Exploitation of wild relatives of pigeonpea and chickpea for resistance to *Helicoverpa armigera*

Nalini Mallikarjuna*, HC Sharma and **HD Upadhyaya**
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India
*Corresponding author: n.mallikarjuna@cgiar.org

Wild relatives continue to evolve in nature, and live by a different rule than the crop plants – which is survival of the fittest. Wild species have evolved to survive droughts, floods, extremes of heat and cold, and have the capability to withstand damage by insect pests and diseases that cause heavy damage to cultivated species. Many improved cultivars of crops are vulnerable to insect pests as they lack defense mechanism of their wild progenitors. The natural defense mechanisms in crop plants have been lost during intense selection for high yield, wider adaptability and improved nutritional quality.

Helicoverpa armigera, commonly known as cotton bollworm, tomato fruitworm, or legume pod borer, is a major pest of several crops including chickpea (*Cicer arietinum*) and pigeonpea (*Cajanus cajan*). Annual yield losses due to this pest have been estimated to be US$ 400 million per annum in India, and over US$ 1 billion in the semi-arid tropics. Chemical control of *H. armigera* has become increasingly difficult due to the development of resistance to commonly used insecticides. Widespread and injudicious use of insecticides to control *H. armigera* has not only led to the development of insecticide-resistant insects, but also has detrimental effects on the environment.

Wild relatives of pigeonpea and chickpea as sources of resistance to *Helicoverpa armigera*

Wild relatives of crops are important sources of resistance to biotic and abiotic constraints. Wild relatives of pigeonpea such as *Cajanus scarabaeoides*, *C. sericeus*, *C. acutifolius*, *C. albicans*, *Rhynchosia aurea*, *R. bracteata* and *Flemingia bracteata* are highly resistant to *H. armigera*. Some of the wild relatives of pigeonpea have also shown resistance to pod fly (*Melanagromyza obtusa*) and pod wasp (*Tanaostigmodes cajaninae*) (Sharma et al. 2003). Wild *Cicer* species such as *C. bijugum*, *C. reticulatum*, *C. judaicum*, *C. pinnatifidium*, *C. microphyllum* and *C. cuneatum* have shown high levels of resistance to *H. armigera* (Sharma et al. 2005). Accessions belonging to *C. bijugum*, *C. pinnatifidum* and *C. echinospermum* have also shown resistance to the bruchid *Collasobruchus chinensis*. Many of these accessions can be exploited to develop resistant crop cultivars.

Interspecific hybridization to transfer genes conferring resistance to *Helicoverpa armigera* from wild species belonging to the compatible gene pool

Wild relatives of pigeonpea and chickpea are classified into different gene pools based on their crossability with cultivated genotypes. Those species, which are cross compatible and set seed using conventional hybridization techniques are placed in compatible gene pool. There are many wild species in the compatible gene pool and many of these are being utilized for the improvement of cultivated species. It is important to select appropriate parents for exploitation of wild relatives of crops in crop improvement. This is particularly true in interspecific hybridization where the aim is to seek transfer of small fragments of genome of the donor parent into agronomically desirable cultivars. *Cajanus scarabaeoides*, a species of Indian origin that has many desirable characters (Upadhyaya 2006), is cross compatible with cultivated pigeonpea, and interspecific gene transfer is possible through conventional hybridization. Derivatives with resistance to *H. armigera* have been successfully produced. *Cajanus acutifolius*, a native of Australia, can also be crossed with pigeonpea as a one-way cross (Mallikarjuna and Saxena 2005). Hybrid embryos abort when *C. acutifolius* is used as the female parent. In vitro interventions are necessary to obtain hybrid plants (Mallikarjuna and Saxena 2002). An advanced generation population from the cross *C. cajan* × *C. acutifolius* has shown considerable variation for pod borer damage under unprotected field conditions (Fig. 1), and the progenies with less than 10% damage have been advanced further.

