ACTA SCIENTIFIC AGRICULTURE (ISSN: 2581-365X)

Volume 3 Issue 2 February 2019

Review Article

Genetic Diversity and Application of DNA Markers in Garden Pea-Review

Arul S[1] and Selvakumar R[2]*

[1]*Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi, India*

[2]*Division of Vegetable and Flower Crops, ICAR-Central Institute of Temperate Horticulture, KD Farm, Old Air Field, Rangreth, Srinagar, Jammu and Kashmir, India*

***Corresponding Author:** Selvakumar R, Scientist, ICAR-Central Institute of Temperate Horticulture, KD Farm, Old Air Field, Rangreth, Srinagar, Jammu and Kashmir, India.

Received: October 24, 2018; **Published:** January 29, 2019

Abstract

Indian olericulture has reached an important juncture in its evolution, with still greater scope for improving both production and productivity. To accord an overriding priority to olericulture, is essentially required to be the feature of the emerging scientific technological interventions in India. Thus, significant multipronged actions are needed to improve and strengthen the infrastructure essential for sustained horticultural growth to fully exploit the individual biological potential of vegetable species. The prime and long term objective of vegetable breeding is to increase productivity to meet the increasing food requirements of people. New varieties with improved horticultural traits have been the major contributing factor to increase food production. Therefore, to plan appropriate garden breeding programme and evolve high yielding garden pea cultivars with resistance to pest and diseases, the vegetable breeder must possess adequate knowledge on variability, genetic divergence, character association and the extent of contribution of each of Garden Pea. This review will help breeders as well as researchers to understand Garden pea improvement mainly depends on the extent of heritable diversity existing in *Pisum* species. Thereby helps in formulating the breeding methodology for handling the segregating garden pa material in the subsequent generations by the exploitation of fixable component.

Keywords: Olericulture; Vegetable; Breeding; Pisum

Introduction

Garden pea (*Pisum sativum* L. subsp. *hortense* (Neilr.) Asch. And Graebn.2n = 14) is an annual herbaceous legume vegetable belonging to the family Fabaceae [1]. It is one of the oldest cultivated plants, grown as a vegetable crop for both fresh and dried seed in cool season globally. It is one of the most nutritious leguminous vegetable, containing high percent of digestible protein, carbohydrates, health promoting phytonutrients, minerals, folate, anti-oxidants, dietary fibre, vitamin 'A', thiamine, riboflavin, ascorbic acid, calcium, phosphorous, iron, respectively [2]. In addition peas are having several nutraceutical compounds like isoflavones linked to reducing hormones related cancers and saponins, which is thought to lower cholesterol as well as have anti-inflammatory, antibacterial and antifungal properties [3]. Garden Pea improvement through suitable breeding techniques is the main option for the breeder to increase the production and productivity. Pea is an autogamous crop, and recombinant breeding is the most appropriate approach to combine various desirable traits like long, dark green pods with high yield potential [4]. The best choice among several breeding methods depends on the genetic architecture of the traits being considered. Furthermore, current development of molecular markers would help the breeders and researcher to understand about recent relevant to its genetics and marker application. The relevant literature on various aspects of garden pea has reviewed under the following heads.

1. Genetic architecture
2. Genetic diversity analysis
3. Genetic diversity based on molecular markers

Genetic architecture

For improvement programme, the best choice among several breeding methods depends upon the genetic architecture of the

traits. Therefore knowledge of the nature of gene action for pod yield traits related to productivity is important for genetic enhancement of complex character like yield. Generation mean analysis is helpful in identifying the nature and magnitude of gene action including non allelic gene interaction in order to formulate breeding strategy. The back ground information has been reviewed and presented as follows.

Chandel and Joshi [5] reported that pod length exhibited the complementary nature of epistasis in most of the crosses. Seeds per pod showed low additive gene effects with complementary epistasis in some crosses. They emphasized the possibility of utilizing additive gene effect in some crosses and complementary gene interaction in majority of the crosses for the improvement of pod length and seeds per pod. Dominance and epistatic effects were important than additive effects alone in the inheritance of number of seeds per pod [6].

Gupta and Dahiya [7] studied the inheritance of pod length and other traits in pea and revealed that additive effects were predominant in pea and revealed that additive effects were predominant for all other characters in a study of F_1, F_2 and F_3 generations involving dwarf, semi dwarf, medium and tall varieties. Khemelev and Rozvadovskii [8] revealed that additive gene effects were more prominent in the medium × medium and medium × tall crosses while dominance effects predominated in the dwarf × medium and dwarf × tall varieties. Relatively high frequency of transgressive segregants was found in dwarf × medium and dwarf × tall crosses.

Gad and El-Swah [9] reported additive and dominance effects to be significant for various morphological traits. Gupta and Lodhi [10] carried out diallel analysis studies over six environments and concluded that additive as well as non-additive gene effects were important for seed yield and 1000-seed weight while, over dominance was the important for the former and partial dominance for the later trait. Singh., *et al.* [11] analysed the data for parental, F_1 and F_2 generations for yield per plant, days to flowering, plant height, pods per plant, seeds per pod, pod length, days to maturity and revealed significant additive and non-additive gene effects for all traits however, the additive component was more important.

