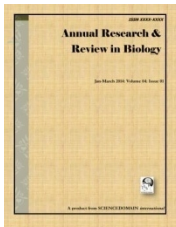

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Application of Physiological Traits Related to Plant Water Status for Predicting Yield Stability in Wheat under Drought Stress Condition

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Authors' contributions

This research was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The objective of the present study was to model the relationship between yield stability index (YSI) and some physiological traits related to plant water status. Fifteen bread wheat (*Triticum aestivum* L.) genotypes with wide range of sensitivity to drought were used in a randomized complete block design with three replications under two different environments (irrigated and rainfed) in 2012-2013 at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran. The results showed that YSI had positive and significant relationship with relative water protection (RWP, r = 0.858**), relative water content (RWC, r = 0.594*), canopy temperature depression (CTD, r = 0.669**), stomata resistance (SR, r = 0.643**) and evapotranspiration efficiency (ETE, r = 0.818**), and negative significant correlation with relative water loss (RWL, r = – 0.822**) and excised leaf water loss (ELWL, r = – 0.543*) under drought stress condition. Also ETE (0.46*) and RWP (0.806) had the highest direct and indirect effects on YSI, respectively. Multiple linear regression analysis indicated that the predicting model for YSI explained 97.9% of the total variation within the measured traits. The residual plots analysis indicated no problem in the model with selected variables. On the other hand, t-test showed that some of the variables are not important to be present in this model. The results of path and stepwise multiple linear

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regression analysis indicated that ETE (R^2 = 73.5%), RWP (R^2 = 11.9%), CTD (R^2 = 6.9%) and RWC (R^2 = 3%) were the best physiological traits related to water status for modeling of YSI.

Keywords: Wheat; YSI; modeling; drought; plant water status; physiological traits.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and it is also the first grain crop in Iran [1-2]. This crop is widely adapted from temperate irrigated to dry and high rainfall areas, and from warm humid to dry cold environments. Undoubtedly this wide adaptation has been possible due to the complex nature of its genome, which provides a fantastic plasticity to the crop [3]. Wheat often experiences drought stress condition during crop cycle. Thus, improvement of wheat production for drought tolerance is a major objective in plant breeding programs for arid and semi-arid regions [4-5].

Drought tolerance is usually quantified by grain yield under stress conditions. Wheat grain yield under drought, however, depends on yield potential as well as the phenology of the genotype [6]. The quantification of drought tolerance has also been approached by a yield stability index (YSI) across environments [7-8] as well as by drought susceptibility indices [9]. These indices are highly dependent on yield potential and crop phenology which are characters with a high genotype x environment interaction [6]. Thus, plant breeders have always looked for appropriate and repeatable indicators to screen germplasms for drought tolerance [10-11]. Physiologists have often suggested that the identification and selection of physiological and/or morphological traits is an effective approach to breeding for higher yield, and could be a valuable strategy for use in conjunction with normal methods of plant breeding [12-13]. Relative water content (RWC), stomata resistance (SR), leaf temperature (LT) and transpiration rate (E) are among the main physiological criteria that influence plant water relations and are used for assessing drought tolerance [14-15]. Relative water protection (RWP) is another important physiological criteria in assessing the degree of water stress. RWP is indicating plant water status related to water stress, as well as reflecting the metabolic activity in tissues [2]. The canopy temperature (T_c) measured with infrared thermometers (IRTs) provides a reliable method for rapid, non-destructive monitoring of plant response to drought stress [14,16]. They also stated that the behavior of T_c both under stress and non-stress conditions provided clues for crop water status and yield performance during drought.

