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Genomic Basis of Defining Heterotic Pools in Pigeonpea (*Cajanus cajan* L.): A Review

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ABSTRACT

Hybrid breeding strategy leading to define heterotic pools expedited execution of hybrid technology for amelioration of yield, quality, wide adaptability and resistance to biotic and abiotic stresses. In case of pigeonpea conventional hybrid breeding programme has yielded good dividends, yet it can be further honed up by understanding the genome wide variations in the hybrid parental lines for defining heterotic pools. Also, combining ability basis of defining heterotic pools offer enormous opportunities for pragmatic exploitation of pigeonpea hybrid technology with high yield advantages realized in farmers' fields.

Key words: Genomics, Heterotic pools, Hybrid breeding, Pigeonpea.

Assuring food security to indomitable population surge in view of ghastly exhausting natural resources including land, labor, energy and above all looming large climate change has become a global concern. Consequent upon environmental pollution and curtailed input response, the production of high-quality food must increase with application of inputs. Thus, task of breeders is very onerous to focus on the quantitative agronomic traits that have the potential to increase yield so as to ensure food and nutritional security to burgeoning surge of global population. Pigeonpea is majorly used as dal and it is important component of diet as they comprise good source of protein that complement well with cereals Varshney *et al*. (2010) and Varshney *et al*. (2012). Its ability to survive and produce high protein food even under stress conditions helps in providing food and nutrition security to subsistence farmers and therefore, it may be considered as the ideal rain fed legume crop of small-holder farmers. Recently, world first grain legume hybrid of pigeonpea ICPH 2671 was released for the commercial cultivation in India and it showed 47% yield advantage over the check variety "*Maruti*" in multi-location testing of 4 years. Unfortunately, to develop such kind of hybrids, breeder used to make thousands of random crosses between cytoplasmic male sterile (CMS) lines and tester lines and hence development of hybrids is a cumbersome process. In such case heterosis breeding has demonstrated a remarkable success story for qualitative and quantitative enhancements of grain production.

In the current era of crop improvement in complementation with emerging science of genomics, it has become amply possible to predict phenotype from the genotype Varshney *et al*. (2009) and Patel *et al*. (2010). The cutting edge large scale DNA sequencing and molecular markers have led to measuring genome wide genetic similarity and dissimilarity in plant species and populations Varshney *et al*. (2017). Molecular markers have been used for studying genetic diversity for the examination of genotype frequencies for deviations at individual loci and characterization of molecular variation within or between populations Varshney *et al*. (2012). Markers based genetic diversity along with the phenotyping data have been widely used for predicting best possible heterotic combinations. In addition to this, heterotic groups identified via genetic diversity analysis are validated through multi-location evaluation of intra-pool and inter-pool crosses, this validates the optimum genetic distance among parental material for attempting maximum pigeonpea hybrid vigor. In this review, we summarize the status of genomic approach for defining heterotic pools in pigeonpea.

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Overview of pigeonpea hybrid breeding technology

The first hybrid was reported in maize (Zea mays) and foresaw the potential of this phenomenon in enhancing crop yields (Shull, 1908). Additionally, plant breeders of cross

pollinated crops designed suitable mating and selection schemes to enhance yields by exploiting hybrid vigour. However, in several other case dominant genes in generally contribute for hybrid vigour, it was considered useful for cross pollinated crops; but later its utility was established in self pollinated crops Saxena *et al*. (2013) and Saxena *et al*. 2018), they reviewed the phenomenon of heterosis in food legumes and concluded that dominance, over dominance, additive and various inter allelic interactions play a significant role in the expression of hybrid vigour. They further postulated that the likelihood of obtaining heterotic crosses in pigeonpea is high because this crop also has a fairly good inherent capacity to carry a considerable genetic load of recessive genes due to partial natural out crossing in the crop. In several of the following review, Varshney *et al*. (2010) showed that in pigeonpea consisting of several of the important economic traits such as seed yield, pods/plant, plant height, seed size and also in several other case seeds/ pod are mainly controlled by both additive as well as non additive genetic variances, in addition to this, the level of realized heterosis for seed yield in pigeonpea is comparable to other crops in which commercial hybrids have already made a mark in global agriculture. Also various report in which mentions that male sterility in pigeonpea germplasm led to the selection of genetic male sterility systems that was controlled by single recessive gene Saxena *et al*. (2013). In fact, various plant breeding programmes was launched to generate valuable data on the extent of hybrid vigour and various other plant specific related issues for large scale hybrid seed production in pigeonpea. Moreover, GMS hybrids showed 25-30% heterosis for seed yield in farmers' fields with wide adaptation, but various seed production difficulties and seed quality concerns did not permit commercialization of these hybrids (Saxena *et al*. 2013 and Saxena *et al*. 2015). The hybrid breeding programme at ICRISAT was then shifted towards developing a more efficient cytoplasmic-nuclear male-sterility (CMS) system.

