CLASSIFICATION OF GENETIC DIVERSITY FOR DROUGHT TOLERANCE IN MAIZE GENOTYPES THROUGH PRINCIPAL COMPONENT ANALYSIS

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Abstract: Water scarcity is a universal environmental constraint for agricultural sustainability and production. Two field experiments were accomplished during the 2012 and 2013 growing seasons in two sites: the experimental farm of Suez Canal University, Ismailia and Romana Province, North Sinai, Egypt to evaluate 21 genotypes of maize comprising six inbred lines and their 15 F_1 crosses for their drought tolerance. The experiments were arranged as a split-plot design with three replications, where moisture levels (100 and 50% of evapotranspiration) and maize genotypes were allocated to main plots and sub-plots, respectively. Results showed reduction in performance for most measured traits in response to water stress with varying degrees with yield plant^{-1} being the most affected. Inversely, proline and relative water content and anthesis-silking interval were increased. Correlation results confirmed the reduced grain yield with the increasing anthesis-silking interval, and suggested kernels row^{-1}, relative water content, peroxidase activity and rows ear^{-1} in Ismailia, and rows ear^{-1}, relative water content, peroxidase activity, kernel weight in Romana were indirect selection criteria for increasing yield in water scarcity environments. Principal component (PC) analysis showed that three PCs having Eigen value >1 explained 70.67 and 70.16%; 69.79 and 71.38% of the total variability among genotypes in control and stress conditions in Ismailia and Romana, respectively. The crosses P1×P3, P4×P6, P3×P5 and P1×P5 were classified as drought tolerant under Ismailia and Romana conditions. On the other hand, P1xP4, P3xP4, and P4 were considered as drought sensitive in Ismailia conditions. In addition, P5, P2×P4, P1×P4 and P5×P6 were the most affected by water deficiency under Romana conditions.

Key words: maize, drought, principle component analysis, yield, correlation.

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Introduction

Crop productivity is faced with environmental stresses such as drought, salinity, nutrient deficiency and heat. Drought is the most disturbing abiotic stress for stable crop production in developing countries. Maize is the third staple cereal crop worldwide after wheat and rice. Rapid changes in populations, society, and economies are expected to increase the demand for maize compared with wheat and rice by 2020 (Pingali, 2001). Different studies have been conducted to identify the most sensitive stages to drought stress in maize. While the flowering period was assigned as the most critical, the seedling stage was identified as influential on plant stand establishment and the resulting yield (Bänziger et al., 2000; Li et al., 2015). In the same context, Lauer (2003) stated that maize yield is most sensitive to water stress during flowering and pollination, followed by grain filling and finally vegetative growth stages. Since drought tolerance is a complex trait, maize researchers and breeders have found that using specific morpho-physiological traits at different growth stages would be extremely useful in the development of new cultivars (Bruce et al., 2002). Consequently, using identified secondary traits in parental lines that were inherited to good yielding hybrids would be ideal (Meeks et al., 2013).

The success of breeding depends generally on the genetic variability among the parental lines as a lack of this variability may limit breeding progress and the gain from selection (Singh et al., 2016). Genetic diversity among maize lines for root architecture under drought stress at the seedling stage was studied by Li et al. (2015). Others stated considerable genetic variation at flowering in modern commercial maize (Barker et al., 2005). In the same context, Bolaños and Edmeades (1996) highlighted the critical importance of the flowering stage in producing kernel number ear^{-1} and improving maize yield under stress. Correlation studies recorded by the same authors revealed the close dependence of yield on kernel number ear^{-1} (up to r = 0.9), and moderate to strong associations of both with anthesis-silking interval (r = −0.4 to −0.7). Araus et al. (2012) confirmed that ASI is the most contributing trait for yield determination under drought compared to any other secondary trait, therefore a significant proportion of variation observed in grain yield was predicted when ASI was measured at flowering. Due to the complexity of drought resistance, constructing an integrated evaluation assay to combine many factors based on investigations of multiple environments is a preliminary task.

Various studies have employed multivariate statistical analysis such as principal component analysis (PCA) to evaluate the magnitude of genetic diversity among the crop germplasm (Brown-Guedira, 2000; Wijewardana et al., 2016) and to reduce a large number of observed traits into a smaller set of

traits that have the maximum contribution in separating the genotypes. Bin Mustafa et al. (2015) found that the first four principal components explained 88.4 and 86.7% of the total variation among maize inbred lines in response to 100 and 40% moisture levels, respectively. Others recorded two PCs that contributed to 94.01% and 91.15% of total variation in root traits of 103 maize inbred lines evaluated in control and water-stressed conditions (Li et al., 2015).

Therefore, the objectives of the present study were to: i) assess the responses of maize genotypes to water stress; ii) classify and rank maize genotypes based on PCA to determine which traits are best suited for screening drought tolerance.

