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Secondary Traits and Selection Environment of Plant Density Tolerance in Maize Inbreds and Testcrosses

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMMAN, RS, MSH and TAE supervised the study and managed the literature searches. Authors AMAM and ASMY managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

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ABSTRACT

Secondary traits in maize are used in screening programs for selecting tolerant genotypes to a specific abiotic stress. Indirect selection would be effective if heritability of the secondary trait is greater than that of the primary trait and correlation between them is substantial. The objectives of the present investigation were to identify secondary trait(s) for plant density tolerance (PDT) in maize and to identify the best selection environment for improving traits related to plant density tolerance. Testcrosses were produced between 23 inbreds and 3 testers. Evaluation of 69 testcrosses and 23 inbreds for 30 traits was carried out in 2016 season under 3 plant densities using a split plot design in 3 replications. Under high density (HD), out of 30 traits, favorable and significant correlation coefficients (r) were exhibited between stress tolerance index (STI) and 23 traits of testcrosses (all 7 yield traits, all 9 tassel traits, penetrated light at ear (PL-E), penetrated light at bottom (PL-B), chlorophyll concentration index (CCI), lower stem diameter (SDL), upper

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stem diameter (SDU), ear leaf area (ELA), and barren stalks (BS). Based on high (r), high heritability and high genetic advance estimates, it is evident that the secondary traits for plant density tolerance in our study were grain yield/plant, grain yield/ha, kernels/plant, kernels/row, rows/ear, ears/plant, SDU, ELA, plant height, tassel dry weight, central spike length, and branch length. The best environment in achieving the highest predicted gain from selection was low density for 8 traits (grain yield/plant, grain yield/ha, 100-kernel weight, kernels/plant, ears/plant, tassel branch number, total spike length and PL-E), medium density for 4 traits (DTS, ear height, SDL and CCI) and HD for the rest of studied traits. These traits could be used by maize breeder as selection criteria for improving PDT.

Keywords: Selection criteria; stress tolerance index; rank correlation; heritability.

1. INTRODUCTION

Commercial maize hybrids used in Egypt are bred and grown under low plant density (ca 47,000 plants/ha) and therefore are subject to yield losses when grown under higher plant densities [1]. Maximization of maize productivity per land unit area could be attained by using high plant density as well as hybrids that can withstand high plant density up to 100,000 plants/ha [2]. Average maize grain yield per land unit area in the USA increased dramatically during the second half of the 20th century, due to the greater tolerance of modern hybrids to high plant densities [3]. Modern maize hybrids in countries achieving higher maize grain productivity from land unit area than Egypt are characterized with plant density tolerance, due to their morphological and phenological adaptability traits, such as early silking, short anthesis silking interval (ASI), less barren stalks (BS) and prolificacy [4]. Radenovic et al. [5] pointed out that maize genotypes with erect leaves are very desirable for increasing the population density due to better light interception. Introducing previously mentioned adaptive traits to Egyptian cultivars is important to enable these cultivars to produce higher grain yield from land unit area than present cultivars.

Correlation coefficient in particular determines the degree of association between traits and how they may enhance selection. It is useful if indirect selection gives greater response to selection for traits than direct selection for the same trait. It is suggested that indirect selection would be effective if heritability of the secondary trait is greater than that of the primary trait and correlation between them is substantial [6]. Similarly, Rosielle and Hamblin [7] also indicated that magnitudes of selection responses and correlated responses will depend on heritabilities and phenotypic standard deviations as well as genetic correlations. The main criterion for plant density tolerance selection is the association of each trait with grain yield under stress conditions [8,9]. A strong phenotypic association between grain yield and grain number/m^2 in both plant density stressed and non-stressed environments was reported by Al-Naggar et al. [10,11]. Bolaños and Edmeades [12] also indicated that variation in grain number has a more pronounced effect on yield rather than grain weight. Similar results were reported by Guei and Wassom [13], who found high associations between grain yield and days to 50% silking, ASI, and EPP under plant density stress. Under plant density stress conditions, yield increases were strongly associated with reduced ASI, reduced barrenness and increased harvest index [8,9].

Traits correlated with grain yield across plant densities would highlight traits and categories of traits that may underlie plant density tolerance (PDT) [14]. They reported that in US maize germplasm evaluated for plant density tolerance, a subset of traits including leaf angle, upper stem diameter, leaf area required to produce one gram of grain, kernel rows per ear, days to canopy closure, barrenness, kernels plant^{-1}, kernel length, leaf number, upper leaf area, stay green, zipper effect, kernels per row, and anthesis-to-silking interval were associated with grain yield across plant densities ranging from 47,000 to 133,000 plants ha^{-1}. Al-Naggar et al. [10,11] reported strong favorable and significant genetic correlations between density tolerance index and each of yield components for inbreds and hybrids and days to anthesis, anthesis silking interval, plant height, ear height, and leaf angle for hybrids; they considered these traits as secondary traits to plant density tolerance.

Whether direct or indirect selection is superior depends upon the heritability of the selected trait in stress and non-stress environments and the genetic correlation between stress and non-stress environments [15]. However, many investigators reported a decline in heritability for grain yield under stress [7]. A number of reports on heritabilities are available for different traits of maize under high density stress conditions [12]. Bänziger et al. [9] concluded that secondary traits are valuable adjuncts in increasing the efficiency of selection for grain yield when broad-sense heritability of grain yield is low. Furthermore, it should be kept in mind that the estimate of heritability applies only to environments sampled [16]. Thus, when planning to improve an adaptive trait to a given stress, priority should be given to estimation of heritability of this trait under targeted environmental conditions. Productivity of the plants in the selection environments and/or a high correlation between yield in the test and the target environments have been used to identify the most appropriate selection environments [15]. The objectives of the present investigation were: (i) to identify secondary trait(s) for tolerance to high plant density in maize inbreds and testcrosses to be used in screening programs for selecting the tolerant genotypes and (ii) to identify the best selection environment for improving traits related to plant density tolerance.