Selection of parental lines

The parents we selected were based on a study investigating genetic diversity of ICRISAT-bred hybrid pigeonpea parents and are considered to be a fair representation of the original parent population as they maintained the same cluster structure and similar allelic variation as those in the original population. Historically, the majority of ICRISAT B- and R-lines developed at earlier stages were derived directly from inbred breeding programs with many common ancestors shared and selected under the same environment with similar agronomic criteria without further breeding, which resulted in a relatively high genetic uniformity among hybrid pigeonpea parents (Varshney *et al*. 2010). This could be one of the reasons for the lower hybrid pigeonpea heterosis observed in the semi-arids environment (Tikka *et al*. 1997). In the last a few years, this issue has been addressed by separating the hybrid breeding from inbred breeding programs and by developing B- and R-line heterotic groups individually to maximize genetic diversity among hybrid pigeonpea parents (Reif *et al*. 2003a).

Improvement of pigeonpea parental lines

The breeding lines require the development of elite parental lines either the three-lines or two- lines system. The three-line hybrid system consist cytoplasmic male sterile line (CMS), maintainer line and restorer line, while the two- lines system include CMS and restorer line. However, an alternative way of exploiting hybrid is to use of the thermo-sensitive genetic male sterile lines (TGMS) as female parents, for the developing two-line hybrids (Pazhamala *et al*. 2015). The development of the superior parental line in terms of disease resistance and grain production has major advantage. Such, type of the parental lines are the prerequisite for the efficient hybrid breeding programme. The use of molecular marker technology has been shown effective in tracking and introducing genes from the resistant donor parents to susceptible parents Table 1, which has significant application for parental lines improvement. In addition to this, various trait mapping for hybrid breeding programmes including fertility restoration, TGMS, wide compatibility, male sterility has major advantage in parental lines improvement Table 2.

Heterotic groups based on markers

Genetic diversity estimates are helpful in classifying germplasm into heterotic groups for hybrid crop breeding. Earlier, the relative performance of inbred lines of known origin and pedigree was commonly used, which largely relies on breeders' empirical experience, to combine parents from different genetic backgrounds to develop heterotic hybrids. Molecular markers have been used in pigeonpea to assess the genetic relationships of pigeonpea ecotypes or sub-

Table 1: Parental line improvement on the basis of molecular marker.

Crop	Parental line	Trait	Gene	Reference
Rice	Elite maintainer line Rongfeng B	Blast and bacterial blight resistance	*Pi1, Pi2* and *Xa23*	Fu *et al*., 2012
Rice	Zhenshan 97A, Zhong 9A, II-32A and Chuanxing 29A,	Brown planthopper	*Bph14, Bph15*	Jie *et al*., 2012
Rice	PPR78	Blast resistance	*Piz-5, Pi54*	Singh *et al*., 2012
Rice	Pusa RH10	Grain aromatic rice	*Xa21*	Basavaraj *et al*., 2010

Table 2: Trait mapping for hybrid breeding program.

Trait	Crop	Name of parents	Associated gene	Associate marker	References
TGMS	Rice	DDR 1S, DDR 4S, DDR 6S, DDR17S, DDR 18S, DDR 19S, DDR 20S DDR 21S, DDR 22S, DDR 23S, DDR 24S, DDR 25S, DDR 26S, DDR 27S, DDR 28S, DDR 29	-	-	Salgotra *et al.*, 2012
	Rice	TS29, COR49	-	SSR	Robin *et al.*, 2010
	Rice	TGMS-Co27, H1493	*UDP-glucose pyrophosphorylase gene1, β-actin, UBQ5, SDHA,*	GeneChip microarrays	Pan *et al.*, 2014
	Rice	Hengnong S-1, Minghui63, T92, T95, R96 and R98	*tms9-1*	SSR	Qi *et al.*, 2014
	Rice	Zhu 1S, Guangzhan 63S, Y58S, C815S, G2011-16S, GS2011-20, DS550, DS552	*lnc R, RNZ*	CAPS	Zhang *et al.*, 2014
	Rice	PA64, NK58, NK58S, 7001S, PA64S, GD 8S and Zhonghua11	*p/tms12-1*	-	Zhou *et al.*, 2014
	Rice	TN2, TN11, TN13, TN15, TN17, TN19, TN23-	-		Cuong *et al.*, 2014
	Rice	GZ63-4S, VE6219	*Pi2, Xa23*		Jiang *et al.*, 2015
	Wheat	BS366	*TAS 3*	miRNA/si RNA	Tang *et al.*, 2012
Fertility restorer	Pigeonpea	ICPA 2089, ICPA 2039, ICPA 2043, PHR 31, ICPR 2438, ICPR 2447, ICPR 2671, ICPR 3467	*Rf*		Kyu *et al.* 2011
	Pigeonpea	ICPA 2043, ICPA 2047, ICPA 2092, ICPA 2052, ICPL 87119, ICPL 20107, ICPL 10928, MAL-9	*Rf*		Dalvi *et al.*, 2008
	Pigeonpea	ICPA 2052, ICPA 2039, ICPA 2067, ICPL 129-3, Nirmal 2, BWR 23, BSMR 736, BSMR 175, BDN 2, BSMR 853	-	-	Wang *et al.*, 2006
	Rice	WA CMS, RT 102 CMS, IR 24	*Rf4, orf* 352	SSR	Cai *et al.*,2013
	Rice	BbA, HJX74, SSSLS, Amol3, BG367, Suyynyo, IR 64, Nayangzhan, Basmati 370, Lianjian 33, IRAT261, Chenglongsuising, Khazar, Lemont, Star bonnet 99, IAPAR9	*rf3, rf4, Rf3, Rf4*	SSR	Ngangkham *et al.*, 2010