Materials and Methods

Genetic materials and selection of parental lines

Twenty maize inbred lines self-pollinated for eight generations, selected from the inbred line development program (Ali, 2004) belonging to local and exotic resources, were evaluated for their drought tolerance at germination (7 days) and seedling stages (20 days) in a pot experiment under greenhouse conditions. The tolerance response was quantified using polyethylene glycol (PEG, MW: 6000, The Panchi Chemicals, Hyderabad, India). Four PEG concentrations (0, 10, 20 and 30%) were applied to pots containing maize grains. Three doses of 300 ml PEG were given to each treatment at three-day intervals. Plants were assessed at germination and early seedling stages based on the following traits: germination percent, survival ability, shoot and root lengths, shoot and root fresh weights and shoot: root ratio. Based on trait means across PEG concentrations, three tolerant (P1, P3 and P5), two sensitive (P2 and P4) and one moderately tolerant (P6) lines were selected for further field experiments. The selected parents P3 and P5 were originated from the local DC-202; P1, P2 and P4 were originated from the cross between local Giza-2 and Ukraine line (AK135) and P6 was originated from the cross between DC-Youpiline (Ukraine) and local OP-Nabelgamal.

Experimental design and treatment application

Diallel crosses were made among the six maize inbreds to develop 15 F_1 crosses in the 2011 season. Field experiments were established in two locations during the 2012 and 2013 growing seasons; the experimental farm of Suez Canal University, Ismailia, and Romana Province, North Sinai, Egypt. Samples were taken from the 0–75 cm soil profile to determine physical properties and chemical compositions of the experimental sites (Table 1).

Table 1. Physical properties and chemical compositions of the experimental sites.

Properties	Ismailia	Romana
Particle size distribution (%)		
Sand	97.65	96.12
Silt	1.51	2.38
Clay	0.84	1.5
Textural class	Sand	Sand
Field capacity (%)	18.0	17.20
PH	7.88	8.19
ECe (dS m^{-1})	1.23	2.35
Soluble cations (meq l^{-1})		
Ca2$^+$	6.2	11.50
Mg^{+2}	4.0	7.50
Na$^+$	2.13	4.93
K$^+$	0.39	0.98
Organic C (g kg^{-1})	1.39	1.72
Available N (mg kg^{-1})	4.10	5.50
Available P (mg kg^{-1})	10.32	11.17

The experiments were laid out in a randomized complete block split-plot design at two drip irrigation rates: 100 (WW) and 50% (WS) of the estimated evapotranspiration and 21 maize genotypes were assigned as the main and split-plots, respectively. Each split-plot consisted of two polyethylene lateral drip lines with a length of 3 m and 0.70 m between lines and 0.3 m between drippers. The total area for each subplot was 4.2 m^2. The total amount of water (I) was determined from the calculated water requirement for maize (mm) as determined from the crop coefficient (kc) and the daily reference evapotranspiration (ETc) using the following equation:

$$I = ET_o k_c \qquad \text{Eq. (1)}$$

Evapotranspiration (ETo) was calculated by the Penman–Monteith equation (Allen et al., 2011) using daily data from a meteorological station located within 500 m from the experimental site. FAO computer programs (CROPWAT Version 7.0 and CLIMWAT) were used to calculate ETo. Accordingly, the total amounts of water applied for irrigation treatments were 6280 and 3140 m^3 ha^{-1}, for 100 and 50% irrigation levels, respectively.

Three seeds around each dripper were sown on May 20 and 25 at Ismailia and Romana in 2012 and 2013, respectively. One week after seeding, plants were thinned to one plant per dripper to obtain a final plant density of 47600 plants ha^{-1}. All agricultural practices were applied as recommended.

Measurements

The anthesis-silking interval (ASI) was calculated on a plot basis as differences between silking and anthesis dates. At harvest, plant height (PH) was measured on ten representative plants from each sub-plot, then ears were separated and the following traits were recorded: rows ear[-1] (RE), kernels row[-1] (KR), 100-kernel weight (KW) and yield plant[-1] (YP). Relative water content (RWC%), peroxidase activity and proline content (μg g dw[-1]) were determined using 0.50 g fresh leaves at 50% silking stage. Relative water content was determined using the method of Mata and Lamattina (2001) and calculated according to the formula: RWC (%) = (FW – DW)/(TW – DW) × 100, where: FW, DW and TW are fresh, dry and turgor weight of leaves. Proline content was estimated by the ninhydrin method of Bates et al. (1975). The chromophore formed was extracted with 4 ml of toluene and the absorbance of the resulting organic layer was measured at 520 nm and calculated using the formula:

$$\text{Proline content} = \frac{34.11 * OD520 * V}{2 * F} \qquad \text{Eq. (2)}$$

where: OD is the optical density; F, leaf sample weight; V, extract volume. Calibrations were made with 1mM L-proline as a standard. Peroxidase activity was assayed according to Liu and Huang (2000), briefly 0.5 g of fresh leaves was homogenized in 4 ml of 50 mM potassium phosphate buffer, pH 7.0 and 1 mM Na_2EDTA. POD reaction solution (3 ml) was assayed at 470 nm every 30 seconds; one unit of peroxidase activity was defined as an absorbance change of mg fresh weight min^{-1}.

