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EVALUATION AND MOLECULAR DETECTION OF ADVANCED INBRED LINES FOR QPM STATUS IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT

A set of 20 advanced inbred lines of maize derived from diverse source populations (CIMMYT, Hyderabad Centre, India and DMR, New Delhi, India), were evaluated for fifteen agro-morphological traits including seed yield. VL1016548 and VL1016453 revealed high yield potential (50 q/ha) as also in case of QPM-11-7-1, QPM-8-3-2 and VL109273. Interestingly, QPM 8-3-2 with medium height plant type and maturing within 97 days, managed to have high yield potential (51.25 q/ha). VL1017524 recorded maximum cob length (17.2 cm) and number of kernels per row (37.1) and 100-kernel weight (32.7gm), but yielded moderately low owing to lower number of kernel rows/cob, total number of kernels/cob and shelling percentage. Cluster analysis based on molecular profiling revealed two highly divergent mono-genotypic clusters containing VL1016548 and VL1017524 respectively and a third cluster with rest 18 inbred lines together. The QPM allele specific marker phi 057 revealed more discriminating power (Rp = 1.8) among the genotypes compared to umc 1066(Rp = 1.2). Among the selected 20 inbreds, QPM11-7-1, QPM 7-3-2, QPM 8-3-2, QPM 10-13-1, VL1016414, VL109476, VL109475, VL1016399, VL1017054, VL109412 and VL1016590 revealed the 169bp QPM allele following PCR with phi 057 and hence, these may be considered as potential QPM inbred lines. These inbred lines may serve as valuable material for QPM breeding.

INTRODUCTION

Maize (*Zea mays* L.) is the queen of cereal crops with highest grain yield potential. It is widely cultivated in about 170 countries. Globally, India ranked fourth in terms of total maize production. Production of maize was 12million tonnes in early 2000s and it was increased to 28.7 million tonnes in 2017-18. However, India is far behind in productivity (3.1 metric tonnes/ha) which is less than half of the world average (5.6 metric tonnes/ha) due to its cultivation mainly in rainfed conditions with inadequate irrigation, quality seed and other inputs. Normal maize grain is very low in two essential amino acids like lysine and tryptophan and very high amount of undesirable amino acids like leucine and imbalanced proportion of isoleucine which results in poor protein quality affecting its biological value. The natural '*opaque-2*' mutants harbour high lysine and tryptophan content (Krivanek *et al.*, 2007) due to decrease in the synthesis of zein proteins and increase in the other seed protein bound lysine and tryptophan (Tripathy *et al.*, 2017), but these genetic stocks were characterized to have soft and opaque endosperm. Such undesirable pleiotropic effects of opaque-2 mutation were subsequently rectified by use of endosperm modifier genes using modified backcrossing and recurrent selection. Vasal *et al.* (1980) combined the opaque-2 allele with QTLs for genetic modifiers and produced elite germplasm with hard kernel and much higher quantity of lysine and tryptophan. The resultant plant types with improved seed quality were designated as Quality Protein Maize (QPM). The QPM version contains higher amount of lysine (>2.4 %) and tryptophan (>0.6 %) with more or less balanced leucine to isoleucine ratio resulting higher biological value of the food products to meet food and nutritional security (Atlin *et al.*, 2011, Tripathy, 2019).

The main objective of the maize breeding programme is the exploitation of heterosis for development of high yielding hybrids. A few promising single cross experimental hybrids have been recently reported (Lenka and Tripathy, 2017) that qualify the minimum standard for QPM with nearly 30% higher seed yield potential than Vivek QPM 9. To achieve a successful hybrid, development and selection of elite inbreds for heterotic cross combination is the key step. As the cross combinations among genetically diverse parents likely to produce high heterotic effect, the knowledge of genetic diversity among maize inbreds is essential for a planned maize breeding programme. Therefore, an attempt was undertaken to evaluate and study the extent of the genetic divergence among twenty advanced inbred lines derived from diverse QPM populations using a few QPM allele specific SSR markers to sort out divergent inbreds and to detect inbreds with QPM status.

