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RESEARCH ARTICLE

Exogenous supply of IAA, GA and cytokinin to salinity stressed seeds of chickpea improve the seed germination and seedling growth

■ **SHWETA TYAGI AND SANJEEV KUMAR**

SUMMARY

When $1x10^{-9}$M NaCl stressed seeds of *Cicer arietinum* cv. SURYA treated with growth hormones (*i.e.* $1x10^{-8}$M of IAA, $1x10^{-5}$M of Kn and $1x10^{-4}$M of GA), it was found that all the phytohormone enhanced seed germination and seedling growth and the level of total nitrogen and enzyme activities *i.e.* amylases, proteases and phosphatases in treated seeds, being maximum at combined treatment of IAA+GA+Kn of these concentration.

Key Words : Salinity, Phytohormones, Auxin, Gibberellins

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Salinity of soil and water is a major environmental stress that drastically affects crop productivity in term of growth and yield, all over world (Zhu, 2001). Pre-soaking seeds with optimal concentration of phytohormones enhance their germination, growth and yield of some crop species of wheat and Barley under condition of environmental stress by increasing nutrient reserves through increased physiological activities and root proliferation (Asana *et al.*, 1985 and Dave and Gaur, 1970). The exogenous application of plant growth regulators, auxins (Khan *et al.*, 2004), gibberellins (Afzal *et al.*, 2006), cytokinins (Gul *et al.*, 2000) reduce the adverse effects of salt stress and also improves germination, growth, development and seed yields and yield quality of *Ceratoides lanta*, *Allenrolfea accidentalis* and *Triticum* species (Egamberdieva, 2009). Further, to extend these studies, salinity stress seeds of *Cicer aeritinum* treated with promotory dose of phytohormones to measure the effect on seed germination and seedling growth along with biochemical changes in imbibed seeds.

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MATERIAL AND METHODS

The experiments was conducted in 2013-14 at D.A.V. College, Muzaffarnagar. The seeds of *Cicer aeritinum* cv. SURYA were collected from IARI, New

Delhi. Seed of uniform size, shape, colour and weight as far as possible, were selected, surface sterilized with 0.1 per cent $HgCl_2$ solution, thoroughly washed with distilled water and treated with inhibitory dose $1x10^{-9}$M of NaCl and promotory dose of growth hormones *i.e.*$1x10^{-8}$M IAA, $1x10^{-4}$M GA and $1x10^{-5}$M Kn and the combination of mentioned dose of all these growth hormones for 24 hr. Simultaneously, seeds treated with $1x10^{-9}$M NaCl, which constituted as controls. After imbibition, the seed germination, seedling growth, the level of biochemical components (total sugar, reducing sugar, total protein and total nitrogen) and total activities of certain enzymes (α-amylase, β- amylase, protease, acid phosphatase and alkaline phosphatase) in imbibed seeds was measured.

Seed germination and seedling growth :

After treatments, seeds were transferred to petridishes having distilled water moistened filter paper and kept for germination and subsequent seedling growth at 25±3°C in dark. Germination was accessed by radical emergence (2-3 mm) and the percentage germination in each case was recorded for dose response relationships, seedling growth was studied at a particular day (*i.e.* 5th day) of germination. After 5 days, seedlings were separated out from each other and length (cm), fresh weight (mg), dry weight (mg) of seedling parts (radicle, plumule and residual cotyledons) were measured. Data were average of 20 seedlings of each treatment and statistically analysed and kept in Table 1.

Estimation of biochemical component :

The level of carbohydrate (Hedge and Hofreiter, 1962), reducing sugar (Bernfeld, 1955), nitrogen (Snell and Snell, 1945), protein (Lowry *et al.,* 1951) in treated seeds of *Cicer aeritinum* was measured and data kept in Table 2.

Determination of enzyme activity :

A common Tris –maleate buffer at 6.8 pH was prepared (Vimla,1983). This was used as extraction cum assay medium for amalyse and protease. Crude enzyme was extracted by homogenising 1 g material in 10 ml buffer and centrifuging the extract to get a clear supernatant, which was made to 20 ml with the buffer. The prepration constituted the crude enzyme extract. Further, each enzyme was assayed as per the method.

~-amylase activity :

Take 1ml of enzyme extract and 1ml substrate *i.e.* starch (0.15%) added to it and then incubated at room temperature for 10 minutes. Now add 3ml of quinching reagent and read O.D. at 620nm with the help of spectrophotometer. Alpha-amylase activity was determined in term of mg starch degraded per minute per g fresh weight (Filner and Varner, 1967).

