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Alleviation of Sodium Chloride Stress by Growth Regulators in Seedlings of Clusterbean (*Cymopsis tetragonoloba* L. Taub)

S.S. RATHORE, N.K. PRUTHI[1] AND G.P.S. RATHORE

College of Horticulture, Mandsaur 458 001
drssrathore@yahoo.co.in

ABSTRACT Clusterbean (*Cymopsis tetragonoloba* L. Taub) is an important arid legume which emerged as a new industrial cash crop. Salinity is the major barrier for crop productivity in India. An experiment conducted to test the seed germination and emergence under NaCl induced salinity and effect of different levels of IAA and GA_3. Different levels of IAA and GA_3 were taken with combinations (0.10, 0.25, 0.50 per cent) of NaCl salinity to observe their effect on different seed quality parameters. Germination percentage decreased with increasing NaCl salinity. It also delayed the germination, root length; shoot length, fresh and dry weight of seedlings decreased with increased salinity. Both IAA and GA_3 alleviated adverse effect of NaCl salinity on germination as well as germination period. It also mitigated the adverse effect of salinity on root length; shoot length, fresh and dry weight of seedlings. GA_3 show high alleviation in comparison to IAA for seedling attributes.

Key words: Clusterbean (*Cymopsis tetragonoloba* L. Taub), NaCl salinity, seedlings.

Cluster bean [*Cymopsis tetragonoloba* (L.) Taub.] is a drought resistant *kharif* crop, which is grown in the arid agro-ecological conditions of India. Its green pods are a rich source of vitamin A, C and iron and are used as vegetable, plant as fodder, seed for extraction of guar gum (galactomannan), which serves as useful source of hydrocolloids Galactomannan is a high molecular weight polysaccharide polymer made up of a large number of mannose and galactose units linked together, is a great value polysaccharide of clusterbean, which has hundreds of uses like in textile, paper, petroleum, mining, cosmetic, oil and pharmaceuticals, explosives, purification of potash, photography, tobacco and food industries.

Salinity problems are very often linked to an excess of NaCl in the soil. Major inhibitory effect of salinity on plant growth and its development has been attributed to osmotic inhibition of water availability, toxic effects of ions causing nutritional and hormonal imbalance [1]. The legumes are most sensitive to salinity and sodicity. Salinity stress alters morphological, physiological and biochemical attributes in plants [2 & 3].

Salinity reduces seed germination and seedling performance. The effects of salt stress are especially adverse during the early seedling stage when it is fully dependent upon the restricted supply of food material from the cotyledon. Plant growth regulators have shown promises in alleviation of salt stress, treatment of IAA and GA_3 significantly mitigated the adverse effects of salinity on growth, tissue ion concentration and ionic balance in soybean [3].

Salinity generally causes a reduction in final germination as well as delays its commencement in rice (*Oryza sativa* L.) [4]. Early stages of growth

[1]Department of Agriculture Chemistry, CCR (PG) College, Muzaffarnagar 251 001.

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are of critical importance as later development and crop production depend upon effective germination and well establishment of seedlings. Numerous studies have shown that length, fresh weight and dry weight of seedling decreased under salt stress [5].

The present investigation was undertaken to study the effect of two growth regulators on germination attribute of salt tolerance in clusterbean.

MATERIAL AND METHODS

Seeds of cluster bean [*Cymopsis tetragonoloba* (L.) Taub.] variety 'RGC-936' was germinated in 25 ml test tubes by paper bridges in sterilized conditions. Tubes were treated with different levels of IAA and GA_3 and different levels of sodium chloride (NaCl) concentrations. A common nutrient solution i.e. Hoagland's solution was given to all treatments along with control [6]. Controlled environmental conditions i.e. temperature, humidity, and light was provided to seedlings for growth in growth chamber.

Samples of control and treated seedlings were taken at every 24h interval up to one week (168 h) after putting the seeds for germination. Percent germination, period of germination, length of radicle and coleoptiles were measured. Fresh weight of seedlings, dry weight of seedlings by drying the samples in an oven initially at 60° C for 48h and finally at 80° C for 24 h or till constant weight was attained and wilting symptoms were noticed.

