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Salt Stress Effects on Respiration and Growth of Germinated Seeds of Different Wheat (*Triticum aestivum* L.) Cultivars

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Abstract: Establishment of seedlings at early growth stages of crop plants as one of the most important determinants of high yield is severely affected by soil salinity. Therefore, high germination rate and vigorous early growth under salty soils is preferred. In this study salt tolerance of wheat cultivars were examined at germination and seedling growth stages. Seeds were germinated and grown in long dark cups using distilled water as control and two levels of salt stress imposed by 9 and 15 ds/m NaCl solution for 48 hours. Coleoptile and root growth was measured as the response of cultivars to salinity. Seedling respiration was expressed as the difference between initial seed weight and seedling dry weight after 48 hours. Significant differences were found among cultivars in terms of coleoptile and root growth under salt stress condition. Differences among cultivars in terms of respiration rate were also significant indicating that genetic variation exists among wheat cultivars. It was also found that seedling respiration was decreased as salinity level was increased. Significant correlation coefficients were found between coleoptile growth and respiration under all condition. Salt stress inhibited coleoptile growth more than root growth. It was concluded that wheat seedling maintenance respiration is higher that what is estimated for C_3 plants.

Key words: Salt stress · Respiration · Germination · Wheat

INTRODUCTION

During their growth crop plants usually exposed to different environmental stresses which limits their growth and productivity. Among these, drought and salinity are the most severe ones [1]. It has been estimated that more than 20% of all cultivated lands around the world containing levels of salts high enough to cause salt stress on crop plants [2-4]. This is particularly the problem of agriculture in arid and semiarid regions of the world [3,5-8]. Soil washing and improving soil drainage are among remedies which are practical only where fresh water is available at low price. Improving salt tolerant varieties on the other hand, is of major importance and efforts should be focused on finding mechanisms which are involved in salinity tolerance. This may lead us to find gene sources [9] as well as methods for screening large number of genotypes for salt tolerance.

Salt stress affects many physiological aspects of plant growth. Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point [10]. Early flowering reduced dry matter, increased root: shoot ratio and leaf size caused by salinity may be considered as possible ways of decreasing yield in wheat under salt stress condition [11-13]. Toxic effects of salts may change enzymatic activity and hormonal balance of plants. It is reported that high transpiration rate at leaf surfaces cause high accumulation of salt in leaves which kill them before full maturity [14].

Net photosynthesis was decreased due to the reduction in photosynthesis and increasing in respiration per unit of leaf area. Studies on physiological salt tolerance mechanisms revealed that plants may reduce detrimental effects of salts by the control of salt uptake [15] reducing damage under excessive ion uptake [4,16] and osmotic adjustment [17].

Growth inhibition by salt stress also occurs due to the diversion of energy from growth to maintenance [18]. To maintain under salt stress plants need to regulate ion concentration in various organs and within the cells

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by synthesis of organic solutes for osmoregulation or protection of macromolecules for maintenance of membrane integrity [14].

It has been reported that increasing salt concentration in growing medium of pea plants increased roots and stems respiration [18,19]. Soaking the seeds before germination in ascorbic acid and thiamin solutions has been suggested as a remedy to increase seedling growth due partly to reduction in dark respiration under saline condition [20]. Nieman [18] found that growth inhibited by salt partly due to the diversion of energy from growth to maintenance.

Salt stress effect on wheat seedling respiration is not well documented. Other environmental stresses such as water stress have been shown to affect electron partitioning and alternative pathway in leaves [21]. However, results of many cases show that respiration decreases under water stress condition [21], though decrease in respiration is much less than photosynthesis. Salt stress was also showed to increase the activity of an alterative pathway along with the cytochrome pathway [21]. A salt tolerant wheat cultivar was showed to produce more ATP than a salt sensitive one [21].

Crop plants are usually seeded within the top 10 cm layer of the soil where it usually contains highest amount of salt [22]. For winter crops such as wheat, soil may contain even more salts at sowing because of high rate of evaporation in the previous summer fallow during which salts migrate to the soil surface (Authors unpublished data). To produce satisfactorily under saline conditions seeds must germinate and seedlings must vigorously pass through the salty layer of the soil and survive [23]. Under such condition vigorous seedling growth is very important for crop establishment. Germination is an important stage in the life cycle of crop plants particularly in saline soils as it determines the degree of crop establishment. Rapid and uniform seed germination under saline condition not only increases early seedling establishment but also has the advantage of higher drought tolerance [24].

