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Collection of genomic tools for pigeon pea crop improvement

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Abstract
Pigeon pea is a legume crop grown under tropical and sub-tropical conditions. In India, Pigeon pea occupies a major portion of vegetarian diet. India stood among the leading producers of Pigeon pea with about 70% share in Pigeon pea production worldwide. It is tolerant to stresses and is very important protein rich crop in India often consumed on daily basis. Pigeon pea emerged from an orphan or least considered crop to a genome resource rich crop whose hybrid studies have been accelerated recently by following genomics assisted breeding approaches like Marker assisted selection (MAS), QTL mapping techniques. In order to provide steady yields under both ideal and stressed environment, it is necessary to integrate different breeding techniques using modern genomic technologies like Marker associated traits study, genome sequencing, genome wide markers, high throughput genotyping assays and germplasm re-sequencing data. Giving importance to selection based on superior adaptation characters and accelerating breeding programs with new breeding tools, it would help in delivering new cultivars to farmers. Practicing new genome sequencing techniques provided a lot of genetic resources which promoted Pigeon pea breeding and the techniques include molecular markers all over genome, transcriptome assemblies, QTL mapping. To improve productivity and remove disparity both genomic research and conventional breeding were initiated at ICRISAT. ICRISAT has ample genome sequence with markers usage which helped in directing research towards locating trait mapping for characters like flowering time, fertility regain, traits contributing to yield and photo-insensitivity. Genomic data helps scientists of biotechnology to identify desired genes having important agronomic characters like biotic and abiotic stress resistance which improves Pigeon pea crop production. Mapping of traits and using genomic tools helps to identify resistant or tolerant genes to those stresses and adopting MAS and other transgenic approaches. It is possible to improve Pigeon pea production in marginal environment which would ensure food security in developing countries. Imparting resistance to biotic stresses like fusarium wilt and sterility mosaic disease, other agronomic traits require genomics assisted breeding via MAS, would accelerate improvement of both varieties as well as hybrids in Pigeon pea. In hybrid breeding specially to develop cytoplasmic male sterile lines, maintainers and hybrids, the mitochondrial genes which are responsible for cytoplasmic male sterility are to be recognized which is possible making use of wide scale genome sequence data and integrating these genome resources in Pigeon pea breeding.

Keywords: Tolerant, marker assisted selection, QTL mapping, CMS lines, genome sequencing, mitochondrial genes, hybrid breeding, cytoplasmic male sterility

Introduction
Pigeon pea (*Cajanus cajan* L. *millspaugh*) is a commercially cultivated legume crop which is naturally cross pollinated by insects accounting for about 20 to 70% of pollination. (Saxena *et al*., 1990) [55]. It became a part of sustainable agriculture as it assures sustainable returns from marginal lands with very limited inputs due to its hardy and drought tolerant nature (Varshney *et al*., 2012) [100]. It is 6th most important legume crop in the world cultivated in 6.97 million ha with a production of 5.05 metric tons say 724 kg/ha as per FAO statistics 2016.about 70% of pigeon peas production and 74% of area under pigeon pea is in our country.it is consumed on daily basis by vegetarians being an important source of protein about 20 to 24% in concentration along with essential amino acids (Bohra *et al*., 2012) [5]. It is a diploid species and often cross-pollinated crop comprising of 833.1mbp in 11 pairs of chromosomes (Varshney *et al*., 2012b) [103, 105]. the natural out crossing in pigeon pea is being utilized in order to develop cytoplasmic genetics male sterile lines in pigeon pea (Saxena *et al*., 2010a; Varshney *et al*., 2010) [3, 104]. In previous decades, productivity remained low consistently being 700 to 800 kg/ha as a result of their exposure to biotic and abiotic stresses and also that it being cultivated in marginal conditions with minimum inputs and using improper agricultural practices (Varshney *et al*., 2012b) [103, 105].

