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Effect of Irrigation with Red Sea Water on Early Growth Characteristics of Saudi Wheat Cultivars (*Triticum aestivum*).

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ABSTRACT

Soil salinity adversely affects large areas of farmland globally. Consequently, it is increasingly important to develop molecular and agricultural solutions for improving saline tolerance of important crops. We aimed to evaluate the behavior of seven selected wheat cultivars (*Triticum aestivum*) from different parts of Saudi Arabia under different conditions of irrigation with Red Sea water to study their saline tolerance and genetic diversity. Random amplified polymorphic (RAPD) markers were used to fingerprint closely related wheat cultivars, and a primer was identified that produced clear bands in all cultivars. Plants were grown in physiological screening units arranged in a completely random design with five replicates and different concentrations of sea water (1.6, 3.1, 6.3, 12.5, 25.0, 50.0, and 100%). Various germination parameters were screened, such as whole weight, shoot length, root length, shoot weight and root weight under various experimental conditions. Cultivar C6 displayed the maximum salt tolerance to 50% sea water. Cultivars C2 and C7 produced recovering effects in comparison to others. Cultivar C3 similarly showed better results in up to 25% sea water. Our results provide some guidelines to local farmers and plant breeders for developing wheat cultivars with enhanced performance under adverse saline conditions.

Keywords: DNA fingerprinting, RAPD, Red Sea, Saudi cultivars, Salt tolerance

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INTRODUCTION

Chronic undernourishment is estimated to affect 870 million people globally [1]. The problem of insufficient food resources is exacerbated by the fact that more than 7% of the total land area used worldwide for farming is affected by increases in salinity [2]. It is important to improve farming outcomes in salt-contaminated areas of the world to meet the growing demand for food [3]. Human activities other than farming also make demands on the fertile agricultural land and further increase the need to cultivate crops in challenging areas, including salt-contaminated areas [3].

Wheat (*Triticum aestivum* L.) is an important food crop for more than 35% of the world's population[4]. In Saudi Arabia, wheat is a vital crop that supplies a large part of the nutritional needs of the low income population[5]. In order to deal with the adverse effects of soil salinity and to develop saline tolerant crop varieties it is essential to study the biochemical, physiological, molecular, and metabolic aspects of salt tolerance in plants [6]. The main factors that influence crop yield are seed germination rates and seedling growth characteristics; monitoring these factors is crucial [7].

Selection of salt tolerant cultivars is usually carried out at the seedling stage because it is easy to monitor different aspects of growth using seedlings, such as germination, leaf growth, and root growth; additionally, the use of seedlings is relatively simple and cost effective [8]. Identification of genotypes with high salinity tolerance and yield among the locally grown wheat cultivars can reinforce breeding programs and can be used for farming in saline areas [9].

Analysis of the gene mechanisms that control specific agronomic and climatic response traits in crops can be achieved using various molecular technologies [10]. In this study, we used random amplified polymorphic (RAPD) markers, as this approach does not require previous information on DNA sequences of the target organism [11]. Additionally, the use of RAPD markers for systematic and taxonomic analyses of plants is universally accepted because of its relative technical simplicity [12].

The aims of the present study were to evaluate and screen the tolerance of seven prominent Saudi cultivars of *Triticum aestivum* to growth under different salinity conditions. To this end, we compared various physiological criteria, such as seed germination, and the development of shoots and roots at seedling stage, and also conducted an RAPD analysis. Our results will be of value to local farmers and plant breeders as they identify appropriate genotypes that are suitable for cultivation in saline areas.

MATERIALS AND METHODS

The specimens were collected from different locations in Saudi Arabia: C1, Gassim; C2, Najran; C3, Jazan; C4, Durma; C5, AL-Jouf; C6, Abha; and C7, Hail. Red Sea water for the study was collected from Jeddah, Saudi Arabia. Shoot and root weights and lengths in the seven cultivars were measured using calipers; the data are presented, Sterilized 90 mm filter paper (Hermann Paulsen, Germany) was used to line petri dishes and was moistened with 2.0 ml of various Red Sea water concentrations (1.6, 3.1, 6.3, 12.5, 25, 50, and 100%). Autoclaved MilliQ water (0% sea water) was used as the control. Plant seeds were soaked in 1% HgCl₂ solution in a 50 ml tube (Corning, Japan) for 60 seconds. The seeds were then washed in distilled water and dried overnight at room temperature. Loss of moisture was prevented by sealing the petri dishes using parafilm. Petri dishes were setup at room temperature ($\pm 25^{\circ}\text{C}$; ± 12 h of sunlight). After 14 days, seedlings were collected and analyzed. This experimental procedure was used for the seeds of the seven cultivars and control. The seedlings were measured for total length and weight of shoots and roots. The data was analyzed using one-way-ANOVA.

