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Effects of GA_3 Treatments on Ion Accumulation in Leaves of Pepper Plants Under Salt Stress

Ozlem Uzal*, Fikret Yasar

Horticulture Department, Agricultural Faculty, Yüzüncü Yıl University, Van, Turkey

Email address:
ozlemuzal@yyu.edu.tr (O. Uzal), fyasar@yyu.edu.tr (F. Yasar)
*Corresponding author

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Abstract: Experiments were conducted with Demre long pepper cultivar in a climate chamber with controlled climate parameters and in a hydroponic system with Hoagland nutrient solution. Three-week old seedlings were subjected to 100 mM NaCl treatments and samples were taken on 10^{th} day of the treatments for physiological and biochemical analyses. With the idea that gibberellic acid reduces negative impacts of salt on plants and regulates ion uptake, thus provide an ion balance, plants were also subjected to Gibberellic acid (GA_3) treatments at different doses (5ppm, 7.5ppm and 10ppm). Then, leaf samples were taken again on 10^{th} day of treatments and Na, K, Ca and Cl analyses were performed on samples. Present findings revealed that GA_3 treatments together with NaCl treatments recessed plant growth and development, but provided significant contributions in regulation of ion uptakes and providing an ion balance. The best GA_3 doses for plant growth and ion balance were identified as 7.5 and 10 ppm.

Keywords: Pepper (*Capsicum annuum* L.), Gibberellic Acid, Ion Uptake, Salt Stress

1. Introduction

Pepper is originated from South America and widespread throughout the world from there. Origin center of various types and forms is tropical regions of South America, especially of Brazil. According to 2012 FAO statistics, world annual production was 461 452 tons [1]. According to TUIK 2015 statistics, annual production in Turkey was 919.004 tons [2]. Pepper (*Capsicum annum* L.) is produced over open fields and under-cover, especially in greenhouses along the Mediterranean coastal zone of Turkey. Salinity problems over pepper-cultivated lands and irrigation-induced salinity problems negatively influence growth and development of salt-sensitive pepper plants [3, 4]. Salinity-induced damages are also observed in open fields. Since pepper plants have a great share in open and under-cover production activities, they are negatively influenced by salt-stress. Yield losses may reach to serious levels because of unavailable conditions. The most effective and instant way of elimination of such negative conditions is to develop salt-tolerant varieties and cultivars and to perform practices able to eliminate negative impacts of salinity on plants.

Growth regulators like GA_3 and cytokinin provided significant contributions in reducing negative impacts of salt stress on plants [5]. Thusly, Lin and Kao (1995) reported that GA_3 treatments reduced the negative impacts of salt stress on germination of salt-treated paddy seedlings [6]. Ashraf et al. (2001) reported significant increases in Na and Cl concentrations in shoot and roots of two wheat genotypes grown in saline growth medium, but increased Na and Cl accumulation in shoot and roots of both genotypes with GA_3 treatments. Researchers reported higher ion accumulation in salt-sensitive Barani-83 than in salt-tolerant SARC-1 genotype. Net CO_2 assimilation continuously decreased in both wheat genotypes with increasing $NaCl_2$ quantities. GA_3 treatments relieved the negative impacts of salt stress on both genotypes [7].

In recent years, previous studies indicated the role of hormones in response of plants to negative environmental conditions and salinity-like abiotic stressors [8, 9]. There is a

general consensus on stimulation of internal hormone levels with external application of different growth regulators [10]. Rodriguez et al. (2006) indicated that potential salt tolerance of paddy seedlings might come from existence of hormones in EP produced by cyanobacteria. As a respond to salt stress, GA_3 treatments stimulated ABA production and reduced the ratio of growth regulators [11]. In another study carried out with eggplants, recessions were observed in plant development with salt and GA_3 treatments as compared to control plants, but GA_3 treatments prevented salt-induced necrosis and deaths. A selective ion uptake was also observed in that study [10].

In present study, possible effects of salt and gibberellic acid treatments on ion uptake of pepper plants were investigated.

2. Material and Method

Experiments were conducted at Physiology Laboratory of Yüzüncü Yıl University Agricultural Faculty Horticulture Department. Demre long green pepper cultivar was used as the plant material of the study. Experiments were conducted in a climate chamber with split air conditioner providing normal atmosphere and in a hydroponic culture.

