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A Predictive Model for Breeding of Pistachio Yield Stability under Water Stress Condition

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ABSTRACT: The present study was conducted to evaluate a statistical model for predicting pistachio yield stability based on physiological traits related to plant water status 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 this study showed that the model developed by multiple linear regression analysis explained 76.8% of the total variation within all the physiological traits while the remaining 23.3% may be due to residual effects. Although analysis of variance (ANOVA) showed goodness of the model ($MS_{Regression}$ = 0.003) when all predictor variables were present in the model, residual plots analysis revealed a right-skewed in the model. Therefore, the model was optimized using stepwise multiple linear regression analysis and the results of the final model demonstrated that about 76.5% of the variability in yield stability index (YSI) could be attributed to relative water protection (RWP), relative water content (RWC) and water retention capacity (WRC) only. The findings of different statistical methods suggested RWP, RWC and WRC as reliable indicators for monitoring drought tolerance and predicting pistachio yield stability.**

Keywords: Pistachio, Water stress, Yield stability, Physiological traits.

INTRODUCTION

Water plays a key role in the life processes of plants. It is the most abundant constituents of most organisms. Water is very essential for plant growth and makes up 75 to 95 percent of plant tissue. A vast amount of water moves throughout the plant daily (Vince and Zoltán, 2011). Plants use water and carbon dioxide to form sugars and complex carbohydrates. Water acts as a carrier of nutrients and also a cooling agent. It also provides an element of support through turgor and as an intercellular reaction medium. Many of the physio-biochemical reactions occur in water and water is itself either a reactant or a product in a large number of those reactions (Ashraf and Harris, 2005; Hasheminasab *et al.*, 2013).

Water stress is one of the major environmental factors limiting plant performance, growth and productivity in arid and semi-arid regions and recent global climate change along with increasing shortage of water resources has made this situation more serious (Blum, 1985; Renu and Devarshi, 2007; Bijanzadeh and Emam, 2012; Hasheminasab *et al.*, 2012). However, certain tolerant crop plants Physiological and metabolic changes occur in response to drought, which prevent the water loss from the leaf and contribute towards adaptation to such unfavorable constraints (Blum, 1985; Golestani and Assad, 1998; Anjum *et al.*, 2011).

Pistachio (*Pistacia vera* L.) is the most important agricultural crop in arid and semi-arid regions of Iran and has high tolerance to drought (Hasheminasab *et al.*, 2014a). But, it does not mean that pistachio trees require less water for optimal performance. The drought tolerance of the pistachio refers to its ability to survive under severe water stress conditions (Goldhamer, 1995; Sepaskhah and Karimi-Goghari, 2005). Increased establishment of irrigated pistachio orchards during the last two decades in Iran has decreased the availability of underground water resources and prolonged drought periods are the major concern for the pistachio producers (Bagheri *et al.*, 2012). The main purpose of the present investigation was to model pistachio yield stability based on physiological traits related to plant water status under water stress condition.

MATERIALS AND METHODS

The current study was conducted to evaluate a statistical model for predicting pistachio yield stability under water stress condition.

Eighteen pistachio (*Pistacia vera* L.) cultivars with wide range of tolerance to drought stress collected from across the orchards of Rafsanjan (It 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.

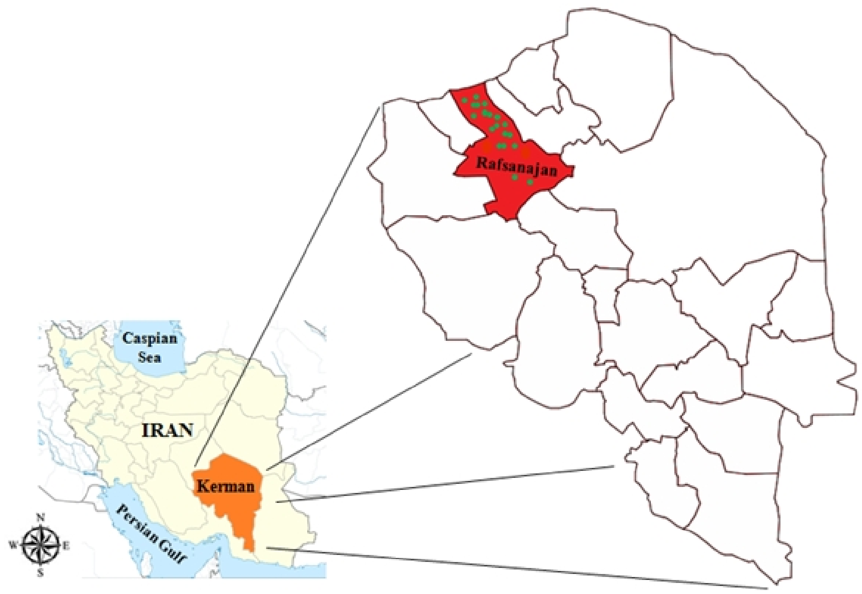

Fig. 1. The geographical locations of nineteen pistachio cultivars collected in this study.

