Mysore J. Agric. Sci., 56 (4) : 386-392 (2022)

Assessing the Potential of Inbred Lines for Rapid Dry Down in Maize (*Zea mays* L.)

Santhoshkumari Banakara[1], H. C. Lohithaswa[2], Sunilkumar Biradar[3], Kalmeshwer Gouda Patil[4], B. C. Keshavamurthy[5], S. Habib Basha[6], Bijjur Amit[7], A. Mohan Rao[8] and Karaba N. Nataraja[9]

[1, 2 & 8]Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru

[3, 4, 5, 6 & 7]Bayer Cropscience, Bengaluru - 561 213, [9]Department of Crop Physiology, CoA, UAS, GKVK, Bengaluru

e-Mail : lohithaswa.chandappa@gmail.com

Authors Contribution

Santhoshkumari Banakara, H. C. Lohithaswa & Sunilkumar Biradar : Conceptualization & design; Kalmeshwer Gouda Patil, B. C. Keshavamurthy, S. Habib Basha & Bijjur Amit : Field experiment & data collection; A. Mohan Rao & Karaba N. Nataraja : Supervision and curation of data.

Corresponding Author :

Santhoshkumari Banakara
Dept. of Genetics and Plant Breeding, CoA, UAS, GKVK, Bengaluru

Received : August 2022

Accepted : October 2022

Abstract

Low kernel moisture content (KMC) at harvest in maize grain is an essential feature to effectively reduce pre- and post-harvest losses and enable mechanical harvesting. To ensure low KMC at harvest, the inbred lines are required to exhibit rapid kernel drying rates (KDR). In the present study, 320 maize inbred lines were evaluated during *kharif* 2021 at two locations to understand the behaviour of these lines for KMC at three intervals *viz.,* 40, 47 and 57 days after silking and the corresponding KDRs by employing the principal component analysis (PCA) and *k*-means clustering. The analysis of variance indicated the presence of substantial variability for KMC and KDR traits. The first three principal components (PCs) explained the cumulative variation of 80.43 per cent of which PC1, PC2, and PC3 explained the variation of 41.6, 19.9 and 19.93 per cent, respectively. The relative positions of inbred lines in terms of PC1 and PC2 scores in PCA plot also indicated the presence of huge genetic diversity in the inbred lines. Kernel moisture content at 40, 47 and 57 days after silking and KDR at 47 days after silking were the major positive contributors to the total variation among the inbred lines. The 320 inbred lines were grouped into five clusters using *k*-means algorithm. Clusters II and III showed the highest inter-cluster distance with cluster V, as they differed in their KMC and KDR behaviour. The inbred lines grouped in the cluster I can be exploited for rapid dry down breeding in maize as high average drying rate of 0.82 per cent was exhibited by these lines.

Keywords : Maize kernel moisture content, Kernel drying rate, Best linear unbiased predictor (BLUP), Principal component analysis, *k*-means clustering

Maize (*Zea mays* L.) is the world's leading crop and is widely cultivated because of its versatility and wider adaptability. It belongs to the family *Poaceae* with chromosome number 2n = 20. Currently, nearly 1147.7 million MT of maize is being produced together by over 170 countries from an area of 193.7 mha with average productivity of 5.75 t/ha (FAOSTAT, 2020).

Mechanical harvesting of maize is the modern key technology that saves time, labour, and cost associated with maize production (Chai *et al.,* 2017). Many developed countries have implemented mechanical harvesting and in contrast, few major maize-producing countries like India and China lack maize cultivars suitable for mechanical harvesting (Liu *et al.,* 2013). Among several constraints, kernel moisture content (KMC) at harvest is one of the important factors that affect the machine performance during harvesting and post-harvest grain handling operations (Xiang *et al.,* 2012; Sala *et al.,* 2016 and Mousaviraad & Tekeste, 2020). Average kernel breakage of 8.63 per cent and total grain yield loss of 16.50 kg/667 m^2 were observed when KMC was >19.90 per cent

(Chai *et al.,* 2017). High KMC at harvest imposes ear sprouting, ear rot, and increases the cost of artificial drying and storage (Li *et al.,* 2017). Hence, breeding cultivars with low KMC at harvest has become an important target of maize breeding programme (Li *et al.,* 2021).