List of treatments:

Treatments			
Control	T_0	0.50% NaCl+100 ppm IAA	T_8
0.10% NaCl	T_1	0.50% NaCl+250 ppm IAA	T_9
0.25% NaCl	T_2	0. 1 0% NaCl+50 ppm GA_3	T_{10}
0.50% NaCl	T_3	0. 10% NaCl+100 ppm GA_3	T_{11}
0.10% NaCl+100 ppm IAA	T_4	0.25% NaCl+50 ppm GA_3	T_{12}
0. 10% NaCl+250 ppm IAA	T_5	0.25% NaCl+100 ppm GA_3	T_{13}
0.25% NaCl+100 ppm IAA	T_6	0.50% NaCl+50 ppm GA_3	T_{14}
0.25% NaCl+250 ppm IAA	T_7	0.50% NaCl+100 ppm GA_3	T_{15}

RESULT AND DISCUSSION

Per cent germination in cluster bean was affected by different NaCl salinity levels (Table 1). Germination percentage significantly decreased with increasing NaCl salinity levels, minimum germination (53.0%) was observed as compared to that in control (89,0%) after 120h at 0.5% NaCl salinity level. IAA and GA_3 increased the germination percentage with different combinations of NaCl salinity. Maximum germination was recorded with treatment T_{11}, (93%) as compared to pure salinity levels T_1 (89%). GA_3 show high alleviation in comparison to IAA for germination. Germination period delayed by increasing NaCl salinity levels, both IAA and GA_3 reduced the germination period. Differences due to treatments, sampling period and their interaction were found statistically significant.

Salinity caused reduction in germination percentage due to NaCl salinity. These findings are in agreement with workers observed the reduction in various crops. Salinity generally causes a reduction in final germination as well as delays its commencement in wheat, barley and triticale [7&8]. At lower levels (0.1% NaCl) salinity delayed the seed germination but did not affect the final germination percentage as seen in clusterbean [9].

GA_3 is more effective than IAA for alleviation of harmful effect of NaCl salinity on germination percentage. These results are more or less concurred with the findings of various workers

Table 1. Effect of different NaCl salinity levels and their interactions with IAA and GA_3 on germination in clusterbean

Treatment		Period						
		24h	48h	72h	96h	120h	144h	168h
Control	T_0	69	81	87	89	89	89	89
0.10% NaCl	T_1	68	80	87	88	88	89	89
0.25% NaCl	T_2	59	72	74	74	74	74	74
0.50% NaCl	T_3	48	52	53	53	53	53	53
0. 10% NaCl+100 ppm IAA	T_4	70	81	87	87	87	87	89
0. 10% NaCl+250 ppm IAA	T_5	72	84	88	89	89	89	89
0.25% NaCl+100 ppm IAA	T_6	61	72	72	72	72	72	72
0.25% NaCl+250 ppm IAA	T_7	63	73	73	73	73	73	73
0.50% NaCl+100 ppm IAA	T_8	50	50	51	51	51	51	51
0.50% NaCl+250 ppm IAA	T_9	51	51	51	51	51	51	51
0.10%NaCl+50 ppmGA_3	T_{10}	75	86	91	91	91	91	91
0.10% NaCl+100 ppm GA_3	T_{11}	81	91	93	93	93	93	93
0.25% NaCl+50 ppm GA_3	T_{12}	66	76	78	78	78	78	78
0.25% NaCl+100 ppm GA_3	T_{13}	71	81	81	81	81	81	81
0.50% NaCl+50 ppm GA_3	T_{14}	54	58	59	59	59	59	59
0.50% NaCl+100 ppm GA_3	T_{15}	56	61	62	62	62	62	62

	SEm±	C.D. at 5%
Treatments (T):	0.4651	1.2890
Periods (P):	0.3076	0.8530
Interaction (TXP):	1.2307	3.4110

who reported that seed treatment with different growth regulators often increased the germination percentage under saline conditions [10&11]. Germination percentage improved by GA_3 in rice seeds [4]. GA_3, IAA and cytokinin simultaneously mitigated the inhibitory effects of salts partially or wholly on the germination of *Cucumus callosus* [12].

