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Variation of grain yield and agronomic traits of *Brassica napus* L. cultivars under salt stress

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Abstract

Salt stress is one of the most critical factors impacting plant growth and yield. The aims of this study were to assess the effect of salt stress on grain yield and some agronomic traits of canola (Brassica napus L.). Five spring canola cultivars including Sarigol, Delgan, Zaffar, Zarfam and RGS003 were selected and treated with 6% and 20% sodium chloride. A factorial experiment based on randomized complete block design (RCBD) with three replications was performed. Main effects of cultivar, NaCl stress and interaction of NaCl stress × cultivar in all agronomic traits were significant at 0.01 probability level. The highest and the lowest seed yield was found in Delgan and Zarfam, respectively. The highest thousand-seed weight of Sarigol, Delgan, Zaffar and RGS003 was found in the control condition while in Zarfam cultivar was found under 6% NaCl treatment. In Sarigol and Zarfam cultivars the largest number of pods were found under 20% and %6 of NaCl treatments. Stepwise multiple linear regression analysis showed that grain weight per pod and number of pods per plant had the highest effect on grain yield and explained 95% of grain yield variations. Factor analysis based on principal component analysis showed that four factors explained 80.81% of total variations. The first factor explained 36.07% of total variance and was named as an effective factor on vegetation growth. The second factor explained 16.28% of the variation and was identified as the grain yield and its components factor. In this research, Sarigol cultivar was introduced as the most tolerant cultivar due to the highest grain yield under NaCl stress conditions.

Key words: Canola, Factor analysis, NaCl stress, Regression analysis

1. Introduction

Canola (Brassica napus L.) is one of the world's major oil seed in recent decades which it's under cultivation field in Iran is 75431 hectares (FAO, 2018). This plant has become an important crop in rotation with wheat that it's under cultivation area is steadily on the rise. However, due to agricultural constraints, it has not yet found its actual status (Ghobadi et al., 2006), One of the main limitations is the abiotic stress such as salinity and drought stresses (Fathi et al., 2003).Salinity stress is one of the most important abiotic stress and its influence on crop production that increased researches with the aim to improve the tolerance of plants. Salinity is the presence of excessive soluble salt and minerals in water and soil leading to accumulation of salt in the root and the absorption of water from the soil is difficult for the plant. Salinity stress is first perceived by the root system and impairs plant growth both in the short term, by inducing osmotic stress that caused by reduced water availability, and in the long term, by salt-induced ion toxicity due to nutrient imbalance in the cytosol (Acosta-Motos et al., 2017). Salt stress considerably reduces Brassica species growth and development by disrupting photosynthesis process, leaf gas exchange (LGE), vegetative, and reproductive growth (Shahzad et al., 2021) Increase NaCl concentration in the soil was prevented water absorption by the root, which was found a severe drought, withering dark spots on the leaves and thick cuticles during different stages of plant growth and also found a decrease in the yield (Sudhir and Murthy, 2004; Rhoades et al., 1992). Due to the inappropriate use of natural resources and technology in agricultural production particularly in relation to irrigation water, a significant portion of arid agricultural land areas face salinity problems (Meloni et al., 2004). Therefore, the selection of tolerant cultivars to salinity stress seems to be necessary. Improved Brassica napus L. cultivars under salt stress have been a challenging task to the plant due to the complexity and multitude of genes that govern them. An aldehyde dehydrogenase gene that encodes an antiquitin enhanced Brassica rapa tolerance to salt stress (Gautam et al., 2020). Brassicaceae family with respect to NaCl tolerance at germination stage, locate among the Poaceae (resistant) and Leguminosae (most sensitive) family (Steppuhn et al., 2001). Several studies were examined the effect of salt stress at different growth stages of canola. Zamani et al., (2009) studied the effect of NaCl stress on four varieties of winter canola, they found that salt stress was significantly reduced grain yield, grain weight, the number of seeds per pod, number of pods per plant, leaf area and plant height. Sakr et al., (2007) reported that most of the growth parameters including weight of dry matter, the number of seeds per pod and the total yield significantly decreased with increasing the levels of. Azimi Gandomani et al., (2008) have evaluated the effect of NaCl stress on physiological characteristics of eight varieties of spring canola. They reported that, biological yield, grain yield and oil content decreased with increasing salinity (NaCl stress). Sharifi (2016) showed that canola root length, germination, shoot length and seedling weight significantly affected by different concentrations of sodium chloride and they observed highest percentage reduction of agronomic traits at a concentration of 17 ds.m^{-1}. Tajali et al., (2011) in a study examined the effects of sodium chloride treatments (0, 3, 6, 9 and 12 ds. m^{-1}) on five cultivars of canola (Opera, Okapi, Madonna, Pioneer and Kobra) and showed that the effect of NaCl stress on canola yield is significant. Alborzi Hagigi et al., (2012) showed the number of leaves, plant height, plant dry weight, leaf area index and potassium concentrations were decreased by increasing salinity (NaCl). Shokri et al., (2002) examined the response of canola cultivars to NaCl stress. They reported that grain yield in Crete cultivar, days to 50% flowering, plant height, number of pods on the main stem, pod length, seed yield per plant, seeds per pod, harvest index, seed weight, number of pods per branch, number of lateral branches were reduced by NaCl stress. Yousefi et al., (2015) studied the effects of salinity (control and salinity of 150 mM NaCl) on two cultivars of canola (Shirali and Hayoula 401) and revealed that seed yield, biological yield, harvest index, plant height, number of branches and relative water content reduced under the NaCl stress.

