

Early production of dry matter and its relationship to grain yield in maize (*Zea mays* L.) populations

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ABSTRACT

Objective: To determine whether early production of dry matter can be considered as a selection criterion, and to analyze the association of the traits in dissimilar environments with grain yield. The genetic material consisted of nine maize populations adapted to the environmental conditions of the southeast of the Coahuila state in Mexico, and the direct crosses between them.

Design/methodology/approach: The genotypes were evaluated for the root dry weight (RDW) and stem dry weight (SDW) under laboratory and greenhouse trials, with and without stress due to salinity, and in the field, for grain yield.

Results: There was no evidence of a significant relationship between dry weight (RDW and SDW) in laboratory trials with the grain yield whereas, in greenhouse trials, a positive RDW and SDW relationship with stress and non-stress was found with grain yield, where the RDW (stress) showed a correlation of $r=0.308^*$. There is a genetic variation among populations for the evaluated traits, which is manifested in their expression, and in the potential of genetic combination of the crosses.

Findings/conclusions: Populations 6R and 7R were identified, and a significant group of crosses showing the combinations of them with positive values of grain yield and root and stem dry weight under salinity stress. Root dry weight (RDW) determined under salinity stress, jointly with RDW and SDW under non-stress conditions in the greenhouse can be considered as potential indicator for early selection of maize genotypes.

Keywords: *Zea mays* L., salinity stress, environmental effects, native populations.

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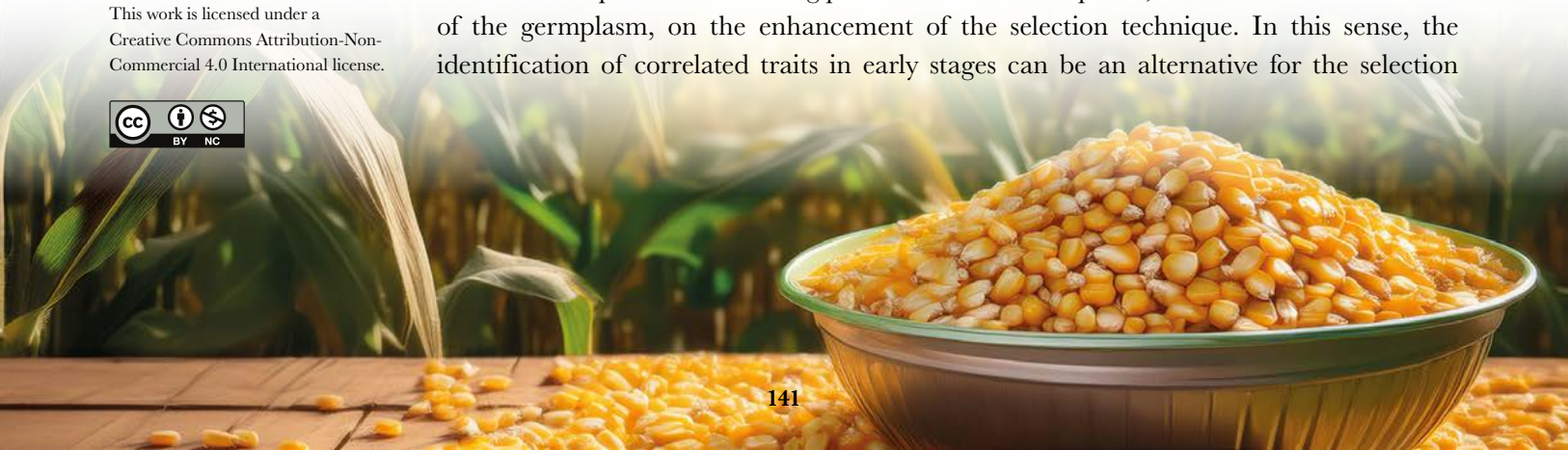
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INTRODUCTION