Gupta., *et al*. [12] studied the inheritance of seed yield in pea and indicated additive as well as non-additive gene effects to be important for the inheritance of seed yield and 1000-seed weight. Singh., *et al*. [13] from generation mean analysis, they revealed that in addition to significant estimates of additive and dominance components, epistatic components of mean [(i) and (l) types] were also important and duplicate type of epistasis was predominant for all the traits in both sets of crosses. They reported that the genetic information obtained from both analyses seems to be complementary rather than alternative modes of inheritance in governing the expression of useful economic traits. Singh [14] studied diallel set of 12 crosses and indicated the importance of both additive and non-additive genetic components of variation for yield and pods per plant.

Singh and Singh [15] in a six generation mean analysis in pea observed that additive that additive –dominance model was adequate for pods per plant and 1000 seed weight. They further showed the evidence of epistasis for most of the quantitative traits though additive-dominance components were significant and seed yield appeared to be predominantly governed by non-additive components. Rana and Gupta [16] reported that additive and dominant components were highly significant for all the traits. However, the magnitude of additive component was appreciably higher than dominance components for all the traits except for green pod

Singh and Sharma [17] studied gene action for yield and its components in three crosses of pea and reported higher estimates of dominance effect (*h*) for almost all the traits were associated with significant epistatic interaction(s) in the respective crosses. Comparing the magnitude of the main effects (*d*) and (*h*) along with their digenic epistatic interactions (*i*, *j* and *I*), the interaction was usually higher. However, the sign of dominance x dominance (*I*) interaction was mostly negative indicating their reducing effect in the expression of almost all the characters. Sharma and Kalia [1] studied a partial diallel analysis for pod yield per plant, pods per plant, pod length, shelling percentage and total soluble solids in ten parental lines in a two environment conditions, reported that gene action for most of the traits had a significant additive and non additive genetic variance. Bhardwaj and Vikram [18] studied genetics of yield components of a garden pea cross (Ageta-6 × DRP-3) by raising six different generations *viz.,* P_1, P_2, F_1, F_2, B_1 and B_2. They revealed that duplicatory type of epistasis controlling the inheritance of shelling percentage, while the additive gene effects were important for node at first flower, number of seeds per pod and pod length.

Singh., *et al.* [19] studied pea for green podyield and protein based on the generation mean analysis and reported the predominance of additive gene effects in one and two crosses for green pod yield and protein respectively. The predominance of dominance gene effects was observed in fourteen crosses for green pod yield. They reported the major role of dominance along with additive × dominance or dominance × dominance components of epistasis in

the inheritance these traits. Sood and Kalia [20] reported the inheritance of seven economic traits *viz*., days to 50% flowering, days to first picking, pods per plant, plant height, seeds per pod, and shelling percentage have governed by additive and non additive gene action. The role of additive, non additive and additive × dominance gene action reported to govern pod yield per plant. Dixit., *et al*. [21] reported the role of epistasis in the inheritance of days to flowering with different set of breeding material. Singh., *et al*. [22] revealed that in addition to significant estimates of additive and dominance components, epistatic components of mean were also important and duplicate type of epistasis was predominant for all the traits in different sets of crosses.

Sharma., *et al.* [24] studied to determine the nature and magnitude of genetic effects for different biometric traits in garden pea. They studied using a ten lines of garden pea were crossed with three testers followed a triple test cross method for estimating the epistasis gen action. The majority of traits show the significant differences in epistasis × location and type × location interaction. Sharma., *et al.* [25] reported the presence of epistatic interaction for majority of the traits in all three crosses viz. Green Pearl × Sugar Giant, DPP 9411 × DPP 9418-06, and Azad P-I × Sugar Giant was observed as reflected by the significance of simple additive-dominance model. They revealed that the nature and magnitude of gene effects differed in different crosses and showed the importance of additive as well as non-additive gene effects in the inheritance of different characters with preponderance of the latter. In view of the parallel role of additive and non-additive gene effects, selection in the segregating generations should be delayed to later generations to diminish the dominance gene effects. They also found duplicate type of epistasis for some of the traits in certain cross combinations whose effect can be eliminated by following sophisticated selection procedure such as reciprocal recurrent selection and/or biparental mating in early segregating generations for the development of high yielding garden pea varieties with desirable horticultural traits.

