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Research Article

The Possibilities of the Prediction of Heterosis in Elite Lines of Onion Based on the Assessment of Genetic Diversity

Dnyaneshwar Vajire, Karishma Thakare, Rahul Solunke, Archana Panche and Aparna Tiwari*

MGM's Institute of Biosciences and Technology, N-6 CIDCO, MGM Hospital Campus, Aurangabad, Maharashtra, India

***Corresponding Author**: Aparna Tiwari, MGM's Institute of Biosciences and Technology, N-6 CIDCO, MGM Hospital Campus, Aurangabad, Maharashtra, India.

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Abstract

The aim of this work was to determine the genetic diversity of elite lines of onion by using random amplified polymorphic DNA (RAPD) molecular marker and predict the future of hybrid performance based on a positive correlation between genetic distance and heterosis. Six different random primers were used which resulted in the amplification of 128 fragments of which 121 being interpretable polymorphic bands. A genetic similarity matrix was created from RAPD data using Jaccard coefficient and a dendogram was produced by UPGMA to study genetic relationship within smaller group of lines. The molecular data grouped the lines into three main clusters where the first major group was made of 3 sub-groups of 4 lines. The range of genetic distance was 0.86 to 0.23 indicating a high degree of variability among lines which enables their use in breeding programs. The highest genetic distance observed between lines 356-2 and 378M, which suggested that crosses between these two lines expressed higher performance if crossed. Finding of this research suggest that molecular characterization of genetic diversity by RAPD markers allow to understand the genetic relationship among the elite lines and aid to make the choice of superior parents for hybridization especially under resource restricted condition.

Keywords: *Allium cepa; RAPD; Genetic Distance; Variability*

Introduction

Onions are the second most valuable vegetable in the world. Majority of the seed market is dominated by open pollinated varieties and number of novel onion varieties and hybrids are miniscule in the market which directly impacts the production cost and economic benefit of farmers. Due to the global culinary and economic significance, demand for high quality onion varieties and hybrids are increasing continuously. Since onion hybrids offer several advantages over open-pollinated cultivar in terms of high productivity, uniform maturity, high quality and earliness [1], breeders give more attention to improve hybridization programme by developing new or improved potential parents for making superior hybrids.

A successful hybridization programme requires development and improvement of open pollinated verities as a source of inbred lines, identification of best combination of inbred line for hybrid seed production, evaluation of their reciprocal crosses at different location and many more. These are very time consuming and expensive process especially when resources are a limiting factor [2,3]. In most of the developing countries, the exploitation and use of hybridization is very lean in onion seed industry due to limited resource availability. Evaluation of genetic diversity and variability of onion resources by using molecular markers facilitate the better selection of parental lines for hybridization and hence improve the breeding efficiency and the quality of the produce. Genetic diversity is essential for hybridization but also crucial to meet several other objectives of plant breeding such as high yield, specific quality parameters, resistance for pest and diseases and many more [39]. Selection of genetically diverse parents and that too without testing all possible hybrid combinations is of immense importance for successful recombination breeding programme [4]. Usually, number of possible hybrid combinations increases

quadratically as the number of parental inbred lines increases. Therefore, it is practically impossible to test all possible hybrids under field conditions. Precise information on the nature and degree of genetic diversity helps the plant breeder to choose the diverse parents for purposeful hybridization to improve the yield and quality. There are considerable evidences that heterosis is proportional to the genetic diversity between the parents of the hybrid [5-8]. This indicates that assessment of genetic diversity is a pre-requisite for the selection of potential parents for effective crossing programme because it reduces not only the chance of evaluating a high number of undesirable crosses but also reduces the total cost of developing good hybrids.

