CHAPTER 10

MOLECULAR GENETICS AND BREEDING OF GRAIN LEGUME CROPS FOR THE SEMI-ARID TROPICS

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Abstract: Grain legumes are important crops for providing key components in the diets of resource-poor people of the semi-arid tropic (SAT) regions of the world. Although there are several grain legume crops grown in SAT, the present chapter deals with three important legumes i.e. groundnut or peanut (*Arachis hypogaea*), chickpea (*Cicer arietinum*) and pigeonpea (*Cajanus cajan*). Production of these legume crops are challenged by serious abiotic stresses e.g. drought, salinity as well as several fungal, viral and nematode diseases. To tackle these constraints through molecular breeding, some efforts have been initiated to develop genomic resources e.g. molecular markers, molecular genetic maps, expressed sequence tags (ESTs), macro-/micro- arrays, bacterial artificial chromosomes (BACs), etc. These genomic resources together with recently developed genetic and genomics strategies e.g. functional molecular markers, linkage-disequilibrium (LD) based association mapping, functional and comparative genomics offer the possibility of accelerating molecular breeding for abiotic and biotic stress tolerances in the legume crops. However, low level of polymorphism present in the cultivated genepools of these legume crops, imprecise phenotyping of the germplasm and the higher costs of development and application of genomic tools are critical factors in utilizing genomics in breeding of these legume crops.

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1. INTRODUCTION

1.1. Importance of Legume Crops

Grain and forage legumes are grown on some 180 million hectares, or 12% to 15% of the Earth's arable surface (source: FAO Database [http://apps.fao.org/page/collections]). They account for 27% of the world's primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen needs of humans (Vance et al., 2000). Grain legumes are key components in the diets of resource-poor people in the developing world; especially those who are vegetarian because of choice or cannot afford to supplement their diets with meat. Grain legumes are a rich source of essential vitamins, minerals, and important amino acids like lysine (Duranti and Gius, 1997; Grusak, 2002). Last but not least, grain legumes can also contribute to the Nitrogen balance of soils where they are grown, making them an indispensable component of the sustainability of the system. Another attractive feature is their ability to fix atmospheric nitrogen in the soil by virtue of their symbiotic association with *Rhizobium* bacteria (Schulze and Kondorsi, 1998; Serraj, 2004), thus reducing the need for N-fertilizers in the cropping systems. Legumes often attract higher market prices than other staple crops, making them an important source of income for farmers.

Legumes belong to the taxonomic family *Fabaceae*, containing over 18,000 species divided into the three sub-families Mimosoideae, Caesalpinoideae and Papilionoideae. Legume species have been cultivated for millennia all over the world because of the nutritional value of their seeds as mentioned above. Among different legumes, soybean (*Glycine max* L.) is the major single contributing species, which is used for multiple applications in the food and feed industries. Others, such as chickpea (*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.), groundnut or peanut (*Arachis hypogaea* L.), cowpea (*Vigna unguiculata* L.) and pigeonpea (*Cajanus cajan* L.) contribute significantly to the diets of large numbers of people in Asia, Africa, and South America. The high nutritional value of legumes is achieved by the presence of a wealth of secondary metabolites and in the capacity of legumes to establish a symbiosis with the soil bacteria *Rhizobium*, which supplies nitrogen to the plant in exchange of carbohydrate supply to the microsymbiont (Dixon and Sumner, 2003, Desbrosses et al., 2005). The symbiosis results in the formation of root outgrowth called nodules, which can have different types of shape depending on plants. That symbiosis gets preferentially established under low N conditions, and gets inhibited under excess nitrogen, although certain species are able to obtain most of their nitrogen from the symbiosis in environments that do contain nitrogen. Nodules host the *Rhizobium* bacteria, which differentiate in the nodules into symbiotic bacteroids, and are the site of catalysis of dinitrogen into ammonia by the microbial enzyme nitrogenase. As an energy source to achieve N fixation, the bacteria obtain dicarboxylic acids from the host plant. By a complex amino-acid cycle the reduced nitrogen is provided to the plant (Lodwig et al., 2003) where it is accumulated into proteins. Thus legumes can also help replenish nutrient-depleted soil.

1.2. Legume Crops in the Semi-Arid Tropics

The semi-arid tropics (SAT) covers parts of 55 developing countries where the 75–180 day growing period has a mean daily temperature of more than 20°C. The dry semi-arid tropics have very short growing seasons, separated by very hot and dry periods in which growth without irrigation or stored soil moisture is impossible. Natural soil fertility is often low, in part because soils are highly weathered by the dry-hot and humid-hot cycles, and pest and disease pressure can be intense. Farmers face further substantive risks, even within the growing season, as there are irregular periods of drought and high evaporative demand which can seriously compromise crop productivity. Based on 1996 statistics, the SAT is home to about 1.4 billion people, of which 560 million (40%) are classified as poor, and 70% of the poor reside in rural areas (Ryan and Spencer 2001).

Although a number of crops are grown in SAT areas, among legume crops, chickpea, groundnut common bean, cowpea and pigeonpea provide key components in the diets of resource-poor people in the developing world. We, at ICRISAT, together with our National Agricultural Research System (NARS) partners are engaged on crop improvement in chickpea, groundnut and pigeonpea, therefore in this article we discuss the advances in the area of genetics and genomics applied to breeding in only these three legume crops. In the first instance, a brief introduction of these crops is given in following sections.

1.2.1. Chickpea (Cicer arietinum L.)

Chickpea is the third most important grain legume globally, and second in importance in Asia. It is also an important legume crop in Eastern and Southern Africa. About 90% of the global area and 88% of production is concentrated in Asia. Chickpea has one of the best nutritional compositions of any dry edible legume, and is mainly used for human consumption. The *desi* type (colored seed coat) is usually de-hulled and split to make *dhal* or flour (*besan*), while *kabuli* types (white or cream-colored seed coat) is often cooked as whole grain. The haulms are used for animal feed. Chickpea improves soil fertility through nitrogen fixation (up to 140 kg N/ha). Chickpea area has slightly decreased globally, but has been stable at 9 M ha in Asia for the past 25 years. However, production in Asia has increased by 39% due to a 32% increase in productivity. Even then, the current average yield in Asia (0.8 t/ha) is low, and far below the potential yield (5 t/ha), or research station yields (3.5 t/ha). The global demand for chickpea in 2010 is estimated at 11.1 Mt (up from the current 8.6 Mt). A combination of productivity enhancement through crop improvement enhanced with biotechnological tools, integrated crop management and expansion of area to new niches and production systems are needed to achieve this target.

According to van der Maesen (1987), the cultivated chickpea has been taxonomically placed in the genus *Cicer*, which belongs to the family *Fabaceae* and its monogeneric tribe *Cicereae* Alef. Presently, the genus consists of 43 species divided into 4 sections, namely *Monocicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer*.

This classification is based on their morphological characteristics, lifestyle and geographical distribution (van der Maesen, 1987). Eight of these *Cicer* species share the annual growth habit with chickpea are of particular interest to breeders.

1.2.2. Groundnut (Arachis hypogaea L.)

Groundnut is an important food and cash crop for the resource-poor farmers in Asia and Africa. It is primarily grown for edible oil (48–50%) as well as for direct consumption as food by people. Groundnut haulms are excellent fodder for cattle, and groundnut cake (after oil extraction) is used as animal feed. It contributes significantly to household food security and cash income through the sale of groundnut products. Groundnut productivity in Western and Central Africa (WCA) and Eastern and Southern Africa (ESA) is below the world average yield of 1.4 t/ha. Although groundnut productivity in Asia (1.8 t/ha) exceeds the world average, it is still lower than the yields in developed countries (3 t/ha). The area under groundnut in ESA has increased dramatically from 2.3 to 3.3 M ha during 2000 to 2004. In Asia, the area under groundnut is increasing in China and Vietnam, but is declining in India during 1991–2004. There has been a slight decline in area in WCA. Although global productivity has shown a positive trend, much more needs to be achieved in future.

The genus *Arachis* belongs to the family *Fabaceae*, subfamily Papillionaceae, tribe Aeschynomenae, subtribe Stylosantheae. Cultivated groundnut (*Arachis hypogaea* L.) can be botanically classified into two subspecies, *hypogaea* and *fastigiata* that are distinguished based on branching pattern and distribution of vegetative and reproductive nodes along lateral branches. Each subspecies is again divided into two botanical varieties; subsp. *hypogaea* into var. *hypogaea* (virginia) and var. *hirsuta* and subsp. *fastigiata* into var. *fastigiata* (valencia), var. *vulgaris* (spanish), var. *peruviana* and var. *aequatoriana* (Karpovickas and Gregory, 1994).

1.2.3. Pigeonpea (Cajanus cajan L.)

Pigeonpea is a versatile and multipurpose crop. It is one of the major food legumes in the tropical and sub-tropical regions in Africa, Asia and the Caribbean countries. Its green pods and seeds are consumed as a vegetable, and the dry grains are cooked whole or after dehulling (as *dhal*). The foliage is used as fodder, and the dry sticks are used for fencing, thatching, and as firewood. It fixes atmospheric nitrogen, and the extensive leaf fall adds organic matter to the soil. Dry grain is also used for animal feed. About 90% of the global pigeonpea area (4.4 M ha) is in Asia (about 86% in India). Other major countries where pigeonpea is grown are Myanmar, Nepal, Bangladesh, Pakistan and China. In Sub-Saharan Africa (SSA), pigeonpea is grown in Uganda, Kenya, Malawi, Mozambique, Zimbabwe, Zambia, South Africa, Sudan and Ethiopia; but reliable statistics are not available. Pigeonpea production has shown only a marginal increase during the past two decades (2.2 to 2.9 million t during 1980–98). However, productivity has remained stagnant at 0.7 t/ha, mostly because it is intercropped with cereals or cotton and receives no or little inputs; or

Table 1. Characteristics and genomics data available for some SAT legumes

	Chickpea	Groundnut	Pigeonpea
Species name	*Cicer arietinum* L.	*Arachis hypogaea* L.	*Cajanus cajan* L.
Ploidy level and chromosome number	2n = 2*x* = 16	2n = 4*x* = 40	2n = 2*x* = 22
Genome size[1]	931 Mbp	2891 Mbp	858 Mbp
SSR markers	~700 (Winter et al., 1999; Huettel et al., 1999; Sethy et al., 2003,2006b; Lichtenzveig et al., 2005; Choudhary et al., 2006; Varshney et al., unpublished; Bhatia et al., unpublished results)	~700 (Hopkins et al., 1999; He et al., 2003; Ferguson et al., 2004; Moretzsohn et al., 2004; Palmieri et al., 2005; Mace et al., unpublished; D. Bertioli, Brazil, pers. commun.; S. Knapp, pers. commun.)	~100 (Burns et al., 2001; Odoney et al. 2007)
BAC libraries	3.8 X (Rajesh et al. 2004), 7 X (Lichtenzveig et al. 2005)	6.5–9.0 X (Yuksel and Paterson, 2005)	–
ESTs	~2000 (NCBI, Buhariwalla et al., 2005)	~7538 (NCBI, Luo et al., 2005; S. Knapp, pers. commun.)	More than 884 (NCBI) (Gaikwad et al. unpublished
Gene arrays	768- features microarray (Coram and Pang, 2005a), SAGE Gene Chip (P. Winter, Germany, pers. commun.)	400 unigene array (Luo et al., 2005)	

[1] As per estimate of Royal Botanic Gardens, Kew, UK (http://www.rbgkew.org.uk/cval/)
[2] NCBI = http://www.ncbi.nlm.nih.gov/

gets relegated to marginal and poor soils, often where no other crop can be grown. Additionally, pigeonpea has also generally a poor harvest index.

Pigeonpea belongs to the *Cajaninae* sub-tribe of the economically important leguminous tribe *Phaseoleae* that contains soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris*) L.) and mungbean (*Vigna radiata* L.) (Young et al., 2003). The genus *Cajanus* comprises 32 species most of which are found in India and Australia although one is native to West Africa. Pigeonpea is the only cultivated food crop of the *Cajaninae* sub-tribe and has a diploid genome.

A brief overview on genome size, ploidy level, existing genomics resources in chickpea, groundnut and pigeonpea is given in Table 1.

2. CHALLENGES IN SUSTAINABLE CROP PRODUCTION OF SAT LEGUMES

2.1. Abiotic Stresses

Abiotic stresses severely limit agricultural production. There is a clear consensus that drought is among the most severe stress for legume production in SAT regions

of Asia and Africa while salinity is the second ranked constraint in the production of these legumes in some Asian countries.