It became difficult for improving hybrids or high yielding cultivars in pigeon pea due to some yield complexities in yield but with the cytoplasmic male sterile system which is a game changer has helped in exploiting heterosis and enhancing yields by developing both varieties and hybrids in pigeon pea. Keeping limiting factors like FW, SMD, virus, pod borers, salinity, water stagnation in view hybrids have been developed using CGMS system. Hybrid breeding technology based on CMS system has been implemented at ICRISA (Saxena *et al*., 2010) [3, 65-68]. Many research activities conducted together have provided in developing necessary genomic tools for improving pigeon pea in last ten years (Varshney *et al*., 2013; Bohra *et al*., 2014; Pazhamala *et al*., 2015) [18, 48, 109]. With advancement in molecular biology the process of breeding is possible to shift from phenotypic based selections to genotype based ones including MAS have accelerated breeding efficiency for decades (Xu & Crouch 2008) [115]. Todays cultivated pigeon pea C. cajan has evolved from interspecific cross between C. scarabaeoides and C. cajanifolius (Pundir and Singh 1985) [41]. Genus cajanus belongs to sub tribe cajaninae, it has about 32 species (Bohra *et al.*, 2010; Pazhamal *et al.*, 2015; Vandermason, 1990) [3, 48]. Among 32 species of genus cajanus only *C. cajan* is domesticated species. However, the use of interspecific species has been limited because of their inability in crossing between the cultivated species other than the closest species like *C. cajanifolia* and *C. scarabaeoids*. For protecting the wide cross hybrids *in vitro* rescue techniques of biotechnology approaches have been tested. There by enabling the transfer of genes from wider species of germplasm to the outside the genus cajanus. Through gene introgression techniques in biotechnology by Varshney *et al*., 2010 [3, 66, 67]. From several decades conventional breeding approaches have been used in order to cope the challenges from biotic and abiotic stresses faced by pigeon pea. But they have very limited success to overcome these challenges and were not able to provide stable yields and crop production (Varshney *et al.*, 2007; Saxena, 2008) [64, 94]. This limited success in addressing production constraints in pigeon pea is mainly due to lack of efficient screening technique to identify the genes. However, with the modern tools that have emerged recently in pigeon pea like sequence based molecular markers, high density sequencing assays, genome maps, transcriptome assemblies most importantly QTL maps and QTLs for important traits assist breeders to choose suitable parents in order to cross them so that novel combinations result in producing elite breeding lines. Pigeon pea is not much exploited using appropriate selection and crossing mating designs which are otherwise used in self-pollinated species. However, methods like pure line selection, population improvement, mutation breeding, interspecific hybridization serve for creating new varieties and thus in improving the potential in yielding of crops. Two genetic male sterility systems have been developed recently in pigeon pea (Reddy *et al*., 1978; Saxena *et al*., 1983) [49]. But the seeds of genetic male sterility hybrids are too expensive to produce though they give 30% higher yields than other than hybrid seeds. As an alternative to GMS keeping in view the hybrid seeds cost Cytoplasmic Genetic Male Sterility system was developed as a result of effective breeding research at ICRISAT and released the first CMS hybrid i.e. GTH-1 in 2004 followed by ICPH-2671 in 2005 (Saxena *et al*., 2008) [64]. It is known as 'Pushkal' by private Pravardhan seeds and was released to cultivate in A.P, Karnataka, M.P, Maharashtra. At present improvement of hybrid technology in terms of boosting yield and focusing on breeding hybrids resistant to stresses in terms of Wilt, Mosaic, Pod borers etc. Thereby, can contribute to enhance returns from farms and maintain sustainability. In this review efforts have been made to highlight the breeding status of pigeon pea in our country and to address both conventional and genomic research in pigeon pea exploring future possibilities of genomic efforts and the challenges of genomics assisted breeding for improving pigeon pea.

Breeding approaches till today

Biotic stress resistance

In diseases, Fusarium wilt (FW) is a serious disease that causes loss in yield for about 30 to 100%. It is expressed by single dominant gene (Pawar and Mayee 1986) [42]. The variety ICP8863 known as 'Maruti' was first released variety which is resistant to Fusarium wilt. Maruti and Asha (ICPL87119) are the most cultivated varieties. ICRISAT, following conventional breeding methods screened about 976 lines under sick plots of wilt and isolated 6 resistant genotypes named ICPL20109, ICPL20096, ICPL 20115, ICPL 20116, ICPL 20102 & ICPL 20094 (Sharma *et al*., 2016) [83]. In genomic assisted breeding using QTL mapping techniques identified 3 wilt resistant traits named qFW11.1, qFW11.2& qFW11.3. by sequencing among rill populations (PRIL b, PRIL c & F_2 populations).

