Assessing genetic diversity of maize genotypes for transpiration efficiency

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Abstract
Transpiration Efficiency (TE), the biomass produced per kilogram of water transpired is an ideal parameter for measuring genetic variation in crop water use efficiency. Assessing the genetic diversity and variability in maize for this character will help us to understand the most efficient genotypes for water use. Studies on 89 maize genotypes for two years using six characters revealed significant differences among the genotypes and wide range of variation for all the characters. High variability was observed for total biomass, seed yield and TE-seed. High heritability and genetic advance as per cent of mean was observed for all the characters except water transpired. Eighty nine genotypes were grouped into 10 clusters and total biomass contributed maximum towards genetic divergence. TE seed was positively and significantly correlated with all the characters studied except water transpired. As there is a lot of variability present in the maize genotypes and the desirable characters reported high coefficients of variability, heritability, genetic advance and positively high correlation among the characters, the chance of selecting superior genotypes are more. Moreover, crossing between different clusters of clusters will enhance the chance of transgressive segregants. Maize genotypes Z 32-87, NSJ-176, DTL-2, Z101-68, HKI-1040-4, NSJ-189, LM-16, HKI-1025, NSJ-2011-26, NSJ-2011-37, DTL-3, DTL 4-1 HKI-1332, Z-60-72 and PSRJ-13038 were found to be superior for TE biomass and TE seed.

Key words
Transpiration efficiency, Maize, Genetic variation

INTRODUCTION
The ability of crop to produce high yield per unit of available water is potentially important in affecting profitability and yield, in both irrigated and rainfed production system. Genetically, increasing crop water use efficiency is an effective strategy for increasing yield in dry environment (Condon et al., 2004; Blum 2009) as forecasts of increasing scarcity of water for agriculture remains a strong motivation for improving crop water use efficiency. Transpiration Efficiency (TE) defined as the biomass production per unit of water transpired is the preferred measure for examining potential genetic variation in crop water use efficiency (WUE). Transpiration efficiency is WUE of plants alone (no soil water losses).

Almost a century ago, Briggs and Shantz (1913) showed that crop specie differ in their transpiration efficiency. Since then, the C3 and C4 photosynthetic pathways have been elucidated, and differences in transpiration efficiency have been related to them. Subsequent studies have identified genetic variation in transpiration efficiency within a specie, example include groundnut (Ratnakumar et al., 2009, Halilou et al., 2015), cowpea (Ismael and Hall 1992, Halilou et al., 2015), wheat (Farquhar and Richards 1984, Blum 2009) and sorghum (Hammer et al., 1997, Xin et al., 2009) and breeding efforts have been made to include TE in the improved germplasm (Udayakumar et al.,1998). Jackson et al., (2015) reported a significant variation in whole plant transpiration efficiency among 51 genotypes of sugarcane (Saccharum spp.) or closely related germplasm in pot culture experiment. Phenotypic values for whole plant TE ranged from 5.7 to 8.6 g/Lin sugarcane. So, it is important to assess the genetic
variation for TE over an entire crop cycle and to determine whether there is a large genotype by water regime interaction for TE in maize.

Maize is a diverse and highly cross pollinated crop. There exist a good number of works on genetic diversity of maize, The genotypes experimented in this study were not studied for TE and water requirement so far. Analysis of genetic diversity is an important step for better understanding and utilization of germplasm. The present investigation was undertaken with a view to quantify the genetic variation for water use and transpiration efficiency in 87 maize genotypes and two varieties, to estimate genetic variance, heritability and genetic advance and to investigate the nature and magnitude of genetic diversity in 89 maize genotypes.

MATERIALS AND METHODS

Eighty seven maize genotypes of diverse origin were used for the study. Seeds of these maize genotypes were obtained from National Bureau of Plant Genetic Resources (NBGPR), Regional Station, Hyderabad, Directorate of Maize Research (DMR), New Delhi, CIMMYT, Regional Centre, Hyderabad, Central Research Institute for Dry Land Agriculture (CRIDA), Hyderabad and Maize Research Station, Hyderabad (Table 1). Two varieties (Harsha and Varun) from Maize Research Centre, PJTSAU, Hyderabad were also used in the experiment. The experiment was laid out in Randomized Complete Block Design with three replications (RCBD). The plants were grown in pots during Kharif -2014 and 2015 (June–Oct.) under rain out shelter facility. The minimum temperature was 19°C and the maximum was 36.6°C and relative humidity and sun shine hours during crop growth period varied from 44.0 % to 96.0 % and 0.0 to 10.3 hrs respectively.

