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Germination and growth response of three wheat cultivars to NaCl salinity

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Abstract

Salinity adversely affects germination and establishment of crop plants. Germination and early seedling growth response of wheat to NaCl salinity was assessed in a laboratory bioassay. Seeds of three different wheat cultivars (Sehar-2006, AARI-2011 and Millat-2011) were sown in Petri dishes and three salinity levels (6.8, 13.2 and 19.0 dS m^{-1}) were imposed by developing NaCl solution concentration of 0.5, 1.0 and 1.5%, respectively. A control (distilled water) was maintained for each cultivar for comparison. Data regarding germination attributes and early seedling growth of wheat were recorded. Results revealed that increasing concentration of NaCl solution resulted in gradual reduction in seed germination and suppression of early seedling growth in all wheat cultivars. However, pronounced differences regarding salinity tolerance were observed among three wheat cultivars. Millat-2011 recorded least (11-40%) suppression in final germination percentage at different salinity levels as compared to 10-86% and 19-72% reduction observed for AARI-2011 and Sehar-2006, respectively. Millat-2011 also suffered less reduction in seedling dry biomass (21-43%) than 29-97% and 14-86% recorded for AARI-2011 and Sehar-2006, respectively. Millat-2011 appeared superior to Sehar-2006 and AARI-2011 due to its better germination and early seedling growth even at high salinity levels. Millat-2011 may tolerate moderate levels of salinity and may be tried for its field appraisal for cultivation on marginal salt affected lands.

Key words: Salt stress, wheat, germination, seedling growth, suppression, tolerance

Introduction

Salinity is one of the major abiotic environmental stresses affecting agricultural productivity (Grewal, 2010). Nearly 7 percent of world's total land area is affected by salinity (Musyimi *et al.,* 2007). It affects various plant growth and development processes resulting in reduced yield and quality (Siddiqui *et al.,* 2008; Basalah, 2010). High level of salinity results in delayed or reduced emergence in many crops (Faheed *et al.,* 2005). The problem of salinity in Pakistan is typical for irrigated agriculture where drainage is inadequate. In Pakistan, nearly 10 million ha area is badly affected by salinity, comprising 12.9 percent of country land (FAO, 2008).

Wheat (*Triticum aestivum* L.) is the staple food for more than 35 percent of world population (Jing and Chang, 2003). In Pakistan, it is the principal staple cereal and occupies 37 percent of total cultivated area. It contributes 12.5 percent to the value added in agriculture and 2.6 percent to GDP. Wheat was cultivated on an area of 8.70 million hectares in 2011-12 with estimated production of 23.50 million tons (Govt. of Pakistan, 2012). Like other crops, salinity adversely affects the growth and yield of wheat crop (Saboora and Kiarostami, 2006; Mehmet, *et al.,* 2006). Numerous studies reported the relative salt tolerance of various cultivars of agricultural crops in Pakistan (Ibrar *et al.,* 2003; Jabeen *et al.,* 2003; Ali *et al.,* 2004; Rahman *et al.,* 2008; Siddiqui *et al.,* 2008). The harmful consequences of salinity for germination and early seedling growth of crops arise presumably due to osmotic stress that prevents water uptake or specific ion toxicity (Wakeel *et al.,* 2011). However, under saline conditions, different plant species may exhibit different responses (Mehmet *et al.,* 2006; Shahid *et al.,* 2011). Crop species as well as their cultivars often differ in their tolerance to salinity. Such differences can be assessed through germination percentage and seedling growth under saline conditions. Such information is crucial for suggesting a suitable crop cultivar for salt affected soils. Present study reports the response of three improved cultivars of wheat to NaCl stress at germination and early seedling growth stage. The objective was to identify a promising wheat cultivar that can fairly tolerate and thrive under saline conditions.

