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Review Article

Functional Genomics Prospective of Chickpea Breeding: Constraints and Future Directions

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Abstract

Chickpea (*Cicer arietinum* L.) is an important legume crop, cultivated in semi-arid and warm temperate zones. It is rich in protein so, one of the most important ingredients of human as well as animal feed. It is grown over 50 countries and traded across 140 countries of the world. The advancement in the development in the genomic resource it made the chickpea enable to adapt the biotic and abiotic stresses. We can create the genetic variability through conventional and non-conventional breeding methods because it is the basic key for breeder. The modern tools of biotechnology and genomic technology in chickpea will improved the breeding program of chickpea and decrease the time to develop new cultivars. However, the efforts have already been directed towards the chickpea improvement by the utilization of the genomics and biotechnological tools. Use of these techniques is expected to be very important in future breeding program. This review covered the genomics perspective of chickpea, constrains and future directions.

Introduction

Chickpea (*Cicer arietinum L.*) is an important legume crop, cultivated in semi-arid and warm temperate zones. It is rich in protein so, one of the most important ingredients of human as well as animal feed. It is grown over 50 countries and traded across 140 countries of the world [1]. Chickpea crop plants have advantageous effect on soil and human health. The progress in improvement of chickpea productivity worldwide has been recognized as very slow. The breeders have emphasized for the improvement of chickpea and its acclimatization to different environmental conditions. The possible situation of chickpea cultivation is changing due to climate shift which create the problems in chickpea breeding and its improvement for high grain yield. Cultivated chickpea has narrow genetic base which limit the breeding program for the enhancement of grain yield and resistance to different biotic and a-biotic stresses. However, wild relatives have wider genetic base and diverse gene pool which contribute to the resistance against the different stresses and breeders are using these resources for several past years. With the advent of modern age, advances in genetic and genomic resources have made the process of chickpea improvement quick and allowed its wider cultivation. In terms of being available genomic resources, chickpea is one of the highly developed grain legumes. Efforts have already been directed towards the chickpea improvement by the utilization of the genomics and biotechnological tools. Use of these techniques is expected to be very important in future breeding program.

Chickpea enhancement through conventional breeding

Conventional breeding refers to the classic or traditional breeding. More than 350 improved cultivars of chickpea were developed through conventional breeding approach which has better adaptability in changing environment and improved productivity and yield to new niches [2]. There are many breeding methods which are used to create the variability, detection of variability and find out the desirable recombinant. Qualitative traits are improved by conventional breeding method but the quantitative traits are improved with difficulty. Over 2000 years ago, farmer selected the superior plant for next generation and not knowing about the systematic breeding method. The systematic breeding was started in the late fifteen in the INIA research institute Madrid. Germplasm collection of chickpea started through several expectation tour of the country. At that time the limiting yield factor was the lack of efficient selection method for chickpea from the field. In the late sixties, the breeding scheme was disband reported in literatures. The breeding of chickpea by systematic way started in 1975 through the collaboration of the interest of the

several farmers to develop good quality of chickpea. At that time the screening against fusarium wilt had done because it was the major yield limiting disease [3]. Selection against fusarium wilt was done at that time on the small scale and some lines of chickpea showed resistance against this disease. In ICRISAT the collaborated work started to develop the resistant strain of chickpea in collaboration with the ICARDA in the 1978 [4]. *C. judaicum*, *C. pinnatifdium*, *C. reticulatum*, *C. microphyllumm*, *C. cuneatum* and *C. bijugum* are the wild types of chickpea which have resistant genes against different diseases and stresses which can be transfer in the cultivated susceptible varieties through conventional and non-conventional breeding methods [5]. Some wild types have cross incompatible with the cultivated form of chickpea at zygotic stage, this problem can be overcome through non-conventional breeding method [6]. So this cross- incompatibility limits the utilization of wild types in chickpea breeding program. In conventional breeding, the pool of available genes and the traits they code for is limited due to sexual incompatibility to other lines of the crop in question and to their wild relatives. This restriction can be overcome by using the methods of genetic engineering, which in principle, allow introducing valuable traits coded for by specific genes of any organism (other plants, bacteria, fungi, animals, viruses) into the genome of any plant. The first gene transfer experiments with plants took place in the early 1980s. Normally, transgenes are inserted into the nuclear genome of the plant cell. Recently it has become possible to introduce genes into the genome of chloroplasts and plastids (small organelles of plant cells which possess a separate genome).