Different statistical methods and multivariate analysis have been used in evaluating and modeling wheat growth and development, including simple correlation, multivariate regression, path analysis and principal component analysis [17-18], But some of them have been used to model the relationship between YSI and physiological traits related to plant water status. Therefore, the main purposes of the present study were to: (i) model the YSI based on physiological traits associated to plant water status and (ii) evaluate the measured traits as selection index for wheat breeding to drought stress.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Conditions

In order to model the relationship between YSI and some physiological traits related to plant water status, fifteen bread wheat (*Triticum aestivum* L.) genotypes with wide range of sensitivity to drought stress listed in Table 1 were used in a randomized complete block design with three replications under two different environments (irrigated and rainfed) at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran (47° 20′ N latitude, 34° 20′ E longitude and 1351m altitude) during 2012-2013. Climate in this region is classified as semi-arid with mean annual rainfall of 478mm and mean annual temperature of 13.8°C. Soil of the Experimental station was of clay-loam texture with EC = 0.550 dS/m and pH = 7.1. The plots consisted of 2m rows and at 15×30 cm inter-plant and inter-row distances, respectively. For measurement of measured attributes, flag leaves of all wheat cultivars at the flowering stage were harvested and weighed.

Table 1. Characteristics of investigated wheat genotypes

Genotype	Code	Pedigree	Reaction to drought
Bahar	1	ICW84-0008-013AP-300L-3AP-300L-0AP	Susceptible
Chamran	2	(Attila.(CM85836-50Y-OM-OY-3M-OY	Tolerant
Shiraz	3	Gv/D630//Ald"s"/3/Azd	Susceptible
Kavir	4	Stm/3/Kal//V534/Jit716	Tolerant
Rijaw	5	PATO/CAL/3/7C//Bb/CNO/5/CAL//CNO/…/Sabalan	Tolerant
Darab2	6	Maya"s"/Nac	Intermediate
Falat	7	Kvz/Buho"s"//Kal/Bb=Seri82	Susceptible
Niknejad	8	"F13471/Crow"s	Intermediate
Roshan	9	Roshan	Intermediate
Azar2	10	Azar2	Tolerant
Tabasi	11	Tabasi	Intermediate
Zarin	12	PK15841	Susceptible
Alamot	13	Kavz/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/3/Pi/Ndr//Hys	Susceptible
Pishtaz	14	Alvand//Aldan/Ias58	Tolerant
Alvand	15	CF1770/1-27-6275	Intermediate

2.2 Relative Water Content (RWC)

RWC was measured using the method of Barrs [19]. A sample of 10 flag 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:

$$RWC = (F_W – D_W) / (T_W – D_W)$$

2.3 Relative Water Protection (RWP)

RWP was determined according to Hasheminasab et al. [2]. Ten randomly selected flag leaves were taken and weighed for fresh weight (F_W). The leaves were then allowed to wilt at

25°C for 8 h and weighed again (Withering weight, W_W). Then the samples were oven-dried at 70°C for 72 h and reweighed (Dry weight, D_W). RWP was calculated using the following equation:

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

2.4 Leaf Water Content (LWC), Relative Water Loss (RWL) and Excised Leaf Water Loss (ELWL)

Randomly selected leaves were weighed spontaneously after their harvesting (W_1). The leaves were then wilted at 25°C and weighed again over 2, 4 and 6 h (W_2, W_3 and W_4). Then the samples were oven-dried at 70°C for 72 h and reweighed (W_D). LWC, RWL and ELWL was worked out using the following formula devised by Clarke and Caig [20], Yang et al. [21] and Manette et al. [22]:

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

2.5 Canopy Temperature Depression (CTD)

The crop canopy temperature (CT) was measured with a portable infrared thermometer (IRT). Four measurements were taken per plot at approximately 0.5 m from the edge of the plot with an approximately 30-60° from the horizontal position. Two to seven days after irrigation in each experiment, canopy temperatures were measured between 12:00 to 14:00 hours on cloudless, bright days. Ambient temperatures (AT) were measured with a common thermometer held at plant height. CTD was worked out according to Dong and Yu [23]:

$$CTD = AT - CT$$

2.6 Stomatal Resistance (SR) and Leaf Temperature (LT)

Stomatal resistance (mmol m^{-2} s^{-1}) and leaf temperature (°C) was measured by Porometer-AP4 (Delta Devices, Cambridge, UK). Three random plants were selected in each plot for determining gas exchange parameters. All measurements were made on the portion of the flag leaf exposed to full sunlight, at about halfway along its length. The measurements were also made over the same time period as for the canopy temperature depression.