MATERIALS AND METHODS

Plant material

The present investigation comprised of 20 advanced inbred lines derived from sixteen CIMMYT (Regional office,

Hyderabad, India) and four DMR (New Delhi, India) quality protein maize (QPM) populations. These were developed by ear to row method and subsequently further purified and maintained by selfing. The inbred lines were evaluated in a two-row plot of 4.0 m length in randomized bock design with two replications during *Rabi* season. Observations were recorded on 10 random plants for 15 quantitative traits and mean values from each replication were used for standard statistical analysis for analysis of variance (Singh and Choudhury, 1985). These inbreds were subjected to SSR analysis to assess molecular diversity and identify the inbreds with QPM characteristics using two gene-specific SSR markers (phi057 and umc1066).

Isolation of DNA and PCR analysis

Genomic DNA of selected inbred lines was extracted using standard CTAB method (Doyle and Doyle, 1990) and each genomic DNA sample was primed and amplified using the gene specific primer 'phi 056' and 'umc 1066' DNA amplification was carried out in the Gene Pro Thermocycler (Bioer Tech. Co., Ltd, Japan), programmed for 5min at 95°C for initial denaturation, 35 cycles of 1min at 94°C for denaturation, 1min at 54°C for annealing and 2min at 72°C for synthesis and final extension for 7 min at 72°C. The gels were scanned by gel doc system (Fire Reader-Uvtec, Cambridge, UK) for detection gene specific alleles. Polymorphic information content (PIC) and Resolving power (Rp) were determined as per Prevost and Wilkinson (1999) using the formula PIC = $\Sigma(1 - p_i^2)/n$, where p_i is the frequency of the i^{th} band amplified by the primer and n = total no. of bands produced by the primer and $R_p = \Sigma I_b$, where I_b (band informativeness) $= 1-[2 \times (0.5-pi)]$.

The binary data matrix of SSR score was analysed using the multivariate analysis program NTSYS-PC 2.1(2000-01) to estimate Jaccard's similarity coefficient (Jaccard, 1908) values. The dendrogram was constructed using Unweighted Paired Group Method with Arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing Sequential Agglomerative Hierarchic and Non-overlapping clustering (SAHN).

RESULTS AND DISCUSSION

Days to 75% dry husk relates to physiological maturity and it ranged from 97.0 days in QPM 8-3-2 to 116.5days VL109288 (Table 1). In the present investigation, there was wide variability in plant height ranging from 82.13 cm to 178.2 cm. VL1017821 exhibited significantly dwarf plant type while, VL10512388 and VL1016453 (177-178 cm) were the tallest. There was wide range of variation in each of the characteristics relating to cob. Cob length ranged from 11.0 in VL109476 to 17.2 cm in VL1017821 and VL1017524, while cob diameter was maximum(16.5 cm) in QPM-10-13-1 followed by QPM-11-7-1 and VL1016453. In maize, no. of rows per kernel, no. of kernels per row, 100-kernel weight and over-shelling percentage are the major determinants for seed yield. In this context, VL1016548 and VL1016453 performed better for all such ancillary traits except 100-kernel weight which was moderately low (20-24 gm). These yielded even more than 50 quintals/ha as also in case of QPM-11-7-1, QPM-8-3-2 and VL109273. Interestingly, QPM 8-3-2 with medium height plant type and maturing within 97 days, managed to have high yield potential (51.25 q/ha). VL1017524 recorded maximum cob length(17.2 cm), number of kernels per row (37.1) and 100-kernel weight (32.7 gm); but yielded moderately (38.12 q/ha) low owing to moderately lower number of kernel rows per cob(13.5), total number of kernels per cob (360.6) and shelling percentage (77.8%).

Twenty quality protein maize inbreds were used to study the level of genetic diversity using SSR markers (Table 2). A set of 10 SSR markers were employed to investigate the polymorphism, out of which 5 revealed clear and consistent amplification profiles. However, three of these SSR markers showed monomorphic profile and hence were not considered for further study. The pattern of polymorphism of two SSR markers (phi057 and umc1066) is presented in Figure 1. The SSR marker, phi057 amplified two different alleles of size 190 bp and 150 bp whereas, the other SSR marker, umc1066 amplified two alleles of size 200 bp and 180 bp. Polymorphism information content (PIC)- a measure of allelic diversity produced by a primer, while resolving power (Rp) refers to the ability of primers to distinguish between genotypes. Using the dataset generated by two SSR primers, PIC value was 0.55 and 0.74 for pi 057 and umc 1066 respectively. However, Rp value of phi 057 (1.8) was appreciably higher than umc 1066 (1.2) indicating relatively more discriminating power of the former than the later.

