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GENETIC AND MULTIVARIATE PHENOTYPIC ANALYSES OF SOME SELECTION INDICES IN PISTACHIO (*Pistacia vera* L.) CULTIVARS UNDER DROUGHT STRESS CONDITIONS

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Hasheminasab H. and M.T. Assad (2017): *Genetic and multivariate phenotypic analyses of some selection indices in pistachio (Pistacia vera L.) cultivars under drought stress conditions*.- Genetika, vol 49, no.2, 705 - 715.
Heritability and Genetic Gain are commonly used by plant breeders to estimate the accuracy of a selection index and used for measuring the response to selection. The aim of the present study was the genetic and phenotypic analyses of some physiological traits to identify the most reliable of them for selective breeding of pistachio cultivars under drought stress condition. Nineteen Pistachio (*Pistacia vera* L.) cultivars with wide range of tolerance to drought stress were collected from across the orchards of Rafsanjan (Iran's center of pistachio cultivation) and were used in randomized complete block design with three replications under two environmental conditions (normal and water stress) in 2011-2012. The results of genetic analysis showed that high magnitude of phenotypic and genotypic coefficient of variation along with broad sense heritability and genetic gain were estimated in relative water protection (RWP), excised leaf water loss (ELWL), relative water content (RWC) and relative water loss (RWL) under drought stress condition, indicating that the inheritance of these traits can be mainly controlled by additive gene effects followed by reflecting the possibility of effective selection for genetic improvement of these traits. Path analysis revealed that RWP had the highest direct and RWL and WRC indirect effects on YSI. Principal component analysis indicated that selected indices were reliable for classification and identification of drought-tolerant pistachio cultivars and identified RWP, RWC and WRC as the best indicators for screening drought tolerant genotypes.
Key words: Pistachio, drought stress, genetic analysis, multivariate phenotypic analysis.

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INTRODUCTION

Heritability is a key concept in quantitative genetics, particularly in selective breeding. It is defined as the proportion of phenotypic variance among individuals in a population that is due to heritable genetic effects and it measures the fraction of phenotype variability that can be attributed to genetic variation. Its main use is for computing the response to selection (FALCONER and MACKAY, 1996; PIEPHO and MOHRING, 2007; FARSHADFAR and AMIRI, 2016). Selection which is the retention of ideal genotypes desirable characteristics and elimination of undesirable ones is an important process in breeding for improvement of one or more plant attributes (CHOPRA, 2000).

Pistachio (*Pistacia vera* L.) is a diploid (2n =30) member of the Anacardiaceae family is the only species in this genus, successfully grown in orchards, which produces edible nuts large enough to be commercially acceptable (HARANDI and GAFFARI, 2001; SARKAR, *et al.*, 2017). The genome size of pistachio is about 600 millions of base pairs (Mb) with a high heterozygosity rate (ZIYA MOTALEBIPOUR *et al.*, 2016). It is a desert plant and has high tolerance to drought. But, it does not mean that pistachio trees require less water for optimal performance and drought is one of the major factors limiting pistachio production in Iran (SEPASKHAH and KARIMI-GOGHARI, 2005; HASHEMINASAB *et al.*, 2014). Improvement of drought tolerance in pistachio can be carried out through: (1) defining drought problem of the target area and proposing ideotypes, (2) identifying drought tolerance traits and developing screening techniques, (3) estimating heritability and genetic gain of traits and their relationship with yield and yield stability, (4) screening germplasm for suitable source of the trait and (5) using suitable source of genetic variability in breeding programmes. Although crop yield is the principle selection index used under drought stress conditions, but breeding for drought tolerance as polygenic and complex trait by selecting solely for yield may not be successful, because the heritability of yield under drought conditions is low, as well as grain yield and drought resistance are controlled at independent genetic loci. Therefore, the identification of physiological traits associated with drought tolerance should be considered in the breeding programs (BLUM, 2005). The main objective of this study was to genetic and phenotypic analyses of some physiological traits to identify the reliable selection indices for breeding of drought-tolerant pistachio cultivars.