2.7 Evapotranspiration Efficiency (ETE)

According to total consumed water through wheat life circle, ETE was calculated by referring to Ehdaie and Waines [24]. The ETE is defined as the ratio of total dry matter (TDM) production to total water use (TWU). TDM was recorded under normal and stress conditions at physiological maturity stage. The physiological maturity stage was considered when 90% of seed changed color from green to yellowish and stopped photosynthetic activity. The ETE was calculated using the following formulae:

$$ETE = TDM / TWU$$

2.8 Yield Stability Index (YSI)

Grain yield was recorded at physiological maturity stage. Yield stability index (YSI) was calculated according to Bouslama and Schapaugh [25] using the following formula:

$$YSI = Ys / Yp$$

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

2.9 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 descriptive statistics, simple correlation coefficients, multiple linear regression, stepwise multiple linear regression and path analysis using SPSS software packages 16 and Minitab version 14.

3. RESULTS AND DISCUSSION

Basis statistics (minimum, maximum and mean values), standard error (SE) and standard deviation (SD) for the measured variables under drought stress (S) and normal (N) conditions are shown in Table 2. The results revealed that drought stress caused a increase in total mean of relative water protection (RWP), relative water content (RWC), canopy temperature depression (CTD), stomatal resistance (SR), and leaf temperature (LE) and fallowed a decrease in leaf water content (LWC), relative water loss (RWL), excised leaf water loss (ELWL) and evapotranspiration efficiency (ETE).

Table 2. Descriptive statistics (minimum, maximum, mean values, standard deviation (SD) and coefficient of variation (CV)) for the measured traits in wheat genotypes under normal (N) and drought stress (S) conditions

Variable	Minimum	Maximum	Mean	S.E.	S. D.
LWC (N)	0.64	0.72	0.6978	0.00565	0.02189
LWC (S)	0.63	0.69	0.6571	0.00519	0.02009
RWP (N)	0.54	0.83	0.6897	0.01929	0.07473
RWP (S)	0.68	0.84	0.7572	0.01333	0.05164
RWL (N)	0.08	0.13	0.1086	0.00312	0.01208
RWL (S)	0.06	0.11	0.0801	0.00336	0.01300
RWC (N)	0.61	0.74	0.6618	0.00852	0.03301
RWC (S)	0.41	0.60	0.5270	0.01167	0.04518
CTD (N)	1.17	3.50	2.0011	0.18180	0.70411
CTD (S)	4.92	7.67	6.3389	0.24145	0.93513
SR (N)	5.27	13.69	10.1402	0.68303	2.64535
SR (S)	18.56	48.33	27.8200	2.28397	8.84578
LT (N)	30.82	32.92	31.8902	0.16443	0.63683
LT (S)	33.90	38.64	35.5869	0.29969	1.16070
ELWL (N)	0.23	0.38	0.3042	0.01012	0.03919
ELWL (S)	0.18	0.30	0.2294	0.00808	0.03131
ETE (N)	2.48	3.47	2.9702	0.08079	0.31289
ETE (S)	2.03	3.14	2.6784	0.08238	0.31906
YSI	0.49	0.86	0.6901	0.03250	0.12587