Initially, 20 inbreds were grouped into three clusters in the dendrogram (Fig 2). The cluster III was the major cluster with 18 genotypes and it was further sub-divided into two sub-clusters with cluster-IIIa having 13 genotypes (VL1052388, VL1016422, VL109273, VL1016399, VL1016590, VL109288, VL1113821, VL109266, VL1017054, VL1016453, VL109412, VL109476 and VL109475) and cluster-IIIb with 5 genotypes(VL1016414, QPM 10-13-1, QPM 8-3-2, QPM 11-7-1 and QPM 7-3-2). But, the inbreds in each such sub-clusters still maintained homology at higher phenon level. This may be traced back to their related pedigree and breeding history or due to use of few number of SSR loci for construction of dendrogram. Interestingly, the rest two genotypes e.g., VL1016548 and VL1017524) were shown to be unrelated and formed two distinctly separate mono-genotypic clusters indicating their status as most divergent inbreds. This may be ascribed to the presence of null-alleles in these inbreds which have not been amplified with the present set of SSR markers. The divergent genotype VL1016548 had also shown high yield potential (^ 50q/ ha) as compared to VL1017524 which yielded moderately (38.12 q/ha).

The gene specific marker phi 057 is reported to be tightly linked to QPM trait (Magulama and Sales 2009). Allelic polymorphism among QPM and normal maize inbreds was surveyed by several workers (Babu *et al.*, 2005; Jompuk *et al.*, 2006, Magulama and Sales, 2009; Gupta *et al.*, 2013, Tripathy *et al.*, 2018) using these gene specific SSR primers and confirmed co-dominant status of phi 057 and umc 1066. A few workers carried out marker assisted selection using these tightly linked markers for conversion of normal maize inbred lines to QPM status (Gupta *et al.*, 2013 and Tripathy *et al.*, 2017). Based on these results we selected marker "phi 057" to verify the selected inbreds for QPM status as it has more

Table 1: Mean performance of 20 inbreds for different agro-economic traits

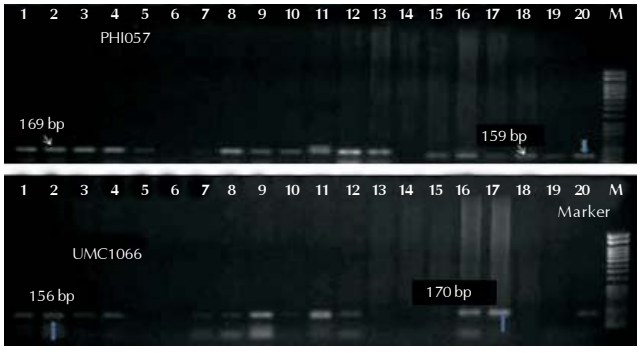
S. No.	Genotype	Source	DH	PHT	CL	CD	KR/C	K/R	K/C	KW	S%	GY
1	VL1016475	CIMMYT	113.5	148.4	12.7	12.5	13.6	24.0	338.7	18.4	80.1	22.81
2	VL1017054	-do-	112.0	145.4	12.9	13.4	13.4	24.8	280.7	22.6	79.0	36.35
3	VL109476	-do-	112.0	147.7	11.0	14.3	15.6	18.6	309.3	25.2	78.1	43.12
4	VL1016399	-do-	112.5	155.7	14.2	13.8	13.4	26.9	351.0	24.1	76.9	30.00
5	VL109273	-do-	114.0	146.9	12.7	12.6	12.8	26.1	385.3	25.4	78.1	50.21
6	VL109288	-do-	116.5	165.7	15.8	12.7	12.6	26.2	258.1	20.1	77.8	38.43
7	VL109266	-do-	111.5	170.4	13.7	13.8	13.6	22.1	296.6	27.4	75.5	41.56
8	VL1016422	-do-	112.0	166.7	14.5	13.8	13.2	23.8	277.7	26.7	80.2	40.21
9	VL1016548	-do-	108.5	161.0	14.3	13.5	15.3	29.8	428.6	20.5	82.0	51.77
10	VL1016453	-do-	108.5	177.0	15.0	15.4	15.4	32.7	426.6	24.5	82.7	51.97
11	VL1016414	-do-	112.0	164.7	15.7	13.4	12.6	30.0	345.5	22.8	79.0	46.67
12	VL109412	-do-	113.0	151.4	15.5	13.4	12.0	27.4	327.1	30.5	82.0	30.83
13	VL10512388	-do-	113.5	178.2	16.3	14.1	13.2	28.8	381.0	31.9	83.7	38.43
14	VL1017821	-do-	110.5	82.13	17.2	13.6	14.0	27.9	347.0	27.8	79.7	29.79
15	VL1017524	-do-	104.5	136.1	17.2	15.1	13.5	37.1	360.6	32.7	77.9	38.12
16	VL1016590	-do-	111.0	175.4	14.5	14.5	15.4	27.8	396.0	24.2	80.0	48.85
17	QPM-7-3-2	DMR	102.5	138.6	13.8	12.5	13.0	26.7	298.5	19.2	81.7	19.33
18	QPM-11-7-1	-do-	108.0	154.4	16.7	16.0	14.0	31.1	394.6	31.1	78.6	52.81
19	QPM-8-3-2	-do-	97.00	137.9	16.3	14.7	14.2	29.6	388.7	26.0	79.7	51.25
20	QPM-10-13-1	-do-	113.0	150.6	16.4	16.5	16.0	25.3	362.6	30.3	78.4	44.79
Mean	110		152.7	14.8	14.0	13.8	27.3	347.7	25.6	79.6	40.36	
CV(%)	0.43		5.16	7.02	4.7	5.68	7.6	10.97	6.65	3.00	7.40	
$CD_{0.05}$	0.97		15.60	2.01	1.3	1.57	4.0	73.99	3.28	4.77	5.86	