MATERIALS AND METHODS

Plant materials and experimental conditions

Nineteen pistachio (*Pistacia vera* L.) cultivars with wide range of tolerance to drought stress listed in Table 1 were collected from across the orchards of Rafsanjan (Rafsanjan is Iran’s center of pistachio cultivation, Fig. 1) and were used in a randomized complete block design with three replications under two different environments (normal and water stress) at the Experimental Orchard in the City of Rafsanjan, Kerman, Iran (30° 24′ 24″ N latitude, 55° 59′ 38″ E longitude and 1469 m altitude) during 2011-2012. Climate in this region is classified as arid and semi-arid with mean annual rainfall of 100 mm and the annual temperature range is between –17°C to 42°C. Soil of the Experimental Orchard was clay-loam texture. For measurement of physiological traits, pinnately compound leaves of all cultivars at the nut filling stage were harvested and weighed.

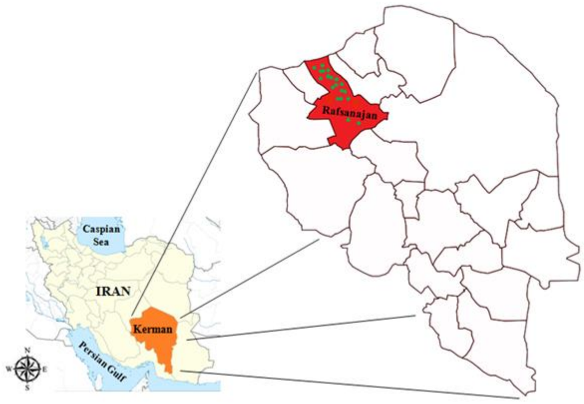

Fig. 1. The geographical locations of nineteen pistachio cultivars (shown in Table 1) collected in this study

Table 1. Characteristics of investigated pistachio cultivars

Cultivar	Code	Origin	Location	Predicting reaction to drought
Kale Ghoochi 1	1	Iran	Rafsanjan-Lotf Abad	susceptible
Kale Ghoochi 2	2	Iran	Rafsanjan-Anar	Susceptible
Kale Ghoochi 3	3	Iran- Rafsanjan-Nogh	Rafsanjan-Nogh	susceptible
Akbari 1	4	Iran	Rafsanjan-Nogh	susceptible
Akbari 2	5	Iran	Rafsanjan-Kashkoieh	susceptible
Akbari 3	6	Iran	Rafsanjan-Kashkoieh	Susceptible
White Akbari	7	Iran-Rafsanjan-Nogh	Rafsanjan-Nogh	susceptible
Ancient Kale Ghoochi	8	Iran-Rafsanjan	Rafsanjan	Intermediate
Kale Ghoochi-Bargi	9	Iran-Rafsanjan-Kashkoieh	Rafsanjan-Kashkoieh	Intermediate
Fandoghi	10	Iran-Rafsanjan-Nogh	Rafsanjan-Nogh	Intermediate
Ahmad-Aghaei	11	Iran	Rafsanjan-Nogh	Intermediate
White Ahmad-Aghaei	12	Iran-Rafsanjan-Nogh	Rafsanjan	Intermediate
Ohadi	13	Iran-Zarand	Rafsanjan	Intermediate
White Ohadi	14	Iran-Rafsanjan-Nogh	Rafsanjan-Anar	Intermediate
Ancient Badami	15	Iran-Rafsanjan-Kashkoieh	Rafsanjan-Lotf Abad	Tolerant
Badami	16	Iran-Rafsanjan-Davaran	Rafsanjan-Davaran	Tolerant
Badami-Self-Female	17	Iran-Rafsanjan-Kashkoieh	Rafsanjan-Kashkoieh	Tolerant
Jafari	18	Iran-Rafsanjan	Rafsanjan-Kashkoieh	Tolerant
Ancient Fandoghi	19	Iran-Rafsanjan-Nogh	Rafsanjan-Lotf Abad	Tolerant

Relative water protection (RWP)

RWP was calculated using the formula suggested by HASHEMINASAB *et al.* (2012). Ten randomly selected pinnately compound leaves were taken and weighed for fresh weight (F_W). The leaves were then wilted at 25°C for 10h (This time can be different for various plant species.) and weighed again, respectively (Withering weight, W_W). Finally, the samples were oven dried at 70°C for 72h and reweighed (Dry weight, D_W). This index is indeed the proportion of water that is protected and not evaporated from the leaves after drying.