When the plants reached maturity, they were harvested at soil level and final pot weight was recorded. Individually plants were partitioned into leaves, stem and cobs. Dry weights were recorded after keeping the plant parts in hot air oven at 60°C till the constant weight were attained. Cobs were sun dried. Seed was separated from cob and seed yield was recorded. Total water transpired was calculated by subtracting the final pot weight from the initial weight and then adding the amount of water that has been applied at regular interval. TE biomass was calculated by dividing the above ground dry biomass by the amount of water transpired. TE seed was calculated by dividing the seed yield by the amount of water transpired.

Table 1. Source of genotypes

| Institute                  | No. | Genotype names                  |
|----------------------------|-----|---------------------------------|
| NBGPR                      | 40  | RJR-049, RJR-55, RJR-42, NSJ-315, RJR-163, NSJ-366, NSJ-245, RJR-159, RJR-270, RJR-198, RJR-288, RJR-208, RJR-247, PSR13187, PSR13255, PSR13247, RJR-328, RJR-363, RJR-385, PSRJ13122, PSRJ13041, PSRJ13007, PSRJ13099, PSRJ13059, NSJ-211, NSJ-176, NSJ-189, NSJ-155, PSRJ13086, PSRJ13038, PSRJ13154, RJR-068, RJR-075, RJR-115, SNJ-2011-37, SNJ-2011-26, SNJ-2011-03, SNJ-2011-104, SNJ-2011-102, SNJ-2011-70 |
| DMR                       | 20  | HKI-161, HKI-163, HKI-164-7-4, HKI-1035-10, HKI-1011, HKI-3-4-6ER, HKI-1025, HKI-209, HKI-1332, HKI-766(0), HKI-577, HKI-1040-4, HKI-46, HKI-325-17AN, HKI-47, HKI-L287, LM5, LM13, LM14, LM16 |
| CIMMYT                    | 19  | Z50-87, Z40-19, Z61-34, Z59-9, Z59-11, Z101-68, Z32-12, Z93-194, Z49-7, Z93-154, Z101-15, Z59-41, Z60-72, Z32-87, Z93-170, Z40-183, Z49-65, Z96-5, Z162-9 |
| CRIDA                     | 8   | DTL-1, DTL-2, DTL-3, DTL-4, DTL-4-1, DTL-10, DTL-11, DTL-12 |
| Maize Research Station, Hyd. | 2   | Harsha, Varun (Composite variety) |

RESULTS AND DISCUSSION

Analysis of variance was carried out separately for two years for the six characters studied in maize and as coefficients of variations were low pooled analysis of variance was carried out and given in Table 2. Pooled analysis of variance revealed highly significant mean sum of squares due to genotypes for all the characters indicating significant differences among 89 genotypes of maize. Significant mean sum of squares due to environment indicates the presence of significant differences among
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The environments for all the characters studied. The estimates of range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability ($h^2$) and genetic advance as per cent of mean are presented in Table 3. Total biomass ranged from 60.45 to 159.78 with a mean of 108.24 gm whereas seed yield ranged from 18.18 to 59.15 with a mean of 37.35 gm. Harvest index ranged from 21.28 to 44.31 with a mean of 34.26%. Range of water transpired in 89 maize genotypes was from 24.45 to 34.15 with a mean of 31.34 kg/plant. The maize genotypes transpired 8.0 to 11.3 kg/plant during pre anthesis stage and 24.5 to 34.1 kg/plant from sowing to grain harvest indicating that 32.6 to 33.1% of water is transpired during pre anthesis stage. TE biomass ranged from 2.55 to 6.24 with a mean of 4.40 g/kg. TE seed ranged from 0.67 to 2.57 with a mean of 1.56 g/kg. Genetic diversity present in the material is evidenced from the wide range of variation observed for all the characters and the probability of selecting desirable genotypes will be