2. MATERIALS AND METHODS

2.1 Experimental Site

This study was carried out at the Agricultural Experiment and Research Station of the Faculty of Agriculture, Cairo University, Giza, Egypt (30°02'N latitude and 31°13'E longitude with an altitude of 22.50 meters above sea level) in 2015 and 2016 seasons.

2.2 Plant Materials

Twenty-three maize inbred lines; al least in the seventh selfing generation, of different origins were chosen on the basis of their adaptive traits to high plant density and/or drought, to be used as females in this study. Seven of them (L14, L17, L18, L20, L21, L28 and L53) were obtained from Agronomy Department, Faculty of Agriculture, Cairo University and 16 inbreds (IL115, IL17, IL24, IL51, IL53, IL80, IL84, IL151, IL171, Sk9, CML67, CML104, Inb174, Inb176, Inb208 and Inb213) were obtained from Agricultural Research Center, Egypt. Three testers of different genetic base were used as males to make all possible testcrosses in 2015 summer season with the 23 inbred females, namely the commercial inbred line Sd7, the commercial single cross hybrid SC 10 and the commercial synthetic Giza 2 (open-pollinated variety).

2.3 Experimental Design and Treatments

In 2016 season, one field experiment was carried out during the early summer. The experiment was conducted to evaluate 100 genotypes, namely 23 inbred lines, three testers, 69 testcrosses and five high-yielding commercial hybrids as checks (the single crosses SC 168, SC 2031, SC 30K9, SC30N11and the three-way cross TWC 1100). A split-plot design in RCB arrangement with three replications was used. The main plots were allotted to three plant densities (low, medium and high) and the sub-plots were devoted to genotypes (100 genotypes). The inbred lines were separated from other studied material in each block, because of their differences in plant height and vigor. The date of planting was the 20th of May. Sub-plots were single rows 4.0 m long and 0.70 m wide, with hills spaced at a distance of 15 cm for the high density (HD), 20 cm for the medium density (MD) and 25 cm for the low plant density (LD) with two plants hill^{-1} and plants were thinned to one plant hill^{-1} before the first irrigation to achieve the plant densities 95,200, 71,400 and 47,600 plants/ha, respectively. All other agricultural practices were followed according to the recommendations of ARC, Egypt. Nitrogen fertilization at the rate of 285.6 kg N/ha was added in two equal doses of Urea before the first and second irrigation. Fertilization with calcium superphosphate was performed with soil preparation and before sowing. Weed control was performed chemically with Stomp herbicide before the first irrigation and just after sowing and manually by hoeing twice, the first before the second irrigation and the second before the third irrigation. Irrigation was applied by flooding after three weeks for the second irrigation and every 12 days for subsequent irrigations. Pest control was performed when required by spraying plants with Lannate (Methomyl) 90% (manufactured by DuPont, USA) against corn borers.

2.4 Soil Analysis and Meteorological Data

The analysis of the experimental soil, indicated that the soil is clay loam (5.50% coarse sand, 22.80% fine sand, 36.40% silt, and 35.30% clay), the pH (paste extract) is 7.92, the EC is 1.66 dSm^{-1}, soil bulk density is 1.2 g cm^{-3}, calcium carbonate is 7.7%, the available nutrients in mg kg^{-1}were Nitrogen (371.0), Phosphorous (0.4), Potassium (398), DTPA-extractable Zn (4.34), DTPA-extractable Mn (9.08) and DTPA-extractable Fe (10.14). Meteorological variables in the 2016 growing season of maize were obtained from Agro-meteorological Station at Giza, Egypt. For May, June, July and August, mean temperature was 27.87, 29.49, 28.47 and 30.33℃, maximum temperature was 35.7, 35.97, 34.93 and 37.07℃ and relative humidity was 47.0, 53.0, 60.33 and 60.67%, respectively.

