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A regression model to depict the influence of physiological and agronomic traits on yield of wheat cultivars under water stress in Tigray, Ethiopia

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ABSTRACT: Although developing drought tolerance is the major objective of plant breeders, it is hampered by the lack of effective selection criteria. Most plant breeders use the single trait approach in identifying the crop response to drought condition, and little priority is given to the physiological traits. Thus, all the possible traits have not been critically evaluated, and correlation among the different traits and their relation to drought has not yet been critically examined. With those facts field and greenhouse, experiments were conducted at Mekelle University to determine the relationship among the different physiological, agronomic, yield and yield component of wheat genotype under water stress. The experiment was laid out in Randomized Complete Block Design using six wheat genotypes and three water regimes with three replications. Pearson's correlation coefficient at 5% indicated that yield was positively and significantly correlated with relative water content, excised leaf water retention, spike length, number of seed per spike, and seed weight in contrast stress susceptibility index and rate of water loss were negatively associated with yield. Regression analysis also showed the rate of water loss, excised leaf water retention, and relative water content explain more of the variation (90%) in grain yield under different water stress regimes. Hence plant breeders should incorporate these physiological traits as a selection criterion in their breeding program for screening water stress on wheat cultivars.

Keywords: Drought tolerance index, genotypes, moisture stress, physiological trait.

INTRODUCTION

Shortage of water is the most important component of life which limits plant growth and crop productivity, particularly in arid regions more than any other single environmental factor (Boyer 1982). Since wheat is grown mostly under rain-fed conditions, where water availability is a limiting factor, it inevitably suffers from drought stress. Balla et al. (2008) reported that drought had a negative effect on physiological processes and agronomic traits of wheat. Studies on plants identification and selection of physiological traits associated with plant water use efficiency (WUE) and drought tolerance under water-limited conditions are important for well-understanding plant physiological characters and taking physiological water-saving measures (Dulai et al., 2006). At the International Maize and Wheat Improvement Center (CIMMYT), researchers have demonstrated associations of some physiological traits, including leaf conductance and photosynthetic rate, the performance of a historic series of cultivars in a high-yielding environment (Nelson et al., 2007). In addition, historical traits associated with drought tolerance have been applied into a number of Australian wheats breeding programmers, including higher transpiration efficiency, greater early vigor and reduced tillering (Reynolds et al., 2009).

Although developing drought tolerance is the major objective of plant breeders, it is hampered by the lack of effective selection criteria (yield other than all available potential markers). Most plant breeders use the single trait approach in identifying the crop response to drought condition, and little priority is given to the physiological traits. Thus, all the possible traits have not been critically evaluated, and correlation among the different traits and their relation to drought has not yet been critically examined. In view of the above facts, this study seeks to determine the nature of associations among physiological and agronomic performances contributing to grain yield and drought tolerance in wheat cultivars; to identify physiological traits that may grant simultaneously high yield potential and constitutive tolerance to water stress.

MATERIALS AND METHODS

Plant material

Six wheat varieties; Hawii, Dandea, Shina, Medawalabu, Mekelle 4 and Mekelle 3 were used in this study (Table 1). Seed for Hawii, Dandea, Shina and Medawalabu was obtained from Alamatta Agricultural Research Center. The seed for Mekelle 4 and Mekelle 3 was obtained from Mekelle Agricultural Research Center. The seeds were sown under irrigated conditions in pots on 25th February 2012 in greenhouse experiment. Second conducted in outdoor and sown on 6th March 2012 and relevant metrological parameters were obtained from the observatory for the field and recorded for the greenhouse.

Experimental design

The experiment was conducted in the field and greenhouse using pots to evaluate the physiological and agronomic response of the six wheat genotypes under three water stress regimes following the methodology of Zadoks et al. (1974). The stages include: (1) well-watered control treatments; (2) water stress at tillering (23 and 20 days after planting in the field and greenhouse experiments, respectively); and (3) stress at booting stage (45 and 40 days after planting in the field and greenhouse experiments, respectively). Pots were arranged in Randomized Complete Block Design (RCBD) in a factorial combination of the six wheat genotypes and three water regimes with three replications. There were total of 18 treatments combination. The combination of the three water stress regimes and the genotype were randomly assigned to the experimental pots in each block. In all water-stressed pots, water stress was maintained by withholding water for ten days at the selected growth stage. The seeds of each genotype were sown in pots of 50 cm deep and 34 cm wide in case of green house and 65 cm deep and 40 cm wide in the field. The pots are equipped with drainage holes, and ten seeds per pot were sown. One liter of water was added to each pot every other day except during the water stress period. All other agronomic management practices (weeding, application of fertilizer, seed rate and soil preparation) were uniformly applied to all experimental pots. All those cultural practices were carried out as recommended for wheat production in this area. The seed rate was 120 kg/ha; hence 1.09 and 1.5 g was used for the greenhouse and field pots respectively.