Materials and Methods

Seed procurement

Seeds of three improved wheat cultivars (Millat-2011, AARI-2011 and Sehar-2006) were collected from Wheat Research Institute, Faisalabad. Seed were surface sterilized with 30% ethanol for 3 min and washed thoroughly with distilled water to remove the traces of ethanol (Srivastava *et*

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al., 2010). Healthy seeds of all the cultivars were used in the experimentation.

Preparation of NaCl solutions

Saline conditions were simulated by employing aqueous NaCl solutions. For this purpose, different concentrations viz., 0.5, 1 and 1.5% (w/v) of NaCl solution were made by dissolving analytical grade NaCl (Merck, USA) in distilled water. The electrical conductivity (EC) of each solution was measured using a digital conductivity meter (HI-9811, Hanna, USA). The EC of 0.50%, 1% and 1.5% NaCl solution was 6.8, 13.2 and 19.0 dS m^{-1}, respectively. A distilled water control was run for comparison.

Bioassay

Seeds (15) of respective each wheat cultivar were evenly placed between two layers of a Whatman no. 41 filter paper in a 9 cm diameter Petri dish. Salinity levels were imposed by exposing these seeds to different concentrations of NaCl solution. NaCl solution (5 mL) of respective concentration was applied to each Petri dish that was arranged in a completely randomized design under factorial arrangement. Half of the solution was applied to filter paper receiving the seed while remaining half was applied to the covering filter paper. Petri dishes were covered with lid and placed on steel racks in well illuminated room with a light/dark period of 10/14 h. The temperature and humidity during the course of study were 20±5ºC and 55±5%, respectively. Equal volume of distilled water was applied to all Petri dishes when their moisture content declined.

Germination of seeds was recorded on daily basis according to AOSA (1990) until a constant count was achieved. Seed was considered to be germinated when radicle length exceeded 2 mm. Time taken to 50 % germination (T_{50}) was calculated according to modified formula of Farooq *et al.* (2005) as under:

$$T_{50} = ti + \left[\frac{N/2 - ni}{nj - ni} \right] \times (tj - ti)$$

Where N is the final number of germinated seeds; and ni and nj are the cumulative number of seeds emerged by adjacent counts at the times ti and tj where ni<N/2<nj. Mean germination time (MGT) was calculated according to Ellis and Robert (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were emerged on day D, and D is the number of days counted from the beginning of germination. Germination index (GI) was calculated as described by AOSA (1983):

$$GI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + --- + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

Final germination percentage (FGP) was taken as the ratio of number of seeds germinated to the total number of seeds sown and is expressed as percentage. Shoot and root length of five randomly selected seedlings from each replication was measured using a measuring tape at 14 days after sowing. Seedling fresh and dry biomass was recorded using a digital balance. For dry biomass, seedlings were oven dried at 70ºC for 48 h. Seedling vigor index was calculated according to the formula of Abdul-Baki and Anderson (1973).

$$SVI = \text{Radicle length (cm)} \times \text{Germination (\%)}$$

Percentage change over control was computed as under;

$$\text{Treatment over control} = \frac{\text{Treatment - Control}}{\text{Control}} \times 100$$

The experiment was replicated four times and repeated twice. Graphical presentation of the data was carried out using MS-Excel and standard error was also computed using the same. Treatment means were separated by employing least significant difference (LSD) test at 0.05 probability level by using STATISTIX 8.1.

Results and Discussion

Germination

Increasing NaCl salinity levels adversely affected germination attributes of the three wheat cultivars (Figure. 1). Significant ($p \leq 0.05$) differences regarding different germination attributes were observed among salinity levels as well as wheat genotypes. Moreover, wheat genotypes respond differentially to different salinity level due to significant interaction ($p \leq 0.05$) between these two factors. Increase in salinity not only decreased the germination percentage but also delayed the germination initiation in all wheat genotypes. Substantial delay in TSG, T_{50} and MGT was observed that varied amongst wheat genotypes. TSG, T_{50} and MGT decreased linearly with increase in salinity levels. At higher salinity levels, TSG was delayed by 1, 2 and 3 days in Millat-2011, AARI-2011 and Sehar-2006, respectively, over control (Figure 1). Nonetheless, Millat-2011 outperformed Sehar-2006 and AARI-2011 at high salinity levels. Likewise, T_{50} was increased in Sehar-2006 (61-97%), AARI-2011 (72-124%), and Millat-2011 (17-