2.4.1 Parameters recorded

1. **Days to 50% anthesis (DTA):** (Number of days from planting to anthesis of 50% of plants), it was measured on all plants $plot^{-1}$.
2. **Days to 50% silking (DTS):** (Number of days from planting to silking of 50% of plants).
3. **Anthesis-silking interval (ASI) (day):** (Number of days between 50% silking and 50% anthesis), it was measured on all plants $plot^{-1}$.
4. **Plant height (PH) (cm):** It was measured at the end of flowering stage on 10 guarded plants $plot^{-1}$ from ground to the point of flag leaf insertion.
5. **Ear height (EH) (cm):** It was measured at the end of flowering stage on 10 guarded plants $plot^{-1}$ from ground to the base of the top most ear.
6. **Barren stalks (BS):** As the percentage of plants bearing no fertile ears relative to the total number of plants in $plot^{-1}$; an ear was considered fertile if it had one or more grains on the rachis.
7. **Leaf angle (LANG) (^{o}):** It was measured as leaf angle between blade and stem for the leaf just above ear using a protractor on 10 guarded plants $plot^{-1}$according to Zadoks et al. [17].
8. **Lower stem diameter (SDL) (mm):** It was measured with caliper from 10 guarded plants/plot as the stem diameter above second node; two measurements were taken. The first measurement was used as a base line with the second measurement recorded after a 90 degree turn of the caliper.
9. **Upper stem diameter (SDU) (mm):** It was measured with caliper from 10 guarded plants/plot as the stem diameter on third internode below flag leaf.
10. **Ear leaf area (ELA) (cm^2):** It was measured on the ear leaf from 10 guarded plants/plot as follows: ELA = Leaf length x maximum leaf width x 0.75 according to Francis et al. [18].
11. **Leaf area to produce 1 g of grain (LA/1Gg) (cm^2):** It was measured as leaf area per plot /grams of grains per plot. At 70 days from sowing date light intensity was measured and then penetrated light inside the canopy was calculated for each genotype. The Lux-meter apparatus was used. The light intensity in (lux) was measured at 12 am (noon time) at the top of the plant and at the base of top-most ear. Penetrated light inside the canopy was measured as a percentage of light penetrated from the top of the plant to the base of top-most ear as follows:
12. **Penetrated light at the base of top-most ear (PLE) (%):** It was calculated from 10 guarded plants/plot as follows**:** PLE =100 (light intensity at the base of top-most ear/light intensity at the top of the plant).
13. **Penetrated light at the bottom of the plant (PLB) (%):** It was calculated from 10 guarded plants/plot as follows: PLB =100 (light intensity at the bottom/light intensity at the top of the plant).
14. **Chlorophyll concentration index (CCI)** (%)**:** It was measured by Chlorophyll Concentration Meter, Model CCM200 as the ratio of transmission at 931 nm to 653 nm through the leaf of top-most ear (http://www.apogeeinstruments.co.uk/apogee-instruments-chlorophyll-content-meter-technical-information/). It was measured on 5 guarded plants/plot.
15. **Tassel fresh weight (TFW) (g):** IT was measured on 5 guarded plants per plot.
16. **Tassel dry weight (TDW) (g):** It was measured on 5 guarded plants per plot.
17. **Total spike length (TSL) (cm):** it was measured as the length from the lowest branch to the tip of spike on 5 guarded plants per plot.
18. **Central spike length (CSL) (cm):** It was measured as the length from highest branch to the tip of spike on 5 guarded plants per plot.
19. **Tassel branch number (TBN):** It was measured as number of branches on 5 guarded plants $plot^{-1}$.
20. **Branching region (BR) (cm):** It was measured as the length from lowest branch to highest branch on 5 guarded plants per plot.
21. **Branch length (BL) (cm):** It was measured as the mean branch length

taken from the lowest, highest, and middle parts of spike on 5 guarded plants per plot.

22. **Tassel size (TS) (cm):** It was measured on 5 guarded plants per plot as follows: TS = TSL+(TBN × BL).
23. **Tassel density (TD) (branch/cm):** It was measured on 5 guarded plants per plot as follows: TD =TS/(1 + TBN). Traits from No. 15 to No. 23 were measured according to Mansfield and Mumm [14].
24. **Number of ears plant^{-1} (EPP):** It was estimated by dividing number of ears plot^{-1} on number of plants plot^{-1}.
25. **Number of rows ear^{-1} (RPE):** Using 10 random ears plot^{-1} at harvest.
26. **Number of kernels row^{-1} (KPR):** Using the same 10 random ear plot^{-1}
27. **Number of kernels plant^{-1} (KPP):** Calculated by multiplying number of ears plant^{-1} by number of rows ear^{-1} by number of kernels row^{-1}.
28. **100-kernel weight (100KW) (g):** Adjusted at 155g water kg^{-1} grain.
29. **Grain yield plant^{-1} (GYPP) (g):** It was estimated by dividing the grain yield plot^{-1} (adjusted at 15.5% grain moisture) on number of plants plot^{-1} at harvest.
30. **Grain yield ha^{-1} (GYPH) (ton):** It was estimated by adjusting grain yield plot^{-1} at 15.5% grain moisture to grain yield ha^{-1}.

2.5 Biometrical Analyses

Analysis of variance of the split-plot design in RCB arrangement was performed on the basis of individual plot observation using the MIXED procedure of SAS ® [19]. The data collected from the experiment was subjected to the standard analysis of variance of split-plot design. Least significant difference (LSD) was calculated to test significance of differences between means according to Steel et al. [20]. Stress tolerance index (STI) modified from equation suggested by Fageria [21] was used to classify genotypes for tolerance to density stress. The formula used is as follows: STI= (Y_1/AY_1) X (Y_2/AY_2), Where, Y_1 = grain yield mean of a genotype at non-stress. AY_1 = average yield of all genotypes at non-stress.Y_2 = grain yield mean of a genotype at stress. AY_2 = average yield of all genotypes at stress. Rank correlation coefficients were calculated between each STI and each of studied traits under each stress environment (medium and high density) for inbreds and testcrosses according to Steel et al. [20]. Phenotypic correlation coefficients were calculated between each pair of studied traits under each environment (low, medium and high density) and combined across all densities for inbreds, testcrosses and across all genotypes according to Steel et al. [20]. Rank correlation coefficients were calculated between STI's and all studied traits for inbred and testcrosses under each stressed environment (high- or medium-density) by using SPSS 17 computer software and the significance of the rank correlation coefficient was tested according to Steel et al. [20]. The correlation coefficient (r_s) was estimated for each pair of any two parameters as follows: r_s =1- (6 $\sum d_i^2)/(n^3$-n), Where, d_i is the difference between the ranks of the i^{th} genotype for any two parameters, n is the number of pairs of data. The hypothesis Ho: r_s= 0 was tested by the r-test with (n-2) degrees of freedom. Data of the testcrosses were further subjected to line × tester analysis according to Kempthorne [22]. The expectations of mean squares due to males, females and male x female are equivalent to the general combining ability for males ($\delta^2_{GCA(m)}$), general combining ability for females ($\delta^2_{GCA(f)}$) and specific combining ability (δ^2_{SCA}), respectively. Estimates of additive (δ^2_A) and dominance (δ^2_D) variances, heritability and genetic advance from selection were calculated according to Sharma [23]. Heritability in the narrow (h^2_n) sense in testcrosses was estimated from the following formulae: h^2_n= 100 (δ^2_A/δ^2_{ph}). The expected genetic advance from selection was calculated as follows: GA = 100 h^2_n k δ_{ph} / $\overline{x}$, Where δ_{ph} = phenotypic standard deviation, k = selection differential (the k value for 10% selection intensity used in this study equals 1.76), $\overline{x}$= mean of the crosses for the respective trait.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance of split plot design (data not presented) showed that mean squares due to plant density (D) for all studied traits were significant (P≤ 0.01) for all studied traits, indicating that the plant density stress has an obvious effect on most studied traits of all studied genotypes in the present experiment. Mean squares due to genotypes (G) were significant (P≤ 0.01) for all studied traits, indicating genetic-background differences among genotypes for all studied traits across the three plant densities (high, medium and low). Mean squares due to genotype × plant density interaction were significant (P ≤ 0.01) for all studied traits, except lower stem diameter, indicating the possibility of selecting genotypes for improved performance under a specific plant density as proposed by previous investigators [1,10,11,24-28]. Mean squares due to genotypes under all environments were significant (P≤ 0.01 or P≤ 0.05)) for all studied traits, indicating the significance of differences among studied genotypes under each of the three plant densities.

