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Research Article

Evaluation of Germination Percentage and Some Physiologic Factors under Salinity Stress and Gibberellic acid Hormone (GA3) Treatments in Wheat (*Triticum aestivum* L.)

Parvaneh Rahdari *[1] and Seyed Meysam Hoseini [2]

[1]Biology Departmant, Islamic Azad University, Tonekabon Branch, Iran.
[2] M.Sc in Islamic Azad University of Tonekabon Branch
*Corresponding author: *Rahdari_parvaneh@yahoo.com*

Abstract

Salinity stress as a major adverse factor can reduce leaf water potential, that leads to reduced turgor and some other responses, and ultimately reduce crop productivity in arid and semiarid zones. Wheat (*Triticum aestivum L.*) is one of the first plant which cultivated by human beings and, now, it is cultivated in extended areas, because of its simplicity in cultivation and its suitability for different climates. Wheat is a mandatory salt tolerant crop and serves as a staple food in 43 countries including Iran, therefore it is grown in a large area. In order to, investigated effect of Gibberelic acid on morphological and physiological factors of wheat under salinity stress. This experiment carried out in salt concentrations (NaCl 0, 100, 200 and 300 mM) and GA3 (200 mg/L) then investigated some physiological activities and germination percent of Wheat (*Triticum aestivum L.*). The results revealed that, NaCl treatments induced drastic reduction in germination percent of seeds wheat plant that in salinity of 250mM showed the most of decrease in germination percent that statistically significant in P<.0/01. Furthermore, concentration of 250mM of salt significantly decreased photosynthetic pigments content include chla and chlb, reducing sugars, protein contents, activity of antioxdent enzymes of peroxidase and catalase ,Which was statistically significant in P<0/01. In this study utilization of concentration of GA3 (200 mg/L) inhibited effects of salinity stress on all studied physiologic factors like pigments content, reducing sugars, protein contents, activity of antioxdent enzymes of peroxidase and catalase.

Keywords: Wheat (*Triticum aestivum L.*), Stress Salinity, Phytohormone, Gibberellic Acid, Morphological Factors, Physiological Factors.

Introduction

A wide range of environmental stresses such as drought, salinity, alkalinity and pathogen infection are harmful to the plants. Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zhu, 2001). Salt stress in soil is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity. Salinity is a soil condition by high content of soluble salts. The problem of soil salinity is increasing. Soil salinity stresses plants in two ways: High concentrations of salts in the soil make it harder for roots to extract water, and high concentrations of salts within the plant can be toxic (Munns and Tester, 2008). Reports by the FAO (2005) indicate that 2% of agriculture land is salt affected. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, that ultimately causing decreased of plant productivity (Sairam and Tyagi, 2004). Wheat is a plant of grasses family (Poacea) and Triticum genus that its scientific name is *Triticum aestivum*. Wheat is one of the oldest cultivated plants for man to cultivate the most extensive and the highest value taken (zaree et al, 2006). It is easy for agriculture to adapt to different weather conditions as one of the major crops in agricultural production and annual consumption of bread is an urgent need to produce more of this product. Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zhu, 2001). Studies carried out in connection with the evaluation of salt tolerance in wheat, this plant is as half-sensitive and half-tolerant to salinity stress was classified (Mehrabi, 2002). Given the importance of the wheat grain in the world is the second most important plant families, this study is importance for food quality and human health. According to the Food and Agriculture Organization of the world (FAO) in 2001, the 217 to 231 million acres under wheat cultivation and production of 590 million tonnes. Stresses result of an unusual combination of biological and physiological processes that impact the environment. Stress can be reduced in a plant growth, yield loss, or death occurs (Jain, 2001). One of the most influential environmental stresses on growth and yield of crops, is soil. Salinity stress had been paid attention by many farmers, and different methods for its solving had been thought which was effective in its time and place. But now days, regarding the progresses in physiology and biochemistry, the negative effects of environmental stresses should be reduced (Ozhan, 2013) that Application of plant growth regulators such as gibberellic acid are from them. Some researchers believe that gibberellin in reducing the negative effects of salinity on plant growth have been useful (Ghorbani et al, 2011). Gibberellin biosynthesis through developmental and environmental stimuli can be controlled (Olszewski, 2002). Some studies have shown that under conditions of biological and Abiological stresses, gibberellin synthesis and accumulation increased in plants (Ghorbani et al, 2011). In one of experiments, it was shown that the application of gibberellin under salinity stress, will enhance the growth factors in Rice (*Oryza sativa*) and Wheat (*Triticum aestivum*) (Varma, 1988). Also, Gomathi and Thandapan in 2005 were showed that salinity decreased the growth and yield of Sugarcane that gibberellin application to reduce these negative effects. Also, some studies have shown that external application of gibberellin in salinity condition by stimulating the synthesis of catalase reduces the negative effects of salinity and improve the growing conditions of the plants will be stress that this has been proven in the Hemp (*Hibiscus sabdariffa* L.) plant (Ali et al, 2012). In this study, with attention to given the importance of wheat in feeding and global population growth, and also little information and researches on the effect of gibberellin on growth of the plants under salinity stress, we decided to use external effects of this hormone on the growth of wheat under salt stress was studied that a major step forward in improving conditions for growing wheat in saline soil to be removed. Application of exogenous treatment of hormone such as Gibberellin could reduce the effects of salinity stress.