Table 2. Analysis of variance for plant traits and TE in pooled data

| Source       | df | Total biomass | Seed Yield | Harvest Index | Water Transpired | TE biomass | TE seed |
|--------------|----|---------------|------------|--------------|------------------|------------|---------|
| Replications | 2  | 24.850        | 8.068      | 1.211        | 0.049            | 0.009      | 0.008   |
| Environment  | 1  | 39720.28      | 674.60     | 1353.63      | 132020.80        | 1967.27    | 290.08  |
| Interaction  | 1  | 2.051         | 0.418      | 0.345        | 0.162            | 0.005      | 0.001   |
| Genotypes   | 88 | 3308.6**      | 807.7**    | 202.9**      | 13.9**           | 4.21**     | 1.31**  |
| Error       | 176| 59.447        | 20.171     | 11.896       | 1.726            | 0.299      | 0.111   |
| SEm         |    | 7.1           | 12.02      | 10.1         | 4.2              | 12.4       | 21.4    |
| CV (%)      |    | 5.1           | 1.83       | 1.4          | 0.5              | 0.2        | 0.1     |
| CD (5%)     |    | 8.7           | 5.10       | 3.9          | 1.5              | 0.6        | 0.4     |

*Significant at 5% level, ** significant at 1% level

Photo 1. Transpiration efficiency experiment setup under rainout shelter facility
high. It is needed to partition the observed variability into heritable and non-heritable components by the means of GCV and PCV to understand the influence of environment on the expression of different characters.

The estimates of PCV were higher than that of GCV for all the characters indicating the effect of environment but the narrow difference between GCV and PCV indicated little influence of environment on these characters. PCV ranged from 6.19 (water transpired) to 35.88 (TE seed) and GCV ranged from 4.56 (water transpired) to 30.67 (seed yield). High genotypic coefficient of variability was observed for total biomass, seed yield and TE seed indicating high variability for these characters among the genotypes and there is a great scope for the improvement of these characters by direct selection among the genotypes. Medium variability was observed for the characters harvest index and TE biomass which indicated the variation for these characters was medium among the genotypes and there is a need for improvement of base population to increase the genetic variability and to fix the favourable alleles. Vashistha et al., (2013) also reported a similar result. Low variability was recorded by water transpired indicated there is a less difference among the genotypes for this character.

For assessing the heritable variation, the magnitude of heritability is the most important aspect in the breeding material which has close bearing on the response to selection. Heritability was high for all the characters except water transpired and it ranged from 54.16 (water transpired) to 90.11 (total biomass) which indicated that these characters were relatively less influenced by environmental conditions and phenotypic selection would be effective for the improvement of these characters. The higher values of heritability of traits are indicative that the selection can be made on the basis of these traits (Ali et al., 2012).

Genetic advance as per cent of mean ranged from 6.91 (water transpired) to 58.82 (seed yield) and it was high for all the characters except water transpired which indicated that these characters are governed by additive gene action and selection would be effective for the improvement of these characters. High heritability coupled with high genetic advance as per cent of mean was observed for all the characters except water transpired which indicated that most likely the high heritability might be due to additive gene effects hence; it could be improved by simple selection methods like pureline selection, mass selection, progeny selection or family selection. Rahman et al., (2015), Vashistha et al., (2013), Nataraj et al., (2014), Haydar et al., (2015), reported analogous kind of result for this trait. Most of the characters in the present study recorded high variability, heritability and genetic advance as per cent of mean indicating additive gene action for the expression of these characters and scope of simple selection for the improvement of these characters.

