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Modeling the relationships between yield stability and its related physio-biochemical traits under water deficit conditions in wheat

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Abstract. Twenty wheat cultivars with a wide range of drought tolerance, including 18 bread wheat (*Triticum aestivum* L.) and two durum wheat (*T. turgidum* L.) were used in two separated field experiments during 2009-2010 growing season. Each experiment was conducted in a randomized complete block design with three replicates. The moisture level in one of the experiments was full field capacity, whereas it was 45% field capacity after anthesis in the second experiment. Data for several physiological traits related to water retention capacity (relative water content, RWC; relative water protection, RWP; relative water loss, RWL; canopy temperature depression, CTD and transpiration rate, *E*) and photosynthetic apparatus (photosynthetic rate, P_n; intercellular CO_2 concentration, C_i; stomatal conductance, g_s; chlorophyll *a*, Chll*a*; chlorophyll *b*, Chll*b* and carotenoids, Car), and biochemical traits (free proline and membrane stability index, MSI) were subjected to an appropriate model for yield stability in wheat. The results of different model selection techniques showed that some of the variables did not fit well in the model, so were not suitable for it. In this study, based on the forward, backward and stepwise selection methods, RWP, RWC, P_n, CTD, MSI, Chll*a* and Chll*b* were found suitable indicators for modeling yield stability under stress conditions. Path analysis revealed that MSI (-1.20134), CTD (1.021) and E (0.652) had the highest direct effects on yield stability index (YSI) and RWP showed the highest correlation (0.678). In general, MSI, RWP, CTD and Pn were found the best traits for indirect selection of wheat cultivars with higher gain yield and more stable.

Key words: modeling, wheat, yield stability, physio-biochemical traits.

Introduction

The ability of Cultivars to produce maximum yield under a variety of environmental conditions is very vital for the farmers (Kilic & Yagbasanlar 2010). It is now well established that the response of a variety to water deficit stress depends on a number of factors such as developmental stage, which water stress is imposed, severity and duration of the stress, and cultivar's genetic make-up (Aliakbari et al. 2013, Saed-Moucheshi et al. 2013). Long ago, Finlay (1968) has written one of the strong views that indicated the stability over varying environmental conditions and potential for high yield do not directly relate to each other. Later on, Blum (2009) advocated that developed a variety through breeding for good performance under water deficit regimes should produce a reasonable yield under optimal growth conditions, on the other hand, a highly stable variety with enhanced yield potential is always desired by the farmers (Finlay & Wilkinson 1963, Smith 1982). The combination of considerable yield stability and high yield potential under water deficit conditions is considered as a prospective indicator for assessing performance of a variety under water deficit conditions (Pinter Jr et al. 1990).

Although several physio-biochemical traits have been employed as effective drought tolerance markers (Blum 1988, Hasheminasab 2014a); however, some traits such as relative leaf water content, stomatal conductance, canopy temperature depression, day length, osmotic adjustment with organic compounds, leaves stay-green, etc. have been given little emphasis during crops breeding for enhanced drought tolerance. Thus, appraisal of an ideal model for assessing yield stability under water deficit conditions using the earlier-mentioned attributes could be useful for achieving high yield stability and enhanced yield potential of wheat under water limited environments. Thus, the present study was conducted to appraise an ideal model for predicting yield stability using some key physio-biochemical traits and detecting relationship of these traits with yield stability of some selected wheat Cultivars under water limited regimes.

Materials and Methods

In order to develop a model for yield stability of wheat under water deficit conditions, 18 bread wheat (*Triticum aestivum* L.) Cultivars including six drought tolerant (Azar2, Pishtaz, Toos, Chamran, Kavir and Koohdasht), six moderately tolerant (Roshan, Alvand, Tabasi, Niknejad, Cross Adl and Darab2) and six drought sensitive (Shiraz, Shiroudi, Flat, Bahar, Zarin and Alamut), as well as two durum wheat (*T. turgidum* L.) Cultivars, Simareh and Yavarus, were used in two separated field experiments during 2009-2010 growing season at the Experimental Station of College of Agriculture, Shiraz University (52° 46' E, 29° 50' N, altitude 1,810 m above sea level). Each experiment was set-up in a field following the randomized complete block design with three replications.

