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Molecular Assisted Breeding for Ascochyta Blight Resistance in Chickpea (*Cicer arietinum* L.) - A Review

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***Ascochyta* Blight is a fungal disease caused by *Ascochyta rabiei* is a devastating disease of chickpea (*C. arietinum*) worldwide. *A. rabiei* completed its cycle in sporophytic and gametophytic both stages. Various approaches have been applied to determine genetics of resistance to *Ascochyta* Blight of chickpea and to map and tag the chromosomal regions using molecular markers. Mild resistance is present in many germplasms of chickpea but resistance clubbed with early flowering is the major breeding objective regarding this disease in chickpea. The RILs are scored for disease reactions in the field and genotyped for polymorphic molecular markers [isozyme, RAPD, SSR, ISSR, SNPs]. The disease occurrence scored quantitatively and QTLs have been analyzed. These DNA markers can be used for marker-assisted selection for A*scochyta* Blight resistance in chickpea and to develop cultivars with durable resistance through gene pyramiding. This review reflects a status and strategy for molecular marker assisted breeding and widening of genetic base in chickpea for *Ascochyta* resistance for future use.**

Keywords: *Ascochyta* Blight, Chickpea, Molecular Markers, RILs, QTL.

Chickpeas (*Cicer arietinum* L.) is a self-polinated, diploid (2n=16) annual grain legume or pulse crop. It is the third most important grain legume crop in the world after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L)[40] with genome size of 738 Mb[47]. The term "pulse" is of Latin origin, meaning "thick soup". Pulse crops like chickpeas, dry beans, dry peas, fababeans, lentils and lupine have unique characteristics to convert nitrogen from the atmosphere into nitrogen nodules in plant roots due to presence of Rhizobia bacteria.

Keeping in view huge benefits of pulses for human health, the United Nations has announced 2016 as the International Pulses Year. Thus, due attention is needed for enhancement in production of pulses not only to meet the dietary requirement of protein but also for creating awareness about pulses for achieving nutritional security, food security and environmental sustainability. Due to wide adaptability of pulses they can be fitted into various cropping systems, being leguminous in nature improve the soil fertility and physical health of soil as they increase the soil porosity due to presence of tap root system. Pulses are most important crop in India having the largest shares about 25% productions, about 33% acreage and about 27% consumption of total pulses of the world.

It is a rich and cheap source of vegetarian protein which is sometimes used as green vegetable dish whereas, other food legumes such as pigeonpea (*Cajanus cajan*), green gram (*Vigna radiata*), blackgram (*Vigna mungo*) and lentils

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(*Lens culinaris*) are essentially consumed after drying and preservation. Chickpea contains significant vitamins and minerals such as Ca, Mg, Zn, K, Fe, and phosphorus[13,49,20,50,22,21] and it is free from anti-nutritional factors so pulses are nutritionally more valuable and getting consumer preference for chickpea. Superior fiber content (12.0g) of chickpea make it a valuable crop over many crops and consumption of even smaller amounts of chickpea improves insulin secretion and controls blood sugar levels.

In 2013 the area of chickpea cultivation increased to 13.5 m ha but production remained at 13.1 MT (FAOSTAT 2015). 89.20% of the chickpea area and 84.47% of production was in Asia, whereas, the contribution of India was recorded as 67.4%[9]. Chickpea is also considered as poor man's meat as they play a significant role in the nutrition of the rural and urban poor in the developing world. Despite the economic importance of pulses chickpea productivity is low because of yield losses due to foliar and soil-borne fungal diseases *(Ascochyta* Blight*, Fusarium* wilt *and Botrytis* grey mould*)*, insect pests (*Helicoverpa* pod borer*)* and abiotic stresses such as drought, cold and salinity. Sources of resistance and tolerance to these constraints exist in the wild *Cicer* germplasm yet remain largely unused by conventional breeding programs[5,16,24,36]. However, *Ascochyta* Blight mainly occurs in north western plains due to favorable climatic conditions for the fungus, while *Fusarium* wilt is mostly restricted to central and southern parts of India[8].

Among many diseases of chickpea crop, *Ascochyta* Blight is the most devastating threat, causing up to 100% yield loss in severely affected fields[26]. The occurrence of Ascochyta Blight has been reported in more than 40 countries across the world[4]. The causal organism of the disease is *Ascochyta rabiei* (Pass.) Labr. In Asia the disease has been spread in major chickpea growing countries like Bangladesh, China, India, Iran, Iraq, Israel, Jordan, Lebanon, Pakistan, Syria and Turkey[30].

Etiology of fungus and symptoms of disease

An enzyme cutinase *is secreted by Ascochyta rabiei* in the culture filtrate when it is induced by cutin or hydroxylated fatty acids. This cutinase is the main esterase in the culture fluids. The molecular weight of this has been reported as 22 kD in SDS-PAGE and cleaved ester bonds of 3H-labelled cutin or p-nitrophenylbutyrate, which is highly active at 8.0 pH. As a serine esterase, cutinase is strongly inhibited by organophosphorous compounds and the most effective inhibitor 2,3,5-trichloropyridine-6-(O-methyl-O-n-butyl)-phosphateester+++ (MAT 9564) shows a Ki value of 0.8 nM. The cutinase gene was cloned from a genomic cosmid library by screening with two oligonucleotides directed against cutinase consensus peptides. The gene was subcloned to a 1.7 Kb SaII/HindIII-insert and sequenced.