Moisture loss from kernels occurs in two phases *i.e.,* before physiological maturity (PM), wherein moisture is reduced due to the accumulation of dry matter (Carbohydrates, oils, proteins, etc.) and after physiological maturity (also called field dehydration rates), in which moisture loss is mainly influenced by the external environmental factors. The moisture loss before PM is mainly controlled by genetic factors and the later is due to both genetic and environmental factors and this process of gradual moisture loss is called kernel drying rate (KDR) (Zohu *et al.,* 2018). Rapid kernel drying rates are the marked features of maize varieties with low KMC at harvest (Sala *et al.,* 2016). Both KMC and KDR are considered as quantitative traits and governed by polygenes (Song *et al.,* 2017 and Wang and Li, 2017).

Genetic variability is the cornerstone to embark on any crop improvement programme thus, assessing the phenotypic variability and genetic diversity for kernel moisture content and kernel drying rates among maize inbred lines is of prime importance. Principal component analysis (PCA), Mahalanobis's D^2 statistics, and *k*-means algorithms are used to assess the genetic and phenotypic diversity. PCA is an unsupervised learning method as it finds patterns in data by reducing relatively a large series of data into a smaller number of components called principal components (PC) without prior knowledge about whether the samples come from different treatment groups or have phenotypic differences (Altman and Krzywinski, 2017). The maximum variation is commonly captured by the first two to three Principal components (PCs) and the traits associated with them are useful in differentiating the genotypes (Guei *et al.,* 2005). Using PCA plots the pattern of distribution of genotypes, the contribution of traits to total variability and also cluster pattern can be understood. The *k*-means is a non-hierarchical clustering method that can split the data into two or more groups (Mac Queen, 1967). Considering the importance of rapid kernel drying, an attempt was made to study the behaviour of maize inbred lines and to identify potential rapid drying inbred lines employing principal component analysis and *k*-means clustering.

Material and Methods

A panel of 320 maize inbreds was sown during *kharif* 2021, in Alpha lattice design with two replications at two locations *viz.,* Mega Breeding Station, Kallinayakanahalli (Gauribidanur taluk, Chikkaballapura district, Karnataka) (MBS_Bangalore, N 13°27', E 77°30') and Muppadighatta (Doddaballapura taluk, Bangalore Rural-district, Karnataka) (MUP_Bangalore, N 13°15, E 77°26'). Each line was sown in 2 m row with a spacing of 0.6 m between rows and 0.2 m between plants within a row. All the agronomic practices were adopted to raise a good crop of maize.

Phenotypic Evaluation

Data were recorded on days to 50 per cent silking, kernel moisture content (%) at 40 (KMC1), 47 (KMC2) and 57 (KMC3) days after 50 per cent silking (DAS) and, kernel drying rate (%) at two intervals *viz.,* between 40-47 DAS (KDR1) and 47-57 DAS (KDR2). In each line, when 50 per cent of the plants started to silk, it was noted as days to 50 per cent silking. For estimating KMC, from each line, two cobs were harvested at three different intervals and fifty kernels from both cobs were taken in butter paper cover and fresh weight (FW) was taken immediately. Then the samples were oven dried at 75^0C to a constant weight and the dry weight (DW) was recorded. Kernel moisture content and kernel drying rate was calculated as follows (Jia *et al.,* 2019).

$$\text{Kernel moisture content (\%)} = \frac{\text{[Fresh weight (FW)-Dry weight (DW)]}}{\text{[Fresh weight (FW)]}} \times 100$$

and,

$$\text{Kernel drying rate (\%)} = \frac{(KMC_x - KMC_y)}{(y - x)} \times 100$$

Where,

x and y represent the days after silking at two intervals

Data Analysis

The phenotypic data was analyzed using R software version 4.2.0. Levene's test (Levene, 1960) was carried out to confirm the homogeneity of error variances. The within and across environments analysis of variance (ANOVA) was carried out in the 'metan' R package. For all the traits, best linear unbiased predictors (BLUPs) were calculated using the META-R software version 6.0. The BLUPs for KMC were adjusted by days to 50 per cent flowering by considering it as covariate as the lines differed for it.