There were six rows 4 m long and spaced 30 cm apart in each plot. The four middle rows were used for determining grain yield, and the two on outer side used for appraising physio-biochemical parameters. Experimental soil was a sandy-clay soil with EC = 0.563 dS m^{-1} and pH = 7.6. The moisture level in one experiment was maintained at 100% field capacity throughout the growing season; while in the other it was kept 45% field capacity after anthesis stage. The climatic conditions during the growing period and amount of water applied are presented in Table 1. For measurement of physio-biochemical attributes, flag leaves of all wheat cultivars at the anthesis stage were harvested and weighed.

<u>Relative water content (RWC)</u>
RWC was measured using the method of Barrs & Kozlowski (1968). A sample of 10 flag leaves was taken randomly from different plants of the same variety and their fresh weight (F_W) measured. The leaf samples were placed in distilled water for 8 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) was measured. However, RWC was calculated using the following formula:

Table 1. Mean temperature, precipitation distribution and total irrigation for each experiment.

Month	Year	Mean temperature (°C)	Rainfall (mm)	Irrigation (mm)	
				Non- stressed	Stressed
November	2009	10.62	10.5	131	131
December	2009	5.66	129	-	-
January	2009	5.1	17	-	-
February	2010	6.13	54.5	-	-
March	2010	10.4	37.5	43	19.35
April	2010	12.23	24.5	70.42	31.69
May	2010	17.04	13	113.1	50.89
June	2010	22.58	0	60.4	27.18
Total			286	417.92	260.11
Total water used				703.92	546.11

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

Relative water protection (RWP)
RWP was determined according to Hasheminasab et al. (2012a). 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 (Hasheminasab et al. 2012a):

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

Relative water loss (RWL)
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). RWL was worked out using the formula devised by Yang et al. (1991).

$RWL = ((W_1 - W_2) + (W_2 - W_4) + (W_4 - W_6)) / ((3 \times W_D (T_1 - T_2))$

Canopy temperature depression (CTD)
These measurements were made using a hand-held infrared thermometer (KaneMay Model Infratrace 800, USA). Four measurements were taken per each plot at approximately 0.5 m from the edge of the plot and approximately 0.5 m above the canopy with an approximately 30-60° from the horizontal position. Two to seven days after irrigation in each experiment, canopy temperatures (CT) were measured between 12:00 to 14:00 o`clock on cloudless, bright days. Ambient temperatures (AT) were measured with a common thermometer held at plant height. CTD was worked out according to Dong et al. (2011):

CTD = AT – CT

Gas exchange parameters
Gas exchange parameters such as photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured with a portable photosynthesis system (L.MAN-LCI Company, USA). 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.

Pigment contents
Pigment contents were determined according to Lichtenthaler & Buschmann (2001). Leaf tissues (0.5 g) were homogenized in 80% acetone solution and then the extract was centrifuged at 3,000×*g* for 10 min and absorbance of the supernatant read at 646.8 nm and 663.2 nm for chlorophyll determination. Pigment contents were calculated using the following formulae:

Chlorophyll $a = (12.25\ A_{663.2} - 2.79\ A_{646.8})$

Chlorophyll $b = (21.21\ A_{646.8} - 5.1\ A_{663.2})$

Carotenoids $= (1000\ A_{470} - 1.8\ \text{Chl}a - 85.02\text{Chl}\ b)/198$

Free proline content
Free proline from the flag leaves of all wheat Cultivars was extracted and determined following Bates et al. (1973). Leaf tissues (0.5 g) were homogenized in 3% sulfosalicylic acid and the homogenate centrifuged at 3,000×*g* for 10 min. The supernatant was treated appropriately with acetic acid and ninhydrin, boiled for 1 h, and the absorbance read at 520 nm. Proline content was worked out using a standard curve and expressed as μmol g^{-1} fresh mass.

Membrane stability index (MSI)
MSI was estimated according to Sairam (1994). Two sets of leaf tissues (0.1 g) were placed in 10 mL of double-distilled water. One set was kept at 40˚C for 30 min and its electrical conductivity recorded using a conductivity meter (C_1), while the second set was kept in a boiling water bath (100˚C) for 10 min and its conductivity recorded (C_2). The membrane stability index and electrolyte leakage were calculated as follows:

$MSI = (1 - (C_1/C_2)) \times 100$

Grain yield (GY) and yield stability index (YSI)
GY was recorded at the maturity stage. YSI was calculated according to Bouslama & Schapaugh (1984) using the following formula:

$$YSI = \frac{Ys}{Yp}$$

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

Statistical analysis
All statistical analysis comprising multiple, backward, forward and stepwise regression, variance inflation, tolerance, path analysis as well as all diagrams were calculated and drawn using the statistical analysis system (SAS) version 9.3.