Root length increased significantly with increasing sampling period (Table 2). NaCl salinity decreased root length significantly. At highest NaCl level (0.5%) root length was minimum (4.2 cm) in comparison to control (6.1 cm) at 168h. IAA and GA_3 increased the root length under salinity with the maximum increase (11.0 cm) was found in GA_3@100ppm+0.1 %NaCl (T_{11}), in comparison to 0.1% NaCl salinity level (6.4 cm) at 168h. GA_3 showed high modulation for adverse affect of NaCl salinity on root length in comparison to IAA. Results were found statistically significant for all treatments and sampling period and their interaction.

Shoot length increased with increasing sampling period (Table 3). At 24-h shoot length was (0.5 cm) increased upto 5.7 cm at 168h in control seedlings. Shoot length significantly decreased with increased NaCl salinity levels. Reduction was higher at higher NaCl salinity level (0.5%) was 2.19 times less than control seedlings. In interaction of IAA and GA_3 and NaCl salinity, both IAA and GA_3 increased shoot length as compared to only NaCl salinity treatments but was more effective than IAA. At 168h interaction of GA_3 @ 100 ppm + 0.50%

Table 2. Effect of different NaCl salinity levels and their interactions with IAA and GA_3 on root length (cm) in clusterbean seedlings

Treatment	Period						
	24h	48h	72h	96h	120h	144h	168h
Control	0.20	0.4	0.70	1.50	3.50	5.10	6.10
0.10% NaCl	0.30	0.5	0.80	1.70	3.70	5.30	6.40
0.25% NaCl	0.20	0.4	0.60	1.30	3.10	4.80	5.70
0.50% NaCl	0.10	0.3	0.45	1.00	2.60	4.00	4.20
0.10% NaCl+100 ppm IAA	0.25	0.55	0.86	1.70	3.80	5.35	6.50
0.10% NaCl+250 ppm IAA	0.25	0.65	0.90	1.80	3.90	5.55	6.75
0.25% NaCl+100 ppm IAA	0.25	0.50	0.65	1.45	3.25	5.00	5.85
0.25% NaCl+250 ppm IAA	0.25	0.65	0.75	1.55	3.50	5.20	5.95
0.50% NaCl+100 ppm IAA	0.15	0.35	0.50	1.67	3.55	5.25	6.05
0.50% NaCl+250 ppm IAA	0.15	0.35	0.50	1.68	3.55	5.25	6.05
0.10% NaCl+50 ppm GA_3	0.35	0.60	1.00	1.95	4.10	5.70	6.70
0.10% NaCl+100 ppm GA_3	0.35	0.65	1.20	2.05	4.40	5.95	6.80
0.25% NaCl+50 ppm GA_3	0.25	0.55	0.95	1.55	3.35	4.95	5.95
0.25% NaCl+100 ppm GA_3	0.25	0.67	1.10	1.75	3.55	5.15	6.15
0.50% NaCl+50 ppm GA_3	0.10	0.35	0.50	1.10	2.65	4.10	4.30
0.50% NaCl+100 ppm GA_3	0.20	0.40	0.60	1.40	2.90	4:60	4.90

	SEm±	C.D. at 5%
Treatments (T):	0.0370	0.1030
Periods (P):	0.0244	0.0680
Interaction (TXP):	0.0979	0.2710