Genetic diversity can be gauged by using agronomical, morphological, pedigree, genotypic traits, or the combination of all [9]. The phenotypic analysis of inbred line can be a complicated and time consuming, limiting its utility for the evaluation of genetic distance [10] while molecular markers are often effective to reduce the ambiguity of phenotypic data [2]. Molecular markers provide an effectual means for inbred lines to be characterized for their genetic diversity and clustered into distinct group. Among all available molecular markers, random amplified polymorphic DNA (RAPD) is found to be superior when simplicity and cost were considered [11]. Fruitfulness of RAPD technique has been proven to assess genetic diversity among onion cultivars [12,13]. Several studies indicate that precise information about genetic diversity assist to predict the best crosses among lines for hybrid development i.e. maize, rice, tomato [14-16]. In spite of all these research, no attempt has been made to identify potential parents of hybridization programme based on the genetic relationship between elite lines of onions. In this research, we have analyzed genetic diversity of elite lines of onion by means of RAPD markers with the aim to identify optimal parental material based on their genetic divergence for attaining maximum hybrid vigor. Result of this research will surely increase the efficiency of hybridization programme to develop genetically superior hybrids for future market and also open the possibility for intensifying production of hybrid in the face of restricted resource availability.

Material and Methods

Plant Materials

Six locally adopted inbred lines named 378-M, 356-1, 356-2, 374, 339, PFRS and one hybrid named as 378-F1 (Table 2) of onion obtained from Jindal Crop Science Pvt. Ltd. at Jalna, Maharashtra (Jalna, Maharashtra) were used in this study. These inbred lines were open pollinated and selected eventually based on their yielding performance, uniformity and quality parameters in each generation. Since onion is a cross pollinated crop, all inbred lines as well F1 hybrid were highly heterozygous and hence F1 was also considered as an inbred line. All inbred line and hybrid were evaluated under the same condition in a randomized, complete block design.

DNA extraction and amplification

Total DNA was extracted using modified CTAB methods [17] and PCR were carried out with six randomly chosen RAPD markers (Table 1). DNA was extraction from leaf tissue collected randomly from 4-week-old plants and quantified in a spectrophotometer (JASCO International Co. Ltd. Japan) by absorbance at 260 nm. RAPD reaction were carried out in a volume of 15 µl containing 1x PCR buffer (75 mM Tris-HCL, 50 mM KCL, 2.0 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 0.3 mM of each dNTP (dCTP, dGTP, dTTP, dATP), 0.4µM of 10-mer primers (Himedia, India: Table 1), 0.7 U of *Taq* polymerase (Himedia, India), and 20 ng of template DNA. The amplifications were performed in a model PT-100 thermocycler (Eppendrof South Pacipic, Austrelia) programmed to an initial stage of 10 min at 94ºC, 1 min cycles at 94ºC, 1 min at 36ºC, 1 min at 72ºC, and a final stage of 10 min at 72ºC. Amplification products were separated by eletrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light (Uviteck, UK).

S.No.	Primer	Sequence 5'-3'	Number of bases	GC content (%)
1	OPB-16	TTTGCCCGGA	10	60.00%
2	OPB-18	CCACAGCAGT	10	60.00%
3	RPI-2	AACGCGTCGG	10	70.00%
4	RPI-24	CCAGCCGAAC	10	70.00%
5	OPA-9	GGGTAACGCC	10	70.00%
6	OPA-3	AGTCAGCCAC	10	60.00%

Table 1: *Six randomly chosen RAPD markers used for PCR amplification with represented here with their base sequences, number of bases and GC content (%).*

Data analysis

PCR amplification profiles of the seven onion lines for each primer were scored by visual observation. By assuming that RAPD product represent a single locus, data were scored as the presence (1) and absence (0) of a DNA band. Pair-wise comparisons of samples were used to estimate Jaccard's similarity coefficients [18]. Dendrogram was constructed based on the UPGMA (unweighted pair-group method using arithmetical average) method of the NTSYs-pc 2.2i software (Numerical Taxonomy and Multivariate Analysis System for personal computers) [19]. Furthermore, polymorphic information content (PIC) was calculated to estimate the relationships between cultivars. The genetic similarity estimate (GS) between each pair of lines was based on similarity coefficient matrix and genetic distances (GD) between pairs of lines were estimated by GD = 1 - GS.