67%) with increase in NaCl concentration against control. At highest salinity level (19 dS m^{-1}), the final germination percentage of Sehar-2006 and AARI-2011 dropped by 69% and 82%, respectively. The respective reduction for Millat-2011 was only 40% (Figure 1). Final germination was inhibited with increase in NaCl concentration and regression analysis revealed that germination percentage of Sehar-2006, AARI-2011 and Millat-2011 varied by 98, 96 and 88%, respectively, owing to salinity (Figure 3). Highest inhibition in MGT was noticed in AARI-2011 (68%) followed by Sehar-2006 (64%) and Millat-2011 (38%) at high salinity over control. Significantly lower germination

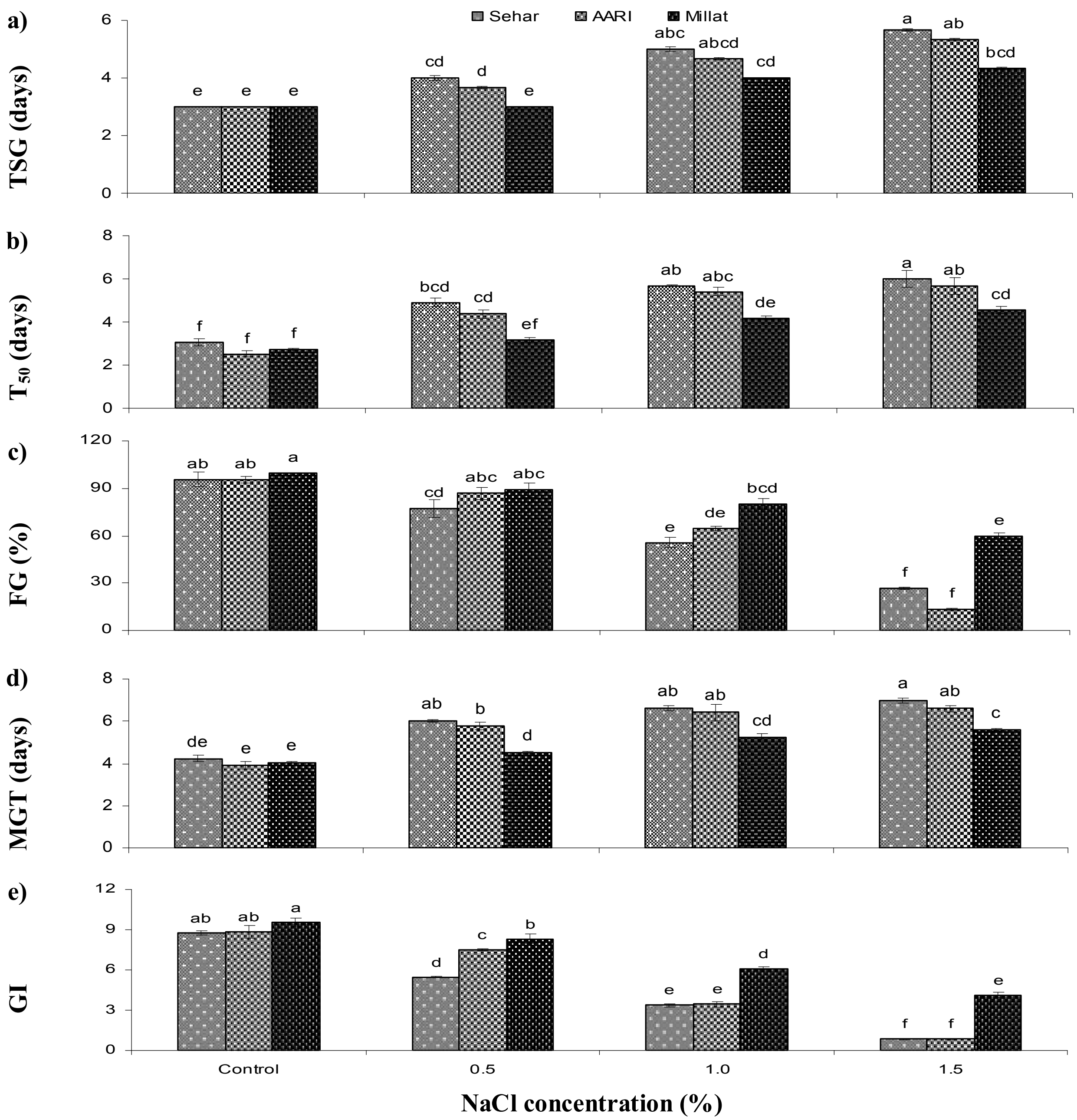

Figure 1: Effect of different NaCl concentrations on (a) time to start germination, (b) time taken to 50% germination, (c) final germination perentage, (d) mean germination time and (e) germination index of three wheat cultivars. Vertical bars above mean denote standard error of four replicates. Means with different letters differ significantly at 0.05 probability level by LSD test. LSD for interaction is (a) 1.027, (b) 1.068, (c) 16.434, (d) 0.841 and (e) 0.912.

indices over control were recorded under the influence of different salinity levels, yet Millat-2011 scored higher GI value at all salinity levels than rest of two wheat genotypes.

