Growth and pigment content of wheat as influenced by the combined effects of salinity and growth regulators

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Abstract

A field experiment was conducted to investigate the effects of presoaking the wheat grains (*Triticum aestivum* L.) in different levels of salinity (33 or 66 mM) and in growth regulators (indolyl-3-acetic acid, IAA at 50 g m^{-3}, gibberellic acid, GA_3 at 100 g m^{-3}, or kinetin at 100 g m^{-3}) on the shoot growth and pigment content of the developing wheat flag leaf. Salinity at 33 or 66 mM led to an insignificant increase in the fresh and dry masses as well as in the shoot diameter and shoot length, but it attenuated the flag leaf area. In the majority of cases, salinity increased the chlorophyll (Chl *a*, Chl *b*) and carotenoid contents as well as the number of chloroplasts per a mesophyll cell. The growth in the wheat shoot of the saline-treated plants was, in general, stimulated in response to presoaking the grains in kinetin or GA_3. On the other hand, IAA + salinity led to a negligible effect on the growth in the wheat plants particularly at the early stages of growth. The presoaking of grains in NaCl at 33 mM + IAA or 66 mM + kinetin induced a marked increase in the pigment content of the wheat flag leaf particularly at the early stages of growth. The interaction between salinity and phytohormones increased the number of chloroplasts; kinetin was the most effective.

Introduction

The recent work related to salinity and plant growth reveals that the suppression of plant growth may be attributed to a decrease in the water potential gradient. This restricts the water absorption by the root system and results in various physiological disturbances in the cell sap (Darra and Saxena 1973). The seed presoaking in growth regulators may provide a better growth and nutrient uptake in the salt substrate conditions (Darra *et al.* 1973). Developmental responses of the plants grown on saline soils have been discussed in recent reviews (Mizrahi 1982, Ball and Farquhar 1984, Singleton and Bohlool 1984, Abdel-Aziz *et al.* 1985).

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The wheat yield is a function of many factors among which the shoot growth and pigment content of the developing wheat flag leaf are the most important ones (Abo-Hamed *et al.* 1987, Mansour et al. 1986). The pigment content which could be regarded as a criterion for photosynthetic activities was markedly increased by salt stress in the maize plants but it was reduced in the safflower plants (El-Deep 1984). Gibberellic acid (GA_3) and indolyl-3-acetic acid (IAA) increased the pigment content of salinized plants (*e.g.*, in grasses Varshney and Baijal 1979, in kidney bean Shaddad and Heikal 1982). On the other hand, Salama *et al.* (1980) demonstrated that the chlorophyll (Chl) content was not significantly influenced by hormonal treatments in the salinized sugar beet.

The present investigation was undertaken to clarify the interactive effects of salinity and phytohormones (*e.g.*, IAA, GA_3 and kinetin) on the growth and pigment content in the developing flag leaf of wheat plants.

Materials and methods

A homogenous lot of wheat (*Triticum aestivum* L., cv. Giza 157) grains was selected. The grains were surface sterilized by soaking in 0.001 M $HgCl_2$ solution for 3 min, washed throughly with distilled water and then soaked in the solutions with different combinations of NaCl and growth regulators; the resulting twelve treatments were marked as follows:

	Treatments 1	2	3	4	5	6	7	8	9	10	11	12
NaCl [mM]	0	33	66	0	33	66	0	33	66	0	33	66
IAA [g m^{-3}]	0	0	0	50	50	50	0	0	0	0	0	0
GA_3 [g m^{-3}]	0	0	0	0	0	0	100	100	100	0	0	0
kinetin [g m^{-3}]	0	0	0	0	0	0	0	0	0	100	100	100

Each group was separately soaked in the appropriate solution for an hour at a room temperature in dark. After soaking, all the grains were throughly washed with water and then sown in ordinary clay soil on 30 November 1989 in the Mansoura district, Egypt. The grains were drilled in singles in rows 15 cm apart using the Afir method of planting. At the bud stage, the crop received 10 g(N) m^{-2} [100 kg(N) ha^{-1}] as ammonium nitrate (33.5 % N_2).

The growth measurements included the length of shoot, shoot diameter, flag leaf area and the fresh and dry matter of shoot per plant. The samples of each treatment (ten replicates for each growth parameter and triplicates for pigment analysis) were taken after 30, 45, 60, 75 and 90 d and 97, 102, 117 d from the sowing date, respectively. The numbers of chloroplasts per mesophyll cells of the flag leaf neighbour to the main vascular bundle in the keel region were counted by microscopy throughout anatomical sections, prepared according to Sass (1951), at one week prnthesis (*i.e.* 90 d from sowing). The results were first subjected to an analysis of variance (ANOVA). When the ANOVA showed a significant ($P \leq 0.05$)

effect, the least significant difference was used to compare the treatments (Snedecor and Cochran 1967). The flag leaf area was estimated using the following equation: area = 0.7 (length x breadth), as described by Quarrie and Jones (1979).