Sterility Mosaic Disease (SMD) is caused by Pigeon Pea Sterility Mosaic Virus (PPSMV). It is transmitted by mite *Aceria cajani*, causes total yield loss under severe infestation and it is governed when one of the two alleles at locus 1&2, or recessive homozygous genes at 3&4 are present (Saxena, 2008) [64]. To reduce use of chemical sprays specially to control mites in pigeon pea, recently Genomics Assisted Breeding (GAB) helps to transfer genes that code for resistance to disease helps in developing disease resistant varieties in pigeon pea (Saxena, Kale *et al*., 2017) [8, 36, 86]. Genome sequencing helps to recognize and read the SNP's and the corresponding candidate genes on CgLG11 loci and it serves as QTL to develop resistant lines for sterility mosaic disease (Saxena, kale *et al*., 2017) [8, 36, 86].

Disease caused by *Phytophthera deschleri f. sp. cajani*, a soil borne fungus governed by single dominant gene PD1 (Saxena, 2008) [64]. The plants infected would dry quickly and under severe infestation would result in 98% loss in yield. Sick plot screening is assumed to be the best out of several methods of screening for studying large germplasm (Singh & Chauhan 1992) [57]. ICP 11376-5, ICP 12730, ICP 12751, ICP 12755, ICPL 20093, ICPL 20100, ICPL 20101, ICPL 20104, ICPL 20105, ICPL 20109 lines proved to be resistant for phytophthera in ICRISAT (Pande *et al*., 2012) [47].

Insect resistance

Helicoverpa armigera is a serious pest of red gram from many years there is no resistant gene identified in present grown species. As a solution to develop resistant gene source for this pest and to improve productivity, pyramiding of 2 genes that are insecticidal in nature and are tissue specific in expression are found to be an approach focused for inducing resistance. Gene CRY 1AcF offers resistance to Helicoverpa (Ramu *et al*., 2012) [53]. Wild relative *Cajanus acutifolius* from secondary germplasm served as male parent ad showed resistance towards pod borers (Jadhav *et al.*, 2012; Mallikarjuna *et al.*, 1997) [21, 32].

Bruchids (*Callosobrochus maculatus f.*) are serious pest under storage and hybrids developed from C. lanciolatus found to offer resistance to bruchids by delaying its life cycle through affecting its antibiosis mechanism (Mallikarjuna *et al.*, 2017; Sreekanth Marri *et al.*, 2017) [36, 89].

Abiotic stress resistance

Drought tolerance mechanism is not clearly understood as it is influenced by seasonal variations (Saxena *et al*., 2015). Moisture is most limiting factor that reduces both nitrogen fixation and productivity Kumar *et al*., 2014) [27]. As yield is influenced by many physiological and agronomic traits like leaf area, relative water content (RWC), tolerance to dehydration and pods per plant, seeds per pod, yield per plant, deep root system (Choudhary *et al.*, 2011). Under genetic studies, out of 51 drought genes 10 genes of U box proteins, H + antiporter proteins and universal stress proteins (A-uspA). These genes provide path for molecular study that causes tolerance to drought (Sinha *et al*., 2016) [84]. In Varshney's research paper it was identified that 111 proteins identified are found to be drought responsive universal stress proteins (Varshney *et al*., 2012) [100]. Prolonged water logging results in results in loss in crop productivity and under severe conditions may cause death of plants due to suffocation and lack of aeration. ICRISAT through suitable screening methods isolated many crosses which are tolerant to ware logging (Sultana *et al*., 2012) [74]. These crosses included some male sterile lines which are ICPB 20243, ICPB 2039& ICPB 2047 and some fertility restores like ICPL 87119, ICPL 149& ICPL 20125. Here resistance es governed by single dominant gene (Perera *et al*., 2001; Sarode *et al.*, 2007) [44]. Lines developed from cross involving C.acutifolius has special features of improving tolerance of pigeon pea under water logging conditions (Hingane *et al*., 2015, Mallikarjuna *et al*., 2017) [20, 36].

Salinity in soil results in accumulation of salts in dry land conditions or where there is scarcity in water. So, under irrigated conditions plants growth and development will be hampered, as soil salinity effects plants physiological and biochemical pathways (Chaudhary *et al*., 2011). Salinity has adverse effects on flowering and 50% flowering is reduced or delay in flowering is caused for about one to two weeks by accumulating higher concentrations of NaCl and Na_2SO_4 in soil. This effects the weight of seeds and reduces the seeds in number per plant (Promila & Kumar 2000) [43]. For variation wild genotypes are examined and C. scarabaeoides, C. albicans, C. platycarpus showed wide genetic variation in terms of salinity tolerance and tolerance is expressed by single dominant genetic trait (Choudary *et al*., 2008).

