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Original article

Comparative Responses of Algerian Tetraploid Wheat Cultivars to Salinity at the Seedling Stage

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Abstract

Salinity is one of the most abiotic stresses restricting wheat growth and productivity in arid and semi-arid regions. This study was carried out to examine the effect of salt stress induced by sodium chloride (NaCl) at different concentration levels (0, 50, 100 and 150 mM) on seed germination rate, root length, roots number, coleoptile length, root and shoot fresh weights of eleven durum wheat varieties. The results revealed significant differences among the genotypes for all the measured parameters. The increase in NaCl concentrations showed concomitant decrease in all morphological attributes, but varied depending on cultivars and levels of salinity. Seed germination rate and root length demonstrated a linear response to NaCl treatment, while significant linear and quadratic regression on salinity for roots number, coleoptile length, root and shoot fresh weights were observed. The cluster analysis based on Ward's method sequestrated the studied genotypes into three clusters. Seed germination rate and root length showed the lowest difference among the groups; and the remaining traits: roots number, coleoptile length, root and shoot fresh weights were the most indicative of salt-tolerance. Waha, Megress and GTA dur were the most tolerant genotypes that could be used as donors of choice in wheat breeding programs targeting the improvement of salinity tolerance during the seedling stage.

Keywords: Genotypic variation, NaCl, Screening, Regression, Tolerance, *Triticum durum*.

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INTRODUCTION

Nowadays, cereals in general and wheat in particular, constitute the main basis of the diet for Algerian consumers (Rastoin and Benabderrazik, 2014). It has a social, economic and political role in most countries around the world. In recent decades, wheat breeders have plaied an increasingly important role in increasing production, both in developed and developing countries (Pfeiffer et al., 2000; De Vita et al., 2007; Aisawi et al., 2015; Gummadov et al., 2015; Zhang et al., 2016; Balota et al., 2017; del Pozo et al., 2019). However, several challenges are actally facing them to make food more accessible than ever in a sustainable way and to meet the needs of an ever-increasing human population (Tester and Langridge, 2010; Benbelkacem, 2013; Shiferaw et al., 2013).

Many biotic and abiotic stresses affect the crop's yield globally and its future growth will most likely come from non-favorable environments where such stresses play a much larger role in yield determination (Buck and Nisi, 2007). Salinity has been identified as the major seedbed factor influencing growth and productivity worldwide (Munns and Tester, 2008; Zörb et al., 2019). Because of salinization, the world loses 10 hectares of cultivable land per minute (Hasan et al., 2015) that could be accelerated by climate change, excessive use of groundwater and low-quality water in irrigation associated with the intensive farming and poor drainage (Machado and Serralheiro, 2017). In the south Mediterranean region, breeding for high salinity thresholds becomes imperative for agricultural production (Paranychianakis and Chartzoulakis, 2005; Maggio et al., 2011). Algeria, which offers all the variants of the Mediterranean climate, is not immune to this phenomenon, where drought, which has been observed a serious risk and a recurrent feature in the last decades, clearly led to the soil salinization process on 3.2 million hectares affected (Benmahioul et al., 2009). Almansouri et al. (2001) revealed that salt and water stresses delayed and inhibited germination processes by acting on different parameters in relation to water uptake, which is reduced in response to water induced stress.

