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Impact of Gibberellic acid under salinity stress on Tomato (*Lycopersicon esculentum* L.)

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Abstract
Tomato has important role in food and commercial utilization in the whole world, which is second to potato in global vegetable production. Susceptibility towards salinity stress has limited the productivity of Tomato. The experiment carried out at different salinity stress level during Rabi 2016-2017 on Tomato variety (P K M 1). To effects of gibberellic acid under salt stress condition of tomato with nine treatments including control with three replicates were laid out in Complete Randomized Design. Salt stress alters a wide array of plant metabolic mechanisms. Different strategies of the application of phytohormones are required to overcome the adverse effects of salt stress. Growth and biochemical attributes i.e. shoot length, leaf area, fresh weight, dry weight, number of fruits; chlorophyll concentration, lycopene ascorbic acid and proline were affected by salinity stress under NaCl treatment. But application of GA_3 in combination was to be proved beneficial in alleviating the adverse effects of salt stress on these growth and biochemical parameters. However, GA_3 applied with salt proved more effective in comparison to control. The results of the experiment revealed that combined application of GA_3 and salt may ameliorate most of the attributes and was proved to be a physiological remedy to increase the tolerance against the ill effects of salt stress.

Keywords: tomato, proline, nacl, ga_3 (gibberellic acid), lycopene, salinity stress, chlorophyll

Introduction
More than 50% reduction in average yield of major crops has been attributed to the abiotic stresses. Although the underlying mechanisms of abiotic stresses may vary depending upon the specific nature and extent of stress, stage and duration of plant exposure, yet the ultimate outcome of exposure to stress is the reduction in germination, growth and final yield of crops**.** Worldwide, soil salinity has adversely affected about 30 % of the irrigated and 6% of total land area (Meena *et al.,* 2017) [13].

Salinity is now widely acknowledged. Although plant response to salinity depends on several factors and their relative importance can vary in time and space, still phytohormones are thought to be the most important endogenous substances that are critical in modulating physiological responses that eventually lead to plant adaptation to an unfavorable environmental, conditions including salinity (Negrao, *et al.*, 2017) [14].

The GA_3 is known to be actively involved in regulating plant responses to the external environment. Rapid accumulation of GA_3 is a characteristic of plants exposed to abiotic stresses. Under abiotic stress specific concentration GA_3 can be beneficial for the physiology and metabolism of many plants, since it regulates the metabolic process such a sugar and anti-oxidative enzymes signaling (Gupta *et al.*, 2013) [9].

Tomato is among one of the moderately salt sensitive crop and higher accumulation of salt in soil may reduce its productivity. Thus for the study tomato is taken as experimental crop because of its importance as food crop and it is grown by 14% of total worldwide production and It contains high quantity of sugar (2.5 - 4.5 %), starch (0.6 - 1.2%) and minerals like potassium, calcium, sodium, magnesium, phosphorus, boron, manganese, zinc, copper, iron, etc. Apart from these, it also contains organic acid such as citric, and acetic acid which are known as health acid in fresh tomato fruit (Dom *et al*., 2005) [7]. So the current study targets on analysis of impact of gibberellic acid under salinity stress condition on growth and yield of tomato with the objectives to study the effect of gibberellic acid under salinity stress on growth, yield and bio chemical parameters of tomato.

Material and method
The experiment entitled "Effects of Gibberellic acid under salinity stress condition on the tomato (*Lycopersicon esculentum* L.)" was carried out during *Rabi* season 2016-2017 as pot experiment at Department of Biological Sciences, Sam Higginbottom University of

Agriculture, Technology & Sciences, Allahabad U.P.

Experimental detail

The experiment contains 10 treatments and 3 replications.

Treatment	Irrigation with GA_3
T0 (-ve control)	Water
T1 (+ control)	GA_3 100ppm
T2	50 mM NaCl
T3	100 mM NaCl
T4	150 mM NaCl
T5	200 mM NaCl
T6	50 mM NaCl +(GA_3) 100ppm
T7	100 mM NaCl +(GA_3) 100ppm
T8	150 mM NaCl +(GA_3) 100ppm
T9	200 mM NaCl +(GA_3) 100ppm

Growth and yield Parameters

The growth and yield parameters viz., plant height, number of leaves and numbers of fruits per plant were recorded of three selected plant.

