CHAPTER - 2

LITERATURE REVIEW

Basic biological aspect of chickpea, salient features, and problems associated with chickpea cultivation, approaches to find remedy and existing knowledge in this regard are reviewed below comprehensively:

2.1 Chickpea: Basic Biology

Chickpea and related crop species are referred to as pulses. Pulses are those grain crops which are basically rich in protein, richer than any grain crop like cereals. Pulses are generally not used as staple food but as major supplement to staple food mainly as source of plant based dietary protein. All the pulses belong to the family Leguminosae. Legumes (Fabaceae) constitute the third largest family of flowering plants comprising more than 650 genera and 18000 species (Zhu et al. 2005). Economically, legumes represent the second most important family of crop plants after Poaceae (grass family), accounting for approximately 27% of the world’s crop production (Zhu et al. 2005). Legumes provide one third of mankind’s protein requirement. In many developing countries legumes serve as the only source of protein (Ashraf et al. 2009). One of the most important attributes of legumes is their unique capacity for symbiotic nitrogen fixation, underlying their importance as a source of nitrogen in both natural and agricultural ecosystems.

The Leguminosae family has enormous diversity as it include every morphophylogical types of plants- viz. herbs, shrubs, small and big tree, climbers, creepers, lianes, annual, perennial etc. Accordingly the family is sub-divided into three sub-families, viz. Mimosoideae, Caesalpinioideae, and Papilionoideae.
Papilionoideae subfamily contains nearly all economically important crop legumes, including soybean (*Glycine max*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and alfalfa (*Medicago sativa*). With the notable exception of peanut, all these important crop legumes fall into two Papilionoid clades, namely, Galegoid and Phaseoloid, which are often referred to as cool season and tropical season legumes, respectively (Zhu et al. 2005).

Chickpea (*Cicer arietinum* L.) is the only cultivated species of the genus *Cicer* which include 43 species. Among them 9 including chickpea are annual. Other 33 species are perennial while one is unclassified. Chickpea is a small bushy type annual plant characterised by unipinnate leaves attaining a height of 30-45 cm. Typically it is grown in semi-arid areas as winter/autumn (Rabi) crop. Its lifespan is 2-3 months and on maturity bears fruit which is called pod with 2-3 seeds per pod. The mature pod along with the plant is harvested and thrashed to collect the seeds. The pods split vertically which is the characteristics of all pulses.

![Atypical chickpea plant at mature stage and seeds of major two classes viz. Desi (upper right) and Kabuli (lower right)](image-url)

Figure 2.1: Atypical chickpea plant at mature stage and seeds of major two classes viz. Desi (upper right) and Kabuli (lower right)
Southwest Asia and the Mediterranean particularly South-East Turkey and Syria are considered as the two primary centres of origin of chickpea (Singh et al. 1997). Ethiopia is regarded as secondary centre of origin. The generally accepted view is that smaller and coarse type of grain referred to as “Desi” evolved first followed by smooth and bigger grain type referred to as “Kabuli” through mutation and selection (Singh, 1997). There is evidence that “Kabuli” type evolved in Afghanistan in the area around the capital Kabul and from there it reached India in 17th century (Van der Maesan, 1972).

Chickpea is consumed as food by humans and used as fodder for livestock. It is consumed as a fresh immature green seed, whole seed, “Daal” and flour. Chickpea pod covers and seed coats can also be used as fodder. In extreme conditions such as crop loss due to drought, people in certain region resort to chickpea plants for food and consumed as vegetables. It is also consumed as a delicacy in India during the chickpea-growing season.

Chickpea seed is a good source of carbohydrates and proteins, which together constitute 80% of the total dry weight of seed. The crude protein content of chickpea varies from 17% to 24% containing the essential amino acids like tryptophan, methionine and cysteine. Thus chickpea serves as a main source of dietary protein for the vegetarians in India. Moreover, they are a good source of calcium, magnesium, potassium, phosphorus, iron, zinc and manganese (Millan et al. 2006). Chickpea also contains high amounts of carotenoids but do not have isoflavones. Chickpea provides more beta-caroteneoids than the genetically engineered “golden rice”. (Millan et al. 2006). As compared to other grain legume, chickpea does not contain anti-nutritive components, which makes it suitable for use as functional food or nutraceutical.
FAOSTAT data shows Chickpea (Cicer arietinum L.) as world’s third most important pulse crop after beans (Phaseolus vulgaris) and peas (Pisum sativum L.). Chickpea is produced all over the world in almost 50 countries covering more than 10 million hectares of cultivated land (Kottapalli et al. 2009). Worldwide annual production of chickpea is 9.1 Mt according to the FAOSTAT data. Among the more than 50 chickpea growing countries major producers are India, Pakistan and Turkey contributing to about 65%, 9.5% and 6.7% of global production (Millan et al. 2006).

2.2. Genetic diversity and production constraints

Evidence shows that chickpea was first cultivated in Turkey, as early as 7000 BC. It is believed to have been domesticated from Cicer reticulatum a close wild relative. After domestication initially in Turkey the crop spread to other region of Middle East, India and Ethiopia (Sharma and Muehlbaur, 2007). In recent times there are such countries like Australia and Canada where it is not consumed much but cultivated exclusively for export to earn foreign exchange (Sethy et al. 2006). It is a self –pollinated crop with diploid genome (2n = 2x = 16). The genome size is estimated to be 740 Mbp (Millan et al. 2006) which is little bigger than model legume Medicago truncatula with 530 Mbp. However, compared to many other legume crop like Soybean, Peanut, Garden pea, Alfalfa and lentil, its genome is relatively smaller (Lichtenveig et al. 2005). This makes genomic study and gene manipulation relatively easier.
All the cultivated chickpea cultivars are broadly grouped into 2 classes – “Desi” and “Kabuli”. Desi chickpeas generally have small, coloured seeds, whereas kabulis produce large, cream coloured ones (Milan et al. 2006). To a certain extent this classification overlaps with the macrosperma and microsperma races proposed by Moreno and Cubero (1978). This classification also reflects utilization. Kabulis are usually utilized as whole grains, desis are decorticated and processed into flour. Kabuli chickpeas probably evolved in the Mediterranean basin from Desi type (Milan et al. 2006). Kabuli × Desi crosses are used in many breeding programs to combine genes for cold tolerance, resistance to Ascochyta blight. Long vegetative growth is
found more frequently in kabuli types along with genes for heat and drought tolerance, resistance to *Fusarium* wilt and genes for early flowering (Singh, 1987).

