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## 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 gene pools 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 Kondorski, 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, Caesalpinioideae 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.

## 01 1.2. Legume Crops in the Semi-Arid Tropics

02 The semi-arid tropics (SAT) covers parts of 55 developing countries where the  
 03 75–180 day growing period has a mean daily temperature of more than 20°C. The  
 04 dry semi-arid tropics have very short growing seasons, separated by very hot and  
 05 dry periods in which growth without irrigation or stored soil moisture is impossible.  
 06 Natural soil fertility is often low, in part because soils are highly weathered by  
 07 the dry-hot and humid-hot cycles, and pest and disease pressure can be intense.  
 08 Farmers face further substantive risks, even within the growing season, as there  
 09 are irregular periods of drought and high evaporative demand which can seriously  
 10 compromise crop productivity. Based on 1996 statistics, the SAT is home to about  
 11 1.4 billion people, of which 560 million (40%) are classified as poor, and 70% of  
 12 the poor reside in rural areas (Ryan and Spencer 2001).

13 Although a number of crops are grown in SAT areas, among legume crops,  
 14 chickpea, groundnut common bean, cowpea and pigeonpea provide key components  
 15 in the diets of resource-poor people in the developing world. We, at ICRISAT,  
 16 together with our National Agricultural Research System (NARS) partners are  
 17 engaged on crop improvement in chickpea, groundnut and pigeonpea, therefore in  
 18 this article we discuss the advances in the area of genetics and genomics applied to  
 19 breeding in only these three legume crops. In the first instance, a brief introduction  
 20 of these crops is given in following sections.  
 21

### 22 1.2.1. Chickpea (*Cicer arietinum* L.)

23 Chickpea is the third most important grain legume globally, and second in impor-  
 24 tance in Asia. It is also an important legume crop in Eastern and Southern Africa.  
 25 About 90% of the global area and 88% of production is concentrated in Asia.  
 26 Chickpea has one of the best nutritional compositions of any dry edible legume,  
 27 and is mainly used for human consumption. The *desi* type (colored seed coat) is  
 28 usually de-hulled and split to make *dhal* or flour (*besan*), while *kabuli* types (white  
 29 or cream-colored seed coat) is often cooked as whole grain. The haulms are used for  
 30 animal feed. Chickpea improves soil fertility through nitrogen fixation (up to 140  
 31 kg N/ha). Chickpea area has slightly decreased globally, but has been stable at 9 M  
 32 ha in Asia for the past 25 years. However, production in Asia has increased by 39%  
 33 due to a 32% increase in productivity. Even then, the current average yield in Asia  
 34 (0.8 t/ha) is low, and far below the potential yield (5 t/ha), or research station yields  
 35 (3.5 t/ha). The global demand for chickpea in 2010 is estimated at 11.1 Mt (up  
 36 from the current 8.6 Mt). A combination of productivity enhancement through crop  
 37 improvement enhanced with biotechnological tools, integrated crop management  
 38 and expansion of area to new niches and production systems are needed to achieve  
 39 this target.  
 40

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

01 This classification is based on their morphological characteristics, lifestyle and  
02 geographical distribution (van der Maesen, 1987). Eight of these *Cicer* species share  
03 the annual growth habit with chickpea are of particular interest to breeders.  
04

#### 05 1.2.2. *Groundnut (Arachis hypogaea L.)*

06 Groundnut is an important food and cash crop for the resource-poor farmers in  
07 Asia and Africa. It is primarily grown for edible oil (48–50%) as well as for  
08 direct consumption as food by people. Groundnut haulms are excellent fodder  
09 for cattle, and groundnut cake (after oil extraction) is used as animal feed. It  
10 contributes significantly to household food security and cash income through the  
11 sale of groundnut products. Groundnut productivity in Western and Central Africa  
12 (WCA) and Eastern and Southern Africa (ESA) is below the world average yield  
13 of 1.4 t/ha. Although groundnut productivity in Asia (1.8 t/ha) exceeds the world  
14 average, it is still lower than the yields in developed countries (3 t/ha). The area  
15 under groundnut in ESA has increased dramatically from 2.3 to 3.3 M ha during  
16 2000 to 2004. In Asia, the area under groundnut is increasing in China and Vietnam,  
17 but is declining in India during 1991–2004. There has been a slight decline in area  
18 in WCA. Although global productivity has shown a positive trend, much more  
19 needs to be achieved in future.

20 The genus *Arachis* belongs to the family *Fabaceae*, subfamily Papilionaceae,  
21 tribe Aeschynomenae, subtribe Stylosantheae. Cultivated groundnut (*Arachis*  
22 *hypogaea* L.) can be botanically classified into two subspecies, *hypogaea* and  
23 *fastigiata* that are distinguished based on branching pattern and distribution of  
24 vegetative and reproductive nodes along lateral branches. Each subspecies is again  
25 divided into two botanical varieties; subsp. *hypogaea* into var. *hypogaea* (virginia)  
26 and var. *hirsuta* and subsp. *fastigiata* into var. *fastigiata* (valencia), var. *vulgaris*  
27 (spanish), var. *peruviana* and var. *aequatoriana* (Karpovickas and Gregory, 1994).  
28

#### 29 1.2.3. *Pigeonpea (Cajanus cajan L.)*

31 Pigeonpea is a versatile and multipurpose crop. It is one of the major food legumes  
32 in the tropical and sub-tropical regions in Africa, Asia and the Caribbean countries.  
33 Its green pods and seeds are consumed as a vegetable, and the dry grains are cooked  
34 whole or after dehulling (as *dhal*). The foliage is used as fodder, and the dry sticks  
35 are used for fencing, thatching, and as firewood. It fixes atmospheric nitrogen, and  
36 the extensive leaf fall adds organic matter to the soil. Dry grain is also used for  
37 animal feed. About 90% of the global pigeonpea area (4.4 M ha) is in Asia (about  
38 86% in India). Other major countries where pigeonpea is grown are Myanmar,  
39 Nepal, Bangladesh, Pakistan and China. In Sub-Saharan Africa (SSA), pigeonpea is  
40 grown in Uganda, Kenya, Malawi, Mozambique, Zimbabwe, Zambia, South Africa,  
41 Sudan and Ethiopia; but reliable statistics are not available. Pigeonpea production  
42 has shown only a marginal increase during the past two decades (2.2 to 2.9 million  
43 t during 1980–98). However, productivity has remained stagnant at 0.7 t/ha, mostly  
44 because it is intercropped with cereals or cotton and receives no or little inputs; or

