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Identification of Diverse Maize Inbred Lines using Molecular and Phenotypic Genetic Divergence Analysis

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ABSTRACT

The experiment was carried out during *rabi* (September, 2018–January, 2019) season under water deficit experimental fields at Annamalai University, Tamil Nadu, India to evaluate the performance of maize inbreds under water deficient conditions and to identify potential diverse inbreds for hybridization programme. Ten quantitative characters were studied for variability studies, whereas genetic diversity was observed at both molecular and phenotypic levels. The genotype, G_{34} showed the highest mean for single plant yield (SPY) (135.6 g), this was followed by G_{11}, G_{32}, G_{38} and G_{48}. While the genotypes G_{20} and G_{50} showed early days to 50% tasseling (DT) and silking (<55 days). High heritability and genetic advance as per cent of mean was observed for the traits leaf area, number of kernels cob^{-1} and SPY, showing the action of additive gene action. Path analysis showed that SPY had positive direct effects from days to DT, plant height, leaf area, cob length, number of kernels and test weight. Cluster analysis based on Mahalanobis (D^2) diversity analysis partitioned the 52 genotypes into 5 clusters, whereas mean Euclidean distance based molecular clustering using the six markers partitioned the selected fourteen genotypes into six clusters. Overall based on both these diversity analyses the genotypes G_{34}, G_{20}, G_{16}, G_{48}, G_{50}, G_{40} and G_{34} can be selected and used as parents in future hybridization programme to get superior high yielding hybrids.

KEYWORDS: Diversity, maize, path analysis, parents, variability

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1. INTRODUCTION

Maize/corn (*Zea mays* L.) (2n=2x=20), known as 'Queen of cereals' is one of the most essential cereal crops next to rice and wheat, all together feeding around 60% of the world population. It is the most adapted cereal, distributed throughout the world serving multi-purposes as livestock feed, biofuel, human food, and as raw material in many industries (Kaushal et al., 2023). Maize is available in different forms as popcorn, sweet corn, dent corn, waxy corn, flint corn, flour corn etc (Kaur et al., 2022). This wide diversity with high outcrossing and utilization of C_4 carbon fixation makes it the world's most dominant and productive crop (Djalovic et al., 2024)

The worldwide production of maize was 1235.73 MMT, the highest among cereals, and in India the maize production was 38 mmt (Anonymous, 2024). In India, major maize growing states are Andhra Pradesh, Tamil Nadu, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Bihar, Uttar Pradesh, Telangana and Gujarat (Yadav et al., 2016). Below-normal monsoon rains and infestation of the fall armyworm, which devastated African corn crops in 2017 have slashed India's corn output and boosted prices, and the government have grant duty-free corn imports for the first time since 2016 (Bengyella et al., 2021). Projected demand for maize production by 2050 in India is around 121 mt and this demand is due to rising number of poultry farms (Shekhar and Singh, 2021). To meet these demands expanding the cultivated area, adopting proper management practises and increasing the productivity is crucial. Hence studies on variability and genetic diversity in the maize genotypes will help to identify suitable lines that can be used in hybridization programmes to develop superior hybrids (Mukri et al., 2022). Variability is the primary interest to the plant breeder as it plays a significant role in framing successful breeding programme (Bharathi et al., 2021). Study of variability, heritability and genetic advance in the germplasm will help to ascertain the real potential value of the genotypes (Neelima et al., 2020). Correlation enables to identify the characters which might be useful as indicator of high yield by way of evaluating relative influence of various characters on yield and among themselves as well (Aman et al., 2020). The direct contribution of each component to the yield and its association with other characters can be elucidated by simple path coefficient analysis. Mahalanobis D^2 analysis is useful tool to assess the genetic divergence among population (Mahalanobis, 1936, Singh et al., 2020). The hybrids of parents with more diversity were expected to exhibit higher heterotic expression and broad spectrum of variability in the following generations (Mukri et al., 2022). Therefore, thorough knowledge on genetic parameters like mean, variability, heritability, genetic advance as per cent of mean, correlation, path analysis and genetic diversity will provide basis for selecting systematic breeding strategy for crop improvement and to increase yield potential of the genotypes (Vishnuvardhan et al., 2021). With the availability of large number of SSR (Simple Sequence Repeats) markers and online resources on gene annotations, many advances were made in crop improvement (Maheswari et al., 2016). Using SSRs and gene-based markers to assess the genetic diversity will strengthen the identification of diverse lines and thereby promote crop improvement (Kumar et al., 2022, Ragi et al., 2022). Hence in the current study maize genotypes collected from various parts of south India were evaluated under water deficit field coastal areas of Tamil Nadu and observations were recorded for ten yield related characters. Genetic diversity was assessed both phenotypically and genotypically to identify potential maize lines that could be used in hybridization programme.

