journal of Plant Pathology **99**: 163-167.

Singh KB and Weigand S. 1994. Identification of resistant sources in *Cicer* species to *Liriomyza cicerina.* Genetic Resources and Crop Evolution **41**: 75-79.

Singh KB and Ocampo B. 1997. Exploitation of wild *Cicer* species for yield improvement in chickpea. Theoretical and Applied Genetics **95**: 418-423.

Singh U and Pundir RPS. 1991. International Chickpea Newsletter **25**: 19-20.

Sujana G, Sharma HC and Rao DM. 2008. Antixenosis and antibiosis components of resistance to pod borer *Helicoverpa armigera* in wild relatives of pigeonpea. International Journal of Tropical Insect Science **28**: 191-200.

Toro Ch, Tohme OJ and Debouck DG. 1990. Wild bean (*Phaseolus vulgaris* L.): description and distribution. International Board for Plant Genetic Resources and Centro Internacional de Agriculture Tropical. Cali, Colombia. Pp 106.

van der Maesan LJG. 1987. Origin, history and taxonomy of chickpea. In: The Chickpea (Eds., MC Saxena and KB Singh). CAB International, Wallingford, UK. Pp 11-34.

Wery J. 1987. In: Drought resistance in plants: physiological and genetic aspects (Eds., L Monti and E Porceddu). CEE, Brussels. Pp 179-202.

Yadav SS, Turner NC and Kumar J. 2002. Commercialization and utilization of wild genes for higher productivity in chickpea. In: Plant Breeding for the 11th Millenium: Proceedings of the 12th Australian Plant Breeding Conference, 15-20 Sept 2002, Perth, Western Australia. Pp 155-160.

Yang S, Pang W, Ash G, Harper J, Carling J, Wenzl P, Hutter E, Zong X and Kilian A. 2006. Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. Theoretical and Applied Genetics **113**: 585-595.