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

	Chickpea	Groundnut	Pigeonpea
04 Species name	<i>Cicer arietinum</i> L.	<i>Arachis hypogaea</i> L.	<i>Cajanus cajan</i> L.
05 Ploidy level and 06 chromosome 07 number	2n = 2x = 16	2n = 4x = 40	2n = 2x = 22
08 Genome size <sup>1</sup>	931 Mbp	2891 Mbp	858 Mbp
09 SSR markers	~700 (Winter et al., 1999; 10 Huettel et al., 1999; 11 Sethy et al., 2003,2006b; 12 Lichtenzveig et al., 2005; 13 Choudhary et al., 2006; 14 Varshney et al., unpublished; 15 Bhatia et al., unpublished 16 results)	~700 (Hopkins et al., 1999; 17 He et al., 2003; Ferguson 18 et al., 2004; Moretzsohn 19 et al., 2004; Palmieri 20 et al., 2005; Mace et al., 21 unpublished; D. Bertioli, 22 Brazil, pers. commun.; S. 23 Knapp, pers. commun.)	~100 (Burns 24 et al., 2001; 25 Odney et al. 26 2007)
27 BAC libraries	3.8 X (Rajesh et al. 2004), 28 7 X (Lichtenzveig et al. 2005)	6.5–9.0 X (Yuksel and 29 Paterson, 2005)	–
30 ESTs	~2000 (NCBI, Buhariwalla 31 et al., 2005)	~7538 (NCBI, Luo et al., 32 2005; S. Knapp, pers. 33 commun.)	More than 884 34 (NCBI) 35 (Gaikwad et al. 36 unpublished)
37 Gene arrays	768- features microarray 38 (Coram and Pang, 2005a), 39 SAGE Gene Chip (P. Winter, 40 Germany, pers. commun.)	400 unigene array (Luo et al., 41 2005)	

<sup>1</sup> As per estimate of Royal Botanic Gardens, Kew, UK (<http://www.rbgekew.org.uk/cval/>)

<sup>2</sup> 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

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

03  
04 *2.1.1. Drought*

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

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

34  
35 *2.1.2. Salinity*

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

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

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

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

## 24 2.2. Biotic Stresses

25 The major biotic factors of SAT legumes are diseases and insect pests. The  
26 chickpea diseases of major importance are ascochyta blight (caused by the  
27 necrotrophic fungus *Ascochyta rabiei* (Pass.) Lab.), fusarium wilt (caused by  
28 *Fusarium oxysporum* f. sp. *ciceris*), Botrytis gray mold and root rots caused by  
29 *Sclerotium* and *Pythium*. Majority of these diseases affect all aerial parts of the  
30 plant. Among the pigeonpea diseases, sterility mosaic (viral disease), fusarium wilt  
31 (caused by the fungus *Fusarium udum* Butler), and phytophthora blight (*Phytoph-*  
32 *thora drechsleri*) are major diseases causing significant losses of pigeonpea yield.  
33 In groundnut, rust, late leaf spot, and early leaf spot are serious diseases worldwide,  
34 which cause 50–60% pod yield loss. Rust and late-leaf spot often occur together  
35 and the pod yield loss can exceed 70% in the crop. Besides adversely affecting  
36 pod yield and its quality, these foliar diseases also affect haulm (fodder) yield and  
37 quality. Whereas the level of resistance available in cultivated groundnut to rust  
38 is very high, for early- and late-leaf spot, it is low. Wild *Arachis* species harbour  
39 many useful resistance genes against various diseases and insect pests. Of the  
40 important biotic constraints specific to sub-Saharan Africa (SSA), the groundnut  
41 rosette disease (GRD), vectored by aphids, is endemic to the continent and its  
42 adjoining islands and epidemics occur often throughout SSA, reducing groundnut  
43 production and crippling rural food security.  
44

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

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### 19 3. UTILIZATION OF PLANT GENETIC RESOURCES (PGRS)

20  
21 Availability and characterization of suitable germplasm is a critical factor for  
22 utilizing genetic variation in crop breeding. Fortunately for all the three legume  
23 species mentioned in the article, a large number of accessions are present in different  
24 genebanks throughout the world (Dwivedi et al., 2006). For instance, ICRISAT,  
25 under an agreement with FAO, holds 16,853 cultivated and 117 wild accessions of  
26 *Cicer* species, whereas the International Centre for Agricultural Research in Dryland  
27 Areas (ICARDA), Syria, under the same FAO agreement, maintains 8,342 cultivated  
28 and 255 wild accessions. Other institutions holding chickpea germplasm are the  
29 National Bureau of Plant Genetics Resource (NBPGR), India (14,566 accessions);  
30 Centre for Legume Improvement in Mediterranean Area (CLIMA) (4,351 acces-  
31 sions) and AusPGRIS (7922 accessions) in Australia; United States Department of  
32 Agriculture (USDA), USA (4,662 accessions); and the Seed and Plant Improvement  
33 Institute, Iran (4,925 accessions). The European *Cicer* database contains 3,700 culti-  
34 vated accessions from 11 countries (Pereira et al. 2001). For groundnut, ICRISAT  
35 holds, under the same agreement with FAO, 14,126 accessions of cultivated peanut  
36 and 293 accessions of wild *Arachis* species from 93 countries. Other institutions  
37 holding large numbers of peanut accessions are the National Research Centre for  
38 Groundnut (NRCG), India (7,935 accessions) and the USDA Southern Regional  
39 Plant Introduction Station, USA (6,233 accessions). In the United States, wild  
40 *Arachis* species are maintained at North Carolina State University, Raleigh (250  
41 accessions) and at the Texas Agricultural Experiment Station (TAMU), Texas (300  
42 accessions). For pigeonpea, ICRISAT holds under the agreement with FAO 12,398  
43 pigeonpea accessions of cultivated and 314 accessions of wild species from 74  
44 countries. Other institutions holding substantial amounts of pigeonpea germplasm



