GROWTH AND MINERAL CONCENTRATIONS OF PEA PLANTS UNDER DIFFERENT SALINITY LEVELS AND IRON SUPPLY

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Summary. The simultaneous occurrence of several abiotic stresses is more the rule than an exception in nature. In this study pea plants were grown as hydroponics under three salinity levels (0, 50 and 100 mM NaCl), and five concentrations of iron under the form of FeEDTA (0, 0.1, 0.25, 2 - optimum concentration, and 10 mg Fe/l). The separate and combined effects of both factors on growth and concentrations of Na, K, Ca, Mg, Fe, Cu, Zn, Mn, P and N in roots and shoots were studied. The combined effect of salinity and inadequate iron supply depended on the stress combination, the levels of individual stresses and the parameter under consideration. Although the decline in shoot growth for a particular combination of stress levels was close to that caused by the stronger individual stress at the same level, an additive effect of both stresses cannot be ruled out since earlier and stronger chlorosis took place under complete Fe deficiency when it was combined with salinity. It might be due to the highest Na, and the lowest Fe and N content in shoots, recorded in the plants when the highest levels of the stresses were combined. Even though salinity lessened the drop of root biomass, caused by Fe deficiency or toxicity, and some of the stress combinations resulted in closer to the control values for some nutrient concentrations compared to individual stresses, a shift in optimum Fe concentration in saline conditions was

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not found, probably because of aggravation of other nutrient disbalances.

Key words**:** growth, iron, nutrient concentrations, peas, salinity, stress combination.

INTRODUCTION

Environmental stresses lead to substantial decrease in plant productivity and crop yield. The simultaneous occurrence of several stresses is more the rule than an exception in nature. Both calcareous soils, causing Fe deficiency, and saline, including sodic soils are widespread among cultivated soils, and a combination of them might be observed, especially in arid regions (Rabhi et al., 2007; Yousfi et al., 2007). There is numerous data in literature about the separate effects of salinity and inadequate Fe supply on plant growth and nutrient uptake, concentration and distribution. There is also some evidence that root or foliar Fe supply might favour sunflower and maize, grown in saline medium (Pakroo and Kashirad, 1981; Salama et al., 1996; Delgado and Sanchez-Raya, 1998). On the other hand, high NaCl might affect iron absorption, and might aggravate Fe deficiency or Fe toxicity (Foy et al., 1978; Rabhi et al, 2007; Yousfi et al., 2007). Often a combination of two abiotic stresses is more harmful to crops, since its deleterious effect exceeds the effect of the individual stresses. According to Mittler (2006) the stress combination should be regarded as a new state of abiotic stress, as the response of plants to it is unique and cannot be directly extrapolated from their response to each different stress.

The objectives of this study were to compare the combined effect of salinity and inadequate iron supply on growth and mineral content of pea plants to the separate effects of the stresses, and to look for a possible shift in optimum Fe concentration in saline conditions.

MATERIALS AND METHODS

Pea plants (*Pisum sativum L.* cv. Ran-1) were grown hydroponically in growth chamber on 1/2 strength Hoagland-Arnon solution I with micronutrient supply according to a modified Hoagland's "A-Z" solution. At day 8 after seed soaking, the plants were supplied with 0, 0.1, 0.25, 2 or 10 $mg.L^{-1}$ iron under the form of FeEDTA. At day 11 the cotyledons were removed in order to reduce the Fe supply from the seeds. At day fourteen 0, 50 or 100 mM NaCl were added to each Fe-treatment. The nutrient solutions were changed every 3-4 days. At day 36 plants were harvested and separated into shoots and roots, their length and fresh biomass were measured, the content of dry biomass in a unite of fresh biomass was determined, plant material was oven dried at 60 ^{0}C, ground and stored for nutrients determination.

The concentrations of Ca, Mg, Fe, Mn, Zn and Cu were measured with an Atomic Absorption Spectrophotometer (AAS 3, Carl Zeiss Jena) after ashing at about 500-550 ^{0}C and dissolving the ash into 20 % HCl. The concentrations of K, Na, N and P were measured after digestion with H_2SO_4 and H_2O_2 on a Kjeldahl apparatus as follows: K and Na with flame photometer; P specrophotometrically according to Kojuharov (1960); N with a chemical analyzer Contiflo, based on indophenol -blue reaction.

