

Original Research Article

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## Variability in Epicuticular Wax Content among Temperate Maize Lines as a Drought Tolerance Related Mechanism

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### ABSTRACT

#### Keywords

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#### Article Info

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Thirty genotypes of Maize which included twenty eight inbred lines and two checks maintained at D(K)ARS, SKUAST-K were used for the study carried out in the Department of Crop Physiology, University of Agricultural Sciences, GKVK Bangalore. In this programme the genetic variability for Epicuticular Wax Content (EWC) was studied. The data of the experiment revealed a significant genetic variability in epicuticular waxes among the germplasm lines of maize. Accordingly, the EWC ranged from 1.56 (KDM-921A)  $\mu\text{g}/\text{cm}^2$  to 7.54 (KDM-954)  $\mu\text{g}/\text{cm}^2$  with a mean of 4.25  $\mu\text{g}/\text{cm}^2$ . The frequency distribution also indicated the spread of genotypes across the mean to suggest the existence of an appreciable genetic variability for EWC in maize genotypes

### Introduction

Maize or Corn (*Zea mays*) is a plant belonging to the family of grasses (*Poaceae*). It is cultivated globally and is one of the most important cereals. Maize grains have long been used as feed, food consumption and industrial applications. India has 5% of maize acreage and contributes 2% of world production. Maize occupies an important place in Indian Agriculture. It is the third most important cereal in India after wheat and rice. The major maize growing states are Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka and Jammu and Kashmir, jointly accounting for over 95% of the national maize production. In India,

about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (starch and oil production) and 1% as seed. In the last one decade, it has registered the highest growth rate among all food grains including wheat and rice because of intervention of single cross heterotic hybrids.

According to advance estimates in India (Anonymus, 2015), Maize was cultivated in 9.1 m ha with a production and productivity of 23.66 (MT) and 26.02 (Q/ha). It contributes nearly 9% in the national food basket and more than 100 billion to the agricultural GDP at current prices apart from

the generating employment to over 120 million man-days at the field and downstream agricultural and industrial sectors. In addition to staple food for human being and quality feed and fodder for animals, maize serves as a basic raw material as an ingredient to thousands of industrial products that includes starch, oil, protein, beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package and paper industries etc.

Drought is a complex syndrome involving timing, intensity, and duration of water deficit; the high variability of these factors makes it difficult to define plant traits required for improved performance under all possible drought situations (Rao and Cramer, 2003). With the unpredictability of drought, geographical and seasonal, including ongoing climate changes, the destructive impact of drought is likely to further increase (Ribaut *et al.*, 2004; Banziger and Araus, 2007). Therefore, crop improvement for limited-water condition is a priority for plant breeding programs worldwide. With most maize in the developing world being grown under rain-fed conditions and the proportion of maize grown in marginal areas increasing, breeding for tolerance to drought has therefore, become a major focus.

### **Materials and Methods**

The material used for the present study consisted of 28 inbred lines and two checks (GM-6 and CML-442) maintained at Dryland (Karewa) Agriculture Research Station, Budgam (SKUAST-K) (Table 1). Plants were raised in specially constructed root structures that measured 150 cm tall, 300 cm wide and 1800 cm long. An additional 150 cm tall wall was built in the middle of the structure all along the length to make two halves each 150 cm wide. Top soil dug out from another field was transported to fill these structures. Soil was compacted to mimic the real field conditions. Seedlings were transplanted in a

randomized block design (RBD) with three replications. Plant population was maintained with 60 x 20 cm spacing, which ensured that plants experienced the inter-plant competition as in field conditions thus leading to more realistic phenotypic expression. The soils in the GKVK campus had been previously estimated to hold 23 percent water (W/W) at 100 percent field capacity. On an average, surface irrigation was provided once every 5 – 7 days to bring the soil to 100 percent FC.

As a prerequisite towards quantifying plant waxes, standard graph has to be developed. A colorimetric based assay was followed for wax estimation (Ebercon *et al.*, 1979). The principle behind this assay is the color change brought about by the reaction of acid dichromate with wax. Wax reagent was prepared by mixing 20g of potassium dichromate with 40ml of deionized water. The resulting slurry was then mixed with 1 lt of concentrated H<sub>2</sub>SO<sub>4</sub> and incubated in a water bath until a clear solution was formed. A wax standard graph was developed using Carnauba wax, which is a plant wax from an Egyptian palm tree. Carnauba wax of 0.5 to 5 mg was used to develop the standard graph. To all these samples, 5 ml of wax reagent was added and boiled for 30 minutes and cooled down afterwards. To this, 12 ml of deionised water was added and allowed for colour development. After the colour development, the samples were filtered using the filter paper and OD was read at 590 nm using spectrophotometer.

