

Full Length Research Paper

The physiological response of wheat plants to exogenous application of gibberellic acid (GA₃) or indole-3-acetic acid (IAA) with endogenous ethylene under salt stress conditions

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Wheat (*Triticum vulgare*) plants were grown without NaCl and under salinization levels of NaCl. Salinity decreased the fresh, dry matter, water content, length and leaf area. Phytohormonal treatments with gibberellic acid (GA₃) or indole-3-acetic acid (IAA) caused a marked increase in these parameters; GA₃ was more effective than IAA. This activation was concomitant with the increase of osmotically active solutes, soluble sugars, soluble protein and amino acids. The accumulation of calcium and magnesium in root of plant treated with GA₃ and in shoot of plant treated with IAA may contributed in osmotic defense systems of wheat plants. The data also reveals that ethylene production was increased in salinity treatments. Spraying wheat plants with GA₃ increased the ethylene evolution while spraying with IAA decreased this evolution under salt stress conditions. Finally, it can be concluded that the GA₃ or IAA regulate the disturbances of metabolites and neglicated the negative effects of the accumulation of ethylene especially in plants treated with IAA under stress conditions which in turn resulted in a pronounced alleviated the drastic effects of salt.

Key words: GA₃, IAA, ethylene, salinity, wheat.

INTRODUCTION

Environmental stresses come in many forms, yet the most prevalent stresses have in common their effect on plant water status. Although plant species vary in their sensitivity and response to the decrease in water potential caused by drought, temperature, or high salinity, it may be assumed that all plants encoded capability for stress perception signaling, and response. First most cultivated species have wild relatives that exhibit excellent tolerance to abiotic stress. Second, biochemical studies include metabolites as nitrogen containing compounds (proline or other amino acids, quaternary amino compounds and polyamine and hydroxyl compounds (sucrose, polyols, and oligosaccharides (McCue and Hanson 1990; and Bohnert et al., 1995). Third, molecular studies have revealed that a

wide variety of species a common set of genes and similar proteins (Skriver and Mundy 1990; and Vilardell et al., 1994). These proteins play active roles in the response to stress.

Plants encounter a variety of external and internal environmental changes. Among the external environmental factors critical for survival of plants are water, temperature, light and other organisms. Internal environmental factors include plant hormones such as ABA, auxin, cytokinins, ethylene, gibberellic acid (GA₃), Jasmonic acid, and brassino-steroids (Hong et al., 1997).

Ethylene evolution is associated with stress and is involved in modulating a broad spectrum of physiological processes such as, senescence, flowering, fruit ripening

Table 1. Effect of salinization and treatment with GA₃ or IAA (200 mg kg⁻¹) on fresh (f.m.), dry matter (d.m., g plant⁻¹) water content and length (cm plants⁻¹) of shoot and root wheat plants.

Treatment	NaCl mM	Shoot		Root		Water content		Length	
		f.m.	d.m.	f.m.	d.m.	Shoot	Root	Shoot	Root
Control	0	0.699	0.091	0.099	0.05	0.608	0.049	28.7	13
	50	0.605	0.143	0.067	0.038	0.462	0.029	33.3*	13.3
	100	0.707	0.155	0.066	0.035	0.552	0.031	31.3	11.0**
	150	0.321*	0.14	0.064	0.032	0.181	0.032	35.0**	13.3
	200	0.357*	0.109	0.028	0.018	0.248	0.01	32.3	8.7**
	250	0.249*	0.068	0.029	0.017	0.181	0.012	24.7*	8.0**
GA ₃	0	1.19**	0.349**	0.191	0.078	0.841	0.113	47.7**	17.5**
	50	1.18**	0.226	0.184	0.075	0.954	0.109	39.3**	15.7**
	100	0.976	0.197	0.064	0.039	0.779	0.028	40.0**	16.7**
	150	0.805**	0.175	0.044	0.028	0.35	0.016	37.3	13.0**
	200	0.567	0.117	0.033	0.014	0.45	0.019	35.7	11.0**
	250	0.27	0.058	0.037	0.019	0.212	0.018	25	6.0**
IAA	0	1.26**	0.194	0.11	0.044	1.07	0.066	35.0**	12.7
	50	0.991*	0.182	0.089	0.046	0.809	0.049	33	14
	100	0.842	0.158	0.078	0.038	0.684	0.046	33	12.7
	150	0.582	0.121	0.066	0.027	0.461	0.039	31.7	9.7**
	200	0.502	0.103	0.037	0.02	0.399	0.017	29	10.7**
	250	0.204	0.046	0.03	0.018	0.158	0.012	20.3	9
L.S.D. (5%)		0.354	0.159	0.186	0.038	3.38	0.148	4.16	1.39
L.S.D. (1%)		0.475	0.248	0.249	0.051	4.49	0.198	5.58	1.86

