

**Research Note****Genetic studies for drought related traits in temperate maize****Z.A. Dar<sup>1</sup>, Mushtaq Ahmad<sup>2</sup>, A.A. Lone<sup>2</sup>, M.S. Sheshsayee<sup>2</sup>, M.D. Pratibha, B.A. Alaie, M.I. Gulzafar, A. Makdoomi, Gazal and S. Gulzar**<sup>1</sup>AICMIP, Srinagar Centre,<sup>2</sup>Department of Crop Physiology, GKVK Bangalore**E-mail:** drmuhtaqdarskuastk@gmail.com

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**Abstract**

Under the INSA Fellowship Programme (2015), thirty genotypes of Maize which included twenty eight inbred lines and two checks maintained at D(K)ARS, SKUAST-K were used for a "Comprehensive Assessment of Drought Tolerance" in the Department of Crop Physiology, University of Agricultural Sciences, GKVK Bangalore. In this programme the genetic variability for Roots, Water Use Efficiency and Photosynthesis related traits studied to identify contrasts for trait based introgression for drought tolerance. Maize accessions revealed a significant genetic variability for roots, WUE ( $\Delta$ 13C). Further significant genetic variability in WUE at single level was also observed. Significant genetic variability in drought tolerance traits was revealed among the maize germplasm lines. The promising lines identified like KDM-932A for root length, KDM-1095 for root volume, KDM-918A for root weight, KDM-954 for epicuticular wax content and KDM- 944A for WUE can be used as trait donor lines to transfer specific traits into a recipient genotype.

**Key words**

Maize, drought tolerance, variability, root, epicuticular wax

Nowadays there is also a strong demand of maize gains for bio-fuel production especially in the two main maize producing countries viz., United State of America and China. The global maize production is predicted to grow continuously as demand and value in the world market increase. About 67% of the total maize production in the developing world comes from low and lower middle income countries; hence, maize plays an important role in the livelihoods of millions of poor farmers. Countries like United States, China, Brazil and Mexico account for 70% of global production. Genetic variation in epicuticular wax is of potential importance in breeding for drought tolerance. In mulberry, significant genotypic variability was noticed for epicuticular waxes. Mamrutha (2008) and Mamrutha *et al.*, (2010) have found a wide variability across accessions of mulberry and found some of the highly promising accessions of mulberry for epicuticular waxes. Further, the moisture retention capacity of those high types were found to be high and in fact, when such leaves were fed to the silkworms, they produced quality cocoons. Being a C<sub>4</sub> species, it is one of the most versatile and emerging crops having wider adaptability under varied agro-climatic conditions and is a high yielding cereal crop for total dry matter production. Maize requires 500-800 mm of water during the life cycle of 80 to 110 days. According to Jamieson *et al.*, (1995), water requirement of maize at the time of tasseling is 135 mm/month (4.5 mm/day) and this requirement may increase up to 195 mm/month (6.5 mm/day) during hot windy conditions. Rather cropping patterns are being changed due to water scarcity. The maize crops may experience reductions of grain yields when subjected to water deficit during the critical period of crop cycle from tasseling stage to initiation of grain filling.

Considering the ongoing climatic changes caused principally by global warming the pressure on food production in water-limited environments will increase in the near future. Selection against silk delay has been the most effective method of breeding for drought tolerance and has been shown to be well correlated with improved yields under drought stress (Troyer, 1983).

Effective approaches to combat current impacts of drought are of utmost importance, more so as the situation is set to become even worse as climate change progresses (Thornton *et al.*, 2009). Nevertheless, a breeding program focused at increasing the frequency of genes that increase productivity under water-limiting conditions is the best route for substantial and sustained gains. Such a program requires a good definition of major drought scenarios in the target environment and needs to be conducted under repeatable experimental conditions reflecting those scenarios. Physiological mechanisms of genetic improvements in grain yield potential are not fully understood. However, it is known that, together with grain yield, some changes were also observed in physiological characteristics controlling the yield during the yield increase process. Physiological characteristics have significant impacts on growth and development of plants grown in production systems (Ahmad *et al.*, 2013). The general structure and function of roots and shoots are so different that the two organs are often conveniently separated for the purposes of research. Functionally, roots absorb water and nutrients, and anchor the plant, while shoots photosynthesize and transpire, and are the site of sexual reproduction. Under the present scenario, especially in the tropical regions of the world, both water and nutrients have emerged the most

