Assessment of maintenance breeding methods in maize (*Zea mays* L.)

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Abstract

An investigation was conducted at IASc. BHU, Varanasi (*Rabi* 2014-15 to *Kharif* 2017) with four maize inbreds using three maintenance methods (selfing, half-sibbing and full-sibbing) for four generations in which a significant amount of genetic correlation was found between the morphological and molecular analyses. The comparison of four inbreds revealed a deviation in the clustering pattern after the four generations of maintenance. A maximum similarity coefficient was recorded between HKI 193-1 FS and LM 10 FS lines; full-sibbing showed the highest similarity between the first and fourth generation. Most of the inbreds followed a similar clustering pattern in morphological as well as in molecular diversity analyses. HKI 1105 is considered as most stable inbred in terms of giving a wide range of partitioning the regression coefficient. The quadratic and cubic trend through the graphical method showed self-ingled to a negative [cb1] response as well as maximum changes whereas, the full-sibbing method recorded with the minimum changes over the generations. Comparison of the combining ability of the inbreds by three methods revealed that CML-161 followed by HKI 1105 recorded maximum and LM 10 recorded minimum significant GCA effects. Through all the experiments it was proved that selfing caused the highest loss of vigour whereas full-sibbing was most stable.

Abbreviations

ASI: Anthesis-silking interval
BP: No. of barren plants in a row
CD: Cob diameter
CP: Cobs plot^{-1}
CTAB; Cetyl Trimethylammonium Bromide
DF: Degree of freedom
DM: Days to Physiological maturity
DNA:Deoxyribonucleic acid
DS: Days to 50 % silking
DT: Days to 50 % tasseling
EH: Ear/cob height
FS: Full sibbing
GCA: General combing ability
He: Expected heterozygosity
HSW: 100 seed weight
HS: Half sibbing
ID: inbreeding depression
KR: kernels row^{-1}
cA: Leaf area
Lx T: Line x Tester mating design
MP: Percent Moisture
NL: No. of leaves
PCR: polymerase chain reaction
PG: Per cent germination
PH: Plant height
PCF: Per cent cob filled
PIC:Polymorphic information content
RCBD: Randomized complete block design
RPC: Kernel rows cob^{-1}
TSS: Total soluble solids
S: Selfing
SCA: Specific combining ability
SSR: Simple sequence repeats
TAE:Tris-acetate-EDTA
TE: Tris EDTA buffer
UPGMA: Unweighted Pair Group Method with Arithmetic Mean
UV:Ultraviolet
YP: Yield plot^{-1}
*, ** significant at 5% and 1% levels, respectively

Introduction

Inbred maintenance follows either selfing or sibling based on the crop's tolerance to inbreeding depression (ID). Maintaining already developed superior inbred line integrity plays a crucial role than developing new inbreds. Production of homogeneous, homozygous inbred lines has provided maize researchers with a large array of uniform, reproducible genotypes. Inbred maintenance with different methods (selfing, full sibbing and half sibbing) over generations helps to study the impact of maintenance methods in inbreds and can identify gradual or drastic changes.

The uniformity of hybrids has been regarded as their

crucial advantage. There are two aspects of hybrid maize uniformity: (*i*) genetic homogeneity and (*ii*) genetic stability. The rapidity of attaining homozygosity is faster through selfing than sibbing as the rate of accomplishing the homozygosity through selfing is three times more than full-sib and six times than half-sib. Extreme inbreeding (selfing) has been recommended to purge genetic load (reduced fitness) and force the adaptation of the endangered population to the inbreeding regime they will experience under human management. Fitness is restored only when the inbred lines are intercrossed to analyze the inbred performance with different maintenance methods. There is evidence that maintained lines may change their performance over a period that may change the hybrid varietal performance over a period and also the poor lines have produced the higher-yielding crosses in some experiments. The yield performance of inbreds has not advanced as rapidly as the performance of hybrids, especially in stressful environments. This is because the inbreds are not to be expected to release as a variety as in the self-pollinated crops but can be used as parental material in hybridization or population development programs. Therefore, modelling a best-inbred maintenance method in the field and the genetic/molecular level is an important task (Russel and Vega, 1973).

Maintenance breeding is essential for continuingthe lines by preserving their original integrity and desirable characters across generations. Adequate maintenance enhances the feasibility of line certification and breeder's rights. In some countries, verification of a detailed description of maize inbred lines must be done before the lines can be used in commercial hybrid seed production. Based on this study, several generations of reproduction may result in strains that no longer fit in entirety the original detailed description. In these situations, certification would be difficult and unreliable. It helps in eliminating off- types and lines can be maintained without alterations. Maintenance by sib mating and selfing (ear to row) over generations helps to study the impact of two methods in inbreds and can identify gradual or drastic changes. The comparable estimates of the genetic changes in long time inbreds that had been maintained by sib mating and selfing help a breeder to choose the best method for the long run to keep the intact genetic integrity of a variety. Because of the number of generations of brother-sister mating involved before the strain was split into sub-lines, it was concluded that few, or none, of the genetic changes observed, could be due to residual heterozygosis but that virtually the whole of it must have arisen by mutation during the course of inbreeding. With this background, the Objectives of the present investigations are 1. Estimation of the genetic diversity of maintained inbred lines with conventional and molecular methods.2. Comparing the variability by sibbing and selfing.3. The effect of inbred maintenance methods on hybrid performance.The reason to conduct this experiment was to assess the changes in maintained lines after a favourable period of maintenance and the effect on the yield and yield-related (quantitative) traits.

Material and methods

The experimental material comprised of the four inbred lines, randomly selected from which four diverse and promising lines maintained (uniform/homozygous lines) for 8-10 generations were selected for making sib mating (Half sibbing and full sibbing) and selfing. The selection of inbred lines is based on the selection of lines through diversity analysis and homozygous lines maintained continuously through selfing or sibbing and assuming the absence of residual heterozygosity. Thus, the method of maintaining the lines permitted maximum opportunity to detect apparent changes. The four inbreds are HKI 193-1, HKI 1105, CML161, LM 10.

Experimental plan

The experiment is done for sixconsecutive seasons. In the first season of the investigation i.e., during Rabi-2014-15 twenty elite inbreds were evaluated for diversity using the molecular (93 polymorphic markers) and conventional approaches (for 10 traits) to select the diverse inbreds for maintenance and diallel experiments. The comparison of the maintenance methods and genetic variability studies were carried out from Kharif 2015 to Rabi 2016-17 with four inbreds. The maintained inbreds for three generations were chosen as a base material to cross with three diverse inbreds (testers) to assess the combining ability of inbreds and their cross combinations during Rabi 2016-17 in the Line Tester design. The four-generation maintained inbreds with three methods as well as F1s of crosses attempted for combining ability studies during Rabi 2016-17 were evaluated in Kharif -2017 and molecular (with 145 polymorphic markers) and diversity analysis (for 18 traits) were also done. The three experiments (correlating genetic diversity, maintenance methods, testing the maintained inbreds combining ability) were carriedout in a randomized complete block design (RCBD) with three replications. Selfing is the process of pollinating a gynoecium or pistil with the same plant's pollen. In full sibbing, a randomly selected plant's pollen is used to pollinate the sister (sib) plant whereas in half sibbing (bulk sibbing) the pollen collected from the plants of many sib plants including the mother plant is mixed and used in artificial pollination means the half sibbing process involves some extent of selfing also A

total of 18 observations were recorded. The first generation was represented as dividing the maintained inbreds (through sibbing) into selfed, half-sibbed and full-sibbed lines in four-meter single rows. Two cobs from each line were harvested separately. Half of the seeds kept as a remnantunder room temperature and the rest part of the seeds were sown in the next season, the process continued for four generations till the final evaluation where all the four generations maintained lines. The reason behind this evaluation to avoid the seasonal differences and soil heterogeneity. The same material's leaf samples were collected for the molecular diversity analysis.

Molecular diversity studies

The diversity of inbreds tested for comparing the three maintenance methods: The experiment consists of four inbreds, tested with three methods and homozygous lines maintained for four generations were planted in a randomizedblock design (48 lines i.e., 4 inbreds, 3 methods, 4 generations).Four inbreds maintained through three methods (S, HS and FS) were subjected to molecular analysis by taking the initial maintenance season and the final season (fourth generation). Out of 258 markers used 145 (Supplementary data, Table S1) proved polymorphic and the complete list of polymorphic markers. DNA was extracted from two samples of each kind and the imbibed seeds for 24 hours, quick-frozen in liquid nitrogen and crushed in CTAB buffer (cetyltrimethyl ammonium bromide) method (according to Doyle and Doyle 1990 with few modifications). The DNA concentration was measured using a Smartspec TM Plus spectrophotometer and the quality of the DNA was determined by electrophoresis in 0.8% agarose. DNA was diluted to 20 ng µL-1 with TE buffer before use. The PCR reaction program was as follows: 94°C for 5 min; 34 cycles of denaturation at 94°C for 1 min, annealing at 40°C -60°C for 2 min, extension at 70°C for 2 min; and hold at 4°C at the end. PCR products were separated on three percent agarose gel in 1×TAE buffer for two hours and the image was captured by using a UV image analyzer. The coded marker data were used to generate a datamatrix in Microsoft Excel 2013.Data were transformed to binary code to obtain a full design matrix of presence versus absence of an allele with missing values represented by 9. The number of alleles per locus was determined and coded with a number ranging from 1 to n (number of alleles). Descriptive statistics PIC and He were estimated by Genetic Data Analysis (GDA) software (Lewis and Zaykin 2001).