Significant difference to control: *P = 0.01; **P = 0.05

(Goeschi et al., 1966; Yang and Hoffman, 1984, Morgan et al., 1990; Narayana et al., 1991 and Raz). Fluhr (1992 reported that water stress induced ethylene production in wheat plant. There are also some evidence that salt stress alters plant growth and tissues which could be due to a decrease in natural growth hormones in plant tissues (Shah and Loomis, 1965; Itia et al., 1978; Walker and Dumbroff, 1991; Shaddad and EL-Tayeb, 1990). In accordance with this, Browning (1973) found that *Coffea arabica* plants which have previously been subjected to water stress, the endogenous cytokinin level in the xylem sap was again elevated after irrigation. Thus the aim of the present work was to test the effect of exogenous treatments with the phytohormones, gibberellic acid or indole acetic acid in counteracting the adverse effects of salinity and sequence of ethylene production of wheat plants under these conditions.

MATERIALS AND METHODS

Wheat (*Triticum vulgare*) plants were grown in plastic pots in the soil without NaCl (control) and under salinization levels corresponding to osmotic potential of NaCl solution of 50, 100, 150, 200 and 250 mM.

Saline solutions were added to the soil in such a way that the soil solution acquired the assigned salinization levels at field capacity. Treatments of plants with saline solutions began when seedlings were two weeks old. The salinized and non-salinized plants were irrigated every other day with 1/10 Pfeffer's nutrient solution for two weeks. Then gibberellic acid (GA₃) and indole-3-acetic acid (IAA) (100 ppm) solutions were sprayed three times (five intervals) by spraying the shoot system of the growing plants (each pot with 10 cm³ of GA₃ or IAA solutions). The control plants were sprayed with distilled water a week after the plants were used for analysis.

Dry matter was determined after drying plants in an aerated oven at 70°C to constant mass. Leaf area was measured by the disk method (Watson and Watson, 1953). Saccharides were determined by the anthrone-sulfuric acids method (Fales, 1951). Free amino acids, proline and a soluble protein contents were measured according to Moore and Stein (1948), Bates et al. (1973) and Lowry et al. (1951) respectively. The osmotic potential of tissue sap was measured by advanced wide-range Osmometer 3W2. Ethylene production was determined by gas-liquid chromatography (GLC) according to Morgan et al. (1990). Calcium and potassium were determined by flame-photometer method (Schwarzenbach and Biedermann, 1948) and phosphorus colorimetrically (Woods and Mellon, 1985).

RESULTS

Growth parameter (fresh, dry matter, length and water con-

Table 2. Effect of salinization and treatment with GA₃ or IAA (200 Kg⁻¹) on soluble saccharides [mg g⁻¹], soluble protein [mg g⁻¹], amino acids [mg g⁻¹] and proline content [mg g⁻¹] of shoot and root wheat plants.