Three independent experiments were carried out. Data are means of one representative experiment run in four or five replications for nutrient determination or morphological parameters, respectively.

RESULTS AND DISCUSSION

Field pea usually copes well with low Fe availability in calcareous soils, but has little salinity tolerance. Control plants in this study were supplied with 2 $mg.L^{-1}$ Fe, which were the optimum concentration in these experimental conditions, and with no NaCl. Two levels of partial Fe deficiency (0.25 and 0.1 $mg.L^{-1}$ Fe), complete Fe deficiency (0 $mg.L^{-1}$ Fe), excess Fe (10 $mg.L^{-1}$ Fe), and two levels of salinity (50 and 100 mM NaCl) were applied.

All three individual stresses reduced shoot growth, the effect being dependent on the strength of the stress (Fig.1). The higher level of salinity and complete Fe deficiency had similar impact on shoot biomass, decreasing it by 35 %, but the former caused a greater decrease in shoot length (35 % vs 12 %). The effect of excess Fe was comparable to the effect of partial Fe deficiency. Root growth was almost unaffected by salinity, decreased by Fe deficiency, and to a smallere extent by excess Fe.

Salinity lessened the drop of root biomass caused by Fe deficiency or toxicity i.e. plants subjected to combined stress did not differ significantly from control plants. As a rule, the decline in shoot growth for a particular combination of stess levels was close to that, caused by the stronger individual stress at the same level e.g. the effect of (0.1 mg.L^{-1} Fe + 100 mM NaCl) was comparable to the effect of 100 mM NaCl alone, since it was stronger than the individual effect of 0.1 mg.L^{-1} Fe. Yousfi et al. (2007) had also found that the interactive effect of salinity and Fe deficiency on barley growth was similar to that of Fe deficiency, which in their case was the stronger stress. On the other hand, Rabhi et al. (2007) had observed an additive effect of both stresses on plant growth and chlorophyll content in *Medicago ciliaris.* In accordance with the latter authors, it was found that the first visual signs of chlorosis (less intensive green colour of the youngest leaves) of plants grown without Fe appered at day 24 in non-saline conditions, and 4 days earlier in saline conditions. Moreover, at the

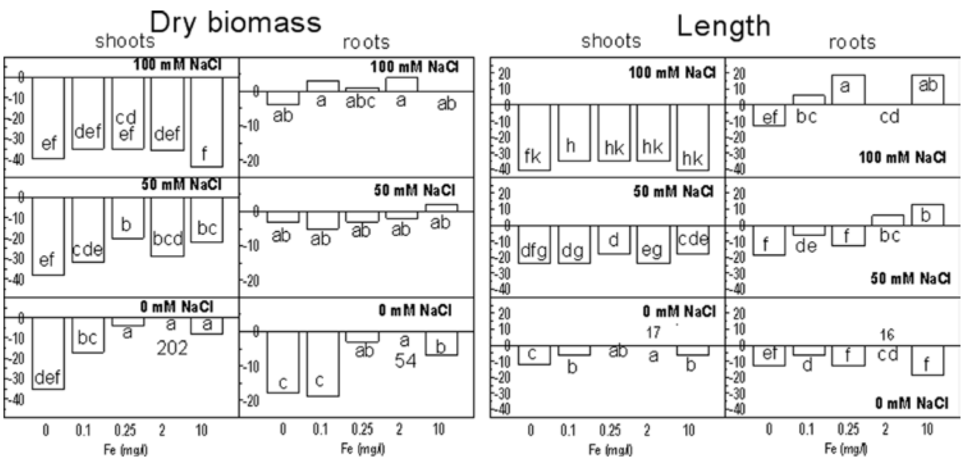

Fig. 1. Effect of iron supply and salinity on shoot and root dry biomass and length in pea plants. Bars represent deviations from the control to the control value in percent. The absolute values of control (in mg.plant^{-1}and cm, respectively) appear as a number in its place. Columns marked with the same letter are not significantly different at P = 0,05 according to Student's t-test.

end of the experiment the latter plants were more chlorotic. There was no visual difference between 50 and 100 mM NaCl. No visual signs of chlorosis were recorded in plants, subjected to partial Fe deficiency, no matter whether NaCl was present or not. Hence, it was obvious that salinity accentuated the harmful effect of Fe insufficiency on chlorophyll content, although it did not affect it when applied separately, as reported by Rabhi et al. (2007).

