Research Article

Evaluation of wheat varieties to salt stress (NaCl) for seed germination and early seedling growth under laboratory conditions

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Abstract

Salinity tolerance during germination and early seedling growth was evaluated of four wheat varieties under four salinity (NaCl) levels that included Control (distilled water) 0, 9, 12 and 16 dSm⁻¹. The seeds of four wheat varieties including Imdad-2005, Mahran-89, TJ-83 and TD-1 were sown under various NaCl levels in Petri dishes. The trial was performed at Seed Testing Laboratory, Department of Agronomy, Sindh Agriculture University, Tandojam Pakistan, following completely randomized design with three replications. The results showed that different salt stress (NaCl) levels had significant effects on germination (%), shoot and root length (cm), shoot and root water content (%), shoot and root fresh weight (g) and shoot and root dry weight (g) of wheat varieties. Among varieties, Imdad-2005 showed tolerance against salt stress for all parameters as compared to other varieties. The Imdad-2005 produced maximum mean germination (93.7%), shoot and root length (14.8 cm and 9.3 cm), shoot and root RWC (80.1 % and 86.2%), shoot and root fresh weight (2.3 g and 1.5 g) and heaviest shoot and root dry weight (0.28 g and 0.13 g). However, for NaCl levels, maximum germination (96.2 %), shoot and root length (15.5 cm, 10.7 cm), shoot and root water content (86.4 %, 87.0%), shoot and root fresh weight (2.3 and 1.5 g) and highest shoot and root dry weight (0.28 g and 0.13 g) was noted at control, where seeds were on sown by using distilled water.

Keywords: Germination; NaCl Tolerance; Seedling growth; Salinity levels; Wheat (*Triticum aestivum* L.)

Introduction

Wheat (*Triticum aestivum* L.) is considered as a major cereal crop and staple food worldwide. Wheat meets the increasing food requirements of the country. Among all cereals, it occupies the significant position and adds 13.1 (%)

value-addition in agriculture and 2.8 (%) in GDP. Wheat contains the fundamental nutrients which are needed in our daily diet. United States consumes wheat as staple food more than any country of world [1]. Wheat is the highly nutritious. It has contained essential fibers, carbohydrates,

protein and vitamins. There are many by-products of wheat such as flour, bread, pastries, cakes and other local foods [2]. Soil salinity is one the main constraints that adversely affect the growth and development of the wheat crop globally. Salinity generally occurs in areas of acute water shortage. Like other countries Pakistan is also greatly facing the issue of soil salinity [3]. Yield of crop declines as the pH of the soil solution crosses 8.5 and the EC value increases upto 4 dSm⁻¹. Increased EC value reduces yield of cultivated crops. Inclusion of salts in water decreases its osmotic potential, which results in the reduced accessibility of water to roots [4]. The micro environment of the plant plays a vital role in creation of saline soils which is characterized by low rainfall, great fluctuations in temperature and high rate of evaporation [5, 6]. Seed germination and seedling growth are known as critical stages to salt stress [7]. That might be due to increased toxicity in ions [8, 9], reported that some plants are sensitive to salinity at shoot growth stage because the tolerance to salinity in the plant is not yet fully developed. Root and shoot length is one of the dominant factors for salt stress because roots are in direct touch in soil and they uptake the water and minerals from soil and supply it to other parts of the plant. Root and shoots traits are important indicators that greatly determines the response of the plant to salt stress [10, 11]. Moreover, these parameters provide valuable clues selections of genotypes for future breeding programs [12, 13]. Assessing genotypic variation under salinity conditions is mandatory in determining the tolerance of crop plants against salt stress [14].

This study aimed the assessment of the tolerance of four wheat varieties to salt stress at the germination and early seedling growth stage.

Materials and methods

Experimental site

The trial was conducted to determine the tolerance of wheat varieties for seed germination and early seedling growth

under various salinity levels during 2013-14 in the Seed Testing Laboratory, Department of Agronomy, Faculty of Crop Production, Sindh Agriculture University Tadojam, Pakistan.