Statistical analysis

Data were averaged across years within sites and analysis of variance was performed to test the effects of irrigation levels, genotypes and their interaction on the measured traits using COSTAT software (version 6.311). The significance of differences was tested using least significant difference tests at $P = 0.05$. Principal component analysis (PCA), which is a tool to identify parameters that best describe the tolerance to response variables, was used to separate genotypes into tolerant groups. PCA was performed on the correlation matrix of 21 genotypes and nine response variables. Eigenvectors generated by PCA were used to identify parameters that best differentiated cultivars for drought tolerance. The first two PC scores, PC1 and PC2 that accounted for maximum variability of the parameters tested, were used to group the genotypes. XLSTAT software (2016.02.275370) was used to perform the analysis and biplot visualization. Eigenvectors generated by PCA were used to rank tested genotypes for their drought tolerance.

Results and Discussion

Responses of maize genotypes to irrigation treatments

Statistical analysis showed the significant differences among the genotypes, irrigation levels, and genotype × irrigation interval combinations for the measured traits in both locations (Tables 2 and 3). The significant variations among genotypes and genotypes × irrigation regimes under two different irrigation conditions indicate that adequate genetic variation existed among the tested maize genotypes (parents and F_1 crosses). Mean values of most traits were decreased after WS compared to WW, except ASI, POD and PC. ASIs of 5.15 and 5.75 d under WS regime in Romana and Ismailia were prolonged compared with 3.59 and 4.50 d of WW regime, respectively. While genotypes showed 16.22, 19.61, 20.62, 27.23, 31.73 and 44.84% reduction in RE, 100-GW, RWC, PH, KR and YP due to water stress; ASI, POD and PC increased by about 27.88, 59.88 and 151.15% compared to regular irrigation in Ismailia. In Romana, water stress reduced mean values for the same traits by 17.03, 19.93, 12.95, 26.53, 31.36 and 50.53%, in contrast, genotypes showed 43.47, 66.73 and 139.46% increases for ASI, POD and PC, respectively. While less reduction due to WS was recorded for ASI and YP in Ismailia, average reduction in RWC was higher in Romana. Morphological and other yield-related traits showed the negligible disparity between sites. Studies show that maize is susceptible to water scarcity during mid to late vegetative development and flowering significantly affects its growth and productivity (Chen et al., 2012; Svačina et al., 2014). Brar et al. (2016) recorded 35.9 and 40.9% increases in maize yield under 100% over 60% replacement of the cumulative pan evaporation in a two-year trial.

Comparing experimental sites, at WS treatments, mean values for measured traits were higher for crosses compared to ILs in Romana. In Ismailia location, inbred lines P1 and P3 recorded the tallest plants, heaviest kernel weight and maximum KR values. Generally, parents exhibited non-visible variation for ASI being P2, and P4 was the most prolonged in ASI. Generally, P4 was the most sensitive parent that showed lower mean values for the majority of traits. Two single crosses, P4×P6 and P3×P5, having the highest GY under WS were in the first order for KR, KW, POD activity and RWC. Two crosses, P1xP3 and P1xP5, showing moderate grain yield were characterized by moderate PH, high RE, KW and RWC. Conversely, crosses P2×P3 and P2×P5 possessed the lowest GY, low RE and KR compared with other crosses. In Romana site, only P1 revealed the highest yielding ability accompanied by the highest values for yield components, PC and RWC. In contrast, P4 was the most sensitive parent to water stress and recorded the lowest values for the majority of traits. While all lines showed similar ASI values, lines 2 and 4 were the latest.

Table 2. Mean values for nine variables measured on maize genotypes evaluated across two growing seasons in Ismailia. Regular and italic letters show values at control and 50% of evapotranspiration.