$RWP = (W_W – D_W) / (F_W – D_W)$

Relative water content (RWC)

RWC was measured using the method of BARRS (1968). A sample of 10 pinnately compound leaves was taken randomly from different plants of the same cultivar and their fresh weight (F_W) measured. The leaf samples were placed in distilled water for 24 h and reweighed to obtain turgid weight (T_W). After that, the leaf samples were oven-dried at 70°C for 72 h and dry weight (D_W) measured. However, RWC was calculated using the following formula:

$RWC = (F_W – D_W) / (T_W – D_W)$

Leaf water content (LWC), relative water loss (RWL) and excised leaf water loss (ELWL)

Randomly selected leaves were weighed spontaneously after their harvesting (W1). The leaves were then wilted at 25°C and weighed again over 4, 6 and 8 h (W2, W3 and W4). Then the samples were oven-dried at 70°C for 72 h and reweighed (WD). LWC, RWL and ELWL was worked out using the following formula devised by CLARKE and CAIG (1982), YANG *et al.* (1991) and MANETTE *et al.* (1988):

$LWC = (W_1 – W_D) / W_1$

$RWL = [(W_1 – W_2) + (W_2 – W_3) + (W_3 – W_4)] / [3 \times W_D (T_1 – T_2)]$

$ELWL = (W_1 – W_3) / (W_1 – W_D)$

Water retention capacity (WRC)

RWC was measured using the method of HASHEMINASAB *et al.* (2013). WRC is a combination of two value indexes, including RWC and RWP, therefore this index is calculated as the ratio of water out of the leaf and the water entering the leaf. WRC is indeed the proportion of actual water that is protected and not evaporated from the leaves after drying, because turgid leaf weight (maximum leaf water capacity) is located in the denominator of the formula. To measure WRC, ten randomly selected leaves were taken and placed in distilled water for 24 h and reweighed to obtain turgid weight (T_W). The leaves were then allowed to wilt at 25°C for 8 h and weighed again (Withering weight, W_W). Finally, the leaf samples were oven-dried at 70°C for 72 h and dry weight (D_W) measured. However, WRC was calculated using the following formula:

$RWC = (W_W – D_W) / (T_W – D_W)$

Yield stability index (YSI)

Yield stability index (YSI) was calculated according to BOUSLAMA and SCHAPAUGH (1984) using the following formula:

YSI =Ys / Yp

Where, Ys and Yp represent yield under stress and non-stress conditions, respectively.

Genetic and statistical analysis of data

The measured data of the YSI and its relative traits were analyzed by the statistical methods including, Principal component and path analysis using SPSS software packages 16. Heritability, phenotypic and genotypic coefficient of variability and phenotypic and genotypic variances were derived from variance component of analysis of variance for measured traits based on the following formulae (FALCONER and MACKAY, 1996):

$V_E = MS_e$

$V_G = (MS_t - MS_e)/r$

$V_P = MS_t + MS_e$

$H^2 (\%) = (V_G / V_P) \times 100$

$GCV (\%) = \sqrt{V_G}/\bar{X} \times 100$

$PCV (\%) = \sqrt{V_P}/\bar{X} \times 100$

$GG = i \times H^2 \times \sqrt{VP}$

Where abbreviations are; MS_t = Treatment mean square, MS_e = Error mean square, V_G = Genotypic variance, V_P = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, H^2 = Heritability, r = Replication, $\bar{X}$ = Mean of traits, GG = Genetic gain, i = Standard selection differential for 5% ($i_{0.05}$ = 2.06).

RESULT AND DISCUSSION

Genetic analyses

Estimates of genetics parameters for measured traits along with means of them are shown in Table 2. The results revealed that phenotypic variances (V_P) and phenotypic coefficient of variation (*PCV*) weren’t much higher than genetic variances (V_G) and genotypic coefficient of variation (*GCV*) for all measured traits expect YSI and LWC. However, the high differences between *PCV* and *GCV* indicate the high influence of environment in the expression of these traits and isn’t desirable in breeding works (BELLO *et al.*, 2012). PIEPHO and MOHRING (2007) stated that the most of the economic characters such as yield and yield stability are controlled by quantitative traits. These characters are affected by many genes that act singly and in interaction with each other and are complex in inheritance along with low selection efficiency.