Experimental details

For this study, Hoagland nutrient solution was used as growing medium. The 1/4th strength of the nutrient solution was used. The chemical combination of nutrient solution is presented in (Table 1). The four local wheat varieties were evaluated under various NaCl levels that included 0, 9, 12, and 16 dSm⁻¹. The seeds were sterilized for ten minutes in 5% sodium hypochlorite and were cleaned with distilled water. Fifteen seeds of each variety were planted on molded plastic sieves placed in plastic bowls at temperature 20-25°C. The seed germination percentage was recorded after five days. The bowls were covered with porous transparent polyethylene bags to avoid excess evaporation. The bowls were placed in controlled growth chamber maintaining the temperature of 20-25°C day and night temperature and 12 hours photoperiod irradiate 22 wv2. The photoperiod period of 12 hours was maintained. Topping of each bowl was done regularly to maintain the same level of moisture with the respective concentration of a nutrient solution.

Statistical analysis

The data was obtained and subjected to the analysis of the variance (ANOVA). The least significant difference test was applied to examine the statistical differences within treatments following the method developed by [15]. After 5 days, germination percent (%) was measured according to the International Seed Testing Association (ISTA) standard method. At the end of the fifth day, the germination percentage was measured according to the following formulas;

$$\text{Formula. 1: GP} = \frac{NGS}{NTS} \times 100$$

Whereas; GP = Germination percentage, NGS = Number of germinated seeds and NTS = Number of total seeds.

Formula. 2: $RWC = \frac{FW}{DW} \times 100$

Whereas; RWC = Relative water content, FW = Fresh weight and DW = Dry weight.

Table 1. Hoagland's nutrient solution formula used for various wheat varieties

Stock solutions	Solution No.1 (ml/l)	
1M.KH ₂ PO ₄	-	1.0
1M.KNO ₃	-	5.0
1M.Ca(NO ₃) ₂ 4H ₂ O	-	5.0
1M.MgSO ₄ 7H ₂ O	-	2.0
Solution No.2 (ml/l)		
1M.KH ₂ PO ₄	-	3.1
1M.KNO ₃	-	6.0
1M.Ca(NO ₃) ₂ 4H ₂ O	-	4.0
1M.MgSO ₄ 7H ₂ O	-	2.0
Micronutrients stock solution	Solution No.3 (g/l)	
H ₃ BO ₄		2.86
MnCl ₂ 4H ₂ O		1.81
ZnSO ₄ 7H ₂ O		0.22
CuSO ₄ 5H ₂ O		0.08
H ₂ MO ₄ H ₂ O		0.02

Results and discussion

The results demonstrated that the all parameters at germination and early seedling growth of wheat varieties were significantly influenced by salt-stress. Our results agreed with [16]. Who observed that increasing levels of salinity had harmful effects on all the parameters.

Seed germination (%)

Germination (%) suppressed under high saline conditions. The salt stress showed adverse effect on seed germination (Table 2), among different NaCl levels, the maximum germination (96.2%) was recorded under control condition (0 dS m⁻¹), followed by (91.5% and 88.2%) that were noted at 9 and 12dS/m⁻¹ levels respectively. However, minimum germination (85.0%) was recorded at 16 dSm⁻¹. Our findings are further confirmed by [17] who described that salt stress causes more ionic toxicity that reduces the absorption of water in the seeds that ultimately leads to less germination (%). [18] also found that a less germination (%) might be due to less osmotic potential to nutrients media. In case of varieties, highest germination (93.7%) was noted in variety Imdad-2005, followed by (91.2% and 89.7%) that was recorded in Mehran-89 and

Tj-83 varieties. However, lowest germination (86.2%) was noted in TD-1. Interaction between varieties and NaCl levels showed that maximum germination (98.0%) was recorded in variety Imdad-2005 under control 0dS/m⁻¹ NaCl level. Moreover, the minimum germination (80.0%) was observed in variety TD-1 at 16dS/m⁻¹ level [19], also found different response of wheat varieties under different salinity levels. The findings of [20], endorse the results of the present investigation, who suggested that salt stress conditions showed adverse effects on germination % of wheat cultivars of wheat. Moreover [21], also demonstrated that most of the cultivars of wheat showed less germination under salt stress conditions.

