

Effect of Salicylic acid on growth and yield of Bread Wheat (*Triticum aestivum* L.) under Salt stress

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Abstract

Bread wheat is one of the most important cereal crops that improved food security at global and regional levels. In this study, the effect of salicylic acid (SA) on growth and development of wheat under salt stress was studied for two consecutive years. The experiment was conducted at Adami Tulu Agricultural Research Center during the 2021 and 2022 seasons to evaluate the application of SA leading to improvement in the growth and yield of wheat under salt stress conditions. A pot experiment was performed in Randomized Completely Block Design with three replications. Analysis of variance revealed highly significant differences among treatments for grain yield (GY), productive tiller (PT), grains per spike (GPS), spike length (SL), plant height (PH), above dry biomass (ADB), and days to maturity (DTM). The highest GY (4.62 g), PT (4.17), GPS (35.67), SL (6.42 cm), PH (51.17 cm), ADB (9.37 g) were observed in treatments in which SA was applied, whereas early maturity date of 79.33 days was recorded in control treatment. The study proved that the application of SA improved wheat growth and yield under salt stress conditions.

Keywords: Bread wheat; Salicylic acid; Salinity; Stress amelioration

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereals crops with high demands for food consumptions across the world. However, wheat is exposed to multiple environmental stresses, such as salinity, drought, high and low temperatures. In particular, salinity is one of the most important environmental constraints affecting plant growth, development, and productivity of wheat crop. Salt stress causes water deficit, ion toxicity, and nutrient deficiency, leading to growth, yield and development reduction and even to plant death [1]. Salinity is a significant constraint for crop quality and production, affecting wheat growth, yield and quality in the major wheat growing regions of the world [2]. Additionally, it has a negative impact on photosynthesis, water management and enzymatic activity [3].

Salinity alters many physiological and biochemical processes such as mineral nutrition, respiration rate, organic solutes/osmolyte synthesis, seed germination, enzyme activities and photosynthesis [4]. Salt stress induces cellular accumulation of reactive oxygen species (ROS), which can damage cell biomolecules [5-8]. Soil salinity refers to the accumulation of water soluble salts mainly sodium, but also potassium, calcium and magnesium which may be chlorides, sulfates or carbonates. All soils contain salts, but salinity becomes a problem only when certain salts concentrate in the crop's rooting zone [9]. Salt can destroy soil structure causing swelling of clays and dispersion of fine particles leading to clogging of the soil pores through which oxygen and water move. Up to 7% of the total land surface of the planet is saline [10]. Out of the total arable lands, one fifth of the land is hit hard by salinity and more area is falling prey to this menace. The potential agricultural land of Ethiopia is also falling prey to this menace of underground saline water and salinity effects. Plants are generally more sensitive to strong alkalinity, where the dominant cation is often sodium, than to strong acidity where the dominant cation is hydrogen. The salts in the rooting medium cause toxicity through absorption of Na⁺ and Cl⁻ ions, disturbing the ionic balance, thereby impacting the physiological processes of plants. Salt stress considerably reduced the chlorophyll content in wheat, bean and maize; however the exogenous application of salicylic acid (SA) improved the chlorophyll content under both saline and non saline conditions.

Salicylic acid is involved in various processes such as growth and development, seed and fruit yield, flowering initiation, glycolysis and defense system to quench the production of ROS. Salicylic acid is an endogenous growth regulator that belongs to a group of phenolic compounds having antioxidant potential. It participates in the regulation of numerous biochemical processes in plants like stomatal closure, ion uptake, transpiration; triggers chlorophyll biosynthesis; enhances photosynthesis; increases total sugar total free proline, oil content also provides protection against biotic and abiotic stresses including salinity. Salicylic acid improved plant performance under normal or stressed conditions through modulation of growth and yield in maize. Although its definite role and the underlying physiological mechanisms have not been fully clarified. In wheat, the application of SA also mitigated the salt-induced adverse effects. The effective concentration of SA is highly dependent upon the plant species, the way of the application, the duration of the treatment, and the environmental conditions. The tolerance against stressful conditions especially salinity, could be augmented by foliar application of certain osmoprotectants, viz., salicylic acid, glycine betaine, ascorbic acid and trehalose in most of the crop plants. Salicylic acid has a key role in abiotic stress tolerance against salinity and drought in wheat. Among various osmoprotectants, SA has been reported to be the most cost-effective and efficient in improving the salt tolerance in majority of crop species. It is now very important to develop salt stress management technologies to cope with the upcoming problem of food security. For this purpose, we have not to wait only for the genetic improvement of salinity tolerance genotypes/cultivars to overcome salt stress. Therefore, it is necessary to develop technologies, methods and strategies to ameliorate deleterious effects of salt stress on plants. However, no research has been done

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on the effect of SA to counteract the growth and yield components of wheat under salt stress conditions in the study area.