Treatment	NaCl mM	Soluble sugar		Soluble protein		Amino acid		Proline	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	0.0	24.9	27.5	35.2	15.1	11.2	8.0	4.6	1.86
	50	36.4**	43.3**	36.9	14.2	11.9	9.1	4.8	3.5
	100	34.9**	37.4**	34.1	16.9	9.3	9.9	5.2	5.1
	150	34.7**	37.7**	38.4*	14.2**	8.2	7.7	4.8	4.1
	200	22.2	25.6**	36.4	13.7	8.4	5.2	3.6	2.9
	250	22.0	23.4	34.9	15.1	5.2	4.7	4.7	2.9
GA ₃	0.0	37.7**	31.8**	46.1**	12.2	14.4	8.3	3.41	3.9
	50	31.9**	49.2**	47.0**	13.8	7.4	14.4**	3.90	2.4
	100	41.9**	49.2**	47.0	14.4*	10.9	9.2	3.5	3.2
	150	44.9**	40.2**	48.4	12.6	13.4**	13.6**	2.1	3.9
	200	35.3**	30.5**	40.9**	13.0	10.0	14.8**	3.8	3.6
	250	34.5**	25.8	40.7**	13.9	13.4**	13.9*	3.3	2.9
IAA	0.0	37.3**	42.9**	42.2**	20.0**	14.1	19.6**	3.9	3.7
	50	34.1	46.3*	38.1	14.6	20.5**	20.9**	3.1	5.6
	100	39.9**	44.5**	39.5	17.4	11.2	19.9**	4.0	3.9
	150	39.4**	41.7**	38.2	19.2**	18.6*	16.9**	4.9	4.4
	200	36.5**	30.3**	36.9	19.5**	14.6**	21.4**	4.4	3.8
	250	27.4**	30.4*	43.1**	22.5**	21.1**	22.3*	3.3	7.8**
L.S.D. (5%)		3.23	2.28	3.09	3.37	5.45	0.889	4.89	3.21
L.S.D. (1%)		4.34	3.06	4.15	4.53	7.32	1.19	6.57	4.31

Significant differences to control: *P = 0.01; **P = 0.05.

tents) of shoots and roots of wheat plants tended to decrease with the increase of NaCl concentration in the culture media (Table 1). This reduction was more pronounced at the higher salinity levels as compared with non-treated plants. The percentage reduction in fresh and dry matter of shoot and root was 45, 2.3, 7 and 3.3% at 250 mM salinity as compared with control plants (100%).

Spraying the vegetative parts of wheat plants with GA₃ (100 ppm) and IAA (100 ppm) resulted in a pronounced increase in the values of fresh and dry matter and water content. This increase was more prominently in GA₃ than in IAA treatments as compared to the corresponding salinized plants (reference control). The percentage reduction in fresh and dry matter of shoot and root are 92, 29, 15.4 and 5.9% of plants treated with GA₃. On the other hand, the percentage reduction in these parameters is 105.6, 14.8, 8 and 2.6% of plants treated with IAA, respectively as compared with control plants (100%).

The data in Table 2 clearly demonstrates that salinity stress resulted in an increase in the soluble saccharide contents in shoot and root of wheat plants up to 150 mM after that a decrease in this content was observed. Salt stress induced non significant changes in soluble protein

content in shoots and roots, the values run overall the value of control (Table 2). Phytohormonal treatments with any of the two hormones GA₃ or IAA resulted in a pronounced accumulation in soluble saccharides and soluble protein in both shoots and roots of wheat plants (Table 2). The compatible compounds (amino acids and proline) showed a variable response with increasing osmotic pressure in the soil. A marked reduction in the amino acids contents was observed while the proline content became more or less unchanged in both shoots and roots of wheat plants as compared with the corresponding untreated plants (Table 2).

Spraying with either GA₃ or IAA induced a marked increase in the amino acids content of both organs shoots and roots (Table 2). On the other side, this treatment has no effect on the proline content as compared with untreated plants.

Moreover, the increase in the concentration of NaCl salt caused an increase in the osmotic pressure value of cell sap of both shoots and roots of wheat plants (Figures 1 and 2). It is worthy to point out that treatments with any of the two different growth regulators (GA₃ or IAA) resulted in a significant increase in the OP values at all levels of NaCl in shoots and roots as compared with con-