Results

Some of the descriptive statistics consisting of means, standard deviations, summation, and also minimum and maximum values for the measured variables used to modeling the yield stability are presented in Table 2. Taking YSI as dependent and other measured physio-biochemical traits as independent variables, a multiple linear regression analysis determined the following prediction equation:

YSI = 106.8728 – 105.7364 MSI - 0.00092 Proline + 0.0078 Car - 0.0257 Chll*b* - 0.0248 Chll*a* - 0.341 RWC + 0.577 RWP - 1.23 RWL + 0.0534 CTD - 1.08 g_s + 0.0592 E + 0.0282 P_n - 0.000976 C_i

The formula can explain 88.1% (R-square = 0.881) of the total variation within the YSI related to physio-biochemical traits.

When all measured variables were presented in the prediction model by multiple regression, analysis of variance (ANOVA) showed that the model was significant (data is not shown). On the other hand, t-test, which calculated for all variables separately, showed that some of the variables were not important to be presented in modeling of YSI (Table 3). Among the variables, Chll*b*, Chll*a*, RWP, CTD, *E*, and *Pn* significantly were contributed to the model at the 5% of probability, while MSI and *C*i were significant at the 10% probability, but the other variables were not significant.

Variance inflation factor and tolerance index showed that there is some co-linearity among variables and the coefficients determined by this model probably are not the best values. Variance inflation for MSI, RWC, CTD, g_s and E were higher than 10 thereby confronting a problem with coefficients for these variables to modeling yield stability.

Variable's selection and introducing best model for yield stability

Since statistical indices such as variance inflation and tolerance experienced a problem with existing all measured variables in the model, so that different statistical and selection methods were used to explain relationship between varia-

Table 2. Descriptive statistics of the variables used in the study.

Variable	*n*	Mean	StdDev	Sum	Minimum	Maximum
MSI	40	0.67448	0.15071	26.979	0.454	0.863
Proline	40	11.285	7.87933	451.4	2.21	29.38
Car	40	5.76925	1.04499	230.77	3.33	7.47
Chl*b*	40	5.154	0.95976	206.16	3.14	7.82
Chl*a*	40	13.035	2.15027	521.4	8.67	17.21
RWC	40	0.60588	0.08254	24.235	0.406	0.778
RWP	40	0.73108	0.0679	29.243	0.539	0.841
RWL	40	0.0935	0.01912	3.74	0.061	0.128
CTD	40	4.32123	2.40256	172.849	1.167	9.083
g_s	40	0.07637	0.04469	3.0546	0.0207	0.19
E	40	3.8615	1.25817	154.46	1.88	7.17
P_n	40	4.9345	1.35956	197.38	2.81	8.64
C_i	40	216.0255	27.47979	8641	153	275.67
YSI	40	0.71057	0.12115	28.42281	0.48681	0.88055

MSI = membrane stability index; Proline= Proline content; Car = Carotenoid content; Chll*b* = Chlorophyll *b* content; Chll*a* = Chlorophyll *a* content; RWC = relative water content; RWP = Relative water protected; RWL = Relative water loss; CTD = Canopy temperature depression; g_s = stomatal conductance; E = transpiration rate; P_n = photosynthetic rate; C_i = intercellular CO_2 concentration; and YSI = Yield stability index.
n = Number of used data, and StdDev = Standard deviation.

Table 3. Multiple regression coefficients and their standard errors for modeling yield stability.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr> |t|	Tolerance	Variance Inflation
Intercept	1	106.8728	61.23803	1.75	0.0937	-	0
MSI	1	-105.7364	61.17112	-1.73	0.0567	8.54E-07	27.1376
Proline	1	-0.000918	0.00222	-0.41	0.6835	0.23624	4.23307
Car	1	0.0078	0.01417	0.55	0.5871	0.33103	3.02083
Chl*b*	1	-0.02574	0.01135	-2.27	0.0325	0.61185	1.63438
Chl*a*	1	-0.02476	0.00888	-2.79	0.0102	0.19909	5.02291
RWC	1	-0.34113	0.39583	-0.86	0.3973	0.06797	14.71241
RWP	1	0.57668	0.22605	2.55	0.0175	0.30793	3.24744
RWL	1	-1.22718	1.02904	-1.19	0.2447	0.1875	5.33335
CTD	1	0.05343	0.0133	4.02	0.0005	0.07106	14.07202
g_s	1	-1.08376	0.71485	-1.52	0.1426	0.07109	14.06729
E	1	0.05922	0.02479	2.39	0.0251	0.07458	13.40869
P_n	1	0.02815	0.0135	2.08	0.0479	0.21525	4.64578
Ci	1	-0.000976	0.0005399	-1.81	0.0833	0.32967	3.03336
R^2 = 0.8814; Adjusted R^2 = 0.8072;							