The descriptive statistics for each trait was estimated using the best linear unbiased predictors (BLUP) (Henderson, 1975). A combination of PCA and *k*-means clustering analysis was carried out to characterize the inbred lines and PCA biplot was plotted using facto extra R package. The PCA scores for genotypes were used for *k*-means cluster analysis. The trait means and variances were estimated in each cluster, tested for their homogeneity across the clusters and cluster means were compared using the 'F' test, 'Levene' test and, t-test, respectively (Kanavi *et al.,* 2020).

Results and Discussion

The significance of ANOVA within each location indicates the presence of substantial variability among the inbred lines and justifies the requirement for assessment of variation for all the traits (Table 1). The significance of MSS for locations and genotypes × locations in pooled ANOVA revealed the influence of environment on the expression of these traits (Table 2). As BLUPs allow the comparison of individuals or species over time (generation, year) and space (location, block) by minimizing their effects (Tajalifar and Rasooli, 2022), BLUPs were calculated within and across locations for all the inbred lines considering six traits and was used in further analysis. The mean, minimum and maximum values for each trait are depicted using the boxplots visualizing the trait variation for pooled and individual locations (Fig. 1). The total length of the box and whisker line represent the range and the dot in each boxplot

Table 1

Analysis of variance of maize inbred lines for six quantitative traits

Source of variation	df	Mean Sum of Squares (MSS)											
		Days to 50 % silking		KMC1 (%)		KMC2 (%)		KMC3 (%)		KDR1 (%)		KDR2 (%)	
		MUP_ Bangalore	MBS_ Bangalore	MUP_ Bangalore	MBS_ Bangalore	MUP_ Bangalore	MBS_ Bangalore	MUP_ Bangalore	MBS_ Bangalore	MUP_ Bangalore	MBS_ Bangalore	MUP_ Bangalore	MBS_ Bangalore
Replication	1	0.019	0.156	2.57	7.67	0.438	1.266	0.72	2.09	0.0181	0.0551	0.035	0.0168
Blocks (replication)	38	1.43 *	1 *	1.07	2.75	1.12	1.68	0.95	1.66	0.0278	0.0373	0.019	0.0185
Genotypes	319	18.6 ***	17.7 ***	23 ***	24.7 ***	18.4 ***	19.5 ***	19.1 ***	25.9 ***	0.246 ***	0.205 ***	0.164 ***	0.169 ***
Error	281	0.628	0.654	1.27	2.08	1.16	1.58	0.963	2.203	0.0272	0.0388	0.015	0.023

TABLE 2

Pooled analysis of variance of maize inbred lines for six quantitative traits

Source of variation	df	Mean Sum of Squares (MSS)					
		Days to 50 % silking	KMC 1 (%)	KMC 2 (%)	KMC 3 (%)	KDR1 (%)	KDR2 (%)
Location	1	2025.07 ***	873.44 ***	137.00 ***	36.42 ***	6.50 ***	3.14 ***
Replication (Location)	2	0.078 *	5.11	0.85	6.14	0.036 *	0.025
Blocks (Replication)	76	1.21 *	1.90	1.40	1.63	0.032	0.019
Genotypes	319	30.66 ***	37.84 ***	29.69 ***	44.62 ***	0.30 ***	0.218 ***
Genotypes × Location	319	5.62 ***	9.89 ***	8.18 ***	12.45	0.14 ***	0.113 ***
Error	562	0.64	1.67	1.36	1.61	0.03	0.019

df - degrees of freedom; *** Significant at P = 0.001; ** Significant at P = 0.01; *Significant at P = 0.05

KMC 1 - KMC at 40 DAS; KMC2 - KMC at 47 DAS; KMC3 - KMC at 57 DAS; KDR1 - Drying Rate (40-47); KDR2 - Drying Rate (47-57 DAS)

represents the mean value of that particular trait. The KMC declined gradually from 40 DAS to 57 DAS, but the variation in KMC for each genotype across two locations displayed distinct trends. A similar trend was observed by Zohu *et al.* (2018) and Li *et al.* (2021). Considering the BLUPs across locations, 14 genotypes had < 26 per cent KMC at 57 DAS and 12 genotypes had > 0.90 per cent average KDR.