A. 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.

$$RWP = (W_W - D_W) / (F_W - D_W)$$

B. 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:

$$RWC = (F_W - D_W) / (T_W - D_W)$$

C. 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):

$$LWC = (W_1 - W_D) / W_1$$

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

$$ELWL = (W_1 - W_3) / (W_1 - W_D)$$

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:

$$WRC = (W_W - D_W) / (T_W - D_W)$$

E. 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.

F. Statistical analysis of data

The measured data of the YSI and its relative traits across the two environment conditions were analyzed by the statistical methods including, multiple linear regression and stepwise multiple linear regression using SPSS software packages 16 and Minitab version 14.

RESULTS AND DISCUSSION

A. Multiple linear regression

In this study, a multiple linear regression equation was defined to predict the relationship between YSI as dependent variable and other measured physiological traits were shown in Table 1 as independent variables (predictors) by fitting a linear model to the observed data.

Table 1: Regression coefficient (b), standard error (S.E.), t-value (t), probability (P-Value), tolerance index (Tolerance) and variance inflation factor (VIF) of the physiological variables for predicting yield stability by the multiple linear regression analysis in pistachio cultivars.

Variable	Unstandardized Coefficients		Standardized Coefficients	t	P-Value	Collinearity	
	b	S.E.	Beta			Tolerance	VIF
(Constant)	0.567	0.232		2.44	0.031		
RWP	0.484	0.109	1.219	4.45	0.001	0.258	3.874
RWC	-0.104	0.096	-0.184	-1.085	0.299	0.671	1.49
RWL	0.692	2.585	0.239	0.268	0.794	0.024	41.236
LWC	-0.125	0.635	-0.088	-0.197	0.847	0.098	10.195
ELWL	-0.048	0.249	-0.133	-0.195	0.849	0.041	24.172
WRC	-0.222	0.183	-0.299	-1.211	0.249	0.317	3.151

The results indicated that the linear regression model for predicting YSI is formulated by using physiological traits as follow:

YSI = 0.567 + 0.484 RWP - 0.104 RWC + 0.69 RWL - 0.125 LWC - 0.048 ELWL - 0.222 WRC

The predictive model explained 76.8% (R^2 = 0.768) of the total variation within the physiological traits while the remaining 23.2% probably be due to residual effects. When many predictors are used in the model, R^2 can be quite large, even when all correlations in the population are actually zero. Therefore, it is important to determine whether R^2 of the prediction model is statistically significant (Wilkinson, 1979). Analysis of variance (ANOVA) for this model was shown in Table 2. ANOVA showed that the statistical model was significant ($MS_{Regression}$ = 0.003**) when all predictor variables were present in the model. On the other hand, t-test calculated for all variables separately showed that some of the variables were not important to be presented in modeling of YSI, because no predictor variables significantly contributed to the model (Table 1).

Table 2: Analysis of variance (ANOVA) for modeling yield stability based on physiological traits in pistachio cultivars.

Model	S.S.	D.F.	M.S.	F	P-Value
Regression	0.069	6	0.011	6.604	0.003
Residual	0.021	12	0.002		
Total	0.089	18			

In an ideal model, independent variables should not be related among themselves, commonly known as the problem of multi-co-linearity, as indicated by their respective values of variance inflation factor (VIF), being above 10, and tolerance index. Tolerance close to 1 indicates the predictor in question is not redundant with other predictors already in the regression equation, while a tolerance close to zero indicates a high degree of redundancy (Belsley et al., 1980; Saed-Moucheshi *et al.,* 2013). VIF and tolerance index showed that there was some collinearity among variables and the coefficients determined by this model probably are not the best values (Table 1). VIF for RWL, LWC and ELWL was higher than 10 thereby confronting a problem with coefficients for these variables for modeling yield stability. Tolerance index also confirmed that there was some collinearity between these traits.

The residuals from the regression model were plotted to demonstrate assumption violations. Residual plot, normal plot, variation plot and normal distribution histogram of the standardized residuals $(y - \hat{y})$ were shown in Fig. 2a–d. A residual plot allows visual assessment of the distance of each observation from the fitted line. The residuals should be randomly scattered in a constant width band about the zero line. Dispersion of residuals above or below the zero line may indicate a non-linear relationship (Belsley et al., 1980; Yang, 2012). In this study, the graphs showed no problem with the residuals of the model with selected variables because the residuals are dispersed almost uniformly around the zero line (Fig. 2a and c). The normal plot of the residuals in Fig. 2b had a straight-line appearance. But histogram with normal overlay of the distribution of the residuals showed that there was a right-skewed or right-tailed in the normal distribution curve (Fig. 2d). These results indicated a partial goodness of the model for predicting YSI using all the physiological variables. Several reports underlined the significant relationship between the ability to maintain leaf water content and drought tolerance in various plants (Turkan *et al.,* 2005; Renu and Devarshi, 2007; Farshadfar *et al.,* 2013). Dong *et al.* (2008) in wheat and 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 and transpiration rate.

