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Molecular characterization and multi-environmental evaluation of field corn (*Zea mays*) inbreds for kernel traits

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ABSTRACT

Kernel size and kernel weight are important yield attributing traits in maize (*Zea mays* L.). Though yield has complex inheritance, understanding and improvement of yield per se becomes relatively easy, when maize breeding is targeted for genetic enhancement of yield component traits. In the present investigation, a set of 45 tropical field corn inbred lines were evaluated under three environments and at different location for kernel length, kernel thickness and kernel weight traits. In a given location, environmental influence on the expression of these traits were negligible as it was evident by exhibition of high heritability (broad sense) for the traits under study, however pooled effect of environments showed some interactions. Based on the AMMI stability value, the inbred lines AI 04 followed by AI 37, AI 18, AI 25 and AI 35 were selected as highly stable genotypes for its yield *per se*. Inbred lines were characterized using gene-based markers linked to kernel traits. It was observed that molecular markers rightly classified the inbred lines into different groups based on their trait means. Furthermore, the makers, *umc1890* and *umc1120* were putatively linked to kernel weight and kernel thickness respectively. These markers may be utilized for identification of suitable donor and genetic improvement of kernel traits driven maize improvement program.

Keywords: AMMI analysis, Gene based markers, Kernel size, Kernel weight, Molecular diversity

The genetic complexity and complex inheritance impede our understanding of the genetic basis and molecular mechanisms underlying grain yield components, especially kernel traits and other yield attributes in maize (*Zea mays* L.) crops. Seed yield is a very complex quantitative trait, whose expression is the cumulative effect of its component traits, viz. kernel row number, cob length, cob girth, kernel numbers, kernel size and kernel weight beard by the genotype, environmental factors and G×E interaction (Bocianowski 2019). The complexity of seed yield is the result of different genotype reactions to fluctuating environmental conditions during plant development. Hence it is a pre-requisite to identify maize inbred lines which are stable across the environments for their successful utilization in hybrid-breeding program to enhance productivity. G×E interaction is often analyzed by the additive main effects and multiplicative interaction (AMMI) model. The AMMI model combines the analysis of variance for the genotype and environment main effects and the principal component analysis (PCA) with multiplicative parameters in a single analysis. Stability analysis helps in understanding the adaptability of a genotype/hybrid over a wide range of environments (Bocianowski *et al.* 2019b).

Many QTLs related to kernel traits have been identified in the maize genome (Xu *et al.* 2015), however, the genetic architecture and molecular mechanisms underlying natural quantitative variation in kernel yield have not been completely elucidated. The most traits such as kernel yield and kernel size are controlled by many genes with small effects (Peiffer *et al.* 2014). Though good amount of molecular information is available with temperate maize germplasm for kernel size and kernel weight, transformation of the information to tropical maize is meager. Present investigation is focused to identify the stable inbred lines for kernel size and kernel weight with the molecular marker associated with them will certainly benefit maize-improvement program, as these inbred lines can be directly utilized as donor for targeted trait improvement in tropical maize.

MATERIALS AND METHODS

Field experiment design and implementation: A set of 45 tropical maize inbred lines were evaluated in randomized complete block design with two replications across the three environments such as *kharif* 2018, *rabi* 2018–19 at IARI, New Delhi and *rabi* 2018–19 at Dharwad. Each genotype

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was sown in two rows of 3 m length with row spacing of 75 cm × 20 cm. The crop management was followed by recommended standard agronomical packages of practice to ensure healthy crop, across the environment.

Trait measurement: The yield components were recorded on the randomly selected five plants of each replication. A randomly selected 100 kernels were weighed to record test weight (g) and randomly selected 10 kernels from a given inbred lines were measured for kernel thickness (mm) and kernel length (mm), using Vernier caliper, and grain yield was recorded on plot basis and converted into yield per ha.

Statistical analysis for morphological traits: The phenotypic data analyzed using software SAS 9.3 version, the components of genotypic and phenotypic coefficients of variability were estimated (Burton and Devane 1953). Heritability in broad sense was calculated (Hanson *et al.* 1956). The AMMI model (Nowosad *et al.* 2016) was used for stability and adaptability analyses.

