

## RESEARCH PAPER

# Response of Some Bread Wheat Genotypes (*Triticum aestivum* L.) to Salinity at Early Growth Stage

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### ABSTRACT:

This study aimed to detect the salinity harsh influence on germination of *Triticum aestivum* L. seeds, seedling growth and some physiological defense mechanisms to determine the salt-tolerant genotype. The study was conducted as a factorial experiment in a completely randomized design with 3 replicates for each treatment. Four bread wheat genotypes; Hawler- 2, Azady, Adana, Rabeaa were subjected to two irrigation patterns; tape water (control) and 100 mM NaCl (salt stress). The highest percent of germination; 46.77 recorded by Rabeaa genotype. Meanwhile Azady and Rabeaa had highest mean germination time (MGT); 16.71 and 16.03 respectively. Longest root was exhibited by Adana; 10.50 cm. while the longest shoot was represented by Hawler- 2; 16.40 cm. Highest dry weight of root was 1.01 g. Root: shoot ratio; 1.63 exhibited by Rabeaa. Hawler- 2 showed minimum chlorophyll a; 0.90 mg/ g. A maximum decrease recorded from chlorophyll b 2.3 mg/ g fresh leaves noticed by Adana and 2.18 mg/ g of fresh leaves of total chlorophyll by Hawler- 2 under salinity condition. Azady and Rabeaa recorded higher MSI%; 43.3 and 43 % respectively as compared to others. Rabeaa recorded highest water content, proline and sugar content; 22.7, 0.31 and 11.56 mg g<sup>-1</sup>. Therefore, it could suggest that Rabeaa and Azady can be successfully grown under 100 mM NaCl saline condition.

KEY WORDS: wheat, salt stress, germination indices, proline, sugar, chlorophyll.

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### 1.INTRODUCTION:

Wheat is the most widely grown and nutrient cereal crop around the world (Qadir et al., 2017). It is in common named the cereal's king because it's the staple food crop for one third and more of the population in the world (Shirazi et al., 2001). It is well known in arid and semi-arid regions for their low primary productivity which is owing to a combination of unpredictable water supply (Qadir et al., 2022). Which decreases the soil moisture and increases the saline soil patches in the soil (Out et al., 2019).

Germination of seeds is a serious problem for crop assemble, due to its vigor to restrict the vegetative area in dry lands (Qadir, 2019). Salinity patches in the soil act as a great threat that adversely influences the physiological and biochemical processes in plant cells which interfere the growth of the plant (Allakhverdiev et al., 2000). NaCl represents most dominant in saline soil to increase Na<sup>+</sup> and Cl<sup>-</sup> level, that affect the uptake Ca, Mg and K by the plant roots and correspondingly the Na<sup>+</sup> and Cl<sup>-</sup> levels increases in the plant cell as a result the physiological drought occurs in susceptible plants (Yassin et al., 2019). Seed is exposed to salinity at germination stage, which inhibits the imbibition of the water by reducing the osmotic potential of the growth media. Salinity also creates a toxic environment which inhibits the enzyme activities and metabolism of protein and nucleic acid and disturbs the utilization of seed reserves (Gupta and

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Huang, 2014). The enzyme which has a major role in seed germination in cereal crops is  $\alpha$ -amylase. It acts to break down the starch in endosperm to degrade sugars to cellular energy and provide nutrients for embryo growth to emerge plumule and radicle. Several investigations documented that most crops had less final percentage of germination under salt stress condition, vice versa, a significant increase in mean time of germination had been recorded. Imbibition process was inhibited due to an interference with water uptake and  $\alpha$ -amylase activity decrease could be the main under saline strain (Asghar, 2012 and El-Hendawy *et al.*, 2019). Many studies found that activity of the  $\alpha$ -amylase decreased sensitive genotypes with an increase of salinity level. The low activity of the enzyme causes slow down the sugar translocation to the embryo during seed germination. As the sugar concentration decreased in the growing cells, the osmotic potential increased and osmosis of water decreased, which retard seed germination Dkhil and Denden, 2010; Nizam, 2011; Hua-long *et al.*, 2014; Zhang *et al.*, 2017; Liu *et al.*, 2018 and El-Hendawy *et al.*, 2019). Important components synthesized acts as an osmotica in the form of sugars and organic compounds for osmotic adjustment for lowering leaf water potential under saline conditions. Proline, an amino acid, helps in osmotic adjustment proline became dominant within the tolerant than inside the sensitive plant (Qadir, 2019). This implied proline ability to support the plant to recover after salt stress as found by Gharsallah *et al.* (2016) and El-Hendawy *et al.* (2019).