Materials and Methods

Seeds of Wheat (*Triticum aestivum* L.) produced from Seeds and Seedlings Center of Iran. The study was done under controlled condition. For antiseptic of seeds, 5% sodium hypochlorite was put them in this solution for 5 to 8 minutes and washed in distilled water (Falahati, 2005). For germination, seeds were put in darkness condition into germinator at temperature of 25 centigrade degree with 16 h light and 8 h darkness conditions. The time required for germination was one week. After the advent of the first leaf, the resulting seedlings were transferred to light environment for 24 h and the green leaves were transferred to hydroponic environment. Then seedlings for one week in a row were consistent with Hoagland solution (Johnson et al, 1957). Once the seedlings have reached a four week period, salinity treatments of 0, 100, 200 and 250 mM of sodium chloride were placed. After a week of stress,was applicated gibberellin (GA3) with a final concentration of 200 mg/lit in Hoagland solution. The salinity treatment and gibberellin treatments were applied for 2 weeks. Plants were kept in grown room with temperature of 20 to 23oC, relative humidity of 70 to 80%, and light/dark period of 17/7 hours. Nutrition solution once a week was replacemented and the pH=6 was adjusted daily. Germination Percent Photosynthetic Pigments (Lichtenthaler, 1987), Carbohydrate Content (Nelson, 1943), Protein Content (Lowry, 1951), Proline Concentration (Bates, 1973) and Antioxidant Enzymes (Chonce et al, 1995) was studied in this research.

Statistical analysis

Data analysis, variance and comparison of means were done by Danken experiment and SPSS software; for drawing of diagrams, Excel software was used.

Results

In this research, in relation with salinity stress effect with levels of 100, 200 and 250 mM of chloride sodium (NaCl) and gibberellic acid (GA3) with level of 200 mg/lit on some morphological factors (germination percent) and physiological factors (proline content, protein content, photosynthetic pigments content, carbohydrate content and antioxidant enzymes) was evaluated.

Germination Percent

In relation to germination percent synchronized with increasing stress levels, its decrease was observed in statistical level 0.01, which was meaningful. The least rate of germination percent was in 250 mM level (Table 1). Also, external gibberellic acid hormone treatment to overcome the damaging effect of salt contributes to improving the germination percentage (Table 2) that this results with data of Sekhar in 1994 on bean was established, was consistent. This study showed that application of the rate of germination than seeds gibberellic acid that are only treated with sodium chloride.

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Germination Percentage (%)	100 a	76 ab	52 ab	18 c

Table 1. Effect of Salinity Stress Levels on Germination Percentage

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Germination Percentage (%)	100 a	95 a	83 ab	36 c

Table 2. Effect of Salinity Stress Levels and Gibberellin Treatment on Germination Percentage

Sugar Content

The concentration of leaf carbohydrate synchronized with increasing of salinity stress level had meaningful decrease and the highest sugar concentration in stress level was blank level and the least concentration was in level of 250 mM of NaCl (Table 3) that because it can inhibit photosynthesis while reducing the content of photosynthetic pigments increased as the level of salinity which is due to reduced leaf surface. This is corresponded to found Maggio et al in 2000 and in *Salvadora persica* plant. Also, the results of this research showed that using and spray levels of external gibberellic acid hormone improved carbohydrate concentration in plant under salinity stress that due to can improving of photosynthetic conditions (Table 4).