All pulses are known for stagnation in production and productivity but more so for chickpea. As per FAO estimate globally chickpea production is stagnating at 0.8t/ha (FAOSTAT, 2005). Compared to number one grain legume Faba Bean (*Vicia faba* L.) at 1.754t/ha followed by second most important pulse pea with 1.569t/ha (Palomino *et al.* 2009).

The production constraints of chickpea are by and large same as that of other pulses. First is their confinement to traditional area of cultivation. Because of specific agro-climatic requirement and soil type various efforts to expand their cultivation in non-traditional or new areas are unsuccessful (Ali and Kumar, 2006). It has been found that in India, area under chickpea /pulse cultivation remained same in the range of 18Mha to 24 Mha over a period of 60 years irrespective of pre green revolution, green revolution or post green revolution period. Particularly, since 1980s the figure is fluctuating from 20-23 M/ha

![Area under pulses cultivation](image)

Figure – 2.4: Area under chickpea cultivation in India from the year 1951-2008
Secondly, unlike cereals, pulses including chickpea have narrow genetic base. Leading cereals like paddy has enormous genetic diversity with the number of genotypes estimated to be between 50,000 to 80,000. Likewise wheat also has very broad genetic diversity. Such a wide gene pool facilitates genetic improvement through conventional as well as molecular breeding strategies. By contrast chickpea gene pool comprises of about 200 cultivars. This narrow genetic background in chickpea hampers crop improvement programs. Despite persistent efforts, the number of improved cultivars with various agronomic traits is limited. Some of the important categories of chickpea genotypes are listed below:

Table – 2.1: Important chickpea genotypes and their utility

<table>
<thead>
<tr>
<th>Category</th>
<th>Important Genotypes</th>
</tr>
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<tbody>
<tr>
<td>Cold Tolerant</td>
<td>ICCV-10, ICCV-88503, ICCV-88506, ICCV-88510</td>
</tr>
<tr>
<td>Drought Tolerant</td>
<td>Anigeri, ICC-4958, ICC-8261</td>
</tr>
<tr>
<td>Helicoverpa Resistant</td>
<td>ICC-506, ICCV-2, I</td>
</tr>
<tr>
<td>Salinity tolerant</td>
<td>Annegiri, ICCV-2, I CCV-10</td>
</tr>
<tr>
<td>Disease Resistant</td>
<td>ICCV-2,</td>
</tr>
<tr>
<td>Fusarium resistant</td>
<td>CPS-1, WR-315, ICCV-2, ICCV-10</td>
</tr>
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</table>

Chickpea like other pulses are exclusively self pollinating crop. This prevents natural cross-pollination and exchange of genetic material and hence limited genetic diversity. Moreover most pulses including chickpea are recalcitrant and hence not much amenable to genetic improvement.
Another major constraint is abiotic and biotic stress. Abiotic stresses like drought, unseasonal rainfall, salinity, cold, frost etc cause considerable damage and hence yield loss. Most chickpea cultivars are susceptible to biotic stresses like pest infestation and diseases that also include storage pests. Among the biotic stresses the most important are *Fusarium wilt, Ascochyta Blight*, Grey mold etc which cause extensive damage to the crop.

Therefore, one approach to address the problem is breeding for disease resistance. In principle, 10% disease prevention will imply 10% increase in overall yield.

### 2.3. Fungal Disease: Overview

Chickpea productivity is limited by several diseases, of which *Fusarium wilt* (FW) caused by *Fusarium oxysporum* f. sp. *ciceris*, *Ascochyta* blight (AB) caused by *Ascochyta rabiei*, and Botrytis gray mold (BGM) caused by *Botrytis cinerea* are predominant (Pande et al. 2007).

Among them *Fusarium* wilt is a vascular disease which cause damage to vascular tissue and eventually the plant die due to wilting. *Ascochyta* blight is a foliar disease that causes wilting and rotting of leaves. Grey mold is caused by Botrytis *cinerea* third most important disease in chickpea.

*Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the major yield limiting factors in chickpea resulting in 10 to 90% yield loss (Sharma and Muehlbauer, 2007). The disease is highly destructive and worldwide in occurrence. It has been reported from almost all chickpea growing areas of the world including the Indian subcontinent, Iran, Peru, Syria, Ethiopia, Mexico, Spain, Tunisia, Turkey and
US as reviewed by Sharma and Muehlbauer (2007). The persistence of the pathogen and capacity to survive in soil without a host for years have, made its control more difficult. The disease to some extent can be managed by use of bio-control agents which provide eco-friendly control of the disease. Some bio-control agents have been reviewed by Hervas et al. 1997; Landa et al. 2001, 2004. But more economic and eco-friendly approach for Fusarium wilt management could be resistance genes (Jimenez-Diaz et al. 1993, Sharma et al. 2005, Sharma and Muehlbauer, 2007) that provide natural and inherent protection against the pathogen.