Evaluation of different accessions of *C. reticulatum* has led to the identification of several accessions for resistance to *H. armigera*. Accessions of *C. reticulatum* were used in the crossing program, and interspecific derivatives evaluated under unprotected field conditions for resistance to pod borer. Damage due to *H. armigera* varied from 0 to 70%. Laboratory bioassay, carried out in 2005, using 3rd instar larvae showed that some of the progenies resulted in reduced larval weight, delayed pupation, and at times failed to pupate. Even if pupae were formed, they took a long time to form pupae, or died before pupation, while those reared on cultivated chickpea, ICCV 10, pupated normally (Table 1). Further experiments will be conducted to check if the progeny exhibits abnormal larval and pupal growth.

Interspecific hybridization using incompatible wild species for *Helicoverpa armigera* resistance

In interspecific hybridization programs involving incompatible wild species, study of barriers to hybridization are essential. Barriers to hybridization in chickpea and pigeonpea wide crosses involving incompatible species is post-zygotic, with pre-zygotic barriers to a lesser extent (Mallikarjuna 1998). Application of growth regulators is mandatory to overcome barriers to crossability in both chickpea and pigeonpea. Hybrid embryo abortion is quite common in wide crosses involving species from the tertiary gene pools in both chickpea and pigeonpea. Reliable embryo rescue and tissue culture techniques are now available for chickpea and pigeonpea (Mallikarjuna 1998), which can be used to save aborting hybrid embryos. Embryo rescue involves the use of tissue culture techniques to save aborting hybrid embryos in vitro. In-ovulo embryo culture is favored rather than directly culturing the hybrid embryos on the medium. This facilitates the growth of the hybrid embryo inside the ovule without the ovule wall participating in the growth process. Once the embryos are big enough, they are separated and individually cultured on the tissue culture medium developed for the crop (Mallikarjuna 2003).

Cajanus platycarpus, a wild species in the tertiary gene pool of pigeonpea, has been successfully crossed with cultivated pigeonpea by hormone aided pollinations and the aborting hybrid embryos rescued in vitro (Mallikarjuna et al. 2006). Tetraploids have been obtained