Wheat is the major cereal crop in Iran. It is cultivated over a wide range of environments because of wide adaptation to diverse environmental conditions. It is a moderately salt-tolerant crop [25]. When the salinity in creased to 100 mM NaCl (about 10 ds/m), wheat performance is decreases marginally compare to other crops liker rice [26]. Therefore, it is a promising crop for cultivation in moderate salty soils of semi arid regions [27]. A collection of bread wheat cultivars were recommended for cultivation during recent years among them some are recommended for saline soil condition. However, little is known about the mechanisms enable them to have higher yield under salt stress condition. The aim of this study was I) to compare wheat cultivars salt stress tolerance at early growth stages and ii) wheat capability to use the energy obtained in respiration for maintenance under salt stress condition.

MATERIALS AND METHODS

Salt tolerance of thirty three wheat cultivars was examined at germination and early growth stage. Table 1 shows some plant features and origins of these cultivars. Seeds were germinated under control and salt stress conditions. Since coleoptile and root growth was intended to be continued for more than what is usually considered enough for germination tests and also to simulate the conditions similar to what is exists in the soil, long dark cups were used as germinating media. There were 20 seeds in each cup. Initially each seed was carefully weighted in gram up to five decimal places using an analytical balance (ALE-40SM- Shimatzu (accuracy 0.00001 g)). Seeds were then soaked for 2 hrs in tap water, surface sterilized with 2% (v/v) commercial bleach solution for 3 min and then were rinsed with distilled water and placed on Watman paper in the cups. At first 12 ml of distilled water, which was enough for imbibitions was added. Two levels of salt stress imposed on seeds. Thirteen ml of NaCl solution whose electrical conductivity (EC) was adjusted at a ds/m was added to one group of cups as low stress. The same amount of 15 ds/m NaCl solution was added to the other group as high stress. Thirteen ml of distilled water was also added to control ones. Final length of coleoptile and roots were measured 48 hrs after application of treatments. All measurements were done with a small transparent ruler which was fixed on the surface of a binocular. Seedlings washed and were then oven dried at 60 °C for 24 hours and weighted. Since some amount of carbohydrates stored in the seeds are used to provide the energy needed for initial growth of seedlings after germination, respiration was expressed as the difference between initial seed weight and seedling dry weight.

Growth and maintenance respiration were estimated statistically. A linear regression analysis was performed to obtain the relationships between mean values of respiration and growth of cultivars under each level of salt stress condition using the standardized data. The line slope was considered as the efficiency of wheat in using energy obtained by respiration for seedling growth.

Table 1: Characteristics of wheat cultivars used in the experiment

Cultivar	1000 grain weight (g)	Response to environmental stresses	Yield (t/ha)	Cultivar	1000 grain weight (g)	Response to environmental stresses	Yield (t/ha)
Ghods	42.0	-	6.0	Omid	39.0	-	4.0
Navid	41.0	Semi-tolerant to cold stress	5.0	Azar2	46.0	Tolerant to drought and relatively tolerant to cold stress	4.0
Hirmand	37.0	Tolerant to salt and drought stress	5.0	Rowshan	32.5	Tolerant to salt and drought stress	4.0
Rasoul	36.0	-	4.0	Khazar	40.0	Susceptible to cold stress	4.3
Alvand	40.0	Tolerant to salt, cold and drought stress	6.5	Toos	38.0	Tolerant to drought and cold stress	6.3
Alamot	40.0	-	4.0	Shahryar	40.0	-	2.7
Mahdavi	49.0	Tolerant to salt stress	7.0	Shiraz	38.0	Tolerant to drought stress	7.4
Zarin	39.0	Relatively tolerant to cold stress	6.4	Dez	42.5	Tolerant to terminal heat stress	6.0
Darab2	37.5	-	5.9	Hamoun	44.5	Tolerant to drought and salt stress	6.6
Tajan	38.0	-	6.3	Pishtaz	30.0	Tolerant to terminal drought stress	4.7
Atrak	35.0	-	5.8	Saisoun	32.0	-	4.0
Niknejad	37.0	Tolerant to drought stress	6.7	Gascojen	39.0	-	4.0
Kavir	38.0	Tolerant to salt and terminal drought stress	6.3	Gaspard	32.0	-	5.0
Chamran	39.0	Tolerant to heat and drought stress	6.3	Rowshan BC (winter)	45.0	-	4.5
Shiroud	38.0	-	6.5	Rowshan BC (spiring)	38.0	-	3.5
Marvdasht	36.0	-	6.7	Falat	35.0	-	4.5
Sardary	39.0	Tolerant to cold stress	3.5				