Genotypes	ASI	PH	RE	KR	KW	POD	PC	RWC	YP
P1	4.3	178.45	14.65	38.85	32.85	37.95	13.50	82.86	126.30
	5.6	142.50	13.65	29.35	27.15	61.50	32.40	69.20	86.55
P2	4.7	166.40	12.65	33.15	30.65	32.45	11.45	70.59	116.00
	6.2	141.05	11.50	26.35	23.9	45.65	21.40	60.63	55.80
P3	3.3	181.55	14.80	34.15	32.90	46.95	11.10	79.74	143.65
	5.5	136.00	11.20	30.35	26.75	69.10	24.50	67.89	77.50
P4	5.0	157.60	13.50	29.70	27.95	26.30	11.10	75.45	81.30
	7.0	120.60	11.00	18.50	28.00	54.35	21.25	60.09	61.55
P5	5.3	181.45	15.15	38.35	36.15	55.75	11.05	85.11	147.20
	5.6	122.05	13.00	24.50	25.50	91.15	24.70	67.90	75.75
P6	4.3	143.50	14.30	31.80	29.55	37.80	10.85	77.74	101.30
	5.3	129.40	11.25	25.50	23.75	65.60	23.60	60.02	68.20
P1xP2	4.0	181.30	15.15	29.00	34.40	38.65	13.20	79.99	98.45
	6.2	143.25	12.50	22.70	26.15	63.90	34.35	57.91	59.15
P1xP3	4.7	233.75	16.00	41.00	35.55	51.85	13.20	81.04	170.60
	6.0	137.95	14.00	26.30	27.70	73.80	43.40	64.93	73.90
P1xP4	4.7	186.35	16.00	32.85	31.35	25.45	12.90	77.92	110.90
	5.6	129.40	12.15	21.65	25.20	62.00	21.40	62.55	61.85
P1xP5	5.0	211.05	16.55	37.65	33.15	35.90	12.35	80.54	168.25
	4.6	144.55	13.35	23.20	27.75	69.20	20.85	63.89	74.90
P1xP6	4.0	175.40	14.35	37.85	35.85	35.15	12.85	80.30	140.60
	5.4	136.95	12.35	27.15	27.75	51.05	36.15	56.97	69.45
P2xP3	5.0	176.70	16.00	35.50	31.80	60.75	12.35	85.90	146.75
	6.2	134.05	14.65	22.00	30.10	77.20	40.95	62.22	55.25
P2xP4	5.7	179.65	15.60	36.50	27.75	48.35	13.25	82.26	128.05
	6.2	130.40	13.15	21.85	25.55	45.85	33.45	62.48	69.65
P2xP5	5.7	170.75	15.35	35.35	30.35	32.65	12.30	84.48	114.40
	5.3	136.35	12.00	20.50	25.60	50.65	29.25	47.77	49.75
P2xP6	3.7	180.45	15.80	37.00	33.75	27.30	12.60	74.38	114.10
	6.5	138.85	11.65	22.15	26.65	46.10	39.65	63.00	68.05
P3xP4	3.7	187.05	14.30	39.50	30.20	64.80	13.05	77.46	93.45
	5.7	141.00	12.50	22.65	20.75	79.85	22.70	61.16	73.80
P3xP5	4.0	214.40	15.35	39.80	35.45	59.20	11.85	86.16	146.00
	5.6	138.70	14.00	27.25	26.65	94.90	28.70	67.15	85.95
P3xP6	3.7	193.35	14.65	42.50	34.05	25.70	13.10	74.59	105.10
	5.4	137.45	13.15	29.30	24.10	40.75	38.45	71.07	72.30
P4xP5	5.3	199.80	15.15	29.30	31.85	42.05	13.00	75.94	95.50
	6.3	136.50	12.85	21.50	25.90	69.25	39.95	59.70	67.60
P4xP6	4.0	229.70	15.50	41.85	34.30	22.40	13.30	82.01	154.25
	4.8	140.70	13.00	27.30	29.70	73.30	40.25	66.85	86.20
P5xP6	4.3	186.30	16.15	36.85	31.55	34.65	10.10	76.95	139.10
	5.7	131.30	13.65	27.80	23.20	61.10	31.75	73.41	63.70
LSD									
≠IL	0.97	3.79	0.44	1.93	0.71	0.19	0.32	1.48	8.94
G	1.82	9.83	1.32	4.38	1.25	0.72	0.84	3.25	18.02
IL*G	3.18	13.30	2.67	5.75	2.42	2.29	1.26	1.42	6.75

[#]IL: Irrigation level, G: Genotypes, IL×G: Irrigation level × Genotype, ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear[-1], KR = Kernels row[-1], KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant[-1].

Table 3. Mean values for nine variables measured on maize genotypes evaluated across two growing seasons in Romana. Regular and italic letters represent values at control and 50% of evapotranspiration.