The causal organism of *Ascochyta* Blight of chickpea exists in both stages as anamorph and a teleomorph. The anamorph, *A. rabiei*, is characterized by the formation of spherical or pear-shaped black fruiting bodies called pycnidia (lifecycle of fungus fig.-1) which contains numerous hyaline unicellular and occasionally bicellular spores called as pycnidiospores or conidia, developed on short conidiophores embedded in a mucilaginous mass. Pycnidiospores are oval to oblong, straight, or slightly bent at one or both ends[27]. The fungus may be grown readily on a variety of nutrient media, the best being chickpea meal dextrose agar. *A. rabiei* generally produces a pale cream colored myceliums in which pale brown to black pycnidia are immersed. Cultures are variable in texture, morphology and color. In culture isolates often produce a prevalence of unicellular conidia. Binucleate asci are cylindrical to subclavate surrounded by paraphyses and contain eight hyaline unequally season, which develops on infected over-wintering chickpea debris, followed by several asexual generations during the parasitic phase of the disease cycle.

The cutinase gene codes for a 223 amino acid protein with strong homology to other fungal cutinase sequences. The purified cutinase is encoded by a single copy gene[41]. Resistance breeding relied on the use of screening technique in nurseries, where disease epidemics created by epiphytotic creation. With this approach, *Ascochyta* Blight resistance sources have been identified and many resistant cultivars have been developed before 2000[33,25,37].

Symptoms of *Ascochyta* Blight can appear on complete aerial parts of the plant. Generally it is seed borne disease but can also

spread through *debris*. In the field, disease can be easily observed at flowering and podding stage as patches of blighted plants. However, the disease can also appear at very early crop growth stage under favorable environmental conditions. The initial symptoms appear as water-soaked lesions on the upper leaves. Later, these lesions become dark brown spots and spread rapidly on aerial parts of the plant *i.e.,* leaves, petioles, flowers, pods, branches and stem. The spots on leaves and pods are circular, while on stem and branches are elongated. The apical twigs, branches and stem often show girdling, and the plant parts above the girdled portion are killed or break off even before drying.

Molecular Studies

Efforts for introgression of resistance to the pathogen into Kabuli germplasm resulted in relatively late flowering germplasm. With the aim to explore the feasibility of combining earliness and resistance, various recombinant inbred lines (RILs) derived from different Kabuli and Desi cultivars which have been evaluated under field conditions and genotyped with polymorphic markers. The identification of a locus linked with resistance and early flowering may account for the correlation observed between these traits[17,23]. Classical genetic studies of *Ascochyta* Blight resistance have advocated that the resistance was governed by single major gene[38]. Further findings indicated that resistance to *Ascochyta* Blight in chickpea was governed by more than one gene[24]. In the year 2000, Tekeoglu *et al.* demonstrated that two complementary recessive genes conferred resistance in chickpea. However, the locations of the genes could not be confirmed. Since multiple genes appear to tune the resistance, knowledge of their genomic locations and linkage to molecular markers would facilitate gene transfer and pyramiding of the genes into acceptable genetic backgrounds through marker-assisted selection. Varshney *et al.*[46] characterized 64 isolates of *Ascochyta rabiei* using AFLP and SSR markers and reported four distinct groups based on STRUCTURE analysis. Further, Kaur *et al.*[14] characterized *Ascochyta* Blight isolates and reported 10 pathotypes based on morphological variation. A large number of QTLs/genes for *Ascochyta* Blight resistance and markers flanking these QTLs have been reported, for instance QTLs for resistance to *Ascochyta* Blight using F2 populations[1] and recombinant inbred line populations[11].

Almost all types of molecular markers have been tested in chickpea including isozymes[7,15], restriction fragment length polymorphism (RFLP)[44], random amplified polymorphic DNA markers (RAPDs)[12], amplified fragment length polymorphisms (AFLPs)[28], sequence characterized amplified regions