Impaired seed germination is the major factor limiting the establishment of plant under salinity (Khan and Gulzar, 2003). The inhibitory effect of salinity on germination attributes of different crops has been reported earlier (Farooq *et al.,* 2011; Elouaer and Hannachi, 2012; Afzal *et al.,* 2012). The decline in germination under salinity has been attributed to combined effect of osmotic pressure (Moud and Maghsoudi, 2008) and toxicity of salts (Saboora and Kiarostami, 2006) or due to the effect of added chlorine ion (Almodares *et al.,* 2007) that gave raise to osmotic stress. Rahman *et al.* (2008) reported that salinity significantly delayed the germination mainly due to altered water relations caused by high salt accumulation in intercellular spaces (Khan and Gulzar, 2003; Zhang *et al.,* 2006) The inability of seeds to germinate under salinity conditions may be due to embryo damage by Na^+/Cl^- ions (Khajeh-Hosseini *et al.,* 2003) or inhibition of seed water uptake (Mehmat *et al.,* 2006; Saboora and Kiarostami, 2006) or exosmosis (Rahman *et al.,* 2008). Salt-tolerant *Triticum* spp. had lower rate of Na^+ accumulation than the salt-sensitive ones (Ali *et al.,* 2004). In present study, AARI-2011 and Sehar-2006 appeared more susceptible to salinity levels (6.8, 13.2 and 19.0 ds m^{-1}) regarding germination attributes as compared to Millat-2011. The better performance of Millat-2011 under salinity suggests its superiority and greater ability to cope with salinity levels. Nevertheless, the synchronized and early germination was strongly correlated with seedling root and shoot length as well as dry biomass in all three wheat cultivars (Table 1) subjected to salinity.