To achieve better knowledge about relationship between salt stress and agronomic characteristics, multivariate statistical analysis such as multiple linear regression analysis and factor analysis can be used. Numerous investigations have used the factor analysis to study relationships among agronomy traits in canola (Ghaffari Nematabad and Tahmasbpour, 2010; Taylor and Smith, 1992). The aims of this study were to assess the effect of NaCl stress on yield and some agronomic traits of Brassica napus L. and to select of the best cultivar out of five Iranian cultivated canola varieties. Study of the relationships among agronomic traits using regression analysis and factor analysis was the other purpose.

2. Materials and Methods

An experiment was conducted based on randomized complete block design with 3 replications in 2019 at the Agriculture Research Station of University of Tabriz. Plant materials included five varieties of canola including Sarigol, Delgan, Zaffar, Zarfam, and RGS003. Salt treatments were three NaCl concentrations of sodium chloride (control, 6% and 20%). Canola cultivars are commercial and currently cultivated in Iran. The seeds were cultivated in the plastic pots with a diameter and height of 30 cm in the greenhouse condition (thermo-period 22\15 ˚C Day\night, relative humidity 50\60% day\night). Soil used in this study was loamy and during vegetation period, irrigation was done weekly using distilled water. Depth of planting seeds was 1-1.5 cm. In order to prevent the accumulation of NaCl in pots, four holes with a diameter of one centimeter in the bottom of the pot for drainage was built and the bottom of each pot was dumped with gravel to a height of 5 cm. Ten seeds were planted in each pot. After the establishment of the plantlets, three plants in each pot retained and the others were eliminated. The NaCl solutions prepared with distilled water. NaCl treatment applied at the flowering stage (55-65 days after planting depending on the cultivar) through irrigation. At the end of the growth period (Seed physiological maturity), of which approximately 50% of the pods per plant were greenish-brown, (after 85-95 days of planting depending on the cultivar) the number of pods per plant and number of seeds per pod were measured. Also, plant height, the number of branches, seed weight per pod, fresh weight, root dry weight, yield per plant (yield of single plant), plant dry weight, thousand-seed weight, and number of leaves were measured. Mean comparison was performed with Duncan's test at the 0.05 level of significance. Factor analysis was conducted based on principal component analysis and Varimax rotating method. Multiple regression analysis was done based on stepwise process. The charts were drawn using Excel 2013 software and data analysis was carried out with MSTATC and SPSS ver.18 software.