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
sugarContent	5.1a	4.4 ab	3.8 b	2.83bc

Table 3.Effect of Salinity Stress Levels on Carbohydrate Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
sugarContent	5.1 a	4.64 ab	4.22 ab	3.52bc

Table 4.Effect of Salinity Stress Levels and Gibberellin Treatment on sugar Concentration

Proline Content

Increasing levels of free amino acids such as proline under salinity stress in extensive of studies have been presented. The free proline rate in Wheat (*Triticum aestivum* L.), also, with increasing stress level had meaningful increase. The highest level of proline in stress was 250mM and the least rate of proline was in blank level (Table 5) its increase was observed in statistical level 0.01, which was meaningful that with found of study on Bean plants with Singh et al in 1994 were compatible that main reason for the reduction was of final nitrogen. In this study it was observed that using of gibberellin hormone level with increased of protein content will decrease of free proline concentration (Table 6) that consistent with results of Ali in 1992.

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Proline Content	14.60 d	72.80 c	90.33 bc	105.2b

Table 5. Effect of Salinity Stress Levels on Proline Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Proline Content	14.60 d	37.49 c	72.44 b	82.86ab

Table 6. Effect of Salinity Stress Levels and Gibberellin Treatment on Proline Concentration

Protein Content

The results of this study showed that simultaneously with increasing of salinity levels, protein content in wheat seedlings was decreased that was significantly in statistical level of 0.01 (Table 7) that caused increasing level of free amino acids and especially proline, which cause of osmotic adjustment. On the other hand, in this study was found that treatment with gibberellin hormone level in wheat seedlings under salinity stress, by adjusting the destructive effects of salinity on seedlings caused of was increased of proteins levels (Table 8). The results of this study with the results of Hamed et al, (1994).

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Protein Content	52.86 a	49.06 a	43.85 ab	22.19c

Table 7. Effect of Salinity Stress Levels on Protein Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Protein Content	52.86 a	52.84 a	48.33 b	28.27bc

Table 8. Effect of Salinity Stress Levels and Gibberellin Treatment on Protein Concentration

Photosynthetic Pigments Content

Analysis of variance showed that photosynthetic pigments content was affected by salinity at 0/01 of probability level. Chlorophyll a and b was decreased under salinity stress that was meaningful in statistical level of 0/01 (Table 9). On the other hand, in this study was found that treatment with gibberellin hormone level in wheat seedlings under salinity stress, by adjusting the destructive effects of salinity on seedlings caused of was increased of chlorophyll levels (Table 10) that due to increase of the number of leaf chloroplasts.

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Chlorophyll a Content	1.36 a	0.97 ab	0.74 b	0.60c
Chlorophyll b Content	0.73 a	0.55 ab	0.43 b	0.35bc

Table 9. Effect of Salinity Stress Levels on Chlorophyll a and b Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Chlorophyll a Content	1.36 a	1.15 ab	0.99 b	0.82bc
Chlorophyll b Content	0.73 a	0.67 ab	0.60 b	0.53bc

Table 10. Effect of Salinity Stress Levels and Gibberellin Treatment on Chlorophyll a and b Concentration

Catalase and Peroxidase Contents

In this study, results showed that antioxidant enzyme activity (Catalase and Peroxidase) was decreased under salinity stress that meaningful in statistical level of 0.01 (Table 11 and 13), that leads to accumulation of toxic levels of hydrogen peroxide in wheat seedlings under stress condition. This data were compatible with results of Kocsy et al in 1991 of the study on wheat plants. Also, increasing of GA_3 levels caused increases in antioxidant enzymes content under salinity condition (Table 12 and 14).