NaCl, shoot length was 1.13 times more than only NaCl salinity level (0.50% NaCl). In same NaCl salinity level IAA@ 250 ppm increased only 1.03 time more shoot length as compared to only NaCl. Results found statistically significant for all treatments and sampling period and their interaction. Seedling fresh weight increased significantly with increasing sampling period (Table 4). Seedling fresh weight was increased upto 280.5mg (at 168h) from 15.4mg (at 24h). Fresh weight of seedlings reduces significantly due to NaCl salinity upto 1.4 times at highest NaCl salinity level (0.5% NaCl). IAA and GA_3 modulated the adverse effect of NaCl salinity significantly when applied in combination of NaCl salinity. IAA at its higher concentration (250 ppm), when interact with 0.5% NaCl salinity increased fresh weight only 1.01 times of only NaCl salinity (0.5% NaCl) treatment at 168h. GA_3 modulated loss of fresh weight due to NaCl salinity was higher in comparison to IAA at 168h. GA_3@ 100 ppm+0.5 per cent NaCl increased 1.13 times of 0.5% NaCl salinity level. Interaction of different treatments and sampling period was found statistically significant.

Seedling dry weight significantly increased with sampling period (Table 5). Seedling dry weight increased at 168h upto 12.72 mg from 24h (0.70 mg). NaCl salinity decreased the seedling dry weight upto 1.41 times than control in 0.5% NaCl level. Seedling dry weight increased upto 1.85 per cent in combination of IAA @ 250 ppm+0.5 % NaCl over pure 0.5% NaCl salinity

Table 3. Effect of different NaCl salinity levels and their interactions with IAA and GA_3 on shoot length (cm) in clusterbean seedlings

Treatment	Period						
	24h	48h	72h	96h	120h	144h	168h
Control	0.15	0.30	0.55	1.30	2.90	4.50	5.70
0.10% NaCl	0.15	0.32	0.57	1.30	2.90	4.50	5.70
0.25% NaCl	0.10	0.20	0.42	1.00	2.30	3.70	4.80
0.50% NaCl	0.10	0.15	0.30	0.65	1.30	1.00	2.60
0.10% NaCl+100 ppm IAA	0.15	0.35	0.60	1.40	3.10	4.70	5.85
0.10% NaCl+250 ppm IAA	0.15	0.35	0.65	1.50	3.20	4.85	5.90
0.25% NaCl+100 ppm IAA	0.10	0.20	0.43	1.05	2.35	3.80	4.90
0.25% NaCl+250 ppm IAA	0.15	0.25	0.50	1.50	3.00	4.15	5.05
0.50% NaCl+100 ppm IAA	0.10	0.15	0.35	0.80	1.40	2.10	2.70
0.50% NaCl+250 ppm IAA	0.10	0.15	0.36	0.85	1.45	2.10	2.70
0.10%NaCH-50 ppm GA_3	0.20	0.35	0.75	1.50	3.20	4.80	6.70
0.10% NaCl+100 ppm GA_3	0.25	0.40	0.85	1.70	3.40	4.50	7.50
0.25% NaCl+50 ppm GA_3	0.15	0.25	0.55	1.60	3.05	4.25	5.10
0.25% NaCl+100 ppm GA_3	0.15	0.25	0.40	1.20	2.70	4.20	5.40
0.50% NaCl+50 ppm GA_3	0.10	0.17	0.37	0.82	1.40	2.15	2.70
0.50% NaCl+100 ppm GA_3	0.10	0.20	0.40	1.05	1.55	2.25	2.95

	SEm+	C.D. at 5%
Treatments (T):	0.0297	0.0820
Periods (P):	0.0196	0.0540
Interaction (TXP):	0.0786	0.2180

level at 168h. GA_3 @ 100 ppm when combined with 0.5% NaCl salinity increased seedling dry weight upto 11.3 per cent of only 0.25 per cent NaCl treatment at 168h (Table 5). Treatments, sampling period and their interaction were found significant.

Reduction in root length, shoot length, fresh and dry weight with increasing level of salinity have also been reported in mungbean [13] pigeon pea [14]; cowpea [15]; chickpea [17]. This has added effect where salt stress not only enforces osmotic but also ionic effects on plants. Increase in dry matter and root growth with GA_3 and IAA in soybean has also been reported earlier [2].

Present study suggests that treatments of GA_3 was more effective than that of IAA to increase root and shoot length, fresh and dry weight and moisture per cent in clusterbean seedlings.