Genetic diversity analysis

For a successful plant breeding programme, genetic divergence is very much essential to classify the experimental material, based on the extent of similarity, into close and divergent types. Genetic diversity can be defined as the variability among different genotypes of a species. The number of classificatory approaches based on multivariate analysis has been proposed by different workers to carry out genetic divergence studies. Genetic diversity arises due to geographical separation or due to genetic barriers to crossabiltiy. D^2 statistics developed by Mahalanobis [25] actually provides measures of magnitudes of divergence between two groups under comparison. It considers the variation produced by any character and their consequent effect that it bears on other character. Its application to the field of botany was started with the work of Nair and Mukharji [26] who applied this method in classifying the natural and plantation teak tree. Its application was extended to taxonomic studies. Murthy and Pavate [27] observed that D^2 analysis can be extended to the situations where overlapping species need to be discriminated and also to the fact that discrimination at subspecies level is needed. They proposed metrograph and index score methods to study the pattern of morphological variation in crop species. The classificatory approaches like principal component, factor analysis and clustering of genotypes overcome the limitations of D^2 statistics. The clusters in broad sense are thought as collection of points which are relatively close, but which are separated by empty regions from other clusters. Cluster can be overlapping or non- hierarchic and over lopping or hierarchic. Following this study the technique has been applied in several vegetable crops. The method of non-hierarchic clustering may be used with larger problems than the hierarchical methods, because it is not necessary to calculate and store the similarity matrix. Non-hierarchical Euclidean cluster analysis was described by Beale [28] and further elaborated by Sparks [29].

Saxena., *et al*. [30] studied the genetic divergence using Mahalanobis D^2 statistics for grain yield and 12 yield component characters in 23 strains of pea. The 23 strains were grouped into 7 clusters. The clustering pattern of strains usually did not follow the geographical distribution. Sureja and Sharma [31] evaluated 30 indigenous and exotic genotypes for genetic divergence utilizing Mahalanobis D^2 statistics on different traits of garden pea. The genotypes were grouped into four clusters. The clusters I, II and III had six genotypes each while cluster IV included 12 genotypes and was the largest cluster. It was suggested from the study that utilizing the genotypes from the cluster I and II as parents in the future breeding programme could breed a high yielding early dwarf plant type with high protein content. Dixit., *et al.* [21] used fifty-three genotypes of field pea to study genetic divergence following D^2 analysis. Genotypes were grouped into 11 different clusters. Clusters I and II consisted of 15 genotypes each. Plant height contributed maximum to the genetic diversity. Intra-cluster was highest in the cluster III followed by cluster I and II. Intra cluster distances were maximum between cluster IV and X followed by clusters IV and XI. The study indicated lack of parallelism between genetic and geographic diversity. The genotypes included in the diverse genetic clusters can

be used as promising parent for hybridization to obtain higher heterotic response and thus better segregates in field pea.

Narayanankutty., *et al.* [32] Studied genetic variability and divergence on thirty seven genotypes of vegetable cowpea revealed significant differences for all the characters under study. In general, the intercluster distances were higher than intracluster distances. The maximum intercluster distance was between clusters VIII and X, followed by clusters VI and X and clusters VIII and IX, respectively. The intracluster distance was maximum in cluster VII. The nature of magnitude of genetic diversity was studied in a set of 45 cowpea (*Vigna unguiculata*) genotypes from indigenous and exotic sources. The genotypes were grouped into five clusters using Mahalanobis D^2 statistics. Cluster-I was the largest with 28 genotypes followed by cluster-II with eleven genotypes and cluster-III with four genotypes. The clusters IV and V were mono genotypic. The maximum inter-cluster distance was observed between clusters III and V followed by distance between clusters II and III. Clusters I and III exhibited the minimum inter-cluster distance. The number of branches per plant, test weight, biomass (dry weight) at harvesting and number of pods per plant had contributed considerably toward divergence.

Yadav., *et al.* [33] revealed wide range of diversity in 45 garden pea germplasm studied for the genetic divergence. These lines were grouped into 15 different clusters with 25 genotypes included in first five clusters. Remaining 20 lines were assigned to next 10 clusters. They evaluated that the genetic constitution rather than the geographical placement of genotypes played major role in clustering pattern of the genotypes. Tiwari., *et al.* [34] evaluated thirty four pea *(Pisum sativum* L.) genotypes, for their genetic diversity, were grouped into six clusters. The cluster V and VI were largest with eight genotypes in each. The first cluster containing only one genotype was the most divergent. The inter cluster distance was minimum (11.84) between cluster III and VI and was maximum (41.77) between clusters I and U. They reported that there was much diversity in the populations of 34 genotypes and genotypes of cluster I, II, III and IV could be exploited for hybridization programme. Singh and Singh [6] evaluated thirty one advanced genotypes of pea Involving six varieties + 25 promising genotypes for assessing genetic divergence for grain yield. Significant differences among the genotypes were recorded for all the characters studied. Genotypes were grouped in six clusters based on D^2 values. Cluster I was more divergent and monogenotypic involving advance genotype KPMR632. Cluster VI was the largest with eight genotypes. The Inter cluster distance was minimum (12.04) between cluster III and VI and maximum (41.35) between cluster I and II closely followed by I and IV. The intermating among genotypes following clusters I, II and III would be of breeding value so as to Improving grain yield.