CMS system - a game changer

Using wild species of pigeon pea (C. scrabaeoides) cytoplasm a new CMS line has been developed first by Reddy and Faris 1981**.** Back crossing followed by extensive selection helped to isolate best CMS lines (Saxena *et al.*, 1996) [56]. Male sterile lines A_1 C. sericius, A_2 Scarabaeoids, A_3 C. volubilis, A_4 C. cajanifolius, A_5 C. cajan, A_6 C. lineatus, A_7 C. platycarpus, A_8 C. reticulatus, A_9 C. lanciolatus were developed out of which A_4 was recognized to be promising having stability over wide agro climate and has many maintainers and restorers (Singh *et al.*, 2017; Singh, Sameer Kumar *et al*., 2017) [8, 37, 86].

Hybrids developed using CMS

At Sardarkrishinagar, Dantiwada Agricultural University, Gujarat the first CMS A2 cytoplasm hybrid GTH-1 was developed in 2004. Due to high environmental influence this has lost it stability and has problems in fertility restoration. Later a commercial hybrid in pigeon pea named ICPH-2671 (A_4) was released worldwide for the first time in 2010 by M.P state government and it showed 47% more yield compared to national check Maruti (Kumar, Wani *et al*., 2016) [29, 82]. There after many hybrids were developed by SAU's and were performing well. ICPH 2740 hybrid with 42% more yield compared to Asha (National check) (Kumar, Wani *et al*., 2016) [29, 82].

Genomic intervention

In India, ICRISAT is in lead in conducting genomic research in pigeon pea in collaboration with international institutes. It became the first crop to have a draft of its genome sequence. Genetic markers and maps help in identifying genes for desirable traits contributing for crop genetic improvement. Trait specific markers for expressing flowering, fertility and resistance to sterility mosaic disease. Yield parameters can be identified using QTL mapping, association mapping for candidate genes, transcriptome assemblies, genome sequencing methods (Mir *et al*., 2017) [37]. Studying mitochondrial DNA sequences would help to understand CMS system and to develop hybrids in pigeon pea. Modern mapping population method Multi Parent Advanced Generation Intercross (MAGIC), Nested Association Mapping population (NAM) are found to be improved over conventional Biparental population method that helped to study QTL associations & linkage analysis (Bohra *et al*., 2017) [8].

Genomic resources

Molecular markers

Genetic markers proved to be efficient in breeding research work by improving genetic gain and fastening work of breeding (Varshney *et al*., 2014a) [6, 110]. Molecular markers of first and second generation used for diversity studies are mentioned in Table 1.

Table 1: Evolution of molecular markers

S. No.	Marker name	Reference
1st Generation molecular markers		
1	RFLP Restricted Fragment length polymorphism	Nadimpalli *et al*., 1993 [38]; Sivaramakrishnan *et al*., 1997 [59]; Lakshmi *et al*., 2000 [30]; Sivaramakrishnan *et al*., 2002 [60].
2	RAPD Random Amplified Polymorphic DNA	Ratnaparkhe *et al*., 1995 [50]; Lohithaswa *et al*., 2003; Choudhury *et al.*, 2008; Malviya & Yadav *et al*., 2010.
3.	AFLP Amplified Fragment Length Polymorphism	Panguluri *et al*., 2005 [45]; Wasike *et al*., 2005 [114]; Aruna *et al*., 2008 [1].
2nd Generation molecular markers		
1.	gSSRs (23, 410) genome sequence SSR	Varshney *et al*., 2012a

2.	ESTs-SSRs (8, 137) Expressed sequence tags	Varshney & Bohra *et al.*, 2011 [91]
3.	BES-SSR (6, 212) Bacterial Artificial Chromosome end sequence	Varshney & Bohra, 2011 [91]; Odeny *et al*., 2017; Aruna *et al*., 2008 [1]; Singh *et al*., 2008 [10]; Saxena *et al*., 2010b [71, 106, 107]; Songok *et al*., 2010 [69]; Upedhyaya *et al*., 2011.
3rd Generation molecular markers (Next generation sequence technology)		
1.	DArTarrays (15360 loci)	Yang *et al*., 2006, 2011 [118]
2.	Goldengate platform (768 SNPs)	
3.	Competitive allele-specific PCR (1, 616 SNPs) KASPr assays	

This unravels the transition of genetic marker technology from gel or hybridization methods (RAPD, RFLP, DArT, SFP's) to SSR and SNP markers which are sequence based. SNP's help to identify haplotypes and blocking such haplotypes would serve as markers which on assessing provide identification of important traits using allele mining techniques.