3. Results and Discussion Analysis of variance and comparison of means

The results of analysis of variance showed that the effects of cultivar, NaCl stress and NaCl stress × cultivar interaction in the probability of 1% in all traits were significant (Table 1). Therefore, the variation among cultivars may be suitable for the selection of cultivars in breeding programs. The mean comparison of NaCl × cultivar effects was done for all traits (Fig. 1). Zaffar cultivar had the highest plant dry weight in the control condition (without NaCl stress) and the lowest was for Delgan under 20% NaCl stress (Fig. 1). In the Delgan cultivar with increasing NaCl concentration the plant dry weight decreased. In accordance with the result of this study, Moradi et al., (2010) reported that the dry matter weight significantly decreases with increasing NaCl concentrations in canola cultivars. The highest number of pods per plant in Delgan, Zaffar and RGS003 varieties were observed at control condition, whereas under two levels of NaCl stress were reduced. However, in Sarigol and Zarfam cultivars the largest number of pods were found under 20% and %6 of NaCl treatments (Fig. 1). This result was similar to Zamani et al., (2009), Shokri (2002) and Tajali et al., (2011). They reported the reduction of the number of pods per plant in the canola under NaCl stress. According to Liu et al., (2004) probably a decrease in the number of pods caused by an increase in the abscisic acid hormone, so the high level of this hormone can cause the death of the pollen, resulting in decreasing the number of inoculum flowers and the number of pods. In Sarigol, Delgan and Zaffar cultivars, the highest grain yield was observed in control condition and grain yield significantly decreased by increasing NaCl concentration while, in Zarfam and RGS003 the highest grain yield was observed in %6 concentration of NaCl. Generally, the highest and the lowest seed yield was found in Delgan and Zarfam, respectively (Fig. 1). This result is consistent with the results of other researchers (Alborzi Haghighi et al., 2012; Azimi Gandomani et al., 2008; Sakr et al., 2007; Shokri et al., 2002; Tajali et al., 2011; Zamani et al., 2009. Dehshiri 1999). They reported that salinity (NaCl) stress was reduced the number of pods per stem and the number of seeds per pods, which led to a decrease in grain yield. One reason for this reduction can be due to reduction in the number of branches and therefore reduction of pods per lateral branches and subsequently decreased grain yield. When plants are exposed to salinity stress, undergo a physiological drought due to osmotic reduction effect. The roots under these conditions increase the amount of abscisic acid. This hormone is transferred to shoot through the transpiration stream. Abscisic acid in shoot decreased stomatal conductance so, transpiration is reduced to comply with it and finally because of the emissions of CO_2, photosynthesis, growth and finally the grain yield decreases (Ashraf, 2001). Accelerate growth of apical offshoot, reduction in the total number of seeds, decline pollen viability, reduction in pollen germination, fertilization and seed filling, are the effect of salinity on the reproductive growth stages. The highest thousand-seed weight of Sarigol, Delgan, Zaffar and RGS003 were found in the control condition while in Zarfam cultivar was found under 6% NaCl stress (Fig. 1).Maas and Grieve (1990) reported that salinity stress affects the yield components, depending on the different phonological stage of the plant. Effect of salinity on grain weight of canola has also been reported by Zamani et al., (2009), Shokri (2002) and Tajali et al., (2012) in the control condition. In the present study, the highest seed weight of Zarfam was found at 6% NaCl, which may be due to reducing the number of seeds per pod which induced by NaCl stress. The other causes of seeds weight loos due to NaCl stress, can be caused by the remobilization of photosynthetic of seed and also reduce the effects of stress on current photosynthesis which finally decrease transferred photosynthetic materials to grains, as a result, seeds become small and wrinkled (Flenet et al., 1996)Responses of canola cultivars to Nacl stress were different with respect to the number of seeds per pod. The number of seeds per pod in Delgan and Zaffar cultivars were reduced with increasing level of NaCl, while other cultivars had not same reactions against Nacl stress (Fig. 1). In accordance with the results of this study, Zamani et al., (2009), Tajali et al., (2011) and Shokri (2002) also reported seeds reduction per pod in canola. Shams Aldin and Farahbakhsh (2008) showed significant positive correlation between the length of pods and seeds per pod. They stated that one of the reasons for reducing the number of seeds per pod in canola under salinity is the reduction of pod length. In all cultivars the highest seed weight per pod was under control treatment (no NaCl). Delgan had the maximum weight of seed per pod under normal condition and Zaffar and Zarfam cultivars had the lowest seed weight per pod under 20% NaCl stress (Fig. 1). In studied cultivars, the highest number of lateral branches was found under normal conditions and at different levels of NaCl significantly reduced the number of lateral branches, which corresponded with the results of Shokri (2002) investigation. The highest plant height (113 cm) was found in Zaffar cultivar under normal condition and the minimum value of plant height (44 cm) was found in RGS003 cultivar under 20% treatment in accordance with the results of other researchers (Alborzi Haghighi et al., 2012; Shokri et al., 2002; Tajali et al., 2011). Under salt stress, cell wall attributes modify and photosynthesis and leaf turgor decline that led to a decrease in leaf area. Furthermore, the stem growth (a component of the aerial part) is also generally reduced by high NaCl concentrations. Decreases in leaf and stem biomass Induces a reduction in all aerial part sizes and in the plant height (Acosta-Motos et al., 2017). The shoot length was considered as a temporary storage tank of non-structural carbohydrate which by the reduction of plant height, provides smaller amount of carbohydrates, especially under salt stress conditions during grain filling stage. Salinity stress decreases the cells division and the cell elongation so, leads to reduction of the plant height and plant dry weight. (Tabatabai et al., 2011). Zaffar cultivar had the highest root dry weight under the normal condition. There was no significant difference between Zarfam under the 6% treatment and Zaffar in the control condition with respect to root dry weight. The root dry weight in Zaffar and Sarigol cultivars significantly increased under 20% NaCl stress compared to 6% treatment. After applying 20% NaCl stress, the highest number of dead leaves (yellow and dried leaves) was observed in Zaffar cultivar. This may indicate that Zaffar cultivar is more sensitive to salt stress than other cultivars.