Gupta and Singh [35] used Mahalanobis D^2analysis study to assess the genetic diversity among 83 genotypes of garden pea *(Pisum sativum* L.) and also the contribution of each character to the total diversity. They revealed that the genotypes varied significantly for all the 18 characters studied. They grouped 83 genotypes into 27 clusters on the basis of D^2 values. Cluster I had the largest number of 17 genotypes. Six genotypes each in clusters II to V, four each in clusters VI to VIII, three each in clusters IX to XI, two each in clusters XII to XVI and one genotype fell in each cluster from XVII to XXVII. The clusters I, II, V, X, XI, XII, XVII, XVIII, XIX, XX and XXI included the F1 crosses only, which showed that the genetic makeup of the crosses was altogether different from the parental lines. The perusal of clustering pattern that the grouping was not influenced by the place of origin, rather genetic back ground influenced their clustering behaviour. They pointed out that clustering of genotypes was random and geographic origin had negligible or no influence on them.

Singh and Mishra [36] studied genetic divergence using Mahalanobis D^2 statistic in 21 genetically diverse genotypes for days to flower, plant height, pods/plant, seeds/pod, pod weight/plant and 1000-seedweight. The genotype was grouped into six clusters. The cluster I was the biggest with 11 genotypes followed by clusters II and III with four and three genotypes, respectively. Cluster IV, V and VI were unique since they had only one genotype. The maximum inter-cluster distance was observed between clusters II and VI and was followed by clusters II and V, and clusters III and VI indicating wide divergence among these clusters, which also suggested that the genetic architecture of the genotypes in one clusters differed entirely from those included in other clusters. The diversity among the genotypes measured by inter-cluster distance (D value) was adequate for improvement of pea by hybridization and selection.

Muhammad., *et al*. [37] studied two hundred and sixty three genotypes of *Pisum sativum* with the prime objective of to investigate the amount genetic diversity in a broad based pea germplasm. All the genotypes were categorized into five clusters using mean variances for linkage. Cluster-I consisted of 73 genotypes, cluster-II of 59, cluster-III of 28, cluster-IV of 37 and cluster-V has 66 genotypes. High genetic distance was observed between cluster-IV and cluster-V, whereas the lowest distance was recorded between cluster-I and cluster-V. These results indicate the scope of selection from various clusters for crop improvement with maximum

diversity among improved cultivars. Katiyar and Dixit [38] studied genetic divergence in pea and obtained wide morphological divergence for all the traits under study using multivariate analysis. Principal component analysis, which transformed all the metric traits into single index of similarity, yielded 8 eigen vector and roots. Based on first 4 principal components (which accounted for 86.51% of the total variation), non-hierarchical Euclidean cluster analysis grouped the 480 field pea accessions into 8 well characterized groups (based on aggregate effect of similarity in traits). There is no parallelism between genetic diversity and geographical origin of accessions.

Dhama., *et al*. [39] studied genetic diversity in pea with 30 genotypes using D^2 statistics and revealed significant differences among the genotypes for yield and its component characters. The genotypes were grouped into 4 clusters in E_1, 4 clusters in E_2, 5 clusters in E_3, 5 clusters in E_4, 4 clusters in E_5, 5 clusters in E_6, 5 clusters in E_7 and 5 clusters in E_8. They reported that clustering pattern of genotypes was not consistence over environments. Number of clusters as well as number of genotypes in the cluster differed from environment to environment. They revealed that the genotypes of the same cluster have little divergence of each cluster with respect to aggregate effects of the characters studied. The hybridization between the genotypes of same cluster, thus, may not provide good recombination and the crosses may be attempted between genotypic clusters of having large inter cluster distances and it is likely to give desirable transgressive segregants. Different methods for selection of parents for a hybridization programme have been suggested in the past for several crops. Usually, it is suggested that parents must be selected on the basis of D^2 analysis of Mahalanobis [25]. In the study they reported D^2 is a useful tool in quantifying the degree of divergence between populations and also helps in the choice of genetically diverse parents to obtain recombinants in the segregating generations.

Yadav., *et al.* [40] investigated sixty-two genotypes of field pea for ten characters to examine the genetic divergence existing among these genotypes. They revealed significant differences for all the characters studied, indicating appreciable amount of variability among the genotypes. All the genotypes were grouped into eighteen clusters. The cluster I had 14 genotypes followed by 9 in cluster II, 8 in cluster III, 4 in cluster V and VI each, 3 in cluster VII and VIII each and 2 in clusters IX and X each. Each of the eight clusters (*viz*., cluster XI, XII, XIII, XIV, XV, XVI, XVII and XIII) were unique having only one genotype. These clusters contained genotypes either of same origin in different clusters or of different origin in same clusters, thereby, indicating no parallelism between genetic and geographic origin. Genetic drift and selection in different environments could cause greater diversity in the geographic distances.