Both nutrient supply and nutrient balance are important factors for plant growth and development. Nutrient interactions consisting of mutual influence on absorption, distribution and functioning exist. As demonstrated in fig.2 and fig.3 and proven by numerous data in literature, most interactions are complex i.e. a nutrient interacts simultaneously with more than one nutrient. Besides the drop of Fe concentration in shoots and roots, Fe deficiency caused a decrease in shoot N, an increase in Mn, Cu, Zn, P and Na in shoots and roots; and an increase only in shoot K and Mg. Excess Fe decreased the shoot concentration of Mn, Zn and Na, and the root concentration of Mn, Cu and Mg. Besides the great increase in Na, salinity was associated with an increase in root P, Cu and Zn, with a decrease in K, Ca, Mg, Fe, Mn in both parts, and a decrease in shoot Cu and P. The interaction between nutrients can occur at the root surface or within the plant and might be due to: i) formation of precipitates and complexes between ions with different chemical properties, and ii) competition between ions with similar properties (Robson and Pitman, 1983; Fageria, 2001). Interactions between Fe and P fall in the first category, while interactions between Fe and Zn, Mn, Cu, and between Na and K, Ca, Mg fall in the second category. More detailed information about the mechanisms of nutrient interactions might be found in Foy et al., 1978; Grattan and Grieve, 1999; Fageria, 2001; Rabhi et al., 2007.

When compared to the values for the normal range of nutrient concentrations published in crop guides (Hill Laboratories, 2002; Bierman and Rosen, 2005), the control plants in this study were a little low on Mg and high on K. Plants grown in saline conditions were above the range for Na, and usually below it for Mg, and those subjected to complete Fe deficiency alone were above the range for K and Cu. With these exceptions, shoot concentrations, including that of Fe, were generally in the adequate range. Still, if ratios between different elements in shoots and shoot to root ratios for a particular nutrient amount were calculated, the nutrient disbalance and altered nutrient distribution would become obvious.

Three combinations among the pairs of individual stresses with regards to their effect on a particular element were found (Fig.2, Fig. 3,Tabl. 1): **A)** Stresses with similar impact on nutrient concentration. **B)** Only one of the