The amounts of Chl *a*, Chl *b* and carotenoids were determined in the flag leaves at different stages of plant development using the spectrophotometric method recommended by Metzner *et al.* (1965).

Results and discussion

Growth vigour of wheat plants (Fig. 1): In the present investigation, water stress induced by NaCl at 33 or 66 mM led in general to an insignificant increase in the fresh and dry matter of wheat shoots at all periods of growth. Moreover, the salinity caused an insignificant increase in both the shoot diameter and the length until 60 and 75 d at the two levels, respectively. On the other hand, a significant increase ($P < 0.01$) in the shoot diameter was observed with the two salinity levels on the 90th day after sowing. Furthermore, the plants treated with salinity at 33 mM showed a marked increase ($P < 0.01$) in the shoot length after 75 and 90 d from sowing. The flag leaf area seemed to be insignificantly affected by the salinity levels (Fig. 2). This may probably be due to the substantially higher ratios of the mesophyll surface area to the leaf area for the *Phaseolus vulgaris* induced by salinity (Longstreth and Park 1979).

In support of the above mentioned results, Heikal (1976) reported that the fresh and dry matter of wheat were not affected by the salinity levels (1000, 3000 and 6000 g m^{-3}). Also, Neals and Sharkey (1981) found that the growth in *Disphyma australe* was stimulated by salinity up to 100 mM. They attributed the simulation of the fresh matter to an increase in the internodes number per plant, while the increments in the dry matter were considered to be a reflection to the increase in the leaf inorganic content. In this respect, Heikal *et al.* (1980) recorded a promotion rather than inhibition in the growth of some salinized plants.

Shoot fresh and dry matters of the saline-treated plants were, in general, greatly stimulated by GA_3 or by kinetin treatments. Furthermore, additional increase in these parameters were observed if compared with those treated with salinity. Salinity (33 or 66 mM) + IAA resulted in a significant increase ($P < 0.01$) in the shoot fresh and dry matter particularly during the early stage of the plant development (after 60 and 75 d from sowing), while an additional increase in the fresh matter was noticeable on the 90th day with the low level of salinity only. The flag leaf area was insignificant increased as a result of the combined effects of salinity and phytohormones.

The changes in the shoot length and shoot diameter induced by presoaking the grains in IAA, GA_3 or kinetin were comparable to the changes in the shoot fresh and dry matter. In the majority of cases, a significant increase ($P < 0.01$) was maintained in the shoot growth above the control levels, when the grains were presoaked in GA_3, kinetin or IAA; the magnitude of the response was pronounced with kinetin or GA_3 and to a lesser extent with IAA. In accord with these results, Tagawa and Bonner (1957), Steward (1959), Shah and Lommis (1965), Heikal *et al.* (1982) and Younis *et al.* (1991) reported a beneficial effect of GA_3 and IAA on the growth of a salinized plant, where in most cases an increase in the water uptake of the treated plants was reported.