DF = Degree of freedom; MSI = Membrane stability index; Proline = Proline content; Car = Carotenoid content; Chll*b* = Chlorophyll *b* content; Chll*a* = Chlorophyll *a* content; RWC = Relative water content; RWP = Relative water protected; RWL = Relative water loose; CTD =Canopy temperature depression; g_s= Stomatal conductance; E =Transpiration rate; P_n = Photosynthetic rate and Ci = Intercellular CO2 concentration.

bles and yield stability of wheat Cultivars under water deficit conditions and to select the important variables to be presented in the prediction model. Table 4 shows some of the best models determining by the Cp method selection, R-square, error sum of squares and Akaike information criteria (AIC) coefficient. Chll*b*, Chll*a*, RWP, CTD, g_s, *E* and P_n were included in all models, but free proline and Car were not included in any of the models. Tables 5 shows the data representing the variables used in stepwise regression analysis related to YSI (as dependent variable) and measured traits (as independent variables). Both RWP (45.97%) and RWC (15.68%) have been selected for modeling YSI by this method. Thus, based on stepwise regression analysis, these traits can be considered the best indicators for appraising drought tolerance in wheat cultivars (Table 5). The predicted equation for YSI using the stepwise regression formula is:

YSI = - 0.586 + 1.29 RWP + 0.585 RWC

From the results of forward selection analysis, the variables selected were RWP, RWC, P_n, CTD, MSI, Chll*a*, Chll*b*, *E*, g_s, C_i and RWL, respectively in the selection steps (Table 6). The F-test suggested that RWP, RWC, CTD, Chll*a*, MSI and *E* had contributed significantly towards yield stability, while the other variables did not do so. Thus, the predicted equation due to forward selection for YSI formula is:

YSI = 0.533 + 1.02 RWP - 0.475 RWC + 0.0512 CTD - 0.0206 Chll*a* - 1.16 LE + 0.0383 *E*

Backward elimination removed variables proline, Car, RWC, RWL, C_i and MSI in different steps, respectively, and these are the variables that are not important to be included in the model by this technique (Table 7). The equation suggested by the backward technique is:

YSI = 0.368 + 0.699 RWP - 0.892 MSI - 0.0158 Chl*a* - 0.0289 Chl *b* + 0.0499 CTD - 1.51 g_s + 0.0669 *E* + 0.0238 P_n

The formula explains 84.5% of the total variation within

Table 4. Some of the results of selection methods for determining best model for yield stability.

n	C(p)	R^2	AIC	SSE	Variables in Model
10	8.5187	0.8689	-229.144	0.07504	MSI; Chl*b*; Chl*a*; RWP; CTD; g_s; *E*; P_n; C_i
9	8.6469	0.8584	-228.056	0.08106	MSI; Chll*b*; Chll*a*; RWP; CTD; g_s; *E*; P_n
11	9.1638	0.8756	-229.242	0.07121	MSI; Chll*b*; Chll*a*; RWP; rwl; CTD; g_s; *E*; Pn
8	9.2919	0.8453	-226.524	0.08855	MSIChll*b*; Chll*a*; RWP; CTD; g_s; *E*; P_n
8	9.3408	0.8451	-226.461	0.08868	MSI; Chll*b*; Chll*a*; RWP; CTD; g_s; *E*; P_n
9	9.8428	0.8525	-226.420	0.08445	MSI; Chll*b*; Chll*a*; RWP; CTD; g_s; *E*; P_n; Ci
11	9.8441	0.8723	-228.175	0.07313	Chll*b*; Chll*a*; RWC; RWP; CTD; g_s; *E*;P_n
9	9.9015	0.8522	-226.342	0.08461	MSI; Chll*b*; Chll*a*; RWP; CTD; g_s; *E*; P_n; Ci

MSI = Membrane stability index; Proline = Proline content; Car = Carotenoid content; Chllb = Chlorophyll b content; Chlla = Chlorophyll a content; RWC = Relative water content; RWP = Relative water protected; RWL = Relative water loose; CTD = Canopy temperature depression; g_s = Stomatal conductance; E = Transpiration rate; P_n = Photosynthetic rate and Ci = Intercellular CO2 concentration.

Cp = Method selection, SSE = Error sum of squares and AIC = Akaike information criteria coefficient.