2.1.1. Drought

The SAT regions are characterized by short and erratic rainfall (and then long periods with virtually no rain), where crops grown under rainfed conditions suffer from both intermittent and terminal drought stress, and crop grown in residual moisture after the rain suffer terminal drought, thus incurring major yield losses. Water deficit is one of the most severe stresses for sustainable crop production. Worldwide, yield losses each year due to drought are estimated to be around US$500 million (Sharma and Lavanya, 2002).

Water capture by roots and water-use efficiency are probably two important components of the yield architecture, as defined by Passioura (1977) that are important for crops growing under terminal drought conditions. These two traits are the classical component of what is called ‘drought avoidance’, and which means getting more water or using it more efficiently). Drought avoidance is considered to be the major trait of interest to expand production to presently uncropped areas and post-rainy fallows in SAT regions. Although roots have already proved to be beneficial for yield under terminal drought (chickpea, Kashiwagi et al., 2004), there is a need to understand better how root traits contribute to drought avoidance, and a need to explore them in those crops where little information on roots has been acquired (e.g., groundnut). Specifically, there is a need to understand the dynamics of roots, how roots contribute to the overall water budget, and more interestingly how they contribute at the time of grain filling, and how they contribute at the time of flowering. Recent studies at ICRISAT indicate that deeper rooting correlates with a higher harvest index (HI) in chickpea in conditions of more severe drought (Kashiwagi et al., 2004, 2006). This might be related to the root being able to supply water during flowering and allowing less flower drop because of water deficit. Water use efficiency (WUE) or more specifically transpiration efficiency (TE) is another trait that is being addressed in groundnut at ICRISAT by using different biotechnological, physiological and breeding approaches. For TE, there is also a need to understand better the mechanisms that lead to better TE, if we ever want to reach the genes involved.

2.1.2. Salinity

Soil salinity is an important limiting factor for crop yield improvement, which affects 5–7% of arable lands, i.e. approximately 77 million ha worldwide. Most crops are sensitive to salt stress at all stages of plant development, including seed germination, vegetative growth and reproductive growth, although the latter stage is certainly the most sensitive across many crops. Legumes, in general, are sensitive to salinity, and within legumes, chickpea, fababean and pigeonpea are more sensitive than other food legumes. The salinity problem is increasing, in particular in areas where irrigation is a common practice (Ghassemi et al., 1995). Though management options exist to alleviate salt effects, these are often in contradiction with the

immediate economic choices of the concerned farmers; thus crop improvement for salt tolerance appears to be the best and economic alternative.

The problem of salinity is basically two-fold. In one case, soil is saturated with sodium (Na) and soil pH remains within an optimal range for crop growth. This type of salinity refers to coastal or dryland salinity. These are soils that get saturated with sodium because an existing saline ground water table rising (proximity to the sea or salt that has accumulated in the soil profile), bringing the salt to the surface. In a second case, soil is both saturated with Na (exchangeable sodium percentage, ESP, > 6) and pH has reached levels above 8.5–9.0. This type of salinity is also called transient salinity, and is thereafter referred to as *sodicity* or *sodic soils*. In this case, the sodium saturation brings about the same effect as salinity, but the high pH dramatically affects the availability of micronutrients (low availability/solubility of micronutrient salts at these pH levels), the soil structure and porosity (poor drainage, tendency for water logging, little oxygenation because of saturation of the exchange complexes in the soil by sodium). In the past, most studies have focused on *salinity*, and only a few on *sodicity*.

Despite the importance of salinity in crop production worldwide and the abundant knowledge on the effect of salinity on plant growth and development, there has surprisingly been little effort to breed for improved salinity tolerance, with the exceptions of wheat, rice, barley, alfalfa and claims of soybean. Breeding tolerant crop varieties is therefore urgently needed.

2.2. Biotic Stresses

The major biotic factors of SAT legumes are diseases and insect pests. The chickpea diseases of major importance are ascochyta blight (caused by the necrotrophic fungus *Ascochyta rabiei* (Pass.) Lab.), fusarium wilt (caused by *Fusarium oxysporum* f. sp. *ciceris*), Botrytis gray mold and root rots caused by *Sclerotium* and *Pythium.* Majority of these diseases affect all aerial parts of the plant. Among the pigeonpea diseases, sterility mosaic (viral disease), fusarium wilt (caused by the fungus *Fusarium udum* Butler), and phythophthora blight (*Phytophthora drechsleri*) are major diseases causing significant losses of pigeonpea yield. In groundnut, rust, late leaf spot, and early leaf spot are serious diseases worldwide, which cause 50–60% pod yield loss. Rust and late-leaf spot often occur together and the pod yield loss can exceed 70% in the crop. Besides adversely affecting pod yield and its quality, these foliar diseases also affect haulm (fodder) yield and quality. Whereas the level of resistance available in cultivated groundnut to rust is very high, for early- and late-leaf spot, it is low. Wild *Arachis* species harbour many useful resistance genes against various diseases and insect pests. Of the important biotic constraints specific to sub-Saharan Africa (SSA), the groundnut rosette disease (GRD), vectored by aphids, is endemic to the continent and its adjoining islands and epidemics occur often throughout SSA, reducing groundnut production and crippling rural food security.

More than 200 species of insects feed on pigeonpea and chickpea, of which pod borer (*Helicoverpa armigera*), spotted pod borer (*Maruca vitrata*), pod fly (*Melanagromyza obtuse*), pod sucking bugs (*Clavigralla* spp., *Nezara viridula*) and the bruchid (*Callosobruchus* spp.) are most important economically (Singh et al., 1990). *Helicoverpa* causes an estimated loss of US$ 317 million in chickpea and pigeonpea (ICRISAT, 1992), and possibly over US$ 2 billion on other crops worldwide. A conservative estimate is that over US$ 1 billion is spent on insecticides to control this pest. Therefore, in addition to the huge economic losses caused directly by the pest, there are several indirect costs from the deleterious effects of pesticides on the environment and human health (Sharma, 2001). These insect pests feed on various plant parts such as leaves, tender shoots, flower buds, and immature seeds. It has been difficult to breed for *Helicoverpa* resistance in chickpea and pigeonpea because sources with a high level of resistance are not available in the cultivated species of these legumes. Recent studies show potential of utilizing the wild species in insect pest resistance breeding programme as these have shown higher levels of resistance.

3. UTILIZATION OF PLANT GENETIC RESOURCES (PGRS)

Availability and characterization of suitable germplasm is a critical factor for utilizing genetic variation in crop breeding. Fortunately for all the three legume species mentioned in the article, a large number of accessions are present in different genebanks throughout the world (Dwivedi et al., 2006). For instance, ICRISAT, under an agreement with FAO, holds 16,853 cultivated and 117 wild accessions of *Cicer* species, whereas the International Centre for Agricultural Research in Dryland Areas (ICARDA), Syria, under the same FAO agreement, maintains 8,342 cultivated and 255 wild accessions. Other institutions holding chickpea germplasm are the National Bureau of Plant Genetics Resource (NBPGR), India (14,566 accessions); Centre for Legume Improvement in Mediterranean Area (CLIMA) (4,351 accessions) and AusPGRIS (7922 accessions) in Australia; United States Department of Agriculture (USDA), USA (4,662 accessions); and the Seed and Plant Improvement Institute, Iran (4,925 accessions). The European *Cicer* database contains 3,700 cultivated accessions from 11 countries (Pereira et al. 2001). For groundnut, ICRISAT holds, under the same agreement with FAO, 14,126 accessions of cultivated peanut and 293 accessions of wild *Arachis* species from 93 countries. Other institutions holding large numbers of peanut accessions are the National Research Centre for Groundnut (NRCG), India (7,935 accessions) and the USDA Southern Regional Plant Introduction Station, USA (6,233 accessions). In the United States, wild *Arachis* species are maintained at North Carolina State University, Raleigh (250 accessions) and at the Texas Agricultural Experiment Station (TAMU), Texas (300 accessions). For pigeonpea, ICRISAT holds under the agreement with FAO 12,398 pigeonpea accessions of cultivated and 314 accessions of wild species from 74 countries. Other institutions holding substantial amounts of pigeonpea germplasm

include the NBPGR (5,454 accessions) in India and the USDA, Southern Regional Plant Introduction Station (4,116) in USA.

3.1. Core and Mini-Core Collections

Despite the availability of a large number of germplasm, only limited numbers of accessions have been used in breeding programme not only in SAT legumes but other crop species as well (Dwivedi et al., 2006). One of the main reasons for this fact may be the large sizes as well as non-availability of information on germplasm collections. Core collections present a manageable and cost-effective entry point into germplasm collections for identifying parental genotypes with new sources of disease and pest resistance or abiotic stress tolerance. Evaluation of core collections is usually the most efficient and reliable means of carrying out an initial search of the germplasm collections. For instance, early evaluation of limited number of germplasm accessions led to premature conclusion that no variability for salinity tolerance existed in chickpea (Saxena, 1984). However, recent screening of large number of germplasm accessions, including the chickpea mini-core collection, revealed very large variation, readily usable for breeding purposes (Vadez et al., 2006). Evaluation of larger amounts of germplasm through multi-location trials is both very expensive and time consuming; large-scale generation of accurate and precise evaluation data from such trials is generally not possible, thus dramatically reducing the probability of identifying desirable material. Core collections usually consist 10% of the entire germplasm collection that represents the collections variability (Brown, 1989). These representative subsample collections are developed from the entire collection, using all available information on accessions including the origin and geographical distribution plus characterization and evaluation data. Ten percent of most crop germplasm collections are a much more feasible amount of material for intensive and precise evaluation.

Most core collections have been designed from global or regional collections held within international agricultural research centers or national program gene banks, while a few have also been developed for wild accessions (Tohme et al., 1996). After evaluating a total of 16,991 chickpea accessions for 13 traits and 14,310 groundnut and 12,153 accessions of pigeonpea for 14 traits each, the core collections of chickpea, groundnut and pigeonpea with 1,956 (Upadhyaya et al., 2001a), 1,704 (Upadhyaya et al., 2003) and 1,290 accessions (Reddy et al., 2005), respectively have been developed at ICRISAT. In addition, the core collection of 505 genotypes of chickpea was developed after analysis of 3,315 genotypes (Hannan et al., 1994). Similarly for groundnut, an USDA core collection with 831 genotypes after evaluating 7,432 accessions for 24 traits (Holbrook et al., 1993) and an Asian core collection based on evaluating 4,738 genotypes for 15 traits (Upadhyaya et al., 2002) are available. Although these core collections have been useful for identifying diverse sources for traits of interests and broadening the genetic base of cultivars for a crop (Upadhyaya et al., 2001b, 2006a; Krishnamurthy et al. 2003;

Serraj et al., 2004), even a core collection can be too large so a further reduction is also valuable providing it is not associated with losing too much of the spectrum of diversity. Upadhyaya and Ortiz (2001) developed a strategy for sub-sampling a core collection to develop a mini-core collection, based on selecting 10% of the core accessions representing the variability of larger collection of species. In this process, the core collection is evaluated for various morphological, agronomic, and quality traits to select a 10% subset from this core subset (i.e., 1% of the entire collection) that captures a large proportion (i.e. more than 80% of the entire collection) of the useful variation. Selection of core and mini-core collections is based on standard clustering procedures used to separate groups of similar accessions combined with various statistical tests to identify the best representatives. The mini-core collection developed at ICRISAT for chickpea consisted of 211 accessions (Upadhyaya and Oritz, 2001), while the groundnut (Upadhyaya et al., 2002) and pigeonpea (Upadhyaya et al., 2006b) mini-core consists of 184 accessions and 146 accessions, respectively. Both core or mini-core germplasm collections have been used for identifying a range of germplasm with beneficial traits for use in breeding programs (see Dwivedi et al., 2006 for references). Increasing concern of trade and food processors for consistent and better quality and physical specifications, however, suggest further characterization of core or mini-core collections for quality and market traits.

3.2. Molecular Characterization of PGRs

The core or mini core collections have been developed based on morphological or agronomic traits; little information is available on molecular genetic diversity present in the germplasm collection. Molecular characterization of germplasm is a particularly useful tool for assisting genebank curators to better manage genetic resources, helping them to identify redundant germplasm and to provide scientists with the most diverse germplasm for applications in research and breeding (Bretting and Widrlechner, 1995; Virk et al., 1995; Brown and Kresovich, 1996; van Treuren et al., 2001; Upadhyaya et al., 2006b). Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles (Tanksley and McCouch, 1997). As a part of the Generation Challenge Programme (GCP) of the CGIAR, molecular characterization of global composite collections of the SAT legumes is in progress at ICRISAT. For example, genotyping of about 3000 chickpea accessions (Upadhyaya et al., 2006a) with 50 SSR markers and 1000 groundnut accessions with 20 SSR markers, in collaboration with ICARDA (Syria) and EMBRAPA (Brazil) respectively has already been completed. Molecular characterization of 1000 pigeonpea accessions at 20 SSR loci is in progress. These studies provide estimates on genetic diversity and the population structure of the germplasm that can be used to define the most diverse collection, called ‘reference collection’ for using in association mapping studies (see later).