DH - Days to 75 % dry husk, PHT - Plant height (cm), CL - Cob length (cm), CD - Cob diameter (cm), KR/C - No. of kernel rows/cob, K/R - No. of kernels/ row, K/E - No. of kernels/cob, KW: 100-kernel Wt. (gm.), S% - Shelling %, GY/P - Grain yield/ha (q/ha)

Table 2: Molecular Characteristics of SSR markers used to analyze maize inbreds

Sl.No.	SSR marker	Sequence of Primer	T_m(°C)	T_{an}(°C)	MAF	maf	No. of alleles	PIC	Rp
1	phi057	F, 5′-CTCATCAGTGCCGTCGTCCAT-3′	59.4	54.0	0.68	0.32	2	0.55	1.8
		R, 5′-CAGTCGCAAGAA ACCGTTGCC-3′	59.5						
2	umc1066	F, 5′-ATGGAGCA CGTCATCTCAATGG-3′	57.3	54.0	0.83	0.17	2	0.74	1.2
		R, 5′-AGCAGCAGCAACGTCTATGACACT-3	60.6						

N.B.: MAF - Major allele frequency, maf - Minor allele frequency, PIC - Polymorphic information content, Rp - Resolving power



Lane-1-20: QPM 11-7-1, QPM 7-3-2, QPM 8-3-2, QPM 10-13-1, VL1016414, VL1016548, VL109266, VL109476, VL109475, VL1016399, VL1017054, VL109412, VL1016590, VL1017524, VL109273, VL109288, VL1113821, VL1016422, VL1052388 and VL1016453; M: 50 bp DNA ladder. 169bp- QPM allele and 159bp-non-QPM allele amplified by phi 057

Figure 1: SSR profile of 20 maize inbred lines generated by two gene specific primer pairs phi 057 and umc 1066

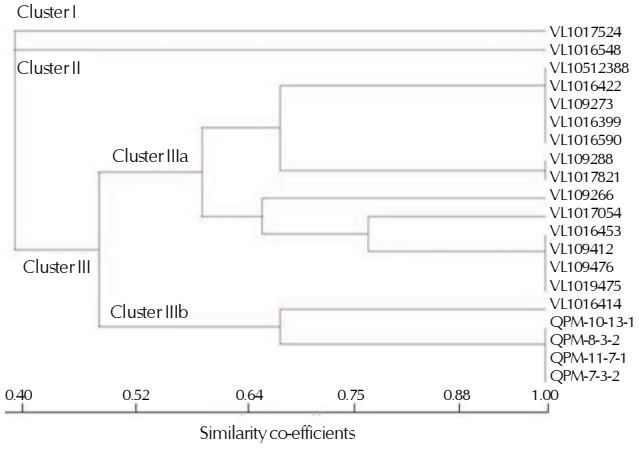


Figure 2: Dendrogram showing genetic relationship of 20 selected inbred lines of maize using gene specific SSR markers (phi 057 and umc 1066)

resolving power than even umc 1066. The inbreds e.g., QPM11-7-1, QPM 7-3-2, QPM 8-3-2, QPM 10-13-1, VL1016414, VL109476, VL109475, VL1016399, VL1017054, VL109412 and VL1016590 revealed the QPM allele 169bp allele and hence, these may be considered as potential QPM inbred lines.

