

Tapping Genetic Diversity in ICRISAT Mandate Legumes Through Wide Hybridization: Progress and Prospects

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Introduction

Wild species form an important component of the genetic diversity of crop plants. At ICRISAT, wild species have had extensive use as donors of disease and pest resistance, of useful agronomic traits and for understanding the crop in terms of its diversity so that it can be effectively manipulated and exploited. Several studies have been conducted to evaluate the wild species of groundnut, chickpea and pigeonpea for important constraints (Kumar et al., 2005; Saxena et al., 1996, Reddy et al., 1996; Subrahmanyam et al., 2001; Prasada Rao et al., 2003; Reddy et al., 2000; Pande and Narayan Rao, 2005; Pande et al., 2006; Sharma et al., 2003; 2006, Srivastava et al., 2006).

Several groundnut lines have been developed and released with desirable characters transferred from wild *Arachis*, such as ICGV 86699 and ICGV 87165. One interspecific derivative, ICGV-SM 86715, was released as a cultivar in Mauritius. In total, more than 150 lines of groundnut have been registered by ICRISAT that are derived from interspecific derivatives. Wild species have been effectively utilized in other groundnut improvement programs and cultivars released (Simpson and Starr, 2001). Cytoplasmic male sterile systems in pigeonpea, were developed utilizing wild *Cajanus* (Saxena and Kumar, 2003; Saxena, et al., 2005; Mallikarjuna and Saxena, 2005) and wild species have given rise to high protein lines. In chickpea, crosses with wild species were utilized for the construction of molecular maps (Tekeoglu et al., 2002).

Disease resistance

ICRISAT mandate legumes are prone to attack by a gamut of fungal pathogens that are economically important. Specific examples of gene transfer by tapping genes from wild species for disease resistance include the following.

Phytophthora blight is a fungal disease of pigeonpea. Two accessions of *Cajanus platycarpus* are the only known sources of resistance to Phytophthora blight highly virulent P3 race (Reddy et al., 1996). Advanced generation interspecific derivatives generated using *C. platycarpus*, were screened for the disease, and the resistance transferred to the derivatives was found to be monogenic and recessive (Mallikarjuna et al., 2005; Mallikarjuna, 2003 a)

Late leaf spot is a serious foliar fungal disease of groundnut in both Asia and Africa and Early leaf spot is an important disease of Africa. Sources of resistance to late leaf spot (LLS) and early leaf spot (ELS) were obtained from crossing wild species *A. cardenasii*. Advanced generation lines resistant to early leaf spot were identified under African target location trial at Samanko, Mali (Genetic Resources and Enhancement Program, Archival Report 1998-99). As a bonus the lines also showed late leaf spot resistance under

ICRISAT field conditions. A total of 5000 interspecific derivatives were screened for LLS using wild *Arachis cardenasii*, *A. diogeni* and *A. stenosperma* for 3 consecutive years (2004-06), and 50 lines consistently showed resistance to LLS (Global Theme Biotechnology Archival Report, 2006). Some of the advanced generation interspecific derivatives with resistance to LLS showed resistance to *A. flavus* infection (Waliyar et al., 2007). *Aspergillus flavus* produces aflatoxin which is carcinogenic, teratogenic and immunosuppressant. This shows that multiple resistance/benefits can be derived depending upon the wild species used in the crossing program.

Botrytis gray mold is an important fungal disease of chickpea in Asia. Wild species of *Cicer* were evaluated for Botrytis gray mold under growth room conditions and many *Cicer* species were found resistant to the disease (Pande et al., 2006). Resistance to Botrytis gray mold was transferred from *Cicer reticulatum*, a wild relative of chickpea, and the resistance was found to be monogenic and recessive (Ramgopal, 2006).

Resistance to sterility mosaic disease (SMD), a major disease of pigeonpea, is limited in cultivated gene pool. *Cajanus albicans*, *C. platycarpus*, *C. cajanifolius*, *C. lineatus*, *C. scarabaeoides* and *C. sericeus* accessions have broad based resistance to multiple isolates of SMD (Kulkarni et al., 2003; LavaKumar et al., 2005). Interspecific hybrids using many of the above mentioned species are available which can be screened for SMD resistance.