Table 5. Summary of Stepwise Selection.

Step	Variable Entered	Variable Removed	Variables in model	Partial R^2	Model R^2	C(p)	F Value
1	RWP	-	1	0.4597	0.4597	73.3074	32.33
2	RWC	-	2	0.1568	0.6165	43.5905	15.12

RWC = Relative water content and RWP = Relative water protected.

Table 6. Summary of Forward Selection.

Step	Variable Entered	Number Variables Entered	Partial R^2	Model R^2	C(p)	F Value	Pr> F
1	RWP	1	0.4597	0.4597	73.3074	32.33	<.0001
2	RWC	2	0.1568	0.6165	43.5905	15.12	0.0004
3	P_n	3	0.0208	0.6373	41.3754	2.07	0.159
4	CTD	4	0.0431	0.6804	34.6557	4.72	0.0367
5	MSI	5	0.0596	0.7400	24.5907	7.8	0.0085
6	Chll*a*	6	0.0455	0.7856	17.3778	7.01	0.0123
7	Chll*b*	7	0.0198	0.8054	15.3684	3.26	0.0804
8	*E*	8	0.0234	0.8288	12.6354	4.24	0.0481
9	g_s	9	0.0205	0.8493	10.491	4.08	0.0525
11	Ci	11	0.0107	0.8723	9.8441	2.35	0.1362
12	RWL	12	0.0066	0.8788	10.513	1.47	0.2365

MSI = Membrane stability index; Chllb = Chlorophyll b content; Chlla = Chlorophyll a content; RWC = Relative water content; RWP = Relative water protected; RWL = Relative water loose; CTD = Canopy temperature depression; g_s = Stomatal conductance; E = Transpiration rate; P_n = Photosynthetic rate and Ci = Intercellular CO2 concentration.

Table 7. Summary of Backward Elimination.

Step	Variable Removed	Variables remained	Partial R^2	Model R^2	C(p)	F Value	Pr> F
1	g_s	14	0.0001	0.8812	14.0278	0.03	0.869
2	proline	13	0.0007	0.8805	12.1705	0.15	0.7032
3	Car	12	0.0017	0.8788	10.513	0.37	0.5491
4	RWC	11	0.0032	0.8756	9.1638	0.72	0.4047
5	RWL	10	0.0067	0.8689	8.5187	1.51	0.2297
6	Ci	9	0.0105	0.8584	8.6469	2.33	0.138
7	MSI	8	0.0131	0.8453	9.2919	2.77	0.1065

MSI = Membrane stability index; Proline = Proline content; Car = Carotenoid content; RWC = Relative water content; RWL = Relative water loose; g_s = Stomatal conductance and Ci = Intercellular CO2 concentration.

Table 8. Multiple regression for all selected variables.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr> |t|	Tolerance	Variance Inflation
Intercept	1	0.50563	0.22957	2.2	0.0355	.	0
MSI	1	-0.96596	0.16836	-5.74	<.0001	0.11216	8.91575
Chll*b*	1	-0.0292	0.01095	-2.67	0.0122	0.65379	1.52954
Chll*a*	1	-0.0157	0.00589	-2.67	0.0122	0.4507	2.21876
RWP	1	0.68479	0.19306	3.55	0.0013	0.41997	2.38113
CTD	1	0.05149	0.01015	5.07	<.0001	0.12136	8.24006
g_s	1	-1.43033	0.61551	-2.32	0.0271	0.09538	10.48415
E	1	0.06278	0.0213	2.95	0.0061	0.10049	9.95092
P_n	1	0.02495	0.0122	2.05	0.0496	0.26247	3.8099
Ci	1	-0.000485	0.0004023	-1.21	0.2369	0.59055	1.69333
R^2 =0.8525; Adjusted R^2 =0.8082							

MSI = Membrane stability index; Chllb = Chlorophyll b content; Chlla = Chlorophyll a content; RWP = Relative water protected; CTD = Canopy temperature depression; g_s = Stomatal conductance; E = Transpiration rate; P_n = Photosynthetic rate and Ci = Intercellular CO2 concentration.

Table 9. Some descriptive statistics and normality test for residuals.

