6 Distant Hybridization and Alien Gene Introgression

Shiv Kumar, Muhammad Imtiaz, Sanjeev Gupta and Aditya Pratap

6.1 Introduction

Chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pigeon pea (*Cajanus cajan* L. Millsp.), green gram (*Vigna radiata* L. Wilczek), black gram (*Vigna mungo* L. Hepper), common bean (*Phaseolus vulgaris* L.) and grass pea (*Lathyrus sativus* L.) are among the important food legume crops grown on 74 million ha area with 64 million tons of global output (FAO, 2010). These crops are an integral part of subsistence agriculture with significant contributions to dietary protein supply, atmospheric nitrogen fixation and agricultural sustainability (Ali and Kumar, 2009). The average productivity of these crops is 846 kg/ha, which is dismally low compared with their potential harvestable yield. This is attributed to their cultivation on poor soils under rainfed conditions by marginal farmers with minimum care and, consequently, these crops suffer severe yield losses not only due to edaphic, abiotic and socio-economic factors but also to confounding effects of various biotic stresses. Yield losses caused by various fungal, bacterial and viral diseases are enormous, besides parasitic weed menace at various growth stages (Dita et al., 2006). Being rich in protein, several insect pests also cause yield losses to food legumes both under field conditions and in storage (Clement et al., 1994, 1999). Among abiotic stresses, drought, temperature extremities and edaphic problems (salinity and mineral toxicities) have great bearing on their harvestable yield (Stoddard et al., 2006). Since plant breeding in practice as an option for crop improvement, efforts have been made to search for genes imparting resistance to these stresses within the cultivated species and, to a limited extent, among their wild relatives, but success has been limited to a few diseases and insect pests, and is confined to major gene(s) from the primary gene pool in few food legume crops (Knott and Dvorak, 1976; Stalker, 1980; Prescott-Allen and Prescott-Allen, 1986, 1988; Ladizinsky et al., 1988; Hajjar and Hodgkin, 2007). To diversify and broaden the genetic base of cultivated germplasm, introgression of alien genes from wild species needs to be persuaded vigorously, not only to minimize the risk of stress epidemics but also to make discernible yield advances in these legume crops. Therefore, pre-breeding efforts are urgently required involving particularly those wild species that carry useful alien genes for improving yield, quality and stress resistance. In this chapter we review the information on the present status of wild gene pools, their evaluation, introgression through distance hybridization and future crossing potential, crossability barriers and means to overcome them, strategies for successful introgressions, and future prospects in the selected legume crops.

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6.2 Wild Gene Pool: Present Status

Wild species are a rich reservoir of useful alien genes that are no longer available within the cultivated gene pool (Hawkes, 1977; Doyle, 1988; Tanksley and McCouch, 1997). Continuous efforts have been under way to collect and conserve wild relatives of various food legume crops in national and international gene banks (Plucknett et al., 1987; FAO, 1996). Over the years, ICARDA has collected and conserved, in its global germplasm repository, 587 accessions representing 6 wild Lens species from 26 countries, 270 accessions of 12 wild Cicer species from ten countries and 1555 accessions of 45 wild Lathyrus species from 45 countries. Similarly, the ICRISAT gene bank is reported to have 308 accessions of 18 Cicer species from 19 countries, 555 accessions of 57 Cajanus species from 41 countries and 478 accessions of 47 Arachis species from 7 countries in its wild gene pool (Upadhyaya, personal communication). The US Department of Agriculture, Agricultural Research Service (USDA-ARS), Western Regional Plant Introduction Station (WRPIS), Pullman, Washington also has a collection of 4602 accessions of chickpea (Hannon et al., 2001). In spite of being the largest collections, these have major germplasm gaps at species and genotype levels (Ferguson and Erskine, 2001), and a continuum in our efforts is very much required to fill these gaps in wild gene pools from the unrepresented areas of diversity in the gene banks.

The gene pool concept of Harlan and De Wet (1971) has been very helpful to plant breeders for initiating a pre-breeding programme for directed crop improvement. Various species of major food legume crops have been grouped into primary, secondary and tertiary gene pools on the basis of crossability, cytogenetic, phylogenetic and molecular data (Table 6.1). The useful genes identified in the primary gene pool are readily usable for crop improvement. However, occurrence of useful genes is much more frequent in the secondary and tertiary gene pools of various food legume crops (Kaiser et al., 1994; Collard et al., 2001; Mallikarjuna et al., 2006; Tullu et al., 2006). This requires the deployment of much more effort and novel techniques for integrating this invaluable resource of nature into crop improvement programmes.

6.3 Evaluation of Wild Gene Pool

Sporadic efforts have been made in the past to screen wild species of food legume crops under field and controlled conditions in order to identify useful alien genes for desired traits. These efforts have resulted in identification of valuable sources of resistance to key diseases and insect pests in addition to useful traits such as protein content, cytoplasmic male sterility, fertility restoration and yield attributes (Table 6.2).

**Chickpea**

Annual Cicer species have been evaluated for reaction to ascochyta blight, fusarium wilt, cyst nematode, leaf miner, seed beetle and cold tolerance at ICARDA (International Centre for Agricultural Research in the Dry Areas), and a high level of resistance to each stress has been identified (Table 6.2). Kumar and Dua (2006) presented a list of possible wild species as a source of useful alien genes for chickpea improvement. Cicer judaicum is reported to have resistance genes for ascochyta blight, fusarium wilt and botrytis grey mould (van der Maesen and Pundir, 1984). Greco and Di Vito (1993) reported valuable sources of resistance to cyst nematode in Cicer bijugum, Cicer pinnatifidum and Cicer reticulatum. Some wild accessions have shown resistance to more than one stress (Singh et al., 1994; Ahmad et al., 2005). For example, ILWC 7-1 of C. bijugum showed resistance to ascochyta blight, fusarium wilt, leaf miner, cyst nematode and cold, and ILWC 33/S-4 of C. pinnatifidum to ascochyta blight, fusarium wilt, seed beetle and cyst nematode. Kaur et al. (1999) reported significantly lower larval density of helicoverpa pod borer on some of the accessions of Cicer echinospermum, C. judaicum, C. pinnatifidum and C. reticulatum. Recently, 150 accessions of wild chickpea have been evaluated for resistance to helicoverpa pod borer under field and greenhouse conditions.
Table 6.1. Different gene pools of selected legume crops

<table>
<thead>
<tr>
<th>Crop</th>
<th>Primary gene pool</th>
<th>Secondary gene pool</th>
<th>Tertiary gene pool</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpea</td>
<td><em>Cicer arietinum</em>, <em>C. reticulatum</em></td>
<td><em>C. bijugum</em>, <em>C. pinnatifidum</em>, <em>C. judaicum</em></td>
<td><em>C. cuneatum</em>, <em>C. chorassanicum</em>, <em>C. yamashtae</em></td>
<td>Ladizinsky and Adler, (1976a, 1976b); Ahmad et al. (1988, 2005); van der Maesen et al. (2007)</td>
</tr>
<tr>
<td></td>
<td><em>C. echinospermum</em>, <em>C. bijugum</em>, <em>C. pinnatifidum</em>,</td>
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<tr>
<td></td>
<td><em>C. cuneatum</em>, <em>C. chorassanicum</em>, <em>C. yamashtae</em></td>
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<tr>
<td>Lentil</td>
<td><em>Lens culinaris ssp. culinaris</em>, *L. culinaris ssp.</td>
<td><em>L. ervoides</em>, <em>L. nigricans</em></td>
<td><em>L. Lamottei</em>, <em>L. tomentosus</em></td>
<td>Ladizinsky et al. (1984); Ladizinsky (1999); Muehlbauer and McPhee (2005)</td>
</tr>
<tr>
<td></td>
<td><em>orientalis</em>, <em>L. odemensis</em></td>
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<tr>
<td>Pigeon pea</td>
<td><em>Cajanus cajan</em>, <em>C. cajanifolius</em></td>
<td><em>C. acutifolius</em>, <em>C. albicans</em>, <em>C. confertiflorus</em>,</td>
<td><em>C. goens</em>, <em>C. heynei</em>, <em>C. kerstingii</em>, <em>C. mollis</em>,</td>
<td>Smartt (1990); Singh et al. (2006)</td>
</tr>
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<td></td>
<td></td>
<td><em>C. taceolatus</em>, <em>C. latisepalous</em>, <em>C. lineatus</em>,</td>
<td><em>C. platycarpus</em>, <em>C. rugosus</em>, *C. volubilis and other</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. reticulatus</em>, <em>C. scarabaeoides</em>, <em>C. sericeus</em>,</td>
<td>species</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. trinervius</em></td>
<td></td>
<td></td>
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<tr>
<td>Mung bean</td>
<td><em>Vigna radiata var. radiata</em>, *V. radiata var.</td>
<td><em>V. angularis</em>, <em>V. dalzelliana</em>, <em>V. giabrescens</em>,</td>
<td></td>
<td>Smartt (1981, 1985); Dana and Karmakar (1990); Chandel and Lester (1991); Kumar et al. (2004)</td>
</tr>
<tr>
<td></td>
<td><em>sublobata</em>, <em>V. radiata var. setulosa</em></td>
<td><em>V. grandis</em>, <em>V. umbellata</em>, <em>V. vexillata</em></td>
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<tr>
<td>Urd bean</td>
<td><em>V. mungo var. mungo</em>, <em>V. mungo var. setulosa</em></td>
<td><em>V. angularis</em>, <em>V. dalzelliana</em>, <em>V. giabrescens</em>,</td>
<td></td>
<td>Dana and Karmakar (1990); Chandel and Lester (1991); Kumar et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. grandis</em>, <em>V. umbellata</em>, <em>V. vexillata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td><em>Phaseolus vulgaris</em></td>
<td><em>P. acutifolius</em>, <em>P. lunatus</em>, <em>other Phaseolus spp.</em></td>
<td></td>
<td>Debouck and Smartt (1995); Debouck (1999, 2000)</td>
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<tr>
<td>bean</td>
<td></td>
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</tbody>
</table>

