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Influence of Salicylic Acid on salinity stress tolerance by seed priming and foliar application on Maize (*Zea Mays*)

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ABSTRACT

This study was intended to measure the impact of salicylic acid (SA) on maize under salt stress. For this purpose salinity effect on various morpho-physiological and biochemical attributes of plants was analyzed by launching a petri dish and pot experiment. The analysis spread out in a totally block design (randomized) with ten replicates for each salt (0 and 120 mM NaCl) and SA (0, 0.1, 0.5 and 1mM) treatment. The commercially available cultivar (cv. Faisal) of maize was planted in earthen pots for 15 days. After fifteen days of growth, seedlings were irrigated with saline water (120 mM NaCl) and SA was applied for 60 days simultaneously. To observe the germination, seed were also primed with same concentrations in petri dishes. After 60 days on final treatment harvesting was carried out, leaf samples were taken for analyzing biochemical attributes (protein contents, antioxidants enzyme activates). A decrease in seed germination percentage from 95.22 (at control) to 25.34% (at 120 mM salt stress) and shoot length from 86.12 (at control) to 42.36 cm (at 120 mM salt stress) was observed. Similar decreasing pattern of growth was observed in case of pot grown plants after 60 days. The results suggested that salt stress drastically reduced length of shoot and root, fresh / dry weight and leaf area and antioxidant enzyme activities while the use of 0.5 mM concentration of SA greatly made good progress in all these growth and biochemical parameters. Production of antioxidant compounds under salt stress is accelerated under the influence of SA. So it causes modifications in antioxidant compounds and hence increases salt tolerance under saline conditions.

Keywords: Antioxidant activities, Priming, Proline, Salinity, Salicylic acid.

INTRODUCTION

Salinity is basically the accumulation of dissolve-able salts present in the soil (Silva and Uchida, 2000). 20% soil is salt affected in the world out of total irrigated area (Pitman and Lauchli, 2002). It has been estimated that the world over salt influenced land is 953 Mha which is approximately 7% of total land area (Abdelfattah *et al*., 2009). Almost 6.3 × 10^6 hectares of irrigated area has become salt stressed to varying degrees in Pakistan (Malik and Shah, 1996). Of this salt affected land 9% in Baluchistan, 40% in Sindhi and half of it in Punjab (province) (Mian and Mirza, 1993). Salinity is viewed a main consideration in constraining plant development and crop yield, and salinization of irrigated and surrounding regions in the parched tropics and sub-tropics has not been reduced. In reality, it continues to increase bit by bit (Rus *et al.*, 2000). As indicated by Saboora *et al*., (2006), it is expected that saline conditions are expanded at a rate of about 10% every year around the world. Pakistan has moderate to very increased salt levels, especially NaCl (Anon, 2001).

According to reports, saline conditions badly affect the plant (growth/development), reducing germination of seeds, growth of seedlings and activity of enzymes (Seckin *et al.*, 2009). Salinity of soil is the real abiotic stress that has adverse impacts on yield and quality of plant mainly

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due to increase of Na^+ and Cl^- ions in plant parts (Shilpi and Narendra, 2005). Salt stress is a real danger to land of agriculture area in arid as well as semiarid regions (El-Hamdaoui *et al.*, 2003). Salinity badly affects the economy of country by reducing the production of crop which is of great value (Ahmed, 2008). Water logged conditions, salt stress and increased level of sodium ions have diminished the drainage capability of the soils resulting in reduced soil fertility, decrease in crop productivity and biodiversity reduction (Zia and Baig, 1986). It has been observed in most saline soil that NaCl is foremost salt specie that leads to death of plant (Munns and Tester, 2008). Different plants have different levels of salt tolerance (Kumar *et al*., 2009) that also varies from species to species. However growth regulators, fertilizers and anti-oxidants can successfully minimize the effects of salt on salt affected plants (Janda *et al*., 1999; Shalata and Neuman, 2001). Reactive oxygen species (ROS) are increased due to saline conditions (Asada, 2006). Oxidative damage of cell due to ROS under salt stress leads to death of cell (Mittler, 2002). During normal growth there is a balance between ROS production and its utility which is regulated by defense system of oxidants (Hameed *et al*., 2011).The plants whose antioxidant enzymes are more active can withstand in salt stress (Gapinska *et al*., 2008). Salinity increases cellular components of plant and other contents like hydrogen peroxide, superoxide dismutase (SOD), ascorbate and peroxidase (POD) are also enhanced, however salt stress also reduces the catalase activity (CAT, Hassanein *et al*., 2009). Some other processes are also affected by salinity in plant body like biochemical, lipid metabolism, protein synthesis, photosynthesis and several enzymes (Parida and Das, 2005). Sodium ion toxicity and oxidative stress in plant is caused by salinity (Sairam and Sarivasta, 2002; Cuin and Shabala, 2007).