Result

Phenotypic observation

Seven elite lines of onion were superior in terms of their independent performance but distinct from each other for their phenotypic behavior (Table 2). All six lines were open pollinated and one line was hybrid; however all were heterozygous in expression; hence treated equally.

S. No.	Lines	No. of leaf	Maturity Time	Bulb Shape	Bulb Colour
1	378 M	8	90	Oval	Dark red
2	339	11	88	Oval	White
3	378F1	9.6	98	Round	Red
4	PFRS	10.2	120	Oval	White
5	356-1	7.2	95	Flat	Dark red
6	374	9.6	80	Round	Light red
7	356-2	8.6	95	Flat	Dark red

Table 2: *Number of leaf, maturity time, bulb shape and colour was recorded from seven elite lines of onion grown under open pollinated condition.*

Molecular analysis

A multiple band profile for each primer was produced by calculating one to five major bands and varying number of minor bands (Figure 1). While scoring, signal strength for major bands was good for majority of primers except few ambiguity observed for minor bands.

The molecular characterization of seven onion lines by using six RAPD primers resulted in total of 128 bands, where 121 were interpretable polymorphic bands and one was monomorphic (OPA-3) (Table 3). Among all RAPD markers, OPB-16 produced maximum

number of bands (30 in all genotypes) followed by OPA-3 (25), RPI-24 (23), RPI-2 (20), OPA-9 (18), and OPB-18 (12) in the genomic pool. The percent polymorphism in banding pattern was highest (100%) in all genotype using six RAPD markers except OPA-3 where level of polymorphism was 72% only (Table 3). Certain amplified bands appeared to be common to all inbred lines while other bands were present in some lines only. The polymorphism information content (PIC) value ranged from 0.75 (OPB-18) to 0.87 (OPA-9) (Table 3).

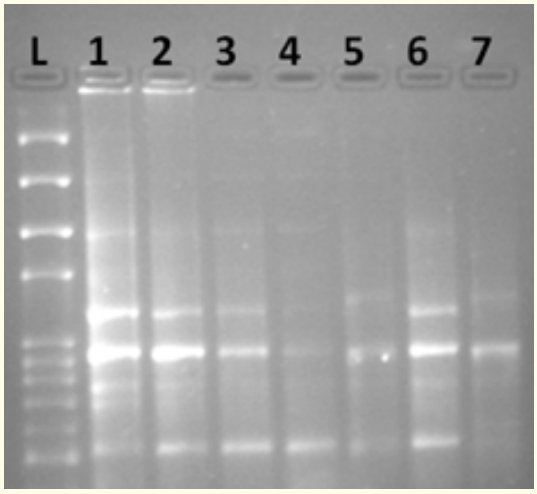

Figure 1: *Single PCR primer on total genomic DNA of onion using random primer OPB16. Lanes 1-7: L- ladder, 1 - 378 M, 2 -339, 3-378-F1, 4 -PFRS, 5 -356_1, 6 -374, 7-356_2.*

S. No.	Primer code	No. of band	No. of polymorphic band	No. of monomorphic bands	Percentage polymorphism	PIC value
1	OPB-16	30	30	0	100.00%	0.82
2	OPB-18	12	12	0	100.00%	0.75
3	RPI-2	20	20	0	100.00%	0.86
4	RPI-24	23	23	0	100.00%	0.86
5	OPA-3	25	18	7	72.00%	0.81
6	OPA-9	18	18	0	100.00%	0.87

Table 3: Total number of DNA bands, number and percentage of polymorphic bands and polymorphic information content (PIC) all RAPD markers used to perform molecular characterization of seven onion lines.

Genetic distance based analysis

From the genetic similarity index (Table 4), the pair-wise genetic similarity coefficients indicated that the variety 339 and 378M were very much close (76.92%) to each other. On the other hand, the lowest similarity (14.28%) was found in between 378M and 356-2 followed by paired of PFRS and 378F1 (15.38%), 356-2 and PFRS (18.18%), 356-2 and 339 (18.75%), 356-2 and 339 (18.75%), 356-1 and 378M (19.35%), 374 and PF (19.35), 356-2 and 378F1 (21.73%), 356-1 and 378F1 (22.22%), 374 and 378M (26.47). It means that 356-2 and 378M are most dissimilar in their genetic level. Since, genetic distance is inversely proportional to the genetic similarity coefficient, lines 356-2 and 378M with least coefficient value had highest genetic distance (0.86) while lines 378M and 339 with highest coefficient value had lowest genetic distance (0.23).