3.2 Trait Interrelationships

Estimates of phenotypic correlation coefficients between each of GYPP and GYPH and other studied traits across the three plant densities were calculated across all inbred lines and across all F_1 testcrosses and presented in Table (1). Grain yield/plant of inbreds showed perfect positive phenotypic association with grain yield/ha (r= 0.99) across all plant densities; that is why the estimates of genetic correlation coefficients between GYPP and other traits are very close to those between GYPH and the same traits.

In general, grain yield (either per plant or per hectare) of inbreds showed very strong and positive phenotypic association with all grain yield components, namely ears/plant, rows/ear, kernels/row, kernels/plant and 100-kernel weight across the three densities; stressed and non-stressed. It is observed that GYPP or GYPH of inbreds showed the strongest correlation with number of kernels/plant (r=0.89 and 0.87, respectively) followed by 100-KW (r=0.81 and 0.84, respectively). Significant and positive correlation coefficients were also observed between GYPP or GYPH and each of SDL (lower stem diameter), SDU (upper stem diameter), ELA (ear leaf area), PL-E (penetrated light at top-most ear), PL-B (penetrated light at bottom), CCI (chlorophyll concentration index), and all tassel traits. Among tassel traits, GYPP or GYPH showed the highest correlation with central spike length (0.52 and .051, respectively) and mean branch length (0.51 and 0.49, respectively). On the contrary, GYPP or GYPH of inbreds showed significant and negative phenotypic correlations with DTA (-0.19 and -0.19, respectively), DTS (-0.22 and -0.22, respectively) and BS (-0.27 and -0.28, respectively) across all densities.

Grain yield per plant or per hectare of inbreds showed very strong and positive association with plant density stress tolerance index (STI) under both MD and HD (Table 2). STI of inbreds showed also a significant and positive correlation with all grain yield components and SDU; with the strongest one between STI and KPP and significant and negative association with DTA, DTS and BS traits under MD and HD.

Grain yield/hectare of crosses had perfect and positive phenotypic associations with grain yield/plant, across the three plant densities. Estimates of phenotypic correlation coefficients between GYPH of crosses and other studied traits are very close in magnitude and sign to those between GYPP and the same other traits (Table 1). Grain yield/plant or GYPH of the testcrosses showed very strong and positive genetic correlation with all grain yield components, namely kernels/plant (0.84 and 0.84), kernels/row (0.78 and 0.79), 100-kernel weight (0.50 and 0.50), rows/ear(0.49 and 0.49) and ears/plant (0.38 and 0.38), respectively across all stressed and non-stressed environments. Significant and positive correlation coefficients were also observed between GYPP or GYPH of testcrosses and each of SDL (lower stem diameter), SDU (upper stem diameter), CCI (chlorophyll concentration index), and five out of nine tassel traits, namely TFW, TSL, CSL, BL and BR. Among tassel traits, GYPP or GYPH showed the highest correlation with mean branch length (0.42 and .041, respectively).

On the contrary, GYPP or GYPH of inbreds showed significant and negative phenotypic correlations with DTA (-0.40 and -0.37, respectively), DTS (-0.41 and -0.39, respectively), PH (0.30 and -0.30, respectively, EH (0.22 and -0.25, respectively) and BS (-0.46 and -0.49, respectively) across all plant densities (Table 1). This indicates the importance of these traits in tolerance to high density.

Grain yield per plant or per hectare of testcrosses showed very strong and positive association with STI under both MD and HD, that is why the estimates of genetic correlation coefficients between GYPP or GYPH and other traits are very close to those between STI and the same traits (Table 2). STI of testcrosses showed significant and positive correlation with all grain yield components and SDL, SDU, LAE, PL-E, PL-B, CCI and all studied tassel traits; with the strongest one between STI and each of KPP and 100-KW and significant and negative association with BS trait under MD and HD. These results are in agreement with those reported by other investigators [29-31]. Significant and negative r values detected between GYPH or GYPP of hybrids and plant

Table 1. Phenotypic correlation coefficients between GYPP or GYPH with other studied traits across all the three plant densities for inbreds and testcrosses