significant constraints that limit agricultural productivity. These constraints would only be exacerbated by the anticipated global climate changes. Increasing agricultural productivity per unit of available water and nutrients appears to be one of the most important challenges that need to be addressed to achieve food and nutrient security. Roots are essential for higher plants for several important reasons. The firm anchorage of the plant in their soil substratum, absorption and effective supply of water and nutrients to the shoot are the most important roles of the root system. Furthermore, a number of plant growth hormones, especially cytokinins and ABA originate in roots, thus having significant influence on growth and development of plants. Thus improving root traits is extremely relevant. Therefore, the present work was aimed to determine the variability for roots, photosynthesis related traits, and Water conservation related traits and epicuticular waxes in maize genotypes.

The material used for the present study consisted of 28 (Table 1) inbred lines and two checks (GM-6 and CML-442) maintained at Dryland (Karewa) Agriculture Research Station, Budgam (SKUAST-K). 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. 20 plants were maintained *per* replication *per* entry. 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. On the 40<sup>th</sup> day after sowing (DAS), the side walls of root structures were dismantled and roots were extracted carefully with a jet of water to wash away the soil from roots. Roots were separated from the shoot and several parameters including root length, root volume were recorded before transferring the plant parts to a drying hot air oven. The samples were oven-dried at 70<sup>o</sup> C and their weights recorded after the dry weights reached constant values. Root structure was dismantled by removing each brick units at 40 days after sowing. High pressure of water was applied to the soil in the structure and roots were taken without damaging along with the whole plant. Roots were separated from the shoot at the node between the shoot and the root.

*Shoot length (cm)*: The shoots were separated from the plants and the shoot length was recorded using a graduated scale.  $\Delta^{13}\text{C}$ : The leaf samples taken for SLA was powdered and used to estimate Carbon isotope ratios at the National facility for stable isotopes at Department of Crop Physiology, UAS, GKVK, Bangalore.

*SCMR*: Leaf nitrogen status is normally manifested with the leaf chlorophyll content. A device developed by Minolta corp, Ramsey, NJ measures the light attenuation at 430nm (the peak wavelength for chlorophyll a and b absorption) and that at 750 nm (near-infrared) with no transmittance.

*Canopy temperature (<sup>o</sup>C)*: Canopy temperature was measured by an infrared thermometer (IRT). Canopies emit long wave infra-red wave radiation as a function of temperature. Measurements were done over a period of two hours between 11 am and 1 pm. Distance, position and angle of measurement were same for the entire experiment.

*Root length (cm)*: The roots were separated from the plants and the root length was recorded using a graduated scale. *Root volume (cm<sup>3</sup>)*: A known volume of water was taken in a measuring cylinder, and the separated roots were immersed into this beaker and then the volume of displaced water was taken as the root volume. *Shoot weight (g)*: All shoots were collected separately and oven dried at 70<sup>o</sup>C for 48 h to determine the dry weight for other biometric analysis. *Root weight (g)*: Roots were washed from the structure, then oven-dried at 80<sup>o</sup>C for 48 h and dry weights were recorded. *Total biomass (g)*: The biomass accumulated during the experimental period was computed by summing up leaf, stem and root dry weights. *Root: Shoot ratio*: It was computed by dividing the root biomass by the shoot biomass.

*Gas exchange*: Gas exchange traits such as net CO<sub>2</sub> assimilation rate (A), stomatal conductance (g<sub>s</sub>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), transpiration rate (T) etc., were measured using portable photosynthesis system LI- 6400XT (LICOR 6400, Lincoln, Nebraska, USA).

*Recording gas exchange parameters*: The gas exchange parameters were recorded for the second fully expanded leaf from the apex. The leaf was clamped to the leaf chamber and the observations were recorded when A, g<sub>s</sub>, T and C<sub>i</sub> reached a steady value. All gas exchange parameters were recorded between 9 am and 12 pm on bright sunny days. The leaf chamber is equipped with a Peltier cooling system that can maintain the chamber temperature. The operational option provided with the system also maintains a constant chamber RH around that of the ambient air.

**Carbon isotope discrimination (CID):** Stable carbon isotope ratio was measured using an Isotope Ratio Mass Spectrometer (Delta plus, Thermo Fischer scientific, Bredmen, Germany) interfaced with an elemental analyzer (NA112, Carlo-Erba, Italy) through a continuous flow device (Conflo-III, Thermo Fischer scientific), installed at the Department of Crop Physiology, UAS, Bengaluru. Dried leaf samples used for estimating SLA were homogenized to a fine powder with a ball mill. Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ), expressed in per mill (‰), was computed as per the notation proposed by Farquhar *et al.*, 1989.

