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Role of calcium chloride on biochemical properties of tomato (*Lycopersicon esculentum*) against different levels of NaCl

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Abstract
Tomato is considered as protective as well as productive food because of its special nutrient value and also wide spread production. It belongs to the family Solanaceae. Salinity has been thread to agriculture in some part of the world for over 3000 years: in recent time, the threat has grown. Calcium play an important role for improving the quality tomato by increasing the firmness, reducing the physiological disorders, delaying ripening process and prolonged the shelf life of tomato. In present study different levels of Calcium chloride has been used against different levels of NaCl. It has been estimated that Calcium Chloride at 2400 ppm showed best result in terms of chlorophyll a, chlorophyll a, carotenoids and lycopene against different levels of NaCl

Keywords: NaCl, calcium chloride, tomato, biochemical properties

Introduction
Tomato is considered as protective as well as productive food because of its special nutritive value and also wide spread production. The fruits are used directly as raw vegetable in sandwiches, salad, etc. Several processed items like paste, puree, syrup, juice, ketchup, drinks, etc. are prepared on a large scale. It is a rich source of lycopene, ascorbic acid, b- carotene and minerals. In recent years, it has attracted the attention due to the anticarcinogenic and antioxidant property of lycopene and ascorbic acid. Lycopene being efficient quencher of singlet oxygen and free radicals provides protection against a broad range of epithelial cancers. In addition, tomato contains a component P3, which by preventing platelet clots helps to cut down deaths from heart diseases and strokes. Tomato is a major contributor of carotenoids (especially hasten or delay ripening, to reduce losses and to improve lycopene), phenolics, vitamin C and small amounts of and maintain the colour and quality by slowing down the vitamin E in daily diets (Barbar and Barber 2002) [1]. Results from the epidemiological studies showed that tomatoes and tomato products may have a protective effect against various organisms by reducing the shriveling which ultimately forms of cancer, especially cardiovascular diseases (Khachik *et al.*, 2002) [4]. Calcium play an important role for improving the quality tomato by increasing the firmness, reducing the physiological disorders, delaying ripening process and prolonged the shelf life of tomato (Sharma *et al.*, 1996) [9]. Elevated Ca^{2+} concentration in nutrient solution mitigated the adverse effect of Nacl by inhibiting the Na^{+} uptake (Kaya *et al.*, 2002) [3] and reducing the membrane leakages (Tuna *et al.*, 2007) [12]. In present study different levels of Calcium chloride has been used against different levels of NaCl.

Materials and methods
The experiment entitled "Role of Calcium chloride on Biochemical properties of Tomato (*Lycopersicon esculentum*) against different levels of NaCl" was carried out separately during the year 2014-15 and 2015-16 under the agro-climatic conditions of Allahabad at Department of Biological sciences, Sam Higginbottom University of Agriculture, Technology and Sciences.

Field preparation
The experimental field was prepared by ploughing with a Tractor drawn disc plough followed by two cross harrowing and planking. The field was thoroughly levelled by a leveller before it was laid out.

Nutrient application

Manures and fertilizers were applied according to recommended doses for cherry tomato, i.e. 10-15 t/ha FYM or compost along with fertilizers N_2: P_2O_5: K_2O @ 120: 50: 50 kg ha^{-1}. FYM was well incorporated in plots at least 20 days before transplanting. Nitrogen (N_2) was applied in three equal splits. One third dose of nitrogen, total phosphorus and potash were applied as basal dressing before transplanting. Balance quantity of N_2 was applied in two split doses of each at 30DAT and 45 DAT as top dressing.

Transplanting

Thirty days old healthy seedlings having 4-5 leaves with a height of 4-6 cm were selected from the nursery and transplanted at the experimental plot and given light irrigation.

Observations recorded

The technique of representative sample was adopted for recording the observations on various morphological characters in tomato. At every observation, five plants from each plot were randomly selected and tagged. The observations were recorded from these samples.
The following observations were recorded at various successive growth stages.

Chlorophyll

One gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 ppm for 5 minute. The supernatant was read under 663, 645 nanometre. The readings were fed in the following formula and results were determined under spectrophotometer.
Amount of chlorophyll a, b and total chlorophyll are calculate as fallow –
Chlorophyll a (mg/g) = 12.9 × A (663 nm) – 2.69 × A (645 nm)
Chlorophyll b (mg/g) = 41.50 × A (645 nm) – 5.10 × A (663 nm)

Total chlorophyll

Total chlorophyll = Chlorophyll ‘a’ + Chlorophyll ‘b’
A – Absorbance of specific wavelength

Carotenoids

The leaves were collected for the carotenoid content estimation. Fresh leaves weighing of 0.5 gm was taken and homogenized in 10 ml of acetone (80% acetone) and centrifuged at 3000rpm at 10 min. The absorbance was recorded at 470 nm.
It is calculated by using formula:
Total carotenoids = [1000 A470 – (2.270 Chl-a - 81.4 Chl-b)]/227

Lycopene

Extraction was performed according to **(**Fish *et al*., 2002) [2]. Samples were first chopped and homogenized in a laboratory homogenizer. Approximately 0.3-0.6 g samples were weighed and 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and 10 mL of hexane were added. The absorbance of the hexane layer (upper layer) was measured at absorbance 503 nm spectrophotometry using the re-determined extinction coefficients (Perkins *et al.*, 2004) [6].