Data collection

Data was measured and recorded on the following physiological, agronomic, yield and yield components during the experimentation. Water related traits were estimated on the first fully expanded leaf (third from top) at vegetative stage and flag leaf at grain filling stage.

Leaf area (cm^2)

Leaf area represents the amount of leaf material in an ecosystem and is geometrically defined as the total one-sided area of photosynthetic tissue per unit ground surface area. Leaf area measurement is a reliable parameter in studying the impact of environment on plants in the disciplines of ecology, genetics, and crop management. Eco-physiologists, geneticist and botanists. Flag leaf was used to measure leaf area in cm^2 using the leaf area meter at the grain filling stage.

Relative water content (RWC %)

RWC was measured at stem elongation using the third leaf from the top and at grain filling stage using flag leaves after imposing drought conditions. Immediately after cutting at the base of lamina, leaves were sealed within plastic bags and quickly transferred to the laboratory. Fresh weight (FW) was determined within 1 hour after excision. Turgid weight (TW) was obtained after soaking leaves in distilled water in test tubes for 16 hours at room temperature (25ºC). After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determining turgid weight. Dry weight (DW) was obtained after oven drying the leaf samples for 72 hours at 70ºC. RWC was calculated from the formula by Slatyer (1967) as given below:

$$\mathrm{RWC}\ \% = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{dry weight}}\ x\ 100$$

Excised leaf water retention (ELWR %)

ELWR was measured at grain filling stage using the flag leaf. The flag leaves were collected and weighed and then kept at 30ºC for 5 hours and reweighed. ELWR was then calculated using the following formula:

$$\mathrm{ELWR} = \left(1 - \frac{\mathrm{W0} - \mathrm{W1}}{\mathrm{W0}}\right) x100$$

Where W0 = weight of fresh leaves and W1 = weight of leaves after 5 hours.

Table 1. Name of genotypes used for drought tolerance assessment.

S. No	Common name	Genotype code	Breeder	Year of release
1	Hawii	HAR 2501	SARC/OARI	1999/2000
2	Shina	HAR 1868	ADARC/ARARI	1998/99
3	Medawalabu	HAR1480	KARC/EIAR	1999/2000
4	Mekelle 3	M17SWASN79	MARC	2012
5	Mekelle 4	FRET1	MARC	2012
6	Dandea	Danphe	KARC	2010

Rate of water loss *(RWL %)*

RWL was computed in percentage using flag leaf at grain filling stage. The flag leaves were collected and weighed. The leaves were wilted at 30ºC and re-weighed three times at an interval of 2 hours (W2, W4, W6) and transferred to an oven for 24 hours and weighed (Wd). RWL was calculated from the formula by McCaig and Romagosa (1989) as given below:

$$\mathrm{RWL} = \frac{(\mathrm{W0} - \mathrm{W2})\ +\ (\mathrm{W2} - \mathrm{W4})\ +\ (\mathrm{W4} - \mathrm{W6})]}{(3 * \mathrm{Wd})\ (\mathrm{t2} - \mathrm{t1})}$$

Where (t2-t1) = time interval between two subsequent measurements, W0 = Fresh weight; W2, W4, W6 = weight after 2 hr, 4 hr and 6 hr respectively; and Wd = dry weight of the flag leaf.

Initial water content (IWC)

IWC was calculated at both stem elongation using the third leaf from the top and at grain filling stage using the flag leaf as:

$$\mathrm{IWC} = \frac{\mathrm{W0} - \mathrm{Wd}}{\mathrm{Wd}}$$

W0 = fresh weight and Wd= leaves placed in an oven at 50ºC for 24 hours and re-weighed.

Agronomic parameters

During the growth period, five randomly selected plants were used to measure plant height (from the ground level to the top of the spike excluding the awns), number of tiller per plant, days to 50% flowering, spike length, grain per spike, 1000 grain weight and grain yield per plant.