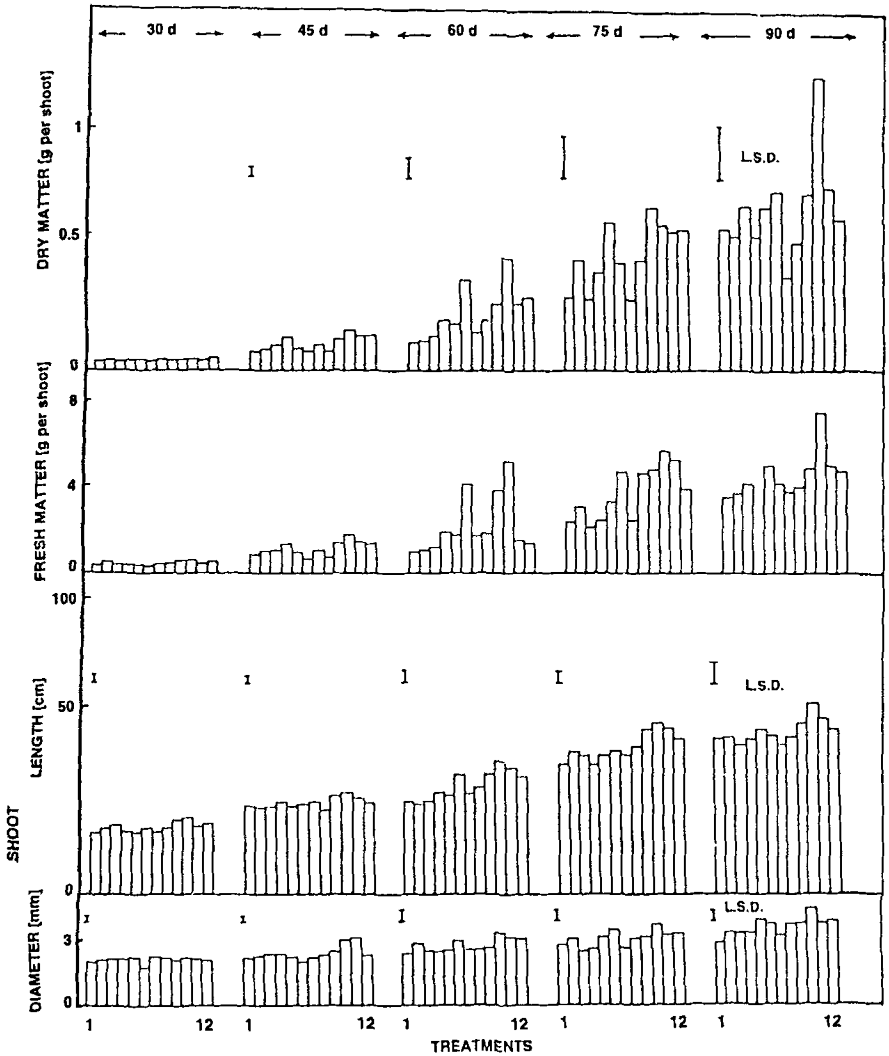

Fig. 1. The effect of grain presoaking in IAA, GA_3 or kinetin on the shoot fresh and dry matter and shoot length and diameter of *Triticum aestivum* plants, stressed with NaCl at different stages of plant development. For the description of treatments 1 to 12 see Material and Methods.

Presumably rather as a consequence than a cause of the cell expansion induced by hormonal treatments. In such situation the level of naturally synthesized hormones may be suppressed and the exogeneous application of phytohormones supplies more or less sufficient quantities which are implicated in the growth promotion. In this connection, Banyal and Rai (1983) concluded that GA_3 induced a recovery from water stress by increasing the water status of lettuce hypocotyl. However, it cannot be ruled out that the observed increase in the fresh and dry matter of the salinized wheat plants in response to a grain hormone presoaking may be the result of an acceleration in the salt absorption. This enables the plant to resist the harmful effects of salinity.

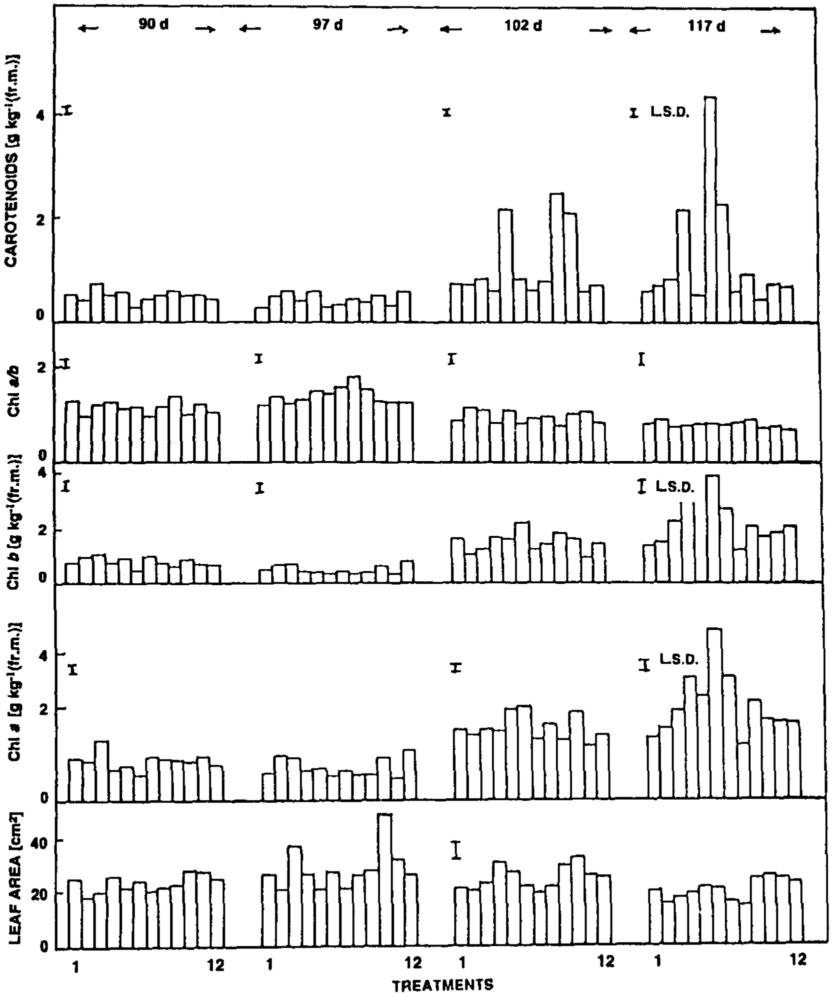

Fig. 2. The effect of grain presoaking in IAA, GA_3 or kinetin on the leaf area, chlorophyll, Chl *a*, Chl *b*, Chl *a/b* and carotenoids of *Triticum aestivum* flag leaf, stressed with NaCl at different stages of leaf development. For the treatments of 1 to 12 see Material and Methods.