Genotypes	ASI	PH	RE	KR	KW	POD	PC	RWC	YP
P1	3.8	167.4	13.0	37.95	27.10	35.40	14.80	78.93	105.70
	4.5	126.5	11.6	23.45	24.05	58.85	35.00	68.39	61.30
P2	3.2	156.5	12.8	33.85	25.60	29.95	15.40	69.66	100.20
	6.2	124.8	10.3	25.55	21.75	42.75	27.15	61.88	50.90
P3	3.4	169.4	13.4	32.70	25.65	44.25	16.30	76.65	108.30
	4.8	120.9	10.4	23.80	22.15	66.40	27.95	67.38	52.70
P4	3.6	147.2	12.1	30.35	26.65	23.60	14.35	70.13	85.70
	6.5	113.9	10.3	20.35	18.80	51.50	22.85	56.38	44.50
P5	3.8	167.6	13.3	37.00	29.05	53.05	16.10	77.03	131.25
	4.5	108.8	11.1	21.30	20.40	88.60	27.80	70.76	52.80
P6	3.3	132.4	12.8	32.70	25.85	35.25	17.15	73.05	101.10
	4.5	112.1	10.4	24.15	19.25	63.10	27.30	62.07	51.05
P1xP2	4.2	159.3	12.8	36.45	27.30	49.55	12.35	73.49	110.30
	5.8	126.4	10.3	26.45	21.15	90.45	23.30	72.29	70.05
P1xP3	3.8	201.3	13.3	36.75	30.10	63.00	13.05	77.70	136.70
	4.5	121.0	11.2	30.10	23.70	86.90	42.65	73.66	60.90
P1xP4	3.3	164.8	13.7	34.15	26.55	45.50	12.75	73.79	112.45
	5.8	113.8	10.4	25.35	21.80	79.80	27.15	63.73	59.60
P1xP5	3.3	181.3	13.6	35.30	31.10	33.95	13.25	74.72	134.20
	4.2	129.4	11.7	26.90	26.85	85.95	29.35	69.88	78.35
P1xP6	3.8	151.5	12.4	32.85	27.90	54.60	12.90	73.47	126.45
	4.7	121.0	10.6	25.40	24.05	94.15	40.70	65.96	66.85
P2xP3	3.6	150.80	13.3	36.80	30.50	37.25	11.65	75.54	121.60
	5.7	117.50	11.4	23.35	22.65	75.50	40.00	63.65	70.60
P2xP4	3.5	149.20	11.6	36.50	26.65	31.20	12.40	73.41	111.55
	6.0	116.1	10.0	22.85	20.55	89.95	23.85	61.08	56.70
P2xP5	4.0	145.6	12.7	33.75	27.00	38.70	12.60	75.96	118.75
	5.5	119.55	10.4	23.35	23.40	61.10	34.70	64.80	62.85
P2xP6	3.8	155.85	13.4	32.45	27.55	53.20	12.75	69.55	114.25
	5.0	122.1	10.7	23.05	23.60	68.55	26.45	67.98	44.80
P3xP4	3.1	153.95	12.0	34.05	25.10	32.80	11.90	74.54	115.10
	5.0	124.30	9.4	21.50	20.85	53.25	33.20	50.03	54.90
P3xP5	3.5	180.9	14.4	39.55	31.55	45.15	11.60	76.99	158.95
	4.5	121.6	11.0	26.90	24.35	82.00	26.40	63.69	61.90
P3xP6	3.8	168.4	12.0	40.25	31.55	60.85	12.30	72.53	99.45
	5.8	121.9	10.4	26.30	24.15	76.75	38.95	64.05	48.85
P4xP5	3.8	169.9	11.3	36.55	28.30	45.50	13.50	69.50	104.35
	5.3	121.2	10.4	23.20	20.95	61.35	40.15	63.70	49.75
P4xP6	3.2	200.2	14.0	39.55	30.65	54.60	13.20	75.94	132.30
	4.5	125.3	11.4	26.75	24.65	87.15	39.75	59.50	62.15
P5xP6	3.3	161.8	12.0	33.75	26.00	64.35	12.00	74.14	148.65
	4.8	115.5	10.7	20.10	21.45	89.40	41.35	64.29	64.05
LSD									
[‡]IL	1.28	3.15	1.09	2.67	2.85	1.49	0.98	1.85	14.38
G	1.15	10.80	1.29	4.44	2.79	4.01	1.27	3.79	13.87
IL×G	3.17	9.68	2.74	5.56	1.09	2.21	1.32	2.48	14.61

[‡]IL: Irrigation level, G: Genotypes, IL×G: Irrigation level × Genotype, ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear[-1], KR = Kernels row[-1], KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant[-1].

The cross P1×P5 exhibited the highest YP (78.35g) closely followed by crosses P2×P3 (70.60g) and P1xP2 (70.05g) with the lowest yield recorded for P1×P4 (59.60g). Similar trends were detected for ASI, PH, RE, KR, KW, POD and RWC, where the cross P1×P5 combined high values for these traits. The minimum values for RE, KR, 100-KW, RWC and POD were observed for the cross P3×P4. Maize is a commercial crop, and most experiments evaluating drought tolerance have been focused on hybrids rather than on inbred lines. In this study, inbred lines were considered sources for excellent genes for drought tolerance and high yield. Overall, it is suggested that promising hybrids combine at least one drought tolerant parent. In accordance with previous results, the inbred lines that make hybrids tolerant could be having favorable alleles for GY under drought (Makumbi et al., 2011). Moreover, Monneveux et al. (2008) stated that three-way hybrids involving two drought tolerant population lines yielded more than those involving one only, indicating the feasibility of gene pyramiding for drought tolerance.