Ascochyta blight (AB) caused by the pathogen Ascochyta rabiei (Pass.) Labr. is one of the most devastating diseases of chickpea causing significant losses in grain yield and quality (Kottapalli et al. 2009). Disease development is favoured by cool and wet weather conditions, often resulting in hundred percent yield loss. The pathogen spreads via airborne spores and although the disease can be controlled by fungicide treatment, this is not economical for farmers of most developing countries (Kottapalli et al., 2009).

2.4. Fusarium wilt: characteristics of the disease and pathogen

Fusarium wilt is a widespread disease caused by the soil born fungal pathogen Fusarium oxysporium f. sp. ciceris. Fusarium wilt has been reported from diverse species from unrelated families. Fusarium wilt is a major constraint in the production of pulse crop, chickpea and lentils in particular (Dita et al. 2005). The disease affects seedlings and adult plants where it causes leaf chlorosis, wilting and death (Dita et al. 2005).
Figure – 2.5: *Fusarium* infested chickpea plant

Figure – 2.6: *Fusarium* infested chickpea in fields

Figure – 2.7: *Ascochyta* infested chickpea
Eight physiological races of *Fusarium oxysporum* f. sp. *ciceris* has been identified till date (Sharma and Muehlbauer, 2007). Four of them were reported from India. Haware and Nene (1982) reported existence of these 4 races using 10 chickpea differential lines which show variable resistance and susceptibility to different races of the pathogen. Races (1, 2, 3 and 4) were found to be prevalent in India causing widespread crop damages. The geographical distribution of races shows regional specificity for their occurrence in different regions of the world. Apart from region specificities, the eight races can also be divided into two groups based on symptomatology of infected plants i.e. yellowing syndrome and wilting syndrome. Out of eight races, six of them (races 1, 2, 3, 4, 5 and 6) cause wilting syndrome. These are more important economically than the others as wilting is more devastating than the yellowing. The plants show wilting syndrome within 3-4 weeks of infestation. However, wilting and yellowing syndromes are considered race-specific (Sharma and Muehlbauer, 2007). Although the pathogen could be classified into several races, overall, genetic makeup of the pathogen is very much similar. All *F. oxysporium* f. sp. *ciceris* isolates were found to belong to a single vegetative compatibility group.

The pathogen penetrates through the roots until it reaches the xylem where it reduces or blocks water transport to aerial parts. The infected plants finally wilt and die. (Halila *et al.* 2009). The infection process is influenced by environment especially the temperature and inoculums load. A temperature of around 25°C and inoculums load of 104–105 micro- or macro-conidia is optimum for disease development. Haware and Nene (1982) reported existence of four physiological races (1, 2, 3 and 4) of *F. oxysporum* f. sp. *ciceris* in India. Their analysis was based on the
reaction of pathogenic races to 10 chickpea differential lines. Two additional races (0 and 5) were later identified from Spain and Tunisia whereas another (race 6) was reported from California, USA as reviewed by Sharma and Muehlbaur (2007). Race 1 was subsequently divided into two races named as race 1A (from India) and race 1B/C (from Spain) based on variation in reaction on differential host lines (Jimenez Diaz et al. 1993). Race 1B/C was also found in USA (California), Syria, Turkey and Tunisia. Thus, a total of eight physiological races of the pathogen have been reported worldwide (Sharma and Muehlbaur, 2007).

Rahman et al. (1998) suggested the occurrence of two additional races in India. If the existence of the new races is confirmed, the total number of races will increase to ten from the present eight (Sharma and Muehlbaur, 2007). The race specificity is basically found to be associated with the geographical distribution. Among eight races, 0, 1B/C, 5 and 6 are primarily found in the Mediterranean region and the USA (Jimenez Diaz et al. 1993). Haware and Nene (1982) found races 1A, 2, 3 and 4 to be restricted to the Indian subcontinent. Symptomatically the races could be divided into two groups, one group showing yellowing syndrome and the other showing wilting syndrome. Of eight races, six (1A, 2, 3, 4, 5 and 6) cause wilting syndrome and are economically more important than races 0 and 1B/C that cause yellowing syndrome (Sharma and Muehlbaur, 2007).

2.5. Genetic basis of wilt resistance

In spite of the fact that the isolates are of monophyletic lineage, geographically isolated populations of the fungus displayed genetic and pathological diversity. Indian populations of the pathogen were also genetically as well as
pathologically distinct as revealed by DNA fingerprinting studies (Sharma and Muehlbauer, 2007).

It is assumed that *F. oxysporium* f. sp. *ciceris* is transported from one plant to another with contaminated seeds and disseminated in different regions evolved as different races. The emergence of geographically distinct virulence appears to be correlated to different chickpea varieties cultivated in these regions (Sharma and Muehlbauer, 2007).

Resistance to *Fusarium* wilt race-1 was first thought to be monogenic (Kumar and Haware, 1982). This was confirmed by Mayer *et al.* (1997) showed race-1 resistance to be governed by a single recessive gene. A second gene was also reported by Upadhyaya *et al.* (1983a, 1983b) and Singh *et al.* (1987). Later the phenomenon of late wilting got importance and was reported as monogenic in nature (Upadhyaya *et al.* 1983). Late wilting was reported to be controlled by three independent loci namely h₁, h₂, and h₃, each of which delayed onset of disease symptoms. Combination of any of the two late wilting genes (h₁h₁, h₂h₂ or h₁h₃ or h₂h₃) was required for complete resistance to race-1 (Upadhyaya *et al.* 1983, Singh *et al.* 1987).