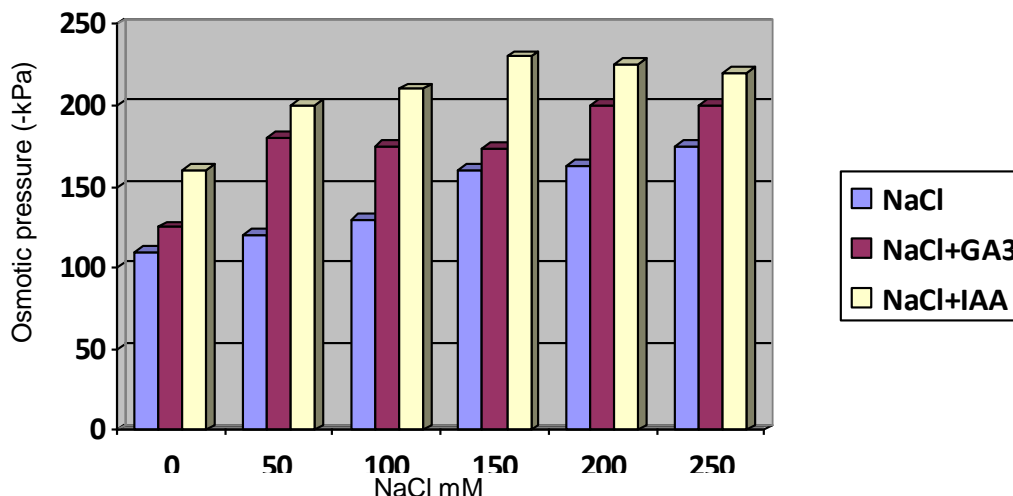


Figure 1. Effect of salinization and treatment with GA₃ or IAA (200 mg g⁻¹) on osmotic pressure (-kPa) of root of wheat plants grown for 45 days.

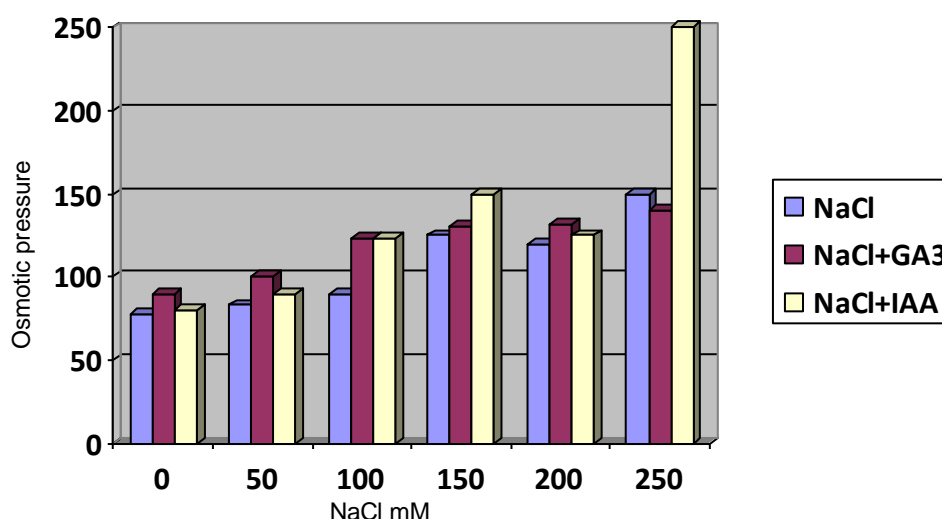


Figure 2. Effect of salinization and treatment with GA₃ or IAA (200 mg g⁻¹) on osmotic pressure (-kPa) of shoot of wheat plants grown for 45 days.

control plants.

The data in Table 3 and Figure 3 represented that ethylene production elevated with increasing the osmotic pressure in the soil. On the other hand, treatments of the wheat plants with GA₃ resulted in a significant reduction in the ethylene evolution while treatments of wheat plants with 100 ppm IAA induced a significant evolution of ethylene at all levels of salinity used as compared with control plants. Moreover the ethylene production was higher at high levels of NaCl in plants of reference control and plants treated with GA₃ or IAA as compared with each of the corresponding control.

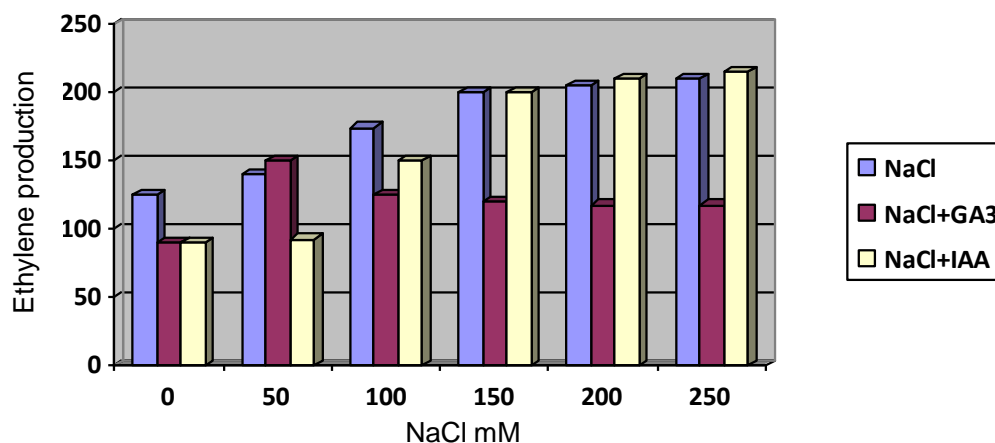
Sodium contents progressively increased with the increasing NaCl concentration in both shoots and roots of