Trait	Inbreds		Testcrosses		Trait	Inbreds		Testcrosses	
	GYPP	GYPH	GYPP	GYPH		GYPP	GYPH	GYPP	GYPH
DTA	-0.19**	-0.19**	-0.40**	-0.37**	**TDW**	0.42**	0.41**	0.18	0.17
DTS	-0.22**	-0.22**	-0.41**	-0.39**	**TS**	0.24**	0.24**	0.14	0.14
ASI	-0.13	-0.13	-0.18	-0.18	**TSL**	0.48**	0.47**	0.29*	0.27*
PH	-0.03	-0.04	-0.30*	-0.30*	**CSL**	0.52**	0.51**	0.26*	0.25*
EH	0.12	0.10	-0.22*	-0.25*	**BL**	0.52**	0.49**	0.42**	0.41**
BS%	-0.27**	-0.28**	-0.46**	-0.49**	**BR**	0.34**	0.32**	0.31**	0.31*
LANG	-0.08	-0.07	0.12	0.1	**TBN**	0.46**	0.44**	0.21	0.21
SDL	0.44**	0.41**	0.22	0.20	**TD**	0.21**	0.21**	0.04	0.05
SDU	0.43**	0.41**	0.36**	0.34**	**EPP**	0.50**	0.45**	0.38**	0.38**
ELA	0.38**	0.36**	0.36**	0.34**	**RPE**	0.58**	0.59**	0.49**	0.49**
LA 1g	-0.02	-0.03	-0.14	-0.16	**KPR**	0.64**	0.63**	0.78**	0.79**
PL-E	0.40**	0.38**	0.17	0.14	**KPP**	0.89**	0.87**	0.84**	0.84**
PL-B	0.43**	0.42**	0.19	0.16	**100-KW**	0.81**	0.83**	0.50**	0.50**
CCI	0.42**	0.40**	0.25*	0.25*	**GYPP / GYPH**	0.99**	0.99**	0.99**	0.99**
TFW	0.36**	0.35**	0.24*	0.24*					

**and ** indicate significant at 0.05 and 0.01 probability levels, respectively*

Table 2. Rank correlation coefficient between stress tolerance indexes (STI) and all studied traits of inbreds and testcrosses in 2016 season

Trait	MD	HD	MD	HD	Trait	MD	HD	MD	HD
	Inbreds		Testcrosses			Inbreds		Testcrosses	
DTA	-0.26*	-0.30*	-0.13	-0.10	**TDW**	0.13	0.02	0.37**	0.48**
DTS	-0.30*	-0.31**	-0.16*	-0.10	**TS**	0.06	0.07	0.21**	0.26**
ASI	-0.16	-0.05	-0.09	-0.004	**TSL**	0.28*	0.02	0.50**	0.36**
PH	-0.16	-0.18	0.08	0.01	**CSL**	0.29*	0.12	0.47**	0.44**
EH	-0.27*	-0.21	0.10	0.08	**BL**	0.44**	0.20	0.49**	0.43**
BS%	-0.32**	-0.50**	-0.25**	-55**	**BR**	0.30*	0.15	0.36**	0.24**
LANG	0.05	0.001	-0.13	-0.01	**TBN**	0.14	0.11	0.42**	0.36**
SDL	0.13	0.18	0.38**	0.36**	**TD**	0.13	-0.08	0.16*	0.21**
SDU	0.31**	0.41**	0.40**	0.42**	**EPP**	0.31**	0.48**	0.28**	0.21**
LAE	0.34**	0.10	0.34**	0.35**	**RPE**	0.53**	0.49**	0.50**	0.53**
LA 1g	-0.08	0.00	-0.06	-0.07	**KPR**	0.64**	0.72**	0.57**	0.65**
PL E	0.29*	0.11	0.36**	0.30**	**KPP**	0.75**	0.83**	0.79**	0.79**
PL B	0.25*	0.18	0.36**	0.37**	**100-KW**	0.46**	0.31*	0.68**	0.67**
CCI	0.17	0.19	0.41**	0.27**	**GYPP**	0.94**	0.93**	0.91**	0.91**
TFW	0.12	0.17	0.31**	0.43**	**GYPH**	0.93**	0.93**	0.92**	0.91**

**and ** indicate significant at 0.05 and 0.01 probability levels, respectively. MD=Medium density, HD=High density* height and ear height traits across all environments (Table 1), indicated that shorter and lower ear placement testcrosses are of high yielding, under high plant density. This conclusion is in agreement with others [32,33]. In contrast, Carena and Cross [34] and Al-Naggar et al. [35] reported that taller inbreds are higher yielding than shorter inbreds under both low and high densities.

Traits correlated with grain yield across plant densities would highlight traits and categories of traits that may underlie plant density tolerance [14]. Other studies have also found kernel number to be associated with final grain yield under high plant density and other stress conditions [12,32]. The number of kernels per row is determined approximately 1 wk before flowering [36], in contrast to kernel rows per ear, which is determined early in the growing season. The combination of rows per ear and kernels per row may be critical to expression of plant density tolerance. These findings suggest that genotypes with high plant density tolerance may be tolerant of early and midseason stress from high plant-to-plant competition that can trigger changes to ear structure. Therefore, unaltered kernel set *(i.e.*, no significant reduction in rows per ear and/or kernels per row) would allow more kernels per plant, which would support high grain yield under high plant density.

The percentage of barren plants in the present study exhibited also negative phenotypic correlation with grain yield; that is to say that as grain yield per unit area increases, the percentage of barrenness decreases. Under high plant density this relationship is important because as plant density increases, competition for resources also increases, which can lead to barrenness [37]. To effectively increase overall productivity, it is essential to have every plant producing an ear to contribute to the final grain yield per unit area. The reduced level of barrenness fits with higher individual plant yield as well as higher grain yield in general.