2. MATERIALS AND METHODS

2.1. Study site, experiment layout and observations recorded

The present investigation was conducted at the Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India in rabi during September 2018–January 2019. The field is situated 15 kms away from the sea shore (Bay of Bengal), 5.2 m above mean sea level, situated at 17° 19' N latitude and 78° 41' E longitude. The soil was clay loamy with a pH of 7 and electrical conductivity was more than 1 (slightly saline), also the soil had poor nitrogen content, whereas phosphorous and potassium were in optimum levels. Sixty-four genotypes were collected from various states of south India viz., Tamilnadu, Andhra Pradesh, Telangana, and Karnataka. They were grown in completely randomized block design (RBD) with three replications and the borders were planted with Salem popcorn hybrid. Each genotype in a replication was sown in rows of 3 m length and a spacing of 60 cm between rows and 25 cm between each plant was maintained. For each genotype, two seeds $hill^{-1}$ was sown and thinning was done 15 days after sowing. Out of these, fifty-two genotypes had sufficient plant population in all three replications (Table 1). Observations were recorded on randomly selected five plants of a genotype in each replication. Ten phenotypical observations viz., Days to 50% tasselling (DT, in days), Days to 50% silking (DS, in days), Plant height at maturity (PH, in cm), Cob height at maturity (CH, in cm), Cob length (CL, in cm), Cob girth (CG, in cm), Number of kernels cob^{-1} (NK, in numbers), Test weight (TW, in g), Single plant weight (SPY, in g) and Leaf area at maturity (LA) were recorded ; LA=lamina length×maximum width×0.75 as (Musa et al., 2016).