This study was done with the following objectives i.e.,

To evaluate SA application to improve the growth and yield of wheat under salt stress and to investigate the physiological mechanism of SA application for salt tolerance.

Materials and Method

Description of the study area

The study was conducted in pots under controlled conditions (inside mesh-wire) to prevent bird attack to wheat in Adami Tullu agricultural research center during the 2021 in Ganna or winter and 2022 in Bona or summer season. The study area is located at a latitude of 7°9'N and longitude of 38°7'E with an altitude of 1650 m.a.s.l, which is 168 km from Addis Ababa and 7 km from Batu/Ziway city.

Treatments and experimental design

The treatments were (10 g L⁻¹ SA, 25 g L⁻¹ NaCl, 10 g L⁻¹ SA + 25 g L⁻¹ NaCl, 50 g L⁻¹ NaCl, 10 g L⁻¹ SA + 50 g L⁻¹ NaCl, 100 g L⁻¹ NaCl, 10 g L⁻¹ SA + 100 g L⁻¹ NaCl, 200 g L⁻¹ NaCl, 10 g L⁻¹ SA + 200 g L⁻¹ NaCl and control) tested for their effects on Kingbird bread wheat cultivar. The experimental design was completely randomized block design with three replicates.

Experimental procedure

Soil was collected from field for each plastic pot of 16 cm length with 26 cm upper and 18 cm lower diameter was filled with soil. The fertilizer rate was depending on Surface area of curved bucket $S_{area} = \pi L(R + r)$ of 0.11 m² incase 1.7 g NPS (150 kg ha⁻¹) and 1.2 g Urea (100 kg ha⁻¹) of (½ at sowing and ½ at tiller initiation stage) applied equally except for control.

The soil in each pot was allowed to hand fed water application with the same amount of water. Ten seeds of wheat cultivar were sown with two rows in each pot with equal depth and distance. After germination, seedlings were thinned to six numbers equally for all pots. The pots were applied water for the crop to attain field capacity (FC) depending on the moisture of the soil. After 35 days after sowing (DAS), different rates of salt treatment (NaCl) were applied. Foliar application of 10 g salicylic acid diluted with one liter of water for each pots was done except to control where normal water was applied. The foliar spray treatments were used twice: first time five days after salt treatment (40 DAS) and second time after ten days of salt treatment (45 DAS).

Experimental soil and water

The same soil type was used for all experimental pots. Before sowing, one representative soil sample was collected and analyzed for physico-chemical parameters such as organic carbon, texture, soil pH, electrical conductivity (EC), cation exchange capacity (CEC), CaCO₃, total N, available K, available P, available S, Fe, Mn, Zn, and Cu. The water used in the study was also analyzed for physico-chemical parameters viz., pH, electrical conductivity, total dissolved solids, sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), alkalinity (CaCO₃), carbonate (CO₃²⁻), bicarbonate (HCO₃⁻), chloride (Cl⁻), nitrate (NO₃⁻), fluoride (F⁻), aluminum (Al), lead (Pb), total nitrogen (N) and total phosphorus (P).

Data collected

Days to 50% heading (DTH): Days to spike heading was

determined as the number of days taken from the date of sowing to the date of 50% heading of the plants from each pot by visual observation.

Days to 90% physiological maturity (DTM): Days to physiological maturity was determined as the number of days from sowing to the date when 90% of the panicle turned yellow in straw color.

Plant height (cm): Plant height was measured from the soil surface to the tip of the spike (awns excluded) of 3 plants at physiological maturity.

Spike length (cm): It was measured from the bottom of the spike to the tip of the spike excluding the awns from 3 plants.

Number of total tillers: Number of total tillers was determined from 3 plants of the pot at physiological maturity by counting the number of tillers.

Number of productive tillers: Number of productive tillers was determined at maturity by counting all kernel bearing spikes from 3 plants of the pot at physiological maturity.

Number of grains per spike: The mean number of grains per spike was computed as an average of 3 plants randomly taken spikes from the pot.

Above ground dry biomass (g pot⁻¹): The aboveground dry biomass was determined from plants harvested from the pot after sun drying to a constant weight.

Grain yield (g pot⁻¹): Grain yield was taken by harvesting and threshing the seeds from the pot. The grain weight of each pot was recorded in gram (g) after sun drying.