Resistance to race-2 of the pathogen was also initially reported to be monogenic in nature by Pathak *et al.* (1975). But Gumber *et al.* (1995) reported two genes involving in race-2 resistance, while Kumar (1998) reported involvement of three genes. Their work implied that complete resistance could be obtained by the combination of aa bb loci (a and b are the first and the second loci). The third loci (C) in either homozygous or heterozygous recessive condition did not influence resistance against race-1 as studied by Gumber *et al.* (1995) and Kumar (1998). However, later works by Sharma *et al.* (2005) confirmed the initial report of Pathak *et al.* (1975) that
resistance to race-2 is monogenic. The discrepancy in the number of genes involved was due to the techniques used by the different workers. Kumar (1998) and Gumber et al. (1995) used sick plots for disease scoring where time of inoculation of all plants could not be uniform. On the other hand, Sharma et al. (2005) ensured uniform inoculation of each plant by dipping roots of each plant in pathogen inoculum containing $1 \times 10^6$ spores per ml. Uniform pathogen inoculation was ensured by cutting the tip of the roots of each plant and dipping it in spore suspension.

Genetics of other races is less studied as mentioned by Sharma and Muehlbauer (2007). Resistance to race-3 of the pathogen was found to be monogenic (Sharma et al. 2004, 2005). But its dominant or recessive nature is not yet ascertained (Sharma and Muehlbauer 2007).

Tullu et al. (1998) and Sharma et al (2005). Reported resistance to Fusarium oxysporium race-4 to be monogenic recessive in nature in some parental lines but found to be digenic in some other lines such as Suratato – 77. Sharma and Muehlbauer (2007) in their review also mentioned the phenomenon of late wilting in case of race-4 infection. For resistance to race-5 and race-0, Sharma and Muehlbauer (2007) found monogenic inheritance. But their dominant or recessive nature could not be determined.

Moreover, it has been observed that wilt resistance is mainly obtained from the “Desi” varieties of chickpea. Races 1, 2, 3, and 4 which occur in India are the most virulent ones while races found in the Mediterranean and the USA are less virulent (Haware and Nene, 1982). Definitely it could be implied from various studies that evolution of virulent races had a relationship with the chickpea genotypes used
for cultivation. Race-1 and race-2 are the two most devastating *Fusarium* races. Most of the studies have been directed towards these two races.

It is already known that resistance to *Fusarium* wilt in chickpea is controlled by at least three independent loci designated as $h_1$, $h_2$ and $h_3$. (Upadhyaya *et al.* 1983a, 1983b, Singh *et al.* 1987, Mayer *et al.* 1997). Partially recessive alleles in homozygous form at either the $h_1$ or $h_2$ locus or the dominant allele at the $h_3$ locus separately delay wilting. But any two of these loci together confer complete resistance. (Mayer *et al.* 1997). Genotypes of parental lines used in this study WR-315 and C-104 are denoted as $h_1h_1h_2h_2h_3h_3$ and $H_1H_1h_2h_2h_3h_3$ respectively which are already known to be segregating at 1:1 Mendelian ratio for $h_1$ locus which is responsible for giving resistance against late wilting.

Genes controlling resistance to *Fusarium* wilt races 1, 2, 3, 4 and 5 have been located in LG2 forming a cluster of genes in a narrow genomic area (Sharma and Muehlbauer 2007). Two genes controlling resistance to *Fusarium* wilt race 0 (*Foc01* and *Foc02*) have been respectively located in LG5 (Cobos *et al.* 2005) and LG2 (Halila *et al.* 2009). The latter was closely linked to the previously cited cluster of resistance genes. Recently a major gene controlling resistance to rust (*Uca1uca1*) has been identified and mapped in the LG7 (Madrid *et al.* 2008).

### 2.6. Molecular genetic basis of disease resistance and R-genes

The exact and detailed molecular and biochemical mechanism of how a resistant cultivar ward off a pathogen is not properly understood. However the genetic basis is known to a good extent. There is a class of genes called resistance genes (R-genes) which confer resistance against microbial pathogen. Resistance may be
monogenic or polygenic. By implication those cultivars that lack R-genes are susceptible. New inventions in the molecular marker technology, emergence of genomics, proteomics and transcriptomics and the establishment of model legume crops such as *Medicago truncatula*, *Lotus japonicas* etc. have enhanced knowledge towards understanding molecular genetic basis of stress resistance and R-gene expression and manipulation (Dita *et al.* 2005). Diploid nature and small genome size of these respective model crops together with short generation time have helped studies in disease responses. Application of powerful genomics and genetic techniques such as whole genome sequencing, expressed sequence tags, microarrays, real time PCR, establishment of genetic and physical maps have helped understanding physiology, genetics and gene expression (Dita *et al.* 2005). It is known that disease resistance genes tend to cluster together in the genome (Palomino *et al.* 2009).

Depending upon the involvement and interaction of several genes, resistance may be complete or partial. In case of complete resistance the host plant directly interferes with symptoms production or with pathogen multiplication. This resistance is basically qualitative in nature. In partial resistance the host interferes with one or more steps in disease progression resulting in slowing down of disease progress or reduction of pathogen multiplication. Partial resistance is basically quantitative in nature. (Bernard *et al.* 2006). Disease incidence and host resistance can be explained on the basis of type of interaction between the host and the pathogen. In specific resistance host resist specific pathogen from infection by the process of race specific R-gene –virulence gene interaction. Race specificity or non-race specificity of resistance is based on the presence or absence of statistically significant interaction between host and pathogen genotypes. Resistance is said to be specific when the
reactions of a set of hosts (called differentials) differ when confronted by different isolates of the pathogen. These specific strains are referred to as races (Bernard et al. 2006). Pathogenicity of the pathogen comprises of virulence and aggressiveness. Aggressiveness can be an important factor in disease development and is defined as the severity of disease and its ability to invade host tissue over a given time period. Pathotype can be defined as an intra-specific group of isolates characterized by similar levels of virulence (or pathogenicity) on a given set of host genotypes (Bernard et al. 2006).