Biochemical parameters

Estimation of chlorophyll content

The chlorophyll content was measured by following the method of Strain *at al.*, (1996). 1 g of fresh leaves were cut into small pieces and homogenized with pure acetone in a mortar with pestle. The supernatant was decanted through Whatmann No. 42 filter paper into a 25 ml volumetric flask. Then 80 per cent acetone was added to the residue in a mortar and the extraction was repeated until residue was decolorized. The volume was made upto 25 ml with 80% acetone and absorbance of the extract was measured at 663, and 645, nm on spectrophotometer (Elico, UV-VIS spectrophotometer CL-54) using 80% acetone as blank. The chlorophyll contents were estimated in leaves at 30, 60, 90, DAS by using the following formula,

The Chlorophyll was calculated by following equation:

$$\text{Chlorophyll 'a'} = (12.7 \times A_{663}) - (2.69 \times A_{645}) \times \frac{V}{1000 \times L \times W}$$

$$\text{Chlorophyll 'b'} = (22.9 \times A_{645}) - (4.68 \times A_{663}) \times \frac{V}{1000 \times L \times W}$$

Total chlorophyll = Chlorophyll 'a' + Chlorophyll 'b'

Estimation of carotenoid

Carotenoid was determined according to (Lichtenthaler and Welburn *at al.*, 1983). 0.5 gm and homogenized in 10 ml of acetone (80% acetone) which centrifuged at 3000 rpm at 10 minutes. The absorbance was recorded at 470 nm.

It is calculated by the formula,

$$\text{Total carotenoid} = \frac{1000 \times A_{470} - (3027 \times \text{Chl-a} + 104 \times \text{Chl-b})}{229}$$

Determination of proline

Proline content was determined by the method adopted from (Delauney *at al.*, 1993) [6]. Fresh 0.5g of leaf sample was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and the homogenate was filtered using what mann No 1 filter paper. Then 2 ml of filtrate was taken in a test tube and 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. This was allowed to react for 1 hr at 100° C in a boiling water bath. The reaction was terminated by placing the test tube in an ice box. Then 6ml of toluene was added to the reaction mixture. The chromophore containing toluene was separated and absorbance was recorded at 520nm wavelength using toluene as blank.

It is calculated by the formula

$$[(\mu g\text{proline/ml X mltoluene}) / 115.5\mu g) / \mu\text{mole}] / [(\text{g sample}) / 5] = \mu\text{moles}]$$

g = gram

Determination of ascorbic acid

Reagents

- Oxalic acid
- Standard dye: dissolve 50 mg of 2, 6 dichlorophenolindophenol in warm distilled water and 42 mg sodium bicarbonate with agitation until dissolved.

The juice was extracted from the fruits and filtered through muslin cloth. 10 ml (W) of this juice was taken with the help of pipette in a 100 ml volumetric flask and the volume (V1) was made up with 1.0% Oxalic acid solution. The flask was then shaken well. The juice was then filtered. Known quantity (V2) of the solution was taken and was titrated against standard dye (V) solution till a pink colour appeared and persisted for 15 seconds. The amount of ascorbic acid was calculated by the formula. (Mehenry *at al.*, 1935).

$$\text{Ascorbic acid mg/ 100 ml of fruit juice} = \frac{1}{\text{Dye factor}} \times V \times \frac{V1}{V2} \times \frac{100}{W}$$

Determination of lycopene

The lycopene was estimated by the method of 1 gram of juice was taken in a stoppered conical flask, to which 20 ml of acetone was added. The contents were shaken well on mechanical shaker (100 rpm) for 30 minutes. Then 40 ml of petroleum ether (grade 60-80) was added to each flask, mixed vigorously to transfer the pigments to petroleum ether phase for measuring the colour intensity. To separate the ether layer from acetone layer, 5ml, of 5% sodium sulphate was added. Petroleum ether layer was separated using Buckner funnel and this solution was used for measuring optical density (colour intensity) in UV-Vis spectrophotometer (ELICO, CL-5) at 503 nm. Lycopene content of the sample was calculated as given below using the relationship that an optical density (OD) of 1.0 = 3.1206 g of lycopene ml^{-1} (Ahmad *at al.*, 2005).

$$\text{Lycopene content (mg/g)} = \frac{3.1206 \times \text{OD} \times \text{Volume}}{\text{Weight of sample}} \times 100$$

Leaf Relative water content (%)

Leaf relative water content (LRWC) was measured using the method of (Yamasaki and Dillenburg *at al.*, 1999). Leaves were sampled from the middle region section of each plant, in order to minimize age effect on variability of results. Individual leaves were removed and then weighed to obtain fresh weight (FW). In order to determine the turgid weight (TW), whole leaves were floated in distilled water inside a closed Petri dish. During the imbibition period, leaf samples were weighed periodically, after gently wiping water from the

leaf surface with tissue paper. At the end of the imbibition period, leaf samples were placed in a pre-heat at 85^0C, for 2 h, in order to obtain dry weight (DW). All mass measurements were made using an analytical balance with precision of 0.0001 g. LRWC was calculated using the equation mentioned below:

$$\text{LRWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

Statistical Analysis

The data recorded during the course of investigation were subjected to statistical analysis as per method of "Analysis of variance" (Fisher *at al.,* 1950). The significance and non-significance of the treatments effect was judged with the help of 'F' variance ration test. The significant differences between the means were tested against the critical difference at 5% level.