For the estimation of variability, heritability and genetic advance as per cent of mean for six characters

| S. No. | Character                        | Range  | Mean | PCV (%) | GCV (%) | h² (%) | GA as % of mean (%) |
|-------|---------------------------------|--------|------|---------|---------|--------|---------------------|
| 1     | Total biomass (g/pl)            | 60.45  | 159.78 | 108.24 | 22.65  | 21.50  | 90.11  | 42.04 |
| 2     | Seed Yield (g/pl)               | 18.18  | 59.15 | 37.35  | 32.94  | 30.67  | 86.68  | 58.82 |
| 3     | Harvest Index (%)               | 21.28  | 44.31 | 34.26  | 19.30  | 16.47  | 72.79  | 28.95 |
| 4     | Water Transpired (kg/pl)        | 24.45  | 34.15 | 31.34  | 6.19   | 4.56   | 54.16  | 6.91  |
| 5     | TE biomass (g biomass/kg water) | 2.55   | 6.24  | 4.40   | 22.15  | 18.34  | 68.53  | 31.27 |
| 6     | TE seed (g seed/kg water)       | 0.67   | 2.57  | 1.56   | 35.88  | 28.77  | 64.29  | 47.52 |

PCV and GCV: Phenotypic and genotypic coefficient of variation, h²: Heritability in broad sense, GA: Genetic Advance

Multivariate analysis using Mahalanobis D² statistic provides a useful tool for measuring the genetic diversity with respect to all the characters considered together. Analysis of variance for dispersion showed a highly significant mean sum of squares due to genotypes. Test of significance using Wilk’s criterion revealed highly significant V statistics. The D² values were computed for all the possible pairs of combinations from the mean values of 89 maize genotypes to 6 characters. Ranking character wise D² values and adding the ranks for each character for all the genotypes had done to identify the characters that contributed maximum towards divergence. Contribution of different characters towards genetic divergence is presented in Table 4. Total biomass contributed the highest (60.80%) towards genetic divergence followed by seed yield (25.20%), water transpired (7.61%), harvest index (3.78%), TE total biomass (0.84%) and TE seed (1.76%). Present results are agreement with those of Marker and Krupakar (2009). High % contribution of total biomass towards genetic diversity indicated this character can be used as parameters in selecting genetically diverse parents for hybridization.
Eighty nine genotypes were grouped into 10 clusters. The compositions of different clusters along with number of genotypes are given in Table 5. Cluster II was the largest with 24 genotypes followed by cluster III with 23 genotypes, cluster I with 16 genotypes, cluster V with 14 genotypes and cluster VIII with 7 genotypes and remaining clusters were solitary. Presence of solitary clusters indicated the extreme phenotypic performance in positive or negative directions for one or the other characters included in the present study. The genotypes from different sources were grouped together in different clusters revealed that there was no parallelism between genetic diversity and geographic diversity. The nature of selection forces operating under one eco-geographical region seemed to be similar to that of other regions since the accessions from different geographical regions were grouped together into same clusters. This would be due to the similarity of objectives and conditions under which the types were bred and domesticated in different localities. By observing the cluster composition, it was evident that the accessions of same source were scattered into different clusters. The existence of wide genetic diversity among the accessions chosen from the same geographical region was thus obvious.

### Table 5. Grouping of genotypes into different clusters by Tocher method

| Cluster | No. | Genotype names |
|---------|-----|----------------|
| Cluster I | 16 | NSJ-176, NSJ-189, Z60-72, Z32-87, Z101-68, Z60-87, HARSHA, HKI-1025, HKI-L-287, SNJ-2011-26, HKI-1332, DTL-1, NSJ-155, LM-15, SNJ-2011-70, SNJ-2011-37 |
| Cluster II | 24 | NSJ-366, HKI-165, HKI-101, PSR-13187, PSRJ-13038, Z49-65, HKI 325-17AN, LM-13, Z32-12, HKI-209, RJR-208, Z40-183, RJR-169, PSRJ-13154, RJR-55, PSRJ-13122, PSRJ13255, RJR-42, PSRJ-13041, Z61-34, PSRJ-13007, RJR-170, PSRJ-13086, RJR-163 |
| Cluster III | 23 | HKI-577, RJR-115, NSJ-245, RJR-328, NSJ-211, HKI-161, RJR-075, HKI-766(0), RJR-049, RJR-247, PSRJ-13099, SNJ-2011-102, RJR-363, HKI-47, RJR-288, PSRJ13059, RJR-068, Z59-11, SNJ-2011-03, RJR-385, NSJ-315, SNJ-2011-104 |
| Cluster IV | 1 | PSR 13247 |
| Cluster V | 14 | HKI-164-4-3, DTL-10, HKI-3-4-8ER, HKI-163, DTL-3, DTL-11, DTL-4, DTL-12, DTL-4_3, HKI-1040-4, VARUN, DTL-2, Z93-170, Z101-15 |
| Cluster VI | 1 | RJR-198 |
| Cluster VII | 1 | LM-5 |
| Cluster VIII | 7 | Z59-9, Z53-154, Z93-194, Z96-5, Z162-9, Z40-19, Z59-41 |
| Cluster IX | 1 | LM-14 |
| Cluster X | 1 | HKI-46 |