(Sharma, 2004). Potential accessions of *C. reticulatum* that can provide genes for high yield have also been reported by various workers (Jaiswal and Singh, 1989; Singh and Ocampo, 1997; Singh et al., 2005).

Lentil

The *Lens* gene pool consists of many wild relatives offering resistance to biotic (Ahmad et al., 1997a, b) and abiotic stresses.
### Table 6.2. Useful wild germplasm for introgression of alien genes in food legume crops

<table>
<thead>
<tr>
<th>Crop</th>
<th>Useful trait(s)</th>
<th>Wild species</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpea</td>
<td>Ascochyta blight resistance</td>
<td><em>C. judaicum</em>, <em>C. montbretii</em>, <em>C. pinnatifidum</em></td>
<td>van der Maesen and Pundir (1984); Singh and Reddy (1993)</td>
</tr>
<tr>
<td></td>
<td>Fusarium wilt resistance</td>
<td><em>C. bijugum</em>, <em>C. judaicum</em>, <em>C. reticulatum</em></td>
<td>van der Maesen and Pundir (1984); Kaiser <em>et al.</em> (1994); Infantino <em>et al.</em> (1996)</td>
</tr>
<tr>
<td></td>
<td>Botrytis grey mould resistance</td>
<td><em>C. pinnatifidum</em>, <em>C. judaicum</em></td>
<td>Singh <em>et al.</em> (1982); van der Maesen and Pundir (1984)</td>
</tr>
<tr>
<td></td>
<td>Cyst nematode resistance</td>
<td><em>C. bijugum</em>, <em>C. pinnatifidum</em>, <em>C. reticulatum</em></td>
<td>Greco and Di Vito (1993); Di Vito <em>et al.</em> (1996)</td>
</tr>
<tr>
<td></td>
<td>Phytophthora root rot resistance</td>
<td><em>C. echinospermum</em>, <em>C. bijugum</em>, <em>C. reticulatum</em>, <em>C. pinnatifidum</em></td>
<td>Knights <em>et al.</em> (2008)</td>
</tr>
<tr>
<td></td>
<td>Cold tolerance</td>
<td><em>C. bijugum</em>, <em>C. echinospermum</em> and <em>C. reticulatum</em></td>
<td>Singh <em>et al.</em> (1990)</td>
</tr>
<tr>
<td></td>
<td>Helicoverpa pod borer tolerance</td>
<td><em>C. bijugum</em>, <em>C. echinospermum</em>, <em>C. judaicum</em>, <em>C. pinnatifidum</em>, <em>C. cuneatum</em></td>
<td>Kaur <em>et al.</em> (1999); Sharma (2004)</td>
</tr>
<tr>
<td></td>
<td>Drought tolerance</td>
<td><em>C. anatolicum</em>, <em>C. microphyllum</em>, <em>C. montbretii</em>, <em>C. oxydon</em> and <em>C. songaricum</em></td>
<td>Toker <em>et al.</em> (2007)</td>
</tr>
<tr>
<td></td>
<td>Yield attributes</td>
<td><em>C. reticulatum</em></td>
<td>Jaiswal and Singh (1989); Singh and Ocampo (1997); Singh <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Grasspea</td>
<td>Low ODAP content</td>
<td><em>L. cicera</em></td>
<td>Aletor <em>et al.</em> (1994); Siddique <em>et al.</em> (1996); Hanbury <em>et al.</em> (1999); Kumar <em>et al.</em> (2010)</td>
</tr>
</tbody>
</table>

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Table 6.2. Continued.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Useful trait(s)</th>
<th>Wild species</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High protein content</td>
<td><em>Cajanus cajanifolius</em>, <em>C. sericeus</em></td>
<td>Akinola et al. (1975); Dalvi et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Sterility mosaic disease resistance</td>
<td><em>C. sericeus</em>, <em>C. albicans</em></td>
<td>Akinola et al. (1975); Singh et al. (1993, 2005)</td>
</tr>
<tr>
<td></td>
<td>Phytophthora blight resistance</td>
<td><em>C. sericeus</em>, <em>C. acutifolius</em>, <em>C. platycarpus</em></td>
<td>Akinola et al. (1975); Mallikarjuna and Saxena, (2002)</td>
</tr>
<tr>
<td></td>
<td>Helicoverpa pod borer resistance</td>
<td><em>C. scarabaeoides</em></td>
<td>Verulkar et al. (1997)</td>
</tr>
<tr>
<td>Vigna</td>
<td>Salinity tolerance</td>
<td><em>C. albicans</em></td>
<td>Subba Rao (1990)</td>
</tr>
<tr>
<td></td>
<td>Earliness</td>
<td><em>C. platycarpus</em></td>
<td>Saxena (2008)</td>
</tr>
<tr>
<td></td>
<td>MYMV resistance</td>
<td><em>V. umbellata</em>, <em>V. trilobata</em>, <em>V. mungo</em></td>
<td>Singh and Dikshit (2002); Pandiya et al. (2008)</td>
</tr>
<tr>
<td>Common bean</td>
<td>Common blight resistance</td>
<td><em>P. acutifolius</em></td>
<td>Singh and Munoz (1999)</td>
</tr>
<tr>
<td></td>
<td>BGYMV resistance</td>
<td><em>P. coccineus</em></td>
<td>Osorno et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Resistance to root rot, anthracnose and angular leaf spot</td>
<td><em>P. coccineus</em></td>
<td>Silbernagel and Hannan, (1992); Mahuku et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Heat tolerance</td>
<td><em>P. acutifolius</em></td>
<td>Federici et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Drought tolerance</td>
<td><em>P. acutifolius</em></td>
<td>Parsons and Howe (1984); Markhart (1985)</td>
</tr>
<tr>
<td></td>
<td>Freezing tolerance</td>
<td><em>P. angustissimus</em></td>
<td>Balsubramanian et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Salt tolerance</td>
<td><em>P. filiformis</em></td>
<td>Bayuelo-Jimenez et al. (2002)</td>
</tr>
</tbody>
</table>

ODAP, β-N-oxalyl-L-α,β-diaminoproptic acid; MYMV, mung bean yellow mosaic virus; BGYMV, bean golden yellow mosaic virus.
Grass pea

The wild gene pool is a rich reservoir of rare alleles for grass pea improvement, which have been evaluated sporadically to identify zero/low ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) lines (Jackson and Yunus, 1984). A total of 1082 accessions belonging to 30 species were evaluated for 21 descriptors and agronomic traits at ICARDA (Robertson and Abd-El-Moneim, 1997). Assessment of ODAP content in wild species of *Lathyrus* indicated that in none of the species is it absent (Aletor et al., 1994; Siddique et al., 1996; Hanbury et al., 1999). On average, the ODAP concentration in *Lathyrus cicera* was lowest, followed by *Lathyrus sativus* and *Lathyrus ochrus* (Aletor et al., 1994; Hanbury et al., 1999). Evaluation of 142 accessions of *L. cicera* at ICARDA showed a range of 0.073–0.513% for ODAP content, which is much lower than that in cultivated species (Kumar et al., 2010). The accessions of *L. cicera* are also a good source of earliness, orobanche tolerance and cold tolerance (Robertson et al., 1996).