Zea mays is an annual crop and belongs to family poaceae. From many years corn is known as staple food across the world and serves as feed crop and it is an important component of global food security (Campos *et al*., 2004). In world the productivity of maize is more than 780 million tons per year as compare to the wheat and rice (FAO, 2013). In Pakistan maize occupies a central position among the cereal crops (Dowswell *et al*., 1996) and ranks 3rd (1st wheat and 2nd rice). In Pakistan the total cultivated area of maize is 1.02 million hectares with per annum production of maize grain is 2.96 million tons (GOP, 2007). The maize crop productivity is about 2,850 kg per hectares (Tariq and Iqbal, 2010). Maize is source of corn flakes, corn starch, syrups, alcohol, glucose and tanning material (GOP, 2007). Its grain has 10 percent protein, 4.8% oil, 58.72% starch fiber, 30% sugars and 1.7% ash (Chaudhry, 1983). Increased concentration of salts in soil has drastic impact on maize growth and yield (Fu *et al*., 2010).

Different techniques were used to minimize the salt stress effect including genetic engineering, tissue culture, exogenous application (Wang *et al*., 2003). External application of different compounds vitamins like ascorbic acid and SA increases growth of plant under saline conditions (Hassanein *et al*., 2009). Moreover SA is now considered as a salt stress alleviating compound (signal molecule) (Horvath *et al*., 2007) that may stimulate the production of ROS under salt stress thus having great importance in salt resistance (Borsani *et al*., 2001). Treatment of SA increases hydrogen peroxide level reducing oxidizing damage of plants (Wahid *et al*., 2007). Many researches on exogenous applications of SA reported that it affects several developmental, physiological and biochemical mechanisms in plants due to which resistance against salt stress and drought has increased remarkably (Tari *et al*., 2002). During salt stress episode it affects fruit yield and seed germination (Raskin, 1992), rate of transpiration, closing of stomata (Rai *et al*., 1986), permeability of membrane (Barkosky & Einhellig, 1993), photosynthesis and growth (Khodary, 2004; El-Tayeb, 2005). Salicylic acid plays important role in photosysnthesis and transpiration (Arfan *et al*., 2007), enhances antioxidant enzymes production (Xu. *et al*., 2008), and reduces accumulation of Na^+ and Cl^- ions (Gunes *et al*., 2007). Exogenous application of SA increases the activities of antioxidant enzymes which reduce the impact of ROS (Erasalan *et al*., 2007). The present work is designed to check whether seed priming and foliar application of SA enhances the growth of maize by minimizing the effect of salt stress.

MATERIALS AND METHODS

Plant material and experimental conditions

The experiments were performed to study the effect of SA by two separate method of its application i.e., seed priming and foliar spray to overcome salinity stress in Maize. Healthy certified seeds were obtained from Pioneer seed company Sahiwal, Pakistan. The experiment was done at Nursery of University of Okara Punjab, Pakistan.

This experiment was performed in totally randomized complete block design under natural conditions from September-November 2014. Average temperature was 28±2^{0}C with average relative humidity 64% during experiment period.

Experimental layout

Seed were soaked in tap water, SA and salt +SA overnight in beaker. Then these seed were placed in petri dishes having cotton at the base. Each petri dish had ten seeds. Five petri dishes were used for this experiment. 1st petri dish was a control, in which seeds were treated with simple tap water. In 2nd petri dish seeds were treated with 120 mM NaCl only. In next three petri dishes (3, 4 and 5) seeds were treated with SA having concentrations 0.1, 0.5 and 1.0 mM with 120 mM of NaCl. The seeds were grown for ten days. Then shoot/root length of seedlings were measured with scale and percentage of growth was also checked.