### Table 6. Inter and intra Cluster Distances on the basis of $D^2$ cluster analysis

| Cluster | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII | Cluster IX | Cluster X |
|---------|-----------|------------|-------------|------------|-----------|------------|-------------|--------------|------------|----------|
| Cluster I | 0.467 | 3.443 | 6.372 | 2.779 | 6.699 | 8.974 | 11.820 | 12.447 | 12.862 |
| Cluster II | 3.443 | 1.303 | 6.372 | 1.761 | 4.177 | 1.825 | 3.022 | 6.392 | 7.635 |
| Cluster III | 6.372 | 1.761 | 1.546 | 3.643 | 11.064 | 3.472 | 3.516 | 4.902 | 12.779 |
| Cluster IV | 2.779 | 4.177 | 11.064 | 0.000 | 5.307 | 0.588 | 1.006 | 4.902 | 5.088 |
| Cluster V | 6.699 | 1.825 | 3.472 | 5.307 | 2.543 | 5.682 | 8.518 | 12.779 | 7.300 |
| Cluster VI | 8.974 | 3.022 | 3.516 | 0.588 | 8.518 | 12.779 | 7.300 | 7.437 | 7.437 |
| Cluster VII | 11.820 | 6.392 | 4.902 | 1.006 | 12.779 | 7.300 | 7.437 | 7.437 | 7.437 |
| Cluster VIII | 12.447 | 7.635 | 5.088 | 4.902 | 7.300 | 7.437 | 7.437 | 7.437 | 7.437 |
| Cluster IX | 12.862 | 7.635 | 11.328 | 5.088 | 11.328 | 7.437 | 7.437 | 7.437 | 7.437 |
| Cluster X | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

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Procedure suggested by Tocher (Rao, 1952) was used to group 89 genotypes into ten clusters. The pattern of distribution of 89 genotypes into various clusters is indicated in Table 5. Inter and intra cluster genetic distance (D) values among five clusters are presented in Table 6. Inter cluster D values ranged from 0.598 (IV and VI) to 25.54 (VIII and X). These findings are in conformity with the findings of Marker and Krupakar (2009). Medium inter cluster distance between cluster III and X and VIII and X suggested genotypes belonging to the clusters separated by the high statistical
distance could be used in recombination breeding for obtaining high heterotic responses and better segregants. Intra cluster values ranged from 0 to 3.62 (VIII). Maximum intra cluster distances were recorded by cluster VIII and V indicated these cluster had the accessions with varied genetic divergence while accessions of clusters III and V showing minimum intra cluster distance genetically resembled to each other and might have come from common gene pool. Intra cluster distances were zero in the solitary clusters. The inter-cluster distances were larger than the intra-cluster distances which indicated wider genetic diversity among the genotypes of different groups. Debnath (1987) obtained a larger inter-cluster distance than the intra-cluster distance in a genetic variability in maize. Similar results were also obtained by Abedin and Hossain (1990) in maize. Clusters with comparatively less magnitude of divergence showed instability, while widely divergent clusters remained distinct in different environments (Raut et al., 1985).