The expected heterozygosity, H_e, was estimated for a locus l as

$$\hat{H}e = \frac{2n(1-\Sigma\,\hat{x}^2\,i)}{(2n-1)}$$

Where, n =number of alleles individuals, where $\hat{x}^2 i$ is the frequency of the i^{th} allele $\hat{x}_i = (\hat{x})_{ii} + \Sigma_i \neq {}_j/2$ (Nei, 1987). Polymorphic information content (PIC) for each SSR marker was calculated as per the formula:

$$PIC = 1-\sum {}^{k}{}_{i=1} P_i^2$$

where, *Pi* is the frequency of the i^{th} allele and *k* is the total number of different alleles at the specific locus. The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. A dendrogram was constructed for 20 inbreds based on Dice similarity coefficients (Dice, 1945). XLSTAT trial version was used to construct the morphological dendrograms based on similarity coefficients and NTSYS 2.02i software with UPGMA (Rohlf, 1998) method for molecular diversity analysis. The estimation of the correlation coefficient and its statistical significance was undertaken using Zt software Version 1.1 (Bonnet and Van de Peer, 2002). Zt software was used to test the correlation between morphological and molecular observations (Mantel's test). A dendrogram was constructed with 48 samples (24 lines) where two plant samples from each line were subjected for the DNA studies and diversity analysis (four inbreds, three methods and two seasons/ generations i.e., initial and final).

Variability studies

The percent changes are calculated by substituting the values in formula $(Y_2\text{-}Y_1)/Y_1\ x100$ where Y_2 is the final (4^{th}) generation and Y_1 is the initial (first) generation mean value. Sib and self-entry sums of squares were partitioned into linear, quadratic, and cubic components for interpretation of significant variation. The data were fitted by orthogonal polynomials (Snedecor and Cochran, 1967) using the equation $Y = b_0 + b_r X,\ldots . + b_i X_i$ Where, b_0 = the overall mean across the sibbed or selfed generations, b_r = the regression coefficients, X_i's =the orthogonal polynomial coefficients. Among sib generations (df = 3) and self-generations (df = 3), and a one-degree-of-freedom comparison between overall sib and self-means. Significance was detected by calculating F-tests at 1% and 5% probability levels. When variation was significant at $P < 0.05$, we assumed that the cause was genetic. Calculation of linear, quadratic and cubic regression is to partition the total variability and to expose the residual variability. This enablesus to find out even the minute genetic changes.

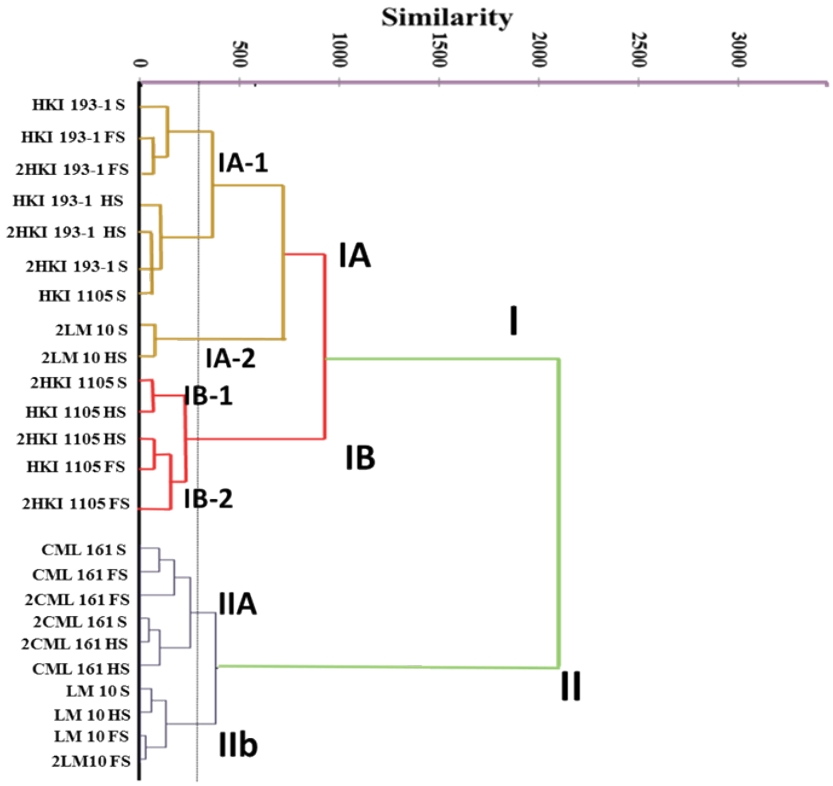

Fig. 1 - A. Dendrogram depicting the morphological diversity of four inbreds in initial and after four generations with three methods.
Note: 2 as a prefix to inbred line indicates line is taken from the final (4th) generation of inbreds compared through three maintenance methods.

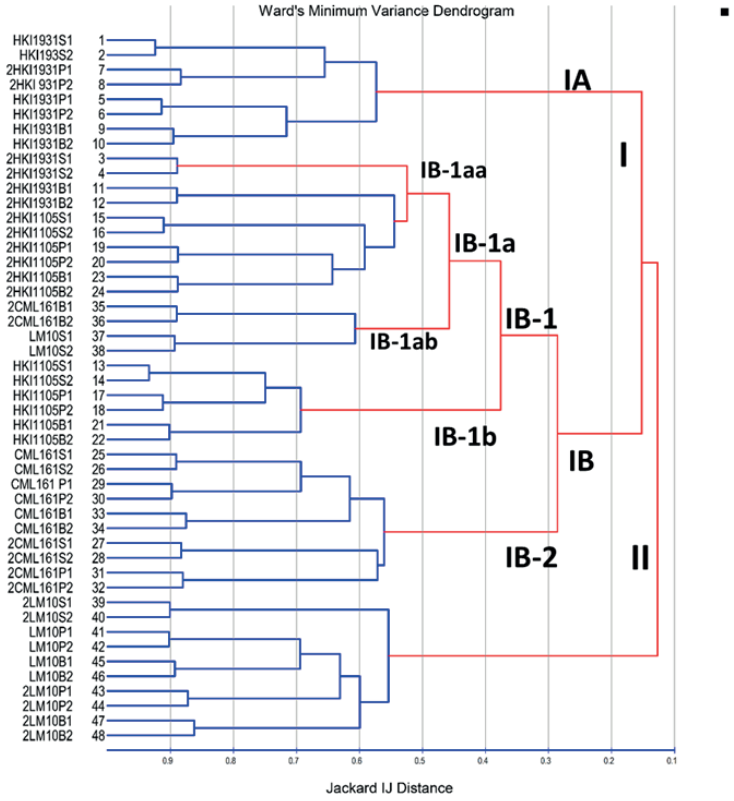

Fig. 1 - B. Dendrogram depicting the molecular diversity of four inbreds in initial and after four generations with three methods
Note: 2 as a prefix to inbred line indicates line is taken from the final (4th) generation of inbreds compared through three maintenance methods.

Combining ability analyses

It includes four inbreds maintained for three consecutive generations of selfing, half sibbing and full sibbing with three inbred testers viz., HUZM 185, HKI 1126 and HKI 323 (crosses made in Line x tester design suggested by Kempthorne (1957). General and specific combining ability were estimated to see the line and cross combination effects.The Analysis of Variance was carried out using mean values. Initially, the test of significance among the genotypes involving crosses and parents was estimated. The treatment sum of the square was partitioned into a sum of squares due to parents, crosses, and parents vs. crosses with an appropriate degree of freedom. Combining ability analysis and Line × Tester procedures. The four inbreds maintained by three methods (S, HS and FS) i.e., 12 lines and three diverse lines selected to cross with them (testers) to estimate the combining ability.A total number of significant effects obtained through selfing, full-sibbing and half-sibbing as well as the maximum tester effect on the crosses were calculated and the total number of significant SCA effects under each method.