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p) / (1 + \delta^{13}\text{C}_p / 1000)$$

Where;  $\delta^{13}\text{C}_a$  and  $\delta^{13}\text{C}_p$  are the carbon isotope composition of atmospheric air and plant sample, respectively. The  $\delta^{13}\text{C}_a$  was considered as -8‰ for the computation.

The analytical uncertainty was better than 0.15% which was determined by using an external standard calibrated against international standards such as ANU-Sucrose (Potato starch, Sigma Aldrich  $\delta^{13}\text{C} = -26.85\%$ ).

**Wax Content:** As a prerequisite towards quantifying plant waxes, standard graph has to be developed. A colorimetric based assay was followed for wax estimation (Ebercorn *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  $\text{H}_2\text{SO}_4$  and incubated in a water bath until a clear solution was formed. A wax standard graph was developed using Carnuba wax, which is a plant wax from an Egyptian palm tree. Carnuba 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.

In Jammu and Kashmir state, 0.51 million quintals of grain were produced from an area of 0.31 million hectares. Though the yields up to 10 t/ha have been realized the state average remained at 1.6 t/ha only. Drought is the primary abiotic stress, causing not only differences between the mean yield and the potential yield, but also causing maize yield instability. When drought stress occurs just before or during the flowering period, a delay in silking is observed resulting in an increase in the length of the

anthesis-silking interval (ASI). This asynchrony between male and female flowering has been recognized as a major source of grain yield decrease. Besides drought stress also severely reduces leaf expression and crop growth rate. It is well recognized that specific traits that help maintain tissue water relations and metabolism under water limited conditions are essential to sustain growth and productivity under drought conditions. It is well known that evident those maize genotypes have the ability to harness water from deeper soil profile, use water efficiently for biomass production for improved productivity under drought conditions.

At a single leaf level, photosynthesis is often considered as a diffusive process.  $\text{CO}_2$  diffuses from atmosphere to the leaf down the concentration gradient, while water vapour diffuses out in to a drier atmosphere. Though diffusion of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  are controlled by respective concentration gradients, the diffusive resistance for the two gaseous molecules significantly alters gas exchange between leaf and atmosphere. Stomata are the prominent structures that have profound influence on overall diffusion of gases. In other words stomata significantly control both  $\text{CO}_2$  diffusion for photosynthesis and evaporation of water during transpiration. Gas exchange parameters like A,  $g_s$ ,  $C_i$  and T were measured using a portable IRGA and the results are described (Table 2.) Assimilation rate (A) varied from  $10.59 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  to  $27.01 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with a mean of  $16.22 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  representing a significant genetic variability among maize germplasm accessions. Transpiration rate (T) also exhibited a significant genetic variability ranging from  $1.29 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  to  $3.20 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with a mean of  $2.13 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Similarly stomatal conductance ( $g_s$ ) varied from  $0.07 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  to  $0.22 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with a mean of  $0.013 \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Intercellular  $\text{CO}_2$  concentration ( $C_i$ ) ranged from 89.09 ppm to 251.73 ppm with a mean of 163.72 showing significant genetic variability in the test material. SCMR a measure of chlorophyll content had a minimum value of 26.1 and a maximum value of 45.8 among the maize accessions. Canopy Temperature (CT) ranged from  $23.47$  to  $29.27$  ( $^{\circ}\text{C}$ ) with a mean of  $26.72$  ( $^{\circ}\text{C}$ ). CCATD which is a reflection of the crop canopy temperature status ranged from 0.01 to  $-5.53$  ( $^{\circ}\text{C}$ ) with a mean of  $-2.28$  ( $^{\circ}\text{C}$ ). WUE ranged from 0.50 to 1.03 with a mean of 0.79. Characterization of natural variation in WUE is an important prerequisite to application of the assembled body of molecular genetic knowledge associated with different crop species to a broader understanding the genetic control of this phenomenon. Genetic variation in WUE in different crop species can be measured at different techniques.