Pest resistance

Pod borer (*Helicoverpa armigera*) is the most important pest of pigeonpea and there is concerted effort to transfer high levels of resistance from wild species. High levels of resistance to *H. armigera* have been observed in wild relatives of pigeonpea namely *Cajanus scarabaeoides*, *C. sericeus*, *C. acutifolius*, and *Flemingia bracteata* (Sharma et al., 2001). Using *Cajanus acutifolius*, a wild species from secondary gene pool, a large number of advanced generation interspecific derivatives were grown in the field for *H. armigera* screening. Screening experiments have shown a small number of progenies with no pod borer damage for the last 3 years and many of the progeny have shown less than 20% damage (Kameshwar Rao et al., 2003; Archival report, 2005). Crosses using *Cajanus platycarpus* gave rise to interspecific derivatives with a range of disease score for *H. armigera*, from 0-73% damage under unprotected field conditions (Archival report, 2005). High levels of resistance to pod borer was reported in *C. scarabaeoides*, with antixenosis, antibiosis and non-preferential oviposition mechanisms of resistance against pod borer (Sharma et al., 2001). Inheritance of antixenosis mechanism was reported in interspecific population using *C. scarabaeoides* (Rupakula et al., 2005).

Cicer reticulatum and *C. echinopsermum* have shown moderate levels of resistance to *H. armigera* (Sharma et al., 2005). It is now known that some of the wild have different mechanisms than those available in the cultivated germplasm, and have a great potential to increase the levels and diversify the basis of resistance to insect pests. *Cicer reticulatum* and *C. echinopsermum* were used to develop interspecific hybrids. Hybrids were screened for *H. armigera* and progeny with low leaf damage, slow and abnormal larval and pupal growth were identified. Efforts are in progress to determine the heritability of these traits.

Spodoptera litura is no more considered as a minor pest of groundnut in India. In addition, it is a major pest of cotton as is *H. armigera*. Hence introduction of resistance to *Spodoptera* may be a good strategy as a precautionary measure. Accessions belonging to *Arachis cardenasii*, *A. duranensis*, *A. kempff-mercadoi* and *A. triseminata* in groundnut have shown resistance to leaf caterpillar (*Spodoptera litura*), leaf miner (*Apraorema modicella*), and the jassid (*Empoasca kerri*) (Sharma et al., 2003; Stevenson et al., 1993). *Arachis kempff-mercadoi* was used in the crossing program and interspecific derivatives generated. Some of the derivatives showed high level of resistance to *Spodoptera litura*. Further investigations confirmed the presence of three bioflavonoids - chlorogenic acid, quercetin and rutin, conferring resistance to *Sopdoptera litura* (Mallikarjuna et al, 2004 a & b). Molecular marker assisted screening can hasten the development of resistant lines and avoid laborious, time consuming and unpredictable field screening due to vagaries of nature.

Accessions belonging to *Sorghum laxiflorum*, *S. australiense*, *S. dimidiatum*, *S. purpureosericeum*, and *S. intrans* are highly resistant to sorghum shoot fly (*Atherigona soccata*) and spotted stem borer (*Chilo partellus*) (Sharma et al., 2005). There is no report of successfully crossing the above-mentioned species with cultivated sorghum.

Agronomic traits

Wild species are not only good sources of resistance to diseases and pests but can also contribute economically good agronomic characters such as CMS and apomixis. To date, five CMS systems have been reported in pigeonpea using cytoplasm from *Cajanus* species (Saxena and Mallikarjuna, 2007): A₁ cytoplasm, derived from *C. sericeus* (Saxena et al., 1996); A₂ cytoplasm, derived from *C. scarabaeoides* (Tikka et al., 1997); A₃ cytoplasm, derived from *C. volubilis* (Wanjari et al., 2001); A₄ cytoplasm derived from *C. cajanifolius* (Saxena et al., 2005); and A₅ cytoplasm derived from cultivated pigeonpea (Mallikarjuna and Saxena, 2005) with nuclear genome from *C. acutifolius*. Apomixis has been identified in chickpea, when crosses are made using wild species from incompatible gene pool as the female parent. Fluorescence microscopy, cytology, anatomy and molecular analysis with RAPDs and SSR were used to confirm apomixis. (Mallikarjuna, 2003b; USAID linkage grant: first year report, 2003). We are in the process in further confirming the trait through molecular analysis.

Broadening the genetic base of groundnut, chickpea and pigeonpea.