Pepper seeds were sown in pumice-filled plastic germination containers as to have 100 seeds per container and they were irrigated with tap water. Following thoroughly wetting of pumice and drainage of excess water, germination containers were placed in a climate chamber with 25±1°C temperature and 70% relative humidity. Irrigations were gradually continued with tap water as to prevent drying of pumice. The seedlings with horizontal cotyledon leaves and the first true leaves were started to be irrigated with Hoagland nutrient solution [12]. The seedlings with the 2nd true leaves were removed from pumice medium and were placed into 25x25x18 cm plastic containers filled with Hoagland nutrient solution (hydroponic culture). Pepper seedlings were wrapped with small sponge pieces and placed over specially perforated plastic panels. Plant roots were immersed into nutrient solution. Solution was aerated with an aquarium pump.

Salt treatments were initiated when the plants had 4-5 true leaves. Experiments were conducted in complete randomized plots design with 3 replications and 10 plants in each replication. For salt treatments, nutrient solution (1/2 Hoagland) was supplemented with NaCl as to have a concentration of 100 mM. Solutions were refreshed every week. In this stage, same salt concentrations were preserved. Together with salt treatments, pepper plants were also subjected to 5.0, 7.5 and 10 ppm Gibberellic acid (GA_3) treatments. There were 8 different treatments as of control, single salt (100 mM), salt (100 mM) + gibberellic acid (5.0, 7.5 and 10 ppm), single GA_3 (5.0, 7.5 and 10 ppm) treatments.

Sampling for measurements and analyses was performed on 10th day of salt treatments. Total plant weights (g) were measured. Leaf samples were taken from mid-sections of the plants and Na, K, Ca, Cl contents of the leaves were determined.

2.1. Mineral Element Analysis

Three leaves from tip to downward were taken and they were kept in deep freezer at –40°C. About 200 g samples were taken from the deep freezer and samples were supplemented with 10 ml 0.1 N HNO_3 (Nitric acid). They were then kept in plastic boxes at dark and room temperature for a week. Samples were shaken in a shaker for 24 hours and resultant extract was subjected to K^+, Cu^+, Zn^+, Fe^+, Mn^+, Mg^+, Cd ion analyses in flame photometer (Eppendorf flame photometer). Fresh leaf ion concentrations were expressed in μg/mg fresh weight [13].

2.2. Statistical Analyses

All results were the means of three replicates, and each replicate consisted of ten plants. Data were analysed statistically and treatment means were separated by Duncan's Multiple Range Test using SAS (1985) software [14].

Experiments were conducted in complete randomized plots design with 3 replications. Resultant data were subjected to statistical analyses with SAS software (SAS Institute, 1985).

3. Results and Discussion

Total plant weights and leaf Na^+, K^+, Ca^{+2} and Cl^- contents (μ g/mg T. A.) of the plants treated with salt and 5, 7.5 and 10 ppm GA_3 are provided in Table 1. With regard to total plant weights, control and all three doses of GA_3 treatments had similar values and the highest levels. As compared to control treatment, total plant weights decreased in NaCl and NaCl+GA_3 treatments and had lower levels. Despite low plant development levels, salt-induced necrosis and damages were not observed in NaCl+7.5ppm GA_3 and NaCl+10 ppm GA_3 treatments. Ashraf et al. (2001) reported decreased shoot and root fresh and dry weights, plant heights and leaf areas with increasing salt treatments, but also reported significant improvements in these parameters with GA_3 treatments. In general, GA_3 treatments stimulated vegetative development in both wheat cultivars under salt stress, but GA_3 resulted in insignificant decreases in grain yield [7]. In the same study, significant increases were reported in Na and Cl concentrations in shoot and roots of two wheat genotypes grown in saline growth medium, but increased Na and Cl accumulation in shoot and roots of both genotypes with GA_3 treatments were also reported. Growth regulators like GA_3 and cytokinin provided significant contributions in reducing negative impacts of salt stress on plants [5]. Lin and Kao (1995) reported that GA_3 treatments reduced the negative impacts of salt stress on germination of salt-treated paddy seedlings [6], Rodriguez et al. (2006) reported significant role of GA_3 treatments in preventing negative impacts of salt stress on growth and development of various plants including paddy [11]. Yaşar et al. (2016) in another study carried out with eggplants reported that plant salt stress and GA_3 treatments inhibited plant growth, but with selective ion

uptake, prevented toxic impacts of salt on plants. Present findings comply with all those earlier ones [9].