Data analysis

Normality of the data was tested using Shapiro-Wilk test for normality in Genstat version 18 and the data were normally distributed. Pearson's correlation coefficient was estimated to identify the relationship between different traits. The rule of thumb was used to interpret the size of correlation coefficient (± 0.9-1 very high correlation; ±0.7-0.9 high correlation; ±0.5-0.7 moderate correlation; ±0.3-0.5 low correlation and 0.00-0.3 negligible correlation). Stepwise regression (criteria: probability-of-F-to-enter =0.05, probability-of-F-to-remove =0.10) were conducted among evaluated traits to test the significance of the independent variables affecting the grain yield using SPSS version 22 and Graphs was drawn using Minitab version 17.

RESULTS

Correlation among agronomic, physiological and yield traits under normal conditions

Under normal conditions grain yield per plant was significantly and positively correlated with seeds per spike (r=0.98), spike length (r=0.45), relative water content (r=0.95), excised leaf water retention (r=0,69), initial water content (r=0.95) and thousand seed weight (r=0.99). Grain yield per plant had a positive but non-significant correlation with days to heading (r=0.07), flowering (r=0.23) and plant height (r=0.13). However, the rate of water loss was negatively associated with spike length (r=-0.46), seed per spike (r=-0.89), thousand seed weight (r=-0.94), relative water content (r=-0.98), excised leaf water retention (r=-0.72) and grain yield (r=-0.93). Significant relation of these agronomic and physiological traits with grain yield indicating their importance for selection with higher yield under normal conditions (table 2).

Correlation among agronomic, physiological and yield traits under water stress at tillering stage

Correlation coefficient for water stress imposed at tillering stage indicated that, grain yield was positively correlated with spike length (r= 0.72), seed per spike (r=0.98), thousand seed weight (r=1.00) and was negatively correlated with rate of water loss (r=-0.92), days to heading (r=-0.32) and flowering (r=-0.22). On the other hand, grain yield was also positively and significantly associated with relative water content at stem elongation (r=0.95), excised leaf water retention (r=0.91), initial water content at stem

Table 2. Correlation coefficient of among the agronomic, water related traits and yield and yield components under normal conditions.

	LA (cm^2)	Tiller	RWCs	IWCs	ELWRg	RWLg	IWCg	RWCg	DH	DF	PH	SL	SPS	TSW	GY
LA (cm^2)	1.00														
Tiller	-0.05	1.00													
RWCs	-0.36	0.07	1.00												
IWCs	-0.40	0.21	0.83	1.00											
ELWRg	-0.19	0.07	0.71	0.71	1.00										
RWLg	0.29	0.03	-0.89	-0.92	-0.72	1.00									
IWCg	-0.30	0.17	0.83	0.95	0.70	-0.93	1.00								
RWCg	-0.23	0.05	0.86	0.91	0.63	-0.98	0.93	1.00							
DH	-0.34	-0.17	0.21	0.14	0.27	-0.12	0.05	-0.05	1.00						
DF	-0.34	-0.20	0.36	0.28	0.41	-0.29	0.23	0.14	0.94	1.00					
PH	-0.13	0.28	0.28	0.15	0.23	-0.07	0.14	0.01	0.64	0.60	1.00				
SL	-0.33	0.01	0.48	0.36	0.28	-0.46	0.29	0.41	0.37	0.41	0.34	1.00			
SPS	-0.29	0.21	0.86	0.93	0.67	-0.89	0.86	0.90	0.03	0.17	0.05	0.48	1.00		
TSW	-0.28	0.17	0.89	0.94	0.66	-0.94	0.95	0.96	0.07	0.25	0.17	0.44	0.94	1.00	
GY	-0.30	0.19	0.89	0.95	0.69	-0.93	0.93	0.95	0.07	0.23	0.13	0.45	0.98	0.99	1.00

Correlation coefficient with ± 0.9-1 indicate very high correlation; ±0.7-0.9 high correlation; ±0.5-0.7 moderate correlation ±0.3-0.5 low correlation and 0.00-0.3 negligible correlation. Where LA= leaf area; TN= Tiller number; RWC= Relative water content; IWC= initial water content; ELWR= Excised leaf water retention; RWL= rate of water loss; DH= Days to heading; DF= Days to flowering; PH= plant height; SL= Spike length; SPS= Seeds per spike; TSW= Thousand Seed weight and GY= Grain yield.

elongation stage (r=0.84). Significant relation of these agronomic and physiological traits with grain yield indicating their importance for selection with higher yield under water stress at tillering stage (Table 3).