To analyse variation patterns among the genotypes, PCA was carried out. To find the optimum number of PCs for extracting useful information scree plot of eigen values for each PC was plotted (Fig. 2). The point at which the curve bends and flattens is the criteria for deciding the optimum number of PCs (Mengistu, 2021).

In this way, the three PCs were considered which accounted for the cumulative variation of 80.43 per cent. The eigen value, *per cent* variation explained by each PC, and the factor loadings for each trait are represented in Table 3 and the distribution of variables and nature of diversity for genotypes is graphically represented in Fig. 3. The PC1 vector captured 41.6 per cent of total variation and the traits KMC1 (%), KMC2 (%) and KMC3 (%) were the major positive

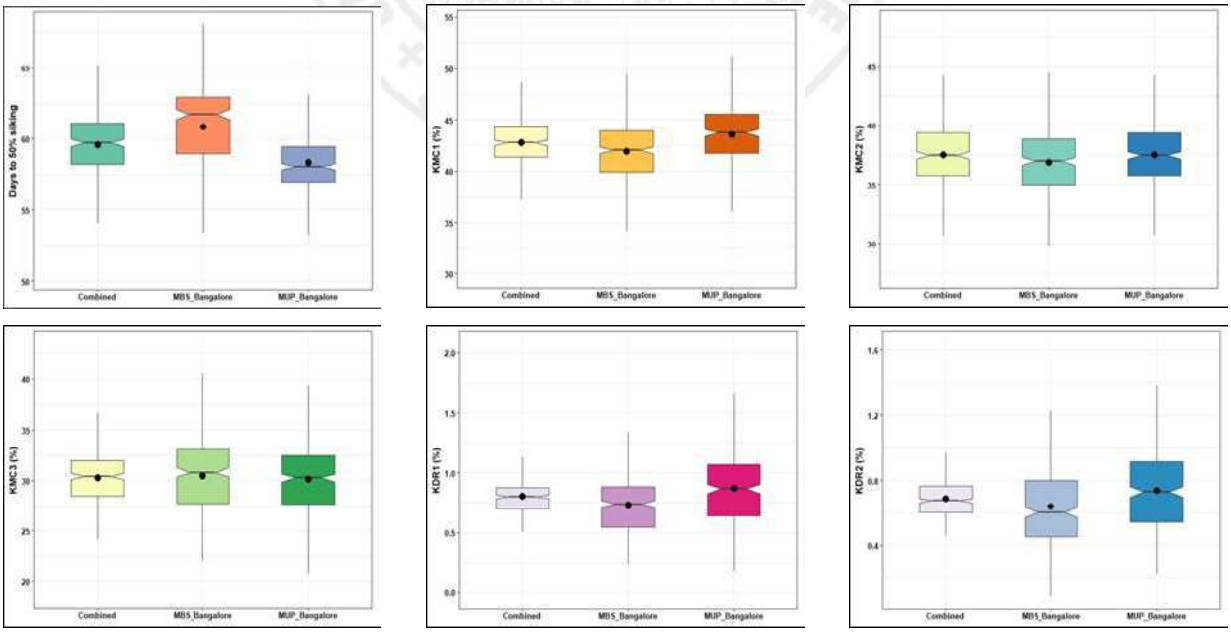

Fig. 1 : Box-Whisker plots showing variation for days to 50% silking, KMC1 (%), KMC2 (%), KMC3 (%), KDR1 (%) and KDR2 (%) within and across locations.