Seedling growth

Significant differences were observed for all seedling growth attributes among salinity levels as well as wheat genotypes. Increasing NaCl concentration recorded a gradual reduction in seedling growth so that a strong negative correlation was observed between seedling growth and NaCl concentration (Figure 3 b, c, d). Regression accounted for over 80% variation in various seedling growth attributes with the exception of Millat-2011 for root length, which showed least suppression in root elongation at higher salt concentrations (Figure 3c). Interaction of wheat genotypes with salinity levels was found to be significant ($p \leq 0.05$). Increasing salinity levels inhibited the shoot length of Sehar-2006 (56-83%), AARI-2011 (58-91%) and Millat-2011 (30-60%) over control. The root length of AARI-2011 and Sehar-2006 was also reduced with the increase in salinity level (Figure 2). Root and shoot length are most important parameter for salt stress because roots are in direct contact with soil and absorbs water and nutrient from soil and shoot supply it to rest of the plant. For this reason, root and shoot length provides an important clue to the response of plant to salt stress (Jamil and Rha, 2004). Seedling biomass in terms of fresh and dry weight was also reduced gradually with increasing NaCl concentration (Figure 2). In comparison with control, maximum reduction in seedling dry biomass was observed in AARI-2011 (30-96%), followed by Sehar-2006 (14-86%) and Millat -2011 (21-43%) with increase in salinity levels. With regard to seedling vigor index (SVI), it was evident that with the increase in concentration of NaCl, the SVI decreased disproportionately in all treatments, as compared to the control variant, the differences being statistically significant ($p \leq 0.05$). Similar trend was observed by other authors on different plants (Ibrar *et al.,* 2003; Jabeen *et al.,* 2003; Rahman *et al.,* 2008 and Basalah, 2010).

Table 1: Correlation coefficients (r) of germination attributes to seedling growth traits in three wheat cultivars under NaCl salinity

	Shoot length	Root length	Seedling dry biomass
	Sehar-2006		
T_{50}	-0.999***	-0.998***	-0.837**
MGT	-0.999***	-0.995***	-0.836**
GI	0.953***	0.945***	0.953***
	AARI-2011		
T_{50}	-0.983***	-0.879**	-0.898**
MGT	-0.982***	-0.839**	-0.867**
GI	0.901***	0.983***	0.970***
	Millat-2011		
T_{50}	-0.892**	-0.975***	-0.967***
MGT	-0.924***	-0.954***	-0.979***
GI	0.928***	0.939***	0.939***

n=4, *** $p < 0.01$, ** $p < 0.05$

Soil salinity affects early seedling growth of plants by altering water relations due to salt accumulation in intercellular spaces (Zhang *et al.,* 2006), injurious effects of toxic ions (Saboora and Kiarostami, 2006), osmotic stress (Almodares *et al.,* 2007) and reduced water use efficiency (Grewal, 2010). Moreover, salinity stress can generate a wave of reactive oxygen species such as super oxide, hydrogen peroxide, hydroxyl radical, resulting in oxidative damage to cell ultra structures (Saboora and Kiarostami, 2006). Almodares *et al.* (2007) stated that some plants are sensitive to salinity at early seedling growth stage because the mechanism of the tolerance to salinity is not yet fully developed. Differential suppression of wheat genotypes under salinity might originate from variable metabolic efficiencies under stress induced carbon deficit and activity of enzymes of anti-oxidative defense as these have been positively correlated with stress tolerance (Siddiqui *et al.*, 2008). Moreover, difference in cell membrane stability and macro molecule stability under salinity might also be the possible cause of differential response.