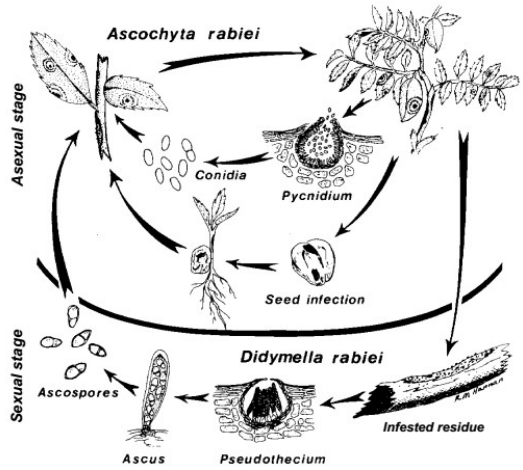

Fig.1. Life cycle of A. *rabiei*

(a) *Ascochyta* Blight infected patches in field of Chickpea at flowering

(b) Chickpeas stem and leaves with lesions of *A*. blight

(c) Chickpeas pod with circular lesions of *A*. blight

(SCARs)[19], inter-simple sequence repeat (ISSRs)[12], simple sequence repeat (STMS or SSR)[18,29], resistance gene analogs (RGAs)[10], DNA amplification fingerprinting (DAF)[2] and expressed sequence tags (ESTs)[31]. However, there is a low level of polymorphism detected in cultivated chickpea using isozyme/allozyme markers[6,7] and RFLP analysis[44,45].

Plenty of polymorphism was achieved through SSR markers within the cultivars of *C. arietinum*[43] and have been routinely utilized for creating genetic linkage maps[39,42,48]. These SSR markers have also been used for integration of the different chickpea linkage groups derived from inter- and intra- specific crosses as reference points[42,48,3]. In general it has been observed that 30–50% of the chickpea SSR markers are polymorphic in any given breeding or intra-specific mapping population, so SSR markers are reported as preferential marker for marker assisted selection in many breeding programs. However, SSR motifs may evolve too rapidly to be valuable. In addition, markers shown to be tightly linked to target genes in interspecific mapping populations may lose their selective power when used in backcross programs based on interspecific derivatives. Thus, there is a need for the development and utilization of gene-based markers.

The most important criteria for new molecular markers are high polymorphism, high reproducibility, detection of co-dominance polymorphism and suitability for rapid large-scale low cost screening. It has been reported by various workers that EST-based markers fulfill these criteria and since they are associated with the coding regions of the genome they also enhance molecular germplasm evaluation by capturing variation across transcribed regions and in genes of known function.

Using microarray technology and a set of chickpea (*Cicer arietinum* L.) unigenes, grasspea (*Lathyrus sativus* L.) expressed sequence tags (ESTs) and lentil (*Lens culinaris* Med.) resistance gene analogues, the *Ascochyta* Blight (*Ascochyta rabiei* (Pass.) L.) resistance response was studied in chickpea genotypes, including resistant, moderately resistant, susceptible and wild relative i.e. *Cicer echinospermum* L. The experimental system minimized environmental effects and was conducted in reference design, in which samples from mock-inoculated controls acted as reference against post-inoculation samples.

It has been proved experimentally that the use of recombinant inbred lines instead of an F_2 population is advantageous for mapping *Ascochyta* Blight resistance genes because nearly homozygous lines are scored rather than individual heterozygous plants. There is little segregation within RILs and this is simplified more for scoring disease reactions. Seed sterility is not a problem in the RILs although the lines are developed from an interspecific cross. Interspecific crosses (*C. arietinum* x *C. reticulatum*) were also used for mapping isozyme and DNA markers in chickpea[7,15,35].

Considerable progress has been made in the last decade in understanding the *Ascochyta* Blight pathogen and its genetics of resistance in chickpea. Resistance to *Ascochyta* Blight has been found in chickpea and breeding for resistance is making progress by identifying new resistance genes. Molecular tools are being integrated with conventional breeding approaches to speed up the process of introgressing genes into chickpea elite genotypes. Molecular markers associated with major QTLs conferring resistance to *Ascochyta* Blight have been located on linkage maps, and these markers can be used for efficient pyramiding of the traits of interest. Efforts, therefore, need to continue to combine high levels of *Ascochyta* Blight resistance with other desirable traits for incorporation into future releases as promising cultivars of different market classes of chickpea in *Ascochyta* Blight-prone environments[34].

CONCLUSION

Productivity in chickpea has became static in last decade therefore, efforts to break the plateau is urgent need which can be achieved through various crop improvement tools. *A*. Blight is a serious disease which can be managed with minor manipulations in crop husbandry practices like change in crop rotation, use of diseased free seeds and adoption of cleanliness practices. But incorporation of resistance genes is stable technique to tackle the problem of this disease.

Considerable progress has been made in the last decade in understanding the *Ascochyta*

Blight pathogen and its genetics for resistance in chickpea. Resistance to *Ascochyta* Blight is available in chickpea and breeding for resistance is progressive with identification of new resistance molecular markers/genes. Molecular tools are being integrated with conventional breeding approaches to speed up the process of introgression of loci/ genes into chickpea genotypes.

On the basis of technologies and methods available it is evident that;

(i) *Ascochyta* Blight resistance of chickpea is quantitative in nature and governed by two major genes and

(ii) Specific molecular markers and effective QTLs linked with A*scochyta* Blight resistance genes have been identified.

(iii) Efforts have been made to develop *A*. Blight based Recombinant Inbred Lines (RILs) which can be exploited directly in marker assisted selection

(iv) Various wild relatives are available having various degrees of resistance against the target disease which can be used in crop improvement programme directly or with distance hybridization.

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