4. MOLECULAR BREEDING FOR SAT LEGUMES

Legume breeders have made major contributions to combat the problem of both abiotic and biotic stresses in the past but the pace and extent of improvements must be dramatically increased to attend to parallel demands. Recent advances in the area of biotechnology have offered the tools in the form of molecular markers to assist the breeding practices (Jain et al., 2002). Molecular markers are powerful diagnostic tools that detect DNA polymorphism both at the level of specific loci and at the whole genome level (reviewed by Azhaguvel et al., 2006). As compared to morphological traits/markers, molecular markers have several advantages as they are phenotypically neutral and are not influenced by pleiotropic and epistatic interactions, and their expression is not dependent on plant age/part (Jones et al., 1997). In fact the use of molecular markers in improving the breeding efficiency in plant breeding was suggested as early as in 1989 (Tanskley et al., 1989; Melchinger, 1990). In this regard, once linkage between a gene for the agronomic trait of interest and marker locus is established, then DNA diagnostic tests can be used to guide plant breeding (Morgante and Salamini, 2003; Gupta and Varshney, 2004). The selection of useful lines for breeding with the help of linked molecular markers is called marker-assisted selection (MAS). Use of MAS is especially advantageous for traits with low heritability where traditional selection is difficult, expensive or lack accuracy or precision.

A variety of molecular markers exist, such as RFLPs (*R*estriction *F*ragment *L*ength *P*olymorphism*s*, Botstein et al., 1980), RAPDs (*R*andom *A*mplification of *P*olymorphic *D*NA*s*, Williams et al., 1990), AFLP*s* (*A*mplified *F*ragment *L*ength *P*olymorphsim*s*, Vos et al., 1995) and microsatellites or SSRs (*S*imple *S*equence *R*epeats, Tautz, 1989). Among the different classes of molecular markers, SSR markers are often chosen as the preferred markers for a variety of applications in breeding because of their multiallelic nature, codominant inheritance, relative abundance and extensive genome coverage (Gupta and Varshney, 2000). More recently, markers such as SNPs (*S*ingle *N*ucleotide *P*olymorphism*s*, Rafalski, 2002) and DArT (*D*iversity *Ar*ray *T*echnology, Killian et al., 2005) have been added to list of preferred marker systems for breeding.

MAS in breeding has revolutionized the improvement of temperate field crops (Koebner, 2004; Varshney et al., 2006) and will have similar impacts on breeding of tropical legume crops, particularly for traits where phenotyping is only possible late in the season, and where screening of traits is difficult or prohibitively expensive. Breeding for enhanced drought and salinity tolerance is notoriously difficult due to the genetic complexity of these traits, the high genotype-by-environment interaction and the difficulties of carrying out precise phenotypic evaluation under field conditions. Part of the problem comes from the difficulty to assess the relative contribution of different traits on the yield under terminal drought. Thus, these are traits where MAS could greatly enhance the effectiveness and impact of plant breeding programs.

4.1. Molecular Tools for SAT Legume Genomics

Molecular markers and molecular genetic linkage maps are the prerequisites for undertaking molecular breeding activities. However, the progress towards development of a reasonable number of molecular markers and molecular genetic maps for cultivated species has been very slow in almost all the three legume crops discussed in this chapter. One of the main reasons for this fact may have been the low level of genetic diversity present in the cultivated gene pools of these species, at least with the detection tools that are currently available. Nevertheless, because of the development of more sophisticated molecular tools, some progress has been made in the area of molecular mapping in these legume species.

4.1.1. Chickpea

The beginnings of the linkage map development in chickpea were based on morphological and isozyme loci. However, their small numbers and the fact that expression of these markers is often influenced by the environment, makes them unsuitable for routine use. Further, there is an extremely low level of polymorphism among genotypes of cultivated chickpea (*C. arietinum* L.). Therefore, interspecific crosses (*C. arietinum* × *C. reticulatum, C. arietinum* × *C. echinospermum*) were exploited for developing genetic linkage maps (Gaur and Slinkard, 1990a, 1990b). The earlier maps were sparse and represented less than 30 loci mapped in a very small portion (about 250 cM) of the chickpea genome (Gaur and Slinkard 1990a, 1990b; Kazan et al. 1993). Integration of molecular markers into genetic linkage maps in chickpea was started with the work of Simon and Muehlbauer (1997). Due to the lack of more recently available molecular markers, Simon and Muehlbauer (1997) employed RFLP and RAPD markers that showed limited polymorphism in the cultivated species (Udupa et al., 1993; Banerjee et al., 1999).

Subsequent development of SSR or microsatellite markers revolutionized genetic analysis and opened new possibilities for the study of complex traits in plant species especially crops like chickpea having a narrow genetic background. As a result, several hundred SSR markers have been developed in chickpea (Huettel et al., 1999; Winter et al., 1999; Sethy et al., 2003, 2006a, 2006b; Lichtenzveig et al., 2005; Choudhary et al., 2006). The majority of these markers have been mapped using interspecific mapping populations (Winter et al., 1999, 2000; Tekeoglu et al., 2002; Pfaff and Kahl, 2003). A genetic map constructed from an interspecific cross, however, may not represent the true recombination distance map order of the cultivated genome due to uneven recombination of homeologous chromosomes and distorted genetic segregation ratios (Flandez-Galvez et al., 2003a). Therefore, in the framework of targeting traits of breeding importance, molecular genetic linkage maps, with SSR markers, have been developed using intraspecific mapping populations from the cultivated gene pool (Cho et al., 2002, Flandez-Galvez et al., 2003a). The genetic linkage maps developed to date with DNA based molecular markers in chickpea are summarized in Table 2.

Table 2. Important genetics maps available for some SAT legume crops

Mapping population	Features of genetic map	Genome coverage	Reference
Chickpea			
F_2, interspecific (*C. arietinum* × *C. reticulatum*) and F_2, interspecific (*C. arietinum* × *C. echinospermum)*	7 linkage groups with 3 morphological and 26 isozymes	200 cM	Gaur and Slinkard, 1990a, 1990b
F_2, interspecific (*C. arietinum* × *C. reticulatum*)	8 linkage groups with 5 morphological and 23 isozymes	257 cM	Kazan et al., 1993
F_2, interspecific (*C. arietinum* × *C. reticulatum*) and F_2, interspecific (*C. arietinum* × *C. echinospermum*)	10 linkage groups with 9 morphological, 27 isozyme, 10 RFLP and 45 RAPD loci	527 cM	Simon and Muehlbauer, 1997
RIL, interspecific (*C. arietinum* 'ICC 4958' × *C. reticulatum* 'PI489777')	11 linkage groups with 120 STMS loci	613 cM	Winter et al., 1999
RIL, interspecific (*C. arietinum* 'ICC 4958' × *C. reticulatum* 'PI489777')	16 linkage groups with 118 SSR, 96 DAF, 70 AFLP, 37 ISSR, 17 RAPD, 8 isozyme, 3 cDNA, 2 SCAR and 3 morphological marker	2,078 cM	Winter et al., 2000
RIL, interspecific (*C. arietinum* 'FLIP 84–92C' × *C. reticulatum* 'PI489777')	9 linkage groups with 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological marker	982 cM	Santra et al., 2000
RIL, interspecific (*C. arietinum* 'ICC 4958' × *C. reticulatum* 'PI489777')	8 linkage groups, integration of 55 SSR and 1 RGA	1,175 cM	Tekeoglu et al., 2002
RIL, intraspecific (*C. arietinum* 'ICCV 2' x *C. arietinum* JG62')	14 linkages groups with 68 SSR, 34 RAPD, 4 ISSR, and 5 morphological markers	297 cM	Cho et al., 2002
RIL, intraspecific (*C. arietinum* 'ILC 1272' × *C. arietinum* 'ILC3279')	8 linkage groups with 52 SSR, 3 *Ascochyta blight* resistance loci	–	Udupa and Baum, 2003
RIL, interspecific (*C. arietinum* 'ICC 4958' × *C. reticulatum* 'PI489777')	incorporated 47 DR gene specific markers to Winter et al. (2000) 2500 cM , total 296 markers, 12 linkage groups	2,500 cM	Pfaff and Kahl, 2003
F_2, intraspecific (*C. arietinum* 'ICC 12004' x *C. arietinum* 'Lasseter')	8 linkage groups with 54 SSR, 3 ISSR, 12 RGA loci	535 cM	Flandez- Galvez et al., 2003a

(*Continued*)

Table 2. (*Continued*)

Mapping population	Features of genetic map	Genome coverage	Reference
F_2 interspecific (*C. arietinum* 'Lasseter' × *C. echinospermum* 'PI527930')	8 linkage groups with 14 SSR , 54 RAPD, 9 ISSR, 6 RGA loci	570 cM	Collard et al., 2003
RIL, intraspecific (*C. reticulatum* 'PI 359075' × *C. arietinum* 'FLIP 84–92C')	11 linkages groups with 53 SSRs	–	Cho et al., 2004
RIL, intraspecific (*C. arietinum*- two populations, CA2139 × JG62, CA2156 × JG62)	10 linkages groups with 118 RAPD, 13 SSR, 3 ISSR, and 4 morphological markers	–	Cobos et al., 2005
RIL, interspecific (*C. arietinum* 'Hadas' × *C. reticulatum* 'Cr205')	9 linkages groups with 91 SSR, 2 CytP450 markers	345 cM	Abbo et al., 2005
Groundnut			
F_2, interspecific (2*x*) (*A. stenosperma* × *A. cardenassi*)	11 linkage groups with 117 RFLP loci	1,063 cM	Halward et al., 1993
BC interspecific (2*x*) (*A. stenosperma* × (*A. stenosperma* × *A. cardenassi*)	11 linkage groups with 167 RAPD loci	800 cM	Garcia et al., 1995
BC interspecific (4*x*) (*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)	23 linkage groups with 370 RFLP loci.	2,210 cM	Burrow et al., 2001
F(2:3), intraspecific (*A. hypogaea*) (4*x*) ICG 12991 (Spanish) × ICGV SM 93541	5 linkage groups with 12 AFLP loci	139.4 cM of the genome	Herselman et al., 2004
F_2 interspecific (A Genome, 2*x*) (*A. duranensis* × *A. stenosperma*)	11 linkage group with 204 SSR loci	1,231 cM	Moretzsohn et al., 2005
F_2 interspecific (B Genome, 2*x*) (*A. ipaensis* × *A. magna*)	11 linkage group with 94 SSR loci	754.8 cM	Gobbi et al., 2006; D. Bertioli, Brazil (pers. communication)
Pigeonpea			
F_2, interspecific (*C. cajan* × *C. scarabaeoides*)	~200 DArT loci	–	A. Killian, Australia (pers. communication)

Two independent interspecific-derived populations have been extensively employed for genetic linkage map development in chickpea: (i) *C. arietinum* 'ICC 4958' × *C. reticulatum* 'PI489777' at the University of Frankfurt, Germany, (ii) *C. arietinum* 'FLIP 84–92C' × *C. reticulatum* 'PI599072' at Washington State University, Pullman, USA. Among the different types of molecular markers developed for chickpea, SSR markers have proved very useful in linkage mapping and formed the basis for the map initially developed by Winter et al. (1999) that spanned a distance of 613 cM and consisted of 120 SSR markers. This map was greatly extended by Winter et al. (2000) and subsequently by Pfaff and Kahl (2003) with his addition of 47 defense response (DR) genes. The extended map covers a distance of 2500 cM arranged in 12 linkage groups and represents the most extensive linkage map in chickpea. Relatively smaller maps derived from intraspecific (within *C. arietinum*) crosses, have been developed and are being extended (Cho et al., 2002,2004; Flandez-Galvez et al. 2003a; Cobos et al., 2005).

In summary, a reasonable number of SSR markers representing the entire chickpea genome are available at present. The repository of SSR markers for chickpea is being extended by serious efforts by developing new microsatellite markers at NIPGR (Sethy et al., 2003; Chaudhary et al., 2006) and ICRISAT, Patancheru. For instance, a set of about 200 SSRs has been developed at NIPGR (Bhatia et al. unpublished). Similarly sequencing of a microsatellite enriched library of a chickpea (*C. arietinum*) genotype ICC 4958 at ICRISAT, in collaboration with University of Frankfurt, provides another set of about 200 SSRs that can be used to develop markers (Varshney et al., unpublished data). Therefore immediate priority should be accorded to saturation of the existing 'reference' intraspecific as well as interspecific genetic maps with the presently available >500 new (unmapped) SSR markers (Lichtenzveig et al., 2005; Sethy et al., 2006a,b; Choudhary et al. 2006; Bhatia et al., unpublished results; Varshney et al., unpublished results).