Association of traits and classification of drought tolerance using PCA

PCA gives two important pictures of association among traits and classification of tested genotypes based on their tolerance/sensitivity to water stress. Since it is not sufficient to consider one of yield-correlated traits as indicative for stress resistance, we considered these traits all together as indicators for drought-resistant maize. The cosine of the angles between vectors shows the magnitude of correlation between traits, whereas the acute angles represent positive correlations, the wide obtuse angles show a negative correlation (Figures 1 and 2). The length of the vectors connecting traits to the origin shows the extent of variability. Water stress treatments at both sites showed that ASI was negatively correlated with all measured traits. A very strong association was recorded for KR, RWC, POD and RE with YP in Ismailia, while in Romana, YP correlated positively with RE, RWC, POD and KW. In Ismailia, RE correlated positively with POD and PC; RWC correlated with KR, on the other hand, KW showed positive correlations with PH and PC. In Romana, increasing GY was associated with increasing RE, KW and POD under WS conditions and KW recorded positive associations with PH, RE, KR and PC. Results by Ziyomo and Bernardo (2013) recorded negative correlations of ASI with YP and PH. The study by Monneveux et al. (2008) suggested that selection for bigger grains and smaller tassels may help to increase grain yield in water-limited environments in the near future.

PCA for nine traits and 21 genotypes at two irrigation treatments and two experimental sites was performed to identify the principal components of measured parameters that best described the response to irrigation treatments and, thus, to identify tolerant and sensitive genotypes (Figures 1 and 2). Results showed that the first three PCs contributed 70.67 and 70.16%; and 69.79 and 71.38% to the total

variation among genotypes at WW and WS in Ismailia and Romana, respectively (Table 4). Bin Mustafa et al. (2015) stated that 59.3 and 55% of the total variation were contributed by the first two PCs when evaluating maize genotypes at 100 and 40% moisture levels. PCA showed that the three factors had eigenvalue > 1, moreover 56.13 and 57.2; 56.22 and 58.94% of the total variability were explained by the first two PCs under the same conditions. Generally, the contributions of PC1 for the nine traits were more than two times that of PC2. The first PC was positively correlated with YP, PH, KR, RE and 100-KW, whereas, POD and RWC were the most important contributing traits to PC2 under WW conditions in Ismailia. At WS treatment, YP, KR, RWC and RE contributed with PC1, while KW and PC were the most important in PC2. In Romana, PC1 presented higher values for PH, KW, YP and KR, while RE and RWC were the most contributing traits for PC2 at WW. At WS, PC2 was related to diversity among maize genotypes due to PH with positive and high loadings and negative loadings of POD, while KW, RE, RWC and YP were the most positive contributors for variation in PC1 and ASI. Previous studies revealed the significant importance of high contributing variables for primary selection in the investigated germplasm and further breeding to generate more variability (Kumar et al., 2016).

Table 4. Eigenvectors (normal values) and factor loadings (italic values) for the principal components obtained for nine traits measured on maize genotypes.

Traits	Ismailia						Romana					
	100%			50%			100%			50%		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
ASI	0.06	-0.14	0.82	-0.43	0.16	-0.04	-0.11	-0.42	0.68	-0.31	0.17	0.56
	0.11	-0.17	0.94	-0.76	0.21	-0.04	-0.21	-0.49	0.74	-0.61	0.20	0.59
PH	0.43	-0.23	-0.20	0.22	0.37	-0.36	0.42	0.07	0.003	0.27	0.63	-0.25
	0.82	-0.28	-0.23	0.40	0.49	-0.40	0.82	0.08	0.003	0.54	0.73	-0.27
RE	0.37	-0.10	0.13	0.32	0.28	0.49	0.31	0.50	0.18	0.36	-0.10	0.42
	0.71	-0.12	0.15	0.58	0.37	0.55	0.60	0.58	0.20	0.70	-0.12	0.447
KR	0.38	-0.07	-0.31	0.40	-0.09	-0.49	0.39	-0.24	0.15	0.36	0.18	-0.14
	0.72	-0.09	-0.36	0.72	-0.12	-0.54	0.75	-0.27	0.16	0.71	0.22	-0.15
KW	0.37	-0.07	0.17	0.20	0.51	0.02	0.40	-0.25	0.15	0.43	0.31	0.10
	0.701	-0.09	0.20	0.358	0.67	0.02	0.79	-0.28	0.16	0.84	0.37	0.11
POD	0.18	0.60	0.06	0.30	-0.23	0.60	0.32	-0.05	-0.54	0.31	-0.51	0.08
	0.337	0.73	0.07	0.542	-0.31	0.66	0.62	-0.06	-0.58	0.61	-0.60	0.09
PC	0.23	-0.57	0.09	0.19	0.56	0.06	0.32	0.32	0.34	0.19	0.20	0.63
	0.445	-0.68	0.10	0.354	0.737	0.06	0.62	0.77	0.37	0.38	0.23	0.67
RWC	0.34	0.43	0.30	0.38	-0.25	-0.11	-0.20	0.59	0.23	0.33	-0.36	-0.01
	0.646	0.51	0.34	0.691	-0.331	-0.12	-0.39	0.69	0.25	0.66	-0.42	-0.01
YP	0.43	0.19	-0.21	0.45	-0.24	-0.11	0.40	-0.01	0.01	0.39	-0.11	-0.12
	0.826	0.23	-0.24	0.824	-0.316	-0.13	0.78	-0.02	0.012	0.78	-0.13	-0.13
Eigenvalue	3.61	1.44	1.31	3.33	1.73	1.22	3.79	1.34	1.18	3.94	1.37	1.12
Contribution%	40.06	16.07	14.54	37.02	19.20	13.57	42.11	14.91	13.14	43.74	15.20	12.44