Result and Discussion

Growth Parameters

The growth parameters viz., (plant height, no. of branches, no. of pods. Total no. of flowers.) Was measured. The plant height and no. of branches were recorded at 30, 60 and 90 days after application of treatment DAT. There was significant difference in plant height among all the treatment in comparison to control**.**

Growth parameters viz., (plant hight, no. of branches, no. of pods. Total no. of flowers.) Significantly decreased with the increasing concentration of the salinity stress but the application of GA_3 significantly increased the plant height for each of salinity concentration. The data on 30 DAT plant heights has been shown in Table 4.1.

The highest plant height among all treatments was recorded for T_1 (GA_3 only) followed by T_0 (control without NaCl and GA_3). A decreasing trend of plant height was observed among all salinity stress concentration and also decrease in height was coping up on application of GA_3 for respective treatments. T6 found better in comparison to other treatments except T0 and T1.

Effect of Gibberellic acid under salinity stress on plant height of *L. esculentum* variety PKM-1.

Treatment	Plant height (cm)before treatment	Plant height (cm)30 DAT	Plant height (cm)60 DAT	Plant height (cm)90 DAT
T0	15.3	21.3^{ab}	30.1^{b}	41.1^{ab}
T1	14.9	22.6^{a}	30.8^{a}	41.1^{a}
T2	14.7	19.7^{cd}	29.2^{c}	40.1^{ad}
T3	14.9	18.7^{de}	28.1^{d}	39.1^{def}
T4	14.4	17.3^{e}	27.1^{e}	38.1^{fg}
T5	14.0	15.6^{f}	25.1^{g}	36.4^{h}
T6	15.1	21.3^{ab}	29.9^{b}	40.4^{bc}
T7	15.0	20.3^{bc}	28.9^{c}	39.4^{de}
T8	14.3	19.3^{cd}	27.9^{d}	38.4^{ef}
T9	14.9	17.6^{e}	26.2^{f}	37.2^{gh}
Mean	14.76	19.37	28.32	39.22
F test	NS	S	S	S
SEm±	0.270	0.506	0.186	0.414
CD (5%)	0.796	1.491	0.548	1.222

Note: Numerical values with same alphabets are non-significant while values with dissimilar alphabets are significant.

Average number of branches per plant decreased significantly with the increasing concentration of the NaCl but the application of GA_3 increased the number for each of salinity concentration. The data on 30 DAT average numbers of branches per plant has been shown in Table 4.1.9, T6 found better in comparison to other treatments except T0 and T1.

Effect of Gibberellic acid under salinity stress on number of branches of *L. esculentum* variety PKM-1.

Treatments	Average number of branches at		
	30 DAT	60 DAT	90 DAT
T0	3.6^{bc}	4.6^{ab}	5.9^{ab}
T1	4.4^{a}	4.9^{a}	6.4^{a}
T2	3.2^{cd}	4.2^{bc}	5.6^{bc}
T3	2.9^{de}	3.9^{cd}	5.2^{cd}
T4	2.6^{ef}	3.6^{d}	4.9^{de}
T5	2.0^{g}	3.0^{e}	4.3^{e}
T6	3.8^{b}	4.2^{bc}	5.9^{ab}
T7	3.4^{bc}	3.9^{cd}	5.6^{bc}
T8	3.0^{d}	3.6^{d}	5.2^{cd}
T9	2.4^{f}	3.1^{e}	4.7^{de}
Mean	3.33	3.89	5.37
F test	S	S	S
SEm±	0.117	0.149	0.189
CD (5 %)	0.344	0.440	0.558

Note: Numerical values with same alphabets are non-significant while values with dissimilar alphabets are significant.

The number of buds per plant of all treatment and control sample was observed and recorded at 35 DAT. There was significant difference in average number of buds among all the treatment in comparison to control.

Effect of Gibberellic acid under salinity stress on number of Pods, Flowers and Leaf relative water content of *L. esculentum* variety PKM-1.