Genetic improvement is a long process and success depends, in addition to the selection of the germplasm, on the enhancement of the selection technique. In this sense, the identification of correlated traits in early stages can be an alternative for the selection



of genotypes with outstanding agronomic behavior, as a basis for the development of new varieties that provide genes associated with resistance to biotic and abiotic factors (Lamz and González, 2013). Exposure of genetic materials to salinity stress conditions may be an alternative for identifying alleles present in genotypes. Salinity affects the growth and development of plants through their impact on photosynthesis, respiration, starch metabolism, water ratios and nutrient absorption; at high levels causes toxicity, reduces plant growth and crop productivity (Torabi *et al.*, 2013; Farooq *et al.*, 2015). In a study under salinity stress conditions, Giaveno *et al.* (2007) reported significant presence of genetic variation, and proposed that the use of vigor traits, such as the weight and growth rate of seedlings under stress conditions may be used as a selection criterion for salt-tolerant in maize breeding programs. In seed quality tests, the dry weight of seedling and seed are considered as vigor tests, as well as the expression of the response of genotypes to different stresses conditions, to determine differences in the physiological potential of seeds (Marcos-Filho, 2015). Considering the needs to identify alleles that can contribute to mitigate fluctuations in the environment, the present research work was developed, with the following objectives: to determine whether dry root and stem weight under salinity stress conditions can be considered as selection criteria; and to analyze the association of the traits in dissimilar environments with grain yield performance. The research work is based on the premise that there is important genetic variation in the genotypes under study, and that early-stage dry matter production traits can be considered as a selection criterion in genetic improvement of maize.

MATERIAL AND METHODS

The genetic material included in the research work consisted of nine native maize populations, representative of five racial groups, adapted to the environmental conditions of the southeast of the Coahuila state in Mexico, which were identified based on a sample of genetic variation and performance potential in the region of study (Espinosa *et al.*, 2019). In the PV-2016 agricultural cycle, direct crosses were made between the nine populations under study. Genotypes (populations and 36 crosses) were subjected to evaluation trials in three stages: 1) Laboratory trials to determine the physiological quality of seeds under conditions with and without salinity stress, 2) Greenhouse trials to analyze early-stage development with two environments, with and without salinity stress, and 3) Field evaluation trial in a target study location in the southeast of the Coahuila state. In laboratory and greenhouse trials, an electrical conductivity of 22.8 mS cm^{-1} was used for salinity stress conditions, obtained with a NaCl concentration of 16.29 g L^{-1} . Electrical conductivity was determined based on an *ad hoc* study to obtain the optimal concentration of salts (unpublished data).

Laboratory trials

Laboratory trials were carried out in the Seed Physiology and Biochemistry Laboratory of the Centro de Capacitación y Desarrollo en Tecnología de Semillas, Universidad Autónoma Agraria Antonio Narro (UAAAN). The physiological quality of the seeds through the standard germination test was determined, according to the ISTA rules (ISTA,

2009), with modification in the sample size and number of replications, since, in some of the crosses not enough seed was obtained.

To know the response of the genotypes to different environmental conditions, two trials were established, one under salinity stress (SL) conditions and one under non-salinity stress (NL) conditions. In both cases, three replicates of 25 seeds were sown on anchor paper, which was moistened with saline solution for the stress conditions, and only distilled water for the non-stress conditions, the latter, used as a check in this study. The paper with the seeds was rolled in the form of a “taco” and placed in polyethylene bags according to the corresponding identification plot number and placed inside a basket, which were subsequently placed in a growth chamber at 25 ± 1 °C for seven days. From the germination test, only the seedlings that had normal development, using as a criterion, those that had a length equal to or greater than three centimeters, both in the stem and the root, were considered. Remnant of the seed was removed from each seedling, separating the stem from the root system. Later, they were dried in a stove at a temperature of 70 °C for 24 h, resulting in the stem dry weight (SDW) and the root dry weight (RDW), expressed in milligrams (mg plant^{-1}).