Pigeon pea

Evaluation of wild species of pigeon pea has shown many desirable characteristics that can be introgressed into cultivated species to make them more adapted and productive. The species with useful traits are listed in Table 6.2. These species have been reported to carry genes for high protein content, salinity tolerance, pod borer tolerance, sterility mosaic resistance, cytoplasmic male sterility. *Cajanus sericeus* and *Cajanus albicans* are rich in protein content, *Cajanus reticulatus* var. *grandifolius* is hardy and fire tolerant (Akinola et al., 1975) and *C. albicans* is tolerant to soil salinity (Subba Rao, 1988).

Vigna crops

A wild accession of *Vigna radiata* var. *sublobata*, PLN 15, has been found to be the potential donor for pods per plant and seeds per pod (Reddy and Singh, 1990). Resistance to mung bean yellow mosaic virus (MYMV) has been reported in *Vigna umbellata*, *Vigna trilobata* and *Vigna mungo* (Nagaraj et al., 1981; Singh and Dikshit, 2002).

Common bean

Wild species of *Phaseolus* have been characterized for biotic stresses. Wilkinson (1983) reported *Phaseolus coccineus* as a potential source of high yield for common bean. Resistance to angular leaf spot (Busogoro et al., 1999), anthracnose (Hubbeling, 1957), ascochyta blight (Schmit and Baudoin, 1992), bean golden mosaic virus (BGMV) (CIAT, 1986; Beebe and Pastor-Corrales, 1991; Singh et al., 1997), bean yellow mosaic virus (BYMV) (Baggett, 1956), common bean blight (CBB) (Mohan, 1982; Schuster et al., 1983; Singh and Munoz, 1999), root rot (Yerkes and Freytag, 1956; Azzam, 1957; Hassan et al., 1971), white mould (Abawi et al., 1978; Hunter et al., 1982) and cold (Bannert, 1979) are found in the secondary gene pool. Some sources of resistance have also been identified in the tertiary gene pool. Resistance to ashy stem blight (Macrophoma phaseolina) and fusarium wilt (Fusarium oxysporum f. sp. phaseoli) (Miklas et al., 1998b), BGMV (Miklas and Santiago, 1996), bruchids (Shade et al., 1987; Dobie et al., 1990; CIAT, 1995, 1996), CBB (Coyne et al., 1963; Schuster et al., 1983; Singh and Munoz, 1999), drought (Thomas et al., 1983; Parsons and Howe 1984; Markhart, 1985; Federici et al., 1990; Rosas et al., 1991), leafhopper (CIAT, 1995, 1996) and rust (Miklas and Stavely, 1998) are found in *Phaseolus acutifolius*.

6.4. Distant Hybridization

Crosses between species of the same or different genera have contributed immensely to crop improvement, gene and genome mapping, understanding of chromosome behaviour and evolution in crops like rice, wheat, maize, sugar cane, cotton, tomato, etc. (Sharma, 1995). The ultimate goal of distant hybridization is to transfer useful genes
from alien species into cultivated species, and this has been very successful in a few crops but not very encouraging for legume crops. Stalker (1980) discussed the gaps between hybridization and utilization, along with approaches for the utilization of wild species in food legumes. However, it is well recognized that gene transfer through wide crosses is a long and tedious process, due to lack of homology between chromosomes of participating species in the cross and pre- and post-zygotic crossability barriers between wild and cultivated species. Utilizing the wild gene pool in breeding programmes may also be constrained by collection gaps in wild species, with no information on genome relationships, poor/limited screening of wild species, linkage drag and genetic complexity of the traits. Therefore, improvement through distant hybridization often takes longer in order to recover genotypes associated with acceptable agronomic background, and thus requires a long-term approach.

Crossability potential

The crossability of cultivars with wild species is a prerequisite for alien gene introgression. A large proportion of wild species are not crossable with cultivated species, and consequently of no use for crop improvement through sexual manipulation. However, variability for crossability has been observed not only among genotypes of cultivated species but also among those of alien species in several crops (Sirkka et al., 1993; Sharma, 1995). Environmental factors can also influence embryo development of interspecific hybrids, and thereby the crossability potential (Percy, 1986; Sirkka et al., 1993; Tyagi and Chawla, 1999). Therefore, an understanding of the extent of crossability is essential for successful production of hybrids and their derivatives. The early work on interspecific hybridization in grain legumes has been reviewed by Smartt (1979). Singh (1990) reviewed a wide spectrum of hybridization work in the genus Vigna, and Ocampo et al. (2000) in cool season legume crops. During the past two decades much information relating to possible gene flow between legume crops and their wild relatives, crossability barriers and methods of overcoming them has been generated. This has greatly enhanced the interest of breeders in distant hybridization. This section summarizes the crossability potential of different food legume crops using various wild and cultivated species.

Chickpea

Of the eight annual wild species, only Cicer reticulatum and Cicer echinospermum have been successfully crossed with chickpea (Ladizinsky and Alder, 1976a; Ahmad et al., 1988, 2005; Verma et al., 1990; Singh and Ocampo, 1993), a technique regularly utilized in the ICARDA chickpea breeding programme (Imtiaz, personal communication). Conventional crossing has been successful in producing interspecific hybrids between Cicer arietinum and C. reticulatum and between C. arietinum and C. echinospermum. Due to the presence of post-zygotic barriers, abortion of the immature embryo occurs for other interspecific crosses involving species from the tertiary gene pool such as C. bijugum and C. judaicum (Ahmad et al., 1988; Clarke et al., 2006). The availability of novel tissue culture techniques and biotechnological tools for circumventing crossing barriers has brightened the prospects of transferring useful traits from the tertiary gene pool (Shiela et al., 1992; Mallikarjuna, 1999; Clarke et al., 2006) and, as a result, hybrids were obtained between C. pinnatifidum and C. bijugum (Mallikarjuna, 1999).

Lentil

Many successful attempts have been made to develop interspecific hybrids, but still many cross combinations are yet to be attempted successfully. As far as the crossability status of wild Lens taxa is concerned, L. c. ssp. orientalis and L. edemensis are crossable with cultivated lentil (Ladizinsky et al., 1984; Abbo and Ladizinsky, 1991, 1994; Fratini et al., 2004; Fratini and Ruiz, 2006; Muehlbauer et al., 2006), although the fertility of hybrids depends on the chromosome arrangement of the wild parent (Ladizinsky, 1979; Ladizinsky et al., 1984). Most accessions of L. c. ssp. orientalis
cross readily with L. culinaris, and both are genetically isolated from other species. *Lens nigricans* and *L. erioides* are not readily crossable with the cultivated lentil using conventional crossing methods, due to hybrid embryo breakdown (Abbo and Ladizinsky, 1991, 1994; Gupta and Sharma, 2005). Crosses are possible between *L. culinaris* and the remaining species, but they are characterized by a high frequency of hybrid embryo abortion, albino seedlings and chromosomal rearrangements that result in hybrid sterility, if these seedlings reach maturity (Abbo and Ladizinsky, 1991, 1994; Ladizinsky, 1993; Gupta and Sharma, 2005). Only four crosses have not resulted in hybrids to date: *L. c. ssp. orientalis* × *L. erioides*; *L. c. ssp. orientalis* × *L. nigricans* (Ladizinsky et al., 1984); *L. c. ssp. tomentosus* × *L. lamottei* (Van Os et al., 1997); and *L. c. ssp. odemensis* × *L. erioides* (Ladizinsky et al., 1984), although viable hybrids have been reported between cultivated species and *L. erioides*. *L. odemensis* and *L. nigricans* with the use of GA3 (Ahmad et al., 1995). Fratini et al. (2006) reported a high correlation between crossing success and phenotypic similarity based on pollen morphology and *in vitro* pollen length, together with pistil and style length, indicating a good predictor of hybridization success between different species.