Treatment	No. of Flower	No. of Pods	L.R.W.C
T0	13.2^{ab}	12.1^{cd}	44.04^{bcde}
T1	14.4^{a}	14.0^{a}	53.71^{cde}
T2	12.7^{bc}	11.8^{de}	46.25^{cde}
T3	12.2^{bcd}	11.4^{def}	44.24^{de}
T4	11.6^{cd}	10.8^{f}	42.61^{e}
T5	10.8^{d}	9.8^{g}	41.94^{e}
T6	14.0^{ab}	13.4^{ab}	51.46^{a}
T7	13.2^{ab}	12.7^{bc}	49.23^{ab}
T8	12.4^{bcd}	12.0^{cd}	47.63^{abc}
T9	11.3^{d}	11.0^{ef}	41.81^{abcd}
Mean	12.61	11.90	46.29
F test	0.312	S	2.175
SEm±	S	0.285	S
CD (5 %)	0.921	0.842	6.415

Note: Numerical values with same alphabets are non-significant while values with dissimilar alphabets are significant.

Biochemical and Yield Parameter

The photosynthetic pigment (Chlorophyll content carotenoids) Lycopene content for all treatments and control sample was observed and recorded at 50 DAT. There were significant differences in chlorophyll 'a' and 'b' and carotenoids content all the treatment in comparison to control.

Effect of Gibberellic acid under salinity stress on Chlorophyll and carotenoids content of *L. esculentum* variety PKM-1.

Treatments	Chlorophyll 'a'	Chlorophyll 'b'	Carotenoids
T0	0.78^{ab}	0.37^{a}	3.7^{ab}
T1	0.82^{a}	1.42^{a}	4.7^{ab}
T2	0.73^{abc}	1.12^{b}	4.1^{bc}
T3	0.66^{bcd}	0.86^{cd}	3.7^{ab}
T4	0.62^{bcd}	0.71^{e}	3.5^{bcd}
T5	0.53^{d}	0.53^{f}	3.4^{ab}
T6	0.75^{abc}	1.00^{bc}	4.3^{b}
T7	0.65^{bcd}	0.88^{cd}	3.9^{ab}
T8	0.57^{d}	0.83^{de}	3.5^{d}
T9	0.53^{d}	0.79^{de}	3.4^{e}
Mean	0.66	0.95	3.81
F test	S	S	S
SEm±	0.051	0.049	0.271
CD (5 %)	0.152	0.145	0.798

Note: Numerical values with same alphabets are non-significant while values with dissimilar alphabets are significant.

Effect of Gibberellic acid under salinity stress on Lycopene ascorbic acid, Proline and Number of fruits per plant of *L. esculentum* variety PKM-1.

T	Lycopene	Ascorbic Acid	Proline	No. of Fruits/ plant
T0	20.9^{e}	21.2^{ab}	3.3^{c}	11.2^{bc}
T1	22.4^{d}	28.4^{a}	3.9^{a}	12.4^{a}
T2	24.9^{cd}	23.9^{bc}	3.4^{b}	10.6^{cde}
T3	25.8^{bc}	24.9^{bcd}	3.8^{bc}	10.0^{ef}
T4	26.1^{abc}	25.8^{cd}	3.9^{a}	9.2^{f}
T5	26.2^{ab}	26.5^{d}	3.9^{a}	7.8^{g}
T6	23.9^{bc}	23.7^{abc}	3.1^{cd}	11.8^{ab}
T7	24.2^{c}	23.9^{cd}	3.1^{cd}	11.0^{bcd}
T8	24.8^{cd}	24.2^{cd}	3.3^{c}	10.3^{de}
T9	25.5^{cb}	24.9^{cd}	3.6^{bc}	9.3^{f}
Mean	24.50	24.76	3.56	10.37
F test	S	S	S	S
SEm±	1.062	1.156	0.182	0.265
CD (5 %)	3.132	3.411	0.538	0.783

Note: Numerical values with same alphabets are non-significant while values with dissimilar alphabets are significant

Discussion

The analysis of variance of the data on growth, yield, and biochemical related parameters as influenced by different gibberellic acid under salinity stress have been discussed here.

Plant Growth parameters

The growth of tomato plant i.e. height of plant, number of leaves and fresh and dry weight of root and shoot was affected by the different salinity stress treatments. Also the adverse effects of salinity stress were coped up by application of Gibberellic acid along with salt stress.

The role of GA_3 in overcoming the harmful effects of salinity on growth may be due to the change in the endogenous growth regulators which affects plant water balance and or decreasing root resistance to water flower. The decrease in water content in stressed plants was increase again by GA_3 application supported these views in tomato (Haleem *at al.,* 2007) [10].