4.1.2. Groundnut

The paucity of DNA polymorphism in cultivated groundnut posed a considerable obstacle to genetic mapping in groundnut. For instance, earlier studies using RAPD and RFLP approaches detected limited DNA variation in *Arachis* species (Kochert et al., 1991; Halward et al., 1992; Paik-Ro et al., 1992). The use of a synthetic amphidiploid TxAG-6 (Simpson et al., 1993) made possible the generation of the first molecular map representing the entire tetraploid genome of groundnut. The discovery of a high level of polymorphism between the cultivar Flourunner and the parents of TxAG-6 by RAPD analysis (Burrow et al., 1996) was followed by RFLP analysis showing 83% polymorphism on a per band basis (Burrow et al., 2001). By using 78 BC_1F_1 lines generated from the cross (TxAG-6 x Florunner), mapping of 220 cDNA probes integrated 370 RFLP loci into 23 linkage groups. The total length of the first tetraploid map was 2210 cM, which was slightly greater than twice the length (1063 cM) of the map previously reported from a cross between two A-genome diploid species (Halward et al., 1993). The common markers mapped

in both crosses showed a high degree of collinearity between the diploid and tetraploid chromosomes (Burrow et al., 2001). These studies have been summarized in the database PeanutMap (http://peanutgenetics.tamu.edu/cmap; Jesubatham and Burrow, 2006).

In terms of mapping the diploid genomes of *Arachis*, the first genetic map was constructed by Halward et al. (1993) based on the 87 F_2 lines derived from a cross of *A. stenosperma* x *A. cardenasii* and contained 117 RFLP loci on 11 linkage groups with a genome coverage of 1400 cM. RFLP analysis is time consuming and labor intensive. RAPD and AFLP were used to detect DNA polymorphism in several studies in different germplasm collections (He and Prakash, 1997; Subramanian et al., 2000; Dwivedi et al., 2001; Raina et al., 2001; Milla et al., 2005), but represent dominant markers with low information content. As a result of extensive efforts of several laboratories, a large number of microsatellite markers have been generated in groundnut (Hopkins et al., 1999; He et al., 2003; Ferguson et al., 2004; Moretzsohn et al., 2004; Mace et al., unpublished; D. Bertioli, Brazil, pers. commun.; S. Knapp, USA, pers. commun.). The availability of more than 500 SSR markers in groundnut provides the opportunity to integrate these markers into a genetic linkage map. However, these markers have been integrate only in the AA- genome map (Moretzsohn et al., 2005) by using an F_2 population obtained from a cross between two diploid species with AA genome (*A. durasenis* and *A. stenosperma*). The genetic map had 80 SSR loci on 11 linkage groups covering 1231 cM. Similar efforts to prepare a genetic map for BB genome are underway in Brazil. As of now, the genotyping of a F_2 population derived from cross between *A. ipaensis* (KG30076) and *A. magna* (KG30097) has resulted in development of 11 linkage groups with 94 markers (Gobbi et al. 2006). As a part of Generation Challenge Programme (GCP) of CGIAR, preparation of the first genetic map for tetraploid cultivated groundnut species is in progress at ICRISAT. However, the lower level of polymorphism between the parental genotypes of existing mapping populations (e.g. TAG24 × ICGV 86031 developed at ICRISAT, GPBD4 × TAG24 developed at UAS Dharwad) poses a serious problem. Nevertheless, we expect to prepare the partial/genome wide map with about 100 SSR loci (Varshney et al., unpublished results). The progress in the area of genome mapping of *Arachis* species is summarized in Table 2.

4.1.3. Pigeonpea

In case of pigeonpea, molecular markers (RFLPs) were used as early as 1994 to study genetic diversity in wild species using nuclear DNA probes (Nadimpalli et al., 1994). Subsequently, Ratnaparkhe et al. (1995) attempted to study DNA polymorphism in cultivars and wild species. The level of polymorphism among the wild species was extremely high, while little polymorphism was detected within *C. cajan* accessions. In order to characterize a few putative cytoplasmic male sterlity lines, maize mitochandrial DNA (mt DNA) specific probes were used in RFLP analysis (Sivaramakrishanan et al., 1997). Recently, AFLP analysis was carried out with a few cultivars and two wild species (*Cajanus volubilis, Rhynchosia bracteata*)

using two *Eco*RI and 14 *Mse*I primers (Punguluri et al., 2006). The two wild species shared only 7% bands with the pigeonpea cultivars, whereas 87% common bands were seen among cultivars. The cluster analysis revealed low polymorphism among pigeonpea cultivars and very high polymorphism between cultivated pigeonpea and its wild relatives. Similar results were obtained in a very recent analysis using DArT markers (Yang et al., 2006).

In terms of development of SSR markers, about 10 SSR markers are available in public domain (Burns et al., 2001). To develop a resource of microsatellite markers for pigeonpea, primer pairs were generated for 39 microsatellite loci at ICRISAT. These markers (19 polymorphic loci) yielded an average of 4.9 alleles per locus while the observed heterozygosity ranged from 0.17–0.80 with a mean of 0.42 per locus (Odeney et al., 2007). However, to the best of our knowledge, there is no report on any genetic mapping in pigeonpea. In collaboration with ICRISAT, some efforts are underway to develop the first generation map for pigeonpea based on DArT markers at DArT Pty. Ltd. (A. Killian, pers. commun.).

4.2. Trait Mapping and Marker-Assisted Selection

Marker-assisted selection (MAS) offers great promise for improving the efficiency of conventional plant breeding. Molecular markers are especially advantageous for traits where conventional phenotypic selection is difficult, expensive, or lacks accuracy or precision. Molecular mapping and identification of molecular markers associated with genes and QTLs for traits are prerequisites for the MAS. As mentioned above, though not excellent, some progress has been made in the area of development of molecular markers or construction of genetic maps in chickpea and groundnut. As a result, molecular markers linked to a few abiotic or biotic stress tolerance/resistance as well as agronomic traits have been identified recently.

4.2.1. Chickpea

Genetic mapping in chickpea has focussed on tagging agronomically relevant genes such as ascochyta blight resistance (Tekeoglu et al., 2002; Udupa and Baum, 2003; Collard et al., 2003; Flandez-Galvez et al., 2003b; Millan et al., 2003; Cho et al., 2004; Iruela et al., 2006), fusarium wilt resistance (Benko-Iseppon et al., 2003; Sharma et al., 2004) and yield-influencing characters such as double podding and other morphological characters (Cho et al., 2002; Rajesh et al., 2002; Abbo et al., 2005; Cobos et al., 2005). Progress in the area of mapping of ascochyta blight resistance has been summarized recently by Millan et al. (2006). Since apparently all major blight resistance QTLs are tagged with SSR markers, pyramiding of resistance genes via MAS should now be feasible and awaits its proof-of-principle. The genetic control of this disease bred into cold tolerant germplasm would be a major breakthrough for yield increases in Mediterranean-type environments in many parts of the world.

In order to address the issue of drought tolerance through molecular markers, more than 1500 chickpea germplasm and released varieties were screened for

drought tolerance at ICRISAT. The most promising drought tolerant variety was ICC 4958 that had 30% more root volume than the popular variety Annigeri (Saxena et al., 1993); therefore, root traits were considered important parameters to improve the drought tolerance (Kashiwagi et al., 2006). Selection for root traits is very difficult, since it involves laborious methods such as digging and measuring root length and density. Molecular tagging of major genes for root traits may enable MAS for these traits and could greatly improve the precision and efficiency of breeding. In this direction, a set of 257 recombinant inbred lines (RILs) was developed from the cross Annigeri × ICC 4958 at ICRISAT and glasshouse-evaluated to identify molecular markers for root traits. After screening the parental genotypes with over 250 STMS and 100 EST markers and the mapping population with 57 poymorphic markers, a QTL flanked by STMS markers TAA170 and TR55 on LG 4A was identified that accounted for maximal phenotypic variation in root length (33%), root weight (33%) and shoot weight (54%) (Chandra et al., 2004). Genotyping of two other mapping populations (ICC 4958 × ICC 1882 and ICC 8261 × ICC 283), which have larger genetic variation than Annigeri × ICC 4958 with SSR markers is in progress at ICRISAT.

For improving cold tolerance, AFLP markers have been linked to the trait using bulked segregant analysis of F_2 progeny of a cross between the chilling sensitive cultivar Amethyst and the chilling tolerant ICCV 88516 (Clarke and Siddique, 2003). Candidate AFLP markers were converted into SCAR markers (Paran and Michelmore, 1993) to overcome the limitations of the dominant AFLP marker system. The most promising primers were based on a 560 bp fragment containing a simple sequence repeat (3 bp repeat microsatellite) with nine repeats in the susceptible parent and ten repeats in the tolerant parent. The three-base difference was visualised on a vertical acrylamide gel, and was very useful in the selection of chilling tolerant progeny derived from crosses between ICCV 88516 and Amethyst. Unfortunately, there has been no success in applying these SCAR markers to other breeding materials.

In the case of flowering, a major gene (*efl-1*) for time of flowering was reported by Kumar & van Rheenen (2000), and another one (*ppd*) by Or et al. (1999). The latter gene controls time to flowering through photoperiod response (Hovav et al., 2003). Cho et al. (2002) mapped a QTL for days to 50% flowering to LG 3. Another QTL was also located on this linkage group in an interspecific RIL population and explained 28% of the total phenotypic variation (Cobos et al., 2005).

In addition to the above mentioned traits, molecular mapping for other traits is in progress in many laboratories. For instance, SSR-based genotyping and phenotyping of one mapping population (ICCV 2 × JG 62) is in progress at NIPGR and ICRISAT to identify the molecular markers associated with salinity tolerance.

4.2.2. Groundnut

There are very few genetic maps available based on cultivated groundnut genotypes. The available maps, based on interspecific crosses, will be useful in locating specific

genes of interest in the interspecific crosses and also providing valuable information about genome organization and evolution. However, these markers will be of less value in elite cultivated germplasm, in which very little polymorphism exist.

Although marker-trait association has been little used within *A. hypogaea*, even with the limitations afforded by present technologies, it has much potential for introgressing genes from closely related *Arachis* species into the cultivated genome. For instance, Garcia et al. (1995) showed introgression of genes from *A. cardenasii* into *A. hypogaea* in 10 of 11 linkage groups on the diploid RFLP map developed by Halward et al. (1993). Subsequently, Garcia et al. (1996) used RAPD and SCAR technologies to map two dominant genes conferring resistance to the nematode by using the mapping population derived from the cross *A. hypogaea* x *A. cardenasii.* Burrow et al. (1996) identified RAPD markers linked to nematode resistance in another interspecific cross involving the species *A. hypogaea*, *A. batizocoi*, *A. cardenasii* and *A. diogoi*. Such linkage of RAPD markers with components of early leaf spot and corn rootworm resistance was shown in another interspecific cross (Stalker and Mozingo, 2001). By using the BSA approach with an F_2 population derived from the cross (ICG 12991 × ICGVSM 93541) and phenotyping the F_3 population, twenty putative AFLP markers were identified of which12 mapped to five linkage groups. Interestingly, mapping of a single recessive gene on linkage group 1 (3.9 cM from a marker originating from the susceptible parent) explained 76% of the phenotype variation for aphid resistance. AFLP markers were used to establish marker-trait association for tomato spotted wilt virus resistance in groundnut (Milla 2003). Marker-trait association studies for several other traits, e.g, water use efficiency (WUE), rust and late leaf spot (LLS) are underway at ICRISAT and UAS Dharwad.

4.2.3. Pigeonpea

Higher level of heterogeneity and very low level of genetic variation in cultivated pigeonpea has hampered development of genetic maps and marker-trait association analysis. Recently, the use of RAPD markers through BSA approach showed association of two RAPD loci with fusarium wilt resistance (Kotresh et al., 2006). It is anticipated that development of higher number of polymorphic SSR markers and DArT arrays (A. Killian, pers. commun.) in pigeonpea will facilitate trait mapping in the near future.

5. NOVEL GENETIC AND GENOMICS APPROACHES

New technologies promise to resolve constraints that have been limiting the impact of linkage based molecular mapping. Such modern genomics approaches have been used in some cereal and other plant species, and legume improvement can be benefited by exploring such approaches.