ASI = Anthesis-silking interval, PH = Plant height, RE = Rows ear^{-1}, KR = Kernels row^{-1}, KW = Kernel weight, POD = Peroxidase activity, PC = Proline content, RWC = Relative water content, YP = Yield plant^{-1}.

Cultivars were classified into four groups based on biplots of PC1 vs. PC2 (Figures 1 and 2). Accordingly, under WS treatment, P1xP3, P4xP6 and P3xP6 were considered tolerant genotypes showing high scores for PC1 and PC2; P1, P3, P1×P5, P3×P5 and P5xP6 were categorized as moderately tolerant genotypes. The remaining genotypes were classified as moderately sensitive to sensitive under Ismailia conditions. In Romana, only P1 was categorized as the tolerant parent, both P4 and P5 were the most sensitive. Regarding crosses, eight crosses were distinguished as tolerant and moderately tolerant with varying degrees, with special regard to P4xP6, P3×P5, P1×P3 and P1×P5, in contrast, P2×P4, P1×P4 and P5×P6 showed the least tolerance to water shortage. According to previous studies (Wijewardana et al., 2015), the scores of PC1 and PC2 together revealed the importance of the variables studied in the cultivar separation for stress tolerance. Therefore, in Romana, genotypes with higher scores for PC1 (P1xP5) tended to have higher values for KR, KW, RE and YP and lower values for PH, PC and ASI. In Ismailia, the cross P4xP6 had higher values for RE, KR, POD, RWC and YP and low values for PH and PC.
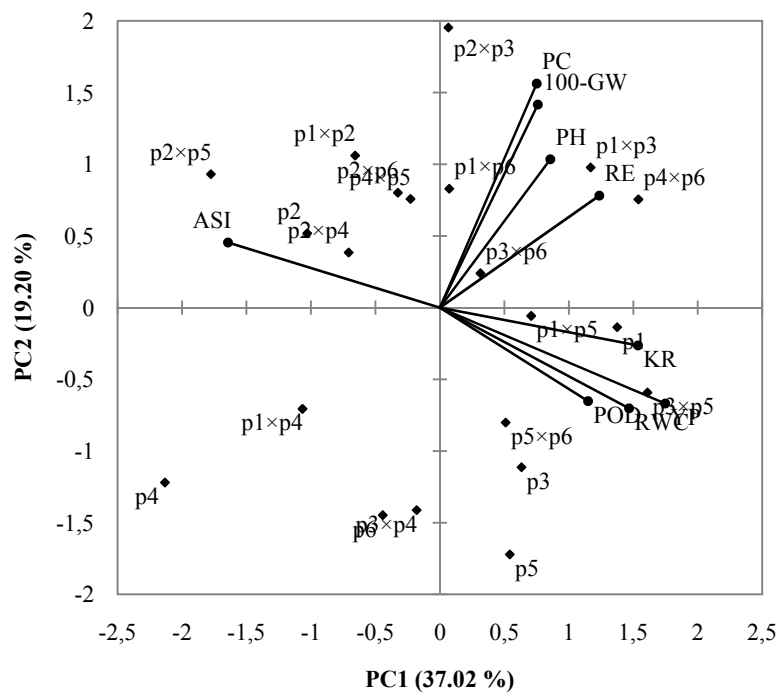