Table 2: Continue...

Table 2: Continue...

	Rice	Pusa6A, IR262829A, Pusa4B, Pusa5B, Pusa6B , Pusa9B , PRR78, PRH 10, IR24, BR827, KMR3, MTU9992, NDR3026, Ajaya R, IR66, C20R, UPR193133	*coxI, coxIII, cob, atp6* and *rps3*	CAPs, RFLP, STS, GNMS	Bazrkar *et al.*, 2008
	Rice	IR58025A, IR42686R, IR36, IR32472, IR32484	*Rf7, Rf4, Rf3, Rf6,*	SSR	Hackauf *et al.*, 2012
Wide compatibility	Rice	(02428 × Nanjing 11) Balilla		RFLP	Liu *et al.*, 1997
	Rice	(02428 × Nanjing 11) Balilla		SSR	Qiu *et al.*, 2005
	Rice	02428 × Dular,02428 × Zhenshan 97		SSR, RFLP	Wang *et al.*, 2005
	Rice	Dular × Nanjing 11,Dular × BalilI,1 02428 × Nanjing 11, 02428 × Balilla	STS, SNPs, CAPs		Qing *et al.*, 2005
	Rice	Nanjing 11 × Balilla		RT-PCR	Qing *et al.*, 2012
	Rice	-	-		Yang *et al.*, 2012
Male sterility	Pearl millet	-	-		Rai *et al.*, 2009
	Pigeonpea	SSR			
				Ccm0021, Ccm0030, CCB9	Patel *et al.*, 2012
	Rice	SSR		-	Yashitola *et al.*, 2002
	Rice	SSR		RM3150, RM1108, RM5373, RM6737, SBD07	Liu *et al.*, 2004 Rice InDels, SNPs
	Cox3a, Cox3b, Cox3c,			orf224a, orf224b, LD-12, LD-24, LD-29, LD-30, atp6, atp6-orf79, orf288, rpsla, rpslb, rps2a, rps2b	Luan *et al.*, 2013

species (Varshney *et al*. 2012) and hybrid pigeonpea parents (Varshney *et al*. 2017; Patel *et al*. 2020 and Shekh *et al*. (2015). However, information is scarce on assessing heterotic groups among semiarid pigeonpea inbred lines and populations and no conclusive study has been conducted to clearly defined heterotic groups of semiarid hybrid pigeonpea parents. Many of those studies investigating genetic diversity in pigeonpea with molecular markers were dealing with large pools of sub-species or ecotypes, such as ICPL 87119, Asha, Maruti from pigeonpea germplasm collections, but with limited value to practical hybrid pigeonpea breeding due to the inability to produce yield heterosis. For example, mass vegetative growth and partial fertility in hybrids between sub-species. Heterotic groups that are applied in breeding and production are different from the parental groups generated from germplasm collections based on molecular markers Table 3. It is still a challenge to find agreement between high, producible yield heterosis and high divergence among pigeonpea sub-species or ecotypes.

Combining ability and heterotic group

It is well known that inbred lines are homogeneous and homozygous in nature. Such, inbred lines when crossed lead to hybrid vigor depending upon appropriate gene action. They differ in combining ability that in turn hinges on type of gene action, being additive for general combining ability and dominance and non-allelic interactions for specific combining ability. Thus the favourable combination depends on the type of additive and non-additive gene action that controls quantitative character (Reif *et al*. 2003a). The inbred lines that combine well with a series of testers, theses inbred line have a good general combining ability (GCA) and inbred lines that combine well with specific cross that inbred lines has specific combining ability (SCA). The selection of parents from different heterotic groups is cardinal to the success of hybrid breeding programme and in this direction the combining ability of the parents entailing both general and specific combining ability is one of the important tools for determining the next phase of breeding strategies (Reif *et al*. 2003a).