5.1. Association Mapping and Advanced Backcross QTL (AB-QTL) Analysis

In general, a low level of polymorphism has been a major constraint in developing genetic maps in the legume crops mentioned in this chapter. Further, species like pigeonpea, which is of regional importance in Asia and Africa, has not been explored at the international level. Non-availability of resistance sources in cultivated genepools of these species for several fungal and viral diseases, e.g., pod borer in chickpea and pigeonpea, sterility mosaic in pigeonpea, aflatoxin in groundnut, and the difficulties of crossing cultivated species with wild species are other barriers that hampered the development of appropriate mapping populations in these legume species. Novel approaches, based on classical genetics, like linkage disequilibrium (LD) based association mapping (Hirschhorn and Daly, 2005), advanced back-cross QTL (AB-QTL) analysis (Tanksley and Nelson, 1996) offers the possibility to overcome at least a few barriers. For instance, an appropriate natural population, genebank or breeding material may be used in LD-based association analysis. In this regard, emergence of novel marker systems such as SNPs and DArTs and developments in this direction for the mentioned legume species would make it possible to undertake candidate gene sequencing (using SNP assays) as well as whole genome scanning (using DArTs) based approaches for association analyses. In contrast to the numerous linkage disequilibrium (LD) studies in human and other mammals, there are very few publications on this topic in agriculturally important crops including legumes (Virk et al., 1996; Beer et al., 1997; Pakniyat et al., 1997; Forster et al., 1997; Igartua et al., 1999; Remington et al., 2001; Thornsberry et al. 2001; Turpeinen et al. 2001; Hansen et al. 2001; Sun et al. 2001, 2003; Skot et al., 2002; Ivandic et al., 2002, 2003; Amirul Islam et al., 2004; Zhu et al., 2003; Simko et al., 2004). Traditionally the plant community has been reticent to use LD mapping believing that it can lead to spurious and non-functional associations due to mutation, genetic drift, population structure, breeding systems and selection pressure (Hill and Weir, 1994; Pritchard et al., 2000). However, most of these limitations are being overcome in recent mammalian studies by following precautions that minimize circumstantial correlations and maximize the accuracy of association statistics (Yu et al., 2006; Yu and Buckler, 2006; Ersoz et al., 2007). Unfortunately the real value of LD mapping in legume species remains to be demonstrated as most of the reports to date are based on small population sizes or a limited number of markers and generally lack validation.

Advanced-backcross QTL analysis (AB-QTL), proposed by Tanksley and Nelson (1996), involves transferring the QTLs of agronomically important traits from a wild species to a crop variety. In this approach, a wild species is backcrossed to a superior cultivar with selection for domestication traits. Selection is imposed to retain individuals that exhibit domestication traits such as non-shattering. The segregating BC_2F_2 or BC_2F_3 population is then evaluated for traits of interest and genotyped with polymorphic molecular markers. These data are then used for QTL analysis, potentially resulting in identification of QTLs, while transferring these QTLs into adapted genetic backgrounds. The AB-QTL approach has been

evaluated in many crop species to determine if genomic regions (QTLs) derived from wild or unadapted germplasm have the potential to improve yield (for a review, see Varshney et al., 2005). However, the wild species chromosome segments masked the magnitude of some of favourable effects that were identified for certain introgressed alleles (Septiningsih et al., 2003). Thus, yield promoting QTL did not have a substantial contribution to the phenotype and the best lines were inferior to commercial cultivars in some studies. In tomato, however, the pyramiding of independent yield promoting chromosome segments resulted in new varieties with increased productivity under normal and stress conditions (Wang D. et al., 2004). One disadvantage is that the value of the wild accession for contributing useful QTL alleles is unknown prior to a major investment in mapping. Nevertheless, the approach holds a great potential to harness the potential of wild species for crop improvement in case of legume species where only low level of genetic variation and source of resistance/tolerance to biotic/abiotic stresses exist in the cultivated gene pool.

5.2. Transcriptomics and Functional Genomics

Functional genomics has revolutionized biological research and is predicted to have a similar impact on plant breeding through the evolution of marker-assisted to genomics-assisted breeding (Varshney et al., 2005). The salient challenge of applied genetics and functional genomics is the identification of the genes underlying a trait of interest so that they can be exploited in crop improvement programmes. Among legume species, much work in terms of development of functional genomics resources such as ESTs, genome sequencing, array development has been done either in model species like lotus (*Lotus japonicus* L.) and medicago (*Medicago truncatula* L.) or major species like soybean. In contrast, only a limited number of ESTs have been generated so far in legume species of SAT (Table 1). These ESTs can be used to develop the molecular markers as shown in chickpea (Buhariwalla et al., 2005) and groundnut (Luo et al., 2005) as well as to develop cDNA arrays. At NIPGR, the chickpea ESTs are being developed from seeds (both developing and maturing) and symbiotic root nodules in association with *Mesorhizobium ciceri*. So far about 1000 seed specific unigenes have been identified (unpublished results). The most striking feature of these ESTs is that, majority of them are putative or unknown proteins. The use of suppression subtractive hybridization (SSH) to prepare the subtracted cDNA library of 7-day old symbiotic root nodules lead to the identification of three putative genes regulated during symbiotic relationship with *M. ciceri*. Further validation with Northern analysis has lead to the identification three putative genes up-regulated during symbiotic association in a temporal manner.

The macro- and micro-arrays based on EST/gene sequence information have been successfully utilized in many plant species for understanding the basic physiology, developmental processes, environmental stress responses, and for identification and genotyping of mutations. Recently in chickpea, a small array with 768 features has been developed (Coram and Pang, 2005a) that has been used to identify genes

responsible for ascochyta blight resistance (Coram and Pang, 2005b, 2006), drought and salinity tolerance (E. Pang, pers. commun.). The candidate genes identified by EST sequencing (and gene prediction) and functional genomics approaches can be further verified through real time PCR analysis (Luo et al., 2005) and genetical genomics/ expression genetics approaches (Jansen and Nap, 2001; Varshney et al., 2005) after conducting gene expression analysis in quantitative fashion using segregating mapping populations. By analyzing the expression levels of genes or clusters of genes within a segregating population, it is possible to map the inheritance of that expression pattern. The QTLs identified using expression data in a mapping population are called e(xpression)QTLs. The eQTLs can be classified as *cis* or *trans* acting based on location of transcript compared to that of the eQTL influencing expression of that transcript (de Konig and Haley, 2005). Because of this feature, eQTL analysis makes it possible to identify factors influencing the level of mRNA expression. The regulatory factor (second order effect) is of specific interest because more than one QTL can be putatively connected to a *trans*-acting factor (Schadt et al., 2003). Thus, the mapping of eQTLs allows multifactorial dissection of the expression profile of a given mRNA or cDNA, protein or metabolite into its underlying genetic components as well as localization of these components on the genetic map (Jansen and Nap, 2001). In recent years, in many plant species, the genetical genomics approach has demonstrated its power (see Kirst and Yu, 2007).

Another powerful approach of gene discovery is 'Serial Analysis of Gene Expression (SAGE)' (Velculescu et al., 1995) that utilizes the advantage of high-throughput sequencing technology to obtain a quantitative profile of gene expression which measures not the expression level of a gene, but quantifies a 'tag' which represents the transcriptome product of a gene. A tag for the purpose of SAGE, is a nucleotide sequence of a defined length, directly adjacent to the 3'-most restriction site for a particular restriction enzyme. The data product of the SAGE technique is a list of tags, with their corresponding count values, and thus is a digital representation of cellular gene expression. Based on the length of tags, several modified forms of SAGE, e.g., MicroSAGE, MiniSAGE, LongSAGE and SuperSAGE, have been developed (Sharma et al., 2007). In fact, by using SuperSAGE methodology, over 220,000 SuperTags describing the differential transcription profiles of chickpea roots and nodules have already been sequenced at University of Frankfurt (G. Kahl, pers. commun.). Targeted gene-expression chips are being developed by adding SuperTag oligonucleotides derived from the most informative genes expressed differentially under stress- and non-stress conditions and from large-versus small root systems to a gene expression chip (P. Winter, pers. commun.).

In groundnut, recent activities in the area of functional genomics have produced a gene chip with 400 unigenes after cluster analysis of 1825 ESTs and used for identifying the genes associated with disease resistance and drought tolerance (Luo et al., 2003, 2005). Further to validate the microarray and EST data by EST-discovery, real-time PCR analysis was conducted for 10 specific genes (Luo et al.,

2005). The use of suppression subtractive hybridization (SSH) to prepare the subtracted cDNA libraries and identify the genes regulated during interaction with the fungus *Cercosporidium personatum* (causing the disease late leaf spot) is in progress in Brazil (Nobile et al., 2006). To understand the molecular mechanisms of drought tolerance, the use of differential expression of mRNA transcripts and proteins are underway at Florida A & M University (Katam et al., 2006). With the development of more functional genomics resources in SAT legumes, it is anticipated that the use of functional genomics and expression genetics approaches may help the community to dissect the complex traits and devise strategies for crop improvement.

5.3. Comparative Genomics

In recent years, the availability of ESTs and genome sequence data for model legumes i.e. medicago (*M. truncatula*), and lotus (*L. japonicus*) and major crop legumes like soybean has opened the possibilities of transfer of information from model to crop legumes and vice-versa (Gepts et al., 2005, Young et al., 2005). Identification of putative orthologs from related genomes will facilitate comparative genomics and comparative genetic mapping. Using 274 unique low copy gene specific markers from *M. truncatula* and *G. max*, Choi et al. (2004, 2006) have demonstrated that gene-specific markers are transferable across *Papilionoid* legume species may find utility in phylogenetic relationship assessment at different, but overlapping, taxonomic levels. Moreover, majority of these markers (85.3%) are also linked to the legume genetic maps. Similarly, Gutierrez et al. (2005) have studied the conservation of 209 EST-SSR markers from the model legume *M. truncatula* in three major European crop legumes i.e. faba bean (*Vicia faba*), pea (*Pisum sativum*) and chickpea and have reported 36%–40% transferability range for this class of markers. Recently, extensive efforts have been made to develop bioinformatics tools and pipelines after exploiting the genomics resources of model species as well as other legume species and as a result about 450 cross species markers have been developed (Fredslund et al., 2005, 2006a, 2006b). For many markers, the map position in lotus and/or medicago is known and in other legume species such as groundnut, soybean, chickpea, these markers are being mapped. These studies will provide more anchor points to relate different legume genomes, Moreover, the identification of the cross-genera transferable legume SSR markers will cut down the cost and labor associated with development of SSR markers in the orphan legumes and will help in comparative mapping and map-based cloning of orthologous genes. Since the EST-SSR markers reveal very less polymorphism in legumes (Gutierrez et al. 2005), the alternative source is the genome specific genomic SSRs. By virtue of their long polymorphic microsatellite repeat stretches and the variable microsatellite flanking region, the genomic microsatellites are a promising source of cross-transferable markers in self-pollinating legume species (Sethy et al., manuscript in preparation). The levels and patterns of conservation of

Cicer genomic SSR markers across model, crop and fodder legumes have demonstrated that the genomic SSRs find a mean average transferability of nearly 25% across *M. truncatula*, *L. japonicus*, soybean, pea, lentil, pigeonpea, blackgram, mungbean and *Trifolium alexandrinum* (Figure 1) and often conserved in the model plant *A. thaliana*. Moreover, the *Cicer* markers have been demonstrated to be