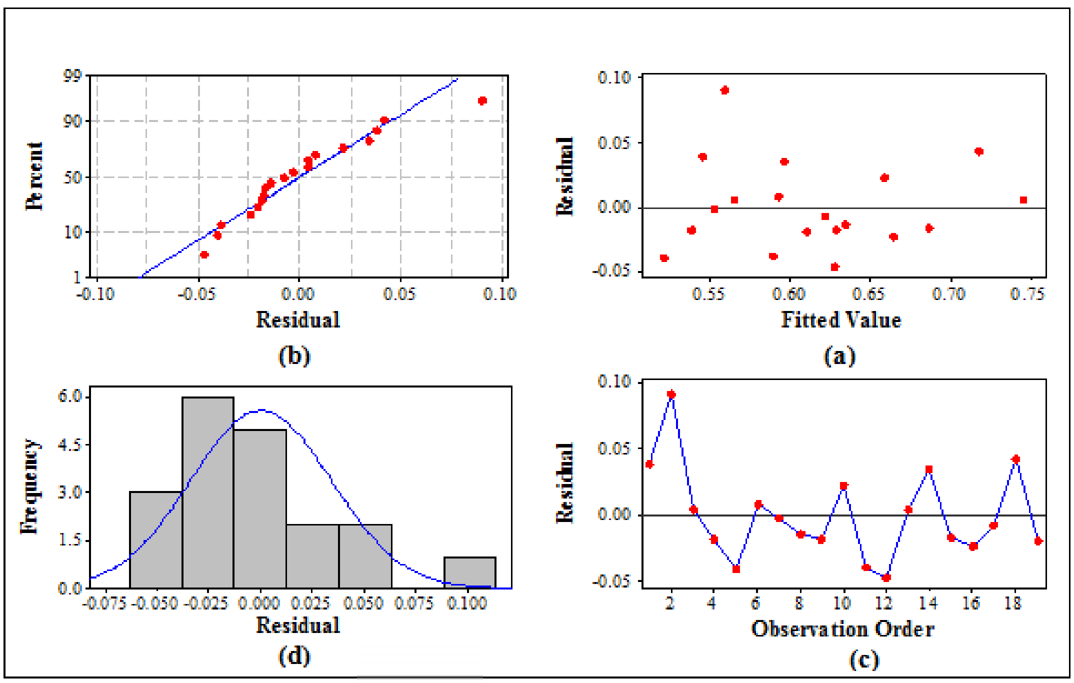

Fig. 2. Scatter plot (a), normal plot (b), variation plot (c) and normal distribution Histogram (d) of the standardized residuals $(y - \hat{y})$ for predicting model of yield stability in pistachio cultivars.

B. Stepwise multiple linear regression

The results of normal distribution curve and collinearity statistics experienced a problem with all measured variables in the model. Thus, as a third step, stepwise multiple linear regression analysis was used to determine the variable accounting for the majority of total YSI variability and to select the best variables for the prediction model of YSI (Wilkinson, 1979; Dong *et al.,* 2008).

Table 3 shows the data representing entered variables from stepwise regression analysis of YSI (dependent) and measured physiological traits (independent) under stress condition. These entered variables were: RWC (R^2 = 71%), WRC (R^2 = 3.3%) and RWC (R^2 = 2.2%) respectively. According to the results, 76.5% of the total variation in YSI could be attributed to these three traits. The other variables were not included in the analysis due to their low relative contributions. Physiologists have often suggested that the detection and selection of physiological traits related to plant water status are reliable methods to breeding for higher yield, and could be a valuable strategy for use in conjunction with normal methods of plant breeding (El Jaafari et al., 1993).

The Stepwise model for predicting YSI was:

YSI = 0.539 + 0.470 RWP - 0.240 WRC - 0.100 RWC

The goodness of fit of the stepwise regression model was measured by the F-test (Table 3). ANOVA for optimized model showed that the model was high significant when all the three predictor variables were present in the model. Dong et al. (2008) and Hasheminasab et al. (2014b) obtained similar results in Stepwise multiple linear regression analysis of some physio-biochemical drought tolerance indicators in wheat.

Table 3: Analysis of variance (ANOVA) and relative contribution (partial and adjusted R^2) for modeling yield stability by the stepwise multiple linear regression analysis in pistachio cultivars.

Model		S.S.	D.F.	M.S.	P-Value	R^2
RWP	Regression	0.063	1	0.063	000	0.710
	Residual	0.026	17	0.002		
	Total	0.089	18			
RWP	Regression	0.066	2	0.033	000	0.743
WRC	Residual	0.023	16	0.001		
	Total	0.089	18			
RWP	Regression	0.068	3	0.023	000	0.765
WRC	Residual	0.021	15	0.001		
RWC	Total	0.089	18			

CONCLUSION

The results of current study showed that predictive model developed by multiple linear regression analysis explained 76.8% of the total variation within all the physiological traits. The residual plots analysis showed no problem in the model with selected variables. On the other hand, t-test and collinearity statistics showed that some of the variables are not important to be present in this model. Therefore, the model was optimized using stepwise multiple linear regression analysis and the results of the final model demonstrated that about 70 % of the variability in YSI could be attributed to RWP, RWC and WRC only and selected these traits as the best indicators for predicting pistachio yield stability under water stress condition.

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