Pot experiment

Maize seeds were grown in earthen pots having soil mixture containing field soil, bhal and manure in the ratio of 1:1:1. The experiment was done in totally randomized fashion in three blocks. Each block consists of three replicates for each concentration. In first fifteen days of experiments simply tap water was given to all pots. Then various levels of SA concentrations SA were used through foliar spray. Surfactant Tween-20 was used for the penetration of SA into leaf tissues. Each block was divided into five groups. Each group had three (one plant/pot) pots for each concentration. The 1st group was designated as control. It was given simple tap water through irrigation with foliar spray of distilled water. The 2nd group was irrigated with water containing 120 mM NaCl. Simple distilled water was also sprayed on leaves of plants of this group. Next three groups were treated foliarly with 0.1, 0.5 and 1.0 mM concentrations of SA with 120 mM NaCl treated through roots drenching for each concentration. Salt treatment through irrigation was given whenever required and spray was also done simultaneously. The treatment was given for sixty days and later on plants were harvested.

Physical characteristics of potting-mix

By using EC and pH meter, EC, TDS (Total dissolve solids) and pH of the potting mix was taken initially before and after the completion of experiment. From pot of each treatment soil sample (15 g) was randomly taken and mixed with little water and then left to set for 5 minutes then separation of water done. This water was used to record EC, TDS and pH.

Table: Physical Characteristics of potting mix

Treatments	pH	EC (mS)	TDS (mg/l)
Control	7.9	1.32	589
Salt (120 mM)	8.7	2.67	782
S+ SA (0.1 mM)	8.6	2.45	1092
S+ SA (0.5 mM)	8.8	3.13	1496
S+ SA (1.0 mM)	8.7	6.21	3310

Harvesting and data recording

The plants were harvested after 60-day of treatment of SA. The length and diameter of shoots was taken before harvesting. For biochemical tests leaf samples were also collected. Then plants were uprooted from each pot carefully and washed water and then dried by the use of blotting paper.

Growth parameters

Shoot length and diameter (cm)

By the use of measuring tape and Vernier caliper the length (cm) and diameter of each shoots was measured after 60 days of treatment. The length of roots / shoots was measured with scale.

Plant biomass (g)

The shoots and roots of plants were separated and washed with tap water to remove soil particles and residues. Then material was dried with blotting paper and fresh weights of plant shoots and roots were recorded. Leaf area was also measured. Then plant material was wrapped in aluminum foil and was dried in oven for 5 days at 65°C to get the dry mass.

Leaf area (cm^2)

Area of leaf of plants was measured by the use of the Image J program (Rosband, W.S., image J,U.S. National Institute of Heath, Bethesa, Maryland, USA, http:/rsb.info.nihigov/IJ/.1997-2008).

Biochemical analysis

Quantitative assay of protein

Fresh tissue of leaf (0.5 g) was crushed in ice-chilled pestle with mortar having 1 ml of 0.1 M phosphate buffer (pH 7.2). Polyvinyl polypyrollidone (PVP) (0.1 g) and 0.5 % (v/v)

Tritone X-100 were also added. The ratio of leaf tissue: buffer was kept at 1:2 (w/v) ratio was kept between leaf tissue and buffer. Then centrifugation of homogenate was done at 14,000 rpm for 30 minutes at 4°C. At the end supernatant was obtained and cooled at 0°C and used for quantitative estimation of proteins. Soluble protein contents estimation was done with the help of Racusen and Johnston's Biuret method (1961).

Quantitative assay of enzymes

A fresh leaves sample (0.5 g) was taken. 1 ml of phosphate buffer 0.1 M (pH 7.2), 0.1 g polyvinyl polypyrollidone (PVP) and 0.5 % (v/v) Tritone X-100 were also added. All material was homogenized and centrifugation was done for 30 minutes at 14,000 rpm at 4°C. The supernatant was separated to store at 0°C which was used further for quantitative enzymes estimation. The method of Racusen and Foote (1965) with some modifications was used to measure activity of peroxidase. For this purpose, Guaiacol (1%), H_2O_2 (0.3 %) and Tris-HCl (0.1 M) solutions were prepared and the enzyme activity was estimated. Superoxide dismutase activity was estimated with the use of method suggested by Maral *et al*. (1977).