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

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### 3.1. Core and Mini-Core Collections

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

30 Most core collections have been designed from global or regional collections held  
31 within international agricultural research centers or national program gene banks,  
32 while a few have also been developed for wild accessions (Tohme et al., 1996).  
33 After evaluating a total of 16,991 chickpea accessions for 13 traits and 14,310  
34 groundnut and 12,153 accessions of pigeonpea for 14 traits each, the core collec-  
35 tions of chickpea, groundnut and pigeonpea with 1,956 (Upadhyaya et al., 2001a),  
36 1,704 (Upadhyaya et al., 2003) and 1,290 accessions (Reddy et al., 2005), respec-  
37 tively have been developed at ICRISAT. In addition, the core collection of 505  
38 genotypes of chickpea was developed after analysis of 3,315 genotypes (Hannan  
39 et al., 1994). Similarly for groundnut, an USDA core collection with 831 genotypes  
40 after evaluating 7,432 accessions for 24 traits (Holbrook et al., 1993) and an Asian  
41 core collection based on evaluating 4,738 genotypes for 15 traits (Upadhyaya et al.,  
42 2002) are available. Although these core collections have been useful for identi-  
43 fying diverse sources for traits of interests and broadening the genetic base of  
44 cultivars for a crop (Upadhyaya et al., 2001b, 2006a; Krishnamurthy et al. 2003;

01 Serraj et al., 2004), even a core collection can be too large so a further reduction is  
02 also valuable providing it is not associated with losing too much of the spectrum  
03 of diversity. Upadhyaya and Ortiz (2001) developed a strategy for sub-sampling  
04 a core collection to develop a mini-core collection, based on selecting 10% of  
05 the core accessions representing the variability of larger collection of species. In  
06 this process, the core collection is evaluated for various morphological, agronomic,  
07 and quality traits to select a 10% subset from this core subset (i.e., 1% of the  
08 entire collection) that captures a large proportion (i.e. more than 80% of the entire  
09 collection) of the useful variation. Selection of core and mini-core collections is  
10 based on standard clustering procedures used to separate groups of similar acces-  
11 sions combined with various statistical tests to identify the best representatives. The  
12 min i-core collection developed at ICRISAT for chickpea consisted of 211 acces-  
13 sions (Upadhyaya and Ortiz, 2001), while the groundnut (Upadhyaya et al., 2002)  
14 and pigeonpea (Upadhyaya et al., 2006b) mini-core consists of 184 accessions and  
15 146 accessions, respectively. Both core or mini-core germplasm collections have  
16 been used for identifying a range of germplasm with beneficial traits for use in  
17 breeding programs (see Dwivedi et al., 2006 for references). Increasing concern of  
18 trade and food processors for consistent and better quality and physical specifica-  
19 tions, however, suggest further characterization of core or mini-core collections for  
20 quality and market traits.

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### 23 **3.2. Molecular Characterization of PGRs**

24  
25 The core or mini core collections have been developed based on morphological  
26 or agronomic traits; little information is available on molecular genetic diversity  
27 present in the germplasm collection. Molecular characterization of germplasm is  
28 a particularly useful tool for assisting genebank curators to better manage genetic  
29 resources, helping them to identify redundant germplasm and to provide scient-  
30 ists with the most diverse germplasm for applications in research and breeding  
31 (Bretting and Widrlechner, 1995; Virk et al., 1995; Brown and Kresovich, 1996;  
32 van Treuren et al., 2001; Upadhyaya et al., 2006b). Accessions with the most  
33 distinct DNA profiles are likely to contain the greatest number of novel alleles  
34 (Tanksley and McCouch, 1997). As a part of the Generation Challenge Programme  
35 (GCP) of the CGIAR, molecular characterization of global composite collec-  
36 tions of the SAT legumes is in progress at ICRISAT. For example, genotyping  
37 of about 3000 chickpea accessions (Upadhyaya et al., 2006a) with 50 SSR  
38 markers and 1000 groundnut accessions with 20 SSR markers, in collaboration  
39 with ICARDA (Syria) and EMBRAPA (Brazil) respectively has already been  
40 completed. Molecular characterization of 1000 pigeonpea accessions at 20 SSR  
41 loci is in progress. These studies provide estimates on genetic diversity and the  
42 population structure of the germplasm that can be used to define the most diverse  
43 collection, called 'reference collection' for using in association mapping studies  
44 (see later).