Transcriptome assembly

It is an efficient cost-effective strategy to improve genetic resources in pigeon pea. As on 26th Dec 2014, 25577 EST's are found to be available for pigeon pea (C. cajan) at NCBI (National Centre for Biotechnology Information). A transcriptome assembly named CcTAv1 with 1, 27, 754 TUS (Tentative Unique Sequences) was developed which was later updated with Illumina GAIIX by 454 platforms to create CcTav2 transcriptome assemblies contigs which has four data groups and 21, 434 transcriptome assembly contigs (TAC's) by (Raju *et al.*, 2010; Dubey *et al*., 2011; Dutta *et al*., 2011; Kudapa *et al*., 2012) [4, 14, 15, 52, 118] available in Legume Information System (LIS; http://cajca.comparative-legumes.org/).

Genetic maps

Unavailability of genetic variation made it difficult to construct maps or develop molecular markers. At first three molecular maps have been developed out of interspecific operation ICP 28 X ICPW 94 (Bohra *et al*., 2011; Yang *et al*., 2011) [4, 17, 118]. DArT markers were helpful to develop molecular maps where 122 DArT loci representing the maternal linkage and172 DArT loci for paternal linkage covering a distance of 270.0 cM and 451.6 cM (Yang *et al.*, 2011) [118]. Intraspecific molecular maps developed with 120 and 467.97 cM distances (Gnanesh *et al*., 2011) [17] by combining 6 molecular maps that are intraspecific in nature including two molecular maps mentioned earlier. The distance of interspecific map developed from ICP 28 X ICPW 94 through pigeon pea KASPar assay (PKAMs) is 1.11cM (Saxena *et al.*, 2012) [5, 21]. Using golden gate SNPs that are taken from cross between Pusa Dwarf x HDMO41 involving 296 loci and distance of 4.95 cM has been produced (Kumawat *et al*., 2012) [25].

QTL's and candidate genes

Resistance to stresses is a major challenge in pigeon pea in India. There has been a lot of research done in it to isolate the genes responsible for expressing resistance to biotic stresses like FW and SMD diseases. Numerous segregating mapping populations which would help to identify the gene loci that contributes resistance to biotic stresses were developed. keeping in view these mapping populations many polymorphic markers were developed (Bohra *et al*., 2011; Saxena *et al*., 2010c) [4, 17, 72]. by conducting thorough screening process of some thousands of plants in wilt sick plots in various regions 2 RAPD markers (Kotresh *et al*., 2006) [23] 4 SCAR markers (Prasanthi *et al*., 2009) [46] 6 SSR markers (Singh *et al*., 2013) [76] have been developed for FW resistance in pigeon pea. For sterility msaic disease 6 QTLs representing 24.72% variation in terms of phenotype have been developed on LG 7 & LG 9 (Gnanesh *et al*., 2011) [17]. About 118 and 33 genes of different kind have been isolated using transcript profiling working with leaves and roots of FW and SMD infected plants (Raju *et al*., 2010; Dubey *et al*., 2011) [4, 14, 52, 118]. For drought tolerant genes candidate genes should be studied to impart tolerance to drought in legumes (Narina *et al*., 2014) [39]. Many approaches based on functional genomics like transcript profiling, micro arrays, homology search help to study the candidate genes and these express resistance to different stresses. This knowledge on candidate genes would help in Genomic Assisted Breeding GAB for crop improvement in pigeon pea for creating multiple stress resistance. Determinacy in pigeon pea is very important adaptive character and 6 DArT's and 19 SNPs were isolated involving DArT arrays and Golden gate assay in pigeon pea (Mir *et al*., 2013). Trait mapping process became easy after developing whole genome re-sequencing techniques like Mut Map & QTL seq (Varshney *et al*., 2014c) [111].