Breeding achievement

India is more participate in chickpea as compare to other countries. More than 350 improved verities of chickpea were developed in the world and about half of these varieties developed in India [7]. The largest national chickpea breeding were run in the ICRISAT (international crop research institute for semi-arid tropics) in India [6,8]. The success stories of the chickpea improvement are available such as; the early maturing varieties of chickpea were developed which escaped the terminal heat stress, drought stress etc. [9,10]. ICCV2 is the landmark early maturing varieties of the chickpea which is mature about 85 days and it consider the world earliest maturing variety of Kabuli chickpea. It is very important varieties which are grow in the short environment season in the Pakistan, India and other chickpea growing countries of the world [7,11]. Many other high yielding early maturing and short duration variety of desi and Kabuli chickpea are cultivated in Myanmar [7,10]. Supper earliest maturing varieties of Kabuli and desi chickpea were developed through further advancement in the chickpea breeding program. ICCV 96029 and ICCV 96030 are two super early maturing varieties of chickpea which mature in the 75 to 80 days and mostly cultivated in the Myanmar and southern India [9,12].

Root biomass and yield have positive relationship and during the drought stress the root biomass decrease ultimately yield is decrease [12,13] so efforts should be made to develop chickpea varieties which have deeper root system and vigorous growth which help to tolerate the drought because chickpea are mostly cultivated in the water deficient area of Pakistan [14]. Genetic variability against heat stress were identified in the chickpea germplasm, by using these variability chickpea heat resistant breeding line ICCV 92944 was developed [8]. Karnal Chana 1 and Genesis 836 are salinity tolerant varieties of chickpea which were developed in India and Australia, recently salt tolerant line were identified in ICRISAT which have more salinity tolerant and gave higher yield as compare to Karnal Chana 1 and Genesis 836 [15,16]. Availability of genetic variability, efficient field screening technique and expert researcher made the excellent breeding program. It is the big challenge to chickpea breeder to develop pod borer resistant chickpea variety due to the unavailability of source of resistant. In the wild type, higher levels of resistant were observed. Efforts should be made to combine the non-preference mechanism in the cultivated chickpea variety which was identified in the wild type, so these resistant mechanisms were observed in ICC 506 EB line and antibiosis mechanism was observed in *C. reticulatum* [5,6].

Success stories of marker assisted breeding in chickpea

Molecular markers consist of specific detectable molecules which show easily difference among different species, a readily detectable sequence of DNA or protein whose inheritance can be monitored. Any chromosome feature, locus or DNA sequences which are detected by cytological, phenotype and other molecular technique is known as marker. The markers are different types such as; morphological, biochemical, cytological and molecular markers [17]. The morphological and biochemical marker are influenced by the environment, but DNA marker are not influenced by environment. The molecular markers are not gene of interest but act as a sign or flag and present near the targeted gene, but the DNA markers are closely linked to the targeted gene [5]. There are several DNA markers which are used in plant breeding. The commonly used markers are Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Sequenced Tagged Sites (STS), Sequence Characterized Amplified Regions (SCAR), Variable Number Tandem Repeats (VNTR), Minisatellites, Microsatellites or Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR). Less in number, confer indistinguishable phenotypes, influenced by the environment, Influenced by the genetic background, Influenced by the ontogeny and No stable inheritance are constraints in using morphological markers and abundant, Ubiquitous, Highly polymorphic, stable inheritance, No environmental influence, No influence of ontogeny of individual and codominant or dominant are the Properties of DNA marker. DNA marker are preferred due to contrasting application such as Diversity analysis at molecular level to characterize the germplasm entries, Markers aided selection for pest resistance in crop improvements, DNA finger printing of crop species from different geographical regions, to establish phylogenetic and taxonomic relationship among individuals, Tagging of major and minor QTLs, Physical mapping and map based cloning of genes for producing transgenic organisms.