Investigating plant response to salt stress is regarded as a key for identifying salt-tolerant genotypes., Germination of seed and seedling growth and development under saline conditions are the most crucial time throughout the entire life of the plants (Katerji *et al.*, 2005). Therefore, the study objectives are to investigate the effect of salt stress on seed emergence indices and growth parameters of seedlings. As well as their physiological response to identifying salt-tolerant and salt-sensitive genotypes to soil salinity at the early life stage.

## 1. MATERIALS AND METHODS

### 2.1 Treatments and design of experiment:

Four genotypes of bread wheat (*Triticum aestivum* L.); Hawler- 2, Azady, Adana, Rabeae were obtained from Agricultural research center in Erbil. They were examined to their ability to salt stress in a glass house experiment at biology department of College of Education, Salahaddin university- Erbil. Seeds surface sterilization done by soaking the seeds in sodium hypochlorite (1%) for 3 mints, later rinsed with tap water. Plastic pots of 3 kg (20 cm height and 30cm diameter) filled with previously sieved sandy loam soil. Ten seeds were sown per pot and kept under the average temperature of 30/25°C day/night. Then thinned to two seedlings per pot after seed emergence. The experiment was laid out as a factorial experiment in a completely randomized design (CRD). Include the interaction between two factors; four bread wheat genotypes and two irrigation patterns; tape water (control) and 100 mM NaCl considered as a (salt stress) as previously tested on bread wheat genotypes by Oyiga (2017) and Saddiq *et al.* (2021). Each replicated three instances. Seeds were considered germinated with the emergence of plumle up to 2mm, and number of germinated seeds was monitored daily for 10 days. Then two weeks after sowing, seedlings were thinned out to five seedlings per pot. Mean traits were compared using Duncan's multiple range test (DMRT).

### 2.2 Studied parameters:

#### 2.2.1 Germination indices and seedling growth characters:

Germination process is monitored daily to measure the indices of germination:

Final Germination Percentage (FGP):

$$FGP \% = \frac{Ng}{Nt * 100}$$

Where, Ng represent the total seed number and Nt number of germinated seeds.

Mean Germination Time (MGT):

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

Where n germinated seed on (D) day, and n is number of days of germination process (Sadeghi *et al.*, 2011; Qadir, 2018).

After one month the final germination growth characters were measured; length of shoot and root (cm) using a ruler, shoot and root dry weight (g) and root: shoot ratio. The seedling dry weight (DW) was obtained by keeping the samples at 72°C for 48 hours (Bağci and Yilmaz, 2003; Qadir *et al.*, 2022).

## 2.2 Physiological response parameters:

Leaf relative water content (LRWC %): fresh leaves were floated on water for 24 hours to saturate and weight, dried at 60 °C until constant weight was reached (Smart and Bingham, 1974).

$$LRWC \% = \frac{(fresh\ weight - dry\ weight)}{(turgid\ weight - dry\ weight)} * 100$$

Membrane stability index (MSI %): 100 mg of leaf discs washed with running tap water with corresponding wash using double distilled water. The discs were heated at 40°C min in 10 ml of double distilled water for 30. The first electrical conductivity (C1) was obtained by EC mater. A second EC read (C2) was obtained by putting the discs for 10 min in a boiling point (100°C) water bath (Hofmann *et al.*, 2003; Qadir *et al.*, 2019).