Table 2. Genetic analysis of measured traits in pistachio cultivars under drought stress condition

Variables	mean	V_G	V_P	H^2 (%)	GCV (%)	PCV (%)	GG
YSI	0.61300	0.02426	0.04519	53.67350	25.40738	34.68007	1.10567
RWP	0.36730	0.00480	0.00615	78.13161	18.86547	21.34294	1.60951
LWC	0.38290	0.00018	0.00036	50.07009	3.49024	4.93249	1.03144
RWC	0.52200	0.00257	0.00417	61.57907	9.70416	12.36634	1.26853
ELWL	0.50770	0.00202	0.00290	69.51148	8.85058	10.61557	1.43194
RWL	0.05420	0.00024	0.00037	65.74376	28.71361	35.41282	1.35432
WRC	0.19130	0.00340	0.00500	68.00000	30.48067	36.96324	1.40080

V_G, Genotypic variance; V_P, Phenotypic variance; H^2, Heritability in broad sense; *GCV*, Genotypic coefficient of variation; *PCV*, Phenotypic coefficient of variation; GG, Genetic gain

Estimate of heritability influences selection procedures used by the plant breeder to determine which selection index would be most effective to improve the character (WAQAR *et al.*, 2008). As seen Table 2, high magnitude of broad sense heritability (H^2) was estimated in RWP, ELWL, WRC and RWL in pistachio cultivars under drought stress condition. ANSARI *et al.* (2004) reported that high heritability percentage indicated the large heritable variance that can offer more possibility of improvement through selection. However, JOHNSON *et al.* (1955) and IDAHOSA *et al.*

(2010) stated that H^2 along with genetic gain (*GG*) are more efficient than H^2 alone to predict the resulting effect of selecting the best genotypes. The finding showed that RWP, ELWL and WRC had the highest *GG* under drought stress condition. In present study, these traits also indicated the highest H^2 among the all measured parameters. SUMATHI *et al.* (2005) reported that the traits with high value of H^2 together with high *GG* were controlled by additive genetic effects. CHANDRASHEKHAR and KERKETTA (2004) estimated genotypic variability, heritability and genetic advance for grain yield and its components. They stated preponderance of additive gene action in the inheritance of Characteristics, which could be improved through direct selection method on the basis of phenotype of the character. FARSHADFAR *et al.* (2013) reported that genetic gain in developing tolerance in bread wheat could be achieved through indirect selection of physiological traits related to leaf water status, because the additive genes mainly controlled these traits.

Genetic path analysis

Standardized regression coefficient (path analysis) in order to determine most important variables on YSI was carried out among the physiological traits (Table 3). The results of path analysis showed that RWP (1.219**), RWL (0.239) and WRC (-0.299) had the highest standardized regression coefficient or direct effect and RWC (0.534), RWL (-0.789) and WRC (0.828) had the highest indirect effect on YSI. RWP (0.842**) contributed positively and ELWL (-0.587**) negatively towards YSI showed the highest total correlation. The results of path analysis were consistent with and stepwise multiple linear regression analysis (Table 3) indicating RWP, RWC and WRC were the best physiological traits for monitoring drought tolerance. ESMAEILPOUR *et al.* (2016) stated that physiological traits related to water status and stomata features had significant relationship with water use efficiency in pistachio cultivars under drought stress condition and recommended these traits as suitable indicators for screening drought tolerance genotypes. BRDAR-JOKANOVIĆ *et al.* (2014) in tomato and ANDJELKOVIC *et al.* (2014) in maize also found that selection indices related to grain yield were reliable to separate drought tolerant genotypes from other ones.

RENU and DEVARSHI (2007), HASHEMINASAB *et al.* (2012) and SAED-MOUCHESHI *et al.* (2015) reported that drought stress tolerant and intermediate tolerant genotypes were superior to susceptible ones in maintaining leaf water content and yield stability under stress condition.

Table 3. Path coefficient (direct and indirect effects) of the measured traits on YSI in pistachio genotypes under drought stress condition

Variables	Path coefficient		Total correlation
	Direct effect	Indirect effect	
RWP	1.219**	-0.377	0.842**
RWC	-0.184	0.534	0.35
RWL	0.239	-0.789	-0.55*
LWC	-0.088	-0.104	-0.192
ELWL	-0.133	-0.454	-0.587**
WRC	-0.299	0.828	0.529*

Principal component analysis (PCA)