Plants have evolved different defense mechanisms to protect themselves against different pathogens. While some of them rely on simple physical or chemical barriers, more sophisticated gene-for-gene interactions (such as R-gene-Avr interaction) are also found in plants. The qualitative capacity of a strain to infect a specific host depends upon the virulence genes of the pathogen and the resistance genes present in the host plant.

Disease resistance is currently a primary objective of most plant breeding programs. Durable and multi-disease resistance is considered as a prerequisite to broad environmental adaptation aiming at stabilizing agricultural systems (Bernard et al. 2006). A combination of two homologous recessive genes and a third dominant gene is necessary for complete resistance to *Fusarium oxysporium* f. sp. *Ciceris* race-1. (Cobos et al. 2005).
2.7. Molecular Structure, characteristics and functions of R-genes and its product

In recent years, a series of plant disease resistance (R) genes have been cloned by transposon tagging or map based cloning from several crops and model species such as Arabidopsis thaliana. A common feature of most R genes is that the encoded proteins recognize target molecules delivered by invading pathogens. The specific interaction of both molecules results in changes in certain components of the plant’s signal transduction pathway, which ultimately leads to localized cell death at the site of infection and thus prevents further growth of the pathogen. The isolated R genes can be classified on the basis of common sequence characteristics of the encoded protein. A majority of R-gene products contain a nucleotide-binding site (NBS) and leucine-rich repeats (LRR). These proteins are further characterized by an N-terminal domain with homology to the Toll/interleukin receptor (TIR). Well known R-genes such as RPS1, I2C-2 have been cloned and characterized. (Huettel et al. 2002). R-genes are also characterised by the presence of conserved motifs such as p-loop, kinase-2a and GLPL (Palomino et al. 2009). The presence of conserved motifs has facilitated designing of primers to amplify resistance gene analogues (RGAs). Such RGAs have facilitated study of genomics of several crop plants.

Plant disease resistance genes (R-genes) play a key role in recognizing proteins expressed by some specific gene in the pathogen (Avr genes). (Sanseverino et al. 2010). R-genes originate from a phylogenetically ancient form of immunity that is common to plants and animals. The vast diversification in plant defence mechanisms resulted during the time of evolution. (Sanseverino et al. 2010). Various studies have reported the genomic basis of R-genes, although they have not been
understood completely. It has been established that proteins encoded by resistance genes display modular domain structures and require several dynamic interaction between specific domains to perform their functions. Some of these domains are necessary for proper interaction with Avr protein and in formation of signaling complexes that activate an innate immune response preventing proliferation of invading pathogen (Sanseverino et al. 2010).

![Diagram of NBS-LRR protein structure](source)

**Figure – 2.8 : Three different domains of NBS-LRR kind of R-genes**

R-genes can be functionally grouped into five distinct classes based on presence or absence of specific domain.

The CNL class comprises resistance genes encoding protein with at least a coiled–coil domain, a nucleotide binding site and a leucine rich repeat
(CC-NB-LRR). The TNL class includes those with a Toll – interleukine receptor like domain, a nucleotide binding site and a leucine –rich repeat (TIR-NB-LRR). While RLP class of R-genes with a receptor serine – threonine kinase like domain and an extra-cellular leucine rich repeat. The RLK class contains a kinase domain and an extra-cellular leucine – rich repeat. The rest of the R-genes which can not be grouped in one particular one, are put into “others” group. Examples of which are “mlo” and “asc-1” (Sanseverino et. al. 2010).

Although many R-genes have been cloned, the exact nature of mechanism of resistance is not understood. This is because of the complexity in understanding their evolution and function. A single R-gene has been evolved through a range of evolutionary mechanisms. Many models such as intra and inter-locus sequence exchange, insertion of transposon elements and base methylation changes etc. has been attributed to the evolution process.

2.8. Genetic marker and molecular markers in chickpea genomics

A genetic marker is a heritable trait that distinguish an individual or a genotype from other. Previously they were visible morphological characteristics referred to as phenotype. But such morphological marker and approach has many limitations and hence they are supplemented with molecular markers which may be protein isozyme profile or DNA markers. If a phenotypic trait is found to be associated with any of the above biomolecule then that will serve as molecular marker. In the context of the present work the phenotypic trait is resistance to fusarium wilt race-1 infection or susceptibility. This is ascertained under field condition or in controlled green house condition after treating the plant with the pathogen. If a particular DNA fragment irrespective of whether it is a coding gene or
part of it or even a non-coding DNA with even undefined function but is found to be associated and co-inherited with the phenotypic trait, then that can be considered as molecular marker. As per conventional and old practice a plant is selected as resistant if after treating with the inoculums of the pathogen it do not show the disease symptom which is possible only under field condition. But if a molecular marker is available such a plant can be identified by laboratory based analysis bypassing cultivation of plant in field and treatment with inoculums etc.

Tankshley et al. (1989) pointed out that such a marker technology can be used to what is called marker assisted selection (MAS) for pyramiding genes of economic interest to high yielding background to make genetic improvement of crop plant speedy and efficient. But the problem of chickpea is that due to limited genetic variability marker development and its use in MAS is slow (Milan et al. 2006).

During the initial period of marker technology, isozyme profile, DNA markers like RFLP, AFLP etc were used which is technically complex, time consuming lengthy process. Presently, most markers used and studied are PCR based markers.