-amylase activity :

Pipette 0.5 ml of respective enzyme dilutions into a series of numbered test tubes. Incubated a blank with 0.5 ml distilled water. Incubated the tubes at 25°C for 3 to 4 minutes to achieved temperature equilibrium. After that added 0.5ml starch solution (1%). Incubated exactly 3 minutes and add 1ml DNS colour reagent to each tube. Incubate all tubes in a boiling water bath at 100ºC for 5 minutes (Bernfeld, 1955). Activity was determined in term of mg maltose degraded per minute per g fresh weight.

Protease activity :

1 ml of enzyme extract was incubated for 1 hr at 40°C with 1 ml substrate (4mg/ml casein in buffer). The reaction was quenched by addition of 2 ml of TCA and chilling for 3 hr. The supernatant was collected by centrifugation, made slightly alkaline by addition of 1 ml 1.5 N NaOH and final volume made to 5ml with buffer. 1 ml of this was mixed with 5 ml of copper sulphate reagent after 10 minutes, 1 ml Folin's reagent (Lowry *et al.,* 1951) was added to the reaction mixture, kept for 30 minutes and then take O.D. at 620nm against blank (Yomo and Varner, 1973). Activity was expressed as mg or µg tyrosine released / h / g fresh weight.

Acid and alkaline phophatase :

Crude enzyme was extracted by homogenizing 50mg plant material in extraction buffer and centrifuging the extract at 6000rpm for 15 minutes to get a supernatant. Now take 50 µl of sample and 25 µl of pNpp were added in it and then make up the volume by 2.925ml of acetate buffer (pH-5) for acid phophatase and tris buffer (pH-7.5) for alkaline phosphatase. Then incubated at 37ºC for 30 minutes. After incubation, 2ml of 0.1N NaOH was added in it. After that O.D. was taken at 430nm with the help of spectrophotometer. The activity of acid phosphatase was determined by (Lea, 1990; Prince and Steven, 1982; Wilson and Walker, 1996 and Sawhney and Singh, 2007) in terms of pNpp as a substrate at 430nm. Activity was determined in term of mg pNpp degraded/min /g fresh weight.

Data are average of 4 separate experiments done in triplicate and mentioned in Table 3.

RESULTS AND DISCUSSION

The seed germination and seedling growth, level of biochemical components (total sugar, reducing sugar, total protein content and total nitrogen) and total activities of some enzymes (α- amylase, β- amylase, protease, acid phosphatase and alkaline phosphatase) in imbibed seeds of *Cicer arietinum* (Surya) are studied and data are compared with inhibitory dose of NaCl.

Table 1 indicate that length of radicle and plumule is increased by the treatment of IAA *i.e.* 124 ,126 per cent of control, GA 134 per cent ,145 per cent of control and Kn *i.e.* 147 per cent, 149 per cent of control, respectively, but it is maximum at combined treatment *i.e.* 223 per cent and 2005 of control, respectively. While, the fresh and dry weight do not show any significant changes.

Table 2 exhibit the biochemical changes in term of protein, total sugar, nitrogen and reducing sugar in treated seeds of *Cicer aeritinum* cv. SURYA. The content of protein, total sugar and reducing sugar do not show significant changes but, the level of total nitrogen is enhanced in all the treat ment *i.e.* at IAA, it is 257 per cent of control. At GA it is 318 per cent of control and at Kn treatment, it is 444 per cent of control and it become maximum at combined treatment *i.e.* 610 per

Table 1: Interactive effect of NaCl dose ($1x10^{-9}$M) and growth hormone doses ($1x10^{-8}$M IAA,$1x10^{-4}$M GA and $1x10^{-5}$ M Kn) on seedling growth of *Cicer arietinum* (Surya) on 5th day of seed germination in dark

Treatments seedling parts		NaCl	NaCl+ IAA	NaCl + GA	NaCl + Kn	NaCl+ IAA+GA+ Kn
Germination %		80	100	100	100	100
Length	Plumule	3.32±0.27	4.12±0.42	4.44±0.46	4.87±0.46	6.64±0.68
(cm±SD)	Radicle	3.35±0.39	4.22±0.44	4.87±0.45	4.98±0.46	7.47±0.77
Fresh weight	Plumule	55.74±5.50	56.41±5.51	56.71±6.51	56.89±5.56	59.25±6.52
(mg±SD)	Radicle	54.12±5.52	55.11±5.55	55.27±5.55	55.78±5.54	57.14±5.53
	Residual cotyledon	237.14±24.11	138.4±23.85	128.49±24.98	121.88±24.61	100.16±24.87
Dry weight	Plumule	19.21±1.19	20.22±2.23	20.49±2.22	20.78±2.20	22.19±2.24
(mg±SD)	Radicle	41.14±4.42	42.12±3.40	42.56±3.41	42.89±3.49	45.66±4.45
	Residual cotyledon	92.14±10.00	73.15±10.26	74.41±10.43	71.87±10.56	50.44±10.51