Results and Discussion

Morphological diversity analysis

Morphological dendrogram depicted the clustering pattern by dividing the total inbreds into two major clusters i.e., Cluster I and cluster II. Cluster I is further subdivided into cluster IA and IB and IA into IA-1 and IA-2. Cluster-I consistedof all the lines belonging to two inbreds i.e., Selfed, half-sib and full-sib lines of HKI 193-1 and HKI 1105 of two generations (first and final generation) and few lines of LM10 (selfed and half-sib lines of final generation). Cluster II consists of all six lines of CML163 and four lines of LM10. By assessing the sub-clusteringpattern it denoted most of the selfed lines from the final generation failed to be placed in asimilar sub-cluster. In other words, the similarity of selfed lines is quite low as compared tothe half and full-sibbing (Fig. 1A). Full-sibbing showed a similar kind of clustering pattern in three out of four inbreds whereas, in HKI 1105 distance between full-sib lines of initial and final generations was different as compared to other inbreds. Further, the grouping pattern of LM10, HKI 193-1 and HKI 1105 revealed more changes could be observed through selfing and half-sibbing (because half-sibbing process also involves a considerable amount of selfing). This finding is in agreement with Russel and Vega (1973) who worked with inbred selfing at the population level confirmed the deleterious effect of selfing (purging) which may deteriorate the maintained line (Good and Hallauer,1977; Hallauer *et al.* 2010)recorded the effect of inbreeding depression and selfing in long-term maintained lines.

Molecular Diversity of maintained inbred lines

A total of 504 alleles found, out of which 391 alleles found to be polymorphic with an average of 2.7 alleles at a locus. The gel electrophoresis documented images are given in Fig. 2.

The PIC value revealed the range of 0.20 to 0.75 with an average of 0.47. bnlg 2162 recorded the maximum PIC, expected heterosis and bnlg 1360 recorded the maximum number of alleles. In contrast to this, bnlg 114 marker showed a minimum PIC, He and the number of alleles. The average PIC value determined in the present investigation agreed well with the earlier findings reported based on SSR marker in maize inbred lines (Heckenberger *et al.* 2002; Senior and Heun, 1998; Patto *et al.* 1975). PIC demonstrates the informativeness of the SSR loci and they are potential to detect differences among the inbred lines based on their genetic relationships. Satua et al, 2018 recorded dinucleotide SSR loci (phi 037, nc003, bnlg619, phi054) identified the largest mean number of alleles (4.8) and mean PIC (0.67) as compared to tri, tetra, and pentanucleotide repeats. The present investigation is also in agreement with previous observations of Legesse *et al.* (2018); Kumari *et al.* (2018); Mushtaq *et al.* (2016); Verma *et al.* (2015); Gupta *et al.*(2012).

i. The Jaccard's similarity coefficient:

Themaximum similarity coefficient was recorded between HKI 193-1 FS and LM10 FS (0.63) and the minimum similarity coefficient was reported between HKI 1105 HS and HKI 1105 S (0.33). Among the three methods, full-sibbing showed maximum average similarity index where as,selfing found with the elevated results (high to low). Jaccard's similarity coefficient indicates the molecular distance or the similarity (diversity between the inbred lines).

The similarity index values of different methods are compared to check the efficacy of maintained inbred lines. Among the three methods of comparison, full-sibbing showed the highest similarity between the inbred lines of the first and fourth generation. For example, in HKI 193-1 similarity coefficient was 0.57 for full-sibbing, whereas the value was 0.44 and 0.50 were found for selfing and half-sibbing respectively. A similar pattern of coefficient values was shown by the other three inbreds also. Two inbreds viz., HKI 1105 followed by CML161 showed minimum differences in similarity coefficients in the three methods of maintenance methods. Similar studies of molecular diversity were reported by Sharma *et al.* (2017); Samanthi *et al.* (2012).

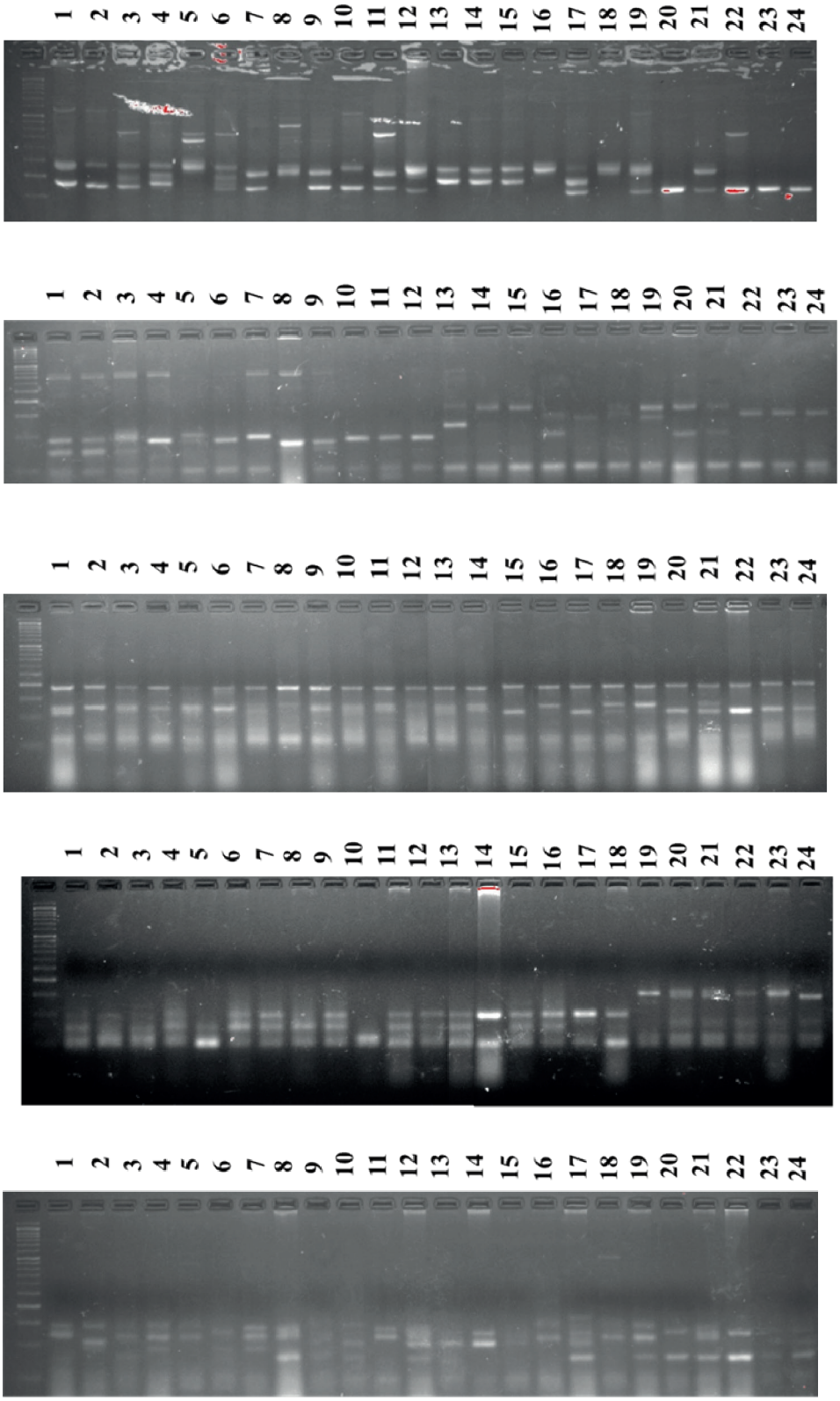

Fig. 2 - Amplification pattern of primer1. bnlg1360 2. phi062 3. bnlg10434. umc16035.bnlg2162for 24 lines maintained through selfing and sibbing. Note: IL Inbred lines; 1. HKI1931S 2. 2HKI1931S 3. HKI1931FS 4. 2HKI1931 FS 5. HKI1931HS 6. 2HKI1931 HS 7. HKI1105S 8. 2HKI1105S 9. HKI1105 FS 10. 2HKI1105 FS 11. HKI1105 HS 12. 2HKI1105 HS 13. CML161S 14. 2CML161S 15. CML161FS 16. 2CML161FS 17. CML161 HS 18. 2CML161 HS 19. LM10S 20. 2LM10S 21. LM10 FS 22. 2LM10 FS 23. LM10 HS 24. 2LM10 HS.2 as a prefix to inbred line indicates line taken from the final (4th) generation of maintaining inbreds.

ii. Cluster analysis:

The lines fall in two major clusters at 0.2 coefficient value i.e., cluster I and cluster II. Cluster I was further subdivided into two sub groups IA and IB. The subcluster IB-1 was further partitioned into IB-1a and IB-1b. Cluster IB-1a was divided into two sub clusters IB-1aa and IB-1ab. This subclustering at different levels enabled to place the lines in different groups or record their grouping pattern (Fig.1B). The maintained inbred lines are given with different letter codes. For example, LM10 means LM10 maintained line is taken from the initial generation and 2LM10 (2 as a prefix) means taken from the final generation. Each inbred is presented in six lines; three from each generation of selfing half-sibbing and full-sibbing.