At single level, gas exchange efficiency is defined as the ratio of the rates of net CO<sub>2</sub> uptake (A) and transpiration (T) besides agronomic approaches. Both uptake and transpiration occur almost exclusively through the stomatal pore in the surface of the Leaf and can be described as diffusion processes. WUE can be improved at the physiological level, where it is often approached using the “intrinsic” WUE i.e., the ratio of net assimilation (AN) to stomatal conductance (gs), which was introduced to compare photosynthetic properties at a common evaporative demand. Differences between the genotypes for the value of the ratio Ci/Ca and WUE have been reported to have a genetic basis, and breeding for high WUE has become a main objective for many crops. However, as pointed out by Blum (2005), genotypic variations in WUE are normally expressed mainly due to variations in water use or gs. Because Net Assimilation (A<sub>N</sub>) shows a direct, curvilinear dependency of gs, this increase in WUE often results in reduced photosynthesis and yield.

Carbon isotope discrimination (CID) measures the ratio of stable carbon isotopes in the plant dry matter compared to that of the CO<sub>2</sub> in atmosphere. The linking of Δ<sup>13</sup>C with the observed variations in WUE is well developed and widely adopted (Sheshshayee, *et al.*, 2003). Commonly, but not always Δ<sup>13</sup>C negatively associates with WUE over the period of biomass accumulation. Under drought stress also Δ<sup>13</sup>C is also a good predictor of stomatal conductance and WUE in crops. Further, Δ<sup>13</sup>C is being used as a surrogate for WUE in different crop species (Sheshshayee, *et al.*, 2003).

Despite the determination of significant genetic variability using a robust, high throughput assessment approach, breeders were not enthusiastic in exploiting the variability in WUE. The inconsistency of the relationship between WUE and biomass which showed, positive, negative and even neutral correlation was the primary concern in large scale breeding programs (Sheshshayee *et al.*, 2012). Udayakumar, *et al.*, (1998) critically explained the hidden secrets of gs dependent (conductance/ stomata mediated) and g<sub>m</sub> dependent (capacity/ mesophyll mediated) WUE. More recently, Sheshshayee *et al.*, (2012) analyzed the sub components of WUE and discussed how WUE can still be a potential trait that can be considered for crop improvement. They demonstrated with experimental data that increasing WUE has tremendous significance after optimizing water use (through root) and /or light interception characters through canopy cover.

Ability to extract water from deeper soil profiles is an extremely important determinant of crop growth under water limited conditions. Presence

of genetic variability among the accessions under study holds the key for maize crop improvement under stress conditions. The maize germplasm accessions were screened for various root and shoot traits in specially built root structures.

Significant genetic variability was observed for various root and shoot traits like shoot length, shoot weight, root length, root volume, root weight, ratio of root to shoot and total biomass. Shoot length varied from 38.33 cm (CML-442) to 82.0 cm (KDM-442) with a mean of 64.09 cm. Root length varied from 23.0 cm (KDM-895A) to 61.67 cm (KDM-932A) with a mean of 36.93 cm. Root length showed a continuous variability and a positively skewed mesokurtic distribution towards the lower values. Root volume varied between a minimum of 11.67 ml (CML-442 and KDM-913A) to a maximum of 51.67 ml (KDM-1095) with a mean of 30.56 ml. Continuous variability was observed for root volume among maize germplasm accessions. Root weight, one of the important root traits that determine root system architecture varied from 1.17 (KDM-332A) to 5.31 g plant<sup>-1</sup> (KDM-918A) with a mean of 2.42 g plant<sup>-1</sup>. Root weight showed a positively skewed mesokurtic distribution towards lower values. Shoot weight varied from 4.17 (KDM-332A) to 15.57 g plant<sup>-1</sup> (KDM-440A) with a mean of 10.25 g plant<sup>-1</sup>.

Total biomass ranged from 3.87 (CML-442) to 20.03 (KDM-440A) gplant<sup>-1</sup> with a mean of 12.33 gplant<sup>-1</sup>. Root: Shoot ratio ranged from 0.12(KDM-892A) to 0.65 (KDM-930A) with a mean of 0.24 (Table 3.). Genotypes which have adaptive traits can maintain higher leaf water potential and relative water content under water limited condition and hence can tolerate stress better. On the other hand “Constitutive traits” are inherent traits expressed all the times irrespective of the stress occurrence. Constitutive traits such as root traits, water use efficiency, water conservation, cellular level tolerance, flowering time, and stay green traits can be routinely screened without a drought challenge. These traits have implications for drought protection and survival (Kamoshita *et al.*, 2008). These constitutive traits besides improving drought tolerance, significantly contribute to growth rates under any given condition. Therefore exploiting such traits has great relevance in improving productivity under water limited conditions (Sheshshayee *et al.*, 2003). To increase growth rates under drought conditions, abilities to mine water associated with roots and WUE becomes crucial.

