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Original Research Article

SEED PRIMING OF WHEAT THROUGH SALICYLIC ACID TO INDUCE SALT STRESS TOLERANCE

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Abstract: *Wheat is an important cereal crop consumed throughout the world. Present study was planned to conduct in greenhouse of IMBB, University of the Lahore for determination of effects of salt stress on growth of wheat seedlings. Seed from selected wheat genotypes (SHAFAQ-2006, ANAJ-2017and Galaxy-2013) were used to grow in 54 pots, which were filled with 2kg pure washed sand. The sand was mixed with 500mg/kg of salt NaCl in each of the pot except of the control pots for wheat sowing. The seed of wheat variety were sown in triplicate pots with all irrigation requirements in equal manners. The use of salicylic acid (SA) for seed priming was more effective for number of roots per plant. The highest root length was recorded for treatment of SA priming + NaCl which indicated that the application of SA induced higher number of roots and long roots under salt stress conditions. The higher root water contents and shoot water contents were recorded under the application of water priming of seeds. The positive contribution of root water contents to root length indicated that the higher root water contents caused increase in shoot length due to higher water, mineral and nutrient absorption and retention which caused increase in photosynthetic rate and vice versa. It was found from results that the genotype GALAXY-2013 performed better under different treatments and SA priming application for root length, shoot length and number of roots per plant. The results suggested that the genotype GALAXY-2013 may be used to develop different wheat genotypes which may show higher grain yield under the applications of SA as seed priming compound while growing wheat in slat stress environment.*

Keywords: wheat, shoot length, root length, salt stress, salicylic acid

Introduction

Wheat is an important cereal crop consumed throughout the world. Along with maize and rice, the wheat is an essential food crops. Wheat is grown on about 220 million hectares globally, covering a land more than any of the other crops. Wheat yield is more challenging with regard to soil conditions and water supply than other all of the cereal crops. Ideally wheat requires dense, deep and humus, but well and highly aerated soils with a greater water capacity with pH between 5.5 to 7.5. The capacity of domesticated wheat to propagate in the wild has limited because of the loss of seed dispersal mechanisms (Sacks et al., 2010; Smith, 1995). In general wheat varieties are divided into 'winter wheat' and 'summer wheat'. Winter wheat can endure temperatures as low as -22°C (-7.6°F) and is sown in October; summer wheat, which yields much less but has relatively higher protein content is sown in early spring. Tolerance to water stress is a complex factor in which crop performance can be swayed by several characteristics (Bray, 1997; Ingram and Bartels, 1996). Tolerance can be divided into two parts comprising drought avoidance and dehydration tolerance. Drought avoidance includes root depth, sufficient use of accessible water by plants, and modifications in plants lifestyle to use rainfall. Capability of plants to moderately dehydrate and grow again when rainfall resumes comprises dehydration tolerance (Dudziak et al., 2019; Maqsood et al., 2012).

The drought stress is one of the major environmental problem and the stress threatening for wheat yield globally. The predicted changes in rain fall and temperature has caused frequent periods for drought conditions. The severity and duration of the stress determine the extent of the productivity losses (Ali and Malik, 2021; Farooq et al., 2014; Sehgal et al., 2018). The production of drought tolerant crop genotypes have became the ultimate source of