Dalsaniya., *et al.* [41] evaluated 60 genotypes of cowpea to study the diversity among the genotypes which were grouped in to 12 clusters revealing the presence of considerable diversity in the material. The clustering pattern of the varieties usually did not confirm to geographical distribution. Inter cluster distance and mean cluster character values indicated that hybridization of cluster-X variety (JCPL-134) with cluster-IV varieties (JCPL-1, JCPL-13 and JCPL-21) and cluster-V varieties (JCPL-50 and JCPL-133) with cluster-III varieties (JCPL-26 and JCPL-131) would exhibit high heterosis and also result in transgressive segregants with higher yield. They reported that characters like plant height, green pod yield per plant, protein content and leaf area were found to contribute much to the total genetic divergence in cowpea.

Genetic diversity based on DNA markers

DNA markers are considered superior to morphological and biochemical marker systems because they analyse polymorphism at the DNA level and allow differentiating genotypes which are not distinguished by other tests and also because nucleotide composition is being directly determined rather than a product of the genome [42]. A number of DNA based markers such as RFLP, RAPD, AFLP, SSRLP, etc. are being used for genetic mapping and diversity analysis.

Molecular markers are useful tools for marker-assisted selection in crop improvement. Random amplified polymorphic DNA (RAPD) [43,44] analysis involves the amplification of random segments of genomic DNA using polymerase chain reaction (PCR) methodology [45]. RAPD analysis has been demonstrated to be an efficient marker detection system for disease resistance genes and plant breeding programs [46,47]. Among the various DNA markers assisted techniques available, RAPD [43] has been most popular because of its speed, low cost, and the use of lower quantities of plant material for analysis. However, the major limitation of this technique is low reproducibility. RAPD marker system does not require any prior knowledge of genome sequence [48] and are being rapidly used by the research community in various field of plant improvement. Samec and Našinec [49] used 42 *Pisum* genotypes representing four wild and cultivated subspecies as templates for RAPD reactions. Amplification with eight decamer primers generated 149 polymorphic products. They reported that RAPD technology is a rapid, precise and sensitive technique for identification of pea genotypes. Lu., *et al.* [50] reported a direct comparison of DNA-based RFLP

with various PCR-based techniques regarding their in formativeness and applicability for genetic diversity analysis. They studied ten pea genotypes for diversity analysis and revealed all PCR-based methods were much more informative than cDNA-RFLP.

Simioniuc., *et al*. [51] studied genetic diversity present within the set of pea cultivars released in Germany, 21 cultivars at DNA level by random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs), as well as for agronomic traits. Twenty RAPD primers amplified a total of 314 scorable bands ranging from about 262 bp to 1996 bp. Of these, 175 fragments (55.7%) were polymorphic. Based on these data, genetic similarity (GS) was estimated between 0.80 and 0.94. Eleven AFLP primer combinations led to the amplification of 949 scorable fragments ranging from 43 to 805 bp and of these, 462 (48.7%) were polymorphic. Genetic similarity based on AFLPs was calculated between 0.85 and 0.94. Correlation of genetic similarity estimated on RAPDs and AFLPs was estimated at r = 0.79 using Spearman's rank correlation coefficient and at r = 0.84 by the Mantel test, respectively. UPGMA cluster analysis carried out on these data separately for RAPDs and AFLPs and on the combined data reflected, to some extent, pedigree relationships and cophenetic correlations indicate a good fit of respective clusters to genetic similarity data. The correlation of cluster analyses to pedigree information and the impact on parental genotype selection is discussed.

Baranger., *et al.* [52] reported the genetic diversity within 148 *Pisum* accessions and including both primitive germplasm and cultivated types using a protein and PCR-based markers. The molecular data from RAPD, ISSR markers revealed the 8 groups consistent with geographical origins and known cultivated types. The genetic diversity largely consistent with the available pedigree data and clearly resolved the different main varietal types according to their end-uses of fodder, food and feed. Yadav., *et al*. [53] investigated fifteen germplasm lines of *Pisum sativum* L. for characterization using Randomly Amplified Polymorphic DNA (RAPD) markers. While 12 random primers were taken, out of them 11 primers gave amplification. These primers gave a total of 133 bands out of which 106 were polymorphic. Genetic similarities of the RAPD profiles were estimated by using Jaccard's coefficient with NTSYS pc 2.0 software. The similarity index values ranged from 0.263 to 0.793 indicating the presence of enormous genetic diversity at molecular level. A dendrogram generated by cluster analysis divided fifteen field pea genotypes into two Groups A and B. Major Group A have five genotypes and major Group B have nine genotypes.

Taran., *et al.* [3] reported the genetic relation among 65 pea varieties and 21 accessions from wild *Pisum* subspecies and are classified based on the molecular markers RAPD, SSR and ISSR, and morphological and physiological characters. The UPGMA cluster analysis and PCA on the marker based grouped the cultivated varieties separately from the silage and specialty varieties regardless of the originating breeding programmes. GS Nei and Lis genetic similarity (GS) estimates calculated using the marker data showed that pair-wise comparison values among the 65 varieties ranged from 0.34 to 1.00. Choudhury., *et al*. (2007) studied twenty-four most popular and widely adapted varieties of pea to find out the genetic relatedness among them using RAPD analysis. They used 60 decamer primers. All the primers used in their study were found to be polymorphic and seven of them showed 100% polymorphism. Out of 579 amplified products, 433 showed polymorphism (74.8%). On an average, 9.65 bands were amplified per primer. Maximum number of 22 amplified products was obtained by primer OPP 13. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all the tall type varieties together, whereas, dwarf types formed two different clusters based upon their pedigree. They revealed that about 10 genotypes can be unambiguously distinguished by employing 60 RAPD primers.