3.1 Simple Correlation Analysis

As a first step in predicting a model for yield stability, linear correlation coefficients (r) among physiological traits with YSI under stress condition were computed individually for all the data sets (Table 3). Simple correlation is an important statistical procedure to clarify the relationship between variables. The r close to 1 indicated that behavior of the two variables was almost identical. Conversely, the r close to – 1 indicated that manner of the two variables were opposite. A coefficient near 0 suggested that the two variables were independent of each other [26-27]. The results from Table 3 revealed that YSI had positive and significant correlation with physiological traits related to plant water status including RWP (r = 0.858**), RWC (r = 0.594*), CTD (r = 0.669**), SR (r = 0.643**) and ETE (r = 0.818**), and negative significant correlation with RWL (r = – 0.822**) and ELWL (r = – 0.543**) under rainfed condition. An increasing number of reports provide evidence on the association between high rate of leaf water retention capacity and sustained yield or biomass under water-limited conditions across different cultivars of crop plants [28-31]. Also the findings of study Sairam [32], Golestani and Assad [33] and Hasheminasab et al. [2] showed a variation in the physiological traits related to plant water status in wheat genotypes and suggested that water stress tolerance was closely associated with these traits. The other traits in this study including LWC (r = –0.421) and LT (r = 0.468) had no significant correlation with YSI. The differential relations of relative physiological traits to YSI may be attributed to environmental effects on plant growth [11,17]. Delacy et al. [34] and Yan [35] stated that genotype by environment interaction is a major problem in the study of quantitative traits because it reduces the association between genotypic and phenotypic values and complicates the process of selecting of genotypes with superior performance. RWP (r = 0.812**), RWL (r = – 0.622*) and ELWL (r = – 0.596*) indicated close correlation with SR. Dong et al. [17] and Siddique et al. [14] in wheat and Yousfi et al. [36] 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.

Table 3. Pearson's correlation coefficients between measured traits in wheat genotypes

Variable	LWC	RWP	RWL	RWC	CTD	SR	LT	ELWL	ETE	YSI
LWC	1									
RWP	-0.154	1								
RWL	0.367	-0.829**	1							
RWC	0.167	0.566*	-0.542*	1						
CTD	-0.192	0.415	-0.516*	0.579*	1					
SR	0.052	0.812**	-0.622*	0.345	0.207	1				
LT	-0.505	0.347	-0.493	0.277	0.25	0.367	1			
ELWL	0.047	-0.659**	0.833**	-0.399	-0.391	-0.596*	-0.412	1		
ETE	-0.623*	0.657**	-0.624*	0.105	0.439	0.466	0.384	-0.414	1	
YSI	-0.421	0.858**	-0.822**	0.594*	0.669**	0.643**	0.468	-0.543*	0.818**	1

** and **: Significant at the 0.05 and 0.01 probability levels, respectively.*

3.2 Multiple Linear Regressions

Multiple linear regression is a valuable method used to model the linear relationship between a dependent variable and some independent variables [17]. As a second step, multiple linear regression and partial coefficient (R^2) were used to model the relationship between YSI as dependent and other measured physiological traits as independent variables by fitting a linear equation to the observed data. The results indicated that the predicting model equation for YSI is formulated by using physiological traits related to water status as follow:

$$YSI = -0.16 - 0.53\ LWC + 0.126\ RWP - 2.46\ RWL + 0.871\ RWC + 0.0211\ CTD + 0.00318\ SR - 0.0018\ LT + 0.844\ ELWL + 0.181\ ETE$$

The statistical model developed by regressing explained 97.9% (R^2 = 0.979) of the total variation within the physiological traits while the remaining 2.1% probably be due to residual effects. Analysis of variance (ANOVA) for this model was shown in Table 4. When all measured variables were present in the prediction model by multiple regression, ANOVA showed that the model was high significant ($MS_{Regression}$ = 0.024**).

Table 4. Analysis of variance for the modeling of yield stability

Source	D.F.	SS	MS	F	*P*
Regression	9	0.217068	0.024119	25.55	0.001
Residual Error	5	0.00472	0.000944		
Total	14	0.221788			

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 (Table 5). Among the variables RWC significantly contributed to the model at the 10% of probability, while ETE was significant at the 5% probability, but the other variables were not significant.

Table 5. Regression coefficient (b), standard error (S.E.), t-value, probability (P), tolerant index (Tolerance) and variance inflation factor (VIF) of the estimated variables in predicting yield stability by the multiple linear regression analysis