PCA of the data in Table 4 showed that four main components together for YSI explained 96.57% of the total variation, which, in conventional analyses. From Fig. 2, it was observed that an increase in the number of the components was associated with a decrease in eigenvalues, which is an important indicator in general genetics and very valuable for evaluating drought tolerant cultivars and also efficient indicators for screening these cultivars. The trend reached its maximum for four components. Thus, it is reasonable to assume that the PCA divided total estimated variables into four main components. CHATTI *et al.* (2017) evaluated genetic diversity of Tunisian pistachio using morphological parameters and reported that the first three components of PCA absorbed 97.23% of the total variability among specimens. Data presented in Table 4 showed that the first component (PC1) explained 55.66% of the total data variation and had a highly positive correlation with ELWL and negative correlation with RWP under stress condition. Therefore the PC1 can be named as crop water stress dimension and it separates the drought susceptible cultivars from tolerant ones. The second component (PC2) explained 18.97% of the total variability and correlated positively with LWC. Therefore, the second component can be named as a component of plant water status with high leaf water content in a stressful environment. In other words, this component was able to separate the cultivars with high tolerant under drought condition. Thus, selection of cultivars that have high PC1 and low PC2 are suitable for water stress condition. The third and fourth dimensions (PC3 and PC4) included RWC and YSI, respectively, which accounted for 12.88 and 9.06% of the total variability in the dependent structure and they were named RWC and YSI factors.

Table 4. Principle component analysis of measured traits in pistachio cultivars under drought stress condition

Variable	Dimension			
	1	2	3	4
RWP	-0.919	0.351	0.038	0.109
RWC	-0.428	0.37	0.798	-0.193
RWL	0.868	0.427	0.076	0.203
LWC	0.389	0.856	-0.277	0.118
ELWL	0.889	-0.014	0.341	0.159
WRC	-0.827	0.366	-0.23	-0.166
YSI	-0.7	-0.134	0.109	0.691
Eigenvalue	3.896	1.328	0.902	0.634
Proportion (%)	55.657	18.972	12.88	9.061
Cumulative (%)	55.657	74.629	87.509	96.57

Therefore PC3 and PC4 can be screening the cultivars with high RWC and YSI under stress condition, respectively. Data obtained of PCA were graphed in a biplot analysis, so that the eigenvalues of PC1 were plotted against those for PC2 for both the cultivars and for the measured traits (Fig. 3). The biplot is a helpful tool for revealing clustering, multicollinearity, and multivariate outliers of a dataset and it can be also used to display Euclidean distances, variances and correlations of variables of large datasets (GABRIEL, 1971; KOHLER and LUNIAK, 2005). In the biplot, the length of the lines approximates the variances of the physiological traits. The longer the line, the higher is the variance. Inferring from Fig. 3, RWL and LWC had the highest variance among the variables in the biplot and RWP and RWC indicated the lowest. The correlation coefficient between any two traits is approximated by the cosine of the angle between vectors drawn from the origin to the trait. An angle of 0° or 180° degrees reflects a correlation of 1 or − 1, respectively, and an angle of 90° represents a correlation coefficient of 0 (GOWER and HAND, 1996). The biplot will differ from correlation analysis among pairs of traits because it explain interrelationships among all traits simultaneously (YAN and RAJCAN, 2002). The angles between RWP, RWC, WRC and YSI were acute (Fig. 3). Therefore, these traits can be classified in similar group. YOUSFI *et al.* (2010) in alfalfa reported that under stress conditions, higher leaf water retention was a resistant mechanism to drought which the result was a reduction in stomatal conductance.

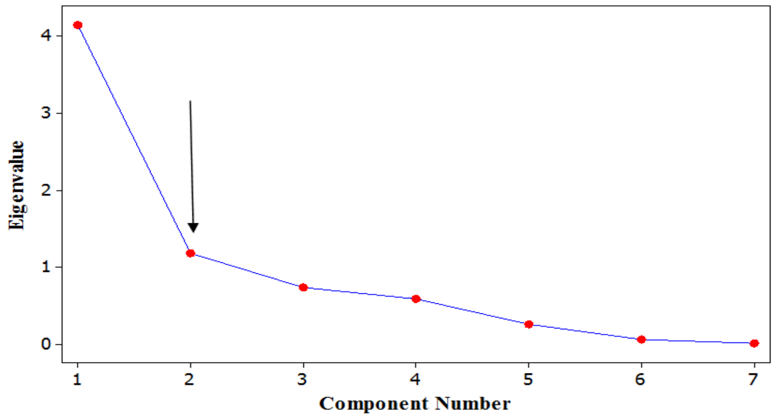

Fig. 2. Eigen values in response to number of components for measured traits in pistachio cultivars