Samatadze., *et al*. [54] used C banding, Ag-NOR staining, FISH with pTa71 (45S rDNA) and pTa794 (5S rDNA), and RAPD PCR analysis to study the genome and chromosome polymorphism in four varieties (Frisson, Sparkle, Rondo, and Finale) and two genetic lines (Sprint-2 and SGE) of pea *Pisum sativum* L. A comparison of the C-banding patterns did not reveal any polymorphism within the varieties.RAPD-PCR analysis revealed high between-variety and low within-variety genomic polymorphism. They reported that chromosomal and molecular markers proved to be promising for genome identification in pea varieties and lines. Cupic., *et al*. [55] studied genetic diversity of European pea (*Pisum sativum* L.) germplasm, to determine differences between *P. sativum* var. *arvense* and *P. sativum* ssp. *sativum* groups, and to estimate genetic variability among and within eighteen *P. sativum* accessions genotypes using morphological traits and molecular markers. Genetic distances estimated by molecular marker (SSR) data in comparison with distances estimated by conventional methods (pedigree and morphologic traits) showed higher similarity with genetic distances estimated by morphological data. They reported that intercrosses between *arvense* and *sativum* accessions as well as inclusion of valuable landraces into breeding programmes might prevent loss of diversity in the *Pisum* gene pool.

Tanveer., *et al.* [56] studied the molecular divergence and developed a DNA finger prints in 24 widely adopted high yielding morphologically diverse and popular cultivars of field pea in India.

The RAPD primers (viz.OPP, OPBA, OPAQ, OPH) showed 75 percent polymorphism in molecular diversity analysis. Yadav *et al.* [40] assessed the genetic diversity among tall and dwarf cultivars/elite lines of pea based on 10 quantitative traits and 282 RAPD markers. The markers viz., OPI11, OPW01 and HU11, or OPQ20 and OPI11 were needed to separate all the 28 lines of pea. In principal component analysis the first three PCs together accounted for 61.8% of the total variation and the grouping was consistent with that of UPGMA method.

Ahmad., *et al*. [57] used four RAPD primers (GM10, GM37, GM52 and GM100) to estimate genetic diversity in five *Pisum* cultivars and scored a total of 16 bands corresponding to an average of 4 bands per primer with 6 bands showing polymorphism (37.5%). One out of 4 primers gave 75% polymorphism. Jaccard's similarity coefficient ranged from 0.7692 to 0.9630. Similarity index reveals the maximum similarity between cultivars KPMR 925(G2) and KPMR 926(G3), KPMR 926(G3) and KPMR 927(G4) *i.e.* 0.9630 and 0.963 respectively while distantly related cultivars were KPMR 922(G1) and KPMR926 (G2) with Similarity index 0.7692. A dendrogram constructed based on the UPGMA clustering method revealed two major clusters. Cluster-I and Cluster-II comprising of two cultivars each. The cultivar KPMR 922(G1) occupies a distinct place as revealed in the dendrogram. Thakur and Singh (2011) evaluated the 15 pea genotypes/varieties based on their biochemical constituents and molecular diversity. They used SDS-PAGE and RAPD analysis which showed that some genotypes are highly diverse. The seed protein profiling revealed a total of 10 protein bands. The dendrogram analysis divided the genotypes in 2 major clusters. The overall similarity coefficient ranged from 0.11 to 1.00.

Conclusion

Genetic diversity analysis at phenotypic level based on morphological traits are not so meaningful as they are environmental dependent and less reliable. The reliability of the genetic marker and its usefulness in genetic diversity analysis are positively associated with the heritability and the level of polymorphism exhibited by molecular marker. DNA based markers are considered to be the best for determining diversity/relationship as they are independent of environmental interactions. So, the study of polymorphism is best done at the level of nucleotide bases in DNA which is the primary source of all biological information. At this level, even seemingly identical accessions could display enormous differences, if only we could employ appropriate DNA profiling techniques. Random Amplified Polymorphic DNA (RAPD) is one such method of identifying polymorphism that can be used to elicit information on genetic differences among individuals of a population between lines or germplasm accessions or any breeding material. The use of molecular markers for diversity analysis serve as an effective tool to discriminate between closely related individuals from different breeding sources and identify genetically diverse parental lines for their use in future breeding programme. This review could help garden pea breeders for broad genetic architecture information as well as molecular application in garden pea.

Bibliography

1. Sharma A and Kalia P. "Genetic analysis for pod yield and its contributing traits in garden pea. (*Pisum sativum* L.)". *Journal of Vegetation Science* 29.2 (2002): 106-109.