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Catalase Content	229 a	184 ab	121 bc	75c

Table 11. Effect of Salinity Stress Levels on Catalase Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Catalase Content	229 d	270 cd	221 cd	308b

Table 12. Effect of Salinity Stress Levels and Gibberellin Treatment on Catalase Concentration

Treatments	Blank	NaCl 100 mM	NaCl 200 mM	NaCl 250 mM
Peroxidase Content	23.5 a	22.1 a	19.2 ab	8.9c

Table 13. Effect of Salinity Stress Levels on Peroxidase Concentration

Treatments	Blank	NaCl 100 mM + GA_3 200 mg/L	NaCl 200 mM + GA_3 200 mg/L	NaCl 250 mM + GA_3 200 mg/L
Peroxidase Content	23.5 ab	26 a	21.8 ab	12.1c

Table 14. Effect of Salinity Stress Levels and Gibberellin Treatment on Peroxidase Concentration

Discussion

A wide range of environmental stresses such as drought, salinity, alkalinity and pathogen infection are harmful to the plants. Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zhu, 2001). Salt stress in soil is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity. Salinity is a soil condition by high content of soluble salts. The problem of soil salinity is increasing. Soil salinity stresses plants in two ways: High concentrations of salts in the soil make it harder for roots to extract water, and high concentrations of salts within the plant can be toxic (Munns and Tester, 2008). Reports by the FAO (2005) indicate that 2% of agriculture land is salt affected. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, that ultimately causing decreased of plant productivity (Sairam and Tyagi, 2004). Application of exogenous treatment of hormone such as Gibberellin could reduce the effects of salinity. Exogenous application of Put has been successfully used to enhance salinity that in this study was investigated. The growth regulator treatments resulted in significant increased growth in Wheat (*Triticum aestivum* L.) under salinity stress.

Germination Percent

Germination process is very complex that begins with water absorption and after a short break made of an protein enzymatic and activated and biosynthesis gibberellin inhibitor active and inhibition of seed germination (Mohammadi et al, 2009). Different studies showed that different levels of salinity stress on germination in a variety of plants which can infer to meaningful decreasing on *Vigna radiata* L. and *Lens culinaris* (Mohammadi et al, 2009), *Elymus Junceus* (Turkyilmaz, 2012), forage sorghum and pearl millet (Sakhabutdinova et al, 2003). Mainly salinity stress by low osmosis potential and ionic poisonous can reduce germination and for this reason it can influence on germination percent more than drought. GA_3 treatments increased germination rate of the plants under salinity condition. Under saline conditions, seed germination and seedlings growth have been improved by application of GA_3 (Sakhabutdinova et al. 2003., Khan et al, 2004., Turkyilmaz, 2012). Gibberellin with increased of cell division and elongated of cells, effects on seed germination and early growth in stress condition (Kaur et al, 1998). One of the most effective factors on seed germination, is release of food storage such as starch, that this process performed byα-Amylase enzyme.GA_3 involved in increase of biosynthesis and secretion of α-Amylase enzyme in plants.

Carbohydrate Content

Data presented in Table 3 show that there is significant and highly significant decreases in reducing sugars and sucrose contents of Wheat (*Triticum aestivum* L.) plant with increasing salinity levels that this is due to the inhibition of photosynthesis which is associated with decline in pigment contents resulted from the reduction in leaf area or due to decrease in leaf organic acid with salinity (Mohammad, 2007). Increase of photosynthetic carbohydrate concentration is a signal in salinity stress, that this causes of decrease in water potential, osmotic pressure adjustment, and inhibition of oxidative stress into stress condition such as salinity stress. But, on the other hand, salinity due to inhibition of photosynthesis causes reduced levels of photosynthetic pigments decreased carbohydrate levels, that Maggio et al in plant *Salvadora persica* in 2000 were concluded. But the use of external gibberellic acid because the device improves photosynthetic and chloroplast structure will lead to increase of carbohydrate concentrations under salinity stress that Aldesuquy in 1992 as a result. Huber et al in 1974, increase of carbohydrate metabolism under salt stress and in effect of treatments of gibberellic acid concentration in *Pennisetum typhoides* was reported that because it increase the level of α-Amylase enzyme and increase starch degradation in cells was reported. This results in Rice (*Oryza sativa*) in 2006 and bye Kim et al, also, was reported. It can be concluded that application of gibberellin leads to the opening of the stomata under salinity stress.