Cereal crops growing in saline soils undergo physiological and biochemical disturbances. Breeding for salt tolerance is a quality widely sought in plants of agronomic interest like wheat to expand their culture in such growing regions, where this kind of stress is largely coupled with water stress (Bartels and Sunkar, 2005; Ahmed et al., 2015). To achieve this goal, it is fundamental to find sources of tolerance that could be available in germplasm collections, landraces or indigenous cultivars of wheat crop. Improving currently used wheat varieties by introgression of exotic alleles/genes from these sources in breeding programs would be an efficient approach to overcome the salt sensitivity. Therefore, screening of this plant material is necessary to identify tolerant parental lines which may be crossed to generate useful segregating lines. A such screening requires knowledge of mechanisms responsible for plant tolerance to salinity which is a very complex phenomenon that involves morphological and developmental features with various physiological and biochemical mechanisms (Munns, 2002; Munns and Tester, 2008). In fact, the degree of response to salinity depends on the species, the variety within the same species, the developmental stage of plant and the salt concentration level (Ben Naceur et al., 2001). Germination percentage and seedling growth are the most sensitive and critical stages in plant development. Salinity generally delays or prevents seed germination and seedling establishment (Aflaki et al., 2017). According to Feyzi (2003), it is recommended to start the screening as soon as germination has occurred. This process may not be helpful in finding tolerant genotypes in subsequent stages of plant growth under conditions of salt stress. The present work aims to (*i*) study the effect of sodium chloride (NaCl) on seed germination and growth parameters of durum wheat at different concentration levels, and (*ii*) identify sources of tolerance to be directly used in breeding programs for efficiently breeding cultivars with improved salinity tolerance.

MATERIALS and METHODS

Plant materials and plant growth conditions

The experiment was implemented at the National Center for seeds and plants Control and Certification (CNCC), regional laboratory of Sétif (Algeria) using a hydroponic system. Eleven durum wheat genotypes were subjected to 4 salt stress levels during germination and seedling growth stages. Name, origin and pedigree of the genotypes studied are shown in Table 1. To assess salt tolerance during germination, 100 seeds per genotype were placed on towel paper in Petri dishes containing 15 ml distilled water (control) or NaCl solutions at three concentrations (50, 100, 150 mM). Seeds were incubated an automatic seed germinator at 22 °C, a 16/8 hours photoperiod, and 85.0% relative humidity average. The experimental unit consisted of a Petri dish. Four dishes were used per genotype, where three dishes served for germination test and the fourth one was intended to assess the effect of NaCl on seedling's growth. For germination test, daily counts of germinated seeds were made during the experiment that ended 7 days after planting. Seeds were considered germinated when their radicle was at least 2 mm out (Adjel et al., 2013). For seedlings growth, the sprouted seeds of the 4^{th} Petri dish (0 mM) were transferred after 48 hours into test tubes containing 25 ml of water at the same intensities of salt stress previously used for the germination test. Cotton flasks were used as a seed carrier in the test tubes. The different treatments were repeated 10 times (1 seed per tube and 10 seeds per treatment and per genotype). The experimental unit consisted of a test tube. The experiment lasted 10 days after transplantation.

Table 1. Name, pedigree and origin of the durum wheat genotypes studied.

No	Name	Pedigree	Origin
1	Boutaleb	GTA dur /Ofanto	Algeria
2	GTA dur	Gaviota/Durum	Cimmyt-Icarda
3	Megress	Ofanto//Waha/Mohamed Ben Bachir	Algeria
4	Oued El Bared	Hedba 3/Ofanto	Algeria
5	Sitifis	Bousselam/Ofanto	Algeria

6	Mohamed Ben Bachir	Local landrace	Algeria
7	Bousselam	Can 2109//Jo/AA/3/S15/Cr	Icarda
8	Waha	Plc/Ruff//Gta/Rtte	Icarda
9	Ofanto	Appulo/Adamello	Italy
10	Simeto	Capeiti 8/Valnova	Italy
11	Guemgoum Rkhem	Local landrace	Algeria

Cimmyt: International Maize and Wheat Improvement Center, Icarda: International Center for Agricultural Research in the Dry Areas.

Growth measurements and statistical analysis

Seed germination percentage (G%) was calculated as follow: %G = 100 (GS/TS), where GS: germinated seeds and TS: total seeds incubated. Seminal roots number (RN) was counted on all the germinated seeds in the test tubes. Maximum root (RL) and coleoptile (CL) lengths were also recorded on the same sample. Shoot (SFW) and root (RFW) fresh weights were also determined per seedling.