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protecting crop against harmful effects caused by drought. Because of its polygenic genetic inheritance as well as the genotype through environmental interactions, the drought tolerance usually has very low heritability status (Ali and Malik, 2021; Azhar et al., 2021; Yahaya and Shimelis, 2022). Severe water shortage causes significant morphological, metabolic and physical variations in plant and eventually reduces crop productivity and quality (Ali and Malik, 2021). In addition, root is the vital organ of plant against drought, because it could absorb water and nutrient from soil (Jatav et al., 2021). The reaction of root growth to water deficit is significant in drought tolerance (Ali et al., 2013; Ali et al., 2016). Therefore, the morphology of plant could show a variety of strategies to adjust to drought stress, which is important for plant to live and develop under drought stress (Ali et al., 2014; Bhargava and Sawant, 2013). The direct drought introduces damages to photosynthetic procedure; it has also lead to light induced oxidative strain by the production of reactive oxygen species in the plant cells (Reddy et al., 2004). If the drought stress prolongs and reactive oxygen species buildup is not reduced by antioxidant, it results in protein oxidation, membrane lipid peroxidation, RNAs and hormonal activities, Inhibition of DNA, ultimately making the cell into state usually called as oxidative stress, which deteriorate the normal growth or even leads to the death of plants (Ali et al., 2016; Ali and Malik, 2021). Under abiotic stress conditions the wheat is highly affected which caused reduction in the yield and productivity. The salt stress caused cell membrane damage, production of toxic chemicals and accumulation of reactive oxygen species in the plant body (Ali et al., 2017; Ali and Malik, 2021; Khaliq et al., 2021; Mustafa et al., 2018; Sarwar et al., 2021). The salt or NaCl stress caused damage in the cell membranes, the use of salicylic acid induce the tolerance in the plant body which may help crop plants to withstand under salt and abiotic stress condition (Aaliya et al., 2016; Ali et al., 2015; Iqra et al., 2020; Majid et al., 2017; Mazhar et al., 2020; Sarwar et al., 2022; Zubair et al., 2016). The use of salicylic acid may be helpful to induce tolerance in wheat plants in our study we have used salicylic acid for the evaluation of what genotypes for salt stress tolerance.

Materials and Methods

Wheat is an important cereal crop grown throughout the world for its grain and other byproducts. It is highly sensitive to abiotic environmental conditions like involving the salt stress as important stress conditions. To evaluate the effects of SA a study was planned to conduct in greenhouse of IMBB, University of the Lahore to determine effects of salt stress on wheat seedling growth. For our study we have selected three wheat varieties SHAFAQ-2006, ANAJ-2017and Galaxy-2013. Seed from selected wheat genotype was used to grow in 54 pots, filled with 2kg pure washed sand. The sand was mixed with 500mg/kg of salt NaCl in each of the pot except of the control pots for wheat sowing. The seed of wheat variety were sown in triplicate pots with all irrigation requirements in equal manners. To carry out our research work we have used following sets for treatment of salt NaCl: 0. Control no priming 1. Water priming 2. SA priming 3. Control + NaCl 4. Water priming + NaCl 5. SA priming + NaCl The treatment of salt NaCl were applied after the germination of wheat seeds and data was recorded for various seedling traits. The treatment was applied and again data was recorded after one week of salt application. The data recorded for two times from two weeks was pooled to carried analysis of variance and all pairwise comparisons for wheat variety and treatments of salt. The data was statistically analyzed and following results were reported for our study. The data was collected for different morphological and physiological traits, viz., number of leaves per plant (NL), leaf area (LA), number of roots per plant (NR), root length (RL), shoot length (SL), shoot water contents (SWC) and root water contents (RWC). The recorded data was analyzed statistically through using the analysis of variance techniques through using SPSS23.1 software.

Results and discussions

It was revealed from results of analysis of variance as given in table 1 that there were significant differences observed among stress and genotypes, interactions between stress and genotypes for all of the studied traits of wheat genotypes under NaCl stress conditions. It was found from results that the genotype GALAXY-2013 performed better under different treatments and SA priming application for root length, shoot length and number of roots per plant (Figure 1). The results suggested that the genotype GALAXY-2013 may be used to develop different wheat genotypes which may show higher grain yield under the applications of SA as seed priming compound while growing wheat in slat stress environment.