Maintenance respiration due to salt stress, assuming that it is negligible under control condition, was expressed as the difference between slopes in the relationship between respiration and growth under control and each level of salt stress.

The layout of the experiment was a factorial based on Randomized Complete Block Design with three replications. Room temperature during the experiment was 22-23°C. Data were subjected to analysis of covariance, taking initial weight of the seeds as covariate. In all cases the covariate was showed to have a significant effect on seedling growth characteristics, therefore, adjusted data were calculated and used for further analysis.

RESULTS

Genotypes and salinity effects were highly significant on all plant characteristics. As a result mean values of genotypes and salinity levels were different from each other (Table 2). Significant genotype by salinity interaction was also found in terms of all plant characteristics.

Under control condition the highest and the lowest coleoptile growth were observed in Rowshan BC (winter type) (24.70 mm) and Shiraz (12.62 mm) respectively. Increasing salinity significantly decreased coleoptile growth. Coleoptile growth under high salt stress was reduced by 67.73% compared to control condition (Fig. 1). Root response to salinity was the same as what was observed in the case of coleoptile. The highest root growth was observed in cultivars Dez, Omid and Shiroudi (37.36 mm), while lowest growth was in Shahriar and Khazar (13.12 mm). Roots were elongated deep to 25.41 mm under control condition, while their growth was averaged to 8.75 mm under high salt stress condition which means a 34.45% reduction in root growth (Fig. 1).

Significant correlation coefficients were found between coleoptile and root growth under both low and high salt stress condition (Table 3). At both salt stress levels, analysis of seedling dry weight data showed highly significant differences among wheat genotypes. Salt stress significantly reduced seedling dry weight at significant rates. Salinity x genotypes interaction indicated that genotypes responded differently to salinity stress. Seedling growth of all genotypes severely reduced under salt stress compared to the control. The highest values of root growth under low stress were observed in cultivars Mahdavi, Rowshan BC (winter type) and

Table 2:Mean squares in the analysis of variance of wheat seedling characteristics germinated and grown under control and salt stress conditions

S. V.	df	Coleoptile growth	Root growth	Respiration	Seedling dry weight
Cultivar	32	26.06**	93.04**	0.0009**	0.0008ns
Salt stress	2	907.06**	1716.16**	0.0004**	0.0005**
Cul* S. stress	64	6.15**	17.91*	0.0008*	0.0007ns
Seed weight	1	4.46ns	18.42ns	0.0005**	0.0001**
Error	195	4.99	14.64	0.0006	0.0002

Table 3: Pearson correlation coefficients between wheat seedling characteristics and respiration of wheat cultivars grown under control and salt stress conditions

		Seedling dry weight			Root growth			Coleoptile growth			Respiration		
		Control	Low stress	High stress	Control	Low stress	High stress	Control	Low stress	High stress	Control	Low stress	High stress
Seedling dry weight	Control	1											
	Low stress	0.29ns	1										
	High stress	0.25ns	0.53**	1									
Root growth	Control	-0.29ns	-0.42*	-0.23ns	1								
	Low stress	-0.46**	-0.42*	-0.16ns	0.87**	1							
	High stress	-0.41**	-0.39*	-0.20ns	0.92**	0.97**	1						
Coleoptile growth	Control	-0.45**	-0.62**	-0.37*	0.74**	0.74**	0.71*	1					
	Low stress	-0.40*	-0.58**	-0.32ns	0.38*	0.49**	0.43*	0.74**	1				
	High stress	-0.43*	-0.66**	-0.37*	0. 31*	0.61**	0.61**	0. 88**	0.84**	1			
Respiration	Control	-0.53**	-0.27 ns	-0.29 ns	.036 ns	0.38 ns	0.36 ns	0.37*	0.35 ns	0.39*	1		
	Low stress	-0.63**	-0.65**	-0.58**	.038 ns	0.60*	0.46**	0.59**	0.57**	0.65**	0.31 ns	1	
	High stress	-0.45**	-0.82**	-0.68**	.038 ns	0.79*	0. 69**	0.61**	0.61**	0.65**	0.22 ns	0.62**	1