Greenhouse trials

The evaluation trial was conducted in the greenhouse number two of the Department of Plant Breeding, located on the Saltillo campus of UAAAN. The greenhouse has passive temperature control, which was recorded every four hours during the experiment, with a daily average of 31.0 °C during the day and 18.3 °C at night. Planting was done in polyethylene bags, using a mixture of peat moss, perlite, and vermiculite as a substrate in a 2:1:1 ratio, respectively. Four replications were set in the evaluation test, and for more efficient handling, each repetition was established on a different planting date. Two seeds were sown at a depth of 3 cm; in the emergency, one of them was removed to leave a plant per bag as an experimental unit, for both non-stress (NG) and salinity stress (SG) conditions.

To subject the seed of the genotypes to different evaluation environments, for the stress condition, the irrigation was performed by applying 100 ml of saline solution, beginning at the planting day, and only distilled water, for testing under non-stress conditions; these settings were applied in subsequent irrigations. In both evaluation environments, 35 days after planting, each plant, through a carefully washing process, the seed residue was removed, and the aerial part or stem was separated from the root system. Subsequently, both parts were subjected to drying in a dehydrating stove at a temperature of 70 °C for 48 h. This resulted in the stem dry weight (SDW) and root dry weight (RDW) traits, expressed in mg plant^{-1} in both environments.

Field evaluation trial

The genotypes were established for agronomic evaluation in the Spring-Summer (2017) agricultural cycle, in the location of El Mezquite, Galeana, N. L. (1890 m; 25° 05' N; 100° 42' W), located 10 km from the limits of Saltillo, Coah., under irrigation conditions. The experiment was established using an incomplete block design with an alpha lattice

arrangement and three replications, and a fertilization dose of 120-60-60 units of N, P and K, respectively. The experimental unit consisted of a 4 m row, with a distance between plants of 0.20 m and a distance between rows of 0.80 m. In this study, only the grain yield was expressed in t ha^{-1} , considering a 15% seed humidity content.

Data analysis

In each evaluation environment, analysis of variance was performed according to the experimental design with the GLM procedure of SAS (SAS Institute, 2022). To identify outstanding genotypes, in each of the traits under study, a decision value was calculated determined by the mean plus one and twice the standard error of the mean ($\mu + Se$; $\mu + 2Se$). A simple Pearson correlation analysis (r) was performed for the studied traits by the trials \times the evaluation environments. The genotype means of each combination of traits by trials \times the evaluation was used to perform a graphical display analysis based on the Principal Component Analysis (PCA) with the GGEbiplot model (Yan and Kang, 2003; Yan, 2014).

RESULTS AND DISCUSSION

An overview of the analysis of variance results for both, root dry weight (RDW) and stem dry weight (SDW) is stated in the trials performed under stress and non-stress conditions in Laboratory and Greenhouse, and the grain yield performance on the field evaluation (Data not shown). In the laboratory trials, significant differences for RDW and SDW ($P \leq 0.01$) were found between the environments, corresponding to the stress and non-stress (check) salinity trails, respectively; while, in the greenhouse trials, no differences were found between environments in the two traits under study. In Genotypes (populations and crosses), statistical differences were only found in the RDW in laboratory, greenhouse and grain yield trials; basically, these differences were observed in the crosses between the populations under study, so in no case there was any statistical evidence of differences between populations. In general, no differences were found in the genotype \times environment interaction, except in the SDW ($P \leq 0.05$) in laboratory trials, which refers to the populations \times environments interaction. In the laboratory trials, there was a reduction of 49.0 and 60.0 % for root dry weight (RDW) and stem dry weight (SDW), respectively, as a response to the salinity stress with respect to the non-stress (check). In greenhouse trials, although there is a numerical difference between stress conditions with respect to the check, those differences are not statistically thought, with a reduction of 5.0 and 13.0 % for RDW and SDW, respectively. Means of grain yield and dry weight of root and stem for populations, and the best 25 crosses from the evaluation environments are presented in Table 1.

Although, populations are different base on their genetic constitution and the area of adaptation source (Espinosa *et al.*, 2019), no differences were found among them, due in part to the relative comparison and confounded effects on the evaluation environments (stress and non-stress) in laboratory and greenhouse; while in the field, possibly to the response to the environment effects, since, with the exception of populations 1CN and 2CN, which are specifically adapted to the evaluation location (transition-highland adaptation area), the

Table 1. Means of grain yield and dry weight of maize populations, and the top 25 crosses evaluated in 2017.