#### 01 4. MOLECULAR BREEDING FOR SAT LEGUMES

02 Legume breeders have made major contributions to combat the problem of both  
03 abiotic and biotic stresses in the past but the pace and extent of improvements  
04 must be dramatically increased to attend to parallel demands. Recent advances  
05 in the area of biotechnology have offered the tools in the form of molecular  
06 markers to assist the breeding practices (Jain et al., 2002). Molecular markers  
07 are powerful diagnostic tools that detect DNA polymorphism both at the level  
08 of specific loci and at the whole genome level (reviewed by Azhaguvel et al.,  
09 2006). As compared to morphological traits/markers, molecular markers have  
10 several advantages as they are phenotypically neutral and are not influenced  
11 by pleiotropic and epistatic interactions, and their expression is not dependent  
12 on plant age/part (Jones et al., 1997). In fact the use of molecular markers in  
13 improving the breeding efficiency in plant breeding was suggested as early as  
14 in 1989 (Tanskley et al., 1989; Melchinger, 1990). In this regard, once linkage  
15 between a gene for the agronomic trait of interest and marker locus is estab-  
16 lished, then DNA diagnostic tests can be used to guide plant breeding (Morgante  
17 and Salamini, 2003; Gupta and Varshney, 2004). The selection of useful lines  
18 for breeding with the help of linked molecular markers is called marker-assisted  
19 selection (MAS). Use of MAS is especially advantageous for traits with low  
20 heritability where traditional selection is difficult, expensive or lack accuracy or  
21 precision.  
22

23 A variety of molecular markers exist, such as RFLPs (*Restriction Fragment*  
24 *Length Polymorphisms*, Botstein et al., 1980), RAPDs (*Random Amplification of*  
25 *Polymorphic DNAs*, Williams et al., 1990), AFLPs (*Amplified Fragment Length*  
26 *Polymorphisms*, Vos et al., 1995) and microsatellites or SSRs (*Simple Sequence*  
27 *Repeats*, Tautz, 1989). Among the different classes of molecular markers, SSR  
28 markers are often chosen as the preferred markers for a variety of applications  
29 in breeding because of their multiallelic nature, codominant inheritance, relative  
30 abundance and extensive genome coverage (Gupta and Varshney, 2000). More  
31 recently, markers such as SNPs (*Single Nucleotide Polymorphisms*, Rafalski, 2002)  
32 and DArT (*Diversity Array Technology*, Killian et al., 2005) have been added to  
33 list of preferred marker systems for breeding.

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

#### 01 **4.1. Molecular Tools for SAT Legume Genomics**

02 Molecular markers and molecular genetic linkage maps are the prerequisites for  
03 undertaking molecular breeding activities. However, the progress towards devel-  
04 opment of a reasonable number of molecular markers and molecular genetic  
05 maps for cultivated species has been very slow in almost all the three legume  
06 crops discussed in this chapter. One of the main reasons for this fact may have  
07 been the low level of genetic diversity present in the cultivated gene pools  
08 of these species, at least with the detection tools that are currently available.  
09 Nevertheless, because of the development of more sophisticated molecular tools,  
10 some progress has been made in the area of molecular mapping in these legume  
11 species.  
12

##### 13 **4.1.1. Chickpea**

14  
15 The beginnings of the linkage map development in chickpea were based on morpho-  
16 logical and isozyme loci. However, their small numbers and the fact that expression  
17 of these markers is often influenced by the environment, makes them unsuitable  
18 for routine use. Further, there is an extremely low level of polymorphism among  
19 genotypes of cultivated chickpea (*C. arietinum* L.). Therefore, interspecific crosses  
20 (*C. arietinum* × *C. reticulatum*, *C. arietinum* × *C. echinospermum*) were exploited  
21 for developing genetic linkage maps (Gaur and Slinkard, 1990a, 1990b). The earlier  
22 maps were sparse and represented less than 30 loci mapped in a very small portion  
23 (about 250 cM) of the chickpea genome (Gaur and Slinkard 1990a, 1990b; Kazan  
24 et al. 1993). Integration of molecular markers into genetic linkage maps in chickpea  
25 was started with the work of Simon and Muehlbauer (1997). Due to the lack of more  
26 recently available molecular markers, Simon and Muehlbauer (1997) employed  
27 RFLP and RAPD markers that showed limited polymorphism in the cultivated  
28 species (Udupa et al., 1993; Banerjee et al., 1999).

29 Subsequent development of SSR or microsatellite markers revolutionized genetic  
30 analysis and opened new possibilities for the study of complex traits in plant species  
31 especially crops like chickpea having a narrow genetic background. As a result,  
32 several hundred SSR markers have been developed in chickpea (Huettel et al.,  
33 1999; Winter et al., 1999; Sethy et al., 2003, 2006a, 2006b; Lichtenzweig et al.,  
34 2005; Choudhary et al., 2006). The majority of these markers have been mapped  
35 using interspecific mapping populations (Winter et al., 1999, 2000; Tekeoglu et al.,  
36 2002; Pfaff and Kahl, 2003). A genetic map constructed from an interspecific  
37 cross, however, may not represent the true recombination distance map order of  
38 the cultivated genome due to uneven recombination of homeologous chromosomes  
39 and distorted genetic segregation ratios (Flandez-Galvez et al., 2003a). Therefore,  
40 in the framework of targeting traits of breeding importance, molecular genetic  
41 linkage maps, with SSR markers, have been developed using intraspecific mapping  
42 populations from the cultivated gene pool (Cho et al., 2002, Flandez-Galvez et al.,  
43 2003a). The genetic linkage maps developed to date with DNA based molecular  
44 markers in chickpea are summarized in Table 2.

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

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

(Continued)

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Table 2. (Continued)