Shoot length (cm)

The results related to shoot length indicated significant (P<0.05) response of wheat cultivars to salt stress. The decreased shoot length was recorded at increasing level of NaCl concentration (Table 3). The maximum shoot length (15.5 cm) was recorded under control condition 0 dS/m⁻¹ NaCl level, followed by (14.8 cm and 13.8 cm) that were noted at 9 dS/m⁻¹ and 12 dS/m⁻¹, respectively. However, minimum (10.6 cm) shoot length was obtained from

16 dS/m⁻¹ level. These findings are supported by [22], who described that soil salinity adversely affects shoot growth of plants by altering water relations due to salt accumulation in intercellular spaces. In case of varieties, longest shoot length (14.8 cm) was recorded in variety Imdad-2005, followed by (13.9 cm and 13.4 cm) that were noted in Mehran-89 and Tj-83, respectively. Moreover, lowest shoot length (12.8 cm) was obtained from variety TD-1. The interaction between varieties and NaCl levels revealed that the maximum shoot

length (16.7 cm) was noted in variety Imdad-2005 under control 0dS/m⁻¹ level. However, minimum shoot length (9.4cm) was observed in variety TJ-83 at 16dS/m⁻¹ NaCl level. Our result are in agreement with [23], who also found that seedling establishment at early growth stages of plants is crucial for getting maximum shoot length and consequently higher yield. The findings of [24], also support the results of current investigation who also observed reduced growth of wheat cultivars under higher level of salt concentrations.

Table 2. Effect of different salinity (NaCl) levels on seed germination (%) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	98.0 a	96.0 bc	93.0 c	88.0 de	93.7 A
Mehran- 89	97.0 ab	93.0 c	89.0 d	86.0 ef	91.2 B
TJ- 83	97.0 ab	89.0 d	87.0 de	86.0 ef	89.7 C
TD- 1	93.0 c	88.0 de	84.0 f	80.0 g	86.2 D
NaCl means	96.2 A	91.5 B	88.2 C	85.0 D	-

S. E. 0.6229

LSD (0.05) 1.2721

Table 3. Effect of different salinity (NaCl) levels on shoot length (cm) of wheat varieties at early seedling stage

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	16.7 a	16.1 ab	14.4 cdef	11.9 g	14.8 A
Mehran- 89	15.6 abc	14.9 bcd	13.5 ef	11.5 g	13.9 B
TJ- 83	15.1 ab	14.6 bcd	14.3 def	9.6 h	13.4 B
TD- 1	14.6 cde	13.7 def	13.2 f	9.4 h	12.8 C
NaCl means	15.5 A	14.8 B	13.8 C	10.6 D	-

S. E. 0.3078

LSD (0.05) 0.6285

Root length (cm)

Root length of wheat varieties significantly affected by different levels of NaCl concentration. The root length of the wheat varieties varied under different salinity levels as shown in (Table 4). The root length decreased with increasing of NaCl level. The maximum root length (10.7 cm) was recorded in 0dS/m⁻¹ level, followed by (9.1cm and 7.0 cm) that were noted at 9dSm⁻¹ and 12dSm⁻¹ levels, respectively. However, minimum root length (5.6 cm) was observed at highest 16 dS/m⁻¹ level of NaCl. The interaction between wheat

varieties and NaCl levels showed that highest root length (14.6 cm) was noted from variety Mehran-89 under control 0dS/m⁻¹ conditions. However, lowest root length (4.9 cm) was observed in variety TD-1 at 16dS/m⁻¹ NaCl level [25]. The other researchers including [26, 27], also observed reduced root length in response to higher salt stress [28], observed that the roots response was very low in both saline and sodic soils due to higher ionic toxicity. Among the varieties, longest root length (9.3 cm) was recorded in Imdad-2005 followed by (8.2 cm and 7.5 cm) that was

obtained from Mehran-89 and Tj-83 varieties, respectively. Moreover, shortest root length (7.2 cm) was recorded in variety TD-1. Since root has a direct contact to soil,

hence it is more vulnerable to salt stress conditions as compared to other parameters.