Four lines viz., HKI 193-1 S, 2HKI 193-1 FS, HKI 193-1 FS and HKI 193-1 HS were grouped in subcluster 1A and their counterparts' 2HKI 193-1 S and HKI 193-1 HS are placed under sub-cluster IB-1aa. All the derived lines of inbred CML161 are placed in sub-cluster IB-2 except CML161 HS. All these three inbreds showed a similar pattern of clustering where the final generation selfed lines are placed away from the rest whereas, inbred HKI 1105 gave contrasting results i.e., all the initial generation's lines (HKI 1105 S, HKI 1105 HS, HKI 1105 FS) were found in subcluster I B-1b and all three lines of the last generation of evaluation were placed in sub-cluster IB-1aa (2HKI 1105 S, 2HKI 1105 HS, 2HKI 1105 FS). In cluster II, all LM10 inbreds comparing three methods are grouped into two sub-clusters except LM10 S, where 2LM10 S falls under a separate cluster (cluster I) and other lines of LM10 i.e., LM10 HS, 2LM10 HS, LM10 FS and 2 LM10 FS, whereas LM10 S found in the cluster I's sub-cluster 1B-1 along with CML161 HS line.

The clustering pattern denotes selfed lines especially selfed lines of the final generation of maintenance evaluation are deviating in their clustering pattern indicating selfing leads to more changes in inbred maintenance than sibbing. The full sibbed lines are found in asimilar cluster in three out of four inbreds depicts the favourable stable performance over the generations of maintenance. The Jaccard's similarity coefficient has also given similar results as the full sibbed lines of the first and fourth generation showed higher similarity values as compared to the other two methods. At the geneticlevel, this was discussed by many researchers. The continuous accumulations of mutations in inbreds and purging at the population level are the major causes (Kashiani *et al.*, 2014; Betran *et al.*, 2003; Tokatlidis *et al.*, 1999; Barrett and Charlesworth, 1991; Donold, 1945). Similar studies to support the changes through maintenance of inbreds at the molecular level in pearl millet restorer lines by Gupta *et al.*, 2012. These groupings, in most instances, revealed evidence of associations related to their pedigree records. This is in agreement with earlier investigators (Reif *et al.*, 2003; Senior and Heun, 1998; Smith *et al.*, 1997), who demonstrated the correspondence of SSR marker distance with pedigree information in maize. Alternatively, groupings of the inbred lines based on their adaptation profiles were also evident based on the cluster analysis. This study is in accordance with Kai *et al.* (2009); Gethi *et al.* (2002); Melchinger *et al.* (1991).

iii. Degree of correspondence between the morpho-molecular diversity of maintained lines

In morphological diversity, the maximum contribution has been observed in inbred LM10 followed by HKI 1105. In molecular diversity analysis, LM10 followed by HKI 193-1 contributed maximum for diversity whereas the lines evaluated through three maintenance methods for two generations (initial and final) fall in separate clusters and recorded with maximum distance.

To test the correspondence (degree) between the genetic distances based on phenotypic data and molecular (SSR) data, the distance matrices were compared using Mantel's (1967) test. The analysis revealed a positive and significant correlation found between the two matrices, with r = 0.63 (P<0.001). Three inbredsviz., HKI 193-1, CML161 and LM10 lines showed asimilar pattern of clustering at a morpho-molecular level whereas, HKI 1105 showed a different clustering pattern. A similar kind of comparisons has been done through Mental's test for inbred diversity analysis by Palumbo *et al.* (2017) and Sharma *et al.* (2010).

Mantel's test through diversity analysis revealed that full-sibbing is the best method of maintenance and inbred CML161 found with minimum differences over the generations among the three methods of comparison. The information obtained from the molecular studies were confirmed with the phenotypic data. It indicates that there is further scope to evaluate the inbreds for multi-seasons and selecting the most suitable parental lines for generating improved maize hybrids. This information can be a preliminary source for allele mining or gene discovery or whole-genome fingerprinting.

The lines which are not following the molecular and morphological clustering pattern similarity otherwise overlapping might be due to the effects of selection, drift, and mutation on the DNA markers or human errors (Warburton *et al.*, 2008). Secondly, an inbred line that is related to two other inbred lines from separate clusters will be grouped with one to which it is most closely related. Overall, this study indicated that SSR markers largely separated the lines into different clu-

Table 1 - Summary of significant F-tests for generations of sib-mated and selfed lines

		PG	PH	EH	LA	NL	DT	ASI	DS		
HKI 193-1 S	Lienar			*	**	*		**			
	Quadratic				***	*		**			
	Cubic				***	*		**			
HKI 193-1 FS	Lienar										
	Quadratic										
	Cubic								*		
HKI 193-1 HS	Lienar				**		**	***	*		
	Quadratic		*	**	**	**	***	***	*		
	Cubic		**	***	*	**	***	***			
HKI 1105 S	Lienar	*		**			**	*	***		
	Quadratic	*		*	*		*	*	**		
	Cubic			*	*				**		
HKI 1105 -FS	Lienar			*		*	*		*		
	Quadratic						*	*	*		
	Cubic						*				
HKI 1105 HS	Lienar	***				***			*		
	Quadratic	***	*			***			**		
	Cubic	**				***			**		
CML161 S	Lienar	*		***	**				*		
	Quadratic		**	**	***		**				
	Cubic		**	**	***		**				
CML 161 FS	Lienar					**			*		
	Quadratic					*					
	Cubic										
CML 161 HS	Lienar				*			**	*		
	Quadratic							*			
	Cubic										
LM10 S	Lienar		**					**			
	Quadratic		**					***			
	Cubic		*				*	***			
LM10 FS	Lienar		*			*	*				
	Quadratic		*								
	Cubic										
LM10 HS	Lienar				*						
	Quadratic										
	Cubic										
		DM	**CP**	**BP**	**CD**	**RPC**	**KR**	**TSS**	**MP**	**YP**	**HSW**
HKI 193-1 S	Lienar		***		*	***				*	
	Quadratic		***		*	***					
	Cubic		***			***					
HKI 193-1 FS	Lienar	**			**			***			
	Quadratic	***			**			**			
	Cubic	**			**		**	*			
HKI 193-1 HS	Lienar		*	**			*			**	
	Quadratic	*	***	**			**			*	
	Cubic	***	***	*			*			*	
HKI 1105 S	Lienar	***		**		**	*		*		
	Quadratic	***		*		***			*		
	Cubic	**				***			***		
HKI 1105 -FS	Lienar	*	*					*			
	Quadratic	*	*			*		*			
	Cubic	*	*		**		*	*		***	
HKI 1105 HS	Lienar		*	*	*				**		*
	Quadratic		*	*					*		
	Cubic		*						*		
CML 161 S	Lienar										***
	Quadratic										***
	Cubic										**
CML 161 FS	Lienar	*		*			*				*
	Quadratic	*		*							
	Cubic	*									
CML 161 HS	Lienar		*	**							
	Quadratic			*							
	Cubic										
LM10 S	Lienar	*	*						*		*
	Quadratic		*						**		*
	Cubic										
LM10 FS	Lienar							***			*
	Quadratic							**			*
	Cubic							**			
LM10 HS	Lienar										
	Quadratic										
	Cubic										

sters, which generally agreed with their morphological records and adaptation regimes.

Variability analyses

Genetic variability and diversity gave more or less similar meaning theoretically but practically both are quite different. Genetic variability mainly occurs at an allelic level. However, diversity is the variations within and among species in the form of genetic makeup. There are two important points to comprehend about the term; one is that it relates to genetic material, and the other is that it could be related toeither one species or more than that. The deviation from the Hardy Weinberg equilibria i.e., the influence of active forces of nature like migration, mutation, selection and random drift are the driving forces behind diversity, whereas sexual reproduction and mutation are the two quenching forces behind genetic variability. In maize, a pool of inbreds and variability (allelic variation) collectively contribute to the diversity; hence, the existence of variations and diversity in genetic materials helps the species to thrive through increased adaptability for the changing environmental conditions. Therefore, in this investigation diversity of the maintained lines and the variability (the study of genetic changes) are emphasized as separate objectives. The amount of genetic change under the methods of selfing and sibbing was evaluated by comparing the means of initial and final generations of lines that had been maintained successively for four generations. Significant variation among the mean of initial and final generations was considered as a cause by genetic change or the effect of random deviation.