$$MSI \% = (1 - \left(\frac{C1}{C2}\right)) * 100$$

Photosynthetic pigments (mg g<sup>-1</sup> fresh weight): pure methanol (99.5%) is used for chlorophyll extraction from the mature leaves at seedling stage; by adding 3ml to 50 mg fresh left in dark condition for 2 hours. The fresh leaf is homogenized to ensure the extraction. Then centrifuged for 10 minutes at 10000 rounds per minute. The absorbance of the supernatant read by UV spectrophotometer (Genesys 10 SUV - Vis spectrophotometer) at two wave lengths; 650 and 665 nm (Horii *et al.*, 2007).

\*1000/(V\*W)

*Chlorophyll a*

$$= (16.5 * A665) - (8.3 * A650)$$

$$* \left( \frac{\quad}{extract\ volume * sample\ weight} \right)$$

*chlorophyll b*

$$= (33.8 * A650)(12.5 * A665)$$

$$* \left( \frac{\quad}{extract\ volume * sample\ weight} \right)$$

*Total chlorophyll*

= *chlorophyll a*

+ *chlorophyll b*

Proline level (mg g<sup>-1</sup> fresh weight): for proline extraction; 0.1 g fresh leaf is kept in 5 ml of sulfosalicylic (3%). Two ml of each of the supernatant, glacial acetic acid and ninhydrin reagent were mixed and left for 1 hour in a boiled water bath (100°C). After 1 hour immediately the mixture transported to an ice bath to stop the reaction. Lastly of toluene (4 ml) was added and vortexed. The supernatant was taken and its absorbance at 520 nm was recorded at using UV-Spectrophotometer- Biochem, 2100 (Bates *et al.*, 1973).

Total soluble sugars (mg g<sup>-1</sup> dry weight): 0.1g of leaf dried powdered used to extract sugar with ethanol ethonal (80%) total sugar sugars estimated using anthrone reagent. The extract absorbance was read at 630 nm. Glucose standard curve is used to estimate total sugar of the extract expressed in mg of sugar per gram of leaf dry weight (Dubois *et al.*, 1956).

## 2.3 Statistical Analysis

Analysis of variance (ANOVA) of the data computed using the Statistical package for the Social Sciences (SPSS) model 26. The Duncan's Multiple Range Test (DMRT) test used to check the variations among the mean values of studied parameters at 5 % level of possibility.

## 3. RESULTS AND DISCUSSION

### 3.1. Germination indices and seedling growth

Significant variation ( $p \leq 0.05$ ) and decrease was observed among bread wheat genotypes germination indices and growth traits under salt stressed condition as shown in table (1). Genomic variation among the genotypes might have a significant influence on germination and seedling growth (Sourour *et al.*, 2014 and Fatih and Kirli, 2018). The highest percent of germination was 46.77 recorded by Rabeaa genotype. Meanwhile Azady and Rabeaa show similar responses by recording highest MGT; 16.71 and 16.03 respectively. Germination process affected due salinity stress indirectly because of osmotic stress. The water uptake was limited and caused ionic toxicity, which might affects the division and expansion of cells, in addition to their

influence for modifying some key enzymes' activity. Ultimately the reduction of food utilization by the embryo (El-Hendawy et al., 2019). Maximum root length was exhibited in Adena; 10.50 cm. while longest shoot represented by Hawler-2; 16.40 cm. The maximum root dry weight: 1.01 g was found in Rabeaa which led to had maximum root: shoot ratio; 1.63 (table 2). It might be inferred the virulence of salinity on germination and seedling growth in a negative manner through affecting the seed imbibition mechanism and retarding the enzymes activity that generated the growth and development reduction and ultimate final yield loss. Results were close agreement with Aflaki et al., 2017; Fellahi et al., 2019 and Panhwar et al., 2021.