Leaf area

The results showed that the coefficient of variance was recorded as 13.31% (CV) which revealed that there was significant consistency for results of leaf area studied trait of wheat. It was persuaded form results (Table 2) that the treatment 3 (control + NaCl

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priming) showed higher effect on leaf area and produced broader leaves with 6.75 cm^2 leaf area followed by treatment 1 (water priming) with 5.5767 cm^2 leaf area. The lowest leaf area 4.4233 cm^2 was recorded for control or no priming. The higher leaf area indicated that the photosynthetic rate will be higher and ultimately leads to improve grain yield of wheat. The broader leaves will have higher chlorophyll contents which may tends to increase organic matter in plants to improve stress tolerance in wheat crop plants. Various researchers have given prime importance to leaf area to develop higher yielding crop plant varieties and hybrids (Iqbal et al., 2006; Mahmood et al., 2009). The seed priming with various chemical compounds like ABA, SA *etc*. helps to develop healthy and higher yielding plants (Farooq et al., 2008; Vijayakumari et al., 2016). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that leaf area (25.879) was positively and highly contributing towards shoot length of wheat as shown by regression equation from table 3.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of leaf area may be helpful to develop stress resistance or tolerance with higher grain yield wheat synthetic varieties and hybrids (Irfan et al., 2005; Jafar et al., 2012).

Table 1: Analysis of Variance Table for different traits of wheat under SA applications

Source	**LA**	**NL**	**NR**	**RL**	**SL**	**RWC**	**SWC**
Replications	0.3493ns	11.44ns	2.111ns	0.16ns	1.441ns	15.675ns	3.291ns
Stress	0.1296*	0.129*	0.136*	0.129*	0.129*	0.129*	0.129*
Genotypes	3.8362*	0.44*	1.978*	36.947*	21.51*	194.54*	40.548*
Stress × Genotypes	5.251*	7.53*	7.431*	3.430*	3.330*	1.328*	2.928*
Error	0.519	0.657	1.263	1.389	2.044	7.498	4.496
Coefficient of variance	13.31	15.67	18.37	6.12	7.50	3.13	2.45
Grand mean	5.415	5.1711	6.1156	19.243	19.071	87.504	86.486

number of leaves per plant (NL), leaf area (LA), number of roots per plant (NR), root length (RL), shoot length (SL), shoot water contents (SWC) and root water contents (RWC), *= Significant at 5% probability level, ns = non-significant

Table 2: All-Pairwise Comparisons Test for different traits of wheat under applications of SA

Source	**LA**	**NL**	**NR**	**RL**	**SL**	**RWC**	**SWC**
Control (0)	4.4233C	5.060AB	6.060C	18.227C	17.227D	83.410CD	85.567BC
Water priming (1)	5.5767B	5.393A	5.7267C	18.127C	20.093B	96.017A	91.133A
SA priming (2)	5.5133B	5.393A	7.060A	16.393D	19.360BC	86.320C	87.633B
NaCl (3)	6.750A	5.060AB	6.060BC	21.327B	22.193B	85.553C	84.963C
Water priming+NaCl (4)	5.440B	5.393A	5.393D	18.260C	18.160CD	85.553C	85.833BC
SA Priming + NaCl (5)	4.7867BC	4.727B	6.393BC	23.127A	17.393D	81.080D	83.787C

SA = Salicylic acid, number of leaves per plant (NL), leaf area (LA), number of roots per plant (NR), root length (RL), shoot length (SL), shoot water contents (SWC) and root water contents (RWC)

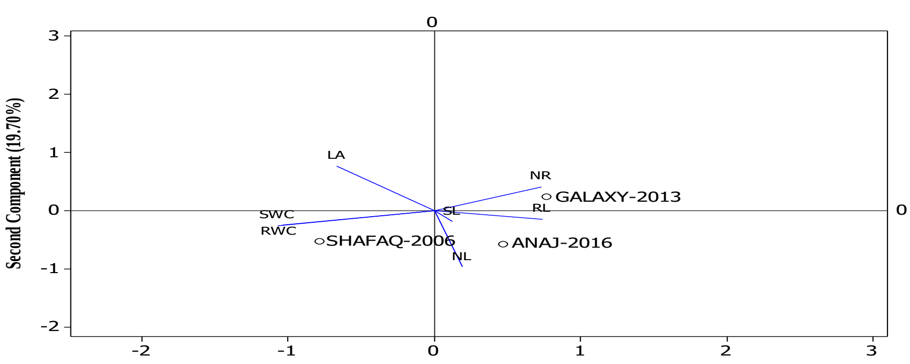

Figure 1: Principal Component analysis of wheat genotypes under different treatments