Table 4. Effect of different salinity (NaCl) levels on root length (cm) of wheat varieties at early seedling stage

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	14.6 a	9.5 bc	7.4 de	5.9 fgh	9.3 A
Mehran- 89	9.7 b	9.4 bc	7.3 de	6.4 efg	8.2 B
TJ- 83	9.5 bc	9.2 bc	6.5 efg	5.1 gh	7.5 BC
TD- 1	9.2 bc	8.2 cd	6.6 ef	4.9 h	7.2 D
NaCl mean	10.7 A	9.1 B	7.0 C	5.6 D	-

S. E. 0.3432

LSD (0.05) 0.7008

Relative water content of shoot (%)

Relative water content of shoot (%) of wheat varieties significantly reduced in the various NaCl levels as compared to control. Shoot water content showed differences among the wheat varieties as affected by different NaCl levels (Table 5). The maximum RWC (86.4%) was observed under the control condition, followed by (83.1 % and 76.1%) that was recorded at 9 dS/m⁻¹ and 12dS/m⁻¹, respectively. Whereas, minimum (67.9%) RWC was obtained from highest level of saline conditions (16dS/m⁻¹). Moreover, among the varieties, the highest RWC (80.1%) was

observed in variety Imdad-2005, followed, by RWC (79.1 % and 78.1%) that was recorded in Mehran-89 and TJ-83 respectively. Moreover, lowest shoot moisture (76.3%) was noted in TD-1. The interaction between wheat varieties and NaCl levels revealed that the maximum RWC (88.2%) was observed in Imdad-2005 under control condition (0 dS/m⁻¹). However, minimum (65.6%) RWC was noted in variety TD-1 at 16 dS/m⁻¹ level. These results are similar with the findings of [29, 30], who investigated that due to drought with PEG and salt- water content in the shoot reduced significantly.

Table 5. Effect of different salinity (NaCl) levels on RWC of shoot (%) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	88.2 a	84.8 abc	78.1 ef	69.4 h	80.12 A
Mehran- 89	86.5 ab	83.6 bcd	77.7 ef	68.7 hi	79.12 AB
TJ- 83	85.8 abc	83.1 cd	75.1 fg	8.1 hi	78.17 B
TD- 1	85.1 abc	80.9 de	73.7 g	65.6 i	76.32 C
NaCl mean	86.4 A	83.1 B	76.15 C	67.95 D	-

S. E. 0.8419

LSD (0.05) 1.7194

Relative water content of root (%)

The data related to relative water content of root revealed highly significant response of wheat varieties to various salinity levels. The interaction between varieties and NaCl levels was also significant (Table 6). The maximum relative water content (RWC) of root (86.9%) was recorded under control conditions (0 dS/m⁻¹) followed by (84.3 % and 81.3%) that was noted at 9 dS/m⁻¹ and 12 dS/m⁻¹, respectively. However, the

minimum RWC (77.2 %) was obtained at highest salinity level of 16 d Sm⁻¹. In case of varieties, the maximum RWC (86.2%) was recorded in variety Imdad-2005, followed by (84.5 % and 81.9%) that was noted in Mehran-89 and TJ-83 varieties, respectively. However, lowest RWC (77.2 %) was noted in variety TD-1. The interaction between varieties and NaCl levels showed that highest RWC (90.3%) was observed in the variety Imdad-2005

under the control conditions (0 dS/m^{-1}). However, minimum RWC (69.3%) was noted in variety TD-1 under highest salinity level (16 dS/m^{-1}). The findings of [31], are in accord with the results of present study, who suggested that salt stress conditions

leads to drought stress in root due to less uptake of water by roots. Moreover, salt stress conditions caused shorter root that unable to reach the soil at deeper level to absorb the moisture.