According to Russel and Vega (1973); Bogenschutz and Russel (1986), under the selfing procedure, a mutated allele or an allele from remnant heterozygosity would have a 25% probability (assuming no selection) of becoming fixed in a progeny plant. If such plant will be mutated, the homozygous locus is harvested in the successive generation, the fixation might cause an alteration in a line performance which can be detected by a significant F-tests among-generations means. Under the sibbing procedure, a deviating allele, introduced by mutation or remnant heterozygosity, should remain near its original low frequency. The probability of fixation under sib-mating with no selection is extremely low (Crow and Kimura, 1970).

The fitting of polynomial models facilitated genetic interpretation of the events that lead to changes and better comparison. Wherever significant variation occurred, the genetic interpretation depended upon the type of significant events observed. Under sibbing, if alleles introduced by mutation accumulate within the line, this would have caused gradual change probably best explained by a linear trend (meant to give clear results). Although the linear model did account for much of the genetic variation there were many instances in which data under selfing, fixation of a deviating allele with measurable effect can appear as a cubic response across successive generations. The actual response will depend on the generation in which the mutation occurs, the magnitude of the effect, and the generation in which the unfavourable allele becomes fully expressed. Therefore, a single-gene event in these experiments can appear as a linear or quadratic as well as a cubic response. Asimilar experimental outcome was also obtained in Russel and Vega, 1973 experiments.

The data used to study the genetic changes by deriving the mean is also considered here to assess the genetic variability in the lines maintained through selfing and sibbing. F-tests are presented in Table 1and summarized in Table 2. Out of the 216 F-tests (18 traits in 12 lines), 183 showed significance among which 73 for selfing, 50 for full sib-mating and 60 for half-sibbing. Further, the F- tests were partitioned into quadratic, cubic and linear regression to partition the variability efficiently and avoid errors. Among the F-tests conducted for selfing, 28 linear events, 26 quadratic and 19 cubic trends were recorded. For full-sibbing the proportion was 21, 16 and 13 for linear, quadratic and cubic and for half-sibbing it was 22 (linear), 22 (quadratic) and 16 (cubic). With the highest range of 22, HKI 193-1 found to be most unstable. For HKI 1105 with the lowest range (six). Rest two inbreds i.e., CML161 and LM10 showed a range of seven and thirteen respectively. That indicates a line with a minimum range is considered as most stable (according toBogenschutz and Russel, 1986). However, the percent contribution of selfing full-sibbing and half-sibbing were 40 %, 27 % and 32.79 % respectively. That denotes with minimum range (difference), HKI 1105 is considered asthe most stable in terms of giving a wide range of partitioning of regression in a better way as well as selfing (40 %) contributed maximum events to the total F- tests which signify that selfing gives more variability followed by half and full-sibbing. In other words,selfing contributed to more loss of plant vigour.

Fitting the values in orthogonal polynomial equation enabled in partitioning the total variability into quadratic, cubic and linear curves. Among the three, the quadratic and cubic pattern has shown similar curves, therefore only linear and quadratic curves are presented in Supplementary data, Figure S1. The coefficient of determination and probability values are given at the left sidetop corner of each figure. As the values of $p< 0.05$ are considered the actual genetic cause for va-

Table 2 - Summary of the number of significant F-tests showing the relative amount of change through selfing and sibbing for 18 characters

Inbreds	Selfing				Full sib				Half-sib (bulk-sibbing)				Inbred comparison
	Linear	Quadratic	Cubic	Total events	Linear	Quadratic	Cubic	Total events	Linear	Quadratic	Cubic	Total events	Total Range
HKI 193-1	8	6	5	19	3	3	4	10	8	13	11	32	**22**
HKI 1105	10	10	6	26	7	7	7	21	8	7	5	20	6
CML- 161	4	5	5	14	6	3	1	10	5	2	0	7	7
LM10	6	5	3	14	5	3	1	9	1	0	0	1	13
Overall significant variables	28	26	19		21	16	13		22	22	16	0	
Per cent contribution		40.00			27.32			32.79			183		

riation, the curves giving only clear-cut results are presented in figures. In the case of selfed lines, increasing trends have been shown not to be a desirable change as plant height should be a constant character over the generations, the similar trend was followed through full-sibbing whereas, half-sibbing showed a decreasing pattern in linear and quadratic distribution. As the probability is <0.05 the cause for this trait is found to be genetic. As the probability value is <0.05 the cause is assumed to be genetic under linear as well as quadratic trends. E.g. Days to 50 percent flowering in inbred HKI 193-1.

Russell & Vega (1973) reported that long-time inbreds maintained by ear-to-row selfing had changed significantly over the successive generations. Bogenschutz and Russell, 1986 showed that lines maintained under selfing had undergone a significant amount of change. Whereas, this investigation with both genetic and molecular studies concluded that selfing was a major cause for the genetic changes in maintained lines. However, for developing inbreds, selfing helps to attain fast homozygosity which enhances the inbreds availability in a short span as well as helps to enrich the gene pool, but in terms of maintenance, sibbing, especially full-sibbing found best as the half-sibbing method also includes selfing to some extent. It also seems logical that breeders' seed of all inbred lines are held in cold storage from which samples can be obtained occasionally for future comparisons and reproduction may enable to recover the inbred line's original genetic integrity (as reported in rice by Peng et al, 2010).

A rapid decrease in percent germination was found in inbred CML161 through selfing whereas, sibbing method showed a slight increase over the generations. The linear, as well as quadratic trends, were recorded with p<0.05 and the cause was genetic. In comparison with earlier research, it suggested that a level of genetic complexity could be unexplainable by simpler models. In a few traits with significant remainders, such as germination and yield of the selfed LM10, large reductions in means occurred in a manner following precisely what is expected from mutation at major loci (Table 2). The other observations indicated that similar results as found in the percent change for each trait of the maintained inbreds for consecutive four generations. Present results as well as the previousstudies

Table 3 - GCA effect for 18 traits

Lines	PG	PH	EH	NL	DT	ASI	DS	DM	BP	CD	PCF	RPC	KR	TSS	MP	YP	HSW	CPP
L 1	-5.56	-10.35**	-8.93**	-1.30**	1.35**	0.29	1.05**	0.94	-0.35	-6.17**	-0.31	-0.87**	-5.91**	1.68**	0.75	-0.22**	-2.79**	-1.16**
L 2	-10.00**	-0.46	1.41	0.028	-1.09**	-0.90**	-0.50	-2.50**	-1.02**	-6.18**	3.01**	0.69**	4.42**	0.75**	-0.60	-0.03	-0.52	0.72
L 3	-1.67	-5.24	-2.59	0.361	-0.20	-0.25	0.05	-0.72	1.65**	12.33**	-4.14**	0.02	1.53	1.35**	-0.42	-0.03	-1.77**	-0.83
L 4	-7.22	-18.90**	-10.48**	-0.86**	3.57**	1.51**	2.056**	1.83**	-0.46	-0.15	-0.42	-1.54**	-4.36**	-0.53	-0.20	-0.16**	-1.10**	-1.38*
L 5	-4.44**	-6.68**	2.63	0.25	-1.20**	-0.37	0.16	-1.39**	-1.13**	-16.59**	1.17	0.46	2.31**	-0.78**	0.11	0.24**	2.53**	1.38**
L 6	-5.00	-14.01**	-4.48**	-0.30	2.24**	0.29	1.94**	0.72	1.32**	9.69**	0.13	-0.20	-2.80**	0.38	-0.14	-0.27**	-0.02	-0.61
L 7	-14.44**	-12.64**	-8.04**	-0.75**	1.13**	-0.25	1.38**	-0.72	-0.46	-5.50**	-0.52	-0.87**	0.64	1.92**	1.70**	0.17**	-0.83**	1.94**
L 8	0.56	17.09**	8.63**	2.36**	-2.09**	-0.81**	-1.27**	-1.61**	-0.91**	-3.89**	2.06	0.91**	5.53**	1.51**	2.14**	0.49**	1.18**	3.27**
L 9	13.89**	12.75**	6.07**	1.13**	-0.20	-0.25	0.05	0.39	1.09**	8.40**	-0.65	0.02	2.81**	0.95**	1.60**	0.24**	-0.86**	1.72**
L 10	11.67**	-4.24	3.63	-1.30**	-0.42	0.63**	-1.05**	2.61**	-1.02**	0.97	-1.20	-0.20	-4.36**	-2.94**	-1.10**	-0.29**	0.38**	-1.72**
L 11	13.33**	12.42**	4.85**	0.47	-3.64**	-0.14	-3.50**	-0.83	-0.13	-1.94	1.60	1.13**	0.98	-2.93**	-1.82**	0.47**	2.53**	-0.50
L 12	8.89**	14.98**	0.30	-0.08	-0.42	-0.03	-0.38	1.28	1.43**	8.88**	-0.74	0.46	-0.80	-1.34**	-2.02**	-0.21**	1.26**	-2.83**