DNA extraction

Wheat seedlings were ground to powder in liquid nitrogen using a sterile mortar and pestle. DNA extraction was carried out using a DNeasy Plant Mini kit with 50 mg powdered wheat seedlings. DNA extraction was carried out using a QIAcube (Qiagen). Gel electrophoresis and a Nanodrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, USA) were used to confirm the quality of the DNA. Extracted DNA was stored at -80°C .

RAPD-PCR analysis

Fifty nanograms of template DNA was used for RAPD-PCR with Primer 2 (5'-GTTTCGCTCC-3'). PCR conditions were 1 cycle of 95°C for 5 min, followed by 45 cycles of 95°C for 1 min, 36°C for 1 min and 72°C for 2 min. A long (20 × 14 cm) 1% agarose gel using 1× TAE buffer containing 0.5 µg/mL ethidium bromide was used for electrophoresis of the products. Gel images were obtained using a Proxima C16 Phi+ (Isogen Life Science) UV transilluminator and Opticom (version 3.2.5; OptiGo) system. Gel image analysis of the RAPD bands and an Amersham 100-bp ladder (GE Healthcare) was performed using TotalLab TL100 1D software (version 2008.01).

RESULTS AND DISCUSSION

Root and shoot lengths are adversely affected by increased salt concentrations and this adverse effect results in decreased plant height. Shoot length is more sensitive to increased salinity compared to the root [13,14]. Soil salinity disturbs early seedling growth in plants by altering water relations due to salt accumulation in intercellular spaces [15]. Osmotic changes are mediated by intracellular compartmentalization and segregation of toxic ions inside the cytoplasm by energy dependent transport into the vacuoles, and these changes are vital for the growth of crops in salty conditions [16]. Two phases of plant growth in saline conditions have been described: an “osmotic phase” in which there is reduced growth of young leaves and an “ionic stage” in which mature leaves senesce sooner than normal [17]. Shoot growth rate is inversely proportional to the concentration of NaCl found in the leaves of the plant. Other factors may also have a role in the response to salinity, e.g., cellular compartmentalization, tissue tolerance, water use effectiveness, and apoplastic ion transport across the root. Ca⁺ changes the apoplastic transpirational by-pass flow and thereby controls the entry of Na⁺ into the shoot. Both saline tolerant and saline sensitive cultivars have this mechanism in operation albeit with different efficiency levels [6].

There are significant changes in Na⁺ transport between saline tolerant and saline sensitive genotypes of durum wheat (*Triticum turgidum durum*). Xylem loading rate is low in the tolerant type, and the leaf sheath's ability to extract and differentiate Na⁺ ions when it penetrates the leaf also differs. It has been found that there is significant variation in root uptake of Na⁺ in different genotypes of durum wheat [18]. Maintenance of a low cytosolic Na⁺ concentration and homeostasis of K⁺ and Na⁺ ions are believed to be critical aspects of salinity tolerance in plants. In addition to this, salt tolerant plants have been shown to have higher than usual concentrations of K⁺/Na⁺ [19]. It has also been observed that leaves and roots of plants with a saline tolerant genotype generally have reduced Na⁺ and Cl⁻ internally in comparison with the saline sensitive genotypes [20].

Physiological and biochemical factors related to salt tolerance include polyamines, companionable solutes, phytohormones, and antioxidants [6,22]. Identification of novel genetic sources related to salt tolerance can be used to enhance the selection process in breeding programs. Data for use in this process can be derived by analyses of physiological parameters and studying gene expression patterns [22]. Biochemical analysis shows that salt tolerance is mostly associated with the potential of plants to isolate Na⁺ into intracellular compartments and at the same time maintain a K⁺ equilibrium [23]. At the molecular level, the mechanism of salt tolerance is complex and most likely involves the simultaneous expression of salt tolerance genes, their interactions, and extrinsic NaCl availability [24].

Our results provide strong evidence that salinity stress greatly affects shoot and root lengths and seedling weight in wheat cultivars. The weights of the different wheat cultivars varied significantly. We found that the cultivar C7 produced bigger seeds (83 ± 0.02 mg) compared to the other varieties. Germination of seeds was observed after 5 days in all sea water concentrations (0–50%). Seeds of all seven cultivars germinated when treated with normal water (Fig. 1). By contrast, none of the seeds of the seven cultivars germinated when treated with 100% sea water. C1 seeds did not germinate in 50% sea water, whereas 50% of C6 seeds germinated at this concentration.