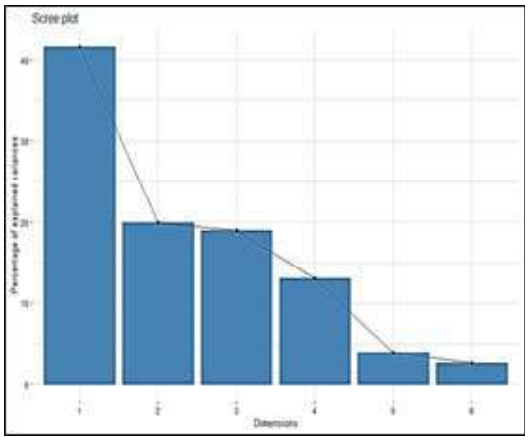

Fig. 2 : Scree plot representing *per cent* variance explained by Principal components (PCs).

contributors to the total genetic variability. The PC2 vector captured 19.9 per cent of variation with days to 50 per cent flowering and KDR1 (%) being the major negative contributors, while the traits KMC2 and KMC3 contributed positively in relatively lesser magnitude. The PC3 captured 18.93 per cent of variation with KDR2 (%) being a major positive contributor.

It can be seen that the factor loading values for each trait are comparable in their contribution to genetic diversity in either positive or negative direction. The PCA clearly explained that some of the studied genotypes were diverse from others while many are similar and positioned near to each other on the biplot (Fig. 3). So, PCA can be used for grouping of

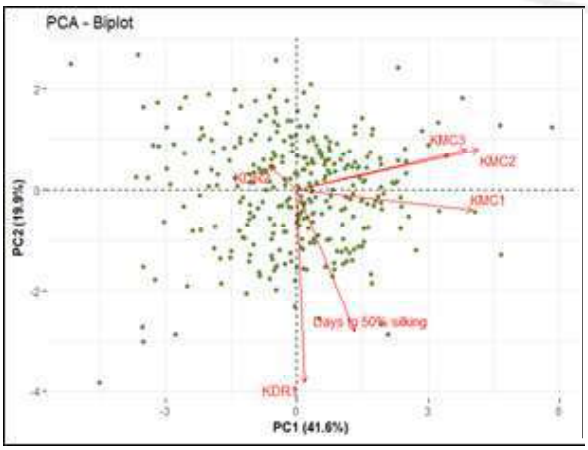

Fig. 3 : Biplot among first two Principal components (PCs) representing relative position of genotypes and factors loadings

TABLE 3

Eigenvalues, *per cent* variance explained and factor loading values of six quantitative traits

Parameters	PC1	PC2	PC3
Eigen Values	2.49	1.19	1.13
Variance explained (%)	41.58	19.88	18.93
Cumulative variance explained (%)	41.58	61.47	80.43
Days to 50% silking	0.18	-0.57	0.25
KMC1 (%)	0.56	-0.08	0.18
KMC2 (%)	0.58	0.15	0.14
KMC3 (%)	0.54	0.15	-0.27
KDR1 (%)	0.02	-0.77	-0.12
KDR2 (%)	-0.09	0.09	0.88

genotypes and the major contributing principal component (PC) scores for genotypes can be used as input variables for the clustering process (Mengistu, 2021).

To group the genotypes based on their similarity and dissimilarity, *k*-means clustering was carried out. The optimum number of clusters considered was five as beyond that there was no significant increase in between sum of squares/total sum of squares ratio. The distribution of 320 inbred lines into five clusters is represented in the PCA plot (Fig. 4).

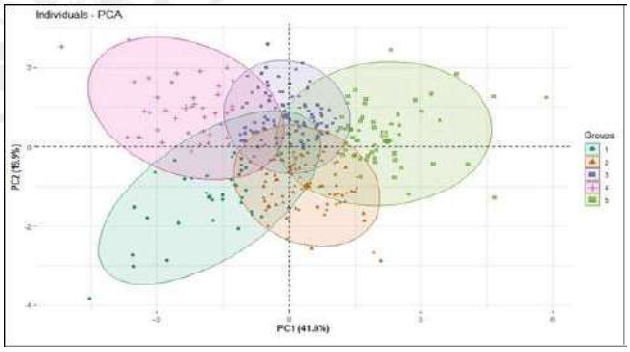

Fig. 4 : PCA plot representing five clusters derived using *k*-means clustering

To examine the significant differences between the clusters one-way analysis of variance was carried out and the results are presented in Table 4. The significance of mean sum of squares for between clusters indicated that each cluster members performed differently for all the traits studied. The