Table 4 - SCA effect of 36 cross combinations for 18 traits

Crosses	PG	PH	EH	LA	DT	ASI	DS
HKI 193-1 S x HUZM 185	2.06	5.54	4.98	0.33	-0.51	0.64	-1.16
HKI 193-1 S x HKI 1126	-1.24	-6.92	-3.54	-1.05	1.03	-0.18	1.22
HKI 193-1 S x HKI 323	-0.82	1.38	-1.43	0.72	-0.51	-0.46	-0.05
HKI 193-1 FS x HUZM 185	5.62	3.99	-6.01	0.33	-1.07	0.53	-1.61
HKI 193-1 FS x HKI 1126	-4.68	-13.14**	-7.45**	0.27	0.48	-0.29	0.77
HKI 193-1 FS x HKI 323	-0.93	3.15	4.56	-0.61	0.59	-0.24	0.83
HKI 193-1 HS x HUZM 185	5.95	5.10	-5.35	0.33	0.03	0.53	-0.50
HKI 193-1 HS x HKI 1126	-4.01	-8.70	0.78	0.61	0.92	-0.20	1.22
HKI 193-1 HS x HKI 323	-1.93	3.60	4.56	-0.94	-0.96	-0.24	-0.72
HKI 1105 S x HUZM 185	-1.93	0.10	3.87	-0.11	-2.07 **	-0.24	-1.83 *
HKI 1105 S x HKI 1126	4.09	8.29	3.00	0.16	1.81*	-0.74	2.55 **
HKI 1105 S x HKI 323	-2.15	-13.39**	-6.88 *	-0.05	0.25	0.98*	-0.72
HKI 1105 FS x HUZM 185	4.17	-0.45	3.09	-0.55	-1.29	-0.68	-0.61
HKI 1105 FS x HKI 1126	-1.46	16.07 **	3.56	0.38	-1.90**	0.48	-1.88 *
HKI 1105 FS x HKI 323	-2.71	-15.62 **	-6.65	0.16	2.70**	0.24	2.50 **
HKI 1105 HS x HUZM 185	3.39	0.21	6.87 *	-0.33	-1.40	-0.68	-0.72
HKI 1105 HS x HKI 1126	1.09	15.74 **	1.67	-0.05	1.48	0.48	1.00
HKI 1105 HS x HKI 323	-4.49	-15.95 **	-8.54*	0.38	-0.07	0.20	-0.27
CML161 S x HUZM 185	-9.38 **	-4.78	-5.90	-0.22	-0.96	0.204	-1.16
CML161 S x HKI 1126	-1.68	-0.92	-2.10	0.05	3.59**	0.03	3.55**
CML161 S x HKI 323	11.06 **	5.71	8.00*	0.16	-2.63**	-0.24	-2.38 **
CML161 FS x HUZM 185	-6.38	-2.56	-3.24	-0.33	-0.74	-0.24	-0.50
CML161 FS x HKI 1126	2.98	-2.03	-2.76	0.611	-0.85	-0.07	-0.77
CML161 FS x HKI 323	3.39	4.60	6.00	-0.27	1.59*	0.31	1.27
CML161 HS x HUZM 185	-8.82 *	-8.23	-2.35	0.22	-1.29	-0.46	-0.83
CML161 HS x HKI 1126	5.53	3.29	-3.54	-0.16	3.25**	-0.29	3.55**
CML161 HS x HKI 323	3.28	4.93	5.89	-0.05	-1.96 *	0.75	-2.72 **
LM10 S x HUZM 185	-1.38	6.10	-3.24	0	3.92 **	-0.01	3.94**
LM10 S x HKI 1126	3.64	-9.70	9.89**	-0.05	-4.51 **	0.48	-5.00 **
LM10 S x HKI 323	-2.26	3.60	-6.65	0.05	0.59	-0.46	1.05
LM10 FS x HUZM 185	2.50	-6.23	2.87	0.22	1.81 *	0.09	1.72
LM10 FS x HKI 1126	-1.46	0.96	-3.99	-0.83	-1.96 *	0.25	-2.22*
LM10 FS x HKI 323	-1.04	5.26	1.12	0.611	0.14	-0.35	0.50
LM10 HS x HUZM 185	4.17	1.21	4.42	0.111	3.59**	0.31	3.27**
LM10 HS x HKI 1126	-2.79	-8.92	-4.43	0.056	-3.85 **	0.14	-4.00**
LM10 HS x HKI 323	-1.38	7.71	0.00	-0.16	0.25	-0.46	0.72
CD 95 %	6.77	10.02	6.78	1.18	1.55	0.79	1.78

of Bogenschutz and Russell (1986); Russell and Vega (1973); Higgs and Russell (1968); Fleming *et al.* (1964); Russell *et al.* (1963) suggested that genetic change in long-time inbred lines is continuous. Several lines have shown consistent levels of genetic variation in different studies, which presumes that mutation and cryptic structural changes during the continuation of crop generations over a prolonged time. Some lines show consistent patterns of stability; for instance, little change was observed for inbred HKI 1105 where major changes were observed in HKI 193-1 and LM10. Based on this evidence, it seems that inbred lines may be inherently stable or unstable, alluding to the presence of mutating systems of inducing instability in certain lines, as presumed by McClintock, 1978; Busch and Russell, 1964.

Combining ability of inbreds

i. GCA of inbred lines - A event of significance in a favourable direction means some traits may be expected to be significant but with negative valuese.g. plant height, ear height, days to 50 % tasselling, ASI, days to 50 % silking, days to physiological maturity, no. of barren plants in a row, per cent moisture etc. and rest of the characters given apart from these characters are expected to be positive with significant effects. overall GCA (of inbred lines) effects are table 3. Among the three methods, full-sibbing has been reported in maximum number i.e., 29 out of 49 total significant effects. Selfing recorded 12 significant GCA effects followed by half-sibbing (8 GCA effects).

Table 4 - SCA effect of 36 cross combinations for 18 traits

Crosses	DM	BP	CD	PCF	RPC
HKI 193-1 S x HUZM 185	1.08	-0.25	0.15	-0.29	-0.40
HKI 193-1 S x HKI 1126	-0.80	0.24	-2.47	1.70	-1.18*
HKI 193-1 S x HKI 323	-0.27	0.01	2.32	-1.41	1.59**
HKI 193-1 FS x HUZM 185	0.52	-0.25	0.40	-0.07	-0.63
HKI 193-1 FS x HKI 1126	1.97	-0.09	1.84	0.86	-0.74
HKI 193-1 FS x HKI 323	-2.50*	0.35	-2.25	-0.79	1.37 *
HKI 193-1 HS x HUZM 185	2.08	1.07 *	0.23	-5.60**	0.03
HKI 193-1 HS x HKI 1126	0.52	-0.75	5.50 *	0.51	-0.74
HKI 193-1 HS x HKI 323	-2.61 *	-0.31	-5.73 *	5.09**	0.70
HKI 1105 S x HUZM 185	-2.47 *	0.18	-10.86 **	0.91	0.25
HKI 1105 S x HKI 1126	0.63	0.35	0.56	2.17	-0.51
HKI 1105 S x HKI 323	1.83	-0.53	10.29**	-3.09	0.25
HKI 1105 FS x HUZM 185	-1.91	-0.14	-0.69	2.10	0.25
HKI 1105 FS x HKI 1126	-0.80	0.35	-3.36	2.47	-0.51
HKI 1105 FS x HKI 323	2.72*	-0.20	6.05*	-4.58 *	0.25
HKI 1105 HS x HUZM 185	-1.69	0.40	-10.50**	-2.45	0.25
HKI 1105 HS x HKI 1126	0.41	0.24	5.27 *	-0.14	-0.51
HKI 1105 HS x HKI 323	1.27	-0.64	5.22 *	2.59	0.25
CML161 S x HUZM 185	-0.25	-0.48	11.98**	0.89	0.25
CML161 S x HKI 1126	1.52	0.01	-4.15	-0.41	0.14
CML161 S x HKI 323	-1.27	0.46	-7.83**	-0.47	-0.40
CML161 FS x HUZM 185	-1.69	-0.03	12.82**	1.85	-0.18
CML161 FS x HKI 1126	2.75*	0.13	-8.01**	-3.00	0.37
CML161 FS x HKI 323	-1.05	-0.09	-4.80	1.14	-0.18
CML161 HS x HUZM 185	-1.36	-0.03	12.09**	2.41	0.03
CML161 HS x HKI 1126	1.08	0.13	-5.79*	0.60	-0.07
CML161 HS x HKI 323	0.27	-0.09	-6.30 *	-3.01	0.03
LM10 S x HUZM 185	2.417 *	0.07	-6.78 **	1.70	0.92
LM10 S x HKI 1126	-2.472 *	0.24	5.16*	-1.82	0.14
LM10 S x HKI 323	0.056	-0.31	1.62	0.12	-1.07
LM10 FS x HUZM 185	2.86 *	-0.81	4.20	-1.64	0.25
LM10 FS x HKI 1126	-2.69 *	0.64	1.09	0.64	1.48**
LM10 FS x HKI 323	-0.16	1.46**	-5.29 *	1.00	-1.74**
LM10 HS x HUZM 185	0.41	0.29	-13.04 *	0.19	-1.07
LM10 HS x HKI 1126	-2.13	-0.20	4.35	-3.61	2.14**
LM10 HS x HKI 323	1.72	-0.09	8.69	3.42	-1.07
CD 95 % SCA	2.31	1.02	5.03	3.79	1.10