Some of statistics value and test of normality for residuals			
Statistics	Statistical Value	Statistics	Statistical Value
Number	40	Sum Weights	40
Mean	0	Sum Observations	0
Standard Deviation	0.0465326	Variance	0.0021653
Skewness	0.0760881	Kurtosis	-0.539887
Uncorrected Sum of Squares	0.0844459	Corrected SS	0.0844459
Range	0.1903	Std Error Mean	0.0073575
Mean	0	Std Deviation	0.04653
Median	-0.00515	Variance	0.00217
Methods for testing normality of the residuals			
Method	Statistics	Statistics Value	p
Shapiro-Wilk	W	0.98529	0.8731
Kolmogorov-Smirnov	D	0.070741	0.15
Cramer-von Mises	W-Sq	0.027888	0.25
Anderson-Darling	A-Sq	0.173735	0.25

YSI describing by not-removed variables.

Table 8 shows the finally selected variables for modeling yield stability determined by different selection methods. These include MSI, Chll*b*, Chll*a*, RWP, CTD, g_s, E, P_n and C_i. These variables also were tested for significance using multiple regression. The only variable non-significant in this model is C_i. Also, in order to better testing of the model and detecting any other problems with the model (if it exists), normality test on the residuals was conducted and the results showed that residuals were absolutely normal (Table 9). Also, Fig. 1 shows no problem with the residuals of the model with selected variables because the residuals are dispersed almost uniformly around the zero line. The observed values, predicted values, standard errors for predicted values, confidence levels for observed and predicted values and residuals for all raw data are presented in Table 10. These results show goodness of the model for predicting yield stability using selected variables.

Path coefficient analysis

Since regression coefficients were affected significantly by the values and units of the raw data of variables, standard-

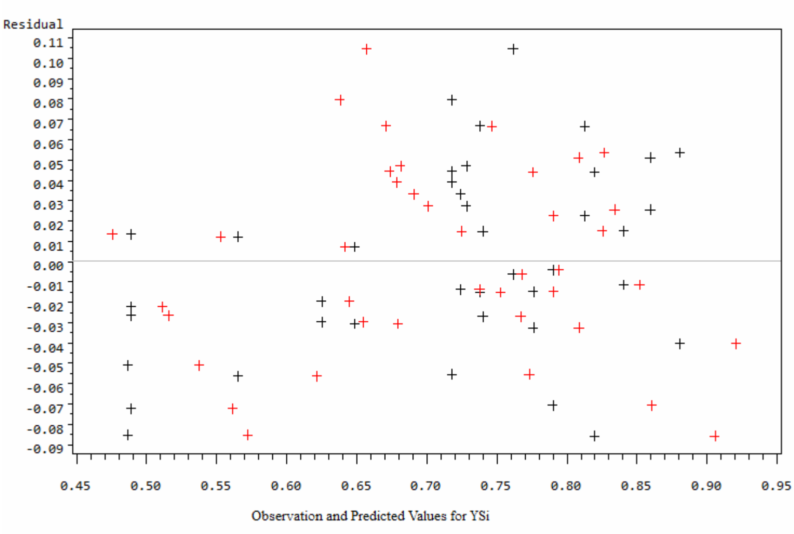

Figure 1. Residuals vs. predicted and observed values of yield stability.

Table 10. Raw data, their predicted values, their confidence levels, and residuals for modeling yield stability.