Mapping population	Features of genetic map	Genome coverage	Reference
$F_2$ interspecific ( <i>C. arietinum</i> 'Lasseter' × <i>C. echinospermum</i> 'PI527930')	8 linkage groups with 14 SSR, 54 RAPD, 9 ISSR, 6 RGA loci	570 cM	Collard et al., 2003
RIL, intraspecific ( <i>C. reticulatum</i> 'PI 359075' × <i>C. arietinum</i> 'FLIP 84-92C')	11 linkages groups with 53 SSRs	—	Cho et al., 2004
RIL, intraspecific ( <i>C. arietinum</i> - 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 ( <i>C. arietinum</i> 'Hadas' × <i>C. reticulatum</i> 'Cr205')	9 linkages groups with 91 SSR, 2 Cyp450 markers	345 cM	Abbo et al., 2005
Groundnut			
$F_2$ , interspecific (2x) ( <i>A. stenosperma</i> × <i>A. cardenassi</i> )	11 linkage groups with 117 RFLP loci	1,063 cM	Halward et al., 1993
BC interspecific (2x) ( <i>A. stenosperma</i> × ( <i>A. stenosperma</i> × <i>A. cardenassi</i> ))	11 linkage groups with 167 RAPD loci	800 cM	Garcia et al., 1995
BC interspecific (4x) ( <i>A. batizocoi</i> × ( <i>A. cardenassi</i> × <i>A. dtogoi</i> ))	23 linkage groups with 370 RFLP loci.	2,210 cM	Burrow et al., 2001
F(2:3), intraspecific ( <i>A. hypogaea</i> ) (4x) 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, 2x) ( <i>A. duranensis</i> × <i>A. stenosperma</i> )	11 linkage group with 204 SSR loci	1,231 cM	Moretzsohn et al., 2005
$F_2$ interspecific (B Genome, 2x) ( <i>A. ipaensis</i> × <i>A. magna</i> )	11 linkage group with 94 SSR loci	754.8 cM	Gobbi et al., 2006; D. Bertoli, Brazil (pers. communication)
Pigeonpea			
$F_2$ , interspecific ( <i>C. cajan</i> × <i>C. scarabaeoides</i> )	~200 DArT loci	—	A. Killian, Australia (pers. communication)

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

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

#### 29 4.1.2. Groundnut

30  
31 The paucity of DNA polymorphism in cultivated groundnut posed a considerable  
32 obstacle to genetic mapping in groundnut. For instance, earlier studies using RAPD  
33 and RFLP approaches detected limited DNA variation in *Arachis* species (Kochert  
34 et al., 1991; Halward et al., 1992; Paik-Ro et al., 1992). The use of a synthetic  
35 amphidiploid TxAG-6 (Simpson et al., 1993) made possible the generation of the  
36 first molecular map representing the entire tetraploid genome of groundnut. The  
37 discovery of a high level of polymorphism between the cultivar Flourrunner and the  
38 parents of TxAG-6 by RAPD analysis (Burrow et al., 1996) was followed by RFLP  
39 analysis showing 83% polymorphism on a per band basis (Burrow et al., 2001). By  
40 using 78 BC<sub>1</sub>F<sub>1</sub> lines generated from the cross (TxAG-6 × Flourrunner), mapping of  
41 220 cDNA probes integrated 370 RFLP loci into 23 linkage groups. The total length  
42 of the first tetraploid map was 2210 cM, which was slightly greater than twice  
43 the length (1063 cM) of the map previously reported from a cross between two  
44 A-genome diploid species (Halward et al., 1993). The common markers mapped

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

05 In terms of mapping the diploid genomes of *Arachis*, the first genetic map was  
 06 constructed by Halward et al. (1993) based on the 87 F<sub>2</sub> lines derived from a cross of  
 07 *A. stenosperma* x *A. cardenasii* and contained 117 RFLP loci on 11 linkage groups  
 08 with a genome coverage of 1400 cM. RFLP analysis is time consuming and labor  
 09 intensive. RAPD and AFLP were used to detect DNA polymorphism in several  
 10 studies in different germplasm collections (He and Prakash, 1997; Subramanian  
 11 et al., 2000; Dwivedi et al., 2001; Raina et al., 2001; Milla et al., 2005), but  
 12 represent dominant markers with low information content. As a result of extensive  
 13 efforts of several laboratories, a large number of microsatellite markers have been  
 14 generated in groundnut (Hopkins et al., 1999; He et al., 2003; Ferguson et al.,  
 15 2004; Moretzsohn et al., 2004; Mace et al., unpublished; D. Bertioli, Brazil, pers.  
 16 commun.; S. Knapp, USA, pers. commun.). The availability of more than 500  
 17 SSR markers in groundnut provides the opportunity to integrate these markers into  
 18 a genetic linkage map. However, these markers have been integrate only in the  
 19 AA- genome map (Moretzsohn et al., 2005) by using an F<sub>2</sub> population obtained  
 20 from a cross between two diploid species with AA genome (*A. duranensis* and  
 21 *A. stenosperma*). The genetic map had 80 SSR loci on 11 linkage groups covering  
 22 1231 cM. Similar efforts to prepare a genetic map for BB genome are underway in  
 23 Brazil. As of now, the genotyping of a F<sub>2</sub> population derived from cross between  
 24 *A. ipaensis* (KG30076) and *A. magna* (KG30097) has resulted in development of  
 25 11 linkage groups with 94 markers (Gobbi et al. 2006). As a part of Generation  
 26 Challenge Programme (GCP) of CGIAR, preparation of the first genetic map for  
 27 tetraploid cultivated groundnut species is in progress at ICRISAT. However, the  
 28 lower level of polymorphism between the parental genotypes of existing mapping  
 29 populations (e.g. TAG24 x ICGV 86031 developed at ICRISAT, GPBD4 x TAG24  
 30 developed at UAS Dharwad) poses a serious problem. Nevertheless, we expect to  
 31 prepare the partial/genome wide map with about 100 SSR loci (Varshney et al.,  
 32 unpublished results). The progress in the area of genome mapping of *Arachis* species  
 33 is summarized in Table 2.