### 3.2. Physiological response:

Data in table (2) explain that salt stress gave rise a significant influence on leaves' chlorophyll. Azady and Rabeaa supported better salt tolerance ability in the two genotypes. While Hawler- 2 showed a significant decrease in chlorophyll a content; 0.90 mg per g fresh weight of leaves. A maximum decrease recorded of chlorophyll b 2.3 mg/ g fresh leaves under salinity condition noticed by Adena and 2.18 mg/ g of fresh leaves of total chlorophyll by Hawler- 2. In contrast the others Adena, Azady and Rabeaa showed no significant difference in their content of chlorophyll a, b and total under salt stress conditions. The declined chlorophyll content due to salt stress in consent with earlier studies on different wheat species by; Rauf et al. (2010); Kumar et al. (2017) and Atlassi and Bahmani (2019). Accumulation of sodium ions have an inverse effect on chlorophyll assemble and photosystem-II in photosynthesis process in plants (Jiang et al., 2017). Despite the low level of Mg<sup>2+</sup> linked to the low chlorophyll level in leaves in the salt-sensitive plants (Zhang et al., 2014).

The decline of the relative water content in leaves (LRWC) might be considered as an early indication of stress effect (fig. 1). All genotypes showed a significant decrease in RWC under salt stressed condition. However, Rabeaa differed significantly as compared to others in recording highest water content in their leaves; 22.7 under the same condition. This decrease trend in RWC in salt- sensitive plants could be linked to a vigor decrease in the plant (Halder and Burrage 2003). Kumar et al. (2018) reported a similar reduction in

RWC intolerant and sensitive cultivars of bread wheat.

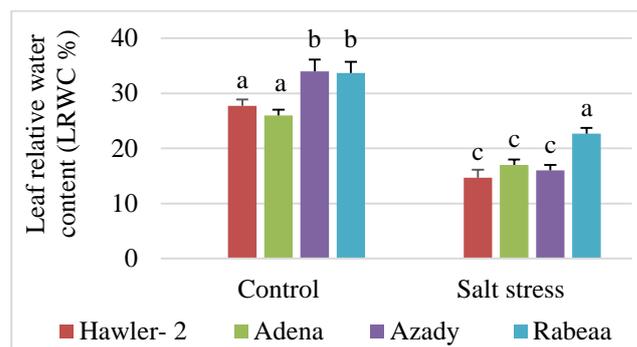


Fig. (1): Variation within leaf relative content (LRWC %) in *Triticum aestivum* L. genotypes under two irrigation patterns.

MSI % reduced extensively at salt stress conditions also confirmed more decline in susceptible genotypes. Azady and Rabeaa had been recorded considerably higher MSI %; 43.3 and 43 % respectively as compared to others (fig. 2). The changes in plasma membrane permeability (electrolyte leakage) are primarily controlled by the membrane transport proteins (Jacobs et al., 2011). The alteration in membrane permeability and ion loss under stress condition is connected with the modulation of in lipid matrix and proteins in the plasma membrane. It has been shown that the extent of membrane injury is mostly pronounced in salt sensitive genotypes as compared to the salt tolerant ones. Which might be maintained via inherited or induced membrane protection mechanisms under salt stress (Mansour, 2013). The found results in assent with those of Rao et al. (2013) and Kumar et al. (2017) whom they reported that salt-tolerant genotypes possess lower peroxidation of membrane lipids and more stable under stressed conditions.