With regard to Na ion uptake of pepper plants, while the lowest Na accumulation levels were observed in control and single GA_3 treatments, the greatest Na accumulation levels were observed in single NaCl and NaCl+5ppm GA_3 treatments. The Na accumulation levels of NaCl+7.5 and 10 ppm GA_3 treatments were lower than single NaCl and NaCl+5ppm GA_3 treatments. Changes were observed in ion uptakes of salt stress-treated plants with GA_3 treatments. GA_3 treatments provided balanced sodium and potassium levels which play a significant role in prevention of salt stress and less K^+ accumulations were observed in plant leaves as compared to control plants. K^+ ions reduce the toxic impacts of salt on plants. The greatest Cl accumulations were observed in NaCl+GA_3 treatments and Cl accumulations of NaCl+7.5 and 10 ppm GA_3 treatments were lower than Cl accumulations of single NaCl and NaCl+5ppm GA_3 treatments. Single GA_3 doses had similar values with the control treatment (Table 1).

Table 1. *Plant total weights (g) and leaf Na^+, K^+, Ca^{+2} and Cl^- contents (µ g/mg T. A.) of NaCl and NaCl+GA_3 treated plants.*

Applications	Total plant weight	Na^+	K^+	Ca^{+2}	Cl^-
Control	7.00 A	2.919 C	12.002 A	13.624 A	0.540 C
NaCl	5.34 B	14.712 A	5.748 C	7.808 C	4.641 A
NaCl+5 GA_3	5.87 B	13.684 A	5.798 C	7.666 C	3.871 A
NaCl+7,5 GA_3	5.42 B	10.556 B	7.747 B	10.28 AB	2.020 B
NaCl+10 GA_3	5.19 B	10.932 B	7.489 B	10.949 AB	1.813 B
5 GA_3	7.68 A	2.343 C	12.011 A	11.49 AB	0.390 C
7,5 GA_3	6.88 A	2.470 C	11.733 A	11.53 AB	0.514 C
10 GA_3	6.96 A	2.796 C	11.958 A	11.82 AB	0.412 C

The differences in means indicated with the same letter in the same column are not significant.

Ashraf et al. (2001) reported significant increases in Na and Cl concentrations in shoot and roots of two wheat genotypes grown in saline growth mediums, increased Na and Cl accumulations in shoots and roots of both genotypes with GA_3 treatments, but decreased accumulations with single salt treatments as compared to control treatments [7]. It was reported in previous studies that growth regulators like GA_3 and cytokinin reduced the negative impacts of salt stress on plants [5, 6]. Similarly in present study, increased Na and Cl ion concentrations were balanced with increasing K and Ca ion uptakes. Then, just because of ion balance, salt damages were not observed at 7.5 and 10 ppm GA_3 doses. However, salt + GA_3 treated plants presented slow and low growth and development levels. Rodriguez et al. (2006) reported that GA_3 treatments reduced growth inhibition effects of salts in paddy plants [11]. On the other hand, Schachtman and Lio (1999) in barley and Iqbal and Ashraf (2013) in wheat plants, reported that salt stress reduced ion uptakes, but reported increased ion uptake with GA_3 treatments [15, 16]. Yasar et al. (2016) reported inhibited plant growth in eggplants with NaCl+GA_3 treatments because of DELLA proteins inhibiting enzyme activities [9]. When GA_3 was applied to plants which were exposed to abiotic stressors, it may synthesize DELLA proteins and thus restrict plant growth and development. Activity of this protein increases with GA_3 treatments and thus may have significant contributions to stress tolerance of the plants since plant growth is restricted under abiotic stress conditions [17, 18, 19]. It was reported in a previous study that increased DELLA activity with GA_3 treatments restricted the production of reactive oxygen species (ROS) under cadmium stress and thus prevented cell deaths [20]. DELLA proteins behave like inner cell suppressor of GA-induced metabolic processes [21, 22, 23, 24, 25].

It was concluded based on current findings that GA_3 treatments together with salt treatments did not have positive contributions to plant growth, but plants presented selective ion uptake with GA_3 treatments to prevent toxic impacts of salt stress on plants. Such an impact of GA_3 was attributed, as it was in previous studies, to synthesis of DELLA proteins when the growth regulator GA_3 hormone as a respond to stressors was applied to stress-exposed plants. This protein inhibited enzyme activities with a great role in plant growth and development and thus resulted in serious recessions in plant growth. GA_3 treatments increased the activity of this protein and restricted plant growth under abiotic stress conditions, but provided significant contributions to stress tolerance of the plants.

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