*and**: significant at 5% and 1%probability level, respectively. ns: Non-significant

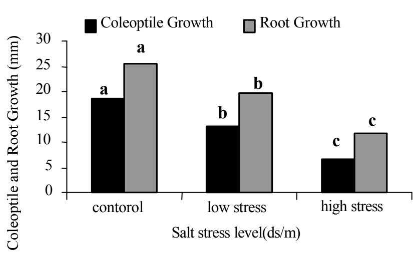

Fig. 1: Mean Coleoptile and Root growth (mm) of wheat cultivars grown under control and salt stress conditions

Hamoun (12.49 mm). The same values under high stress were observed in Atrak, Mahdavi and Dez (26.16 mm). The highest coleoptile growth under low and high salt stress levels observed in Rowshan (18.44 mm) and Alvand (9.18 mm), respectively. Root: shoot ratio, increased under salt stress condition (Fig. 2b).

Genotypes respiration, however, increased under salt stress condition (Fig. 2a). Maximum and minimum values of respiration rate were found in Alvand (4.1 mg/day) and

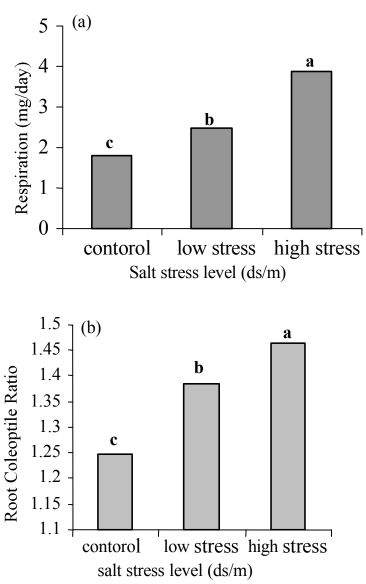

Fig. 2: Mean values of respiration (mg/day) (a) and ratio of Root to Coleoptile growth (b) of wheat cultivars were grown under control and salt stress conditions