The combining ability a priori between two inbred lines is explained by the genetic distance of the parents, their mode of pollination and mid parent heterosis (MPH) values. The traditional plant breeding method is hamstrung by lack on information one genetic relationship of parents, morphological characteristic and geographic origin of parent with their wild relative germplasm for obtaining parents from different heterotic groups with good combining ability (Reif *et al*. 2010). Conventional plant breeding based combining ability depend on the test cross performance. Compare to such traditional method, molecular marker based selection of parental line has provided a new way to get a good combination. However, molecular marker based genetic distance and heterotic group used for the selection of the parental lines could not accurately predict parental combination unless DNA based molecular markers was linked to genes affecting a trait. Thus, marker based genetic diversity together with phenotyping data have been used for predicting heterosis and developing heterotic group.

Genetic distance and heterosis

Genetic distance used for the measure the genetic difference between different plant species within population. Such, population have many similar genes with small genetic distance. It indicates that they are closely related and have a recent common ancestor (Reif *et al*. 2013). The genetic distance is useful for the reconstructing the history of the common populations and understanding the origin of biodiversity (Xie *et al*. 2014). For example, the genetic distance between different inbreeds are often investigated in order to determine which breeds should be protected to maintain genetic diversity.

High degree of the heterozygosity in the genome at the homologous chromosomes is responsible for heterosis (Bansal *et al*. 2012). The heterozygosity can be increased by the crossing genetically distinct parental materials, *i.e*. materials belonging to genetically divergent parents Reif *et al*. (2003b). Such, genetically distinct parental combination have great interest for the breeders. However, identifying

Table 3: Reports related to heterotic group construction.

Crop	Number of genotype	Molecular marker	Number of molecular markers	Statistical tools	Statistical methods	Reference
Maize	14	RFLP	-	-	Modified rogers distance	Dudley *et al*., 1991
	32	RFLP	-	-	Roger's distance, Principal component analysis	Melchinger *et al*., 1991
	7	-	-	-	ANOVA, F-test	Vasal *et al*., 1992
	92	SSR	83	-	ANOVA, F-test, Modified rogers distance	Reif *et al*., 2003b
	96	AFLP	9		Genetic similarities, Jaccards similarity coefficient, UPGMA	Oliveira *et al*., 2004
	30	SSR	55	R package	K-means clustering algorithm, Principal coordinate analysis, Modified rogers distance	Reif *et al*., 2005

specific gene located at a specific place is one of important task in plant breeding. The allelic variations at these loci cause phenotypic variations between different plant species (Riedelsheimer *et al*. 2012). The random fluctuation of allele frequencies also produces genetic difference between different populations, this process is known as genetic drift., In addition to this polymorphic markers measure genetic diversity between closely related inbreeds lines. Moreover, SSR and SNPs basis QTL mapping have high applicability to identify diverse parental line from the evolution rate, this evolution rate particularly useful for working out relationships among closely related parental lines for the next generation hybrid breeding programmes.

The development of heterotic pigeonpea hybrid using genomic which include different types of molecular markers; next generation sequencing (NGS) based genotyping widely apply for the selection of the diverse parents. The construction of heterotic pool from the different cytoplasmic male sterile line (CMS), maintainer line, restorer lines are applied for efficient hybrid breeding