Variable	b	S.E.	t	*P*	Collinearity Statistics	
					Tolerance	VIF
(Constant)	-0.157	1.115	-0.141	0.894		
LWC	-0.532	1.202	-0.442	0.677	0.116	8.65
RWP	0.126	0.542	0.233	0.825	0.086	11.616
RWL	-2.463	2.682	-0.918	0.401	0.055	18.024
RWC	0.871	0.428	2.037	0.097	0.18	5.541
CTD	0.021	0.014	1.561	0.179	0.422	2.37
SR	0.003	0.002	1.454	0.206	0.18	5.569
LT	-0.002	0.013	-0.14	0.894	0.312	3.202
ELWL	0.844	0.751	1.124	0.312	0.122	8.191
ETE	0.181	0.058	3.109	0.027	0.194	5.146

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 [37]. VIF and tolerance index showed that there was some co-linearity among variables and the coefficients determined by this model probably are not the best values (Table 5). VIF for RWP and RWL was higher than 10 thereby confronting a problem with coefficients for these variables to modeling yield stability.

The residuals from the regression model were plotted to demonstrate assumption violations [18]. Residual plots, normal plot and normal distribution histogram of the standardized residuals (y – ŷ) are shown in Fig. 1. 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 [37-38]. 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. 1a and c). The normal plot of the residuals in Fig. 1(b) had a straight-line appearance. Also histogram with normal overlay of the distribution of the residuals showed that the measurement errors in the response variable (YSI) were normally distributed (Fig. 1d). These results indicated goodness of the model for predicting YSI using selected variables.

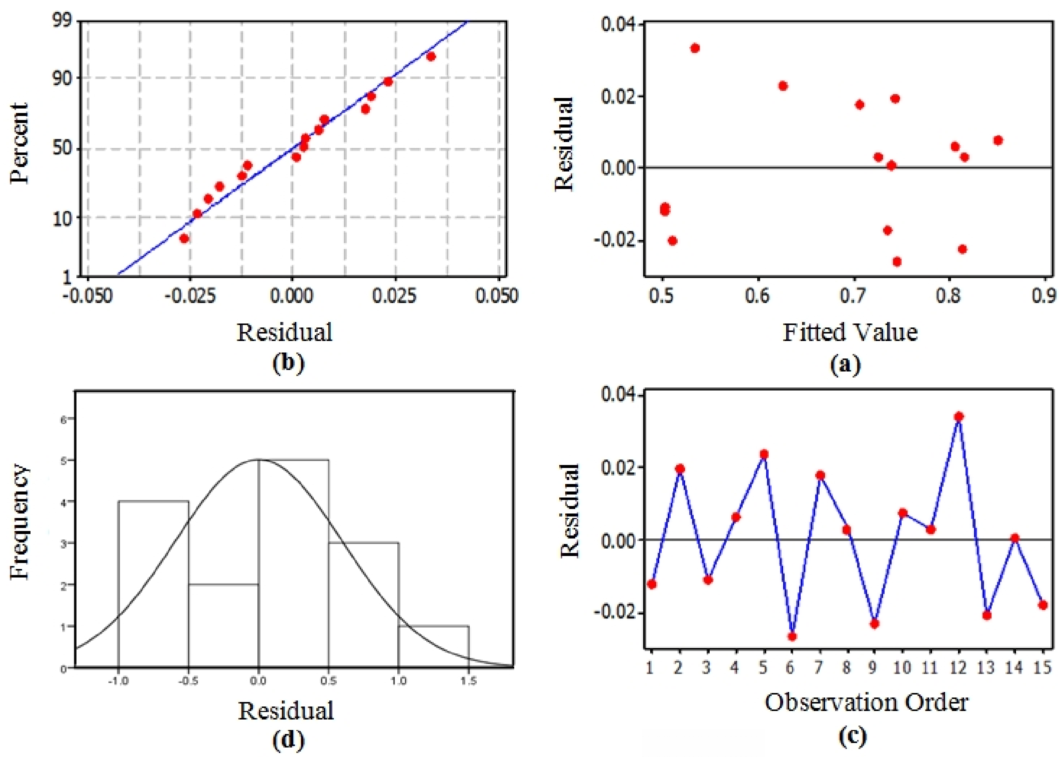

Fig. 1. Scatter plot (a), normal plot (b), variation plot (c) and normal distribution Histogram (d) of the standardized residuals (y – ŷ) for predicting model of yield stability in wheat

3.3 Stepwise Multiple Linear Regression

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 [17], because the results of t-test and VIF experienced a problem with all measured variables in the model.