Cultivated groundnut is a polyploid species (amphidiploid). It has been reproductively isolated from its progenitor and other related species, leading to a narrow genetic base. Development of synthetic groundnuts by combining the genomes that gave rise to amphidiploid groundnut as well as between various other diploid species has been undertaken. These efforts to develop synthetic groundnut, will broaden the genetic base of the crop and lead to the introduction of useful genes/traits lost during the course of evolution.

We have taken the lead in understanding the constraints and developing methods to cross wild species from section *Arachis* to which cultivated groundnut belongs, and other sections of *Arachis*, which were never crossed before. As a result, hybrids are available between members of section *Arachis* and wild species *A. glabrata* (section *Rhizomatosae*). *A. glabrata* is an important species because it has multiple resistance to

fungal foliar pathogens (ELS, LLS and rust), insect pests (thrips, aphids and jassids) and viral diseases (peanut mottle, peanut bud necrosis and peanut stripe). Resistances to foliar fungal and viral diseases were successfully transferred to the hybrids (Mallikarjuna, 2003a; Mallikarjuna and Sastri, 2002; Mallikarjuna, 2002). *Arachis kretschmeri* (resistant to LLS, rust and rosette) and *A. chiquitana* (resistant to LLS, *Aspergillus flavus* colonization and aflatoxin production) belong to section *Procumbentes*. Tissue culture techniques developed to save aborting embryos from interspecific crosses (Mallikarjuna and Sastri, 2002) were applied to save hybrid embryos from *A. hypogaea* x *A. kretschmeri* and *A. hypogaea* x *A. chiquitana* crosses and fertile interspecific hybrids were produced (Mallikarjuna, 2003a; 2007). Initial screening experiments using *A. hypogaea* x *A. chiquitana* F₁BC₁ hybrids have shown some of the seeds devoid of *Aspergillus flavus* infection (Mallikarjuna, 2007).

Various studies have shown that chickpea also has a narrow genetic base (Millan et al, 2006), and this is one of the reasons for the absence of resistance to various diseases and pests such as *Ascochyta* blight (AB), *Botrytis* gray mold (BGM), and resistance to *Helicoverpa armigera* (pod borers). ICRISAT has taken the lead and developed techniques to cross incompatible wild *Cicer* (Mallikarjuna, 1999; 2007) to produce hybrids (Mallikarjuna et al., 2005).

Although pigeonpea exhibits morphological diversity, the same cannot be said at the molecular level (Yang et al., 2006). A major research initiative to cross wild species from different gene pools was undertaken to introduce resistances to diseases and pests, thus broadening the genetic base of pigeonpea. *Cajanus platycarpus* is a wild species from the tertiary gene pool of *Cajanus*. Embryo rescue and tissue culture techniques were developed in house and used to obtain interspecific derivatives. The derivatives showed extra early flowering, variation for plant type, pollen fertility, CMS, nature and color of flowers and pods (Cherian et al., 2005). It is known that wide crosses can provide novel genetic variation (Hoisington et al., 1999). Knowledge on the barriers operating to access genes from tertiary gene pool of *Cajanus* and methods to overcome them are now available (Mallikarjuna, 1998, 2003 a; Mallikarjuna et al., 2006).

Prospects

As in the case of stem rust (Ug 99) of wheat (CIMMYT E-News, vol 3 no. 12, December 2006) and *Phytophthora* blight of potato (<http://www.cipotato.org/glib>), wild species continue to offer useful genes for the important abiotic and biotic constraints caused by fungi, bacteria, viruses, insect pests and climate change. There are various instances where unintentionally many more useful genes/traits are transferred when wide crosses are attempted. Wide crosses are useful to transfer polygenic characters such as rust and late leaf spot resistance in groundnut in one step and the major constraint of linkage drag can be overcome by the use of molecular markers and linkage maps.

Development of synthetic groundnuts will generate an array of amphidiploid population with desirable genes/traits as witnessed in wheat and brassica, thus bringing in variation into the groundnuts, which has a monophyletic origin.

Wild species in secondary gene pool are not as difficult to cross as those in the tertiary gene pool. ICRISAT was a pioneer in developing techniques to cross wild species in the tertiary gene pools of groundnut, pigeonpea and chickpea (Mallikarjuna, 1998,1999;

Mallikarjuna and Sastri, 2002). Using these techniques genetic diversity from tertiary gene pool can be captured.

Evaluation of wild species for various constraints is an on-going process at ICRISAT which is essential for effective utilization of wild species to overcome important biotic and abiotic constraints of groundnut, chickpea and pigeonpea, as and when they arise due to various reasons including climate change.

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