Table 6. Effect of different salinity (NaCl) levels on RWC of root (%) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	90.3 a	86.9 abc	85.2 bcd	82.5 de	86.22 A
Mehran- 89	87.7 ab	85.8 bcd	84.1 bcde	80.7 e	84.57 A
TJ- 83	86.1 bcd	83.9 bcde	81.1 e	76.5f	81.91 B
TD- 1	83.7 cde	80.8 e	75.1 f	69.3 g	77.20 C
NaCl mean	86.95 A	84.35 B	81.35 C	77.25 D	-

S. E. 0.9442

LSD (0.05) 1.9283

Fresh Shoot weight (g)

Shoot fresh weight was affected by the different concentrations of NaCl levels (Table 7), among various NaCl levels, the maximum shoot fresh weight (3.0 g) was recorded under control condition (0 dS/m^{-1}), followed by (1.9 g and 1.7 g) shoot fresh weight that was noted under salt stress conditions of 9 dS/m^{-1} and 12 dS/m^{-1} , respectively. While, minimum shoot fresh weight (1.5 g) was observed under highest salinity level of 16 dS/m^{-1} . In case of varieties, highest shoot fresh weight (2.3 g) was obtained from variety Imdad-2005, followed by (2.1 g and 1.9 g) that was noted in Mehran-89 and Tj-83, respectively.

Moreover, lowest shoot fresh weight (1.7 g) was recorded in TD-1. The results of [32], are also in agreement with the findings of current study, who reported that the shoot fresh weight was induced by salinity stress [33], also observed that the root fresh weight was not affected by salt stress however, shoot fresh weight was significantly affected by salinity. The interaction of varieties and NaCl levels showed that the maximum shoot fresh weight (3.6 g) was recorded in variety Imdad-2005 under control conditions (0 dS/m^{-1}). However, minimum shoot fresh weight (1.3 g) was noted in variety TD-1 at 16 dS/m^{-1} salt condition.

Table 7. Effect of different salinity (NaCl) levels on fresh Shoot weight (g) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	3.6 a	2.2 cd	1.9 cdef	1.6 defg	2.3 A
Mehran- 89	3.2 a	2.0 cde	1.7 defg	1.4 fg	2.1 AB
TJ- 83	2.8 ab	1.8 defg	1.5 efg	1.5 efg	1.9 BC
TD- 1	2.4 bc	1.6 efg	1.5 efg	1.3 g	1.7 C
NaCl mean	3.0 A	1.9 B	1.7 BC	1.5 C	-

S.E. 0.1385

LSD (0.05) 0.2828

Root fresh weight (g)

The results related to fresh weight of wheat varieties as influenced by various NaCl levels are given in (Table 8). The result showed that the highest root fresh weight (2.2 g) was recorded in control treatment (0 dS/m^{-1}), followed by root fresh weight (1.2 g and 1.1 g), observed at salt stress levels of

9 dS/m^{-1} and 12 dS/m^{-1} levels, respectively. While, lowest root fresh weight (1.0 g) was noted at 16 dS/m^{-1} NaCl level [34]. Among the wheat varieties, highest root fresh weight (1.5 g) was recorded in variety Imdad-2005, followed by (1.4 g and 1.3 g), observed in Mehran-89 and Tj-83 varieties. The lowest root fresh weight (1.2 g) was

noted in TD-1. The interaction between varieties and NaCl levels showed that the maximum root fresh weight (2.7 g) was recorded in variety Imdad-2005 under

control (0 dSm⁻¹) salinity level. However, minimum root fresh weight (0.9 g) was observed in variety TD-1 at 16 dSm⁻¹ salinity level.

Table 8. Effect of different salinity (NaCl) levels on root fresh weight (g) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	2.71 a	1.25 e	1.12 gh	1.05 ghi	1.5 A
Mehran- 89	2.32 b	1.23 ef	1.11 gh	1.01 hij	1.4 B
TJ- 83	2.03 c	1.12 fgh	1.10 ghi	0.99 ij	1.3 C
TD- 1	1.81 d	1.15 fg	1.07 ghi	0.92 j	1.2 D
NaCl mean	2.2 A	1.2 B	1.1 C	1.0 D	-

S.E. 0.0275

LSD (0.05) 0.0561

Shoot dry weight (g)