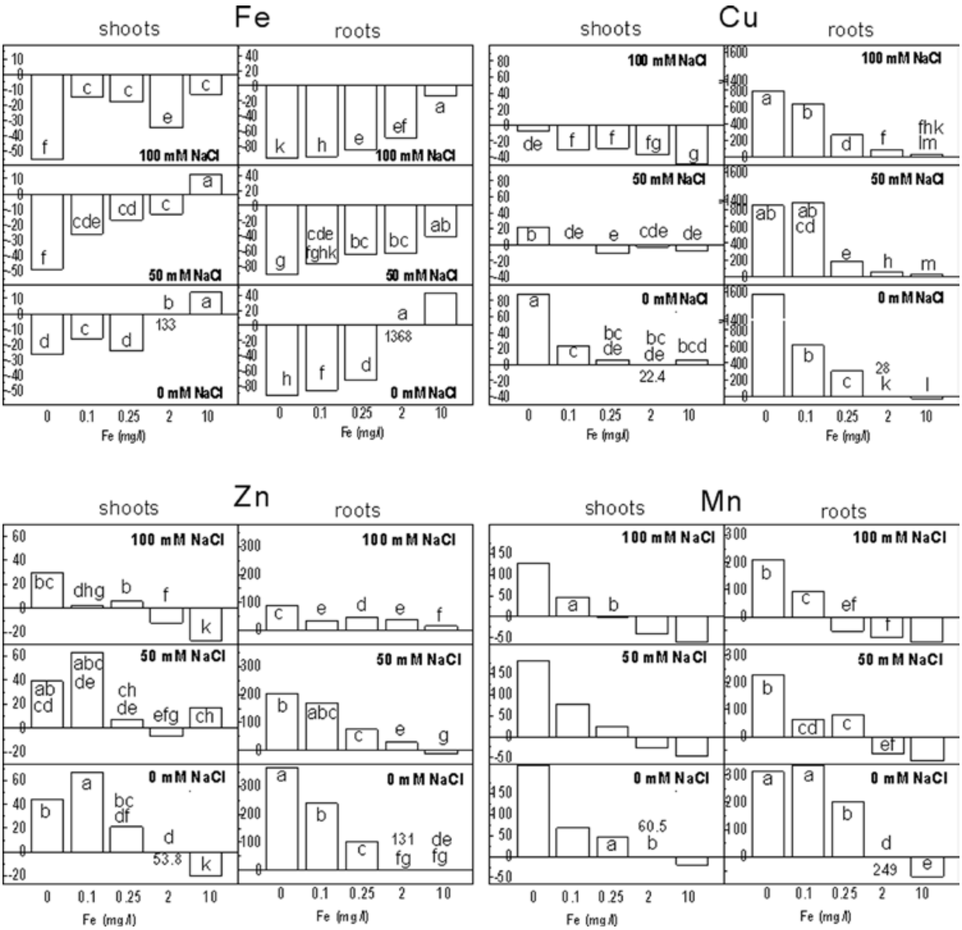

Fig. 2. Effect of iron supply and salinity on Fe, Cu, Zn and Mn concentrations in pea plants. Bars represent deviations from the control to the control value in percent. The absolute value of control (in $\mu g.g^{-1}$ dry biomass) appears as a number in its place. Columns marked with the same letter are not significantly different at P = 0,05 according to Student's t-test.

stresses with impact on concentration, while the other with no effect.
C) Stresses with opposite impact on nutrient concentration.

The two stresses from the first group changed in a similar direction the concentration, but usually acted with different strength. In some instances they also significantly altered the uptake. For example, both Fe deficiency and salinity decreased the concentration and uptake of Fe in shoots and roots, and increased Zn concentration and uptake in roots. Both excess Fe and salinity decreased the concentration and uptake of Mn in roots and shoots, and of Mg in roots. In other cases, one of the stresses had no significant impact on uptake and the observed increase in nutrient concentration might be attributed mainly to a concentration effect due to growth restriction. For example, Fe deficiency did not rise significantly the uptake of Na in shoots and roots and of P in roots, while the concentrations of these elements were increased by both Fe deficiency and salinity. The root concentrations of K and Ca, and the shoot concentration of Ca under salinity and Fe deficiency might illustrate the combinations from group B. Salinity decreased them strongly, while the suboptimal Fe supply had little effect on them. Group C might be illustrated by the rise of Cu and Mg in shoots, and of Mn in shoots and roots under Fe deficiency, and their drop caused by salinity. Another example would be the rise of Fe concentrations in both parts due to excess Fe, and their decrease due to salinity.

The simultaneous action of two stresses might aggravate or partially alleviate some of the nutrient disbalances, the effect being dependent on element and tissue under consideration as well as on stress combination and level. Two extreme effects of stress combinations were observed: i) Enhanced effect, when the impact of the combination exceeded the impact of the stronger of the individual stresses, and ii) Softened effect, when the effect of the combination was lower than the weaker of the individual stresses.