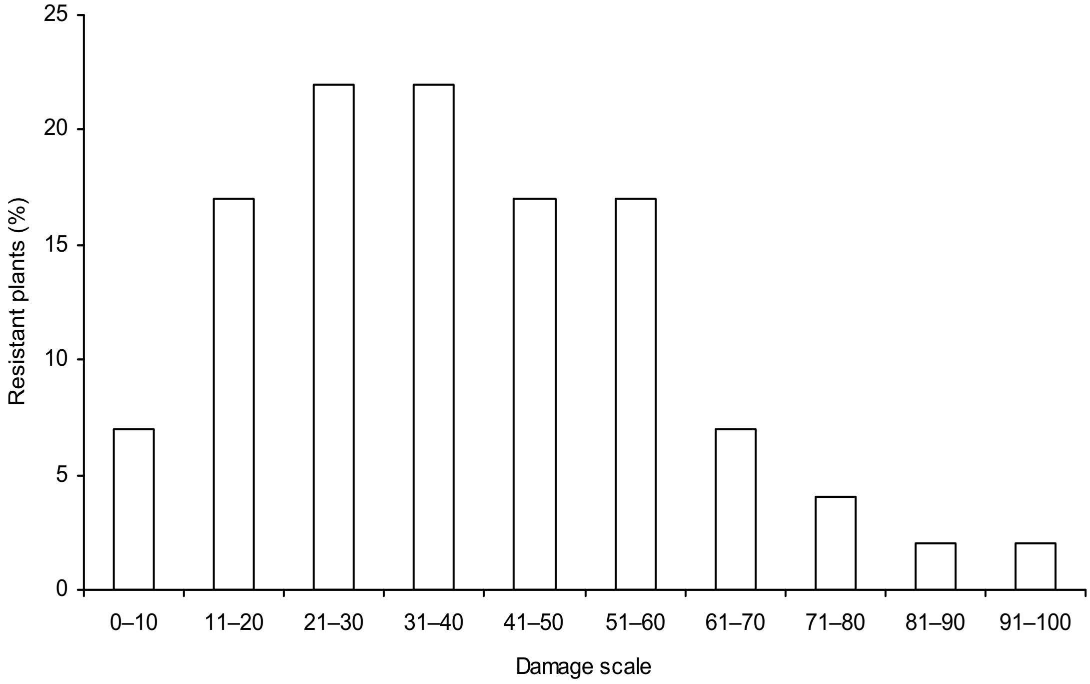

Figure 1. Field evaluation of advanced generation interspecific derivatives using *Cajanus acutifolius* for *Helicoverpa armigera* damage.

Table 1. Larval and pupal transformations on interspecific derivatives of cultivated chickpea (*Cicer arietinum*).

Identity	Initial weight of larva (mg)	Final weight of larva (mg)	Weight gain (mg)	Days to pupation[1]	No. of larvae pupated[2]
CP290-03	11.38	62.90	51.63	22 (3)	1 (2)
CP290-14	10.98	55.88	44.90	0 (5)	0
CP290-15	10.88	97.70	86.83	22 (2)	1 (2)
CP291-22	9.54	53.46	43.92	22 (1)	2 (2)
CP291-35	10.42	79.06	68.64	22 (3)	0 (2)
ICCV 10 (cultivated species)	10.48	135.58	125.10	18 (1)	4
ICC 3137 (cultivated species)	10.98	215.82	204.84	20	5
C. reticulatum (wild species)	10.40	66.30	56.00	22	0 (5)

1. Number of larvae is given in parentheses.
2. Number of dead larvae is given in parentheses.

by treating the hybrids with colchicine as the F_1 hybrids were 100% pollen sterile. The convertibility of diploids to tetraploids using colchicine treatments was less than 3%. Tetraploids at the F_3 and F_4 generations were screened for resistance to *H. armigera* under field conditions and pod damage was found to be quite low (<10%). Advanced generation diploid hybrids (F_1BC_4) were screened for resistance to *H. armigera* under field conditions and considerable variation (0 to 68%) was observed for *H. armigera* damage.

Levels of resistance to *H. armigera* in *C. judiacum*, *C. bijugum* and *C. pinnatifidum* are quite high (Sharma et al. 2005, 2006), but these are incompatible with the cultivated chickpea. There is some information on the nature of barriers to crossability in the annual wild *Cicer* species and methods are available to overcome them (Mallikarjuna 1998, Mallikarjuna et al. 2005a). A large number of *Cicer* species are perennials; however, none of these have been exploited for chickpea improvement. As reported in crosses with annual *Cicer* species, crossability barriers between chickpea and perennial *Cicer* species are post-zygotic (Mallikarjuna 2001, Babb and Muehlbauer 2005), and there is ample scope to use them in the improvement of cultivated chickpea.

The information generated so far has shown that wild relatives of chickpea and pigeonpea have high levels of resistance to *H. armigera*, and these can play a major role in developing cultivars with resistance to *H. armigera* for use in integrated pest management. There is considerable potential for transferring *H. armigera* resistance genes through interspecific hybridization using both compatible and incompatible species. Since incompatible species have separated from the crop plants over time and space, it is likely that the mechanisms of resistance or the components of resistance may be different in the two groups, which can be exploited for diversifying the basis of resistance to *H. armigera*. Many of the wild species have shown multiple resistance to insects and diseases. An interspecific population generated for *H. armigera* resistance may show resistance to other diseases too, as seen in the case of *C. platycarpus* derivatives, which showed resistance to Phytophthora blight, a fungal disease of pigeonpea (Mallikarjuna et al. 2005b). Often wide crosses can provide novel genetic variation (Hoisington et al. 1999) due to genome recombination not seen in the wild species, which will be highly useful for crop diversification.

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