Data collected were statistically analyzed using the balanced analysis of variance (ANOVA) with two criteria of classifications to compare the different levels of salt stress as well as the behavior of the varieties evaluated with respect to this environmental constraint. Whenever ANOVA showed significant differences, the treatment's means were compared using the least significant difference test at the 0.05 probability level (LSD 0.05). Where 'salinity' effect was significant, linear and curvilinear (quadratic) regression analysis were applied. In addition, discriminating traits between the tested varieties for the response to NaCl were identified through a univariate clustering analysis of the tolerance indices data matrix generated by ranking the tested genotypes in each level of salinity and for each trait measured. Statistical analyzes were performed using R Studio, Inc. version 1.2.5033 © 2009-2019 and the graphs were generated with custom R scripts based on the "ggplot2" package (Wickham, 2009).

RESULTS and DISCUSSION

The *F*-test of the analysis of variance indicated significant 'genotype' and 'salinity' main effects and significant 'genotype*salinity' interaction for the measured traits, with the exception of germination percentage (Table 2). Salinity levels followed by genotype effect were found highly significant as sources of variance for all measured traits although the first source explained almost all the genetic variation observed in the evaluated plant material (Table 2). Aflaki et al. (2017) and Fellahi et al. (2019) also found that the salinity marked the major effect when compared to the genotype in spring wheat. Conversely, Yildirim et al. (2015) showed that the total variation observed in durum wheat was better explained by genotype main effect when using the same NaCl concentrations (0, 50, 100, 150 mM). This divergence could be attributed to the genetic background of the plant material tested.

Table 2. Mean squares of the analysis of variance of traits measured the durum wheat studied.

SV	df	%G	RL	RN	CL	RFW	SFW
Genotype (G)	10	138.0*** (39.5%)	74.2*** (7.8%)	8.0*** (11.4%)	7.9*** (14.6%)	484.9*** (10.8%)	10966.3*** (6.5%)
Salinity (S)	3	194.5*** (55.7%)	782.0*** (82.5%)	58.3*** (83.0%)	39.8*** (74.0%)	3742.6*** (83.0%)	152541.2*** (90.7%)
Linear	1	573.1*** (98.2%)	2322.0*** (99.0%)	162.3*** (92.8%)	103.7*** (86.8%)	10221.2*** (91.0%)	429868.2*** (93.9%)
Quadratic	1	10.3^{ns} (1.8%)	24.0^{ns} (1.0%)	12.5*** (7.2%)	15.8*** (13.2%)	1006.7*** (9.0%)	27755.3*** (6.1%)
G*S	30	8.8^{ns} (2.5%)	83.3*** (8.8%)	3.0*** (4.3%)	5.3*** (9.9%)	214.1*** (4.8%)	3864.4*** (2.3%)
Error	88/396¤	7.9 (2.3%)	8.0 (0.9%)	0.9 (1.3%)	0.8 (1.5%)	70.0 (1.6%)	893.5 (0.5%)

%G: Seed germination percentage, RL: Root length, RN: Roots number, CL: Coleoptile length, RFW: Root fresh weight, SFW: Shoot fresh weight, ^{ns} and *** indicate non significance and significance at $p < 0.001$, respectively; ¤: 88 and 396 are the degrees of freedom for seed germination percentage and growth parameters, respectively.

Averaged over salinity levels, our findings showed that the mean seed germination percentage varied from 88.17% (Simeto) to 98.75% (GTA dur and Megress), the root length from 4.70 cm (Ofanto) to 9.85 cm (GTA dur), the number of roots from 2.93 roots (Sitifis) to 4.70 roots (GTA dur) per germinated seed, the coleoptile length from 1.89 cm (Sitifis) to 3.16 cm (Guemgoum Rkhem), the root and shoot fresh weights from 5.78 mg (Sitifis) 17.18 mg (GTA dur) and 47.40 mg (Sitifis) to 98.45 (GTA dur), respectively (Table 3). Genotype main effect indicated that GTA dur was tolerant to salinity, based on all the traits measured while Sitifis was highly sensitive (Table 3).

Table 3. Genotype main effects of traits measured in the durum wheat genotypes studied.