The first genetic map of chickpea was developed by combining the mapping results from three inter-specific mapping populations (Simon and Muehlbauer 1997). The map comprised of 28 isozymes, 44 RAPD and 9 FRLP and 6 other markers distributed into 9 linkage groups. Isozyme markers in chickpea were reported by Gaur and Slinkard (1990a, b), Kazan et al. (1993) etc. Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers were mapped by Simon and Muehlbauer, (1997). The first comprehensive molecular marker map of chickpea was reported by Winter et al (2000). They used an inter-specific cross C. arietinum and the wild relative C. reticulatum. The markers included
simple sequence repeats (SSR), DNA amplification fingerprints (DAF), amplified fragment length polymorphisms (AFLP), inter-simple sequence repeats (ISSR), RAPD, isozymes, sequence characterized amplification regions (SCAR) and disease resistance analogues. Forty-seven gene specific markers were subsequently added to this map by Pfaff and Kahl (2003). Simple Sequence Repeats (SSR) markers from this reference map have been extensively used for linkage analysis of disease resistance and agronomically important traits in chickpea by various workers (Tekeoglu et al. 2002; Benko-Iseppon et al. 2003; Rakshit et al. 2003; Pfaf and Kahl 2003; Abbo et al. 2005). Similar STMS markers were developed by Sethy et al. (2006) for cicer.

Sethy et al. (2006) reported construction of microsatellite enriched library from the *C. arietinum* L. A total of 92 new microsatellites were identified, from which 74 functional STMS primer pairs were developed.

Kottapalli et al. (2009) reported an intraspec linkage map of cultivated chickpea. The map was constructed using an F2 population derived from a cross between an *Ascochyta* blight (AB) susceptible parent ICC 4991 (Pb-7) and an *Ascochyta* blight (AB) resistant parent ICCV 04516. The resultant map consisted of 82 simple sequence repeat (SSR) markers and 2 expressed sequence tag (EST) markers covering 10 linkage groups, spanning a distance of 724.4 cM with an average marker density of 1 marker per 8.6 cM.

Collard et al. (2003) reported another inter-specific linkage map (*C. arietinum* x *C. echinospermum*), developed using RAPD, SSR, ISSR and RGA markers. Intra-specific maps have been generated utilizing SSR, DAF, AFLP, STMS, ISSR and RGA markers and are being increasingly applied in chickpea mapping (Cho et al. 2002; Flandez-Galvez et al. 2003; Udupa and Baum 2003; Cho et al. 2004).
New SSR markers are being developed from ESTs and BAC library resources. (Sethy et al. 2003; Lichtenzveig et al. 2005; Tar’an et al. 2007).

For traits governed by polygenes, the preferred approach is quantitative trait loci (QTLs). QTL analysis in chickpea for various traits of interest has been reported by number of workers. Cho et al. (2002) reported mapping of genes for double podding in chickpea. An intra-specific linkage map was constructed by evaluating RILs with STMS, ISSR and RAPD markers. QTL analysis located gene for double podding in linkage group 6 of the chickpea reference map. QTLs for pathotype specific genetic factors in chickpea for resistance to *Ascochyta* blight were identified in chromosome number 4 and 6. Mapping was done again using STMS markers (Cho et al. 2004).

Quantitative trait loci governing carotenoid concentration and seed weight was reported by Abbo et al. (2005). They used STMS markers for QTL mapping. Among the various traits, QTLs for *Ascochyta* blight resistance attracted attention of most researchers because of economic importance associated with the disease. Iruela et al. (2006) detected 2 QTLs for resistance to *Ascochyta* blight in an intra-specific cross. One QTL was located on LG4 on a highly saturated region covering 0.8 cM and containing 9 markers. The markers included 3 SCAR, 2 STMS and 4 RAPDs. This finding could be useful in molecular breeding and marker assisted selection (MAS) for *Ascochyta* resistance in chickpea. Again Iruela et al. (2007) reported association of QTL for resistance to *Ascochyta* blight to *Fusarium* wilt resistance loci for race-5 in chromosome number 2 of chickpea. Linkage relationship was studied using a RIL population segregating for both the diseases and genotyped by STMS markers.
Lichtenzveig et al. (2005) mapped QTLs for Ascochyta blight resistance. RIL population derived from a cross between a Desi and a Kabuli variety were evaluated phenotypically and genotypically by SSR markers. Three quantitative trait loci with significant effects on resistance were identified. In recent years Kottapalli et al. (2009) have done QTL mapping for Ascochyta blight resistance using molecular markers particularly, STMS. Markers TA146 and TR20 were linked to QTL-2 for Ascochyta blight resistance.

Madrid et al. (2008) reported mapping of rust resistance in chickpea in interspecific cross between C.arietinum and C.reticulatum using STMS markers. Rajesh et al. (2002) utilized conserved region of the resistance genes to design resistance gene analogue primers to map Ascochyta blight resistance in chickpea. Bulk segregant analysis (BSA) approach was employed to genotype the segregating population and for mapping the RGA loci. Huttel et al. (2002) reported mapping of RGA marker to Fusarium resistance loci in LG2 of the concensus map. They employed RFLP and CAPs analysis approach to map RGA close to Fusarium resistance loci in chickpea.

Among all the different types of molecular markers simple sequence repeat (SSR) are most convenient, effective and widely used. SSR are tandem repeats of di, tri or tetra-nucleotide sequence motifs flanked by conserved sequences. As different alleles vary in length, depending upon the number of repeats, PCR with primers complementary to the flanking conserved region detects length polymorphism. They are highly polymorphic, PCR based and widely dispersed in the genome. SSR markers have been the DNA marker of choice for many crop plants. SSR markers have been documented in several crop species to overcome the low polymorphism of
other marker types (Litchenzveig et al. 2005). Another very common marker type is the Sequence Tagged Microsatellite Markers (STMS), which is also based on microsatellite repeat motifs of DNA. Like SSR markers STMS is also characterised by co-dominant nature, PCR based assay system and non-radioactive detection method. Because of its wide distribution in genome and high polymorphic nature it has found applications in many crop improvement programs as marker of choice for MAS and map based cloning. These repeats based markers are attributed with high allelic variation, co-dominant Mendelian inheritance and easy conversion to PCR based assays, which makes them ideal for many breeding purposes (Sethy et al. 2006).