These results are in agreement with those obtained by Abdel-Aziz *et al.* (1985) who found that seed pretreatment with IAA induced a significant stimulation in the fresh and dry matter of the stressed soybean plants. Wu *et al.* (1983) found that in tomato both 100 g m^{-3} GA_3 and 1 g m^{-3} kinetin increased the plant height, leaf area and stem fresh and dry matter. Also Wilkins (1978) stated that IAA increased the cell division in some tissues. Furthermore, Darra *et al.* (1973) and Vidhu and Murty (1985) stated that the presoaking of seeds with optimal concentrations of certain phytohormones was beneficial to the growth and yield of some crop species grown under the saline conditions.

Pigment content and number of chloroplasts in wheat flag leaf (Fig. 2 and Table 1): The leaf growth is more sensitive to salinity than that one of a root (Munns and Termaat 1986). The flag leaf in cereal plants plays an essential role in exporting the photosynthates to the developing grains (Thorne 1966, Palit *et al.* 1976, Rao and Murty 1976, Marour *et al.* 1988). Thus it was of particular interest to investigate in what way does the interaction between the salinity and hormones affect the photosynthetic machinery of this leaf.

Our results revealed that salinity at both levels (33 or 66 mM) stimulated the formation of pigments. Salinity caused a significant increase in the Chl *a* and *b* contents during the flag leaf growth, but at full expansion these pigments showed an irregular pattern of changes. In the majority of cases, salinity caused a marked increase ($P < 0.01$) in the carotenoids content during the flag leaf growth particularly at a higher salinity (66 mM). Salinity lowered the Chl *a/b* ratio especially at one week before the anthesis (*i.e.* 90) d from sowing, which was followed by a significant increase ($P < 0.01$) from the anthesis date (*i.e.* 97 d from sowing) till two weeks after the anthesis. Thereafter a significant increase ($P < 0.05$) or aninsignificant decrease in response to 33 or 66 mM was obtained respectively. The stimulative effect of salinity on the pigment content of the wheat flag leaf might be due to the fact that salinity lead to an increase in a number of chloroplasts (Table 1). The observed increase in the pigment content of the saline-treated plants was in accordance with the results of Chavan and Karadge (1980) on *Arachis hypogea*.

The interactive effect of salinity and kinetin resulted in a marked increase in both Chl *a*and *b* particularly at the anthesis and one month later. Also the saline treated plants exhibited an abvious increase in the pigment content in response to the grain presoaking in GA_3 particularly at the late stage of the leaf development. On the other hand, salinity + IAA led to an obvious increase in the pigment content during the flag leaf development paiticularly with a low level of salinity and at maturity with a high level of salinity.

The interaction between the salinity and these hormones greatly accelerated the pigment content particularly in the fully expanded flag leaf. Thus the priming in these hormones may retard the senescence of the flag leaf and this advantage will keep it for a prolonged time.

The presoaking of grains in IAA, GA_3 and namely kinetin induced an additional increase in the number of chloroplasts in the mesophyll cells of the flag leaf of the saline-treated plants (Table 1). The ratio of Chl *a/b* was greatly affected by the combined effect of salinity and phytohormones particularly at the anthesis and two weeks later.

Table 1. The effect of grain presoaking in IAA, GA3 or kinetin (for experimental variants see Material and methods) on the number ofchloroplasts in the mesophyll cells of the flag leaf on wheat plants stressed with two NaCl levels. The values listed are the means of 24 determinations per treatment

Variants	1	2	3	4	5	6	7	8	9	10	11	12	L.S.D 5%	L.S.D 1%
Number of chloroplasts per mesophyll cell	26	47	41	98	49	4 5	61	60	59	133	105	48	5.807	7.723

The stimulative effect of presoaking the grains in NaCl (especially at 66 mM) + kinetin on the pigment content of the wheat flag leaf may presumably be due to the fact that the cytokinin supply had a much greater influence on the plastid biogenesis than on the leaf growth in general (Felerabend and Boer 1979).

The alterations in the growth and pigment content of the saline-treated plants induced by grain priming with IAA, GA_3 or kinetin seemed to depend on: (1) the salinity level, (2) the type of hormone used, and (3) the age of the plant used. The interaction between salinity and hormones thus increased the resistance of wheat plants toward salinity.

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