#### 34 4.1.3. *Pigeonpea*

35 In case of pigeonpea, molecular markers (RFLPs) were used as early as 1994  
 36 to study genetic diversity in wild species using nuclear DNA probes (Nadimpalli  
 37 et al., 1994). Subsequently, Ratnaparkhe et al. (1995) attempted to study DNA  
 38 polymorphism in cultivars and wild species. The level of polymorphism among the  
 39 wild species was extremely high, while little polymorphism was detected within  
 40 *C. cajan* accessions. In order to characterize a few putative cytoplasmic male sterility  
 41 lines, maize mitochondrial DNA (mt DNA) specific probes were used in RFLP  
 42 analysis (Sivaramakrishanan et al., 1997). Recently, AFLP analysis was carried out  
 43 with a few cultivars and two wild species (*Cajanus volubilis*, *Rhynchosia bracteata*)  
 44



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

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

## 17 **4.2. Trait Mapping and Marker-Assisted Selection**

18  
19 Marker-assisted selection (MAS) offers great promise for improving the efficiency  
20 of conventional plant breeding. Molecular markers are especially advantageous  
21 for traits where conventional phenotypic selection is difficult, expensive, or lacks  
22 accuracy or precision. Molecular mapping and identification of molecular markers  
23 associated with genes and QTLs for traits are prerequisites for the MAS. As  
24 mentioned above, though not excellent, some progress has been made in the area of  
25 development of molecular markers or construction of genetic maps in chickpea and  
26 groundnut. As a result, molecular markers linked to a few abiotic or biotic stress  
27 tolerance/resistance as well as agronomic traits have been identified recently.

### 28 **4.2.1. Chickpea**

29  
30 Genetic mapping in chickpea has focussed on tagging agronomically relevant genes  
31 such as ascochyta blight resistance (Tekeoglu et al., 2002; Udupa and Baum, 2003;  
32 Collard et al., 2003; Flandez-Galvez et al., 2003b; Millan et al., 2003; Cho et al.,  
33 2004; Iruela et al., 2006), fusarium wilt resistance (Benko-Iseppon et al., 2003;  
34 Sharma et al., 2004) and yield-influencing characters such as double podding and  
35 other morphological characters (Cho et al., 2002; Rajesh et al., 2002; Abbo et al.,  
36 2005; Cobos et al., 2005). Progress in the area of mapping of ascochyta blight  
37 resistance has been summarized recently by Millan et al. (2006). Since apparently  
38 all major blight resistance QTLs are tagged with SSR markers, pyramiding of  
39 resistance genes via MAS should now be feasible and awaits its proof-of-principle.  
40 The genetic control of this disease bred into cold tolerant germplasm would be  
41 a major breakthrough for yield increases in Mediterranean-type environments in  
42 many parts of the world.

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

01 drought tolerance at ICRISAT. The most promising drought tolerant variety was  
02 ICC 4958 that had 30% more root volume than the popular variety Annigeri (Saxena  
03 et al., 1993); therefore, root traits were considered important parameters to improve  
04 the drought tolerance (Kashiwagi et al., 2006). Selection for root traits is very  
05 difficult, since it involves laborious methods such as digging and measuring root  
06 length and density. Molecular tagging of major genes for root traits may enable MAS  
07 for these traits and could greatly improve the precision and efficiency of breeding.  
08 In this direction, a set of 257 recombinant inbred lines (RILs) was developed from  
09 the cross Annigeri × ICC 4958 at ICRISAT and glasshouse-evaluated to identify  
10 molecular markers for root traits. After screening the parental genotypes with over  
11 250 STMS and 100 EST markers and the mapping population with 57 polymorphic  
12 markers, a QTL flanked by STMS markers TAA170 and TR55 on LG 4A was  
13 identified that accounted for maximal phenotypic variation in root length (33%),  
14 root weight (33%) and shoot weight (54%) (Chandra et al., 2004). Genotyping of  
15 two other mapping populations (ICC 4958 × ICC 1882 and ICC 8261 × ICC 283),  
16 which have larger genetic variation than Annigeri × ICC 4958 with SSR markers  
17 is in progress at ICRISAT.

18 For improving cold tolerance, AFLP markers have been linked to the trait using  
19 bulked segregant analysis of F<sub>2</sub> progeny of a cross between the chilling sensitive  
20 cultivar Amethyst and the chilling tolerant ICCV 88516 (Clarke and Siddique,  
21 2003). Candidate AFLP markers were converted into SCAR markers (Paran and  
22 Michelmore, 1993) to overcome the limitations of the dominant AFLP marker  
23 system. The most promising primers were based on a 560 bp fragment containing  
24 a simple sequence repeat (3 bp repeat microsatellite) with nine repeats in the  
25 susceptible parent and ten repeats in the tolerant parent. The three-base difference  
26 was visualised on a vertical acrylamide gel, and was very useful in the selection of  
27 chilling tolerant progeny derived from crosses between ICCV 88516 and Amethyst.  
28 Unfortunately, there has been no success in applying these SCAR markers to other  
29 breeding materials.

30 In the case of flowering, a major gene (*efl-1*) for time of flowering was reported  
31 by Kumar & van Rheenen (2000), and another one (*ppd*) by Or et al. (1999). The  
32 latter gene controls time to flowering through photoperiod response (Hovav et al.,  
33 2003). Cho et al. (2002) mapped a QTL for days to 50% flowering to LG 3. Another  
34 QTL was also located on this linkage group in an interspecific RIL population and  
35 explained 28% of the total phenotypic variation (Cobos et al., 2005).

36 In addition to the above mentioned traits, molecular mapping for other traits  
37 is in progress in many laboratories. For instance, SSR-based genotyping and  
38 phenotyping of one mapping population (ICCV 2 × JG62) is in progress at  
39 NIPGR and ICRISAT to identify the molecular markers associated with salinity  
40 tolerance.