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Amplified Fragment Length Polymorphism (AFLP) Direct marker is one of the types of the molecular markers which are present within the gene of interest and very difficult to find out the this type of marker and very uncommon type of molecular marker and linked marker is the other type of markers which are present near the targeted gene of interest and not part of the gene of interest. the functional markers are those markers which are developed from the gene sequence of known expression such as EST-SSR. MAS (marker assisted section) is used when the gene of interest present in the gene pool of the targeted plant and the transgenic technique is used when the trait of interest without of the gene pool of targeted plant. There are many success stories of the marker assisted selection some example is given below 33 different genes of disease resistant were identified through MAS and through this herbicide tolerant and pest resistant plant of the chickpea and many other crops were developed Sudupak 2002. The speed and efficiency of the selection can be increase through selecting the plant on genotypic bases as compare to phenotype bases. The first isozyme marker used in tomato to speed up the introgression of monogenic traits and Rich in 1980. The DNA marker only allow the breeder to introduce the gene of interest from related species, but the conventional breeding allow the transfer of the whole genome along with undesirable gene. Marker assisted pyramiding is a technique of combing several targeted gene in the single genotype Teresa 2006. It is also possible through conventional method but the identification of the plant which has more than one genos is very difficult task because the recessive resistant not identify in heterozygous condition. The DNA marker is the best source to pyramiding the resistant gene in the susceptible elite cultivar of the chickpea [5].

The genomic resources are available for chickpea breeding and people have been re-examine at different times [18,19]. Marker-assisted selection (MAS) has been used for better targeting of the desired genes Teresa 2006. There are following features of the ideal marker such as; polymorphic, co-dominant microsatellite-based markers reproducible etc. If we consider the method of detection of the sequence variation then the molecular markers can be classified as PCR-independent (hybridization based), PCR dependent and micro-array based markers [1]. RFLP markers were the first PCR dependent highly reproducible, co-dominant, locus specific markers employed for plant genome analysis during 90's. Molecular markers along with isozyme markers are used for the construction of first genetic map [20]. RFLP markers and microsatellite-derived RFLP markers were also used for genetic diversity studies in chickpea [21]. There are two types of hybridization based (PCR dependent) marker systems. 1) Markers which are non-sequence specific which include RAPD and AFLP markers, and (2) sequence tagged PCR-dependent markers which include cleaved amplified polymorphic sequence (CAPS), sequence tagged site (STS) and SSR markers [1]. RAPD markers were also used to characterize germplasm. At present these markers are not consider desirable for any type of genetic analysis in chickpea due to their dominant nature of inheritance and non-reproducibility. Despite of this, usage of RAPD markers can be intensified by converting these into more reproducible informatory marker types. AFLP marker system was developed by selective amplification of DNA fragments obtained by restriction enzyme digestion to overcome the limitations associated with RAPD, AFLP marker system (low level of reproducibility). AFLP markers have been used for genetic diversity estimation in cultivated chickpea and its wild relatives in order to discover the origin and history of chickpea, AFLP markers are used [22,23]. The chickpea resistant cultivar against different disease e.g.; fusarium wilt, were developed through molecular marker [17]. As highlighted earlier, various marker systems have different strengths and constraints. However, SSRs and AFLPs offer distinct advantages over RAPDs and RFLPs, and are widely preferred currently for fingerprinting, genetic diversity analysis and mapping experiments in various crop species.