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Number of leaves per plant

The results indicated that the coefficient of variance was recorded as 15.67% (CV) which revealed that there was significant consistency for results of number of leaves per plant studied trait of wheat. It was persuaded form results (Table 2) that the treatment 4 (water priming + NaCl) showed higher effect on number of leaves per plant and produced higher number of leaves 5.3933 leaves per plant followed by treatment 1 (water priming) with 5.3933 and treatment 2 (SA priming) 5.3933 leaves per plant. The lowest 4.7267 number of leaves per plant was recorded for treatment of SA priming + NaCl. The large number of leaves per plant indicated that the large number of leaves may cause higher photosynthetic rate and ultimately leads to improve grain yield of wheat (Iqbal and Ashraf, 2006; Iqbal and Ashraf, 2007). The large number of leaves per plant showed higher chlorophyll contents in plants which may tends to increase organic matter in plants to improve stress tolerance in wheat crop plants. Various researchers have found that large number of leaves per plant may be helpful to develop higher yielding crop plant varieties and hybrids. The seed priming with various chemical compounds like ABA, SA etc. helps to develop healthy and higher yielding plants (Bakht et al., 2011; Bernstein et al., 1993; Karimi et al., 2005; Xue et al., 2004). The lower number of leaves per plant under SA priming + NaCl indicated that there may be some stress on physiological aspects of seed to develop higher number leaves per plant (Farooq et al., 2008; Jafar et al., 2012). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that number of leaves per plant (2.299) was positively and highly contributing towards shoot length of wheat as shown by regression equation from table 4.13.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of number of leaves per plant may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Chiba et al., 2003; Slafer et al., 1994).

Number of roots per plant

The results indicated that the coefficient of variance was recorded as 18.37% (CV) which revealed that there was significant consistency for results of number of roots per plant studied trait of wheat. It was persuaded form results (Table 2) that the treatment 2 (SA priming) showed higher affect on number of roots per plant and produced 7.06 roots per plant followed by treatment 5 (SA priming + NaCl) with 6.3933 and treatment 3 (control + NaCl) 6.06 roots per plant. The lowest number of roots per plant 5.3933 was recorded for water priming + NaCl. The large number of roots per plant indicated that the absorption of water, minerals and nutrients will be higher which lead to increase photosynthetic rate and ultimately leads to improve grain yield of wheat. The large number of leaves per plant showed higher stress tolerance in wheat crop plants (Brooking et al., 1995). Various researchers have found that large number of roots per plant may be helpful to develop drought resistant higher yielding wheat crop plant varieties and hybrids. The seed priming with various chemical compounds like growth regulators, ABA, SA etc. helps to develop healthy and higher yielding plants (Bakht et al., 2011; Karimi et al., 2005; Vijayakumari et al., 2016). The higher number of roots per plant under SA priming + NaCl indicated that there may be some useful effects on the physiological aspects of seed to develop higher number roots per plant (Farooq et al., 2008; Jafar et al., 2012). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that number of roots per plant (-6.413) was negatively and highly contributing towards shoot length of wheat as shown by regression equation from table 4.13. The negative contribution of number of roots per plant to shoot length indicated that the less root number caused reduction in shoot length due to low water, mineral and nutrient absorption which caused reduction photosynthetic rate and vice versa.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of number of roots per plant may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Brooking et al., 1995; Siddique et al., 1989).

Root length (cm)

The results indicated that the coefficient of variance was recorded as 6.12% (CV) which revealed that there was significant consistency for results of root length studied trait of wheat. It was persuaded form results (Table 2) that the treatment 3 (control + NaCl) showed higher affects on root length and produced 21.327cm root length followed by treatment 4 (water priming + NaCl) with 18.260cm and treatment 5 (SA priming + NaCl) 23.127cm root length. The lowest root length 16.393cm was recorded for SA priming.