wheat plants. This accumulation was more pronounced in shoots than in roots (Table 3). On the other hand, potassium content became more or less unchanged at all salinization levels in both shoots and roots organs (Table 3). However, the lower and moderate salinity levels exhibited an increase in K⁺ content of shoot organ. In general, salinity stress did not induced any significant change in Ca²⁺, Mg²⁺ and P content in shoot and root of wheat plants.

Spraying with GA₃ or IAA induced a marked reduction in the accumulation of Na⁺ in shoots and roots of wheat plants (Table 3). This reduction was more prominent in shoots than in roots, whatever the salinization levels used. Also, phytohormonal treatments exhibited an increase of

Table 3. Effect of salinization and treatment with GA₃ or IAA (200 mg kg⁻¹) on mineral contents (mg g⁻¹) of shoot and root of wheat plants.

Treatment	NaCl mM	Shoot					Root				
		Na	K	Ca	Mg	P	Na	K	Ca	Mg	P
Control	0.0	7.0	1.0	4.0	3.0	1.2	4.0	1.8	4.7	0.45	5.2
	50	8.5	1.8	4.0	1.8	2.1	5.5	1.3	4.0	0.3	4.9
	100	9.0	2.1	4.0	1.4	1.7	5.2	1.0	4.5	0.3	5.7
	150	17.0**	3.4	4.7	1.2	1.9	4.0	1.6	3.5	0.3	6.2
	200	18.0**	1.4	4.3	1.8	1.4	9.0**	1.68	3.0	0.3	4.6
	250	18.0**	2.4	4.0	1.4	2.1	9.2**	1.2	3.0	0.3	4.9
GA ₃	0.0	7.0	5.5**	6.0**	1.8	1.7	3.0	2.7	3.5	1.7	3.9
	50	7.0	3.1	5.0	2.6	1.7	3.5	1.7	2.7	1.4	5.4
	100	6.0	2.4	4.0	1.8	1.5	4.5	1.2	2.2	1.2*	6.2
	150	7.5**	5.2	5.7	1.2	3.1	6.0	2.6	2.5	2.0**	6.2
	200	9.5**	3.1	4.7	1.2	1.4	3.0	1.6	2.8	0.8	7.6*
	250	11.0*	2.1	6.0**	1.2	1.3	1.5*	1.0	3.5	0.8	5.7
IAA	0.0	8.7	2.8*	5.3*	1.8	1.5	4.0	1.4	2.5	1.7*	5.0
	50	9.0	2.1	5.0	2.4	1.9	3.3	1.0	3.0	0.3*	5.4
	100	8.0	2.8	4.0	2.8	1.3	4.5	1.3	2.7	0.3	6.1
	150	9.0**	2.8	5.3	2.0	1.5	3.0	1.4	2.0	0.3	4.2
	200	9.5**	2.4	3.3	1.2	2.2	3.0	1.2	2.0	0.3	6.7
	250	11.5**	1.7	3.3	0.6	1.3	2.5	0.86	2.0	0.3	8.0*
L.S.D. (5%)		3.34	2.08	1.13	2.4	2.63	4.35	1.23	2.63	0.86	2.62
L.S.D. (1%)		4.47	2.80	1.52	3.25	3.52	3.67	1.66	3.52	1.15	3.52

**Figure 3.** Effect of salinization and treatment with GA₃ or IAA (200 mg g⁻¹) on ethylene production (n moe⁻¹ h⁻¹) of wheat plants grown for 45 days.

Ca²⁺ content in shoots while this content become more or less unchanged in roots at all salinization levels as compared with control plants (Table 3).

Spraying with GA₃ resulted in no change in Mg²⁺ content of shoots while in roots, this content tended to increase. IAA treatments accumulated Mg²⁺ fractions in shoots and

induced no marked change in roots as compared with reference control plants (Table 3).