#### 41 4.2.2. *Groundnut*

42 There are very few genetic maps available based on cultivated groundnut genotypes.  
43 The available maps, based on interspecific crosses, will be useful in locating specific  
44

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

05 Although marker-trait association has been little used within *A. hypogaea*, even  
06 with the limitations afforded by present technologies, it has much potential for  
07 introgressing genes from closely related *Arachis* species into the cultivated genome.  
08 For instance, Garcia et al. (1995) showed introgression of genes from *A. cardenasii*  
09 into *A. hypogaea* in 10 of 11 linkage groups on the diploid RFLP map developed  
10 by Halward et al. (1993). Subsequently, Garcia et al. (1996) used RAPD and SCAR  
11 technologies to map two dominant genes conferring resistance to the nematode by  
12 using the mapping population derived from the cross *A. hypogaea* x *A. cardenasii*.  
13 Burrow et al. (1996) identified RAPD markers linked to nematode resistance in  
14 another interspecific cross involving the species *A. hypogaea*, *A. batizocoi*, *A. carde-*  
15 *nasii* and *A. diogoi*. Such linkage of RAPD markers with components of early  
16 leaf spot and corn rootworm resistance was shown in another interspecific cross  
17 (Stalker and Mazingo, 2001). By using the BSA approach with an F<sub>2</sub> population  
18 derived from the cross (ICG 12991 × ICGVSM 93541) and phenotyping the F<sub>3</sub>  
19 population, twenty putative AFLP markers were identified of which 12 mapped to  
20 five linkage groups. Interestingly, mapping of a single recessive gene on linkage  
21 group 1 (3.9 cM from a marker originating from the susceptible parent) explained  
22 76% of the phenotype variation for aphid resistance. AFLP markers were used  
23 to establish marker-trait association for tomato spotted wilt virus resistance in  
24 groundnut (Milla 2003). Marker-trait association studies for several other traits, e.g,  
25 water use efficiency (WUE), rust and late leaf spot (LLS) are underway at ICRISAT  
26 and UAS Dharwad.

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#### 29 4.2.3. *Pigeonpea*

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

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### 39 5. NOVEL GENETIC AND GENOMICS APPROACHES

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

### 01 **5.1. Association Mapping and Advanced Backcross QTL (AB-QTL)** 02 **Analysis**

03 In general, a low level of polymorphism has been a major constraint in devel-  
04 oping genetic maps in the legume crops mentioned in this chapter. Further, species  
05 like pigeonpea, which is of regional importance in Asia and Africa, has not been  
06 explored at the international level. Non-availability of resistance sources in culti-  
07 vated gene pools of these species for several fungal and viral diseases, e.g., pod borer  
08 in chickpea and pigeonpea, sterility mosaic in pigeonpea, aflatoxin in groundnut,  
09 and the difficulties of crossing cultivated species with wild species are other barriers  
10 that hampered the development of appropriate mapping populations in these legume  
11 species. Novel approaches, based on classical genetics, like linkage disequilibrium  
12 (LD) based association mapping (Hirschhorn and Daly, 2005), advanced back-cross  
13 QTL (AB-QTL) analysis (Tanksley and Nelson, 1996) offers the possibility to  
14 overcome at least a few barriers. For instance, an appropriate natural population,  
15 genebank or breeding material may be used in LD-based association analysis. In  
16 this regard, emergence of novel marker systems such as SNPs and DArTs and  
17 developments in this direction for the mentioned legume species would make it  
18 possible to undertake candidate gene sequencing (using SNP assays) as well as  
19 whole genome scanning (using DArTs) based approaches for association analyses.  
20 In contrast to the numerous linkage disequilibrium (LD) studies in human and other  
21 mammals, there are very few publications on this topic in agriculturally important  
22 crops including legumes (Virk et al., 1996; Beer et al., 1997; Pakniyat et al., 1997;  
23 Forster et al., 1997; Igartua et al., 1999; Remington et al., 2001; Thornsberry et al.  
24 2001; Turpeinen et al. 2001; Hansen et al. 2001; Sun et al. 2001, 2003; Skot  
25 et al., 2002; Ivandic et al., 2002, 2003; Amirul Islam et al., 2004; Zhu et al., 2003;  
26 Simko et al., 2004). Traditionally the plant community has been reticent to use  
27 LD mapping believing that it can lead to spurious and non-functional associations  
28 due to mutation, genetic drift, population structure, breeding systems and selection  
29 pressure (Hill and Weir, 1994; Pritchard et al., 2000). However, most of these limita-  
30 tions are being overcome in recent mammalian studies by following precautions  
31 that minimize circumstantial correlations and maximize the accuracy of association  
32 statistics (Yu et al., 2006; Yu and Buckler, 2006; Ersoz et al., 2007). Unfortunately  
33 the real value of LD mapping in legume species remains to be demonstrated as  
34 most of the reports to date are based on small population sizes or a limited number  
35 of markers and generally lack validation.

36 Advanced-backcross QTL analysis (AB-QTL), proposed by Tanksley and Nelson  
37 (1996), involves transferring the QTLs of agronomically important traits from a  
38 wild species to a crop variety. In this approach, a wild species is backcrossed  
39 to a superior cultivar with selection for domestication traits. Selection is imposed  
40 to retain individuals that exhibit domestication traits such as non-shattering. The  
41 segregating  $BC_2F_2$  or  $BC_2F_3$  population is then evaluated for traits of interest  
42 and genotyped with polymorphic molecular markers. These data are then used for  
43 QTL analysis, potentially resulting in identification of QTLs, while transferring  
44 these QTLs into adapted genetic backgrounds. The AB-QTL approach has been

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

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## 5.2. Transcriptomics and Functional Genomics

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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

01 responsible for ascochyta blight resistance (Coram and Pang, 2005b, 2006), drought  
02 and salinity tolerance (E. Pang, pers. commun.). The candidate genes identified by  
03 EST sequencing (and gene prediction) and functional genomics approaches can be  
04 further verified through real time PCR analysis (Luo et al., 2005) and genetical  
05 genomics/ expression genetics approaches (Jansen and Nap, 2001; Varshney et al.,  
06 2005) after conducting gene expression analysis in quantitative fashion using segre-  
07 gating mapping populations. By analyzing the expression levels of genes or clusters  
08 of genes within a segregating population, it is possible to map the inheritance of  
09 that expression pattern. The QTLs identified using expression data in a mapping  
10 population are called e(xpression)QTLs. The eQTLs can be classified as *cis* or  
11 *trans* acting based on location of transcript compared to that of the eQTL influ-  
12 encing expression of that transcript (de Konig and Haley, 2005). Because of this  
13 feature, eQTL analysis makes it possible to identify factors influencing the level  
14 of mRNA expression. The regulatory factor (second order effect) is of specific  
15 interest because more than one QTL can be putatively connected to a *trans*-  
16 acting factor (Schadt et al., 2003). Thus, the mapping of eQTLs allows multifac-  
17 torial dissection of the expression profile of a given mRNA or cDNA, protein  
18 or metabolite into its underlying genetic components as well as localization of  
19 these components on the genetic map (Jansen and Nap, 2001). In recent years, in  
20 many plant species, the genetical genomics approach has demonstrated its power  
21 (see Kirst and Yu, 2007).