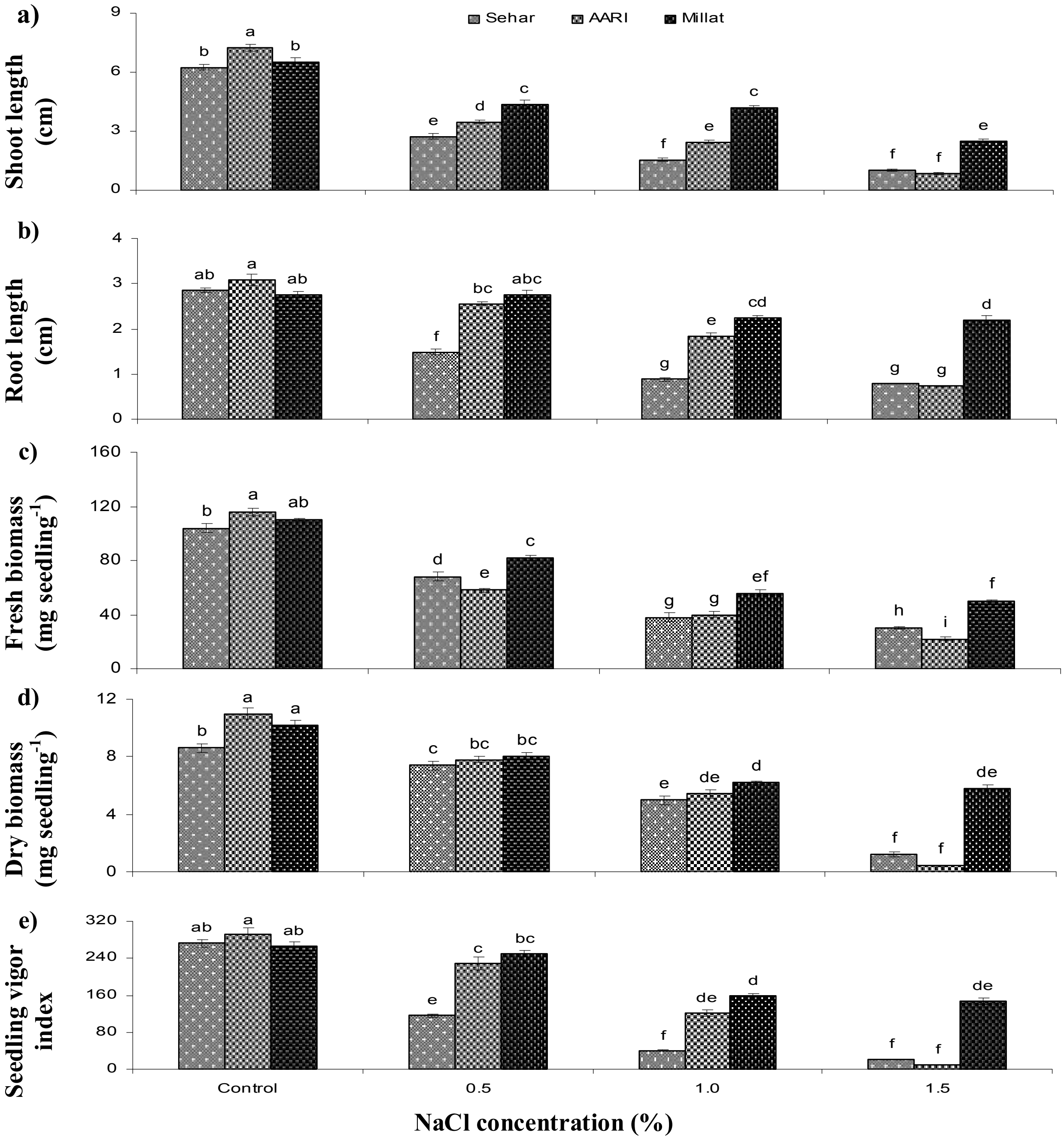

Figure 2: Effect of different NaCl concentrations on early seedling growth of three wheat cultivars **Vertical bars above mean denote standard error of four replicates. Means with different letters differ significantly at 0.05 probability level by LSD test. LSD for interaction is (a) 0.7167, (b) 0.471, (c) 6.6234, (d) 0.923 and (e) 41.406**