Quantitative assay of proline

Estimation of proline was done by the method proposed by Bates *et al*. (1973). Sample of leaf tissue (0.5g) was taken along with 10 ml of 3 % sulfosalicylic acid. Then material was homogenized and centrifuged of homogenate at 13000 rpm for 10 minutes at 4ºC. Then in a test tube 2 ml acid ninhydrin and 2 ml of glacial acetic acid and 2ml of supernatant were mixed and allowed to stand for 1 hour at 100°C and then allowed to cool at room temperature. At the end, 4 ml of toluene was allowed to mix vigorously and then the sample was left to stand for 10 minutes for the separation of toluene and aqueous phases. Upper toluene layer was separated with care and by the use of toluene its absorbance was recorded at 520 nm.

Statistical analysis

The data was analyzed statistically using one way analysis of variance by SPSS (version 12.0.0) computer program. Duncan multiple range test (at 0.05% probability level) was used to take the mean values.

RESULTS

Effect of seed priming with salicylic acid on seed germination, shoot/root length emerging from seeds under salt stress

Seed germination

The seed germination was gradually reduced by the application of salt to very much extent as obvious in table 1. The maximum germination was observed in control (95.22%) which was reduced under salt stress to 25.34%. But %age germination of seed increased significantly by priming with SA. In case of priming, the germination %age was maximum at 0.5 mM with saline conditions. However when the plants were subjected to a higher concentration of SA (1.0 mM) percentage of germination was decreased as compared to control. The percentage germination recorded for 0.1, 0.5 and 1.0 mM SA priming seed under salinity stress was 40.53, 76.46 and 70.10%, respectively.

Shoot length and root length

In case of length of shoot, it was maximum in control (10.15cm) but it was drastically reduced under salt stress (1.86 cm). Priming of seed significantly reduced the effect of salinity and was clear improvement in shoot length. Maximum increase in length (shoot) was observed at 0.5 mM priming of SA. Shoot length after seed priming at concentration levels 0.1, 0.5 and 1.0 mM of SA was 2.89, 5.76 and 4.33 cm, respectively. Same results were observed in case of root length. Control seeds showed maximum growth with root length of 11.47 cm which was greatly reduced (2.10cm) in seeds under 120 mM salt stress. Seed priming (with salicylic acid) reduced the adverse effects of salts and there was remarkable increase in root length. Maximum increase in length (root) was observed in 0.5 mM seed priming plant under salt stress. Priming with concentration levels 0.1, 0.5 and 1.0 mM of SA have root length 4.13, 6.76, and 5.89 cm, respectively.

Table I: Effect of SA (Salicylic acid) on maize seed priming under salt stress in terms of germination, shoot length/root length.

Treatments	Germination %age	Shoot Length (cm)	Root Length (cm)
Control	95.22 ± 2.00^{d}	10.15 ± 2.20^{e}	11.47 ± 2.28^{e}
Salt (120 mM)	25.34 ± 3.50^{a}	1.86 ± 0.40^{a}	2.10 ± 0.55^{a}
S + SA (0.1 mM)	40.53 ± 3.2^{b}	2.89 ± 0.88^{b}	4.13 ± 0.82^{c}
S + SA (0.5 mM)	76.46 ± 3.66^{c}	5.76 ± 1.63^{d}	6.76 ± 1.59^{d}
S + SA (1.0 mM)	70.10 ± 2.91^{c}	4.33 ± 0.74^{c}	5.89 ± 1.13^{b}
Significance with df 4 and 49	*	*	*

The results are based on 10 replicates for each treatment.
Duncan multiple range test was applied to get mean values
(*) Significant or (NS) non-significant at 0.05% probability level
S= Salt (120 mM NaCl) and SA= salicylic acid

Effect of foliar application of salicylic acid on shoot length, shoot diameter and leaf area under field or pot condition

Shoot length and shoot diameter (cm)

There was gradual increase in shoot length and diameter by increasing salicylic acid concentration. There was maximum increase at concentration of 0.5 mM of SA. Salt stressed plants have shoot length of 42.36cm. It was increased maximum up to 74.35cm at 0.5 mM SA with 120 mM salt treatment. Similarly, shoot diameter was also increased up to maximum at 0.5 mM concentration of SA. Shoot diameter was 2.64, 1.25, 1.65, 2.24 and 2.05cm at control, 120 mM salt, 0.1, 0.5 and 1.0 mM of salicylic acid treatments (Fig. 1a-e).