Observation	Dependent Variable	Predicted Value	Standard Error of Mean Predict	95% CL Mean		95% CL Predict		Residual
1	0.4891	0.4755	0.0327	0.4087	0.5422	0.3482	0.6027	0.0137
2	0.7616	0.6571	0.0355	0.5845	0.7296	0.5267	0.7874	0.1046
3	0.6250	0.6547	0.0187	0.6165	0.6929	0.5398	0.7696	-0.0297
4	0.4891	0.5613	0.0370	0.4857	0.6370	0.4292	0.6935	-0.0722
5	0.8129	0.7463	0.0333	0.6782	0.8144	0.6184	0.8743	0.0666
6	0.7378	0.7528	0.0268	0.6981	0.8075	0.6314	0.8741	-0.015
7	0.7762	0.8087	0.0296	0.7483	0.8690	0.6847	0.9327	-0.0325
8	0.6488	0.6415	0.0227	0.5950	0.6879	0.5236	0.7594	0.00734
9	0.8805	0.8268	0.0246	0.7766	0.8771	0.7074	0.9463	0.0537
10	0.7179	0.6736	0.0273	0.6179	0.7293	0.5518	0.7954	0.0443
11	0.7239	0.6906	0.0183	0.6533	0.7280	0.5760	0.8052	0.0333
12	0.8200	0.9057	0.0248	0.8551	0.9564	0.7861	1.0253	-0.0857
13	0.7900	0.8606	0.0337	0.7917	0.9294	0.7322	0.9890	-0.0706
14	0.8596	0.8084	0.0261	0.7552	0.8617	0.6877	0.9292	0.0511
15	0.7283	0.7009	0.0255	0.6488	0.7529	0.5807	0.8211	0.0274
16	0.5654	0.6217	0.0276	0.5654	0.6781	0.4996	0.7439	-0.0563
17	0.4868	0.5721	0.0265	0.5180	0.6262	0.4510	0.6932	-0.0853
18	0.8406	0.8518	0.0401	0.7699	0.9338	0.7160	0.9877	-0.0113
19	0.7401	0.7670	0.0190	0.7282	0.8058	0.6519	0.8820	-0.0269
20	0.7178	0.6381	0.0214	0.5945	0.6817	0.5213	0.7549	0.0797
21	0.4891	0.5111	0.0240	0.4622	0.5601	0.3922	0.6300	-0.022
22	0.7616	0.7678	0.0267	0.7131	0.8224	0.6464	0.8891	-0.0061
23	0.6250	0.6446	0.0160	0.6119	0.6773	0.5314	0.7578	-0.0196
24	0.4891	0.5156	0.0227	0.4693	0.5620	0.3978	0.6335	-0.0265
25	0.8129	0.7902	0.0290	0.7310	0.8495	0.6667	0.9137	0.0227
26	0.7378	0.6710	0.0253	0.6193	0.7228	0.5509	0.7911	0.0668
27	0.7762	0.7907	0.0269	0.7357	0.8457	0.6691	0.9122	-0.0145
28	0.6488	0.6792	0.0196	0.6392	0.7192	0.5637	0.7947	-0.0304
29	0.8805	0.9209	0.0270	0.8658	0.9761	0.7994	1.0425	-0.0404
30	0.7179	0.6788	0.0217	0.6345	0.7231	0.5617	0.7959	0.0391
31	0.7239	0.7376	0.0179	0.7011	0.7742	0.6233	0.8520	-0.0137
32	0.8200	0.7759	0.0188	0.7374	0.8144	0.6609	0.8909	0.0441
33	0.7900	0.7942	0.0240	0.7451	0.8433	0.6752	0.9131	-0.0041
34	0.8596	0.8340	0.0289	0.7751	0.8930	0.7107	0.9574	0.0255
35	0.7283	0.6813	0.0247	0.6307	0.7318	0.5617	0.8008	0.0470
36	0.5654	0.5532	0.0299	0.4921	0.6143	0.4288	0.6776	0.0122
37	0.4868	0.5378	0.0274	0.4818	0.5937	0.4158	0.6597	-0.051
38	0.8406	0.8255	0.0282	0.7680	0.8830	0.7028	0.9481	0.0151
39	0.7401	0.7251	0.0282	0.6675	0.7827	0.6024	0.8478	0.0150
40	0.7178	0.7731	0.0210	0.7303	0.8160	0.6566	0.8897	-0.0554

ized regression coefficient (path analysis) to determine the most important variables on yield stability were carried out among the selected variables (Table 11). The results of the path analysis showed that MSI, Chll*b*, Chll*a*, g_s and C_i had a negative direct effect, but RWP, CTD, *E* and P_n had a positive direct effect. Highest standardized regression coefficient or direct effect on YSI had been found due to MSI (-1.20) and CTD (1.02), followed by *E* (0.652), but the lowest were determined for C_i (-0.11). Also the highest indirect effects on YSI were observed with MSI (1.06), CTD (-0.8) and *E* (-0.71). RWP contributed positively towards YSI.

Table 11. Standardized estimate or path analysis for modeling the variables.

Variable	Direct effect	Indirect effect	Correlation with YSI
MSI	-1.20134	1.05648	-0.14486
Chll*b*	-0.23134	0.04332	-0.18802
Chll*a*	-0.27863	0.03495	-0.24368
RWP	0.38381	0.29419	0.678
CTD	1.02100	-0.79962	0.22138
g_s	-0.52762	0.37041	-0.15721
E	0.65200	-0.7123	-0.0603
P_n	0.28001	-0.11795	0.16206
Ci	-0.11013	0.18448	0.07435

MSI = Membrane stability index; Chllb = Chlorophyll b content; Chlla = Chlorophyll a content; RWP = Relative water protected; CTD =Canopy temperature depression; g_s =Stomatal conductance; E =Transpiration rate; P_n = Photosynthetic rate and Ci = Intercellular CO2 concentration.