Genotypes	Yield (t ha ⁻¹)	Greenhouse trials		Laboratory trials	
		RDW (mg)	SDW (mg)	RDW (mg)	SDW (mg)
7R [†]	9.25 *	375.7	2,343.6	23.9	43.1 *
8T	9.10	432.6	2,264.2	26.4	37.4
6R	8.13	333.3	2,436.6	25.8	42.0 *
5TN	8.09	380.8	1,960.6	21.3	38.4
3C	7.61	371.2	2,303.1	27.2	40.4
2CN	7.18	317.7	2,146.5	28.9 *	36.5
9T	6.99	350.8	2,295.6	17.6	37.6
1CN	6.97	377.6	2,269.8	24.3	40.1
4R	6.75	434.6	2,341.8	25.2	38.1
Populations means	7.79	374.9	2262.4	24.5	39.3
8×9	10.22 **	454.7	2,635.6	27.6	39.8
5×7	10.21 **	423.9	2,225.6	19.7	37.0
4×7	9.89 *	440.2	2,599.9	28.3	42.1 *
6×9	9.78 *	349.7	2,241.2	22.8	45.0 **
2×7	9.69 *	355.3	2,260.8	25.9	38.8
4×8	9.49 *	322.0	2,111.6	28.4 *	37.7
7×9	9.46 *	573.2 **	2,823.1 *	34.5 **	41.4
6×7	9.38 *	463.5	2,440.8	27.1	43.3 *
1×7	9.34 *	330.4	2,453.5	24.3	38.0
5×6	9.33 *	373.0	2,458.7	25.7	39.3
3×9	9.32 *	410.0	2,539.8	31.4 **	42.6 *
2×6	9.25 *	346.1	2,429.4	26.0	36.8
3×8	9.13	405.5	2,623.1	27.4	38.8
7×8	9.11	375.9	2,462.7	21.8	37.2
4×9	9.07	385.6	2,087.2	18.7	34.2
2×9	8.91	320.0	2,361.8	16.1	35.3
5×9	8.83	476.4 *	2,573.2	26.3	46.5 **
3×7	8.79	446.4	2,219.7	22.7	35.0
4×6	8.75	373.8	2,614.8	26.0	35.4
6×8	8.73	472.1 *	2,669.7 *	25.7	39.8
1×9	8.70	411.9	2,652.6 *	22.1	37.6
2×8	8.65	446.8	2,330.8	24.7	37.3
3×6	8.59	445.7	2,345.9	31.0 *	39.8
2×5	8.27	369.5	2,050.6	24.3	39.0
3×4	8.16	504.1 *	2,693.1 *	28.9 *	39.9
Crosses means	8.60	397.2	2,393.5	25.5	38.8
Genotypes means	8.43	392.7	2,367.3	25.3	38.9
Se	0.81	75.2	281.9	3.1	2.7

*, **, Selection base on $\mu + Se$ and $\mu + 2Se$, respectively; [†] The first digit indicates the population number and the letter, the race id; C=Celaya, CN=Cónico Norteño, R=Ratón, T=Tuxpeño and TN=Tuxpeño Norteño; RDW, SDW=Root and Stem dry weight, respectively; Se=Standard error of the mean.

rest of the populations come from intermediate adaptation area, within the study region. However, by comparing the average performance of the crosses against populations, except for SDW in the laboratory trial, where a reduction of 1.3% was obtained, in the rest of the cases, a positive response of the crosses was found with 11.4% increase in grain yield, with 4.1 and 6.0% of RDW in laboratory and greenhouse tests, respectively. Of the 12 outstanding crosses in grain yield, six involve the population 7R, and four the population 9T, corresponding to the Ratón and Tuxpeño races, respectively, suggesting a positive response of the genetic combination of these two populations. In particular, the 7R×9T (Ratón×Tuxpeño) cross with outstanding grain yield, also achieved significant values in RDW in laboratory and greenhouse trials. The rest of the upper crosses in grain yield, generally, also showed higher values in the average crosses in RDW, both, in laboratory and greenhouse trials. It can be verified from Table 1 that populations with higher values in grain yield, in general, also showed positive relationship in RDW values in the evaluation environments.