Leaf area

In control the leaf area is 386cm^2 while the leaf area of the plants grown under salt stress was 157 cm^2. There was a gradual increase in leaf area with gradual increase of salicylic acid concentration. Maximum leaf area was observed when the plants were subjected to concentration of 1.0 mM of SA. Leaf area at 0.1, 0.5, 1.0 mM is 245, 297, 305cm^2, respectively.

Table II: Effect of foliar application of SA (Salicylic acid) on shoot length/diameter and leaf area of salt-stressed maize plants.

Treatments	Shoot length (cm)	Stem diameter (cm)	Leaf area (cm^2)
Control	86.12 ± 5.42^{e}	2.64 ± 0.67^{e}	386 ± 21.63^{e}
Salt (120 mM)	42.36 ± 2.45^{a}	1.25 ± 0.37^{a}	157 ± 9.77^{a}
S + SA (0.1 mM)	51.43 ± 3.86^{b}	1.65 ± 0.60^{b}	245 ± 22.43^{b}
S + SA (0.5 mM)	74.35 ± 2.95^{d}	2.24 ± 0.87^{d}	297 ± 24.65^{c}
S + SA (1.0 mM)	67.52 ± 3.57^{c}	2.05 ± 0.49^{c}	305 ± 20.21^{d}
Significance with df 4 and 49	*	*	*

The results are based on 10 replicates for each treatment.
Duncan multiple range test was applied to get mean values
(*) Significant or (NS) non-significant at 0.05% probability level
S= Salt (120 mM NaCl) and SA= salicylic acid

Fig. 1a: Control of maize grown with tap water showing growth without salt stress.

Fig., 1b: Plant of maize showing reduction in growth primed with 1.0 mM of Salicylic Acid under salt stress.

Fig., 1c: Plants grown under salt stress of 120 mM of NaCl showing reduced growth

Fig. 1d: Plants grown under salt stress of 120 mM with foliar application of SA of 0.5 mM showing significant improvement in growth.

Fig., 1e: Plants under salt stress with Salicylic acid of 1.0 mM showing little decrease in growth.

Effect of foliar application of salicylic acid on plant biomass production

Shoot fresh and dry weights

Foliar spray with salicylic acid resulted in an increase in the biomass of maize plants that were grown under saline conditions (Table III). There is gradual increase of biomass with gradual increase of SA concentration. Fresh weight of shoot was maximum (389.24 g) of control and that of under 120 mM salt stress was 211.54g. When SA was applied to salt treated plants it increase the fresh biomass of plant as compared to salt treated ones. However, there was gradual increase in fresh weight with increasing SA level. But it was maximum at 0.5 mM concentration of SA. Shoot fresh weight was 209.23, 341.10, 321.75g at concentration levels 0.1, 0.5 and 1.0 mM of SA, respectively. Same pattern was seen in case shoot dry weight. Shoot dry weight was maximum at 0.5 mM of SA which was 55.88 g. Shoot dry weight of control is 72.56g and that of under salt stress was 30.21g. Then there was gradual enhancement in dry weight of shoot with increase in SA treatment concentrations.

Root fresh and dry weights

A little improvement in was seen fresh and dry weight of root with increasing concentrations of SA under salt stress in maize plants. Root fresh weight was 105.52g of control plant and that of under 120 mM salt stress was 48.88g. There was little increase in fresh weight with increasing concentrations of SA under salt stress. Root fresh weight was maximum at 0.5 mM of SA. Fresh weights were 58.36, 66.51 and 61.60 g at concentrations 0.1, 0.5 and 1.0 mM of SA. In the same pattern, there was a small enhancement in dry weight of root with increasing levels of SA. Dry weight of root of control was 19.25g and that of salt stressed plants was 8.48g. A little increase in dry weight with increase in SA concentrations was noticed. Dry weight of root was maximum at 0.5 mM (12.26g) treatment of SA under saline conditions.