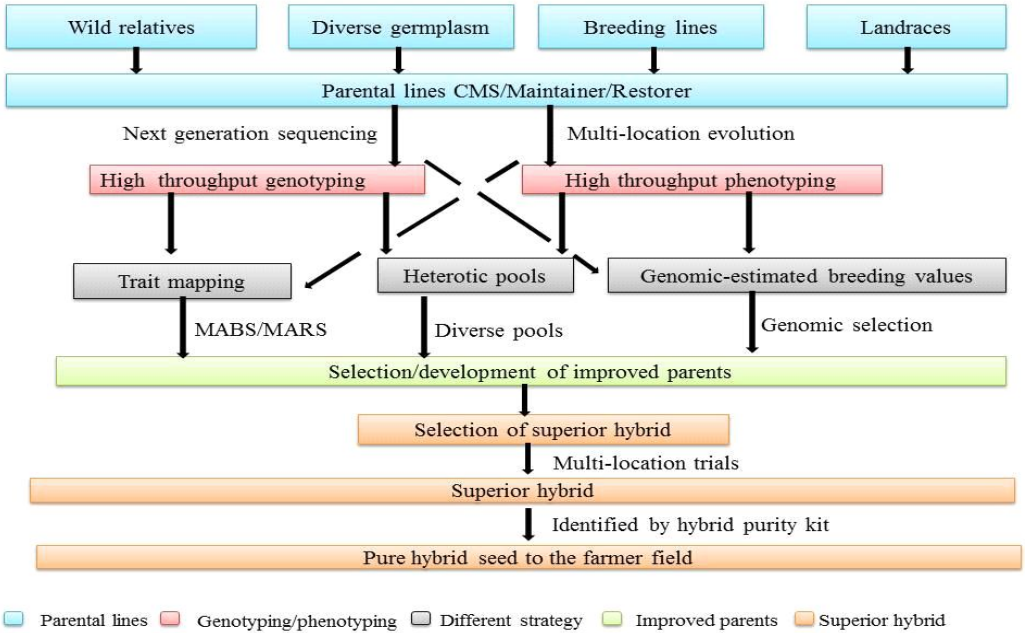

Fig 1: An overview of genomic strategy for pigeonpea hybrid breeding. The genomic and phenotyping strategies such as trait mapping, heterotic pools and genomic selection used for the development superior hybrid. Further, high throughput genotyping and high throughput phenotyping play a key role in superior hybrid development. Abbreviations: CMS, Cytoplasmic male sterile line, MABS, marker assisted backcross selection, MARS, marker assisted recurrent selection.

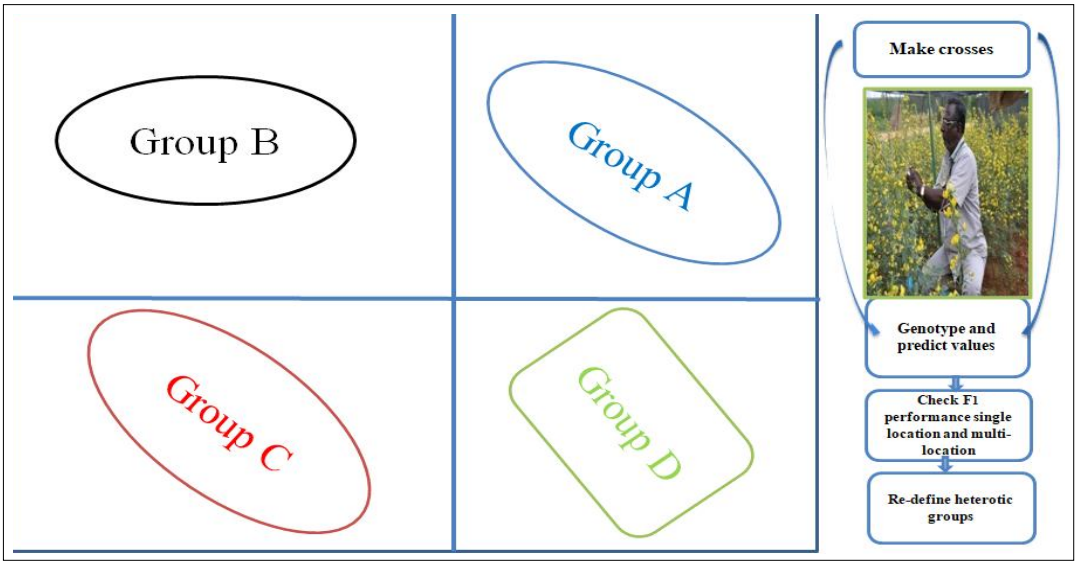

Fig 2: Explaining heterotic groups in pigeonpea.

programme (Reif *et al*. 2010) and it will change the future breeding strategies. The trait mapping approach which include fertility restoration, temperature sensitive genetic male sterility (TGMS), wide compatibility and combining ability are widely apply for the hybrid breeding programme. Although, genomic selection using best linear unbiased prediction (BLUP) and genomic estimated breeding values applies for the selection of superior lines and these all strategies for heterotic hybrid combination are described in Fig 1 and 2.

Conclusion

The defining of heterotic pools in pigeonpea confirms the efficiency of NGS-based technology for improvement of yield, quality and resistance to biotic and abiotic stress. Further use of genome sequence information in conjunction for defining heterotic pools provide an alternative approach for development of hybrid breeding programs. This approach has provide almost all possible sequence variations in the target region and will be useful in future hybrid breeding efforts. On the other hand, development of hybrid with phenotyping and utilization of traditional method is time consuming and labour intensive.

Limitation and Future Aspect

The constraint in pigeonpea hybrid breeding as recognized now are i) long generation turnover time, ii) determination of genetic diversity is another factor that limits selection of heterotic hybrid parents, iii) on-farm seed production practice and iv) genetic purity. To deliver the advantages of hybrid technology to farmers, it is imperative that the process of breeding new hybrids be enhanced and seed technology is simplified. It is envisaged that the new advancements in genomics science can help in understanding/ resolving many of the above mentioned issues. In additions to this, various next generation sequencing platforms has allowed the large scale discovery of the single nucleotide polymorphic markers (SNPs) for the advance selection of the parental lines, which go through many changes to assess widely adopted hybrid. The new marker system combines with efficient, high-throughput phenotypic will provide leverage information for defining heterotic pools in case of pigeonpea.