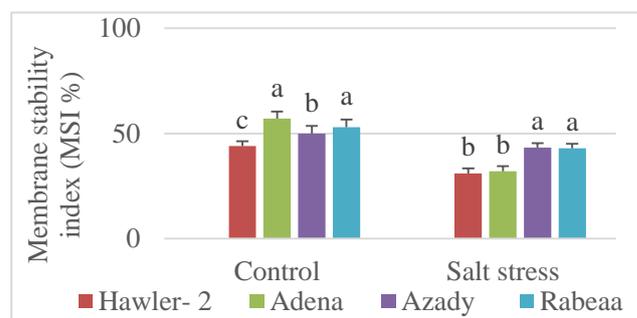


Fig. (2): Variation within membrane stability index (MSI %) in *Triticum aestivum* L. genotypes under two irrigation patterns.

Compatible solutes: during abiotic stress; sugars and prolines act in efficient role in osmotic adjustment and structural stability (Romero-Aranda et al., 2006 and Qadir et al., 2019). The

results in (fig. 3) shows a considerable increase in proline content of the four genotypes. The maximum increase 0.31 mg g<sup>-1</sup> fresh weight of proline was observed in Rabeaa, while the minimum was observed in Hawler- 2 and Adena. Salt stress caused a significant increase in total sugar content in leaves of Rabeaa; 11.56 mg g<sup>-1</sup> dry weight as compared to the other three genotypes (fig. 4). Soluble sugars act as potential osmo-regulators. Elevated sugar levels under stress conditions might contribute towards turgor maintenance (Kumar et al., 2017). In fact, under stress conditions, cells release more compatible solutes to maintain their redox potential. Proline not only acts as an osmolyte but it has other functions as well. It is accumulated under stressed conditions supplies energy for growth and survival, might have the function of scavenger of reactive oxygen species or act as an osmolyte and thereby helps the plant to tolerate stress (Mafakheri et al., 2010). Increased accumulation of soluble sugar and proline agreed with the earlier observations of Romero-Aranda et al. (2006) and Kumar et al. (2017). The key factor of desiccation and salinity resistance might belong to the accumulation of compatible solutes to increase their defense mechanism through osmotic adjustment. (Lata et al., 2017).

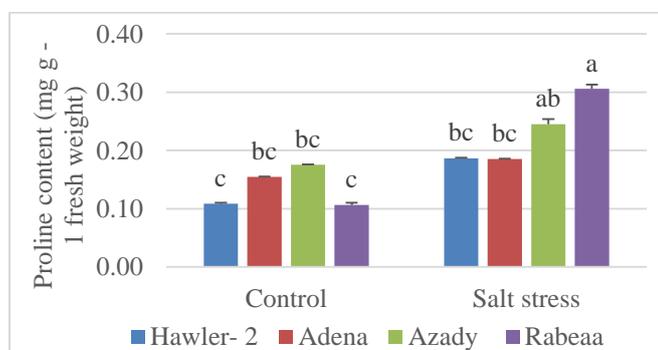


Fig. (3): Variation within proline content (mg g<sup>-1</sup> fresh weight) in *Triticum aestivum* L. genotypes under two irrigation patterns.

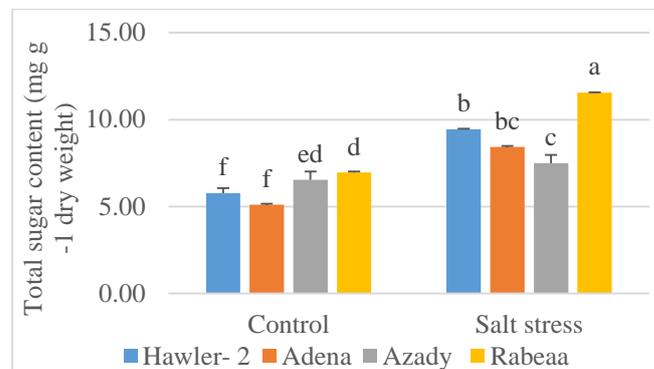


Fig. (4): Variation within total sugar content (mg g<sup>-1</sup> dry weight) in *Triticum aestivum* L. genotypes under two irrigation patterns.