Table 7. Cluster-wise mean table for the six traits studied in 89 genotypes of maize

| Cluster | Total biomass | Seed Yield | Harvest Index | Water Transpired | TE biomass | TE seed |
|---------|---------------|------------|---------------|------------------|------------|---------|
| Cluster I | 128.763 | 52.497 | 40.956 | 31.879 | 5.081 | 2.130 |
| Cluster II | 108.060 | 36.490 | 33.860 | 31.684 | 4.384 | 1.520 |
| Cluster III | 80.662 | 27.452 | 34.289 | 30.807 | 3.414 | 1.204 |
| Cluster IV | 102.750 | 26.533 | 26.562 | 31.807 | 3.977 | 1.105 |
| Cluster V | 136.037 | 46.599 | 34.103 | 32.049 | 5.411 | 1.923 |
| Cluster VI | 103.683 | 27.250 | 26.275 | 31.287 | 3.709 | 0.940 |
| Cluster VII | 93.950 | 22.000 | 23.305 | 33.370 | 3.630 | 0.810 |
| Cluster VIII | 83.540 | 22.162 | 26.312 | 28.131 | 3.709 | 0.940 |
| Cluster IX | 130.600 | 27.800 | 21.280 | 33.710 | 5.085 | 1.075 |
| Cluster X | 156.300 | 41.300 | 26.430 | 34.470 | 6.000 | 1.575 |

Table 8. Phenotypic (upper diagonal) and genotypic (lower diagonal) correlation matrix.

| Character | Total Biomass | Seed Yield | Harvest Index | Water Transpired | TE (biomass) | TE (seed) |
|-----------|---------------|------------|---------------|------------------|--------------|-----------|
| Total biomass | 1.000 | 0.831** | 0.256 | 0.449 | 0.887** | 0.691** |
| Seed Yield | 0.859** | 1.000 | 0.733** | 0.315 | 0.768** | 0.902** |
| Harvest Index | 0.314 | 0.745** | 1.000 | 0.024 | 0.270 | 0.751** |
| Water Transpired | 0.579 | 0.429 | 0.101 | 1.000 | 0.257 | 0.191 |
| TE Biomass | 0.989** | 0.858** | 0.324 | 0.431 | 1.000 | 0.795** |
| TE seed | 0.820** | 0.999** | 0.797** | 0.334 | 0.824** | 1.000 |

*Significant at 5% level, ** significant at 1% level

Cluster mean values were given in Table 7 and ranged from 80.66 (III) to 156.30 g/pl (X) for total biomass. Seed yield ranged from 22 (VII) to 52.5 g/pl (I). Harvest index ranged from 21.28 (IX) to 40.96 (I). Water transpired ranged from 28.13 (VIII) to 33.71 Kg/pl (IX). TE Biomass ranged from 3.41 (III) to 6.00 g/kg (X). TE seed ranged from 0.81 (VII) to 2.13 g/kg (I). Based on cluster means Singh and Chaudhari (2001) also reported a wide range of variation for grain yield and its components in maize. Similarly, Marker and Krupakar (2009) have also assessed the range of variability of 16 genotypes for 14 different traits in maize.

Knowledge about the nature and extent of association among different biometrical characters will be useful to identify the key characters for which the selections can be fruitfully made. Genotypic and phenotypic correlation coefficients were estimated and given in Table 8. Magnitude of genotypic correlations were high compared to corresponding phenotypic correlations for all the characters which indicated that there was a strong inherent genetic association between the various characters studied and phenotypic correlations were reduced by significant interaction of environment. TE seed is significantly positively correlated with total biomass (0.82), seed yield (0.99), harvest index (0.80) and TE Biomass (0.82). TE biomass was positively and highly significantly correlated with total biomass (0.99) and seed yield (0.86). Seed yield (0.75) was positively highly significantly correlated with harvest index and total biomass (0.86) was highly significantly positively correlated with seed yield. High magnitude of genotypic correlations indicated that there was a strong inherent genetic association between the various characters studied. Correlation among component characters revealed that strong positive associations among desirable component characters, hence the selection criteria should consider all these characters. Maize genotypes Z 32-87,
Superior for TE biomass and TE seed. HKI-1025, NSJ-2011-26, NSJ-2011-37, DTL-3, DTL-4-1 HKI-1332, Z-60-72 and PSRJ-13038 were found to be superior for TE biomass and TE seed.

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