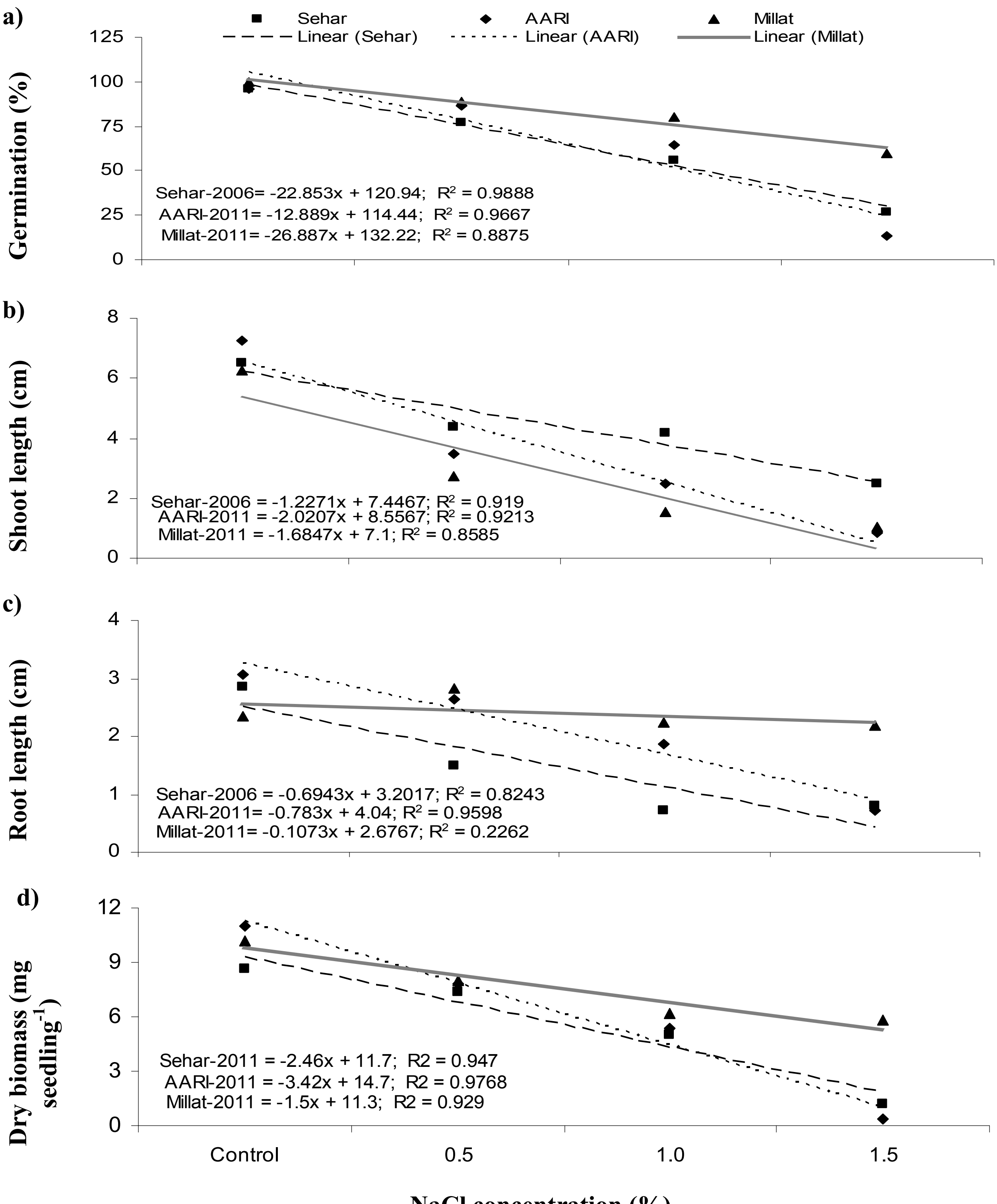

Figure 3: Relationship between NaCl solution concentration and (a) germination (b) shoot length (c) root length and (d) seedling dry biomass in three wheat cultivars

Conclusion

In this preliminary study, Millat-2011 appeared relatively salt tolerant than rest of wheat cultivars. Whether such results can be reproduced under natural settings necessitates the significance of field appraisal through screen house and field trials. Moreover, comparative physiological and biochemical basis of such tolerance amongst cultivars also needs to be worked out.

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