Regression analysis

The results of linear regression between grain yield (as the dependent variable) and measured traits (as the independent variables) (Tables 2 and 3) showed that there is a significant relationship among grain yield per plant and seed weight per pod, number of pods per plant, number of seeds per pod and number of lateral branches (Equation 1). Adjusted R^2 in this model was 95 percent.

Equation 1: $y= -0.858 + 1.45 X_1 + 0.49X_2 - 0.013X_3 - 0.02 X_4$

Where in;

Grain yield per plant (gr) = y

Seed weight per pod (gr) = x_1

The number of pods per plant = x_2

The number of seeds per pod =x_3

The number of lateral branches =x_4

Equation 1 indicates that by increasing the seed weight per pod and number of pods per plant, grain yield increases. Sheikh et al., (1999) showed that in canola cultivars, grain yield changes were explained by the number of branches, number of pods per plant and seed weight. In the other study stepwise regression analysis showed that 56 percent of the variations in grain yield of canola were explained by the weight of one thousand seeds and plant height under water deficiency stress (Sabaghnia et al., 2010). Fathi et al., (2003) also found that the number of pods per plant, biological yield and number of seeds per pod were the best choice for improving grain yield. Baradaran et al., (2006) similar to the results of this study showed that the number of pods per plant, seeds per pod, seed weight and number of nodes on the stem explained the 64 percent of the grain yield variations in canola cultivars. Moradi and Godrati (2010) showed that seed yield variations were explained by seed weight and number of seeds per pod and these traits explained 93% of grain yield variations. The results of regression between leaf weight (as the dependent variable) and measured traits (as the independent variables) (Tables 4 and 5) showed that there is a significant relationship between leaf weight and the number of lateral branches, yield per plant, plant dry weight, thousand-seed weight and number of pods (Equation 2). Adjusted R^2 in this model was 92 percent.

Equation 2: $y= -2.713 + 1.626X_1 + 0.654X_2 - 0.86X_3 + 0.179 X_4 - 0.024X_5$

Where in;

Leaf weight = y

The number of lateral branches = X_1

Yield per plant= X_2

Plant dry weight= X_3

One thousand seed weight= X_4

Number of pods per plant= X_5

This relationship indicates that by increasing the number of lateral branches, yield per plant and one thousand seed weight, leaf weight increases.