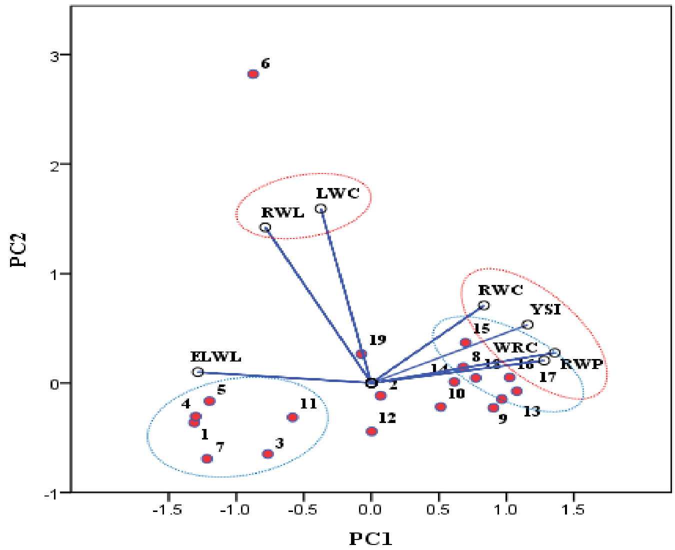

Fig. 3. Biplot of principal component analysis for measured traits and nineteen pistachio cultivars. Numbers 1 to 19 are the codes of studied pistachio cultivars, respectively (shown in Table 1)

RWL and LWC were placed in the second group with an acute angle between them. The biplot in Fig. 3 showed a strong relationship between RWP with WRC and RWL with LWC and a weak relationship (90°) between YSI and LWC. The distance from a cultivar to a trait name is an indication of the rank of that trait for that cultivar. Cultivar can be compared by determining their position relative to each other and to a trait name (YAN and RAJCAN, 2002) Therefore, cultivars 8, 13, 14, 15, 16, 17 and 18 with high PC1 and low PC2 had highest relationship with the first group and confirmed these cultivars are superior for stress environment. Biplot presentation depicted cultivars 1, 3, 4, 5, 7 and 11 were close to ELWL and away from the other drought tolerance indicators, and were identified as susceptible cultivars with low PC1 and PC2. According to Fig. 3, cultivar was selected as the most susceptible cultivars with highest PC2 and low PC1.

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GENETIČKA I MULTIVARIJETNA FENOTIPSKA ANALIZA NEKIH SELEKCIONIH INDEKSA U KULTIVARIMA PISTAČIJA (*Pistacia vera* L.) U USLOVIMA SUŠE

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Izvod

Heritabilnost i genetička dobit najčešče koriste oplemnjivači da odrede tačnost selekcionih indeksa i kao meru odgovora na selekciju. Cilj ovog rada je genetička i fenotipska analiza nekih fizioloških svojstva da se odrede koji su od njih najpogodniji za selekciju kultivara pistačija u uslovima suše. Devetnest kultivara sa širokim opsegom toleratnosti na sušu su sakupljeni sa različitih zasada u Rafsanjanu (Iranski centar za gajenje pistacija) i koriščen je RCB dizajn sa tri ponavljanja u dve sredine (normalna i stres vode) tokom 2011-2012 godine. Rezultati genetičke analize su pokazali da visoke magnitude fenotipskih i genotipskih koeficijenata variranja zajedno sa širokom heritabilnosti i genetičkom dobiti su izračunate za relativnu vodenu zaštiti (RWP), pojačano gubljenje vode u listovima (ELWL), relativni sadržaj vode (RWC) i relativni gubitak vode (RWL) u uslovima suše, ukazujući da nasleđivanje ovih svojstava može uglavnom biti pod konrolom aditivnih gena praćeno mogućnostima efikasne selekcije za ova svojstva. Path analiza otkriva da RWP ima najveći direktan i RWL i WRC indirektan efekat na YSI. Principijalna komponentna analiza ukazuje da selekcioni indeksi su pouzdani za klasifikaciju i identifikaciju kultuivara koji su otporni na sušu i identifikaciju RWP, RWC i WRC kao najbolje indikatore za skrining toleratnih genotipova.

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