Result and discussion

Chlorophyll a (mg/g)

The maximum chlorophyll a content was shown by T_6 treatment during both the trials as (2.38 mg/g) and (2.42 mg/g) respectively and the minimum chlorophyll a content was recorded in treatment T_3 as (0.95 mg/g) and (0.98 mg/g) respectively. Individually Calcium is known to play a special role in tolerance under salinity. Ca with magnesium increasing the chlorophyll contents, membrane stability under stress conditions. The same report has been reported by (Sagi, 2005) [8].

Table 1: Effect of NaCl and $CaCl_2$ salinity on the Chlorophyll a content (mg/g) of Tomato leaves

Treatments		Chlorophyll a (mg/g)		
		1st trial	2nd trial	Pooled
T_0	Control	1.54	1.55	1.55
T_1	NaCl 100ppm	1.19	1.21	1.20
T_2	NaCl 200ppm	1.12	1.14	1.13
T_3	NaCl 300ppm	0.95	0.98	0.97
T_4	$CaCl_2$ 1200ppm	1.68	1.68	1.68
T_5	$CaCl_2$ 1800ppm	1.78	1.84	1.81
T_6	$CaCl_2$ 2400ppm	2.38	2.42	2.40
T_7	NaCl 100ppm +$CaCl_2$ 1200ppm	1.37	1.39	1.38
T_8	NaCl 100ppm +$CaCl_2$ 1800ppm	1.42	1.44	1.43
T_9	NaCl 100ppm +$CaCl_2$ 2400ppm	1.55	1.61	1.58
T_{10}	NaCl 200ppm +$CaCl_2$ 1200ppm	1.21	1.23	1.22
T_{11}	NaCl 200ppm +$CaCl_2$ 1800ppm	1.29	1.28	1.29
T_{12}	NaCl 200ppm +$CaCl_2$ 2400ppm	1.34	1.34	1.34
T_{13}	NaCl 300ppm +$CaCl_2$ 1200ppm	1.04	1.39	1.22
T_{14}	NaCl 300ppm +$CaCl_2$1800ppm	1.2	1.34	1.27
T_{15}	NaCl 300ppm +$CaCl_2$ 2400ppm	1.22	1.04	1.13
F- test		S	S	S
S. Ed. (±)		0.076	0.074	0.074
C. D. (P = 0.05)		0.157	0.154	0.153

Chlorophyll b (mg/g)

Chlorophyll b content was shown by T_6 treatment during both the trials as (1.07mg/g) and (1.09mg/g) respectively and the minimum chlorophyll b content was recorded in treatment T_3 as (0.46mg/g) and (0.47mg/g) respectively. The phenomena is same as reported in chlorophyll a and the similar results has been reported by (Sagi, 2005) [8].

Table 2: Effect of NaCl and $CaCl_2$ salinity on the Chlorophyll b content (mg/g) of tomato leaves

Treatments		Chlorophyll b (mg/g)		
		1st trial	2nd trial	Pooled
T_0	Control	0.89	0.91	0.900
T_1	NaCl 100ppm	0.57	0.59	0.580
T_2	NaCl 200ppm	0.52	0.53	0.525
T_3	NaCl 300ppm	0.46	0.47	0.465
T_4	$CaCl_2$ 1200ppm	0.96	0.94	0.950
T_5	$CaCl_2$ 1800ppm	0.97	0.98	0.975
T_6	$CaCl_2$ 2400ppm	1.07	1.09	1.080
T_7	NaCl 100ppm +$CaCl_2$ 1200ppm	0.76	0.78	0.770
T_8	NaCl 100ppm +$CaCl_2$ 1800ppm	0.79	0.8	0.795
T_9	NaCl 100ppm +$CaCl_2$ 2400ppm	0.85	0.86	0.855
T_{10}	NaCl 200ppm +$CaCl_2$ 1200ppm	0.70	0.72	0.710
T_{11}	NaCl 200ppm +$CaCl_2$ 1800ppm	0.73	0.74	0.735
T_{12}	NaCl 200ppm +$CaCl_2$ 2400ppm	0.77	0.76	0.765
T_{13}	NaCl 300ppm +$CaCl_2$ 1200ppm	0.66	0.78	0.720
T_{14}	NaCl 300ppm +$CaCl_2$1800ppm	0.68	0.76	0.720
T_{15}	NaCl 300ppm +$CaCl_2$ 2400ppm	0.71	0.66	0.685
F- test		S	S	S
S. Ed. (±)		0.026	0.030	0.027
C. D. (P = 0.05)		0.053	0.063	0.056

Carotenoids (mg/g fresh weight)

The maximum carotenoids content was shown by T_6

treatment during both the trials as (4.40 mg/g fresh weight) and (4.31 mg/g fresh weight) respectively and the minimum carotenoids content was recorded in treatment T_3 as (3.02 mg/g fresh weight) and (3.19mg/g/fw) respectively. The application of $CaCl_2$ was found to be better to improve carotenoid and lycopene, index of tomato fruits under stress conditions. The similar report has been reported by (Subiah and Perumal, 1990) [11].