Similar conclusions were reported by several investigators [38-40]. The strong relationships between grain yield and all yield components under high plant density are in harmony with other reports [40,41]. Mansfield and Mumm [14] reported that phenotypic trait correlations revealed a subset of traits associated with grain yield across plant densities, with all five categories of traits implicated directly; the subset included leaf angle, upper stem diameter, leaf area required to produce a gram of grain, kernel rows per ear, days to canopy closure, barrenness, kernels per plant, kernel length, leaf number, upper leaf area, stay-green, zipper effect, kernels per row, and anthesis–silking interval.

3.3 Heritability and Genetic Advance

Estimates of broad-sense heritability (h^2_b) (Table 3) were generally higher under low-density for only two traits (ASI and TD), under high density for seven traits (DTA, LA/1gG, PL-E, PL-B, CSL, EPP and RPE) and under medium density for 20 traits (the rest of studied traits). This may be due to the greater genetic variance under elevated density than under the low density. The h^2_b estimate ranged from 51.89% for ASI under HD to 99.66% for EPP under HD.

The highest environment in narrow-sense heritability (h^2_n) was high density for 13 traits (DTA, ASI, PH, LANG, LA/18G, PL-B, PL-E, TFW, TDW, TS, BR, RPE and KPR), medium density for 10 traits (DTS, EH, SDL, SDU, LAE, CCI, TS, CSL, TBN and TD) and low density for six traits (TSL, EPP, KPP, 100-KW, GYPP and GYPH). The highest estimate of h^2_n (88.15%) was shown by SDU under MD followed by (83.85%) which was shown by EPP under LD, while the lowest estimate (0.00%) was shown by ASI under LD and MD due to the absence of additive genetic variance.

The results of this investigation (Table 3) indicated that the best environment in achieving the highest predicted gain from selection (GA%) was the high plant density environment for 17 traits (DTA, ASI, PH, LANG, SDU, LAE, LA/1gG, PL-B, TFW, TDW, TS, CSL, BL, BR, TD, RPE and KPR), followed by the low plant density environment for eight traits (GYPP, GYPH, 100-KW, KPP, EPP, TBN, TSL and PL-E) and the medium density environment for four traits (DTS, EH, SDL and CCI). The highest GA under each environment was achieved by PL-E under low density (32.13%) and PL-B under medium (26.75%) and high density (42.38%). On the contrary, the lowest GA estimate was shown by ASI (0.00%) under low and medium density and by DTS (1.21%) under high density.

Table 3. Estimates of broad (h^2_b) and narrow (h^2_n) sense heritability calculated from line × tester analysis traits under low (LD), medium (MD) and high (HD) plant density in 2016 season

Parameter	LD	MD	HD	LD	MD	HD	LD	MD	HD
		DTA			**DTS**			**ASI**	
h^2_b (%)	90.49	89.46	91.16	89.81	90.50	89.71	66.20	55.64	51.89
h^2_n (%)	4.97	28.02	40.14	8.66	37.47	35.20	0.00	0.00	24.42
GA (%)	0.20	0.99	1.53	0.36	1.37	1.21	0.00	0.00	7.26
		PH			**EH**			**BS%**	
h^2_b (%)	57.78	78.35	73.31	86.48	94.02	92.37	-	-	62.81
h^2_n (%)	7.21	24.00	48.17	21.29	47.62	28.08	-	-	0.00
GA (%)	0.70	1.78	4.12	2.80	5.01	2.98	-	-	0.00
		LANG			**SDL**			**SDU**	
h^2_b (%)	91.02	95.97	94.88	92.22	97.14	91.41	95.62	98.55	97.83
h^2_n (%)	55.64	63.61	66.54	71.50	75.32	64.62	76.85	88.15	86.30
GA (%)	15.77	18.61	25.00	13.62	13.78	13.40	25.21	31.73	36.95
		LAE			**LA/1g**			**PL-E**	
h^2_b (%)	92.04	96.44	93.11	87.93	93.35	93.52	86.32	88.89	91.71
h^2_n (%)	46.22	65.00	64.37	6.84	6.89	20.17	35.39	44.16	48.93
GA (%)	10.26	19.08	23.13	2.07	1.70	5.10	32.13	22.84	22.04
		PL-B			**CCI**			**TFW**	
h^2_b (%)	92.26	95.71	95.91	89.96	97.21	95.03	94.17	97.48	96.40
h^2_n (%)	49.42	49.49	60.10	37.18	37.48	25.88	0.00	1.88	23.15
GA (%)	26.48	26.75	42.38	4.44	5.62	5.17	0.00	0.80	12.25
		TDW			**TS**			**TSL**	
h^2_b (%)	94.32	98.69	97.75	-289.00	97.95	97.48	92.14	96.69	92.18
h^2_n (%)	29.85	44.40	61.56	1.47	14.82	22.24	36.45	30.34	11.50
GA (%)	10.45	17.58	29.00	0.06	5.23	9.54	5.39	4.34	2.01
		CSL			**BL**			**BR**	
h^2_b (%)	98.54	97.21	95.90	96.32	98.64	96.99	94.17	96.21	95.19
h^2_n (%)	0.00	57.19	53.57	29.58	68.99	62.48	31.38	31.66	45.68
GA (%)	0.00	10.01	12.60	6.51	15.33	17.40	7.69	8.67	17.36
		TBN			**TD**			**EPP**	
h^2_b (%)	94.64	97.09	94.67	97.41	96.88	94.73	96.89	96.05	99.66
h^2_n (%)	35.79	36.78	28.11	34.61	50.39	48.90	83.85	0.00	40.27
GA (%)	6.56	5.99	6.23	16.51	22.34	23.70	20.81	0.00	10.11
	RPE			**KPR**			**KPP**		
h^2_b (%)	90.51	92.97	95.46	90.49	94.32	93.82	91.33	94.51	94.70
h^2_n (%)	16.65	10.73	27.98	11.27	22.71	29.33	36.58	22.22	32.14
GA (%)	1.60	1.16	3.44	1.35	2.68	4.10	7.57	3.88	7.08
	100-KW			**GYPP**			**GYPH**		
h^2_b (%)	95.05	98.31	97.55	94.88	97.54	97.16	94.92	97.46	97.17
h^2_n (%)	42.27	28.49	27.53	50.93	42.53	43.18	50.98	42.57	43.17
GA (%)	6.19	4.86	5.32	16.38	12.97	16.07	16.38	12.99	16.07