Factor analysis

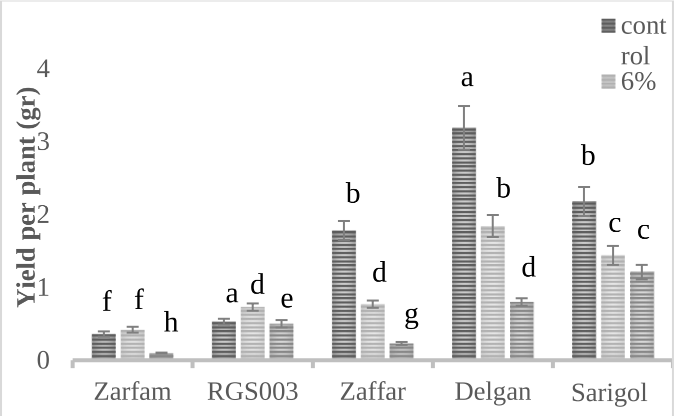
The significance of the Bartlett's test of sphericity indicates that the correlation values of the initial variables were sufficient for factor analysis. Based on the results of the factor analysis (Table 6), primary variables were defined in four factors which explained 80.81 percent of the total variation. Based on the results of factor analysis, most traits had a high amount of communality values, that indicates the number of selected factors were appropriate and the selected factors have been able to justify the changes of traits. The first factor explained the highest percentage of the total variance (% 36.07). Plant height, fresh leaf weight, root dry weight and plant dry weight had the highest positive coefficients in factor one, so it can be called as growth factor. In second factor that justified 16.28% of the changes, the number of pods per plant, the number of seed per pod, seed weight per pod, grain weight and one thousand grain yield had high positive coefficient. It means that by increasing the number of pods per plant, number of seeds per pod, seed weight per pod, plant yield increases. Therefore, this factor can be called yield factor and its components. The third factor explained 14.80% of the total variation. In this factor, the root dry weight had high positive and was named as root dry weight factor. The fourth factor justified 9.66 percent of the total variance and in this factor the one thousand grain weight had the high positive coefficient while number of pods per plant had the high negative coefficient. Therefore, this factor was named as the thousand seed weight. In accordance with the results of this study, Ghaffari Nematabad and Tahmasbpour (2010) by using factor analysis reduced 12 traits of canola into 5 factors which explained 81 percent of the total variance.

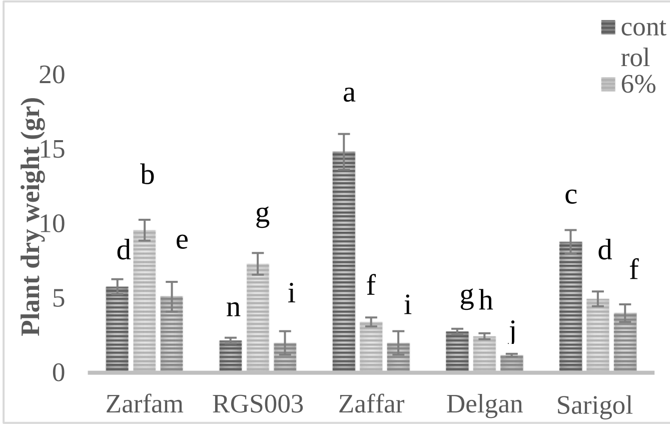
Conclusion

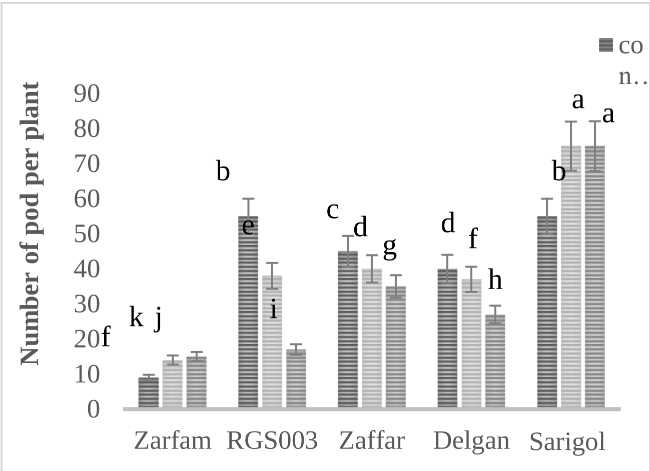
The results showed that NaCl stress significantly reduced the important agronomic traits. Also, the highest effect of NaCl stress on seed yield was under 20% NaCl stress. In all cultivars, the highest grain yield was observed in control group and by increasing NaCl stress, grain yield significantly decreased. Delgan cultivar had the highest grain yield in control condition while, Zarffam had the lowest grain yield in 20% NaCl solution. From the results of the regression analysis in canola varieties, it can be found that for genetic improvement of grain yield, grain weight per pod and pod number per plant can be used as indirect selection criteria in breeding programs. Based on the results of the factor analysis, first four factors were explained 80.81 percent of the total variation. Sariogel cultivar with the highest grain yield under NaCl stress can be introduced as tolerant cultivar. Number of pods per plant in sariogel and zarffam was higher when treated with 6 and 20 % sodium chloride. Constant advances are being made to identify traits that are associated with salinity tolerance, such as measurements of physiological traits allowing us to get a better understanding of the complex network of traits that contribute to salinity tolerance.