The differences found between populations and crosses of 4.1 and 6.0% in root dry weight in laboratory and greenhouse trials, and 11.4% in grain yield, suggests that the potential of the genetic combination between populations, as an indicator of genetic divergence and the average heterotic effects between them (Reif *et al.*, 2005). The positive genetic combinations identified on the traits in this study may be useful to transfer favorable alleles to adapted population, and through recurrent selection schemes, to develop new genetic materials. Although, the performance of 7R and 9T populations in genetic combinations is also emphasized, which, according to their contribution on the best crosses in grain yield, reveals their overall combined ability, but also, the cross of both (7R×9T), with outstanding values in grain yield, obtained significant values in root dry weight in laboratory and greenhouse tests (Table 1). In much of the upper crosses, a positive association of root dry weight values in the evaluation environments is shown, which represent a source of alleles that may be useful to incorporate them into the selection process, complementing grain yield (Mercer and Perales, 2010). Results in Table 1 suggest analyzing the association of the traits under study to the evaluation environments, including grain yield. The results of Pearson's correlation analysis are presented in Table 2.

It is apparent from the results of Table 2 that, except for the laboratory stress trial, the relationship between RDW and SDW is significant ($P \leq 0.01$) within trials, and within stress and non-stress conditions. In the case of the dry weight, the relationship between laboratory and greenhouse trials, only under non-stress laboratory conditions, a positive and significant correlation between RDW and SDW was found under greenhouse stress conditions. No evidence of a significant relationship between dry weight (RDW and SDW) was found in laboratory trials with grain yield. In the greenhouse trials, a positive relationship, although a low value, for dry weight with grain yield was found, where RDW under stress conditions (RDW_SG) obtained a correlation of $r=0.308^*$. This is partially consistent with Giaveno *et al.* (2007), who suggest that seedling weight can be used as a selection criterion, however, in this study, RDW and SDW under non-stress conditions in laboratory trials showed positive correlation with RDW and SDW in the greenhouse trials. On the other hand, under greenhouse stress conditions the RDW showed a positive

Table 2. Grain yield and dry matter correlation coefficients across environments based on the genotypes evaluated in 2017.

	Laboratory trials			Greenhouse trials				Yield
	SDW_SL [†]	RDW_NL	SDW_NL	RDW_SG	SDW_SG	RDW_NG	SDW_NG	
RDW_SL	0.028	0.304 *	0.223	0.044	0.208	-0.132	-0.061	-0.192
SDW_SL		-0.288	-0.099	0.010	-0.078	0.117	0.237	0.126
RDW_NL			0.465 **	0.486 **	0.366 *	0.224	0.090	0.102
SDW_NL				0.401 **	0.335 *	0.168	0.276	0.079
RDW_SG					0.549 **	0.281	0.386 **	0.308 *
SDW_SG							0.182	0.234
RDW_NG							0.408 **	0.157
SDW_NG								0.148

*, **, Significant at 0.05 and 0.01 probability levels, respectively; RDW, SDW=Root and Stem dry weight, respectively; [†] In the name of the variables, S and N, refers to the salinity stress and non-stress (Check) trials, respectively; L and G, Laboratory and Greenhouse trials, respectively.

correlation with SDW ($r=0.549^{**}$), and positive correlations of $r=0.281$ and $r=0.386^{**}$ for the RDW and SDW, respectively, in the greenhouse trial under non-stress conditions.

The genetic variation of populations and their crosses regarding the study traits (Table 1) and the relationship to the evaluation environments (Table 2) can be graphically analyzed to understand the interrelationships between genotypes and combinations of traits with environments and grain yield performance. Figure 1 presents the graphical display of the interaction of genotypes with the traits of this study, based on the principal component analysis, using the GGEbiplot model for graphical analysis (Yan 2014).