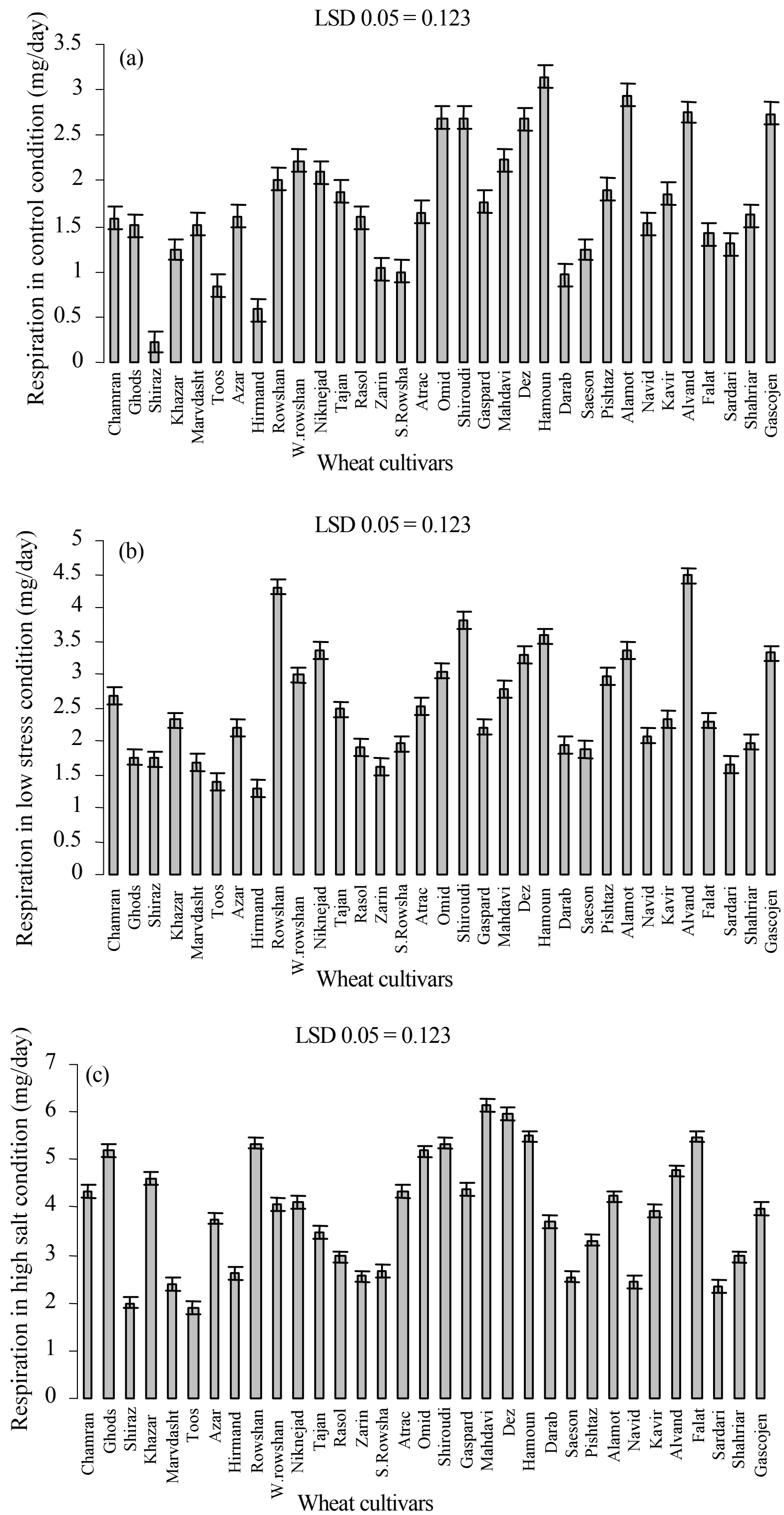

Fig. 3: Mean values of respiration (mg/day) of wheat cultivars grown under control (a), low salt stress (b) and high salt stress (c) conditions

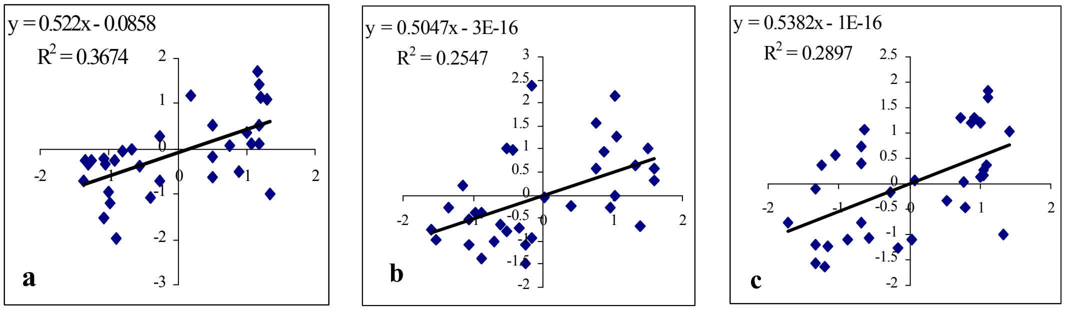

Fig. 4: Growth respiration of wheat seedlings estimated as the slope of linear relationship between seedling growth and seedling respiration (expressed as the difference between initial seed weight and seedling dry weight) under control (a), low stress (b) and salt stress (c) conditions

Shiraz (2.25 mg/day), respectively. Meanwhile, under low and high stress levels they were 6.1 in Mahdavi and Dez, 4.3 in Rowshan, 1.9 in Tous and 1.25 in Hirmand (Fig 3. a, b, c).