Conflict of Interest: None

References

Bansal, P., Banga, S., Banga S.S. (2012). Heterosis as investigated in terms of polyploidy and genetic diversity using designed *Brasica juncea* amphiploid and its progenitor diploid species. PLoS ONE 7: e29607. doi: 10.1371/ journal. pone. 0029607.

Basavaraj, S.H., Singh, V.K., Singh, A., *et al.* (2010). Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Molecular Bbreeding. 26: 293-305.

Bazrkar, L., Ali, A.J., Babaeian, N.A., Ebadi., A.A., Allahgholipour, M., Kazemitabar, K., Nematzadeh G. (2008). Tagging of four fertility restorer loci for wild abortive cytoplasmic male sterility system in rice (*Oryza sativa* L.) using microsatellite markers. Euphytica. 164: 669-677.

Cai, J., Liao, Q.P., Dai, Z.J., Zhu, H.T., *et al*., (2013). Allelic differentiations and effects of the *Rf3* and *Rf4* genes on fertility restoration in rice with wild abortive cytoplasmic male sterility. Biologia Plantarum. 57: 274-280.

Cuong., P.V., Cuong, H.V., Hanh, T.T., *et al*.., (2014). Heterosis for photosynthesis and dry matter accumulation in F_1 hybrid rice (*Oryza sativa* L.) produced from thermo-sensitive male sterile line under drought stress at heading stage. Journal of Factorial Agriculture. 59: 221-228.

Dalvi, V.A., Saxena, K.B., Madrap, I.A., (2008). Fertility restoration in cytoplasmic-nuclear male-sterile lines derived from 3 wild relatives of pigeonpea. Journal of Heredity. 99: 671-673.

Dudley, J.W., Saghai, M.A., Rufener, G.K. (1991). Molecular Markers and Grouping of Parents in Maize Breeding Programs. Crop Science. 31: 718-723.

Fu, C., Wu, T., Liu, W., Wang, F., Li, J., Zhu, X., *et al*., (2012). Genetic improvement of resistance to blast and bacterial blight of the elite maintainer line Rongfeng B in hybrid rice (*Oryza sativa* L.) by using marker-assisted selection. African Journal of Biotechnology. 11: 13104-13114.

Hackauf, B., Korzun, V., Wortmann, H., Wilde, P., Wehling, P. (2012). Development of conserved ortholog set markers linked to the restorer gene Rfp1 in rye. Molecular Breeding. 30: 1507-1518.

Hu., J., Li., X., Wu., C., Yang., C., Hua., H. Gao., G., Xiao, J., He, Y. (2012). Pyramiding and evaluation of the brown planthopper resistance genes Bph14 and Bph15 in hybrid rice. Molecular Breeding. 61-69.

Jiang, J., Yang, D., Ali, J., *et al*., (2015) Molecular marker-assisted pyrimiding of broad-spectram disease resistance genes, *Pi2* and *Xa23*, into *GZ63-4S*, an elite thermo-sensitive genic male sterile line in rice. Molecular Breeding. 35: 83.

Kyu, K.L., Saxena, K.B., Kumar, R.V. and Rathore, A. (2011). Prospects of hybrids in enhancing production and productivity of pigeonpea in Myanmar. Journal of Food Legumes. 24: 1-7.

Liu, B., Zhang, S., Zhu, X., Yang, Q., Wu, S., Mei, M., *et al..,* (2004). Candidate defense genes as predictors of quantitative blast resistance in rice. Molecular Plant-Microbe Interactions. 17: 1146-1152.

Liu, K.D., Wang, J., Li, H.B., Xu, C.G., Liu, A.M., Li, X.H. and Zhang, Q. (1997). A genome-wide analysis of wide compatibility in rice and the precise location of the S5 locus in the molecular map. Theoretical and applied Applied genetics Genetics: . 95;: 809-814.

Luan, J., Liu, T., Luo, W., Liu W, *et al*., (2013). Mitochondrial DNA genetic polymorphism in thirteen rice cytoplasmic male sterile lines. Plant Cell Report. 32: 545-554.

Melchinger, A.E., Messmer, M.M., Lee, M., *et al*., (1991) Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms, Crop Science. 31: 669-678.

Ngangkham, U., Parida, S.K., De, S., Kumar, K.A.R., *et al.* ,(2010). Genic markers for wild abortive (WA) cytoplasm based male sterility and its fertility restoration in rice. Molecular Breeding. 26*:* 275-292.

Oliveira, M., Laborda, K., Augusto, P., *et al.* ,(2004). Evaluating genetic relationships between tropical maize inbred lines by means of AFLP profiling. Hereditas. 140: 24-33.

Pan, Y., Li, Q., Wang, Z., Wang, Y., Ma, R., Zhu, L., He, G., Chen, R. (2014) Genes associated with thermosensitive genic male sterility in rice identified by comparative expression profiling. BMC Genomics. 15: 1-17.