Table 6 shows the data representing entered variables from stepwise regression analysis of YSI (dependent) and measured physiological traits (independent) under stress conditions. These entered variables were: ETE (R^2 = 73.5%), RWP (R^2 = 11.9%), CTD (R^2 = 6.9%) and RWC (R^2 = 3%) respectively. According to the results, 95.3% 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. The variables ETE, RWP, CTD and RWC are important characteristics that demonstrate crop water stress. These traits are considered as a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance [15,33].

Table 6. Relative contribution (partial and adjusted R^2) in predicting yield stability, standard error (S.E.) of estimates by the stepwise multiple linear regression analysis.

Step	Model	R	Partial R^2	Adjusted R^2	S.E. of the Estimate
1	ETE	0.858	0.735	0.715	0.06718
2	RWP	0.924	0.854	0.829	0.05201
3	CTD	0.961	0.923	0.902	0.03944
4	RWC	0.976	0.953	0.934	0.03225

The predicted equation for YSI was:

$$YSI = -0.948 + 0.648\ RWP + 0.0190\ CTD + 0.217\ ETE + 0.847\ RWC$$

These findings are in accordance with the results obtained by Siddique et al. [14], Dong et al. [17] and Hasheminasab et al. [1,39]. They suggested RWC, ETE and CTD as the best indicators for modeling of crop water stress.

3.4 Path Analysis

Since regression coefficients are affected by the values and unit of the raw data of variables, as a fourth step, standardized regression coefficient (path analysis) in order to determine most important variables on YSI was carried out among the physiological traits (Table 7). The results of path analysis showed that RWP, RWC, CTD, SR, ELWL and ETE had positive direct effect but LWC, RWL and LT had negative direct effect on YSI. The highest standardized regression coefficient or direct effect on YSI belonged to ETE (0.46*) but the lowest was determined for LT (0.016). Also the highest indirect effects on YSI were observed with RWP (0.806) and ELWL (– 0.753). RWP contributed positively towards YSI showed the highest total correlation (0.858). The results of path analysis were consistent with stepwise multiple linear regression analysis (Table 6) indicating RWP, RWC, CTD and ETE were the best physiological traits related to water status for modeling of YSI. Farshadfar and Hasheminasab [11] reported that genetic gain in developing tolerance in bread wheat could be achieved through indirect selection of physiological indicators related to water status, because the additive genes mainly controlled these traits.

Table 7. Path coefficient (direct and indirect effects) of the measured traits on yield stability in wheat genotypes under drought stress condition

Variable	Direct effects	Indirect effects	Total correlations
LWC	-0.085	-0.336	-0.421
RWP	0.052	0.806	0.858
RWL	-0.254	-0.568	-0.822
RWC	0.313	0.281	0.594
CTD	0.157	0.512	0.669
SR	0.224	0.419	0.643
LT	-0.016	0.484	0.468
ELWL	0.21	-0.753	-0.543
ETE	0.46*	0.358	0.818

**: Significant at 0.05 probability levels.*

4. CONCLUSION

The results of the present study showed that YSI had positive and significant relationship with RWP (r = 0.858**), RWC (r = 0.594*), CTD (r = 0.669**), SR (r = 0.643**) and ETE (r = 0.818**), and negative significant correlation with RWL (r = – 0.822**) and ELWL (r = – 0.543**) under drought stress condition. Multiple linear regression analysis indicated that the predicting model for YSI explained 97.9% of the total variation within the measured traits. The residual plots analysis showed no problem in the model with selected variables. On the other hand, t-test showed that some of the variables are not important to be present in this model. Path analysis revealed that ETE (0.46*) and RWP (0.806) had the highest direct and indirect effects on YSI, respectively. Path and stepwise analysis selected ETE (R^2 = 73.5%), RWP (R^2 = 11.9%), CTD (R^2 = 6.9%) and RWC (R^2 = 3%) as the major contributing indicators for modeling YSI under stress condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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