Genomic resource and mapping population in chickpea

The basic set of the chromosome is known as genome. The cultivated chickpea has 16 chromosome and diploid in nature and these are numbered in decreasing ordered from 1 to 8. 353.53mb are the total length of the chickpea chromosome and its size ranged from 30.53 to 58.05 recorded by Ahmad and Hymowitz [24,25]. The karyotype analysis of the chickpea is very difficult due to small size of chromosome. Cytological investigation revealed that the karyotype of the chickpea have various features such as; one pair of the chickpea chromosome was very long along distinct satellite and sub- metacentric, metacentric to sub metacentric are six pairs and one pair of the chickpea chromosome are very short and metacentric [24,26,27]. Gupta et al. [24] also reported the autotetraploid of the chickpea which was developed due to spontaneous or artificial sources. Sharma et al [21] developed the artificial autotetraploid of the chickpea by the seed treatment with the colchicine at the 0.1 to 0.25% concentration and identified the bivalent pairing at anaphase 1 [21]. Mapping population, molecular marker, linkage map quantitative trait loci (QTLs), genetic stocks and breeding material are the genetic resources which are used in the breeding program and genetic studies of the chickpea [1]. The first approach which is used to find out the position of the genes and QTLs on the chromosome mapping is required. Two genetically divergent parents are required during the producing mapping populations which have one or more than one trait of interest [1]. The divergent parents should be used and distant parents should not be used because it causes the sterility problem during segregating population. Population from DH (double haloid) F2 generation, RILs (recombinant inbred line), NILs (near isogenic line) and back crosses are used during constructing the genetic map in chickpea [1,28]. So the F2 population is produced by selfing the F1 population and BC (back cross) population is developed by crossing F1 to any one of the parent [29]. More than 99% genome of the recurrent parent is recovered by repeated BC upto six generation. The homozygous line to target gene was developed by selfing the BC6 0r BC7 generations and these line is called nearly isogenic line (NILs) which are generated for QTLs mapping. The

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RILs are developed through SSD breeding method and the selection of this line is done in F6 or F7 generation after the advancement into F2 generation and the lines which are developed by this process known as RILs which are used in the QTLs mapping [29].

The seeds of the RILs are homozygous which are growing on the different places and construct the several years map of the chickpea. In the ICRISAT the linkage map of the chickpea was developed through RILs. Targeting Induced Local Lesions IN Genomes (TILLING) are used to find out the allelic variation in the ICRISAT India population from chickpea accession ICC 4958 and the EMS (ethyl methane sulphonate) used as mutagen to create mutation in this accession. More than 1200 line are developed in the ICRISAT through MAGIC (multi-parent advanced generation inter cross population and it was developed through 8 parents from Africa to India. The MAGIC population can be used directly as improved cultivar of the chickpea [1,29].

Sequence information

Next generation sequences are the new biotechnological technique which has ability to sequence the plant genome and through NGS sequence the transcriptomes of the several plants included chickpea nucleotides sequence [30]. 97836 nucleotides of the chickpea are sequence and it is available NCBI data base against limited number of EST tags [31].

Genetic map of chickpea

In 1990 the first genetic map of chickpea was constructed which had consisted three morphological trait loci and 26 isozyme [11]. After that several morphological trait loci and isozyme were mapped and these mapped constructed excellently by using DNA marker. The first linkage map of the chickpea was reported in 1997 which consists of 45 RAPD and 10 RFLP markers [20,32]. The chickpea first map which construct by using RILs in 2000, consists of 96 DNA amplification fingerprinting (DAF), 118 STEMS, 37 inter simple sequence repeats (ISSR), 70 AFLP, 17 RAPD, 2 SCAR, 3 cDNA and 8 isozyme marker [33]. The earlier studies showed the interspecific mapping population was used for the linkage studies and to tag the fusarium resistant gene molecular map developed based on interspecific cross and find out that two RAPD and SCAR were associated to race 1 and 1 ISSR associated to race four [20]. A large scale of markers are available in ICRISAT which developed with the collaboration of the other partner throughout the globe and at this time both inter and intra specific map have been developed in ICRISAT [1].