Shoot lengths in all cultivars were similar in normal water (F = 0.61, P = 0.72). However, when cultivated in sea water, shoot lengths varied among different cultivars (F = 2.95, P < 0.01) (Fig. 2). The highest shoot lengths were present in cultivar C6 (15.5 ± 0.75 cm.). Shoot lengths did not differ significantly in plants grown on lower concentrations of sea water: 1.6% (F = 1.5, P = 0.21); 3.1% (F = 0.63, P = 0.71); 6.3% (F = 1.31, P = 0.28); 25% (F = 1.6, P = 0.19); and 50.0% (F = 1.3, P = 0.27). Significant variation was observed at 12.5% (F =

3.3, $P < 0.01$). Similarly, shoot weight, did not vary in normal water ($F = 1.03$, $P = 0.42$), but did differ among cultivars in different concentrations of sea water ($F = 2.7$, $P < 0.02$) (Fig 3). There were no variations among seedlings in 1.6% ($F = 1.04$, $P = 0.42$) and 50% sea water ($F = 0.99$, $P = 0.44$). However, significant variations were seen in 3.1, 6.3, and 25% sea water.

Some differences in root and shoot lengths were observed among cultivars ($F = 2.1$, $P = 0.09$) even in normal water (Fig 4). A mean root length of 11.68 ± 0.96 cm was found in normal water. Cultivar C3 had the longest root length (21.3 cm) in 1.6% sea water. Root lengths differed among cultivars in sea water ($F = 1.80$, $P = 0.10$) (Fig. 4). Root lengths did not differ significantly in the following treatments: 1.6% ($F = 1.6$, $P = 0.17$); 6.3% ($F = 0.1$, $P = 0.44$); 12.5% ($F = 2.13$, $P = 0.08$); 25% ($F = 0.89$, $P = 0.52$); and 50% sea water ($F = 1.3$, $P = 0.2$). However, significant variations were observed for in 3.1% sea water ($F = 3.5$, $P < 0.01$).

Mean root weight was 44 ± 3.7 mg for all cultivars. Similarly to root lengths, root weights also varied among ($F = 0.78$, $P = 0.59$) in normal water (Fig. 5). Overall, sea water had a significant effect on root weights of the different cultivars ($F = 1.10$, $P = 0.37$). Roots weight did not differ significantly in the following treatments: 1.6% ($F = 2.1$, $P = 0.09$); 3.1% ($F = 1.6$, $P = 0.19$); 6.2% ($F = 0.42$, $P = 0.86$); 12.5% ($F = 1.97$, $P = 0.10$); 25% ($F = 2.0$, $P = 0.09$); and 50% sea water ($F = 1.7$, $P = 0.15$).

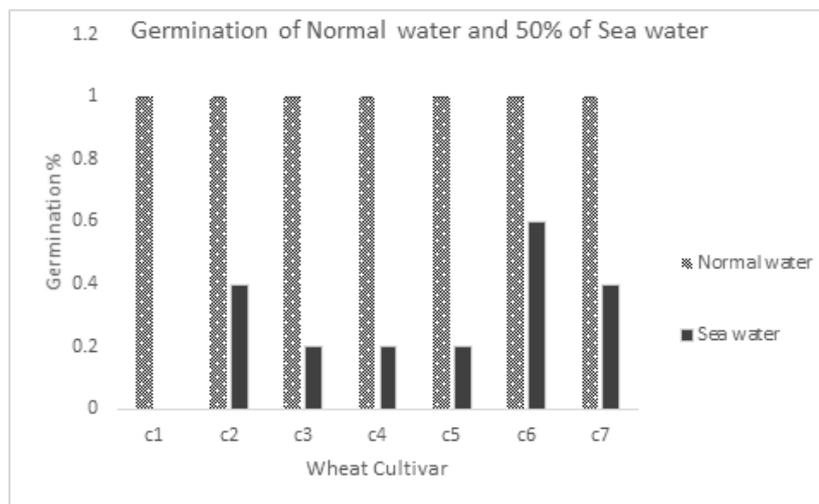


Figure 1: Germination (%) of different wheat cultivars (C1-C7) treated with normal or sea water.