Molecular markers have been used in identifying and localizing important genes controlling qualitatively and quantitatively inherited traits. PCR based codominant marker, SSRs are used in these studies to evaluate yield seed quality, biotic and abiotic stress resistance etc. (Radhika et al. 2007).

2.9. Tagging markers to trait of interest and its significance

Mere identification or development of a marker is not enough if it is not tagged or associated with the loci of interest. In the context of the present study the target loci is resistance to Fusarium wilt. Again tagging of markers to loci of interest is not enough, it is the degree of linkage which will determine the practical use of the marker. In practice only very closely linked markers are of use in MAS and map based cloning of trait genes. Moreover, such markers which flank the target loci are still superior in effect for selection at molecular level. An ideal marker should have the following properties:
i. Highly polymorphic in nature.

ii. Co-dominant inheritance – determination of homozygous and heterozygous composition in diploid organism.

iii. Frequent occurrence in genome.

iv. Reproducibility and easy and fast assay system.

v. Easy access – easy exchange of data between laboratories.

Once the marker is found to be linked or tagged to target loci, the next step is to estimate the distance between target loci and the marker(s). Based on this the linkage map is prepared which finds use in MAS and map based cloning strategies.

First elaborate molecular marker linkage group was reported by Winter et al. (2000) with 354 markers spanning 740 centi Morgan into 17 linkage groups. The work included the trait for resistance to Fusarium wilt which was found linked to different markers. Many traits of interests were also mentioned in this report. Mayer et al. (1997) reported that race-1 resistance is governed by a single recessive gene linked to an RAPD marker Cs27700. Ratnaparkhe et al. (1998) have reported ISSR marker UBC 855500 to be associated with race-4 resistance. Tullu et al. (1998) have reported tagging of RAPD marker Cs 27700 to race-4 resistance.

Sharma et al. (2004) reported association of STMS markers TA96, TA27 and TA194 to race-3 resistance loci against Fusarium wilt in chickpea. Cobos et al. 2005 used STMS markers for resistance mapping of race-0 resistance. They used an intra-specific cross to tag STMS markers to race-0 resistance loci. Besides Collard et al.
2003 developed a linkage map from an inter-specific cross between *Cicer arietinum* and *Cicer echinospermum* using various marker system.

Beneko-Isepon *et al.* (2003) reported tagging of DAF and SCAR markers to race-4 and race-5 resistance. They further reported the occurrence of similar loci in Arabidopsis chromosome no 1 and 5 respectively indicating homology between chickpea and Arabidopsis for resistance genes.

Halila *et al.* (2009) reported a second gene conferring resistance to the chickpea fusarium wilt for race-0 which has been mapped to linkage group-2 of the chickpea genetic map. This data was revealed by genetic mapping of segregating RIL population using STMS markers.

Markers based on resistance gene analogs (RGA)s have also been used as candidate gene approaches to identify chromosomal elements responsible for resistance against many pathogens in many crop species (Flandez-Galvez, 2003). Huttel *et al.* (2002) reported mapping of RGA marker to *Fusarium* resistance loci in linkage group 2. They employed RFLP and CAPs analysis approaches to map RGAs close to *Fusarium* resistance loci in chickpea.

In recent years Kottapalli *et al.* (2009) have done QTL mapping in chickpea for *Ascochyta* blight resistance using molecular markers of which most of them were STMS markers. Markers TA146 and TR20 were linked to QTL-2 of the analysis.

Madrid *et al.* (2008) utilized conserved region of the resistance genes to design Resistance Gene Analogs (RGA) primers to map *Ascochyta* blight resistance in chickpea. Bulk segregant analysis (BSA) approach was employed to genotype the segregating population and for mapping the RGA loci.
2.10. Genomics approach for genetic improvement of chickpea

Study of genomics is gaining momentum not only because it contribute to the advancement of our knowledge about gene complement of a crop species but also its application in making genetic improvement rapid and more effective. Practical genomics comprise of identifying and characterizing genes of economic importance; tracking them during the course of inheritance with the help of molecular markers. Molecular markers enable us to identify a desired type of plant with improved characteristics without looking at the fully grown plant in field. In terms of tools and techniques it involve marker designing, genotyping by PCR assay, linkage map construction and its application in MAS, map based cloning, transcript profiling and expression profiling.

Mapping in chickpea was hindered by the low polymorphism among cultivated chickpea (Kottapalli et al. 2009). Although several genetic linkage maps using various markers and genomic tools have become available, sequencing efforts and their use are limited in chickpea genomics research. Current knowledge of the chickpea genome is based mostly on seven linkage maps that are comprised of mainly RAPD, ISSR, AFLP and SSR markers. (Rajesh et al., 2008).

Resistance Gene Analogues (RGA)s of chickpea were isolated by different PCR approaches and mapped in an inter-specific cross, *C. arietinum* x *C. reticulatum* segregating for *Fusarium* wilt by Huettel et al. (2002). The study provided a starting point for the characterization and genetic mapping of candidate resistance genes in *Cicer*. 
Molecular markers are being used as tools for genetic mapping, diversity analysis, tagging genes; marker assisted selection and for map based cloning. Genetic mapping by linkage map construction using molecular markers is an important aspect in every crop improvement programs. Various crop improvement programs worldwide have concentrated on the major species like wheat, rice, maize, tomato, soybean etc.