Correlation among agronomic, physiological and yield traits under water stress at booting stage

A significant correlation among the traits was also obtained when water stress was imposed at booting stage. Grain yield was positively correlated with seeds per spike(r=0.98), thousand seed weight (r=0.99), plant height (r=0.60), relative water content at grain filling period(r=0.93), initial water content at grain filling stage (r=0.98), excited leaf water retention (r=0.95), spike length (r=0.86) and a negative relation with the rate of water loss (r=-0.94) (Table 4).

Multiple regression analyses

Stepwise regression is a combination of the forward and backward selection techniques. It was very popular and usually better in Multivariate Variable Selection procedure. Stepwise regression is a modification of the forward selection so that after each step in which a variable was added, all candidate variables in the model are checked to see if their significance has been reduced below the specified tolerance level. Results from the stepwise regression analysis under normal conditions showed that seed per spike and initial water content at grain filling stage explain 95.7 and 86.2% of the variation for grain yield per plant respectively. While both seed per spike and initial water content at grain filling stage cumulatively explained 98.7% of the variation in the grain yield. These traits are the best traits that could explain more variation for the grain yield per plant under normal conditions (Table 5). When water stress was imposed at tillering stage 89.5% of the variation in the grain yield was accountable for by rate of water loss at grain filling stage and initial water content at stem elongation. While water stress at booting stage 89% of the variation was explained by excited leaf water retention (Figure 1c). when water stress was imposed at tilering stage 83.2 and 69 % of the variation in grain yield was explained by rate of water lose and initial water content respectively (Figure 1a) while at normal condition 95.7 and 86.2 % of the variation in grain yield was explained by seed per spike and initial water content at grain filling stage respectively (Figure 1b).

Table 3. Correlation coefficient among agronomic, water related traits and yield and yield components at water stress during tillering stage.

	LA (cm^2)	TN	RWCs	IWCs	ELWRg	RWLg	IWCg	RWCg	DH	DF	PH	SL	SPS	TSW	GY
LA (cm^2)	1.00														
Tiller	-0.01	1.00													
RWCs	0.18	0.52	1.00												
IWCs	-0.21	0.50	0.77	1.00											
ELWRg	0.26	0.37	0.95	0.70	1.00										
RWLg	-0.10	-0.40	-0.94	-0.73	-0.94	1.00									
IWCg	0.09	0.43	0.94	0.85	0.94	-0.93	1.00								
RWCg	0.18	0.33	0.95	0.75	0.94	-0.96	0.93	1.00							
DH	-0.21	0.31	-0.34	-0.14	-0.38	0.40	-0.30	-0.46	1.00						
DF	0.00	0.38	-0.24	-0.13	-0.26	0.30	-0.22	-0.36	0.95	1.00					
PH	-0.06	0.43	0.13	0.28	-0.03	0.02	0.15	-0.08	0.18	0.14	1.00				
SL	0.09	0.66	0.84	0.69	0.70	-0.71	0.70	0.75	-0.09	-0.06	0.28	1.00			
SPS	0.09	0.48	0.95	0.81	0.90	-0.91	0.92	0.94	-0.38	-0.28	0.04	0.76	1.00		
TSW	0.09	0.48	0.94	0.83	0.91	-0.91	0.96	0.92	-0.27	-0.17	0.12	0.71	0.97	1.00	
GY	0.07	0.47	0.95	0.84	0.91	-0.92	0.96	0.93	-0.32	-0.22	0.11	0.72	0.98	1.00	1.00

Correlation coefficient with ± 0.9-1 indicate very high correlation; ±0.7-0.9 high correlation; ±0.5-0.7 moderate correlation ±0.3-0.5 low correlation and 0.00-0.3 negligible correlation. Where LA= leaf area; TN= Tiller number; RWC= Relative water content; IWC= initial water content; ELWR= Excised leaf water retention; RWL= rate of water loss; DH= Days to heading; DF = Days to flowering; PH= plant height; SL= Spike length; SPS= Seeds per spike; TSW= Thousand Seed weight and GY= Grain yield.