#### 4. CONCLUSION

Our comprehensive analysis is based on germination indices, seedling growth characters' output to identify the most salt-tolerant bread wheat genotypes; Rabea and Azady. As well as mostly related traits to salt-tolerant genotypes restrict at the early growth stage were membrane stability index, chlorophyll content, higher accumulation of osmolytes, proline and sugar.

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#### Conflict of Interest (1)

The authors don't have conflict of interest for this publication.

Table (1): Variation within germination indices and seedling growth characters in *Triticum aestivum* L. genotypes under two irrigation patterns.

Characters	Irrigation pattern	Genotypes			
		Hawler- 2	Adena	Azady	Rabeaa
FG %	Control	73.33 ± 3.44 b	90.12 ± 6.76 a	46.71 ± 3.45 c	76.7 ± 3.56 b
	Stress	40.25 ± 2.45 b	33.33 ± 2.24 c	43.33 ± 3.01 b	46.77 ± 3.14 a
MGT	Control	8.31 ± 0.54 a	7.01 ± 0.39 b	8.32 ± 0.49 a	6.21 ± 0.32 b
	Stress	12.03 ± 1.13 c	14.31 ± 1.06 b	16.71 ± 2.01 a	16.03 ± 1.98 a
Root length (cm)	Control	9.40 ± 1.32 d	25.10 ± 3.13a	12.80 ± 1.87 c	22.40 ± 3.03 b
	Stress	8.10 ± 1.03 b	10.50 ± 1.32 a	6.10 ± 0.76 c	7.20 ± 0.32 c
Shoot length (cm)	Control	19.70 ± 2.12 b	24.60 ± 4.12 a	20.90 ± 2.65 b	24.30 ± 4.12 a
	Stress	16.40 ± 3.12 a	13.60 ± 2.10 b	12.00 ± 1.23 b	13.90 ± 2.11 b
Root dry weight (g)	Control	0.91 ± 0.01 b	1.31 ± 0.04 a	1.24 ± 0.05 a	1.12 ± 0.03 b
	Stress	0.71 ± 0.08 b	0.74 ± 0.07 b	0.75 ± 0.08 b	1.01 ± 0.11 a
Shoot dry weight (g)	Control	0.81 ± 0.06 c	1.01 ± 0.09 b	0.93 ± 0.08 b	1.12 ± 0.09 a
	Stress	0.71 ± 0.07 a	0.67 ± 0.06 a	0.72 ± 0.06 a	0.62 ± 0.05 a
Root: shoot	Control	1.12 ± 0.91 b	1.30 ± 0.93 a	1.33 ± 0.89 a	1.00 ± 0.78 b
	Stress	1.00 ± 0.83 b	1.10 ± 0.85 b	1.04 ± 0.75 b	1.63 ± 0.91 a

Table (2): Variation within chlorophyll content (mg g<sup>-1</sup> fresh weight) in *Triticum aestivum* L. genotypes under two irrigation patterns.

Photosynthetic pigments	Irrigation pattern	Genotypes			
		Hawler- 2	Adena	Azady	Rabeaa
Chlorophyll a	Control	2.22 ± 0.21 a	2.77 ± 0.24 a	2.76 ± 0.24 a	2.84 ± 0.27 a
	Stress	1.28 ± 0.09 a	2.3 ± 0.08 b	2.48 ± 0.06 a	2.58 ± 0.07 a
Chlorophyll b	Control	1.07 ± 0.12 a	1.4 ± 0.09 a	1.59 ± 0.16 a	1.62 ± 0.08 a
	Stress	0.90 ± 0.02 b	1.24 ± 0.03 a	1.29 ± 0.06 a	1.32 ± 0.07 a
Total chlorophyll	Control	3.29 ± 0.43 b	4.17 ± 0.67 a	4.35 ± 0.53 a	4.46 ± 0.57 a
	Stress	2.18 ± 0.81 b	3.54 ± 0.74 a	4.13 ± 0.65 a	4.17 ± 0.73 a

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