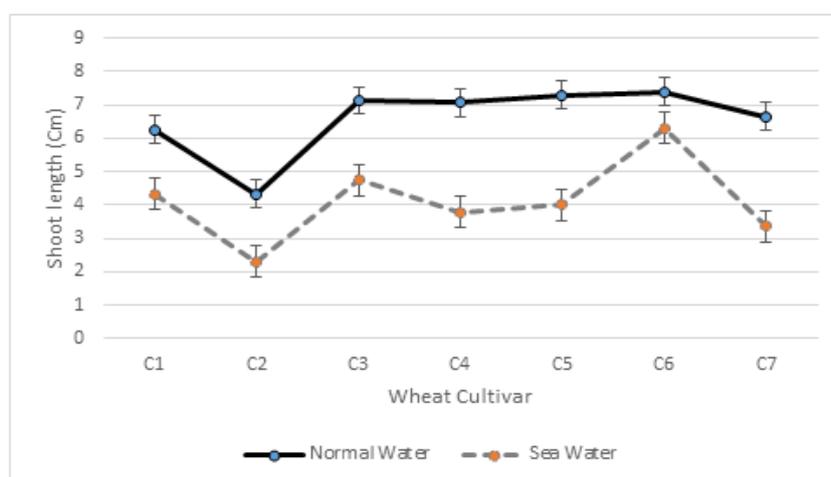


Figure 2: Shoot-length of different wheat cultivars (C1-C7) treated with normal or sea water. Bar, standard error of the mean (SE). Normal water $F = 0.61$, $P = 0.72$. Sea water $F = 2.95$, $P < 0.01$.

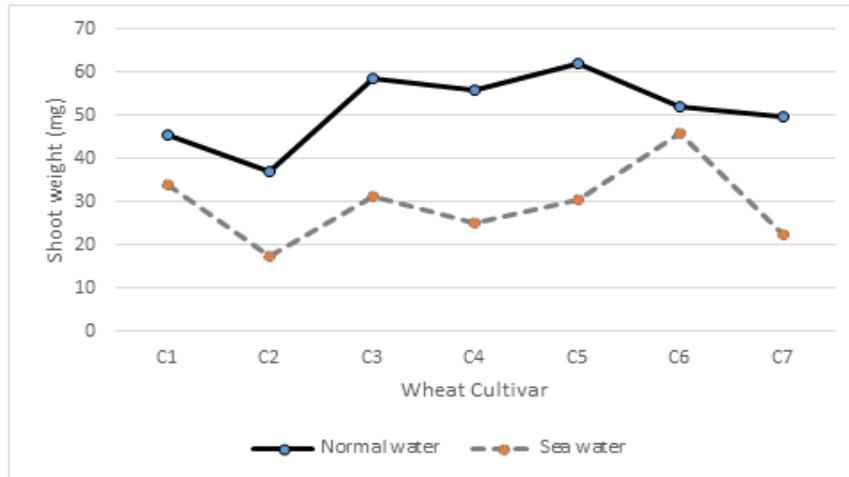


Figure 3: Shoot-weight of different wheat cultivars (C1–C7) treated with normal or sea water. Bar, SE. Normal water $F = 1.03$, $P = 0.42$. Sea water $F = 2.7$, $P < 0.02$.

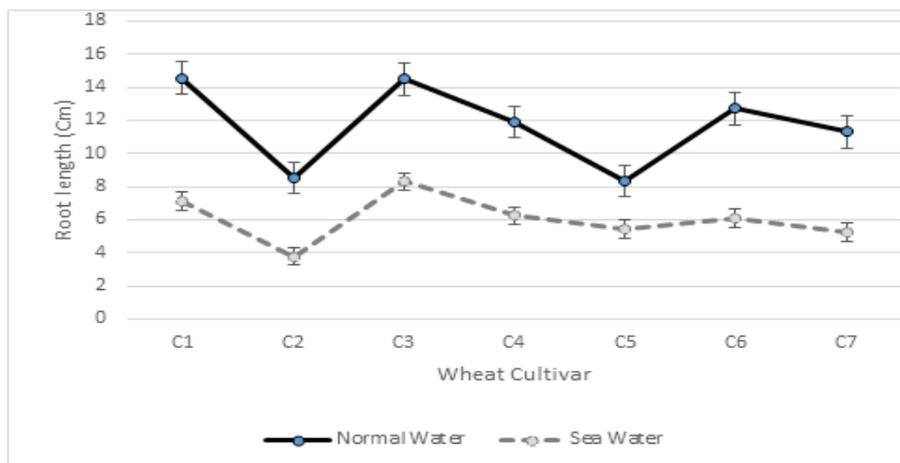


Figure 4: Root lengths in different wheat cultivars (C1–C7) treated with normal or sea water. Bar, SE. Normal water $F = 2.1$, $P = 0.09$. Sea water $F = 1.80$, $P = 0.10$.