In Figure 1, vectors representing the combination of traits with environments originate from the origin, and the magnitude of these, is an indicator of the proportion of variation associated with the traits and environments; the cosine of the angle between vectors approximates the correlation coefficient and indicates the level of association between them (Yan and Kang, 2003). Similarly, each genotype projects a vector from the origin, and therefore the relationship to the traits and environments will depend on the place in the quadrant. In this sense, the interrelationships between the genotypes and the traits×environments can be used as a process of indirect selection of genotypes according to the traits of interest. In this analysis, the two first principal components (PC1 and PC2) were considered, which are those that most explain the cumulative variation presented in the original traits (48.8%).

According to the projection and angles of the vectors, grain yield is highly and positively correlated with the dry root and stem weight under non-stress greenhouse conditions (RDW_NG and SDW_NG); however, these values being $r=0.157$ and $r=0.148$, respectively (Table 2). There is also a positive correlation between grain yield and RDW under stress conditions in the greenhouse (RDW_SG), being $r=0.308^*$ in Table 2.

The discrepancy between the projection of the vectors in Figure 1 and the correlation results (Table 2) in these traits, is due to the fact that, in Table 2, 100.0% of the cumulative variation was used, while in Figure 1, only 48.8% of that variation was exploited; but data on Table 2 explain the phenotype values ($P = G + E + GE$), while Figure 1 means