Table 3: Effect of NaCl and $CaCl_2$ salinity on the Carotenoids (mg/g fresh weight) in tomato leaves

Treatments		Carotenoids (mg/g fresh weight)		
		1st trial	2nd trial	Pooled
T_0	Control	3.22	3.39	3.31
T_1	NaCl 100ppm	3.60	3.78	3.69
T_2	NaCl 200ppm	4.12	4.30	4.21
T_3	NaCl 300ppm	3.02	3.19	3.11
T_4	$CaCl_2$ 1200ppm	4.32	4.47	4.40
T_5	$CaCl_2$ 1800ppm	4.09	4.14	4.12
T_6	$CaCl_2$ 2400ppm	4.40	4.31	4.36
T_7	NaCl 100ppm +$CaCl_2$ 1200ppm	4.13	4.19	4.16
T_8	NaCl 100ppm +$CaCl_2$ 1800ppm	3.40	3.55	3.48
T_9	NaCl 100ppm +$CaCl_2$ 2400ppm	3.90	4.01	3.96
T_{10}	NaCl 200ppm +$CaCl_2$ 1200ppm	3.92	4.11	4.02
T_{11}	NaCl 200ppm +$CaCl_2$ 1800ppm	4.10	4.22	4.16
T_{12}	NaCl 200ppm +$CaCl_2$ 2400ppm	3.84	4.01	3.93
T_{13}	NaCl 300ppm +$CaCl_2$ 1200ppm	4.16	4.30	4.23
T_{14}	NaCl 300ppm +$CaCl_2$1800ppm	3.85	4.12	3.99
T_{15}	NaCl 300ppm +$CaCl_2$ 2400ppm	3.94	3.89	3.92
F- test		S	S	S
S. Ed. (±)		0.113	0.113	0.111
C. D. (P = 0.05)		0.137	0.138	0.134

Lycopene (mg/gm fresh weight)

Maximum lycopene content was shown by T_6 treatment during both the trials as (3.07) (mg/g fresh weight) and (3.18) (mg/g fresh weight) respectively and the minimum lycopene content was recorded in treatment T_3 as (2.11) (mg/g fresh weight) and (2.22) (mg/g fresh weight) respectively. As already mentioned that the application of $CaCl_2$ was found better to improve the carotenoid and lycopene, index of tomato fruits under stress conditions. The same report has been reported by (Subiah and Perumal 1990) [11].

Table 4: Effect of NaCl and $CaCl_2$ salinity on the Lycopene content (mg/g fresh weight) of tomato fruit

Treatments		Lycopene (mg/gm fresh weight)		
		1st trial	2nd trial	Pooled
T_0	Control	2.25	2.36	2.31
T_1	NaCl 100ppm	2.52	2.63	2.58
T_2	NaCl 200ppm	2.88	2.99	2.94
T_3	NaCl 300ppm	2.11	2.22	2.17
T_4	$CaCl_2$ 1200ppm	3.02	3.13	3.08
T_5	$CaCl_2$ 1800ppm	2.86	2.97	2.92
T_6	$CaCl_2$ 2400ppm	3.07	3.18	3.13
T_7	NaCl 100ppm +$CaCl_2$ 1200ppm	2.88	2.99	2.94
T_8	NaCl 100ppm +$CaCl_2$ 1800ppm	2.38	2.49	2.44
T_9	NaCl 100ppm +$CaCl_2$ 2400ppm	2.71	2.82	2.77
T_{10}	NaCl 200ppm +$CaCl_2$ 1200ppm	2.71	2.82	2.77
T_{11}	NaCl 200ppm +$CaCl_2$ 1800ppm	2.85	2.96	2.91
T_{12}	NaCl 200ppm +$CaCl_2$ 2400ppm	2.69	2.80	2.75
T_{13}	NaCl 300ppm +$CaCl_2$ 1200ppm	2.90	3.01	2.96
T_{14}	NaCl 300ppm +$CaCl_2$1800ppm	2.47	2.58	2.53
T_{15}	NaCl 300ppm +$CaCl_2$ 2400ppm	2.74	2.85	2.80
F- test		S	S	S
S. Ed. (±)		0.069	0.068	0.068
C. D. (P = 0.05)		0.142	0.140	0.139

Conclusion

Based upon the present study, it was found that the treatment T_6 ($CaCl_2$ 2400 ppm) showed better results in terms of chlorophylla, chlorophyll b, carotenoids and lycopene against different levels of NaCl.

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