Table 1: List of fifty-two maize genotypes used in the study

Code	Name	Type	Source	Code	Name	Type	Source
G_1	16K1816	Inbreds	AP	G_{28}	17PRC1019	Inbreds	TG
G_2	16K1818	Inbreds	AP	G_{29}	17PRC1020	Inbreds	TG
G_3	16K1887	Inbreds	AP	G_{30}	17PRC1021	Inbreds	TG
G_4	16K1899	Inbreds	AP	G_{31}	17PRC1022	Inbreds	TG
G_5	16K1900	Inbreds	AP	G_{32}	17PRC1023	Inbreds	TG
G_6	16K1901	Inbreds	AP	G_{33}	17PRC1024	Inbreds	TG
G_7	16K1904	Inbreds	AP	G_{34}	17PRC1025	Inbreds	TG
G_8	16K1914	Inbreds	AP	G_{35}	AU18M01	Inbreds	TN
G_9	16K1915	Inbreds	AP	G_{36}	AU18M02	Inbreds	TN
G_{10}	17PRC1001	Inbreds	TG	G_{37}	AU18M03	Inbreds	TN
G_{11}	17PRC1002	Inbreds	TG	G_{38}	AU18M04	Inbreds	TN
G_{12}	17PRC1003	Inbreds	TG	G_{39}	AU18M05	Inbreds	TN
G_{13}	17PRC1004	Inbreds	TG	G_{40}	AU18M06	Inbreds	TN
G_{14}	17PRC1005	Inbreds	TG	G_{41}	AU18M07	Inbreds	TN
G_{15}	17PRC1006	Inbreds	TG	G_{42}	AU18M08	Inbreds	TN
G_{16}	17PRC1007	Inbreds	TG	G_{43}	AU18M09	Inbreds	TN
G_{17}	17PRC1008	Inbreds	TG	G_{44}	AU18M10	Inbreds	TN
G_{18}	17PRC1009	Inbreds	TG	G_{45}	AU18M11	Inbreds	TN
G_{19}	17PRC1010	Inbreds	TG	G_{46}	AU18M12	Inbreds	TN
G_{20}	17PRC1011	Inbreds	TG	G_{47}	AU18M13	Inbreds	TN
G_{21}	17PRC1012	Inbreds	TG	G_{48}	AU18M14	Inbreds	TN
G_{22}	17PRC1013	Inbreds	TG	G_{49}	Vilupuram L.	Selfed OPV	TN
G_{23}	17PRC1014	Inbreds	TG	G_{50}	PeruRani	Selfed OPV	TN
G_{24}	17PRC1015	Inbreds	TG	G_{51}	Karnataka L.	OPV	KN
G_{25}	17PRC1016	Inbreds	TG	G_{52}	Sivagangai L.	OPV	TN
G_{26}	17PRC1017	Inbreds	TG	Border	Salem popcorn	OPV	TN
G_{27}	17PRC1018	Inbreds	TG				

TN: Tamil Nadu; AP: Andhra Pradesh; TG: Telangana; KN: Karnataka; OPV: Open pollinated variety; L: Local variety

2.2. Statistical analysis

Genotypic (GCV) and phenotypic (PCV) co-efficient of variation were calculated as, PCV=√Vp/Mean×100; GCV=√Vg/Mean×100 (Burton, 1952). Where, 'Vp' and 'Vg' are the phenotypic and genotypic variance obtained from analysis of variance (ANOVA). Heritability in broad sense (h^2) was calculated as h^2=(Vg)/Vp×100, genetic advance (GA)=h^2×√(Vph)×K, where, 'K' is the 2.06 at 5% selection intensity (2.06) and genetic advance as per cent of mean (GA(%)=(Genetic advance/Grand mean)×100.

Mahalanobis D^2 statistic was used for estimating the genotypic divergence as, $D^2p= \sum^{p}_{i=1}\sum^{p}_{j=1}(\lambda ij)\sqrt{i}\sqrt{j}$, where, 'ij' is the reciprocal matrix to the pooled common dispersion obtained from the error matrix, 'i' is the difference in mean values for the i^{th} character of the two populations, 'j' is the difference in mean values for the j^{th} character of the two populations. The D^2 analysis was performed in TNAUSTAT (Manivannan, 2014)

The genotypic correlation co-efficient were worked out as $(rg_{xy})=(Cov(g_x \times g_y/\sqrt{(\sigma_{gx}\times)\sigma^2}_{gy})$; where, 'Cov (gx. gy)' is the genotypic covariance between character x and y, '$\sigma^2 g_x$' and '$\sigma^2 g_y$' are the genotypic variance of character x and y, respectively. Path co-efficient analysis were calculated through path co-efficient analysis as suggested by Dewey and Lu (1959). The path diagram was drawn manually in MS Powerpoint v. 2021.