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The large root length indicated that the absorption of water, minerals and nutrients through long and with good health of roots caused increased growth and development of shoot which may lead to increase stress tolerance in wheat (Blum, 2005; Sayar et al., 2007; Siddique et al., 1990). The longer roots can penetrate deeper for mineral nutrients to withstand under stress environments. The higher root length indicated that the wheat crop plant with higher root length showed drought tolerance and can survive under harsh environmental conditions. Various researchers have found that large root length may be helpful to develop drought resistant higher yielding wheat crop plant varieties and hybrids. The seed priming with various chemical compounds like growth regulators, ABA, SA etc. helps to develop healthy and higher yielding plants (Bakht et al., 2011; Kalayci et al., 1999; Karimi et al., 2005; Vijayakumari et al., 2016; Zeidan et al., 2010). The lower root length under SA priming indicated that there may be some harmful or adverse effects of SA on the morpho-physiological aspects of seed to develop healthy long roots. Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that root length (-6.241) was negatively and highly contributing towards shoot length of wheat as shown by regression equation from table 3. The negative contribution of root length to shoot length indicated that the higher root length caused increase in shoot length due to higher water, mineral and nutrient absorption which caused increase in photosynthetic rate and vice versa.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of root length may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Sayar et al., 2007).

Shoot length (cm)

The results indicated that the coefficient of variance was recorded as 6.12% (CV) which revealed that there was significant consistency for results of shoot length studied trait of wheat. It was persuaded form results (Table 2) that the treatment 3 (control + NaCl) showed higher affects on shoot length and produced 22.193cm shoot length followed by treatment 2 (SA priming) with 19.360cm and treatment 1 (water priming) 20.093cm shoot length. The lowest shoot length 17.227cm was recorded for control or no priming. The large shoot length indicated that the absorption of water, minerals and nutrients through long and with good health of roots caused increased growth and development of shoot which may lead to increase stress tolerance in wheat (Sayar et al., 2007; Siddique et al., 1990). The higher shoot length indicated that the wheat crop plant with higher shoot length showed drought tolerance and can survive under harsh environmental conditions. Various researchers have found that large shoot length may be helpful to develop drought resistant higher yielding wheat crop plant varieties and hybrids (Gregory et al., 1992; Pleijel et al., 2006; Sayar et al., 2007). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that the traits leaf area (LA), number of leaves per plant (NL), root fresh weight (RFW), root length (RL), shoot length (SL), shoot dry weight (SDW), shoot root dry weight ratio (SRDWR), shoot water contents (SWC) and root water contents (RWC) contributed positively towards shoot length while the traits number of roots per plant (NR), shoot fresh weight (SFW) and dry root dry weight (RDW) contribute negatively towards shoot length of wheat as shown by regression equation from table 3.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of shoot length may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Acharya and Sharma, 1994; Pleijel et al., 2006; Quarrie et al., 2005).

Root water contents (%)

The results indicated that the coefficient of variance was recorded as 6.12% (CV) which revealed that there was significant consistency for results of root water contents studied trait of wheat. It was persuaded form results (Table 2) that the treatment 3 (control + NaCl) showed higher affects on root water contents and produced 92.64% root water contents followed by treatment 1 (water priming) with 96.017% and treatment 2 (SA priming) 86.320% root water contents. The lowest root water contents 81.080% was recorded for SA priming + NaCl. The large root water contents indicated that the absorption of water, minerals and nutrients through roots caused increased growth and development of shoot which may lead to increase stress tolerance in wheat. The higher water contents indicated that the roots have ability to retain water/moisture under harsh environmental conditions and can help plants to withstand under stress environments (Blum, 2005; Gregory et al., 1992; Jafar et al., 2012). Various