Generally seedlings respired 0.4622 for growth activities. Meanwhile, growth respiration values were 0.5047 and 0.5382 under low and high stress levels, respectively (Fig. 4. a, b, c).

Significant correlation coefficients were found between coleoptile growth, root growth and respiration under both low and high salt stress condition, while significant negative correlation coefficients were found between seedling dry weight and respiration under both low and high salt stress condition (Table 3).

DISCUSSION

Wheat cultivars seedling responses were different to salt stress though responses to both levels of salinity were reflected in a decrease in coleoptile and root growth. These results indicate that genetic variation exists among them in terms of early seedling growth rate under salt stress condition. It may be concluded that germination and emergence rates, as well as coleoptile and root length could be used as selection criteria for salt stress tolerance at early growth stages. Under salt stress condition elongation rate of coleoptile may decrease by low soil water potential [12] and seedlings may not be established well due to weak coleoptile and root growth. Reduced seedling growth has also been reported by Huang and Reddman [28] on barley, Foolad and Jones [29] on tomato and Jeannette *et al*. [30] on phaseolus under salt stress condition. It can be concluded that to select cultivars for better salt stress tolerance at seedling stage coleoptile and root elongation may be used as breeding criterions.

More vigorous cultivars like Rowshan and Alvand could be considered as plant materials which are useful to breeders for future development of salt tolerant wheat cultivars. Longer coleoptiles may increase their potential to emerge under salt stress condition. Results therefore could be used for prediction of sowing rates depending upon expected conditions. Results may also suggest that in salty soils, seeds are better to be sown less deep compared to normal soil condition. Higher seedling growth under salt stress condition also suggests that they may have higher performance in salty soils.

This study showed that salt stress inhibits coleoptile growth more than root growth. Similar results were found by Foolad [31], Keiffer and Ungar [6], Huang and Reddman [28] and Jeannette *et al*., [30]. Inhibitory effects of salt stress on root and shoot growth increased root: shoot ratio. This may in turn have the advantage of increased ratio of water absorption to transpiration area a plant feature which is useful for dry land condition if last longer during other growth stages.

Plant respiration was been studied using different approaches such as manometry, O_2 absorption rate, O_2 exchange rate in darkness [32, 33], PH variation in cell suspension medium [34], polarography and gas exchange measuring apparatus at different plant levels including mitochondrial fractions, whole cell, tissue segments and leaves, but there are no reports on the changes of germinated seeds dry weight as a measure of respiration, to our knowledge. However, this technique seems to be promising as a method for estimation of respiration. Since the coefficient of variation (cv) was low (2.84) and the results were in accordance with what has been obtained by Miquel *et al.* [21] and Shimazaki and Zeiger [34].

In this experiment significant correlation coefficients were found between root growth and respiration. Coefficients were highly significant under salt stress conditions. Respiration values were more closely correlated with coleoptile growth under all growing conditions (Table 3). These results suggesting that increasing respiration rate may increase seedling growth.

In this study seedling respiration was decreased in accordance with seedling growth as salt stress level increased. However correlation between seedling growth characteristics and respiration shows that under salt stress condition more respiration results in more vigorous seedlings, which may increase the degree of crop establishment.

Germinating seeds do not need to use stored carbohydrate sparingly. Therefore, the more the rate of respiration, the more expected to be the growth rate of seedlings. However, if this high rate of respiration persists long during other growth stages, it may have converse effects on final crop performance.

Significant differences between cultivars in terms of germinating seed and respiration under salt stress condition which were found in this study indicate that breeding cultivars for higher rates of respiration is possible.

Maintenance respiration was estimated to be about 0.015 in C_3 plants [35]. In germinating as heterotrophic organisms, respiration is expected to be high. Moreover, seedlings respiration under environmental stress condition also expected to be increased [35]. Values obtained in this experiment showing considerably higher values of maintenance respiration in growing seedlings compared to the above mentioned consensus value for C_3 plants. This indicates that wheat seedlings use high amounts of stored carbohydrates for maintenance of developed organs under salt stress condition. More studies are needed to determine if there are any differences among cultivars in terms of maintenance respiration under salt stress conditions.

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