The results showed that shoot dry weight decreased significantly with increasing salinity levels in all varieties. The interaction between varieties and salt stresses was also significant ($p>0.5$) in (Table 9). The highest shoot dry weight (0.31g) was recorded at 0 dSm⁻¹ level, followed by (0. 25 g and 0.22 g) that were noted at 9 dS m⁻¹ and 12 dSm⁻¹ respectively. While, minimum shoot dry weight (0.19 g) was noted from the highest level of NaCl (16 dSm⁻¹). In case of varieties, the highest

shoot dry weight (0.28 g) was recorded in Imdad-2005, followed by (0. 25 g and 0.22 g) that were obtained in Mehran-89 and TJ-83. While lowest shoot dry weight (0.21 g) was noted in TD-1. Interaction of varieties and NaCl revealed that the heaviest shoot dry weight (0.35 g) was observed in variety Imdad-2005 at the control. However, lowest shoot dry weight (0.15 g) was noted in variety TD-1 at 16 dS m⁻¹ level. This study agreed to those reported by [35, 36], they also found adverse effect of salt stress on shoot dry weight.

Table 9. Effect of different salinity (NaCl) levels on shoot dry weight (g) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad- 2005	0.35 a	0.29 ab	0.26 bc	0.23 cd	0.28 A
Mehran- 89	0.31 ab	0.25 bcd	0.23 cd	0.21 cd	0.25 B
TJ- 83	0.29 ab	0.24 cd	0.22 cd	0.20 cde	0.22 BC
TD- 1	0.26bc	0.23 cd	0.20 de	0.15 e	0.21 C
NaCl mean	0.31 A	0.25 B	0.22 BC	0.19 C	-

S.E. 0.0139

LSD (0.05) 0.0284

Root dry weight (g)

Root dry weight showed a significant reduction with increasing salinity levels. In this study, NaCl levels significantly affected on root dry weight of different wheat varieties (Table 10). The maximum root dry weight (0.13 g) was recorded at 0 d Sm⁻¹ level, followed by (0.10 g and 0.8 g), recorded at salt stress levels of 9 dSm⁻¹ and 12 dSm⁻¹, respectively. While minimum root dry weight (0.6 g) was obtained at 16 dSm⁻¹ NaCl level. Among the different wheat varieties, highest root dry weight

(0.15 g) was noted in Imdad 2005, followed by (0.11 g and 0.8 g) that was obtained in Mehran-89 and Tj-83. However, lowest root dry weight (0.05 g) was noted in TD-1. The interaction of varieties and NaCl concentrations reveals that maximum root dry weight (0.2 g) was recorded in the variety Imdad-2005 at control (0 dSm⁻¹). The minimum root dry weight (0.4 g) was noted in variety TD-1 under 16 dSm⁻¹ salt level. The trend in decrease in root dry weight under high salinity level was also reported by [37, 38], who suggested that the

salinity had a significant adverse effect on root dry weight.

Table 10. Effect of different salinity (NaCl) levels on root dry weight (g) of wheat varieties

Wheat varieties	Salinity levels (dS/m)				Varietal means
	0 dS/m	9 dS/m	12 dS/m	16 dS/m	
Imdad-2005	0.23 a	0.13 bc	0.10 cde	0.07 efgh	0.13 A
Mehran- 89	0.15 b	0.11 cd	0.09 defg	0.06 fgh	0.10 B
TJ- 83	0.13 bc	0.09 cdef	0.07 defgh	0.05 gh	0.08 B
TD- 1	0.09 cdef	0.08 defgh	0.05 gh	0.04 h	0.06 C
NaCl mean	0.15 A	0.11 B	0.08 C	0.05 D	-

S.E. 9.4223

LSD (0.05) 0.0192

Conclusion

It is concluded that different salinity levels had adverse effects on all the studied parameters. The control where no salt concentration was applied revealed positive effect on germination, shoot and root related attributes. Among varieties, Imdad-2005 showed more tolerance to salt stress as compared to other varieties viz; Mehran-89, TJ-83, TD-1.

Authors' contributions

Conceived and designed the experiments: TA Bhutto, NA Wahocho & AQ Gola, Performed the experiments: MH Bhutto & M Buriro, Analysis the data: SA Wahocho & M Buriro, Contributed reagents/materials/ analysis tools: NA Wahocho & TA Bhutto Wrote the paper: TA Bhutto & AQ Gola.

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