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researchers have found that large root water contents may be helpful to develop drought resistant higher yielding wheat crop plant varieties and hybrids. The seed priming with various chemical compounds like growth regulators, ABA, SA etc. helps to develop healthy and higher yielding plants (Bakht et al., 2011; Karimi et al., 2005; Zeidan et al., 2010). The lower root water contents under SA priming + NaCl indicated that there may be some harmful or adverse effects of SA and NaCl on the morpho-physiological aspects of seed to develop healthy long roots that can retain water/moisture for long time under stressful environmental conditions (Kalayci et al., 1999; Vijayakumari et al., 2016). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that root water contents (21.076) was positively and highly contributing towards shoot length of wheat as shown by regression equation from table 3. The positive contribution of root water contents to shoot length indicated that the higher root water contents caused increase in shoot length due to higher water, mineral and nutrient absorption and retention which caused increase in photosynthetic rate and vice versa.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of root water contents may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Kang et al., 2002; Shearman et al., 2005).

Shoot water contents (%)

The results indicated that the coefficient of variance was recorded as 2.45% (CV) which revealed that there was significant consistency for results of shoot water contents studied trait of wheat. It was persuaded form results (Table 2) that the treatment 4 (water priming + NaCl) showed higher affects on shoot water contents and produced 85.833% shoot water contents followed by treatment 1 (water priming) with 91.133% and treatment 2 (SA priming) 87.633% shoot water contents. The lowest root water contents 83.787% was recorded for SA priming + NaCl. The higher shoot water contents indicated that the absorption of water, minerals and nutrients through roots caused increased growth and development of shoot which may lead to increase stress tolerance in wheat. The higher water contents indicated that the shoot have ability to retain water/moisture under harsh environmental conditions and can help plants to withstand under stress environments (Doorenbos and Kassam, 1979; Kang et al., 2002). Various researchers have found that higher shoot water contents may be helpful to develop drought resistant higher yielding wheat crop plant varieties and hybrids. The lower shoot water contents under SA priming + NaCl indicated that there may be some harmful or adverse effects of SA and NaCl on the morpho-physiological aspects of seed to develop healthy long roots that can retain water/moisture for long time under stressful environmental conditions (Baumhardt and Jones, 2002; Sionit et al., 1980; Vijayakumari et al., 2016). Regression analysis was carried out to find out the traits which were highly contributing towards shoot length in order to develop stress tolerance wheat genotypes. It was found that shoot water contents (0.138) was positively and highly contributing towards shoot length of wheat as shown by regression equation from table 3. The positive contribution of shoot water contents to shoot length indicated that the higher shoot water contents caused increase in shoot length due to higher water, mineral and nutrient retention in shoots which caused increase in photosynthetic rate and vice versa.

Y = -126.943 – 6.241 (RL) + 2.299 (NL) – 6.413 (NR) + 25.879 (LA) + 0.138 (SWC) + 1.031 (RWC)

The results showed that the selection of wheat genotypes on the basis of shoot water contents may be helpful to develop stress resistance or tolerance with higher plant biomass and grain yield wheat synthetic varieties and hybrids (Baumhardt and Jones, 2002; Doorenbos and Kassam, 1979; Zhang et al., 2008).

Table 3: Stepwise regression analysis for Shoot length as response trait

Traits	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
RL	-6.241	3.249	-1.921	0.3055	-47.5234	35.04122
NL	2.299	1.142	2.013	0.2934	-12.2134	16.81299
NR	-6.413	4.263	-1.504	0.3734	-60.5755	47.7493
LA	25.879	12.215	2.119	0.2807	-129.332	181.0914
SWC	0.138	0.163	0.845	0.5535	-1.93873	2.214777
RWC	1.031	0.535	1.926	0.3048	-5.76725	7.828384

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Intercept (Y) = -126.943, Multiple R^2 = 78.77%, R^2 = 62.05%, Adjusted R^2 = 35.49%, Standard Error = 1.7171, leaf area (LA), number of leaves per plant (NL), number of roots per plant (NR), root length (RL), shoot length (SL), shoot water contents (SWC), root water contents (RWC)

Conflict of interest

The authors declared absence of conflict of interest.

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