2.3. Molecular diversity analysis

Extraction of total genomic DNA was carried out by using the method described by Doyle and Doyle (1987). In this study five SSR markers viz., Phi037, P-umc2189, P-bnlg1179, Umc1545, bnlg1812 (Maheswari et al., (2016)) and a gene-based marker for Drought and salt tolerance protein (LOC100382689) was developed using primer 3 tool (Untergasser et al., 2012) and used in this study. These markers were studied in a panel of fourteen selected inbred lines based on diversity analysis. Basic polymorphic information's were obtained using POWER MARKER version 3.23 (Liu and Muse, 2005). Genetic diversity was estimated using dice dissimilarity co-efficient d_{ij}=b+c/2a+(b+c) Where, 'a' denotes both allele present, 'b' and 'c' denotes either of allele present. A binary matrix (0,1) from scoring of gel was then transformed to genetic similarity coefficient matrix for the selected 14 genotypes using Jaccard's coefficient. A dendrogram based on similarity coefficient was prepared by using unweighted neighbour joining (NJ) method in DARWIN software version 6.0 (Perrier and Jacquemound-Collet, 2006). Bootstrap analysis was carried out to statistically support the cluster branches with 7000 replicates. The clads showing more than 70% of the bootstrap were considered arbitrarily a strong cluster.

3. RESULTS AND DISCUSSION

3.1. Performance of the studied maize genotypes

Analysis of variance (ANOVA) for the 52 lines of maize genotypes for 10 quantitative characters viz., DT, DS, PH, CH, CL, CG, NK, TW, SPY and LA revealed highly significant variations among the genotypes. Similar results of significant variations for the studied characters were observed by Magar et al., 2021 and Mukri et al., 2022. DT exhibited a range of 50.00 to 71.30 days and the overall mean was 57.33 days. Out of 52 genotypes, 19 genotypes had DT significantly lower than the mean value. DS ranged from 53.7 to 81.00 days in with a population mean of 63.00 days. Among the genotypes, 19 genotypes had significantly lower value than the general mean. PH ranged from 80.8 cm in G_{33} to 170.7 cm in G_{48} with an average mean of 130.5 cm. Among the 52 genotypes, 20 genotypes had significantly higher value than the general mean. CH ranged from 18 cm in G_{47} to 60.1 cm in G_{6} with a population mean value of 38.2 cm. Among the genotypes, 19 genotypes had significantly lower value than the general mean. LA ranged from 164.2 cm^2 in G_{45} to 573.9 cm^2 in G_{20} with a general mean value of 294.8 cm^2. CL exhibited a range from 7.6 cm in G_{41} to 19.3 cm in G_{34} with an average value of 11.2 cm. Among the genotypes 16 genotypes had significantly higher value than the general mean. For CG the genotypes exhibited a range of 3.0 cm in G_{40} to 4.2 cm in G_{34} and G_{38} and the mean was 3.6 cm. NK ranged from 98 kernels in G_{9} to 339.1 kernels in G_{34}. The average TW was 20.6 g and it ranged from 15.9 g in G_{12} to 28 g in G_{49}. SPY ranged from 32.9 g in G_{45} to 135.6 g in G_{34} with an average of 65.1 g. Among the 52 maize genotypes, 21 of them had significantly higher plant yield than the concerned general mean. Based on the mean performance the accessions identified to be superior for the ten quantitative characters are given in table 2.

Table 2: List of superior genotypes based on mean performance

Characters	Range	Genotypes
Days to 50% tasseling	Less than 51 days	G_{26}, G_{15}, G_{21}, G_{50}
Days to 50% silking	Less than 56 days	G_{20}, G_{50}, G_{21}, G_{34}, G_{16}, G_{15}, G_{48}
Plant height	Below 90 cm	G_{33}, G_{1}
Cob height	Below 22 cm	G_{47}, G_{30}, G_{44},
	Above 55 cm	G_{6}, G_{2}, G_{31}, G_{50}
Leaf area	More than 420 cm^2	G_{20}, G_{34}, G_{52}, G_{38}, G_{16}
Cob length	More than 15 cm	G_{34}, G_{32}, G_{12}, G_{38}, G_{30}
Cob girth	More than 4.1 cm	G_{38}, G_{34}, G_{50}, G_{49}, G_{27}, G_{22}, G_{13}
No. of kernels	More than 300 kernels	G_{34}, G_{11}
Test weight	More than 26 g	G_{49}, G_{19}
Single plant yield	More than 100 g	G_{34}, G_{11}, G_{32}, G_{32}, G_{38}, G_{48}

2.2. Variability among the studies traits

It is apparent from the table 3, that there is almost perfect relation between PCV and GCV of each character. The higher magnitudes of both PCV and GCV show that these characters were under the influence of genetic control.