Polymorphism survey in a set of genotypes: Genotyping was done using 20 gene based markers (SSR) linked to kernel traits, which was obtained from Maize Genetics and Genomics Database (MaizeGDB). DNA was extracted from fresh young leaves of genotypes by CTAB (Cetyl-trimethyl ammonium bromide) method (Saghai-Maroof *et al.* 1984).The PCR was performed with 1 unit of Taq DNA polymerase, 10× reaction buffer supplied by the manufacturer, 0.1 mM dNTPs, 10 pmol/µl each primer and 50 ng DNA template in a total reaction volume of 25 µl. The PCR amplification conditions were, initial denaturation at 94ºC for 5 min followed by 35 cycles consisting of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, extension at 72ºC for 60 s and a final extension of 7 min. at 72ºC. The PCR amplified fragments were resolved on 3.5% agarose gel (HiMedia) and gel pictures were archived in a gel documentation framework. The amplified product was scored and data was computed using software NTSys (Rohlf 1997) and also to PIC (polymorphism information content) values (Anderson *et al.* 1993).

RESULTS AND DISCUSSION

Stability analysis of inbred lines: To identify stable genotypes, both GGE biplot and AMMI analyses are the most frequently used methods in analyzing GEI pattern of multi-environment data set. AMMI has been used to analyze GEI in maize over the decades (Oyekunle *et al.* 2017). Genotypes best suited to precise environmental conditions can be detected based on AMMI parameters which permit estimation of the genotype interaction effect in each environment. In the present investigation, significant G×E was detected across the tested environment. Tested location comprises IARI, New Delhi (two seasons) and RRC, Dharwad (one season) were geographically isolated and had distinct weather condition in a given season, had impacted on trait expression of the inbred lines. Variability studies indicated the presence of ample amount of phenotypic variance for kernel length, kernel thickness, test weight and grain yield among the inbred lines. This supports effective selection of the inbred lines for the trait-specific crop improvement. However, systematic understanding of G×E interaction could give opportunity to improve accuracy and precision in the assessment of both genetic and environmental influences on phenotypic expression of yield and yield component traits (Bocianowski *et al.* 2019a) followed by their selection.

The combined analysis of variation for kernel traits and yield of 45 tropical inbred lines showed significant difference all three seasons and across the locations. The pooled variance by AMMI analysis showed that across the environment's performance of genotypes as well as genotype × environment interaction was highly significant (Table 1). The range for kernel length was observed lowest (7.35 mm) and highest (11.20 mm) in environment II compared to rest of environments. Similarly, for trait kernel thickness was lowest (4.45 mm) in environment I and highest (8.90 mm) in environment II. A wide range of test weight is appeared in environment I (14.90–30.90g) and environment III (14.85–31.95 g) compared to environment II (16.50–30.0 g). The phenotypic coefficient of variation for kernel length, kernel thickness and test weight were more than the genotypic coefficient of variation for respective traits, in all the studied environments. Heritability (broad sense) was high for all kernel traits under study (Table 2).

The stability of the tested genotypes can be evaluated using the biplot for different traits. The average environment coordination of GGE biplot depicted the better performing genotype across the environment along with their adaptability (Fig 1). The PCA for the traits under consideration, viz. kernel length, kernel thickness, test weight and grain yield were 100%. A set of inbred lines like AI 39, AI 14, AI 23, AI 32 and others like AI 33, AI 32, AI 26, AI 15, AI 18, AI 30 appear to be stable with high mean value for both the traits like kernel length and kernel thickness respectively. Furthermore, inbred lines such as AI 44, AI 09, AI 21, AI 36, AI 10 and lines like AI 04, AI 37, AI 18, AI 35 and AI 25 were found to be stable across the environments for both traits such as test weight and grain yield respectively. Along with the component traits, understanding interaction of final grain yield with the environment is most important to select line with high productivity. Further, it was also

Table 1 Pooled AMMI analysis of variance for genotypes evaluated across three environments

Source	Df	Kernel length	Kernel thickness	Test weight	Grain yield
Treatment	134	525.30**	64.68**	40.56**	0.835**
Genotypes	44	1073.10**	171.26**	84.86**	1.536**
Environment	2	369.70**	3.31	19.08	5.489**
Block	3	10.70	1.56	5.28	0.07
Interaction	88	254.90**	12.78**	18.90**	0.38**
Error	132	5.70	1.25	1.86	0.03

* Significance at 5% level of probability; **significance at 1% level of probability

Table 2 Variability parameters for kernel traits and grain yield across the locations

Parameter	Environment I				Environment II				Environment III			
	KL (mm)	KT (mm)	TW (g)	GY (t/ha)	KL (mm)	KT (mm)	TW (g)	GY (t/ ha)	KL (mm)	KT (mm)	TW (g)	GY (t/ ha)
Mean	8.94	6.82	21.89	2.74	8.96	6.98	21.57	2.70	9.06	6.89	21.37	3.15
Range	7.60-9.85	4.45-8.25	14.9-30.90	1.22-3.83	7.35-11.20	5.50-8.90	16.5-30	1.37-3.69	7.75-11.15	5.55-8.60	14.85-31.95	1.03-3.90
PCV	7.16	11.48	18.21	24.57	8.58	12.29	15.89	24.62	7.58	10.07	16.96	16.88
GCV	6.47	9.71	17.12	24.39	8.29	12.06	14.63	23.61	6.61	9.11	15.26	15.91
H^2_{BS}	81.70	71.50	88.40	98.50	93.20	96.40	84.90	92.00	75.80	81.80	81.00	88.90