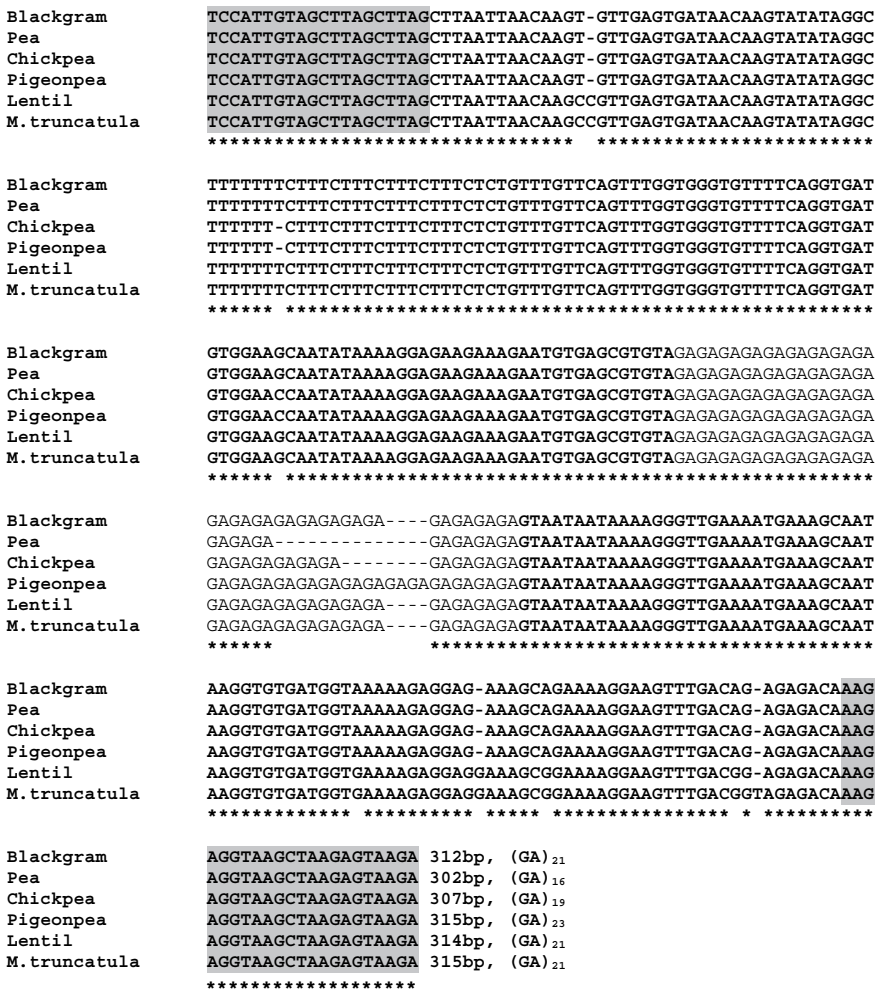

Figure 1. Multiple sequence alignment of the size variant alleles of the legume accessions at the chickpea STMS marker NIPGR19 locus. Accessions of *M. truncatula* (SA27783), blackgram (IC342955), lentil (IC383669), pea (RFP16) and pigeonpea (IC347150) along with chickpea (Pusa362) are analyzed. The *asterisks* indicate similar sequences and '–' indicate alignment gaps. The repeat region is indicated in boldface and shadowed boxes indicate conserved primer binding sites. Allele sizes and repeat motifs are mentioned at the end of the sequence

polymorphic even within *M. truncatula*, soybean and blackgram opening the possibility of comparative mapping and generation of a consensus legume genetic map (Sethy et al., unpublished results).

6. TOWARDS A BRIGHT FUTURE OF MOLECULAR BREEDING IN SAT LEGUMES

Traditional cropping systems across the world have depended on the rotation of cereal and legume crops. However, with increasing intensification of agriculture during the twentieth century, there has been a substantial emphasis on cereals as the pre-eminent food commodity in national production and international trade. In turn, this has been reflected by a continuous and cumulative increase in funding for research and breeding of cereal crops (Goff and Salmeron, 2004) that has resulted in the state-of-the-art in legumes falling further and further behind. Nevertheless, progress in the genomics of two legume species, medicago and lotus, as model genomes offers the potential for real technological leap-frogging amongst legume crops.

Although during the past few years, significant progress has been made in the area of genomics of SAT legume crops as a large number of molecular (SSR) markers and ESTs have been developed, there is still a need to develop more SSR, SNP or DArT markers and dense genetic maps for the species mentioned in this chapter. Further the generation of some BAC and BIBAC libraries in case of chickpea and groundnut offers the possibility to develop genome wide or local physical maps to isolate genes for resistance/tolerance to biotic/abiotic stresses as well as agronomic traits (Yuksel et al., 2005). Thus molecular breeding through existing tools in combination with continuous incremental changes such as improvements in genetics and biometrics, plus revolutionary changes including automation of breeding trials and computerization of phenotyping will be very useful for legume improvement (Dwivedi et al., 2006). In addition to linkage based trait mapping, several other approaches such as LD-based association mapping, AB-QTL analysis, transcriptomics and functional genomics can be used to identify the molecular markers or candidate genes for traits of interest in breeding. Beyond its increased power of selection, marker or genomics-assisted breeding offers additional advantages in the economics of scale both in terms of cost and time as very different traits can be manipulated using the same technology. The proof-of-function of candidate genes can be obtained by using TILLING (Targeting Induced Local Lesions In Genomes, see Till et al., 2007) population, while the EcoTILLING approach may be used for allele mining to improve the traits. Allele mining for candidate genes should provide superior alleles and haplotypes for the traits (Varshney et al., 2005).

Recent studies show strong correlation between the degree of synteny and phylogenetic distance in legumes (Young et al., 2003; Wang M.L. et al., 2004; Choi et al., 2004). Therefore, advances in the area of genomics of medicago and lotus may be used to transfer information on genes involved in nitrogen fixation and other physiological processes of agronomic importance in SAT legume crops by utilizing

the comparative genomics approach combined with bioinformatics. However, the extent to which genetic knowledge from model systems will readily translate into economic impact in related crops remains to be empirically demonstrated (Thro et al., 2004; Koebner and Varshney, 2006). Genomics research in the legume crops together with model systems will soon routinely define the location of genomic regions controlling a target trait as well as identify underlying candidate genes and their sequences through mapping, mutation analysis and transcriptomics. Based on this new knowledge it will be possible to develop highly precise DNA markers for selection or introgression of desired traits. While the newly developed genetic and genomics tools will certainly enhance the prediction of phenotype, they will not entirely replace the conventional breeding process.

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REFERENCES

Abbo S, Molina C, Jungmann R, Grusak MA, Berkovitch Z, Reifen R, Kahl G, Winter P, Reifen R (2005) QTL governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor Appl Genet 111:185–195

Amirul Islam FM, Beebe S, Muñoz M, Tohme J, Redden RJ, Basford KE (2004) Using molecular markers to assess the effect of introgression on quantitative attributes of common bean in the Andean gene pool. Theor Appl Genet 108:243–252

Azhaguvel P, Vidya Saraswathi D, Sharma A, Varshney RK (2006) Methodological advancement in molecular markers to delimit the gene(s) for crop improvement. In: Teixera da Silva J. (ed) Floriculture, ornamental and plant biotechnology: Advances and topical issues, Global Science Books, London, UK, pp 460–499

Banerjee H, Pai RA, Sharma RP (1999) Restriction fragment length polymorphism and random amplified polymorphic DNA analysis of chickpea accessions. Biol Plant 42:197–199

Beer SC, Siripoonwiwat W, O'Donoughue LS, Souza E, Matthews D, Sorrells ME (1997) Association between molecular markers and quantitative traits in an oat germplasm pool: can we infer linkages. Jour Agr Genom 3 (http://wheat.pw.usda.gov/jag/papers97/paper197/jqtl1997–01.html)

Benko-Iseppon AM, Winter P, Huettel B, Staginnus C, Muehlbauer FJ, Kahl G (2003) Molecular markers closely linked to *Fusarium* resistance genes in chickpea show significant alignments to pathogenesis-related genes located on *Arabidopsis* chromosomes 1 and 5. Theor Appl Genet 107:379–386

Botstein D, White DL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Bretting PK, Widrlechner MP (1995) Genetic markers and plant genetic resource management. Plant Breed Rev 31:11–86

Brown AHD (1989) Core collections: a practical approach to genetic resources management. Genome 31:818–824

Brown SM, Kresovich S (1996) Molecular characterization for plant genetic resources conservation. In: Paterson AH (ed) Genome mapping of plants. Academic Press, San Diego, pp 85–93

Buhariwalla HK, Jayashree B, Eshwar K, Crouch JH (2005) ESTs from chickpea roots with putative roles in drought tolerance. BMC Plant Biol 5:16

Burns MJ, Edwards KJ, Newbury HJ, Ford-Lloyd BV, Baggott CD (2001) Development of simple sequence repeat (SSR) markers for the assessment of gene flow and genetic diversity in pigeonpea (*Cajanus cajan*). Mol Ecol Notes 1:283–285

Burow MD, Starr JL, Simpson CE, Paterson AH (1996) Identification of RAPD markers in peanut (*Arachis hypogaea* L.) associated with root-knot nematode resistance derived from *A. cardenasii*. Mol Breed 2:307–319

Burrow MD, Simpson CE, Paterson AH, Starr JL (1996) Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. Mol Breed 2:369–379

Burrow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): broadening the gene pool of a monophyletic polyploid species. Genetics 159:823–837

Chandra S, Buhariwalla HK, Kashiwagi J, Harikrishna S, Rupa Sridevi K, Krishnamurthy L, Serraj R, Crouch JC (2004) Identifying QTL-linked markers in marker-deficient crops. In new directions for a diverse plant: Proceedings of the 4th international crop science congress brisbane, Australia, 26 September–1 October 2004

Cho S, Kumar J, Shultz JF, Anupama K, Tefera F, Muehlbauer FJ (2002) Mapping genes for double podding and other morphological traits in chickpea. Euphytica 125:285–292

Cho S, Chen W, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733–739

Choi HK, Mun JH, Kim DJ, Zhu H, Baek JM, Mudge J, Roe B, Ellis N, Doyle J, Kiss GB, Young ND, Cook DR (2004) Estimating genome conservation between crop and model legume species. Proc Nat Acad Sci 101:15289–15294

Choi HK, Luckow MA, Doyle J, Cook DR (2006) Development of nuclear gene-derived molecular markers linked to legume genetic maps. Mol Gen Genome 276:56–70

Choudhary S, Sethy NK, Shokeen B, Bhatia S (2006) Development of sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Mol Ecol Notes 6:3–95

Clarke H, Siddique KHM (2003) Chilling tolerance in chickpea – novel methods for crop improvement. In: Sharma RN, Yasin M, Swami SL, Khan MA, William AJ (ed) International Chickpea Conference, Indira Gandhi Agricultural University, Raipur, India, pp 5–12

Cobos MJ, Fernandez MJ, Rubio J, Kharrat M, Moreno MT, Gil J, Millan T (2005) A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli × Desi crosses: location of genes for resistance to *fusarium wilt* race 0. Theor Appl Genet 110:1347–1353

Collard BCY, Pang ECK, Ades PK, Taylor PWJ (2003) Preliminary investigation of QTLs associated with seedling resistance to ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. Theor Appl Genet 107:719–729

Coram TE, Pang ECK (2005a) Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part I. Generation and analysis of an expressed sequence tag (EST) library. Physiol Mol Plant Pathol 66:192–200

Coram TE, Pang ECK (2005b) Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part II. Microarray expression analysis of putative defence-related ESTs. Physiol Mol Plant Path 66:201–210

Coram TE, Pang ECK (2006) Expression profiling of chickpea genes differentially regulated during a resistance response to *Ascochyta rabiei*. J Plant Biotechnol 4:647–666

De Koning DJ, Haley CS (2005) Genetical genomics in humans and model organisms. Trends Genet 21:377–381

Desbrosses GG, Kopka J, Udvardi MK (2005) *Lotus japonicus* metabolic profiling. Development of gas chromatography-mass spectrometry resources for the study of plant–microbe interactions. Plant Physiol 137:1302–1318

Dixon RA, Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. Plant Physiol 131:878–885

Duranti M, Gius C (1997) Legume seeds: protein content and nutritional value. Field Crops Res 53:31–45

Dwivedi SL, Gurtu S, Chandra S, Yuejin W, Nigam SN (2001) Assessment of genetic diversity among selected groundnut germplasm. I: RAPD analysis. Plant Breed 120:345–349

Dwivedi SL, Blair MW, Upadhyaya HD, Serraj R, Balaji J, Buhariwalla HK, Ortiz R, Crouch JH (2006) Using genomics to exploit grain biodiversity in crop improvement. Plant Breed Rev 26: 171–310

Ersoz ES, Yu J, Buckler ES (2007) Applications of linkage disequillibrium and association mapping in crop plants. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement Vol 1: Genomics approaches and platforms, Springer, The Netherlands, pp 97–120

Ferguson ME, Burrow M, Schulze S, Bramel PJ, Paterson A, Kresovich S, Mitchell S (2004) Microsatellite identification and characterization in peanut (*A. hypogaea* L.). Theor Appl Genet 108:1064–1070

Flandez-Galvez H, Ford R, Pang ECK, Taylor PWJ (2003a) An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged based microsatellite site and resistance gene analog markers. Theor Appl Genet 106:1447–1453

Flandez-Galvez H, Ford R, Taylor PWJ (2003b) Mapping QTLs governing resistance to ascochyta blight in chickpea. Theor Appl Genet 107:1257–1265

Forster BP, Russell JR, Ellis RP, Handley LL, Robinson D, Hackett CA, Nevo E, Waugh R, Gordon DC, Keith R, Powell W (1997) Locating genotypes and genes for abiotic stress tolerance in barley: a strategy using maps, markers and the wild species. New Physiol 137:141–147