**Grass pea**

Interspecific hybridization has been successful between *L. sativus* and two wild *Lathyrus* species (*L. cicera* and *L. amphicarpus*) with viable seeds (Davies, 1957, 1958; Khawaja, 1985; Yunus, 1990). Yunus (1990) crossed 11 wild species with *L. cicera* and found viable seeds with *L. cicera* and *L. amphicarpus* only. Other species formed pods but did not give fully developed viable seeds (Yamamoto et al., 1989; Yunus, 1990; Kearney, 1993). Some other successful interspecific hybrids reported in the genus *Lathyrus* were *L. annuus* with *L. hierosolymitanus* (Yamamoto et al., 1989; Hammett et al., 1994, 1996); *L. articulatus* with *L. clymenus* and *L. ochrus* (Davies, 1958; Trankovskij, 1962); *L. cicera* with *L. blepharicarpus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera* (Yamamoto et al., 1989, 1993; L. gorgoni with *L. pseudocicera* (Yamamoto et al., 1989; Kearney, 1993); *L. hirsutus* with *L. odoratus* (Davies, 1958; Trankovskij, 1962; Khawaja, 1988; Yamamoto et al., 1989); *L. marmoratus* with *L. blepharicarpus* (Yamamoto et al., 1989; Kearney, 1993); *L. odoratus* with *L. belinenses* (Hammett et al., 1994, 1996); *L. rotundifolius* with *L. tuberosus* (Marsden-Jones, 1919); and *L. sylvestris* with *L. latifolius* (Davies, 1957).

**Pigeon pea**

Hybridization studies have shown that *C. cajan* can be successfully crossed with *C. albicans*, *C. cajani*, *C. cajanifolius*, *C. scarabaeoides*, and *C. lineatus* (Reddy, 1981; Reddy and De, 1983; Kumar et al., 1985; Pundir and Singh, 1985). Reddy et al. (1981) reported that five species of *Cajanus* (*C. cajanifolius*, *C. scarabaeoides*, *C. albicans*, *C. trinervius* and *C. cajanifolius*) were crossable with pigeon pea cultivars. However, *C. cajan* var. *cassius* and *C. platycarpus* cannot be crossed. With the help of *in vitro* embryo rescue technique, a *C. cajan* × *C. platycarpus* cross has also been successfully engineered (Dhanuj and Gill, 1985; Kumar et al., 1985; Mallikarjuna and Moss, 1995; Mallikarjuna et al., 2006; Saxena et al., 1996). Shahi et al. (2006) attempted crosses between *C. cajan* and *C. platycarpus* to diversify the existing gene pool. Since the pollen of *C. platycarpus* failed to germinate on the stigma of *C. cajan*, the former was used as the female parent. However, hybrids of *C. platycarpus* with two cultivars of *C. cajan* var. Bahar and Pant A3 survived through embryo culture. Mallikarjuna et al. (2006) were also able successfully to cross *C. platycarpus* with cultivated pigeon pea by hormone-aided pollinations, rescuing the hybrid embryos *in vitro* and treating the hybrids with colchicines as these were 100% sterile. Nevertheless, *Cajanus scarabaeoides* has several undesirable characteristics (Upadhyaya, 2006), but is cross-compatible with cultivated pigeon pea and interspecific gene transfer is possible through conventional hybridization. *C. acutifolius* can also be successfully crossed with pigeon pea as a one-way cross (Mallikarjuna and Saxena, 2005).

**Vigna species**

A number of studies undertaken on crossability among different *Vigna* species have
been reviewed by Dana and Karmakar (1990) and Singh (1990). Most reports indicate that *V. radiata* produced successful hybrids as seed parent with *V. mungo*, *V. umbellata* and *V. angularis*, although their reciprocal cross hybrids were not viable. However, by using sequential embryo rescue methods, the reciprocal hybrids between *V. mungo* and *V. radiata* could be successfully produced (Gosal and Bajaj, 1983a; Verma and Singh, 1986). *V. mungo* was also successfully crossed with *V. delzelliana* (Chavan et al., 1966), *V. glabrescens* (Dana, 1968; Krishnan and De, 1968) and *V. trilobata* (Dana, 1966). In some cases, hybrid plants could be obtained only through embryo rescue technique, e.g., *V. mungo × V. umbellata* (Biswas and Dana, 1975; Chen et al., 1983). Mung bean × rice bean crosses were generated to incorporate MYMV resistance and other desirable traits into mung bean (Verma and Brar, 1996). However, genotypic differences were observed in successful crosses. Furthermore, four amphidiploids of mung bean (ML 267 and K 851) × rice bean (RBL 33 and RBL 140) crosses were successfully produced and evaluated for different characters (Dar et al., 1991). Singh et al. (2003) also produced successful hybrids between *V. radiata* and *V. umbellata*, and the hybrids possessed intermediate morphology with MYMV resistance. Similarly, Pal et al. (2005) were also successful in producing interspecific crosses between *V. mungo* and *V. umbellata*. Interspecific hybridizations between cultivated cowpea (*V. unguiculata* ssp. *unguiculata* and *V. u. ssp. biflora*) and wild forms of cowpea (*V. u. var. spontanea*, *V. u. ssp. alba*, *V. u. ssp. stenophylla*, *V. u. ssp. pawekiae* and *V. u. ssp. baoulensis*) were attempted by Kouadio et al. (2007), and the highest success rate was obtained in crosses between cultivated and annual inbred forms, although hybridization between cultivated and wild allogamous forms gave an intermediate rate of success. The success rate was lower when *V. u. ssp. baoulensis* was crossed with cultivated forms.

### Crossability barriers

Crossability barriers developed during the process of speciation frustrate breeders’ efforts in successful hybridization between species of different gene pools. Reproductive isolation, embryo or endosperm abortion, hybrid sterility and limited levels of genetic recombination are significant obstacles to the greater use of wild germplasm. These obstacles are in addition to those of undesirable linkages to non-agronomic traits once gene flow has been achieved. These barriers can prevent fertilization, reduce the number of hybrid seeds, retard the normal development of hybrid endosperm leading to embryo death or can cause hybrid sterility. In nature, there is selection bias towards strengthening these barriers to avoid extinction of the species by chaotic hybridization. In food legume crops several crossability barriers have been reported, the most common being cross incompatibility, embryo abortion at early growth stage, inviability of F₁ hybrids and sterility of F₁ hybrid and subsequent progenies (Kumar et al., 2007). The pre-fertilization cross incompatibility between parent species arises when pollen grains do not germinate, the pollen tube does not reach the ovary or the male gametes do not fuse with the female (Chowdhury and Chowdhury, 1983; Shanmugam et al., 1983).

### Chickpea

Both pre-zygotic and post-zygotic barriers to interspecific hybridization in chickpea have been reported (Croser et al., 2003). In the case of pre-zygotic barriers, Mercy and Kakar (1975) attempted to clarify incompatibility barrier(s) present among *Cicer* genus. They found the evidence of a low molecular weight inhibitory substance, possibly a protein present in the stylar and stigmatic tissues, inhibiting the germination and tube growth of the pollen. One of the reasons reported for the failure of interspecific crosses is the presence of localized sticky stigmatic secretion at the time pollen needs to be placed directly on the most receptive part of the stigma (Croser et al., 2003). However, Ahmed et al. (1988) and Ahmed and Slinkard (2004) demonstrated a post-zygotic barrier(s) to crossing incompatibility rather than a pre-zygotic. They used seven of the eight wild annual *Cicer* species, belonging to the secondary and tertiary gene
problems can occur in the F₁ and also persist in L. culinaris × L. nigricans (Goshen et al., 1993). In contrast, Abbo and Ladizinsky (1991) showed no sign of disintegration (Ladizinsky, 1979). In some L. culinaris × L. culinaris ssp. orientalis crosses, the hybrid embryo ceased growing but the endosperm shows no sign of disintegration (Ladizinsky, 1993). In contrast, Abbo and Ladizinsky (1991) observed that the endosperm was either abnormal or lacking in L. culinaris × L. c. ssp. orientalis crosses. Hybrids showed varying degrees of fertility, usually due to chromosome translocations and subsequent problems with chromosome pairing at meiosis, in Lens culinaris × L. nigricans (Goshen et al., 1982; Ladizinsky et al., 1984). Fertility is often very low, with little viable pollen produced in anthers, and varies depending on the accession in L. culinaris × L. c. ssp. orientalis crosses from 2% to 69% (Ladizinsky et al., 1984). These problems can occur in the F₁, and also persist in later generations, causing partial or complete sterility. Albino seedlings can also occur in the F₁ generation and thus prevent hybridization success (Ladizinsky and Abbo, 1993). Another common problem is that hybrid embryos cease to grow about 7–14 days after pollination due to endosperm degeneration, and thus need rescuing in order to obtain viable hybrids (Ladizinsky et al., 1985; Ahmad et al., 1995). Hence, L. culinaris × L. ervoides or L. culinaris × L. nigricans crosses need embryo rescue techniques in order to develop mature hybrid plants (Cohen et al., 1984; Abbo and Ladizinsky, 1991).