TABLE 4

Analysis of variance for different traits between the clusters

Source of variation	df	Days to 50 % silking	KMC 1 (%)	KMC 2 (%)	KMC 3 (%)	KDR1 (%)	KDR2 (%)
Between clusters	4	135.87 ***	245.48 ***	218.55 ***	290.72 ***	0.69 *	0.03 ***
Within clusters	315	3.50	2.30	1.36	2.33	0.01	0.01

df- degrees of freedom, *** Significant at P= 0.001; * Significant at P= 0.05

TABLE 5

Intra and inter cluster distances among the five clusters obtained through *k*-means

Clusters	I	II	III	IV	V
I	0				
II	6.50	0			
III	7.66	6.60	0		
IV	6.03	9.06	6.79	0	
V	7.01	11.10	11.55	7.10	0

intra and inter-cluster distances are represented in Table 5.

The number of genotypes within each cluster, cluster means and mean comparison between clusters for each trait are represented in Table 6. The inter-cluster distance was maximum between clusters II, III with cluster V indicating the higher genetic distance between them followed by cluster I and III. The members belonging to cluster II and III differed in their KMC and KDR at different intervals in comparison to cluster V members. The members belonging to cluster I were faster drying with average drying rate of 0.82% with low KMC (27.49%), while the members belonging to cluster III are the slower drying ones (0.69%) with relatively high KMC at 57 DAS (30.45%).

It is observed that the genotypes belonging to cluster I had relatively low kernel moisture content at 57 DAS (27.49%) and have a high average rapid drying rate of 0.82% between 40 DAS to 57 DAS followed by cluster II with an average drying rate of 0.78 per cent compared to other clusters. These genotypes have the potential usage in breeding maize cultivars harbouring rapid drying ability.

To promote mechanical harvesting with minimum kernel breakage there was a requirement of inbred lines with minimum KMC at harvest and rapid KDR. Hence, 320 inbred lines were evaluated for traits implicated in rapid dry down. The performance of genotypes studied for KMC and KDR was significantly different and was found to be highly influenced by the environment. The diversity among the genotypes was evident through PCA and *k*-means clustering approaches. The traits, KMC and KDR contributed significantly to the total variation. It is evident from the cluster analysis that the genotypes belonging to cluster I have high KDR and can be exploited for rapid dry down breeding in maize.

TABLE 6

Number of genotypes in a cluster, cluster means and mean comparison for the different traits

Cluster	No. of entries	Days to 50 % silking	KMC 1 (%)	KMC 2 (%)	KMC 3 (%)	KDR1 (%)	KDR2 (%)
I	37	60.54 a	40.47 a	34.71 a	27.49 a	0.93 a	0.70 a
II	87	61.15 ab	43.53 b	37.32 b	30.40 b	0.90 a	0.67 ab
III	100	58.73 b	42.45 c	37.41 b	30.45 b	0.71 b	0.68 b
IV	39	57.17 c	39.93 d	34.54 c	27.41 c	0.69 c	0.73 b
V	57	59.81 d	45.52 d	39.80 c	33.45 c	0.78 c	0.67 b

The Mysore Journal of Agricultural Sciences

Acknowledgement : The author gratefully acknowledges the M/s Bayer Cropscience Pvt. Ltd., (India) for providing resources and financial support for conducting research and the Directorate of Postgraduate Studies, University of Agricultural Sciences, Bangalore, India, for permitting the first author to conduct research in the facility of M/s Bayer Cropscience Pvt. Ltd.

REFERENCES

ALTMAN, N. AND KRZYWINSKI, M., 2017, Principal component analysis. *Nat. Methods,* **14** : 545 - 554.

CHAI, Z., WANG, K., GUO, Y., XIE, R., LI, L., MING, B. AND LI, S., 2017, Current status of maize mechanical grain harvesting and its relationship with grain moisture content. *Sci. Agric. Sin.*, **50** (11) : 2036 - 2043.