Plant Height- Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is reutilized when stress is over and may play a role in osmotic adjustment (Ashraf and Harris *et al.,* 2004) [2]. A higher content of soluble protein due to the salt stress has been observed in tomato (Ashraf and Tufail *et al.,* 1995) [3].

Number of Branches- Average number of branches per plant decreased significantly with the increasing concentration of the NaCl but the application of GA_3 increased the number for each of salinity concentration. The data on 30 DAT average number of branches per plant.

Since the nitrate reductase is sensitive to NaCl ions (Gouia *et al.,* 1994) [8], it is an indicator of the damaging effects of NaCl. The in vivo assayed NRA gradually decreased, in young and old branches, by increasing NaCl concentration in the rooting medium.

Significant difference was observed in the stage of growth at 40 days. More number of buds/flowers be due to concentration of gibberellic acid compare to other all treatments. (Sharma *at al.,* 2016) [16]

Relative water content- Salt treatment induced a reduction in leaves' LRWC (%), which indicates a loss of turgor that resulted in limited water availability for cell extension process. Thus, the growth inhibition in less tolerant plants could be related to the decrease of LRWC (%) provoked by the salt treatment.

Pigment activity- The chlorophyll content showed corresponding decrease as the concentration of salt was increased from 50 to 200 mM concentration of salt. Such reduction in chlorophyll content coupled with increased salt concentration is also reported by (Croser *et al.,* 2001) **[5]**. The loss of chlorophyll content could be associated with the accumulation of Na^{+} in the leaves.

According to (Choudhury and Choe *et al.,* 1996) [4] decline in photosynthetic rate with increased concentration of NaCl may be associated with decreased pigmentation concentration. For maintaining the ionic balance in the vacuoles, cytoplasm accumulates low molecular mass compounds termed compatible solutes because they do not interfere with normal biochemical reactions, rather they replace water in biochemical reactions.

Chlorophyll 'a' & 'b'- Both chlorophyll a and b contents decreased in response to salinity stress. The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez *et al,* 1978) [15] as well as damaging to the photosynthetic apparatus (**Yasseen *et al,* 1983**). Application of GA to the saline stressed plants increased the pigment level. GA3 also plays a vital role in tolerance to salt stress by improving plant growth and chlorophyll synthesis. In addition, the inhibitory effect of GA3 on chlorophyll catabolism might be partly due to the down regulation of the activities of enzymes involved in chlorophyll catabolism and the alleviation of oxidative chlorophyll bleaching.

Carotenoids- The carotenoids content per gram leaf decreased with the increasing concentration of the NaCl but

the application of GA_3 increased the carotenoids content for each of salinity concentration. The data on carotenoids content per gram leaf (Hayssam *at al.,* 2012) [11].

Proline content- Proline, which occurs widely in higher plants, does accumulate in large amounts than other amino acids in salt-stressed plants (Ali *et al.,* 1999) [1]. Proline accumulation under stress conditions may either be caused by induction or activation of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilization of proline in protein synthesis and enhanced protein turnover (Delauney and Verma *et al.,* 1993) [6].
Salinity stress stimulated the accumulation of proline. It has been widely reported that proline may

Lycopene content- The highest lycopene among all treatments was recorded for T_1 (GA_3 only) followed by T_0 (control without NaCl and GA_3). A decreasing trend of lycopene content per gram fruit juice was observed with increasing NaCl concentration and application of GA_3 helped in coping up decrease in lycopene content for respective treatments. (Ashraf, and Tufail, *et al.,* 1995) [3].
Salinity stress stimulated the accumulation of lycopene. It has been widely reported that lycopene may play a role in stress adaptation within the cell.

Ascorbic acid- The highest ascorbic acid content among all treatments was recorded for T_1 (GA_3 only) followed by T_0 (control without NaCl and GA_3). A decreasing trend of ascorbic acid content per 1 gram fruit juice was observed with increasing NaCl concentration and application of GA_3 helped in coping up decrease in ascorbic acid content for respective treatments. (Delauney and Verma *et al.,* 1993) [6].
Salinity stress stimulated the accumulation of ascorbic acid. It has been widely reported that ascorbic acid may play a role in stress adaptation within the cell.

Fruits yield and its components- The number of tomato plant fruits as well as the valve of total soluble solid of fruits tissue as affected by the salt stress. The influence of studied Gibberellic acid treatments on tomato plant fruits yield and its components, the illustrated by data shows clearly, that, GA_3 treatment caused an increment in total fruits yield as/ gram as well as number/ plant fruits compared with control plants which is confirmatory by (Kumar, *et al.,* 2014) [12].

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