KL-Kernel length, KT-Kernel thickness, TW-Test weight, GY-Grain yield, PCV-Phenotypic coefficient of variation, GCV- Genotypic coefficient of variation, H^2_{BS}- Heritability broad sense. Environment I: *Kharif* 2018 at IARI, New Delhi, Environment II: *Rabi* 2018-19 at IARI, New Delhi, Environment III: *Rabi* 2018–19 at RRC Dharwad.

observed range of PCA values across the tested environment. Inbred line, AI 38 had highest IPCA1 value (0.63) and AI 42 had lowest IPCA1 value (-0.57). The average stability value (ASVi) with lowest value was recorded in AI 04 followed by AI 37, AI 18, AI 25 and AI 35 in ascending order and the highest ASVi value recorded by inbred line AI 38 (13.70). Kernel size and kernel weight plays important role in deciding final yield of maize (Zhang *et al.* 2016). Selection of inbred lines having stable expression of these traits across the tested environments may pay much role in improving maize productivity. Out of 45 inbred lines, five lines (AI 04, AI 37, AI 18, AI 25 and AI 35) were selected based on their average stability value and/or average environment coordination (AEC) based on environment focused scaling for the mean performance vs adaptability (Bocianowski *et al.* 2019).

Molecular characterization: In maize, mutant's analysis has identified several genes in key pathways involved in kernel development, such as *Mn1, o2, sh2, gln1-4, o1* and others (Chen *et al.* 2016). In this study, 20 gene-based SSR markers associated with different traits such as kernel length, kernel thickness and test weight were used for the characterization of inbred lines to understand the molecular diversity and the putative association among markers and kernel traits. The molecular markers showed an average PIC value of >0.5 with range of 0.56-0.99, which confirms that markers are highly informative (Botstein *et al.* 1980). It was observed that there exists allelic variability among the markers. The markers like *bnlg1953*, *umc1560* and *umc1551* recorded three alleles and rest of the markers showed two alleles among the inbred lines. The inbred lines were grouped into seven clusters based on the molecular diversity prevailed among them. It clearly indicated that markers associated with kernel traits had effectively classified the