Table 2: Interactive effect of NaCl dose ($1x10^{-9}$M) and growth hormone doses ($1x10^{-8}$M IAA,$1x10^{-4}$M GA and $1x10^{-5}$ M Kn) on the level of biochemical component in imbibed seed of *Cicer arietinum* cv. SURYA

Treatments parameters		NaCl	NaCl+ IAA	NaCl + GA	NaCl + Kn	NaCl+ IAA+GA+ Kn
Level of biochemical components	Protein content (mg/g fresh weight ±SD)	51.23±5.87	53.21±4.28	53.49±5.40	54.81±5.75	58.47±6.45
	Total sugar (mg/g dry weight ± SD)	150.01±15.31	153.04±14.22	152.47±15.47	152.78±15.49	158.14±16.27
	Reducing sugar (mg/g fresh weight ± SD)	75.42±7.69	77.42±7.27	76.54±8.12	78.14±8.74	81.56±8.97
	Total nitrogen (mg/g dry weight ± SD)	0.38±0.32	0.98±0.21	1.21±0.43	1.69±0.34	2.32±0.42

Table 3: Interactive effect of NaCl dose ($1x10^{-9}$M) and growth hormone doses ($1x10^{-8}$M IAA,$1x10^{-4}$M GA and $1x10^{-5}$ M Kn) on total activity of certain enzymes in imbibed seed of *Cicer arietinum* cv. SURYA

Treatments		NaCl	NaCl+ IAA	NaCl + GA	NaCl + Kn	NaCl+ IAA+GA+ Kn
Activities of Enzymes (Per g fresh weight ± SD)	α-amylase activity (mg starch degraded/min.)	19.21±1.13	22.27±2.41	21.54±2.24	21.01±2.21	25.74±3.41
	β- amylase activity (mg maltose degraded/ min.)	15.59±1.17	16.54±1.18	17.54±1.17	16.88±1.16	20.11±2.26
	Protease activity (mg tyrosine released/hr.)	4.39±0.35	5.54±0.56	6.13±0.81	5.88±0.53	8.78±0.67
	Acid phosphatase activity (mg pNPP degraded/min.)	12.13±1.13	13.13±1.14	13.58±1.24	14.44±1.25	17.88±1.17
	Alkaline phosphatase activity (mg pNPP degraded/min.)	9.65±0.54	10.32±1.18	11.21±1.50	11.87±1.19	14.33±1.12

cent of control. It shows that nitrogen metabolism is directly linked to overcome the toxicity of NaCl.

Table 3 express the stimulation in activities of amylases, proteases and phosphatases by the treatment of phytohormones. Activities of amylases is 116 per cent of control, protease is 126 per cent and phophatase is 108 per cent of control at treatment of IAA. It also become maximum at combined treatment (NaCl+IAA+ GA+Kn) *i.e.* amylase activity is 134 per cent of control, protease activity is 200 per cent of control and phosphatase activity is 148 per cent of control, respectively.

Above results shows that level of nitrogen and enzyme activities are enhanced by phytohormones treatment. So, it is interesting to mention that the stimulation is linked with the metabolic pathway involving nitrogen content and activities of amylases, proteases and acid and alkaline phosphatases.

Similarly, Phytohormones have also been shown to influence salinity tolerance through modulating several physiological processes and biochemical mechanisms (Fatma *et al.*, 2013). Darra *et al.* (1973) suggested that plant hormones increase the rate of absorption of water and available nutrients thereby resulting in better growth. The hormones might also have substantially enhanced cell enlargement and rapid increase in cell division (Magome *et al.*, 2004). Naseer *et al.* (2001) have reported that application of growth regulators (IAA, 25 mg/lit.) either at the time of salinization proved beneficial in alleviating the adverse effect of salinity on growth and yield parameter. When NaCl stressed seeds of different plants is treated with IAA and GA in rice, the total sugar, reducing sugar and the activity of α- amylase increased (Kim *et al*., 2006). Sadak *et al.* (2013), observed that, when salt stressed faba bean seed treated with IAA and Kn treatment then total carbohydrate, free amino acid, proline and phenolic compounds were increased. Researchers found that both under stress and non-stress conditions, N compounds, including nitrous oxide can improve seed germination through enhancing amylase activities (Zhang *et al.*, 2005; Hu *et al.*, 2007 and Zheng *et al.*, 2009). Through, decreasing the production of O_2 and H_2O_2, such products can also alleviate the stress by controlling the likely oxidative damage, similar to the effects of antioxidant enzymes including superoxide dismutase (SOD), catalse (CAT) and peroxidase (POD) on plant growth undervarious stresses (Song *et al.*, 2006; Tian and Lei, 2006; Tseng *et al.*, 2007; Li *et al.*, 2008; Tuna *et al.*, 2008; Zheng *et al.*, 2009 and Sajedi *et al.*, 2011). Increase of enzyme activities is still remain a question, it need further kinetic studies.

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