The enhanced effect was usually observed for combinations of individual stresses which belonged to group A or B. For example, when salinity and excess Fe were considered, the lowest concentrations of Mn in shoots and roots, and of Cu in shoots were registered under the combined action of (10 $mg.L^{-1}$ Fe + 100 mM NaCl). The shoot Mn concentration was close to the lowest normal value, bordering with insufficiency for this element. Plants

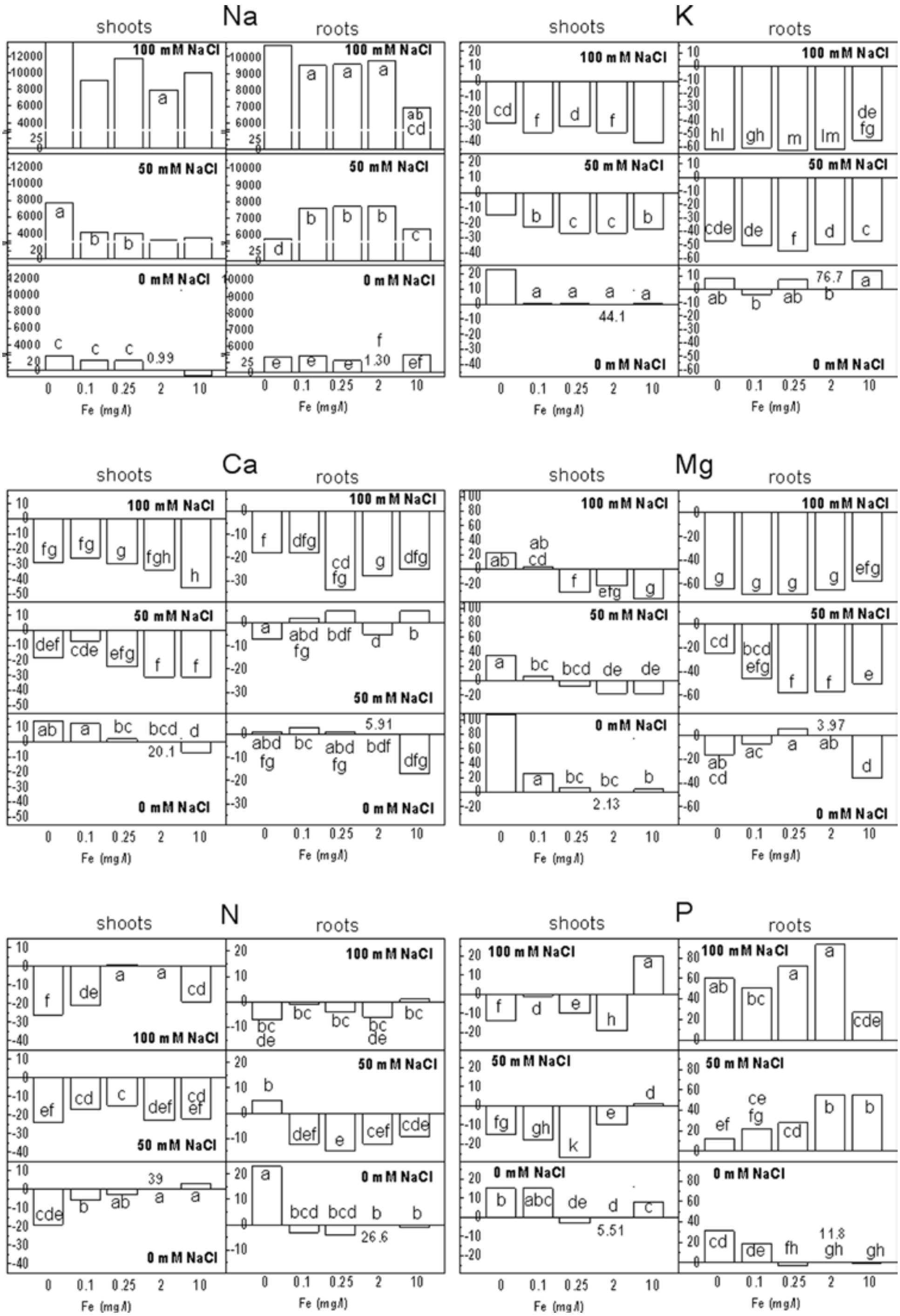
Na
K
Ca
Mg
N
P
shoots
roots
100 mM NaCl
50 mM NaCl
0 mM NaCl
0 0.1 0.25 2 10
Fe (mg/l)

Fig. 3. Effect of iron supply and salinity on Na, K, Ca, Mg, N and P concentrations in pea plants. Bars represent deviation from the control to the control value in percent. The absolute value of control (in $mg.g^{-1}$ dry biomass) appears as a number at its place. Columns marked with the same letter are not significantly different at P = 0,05 according to Student's t-test.