So, the characters can be relied upon and simple selection is effective for further improvement. PCV had a wide range from 7.56% in DT to 43.92% in SPY. High PCV and GCV were observed for SPY, NK, LA, CH and CL whereas, low PCV and GCV were observed for DS and DT. This was similar to the findings of Al-Naggar et al., 2021, Bharathi et al., 2021. The difference between the estimates of PCV and GCV were low for all traits showing low environmental effects in the expression of these characters (Magar et al., 2021). High heritability was observed for all the characters, except cob girth indicating that they were least influenced by the environmental effects. Cob girth showed moderate heritability, it shows there was a little environmental influence on the character. High heritability

Table 3: variability parameters studied for the ten quantitative traits among the 52 maize genotypes

Characters	GCV	PCV	h^2	GAM
Days to 50% tasseling	7.33	7.56	0.94	14.64
Days to 50% silking	8.54	8.78	0.94	17.13
Plant height	16.74	17.12	0.95	33.7
Cob height	25.74	26.84	0.91	50.86
Leaf area	30.02	30.35	0.97	61.18
Cob length	24.54	25.22	0.94	49.17
Cob girth	7.72	10.44	0.54	11.75
Number of kernels	33.42	34.71	0.92	66.30
Test weight	13.88	14.44	0.92	27.50
Single plant yield	41.21	42.38	0.94	82.56

GCV: Genotypic coefficient of variance; PCV: Phenotypic coefficient of variance; h^2: Heritability in broad sense; GAM: Genetic advance as percentage of mean

with moderate genetic advance was recorded for DT and DS. Moderate heritability with moderate genetic advance was observed for CG. These traits appear to be under the control of both additive and non-additive gene actions. In the present investigation, high heritability coupled with high genetic advance was observed for SPY, NK, LA, CH, CL and PH.

Thus, these traits are predominantly under the control of additive gene action and hence these characters can be improved by pedigree method of breeding. Similar results of higher PCV, GCV, heritability and genetic advance as per cent of mean for LA, NK and SPY were observed by Al-Naggar et al., 2021.

3.3. Correlation and path analysis among the studied traits

SPY had significantly positive association with NK, CL, TW, PH and CG, whereas SPY had significant negative association with DS and DT (Bharathi et al., 2021, Sabitha et al., 2024). Single plant yield had non-significant positive association with CH and LA. NK had significantly positive association with CL, TW, CG and PH. DT and DS had significant and nonsignificant associations with all the studied traits. Path analysis revealed that NK had high positive direct effects, DT and PH had moderate positive direct effects, whereas, LA, CL and TW had low and negligible direct effects towards SPY. Traits like DS, CG and CH had negative direct effects towards SPY (Figure 1). These were slightly in contrast to the findings of Aman et al. (2020), where he did not observe any significant direct effect of NK but he reported similar positive direct effects of DT for SPY. Based on correlation and path analysis, the main SPY contributing characters in maize are NK, CL, PH,