Patel, K.A., Acharya, S., Prajapati, N., Patel, J.B. (2012). Molecular identification of cytoplasmic male sterility based hybrid GTH 1 and its parents in pigeon pea. Indian Journal of Genetics. 72: 94-96.

Patel, K.A., Acharya, S., Vaghela, K.O., Patel, J.B., Patel, B.T., Kanbi, V.H., Sheikh, W. (2010). Modified efficient CTAB DNA isolation protocol for cultivated and wild backgrounds pigeonpea. GAU Research Journal. 35: 75-79.

Patel, K.A., Panchchigar, K., Saxena, R.K., Varshney, R.K., Bohra, A. (2020) Understanding molecular divergence and population structure of parental lines of CMS hybrids in pigeonpea, Journal of Food Legumes. 33: 82-92..

Pazhamala, L., Saxena, R.K., Singh, V.K., Sameerkumar, C.V., Kumar, V., Sinha, P., *et al.* (2015). Genomics-assisted breeding for boosting crop improvement in pigeonpea. Frontiers in Plant Science. 6: 50.

Qi., X., Kimatu, J.N., Li, Z., *et al.*, (2014). Heterotic analysis using AFLP markers reveals moderate correlations between specific combining ability and genetic distance in maize inbred lines, African Journal of Biotechnology. 9: 1568-1572.

Qing, J., Lu, J., Chao, Q., Gu, M., Xu, M. (2005). Delimiting a rice wide-compatibility gene S 5 n to a 50 kb region. Theoretical and Applied Genetics. 111: 1495-1503.

Qing, Y.A.O., Jun, L.V., Liu, Q.J., Diao, G.Q., Yang, B.J., Chen, H.M., Jian, T.A.N.G. (2012). An insect imaging system to automate rice light-trap pest identification. Journal of Integrative Agriculture. 11: 978-985.

Qiu, S.Q., Liu, K., Jiang, J.X., Song, X., Xu, C.G., Li, X.H., Zhang, Q. (2005). Delimitation of the rice wide compatibility gene S5 n to a 40-kb DNA fragment. Theoretical and Applied Genetics. 111: 1080-1086.

Rai, K.N., Khairwal, I.S., Dangaria, C.J., Singh, A.K., Rao, A.S. (2009). Seed parent breeding efficiency of three diverse cytoplasmic-nuclear male-sterility systems in pearl millet. Euphytica. 165: 495-507.

Reif, J.C., Fischer, S., Schrag, T.A., Lamkey, K.R., Klein, D., Dhillon, B.S., *et al.* ,(2010). Broadening the genetic base of European maize heterotic pools with US Cornbelt germplasm using field and molecular marker data. Theor. Appl. Genet. 120: 301-310.

Reif, J.C., Hamrit, S., Heckenberger, M., *et al.*,(2005). Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. Theoretical Applied Genetics. 111: 838-845.

Reif, J.C., Melchinger, A.E., Xia, X.C., Warburton, M.L., Hoisington, D.A., Vasal, S.K., Srinivasan, G., Bohn, M. and Frisch, M. (2003). Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Science. 43: 1275-1282.

Reif, J.C., Melchinger, A.E., Xia, X.C., *et al.* (2003). Use of SSRs for establishing heterotic groups in subtropical maize. Theoretical Applied Genetics. 107: 947-957.

Riedelsheimer, C., Czedik-Eysenberg, C., Grieder, C., Lisec, J., Technow, F., Sulpice, R., Altmmann, T., Stitt, M., Willmitzer, L. and Melchinger, A.E. (2012). Genomic and metaboloic prediction of complex heterotic traits in hybrid maize. Nat. Genet: 44: 2.

Robin, S., Kavithamani, D., Manonmani, S., Sundaram, K.M., Thiyagarajan, K. (2010). Molecular Tagging of a Thermo-Sensitive Genic Male Sterile Gene and Identifyng New TGMS Lines in Rice, 28th International Rice Research Conference, 8-12. Hanoi Vietnam. OPI2: Molecular Biology and ''Omics'' Technologies.

Salgotra, R.K., Gupta, B.B., Ahmed, M.I. (2012). Characterization of thermo-sensitive genic male sterility (TGMS) rice genotypes (*Oryza sativa* L.) at different altitudes. Australian Journal of Crop Science. 6: 957-962.

Sang,. X., Yang, Z., Zhong, B., Li, Y., Hou, L., *et al.*, (2006). Assessment of purity of rice CMS lines using cpDNA marker. Euphytica. 152: 177-183.

Saxena, R.K., Patel, K.A., C, V, Sameer Kumar. , C.V.S., Tyagi, K., Saxena., K.B., Varshney, R.K. (2018) Molecular mapping and inheritance of restoration of fertility (Rf) in A4 hybrid system in pigeonpea [*Cajanus cajan* (L.) Millsp.] Theoretical and Applied Genetics. https://doi.org/10.1007/s00122-018-3101-y.