ii. Effect of maintenance methods on SCA -A set of four inbreds compared through three maintenance methods wassubjected to specific combining ability analysis (9), yielding 36 cross combinations (four inbreds x three methods). Due to the limited base material, only a few significant events were recorded for the different traits. A maximum number of cross combinations with a significant effect of SCA (Table 4) were recorded in full-sibbing (31 effects) followed by selfing (28 SCA effects) and half-sibbing (26 effects). Similarly, among the three testers taken, inbred line HKI 323 found to be a better combiner (selection based on several crosses involving HKI 323 as a common tester) however, GCA failed to differentiate the tester effects. The best three combinations found through combining ability analysis (irrespective of maintenance methods used to compare the inbreds) based on yield and yield-related traits.

The estimation of combining ability denotes, the full-sibbing is a better method followed by selfing and half-sibbing. The probable cause for this kind of outcome might be the minimum deviation of allelic integrity in full-sibbing, as in the sister plant mating and the intensity of accumulation of deleterious alleles are quite less as it mimics the natural outcrossing process. Whereas, half-sibbing failed to give a favourable number of GCA effects, maybe due to unfavourable allelic effect in the long run and the deleterious allelic load could not be eliminated immediately after one generation

Table 4 - b - SCA effect of 36 cross combinations for 18 traits

Crosses	KR	TSS	MP	YP	HSW	CP
HKI 193-1 S x HUZM 185	-3.39*	-3.45**	-0.51	-0.28 **	-0.86	-0.47
HKI 193-1 S x HKI 1126	-0.11	0.90	0.77	-0.20*	0.04	-0.69
HKI 193-1 S x HKI 323	3.50*	2.54**	-0.25	0.49**	0.81	1.16
HKI 193-1 FS x HUZM 185	2.27	-2.51**	-0.89	-0.26**	-1.66**	-0.69
HKI 193-1 FS x HKI 1126	-0.11	1.03	0.85	-0.25 **	1.71 **	-1.58
HKI 193-1 FS x HKI 323	-4.15**	1.48 *	0.03	0.51**	-0.05	2.27*
HKI 193-1 HS x HUZM 185	-0.50	-0.25	-0.01	-0.01	-0.47	1.19
HKI 193-1 HS x HKI 1126	0.10	1.67**	0.43	-0.33**	-0.57	-2.69**
HKI 193-1 HS x HKI 323	0.39	-1.42 *	-0.42	0.34**	1.05	1.50
HKI 1105 S x HUZM 185	-4.28**	3.96**	-1.22	0.175	0.55	1.08
HKI 1105 S x HKI 1126	0.32	-1.48*	0.22	0.36	0.95	0.19
HKI 1105 S x HKI 323	3.95**	-2.47**	1.00	-0.21*	-1.50 *	-1.27
HKI 1105 FS x HUZM 185	0.05	3.80**	-2.03*	-0.12	2.35**	-2.02*
HKI 1105 FS x HKI 1126	-4.67**	-2.04 **	0.94	-0.16	0.40	0.41
HKI 1105 FS x HKI 323	4.62 **	-1.76 **	1.09	0.28**	1.30 *	1.61
HKI 1105 HS x HUZM 185	-3.50 *	1.71 **	-0.52	0.19 *	0.34	-0.02
HKI 1105 HS x HKI 1126	-0.22	-0.59	-0.94	0.25	1.07	-0.25
HKI 1105 HS x HKI 323	3.73**	-1.12	1.46	-0.06	-1.41 *	0.27
CML161 S x HUZM 185	4.05**	1.07	1.77	-0.17	0.61	-1.91 *
CML161 S x HKI 1126	-0.00	-0.80	-0.67	0.36**	0.58	3.52**
CML161 S x HKI 323	-4.04**	-0.26	-1.09	-0.18	-1.20 *	-1.61
CML161 FS x HUZM 185	6.16**	-0.05	2.32 *	0.06	-0.26	-0.25
CML161 FS x HKI 1126	-4.56**	-0.86	-1.28	0.29 **	0.84	1.52
CML161 FS x HKI 323	-1.60	0.91	-1.04	-0.36**	-0.58	-1.27
CML161 HS x HUZM 185	4.88**	-0.25	2.37*	-0.12	0.37	2.30 **
CML161 HS x HKI 1126	1.82	-0.12	-0.77	0.55**	1.17	0.41
CML161 HS x HKI 323	-6.71**	0.38	-1.59	-0.43**	-1.55*	-2.72 **
LM10 S x HUZM 185	0.38	-1.59*	-1.62	-0.0	0.07	-2.25 *
LM10 S x HKI 1126	-0.00	1.59 *	-0.50	0.02	-0.49	2.19 *
LM10 S x HKI 323	-0.38	0.00	2.13*	0.03	0.41	0.05
LM10 FS x HUZM 185	-1.61	-2.17**	0.18	0.47**	-0.84	2.52**
LM10 FS x HKI 1126	1.66	0.81	0.43	0.05	-2.67**	-2.36 **
LM10 FS x HKI 323	-0.04	1.35*	-0.62	0.42**	3.52**	-0.16
LM10 HS x HUZM 185	-4.50**	-0.26	0.18	0.12	-0.20	0.52
LM10 HS x HKI 1126	5.77**	-0.10	0.50	-0.13	-1.60**	-0.69
LM10 HS x HKI 323	-1.26	0.36	-0.68	0.22	1.80**	0.16
CD 95 % SCA	2.69	1.21	1.83	0.19	1.19	1.72

of outcrossing. As per Falconer, 1979 and previous reports of several researchers, the effects of selfing on the inbreds are immediately overcome by a single generation of cross-pollination. But in the present investigation response of selfed lines to hybridization was found to be lower than full sibbed lines. The studies on combining ability through L x T was also done by many scientists but there are no reports on comparing the maintenance methods through combining ability analysis. The diversity analysis, estimation of genetic changes, variability studies and combining ability analyses showed that full-sibbing is the best method of inbred maintenance. Italso concludes that inbred line HKI 1105 followed by CML161 were the stable inbreds in terms of genetic changes across the generations irrespective of the methods used to maintain them.

Conclusions

The investigation to assess the changes in maintained lines after a favourable period of maintenance and effect on the yield and yield-related (quantitative) traits revealed that selfing caused the highest loss of vigour whereas the full sibbing method was most stable. The probable cause for this kind of outcome might be the minimum deviation of allelic integrity in full-sibbing, as in the sister plant mating and the intensity of accumulation of deleterious alleles are quite less as it mimics the natural outcrossing process. Whereas, half-sibbing failed to give a favourable number of GCA effects, may be due to unfavourable allelic effect in the long run and the deleterious allelic load could not be eliminated immediately after one generation of outcrossing. The

study of comparing the inbred maintenance method helped to identify the best-inbred maintenance method not only through morphological observations but also boosted through molecular studies which revealed that inbred maintenance through full sibbing is the best ever method and continuous selfing is least preferred as the method proved to be detrimental to the genetic constitution of inbreds.