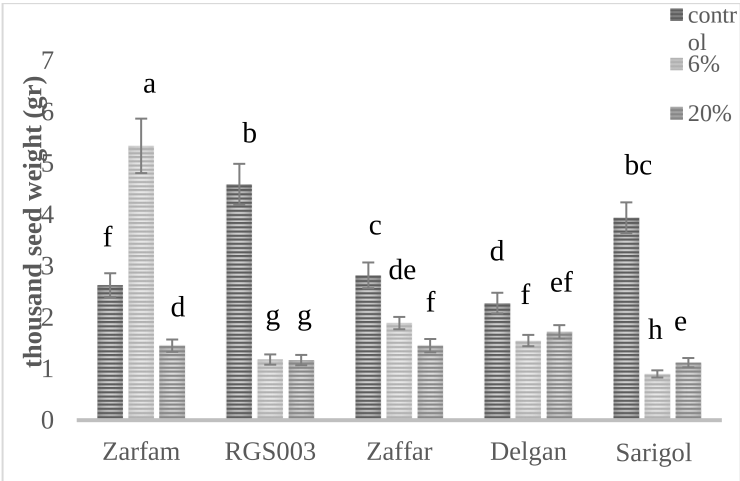
Table 1. Analysis of variance for agronomic traits in canola cultivars. plant height: h, Number of pods per plant: npp, Number of seeds per pod: nsp, Seed weight per pod: sw, One thousand seed weight: tsw, Biomass dry weight (gr): bdw, Yield per plant (gr): yp, Number of dead leaves after NaCl stress: ndl, Fresh leave weight: flw, Root dry weight: rdw, Number of lateral branches and nb Number of lateral branches: nb.

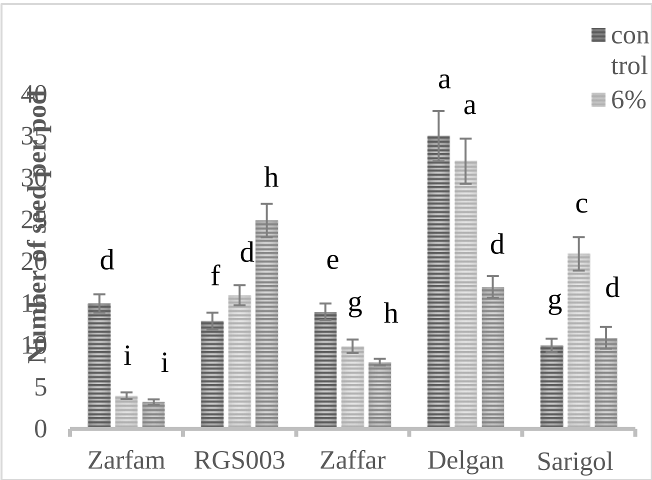
ource of variation	Degree of freedom	Mean of squares										
		h	npp	nsp	sw	tsw	bdw	yp	ndl	flw	rdw	nb
p-value		0.001	0.004	0.005	0.009	0.006	0.003	0.008	0.002	0.007	0.0098	0.001
Replication	2	0.057**	15.2**	0.429 *	0.017	0.295	14.506	0.673	0.657 **	1.093**	0.673 **	0.383 **
Cultivar	4	8.412**	3549.8 **	7.442 **	0.010 **	0.343 **	69.14 **	1.149 **	0.18 **	1.609**	0.368 **	0.837 **
NaCl stress	2	1.615**	245 **	0.301 **	0.032 **	1.2 **	10.697 **	1.519 **	0.276 **	0.044	0.046	2.287 **
cultivar × NaCl	8	2.415 **	371.75 **	2.239 **	0.003 **	0.797 **	39.708 **	0.221 **	0.212 **	0.595**	0.258 **	10.19 **
Error	28	0.1	0.2	0.26	0.0003	0.38	0.6	0.25	0.7	0.38	0.38	0.12
CV (%)	-	0.45	1.16	3.14	50.94	27.16	11.92	45.46	14.16	40.82	65.58	3.21