Table 4. Correlation coefficient of physiological traits and drought tolerance indices under water stress at booting stage.

	LA (cm^2)	Tiller	RWCs	IWCs	ELWRg	RWLg	IWCg	RWCg	DH	DF	PH	SL	SPS	TSW	GY
LA (cm^2)	1.00														
Tiller	-0.23	1.00													
RWCs	-0.05	0.31	1.00												
IWCs	-0.05	0.24	0.94	1.00											
ELWRg	-0.09	0.35	0.97	0.94	1.00										
RWLg	0.09	-0.32	-0.96	-0.94	-0.98	1.00									
IWCg	-0.05	0.20	0.94	0.98	0.95	-0.96	1.00								
RWCg	-0.12	0.32	0.95	0.93	0.96	-0.99	0.95	1.00							
DH	-0.11	0.28	0.26	0.12	0.24	-0.15	0.09	0.07	1.00						
DF	-0.19	0.32	0.34	0.19	0.32	-0.22	0.17	0.14	0.97	1.00					
PH	0.22	-0.28	0.55	0.55	0.49	-0.42	0.53	0.37	0.49	0.52	1.00				
SL	-0.13	0.42	0.89	0.83	0.90	-0.88	0.81	0.87	0.47	0.49	0.50	1.00			
SPS	-0.07	0.33	0.96	0.96	0.94	-0.94	0.94	0.96	0.14	0.21	0.49	0.88	1.00		
TSW	-0.08	0.16	0.92	0.97	0.93	-0.92	0.97	0.91	0.20	0.28	0.64	0.85	0.95	1.00	
GY	-0.06	0.23	0.95	0.99	0.95	-0.94	0.98	0.93	0.17	0.25	0.60	0.86	0.98	0.99	1.00

Correlation coefficient with ± 0.9-1 indicate very high correlation; ±0.7-0.9 high correlation; ±0.5-0.7 moderate correlation ±0.3-0.5 low correlation and 0.00-0.3 negligible correlation. Where LA= leaf area; TN= Tiller number; RWC= Relative water content; IWC= initial water content; ELWR= Excised leaf water retention; RWL= rate of water loss; DH= Days to heading; DF= Days to flowering; PH= plant height; SL= Spike length; SPS= Seeds per spike; TSW= Thousand Seed weight and GY= Grain yield.

Table 5. Single variable and stepwise regression between grain yield (GY, Y) and agronomic traits of seed per spike and physiological trait of initial water content at grain filling stage.

Water stress	Traits	Regression Equation	R^2	p
Control	Seed/spike (x1)	Y= 0.07829x1 - 1.461	95.7	<0.001
	IWCg (x2)	Y=0.4464(x2) + 0.3964	86.2	<0.001
	Seed/spike, IWCg	Y= 0.05477(x1) + 0.1630(x2)- 0.963	98.7	<0.001
At tillering stage	RWLg (x1)	Y= 2.111 - 0.03086 x1	83.2	<0.001
	IWCs (x2)	Y=0.793 x2 -0.899	69.1	<0.001
	RWLg, IWCs	Y= -0.02178 (x1) + 0.351 (x2) + 0.964	89.5	<0.001
at booting stage	ELWRg (x1)	Y= 0.03720 (x1) - 0.4761	89	<0.001

