Chapter 1  

Introduction

Chickpea (Cicer arietinum L., 2n=16) is an autogamous annual cool season grain legume cultivated in arid and semi-arid areas across the world (Iruela et al. 2007). It is the third most important food legume in the world after dry beans and peas (FAOSTAT, 2004). It is an extremely self-pollinated crop with an out-crossing rate of less than 1% (Singh et al. 2008). The genus Cicer belongs to family Leguminoseae and includes 9 annual and 34 perennial wild species (Van der Maesen, 1972, 1987; Muehlbauer, 1993). Of the 9 annual species, chickpea (Cicer arietinum L.) is the only one which is cultivated, while the other 8 annual species of chickpea are wild and include C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum, C. cuneatum, C. chorassanicum and C. yamashitae. The cultivated chickpea has many other common names like bengal gram (Indian), garbanzo (Latin America), homes, hamaz (Arabian), nohud, lablabi (Turkey) and shimbra (Ethiopia) (Muehlbaeur and Tullu, 1997).

Chickpea was one of the first grain legumes to be domesticated in the old world (Van der Maesen, 1987). This primordial crop probably originated 7000 years ago in an area of present-day south-eastern Turkey and adjoining areas of Syria, and spread from there to Middle East, South Asia and North Africa, where it become an imperative crop. Ladizinsky and Adler (1976) considered C. reticulatum as the wild progenitor of cultivated chickpea as supported by seed protein electrophoresis. Although there is still controversy on the wild progenitor of chickpea, but the investigations of interspecific hybridization (Singh and Ocampo, 1993), karyotype (Ocampo et al. 1992) and isozyme patterns (Labdi et al. 1996) among nine annual Cicer species strongly supports the findings of Ladizinsky and Adler (1976).

The plant of chickpea stands between 20 cm and 1 m tall (Muehlbauer and Tullu, 1997), with a genome size of 740 Mbp (Arumuganathan and Earle, 1991; Coram et al. 2007), slightly less than the well-characterized tomato genome (950 Mbp). Nearly 90% of the crop is cultivated under rainfed conditions mostly on receding soil moisture. Chickpea being a rabi crop is normally sown in the month of October and harvested in March, whereas in northeastern Australia, it is sown in May/June and harvested from October to December (Knights, 1993).
Chickpea has high nutritive value and serves as an important cheap source of protein in developing countries diet in addition to improving land fertility (Saeed et al. 2011). The main use of chickpea seeds is for human consumption, especially for strictly vegetarian people, as they are free from any anti-nutritional factors and are rich in phosphorus, calcium and digestible proteins (Rana et al. 2003). They may be consumed whole as dhal/flour, or the juvenile shoots may be eaten as a vegetable (Muehlbauer and Tullu, 1997). It is the most economical and easily available source of carbohydrates (57-60%), protein (19.5%), fats (1.4%), moisture (4.9-15.59%) and ash (4.8%) (Huisman and Van der Poel, 1994) and it is a good source of calcium, magnesium, potassium, phosphorus, iron, zinc and manganese (Ibrikci et al. 2003). It also makes up the deficiency of cereal diets (Ali et al. 2011). Moreover, chickpea pod covers and seed coats are also used as fodder (Tahir and Karim, 2011). Chickpea is also used as a medicine for aphrodisiac, bronchitis, cataract, cutamenia, cholera, constipation, diarrhea, dyspepsia, flatulence, snake bite, sunstroke and warts (Duke, 1981). Further, it was reported to be most hypocholesteremic agent (Geervani, 1991). Thus, it is an important food for the people to improve major food-related health problems (Agharkar, 1991; Charles et al. 2002; McIntosh and Topping, 2000).

Chickpea can grow well despite the low inputs under edaphic and arid environments, which make it an important component of the cropping system of subsistence farmers in the Indian subcontinent, West Asia and North Africa. It can manage to squeeze out 70% of its nitrogen requirements from symbiotic nitrogen fixation and as a consequence, provides an attractive option for intercropping. More than 50 countries grow chickpea; however, India, Turkey, Pakistan, Canada, Mexico, Myanmar, Iran, Ethiopia and Australia together contribute 93.1% of the global chickpea production (Upadhyaya et al. 2008). India produces 5.97 million tonnes of chickpea during 2008, which was about 75% of the total world’s production (FAO, 2008). The yield potential of present-day chickpea varieties exceeds 4 t/ha; however, actual yield is less than 0.8 t/ha (Madrid et al., 2008). The gap between potential and actual average yield is mainly due to diseases and poor management. Chickpea yield can vary considerably due to combination of diseases and stresses, resulting in regular crop failures in many areas (Knights and Siddique, 2002). Fungal diseases, such as Ascochyta blight, Rhizoctonia root rot, Pythium rot and Fusarium wilt, as well as bacterial blight and certain viruses can cause considerable damage to the crop.
In the field, the two most incurable chickpea diseases on a global scale are *Fusarium* wilt and *Ascochyta* blight (Nene and Reddy, 1987) and accounts for major (10-90%) crop losses every year. Another major reason for low productivity of cultivated chickpea is its narrow genetic base and its sexual incompatibility with other *Cicer* wild types in natural interspecific crosses (Sant et al. 1999).