Categorization of genomics assisted breeding

In red gram for crop improvement among varieties many markers are being used for various traits. For traits that are heritable FW and SMD disease resistance marker assisted back cross can be used. MAGIC & NAM populations have been popularized for their capability in improving large data on various loci for GAB involving multiple parents apart from biparental crosses trait mapping, it is clearly shown in fig:1 how various approaches to implement GAB in pigeon pea. For hybrid breeding 7 CMS systems including A1 to A7 have been developed from wild species and CGMS hybrid system have been developed from A4 cytoplasm (Saxena *et al.*, 2002; Saxena & Kumar, 2003) [61] which included three hybrids ICPH 2671, ICPH 2740, ICPH 3762 producing 30 to 48% higher yields than checks under multilocation trails (Saxena and Nadarajan 2010) [3, 65-68]. With the availability of markers the genome of mitochondria of a male sterile cytoplasm of line ICPA 2039 genome of maintainer ICPB 2039 and C.cajanifolius as restorer ICPW 29 can be sequenced. This genomic sequence study revealed 9 sets of wild accessions and CMS line, 22 sets of CMS & maintainer lines, 34 genes that code for different proteins. Their presence absence variations PAV's in 29 regions (Tuteja *et al*., 2013) [90]. The basic principle in identifying proteins is the variation produced due to rearrangements or due to differences in structures being replaced under study. This variation is due to change in the respiration of mitochondria resulting in abnormal protein production (Ma 2013) by isolating and studying genes that produced change in mitochondrial

respiration reveals the mechanism responsible for causing CGMS in pigeon pea. More advanced SSR markers for testing genetic purity have been developed in pigeon pea (Saxena *et al*., 2010a; Bohra *et al*., 2014b) [7, 104] instead of traditional grow out tests being conducted. These 3 line system of hybrid development is laborious and expensive to maintain all the three lines to get good restorers as an alternative 2 line system of hybrid development has been developed where male sterile acts as fertile under environmental influence. Identifying various male sterile lines that are temperature sensitive and various parental combinations that would offer important resistance to various diseases and yield higher. Seven Heterotic pools have been developed for different regions and would offer resistance to different stress (Saxena & Sawargaonkar 2014) [77, 78, 80]. For studying polygenic traits GWS is used in which GEBW-Genomics Estimated Breeding Value is estimated using markers and profiling by studying the genome sequence, by surveying extensive phenotypic data of various traits then the data is evaluated using markers (Hafner *et al.,* 2009). This gives data on the genotype and its use as parent before evaluating over several years conventionally. This reduces the breeding cycle and improves genetic gain (Moose & Mumm 2008) [33].

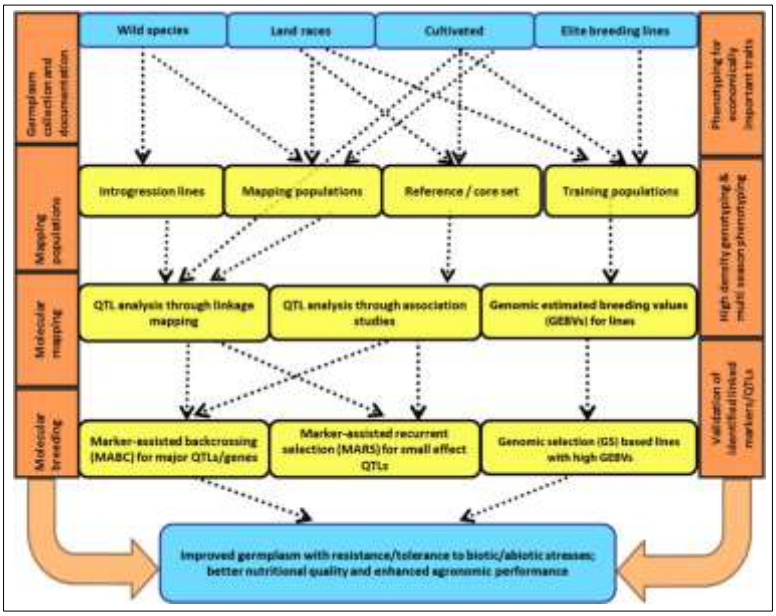

Fig 1: Use of integrated genomics and breeding approaches in systematic manner for genetics and breeding applications in pigeon pea