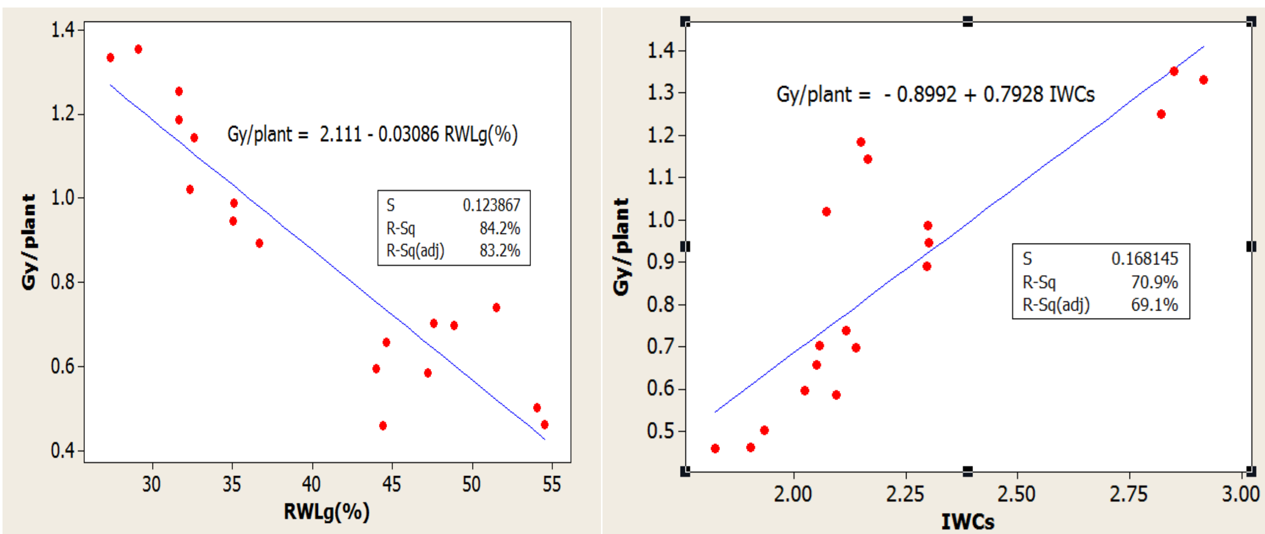

Figure 1a. Regression line for grain yield under water stress at tillering stage.

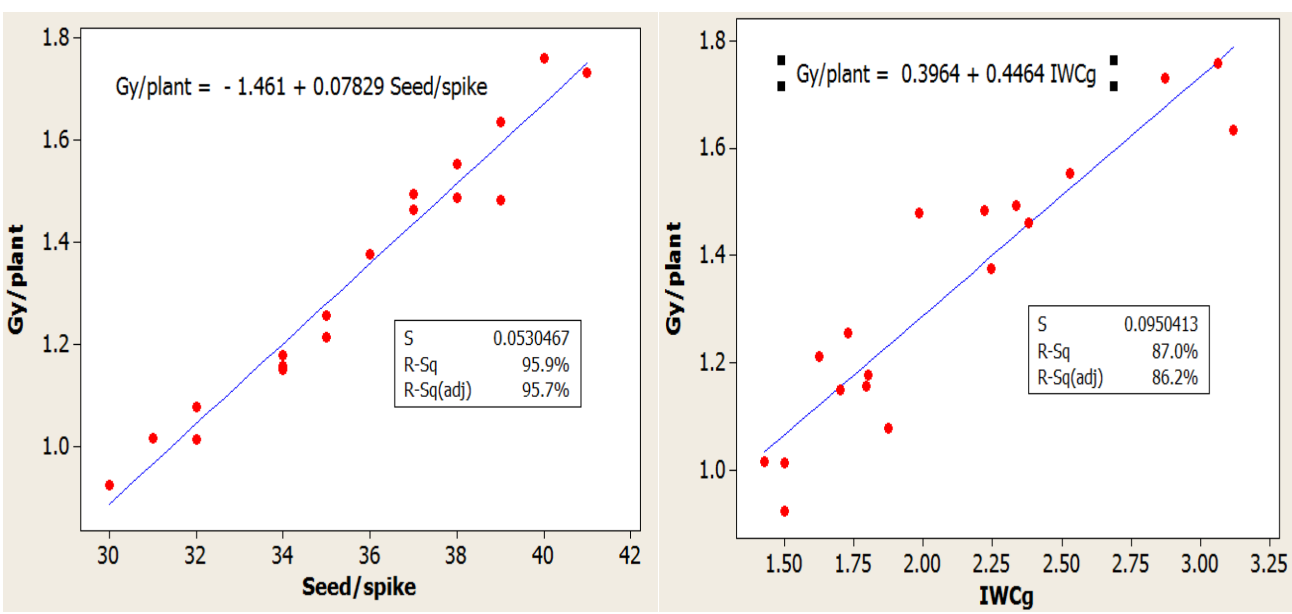

Figure 1b. Regression line for grain yield under normal condition.