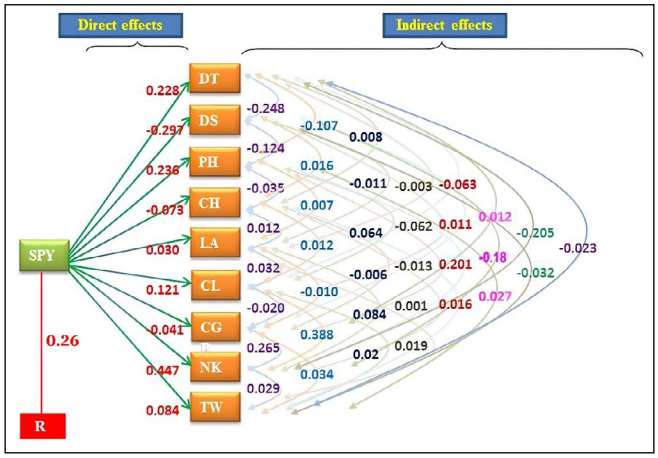

Figure 1: Path diagram for the nine dependant characters towards single plant yield; DT: Days to 50% tasselling; DS: Days to 50% silking; PH: Plant height; CH: Cob height; LA: Leaf area; CL: Cob length; CG: Cob girth; NK: Number of kernel; TW: Test weight; SPY: Single plant yield; R: Residual effect

TW, LA and DT whereas DS, CH and CG have negative contributions towards yield. The results thus emphasized the need for selection based on plant type with higher NK, larger CL, optimum PH, high TW, larger LA, less DT, very low DS, low CH and optimum CG.

3.4. Phenotypic genetic diversity

The fifty-two genotypes of maize were grouped into 5 clusters. Cluster 1 comprised of twenty-one genotypes was the largest cluster. This was followed by cluster 2 with fifteen genotypes and cluster 4 had the lower of 2 genotypes. The compositions of different genotypes in each cluster are presented in Table 4. The clustering pattern of the genotypes indicated that there was no association between genetic and geographical diversity (Alam et al., 2022). Based on genetic distance and field performance fourteen genotypes

Table 4: Composition of 52 genotypes in different clusters

Cluster number	Genotypes	No. of genotypes
Cluster 1	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8, G_9, G_{10}, G_{11}, G12, G_{13}, G_{14}, G_{15}, G_{16}, G_{17}, G_{18}, G_{19}, G_{41}, G_{44}	21
Cluster 2	G_{20}, G_{21}, G_{22}, G_{23}, G_{24}, G_{25}, G_{26}, G_{27}, G_{28}, G_{29}, G_{30}, G_{31}, G_{32}, G_{33}, G_{36}	15
Cluster 3	G_{34}, G_{35}, G_{37}, G_{38}, G_{39}, G_{40}, G_{42}, G_{43}, G_{45}, G_{46}, G_{47}	11
Cluster 4	G_{49}, G_{50}	2
Cluster 5	G_{48}, G_{51}, G_{52}	3

were selected and subjected to molecular diversity analysis. The intra cluster distance ranged from 16.773 to 27.137. Cluster 4 showed minimum intra cluster distance (16.773) and maximum intra cluster distance was exhibited by cluster 5 (27.137). The maximum inter cluster distance was found between clusters 2 and 5 (28.101) followed by clusters 4 and 5 (27.13). The minimum inter cluster distance was observed between clusters 3 and 4 (20.119). Based on cluster means, the important clusters are cluster 2 for lowest DT and days to 50% silking. The prominent traits in cluster 5 are lowest cob height, highest plant height, cob length and single plant yield respectively. Cluster 4 also had important characters like highest leaf area, cob girth, number of kernels, test weight and single plant yield. Cluster 3 is found to have moderate mean performances for all the characters.

3.5. Molecular genetic diversity

Genetic diversity of the selected fourteen genotypes were analyzed using five SSR and one gene-based markers. All the six markers showed amplification and were polymorphic. The obtained polymorphism is highly significant as comparable to the results of Maheswari et al. (2016). The range of PIC value varied from 0.61 (P-bnlg1179) to 0.27 (Bnlg1812). Heterozygosity ranged between 0.32 (Bnlg1812) to 0.67 (P-bnlg1179) with the mean value of 0.52. The major allele frequency ranged between 0.81 (Bnlg1812) to 0.40 (P-bnlg1179) and the overall average was 0.63. Gene diversity, ranged between 0.66 (P-bnlg1179) to 0.30 (Bnlg1812) and the mean of all the primers for gene diversity was 0.48. A similarity matrix based on mean euclidean distance method was obtained and a tree was constructed using neighbour joining (NJ) method (Figure 2).