**Vigna crops**

In Vigna crops a slow rate of pollen growth, in addition to abnormalities in stigmatic and stylar regions, could be one of the major causes for low percentage of pod set in V. radiata × V. umbellata and V. mungo × V. umbellata crosses (Thiyagu et al., 2008). However, the ploidy level and style length difference may not be major barriers in the case of Vigna species, as the long-styled female parent V. radiata could be successfully crossed with the short-styled male parent V. trilobata. Crosses between diploid × tetraploid (V. radiata × V. glabrescens) (Krishnan and De, 1968; Chen et al., 1989) and tetraploid × diploid (V. glabrescens × V. umbellata) were also successful. In many studies crossability was genotype dependent (Rashid et al., 1988). It was observed that strong pre-fertilization barriers were present in the cross between V. radiata and V. umbellata, and growth and lethality of interspecific hybrid seedlings were influenced by the genotypes of both parental species (Kumar et al., 2007). Male sterility in F₁ plants and subsequent generations in interspecific crosses of Vigna could be attributed to meiotic irregularities: for example, unequal separation of tetrads and female sterility to degeneration of megaspores during megasporogenesis (Pandiyan et al., 2008). One fertile pod with two hybrid seeds was obtained when V. angularis was used as a male parent; consequently, a partly fertile interspecific hybrid was obtained. Among the post-fertilization barriers, production of shrivelled hybrid seed with reduced or no germination (hybrid inviability), development of dwarf and non-vigorous plants and death of F₁ plants at critical stages of development (hybrid lethality) are the most common crossability barriers (Biswa and Dana, 1975). These barriers were of varying degrees in most of the interspecific crosses (Đana, 1964; Al- Yasiri and Coyne, 1966; Biswas and Dana, 1976; Chowdhury and Chowdhury, 1977; Machado et al., 1982; Chen et al., 1983; Gopinathan et al., 1986). Sidhu (2003) produced interspecific hybrids...
of *V. radiata* with *V. mungo* and *V. trilobata*. Although the crosses between *V. radiata* and *V. trilobata* were successful, the seeds produced between *V. mungo* and *V. trilobata* had very poor germination and the germinated seedlings did not survive. Cytological analysis revealed irregular chromosome behaviour at diakinesis/metaphase I. In some of the interspecific crosses of *Vigna*, hybrid sterility has been observed to be of segregational type and was due mainly to interchange, inversion and possibly the duplication-deficiency type of structural heterozogosities in the F₁ individuals (Dana, 1964; Biswas and Dana, 1975; Karmakar and Dana, 1987).

**Strategy to overcoming crossability barriers**

With better understanding of the processes involved in pollen germination, pollen tube growth and fertilization, the opportunities to manipulate these processes toward the development of viable and fertile interspecific hybrids have improved considerably. Various measures to crossability barriers were reviewed by various workers (Sharma and Satija, 1996; Singh and Munoz, 1999), and are summarized in Table 6.3.

**Embryo rescue protocols**

The advent of *in vitro* techniques such as embryo and ovule culture, coupled with *in vivo* hormonal treatments, has greatly increased the scope of distant hybridization in food legume crops where post-fertilization barriers (zygotic abortion mechanisms) are common (Gupta and Sharma, 2005; Clarke *et al.*, 2006; Fratini and Ruiz, 2006; Mallikarjuna *et al.*, 2006). In wide crosses where few embryos are produced, the efficiency of recovering viable hybrid plants may also be enhanced by callus induction from the embryo and subsequent regeneration of plantlets. These procedures are also directed towards obtaining more efficient survival of embryos in situations where very immature embryos are to be cultured. Wide crosses that do not produce viable seeds could also be obtained through embryo cal- lus production and subsequent regeneration and rooting of the callus. The possibility of increasing crossability also exists by predisposing crop embryos to alien endosperm and then using plants raised from those embryos to cross with the alien species. Hybridization of cultivated lentil with *L. ervoides* and *L. nigricans* results in pod development that is arrested within 10–16 days after pollination and finally yields shrivelled, non-viable seeds (Ladizinsky *et al.*, 1985), but can be rescued by a two-step *in vitro* method of embryo–ovule rescue to obtain successful distant hybrids (Cohen *et al.*, 1984). However, Ahmad *et al.* (1995) and Gupta and Sharma (2005) could not produce hybrids using the same technique. Fratini and Ruiz (2006) developed a protocol in which hybrid ovules were rescued 18 days after pollination. Fiala (2006) also obtained *L. culinaris × L. ervoides* hybrids using the Cohen *et al.* (1984) protocol. In addition, one viable *L. culinaris ssp. culinaris × L. lamot- tei* hybrid was also produced in this study. In chickpea, Clarke *et al.* (2006) suggested that the appropriate time to rescue *C. arietinum × C. bijugum* hybrids is the early globular stage of embryogenesis (2–7 days). In contrast, *C. arietinum × C. pinnatifidum* hybrids abort later (15–20 days) at the heart-shaped or torpedo stages, and are easier to rescue *in vitro*. Genotype also plays a significant role in the ability of immature selfed ovules to germinate *in vitro*. Thus the development of appropriate and efficient *in vitro* protocols for rescuing immature hybrid embryos is a necessity for these legume crops to secure alien gene resources available for their improvement.

**Chromosome doubling**

Colchicine-induced allopolyploids have been raised from most of the semi-fertile and completely seed-sterile F₁ hybrids in *Vigna* having high pollen fertility and seed set (Dana, 1966; Pande *et al.*, 1990), and some of these allopolyploids were used as a bridge species in wide crosses. In pigeon pea, Mallikarjuna and Moss (1995) attempted chromosome doubling of diploid F₁ hybrids of *Cajanus platycarpus × C. cajan* to obtain tetraploid F₁ hybrids. Selfing in successive generations had given rise to mature seeds with introgression of a resistance gene to phytophthora blight
Table 6.3. Methods of overcoming crossability barriers in food legumes