Harvest index (HI): Harvest index was calculated as ratio of grain yield per plot to total aboveground dry biomass yield per plot.

Data Analysis

Data was analyzed using GenSTAT statistical software package and mean values or Least Significant Differences (LSD) were compared using Duncan's test at 5% level of significance.

Results and Discussion

From total ten numbers of treatments with three replications, the data were available only for three treatments because the plants from other treatments got totally damaged due to salt except the minimum concentration of 10 g L⁻¹ SA + 25 g L⁻¹ NaCl, 10 g L⁻¹ SA and control treatments. This may be due to the addition of NaCl to growth medium leading to reduced net CO₂ assimilation rate, transpiration rate, stomatal conductance and sub-stomatal CO₂ concentration.

Physico-chemical properties of the soil used for the experiment

According to the laboratory analysis result of soil, the soil texture of the experiment is loam for two years. However, the low level of organic matter and Nitrogen content of the experimental soil could influence the availability of nutrients for the crops (Table 1).

Physico-chemical properties of laboratory result of water used in the experiment

The chemical composition of water used is varying in which some values are below the approximate standard of world health organization (WHO) and the others are above the standard of maximum allowable concentration of WHO.

Table 1: Physico-chemical properties of the soil experiment before sowing.

No	Soil characters	Values		Source
		2021	2022	
1	Soil texture \mathbb{J}			
	Sand (%)	47.62	38.71	
	Clay (%)	23.05	19.02	
	Silt (%)	29.33	42.27	
	Texture Class	Loam	Loam	
2	pH-Water (H_2O) \mathbb{W}	8.64 Strongly alkaline	8.05 Moderately alkaline	[37]
3	EC(mS/cm) \mathbb{C}	0.26 Non-saline	0.75 Non-saline	[38]
4	CEC(meq/100 gm soil) \mathbb{t}	36.36 high	38.94 High	[38]
5	Organic carbon (OC) (%) \mathbb{f}	1.22 Low	1.66 Low	[39]
6	Total nitrogen (N) (%) \mathbb{T}	0.07 Poor	0.09 Poor	[40]
7	Available potassium (K) (mg K_2O /kg soil) \mathbb{Y}	708.01 high	1837.34 very high	[39]
8	Available phosphorus(P) (mg P_2O_5 /kg soil) \mathbb{J}_p	30.34 Very high	19.44 High	[41]
9	Available sulfur (S) (mg/kg soil) \mathbb{S}	13.89 Low	95.70 High	[39]
10	Calcium Carbonate ($CaCO_3$) (%) \mathbb{p}	1.30	1.85	
11	Iron (Fe) mg/kg soil \mathbb{Q}	8.81 High	5.46 Medium	[I]
12	Magnesium (Mn) mg/kg soil \mathbb{Q}	6.23 High	7.09 High	[I]
13	Copper (Cu) mg/kg soil \mathbb{Q}	0.96 Very high	0.97 Very high	[I]
14	Zink (Zn) mg/kg soil \mathbb{Q}	0.99 Medium	2.84 Very high	[I]

Days to heading (DTH)

The combined treatments mean effect on days to 50% heading of wheat was non-significant (Table 4). The highest delayed duration to reach days to flowering was observed in response to SA application of 47 days. However, the minimum duration of days to flowering (46 days) was obtained from control. The reason may be that wheat seedlings treated with 50 μ M SA developed larger spike due to enhanced cell division within the apical meristem of seedling roots. The increase in growth parameters of water stressed plants in response to SA may be related to the induction of antioxidant responses that protect the plant from damage. SA plays a key role in physiological processes like flower induction, photosynthesis and protein synthesis. SA has proved to have an important contribution to growth stimulation in wheat seedlings by an enhancement of cell division and extension of root cells. In mung bean also, exogenous SA application alleviated the harmful effects of salt stress through the improvement of plant photosynthesis, growth and also enhancing antioxidant system.

Days to maturity (DTM)

The combined treatments mean effect on days to maturity was highly significant ($P < 0.05$) (Table 4). The late maturity days of (80.67 days) was recorded from SA+NaCl treatment, while the early maturity days of (79.33 days) were recorded from control. The earliness of maturity days for control treatments, while the delay maturity days of treatment SA+NaCl may be due to time taken to restructuring the impact of salt stress. Salicylic acid treatment (1 mM) ameliorative impact of calcium chloride (5 mM) on the growth and development of 3 mg/L boron stressed barley and enhanced the growth of wheat. SA also counteracted salt stress-induced growth inhibition and enhanced the net photosynthetic rate in wheat. Salicylic acid could generate a high level of ionic competition with Na^+ and Cl^- , thus avoiding the ionic phytotoxicity in plant tissues, allowing an effective development at the cell and tissue levels.