subjected to the combined effect of complete Fe deficiency and salinity were characterized by the highest shoot and root Na content and amount. Moreover, these plants had the lowest concentrations and uptake of shoot Fe and N. One might assume that this fact contributed significantly to the earlier appearance of chlorosis and to its greater strength. According to Rabhi et al. (2007), when applied simultaneously with Fe deficiency, salinity limited the aptitude of *Medicago ciliaris* to take up Fe traces from the solution, probably through salt decrease in root acidification, normally induced by Fe deficiency. In our experiments under partial Fe deficiency salinity additionaly decreased the Fe uptake in shoots, while the Fe content was not always significantly reduced.

The softened effect was observed more often for combinations of individual stresses which belonged to group C. In this case, frequently the maximum and minimum concentrations and amounts of the elements were found at the highest level of the individual stresses. For example, when Fe deficiency and salinity were considered, the highest concentrations of Cu and Mg in shoots, and of Mn in shoots and roots were recorded at (0 $mg.L^{-1}$ Fe + 0 mM NaCl), and the lowest at (2 $mg.L^{-1}$ Fe +100 mM NaCl). In these instances nutrient concentrations in plants subjected to combined stress were often closer to control values than those in plants subjected to individual stresses. The levels of both stresses in combination were of particular importance. The shoot content of K, Ca, Mg and N were also closer to the control values when plants were subjected to some of the studied combinations between salinity and Fe deficiency, compared to salinity alone. The same was true for Fe concentration in shoots and roots, when salinity was combined with excess Fe. In sunflower Fe application counteracted the effects of salinity on germination, growth and uptake of some nutrients, the efficacy depending on Fe form and level, as well on salinity level (Pakroo and Kashirad, 1981; Delgado and Sanchez-Raya,

Table 1. Effect of iron supply and salinity on Fe, Cu, Zn, Mn, P, Na, K, Ca, Mg and N uptake in pea plants.

Fe (mg/L)	Na Cl (mM)					
	0	50	100	0	50	100
	shoots			**roots**		
Fe (μg/plant)						
0	12.81	8.50	7.31	3.96	6.67	2.65
0.1	18.63	13.57	14.80	8.32	16.10	4.62
0.25	19.84	17.89	14.50	19.58	25.38	11.14
2	**26.96**	16.65	11.16	**73.71**	25.82	23.99
10	28.26	23.93	13.18	97.63	44.30	64.41
Cu (μg/plant)						
0	5.48	3.40	2.53	21.01	14.20	13.12
0.1	4.61	3.07	2.02	8.91	14.39	11.63
0.25	4.64	3.21	2.09	5.92	4.17	5.61
2	**4.53**	3.13	1.82	**1.53**	2.26	2.85
10	4.42	3.26	1.30	1.03	1.93	2.02
Zn (μg/plant)						
0	10.12	9.33	8.55	27.00	20.66	12.73
0.1	15.02	12.06	7.21	19.45	18.08	9.78
0.25	12.67	9.26	7.50	13.83	12.04	10.43
2	**10.87**	7.23	6.11	**7.08**	8.90	10.12
10	8.01	10.01	4.48	6.75	6.35	8.22
Mn (μg/plant)						
0	24.98	21.15	16.79	45.05	42.56	39.81
0.1	16.96	14.79	11.66	47.20	20.84	26.85
0.25	17.33	12.06	7.82	39.41	23.61	6.55
2	**12.23**	6.37	4.55	**13.41**	5.27	3.34
10	9.02	5.20	2.79	3.50	1.51	1.00
P (mg/plant)						
0	0.83	0.58	0.58	0.68	0.69	0.98
0.1	1.06	0.62	0.72	0.62	0.74	0.99
0.25	1.04	0.65	0.65	0.60	0.79	1.10
2	**1.11**	0.71	0.57	**0.64**	0.96	1.27
10	1.11	0.88	0.75	0.59	1.01	0.81