CONCLUSIONS

Maize being a predominantly cross pollinated crop, development of hybrids seems to be the major breeding programme. Cross combination of Inbreds with diverse traits can make it successful. Diversity analysis based on molecular

profiling confirmed high genetic divergence of inbreds 'VL1016548' and 'VL1017524'. However, the divergent inbred VL1016548 revealed high yield potential (^ 50 q/ha) as compared to VL1017524 which yielded moderately (38.12 q/ha). Such elite genotypes may be chosen as parents in suitable cross combination to exert effective and high throughput heterotic performance in seed yield. Besides, few inbred lines which confirmed to have QPM status, may serve as valuable material for QPM breeding.

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REFERENCES

Atlin G. N., Palacios N., Babu R., Das B. and Twumas-Afriyie S. 2011. Quality protein maize: Progress and prospects. *Plant Breed. Rev*. 34: 83-130. doi: 10.1002/9780470880579.ch3

Babu R., Nair S. K., Kumar A., Venkatesh S., Sekhar J.C., Singh N. N., Srinivasan G. and Gupta H.S. 2005. Two generation marker aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). *Theor. Appl. Genet*. **111:** 888-897.

Doyle J. J. and Doyle J. L. 1990. Isolation of DNA from plant tissue. *Focus* **12:** 13-15.

Gupta H. S., Babu R., Agrawal P. K., Mahajan V., Hossain F. and Thirunavukkarasu N. 2013. Accelerated development of quality protein maize hybrid through marker assisted introgression of opaque 2 allele. *Plant Breeding* **132(1):** 77-82.

Jaccard P. 1908. Nouvelles recherches Sur la distribution florale. *Bulletin Society Vaud Science National*. **44:** 223-270.

Jompuk P., Wongyai W., Jampatong C. and Apisitvanich S. 2006. Detection of quality protein maize (QPM) using simple sequence repeat (SSR) markers and analysis of tryptophan content. *Kasetsart J. (Natl. Sci.).* **40:** 768-774.

Krivanek A., Groote H., Gunaratna N., Diallo A., Freisen D. 2007. Breeding and disseminating quality protein maize for Africa. *Afr. J. Biotech.* **6:** 312-324.

Lenka D. and Tripathy Swapan K. 2017. Development of high yielding quality protein maize (QPM) hybrids and their molecular detection. *Res. J. of Biotech.* **12(4):** 72-78.

Magulama E. and Sales E.K. 2009. Marker assisted introgression of opaque-2 gene into elite maize inbred lines. *Academia.Edu.USM R & D.* **17(2):** 131-135.

Prevost A and Wilkinson. M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet*. **98:** 107-112.

Singh R. K. and Choudhury B. C. 1985. Biometrical Techniques in Genetics and Plant Breeding. International Bioscience Publisher, India. p. 63-68.

Sneath P. H. A. and Sokal R. R. 1973. Numerical taxonomy. *Freeman, San Fransisco, Calif.*

Tripathy Swapan K, Ithape D. M., Maharana M. and Prusty A. M. 2017. Quality protein maize (QPM): Genetic basis and breeding perspective. *Tropical Plant Res*. **4(1):** 145-152.

Tripathy Swapan K., Lenka D., Ithape D. M. and Maharana M. 2017. Development of opaque-2 introgression line in maize using marker assisted backcross breeding. *Plant Gene*. **10:** 26-30.

Tripathy Swapan K., Lenka D., Maharana M., Ithape D.M. 2018. Biochemical analysis and validation of molecular markers for identification of quality protein maize. *Plant Gene*. **14:** 69-73.

Tripathy Swapan K. 2019. Quality protein maize (QPM): A way forward for food and nutritional security. *Genomics and Applied Biology*. **10(2):** 10-19.

Vasal S. K., Villegas E., Bjarnason M., Gelaw B., Goertz P. 1980. Genetic modifiers and breeding strategies in developing hard endosperm opaque 2 materials. In: Improvement of quality traits of maize for grain and silage use (Eds. W.G. Pollmer and R.H. Phillips). Nijhoff, London. pp. 37-43.