Genotypes	%G	RL	RN	CL	RFW	SFW
Boutaleb	95.83^{b}	8.89^{ab}	3.95^{cd}	1.98^{de}	11.78^{bc}	74.31^{cd}
GTA dur	98.75^{a}	9.85^{a}	4.70^{a}	2.88^{ab}	17.18^{a}	98.45^{a}
Megress	98.75^{a}	7.16^{cd}	4.33^{abc}	2.30^{cd}	11.00^{bc}	62.58^{def}
Oued El Bared	92.67^{c}	8.20^{bc}	4.19^{bcd}	1.98^{de}	7.00^{de}	70.95^{cde}
Sitifis	92.92^{c}	7.60^{cd}	2.93^{e}	1.89^{e}	5.78^{e}	47.40^{g}
Mohamed Ben Bachir	97.83^{ab}	8.90^{ab}	4.20^{bcd}	2.94^{ab}	8.88^{cde}	61.75^{def}
Bousselam	97.42^{ab}	7.39^{cd}	4.28^{bcd}	2.93^{ab}	11.60^{bc}	63.28^{def}
Waha	96.83^{ab}	6.63^{d}	4.15^{bcd}	2.68^{bc}	8.15^{cde}	50.98^{fg}
Ofanto	92.08^{c}	4.70^{e}	4.03^{bcd}	2.41^{c}	6.35^{de}	58.68^{efg}
Simeto	88.17^{d}	7.56^{cd}	4.40^{ab}	2.40^{c}	9.90^{cd}	94.02^{ab}
Guemgoum Rkhem	93.00^{c}	7.12^{cd}	3.90^{d}	3.16^{a}	14.30^{ab}	82.98^{bc}
Average	94.93	7.63	4.09	2.50	10.17	69.58
$LSD_{0.05}$	2.28	1.25	0.42	0.40	3.68	13.14

%G: Seed germination percentage (%), RL: Root length (cm), RN: Roots number (No), CL: Coleoptile length (cm), RFW: Root fresh weight (mg), SFW: Shoot fresh weight (mg), similar letters are not significantly different at 0.05 probability level of LSD.

Salt affected all the measured traits as indicated by the significant salinity main effect of the analysis of variance. Furthermore, the *F*-test of ANOVA exhibited a highly significant linear regression ($p < 0.001$) for seed germination percentage and roots number, but there were no significant quadratic trends (Table 2). Both linear and curvilinear (quadratic) regression were significant ($p < 0.001$) for root length, coleoptile length, root and shoot fresh weights, which gave evidence of an inverse relation between these growth parameters and salinity levels (Table 2). The presence of both significant negative linear and quadratic regression trends indicates that not only has the traits mean values increased over salinity, but it has also increased at an increasing rate. Linear regression mean squares, accounted for 98.2, 99.0, 92.8, 86.8, 91.0 and 93.9% of the variation in %G, RL, RN, CL, RFW and SFW, respectively. Mean squares due to curvilinear regression accounted only for 1.8, 1.0, 7.2, 13.2, 9.0, 6.1 % of the total sum of squares of salinity main effect, respectively (Table 2).

Averaged over genotypes, the data in Table 4 indicate that mean germination percentage decreased from 97.42% for the control to 91.97% under the supply of 150 mM NaCl. Intake of 150 mM NaC1 solution decreased progressively root length from 10.62 to 4.67 cm. Similarly, the number of roots ranged from 4.98 to 3.45 roots seedling^{-1} under the same treatments. The coleoptile length regressed from 3.06 to 1.93 cm, and the root and shoot fresh weights from 18.00 to 5.32 mg and 110.65 to 33.52 mg per germinated seed, respectively as salinity increased from none to 150 mM NaCl treatment (Table 4).

Table 4. Salinity main effects of traits measured in the durum wheat genotypes studied.