S. No.	Lines	378M	339	378F1	PFRS	3561	374	3562
1	378M	0						
2	339	0.23	0.00					
3	378F1	0.62	0.69	0.00				
4	PFRS	0.69	0.71	0.85	0.00			
5	356-1	0.81	0.70	0.78	0.65	0.00		
6	374	0.74	0.64	0.56	0.81	0.67	0	
7	356-2	0.86	0.81	0.78	0.82	0.79	0.6	0

Table 4: *Genetic similarity index between elite line of onion (378M, 339,378F1, PFRS, 356-1, 374, 356-2) calculated through UPGMA method.*

Cluster Analysis

The data obtained by RAPD markers was analyzed by NTSYs-pc 2.2i software and dendogram was constructed by using Jaccard's similarity coefficient value to estimate the genetic similarity of the onion genotype. A dendrogram based on UPGMA cluster analysis (Figure 2) of the RAPD data showed two clusters of seven onion inbreed line where lines 378M, 339, 378 F1, 374 were in the same cluster; however line 339 and 378M were more closer to each other. Second cluster consist of only two lines: PFRS and 356-1. The variety 356-2 was found to be an out-grouped in the UPGMA tree, representing a completely different line at genetic level. The dendogram formed using seven lines of onion (Figure 2) showed that the lines which were closely related had highest genetic similarity index (0.77 between 339 and 378M) while those lines which were more diverse showed lowest genetic similarity index (0.14 between 378M and 356-2).

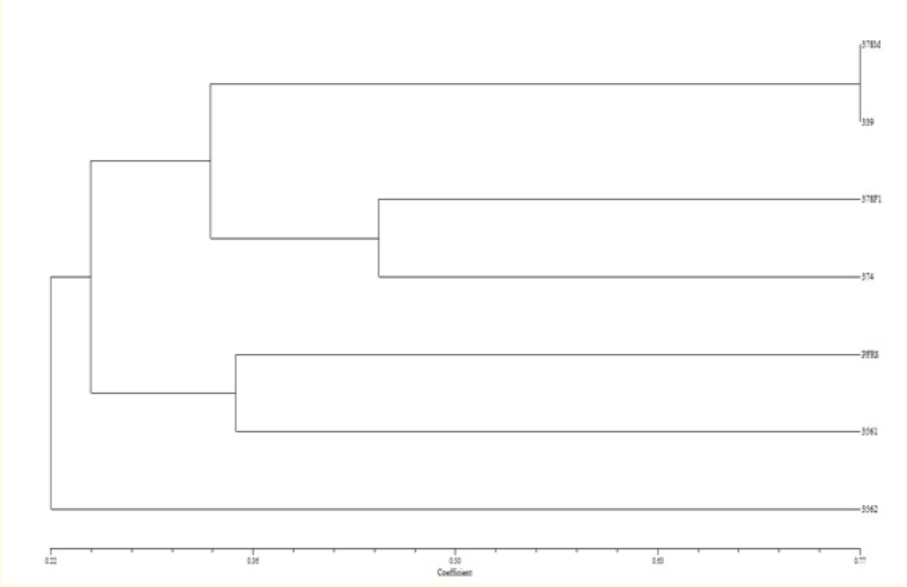

Figure 2: *Association between seven onions inbred line revealed by UPGMA cluster analysis of the Jaccard genetic similarity coefficient calculated from 232 RAPD amplification products generated by 6 primers.*