Fredslund J, Schauser L, Madsen LH, Sandal N, Stougaard J (2005) PriFi: using a multiple alignment of related sequences to find primers for amplification of homologs. Nucl Acids Res 33: W516–W520

Fredslund J, Madsen LH, Hougaard BK, Nielsen AM, Bertioli D, Sandal N, Stougaard J, Schauser L (2006a) A general pipeline for the development of anchor markers for comparative genomics in plants. BMC Genome 7:207

Fredslund J, Madsen LH, Hougaard BK, Sandal N, Stougaard J, Bertioli D, Schauser L (2006b) GeMprospector – Online design of cross-species genetic marker candidates in legumes, grasses. Nucl Acids Res 34:W630–W640

Garcia GM, Stalker HT, Kochert G (1995) Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers. Genome 38:166–176

Garcia GM, Stalker HT, Shroeder E, Kochert GA (1996) Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* to *A. hypogaea*. Genome 39:836–845

Gaur PM, Slinkard AE (1990a) Genetic control and linkage relations of additional isozymes markers in chickpea. Theor Appl Genet 80:648–653

Gaur PM, Slinkard AE (1990b) Inheritance and linkage of isozyme coding genes in chickpea. J Hered 81:455–459

Gepts P, Beavis WD, Brummer EC, Shoemaker RC, Stalker HT, Weeden NF, Young ND (2005) Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. Plant Physiol 137:1228–1235

Ghassemi F, Jackman AJ, Nix HA (1995) Salinization of land and water resources. CAB international, Wallingford, UK, p 526

Gobbi A, Teixeira C, Moretzsohn M, Guimaraes P, Leal-Bertioli S, Bertioli D, Lopes CR, Gimenes M (2006) Development of a linkage map to species of B genome related to the peanut (*Arachis hypogaea* – AABB). Plant and animal genomes XIV conference, San Diego, CA, USA, P 679 (http://www.intl-pag.org/14/abstracts/PAG14_P679.html)

Goff SA, Salmeron JM (2004) Back to the future of cereals. Sci Am 291:42–48

Grusak, MA (2002) Enhancing mineral content in plant food products. J Am Coll Nutr 21:178S–183S

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Gupta PK, Varshney RK (2004) Cereal genomics. Kluwer Academic Publishers, Dordrecht, The Netherlands

Gutierrez MV, Vaz Patto MC, Huguet T, Cubero JI, Moreno MT, Torres AM (2005) Cross-species amplification of *Medicago truncatula* microsatellites across three major pulse crops. Theor Appl Genet 110:1210–1217

Halward TM, Stalker HT, Kochert G (1993) Development of an RFLP linkage map in diploid peanut species. Theor Appl Genet 87:379–384

Halward TM, Stalker HT, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol Biol 18:315–325

Hannan RM, Kaiser WJ, Muehlbauer FJ (1994) Development and utilization of the USDA chickpea germplasm core collection. Agron Abstr, ASA, Madison, WI, p 217

Hansen M, Kraft T, Ganestam S, Sall T, Nilsson NO (2001) Linkage disequilibrium mapping of the bolting gene in sea beet using AFLP markers. Genet Res 77:61–66

He G, Prakash CS (1997) Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97:143–149

He G, Meng R, Newman M, Gao G, Pittman RN, Prakash CS (2003) Microsatellites as DNA markers in cultivated peanut (*A. hypogaea* L.). BMC Plant Biol 3:3

Herselman L, Thwaites R, Kimmins FM, Courtois B, van der Merwe PJA, Seal SE (2004) Identification and mapping of AFLP markers linked to peanut (*Arachis hypogaea* L.) resistance to the aphid vector of groundnut rosette disease. Theor Appl Genet 109:1426–1433

Hill WG, Weir BS (1994) Maximum-likelihood estimation of gene location by linkage disequilibrium. Am J. Hum Genet 54:705–714

Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 6:95–108

Holbrook CC, Anderson WF, Pittman RN (1993) Selection of a core collection from the US germplasm collection of peanut. Crop Sci 33: 859–861

Hopkins MS, Casa AM, Wang T, Mitchell SE, Dean RE, Kochert GD, Kresovich S (1999) Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci 39: 1243–1247

Hovav R, Upadhyaya KC, Beharav A, Abbo S (2003) Major flowering time gene and polygene effects on chickpea seed weight. Plant Breed 122:539–541

Huettel B, Winter P, Weising K, Choumane W, Weigand F, Kahl G (1999) Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217

Igartua E, Casas AM, Ciudad F, Montoya JL, Romagosa I (1999) RFLP markers associated with major genes controlling heading date evaluated in a barley germplasm pool. Heredity 83:551–559

Iruela M, Rubio J, Barro F, Cubero JI, Millan T, Gil J (2006) Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. Theor Appl Genet 112:278–287

Ivandic V, Thomas WTB, Nevo E, Zhang Z, Forster BP (2003) Associations of simple sequence repeats with quantitative trait variation including biotic and abiotic stress tolerance in *Hordeum spontaneum*. Plant Breed 122:300–304

Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP (2002) Analysis of simple sequence repeats (SSRs) in wild barley from the fertile crescent: associations with ecology, geography, and flowering time. Plant Mol Biol 48:511–527

Jain SM, Brar DS, Ahloowalia BS (2002) Molecular techniques in crop improvement. Kluwer Academic Publishers, Dordrecht, The Netherlands

Jansen RC, Nap JP (2001) Genetical genomics: the added value from segregation. Trends Genet 17: 388–391

Jesubatham AM, Burrow MD (2006) Peanut Map: an online genome database for comparative molecular maps of peanut. BMC Bioinformatics 7:375

Jones N, Ougham H, Thomas H (1997) Markers and mapping: we are all geneticists now. New Phytol 137:165–177

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V, Serraj R (2004) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213–222

Kashiwagi J, Krishnamurthy L, Crouch JH, Serraj R (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Fields Crop Res 95:171–181

Katam R, Vasanthaiah HKN, Basha SM (2006) Differential expression of mRNA transcripts and proteins in leaf tissue of peanut genotypes to water stress. In: Plant and animal genome XIV conference, San Diego, CA, USA, P446 (www.intl-pag.org/14/abstracts/PAG14_P446.html)

Kazan K, Muehlbauer FJ, Weeden NE, Ladizinsky G (1993) Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor Appl Genet 86: 417–426

Killian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska K, Uszynski G, Cayla C, Patarapuwadol S, Xia L, Yang S, Thomson B (2005) The fast and the cheap: SNP and DArT – based whole genome profiling for crop improvement. In: Proceedings of the international congress, 'In the wake of double helix: from the green revolution to the gene revolution', 27–31 May 2003, Bologna, Italy, pp 443–461

Kirst M, Yu Q (2007) Genetical genomics: successes and prospects in plants. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement Vol 1: genomic approaches and platforms, springer, The Netherlands, pp 245–265

Kochert G, Halward T, Branch WD, Simpson CE (1991) RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor Appl Genet 81:565–570

Koebner RMD (2004) Marker assisted selection in the cereals: the dream and the reality. In: Gupta PK, Varshney RK (eds) Cereal genomics. Kluwer Academic Publishers, Netherlands, pp 199–252

Koebner RMD, Varshney RK (2006) The development and application of genomic models for large crop plant genomes. In: Varshney RK, Koebner RMD (eds) Model plants and crop improvement CRC Press, FL pp 1–10

Kotresh H, Fakrudin B, Punnuri S, Rajkumar B, Thudi M, Paramesh H, Lohithswa H, Kuruvinashetti M (2006) Identification of two RAPD markers genetically linked to a recessive allele of a *Fusarium* wilt resistance gene in pigeonpea (*Cajanus cajan* (L.) Millsp.). Euphytica 149:113–120

Krapovikas A, Gregory WC (1994) Taxonomy of genus *Arachis* (Legumonisae). Bonplandia 8:1–186

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Serraj R (2003) Genetic diversity of drought avoidance root traits in the mini core germplasm collection of chickpea. Intl Chickpea Pigeonpea Newsl 10: 21–24

Kumar J, Van Rheenen HA (2000) A major gene for time of flowering in chickpea. J Hered 91:67–68

Lichtenzveig J, Scheuring C, Dodge J, Abbo S, Zhang HB (2005) Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea (*Cicer arietinum* L.). Theor Appl Genet 110:492–510

Lodwig EM, Hosie AH, Bourdes A, Findlay K, Allaway D, Karunakaran R, Downie JA, Poole PS (2003) Amino-acid cycling drives nitrogen fixation in the legume–Rhizobium symbiosis. Nature 422:672–674

Luo M, Dang P, Guo BZ, He G, Holbrook CC, Bausher MG, Lee RD (2005) Generation of expressed sequence tags (ESTs) for gene discovery and marker development in cultivate peanut. Crop Sci 45:346–353

Luo M, Dang P, Guo BZ, Holbrook CC, Bausher M (2003) Application of EST technology in functional genomics of *Arachis hypogaea* L. Phytopathology 94:S55

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Milla SR (2003) Relationships and utiligation of Arachis germplasm in peanut improvement. PhD Thesis, North Carolina State University, USA, pp 1–150

Milla SR, Isleib TG, Stalker HT (2005) Taxonomic relationships among *Arachis* sect. *Arachis* species as revealed by AFLP markers. Genome 48:1–11

Millan T, Rubio J, Iruela M (2003) Markers associated with Ascochyta blight resistance in chickpea and their potential in marker-assisted selection. Field Crops Res 84:373–384

Millan T, Clarke HJ, Siddique KHM, Buhariwalla HK, Gaur PM, Kumar J, Gil J, Kahl G, Winter P (2006) Chickpea molecular breeding: new tools and concepts. Euphytica 147:81–103

Moretzsohn MC, Hopkins MS, Mitchell SE, Resovich SK, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol 4:11

Moretzsohn MC, Leoi L, Proite K, Guimara PM, Leal-Bertioli SCM, Gimenes MA, Martins WS, Valls JFM, Grattapaglia D, Bertioli DAJ (2005) Microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). Theor Appl Genet 111:1060–1071

Morgante M, Salamini F (2003) From plant genomics to breeding practice. Curr Opin Biotech 14: 214–219

Nadimpalli RG, Jarret RL, Phatak SC, Kochert G (1994) Phylogenetic relationships of the pigeon pea (*Cajanus cajan* L.) based on nuclear restriction fragment length polymorphism. Genome 36:216–223

Nobile PM, Lopes CR, Barata T, Barsalobres C, Guimaraes P, Leal-Bertioli S, Gimenes M (2006) Identification and characterization of peanut (*Arachis hypogaea* L.) ESTs regulated during interaction with *Cercosporidium personatum* (Berk and Curt) Deighton In: XIV international plant and animal genome conference, San Diego, CA, USA, P680 (wwwintl-pagorg/14/abstracts/PAG14_P680html)

Odeny DA, Jayashree B, Ferguson M, Hoisington D, Crouch J, Gebhardt C (2007) Development, characterization and utilization of microsatellite markers in pigeonpea. Plant Breed 126:130–136

Or E, Hovav R, Abbo S (1999) A major gene for flowering time in chickpea. Crop Sci 39:315–322

Paik-Ro OG, Smith RL, Knauft DA (1992) Restriction fragment length polymorphism evaluation of six peanut species within the Arachis section. Theor Appl Genet 84:201–208

Pakniyat H, Powell W, Baired E, Handley LL, Robinson D, Scrimgeour CM, Novo E, Hackett CA, Caligari PDS, Foster BP (1997) AFLP variation in wild barley (*Hordeum spontaneum* C Koch) with reference to salt tolerance and associated ecogeography. Genome 40:332–341

Palmieri DA, Bechara MD, Curi RA, Gimenes MA, Lopes CR (2005) Novel polymorphic microsatellite markers in section *Caulorrhizae* (*Arachis*, Fabaceae). Mol Ecol Notes 5:77–79

Paran I, Mitchelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance gene in lettuce. Theor Appl Genet 85:985–993

Passioura J (1977) Grain yield, harvest index and water use of wheat. J Aust Inst Agric Sci 43:117–120

Pereira MG, de Sousa MMT, Duarte IM (2001) Status of European *Cicer* database In: Magioni L, Ambrose M, Schachl R, Duc G, Lipman E (compilers) Report of a working group on grain legumes. Third Meeting, 5–7 July 2001, Krakow, Poland IPGRI, Rome, Italy, pp 45–46

Pfaff T, Kahl G (2003) Mapping of gene-specific markers on the genetic map of chickpea (*Cicer arietinum* L). Mol Gen Genomics 269:243–251

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotyping data. Genetics 155:945–959

Punguluri SK, Janaiah K, Govil JN, Kumar PA, Sharma PC (2006) AFLP fingerprinting in pigeonpea (*Cajanus cajan* (L.) Millsp) and its wild relatives. Genet Resour Crop Evol 53:523–531