Proline Content

Under salinity stress, plants have mechanisms against with that which accumulation of solution components such as proline one of the primary responses of plant proportion to salinity. Yazici et al in 2007 was performed of Purslane, he was observed that with increasing of salinity imposed, free proline content in leaves was increased which confirm this result. In other research on Barley (*Hordeum vulgare* L.) was observed that proline as a reducer component of osmosis pressure in response to increase of salinity (Ueda et al, 2007). Also, increasing of proline content in cotton (Desingh et al, 2007), *Paulownia imperialis* (Astorga et al, 2010) and wheat (Khan et al, 2009) was synchronised with increasing of salinity level is reported. Increasing of proline level under salinity stress was recognized due to that proline is consistent smolite, macromolecules conserver and remover of active oxygen producing in during of environmental stress. Studies showed that proline accumulation in plants was performed by different methods (Yordanov et al, 2003):

1) Stimulating of synthesis from pre-material,
2) Reducing of Proline Oxidas activity,
3) Proteins destruction
4) Reducing of proteins structure partnership.

Application of gibberellin concentrations under salinity stress caused of increase the nitrogen and protein level and decrease of free proline level in plants. This results by Shahba et al in 2010 and studied in Tomato and by sakhabutdinova et al in 2010 and in Wheat (*Triticum aestivum* L.) seedlings, was reported.

Protein Content

Several reports on the increase and decrease protein levels during salinity stress. In this research, proteins concentrations with increasing of salinity level showed reduction, of course in relation with salinity effect on protein synthesis, showed different results which depended on plant species. Terminal content decreasing of solution protein in plants such as *Helianthus annuus* and *Colcus blumei* (Yu et al, 1999), *Vicica faba* and *Amaranthus* (Doganlar et al, 2010), and also increasing of protein level in plants such as cotton(Jiang et al, 2005), *Pancratium maritimum* (Khedr et al, 2003) under salinity stress was reported. Vibration in protein solution terminal content, was resourced synthesis of stress proteins (Doganler et al, 2010). In general, decrease in the salinity of surface protein breakdown and production of free amino acids such as proline in plant tissue is. On the other hand, gibberellin treatments can lead to increased levels of protein during the stress condition that With the increase in Nitrate Reductase enzyme activity in the protein surface tension is increased was performed. This results was reported with Masroor et al in 2006 in Tomato and El-Tayeb in 2005 in Wheat (*Triticum aestivum* L.).

Photosynthetic Pigments Content

Salinity has toxic effects on plants and causes of changes in metabolic activity such as reduced activity of chloroplasts, reduced photosynthetic pigments, reducing the rate of photosynthesis and increase of respiration rateWhich ultimately leads to increased production of reactive oxygen species in plant will be. With changing of leaf chlorophyll content by salinity stress, terminal photosynthesis level, distillation speed and stomatal transport was under control meaningfully (Doganlar et al, 2010).Content reduction of chlorophyll in plants such as *Poulownia imperialis* (Astorga et al, 2010),Bean (Beinsan et al, 2003) and *Carthamus tinctorius* (Siddiqi et al, 2009) was reported. The cause of this reduction was the increasing of destructive enzymes called chlorophyllase. Plant hormones such as gibberellin, has special effects on leaf anatomy and chloroplast structure. salinity Through the influence of on peroxidase enzymes and chlorophyll degradation can be induces catabolism that Gibberellin reducesof activity of these enzymes. This results was reported with El-Tayeb in 2005 andin Wheat, Younis in 1991 in *Pisium sativum*, Aldesuquy in 1992 in Wheat and El-Bastawisy in 1999 in Wheat.

Catalase and Peroxidase Content

Under salt stress, antioxidant enzymes activity reduced of that due to reduced activity of catalase and peroxidase in plants under stress can increase levels of sodium and chloride ions. Decrease of catalase and peroxidase activity caused of accumulate toxic levels of hydrogen peroxide followed. This results was reported by Kocsy et al in 1991 and in studied on Wheat and also, Lee in 2001 in Rice. The study found that the use of external concentrations of gibberellin increased antioxidant enzymes levels in the plants and will reduce the level of hydrogen peroxide. This results was reported by Mohammed et al in 2007 in Wheat, Nandini et al in 2002 in *Vigna radiata*, Chakabarti and Mukherji in 2003 in *Vigna radiata.*

Conclusion

Accordingly, bad effects of salinity on the vegetative growth significantly reduced by gibberellin treatments.

Gibberellin are involved in plant defense to environmental stresses. In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance gibberellin biosynthesis in response to environmental stresses including salinity (Amir et al, 2011). On the other hand, exogenous GA_3 application alleviated growth inhibition of Wheat and improved grain yield of plants under salinity. These results indicate that the accumulation of GA_3 may be detrimental for growth and development of plants.

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