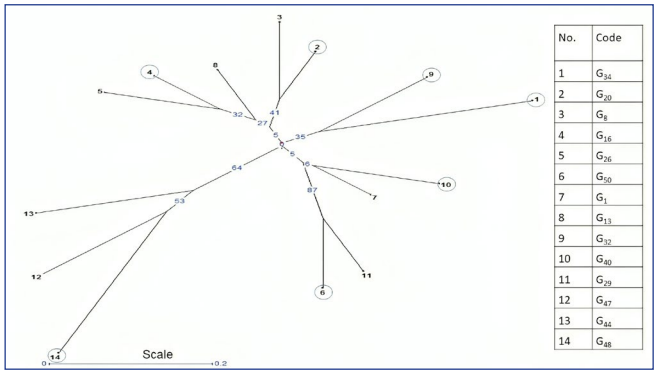

Figure 2: Mean eucledian distance based NJ-tree for the selected maize genotypes; The genotypes highlighted in blue circles can be selected as parents in crossing programme

A representative gel pictures showing amplifications of the selected lines were given in figure 3. There was a moderate correlation between clustering pattern based on markers and phenotypic data (de Faria et al., 2022), because these grouping have been carried out based on all markers located in drought linked QTL region. The polymorphism for the studied markers in the selected genotypes denotes higher probability of the presence of drought tolerance in some of the selected lines. The NJ tree showed six clusters based on the marker allele distribution. Cluster I consists of 2 genotypes viz. G_{40} and G_{1}, cluster II had 2 genotypes, G_{29} and G_{50}, cluster III consists of 3 genotypes G_{48}, G_{47} and G_{44}, cluster IV also had 3 genotypes viz. G_{26}, G_{16} and G_{13}, while cluster V and cluster VI had two genotypes each viz. G_{8}, G_{20} and G_{32}, G_{34} respectively. The maximum mean Euclidean distance was observed between the genotypes G_{48} and G_{50}, G_{48} and G_{32} and G_{48} and G_{29}. If one genotype can be selected in each cluster the genotypes viz., G_{34}, G_{20}, G_{16}, G_{48}, G_{50} and G_{40} can be selected. Even though G_{32} occurs in a cluster along with G_{34}, both were high yielding inbreds and thus can be selected. Therefore, all these seven inbred can be selected for studying the combining ability, followed by which they can be used as parents in hybrid development. The selected seven inbreds were distributed in all the clusters of both D^2 analysis (five clusters) and molecular clustering (six clusters), thus they show high diversity under the water deficit conditions.

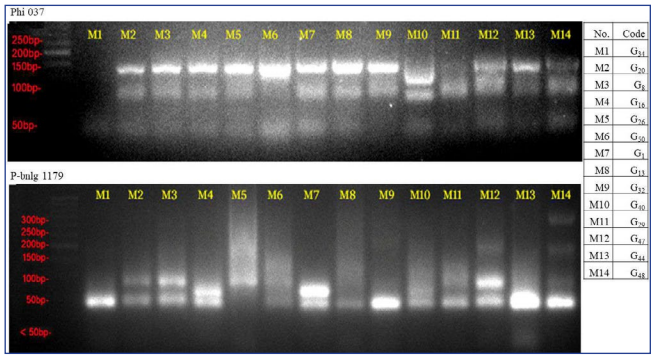

Figure 3: A representative figure of markers, Phi 037 and P-bnlg 1179 among the selected fourteen lines;

4. CONCLUSION

Variability study revealed high PCV, GCV, h^2 and GAM for LA, NK and SPY, showing less environmental effects on these traits. Path analysis showed that DT, PH, LA, CL, NK and TW had positive direct effects whereas DS, CH and CG had negative direct effects towards SPY. Based on molecular and phenotypic genetic divergence, the inbred lines G_{34}, G_{32}, G_{20}, G_{16}, G_{48}, G_{50} and G_{40} were considered diverse and could be used as parents in future hybridization programmes.

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