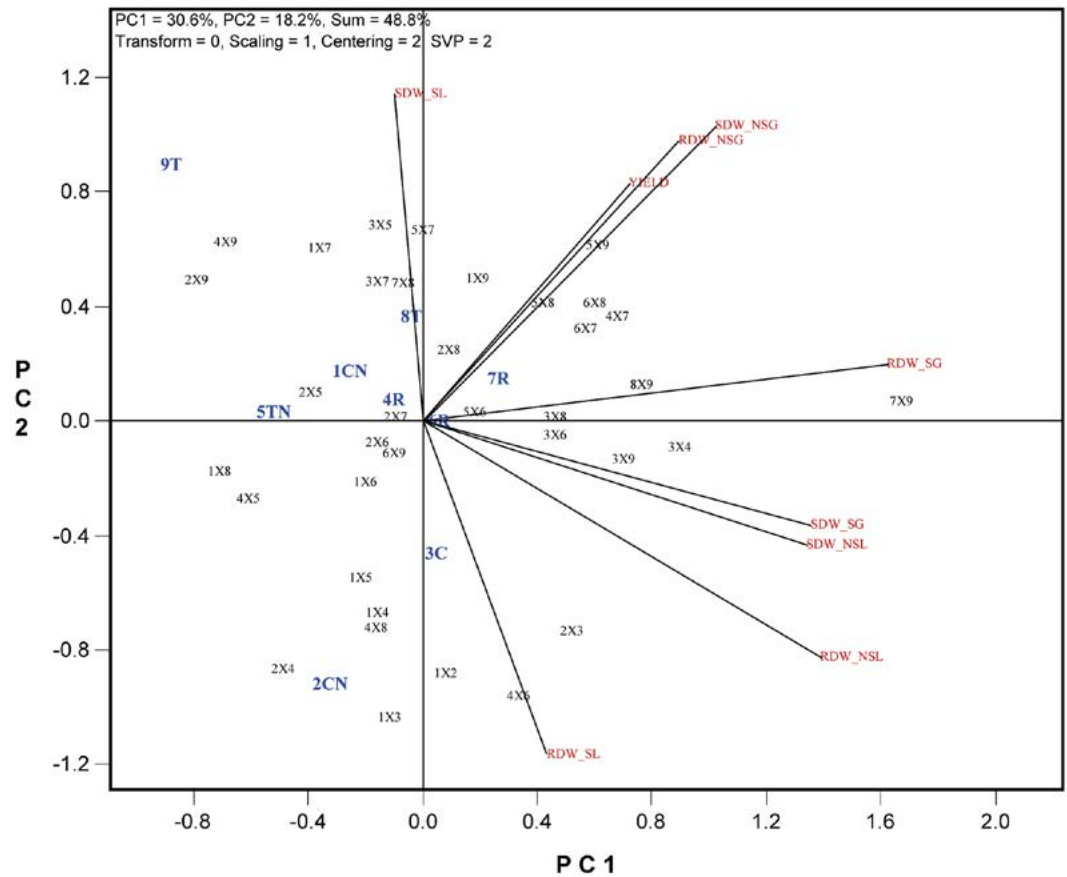


Figure 1. Graphical dispersion of the interaction of maize populations and their crosses with dry weight and grain yield traits; RDW, SDW=root and stem dry weight, respectively; SL and SG, stress conditions in laboratory and greenhouse, respectively; NL and NG, non-stress conditions test in laboratory and greenhouse, respectively; in populations, the first digit indicates the population number and the letters, the identification of race: C=Celaya, CN=Cónico Norteño, R=Ratón, T=Tuxpeño y TN=Tuxpeño Norteño.

only to the $G + GE$, referred as the GGE model (Yan and Kang, 2003; Yan, 2014). The association of RDW and SDW in the laboratory trials under non-stress conditions with RDW and SDW under greenhouse stress conditions, as shown in Table 2, is also revealed in Figure 1. The 48.8% of the cumulative variation through the GGEbiplot model (Figure 1) regarding the phenotype relationship of the traits under study (Table 2), can be analyzed to understand the combination of traits \times evaluation environments with the genotypes under study.

The dispersion analysis of the GGE model (Figure 1) can validate the variation that exists between the populations under study, which are sorted according to the interactions between the traits \times evaluation environments. The genotypes \times traits in the environments shows that 7R, 6R and 8T populations create a group, which positively correlates with grain yield and RDW and SDW under greenhouse non-stress conditions; the rest of the populations, except for 9T, are negatively associated forming another group. Similarly, the crosses 5 \times 9, 6 \times 8, 4 \times 7, 6 \times 7, 5 \times 8, 1 \times 9, set up a group closely related to this group of traits.

In these results, RDW and SDW stands out under stress conditions in the greenhouse trial, which correlate positively with grain yield (Table 2) and based on the interaction with genotypes (Figure 1), the 6R, and 7R populations can be identified, as well as a significant group of crosses showing the combinations of these populations, additionally, five of outstanding crosses, involve the 9T population. On the other hand, populations 4T, 5TN, 1CN and 2CN, and four of the crosses involving the 2CN population, correlate negatively with the RDW under stress conditions in the greenhouse. Based on the above results, the RDW under greenhouse stress conditions, jointly with RDW and SDW under non-stress conditions in the greenhouse, can be considered as potential indicator for early selection of maize genotypes, which strengthens improvement strategies, as pointed out by Lamz and González (2013).

In genetic improvement, it is important to identify attributes that determine quality levels in early stages of development, and that can be considered as a selection criterion. However, physiological quality indices are commonly not associated with good agronomic behavior. In a trial of lines and their crosses in maize, Antuna *et al.* (2003) found no direct association between seed physiological quality and agronomic traits, including grain yield. In a study to analyze the ratio of seedling dry weight as an indicator of vigor with maize growth and yield, TeKrony *et al.* (1989) conclude that the vigor of the seed had no effect on grain yield. Also, in a review of seed vigor, basically referring to the dry weight of seed in maize, TeKrony and Egli (1991) conclude that, in general, there is no relationship of seed vigor with grain yield, because in crops with complete reproductive maturity, yield is generally not closely associated with vegetative growth. From a trial under salinity stress conditions, Giaveno *et al.* (2007) suggested that vigor traits such as seedling weight and growth rate can be used as a selection criterion in maize with salinity tolerance.

CONCLUSIONS

Base on the conditions under which the study was developed, it is verified that there is genetic variation between populations for the traits included in the study, which manifests itself in the expression of the potential of the genetic combination in crosses. Populations 6R and 7R were identified, and a significant group of crosses showing the combinations of them with positive values of grain yield and root and stem dry weight under salinity stress. Root dry weight (RDW) under salinity stress, jointly with RDW and stem dry weight under non-stress conditions in the greenhouse can be considered as a potential indicator for early selection of maize genotypes.

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