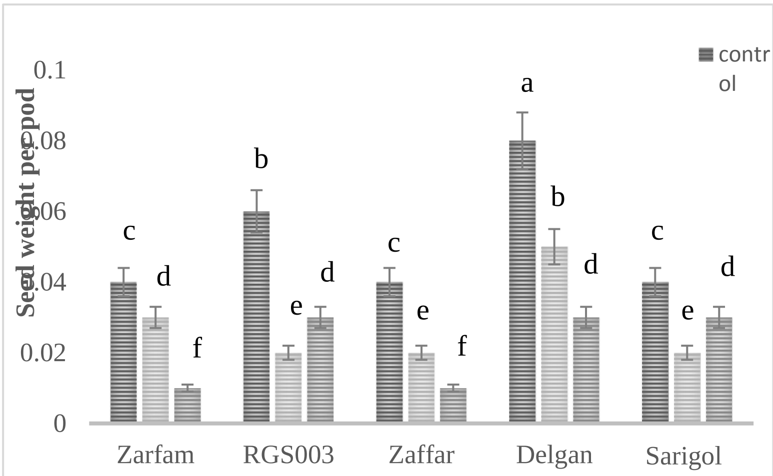

Yield per plant (gr)
control
6%
0
1
2
3
4
Zarfam
RGS003
Zaffar
Delgan
Sarigol


Plant dry weight (gr)
control
6%
0
5
10
15
20
Zarfam
RGS003
Zaffar
Delgan
Sarigol


Number of pod per plant
co
n..
0
10
20
30
40
50
60
70
80
90
Zarfam
RGS003
Zaffar
Delgan
Sarigol


thousand seed weight (gr)
control
6%
20%
0
1
2
3
4
5
6
7
Zarfam
RGS003
Zaffar
Delgan
Sarigol


Number of seed per pod
control
6%
0
5
40
Zarfam
RGS003
Zaffar
Delgan
Sarigol


Seed weight per pod
control
0
0.02
0.04
0.06
0.08
0.1
Zarfam
RGS003
Zaffar
Delgan
Sarigol