<table>
<thead>
<tr>
<th>Method</th>
<th>Cross combination</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>Reciprocal crosses</td>
<td><em>Vigna radiata</em> × <em>V. mungo</em></td>
<td>Verma and Singh (1986), Ravi et al.</td>
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<td></td>
<td><em>Phaseolus vulgaris</em> × <em>P. coccineus</em></td>
<td>Rabakoarihanta et al. (1979)</td>
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<td></td>
<td><em>P. vulgaris</em> × <em>P. lunatus</em></td>
<td>Leonard et al. (1987)</td>
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<td></td>
<td><em>V. radiata</em> × <em>V. umbellata</em></td>
<td>Gupta et al. (2002)</td>
</tr>
<tr>
<td>Growth regulators</td>
<td><em>V. mungo</em> × <em>V. umbellata</em></td>
<td>Chen et al. (1978)</td>
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<td></td>
<td><em>Cajanus cajan</em> × <em>C. cajanifolius</em></td>
<td>Singh et al. (1993)</td>
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<td></td>
<td><em>C. cajan</em> × <em>P. acutifolius</em></td>
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<td></td>
<td><em>P. vulgaris</em> × <em>P. acutifolius</em></td>
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<td></td>
<td><em>C. cajan</em> × <em>C. scarabaeoides</em></td>
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<tr>
<td>Embryo rescue</td>
<td><em>C. cajan</em> × <em>C. acutifolius</em></td>
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<td></td>
<td><em>C. marina</em> × <em>C. luteola</em></td>
<td>Palmer et al. (2002)</td>
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<td></td>
<td><em>V. glabrescens</em> × <em>V. radiata</em></td>
<td>Chen et al. (1990)</td>
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<td></td>
<td><em>C. luteolus</em> × <em>C. luteolus</em></td>
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<td></td>
<td><em>V. vexillata</em> × <em>V. unguiculata</em></td>
<td>Gomathinayagam et al. (1998)</td>
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<td></td>
<td><em>V. unguiculata</em> × <em>V. mungo</em></td>
<td>Shrivastava and Chawla (1993)</td>
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<td></td>
<td><em>Lens culinaris</em> × <em>L. orientalis</em></td>
<td>Ladizinsky et al. (1985), Ahmad et al.</td>
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<td></td>
<td><em>C. arietinum</em> × <em>C. echinospernum</em></td>
<td>Ladizinsky and Adler (1976a, b)</td>
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<td></td>
<td><em>C. arietinum</em> × <em>C. reticulatum</em></td>
<td>Ladizinsky and Abbo (1993)</td>
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<td></td>
<td><em>C. arietinum</em> × <em>C. bijugum</em></td>
<td>Pundir and Mengesha (1995)</td>
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<td><em>C. arietinum</em> × <em>C. pinnatifidum</em></td>
<td>Mallikarjuna (1999)</td>
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<td></td>
<td><em>C. arietinum</em> × <em>C. cajanifolius</em></td>
<td>Clarke et al. (2006)</td>
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<td><em>L. culinaris</em> × <em>L. angustifolius</em></td>
<td>Dana (1966)</td>
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<td><em>L. culinaris</em> × <em>L. ervoides</em></td>
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<td><em>C. radiata</em> × <em>V. mungo</em></td>
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<td><em>C. radiata</em> × <em>V. trilobata</em></td>
<td>Gupta et al. (2002)</td>
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<td><em>C. radiata</em> × <em>V. radiata</em> × *V.</td>
<td>Sharma and Satija (1996)</td>
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<td><em>trilobata</em></td>
<td>Sharma and Satija (1996)</td>
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<td><em>C. radiata</em> × <em>V. radiata</em></td>
<td>Sharma and Satija (1996)</td>
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<td><em>V. mungo</em> × <em>V. radiata</em> × *V.</td>
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<td><em>radiata</em> × <em>V. radiata</em> × *V.</td>
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<td><em>radiata</em> × <em>V. trilobata</em></td>
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<td><em>C. cajan</em> × <em>C. scarabaeoides</em></td>
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<td><em>C. cajan</em> × <em>C. acutifolius</em></td>
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<td><em>P. vulgaris</em> × <em>P. acutifolius</em></td>
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<td><em>P. vulgaris</em> × <em>P. acutifolius</em></td>
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<tr>
<td>Chromosome doubling</td>
<td><em>V. radiata</em> × <em>V. trilobata</em></td>
<td>Dana (1966)</td>
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<td>using colchicine</td>
<td><em>(V. mungo × V. radiata)</em> × *V.</td>
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<td>Use of bridge species</td>
<td><em>(V. radiata)</em> × <em>V. radiata</em></td>
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<td>*(V. mungo × V. radiata) × *V.</td>
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disease from \textit{C. platycarpus}. In cases where cultivated species cannot tolerate a large portion of alien chromosome, irradiation techniques have been successfully used. Among food legumes, irradiation techniques have been successful in recovering fertile plants in F\textsubscript{1} and subsequent generations in interspecific crosses in \textit{Vigna}. Pandiyan \textit{et al}. (2008) reported increased pod set in interspecific \textit{V. radiata} × \textit{V. umbellata} crosses developed from gamma ray-irradiated parental lines.

\textbf{Reciprocal crossing}

Reciprocal differences in wide crosses are also very common, and can be due to chromosomal imbalance in the endosperm, the role of the sperm nucleus in differential endosperm development or the alteration of endosperm development by pollen through the effects of antipodal cells, which are assumed to supply nutrients during early endosperm development (Beaudry, 1951). If disharmony between the genome of one species and cytoplasm of the other is a cause of a fertilization barrier, reciprocal crosses can be successful in recovery of hybrids. For example, while a \textit{V. mungo} × \textit{V. radiata} cross was unsuccessful, its reciprocal cross, \textit{V. radiata} × \textit{V. mungo}, produced successful hybrids (Verma and Singh, 1986; Ravi \textit{et al}.., 1987). Interspecific hybridization between \textit{V. nakashimae} and \textit{V. angularis} was successful in both directions and viable seeds were produced, while \textit{V. riukinensis} produced successful hybrids when used as male parent only with \textit{V. angularis} and \textit{V. umbellata} (Siriwardhane \textit{et al}.., 1991). In general, using a female parent with higher chromosome number is more successful than the reciprocal method.

\textbf{Use of bridge species}

When useful genes are available in secondary and tertiary gene pools and direct hybridization between cultivated and wild species does not result in fertile hybrids, involvement of a third species as a bridge species has often been used for introgression of alien genes. For example, attempts at hybridizing \textit{Lens culinaris} with \textit{L. lamottei} and \textit{L. nigricans} have not yielded fertile hybrids. This offers the possibility of transferring the genes for resistance to ascochyta blight and anthracnose to \textit{L. culinaris} by using \textit{L. ervoides} as a bridge species, with the embryo rescue technique as a means of broadening the resistance gene base in the cultivated species (Ye \textit{et al}.., 2002; Tullu \textit{et al}.., 2006). Transfer of bruchid resistance from wild \textit{Vigna} species is difficult due to cross incompatibility. By using the bridge species \textit{V. nakashimae}, the bruchid resistance of \textit{V. umbellata} is transferred to azuki bean (Tomooka \textit{et al}.., 1992, 2000). However, bridge crosses will work only under the condition where species A hybridizes with species B but not with species C, and species B and C form a viable hybrid. Based on the close relationship reported in perennial \textit{Cicer anatolicum}, \textit{C. reticulatum} and \textit{C. echinospermum}, the bridge-crossing approach deserves further attention.

\textbf{Growth hormones}

In wide crosses, if the hybrid seeds die when their embryos are too small to be cultured, post-pollination application of growth regulators such as gibberellic acid, naphthalene acetic acid, kinetin or 2, 4-D (dimethylamine), singly or as in combination, may be helpful in maintaining the developing seeds by facilitating division of the hybrid zygote and endosperm. Mallikarjuna (1999) observed that the only way to obtain interspecific hybrid in chickpea is by the application of growth regulators to pollinated pistils, to prevent initial pod abscission and to save the aborting hybrid embryos by embryo rescue techniques. Some interspecific crosses have been successful in \textit{Phaseolus} (Stalker, 1980), \textit{Cajanus} (Singh \textit{et al}.., 1993) and \textit{Cicer} (Shiela \textit{et al}.., 1992) by application of growth regulators after pollination. This suggests that further breakthroughs in wide crossing may be possible through the exploitation of growth regulators followed by embryo rescue. \textit{In vivo} hormonal treatments have also greatly helped in recovery of interspecific hybrids in \textit{Vigna}. A true-breeding \textit{Vigna mungo} × \textit{V. radiata} derivative was reciprocally crossed with \textit{V. angularis}, and the pollinated pistils were treated with after GA3 24 and 78 h of pollination.
Backcrossing

In wide crosses, plants in initial generations are generally of inferior nature with poor expression of desired traits. This requires advancing the cross populations up to F8/F9 generations for recovery of desired types. In many cases the crosses are abandoned midway due to various reasons, in spite of reports that useful recombinants could be recovered in later generations (F10–F12) of an interspecific cross (Singh and Dikshit, 2002). Therefore, delayed segregation often causes problems in identification and utilization of useful recombinants in interspecific crosses. This problem can be overcome through backcrossing F1 hybrids with cultivated species in early generations. Mallikarjuna et al. (2006) introgressed the Cajanus platycarpus genome into cultivated pigeon pea by backcrossing embryo-rescued F1 hybrids with cultivated pigeon pea followed by in vitro culture of aborting embryos of BC1 progeny. Similarly, one or more backcrosses to the recurrent parent are often required in common bean to restore fertility of hybrids when crossed with Phaseolus acutifolius and P. parvifolius. Using P. acutifolius as female parent of the initial F1 cross, and/or first backcrossing P. vulgaris × P. acutifolius hybrid on to P. acutifolius, is often more difficult than using P. vulgaris as the female parent of the initial cross and backcrossing the interspecies hybrid on to P. vulgaris (Mejia-Jimenez et al., 1994). The choice of parents (Parker and Michaels, 1986; Federici and Waines, 1988; Mejia-Jimenez et al., 1994) and use of the congruity backcross (i.e. backcrossing alternately to each species) over recurrent backcrossing (Haghighi and Ascher, 1988; Mejia-Jimenez et al., 1994) facilitate interspecific crosses of common and tepary beans, in addition to recovery of fertility and more hybrid progenies.