### **Results and Discussion**

Sufficient amount of genotypic variability for the trait of interest must be available among the genotypes for subsequent use in crop improvement programme. Therefore in the present study, attempts were made to look for genotypic variability for epicuticular wax content in maize inbred lines. A set of 28 inbred lines and 2 checks were selected and

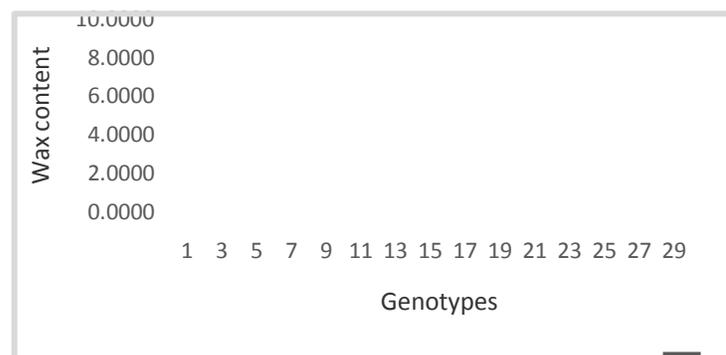
phenotyped in the present study for the variability in epicuticular wax content (EWC). Using carnauba wax, which is extracted from the leaves of *Copernicia prunifera*, a standard curve was developed. Following the standard protocol, carnauba wax standards were prepared and OD was read at

592 nm and a standard curve with a regression value ( $R^2$ ) of 0.987 was developed. Following the standardized protocol to extract epicuticular waxes in rice, the EWC was quantified in all the 30 genotypes of maize.

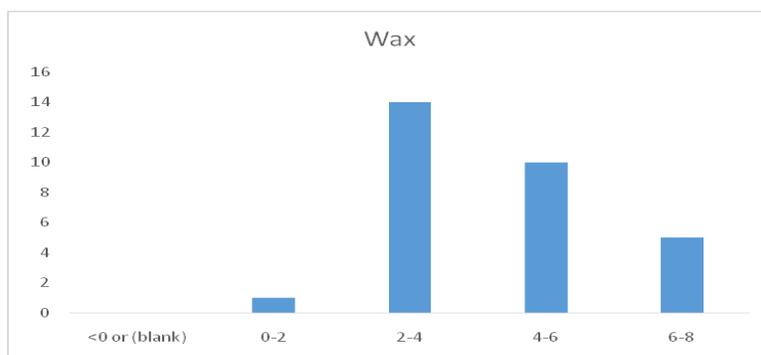
**Table.1** List of inbreds and checks used in the study

S.No	Accessions
1.	KDM 927A
2.	KDM 930A
3.	KDM 332A
4.	KDM 921A
5.	KDM 408
6.	KDM 1156
7.	KDM 941A
8.	KDM 932A
9.	NGB17094-1
10.	KDM 892A
11.	KDM 362A
12.	NGB 17099-1
13.	KDM 895A
14.	KDM 1095
15.	KDM 343A
16.	KDM 329
17.	KDM 945
18.	KDM 963A
19.	KDM 925B
20.	KDM 440A
21.	KDM 926B
22.	NGB 17097-1
23.	KDM 954
24.	KDM 913A
25.	KDM 443
26.	KDM 918A
27.	KDM 944A
28.	NGB13905
29.	GM-6 (C)
30.	CML-442 (C)

**Fig.1** Genetic variability in EWC in maize genotypes



**Fig.2** Frequency distribution of EWC in maize genotypes



The data of the experiment revealed a significant genetic variability in epicuticular waxes among the germplasm lines of maize (Fig. 1). Accordingly, the EWC ranged from 1.56 (KDM-921A)  $\mu\text{g}/\text{cm}^2$  to 7.54 (KDM-954)  $\mu\text{g}/\text{cm}^2$  with a mean of 4.25  $\mu\text{g}/\text{cm}^2$ . The frequency distribution also indicated the spread of genotypes across the mean to suggest the existence of an appreciable genetic variability for EWC in maize genotypes (Fig. 2).

A simple colorimetric method of extracting waxes from the chloroform and assessing the chromophore developed by waxes with appropriate reagent was developed to assess the genetic variability in epicuticular wax content. A procedure of dipping the leaves in chloroform for exactly 15 seconds was standardized. Dipping the leaves for more than 15 seconds leads to the dissolution of

cuticular and membrane waxes besides the epicuticular waxes. This protocol was adopted to assess the EWC among 86 genotypes which were selected based on the difference in rate of water loss (RWL) measured in the previous experiment. Such standardization is required in the species where EWC is to be determined. While characterizing mulberry accessions for EWC, Mamrutha (2008) standardized a dipping time of 15 seconds to extract epicuticular waxes. Therefore it appears that, depending on the species, dipping duration also varies and accordingly, needs standardization of extraction protocol.

In the present study with maize inbred lines, a significant genetic variability was noticed for EWC which ranged from 1.56 to 7.54  $\mu\text{g}/\text{cm}^2$ . Significant genetic variability for EWC was also noticed by Mamrutha (2008) and Mamrutha *et al.*, (2010) in mulberry where

they have showed that, the EWC of mulberry accessions remained stable across seasons and locations. All these evidences therefore clearly emphasize the need for including epicuticular wax content as a trait in a breeding program to enhance the water conservation.

In conclusion the promising lines identified like KDM-954 for epicuticular wax content can be used as trait donor lines to transfer specific traits in to a recipient genotype. The contrasting lines will be useful in developing trait specific mapping populations as well as for greater understanding of molecular regulation of the trait.

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