Table III: Effect of foliar spray of Salicylic Acid on biomass production of salt-stressed maize plants

Treatments	Shoot weight (g)		Root weight (g)	
	Fresh wt. (g)	Dry wt. (g)	Fresh wt. (g)	Dry wt. (g)
Control	389.24 ± 15.12^{e}	72.56 ± 3.44^{e}	108.52 ± 4.27^{e}	19.25 ± 1.28^{e}
Salt (120 mM)	211.54 ± 8.73^{a}	30.21 ± 1.23^{a}	48.88 ± 3.85^{a}	8.48 ± 1.67^{a}
S+ SA (0.1 mM)	290.23 ± 7.14^{b}	38.33 ± 2.40^{b}	58.36 ± 2.33^{b}	10.34 ± 1.16^{b}
S+ SA (0.5 mM)	341.10 ± 10.45^{d}	55.88 ± 4.38^{d}	66.51 ± 3.41^{d}	12.26 ± 1.77^{d}
S+ SA (1.0 mM)	321.75 ± 6.47^{c}	52.43 ± 2.45^{c}	61.60 ± 2.28^{c}	11.22 ± 1.14^{c}
Significance with df 4 and 49	*	*	*	*

The results are based on 10 replicates for each treatment.

Duncan multiple range test was applied to get mean values.

(*) Significant or (NS) non-significant at 0.05% probability level.

Effect of foliar application of salicylic acid on soluble protein contents

SA effect on protein contents in maize. Table 4.4 shows that there is significant effect of SA on protein contents through foliar application. The table 4.4 shows that there was significant increase of protein contents under salt stress. Protein contents of control plants were 0.23mg/g of tissue and that of plant under salt stress was 0.34mg/g tissue. But there was gradual decrease in protein contents with increasing SA level. Contents of protein were 0.32, 0.28 and 0.26 mg/g tissue at concentration levels 0.1, 0.5 and 1.0 mM of SA.

Effect of foliar application of salicylic acid on peroxidase activity

There is clear impact of SA on peroxidase activity of plants under saline conditions. In control plants perodidase activity was 0.16mg/g tissue and in salt stressed plants was 0.13 mg/g tissue. This result shows that there is decrease in activity under salt stress. But with treatments of SA there is positive effect on peroxidase activity. Its value is 0.16, 0.16 and 0.15 mg/g tissue with concentration levels (0.1, 0.5 and 1.0 mM) of salicylic acid, respectively (Table IV).

Effect of foliar application of salicylic acid on activity of superoxide dismutase

Data shows that superoxide dismutase (SOD) activity of maize plants increased significantly under salt stress. In control plants it was 55.43U/mg of protein and in salt stressed plant it was 81.24U/mg of protein. But there was little decrease in SOD activity with increasing SA level in salt stressed plants. SOD activity was 72.36, 69.44 and 66.5 U/mg of protein noticed at 0.1, 0.5 and 1.0 mM concentrations of SA, respectively.

Effect of foliar application of salicylic acid on proline contents

There was significant impact of salts on proline contents of maize plants. In control (plants) its value was 16.19 µmol/g of FW but under salt stress its value increased up to 38.26 µmol/g of FW. These contents were decreased at 0.1 mM concentration of SA but at higher concentrations of SA proline contents were again increased. Proline contents at 0.1, 0.5 and 1.0 mM concentrations of SA were 30.53, 47.44 and 58.69 µmol/g of FW, respectively (Table IV).

Table IV: Effect of foliar spray of SA (Salicylic acid) on soluble protein contents, peroxidase, superoxide dismutase activities and proline contents of salt-stressed maize plants.

Treat-ments	Soluble Protein Contents (mg/g tissue)	Peroxidase activity (mg/g tissue)	Superoxide dismutase activity (U/mg protein)	Proline Contents (µmol/g FW)
Control	0.23± 0.006^{a}	0.16 ± 0.007^{c}	55.43 ± 12.12^{a}	16.19 ± 0.742^{a}
Salt (120 mM)	0.34 ± 0.014^{d}	0.13 ± 0.005^{a}	81.24 ± 8.68^{e}	38.26 ± 3.24^{c}
S+ SA (0.1 mM)	0.32 ± 0.009^{c}	0.16 ± 0.004^{c}	72.36 ± 11.43^{d}	30.53 ± 5.66^{b}
S+ SA (0.5 mM)	0.28 ± 0.004^{b}	0.16 ± 0.005^{c}	69.44 ± 4.27^{c}	47.44± 6.22^{d}
S+ SA (1.0 mM)	0.36 ± 0.006^{e}	0.15 ± 0.006^{b}	66.57 ± 5.09^{b}	58.69 ± 5.46^{e}
Significance with df 4 and 49	*	*	*	*

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