In the literature, two groups of researchers reported two contrasting conclusions; the first group reported that heritability and expected genetic advance is higher under stress than non-stress conditions, and that selection should be practiced in the target (stressed) environment to obtain higher genetic advance [42-46]. The second group of researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress [7,9,47,48]. Our results for grain yield and its components, TBN, TSL and PL-E are in agreement with the second group, but for other studied traits our results are in agreement with the first group.

Based on the rank correlations (r) between studied traits and STI under high plant density and their corresponding estimates of narrow-sense heritability, it is evident that the best secondary traits for plant density tolerance in our study are: GYPH, GYPP, followed by KPP, KPR, RPE and EPP, SDU, LAE, PH, TDW, CSL and BL traits, since they show high (r) values, high (h^2_n) estimates and high GA estimates.

4. CONCLUSIONS

Out of 30 studied traits, 23 traits of maize testcrosses (GYPH, GYPP, KPP, KPR, RPE, 100-KW and EPP, TFW,TDW, TSL, CSL, TBN, BR, BL, TS, TD, PL-E, PL-B, CCI, SDL, SDU, ELA and BS) and 11 traits of inbreds (GYPH, GYPP, KPP, KPR, RPE, 100-KW and EPP, DTA, DTS, BS and SDU) exhibited favorable and significant correlations with density stress tolerance index (STI). The study concluded that the traits GYPH, GYPP, followed by KPP, KPR, RPE, EPP, SDU, LAE, PH, TDW, CSL and BL traits could be considered secondary traits for plant density tolerance (PDT). One or more of these traits could be used by maize breeder as selection criteria for improving PDT. Also the study concluded that the best environment for achieving the highest predicted gain from selection was LD for GYPP, GYPH, 100KW, KPP, EPP, TBN, TSL and PL-E under MD and DTS, EH, SDL and CCI under HD for the rest of studied traits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH. Maize response to elevated plant density combined with lowered N-fertilizer rate is genotype-dependent. The Crop J. 2015;(3):96-109.
2. Huseyin G, Omer K, Mehmet K. Effect of hybrid and plant density on grain yield and yield components of maize (*Zea mays* L.). Indian J. Agron. 2003;48(3):203-205.
3. Duvick DN, Cassman KG. Post-green revolution trends in yield potential of temperate maize in the North-Centeral United States. Crop Sci. 1999;39:1622-30.
4. Duvick D, Smith J, Cooper M. Long-term selection in a commercial hybrid maize breeding program. Plant Breeding Reviews, J. Janick (ed). John Wiley and Sons: New York, USA; 2004
5. Radenovic C, Konstantinov K, Delic N, Stankovic G. Photosynthetic and bioluminescence properties of maize inbred lines with upright leaves. Maydica. 2007;52(3):347-356.
6. Falconer AR. Introduction to quantitative genetics. Third edition. Longman, New York; 1989.
7. Rosielle AA, Hamblin J. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci. 1981; 21:943-946.
8. Edmeades GO, Bolaños J, Chapman SC. Value of secondary traits in selecting for drought tolerance in tropical maize. In Edmeades GO, Bänziger M, Mickelson HR, Pena-Valdiva CB. (Eds.). Developing drought and low-n tolerant maize. Proceedings of a symposium March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico, D.F. CIMMYT. 1997;222-234.
9. Banziger M, Betran FJ, Lafitte HR. Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Sci. 1997;37:1103-1109.
10. Al-Naggar AMM, Atta MMM, Ahmed MA, Younis ASM. Maximizing maize (*Zea mays* L.) crop yield via matching the appropriate genotype with the optimum plant density. Journal of Applied Life Sciences International. 2016; 5(4):1-18.
11. Al-Naggar AMM, Atta MMM, Ahmed MA, Younis ASM. Differential responses of maize (*Zea mays* L.) genotypes to elevated plant density combined with deficit irrigation. Journal of Advances in Biology & Biotechnology. 2016;7(2):1-20.
12. Bolanos J, Edmeades GO. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Res. 1996;48:65-80.
13. Guei RG, Wassom CF. Inheritance of drought adaptive traits in maize. Interrelationships between yield, flowering, and ears per plant. Mydica. 1992;37:157-164.
14. Mansfield BD, Mumm RH. Survey of plant density tolerance in U.S. maize germ plasm. Crop Sci. 2014;54:157-173.
15. Zavala-Garcia F, Bramel-Cox PJ, Eastin JD, Witt MD, Andrews DJ. Increasing the efficiency of crop selection for unpredictable environments. Crop Sci. 1992;32:51-57.
16. Hallauer AR, Miranda JB. Quantitative genetics in maize breeding, 2nd edn. Iowa State University Press. Ames; 1988.
17. Zadoks JC, Chang TT, Konzak CF. Decimal code for the growth states of cereals. Eucarp. Bull. 1974;7:42-52.