REFERENCES

1. WHY-JONES, R.C. (1981). Salt tolrance in plants. *In*: Jonson , C.B. (ed) *Physiological process limiting plant productivity*, Butter- worths, London, pp 271-292.

2. MALIBARI, A.A. (1993). The interactive effects between salinity, Abscissic acid and kinetin on transportation, chlorophyll content and growth of wheat plant. *Indian J. Plant Physiol.*, **36**: 232-35.

3. ZAIDI, P.H. & B.B. SINGH (1996). Modulation of

Table 4. Effect of different NaCl salinity levels and their interactions with IAA and GA_3 on fresh weight (mg) in clusterbean seedlings

Treatment	Period						
	24h	48h	72h	96h	120h	144h	168h
Control	15.4	33.8	81.40	149.60	231.00	242.00	280.50
0.10% NaCl	14.70	36.00	85.80	169.50	244.20	264.00	289.70
0.25% NaCl	13.00	27.50	59.40	111.10	189.20	214.50	237.60
0.50% NaCl	10.0	21.00	37.40	86.90	147.40	271.60	196.90
0.10% NaCl+100 ppm IAA	14.65	35.70	89.20	147.40	243.10	266.20	290.20
0.10% NaCl+250 ppm IAA	14.60	35.70	81.40	151.80	254.50	283.10	305.80
0.25% NaCl+100 ppm IAA	13.00	27.50	59.80	110.50	190.50	214.70	238.70
0.25% NaCl+250 ppm IAA	14.65	28.50	62.40	112.20	201.00	216.70	245.10
0.50% NaCl+100 ppm IAA	11.10	22.10	38.30	87.20	148.30	172.40	197.00
0.50% NaCl+250 ppm IAA	11.10	22.30	38.60	87.90	149.00	173.20	197.40
0.10% NaCl+50 ppm GA_3	13.00	32.00	84.00	151.80	251.50	275.00	305.10
0.10% NaCl+100 ppm GA_3	13.00	32.00	83.50	149.60	247.50	273.90	290.10
0.25% NaCl+50 ppm GA_3	12.00	29.00	40.00	112.50	187.00	216.70	240.10
0.25% NaCl+100 ppm GA_3	14.00	35.70	81.00	153.00	233.20	258.50	260.10
0.50% NaCl+50 ppm GA_3	9.00	36.00	82.00	86.90	148.50	171.00	190.10
0.50% NaCl+100 ppm GA_3	9.00	35.50	81.00	89.00	154.00	182.00	201.50

	SEm±	C.D. at 5%
Treatments (T):	1.9478	5.3980
Periods (P):	1.2883	3.5710
Interaction (TXP):	5.1535	14.2830

adverse effect of salinity by growth regulators in soyabean II growth nutrient concentrations and balances. *Pl. Physiol. and Bio-chem.* **23**(2): 139-144.

4. RAHMAN, M.S., H. MIYAKE & Y. TAKEOKA (2001). Effect of sodium chloride on seed germination and early seedling-growth of rice (*Oryza sativa* L.). *Pak. J. Biol. Sci.*, **4**(3): 351-355.

5. SHARMA, P.K., S.K. VERMA, K.S. DATTA, B. KUMAR & R. ANGARISH (1996). Differential response of wheat to chloride and sulphate salinities at germination and early seedling growth. *Haryana Agric. Univ. J. Res.*, **26:** 1-7.

6. HOAGLAND, D.R. & D.I. ARNON (1950). Water culture method for growing plants without soil. *Univ. Calif. Agric. Exp. Star Cire*, **347**: 1-39.

7. LARIK, A.S. & Y.A. Al-SAHAEAL (1986). Effect of salt stress on germination and seedling growth of wheat, triticale and barley. *J. College Sci. King Saud Univ.*, **17**: 37-44.