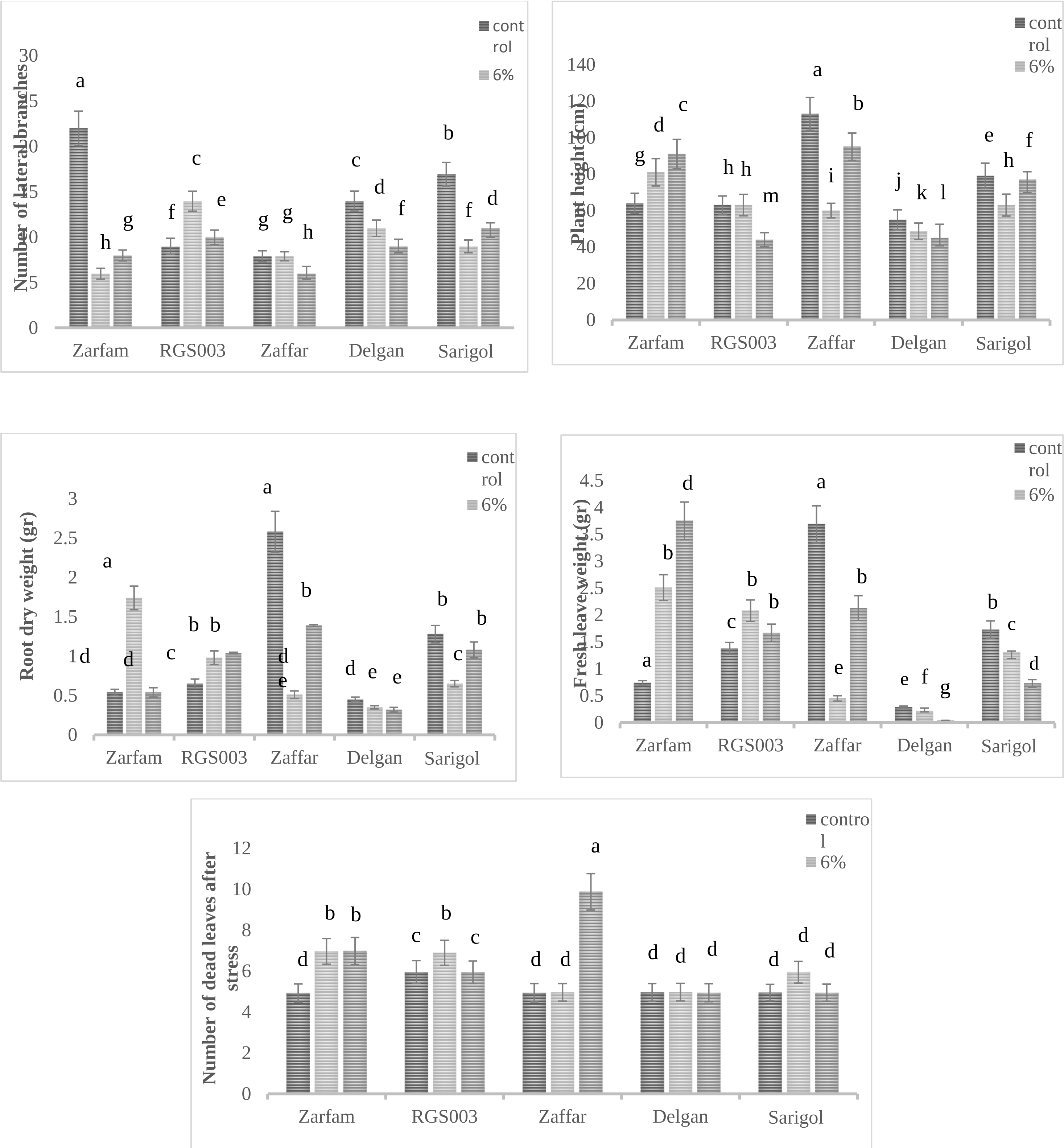


Figure 1. Mean comparison of Nacl×cultivar interaction effects on agronomic traits (Compared in 5% probability level using Duncan's multiple range test.)

Table 2. ANOVA for regression 0f grain yield as dependent variable and other agronomic traits as independent variables

Sources of variation	Degree of freedom	Mean of squares
Regression	4	12.352**
Residual	40	0.065
** significant at 0.01 probability level		Adjusted R^2= 0.951 R^2= 0.958

Table 3. Results of stepwise regression for grain yield as dependent variable and other agronomic traits as independent variables

Model	Nonstandard coefficients	Standardized coefficients (β)
Constant(α)	-0.858	
Seed weight per pod (gr) (X_1)	1.45	0.837
Number of pods per plant (X_2)	0.49	0.535
Number of seeds per pod (X_3)	-0.013	-0.105
Number of lateral branches (X_4)	-0.020	-0.080

Table 4. ANOVA for regression of leaf weight as dependent variable and other agronomic traits as independent variables

Sources of variation	Degrees of freedom	Mean of squares
Regression	5	101.160**
Residual	39	1.160
** significant at 0.01 probability level		Adjusted R^2= 0.922 R^2= 0.936

Table 5. Results of stepwise regression for leaf weight as dependent variable and other agronomic traits as independent variables

Model	Nonstandard coefficients	Standardized coefficients (β)
Constant(α)	-2.713	
The number of lateral branches (X_1)	1.626	0.566
Yield per plant (gr) (X_2)	0.654	0.403
Plant dry weight (gr) (X_3)	-0.86	-0.355
The weight of one thousand seeds (gr) (X_4)	0.179	0.202
Number of pods per plant (X_5)	-0.024	-0.126

Table 6. Rotated factor loadings, the proportion of variation explained by each factor, Cumulative percentage of variance and Eigen values

Traits	First factor	Second factor	Third factor	Fourth factor	Communality
Number leaves	0.081	-0.209	0.417	0.169	0.876
Number of dead leaves after stress	0.139	-0.024	0.26	0.259	0.670
Plant height (cm)	0.989	0.126	-0.072	-0.293	0.864
The number of lateral branches	-0.096	0.249	-0.077	0.225	0.495
Number of pods per plant	-0.043	0.698	0.138	-0.752	0.891
Number of seeds per pod	-0.168	0.726	0.261	0.075	0.813
Seed weight per pod (gr)	-0.108	0.696	0.011	0.132	0.842
Fresh leaf weight (gr)	0.796	0.153	0.058	0.081	0.828

Root dry weight (gr)	0.757	0.256	0.649	-0.046	0.786
Yield per plant (gr)	-0.109	0.833	0.629	-0.010	0.773
Plant dry weight (gr)	0.696	0.152	0.046	-0.004	0.812
weight of one thousand seeds (gr)	0.070	0.602	-0.296	0.982	0.567
Eigen values	4.4	1.90	1.70	1.20	
Percent of variance	36.07	16.28	14.80	9.66	
Percent of cumulative variance	36.07	52.35	67.15	80.81	

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