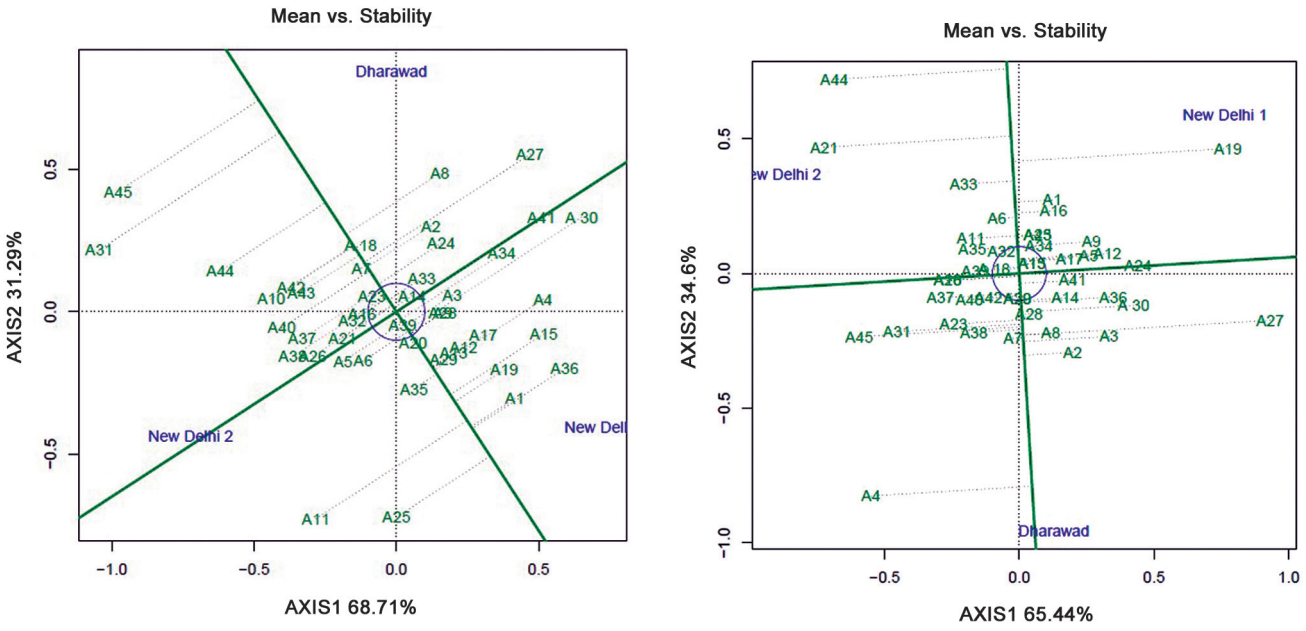

Fig 1 Average environment coordination (AEC) view of GGE bi-plot based on environment – focused scaling for the mean performance vs. adaptability for Kernel related traits (a, b, c & d) across three environments.

inbred lines into distinct category in relation to their trait means. The marker *umc1890* was monomorphic among inbred lines with high kernel weight or low kernel weight traits, but polymorphic between them. Similar observations were made on *umc1120*, where marker was monomorphic among high kernel thickness or low kernel thickness but polymorphic between the contrasts. The inbred lines AI 12 and AI 06 had lowest mean kernel thickness and lowest kernel weight, respectively, were differentiated by markers, *umc1120* and *umc1890,* respectively from their counterpart with high mean value (Mikic *et al.* 2016). These markers can be effectively utilized for the selection of inbred lines for kernel thickness and kernel weight.

The polymorphic information (PIC) value was more than 0.5 for all the markers and it was ranged from 0.56 for umc2041 to 0.99 in *umc1890*. The marker *umc2061* showed mono-morphic allele between AI 01 and AI 02 and/or AI 03 and AI 04, whereas it was polymorphic between AI01 and AI 03 and /or AI02and AI 04. The marker *umc1890* was found monomorphic among AI 44 and AI 15 or AI 06 and AI 12, but it was polymorphic between AI 44 and AI 06 or AI 12 and AI 15 and AI 06 or AI 12. Similarly, *umc1120* was found monomorphic among AI 02, AI 39, AI 42 and AI 24, whereas it was polymorphic between AI 02 and AI 06 or AI 12, AI 39 and AI 06 or AI 12, AI 42 and AI 06 or AI 12 and AI 24 and AI 06 or AI 12. Molecular diversity analyses indicated that genotypes under study were diverse among each other and based on this information they were grouped into seven clusters, having 4, 13,11,4,1,11 and 1 inbred line serially in the groups. The two-solitary cluster, cluster V and cluster VII had lowest mean kernel thickness (5.98 mm) and lowest kernel length (7.81 mm), respectively. The cluster II and cluster III had equal number of inbred lines with highest kernel length (9.11 mm) and kernel thickness (7.40 mm), respectively. Among the stable inbred lines identified for kernel length, three inbred lines belonged to cluster II (AI 14, AI 23 and 39) and two belonged to cluster III (AI 32 and AI 33), where these cluster had highest cluster mean for kernel length of more than 9 mm. Similar observations on kernel thickness were also made, where two inbred lines each were belonged to cluster II (AI 18 and AI 30) and cluster III (AI 26 and AI 32), which had highest cluster mean for kernel thickness (>7 mm). For the test weight, except AI 44 and remaining four other stable inbred lines (AI 10, AI 09, AI 21 and AI 36) were grouped in cluster VI which had highest cluster mean (22.12 g) for test weight compared to other clusters (Rafique *et al.* 2018). These observations clearly indicate the efficiency of markers for grouping inbred lines based on their field potentiality. As grouping of inbred lines were done based on the molecular diversity and stable inbred lines also classified according to molecular marker information, the markers selected for the study can be effectively utilized for the indirect selection of inbred lines for the kernel, trait improvement in maize. The stable inbred lines so obtained for each trait separately can be used for targeted trait improvement in maize.

Kernel traits comprising kernel length, thickness and weight are the function of grain yield can be given weightage for final selection through by identifying its final impact on grain yield. The stable genotypes for yield component traits, viz. AI 14, AI 23, AI 32, AI 33 and AI 39 can be used further yield improvement in maize. The genotypes AI 06 and AI 34 appears to be divers in comparison with genotype AI 01, AI 02, AI 15 and AI 28. These may be better targeted to obtain heterosis for yield component traits. The markers, *umc1120* and *umc1890* clearly differentiate genotypes with differential kernel thickness and kernel weight. These markers may be used in identification of suitable donor for the trait driven maize improvement program.

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