A more comprehensive improvement of growth rates can be achieved only when constitutive traits are introgressed on to a genetic background with

reasonably high cellular level tolerance. In other words, it is important to pyramid “constitutive QTL” on to a background of superior adaptive traits. Clearly, the challenge of evolving improved cultivars with high yield potential under drought conditions appears to be a formidable approach. As per the Passioura model, crop growth rate is a function of the ability of the plant to harness water from deeper soil profile to meet the evaporation demand of the plant (Roots) and the efficiency of the plant to use water for biomass production (WUE) (Passioura, 1986). Though plants have evolved naturally increase WUE, it is generally achieved through a reduction in transpiration, because of a strong link between transpiration and photosynthesis, any reduction in transpiration would have an adverse effect on growth rates (Sheshshayee *et al*, 2003) Hence increase in WUE must be achieved without a substantial reduction in transpiration. Thus, evolving a tolerant genotype to sustain productivity under drought condition, diverse traits need to be pyramided to a single background.

However, all these traits are quantitatively inherited polygenic traits and their introgression can be effectively achieved only through a focused molecular breeding approach. Conventionally DNA based molecular markers flanking QTL are discovered using a bi-parental mapping population segregating for a specific trait. Since diverse trait are needed for improving drought tolerance, the bi-parental linkage mapping strategy become very cumbersome and laborious. Thus attempts to increase root traits and WUE have great relevance.

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 phenotype 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. 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 chromospheres 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.

The root system architecture is very plastic and able to adapt to different environmental conditions. Hammer *et al.*, (2009) showed that change in root system architecture and exploitation of soil water had a direct effect on the accumulation of maize biomass and contributed to the increasing yield. Remarkable genotypic diversity of root system architecture has been reported in artificial systems and in the field for young and fully developed plants. Information about root system architecture is mainly derived from plants growing under controlled conditions: Significantly different nodal root angles at the two-leaf stage were observed among nine inbred lines and two hybrids (Singh *et al.*, 2010). Hund (2010) also confirmed the positive effect of steeper roots on rooting depth during early growth. A study with 74 maize inbred lines showed differences in root numbers 14 days after planting: nodal root numbers ranged from 1.5 to 5.8 per plant; lateral root numbers ranged from 40.8 to 235.7 per plant (Kumar *et al.*, 2012). Ristic and Jenks (2002) measured epidermal water loss from 16-day-old plants by measuring water loss in

shoots and detached leaves (leaf blades) under dark conditions. The results revealed an inverse relationship between the epidermal water loss and cell-wall and cuticle thickness. Monneveux *et al.* (2007) carried out isotope discrimination studies in maize and reported that under drought, tolerant inbred lines showed significantly higher  $\Delta^{13}\text{C}$  than susceptible ones. There was a significant positive correlation between leaf, ear and silk  $\Delta^{13}\text{C}$ , and ear dry weight at flowering, a trait closely associated to grain yield. Drought tolerant hybrids had significantly higher grain yield than the used checks. No correlation was found, however, between  $\Delta^{13}\text{C}$  and grain yield within tolerant hybrids.

It is also evident that for a comprehensive improvement in productivity, it is essential to pyramid several of these traits in to a single genetic background. Therefore a systematic assessment of genetic variability in specific drought adaptive traits is an essential pre-requisite for breeding to improve drought adaptability in Maize.

Significant genetic variability in drought tolerance traits was revealed among the maize germplasm lines. The promising lines identified like KDM-932A for root length, KDM-1095 for root volume, KDM-918A for root weight, KDM-954 for epicuticular wax content and KDM- 944A for WUE can be used as trait donor lines to transfer specific traits into 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 these traits.

The contrasting genotypes differing in specific traits forms the basis to develop greater understanding on the factors that cause the variations in the trait both at the physiological level as well as molecular level. Mining superior alleles of genes which control root growth, epicuticular wax formation is extremely crucial in developing focused translational research activities either through breeding or transgenic. The contrast groups of genotypes for these traits form an excellent material for allele mining. Development of segregating populations for specific traits is still the most effective strategy for identifying Novel genes and alleles by map based cloning or positional cloning. The contrasts for specific traits are hence an excellent source for developing such trait specific populations, which can be used for QTL mapping as well as gene discovery.