Salinity (mM)	%G	RL	RN	CL	RFW	SFW
0	97.42^{a}	10.62^{a}	4.98^{a}	3.06^{a}	18.00^{a}	110.65^{a}
50	96.30^{a}	8.96^{b}	4.40^{b}	2.98^{a}	11.22^{a}	91.16^{b}
100	94.03^{b}	6.28^{c}	3.55^{c}	2.04^{b}	6.15^{b}	42.98^{c}
150	91.97^{c}	4.67^{d}	3.45^{c}	1.93^{b}	5.32^{c}	33.52^{d}
$LSD_{0.05}$	1.37	0.75	0.25	0.24	2.21	7.92

%G: Seed germination percentage (%), RL: Root length (cm), RN: Roots number (No), CL: Coleoptile length (cm), RFW: Root fresh weight (mg), SFW: Shoot fresh weight (mg), similar letters are not significantly different at 0.05 probability level of LSD.

Relatively to the control (0 mM), the results of the present study indicated that salinity effect varied according to the trait measured and the intensity of stress applied (Fig. 1). Low-dose salt stress (50 mM) had mild effect on the measured variables, the rate growth of seedlings continued relatively and reductions registered did not exceed 20%, except for RFW, suggesting tolerance to this salt stress level. However, significant decreases were exhibited under moderate (100 mM) and severe (150 mM) levels of sodium chloride.

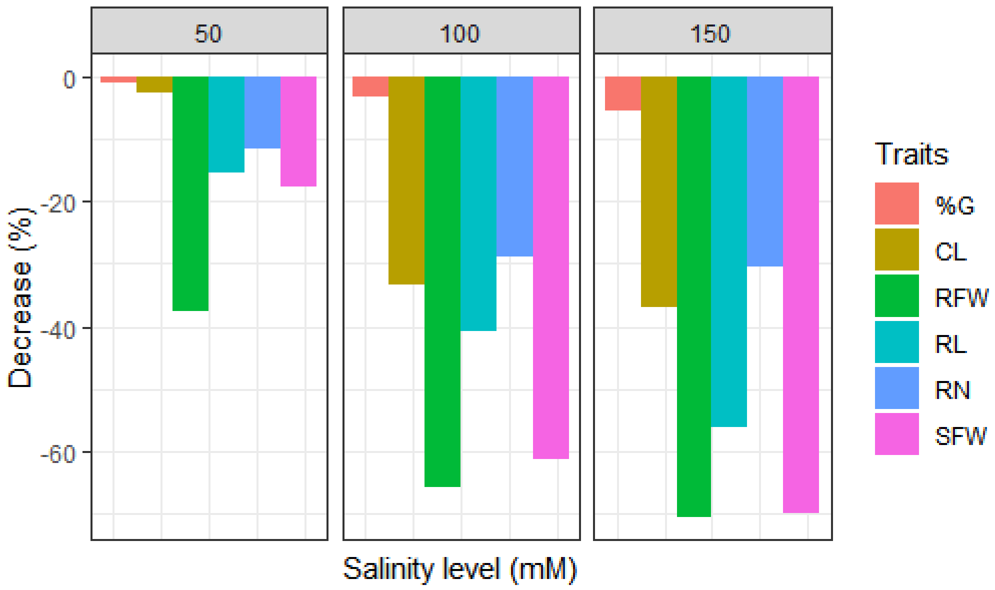

Figure 1. Relative decreases (%) in durum wheat traits relatively to the control.

%G: Seed germination percentage, RL: Root length, RN: Roots number, CL: Coleoptile length, RFW: Root fresh weight, SFW: Shoot fresh weight, means within each column followed by the same letter are not significantly different from each other based on the 0.05 probability level of LSD.

Our results showed that %G, RL, RN, CL, RFW and SFW estimates declined, as compared to the control non treated, by 5.6, 56.1, 30.7, 37.0, 70.5 and 69.7%, respectively (Fig. 1). Root length, root and shoot fresh weights were the most affected among the other traits studied. The decrease in root and shoot development may be attributed to toxic effects of the higher salt stress intensity as well as unbalanced nutrient uptake by the seedlings. The results are in harmony with those of Yildirim et al. (2015) and Fellahi et al. (2019) who also showed that germination percentage, coleoptile, root and shoot length and fresh weights decreased in wheat varieties with increasing salinity level. These decreases are brought about by modification of ionic balance, water status, mineral nutrition, and carbon allocation and utilization as indicated by Munns and James (2003).