Discussion

This research was conducted to characterize the extent of genetic diversity among seven elite lines of onion by using RAPD molecular markers. A variety of molecular marker methods have been successfully used to resolve the questions of genetic diversity but RAPD is found to be useful for the assessment of genetic diversity for small population of intra-sub specific hybrids especially under resources constrained condition [11]. Effectiveness of RAPD for the evaluation of genetic diversity to species level has also been proven successful in several species of *Allium* [12,20,21]. Our result showed high degree of polymorphism indicating a considerable degree of variation available among inbred lines of onion. All 6 primers generated one or more polymorphic DNA markers, and the frequency of polymorphic markers was as high as 100% (Table 3). These observations are consistent with the broad range of genetic diversity reported in onion population by Bark and Havey [22] and Susana., *et al* [23]. The polymorphism information content (PIC) value ranged from 0.75 (OPB-18) to 0.87 (OPA-9) (Table 3) suggesting a wide range of variability among markers as PIC is the estimate of marker's discriminatory strength and is synonymous with genetic diversity. Our results are in accordance with results observed by Amorim and Souza [24] and Bered., *et al*. [25] in sweet corn and by Patto., *et al*. [26] and Terra., *et al*. [27] in maize.

The UPGMA dendogram based on the similarity index matrix resulted into two closely associated clusters of three and two lines and one line was obtained as an out-group indicating a much diverse variety. Cluster analysis indicates the establishment of groups with high internal homogeneity and high external heterogeneity for a certain trait of interest [28]. Lines which are in one cluster are genetically most similar to each other while lines in different cluster are genetically different. Data from phenotype revealed that line 378M had dark red bulb while line 339 had white bulb. However, from the dendogram analysis, both lines (with white bulb and dark red bulb) grouped together as one cluster with a similarity coefficient of 0.77. Phenotypically, both lines are distinct for a specific attribute, but genetically they are very homogeneous and hence clustered together. This observation suggested that morphological features, because of their dependency on environmental condition, are independent of their genetic relationship. These observations are in accordance with Susana., *et al*. [23] who reported the same in onion landraces grown in North West of Spain.

The genetic divergence between inbred line 378M and 356-2 was largest with the lowest similarity coefficient (0.14) while it was lowest between inbred line 378M and 339 with largest similarity coefficient (0.77) (Table 4). Several researchers have found that the level of heterosis exhibited by hybrids is directly related with the genetic distance between their parental lines as reported in pepper [29], rice [30,31], wheat [32,33], maize [15,34], chickpea [35], alfalfa [36], tomato [16] and many more. According to Fuzatto., *et al*. [37], heterosis of two inbred line to cross depends on the existence of dominance in controlling the character, and genetic diversity between them, so that when parents are selected, preference should be given to parents that are divergent and already adapted. In general, heterosis is thought to be higher in hybrids with genetically diverse parents [38] suggesting that lines 356-2 and 378M which are most dissimilar in their genetic level could be used in breeding for more productive crosses and heterotic hybrids. Other recommendation could also be given that lines 378M and 339 which are genetically most similar and hence could not be taken in the crossing programme to create genetic variability. Our results are supported by the finding of Hallauer and Miranda [38] where they reported that degree of divergence between parents in maize is proportional to the magnitude of heterosis. Finding of this research is helpful not only for the selection of genetically diverse parents and that too without testing all possible hybrid combinations but also critical to meet several other objectives of plant breeding such as breeding for high yield, for specific quality parameter, for resistance to pest and disease and many more. Molecular characterization of genetic diversity within a given set of onion elite lines are useful for understanding the genetic relationships among the breeding materials and for further improvement, such as selecting parental lines for hybridization, assigning heterotic groups and creating a core set of germplasm. Genetic divergence based prediction of heterosis might not be universally accepted phenomenon in all circumstances therefore it is advisable to verify genetic distance based recommendation by performing combining ability. However, it is still very effective method for predicting hybrid performance especially under resource limited condition and thus open a new horizon for the expansion of hybrid onion seed market.