2. Singh N and Dhillon GG. "Genetic analysis of pod yield and quality traits in garden pea. (*Pisum sativum* L. *sub. sp. hortense*)". *Journal of Vegetation Science* 30.1 (2003): 79-80.

3. Tar'an B., *et al*. "Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological character". *Genome,* 48 (2005): 257-272.

4. Thakur N and Singh N. "Biochemical evaluation and molecular characterization of garden pea (*Pisum sativum* L.)". *Indian Journal of Agricultural Biochemistry* 24.1 (2011): 49-54.

5. Chandel KPS and Joshi BS. "Gene action for some yield contributing characters in peas (*Pisum sativum* L.)". *Genetic Agriculture* 32 (1978): 173-184.

6. Singh JD and Singh JP. "Genetic divergence in advanced genotypes for grain yield in field pea *(Pisum sativum* L.)". *Legume Research* 29.4 (2006): 301-303.

7. Gupta KR and Dahiya BS. "Inheritance of pod related traits in pea". *Crop Improvement* 11 (1984): 45-48.

8. Khemdev BI and Rozvadovskii AM. "Inheritance of stem length in pea". *Tsitologiyai - Genetika* 18.2 (1984): 101-105.

9. Gad AA and El-Swah MH. "Diallel analysis of pea crosses. Inheritance of some morphological traits". *Egyptian Journal of Genetics and Cytology* 14 (1985): 265-274.

10. Gupta KR and Lodhi GP. "Diallel analysis over environments for seed yield and 1000-seed weight in pea". *Crop Improvement* 12 (1985): 175-178.

11. Singh KN., *et al*. "Combining ability and components of heterosis in pea". *Crop Improvement* 12 (1985): 94-97.

12. Gupta KR., *et al*. "Inheritance of seed yield and quality traits in peas". *Theoretical and Applied Genetics* 69 (1986): 133-137.

13. Singh BB., *et al*. "Genetic analysis of yield and yield components in field peas". *The Journal of Agricultural Science* 109 (1987): 67-71.

14. Singh KN. "Genetics of harvest index in field pea; certain released genetic models in a diallel cross". *Indian Journal of Pulses Research* 3.1 (1990): 19-24.

15. Singh BB and Singh DP. (1994). "Generation mean analysis for yield and yield components in pea". *Indian Journal of Pulses Research* 7(2): 108-111.

16. Rana JC, and Gupta VP. "Genetic analysis of green pod yield and phonological traits in pea". *Legume Research* 17.2 (1994): 105-108.

17. Singh TH and Sharma RR. "Gene action for yield and its components in three crosses of pea *(Pisum sativum* L.)". *Indian Journal of Genetics* 61.2 (2001): 174-175

18. Bhardwaj RK and Vikram A. "Genetics of yield and other characters in a cross of garden pea. (*Pisum sativum* L.)". *Indian Journal of Agricultural Research* 38.2 (2004): 154-156.

19. Singh P., *et al*. "Generation mean analysis for green pod yield and protein content in pea". *Indian Journal of Pulses Research* 18.1 (2005):72-73.

20. Sood M and Kalia P. "Gene action of yield related traits in garden pea. (*Pisum sativum* L.)". *SABRAO* 38.1 (2006): 1-17.

21. Dixit GP., *et al*. "Genetic divergence study in field pea". *Legume Research* 25 (2002): 199-201.

22. Singh H., *et al*. "Estimation of gene effects of yield and quality characters in pea (*Pisum sativum* L)". *Environment and Ecology* 24.2 (2006): 445-448.

23. Sharma A., *et al*. "Detection of variation for biometrical traits in garden pea". *International Journal of Vegetation Science* 14.1 (2008): 67-81.

24. Sharma A., *et al*. "Generation mean analysis to estimate genetic parameters for desirable horticultural traits in garden pea (*Pisum sativum* L)". *Indian Journal of Agriculture Science* 82.3 (2012): 201-206.

25. Mahalanobis PC. "On the generalized distance in statistics". *Proceedings of National Academic Science* 2 (1936): 55-79.

26. Nair KR and Mukharji HK. "Classification of natural and plantation teak (*Tectona grandis*) grown at different localities of India, Burma with respect to its physiological and mechanical properties". *Sankhya* 22 (1960): 1-20.

27. Murthy MGS and Pavate MV. "Studies on quantitative inheritance in *Nicotiana tabacum* L., varietal classification and selection by multivariate analysis". *Indian Journal of Genetics* 22 (1962): 68-71.

28. Beale A. "Euclidean cluster analysis". *Bulletin of the International Statistical Institute* 43 (1969): 92-94.

29. Sparks DN. "Euclidean cluster analysis algoritham". *Journal of Applied Statistics* 22 (1973): 126-130.

30. Saxena JK., *et al.* "Genetic divergence in pea". *Crop Improvement* 12 (1985): 120-122.

31. Sureja AK and Sharma RR. "Genetic divergence in garden pea (*Pisum sativum* L. *Sub. Sp. hortense* Asch and Graebn)". *Journal of Vegetation Science* 28.1 (2001): 63-64.