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

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

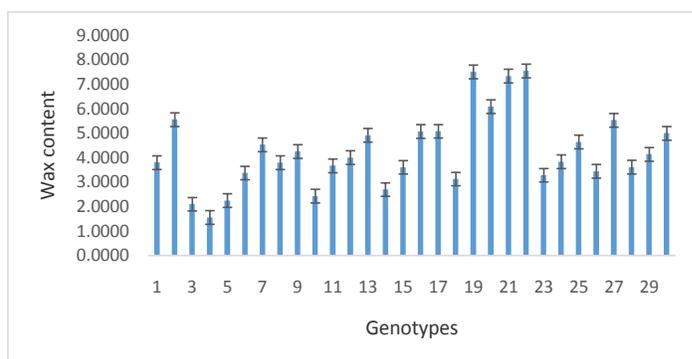
**Table 2. Genetic variability for physiological parameters in maize genotypes**

Traits	Mean	Minimum	Maximum	Std. Dev
A	16.22	10.59	27.01	0.07
G <sub>s</sub>	0.13	0.07	0.22	0.002
Ci	163.72	89.09	251.73	1.53
T	2.13	1.29	3.20	0.01
Δ <sup>13</sup> C (WUE)	0.79	0.50	1.03	0.04
SCMR	37.9	26.1	45.80	1.88
CT(°C)	26.72	23.47	29.27	0.81
CCATD(°C)	-2.28	-5.53	- 0.01	1.51
A/T	7.61	8.20	8.46	7.0
Ci/g <sub>s</sub>	1259.38	1272.71	1144.22	765.0
A/g <sub>s</sub>	124.76	151.28	122.77	35.0

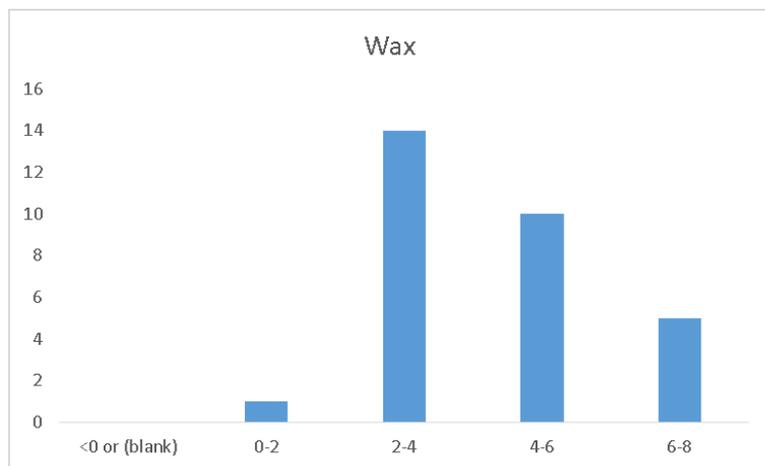
Ci – Intercellular CO<sub>2</sub> concentration (ppm), A – Assimilation rate ( $\mu\text{ mol m}^{-2} \text{ s}^{-1}$ ), T – Transpiration rate ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), g<sub>s</sub> - Stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), A/T – Ratio of Assimilation to transpiration rate ( $\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$ ), A/g<sub>s</sub> – reflection of WUE at single leaf level ( $\mu\text{mol CO}_2.\text{molH}_2\text{O}^{-1}$ ), Ci/g<sub>s</sub> – reflection of mesophyll efficiency at single leaf level

**Table 3. Genetic variability in root and shoot traits among maize genotypes**

Traits	Mean	Minimum	Maximum	Std. Dev
Shoot Length (cm)	64.09	38.33	82.00	3.15
Root Length (cm)	36.93	23.00	61.67	3.10
Root Volume (ml)	30.56	11.67	51.67	1.10
Shoot Weight (gplant <sup>-1</sup> )	10.25	4.17	16.63	0.78
Root Weight (gplant <sup>-1</sup> )	2.42	1.15	5.31	0.14
Root:Shoot Ratio	0.24	0.12	0.65	0.16
Total Biomass (gplant <sup>-1</sup> )	12.33	3.87	20.03	2.11



**Fig.1. Genetic variability in EWC in Maize Genotypes**



**Fig. 2. Frequency distribution of EWC in Maize Genotypes**