Phosphorus content was elevated in root organ of both treated plants. Phytohormonal treatment of GA₃ or IAA was increased in the root while this content became more or less unchanged in shoots of both GA₃ and IAA treatments

as compared with control plants (Table 3).

DISCUSSION

This report confirms that wheat plants could tolerate the saline injury to some extent in fresh, dry matter, length, leaf area and water content up to 100 mM of NaCl concentrations after which a sharp reduction was observed. This reduction was due to decreasing the rate of water uptake due to osmo- effects through toxic effects or through a nutritional imbalance resulting to inter-element antagonism (Levitt 1980; Quayum et al., 1991; Hamdia 1993, 1994 and Hamdia and Shaddad, 1996; Tester and Devenport, 2003; Parida and Das, 2005, Munns, 2008).

The observed losses in soluble saccharides in roots as well as proteins in shoots and roots of salt stressed maize plants were accompanied with a marked decrease in total amino acids content and increase in the osmotic pressure value. These results are in accordance with previously reported findings of Barnett and Naylor (1966), Handa et al. (1983), Devitt et al. (1987), Hamdia and El-Komy (1997) and Hamdia et al., (2010). Osmotic adjustment helps cells of higher plants to with stand salt stress and water deficit by maintaining sufficient turgor for growth to proceed (Zimmermann, 1978) and involves transportation, accumulations and compartmentation of inorganic ions and organic solutes (Wyn Jones, 1981, Weimberg et al., 1984; Voetberg and Sharp, 1991; Spickelt et al., 199; Rodriguez et al., 1997; Bu et al., 2012).

The increase of osmotic pressure of cytoplasm with treatments with any of the two different hormones, GA₃ or IAA was concomitant with the increase of osmotically active solutes soluble sugars, soluble protein and amino acids. This promotion in these contents reflected the great production in fresh, dry matter, length leaf area and water content of both shoots and roots of wheat plants (Shaddad and El-Tayeb, 1990; Hamdia, 1991). It is worthy to point out that hormonal treatments increase the accumulation of Ca and Mg in root with GA₃ treatment and in shoot of IAA treatments. This would suggest the osmoregulatory role of these minerals (Shaddad and El-Tayeb, 1990; Hamdia, 1991).

Ethylene production by plants is increased by a number of biotic and abiotic stress (Abeles, 1973). The phenomenon is so common, it is referred to as stress ethylene production. Plant water deficit is one stress which has been extensively associated with elevated release of ethylene (El-Beltagy and Hall, 1974; Guinn, 1976; McKeon et al., 1982, Hoffman et al., 1983). The impact of water stress on ethylene synthesis is of interest because the ethylene could be responsible for senescence and abscission induced by water stress (McMichael et al., 1973 and El-Beltagy, 1974). This observation was in agreement with the results obtained in this report, that ethylene production increase with increasing salinity of wheat plants. Water stress reduces the transpiring surface and prevents dehydration of the plant to drastic

levels (Adicott, 1982). Confirming the above statement, when wheat plants treated with GA₃, the level of ethylene production progressively retarded at the all salinization levels, when concomitant with the progressively increase in dry matter production resulting in the complete alleviation of the inhibitory effect of the salt at the higher salinization levels and the obvious stimulation at the lower salinity as compared with the control plants. Under drought conditions, depleted flow of materials from roots would reduce the availability of GA₃ (Jones and Philips, 1966). Thus it would suggest that, exogenous application of GA₃ retarded the synthesis of ethylene which in turn increased the tolerance of wheat plants under stress conditions.

The opposite effect was observed in plants treated with IAA, although, there was an obvious stimulatory effect in mass production. However ethylene production was vigorously stimulated especially at the higher salinization levels. Auxin stimulates ethylene production in a wide variety of plant tissue under normal conditions (Yang and Hoffmann, 1984; Yoshii and Imaseki, 1982; Kim et al., 1997). Therefore, it can be said that exogenous application of IAA decreased the sensitivity of plant towards the accumulation of ethylene.

Finally, it can be observed that any of the two phytohormones used regulate the disturbances of metabolites and neglect the negative effects of the accumulation of ethylene especially in plants treated with IAA under stress conditions which in turn resulted in a pronounced alleviated drastic effect of salt.

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