6.5. Successful Examples of Alien Gene Introgression in Food Legumes

Successful examples of alien gene introgressions in food legumes are limited to a few, for various reasons (Table 6.4). Genes for disease and insect resistance, male sterility and fertility restoration, and yield attributes have been transferred into cultivated species of various legume crops. For example, successful introgression of drought tolerance from Cicer reticulatum (Hajjar and Hodgkin, 2007), yield genes from C. reticulatum (Singh et al., 2005) and tolerance to ascochyta blight, cyst nematode and leaf miner have been documented. In lentil, some progress has been made in introgression of alien genes for resistance to ascochyta blight, anthracnose and cold in cultivated lentil (Hamdi et al., 1996; Ye et al., 2002; Fiala, 2006). Successful examples of using crossable wild species in pigeon pea breeding include development of a highly cleistogamous line (Saxena et al., 1992); genetic dwarfs (Saxena and Sharma, 1995); phytophthora blight resistance (Reddy et al., 1996; Mallikarjuna and Saxena, 1994).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Wild relatives</th>
<th>Character</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpea</td>
<td>Cicer reticulatum</td>
<td>Cyst nematode</td>
<td>Di Vito et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>C. reticulatum</td>
<td>Yield</td>
<td>Jaiswal and Singh (1989), Singh et al. (2005)</td>
</tr>
<tr>
<td>Lentil</td>
<td>C. reticulatum</td>
<td>Cold tolerance</td>
<td>Singh et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Lens orientalis</td>
<td>Cold tolerance, Agronomic traits</td>
<td>Hamdi et al. (1996), Abbo et al. (1992), ICARDA (1995)</td>
</tr>
<tr>
<td></td>
<td>Lens ervoides</td>
<td>Anthracnose resistance</td>
<td>Fiala (2006); Tullu et al. (2006)</td>
</tr>
<tr>
<td>Pigeon pea</td>
<td>Cajanus sericeus</td>
<td>Male sterility</td>
<td>Ariyanayagam et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>C. scarabaeoides</td>
<td>Male sterility</td>
<td>Tikka et al. (1997)</td>
</tr>
<tr>
<td>Mung bean</td>
<td>Vigna mungo</td>
<td>YMV resistance, plant type traits</td>
<td>Singh and Dikshit (2002)</td>
</tr>
</tbody>
</table>
2002); high-protein lines (Saxena et al., 2002); cytoplasmic male sterile (CMS) lines (Saxena et al., 2006); cyst nematode resistance (Saxena et al., 1990); salinity resistance (Subba Rao et al., 1990); and helicoverpa tolerance (Reed and Lateef, 1990). Some successful examples of alien gene introgression in food legume crops are described below.

**Yield genes**

The notion that wild relatives are a prospective source of genes for biotic stress tolerance only has been dismantled with convincing evidence of introgression of yield QTLs from the wild progenitors in some crops, including oats (Frey et al., 1983), rice (Xiao et al., 1996) and tomato (Tanksley et al., 1996; Fulton et al., 2000). The possibilities of introgression of desirable alien genes from wild to cultivated chickpea have been explored (Jaiswal and Singh, 1989; Verma et al., 1990; Singh et al., 2005). Studies have shown that, besides disease resistance and drought tolerance, wild *Cicer* species have genes for desirable yield components such as high number of fruiting branches and pods per plants (Singh et al., 1994). In chickpea, alien genes for productivity have been transferred from *Cicer echinospermum*, *C. reticulatum* (Singh and Ocampo, 1997) and *C. reticulatum* (Singh et al., 2005). Singh and Ocampo (1997) transferred some genes from *C. echinospermum* and *C. reticulatum* into cultivated chickpea and observed up to 39% increase in seed yield following the pedigree method. Singh et al. (2005) also reported introgression of yield genes and disease resistance genes from *C. reticulatum* to cultivated variety L550, with interspecific derivatives showing 6–17% yield advantage. A cross between Pusa 256 and *C. reticulatum* was made and their F1 was again crossed with the wilt-resistant variety Pusa 362. Further selection concluded with the development of Pusa 1103, which is a high-yielding early variety with resistance to wilt, root rot and stunt virus and tolerance to drought and heat (Hajjar and Hodgkin, 2007; Kumar et al., 2010). Singh and Dikshit (2002) introgressed yield genes in mung bean from urd bean with 15–60% yield advantage. The derivatives from mung bean × urd bean crosses exhibit many other desirable features such as lodging resistance, synchrony in podding and non-shattering (Reddy and Singh, 1990).

**Disease resistance**

In chickpea, introgression of resistance to cyst nematode from *Cicer reticulatum* has been reported, with promising lines under evaluation at ICARDA (Di Vito et al., 1996; Ocampo et al., 2000). Recently, resistance to anthracnose found in *Lens ervoides* germplasm has been exploited in Canada by introgressing resistance genes into cultivated backgrounds (Fiala, 2006; Tullu et al., 2006). This successful use of *L. ervoides* holds promise as a source of genes for resistance to other diseases, and possibly for plant habit, biomass production and other important agronomic and marketing traits. Further exploitation of *L. ervoides* and the other wild *Lens* species is warranted. Derivatives from mung bean × urd bean crosses exhibit a higher level of MYMV resistance (Gill et al., 1983). A few mung bean × ricebean and mung bean × *Vigna radiata* var. *sublobata* crosses having a high degree of resistance to MYMV were also recovered (Verma and Brar, 1996). Three mung bean cultivars, HUM 1, Pant Moong 4 and IPM99-125, and one urd bean cultivar, Mash 1008 (Sandhu et al., 2005) have been developed from mung bean × urd bean crosses. These cultivars have improved plant types, in addition to higher MYMV resistance and synchronous maturity. In common bean, successful introgressions of alien genes imparting CBB (Freytag et al., 1982; Park and Dhanvantari, 1987; Miklas et al., 1994a, b), fusarium root rot (Wallace and Wilkinson, 1965) and white mould (Abawi et al., 1978; Dickson et al., 1982; Lyons et al., 1987; Miklas et al., 1998a) from *Phaseolus coccineus* have been reported. In contrast, resistance to halo blight from the common bean was incorporated into *P. coccineus* (Ockendon et al., 1982). A high level of resistance to CBB was transferred from tepary to common bean (Coyne et al., 1963; McElroy, 1985; Scott and Michaels, 1992; Singh and Munoz, 1999).
Insect pest resistance

The major production constraint of food legumes is susceptibility to bruchids (Callosobruchus chinensis L.) that eat seeds in storage. One accession of wild mung bean (Vigna radiata var. sublobata) exhibited complete resistance to azuki bean weevils and cowpea weevils (Fujii et al., 1989), which has successfully been used in breeding programmes (Tomooka et al., 1992). Vigna mungo var. silvestris is also reported to be immune to bruchids (Fujii et al., 1989; Dongre et al., 1996). Recently, rice bean (V. umbellata) has been identified as being of use because many accessions show complete resistance to bruchids and it is a cultivated species. Efforts are in progress at AVRDC to utilize V. r var. sublobata for resistance to bruchids. Similarly, sources of resistance to leaf miner were used successfully in a chickpea breeding programme at ICARDA to develop promising breeding lines with leaf miner resistance for North Africa and West Asia (Singh and Weigand, 1996).