Saxena, R.K., Saxena , K.B., Pazhamala, L.T., Patel, K.A., Parupalli, S., Sameerkumar, C.V., Varshney, R.K. (2015). Genomics for greater efficiency in breeding hybrid pigeonpea. Frontiers in Plant Science. 6: 793.

Saxena. K.B., Ravikoti, V.K., Tikle, A.N., Saxena, M.K., Singh, V.G., Rao, S.K., Khare, K., *et al.* (2013) ICPH 2671 - the The world's first commercial food legume hybrid. Plant Breeding; DOI: 10:1111/pbr.12045.

Shekh, W., Acharya, S., Patel, J.B., Kalaskar, S.R., Shinde, A.S., Patel, K.A. (2015). Genetic fingerprinting of A and R lines of pigeonpea [*Cajanus cajan* (L.] Millsp.] using RAPD and SSR markers. Indian Journal of Biotechnology. 14: 328-333

Shull, G. H., (1908). The composition of a field of a maize. Report Am. Breeders's Assoc. 4: 296-301.

Singh, V.K., Singh, A., Singh, S.P., *et al.*, (2012). Incorporation of blast resistance into "PRR78", an elite basmati rice restorer line, through marker assisted backcross breeding. Field Crops Research. 128: 8-16.

Tang, Z., Zhang, L., Xu, C., *et al.*, (2012). Uncovering small RNA-mediated responses to cold stress in a wheat thermo sensitive genic male-sterile line by deep sequencing. Plant Physiology. 159.

Tikka, S.B.S., Parmar, L.D., Chauhan, R.M. (1997). First record of cytoplasmic-genic male-sterility system in pigeonpea [*Cajanus cajan* (L.) Millsp.] through wide hybridization. Gujarat Agricultural University Research Journal. 22: 160-162.

Varshney, R. K. *et al.* (2017). Whole-genome re-sequencing of 292 pigeonpea accessions identifies genomic regions associate with domestication and agronomic traits. Nat. Genet. doi: 10.1038/ng.3872.

Varshney, R.K., Chen, W., Li, Y., Bharti, A.K., Saxena, R.K., Schlueter, J.A., Donoghue, M.T.A., Azam, S., *et al.* (2012). Draft genome sequence of pigeonpea (*Cajanus cajan* L), an orphan legume crop of resource-poor farmers. Nature Biotechnology. 30: 83-89.

Varshney, R.K., Nayak, S.N., May, G.D., *et al*., (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends biotechnology. 27: 522-30.

Varshney, R.K., Penmetsa, R.V., Dutta, S., Kulwal, P.L., Saxena, R.K., Datta, S., Sharma, T.R., *et al*. (2010) Pigeonpea genomics initiative (PGI): An international effort to improve crop productivity of pigeonpea (*Cajanus cajan* L.). Molecular Breeding. 26: 393-408.

Vasal, S.K., Srinivasan, G., Crossa, J., Beck, D.L. (1992). Heterosis and combining ability of CIMMYT's subtropical and temperate early-maturity maize germplasm. Crop Science. 32: 884-890.

Wang, G.W., He, Y.Q., Xu C.G., Zhang, Q. (2005). Identification and confirmation of three neutral alleles conferring wide compatibility in inter-subspecific hybrids of rice (*Oryza sativa* L.) using near-isogenic lines. Theoretical and Applied Genetics Genetics. 111: 702-710.

Xie, F., He, Z., Esguerra, M.Q., Qiu, F. and Ramanathan, V. (2014). Determination of heterotic groups for tropical Indica hybrid rice germplasm. Theoretical and Applied Genetics. 127: 407-417.

Yang, J., Zhao, X., Cheng, K., Du, H., Ouyang, Y., *et al*., (2012). A killer-protector system regulates both hybrid sterility and segregation distortion in rice. Science. 337: 1336-1340.

Yashitola, J., Thirumurugan, T., Sundaram, R.M., Naseerullah, M.K., Ramesha, M.S., Sarma, N.P., Sonti, R.V. (2002). Assessment of purity of rice hybrids using microsatellite and STS markers. Crop Science. 42: 1369-1373.

Zhang, D., Wang, Z., Wang, N., Gao, Y., Liu, Y., Wu, Y., Bai, Y., Zhang, Z., Lin, X., Dong, Y., Ou, X. (2014). Tissue Culture-induced hHeritable gGenomic vVariation in rRice and their Phenotypic iImplications. PloS One. 9: p.e96879.

Zhou, H., Zhou, X., Zeng., M., Liao, B.H., Liu, L., Yang, W.T., Wu, Y.M., Qiu, Q.Y., Wang, Y.J. (2014). Effects of combined amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on contaminated paddy soil. Ecotoxicology and Environmental Safety. 101: 226-232.