Discussion

Based on the results of variance inflation factor and tolerance index, all measured variables cannot be concluded in the modeling yield stability and fewer variables can explain its variability. Using Cp statistic, R-square, error sum of squares and Akaike information criteria (AIC) coefficient revealed that Chll*b*, Chll*a*, RWP, CTD, g_s, *E* and P_n are the most important variables which were included in all models determined by mentioned selection methods, but proline and Car were not included in any of the models.For better understanding the relationships and introducing best model for yield stability, stepwise and forward selection and also backward elimination were used. Also these multiple linear regressions proved to be very effective in determining the predictive equation for yield stability under drought stress conditions (Dong et al. 2011). In stepwise selection, according to the results, 61.65% of the total variation in YSI could be attributed to RWP and RWC. The other variables were not included in the stepwise model due to their low relative contributions showing high effect of these factors on YSI. Dong et al. (2011), Geravandi et al. (2011) and Hasheminasab (2014b) obtained similar results from the stepwise multiple linear regression analysis of some physio-biochemical indicators for drought tolerance in wheat. The predicted model in forward selection explained 78.2% of the total variation within YSI where RW, RWC, P_n, CTD, MSI, Chll*a*, Chll*b*, *E*, g_s, *Ci* and RWL were selected as most important variables. With the regards to the backward elimination, g_s, proline, Car, RWC, RWL, *Ci* and MSI were removed from the model, while other variables could be stayed in the model where total R-square for determination of variability explaining of the model were 0.84%. Overall results of all used model selection techniques results in a final model with MSI, Chll*b*, Chll*a*, RWP, CTD, g_s, *E*, P_n and C_i as most important independent variables contributing YSI. Variance inflation factor and tolerance index in this model did not experience any problem with these variables. The adjusted R-squares (0.81) showed that this model is a fitted model for yield stability.

Cp statistic, R-square, error sum of squares and AIC coefficient revealed MSI, CTD, *E* and RWP as most important variables for yield stability. This result is in agreement with that of Zhao et al. (2006) in wheat and Razi & Assad (1999) in sunflower. The variables RWP, CTD and MSI are important characteristics that demonstrate plant water retention capacity. 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 (Araghi & Assad 1998, Anjum et al. 2011, Hasheminasab et al. 2012b and 2014b). Chaves (1991) and Cornic (2000) stated that drought-related reduction in yield and yield components of plants could be attributed to stomatal closure under low soil water content, which decreases the intake of CO_2 thereby leading to reduced photosynthesis. Previous studies have demonstrated that drought-resistant wheat genotype acclimated better than susceptible genotype by maintaining higher water relations, and low membrane injury, pigment photo-oxidation and chlorophyll degradation by up-regulating antioxidant defense system, which results in increased photosynthesis and yield stability under stressful environments. Since MSI, Chll*b*, Chll*a*, RWP, CTD, g_s, *E*, P_n and C_i are variables consisted in the final model,our findings are in line with those reported by Hui et al. (2008) and Dong et al. (2011) who found that C_i, P_n, *E*, RWL and g_s were associated significantly with leaf water use efficiency in wheat.

Proline and Car are the major non-enzymatic antioxidants that have a role in defense against water stress by scavenging singlet oxygen and suppressing lipid peroxidation in photosynthetic organisms (Anjum et al. 2011). However, several earlier published reports show that proline and Car are not reliable indicators in screening wheat genotypes for drought tolerance (Geravandi et al. 2011). Also, our results showed that these two variables are important variables contributing YSI variability.

Since regression coefficients were affected significantly by the values and units of the raw data of variables, standardized regression coefficient (path analysis) to determine the most important variables on YSI were carried out among the all selected variables. The highest negative direct effects were belonging to MSI and g_s, while the highest positive effect was obtained for RWP. These results confirmed obtained results of selection techniques showing that selected variables can be used for predicting YSI.

Conclusion

The results obtained from several statistical procedures used in the study showed that some of the variables were not important to be used in the model. Forward, backward and stepwise multiple linear regression analyses selected RWP, RWC, P_n, CTD, MSI, Chll*a* and Chll*b* as the suitable indicators for modeling yield stability under stress conditions. Path analysis revealed that MSI (-1.20), CTD (1.02) and *E* (0.65) had high direct effect on yield stability index (YSI), while RWP had the highest total correlation (0.678). Also the highest indirect effects on YSI were observed with MSI (1.06), CTD (-0.79962) and *E* (-0.7123). Overall, it can be suggested that MSI, RWP, CTD and P_n are the best traits for indirect selection for revealing new wheat genotypes with high yield and resistance against drought stress.

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