Conclusion

The most expensive, time consuming and crucial phase in a hybrid seed production is the identification of parental lines that would give superior heterosis effect when crossed. This labour some process of hybrid testing can be avoided by efficient use of molecular marker for the evaluation of genetic relationship among lines. In this research paper, genetic diversity of seven elite lines of onion was determined with the help of RAPD markers. Our result showed that line 378M and 356-2 are genetically most diverse, suggested that crosses between these two parents may increased heterosis if crossed as genetic divergence among cultivar considered as the expression of heterosis. Research finding of this paper strongly suggest that molecular characterization of genetic diversity by RAPD markers allow to understand the genetic relationship among the elite lines and help to make several choices in the breeding programme. Since parental selection for hybridization is an expensive and laboursome process, this type of research can aid in the selection of superior parents for hybridization and hence give a new dimension to hybrid seed production especially when concern is to save time and resources.

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Bibliography

1. Currah L. "Leek breeding: a review". *Journal of Horticultural Science* 61.4 (1986): 407-415.

2. Riaz A., *et al*. "Genetic diversity of oilseed Brassica napus inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance". *Plant Breeding* 120.5 (2001): 411- 415.

3. Mienie CMS and Fourie AP. "Genetic diversity in South African maize (Zea mays L.) genotypes as determined with microsatellite markers". *African Journal of Biotechnology* 12.2 (2013): 123-133.

4. Singh SR., *et al*. "Determination of genetic diversity in onion (Allium cepa L.) by multivariate analysis under long day conditions". *African Journal of Agricultural Research* 8.45 (2013): 5599-5606.

5. Ali M., *et al*. "Relationship between genetic distance and heterosis for yield and morphological traits in winter canola (Brassica napus L.)". *Theoretical and Applied Genetics* 91.1 (1995): 118-121.

6. Qian W., *et al*. "Heterotic patterns in rapeseed (Brassica napus L.): I. Crosses between spring and Chinese semi-winter lines". *Theoretical and Applied Genetics* 115.1 (2007): 27-34.

7. Girke A., *et al*. "Extending the rapeseed gene pool with resynthesized Brassica napus II. Heterosis". *Theoretical and Applied Genetics* 124.6 (2012): 1017-1026.

8. Xing N., *et al*. "Parental selection of hybrid breeding based on maternal and paternal inheritance of traits in rapeseed (Brassica napus L.)". *PLoS one* 9.7 (2014): e103165.

9. Abdel-Ghani AH., *et al*. "Genotypic variation and relationships between seedling and adult plant traits in maize (Zea mays L.) inbred lines grown under contrasting nitrogen levels". *Euphytica* 189.1 (2013): 123-133.

10. Langer SM., *et al*. "Flowering time control in European winter wheat". *Frontiers in Plant Science* 5 (2014): 537.

11. dos Santos JB., *et al*. "Comparison of RFLP genetic markers in determining genetic similarity among Brassica oleracea L. genotypes". *Theoretical and Applied Genetics* 87.8 (1994): 909-915.

12. Tanikawa T., *et al*. "Cultivar identification and genetic diversity in onion (Allium cepa L.) as evaluated by random amplified polymorphic DNA (RAPD) analysis". *Japanese Society for Horticultural Science* 71.2 (2002): 249-251.

13. Kutty MS., *et al*. "Analysis of genetic diversity among Indian short-day onion (Allium cepa L.) cultivars using RAPD markers". *The Journal of Horticultural Science and Biotechnology* 81.4 (2015): 774-778.

14. Lanza LLB., *et al*. "Genetic distance of inbred lines and prediction of maize single cross performance using RAPD markers". *Theoretical and Applied Genetics* 94.8 (1997): 1023-1030.

15. Fernandes EH., *et al*. "Genetic diversity in elite inbred lines of maize and its association with heterosis". *Genetics and Molecular Research* 14.2 (2015): 6509-6517.

16. Shapturenko MN., *et al*. "The possibilities of the prediction of the genetic potential of the tomato (Solanum lycopersicum L.) F1 based on the assessment of a simple sequence research polymorphism". *Russian Journal of Genetics: Applied Research* 5.5 (2015): 486-493.