Fe (mg/L)	Na Cl (mM)					
	0	50	100	0	50	100
	shoots			**roots**		
Na (mg/plant)						
0	0.18	9.69	16.61	0.08	3.99	7.26
0.1	0.21	5.84	11.92	0.08	5.14	6.91
0.25	0.24	6.74	15.49	0.09	5.32	6.80
2	**0.20**	4.72	10.18	**0.07**	5.38	7.17
10	0.16	5.63	11.41	0.09	4.62	4.95
K (mg/plant)						
0	7.07	4.68	3.85	3.66	2.11	1.51
0.1	7.46	4.66	3.82	3.21	1.91	1.65
0.25	8.68	5.18	4.05	4.30	1.80	1.53
2	**8.90**	4.65	3.72	**4.14**	2.02	1.61
10	8.25	5.28	2.96	4.36	2.26	1.88
Ca (mg/plant)						
0	3.00	2.05	1.74	0.26	0.29	0.25
0.1	3.76	2.58	1.94	0.27	0.31	0.27
0.25	4.01	2.46	1.85	0.31	0.32	0.21
2	**4.07**	2.01	1.71	**0.32**	0.30	0.24
10	3.47	2.19	1.23	0.25	0.34	0.24
Mg (mg/plant)						
0	0.58	0.36	0.32	0.15	0.16	0.07
0.1	0.45	0.31	0.29	0.16	0.11	0.07
0.25	0.44	0.32	0.19	0.22	0.09	0.07
2	**0.43**	0.25	0.21	**0.21**	0.09	0.08
10	0.41	0.28	0.14	0.13	0.11	0.09
N (mg/plant)						
0	4.11	3.67	3.54	1.43	1.46	1.28
0.1	6.13	4.44	4.06	1.13	1.20	1.46
0.25	7.41	5.31	5.21	1.32	1.18	1.38
2	**7.88**	4.31	5.02	**1.43**	1.23	1.39
10	7.44	4.84	3.58	1.31	1.32	1.44

1998). Our results indicated that higher than optimum Fe concentration in nutrient solution was unable to diminish the effect of salinity despite its beneficial effect on Fe content. This fact might be at least partially explained by aggravation of some other nutrient disbalances e.g. Mn concentration. On the other hand, according to Foy et al. (1978), high NaCl concentrations might aggravate Fe toxicity disorder by causing physiological drought.

Between those two extreme effects of stress combinations some other relations were also observed. No strict limits might be drawn between them and their interpretation might be subjective. The stress combination might increase the effect of one of the stresses and decrease the effect of the other. For example, when salinity and Fe deficiency were combined, the root P concentration was higher compared to its level under Fe deficiency alone, but lower than its level under salinity alone. The opposite was true for root Cu content. Another possibility was the lack of significant modification of the effect of an individual stress by its combination with another stress e.g. Fe deficiency, alone or in combination, had small effect on root K concentration, which was decreased by salinity.

In a previous study Dahiya and Singh (1974) subjected soil grown pea plants to separate and combined effects of increasing alcalinity (achieved by adding $NaHCO_3$), increasing salinity (achieved by adding sulphates and chlorides of Na, K, Mg and Ca) and Fe addition. The content and uptake of Fe, Mn, P and Na, without separating the plants on shoots and roots, as well as dry matter yield were given. The differences in experimental design between their work and our study make impossible the comparison of the obtained results, despite the common plant object.

In conclusion, it could be summarized that the combined effect of salinity and inadequate iron supply depended on the stress combination, the levels of individual stresses and the parameter under consideration. Although the decline in shoot growth for a particular combination of stress levels was close to that caused by the stronger individual stress at the same level, an additive effect of both stresses cannot be ruled out since earlier and stronger chlorosis took place under complete Fe deficiency when it was combined with salinity. It might be due to the highest Na, and the lowest Fe and N content in shoots, recorded in the plants when the highest levels of the stresses were combined. Even though salinity lessened the drop of root biomass caused by Fe deficiency or toxicity, and some of the stress combinations resulted in closer to the control values for some nutrients compared to individual stresses, a shift in optimum Fe concentration in saline conditions was not found, probably because of aggravation of other nutrient disbalances.

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