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Table S1 - List of polymorphic markers, PIC value and heterozygosity value

S. No.	Marker	No. of alleles	He	PIC	S. No.	Marker	No. of alleles	He	PIC
1	umc1842	3	0.39	0.35	38	umc 1652	2	0.46	0.35
2	bnlg1586	2	0.50	0.37	39	phi 059	3	0.64	0.57
3	Umc1088	2	0.50	0.37	40	umc 1035	3	0.55	0.47
4	umc1586	2	0.49	0.37	41	umc 226	3	0.65	0.58
5	umc1130	3	0.57	0.50	42	umc 1015	2	0.50	0.37
6	bnlg1360	7	0.77	0.74	43	bnlg 1520	3	0.64	0.57
7	bnlg1867	4	0.71	0.66	44	nc 009	2	0.50	0.38
8	bnlg1714	2	0.26	0.22	45	umc 0063	2	0.41	0.33
9	umc1492	2	0.42	0.33	46	phi 0057	2	0.50	0.38
10	bnlg1712	2	0.42	0.33	47	phi 034	2	0.48	0.37
11	phi027	2	0.50	0.37	48	bnlg 1152	3	0.65	0.57
12	bnlg1079	2	0.47	0.36	49	umc 1896	2	0.50	0.38
13	bnlg 1265	2	0.49	0.37	50	umc 1969	2	0.35	0.29
14	bnlg 1126	2	0.38	0.30	51	bnlg1937	2	0.50	0.38
15	phi 119	2	0.46	0.35	52	umc2061	2	0.50	0.37
16	bnlg 2162	5	0.79	0.76	53	umc1142	2	0.50	0.37
17	bnlg 1046	3	0.53	0.43	54	umc1303	2	0.26	0.22
18	bnlg 1839	2	0.44	0.35	55	umc1031	2	0.46	0.35
19	bnlg 1185	4	0.60	0.53	56	umc1276	2	0.45	0.35
20	umc 1654	2	0.38	0.31	57	bnlg1257	2	0.47	0.36
21	umc 1380	2	0.42	0.33	58	bnlg1124	3	0.53	0.43
22	bnlg 2086	2	0.47	0.36	59	phi086	3	0.53	0.43
23	bnlg 2277	2	0.46	0.35	60	umc2208	2	0.44	0.35
24	bnlg 1754	4	0.69	0.63	61	umc1932	5	0.76	0.72
25	bnlg 1070	3	0.58	0.52	62	umc1770	2	0.50	0.38
26	phi112	2	0.32	0.27	63	umc1329	2	0.39	0.32
27	umc 1422	5	0.75	0.70	64	umc1106	2	0.44	0.34
28	umc 2319	2	0.38	0.30	65	umc1692	2	0.50	0.38
29	bnlg 1523	3	0.66	0.58	66	phi035	2	0.49	0.37
30	bnlg 2082	3	0.57	0.51	67	umc1085	4	0.68	0.63
31	bnlg 1287	2	0.48	0.36	68	umc2250	2	0.39	0.32
32	bnlg 339	2	0.35	0.29	69	umc1657	2	0.39	0.32
33	umc 2190	2	0.32	0.27	70	phi328189	4	0.68	0.63
34	umc 2284	3	0.54	0.48	71	bnlg1434	2	0.39	0.32
35	bnlg 1452	2	0.50	0.37	72	umc2288	5	0.76	0.72
36	umc1858	2	0.50	0.37	73	umc1482	2	0.39	0.32
37	umc 1395	2	0.42	0.33	74	bnlg 1014	2	0.46	0.35
75	bnlg 1866	2	0.42	0.33	111	phi065	2	0.39	0.32
76	bnlg 1067	2	0.50	0.37	112	umc2365	3	0.53	0.43
77	umc 2088	2	0.42	0.33	113	umc2101	4	0.68	0.63
78	umc 1259	2	0.46	0.35	114	umc1991	3	0.53	0.43
79	umc 1633	2	0.50	0.37	115	phi364545	4	0.68	0.63
80	bnlg 114	2	0.23	0.20	116	bnlg279	2	0.39	0.32
81	umc 2056	2	0.48	0.36	117	umc1543	4	0.68	0.63
82	umc 1165	3	0.65	0.58	118	Phi96100	3	0.64	0.56
83	bnlg 1160	3	0.64	0.57	119	bnlg1297	4	0.68	0.63
84	bnlg 1012	2	0.50	0.37	120	umc 1465	3	0.64	0.56
85	phi 085	2	0.50	0.37	121	Umc1446	2	0.39	0.32
86	umc 1669	2	0.50	0.37	122	umc1859	3	0.64	0.56
87	umc 2173	2	0.50	0.37	123	umc 1824	4	0.68	0.63
88	umc 1539	2	0.43	0.34	124	umc1320	2	0.39	0.32
89	umc 1117	2	0.38	0.30	125	umc050	2	0.39	0.32
90	bnlg 2323	3	0.60	0.51	126	phi084	2	0.39	0.32
91	phi 101049	2	0.48	0.37	127	Umc1934	3	0.64	0.56

S. No.	Marker	No. of alleles	He	PIC	S. No.	Marker	No. of alleles	He	PIC
92	bnlg 1893	2	0.40	0.32	128	umc1178	3	0.64	0.56
93	umc 1550	2	0.49	0.37	129	bnlg238	4	0.68	0.63
94	umc 1066	2	0.26	0.22	130	umc1221	4	0.68	0.63
95	phi 113	4	0.66	0.62	131	umc2161	5	0.76	0.72
96	umc 1277	2	0.50	0.37	132	phi 1001	4	0.68	0.63
97	phi 0128	2	0.50	0.38	133	phi062	5	0.76	0.72
98	phi 129	2	0.46	0.35	134	umc2276	2	0.39	0.32
99	umc 1735	2	0.42	0.33	135	umc1593	3	0.64	0.56
100	umc 1069	2	0.50	0.37	136	umc1634	3	0.64	0.56
101	umc 1037	2	0.50	0.37	137	bnlg2244	2	0.39	0.32
102	bnlg 602	4	0.62	0.57	138	bnlg1711	2	0.39	0.32
103	phi 093	2	0.49	0.37	139	bnlg1043	5	0.76	0.72
104	umc 1662	2	0.50	0.38	140	umc1603	5	0.76	0.72
105	umc 1433	2	0.32	0.27	141	umc308	2	0.39	0.32
106	bnlg 1065	2	0.50	0.37	142	umc1122	4	0.68	0.63
107	umc1479	4	0.68	0.63	143	umc1419	2	0.39	0.32
108	Umc1852	5	0.76	0.72	144	umc2258	6	0.72	0.70
109	dupssr23	5	0.76	0.72	145	phi027	3	0.64	0.56
110	umc1250	3	0.53	0.43					

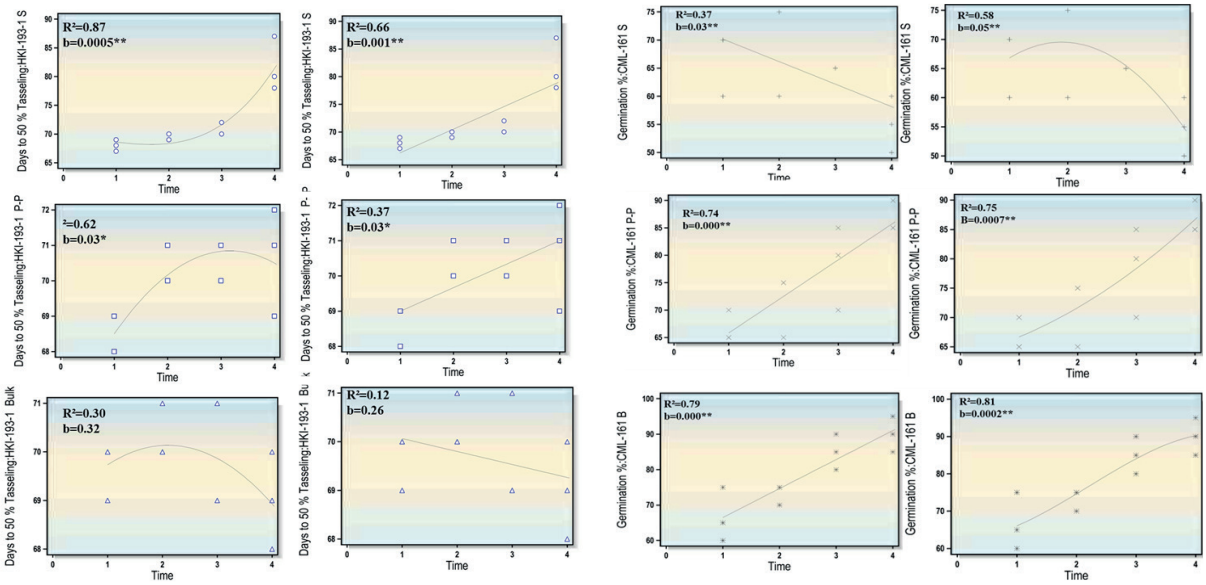

Fig. S1 - A. Quadratic and Linear trends depicting different traits for selfing, half-sib and full-sib with a significance level of p<0.05 in HKI 193-1 inbred.
B. Linear and quadratic trends depicting per cent germination traits for selfing, half-sib and full-sib in CML- 161. Note: P-P indicates full-sibbing and Bulk indicates half-sibbing