Figure 1. A biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of maize genotypes for drought tolerance in Ismailia. Diamond and circle symbols refer to genotypes and variables, respectively.
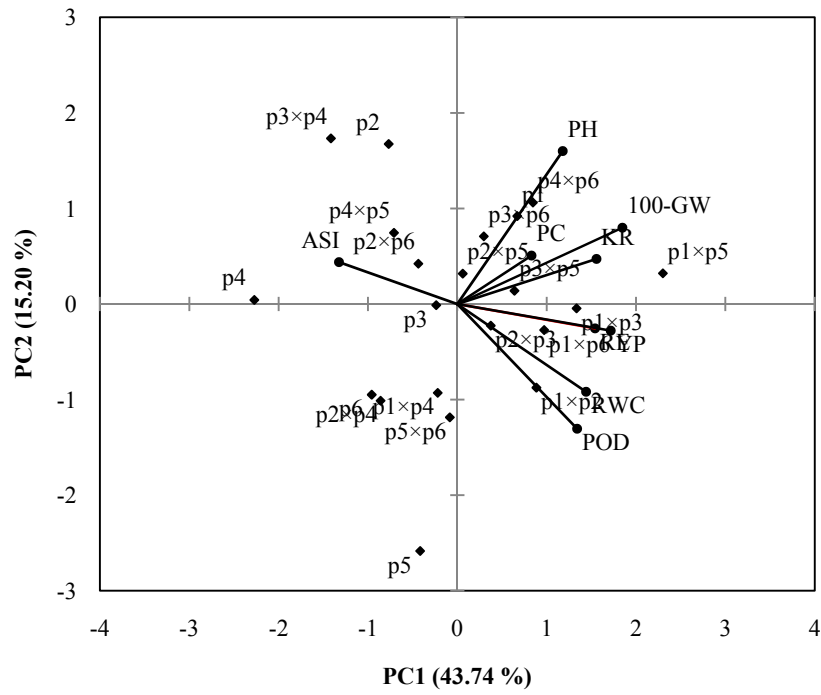
Figure 2. A principal component analysis (PCA) biplot for the first two principal component (PC) scores, PC1 vs. PC2, related to the classification of 21 maize genotypes for drought tolerance in Romana.

Conclusion

The tested maize genotypes exhibited substantial variability in their responses for all measured traits. PCA identified P1xP3, P4xP6, P3xP5 and P1xP5 as tolerant crosses for drought effects at both sites. Moreover, ASI, KR, RWC, POD and RE in Ismailia; and RE, RWC, POD, KW in Romana were ascribed as variables to best describe drought tolerance of investigated genotypes due to their strong correlations with YP. Experiments evaluating drought tolerance have been focused more on hybrids than on inbred lines. In this study, inbred lines were considered as sources for excellent genes for drought tolerance and high yield. Overall, it is suggested that promising hybrids combine at least one drought tolerant parent. In accordance with previous results, the inbred lines that make tolerant hybrids could be having favorable alleles for GY under drought. Moreover, analysis shows that three-way hybrids involving two drought tolerant population lines yielded more than those involving only one line, indicating the feasibility of gene pyramiding for drought tolerance.

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KLASIFIKACIJA GENETIČKE DIVERGENTNOSTI GENOTIPOVA KUKURUZA ZA TOLERANCIJU PREMA SUŠI PUTEM ANALIZE GLAVNIH KOMPONENTI

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R e z i m e

Nestašica vode predstavlja univerzalno ograničenje životne sredine za održivu poljoprivrednu proizvodnju. Sprovedena su dva poljska ogleda tokom 2012. i 2013. godine na dva lokaliteta: na eksperimentalnom gazdinstvu Univerziteta Suecki kanal, Ismailija i u pokrajini Romana, Severni Sinaj, Egipat. U ogledu je izvršeno procenjivanje 21 genotipa kukuruza koji obuhvataju šest inbred linija i njihovih 15 F_1 hibrida, radi ispitivanja njihove tolerantnosti prema suši. Ogledi su bili koncipirani kao sistem podeljenih parcela sa tri ponavljanja, gde su nivoi vlažnosti (100 i 50% evapotranspiracije) i genotipovi kukuruza dodeljeni glavnim parcelama odnosno potparcelama. Rezultati su pokazali smanjenje učinka za većinu merenih osobina kao odgovor na različite stepene vodnog stresa i sa prinosom po biljci kao najviše pogođenom osobinom. Nasuprot tome, prolin i relativni sadržaj vode i povećanje intervala metličanje-svilanje su bili povećani. Rezultatima korelacione analize su uvrđeni smanjeni prinos zrna sa povećanim intervalom metličenje-svilanje, i sugerisano je da su broj zrna u redu, relativni sadržaj vode, aktivnost peroksidaze i broj redova po klipu u Ismailiji, i broj redova po klipu, relativni sadržaj vode, aktivnost peroksidaze, masa zrna u Romani bili indirektno selekcioni kriterijumi za povećanje prinosa u sredinama sa nestašicom vode. Analiza glavnih komponenti (engl. *principal component* – PC) pokazala je da tri glavne komponente sa vrednošću >1 objašnjavaju 70,67 odnosno 70,16%; 69,79 odnosno 71,38% ukupne varijabilnosti među genotipovima u kontrolnim i uslovima stresa u Ismailiji odnosno Romani. Hibridi P1×P3, P4×P6, P3×P5 i P1×P5 klasifikovani su kao tolerantni prema suši u Ismailiji i Romani. S druge strane, P1×P4, P3×P4, i P4 smatrali su se osetljivim prema suši u Ismailiji. Pored toga, P5, P2×P4, P1×P4 i P5×P6 najviše su bili pogođeni nestašicom vode u Romani.

Ključne reči: kukuruz, suša, analiza glavnih komponenti, prinos, korelacija.

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