32. Narayanankutty C., *et al*. "Variability and genetic divergence in vegetable cowpea". *Journal of Maharashtra Agriculture University* 28.1 (2003): 26-29.

33. Yadav PK., *et al*. "Genetic divergence studies in garden pea". *Haryana J. Hort.Sci.,* 33.1/2 (2004): 102-105.

34. Tiwari SK., *et al*. "Genetic diversity analysis in pea *(Pisum sativum* l.)". *Indian Journal of Agricultural Research* 38.1 (2004): 60-64.

35. Gupta AJ and Singh YV. "Genetic divergence in garden pea (*Pisum sativum* L.)". *Indian Journal of Human Genetics* 66.4 (2006): 341-342.

36. Singh D and Mishra VK. "Studies on genetic divergence in pea *(Pisum sativum* L.)". *Agricultural Science Digest* 28.1 (2008): 77-78.

37. Muhammad S., *et al*. "Genetic diversity for determining yield potential and selection criteria in *Pisum sativum* L. genetic resources". *Pakistan Journal of Botany* 41.6 (2009): 2987-2993.

38. Katiyar PK and Dixit GP. "Multivariate analysis for genetic divergence in field pea (*Pisum sativum*) germplasm". *Indian Journal of Agricultural Science* 79.3 (2009): 181-183.

39. Dhama SK., *et al*. "Studies on genetic diversity under varying environments in pea (*Pisum sativum* L.)". *Legume Research* 32.3 (2009): 173-179.

40. Yadav K., *et al*. "Analysis of genetic divergence in pea (*Pisum sativum* L.) using quantitative and RAPD markers". *Indian Journal of Human Genetics* 70.4 (2010): 363-369.

41. Dalsaniya SB., *et al*. "Genetic divergence in cowpea (*Vigna unguiculata* (L.) walp.)". *Legume Research* 32.4 (2009): 250-254.

42. McDonald MB. "*Genetic purity*: From protein electrophoresis to RAPDs". Proceedings of the fiftieth annual corn and sorghum industry research conference, American Seed Trade Association, Washington D.C., (1995): 256-271.

43. Williams JGK., *et al*. "DNA polymorphism amplified bys arbitary primers are useful as genetic markers". *Nucleic Acids Research* 18 (1990): 6531-6535.

44. Welsh J and Mcclelland M. "Fingerprinting genomes using PCR with arbitrary primers". *Nucleic Acids Research* 18 (1990): 7213-7218.

45. Saiki RK., *et al*. "Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase". *Science* (Washington, D.C.), 239 (1988): 487-491.

46. Michelmore RW., *et al*. "Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations". *Proceedings of the National Academy of Sciences of the United States of America* 88 (1991): 9828-9832.

47. Penner G., *et al*. "Identification of a RAPD marker linked to the oat stem rust gene *pg3"*. *Theoretical and Applied Genetics* 85 (1993a): 702-705.

48. Godwin ID., *et al*. "Application of inter-simple sequence repeat (ISSR) markers to plant genetics". *Electrophoresis* 18 (1997): 1524-1528.

49. Samec P and Našinec B. "The use of RAPD technique for the identification and classification of *Pisum sativum* L". Genotypes *Euphytica* 89 (1996): 229-234.

50. Lu J., *et al*. "Comparative analysis of genetic diversity in pea assessed by RFLP and PCR-based methods". *Theoretical and Applied Genetics* 93 (1996): 1103-1111.

51. Simioniuc D., *et al*. "Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs". *Plant Breed* 121 (2002): 429-435.

52. Baranger A., *et al*. "Genetic diversity within *Pisum sativum* using protein-and PCR based markers". *Theoretical and Applied Genetics* 108 (2004): 1309 -1321.

53. Yadav VK., *et al*. "Measurement of genetic dissimilarity in field-pea (*Pisum sativum* L.) genotypes using RAPD markers". *Genetic Resources and Crop Evolution* 54 (2007): 1285-1289.

54. Samatadze TE., *et al*. "Comparative genome analysis in pea (*Pisum sativum* L.) varieties and lines with chromosomal and molecular Markers". *Russian Journal of Genetics* 44.12 (2008): 1424-1430.

55. Cupic T., *et al*. "Genetic diversity of pea (*Pisum sativum* L.) genotypes assessed by pedigree, morphological and molecular data". *Journal of Food Agriculture and Environment* 7.3-4 (2009): 343-348.

56. Tanveer H., *et al*. "Detection of molecular divergence and development of DNA fingerprints in field pea cultivars". *Indian Journal of Human Genetics* 70.2 (2010): 132-139.

57. Ahmad G., *et al*. "Evaluation of genetic diversity in pea (*Pisum sativum* L) using RAPD analysis". *Genetic Engineering and Biotechnology Journal* 16.1 (2010): 1-5.

Volume 3 Issue 2 February 2019