18. Francis GA, Rutger JN, Palmer AFE. A rapid method for plant leaf area estimation in maize (*Zea mays* L.). Crop Sci. 1969;9: 537- 537.
19. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS system for mixed models. SAS Inst, Cary, NC; 1996.
20. Steel RGD, Torrie GH, Dickey DA. Principles and procedures of statistics: a biometrical approach. 3rded. McGraw-Hill, New York, USA. 1997;450.
21. Fageria NK. Maximizing crop yields. Dekker. New York; 423.Kempthorne O 1957. An Introduction to Genetic Statistics. John Wiley and Sons, Inc. New York; 1992.
22. Kempthorne O. An Introduction to genetic statistics. John Wiley and Sons, Inc., New York; 1957.
23. Sharma RJ. Statistical and biometrical techniques in plant breeding. New Delhi, Second Edition; 2003;432.
24. Shakarami G, Rafiee M. Response of corn (*Zea mays* L.) to planting pattern and density in Iran. Agric. J. Environ. Sci. 2009;5(1):69-73.
25. Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH. Genetic parameters controlling some maize adaptive traits to elevated plant densities combined with reduced N-rates. World Research Journal of Agronomy. 2014;3(2):70-82.
26. Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH. Regression of grain yield of maize inbred lines and their diallel crosses on elevated levels of soil-nitrogen. International Journal of Plant & Soil Science. 2015;4(6):499-512.
27. Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH. Optimum plant density for maximizing yield of six inbreds and their f1 crosses of maize (*Zea mays* L.). Journal of Advances in Biology & Biotechonology. 2015;2(3):174-189.
28. Al-Naggar AMM, Atta MMM. Elevated plant density effects on performance and genetic parameters controlling maize (*Zea mays* L.) agronomic traits. Journal of Advances in Biology & Biotechnology. 2017;12(1):1-20.
29. Banziger M, Edmeades GO, Lafitte HR. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Res. 2002;75:223-233.
30. Al-Naggar AMM, Shabana R, Rabie AM. The genetic nature of maize leaf erectness and short plant stature traits conferring tolerance to high plant density. Egypt. J. Plant Breed. 2012;16(3):19 -39.
31. Haegele JW, Cook KA, Nichols DM, Below FE. Changes in nitrogen use traits associated with genetic improvement for grain yield of maize hybrids released in different decades. Crop Sci. 2013;53:1256-1268.
32. Sangoi L, Gracietti MA, Rampazzo C, Biachetti P. Response of Brazilian maize hybrids from different ears to changes in plant density. Field Crops Res. 2002; 79:39-51.
33. Sofiatti V, Cargnin A, Silva LVBD, Galvao JCC. Maize population increase and reduced spacing between plant rows. Revista Cientifica Rural. 2007;12(1):131-139.
34. Carena MJ, Cross HZ. Plant density and maize germ plasm improvement in the Northern Corn Belt. Maydica. 2003;48(2): 105-111.
35. Al-Naggar AMM, Shabana R, Rabie AM. Genetics of maize rapid of silk extrusion and anthesis-silking synchrony under high plant density. Egypt. J. Plant Breed. 2012; 16(2):173-194.
36. Abendroth LJ, Elmore RW, Boyer MJ, Marlay SK. Corn growth and development. Iowa State University Extension, Ames. IA, USA; 2011.
37. Sangoi L. Understanding plant density effects on maize growth and development: An important issue to maximize grain yield. Ciencia Rural. 2001;31(1):159-168.
38. Edmeades GO, Bolanos J, Chapman SC, Lafitte HR, Banziger M. Selection improves drought tolerance in tropical maize populations. I. Gains in bio-mass, grain yield and harvest index. Crop Sci. 1999;39: 1306–1315.
39. Gebre BG. Genetic variability and inheritance of drought and plant density adaptive traits in maize. Ph.D. Thesis, Fac. Agric. Free State Univ. South Africa. 2006; 189.
40. Al-Naggar AMM, Shabana R, Rabie AM. Inheritance of maize prolificacy under high density. Egypt. J. Plant Breed. 2012;16(2): 1- 27.
41. Andrade FH, Vega CRC, Uhart S, Cirilo A, Cantarero M, Valentinuz O. Kernel number determination in maize. Crop Sci. 1999;39: 453-459.
42. Hefny MM. Estimation of quantitative genetic parameters for nitrogen use

efficiency in maize under two nitrogen rates. Int. J. Pl. Breed. Genet. 2007;1:54-66.

43. Al-Naggar AMM, Shabana R, Mahmoud AA, Abdel El-Azeem MEM, Shaboon SAM. Recurrent selection for drought tolerance improves maize productivity under low-N conditions. Egypt. J. Plant Breed. 2009;13: 53-70.
44. Al-Naggar AMM, Shabana R, Al Khalil TH. Tolerance of 28 maize hybrids and populations to low-nitrogen. Egypt. J. Plant Breed. 2010;14(2):103-114.
45. Al-Naggar AMM, El-Lakany MA, El-Sherbieny HY, El-Sayed WM. Combining abilities of newly-developed quality protein and high-oil maize in breds and their testcrosses. Egypt. J. Plant Breed. 2010; 14(2):1-15.
46. Naggar AMM, El-Lakany MA, El-Sherbieny HY, El-Sayed WM. Diallel analysis of maize inbred lines with contrasting protein contents. Egypt. J. Plant Breed. 2010; 14(2):125-147.
47. Shabana R, Bailey T, Fery KJ. Production traits of oats selected under low; medium and high productivity. Crop Sci. 1980;20: 739-744.
48. Worku M. Genetic and crop-physiological basis of nitrogen efficiency in tropical maize. Ph.D. Thesis. Fac. Agric. Hannover Univ. Germany. 2005;122.

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