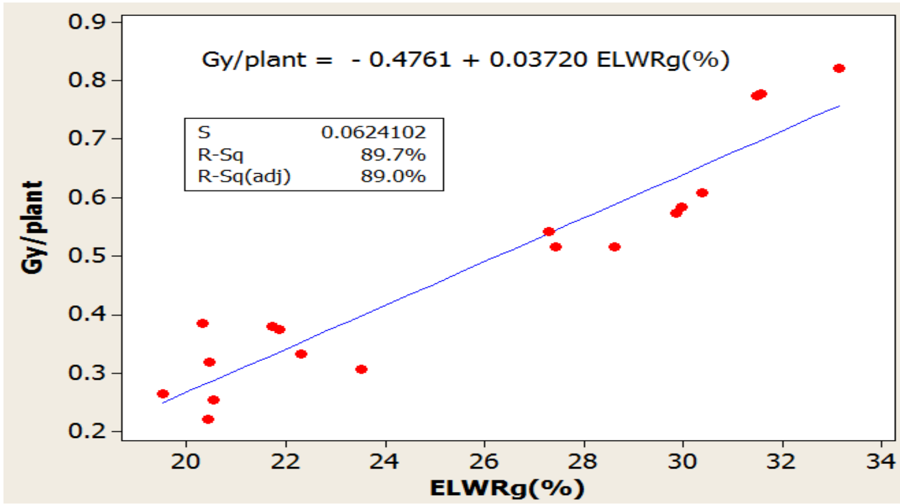

Figure 1c. Regression line for grain yield under water stress at booting stage.

DISCUSSIONS

Responses of wheat growth to water deficits vary depending on wheat species and growth stages. Jalota et al. (2006) reported that the anthesis to grain development period is the most sensitive stage to water stress in wheat in Northwest India. In China, Zhang et al. (2006) concluded from experiments that water stress should be avoided at the booting and heading of spring wheat. In addition, growth parameters have manifested certain differences during deficit irrigation.

Traits such as ELWR, RWC, and IWC had positive and significant correlation coefficient and traits RWL had negative and significant correlation coefficient with grain yield. These correlations indicate that increment in relative water content, initial water content and excised leaf water retention and reduction in rate of water loss could simultaneously increase yield traits in water stress conditions. These results were similar to those of Ahmadizadeh et al. (2011) who showed that seed per spike had more direct positive effects on grain yield under non stressed conditions. Rangare et al. (2010) also revealed that grain weight showed a direct relationship with grain yield. Gulmezoglu et al. (2010) discovered that grain yield of wheat is depended on plant height, length of spike and spike weight. Shamsi et al. (2011) also showed that the most important yield component on grain yield was grain weight.

Also, the result obtained corollate the findings of Jaradat (2009) and Kozak (2009) who observed a positive and significant correlation between excised leaf water retention and yield in wheat. Clarke et al. (1984) also concluded that low rate of water loss (high leaf water retention) was associated with high grain yield potential under drought conditions. Clarke et al. (1989) reported the association of low rate of excised leaf water loss with improved yields under very dry environments in wheat. Taheri et al. (2011) who obtained a positive and significant correlation between stress tolerance index with grain yield and RWC in stress conditions. It means there is a greater scope of using physiological traits in selection for improving yield in wheat. Similar results were also achieved by Gupta et al. (2001). Jatoi et al. (2011) reported that, it is a well-known fact that any increase or decrease in agronomic traits is caused by variable response of wheat genotypes via physiological changes. Thus, the development of cultivars for water limited environments would involve selection and incorporation of both physiological and morphological mechanisms of drought resistance through traditional breeding programmes. Hence, to increase grain yield under normal and water stress condition, the more focus should be on physiological traits such as relative water content, initial water content, excised leaf water retention and rate of water loss which have a high correlation with grain yield and also should utilize them in drought resistance breeding programs.

Conclusions and recommendations

It is concluded from the results of this study that water stress significantly reduced the yield and mean value of the studied traits in wheat genotypes. Thus, wheat, a staple food, appears to be suffering yield losses due to deficiency of water at any critical stage.

Under stress and non-stress condition, ELWR, RWC, and IWC had positive and significant correlation coefficient with grain yield while traits RWL had negative and significant correlation coefficient with grain yield indicating their importance for selection with higher yield and drought tolerance. Physiological traits like RWC, ELWR, IWC, RWL, Plant height, and Days to flowering, was effective in identifying suitable wheat genotypes for moisture stresses conditions. Genotypes with high ELWR, RWC and IWC and low RWL can be regarded as high yielders and water stress tolerant varieties. Therefore, these traits could be used as selection criteria for improving wheat grain yield under normal and water stress at tillering and booting stages. Therefore, breeder should strongly consider these traits in crop improvement program.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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