17. Saghai-Maroof MA., *et al*. "Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal localization and population dynamics". *Proceedings of the National Academy of Sciences of the United States of America* 81.24 (1984): 8014-8018.

18. Jaccard P. "Nouvelles recherches sur la distribution florale". *Bulletin de la Société Vaudoise des Sciences Naturelles* 44 (1908): 223-270.

19. Rohlf FJ. "NTSYS pc: Numerical Taxonomy and Multivariate Analysis System". Version 2.1. Exeter Software, New York (2000).

20. Dennequin ML., *et al*. "Assessment of genetic relationships among sexual and asexual forms of Allium cepa using morphological traits and RAPD markers". *Heredity* 78.4 (1997): 403-409.

21. Bradeen JM and Havey MJ. "Randomly amplified polymorphic DNA in bulb onion and its use to assess inbred integrity". *Journal of the American Society for Horticultural Science* 120.5 (1995): 752-758.

22. Bark OH and Havey MJ. "Similarities and relationships among populations of the bulb onion as estimated by nuclear RFLPs". *Theoretical and Applied Genetics* 90.3-4 (1995): 407-414.

23. Susana GP., *et al*. "Exploring genetic diversity and quality traits in a collection of onion (Allium cepa L) landraces from north-west Spain". *Genetika* 47.3 (2015): 885-900.

24. Amorim EP and Souza JC. "Evaluation of inter and intra-population maize hybrids from S0 progenies of commercial single cross hybrids". *Bragantia* 64.4 (2005): 561-567.

25. Bered F., *et al*. "Genetic variation among and within sweet corn populations detected by RAPD and SSR markers". *Crop Breeding and Applied Biotechnology* 5 (2005): 418-425.

26. Patto MCV., *et al*. "Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers". *Euphytica* 137.1 (2004): 63-72.

27. Terra TF., *et al*. "Genetic variability in maize and teosinte populations estimated by microsatellites markers". *Ciência Rural* 41.2 (2011): 205-211.

28. Laude TP and Carena MJ. "Genetic diversity and heterotic grouping of tropical and temperate maize populations adapted to the northern US Corn Belt". *Euphytica* 204.3 (2015): 661-677.

29. Costa MP., *et al*. "Characterization and genetic diversity of pepper (Capsicum spp) parents and interspecific hybrids". *Genetics and Molecular Research* 15.2 (2016).

30. Xiao J., *et al*. "Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers". *Theoretical and Applied Genetics* 92.6 (1996): 637-643.

31. Kwon SJ., *et al*. "Relationship between genetic divergence and hybrid performance in japonica rice grown in a cold water-irrigated field". *Euphytica* 128.3 (2002): 389-396.

32. Barbosa-Neto JF., *et al*. "Prediction of heterosis in wheat using coefficient of parentage and RFLP based estimates of genetic relationship". *Genome* 39.6 (1996): 1142-1149.

33. Burkhamer RL., *et al*. "Predicting progeny variance from parental divergence in Hard Red Spring wheat". *Crop Science* 38.1 (1998): 243-248.

34. Benchimol LL., *et al*. "Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid performance determined by RFLP markers". *Plant Breeding* 119.6 (2000): 491-497.

35. Sant VJ., *et al*. "Potential of DNA markers in detecting divergence and in analyzing heterosis in Indian elite chickpea cultivars". *Theoretical and Applied Genetics* 98.8 (1999): 1217-1225.

36. Riday H., *et al*. "Comparison of genetic and morphological distance with heterosis between Medicago sativa and subsp. Falcata". *Euphytica* 131 (2003): 37-45.

37. Fuzatto SR., *et al*. "Divergência genética e sua relação com os cruzamentos dialélicos na cultura do milho". *Ciência and Agrotecnologia* 26 (2002): 22-32.

38. Hallauer AR and Miranda Filho JB. "Quantitative genetics in maize breeding". Ames: Iowa State University Press (1988): 468.

39. Aparna Tiwari. "Plant Breeding: A Prospect in Developing World". *EC Microbiology* 8.5 (2017): 272-278.

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