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94–100

Raina SN, Rani V, Kojima T, OgiharaY, Singh KP (2001) RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea* L.) cultivars and wild species. Genome 44:763–772

Rajesh PN, Tullu A, Gil J, Gupta VS, Ranjekar PK, Muehlbauer FJ (2002) Identification of an STMS marker for the double-podding gene in chickpea. Theor Appl Genet 105:604–607

Rajesh PN, Coyne C, Meksem K, Sharma KD, Gupta V, Muehlbauer FJ (2004) Construction of a *Hind* III bacterial artificial chromosome library and its use in identification of clones associated with disease resistance in chickpea. Theor Appl Genet 108:663–669

Ratnaparkhe MB, Gupta VS, Ven Murthy MR, Ranjekar PK (1995) Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp] and its wild relatives using RAPD markers. Theor Appl Genet 91:893–898

Reddy LJ, Upadhyaya HD, Gowda CLL, Singh S (2005) Development of core collection in pigeonpea (*Cajanus cajan* (L.) Millasp). Genet Resour Crop Evol 52:1049–1056

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc Natl Acad Sci USA 25: 11479–11484

Ryan JG, Spencer DC (2001) Future challenges and opportunities for agricultural R&D in the semi-arid tropics, International crops research institute for the semi-arid tropics, Patancheru 502 324, Andhra Pradesh, India, p 83

Santra DK, Tekeoglu M, Ratnaparkhe MB, Gupta VS, Ranjekar PK, Muehlbauer FJ (2000) Identification and mapping of QTLs conferring resistance to Ascochyta blight in chickpea. Crop Sci 40: 1606–1612

Saxena NP (1984) Chickpea in the physiology of tropical field crops. In: Goldworthy PR, Fisher NM (eds) Wiley, New York, pp 419–452

Saxena KB, Singh L, Ariyanagam RP (1993) Role of partially cleistogamy in maintaining genetic purity of pigeonpea. Euphytica 66:225–229

Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff T G, Milligan SB, Lamb JR, Cavet G, Linsley PS, Mao M, Stoughton RB, Friend SH (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422:297–301

Schultze M, Kondorosi A (1998) Regulation of symbiotic root nodule development. Annu Rev Genet 32:33–57

Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O rufipogon*. Theor Appl Genet 107:1433–1441

Serraj R (2004) Symbiotic nitrogen fixation:challenges and future prospects for application in tropical agroecosystems. Oxford & IBH, New Delhi, India

Serraj R, Krishnamurthy L, Upadhyaya HD (2004) Screening chickpea mini-core germplasm for tolerance to salinity. Intl Chickpea Pigeonpea Newsl 11:29–32

Sethy NK, Shokeen B, Bhatia S (2003) Isolation and characterization of sequence-tagged microsatellite sites markers in chickpea (*Cicer aeritinum* L.). Mol Ecol Notes 3:428–430

Sethy NK, Choudhary S, Shokeen B, Bhatia S (2006a) Identification of microsatellite markers from *Cicer reticulatum*:molecular variation and phylogenetic analysis. Theor Appl Genet 112:347–357

Sethy NK, Shokeen B, Edwards KJ, Bhatia S (2006b) Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). Theor Appl Genet 112:1416–1428

Sharma HC (2001) Cytoplasmic male-sterility and source of pollen influence the expression of resistance to sorghum midge, *Stenodiplosis sorghicola.* Euphytica 122:391–395

Sharma KK, Lavanya M (2002) Recent developments in transgenics for abiotic stress in legumes of the semi-arid tropics In: Ivanaga M (ed) Genetic engineering of crop plants for abiotic stress, JIRCAS Working Report No 23:61–73 Tsukuba, Japan

Sharma KD, Winter P, Kahl G, Muehlbauer FJ (2004) Molecular mapping of *Fusarium oxysporum* f.sp. *ciceris* race 3 resistance gene in chickpea. Theor Appl Genet 108:1243–1248

Sharma PC, Matsumura H, Terauchi R (2007) Use of serial analysis of gene expression (SAGE) for transcript analysis. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement Vol I: genomics approaches and platforms, Springer, the Netherlands, pp 227–244

Simko I, Costanzo S, Haynes KG, Christ BJ, Jones RW (2004) Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach. Theor Appl Genet 108:217–224

Simon CJ, Muehlbauer FJ (1997) Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Hered 88:115–119

Simpson CE, Nelson SC, Starr JL, Woodard KE, Smith OD (1993) Registration of TxAg-6 and TxAg-7 peanut germplasm lines. Crop Sci 33:1418

Singh KB, Kumar J, Haware MP, Lateef SS (1990) Disease and pest resistance breeding: which way to go in the nineties. In Chickpea in the nineties: Proceeding of the second international workshop on Chickpea improvement, 4–8 December 1989, International crops research institute for the semi-arid tropics, Patancheru 502 324, Andhra Pradesh, India, pp 223–238

Sivaramakrishnan S, Seetha K, Nageshwar Rao A, Singh L (1997) RFLP analysis of cytoplasmic male-sterile lines of pigeonpea [*Cajanus cajan* (L.) Millsp] developed by interspecific crosses. Euphytica 93:307–312

Skot L, Hamilton NRS, Mizen S, Chorlton KH, Thomas ID (2002) Molecular genecology of temperature response in *Lolium perenne*: 2 Association of AFLP markers with ecogeography. Mol Ecol 11: 1865–1876

Stalker HT, Mozingo LG (2001) Molecular markers of *Arachis* and marker assisted selection. Peanut Sci 28:117–123

Subramanian V, Gurtu S, Nageswara Rao RC, Nigam SN (2000) Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Genome 43:656–660

Sun GL, William M, Liu J, Kasha KJ, Pauls KP (2001) Microsatellite and RAPD polymorphisms in Ontario corn hybrids are related to the commercial sources and maturity ratings. Mol Breed 7:13–24

Sun G, Bond M, Nass H, Martin R, Dong Z (2003) RAPD polymorphisms in spring wheat cultivars and lines with different level of *Fusarium* resistance. Theor Appl Genet 106:1059–1067

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Nelson JC (1996) Advance backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTL from unadapted germplasm to the elite breeding lines. Theor Appl Genet 92:191–203

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Biotechnology 7:257–264

Tautz D (1989) Hypervariablity of simple sequences as a general source of polymorphic DNA markers. Nucl Acids Res 17:6463–6471

Tekeoglu M, Rajesh PN, Muehlbauer FJ (2002) Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theor Appl Genet 105:847–854

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler IV ES (2001) *Dwarf8* polymorphisms associate with variation in flowering time. Nature Genet 28:286–289

Thro AM, Parrott W, Udall JA, Beavis WD (2004) Genomics and plant breeding: the experience of the initiative for future agricultural and food systems. Crop Sci 44:1893–1919

Till BJ, Comai L, Henikoff S (2007) TILLING and EcoTILLING for crop improvement. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement Vol I: Genomics Approaches and Platforms, Springer, The Netherlands, pp 333–350

Tohme J, Gonzalez OD, Beebe S, Duque MC (1996) AFLP analysis of gene pools of a wild bean core collection. Crop Sci 36:1375–1384

Turpeinen T, Tenhola T, Manninen O, Nevo E, Nissila E (2001) Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. Mol Ecol 10:1577–1591

Udupa SM, Baum M (2003) Genetic dissection of pathotype-specific resistance to ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106: 1196–1202

Udupa SM, Sharma A, Sharma AP, Pai RA (1993) Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. J. Plant Biochem Biotechnol 2:83–86

Upadhyaya HD, Ortiz R (2001) A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources. Theor Appl Genet 102:1292–1298

Upadhyaya HD, Bramel PJ, Singh S (2001a) Development of a chickpea core subset using geographic distribution and quantitative traits. Crop Sci 41:206–210

Upadhyaya HD, Nigam SN, Singh S (2001b) Evaluation of groundnut core collection to identify sources of tolerance to low temperature at germination. Indian J Plant Genet Resour 14:165–167

Upadhyaya HD, Bramel PJ, Ortiz R, Singh S (2002) Developing a mini core of peanut for utilization of genetic resources. Crop Sci 42:2150–2156

Upadhyaya HD, Ortiz R, Bramel PJ, Singh S (2003) Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. Genet Resour Crop Evol 50:139–148

Upadhyaya HD, Gowda CLL, Buhariwalla HK, Crouch JH (2006a) Efficient use of crop germplasm resources: Identifying useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches. Plant Genet Resour 4:25–35

Upadhyaya HD, Reddy LJ, Gowda CLL, Reddy KN, Singh S (2006b) Development of a mini core subset for enhanced and diversified utilization of pigeonpea germplasm resources. Crop Sci 46:2127–2132

Vadez V, Krishnamurthy L, Gaur PM, Upadhyaya HD, Hoisington DA, Varshney RK, Turner NC, Siddique KHM (2006) Tapping the large genetic variability for salinity tolerance in chickpea. Proceeding of the Australian society of agronomy meeting, 10–14 September 2006 (http://wwwregionalorgau/au/asa/2006/ concurrent/environment/4561_vadezhtm)

Van der Maesen, LJG (1987) Origin, history, and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) The Chickpea, CABI/ICARDA, Wallingford, UK, pp 11–43

van Treuren R, van Soest LJM, van Hintum ThJL (2001) Marker-assisted rationalization of genetic resource collections: a case study in flax using AFLPs. Theor Appl Genet 103:144–152

Vance CP, Graham PH, Allan DL (2000) Biological nitrogen fixation phosphorus: a critical future need In: Pedrosa FO, Hungria M, Yates MG, Newton WE (eds) Nitrogen fixation: from molecules to crop productivity. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 506–514

Varshney RK, Graner A, Sorrells ME (2005) Genomics-assisted breeding for crop improvement. Trends Plant Sci 10:621–630

Varshney RK, Hoisington D, Tyagi AK (2006) Advances in cereal genomics and applications in crop breeding. Trends Biotechnol 24:490–499

Velculescu VE, Hang LZ, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270:484–487

Virk PS, Newbury HJ, Jackson MT, Ford-Lloyd BV (1995) The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. Theor Appl Genet 90:1049–1055

Virk PS, Ford-Lloyd BV, Jackson MT, Pooni HS, Clemeno TP, Newbury HJ (1996) Predicting quantitative variation within rice germplasm using molecular markers. Heredity 76:296–304

Vos P, Hogers R, Bleeker M, Reijans M, Lee T van de, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23:4407–4414

Wang D, Graef GL, Procopiuk AM, Diers BW (2004a) Identification of putative QTL that underlie yield in interspecific soybean backcross populations. Theor Appl Genet 108:458–467

Wang ML, Gillaspie AG, Newman ML, Dean RE, Pittman RN, Morris JB, Pederson GA (2004b) Transfer of simple sequence repeat (SSR) markers across the legume family for germplasm characterization and evaluation. Plant Genet Resour 2:107–119

Williams JGK, Kubelik ARK, Livak JL, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by random primers are useful as genetic markers. Nucl Acids Res 18:6531–6535

Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC, Sahi S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ, Kahl G (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol Gen Genet 262:90–101

Winter P, Benko-Iseppon AM, Huttel B, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for Fusarium wilt races 4 and 5. Theor Appl Genet 101: 1155–1168

Yang S, Pang W, Ash G, Harper J, Carling J, Wenzl P, Huttner E, Zong X, Kilian A (2006) Low level of genetic diversity in cultivated Pigeonpea compared to its wild relatives is revealed by diversity arrays technology. Theor Appl Genet 113:585–595

Young ND, Mudge J, Ellis THN (2003) Legume genomes: more than peas in a nod. Curr Opin Plant Biol 6:199–204

Young ND, Cannon SB, Sato S, Kim D, Cook DR, Town CD, Roe BA, Tabata S (2005) Sequencing the genespaces of *Medicago truncatula* and *Lotus japonicus*. Plant Physiol 137:1174–1181

Yu J, Buckler IV ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155–160

Yu J, Pressoir G, Briggs WH, Vroh BI, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

Yüksel B, Paterson AH (2005) Construction and characterization of a peanut *Hin*dIII BAC library. Theor Appl Genet 111:630–639

Yüksel B, Bowers JE, Estill J, Goff L, Lemke C, Paterson AH (2005) Exploratory integration of peanut genetic and physical maps and possible contributions from *Arabidopsis*. Theor Appl Genet 111: 87–94

Zhu YL, Song QJ, Hyten DL, Van Tassell CP, Matukumalli LK, Grimm DR, Hyatt SM, Fickus EW, Young ND, Cregan PB (2003) Single-nucleotide polymorphisms in soybean. Genetics 163:1123–1134