The significant ‘Genotype*Salinity’ interaction of the traits measured indicated that the responses of the tested set of genotypes varied among salt treatments (Table 2). The range and mean values of all the studied characters indicated wide ranges of variation which revealed possible amount of variability among the genotypes (data not shown). The decrease or increase in the traits measured, over the 3 salt treatments, varied among the tested cultivars, between 0 and -15% for %G, 199 and -80% for RL, 4 and -64% for RN, 14 and -88% for CL, 52 and -95% for RFW, 3 and -96% for SFW. High salt treatment was more discriminating between salt tolerant and salt sensitive genotypes than the low salt treatment. In general, the pattern of cultivar responses varied within each trait and between traits. This leads to the variation of the tolerance among cultivars according to the various traits used as selection criteria. A given cultivar is tolerant or sensitive depending on the trait used for its classification. These results suggested that salinity tolerance is a complex trait, affected by a large number of mechanisms as mentioned by Ashraf and Haris (2004) as well as Munns and Tester (2008).

In order to summarize the results obtained and properly judge the behavior of the genotypes tested and identify the most tolerant that had the capacity to accumulate more fresh matter in the shoots and roots over the entire levels of salinity, we adopted the following method. Initially, all genotypes were ranked for each trait and each salt treatment based on the discrepancy's values (decrease or increase compared to the control) previously discussed, in which the genotype with the highest value was ranked first. Subsequently, the sum of ranks per genotype was calculated to determine the new rank, in which the genotype with the lowest value was the most tolerant. The order of classification and the sum of the ranks obtained are given in Table 5. The results indicated that a given genotype did not keep the same position for all traits measured and all the levels of stress applied. The sum of ranks ranged between 62 for Waha and 169 for Sitifis. Finally, the obtained values were subjected to a univariate clustering, in which we fixed a number of classes equal to three. This approach clustered Waha, Megress and GTA dur together (cluster 1). Boutaleb, Oued El Bared, Mohamed Ben Bachir, Bousselam, Ofanto, Simeto and Guemgoum Rkhem formed the second cluster (cluster 2), while Sitifis formed the third cluster (cluster 3).

Table 5. Stress tolerance indices of traits measured the durum wheat genotypes studied.

Traits	%G			RL			RN			CL			RFW			SFW			Σ ranks
Genotypes	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	
Boutaleb	1	3	2	6	4	9	5	10	9	7	9	8	11	10	10	1	7	10	122
GTA dur	3	1	3	8	7	7	4	3	1	5	1	1	10	8	4	2	2	2	72
Megress	4	2	4	5	5	6	1	5	4	1	5	4	1	2	3	4	6	4	66
Oued El Bared	11	9	8	9	10	10	2	4	8	3	2	7	6	3	8	10	3	7	120
Sitifis	5	8	7	7	8	11	11	11	11	10	10	11	9	11	11	7	10	11	169
Mohamed Ben Bachir	7	4	1	10	11	8	10	1	6	11	6	9	8	4	6	11	9	8	130
Bousselam	2	5	5	11	9	5	7	2	5	9	3	2	7	7	5	8	5	3	100
Waha	6	6	6	1	1	1	8	8	3	6	4	3	2	1	1	3	1	1	62
Ofanto	10	7	9	3	2	2	9	6	7	8	8	6	3	6	2	9	4	5	106
Simeto	8	11	11	4	6	4	3	7	2	2	7	5	4	5	7	6	8	6	106
Guemgoum Rkhem	9	10	10	2	3	3	6	9	10	4	11	10	5	9	9	5	11	9	135

%G: Seed germination percentage, RN: Roots number, RL: Root length, CL: Coleoptile length, RFW: Root fresh weight, SFW: Shoot fresh weight.