Male sterility and fertility restoration

Several wild relatives were used in hybridization with Cajanus cajan, and male sterile plants were isolated from the segregating populations. Ariyanayagam et al. (1995) crossed C. sericeus with C. cajan and isolated male sterile plants from the BC$_3$F$_1$ population. Tikka et al. (1997) developed a CMS line using C. scarabaeoides cytoplasm. Male sterile plants were also isolated from an interspecific cross of C. cajanifolius with C. volubilis. Saxena and Kumar (2003) developed a CMS sterile line, cms 88039A, using C. scarabaeoides (ICPW 89) and an early-maturing line of C. cajan (ICPL 88039). Similarly, two CMS lines, COR9 900052A and COR9 990047A, were developed by interspecific hybridization of C. cajan and C. scarabaeoides (Kalaimagal et al., 2008). Experimental hybrids based on cytoplasmic male sterility derived from C. scarabaeoides and C. sericeus in pigeon pea are currently being evaluated in multi-environment trials. One recently released hybrid, GTH 1, has male sterile cytoplasm from C. scarabaeoides.

6.6 Future Strategy for Alien Gene Introggression

Advanced backcross-QTL strategy

Since the mid-1990s, convincing evidence at both morphological and molecular levels has accumulated for the utility of wild progenitors and related species as donors of productivity alleles. Productivity-enhancing genes/QTLs (quantitative-trait loci) have been introgressed in oats from Avena sterilis (Frey et al., 1983), in tomato from Lycopersicon pimpinellifolium and L. parviflorum (Tanksley et al., 1996; Fulton et al., 2000), in rice from Oryza rufipogon (Xiao et al., 1996) and in chickpea from Cicer reticulatum (Singh et al., 2005). Novel breeding strategies such as AB-QTL (advanced backcross-QTL) have been deployed to exploit the worth of the progenitor and related species as this helps minimize the negative effect of linkage drag associated with alien gene introgression (Tanksley and Nelson, 1996). The related species of mung bean, such as Vigna umbellata and V. angulata, have comparatively higher productivity and their relationship with mung bean offers an opportunity for the introgression of some productivity alleles using AB-QTL strategy. Another related species, V. mungo, and the wild progenitor of mung bean, V. radiata var. sublobata, may also contribute some productivity alleles to the elite mung bean lines using the same approach.

Looking for genes based on molecular maps

The traditional approach in utilizing exotic germplasm is to screen the phenotype of entries from a gene bank for a clearly defined character and to use them in a crossing programme in order to introduce the genes into cultivated germplasm. Although effective for qualitative traits, only a small proportion of the genetic variation has been exploited for crop improvement as a result of this strategy (Tanksley and McCouch, 1997). Availability of genetic linkage maps based on molecular markers has opened up new opportunities in
the utilization of hitherto unexploitable exotic germplasm. This requires a paradigm shift from selecting potential parents on the basis of phenotype to evaluating them directly for the presence of useful genes, through the integration of molecular tools. A gene-based approach to screening exotic germplasm has already been successfully used in rice and tomato for improving yield levels (Tanksley et al., 1996; Xiao et al., 1996). Recently, good progress has been made in generating genomic resources for food legume crops that will be very useful in genetic mapping and QTL analysis in these crops (Varshney et al., 2009). With the use of DNA profiles, the genetic uniqueness of each accession in a gene bank can be determined and quantified. Molecular marker technology allows a targeted approach to the selection and introgression of valuable genes from a range of genetic resources while retaining the integrity of valuable genetic background through forward and background selection.

**Recombination DNA technology**

Transgenic approaches provide new options for broadening the genetic base in those cases where current options are lacking in their efficacy or existence. Plant genetic transformation techniques such as Agrobacterium-mediated transformation and direct gene delivery system (biolistics) allow the precise transfer of genes from any organism into either plant nuclear or chloroplast genomes. Many isolated plant genes are now being transferred between sexually incompatible plant species. In chickpea and pigeon pea, helicoverpa pod borer is a major insect pest for which no genetic solution exists. This requires development of transgenics having Cry genes from the soil bacterium Bacillus thuringiensis to combat the menace of helicoverpa pod borer. The recent report of a Bt. chickpea is an encouraging step towards improvement of food legumes for difficult traits such as pod borer resistance (Acharjee et al., 2010). Similar is the case for botrytis grey mould in chickpea, where efforts are under way to construct a resistance against this disease. For gene introgression purposes, difficult species falling in tertiary and quaternary gene pools may turn out to be important sources of alien genes. For example, identification and cloning useful genes from Phaseolus filiformis, P. angustissimus and P. lunana and successful regeneration and transformation of common bean may facilitate gene introgression in the future.

**Protoplast technology**

Somatic hybridization using protoplast fusion has potential to overcome pre- and post-zygotic barriers to interspecific hybridization (Powers et al., 1976; Davey et al., 2005). It is possible to regenerate plants from a number of legume species, including Pisum (Ochatt et al., 2000), Trifolium (Gresshoff, 1980), Lotus (Ahuja et al., 1983) and Melilotus (Luo and Jia, 1998), and asymmetric protoplast fusion has been used for Medicago improvement (Tian and Rose, 1999; Yuko et al., 2006). However, only a few reports of successful regeneration of plantlets are available in legumes (Li et al., 1995). Initially, protoplast-derived tissues in rice bean were obtained although no shoot regeneration could be obtained. Shoot regeneration from protoplasts of Vigna sublobata has more recently been reported by Bhadra et al. (1994), with the maximum protoplast yield being obtained from 5-day-old seedlings. There are no reports at the time of writing of successful growth or regeneration of protoplasts from Lens species. Rozwadowski et al. (1990) cultured protoplasts from lentil epicotyl tissue, and around 6% of protoplasts developed into cell colonies.

**Doubled haploids**

Doubled haploid breeding is an important approach in many crop species, including wheat, barley, rice, maize and canola, to fix the hybrid immediately. Implementation of doubled haploids increases selection efficiency and allows new varieties to be bred up to 5 years faster than with conventional breeding methods alone. Haploids may be produced from either immature pollen cells, immature
egg cells or following asymmetric chromosome elimination after interspecific hybridization. Several attempts have been made to develop anther and microspore culture systems for chickpea (Huda et al., 2001; Vessal et al., 2002; Croser et al., 2006), common bean (Peters et al., 1977; Munoz-Florez and Baudoin 1994a, b), field pea (Croser et al., 2006) and pigeon pea (Pratap et al., 2009). In chickpea, cultivars responsive to isolated microspore cultures have been identified and the induction of sporophytic development achieved in uninucleate microspores via the application of heat stress (32.5°C) pre-treatment to the buds (Croser et al., 2006). Due to difficulty in derivation of green haploid regenerants these species have been defined as recalcitrant to androgenesis, although some progress has been made towards standardizing callus induction media and culture conditions in some of these crops. However, the production of a successful double haploid system in chickpea has been reported (Grewal et al., 2009). A review of the literature on doubled haploid production in Fabaceae (Croser et al., 2006) indicated that none of these approaches had been successful in producing haploid plants in food legumes, but the early stages of isolated microspore division have been observed.

### 6.7 Prospects

Productivity of food legume crops is affected by various biotic and abiotic stresses. There is thus an urgent need to widen the cultivated gene pool of these crops by incorporating genes for economically important traits from diverse sources. Wild species have proved to be an important reservoir of useful genes, and offer great potential for the incorporation of such genes into commercial cultivars. Many of the useful alien genes are expected to be different from those of the cultivated species, and are thus useful in broadening the base of resistance to various stresses. Recently, OTLs (oligogenic traits) that have been identified for yield traits in wild species of pulse crops may enhance agronomic and market values of cultivated varieties. The molecular marker technique can also be used for authentication of interspecific hybrids (Yamini et al., 2001). There is a need to identify high-crossability genes in food legumes, as has been identified in wheat cultivars such as Chinese Spring (Luo et al., 1993; Sharma, 1995). Identification of such genes in food legumes can bring non-crossable species within the ambit of alien gene transfer technology. There are major gaps in germplasm collections of wild species and their evaluation in food legumes that need to be filled, in order to progress further inroads in alien gene introgression. Continuing advances in wide-crossing techniques, such as embryo culture and development of novel crossing strategies, are creating greater accessibility in wild gene pools of many crops. The success rate of gene transfer in such wide crosses can be increased by knowledge of chromosome pairing mechanisms and their genetic control. The modern tools of molecular biology, such as monoclonal antibodies and in situ hybridization using various DNA probes, may soon make it possible to study the switching on and off of various genes in diverse tissues of the fertilized ovule, and control over the levels and movements of both exogenous and endogenous growth substances within the developing seed. It is likely that continuing advances in structural genomics and genetic engineering will result in new strategies for alien gene introgression.

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