Functional and comparative genome

The sequencing of the transcriptomes and the sequence the genome is done through advances the next generation sequencing technology, by using this technique many genes and EST are identified through studies of transcriptomes and proteomes and these are responsive the biotic and abiotic stress [31,34,35]. In the chickpea many functional transcriptomes which responsive to abiotic stress are studies through following technique such as; Supers AGE (super serial analysis of gene expression), SSH (suppression subtractive hybridization), EST sequencing and microarray [31,35]. Molina [35] identified the transcriptomes which responsive to nodules formation and salt stress. The up and down regulation of gene expression against drought were studied in the drought tolerant and drought susceptible verities of chickpea. The sequence the De novo gene and assembly of the chickpea transcriptomes [36].

Major constraints to chickpea production

The major limiting factor in the chickpea production is biotic and a-biotic stress [31]. 50% yield reduction in the world is due to the drought stress at terminal stages because these stages are very sensitive to these stresses [18]. This problem occurs in those crops which sow on the conserved soil moisture in the rain fed area of Pakistan. The other limiting factor is heat stress at reproductive stage which limits the yield of chickpea [31,37]. These constraints are due to the large shift in chickpea production area from long cooling season to short wormer season, due to the more cropping intensity, increase area under late sown cultivation of chickpea and global warming [13,15,16]. Soil salinity is also major constraint in chickpea production globally [38,39]. Some important root disease such as dry root rot, fusarium wilt and collar rot are the major limiting factor in the chickpea production in dry areas of the world [39]. Recently reported that the dry root rot cause the plant mortality at seedling stage but the collar rot and late fusarium cause mortality at later and grain filling stage. When the chickpea is cultivated in the cooler and humid areas it affected by botrytis grey mold (BGM) and Ascochyta blight (AB) at foliar stage [39,40]. AB is the important disease of Pakistan, India, Africa, North America, Australia and Central and West Asia. BGM is the important disease of North India, Bangladesh and Nepal [41,42]. In the cultivated and wild species absolute source are not available [43]. Pod Borer is another polyphagus pest of chickpea which feed at tender leaves, immature seed flower bud etc. in the some chickpea growing area chickpea crop are infected by viral disease, rust, root nematodes, crown rust, root rot and leaf minor [34].

Future Directions and prospective in chickpea improvement

The phenology, adaptation and duration of the chickpea are the very important component in the range of the environment. The main objectives of the chickpea breeder to develop short duration cultivar which escape the terminal drought stress and expand the chickpea production area in the semi- arid tropics [44]. The era early maturing varieties of the chickpea are expected to develop in future in both Kabuli and desi chickpea cultivar [2]. The resistant and early maturing traits are present in separate line and objective in future is to combine all the traits in the single line. The immature of the pods of the chickpea are used as vegetable, to develop early podding verities which delivers in market and give high income to farmer. Developments of the chickpea varieties which perform well when sown in late become the northern area are available in late season which help expand the chickpea area. In these condition heat tolerant and early maturing cultivars of the chickpea are required and these development program are started in ICRISAT.

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The chickpea plants are infected by more than 60 pests which reduce the survival of chickpea and this damage can be manage by culture practices, proper plant spaces, time of sowing, flooding, intercultural practices, trap crops and fertilizer applications [44-46]. It can also be reduced adopted mixed cropping systems such as; sowing with sunflower, linseed, mustard etc. IPM (integrated pest management) is the best method to control the pest and weeds because it decreases the toxic effect of chemicals on environment. Collection and evaluation of genetic variability, for increase variation, for high yield, for new or unexploited cultural regime, tolerance to iron chlorosis and drought and for photoperiod and thermos sensitivity are the main future thrust of chickpea.

Conclusion

It is concluded that through the advancement in the development in the genomic resource it made the chickpea enable to adapt the biotic and abiotic stresses. We can create the genetic variability through conventional and non-conventional breeding methods because it is the basic key for breeder. The modern tools of biotechnology and genomic technology in chickpea will improved the breeding program of chickpea and decrease the time to develop new cultivars. The cross incompatibility limits the transfer resistant genes from wild relatives to cultivated species of chickpea and this problem can be overcome through biotechnological tools. Marker assisted breeding is the efficient source of the chickpea improvement.

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