Plant height (cm)

The analysis of variance indicated that the combined treatments

mean effect of plant height was highly significant ($P < 0.05$) (Table 4). The plant height was less pronounced as compared to open field crops. However, the tallest plant height of (51.17 cm) was recorded from SA treatment, while the shortest plant height (44.75 cm) was obtained in NaCl + SA treatment.

Spike length (cm)

The analysis of variance indicated that the combined treatments mean effect of spike length was highly significant ($P < 0.001$) (Table 4). Thus, the longest spikes (6.42 cm) were recorded from SA treatments. Whereas the shortest spikes length (5.15 cm) was recorded from control (Table 4). This may be due to the reason that SA acts as a potential osmoprotectant to mitigate the adverse effects of salinity, thereby improving the physiological and biochemical attributes and also enhanced uptake of K^+ ion while depressing Na^+ and Cl^- ions in plant system.

Number of total tillers

The combined treatments mean effects on number of total tillers of wheat was non-significant (Table 4). The highest (4.83) number of total tiller was recorded from SA and the lowest (3.50) was recorded from control. The number of total tillers decreased as compared to open field wheat crop. The number of tillers ranged from 3 to 12 with 6 on average, while under control conditions the average was eight [2]. The reduction in the number of tillers in wheat under salt stress has been reported previously.

Number of productive tillers

The combined treatments mean effect on number of productive tillers was highly significant ($P < 0.01$) (Table 4). The highest (4.17) number of productive tillers was recorded from SA and the lowest (2.50) was recorded from control treatment. The number of fertile spikes can be considered as one of the main traits to evaluate salt tolerance in wheat because only tolerant varieties could produce fertile spikes, while the susceptible ones produce sterile spikes [2]. SA has an important competition with ions such as Na^+ at the membrane

transport, has positive effects on ion uptake and inhibitory effects on Na^+ and Cl^- uptake where it is responsible for managing salinity in maize plants.

Number of grains per spike

The combined treatments mean effect on number of grains per spike was highly significant ($P < 0.01$) (Table 4). The highest (35.67) grains per spike were recorded from SA and the lowest (28.92) were recorded from control. The number of grains per panicle, number of filled grains per panicle, 1000-grain weight were also increased by SA application to the high saline soil in both the rice varieties (ASD16 and BR26). The previous studies, reported that SA strongly inhibited Na^+ and Cl^- accumulations, but also stimulated N, Mg, Fe, Mn, and Cu concentrations in stressed maize plants.

Above ground dry biomass (g pot⁻¹)

The combined treatments mean effect on above ground dry biomass was highly significant ($P \leq 0.01$) (Table 4). The highest (9.37 g) above ground dry biomass was recorded from SA, while the lowest (6.37 g) was recorded from control. Salicylic acid alone applied enhanced vegetative growth as compared to salt-induced treatments by increasing fresh and dry biomass. However, salt-induced treatments have higher above ground dry biomass than control. These findings correlate with; the foliar application of SA enhanced biomass production in barley and wheat. They concluded that the increase in growth biomass in response to SA under salinity stress may be due to protective role of SA on cell membranes that might be responsible for increasing plant salt tolerance. Others author also observed that foliar application of SA increased the stomatal conductance under drought stress. The foliar spray of SA enhanced the relative water content, biological yield, soluble protein, proline content and photosynthetic pigments in wheat seedlings.

Grain yield (g pot⁻¹)

The combined treatments mean effect on grain yield was highly significant ($P \leq 0.01$) (Table 3). The highest (4.62 g) grain yield was recorded from SA and the lowest (2.77 g) was recorded from control. This may be due to time taken to take action against salt stress may decreased grain yields. This suggests that the increases in photosynthetic rates following foliar spray of certain phenolic compounds such as SA could be the result of increased enzyme activity related to CO_2 uptake at the chloroplast level, rather than simple increases in stomatal opening. Foliar application of SA enhanced the values of net CO_2 assimilation rate in salt treated and untreated plants.

Similar conclusion was found during salt stress, as the improvement in growth and grain yield due to SA application was associated with improved photosynthetic capacity, which was not due to stomatal limitations, but was associated with metabolic factors, other than photosynthetic pigments and leaf carotenoids. However, the exact mechanism of Salicylic acid mediated photosynthesis enhancement is still unclear. In another study, it was claimed that faba bean yield was improved by foliar spraying the plants with SA under both greenhouse and open field conditions. The unfavorable environmental conditions have shown to increase the endogenous SA level in plants. SA inhibits seed germination in favorable conditions, but promotes growth under abiotic stress. It has long been suggested that different stressors, such as UV light, ozone fumigation or virus infection can activate a common signal transduction pathway that leads to SA accumulation and increased stress resistance.