One of the first crops where the knowledge and tool of genomics were successfully applied is Maize. This is because, apart from being a major food crop, maize is an ideal model plant for both classical and molecular genetics. Even before the advent of molecular biology maize gene mapping was started using morphological traits and based on recombination frequency data. In recent times it is vastly improved through preparation of linkage map based on target loci tagged to almost all types of molecular markers. The resolution of maize genetic map has now improved with 1000 RFLP and 1000 SSR markers (Xu et al. 2009).

Molecular marker technology is extensively used for study of various aspects of wheat genomics under the initiatives of International Triticeae Mapping Initiative (ITMI). These include high density maps for chromosomes belonging to each of the seven different homologous groups using markers like SSR, AFLP, SNP, DAvT etc. Many economically important genes have been tagged with markers. The present status of wheat genomics has been reviewed by Gupta et al. (2008). Barley is another crop where substantial progress has been made using molecular marker technology and the same is reviewed by Sreenivasulu et al. (2008).

Cultivated tomato, *Lycopersicon esculentum*, is the second most consumed vegetable worldwide. High density genetic map with 2200 markers has been
generated using RFLPs, AFLPs, SSRs, CAPS, RGAs, ESTs, and COSs and mapped onto the 12 tomato chromosomes. The classical genetic map reported in 1968 included 153 morphological and physiological markers. Next generation mapping was involved isozyme markers. The latest published classical linkage map of tomato consists of around 400 morphological, physiological, isozyme, and disease resistance genes mapped onto the 12 tomato chromosomes using all type of markers like RAPD, RFLP, AFLP, SSR, CAPS, SCARs, SSCP, EST, COS etc. Till 2007 there were 2200 markers on the tomato genetic map with an average marker density of less than 1 cM per marker. (Foolad, 2007).

Over the last decade an enormous amount of genomics research in rice, including the identification of thousands of QTLs for agronomically important traits, the generation of large amounts of gene expression data, and cloning and characterization of new genes, including the detection of single nucleotide polymorphisms has been generated. Molecular marker application in rice genomics started in mid 1990s with the application of RFLP and AFLP markers. DNA markers in rice breeding with marker-assisted selection (MAS) could greatly improve the precision and efficiency of selection, leading to the accelerated development of new crop varieties (Collard et al. 2008).

Buhariwalla et al. (2005) have reported generation of chickpea EST resources for genomics applications. A total of 106 EST-based markers were designed from 477 sequences with functional annotations. Lichenzveig et al. (2005) reported construction of high capacity BAC library for chickpea. SSR markers were designed by screening the library with synthetic SSR oligos. Two hundred and thirty-three new chickpea SSR markers were made available for the public.
Varshney et al. (2007) utilized existing chickpea EST resources at ICRISAT to generate SNP marker for chickpea genotyping. For validating the SNPs, CAPS analysis was performed.

Radhika et al. (2007) reported a composite intra-specific linkage map of chickpea by integrating individual maps developed from two RIL populations with one common parent. RAPD, ISSR, RGA, SSR and ASAP markers were analyzed along with three yield related traits: double podding, seed per pod and seed weight. Gene specific SSR markers were designed by Choudhury et al. (2009) from a collection of EST resources. Two hundred and forty six SSR motifs were identified from which 183 primer pairs were generated. The markers show cross-specific transferability. There have been efforts to generate new SSR based markers for chickpea genomics. Sethy et al. (2006) reported generation of 74 functional STMS primer pairs from a genomic library of chickpea. A total of 159 alleles were detected with an average of 6.4 alleles per locus.

SNP markers have been used for mapping and tagging by various workers: Kottapalli et al. (2009), Taran et al. (2007), Flandez-Galvez et al. (2003), Sethy et al. (2006), Tekeglu et al. (2002), Cho et al. (2002), Ratnaparkhe et al. (1998). Rao et al. (2007). SNP and insertion deletion markers were generated from coding and genomic region of two differential lines. SNP and InDels were validated by CAPS analysis. (Rajesh and Muehlbauer, 2008). They have reported validation of SNPs by developing CAPS and dCAPS.

Abundance and chromosomal location of major repetitive elements in chickpea genome was initially studied by Staginnus et al. (1999).
BAC library constructed from chickpea accession has been reported by Rajesh et al. (2004). The library was screened by closely linked STMS markers to Fusarium wilt race-3, TA96 for map based cloning of Fusarium wilt resistance R-gene. Besides, the library could be used for generating new SSR markers and for physical mapping. Chickpea genome organization, composition and micro-synteny were studied using 500 kb BAC sequence by Rajesh et al. (2008). This 500 kb genome was derived from 11 BAC clones which included *Ascochyta* blight resistance loci also. This information could be very useful in chickpea genome mapping in future.

Mantri et al. (2007) reported transcriptional profiling of chickpea genes differentially regulated in response to abiotic stresses like drought, salinity and cold. QTLs for pathotype specific genetic factors in chickpea for resistance to *Ascochyta* blight were identified in chromosome number 4 and 6. Mapping was done again using STMS markers (Cho et al. 2004).

Map positions of a series of genes involved in plant defence response was determined by linkage mapping (Pfaff and Khal, 2003). Using sequence data from Genebank, primers were designed from genes known to involve in plant defence and were mapped using an intra-specific cross. 47 gene specific loci were located on different chromosomes of chickpea genome.

It has also been mentioned by Bernard et al. (2006) that emerging information on the model legume crop *Medicago truncatula* which has various degrees of synteny to the other legumes, has promises for identification of closely linked markers to agronomical traits for map based cloning. Physical mapping is fundamental to any progress towards a more complete understanding of the structure, composition and function of genomes. Isolation of genes of agronomical importance (e.g. genes coding
for receptor kinases, genes for signal transduction, transcription factors, regulatory elements, small regulatory RNAs or genes of defence pathways) inevitably necessitates a physical map. An elaborate genetic map is the first step in achieving this goal.