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

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

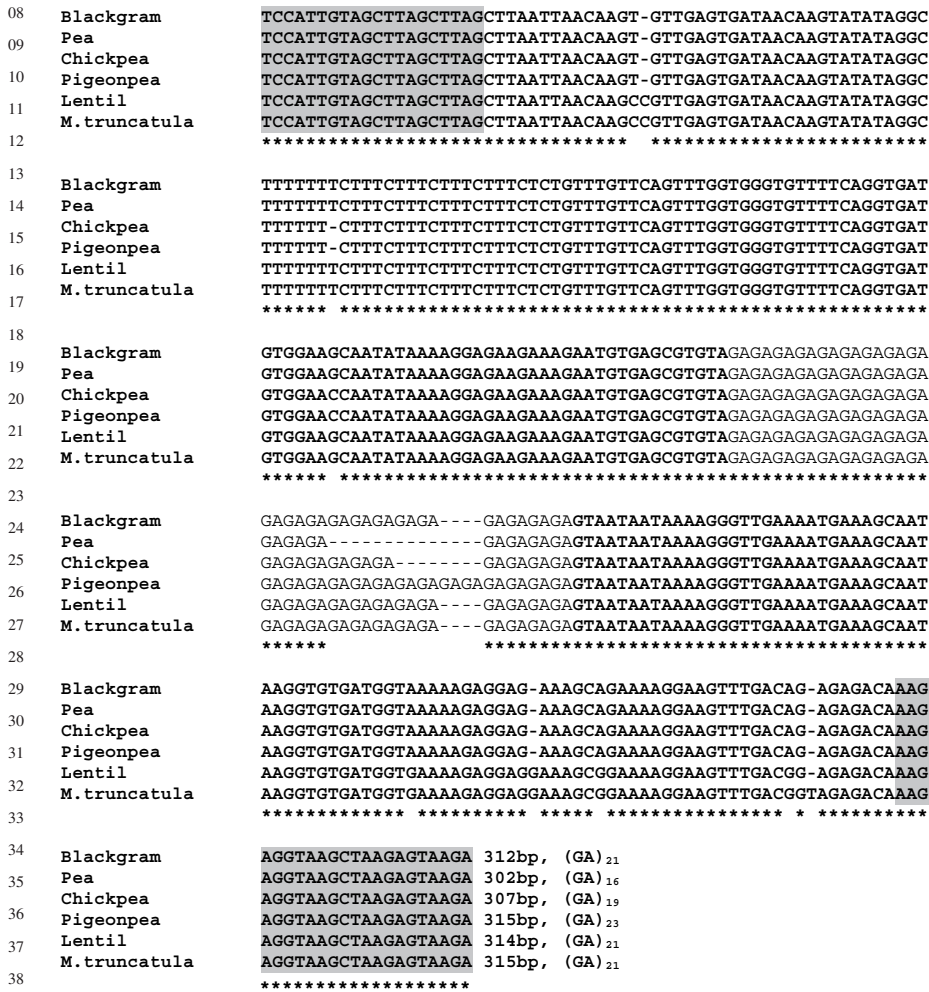
01 2005). The use of suppression subtractive hybridization (SSH) to prepare the  
02 subtracted cDNA libraries and identify the genes regulated during interaction with  
03 the fungus *Cercosporidium personatum* (causing the disease late leaf spot) is in  
04 progress in Brazil (Nobile et al., 2006). To understand the molecular mechanisms  
05 of drought tolerance, the use of differential expression of mRNA transcripts and  
06 proteins are underway at Florida A & M University (Katam et al., 2006). With the  
07 development of more functional genomics resources in SAT legumes, it is antici-  
08 pated that the use of functional genomics and expression genetics approaches may  
09 help the community to dissect the complex traits and devise strategies for crop  
10 improvement.

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### 13 5.3. Comparative Genomics

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

01 *Cicer* genomic SSR markers across model, crop and fodder legumes have demon-  
 02 strated that the genomic SSRs find a mean average transferability of nearly 25%  
 03 across *M. truncatula*, *L. japonicus*, soybean, pea, lentil, pigeonpea, blackgram,  
 04 mungbean and *Trifolium alexandrinum* (Figure 1) and often conserved in the model  
 05 plant *A. thaliana*. Moreover, the *Cicer* markers have been demonstrated to be  
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 40 *Figure 1.* Multiple sequence alignment of the size variant alleles of the legume accessions at the chickpea  
 41 STMS marker NIPGR19 locus. Accessions of *M. truncatula* (SA27783), blackgram (IC342955), lentil  
 42 (IC383669), pea (RFP16) and pigeonpea (IC347150) along with chickpea (Pusa362) are analyzed. The  
 43 asterisks indicate similar sequences and ‘-’ indicate alignment gaps. The repeat region is indicated in  
 44 boldface and shadowed boxes indicate conserved primer binding sites. Allele sizes and repeat motifs  
 are mentioned at the end of the sequence



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

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## 06 6. TOWARDS A BRIGHT FUTURE OF MOLECULAR BREEDING 07 IN SAT LEGUMES

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

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

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

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

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