In recent years, major emphasis in breeding programmes has been on the stabilization and enhancement of yield. For this purpose, the chickpea cultivars have been evaluated for a range of diseases and stresses, and causes of resistance have been identified. The identification and tagging of resistance genes for *Ascochyta* blight and *Fusarium* wilt would be invaluable tools for the development of resistant chickpea cultivars through marker-assisted selection (MAS). Quantitative trait loci (QTL) mapping is a highly effective approach to identify and tag disease resistance genes in plants (Young, 1996). QTL mapping may be appropriate for identifying the number and position of genes conferring disease resistance, as resistance is reported to be controlled by multiple genes (Tekeoglu et al. 2000). Knowledge of the extent of genetic diversity within a species is a critical factor while selecting parents for QTL mapping, because sufficient DNA polymorphism must exist between parents for segregation analysis and genetic mapping (Collard et al. 2003). Subsequently, genetic diversity among the parents is a prerequisite to ensure the chances of improved segregate selection for various characters (Dwevedi and Gaibriyal, 2009).

Genetic diversity studies have traditionally been through morphological and biochemical markers which are rather less effective and are influenced by environmental factors as well as developmental stage. The genetic diversity among chickpea cultivars is limited; therefore chickpea breeders are keen to search for new approaches for the analysis of genetic variability. Further, due to obligatory self-pollination and thousands of years of selection, the genome of chickpea (*Cicer arietinum* L.) has been streamlined and became extensively monotonous. Therefore, it’s the time to explore new sources of variation that might be used in plant breeding programs. The basic criterion of phylogenetic relationship is the gene homology, which in majority of cases cannot be measured directly because of reproductive barriers between species (Nisar et al. 2007). Conventional breeding programs have not greatly helped in improving the grain yield. All over the world chickpea breeders...
are focusing on increasing yield by pyramiding genes for resistance/tolerance into elite germplasm (Bharadwaj et al. 2010). The advances in molecular marker technology have speed up the progress of genome mapping and MAS in chickpea. Molecular marker approach help in improving the effectiveness of molecular breeding to ten times because selection is based on the molecular marker closely related to trait of interest and therefore, new combinations are generated continuously especially where the scoring of morphological characters are near to impossible.

Several different molecular markers are available for the identification of cultivars and analysing genetic diversity. Randomly amplified polymorphic DNA (RAPD) marker-system has been used previously for evaluating genetic diversity in chickpea (Moussa et al. 1996; Ahmad, 1999; Sant et al. 1999; Sudupak et al. 2002; Collard et al. 2003). However, little genetic diversity was detected using RAPD markers (Simon and Muehlbauer, 1997; Singh et al. 2002). ISSR (inter simple sequence repeats) technique is more consistent than the RAPD technique as it generates larger number of polymorphic loci per primer (Aggarwal et al. 2011; Ratnaparkhe et al. 1998 a, b). Unlike RAPDs, ISSR markers are detected using comparatively longer semi-arbitrary SSR primers at highly stringent conditions in PCR; therefore, they are reproducible and highly polymorphic DNA markers (Rafalski et al. 1996; Bornet and Branchard, 2001). ISSR is based on amplification of genomic segments flanked by inversely oriented and closely spaced SSR loci using microsatellite core unit bearing oligonucleotide primers which could be either nonanchored or anchored to 5’ or 3’ end of the repeats with 1-3 random nucleotides (Zietkiewicz et al. 1994; Gupta et al. 1994; Rafalski et al. 1996; Sudupak, 2004a). This technique is fast, cost-efficient and does not need prior knowledge of sequence.

Currently, markers generated by sequence tagged microsatellite site (STMS) primer pairs are also predominantly appropriate for genetic diversity analysis. They consist of 1-6 bp tandem repeat regions that subsist all over the genome uniformly. Aside from being highly polymorphic, STMS markers are PCR-based and can afford single-locus detection, may be co-dominantly inherited and offer the potential for automated application in plant breeding (Mansfield et al. 1994). In recent years, STMS markers have been used broadly in genetic diversity analysis and DNA fingerprinting (Choumane et al. 2000; Abe et al. 2003; He et al. 2003). Globally, in chickpea a lot of research efforts have led to identification and characterization of a
variety of microsatellite markers (Huttel et al. 1999; Winter et al. 1999; Sethy et al. 2003; Lichtenzveig et al. 2005; Choudhary et al. 2006; Sethy et al. 2006b; Castro et al. 2011) and their management for genome mapping and phylogenetic analysis (Winter et al. 2000; Sethy et al. 2006a). Moreover, inter and intraspecific polymorphism studies were conducted to provide new dimensions for the advancement of linkage maps (Chodhary et al. 2009; Choudhary and Abhishek, 2010; Gaur et al. 2011).

In order to make optimal use of any desirable traits detected within novel germplasm, the genomic location of the governing genes should be identified. Also, the large amount of genetic variation can be used efficiently for gene tagging and genome mapping of crosses to introgress the favourable traits such as high yield potential, disease and insect resistance into the cultivated genotypes. Therefore, use of molecular markers to access genetic diversity and genome mapping via genetic map will be valuable for identification of genes or QTLs associated with various diseases like wilt/blight resistance. Thus, to explore the genetic diversity in chickpea cultivars and to find out any possible molecular markers linked with resistance against wilt/blight, the present investigation entitled “Genetic divergence and molecular characterization of chickpea (Cicer arietinum L.) genotypes using molecular markers” has been proposed with the following objectives:

1) To prepare DNA fingerprint database of chickpea genotypes using ISSR and STMS markers.

2) To determine genetic diversity/relatedness among chickpea genotypes using suitable software programmes.

3) To identify molecular markers linked to genes of disease resistance in chickpea.