FAO, 2020, FAOSTAT. In : *FAO* [online]. http://www.fao.org/faostat/en/#data/QC

GUEI, R. G., SANNI, K. A. AND FAWOLE, A. F. J., 2005, Genetic diversity of rice (*O. sativa* L.). *Agron. Afr.,* **5** : 17 - 28.

HENDERSON, C. R., 1975, Best linear unbiased estimation and prediction under a selection model. *Biometrics*, **31** : 423.

JIA, T. J., LI, P. F., WANG, L. F., ZHANG, Y. L., LI, J. J., CAO, Y. Y., MA, J. AND LI, H. Y., 2019, Investigation of grain dehydration rate in 428 inbred lines. *J. Plant. Genet. Res.,* **20** (2) : 406 - 411.

KANAVI, P. M. S., KOLER, P., SOMU, G. AND MARAPPA, N., 2020, Genetic diversity study through *k*-means clustering in germplasm accessions of green gram [*Vigna radiata* (L.)] under drought condition. *Int. J. Bio-resour. Stress Manag.,***11** (2) : 38 - 147.

LEVENE, H., 1960, Contributions to probability and statistics : Essays in honor of harold hotelling. Stanford University Press, Palo Alto, pp. : 278 - 292.

LI, L. L., LEI, X. P., XIE, R. Z., WANG, K. R., HOU, P., ZHANG, F. L. AND LI, S. K., 2017, Analysis of influential factors on mechanical grain harvest quality of summer maize, *Sci. Agric. Sin.,* **50** : 2044 - 2051.

LI, W., YU, Y., WANG, L., LUO, Y., PENG, Y., XU, Y., LIU, X., WU, S., JIAN, L., XU, J., XIAO, Y. AND YAN, J., 2021, The genetic architecture of the dynamic changes in grain moisture in maize. *Plant Biotech. J.,* **19** (6) : 1195 - 1205.

LIU, F. H., WANG, K. R., JIAN, L., WANG, X. M., SUN, Y. L. AND CHEN, Y. S., 2013, Factors affecting corn mechanically harvesting grain quality. *Crops,* **4** : 16 - 119.

MAC QUEEN, J. B., 1967, Some methods for classification and analysis of multivariate observations, *Proceedings* of In *5th Berkeley Symp. Math. Statist. Probability*, Berkeley, University of California Press, **1** : 281 - 297.

MENGISTU, S., 2021, Maize germplasm characterization using principal component and cluster analysis. *Am. J. Bio. Sci.*, **9** (4) : 122.

MOUSAVIRAAD, M. AND TEKESTE, M. Z., 2020, Effect of grain moisture content on physical, mechanical and bulk dynamic behaviour of maize. *Biosystems Engineering*, **195** : 186 - 197.

SALA, R. G., ANDRADE, F. H., CAMADRO, E. L. AND CERONO, J. C., 2016, Quantitative trait loci for grain moisture at harvest and filed grain drying eate in maize (*Zea mays* L.). *Theor. Appl. Genet.,* **12** : 462 - 471.

SONG, W., SHI, Z., XING, J. F., DUAN, M, X. AND ZHANG, Y. R., 2017, Molecular mapping of quantitative trait loci for grain moisture at harvest in maize. *Plant Breed.,***136** : 28 - 32.

TAJALIFAR, M. AND RASOOLI, M., 2022, Importance of BLUP method in plant breeding. *J. Plant. Sci. Phytopathol.,* **6** : 40 - 42.

WANG, K. R. AND LI, S. K., 2017, Analysis of influence factors on kernel dehydration rate of maize hybrids. *Sci. Agric. Sin.,* **50** : 2027 - 2035.

XIANG, K., REID, L. M., ZHANG, Z. M., ZHU, X. Y. AND PAN, G. T., 2012, Characterization of correlation between grain moisture and ear rot resistance in maize by QTL meta-analysis. *Euphytica,* **183** : 185 - 195.

ZHOU, G., HAO, D., XUE, L., CHEN, G., LU, H., ZHANG, Z., SHI, M., HUANG, X. AND MAO, Y., 2018, Genome-wide association study of kernel moisture content at harvest stage in maize. *Breed. Sci.,* **68** (5) : 622 - 628.