Limitations in GAB

There are specific constraints in pigeon pea crop improvement. Among them first constraint is narrow genetic diversity. Breeders have to depend on wild forms of plants which are in secondary, tertiary and quaternary gene pools. Due to lack of diversity in primary gene pool using suitable techniques for transferring genes. Though wild relatives have high variability for traits their use is limited due to lack of easy methods to identify desirable traits and their isolation which requires much research to be carried out (Goodman 1990) [16]. Genetic advancement is done using wild relatives and from very limited sources if there is combining of poor agronomic traits then it would be a failure (Saxena 2014) [77, 78, 80]. Second constraint is in response to temperature and light. Pigeon pea is a short-day plant (Silim *et al.,* 2006; Vales *et al*., 2012) [62, 74]. Interaction of day and night temperature and suitable photoperiod makes pigeon pea to adopt within 30 degrees north and 30 degrees southern latitudes (Saxena 2008) [64]. This thermo & photo sensitivity of pigeon pea limited its adaptation within lower latitudes which does not permit it. Being a part of integrated cropping system (Wales *et al*., 2012). 3rd constraint is undesirable linkage. While transferring genes within plants along with targeted genes other genes that are not targeted also descend into the transformed plants which may exhibit undesirable characters and delay breeder work. In pigeon pea it took 12 to 14 years to transfer genes from Scarabaeoids and C. albicans into commercial cultivar when targeted for high protein and yield parameters (Saxena & Sawargoankar 2016) [29, 85]. Lack of support and financial support is the 4th constraint. There is no support for pigeon pea research and had slowed down the process of development of varieties and the pioneer of Microsoft corporation Mr. Bill Gates also concentrated in funding and supporting private sector for research and development in pigeon pea at his visit to ICRISAT (Varshney *et al*., 2017; Varshney *et al*., 2017) [8, 36, 37].

Challenges for implementing GB in pigeon pea

Due to its lengthy life-cycle it permits only one season for crop production in pigeon pea. There should be ample resources for raising off season crop. It exhibits heterozygosity being often cross-pollinated crop. This limits its program in crossing by reducing the number of mapping population comparatively. Other impeding characters are exhibiting less genetic polymorphism, less heritability and sensitivity to light and temperature which pose limitations for GAB in pigeon pea.

Prospects

1. Sequencing and re-sequencing techniques

Making use of variation available using these techniques is possible. As there is very limited diversity resent in pigeon pea. There is need to introduce novel genetic variation by mutations or collecting its wild relatives but linkage drag may not permit transfer of favorable traits from wild species to the commercial cultivars. Here NGS, draft genome sequencing serves the purpose of studying molecular level variation in species and their relation with phenotypic variation (Varshney

et al., 2012) [100]. Re sequencing helps to study the existing variation and genes associated with phenotypes. Making use of the available genetic diversity, it is possible to develop new superior genotypes (Varshney *et al*., 2017; Varshney *et al*., 2017) [8, 36, 37].

2. QTL mapping approach

Though it is time consuming and resource intensive process QTL mapping helps in identifying best parents and to now their gene sequence using polymorphic markers (Abe *et al*., 2012) [2]. BSA Bulked Segregant Analysis helps in screening parents and gives trait linked markers. These both would make use in rapid trait mapping accurately in future in pigeon pea.

3. Next generation breeding

Current methods used in pigeon pea for introgression of resistant traits into elite and commercial cultivated varieties or marker assisted purity test of hybrids, parents and DNA based finger printing, Genomics Assisted Breeding (Singh *et al*., 2017; Singh *et al*., 2017) [8]. Now the whole genome sequence of pigeon pea is available (Varshney *et al*., 2012c) at ICRISAT. In future combination of traditional breeding with genetic approaches like Next Generation Sequence, high throughput genotyping used for screening in early generation, Marker Assisted Back Crossing & Marker Assisted Selection would help to lift pigeon pea breeding.

Conclusion

In response to the happening rapid climate change globally, resulting in depletion of land as well as water resources. There is a need to develop crops that are tolerant to drought. Giving importance to the crops that are providing food security in semi-arid tropics under limited resources pigeon pea has major role. In spite of its limitations that it is affected by various pests and diseases and its limited genetic variability, efforts have been made in developing genetic resources with appropriate marker system for trait mapping. New techniques like transcriptome assembly, MAGIC, NAM populations were created to focus on trait linked marker study. Identification of candidate genes and marker assisted selection for biotic stress resistance, for FW & SMD. Tolerance to abiotic stresses like drought, salinity & water logging; adaptive traits like plant type, determinacy & earliness. With advanced AB-QTL techniques, it is possible for Introgression of genes from wild species to the commercially cultivated varieties. Efforts should be made to focus on cost effective, high throughput and efficient phenotypic techniques in future. Marker assisted selection and genome sequencing techniques should be worked out solely or in combination to each other to enhance productivity in pigeon pea.

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