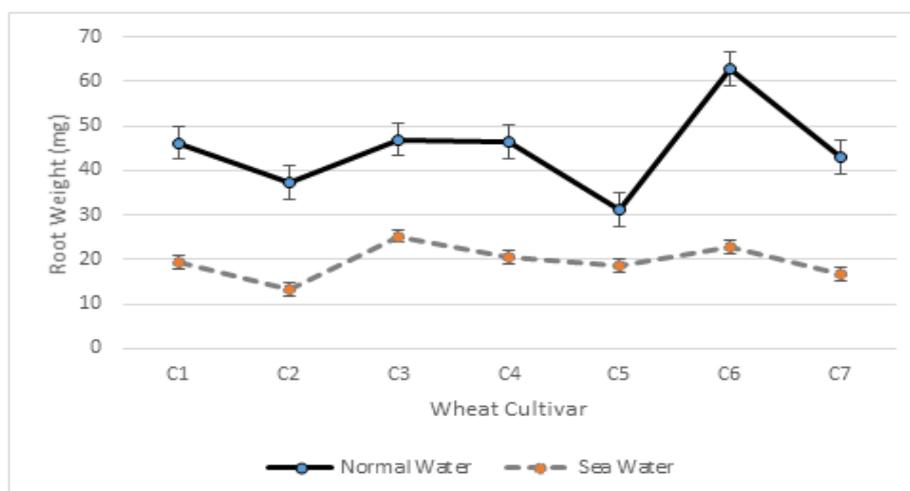


Figure 5: Root weights of different wheat cultivars (C1–C7) treated with normal or sea water. Bar, SE. Normal water $F = 0.78$, $P < 0.59$. Sea water $F = 1.10$, $P = 0.37$.

Genetic constitution can differentiate some cultivars from others with respect to salinity stress resistance or tolerance; this effect is one that needs to be clarified by further research [25]. RAPD analysis has become a popular tool in genetic research for differentiation and determination of phylogenetic relationships among cultivars and detection of genetic differences among species. RAPD is the most widely used technique to determine the gene level diversity in the genus *Triticum* [26,27,28]. Cultivar identification is generally done by using RAPD markers [29]. Our previous study showed that primer 2 produced consistent results for RAPD analyses in plants [30]. Here, we selected primer 2 to investigate genetic variation among the selected wheat cultivars and it produced similar banding patterns for all wheat samples (Fig 6). In comparison to conventional selection and breeding techniques by which many plant breeders have successfully developed saline tolerant crops, it is widely accepted that selection will be enhanced if the desired crop displays specific salt tolerance indicators [6].

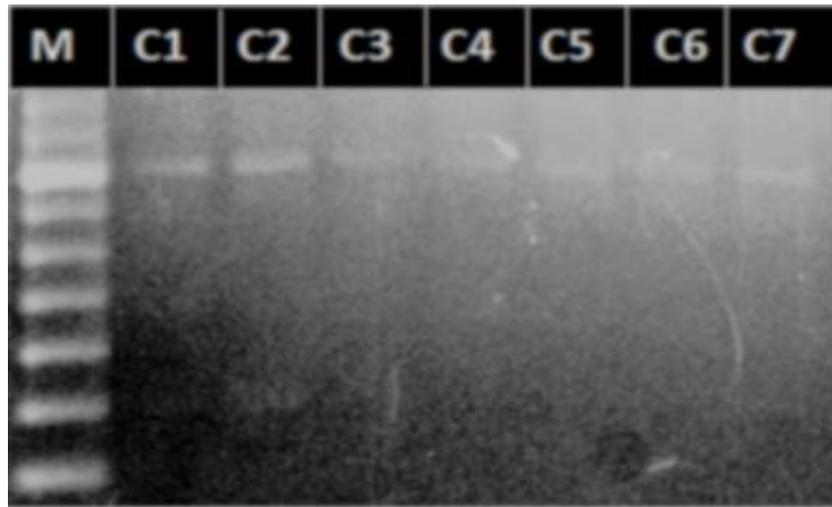


Figure 6: RAPD-PCR product profile of different wheat cultivar (C1-C7). Lane M, 100 bp molecular weight, Ready-To-Go RAPD analysis primer (P2) was used for determination.

CONCLUSION

Our results show that different wheat cultivars responded differently to a range of salt concentrations at the stage of germination. This also indicates that the cultivars can be ranked in accordance to their salt tolerance based on the growth rate, root, and shoot length. Cultivar C6 proved to be the most salt tolerant in 50% sea water. Cultivar C2 and C7 were also better when compared with other cultivars. Similarly, C3 was proven to be a better salt tolerant cultivar in 25% sea water. Our results will be of value for producing guidelines to local farmers and plant breeders for development of new wheat cultivars with improved germination under salt stress.

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