Table 5. Effect of different NaCl salinity levels and their interactions with IAA and GA_3 on dry weight (mg) in clusterbean seedlings

Treatment	Period						
	24h	48h	72h	96h	120h	144h	168h
Control	0.70	1.55	3.70	6.80	10.50	11.60	12.72
0.10% NaCl	0.65	1.65	3.90	7.70	11.10	12.00	13.17
0.25% NaCl	0.55	1.20	2.70	5.05	8.60	9.75	10.80
0.50% NaCl	0.40	1.90	1.70	3.95	6.70	7.80	8.98
0.10% NaCl+100 ppm IAA	0.65	1.60	3.60	6.70	11.05	12.10	13.20
0.10% NaCl+250 ppm IAA	0.65	1.60	3.70	6.90	11.57	12.87	13.90
0.25% NaCl+100 ppm IAA	0.55	1.20	2.70	5.05	8.66	9.76	10.85
0.25% NaCl+250 ppm IAA	0.60	1.25	2.75	5.10	8.70	9.85	10.90
0.50% NaCl+100 ppm IAA	0.43	0.96	1.73	3.98	6.72	7.86	9.04
0.50% NaCl+250 ppm IAA	0.44	0.98	1.78	4.03	6.80	7.91	9.12
0.10%NaCH-50 ppmGA_3	0.55	1.50	3.75	6.89	11.43	12.50	13.75
0.10% NaCl+100 ppm GA_3	0.55	1.50	3.65	6.80	11.25	12.45	13.50
0.25% NaCl+50 ppm GA_3	0.45	1.30	2.85	5.10	8.50	9.85	10.90
0.25% NaCl+100 ppm GA_3	0.60	1.60	3.78	6.90	10.60	11.75	12.85
0.50% NaCl+50 ppm GA_3	0.35	0.92	1.70	3.95	6.75	7.80	8.95
0.50% NaCl+100 ppm GA_3	0.35	0.95	1.80	4.085	7.00	8.05	9.15

	SEm+	C.D. at 5%
Treatments (T):	0.0861	0.2390
Periods (P):	0.0569	0.1580
Interaction (TXP):	0.2278	0.6320

8. PARASHAR, A. & S.K. VERMA (1988). Effect of presowing seed soaking in gibberellic acid, duration of soaking, different temperatures and their interaction on seed germination and early seedling growth of wheat under saline conditions. *Plant Phyisol & Biochem.*, **15**: 189-97.

9. KHAN, M.A. & I.A. UNGAR (1996). Influence of salinity and temperature on the germination of *Haloxyton vecurvum* Bunge ex Boiss. *Ann Bot.*, **78**: 547-51.

10. KHAN, A. &I.A. UNGER (1985). The role of hormones in regulating the germination of polymorphic seeds and early seedling growth of *Atriplex transgulars* under saline conditions. *Physiol. Plant*, **63**(1): 109-13.

11. SHAH, S.H. (2007). Effect of salt stress on mustard as affected by gibberellic acid applications. *Gen. Appl. Plant Physiol.*, **33**(1-2): 97-106.

12. DADHEECH, P.L., A. UPADHYAYA, A. TIKKU, D. SHANKHLA & N. SHANKHLA (1980). Ecophysiological studies on cucurbits of Indian desert I. Role of salt and bioregulants in seed germination of *Cucumus callosus*. *Trans. Indian Soc. Desert Technol.*, **5**: 89-90.

13. SEKHAR, M.R. (1994).Salt tolerance of mungbean (*Vigna radiata* L. Wilczek) at germination stage. *Ann. Agril. Res.*, **15**(1): 90-91.

14. GILL, K.S. & P.C. SHARMA (1993). Mechanism of salt injury at seedling and vegetative growth stages in *Cajanus cajan* (L.) Millsp. *Pl. Physiol. and Bio-chem.*, **20**(1) : 49-52.

15. REMA DEVI, G. & P.K. GOPALKRISHAN (1997). Effect of sodium chloride and calcium chloride salinity and the seedling growth of cowpea. *Ind. J. Pl. Physiol.*, **2**(1): 79-80.

16. JAIWAL, P.K., S. BHAMBIE & K. MEHTA (1983). Effect of salinity on seed germination and seedling growth of chickpeas. *International Chickpea Newsletter*, **9**: 15-16.