Genotypes of cluster 1 were the most tolerant over the whole range of salt treatments tested, in descending order (Fig. 2). Such finding suggests that these genotypes possess sufficient plasticity to respond to soil salinity constraint as well as an implication of significant salt tolerance mechanisms as reported by Allel et al. (2019). Sitifis in cluster 3 was the most sensitive cultivar, while the second cluster contained the remaining genotypes that had an intermediate behavior regarding their tolerance to NaCl treatment (Fig. 2). Genotypes in clusters 2 and 3 decreased their germination percentage and all the other growth parameters compared to those of cluster 1 (Fig. 2). Root and shoot fresh weights, and to a lesser extend roots number and coleoptile length discriminated efficiently between genotypes as far as tolerance to salinity is concerned. Some studies revealed that seed germination rate could be used as a valuable criterion for the screening of salinity resistance. Adjel et al. (2013) reported that root length and germination percentage were the most discriminating traits between salt-sensitive and tolerant barley cultivars. Our results agree with those given by Almansouri et al. (2001) who demonstrated that germination percentage in stress conditions do not appear to be used as a valuable selection criterion for the improvement of salinity tolerance in durum wheat.

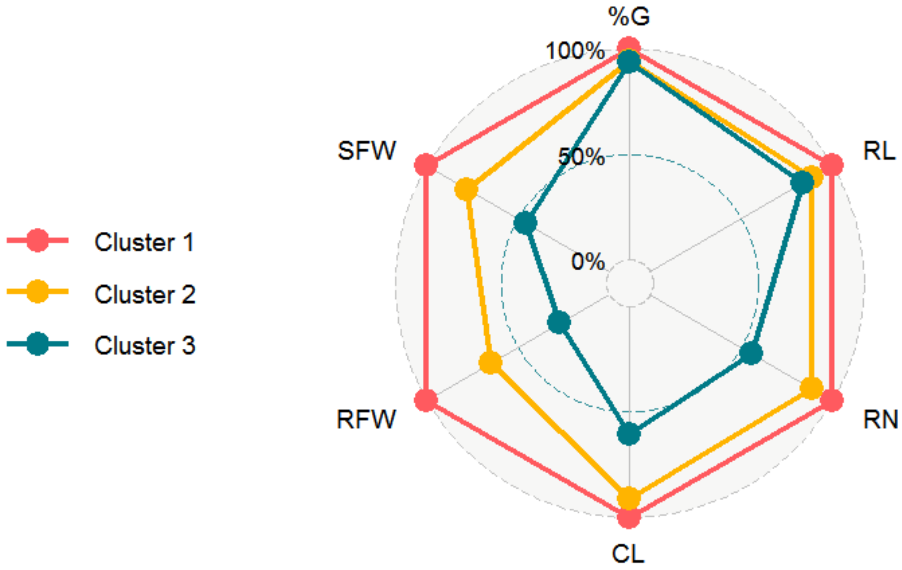

Figure 2. Radar chart comparing the traits measured of the three genetic clusters identified.

%G: Seed germination percentage, RL: Root length, RN: Roots number, CL: Coleoptile length, RFW: Root fresh weight, SFW: Shoot fresh weight.

Conclusion

The present work focused on the effects of sodium chloride (NaCl) at germination and seedling growth stages of some durum wheat genotypes. The results showed decreases in the measured traits at both growth stages as salt levels increased, however, the NaCl had greater inhibitory effects on seedling growth than on seed germination rate. The seed germination rate and root length showed only a significant and negative linear component over salinity with no significant quadratic regression component. However, roots number, coleptile length, root and shoot fresh weights showed a downward linear trend with a curvilinear component, but the linear regression was to be the more important regression since it accounts for a greater portion of the salinity variation. High salinity treatment had a very good ability to discriminate between the genotypes tested. Waha, Megress and GTA dur were the most tolerant genotypes. These genetic resources remained the donors of choice for improving salinity tolerance during the seedling stage in future breeding programs.

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