Harvest index (HI)

The combined treatments mean effects on harvest index of wheat was non-significant (Table 2). The highest harvest index (49 %) was obtained from SA, whereas the lowest harvest index (43%) was recorded

Table 2: Physico-chemical parameters of water used for this study.

No	Tests	Test results	Unit	Test method	WHO Maximum allowable concentration (mg/L)
1	pH	7.64	-	Potentiometric	6.5-8.5
2	Electrical Conductivity	500.00	$\mu\text{S}/\text{cm}$	Potentiometric	-
3	Total dissolved soil	275.00	mg/L	Potentiometric	1000.0
4	Sodium (Na^+)	73.8	mg/L	Flame photometric	200.0
5	Potassium (K^+)	10.5	mg/L	Flame photometric	-
6	Calcium (Ca^{2+})	16.32	mg/L	Titrimetric	200.0
7	Magnesium (Mg^{2+})	4.90	mg/L	Titrimetric	150.0
8	Alkalinity (CaCO_3)	192.9	mg/L	Titrimetric	-
9	Carbonate (CO_3^{2-})	Nil	mg/L	Titrimetric	-
10	Bicarbonate (HCO_3^-)	235.28	mg/L	Titrimetric	-
11	Chloride (Cl^-)	16.9	mg/L	Mohr Argentometric	250.0
12	Nitrate ($\text{NO}_3\text{-N}$)	ND	mg/L	Cadmium Reduction method	10
13	Fluoride (F^-)	2.1	mg/L	Ion-Selective Electrode	1.5
14	Aluminum (Al)	ND	mg/L	Aluminon	0.2
15	Lead (pb)	ND	mg/L	Flame AAS	0.01
16	Total kjeldahl Nitrogen (N)	1.71	mg/L	Keldjahal	80.0
17	Total Phosphorus (P)	0.34	mg/L	Ascorbic acid	10.0

Table 3: The combined mean square values of ANOVA for salicylic acid and NaCl on wheat at Adami Tulu Agricultural Research Center tested for two years.

SoV.	df	GY(g)	DTH	DTM	TT	PT	GPS	SL	PH	HI	ADB
Rep.	2	2.59NS	0.17NS	0.06NS	1.26NS	0.22NS	55.10NS	0.14NS	127.76NS	16.97 NS	7.48NS
Trt.	2	5.22**	1.50NS	2.72**	3.01NS	4.39***	72.60***	2.71***	73.85**	43.21NS	13.66**
Year	1	0.06NS	4.50NS	20.06***	2.35NS	3.56**	78.13***	0.13NS	13.35NS	17.07NS	0.93NS
Trt.Year	2	0.38NS	0.17NS	0.72NS	0.18NS	0.06NS	5.38NS	0.02NS	5.01NS	14.38NS	1.89NS

Table 4: The combined means of salicylic acid and NaCl treatments on wheat tested at Adami Tulu Agricultural Research Center for two years.

Treatments	Combined means for each parameters									
	GY(g)	DTH	DTM	TT	PT	GPS	SL	PH	HI	ADB
Control	2.77a	46.00	79.33a	3.50	2.50a	28.92a	5.15a	45.50a	43	6.37a
NaCl +SA	3.48a	46.50	80.67b	3.75	3.00a	30.83a	5.39a	44.75a	46	7.58a
SA	4.62b	47.00	79.83ab	4.83	4.17b	35.67b	6.42b	51.17b	49	9.37b
LSD (0.05)	1.11**	NS	0.99**	NS	0.84***	2.91***	0.44***	4.31**	NS	1.77**
CV (%)	23.9	2.4	1.0	22.2	20.2	7.1	6.1	7.1	11.7	17.7

from control (Table 3). The higher the harvest index value, the greater the physiological potential of the crop for converting dry matter to grain yield. The highest harvest index of (50.4 %) was recorded from Ogocho bread wheat cultivar.

Conclusions and Recommendations

The study proved that the application of salicylic acid (SA) improved wheat growth and yield under salt stress conditions of treatment containing 10 g L⁻¹SA + 25 g L⁻¹NaCl, but not under 25 g L⁻¹ NaCl alone. The present study revealed that the optimum level of SA application minimizes the deleterious effects of salt stress in wheat. It was observed that SA application improved the growth and yield of wheat plants under salt stress conditions.

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