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Effects of different mineral nutrients on abscisic acid in maize (*Zea mays*)

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The effects of different concentrations of various macroelements on growth and endogenous ABA (absisic acid) levels in root, stem, leaf and flower tissue of maize (*Zea mays*) were studied. Plants were cultivated in sand and supplied twice a week with a nutrient solution containing optimum, excessive or deficient concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and iron. Plants were harvested at three different stages: vegetative (4–5 leaves), flowering, and fruiting. Fresh weight, leaf and stem size, leaf number and ABA concentrations differed remarkably between plants cultured in abnormal concentrations of macroelements compared to the controls. In general, deprivation of macroelements caused an increase in ABA levels. Deficiency of N, P, K, S and Fe in the nutrient solution resulted in marked increases in the levels of ABA extracted from root, leaf, stem and flower at the three developmental stages. Excessive concentrations of these macroelements resulted in a decrease in ABA levels in all parts of plants at all three stages as compared with their respective controls. ABA levels in roots, stems, and leaves were elevated from 3% to 159% in N, P, K, and S limited plants whereas the levels of ABA in these same organs were reduced from 1% to 98% in the presence of excessive levels of N, K, P and S.

Key words: abscisic acid, ABA, HPLC, mineral nutrition, plant physiology

Introduction

Each of the mineral nutrients N, P, K, S, Ca, Fe and Mg performs a vital role in the growth and development of plants. They are constituents of cell organelles and molecules, necessary for cell division, activators of enzymes essential for photosynthesis, and involved in protein and carbohydrate metabolism and membrane synthesis. Deficient or excessive concentration of these elements results in abnormal or restricted growth (FAO 1984). Plant hormones are involved in all developmental stages of plants. Therefore, endogenous plant hormone levels may be affected by the changes in the concentration of nutrient elements, which affect the growth process. Mineral deficiency causes slower growth in seed and fruit, chlorosis, necrosis, defoliation or death of leaves, reduces flowering, restricts root development and weakens stem structure (Kuiper *et al*. 1989, Galleago *et al*. 1991). Excessive concentration of nutrients accumulated in plants may inhibit growth because of toxicity (Aydemir & Ince 1988).

Hormonal interaction between root and shoot is influenced by mineral imbalance in the soil (Galleago *et al*. 1991, McKersie & Lesham 1994, Liya *et al*. 1998, Jeschke *et al*. 2000). Ample data may be found in the literature showing that moderate changes in the concentration of mineral ions present in the nutrient solution such as K^+, PO_4^{3+}, NO_3^- or NH_4^+ affect the concentration of endogenous phytohormones (Amzallag *et al*. 1992).

Abscisic acid (ABA) is a naturally occurring plant hormone, which can be affected by exogenous and endogenous factors such as various concentrations of mineral nutrients (Trewavas 1981). ABA plays an important role in protein and nucleic acid synthesis and can reverse the effect of growth promoters such as GA, auxins and cytokinins (Fosket 1994). ABA is known as a growth inhibiting substance able to cause bud and seed dormancy and defoliation (Radin 1984). It also acts as a stress signal under conditions of water limitation, increased salt concentration, high pH and perhaps reduced nutrient supply. A series of papers have been published dealing with the effects of nutrient deficiency and nutritional disorder on plant development including the stress signal ABA in several plants. The effects of nitrogen source (Peuke & Jeschke 1993), salt stress (Peuke & Jeschke 1995), phosphorus deficiency (Jeschke *et al*. 1996, 1997a), and potassium levels (Peuke *et al*. 2002) on ABA levels have been reported.

Nitrogen and phosphorus deficiency relative to ABA were studied by Radin (1984), Peuke *et al*. (1994a) and Jeschke *et al*. (1997b). These authors reported increased ABA concentrations in various tissues. Decreased ABA concentrations have been reported in potassium-limited sorghum and capsicum plants (Benech-Arnold *et al*. 1995, Marrush *et al*. 1998). In contrast, elevated ABA concentrations have been reported in *Pinus sylvestris* (Michniewicz & Stopińska 1980), wheat (Haeder & Beringer 1981) and potato (Bhargava *et al*. 1995). ABA accumulation was observed in pea plant leaf (*Pisum sativum* cv. *Little Marvell*) grown in low nitrogen supply (Zdunek & Lips 2001). ABA markedly accumulated in xylem sap of *Ricinus communis* grown on sulphate and phosphate free medium (Peuke 2000). Leaf ABA levels decreased with increasing N and K rates in tobacco (Lin *et al*. 1999). High Ca concentration decreased ABA levels in wheat plant (Zielinska & Michniewicz 2001). An increase in ABA content was observed in floral bud of cucumber when Fe concentration was high in tissues (Wang *et al*. 2000).

The different nitrogen-containing compounds did not change ABA level in wheat (*Triticum*; Chen *et al*. 1998). Relatively high concentration of NaCl raised ABA levels in tomato (*Lycopersicum esculentum*; Dunlap & Binzel 1996). It was shown that a high nitrogen concentration caused a small rise in ABA levels in two hybrid poplar clones (*Tristis* and *Eugenei*; Liu & Dickman 1992). Conversely, high concentrations of NO_3^- and NH_4^+ increased ABA levels in the leaves, stems, roots of *Ricinus communis*, but decreased ABA levels in xylem exudates (Peuke *et al*. 1994b). Cotton (*Linum*) under P-deficient conditions, produced more ABA than plants treated with high concentrations of phosphorus (Radin 1984). Haeder and Beringer (1981) demonstrated that the ABA levels in the seeds of wheat (*Triticum*) plants decreased when the plants were treated with a high concentration of potassium. Adjustment of nutrient concentrations may induce the synthesis of endogenous ABA concentration as a response to stress conditions.

The aim of this paper is to investigate the impact of limited and excessive concentrations of macronutrients on growth and ABA concentrations in different organs in different developmental stages of maize (*Zea mays*).

Material and methods

Maize plants were grown in six-liter pots containing cleaned silica sand (0.8–1.2 mm diameter). The pots were divided into 15 groups, each with three pots. The pots were as follows:

1. Control group (containing all nutrients at optimum levels).
2. –N, –P, –K, –Ca, –Mg, –S, –Fe (deficiency of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and iron).
3. +N, +P, +K, +Ca, +Mg, +S, +Fe (excessive concentration of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and iron).

The optimum concentrations of nutrient solutions were prepared according to Witham *et al.* (1971). Deficient and excessive levels of nutrient solutions were modified from Battal and Tileklioğlu (2001). Deficient, optimum, and excessive levels of nutrient solutions are indicated in Table 1. Microelements boric acid, copper chloride, manganese chloride, zinc chloride and molibdic acid were added into the nutrition solution in each group at 2.32×10^{-3} mM, 2.2×10^{-4} mM, 4.54×10^{-3} mM, 8.8×10^{-4} mM and 4.8×10^{-5} mM concentrations, respectively. In addition, the mixture of EDTA (7.2×10^{-3} mM) and iron sulphate (5×10^{-4} mM) were added to each nutrition solution except the Fe-deficiency treatment. 150 ml of nutrient solutions was poured into each pot twice per week. Each pot was watered with 150 ml of distilled water a day. The plant samples were harvested at three different stages: vegetative (4–5 leaves; 4 weeks), flowering (12 weeks), and fruiting (19 weeks). 2 g of samples from each organ (roots, stems, leaves, and flowers) of the plants was harvested at the end of the first (4–5 leaf), second (flowering) and third stage (fruiting). Harvested samples were stored at –80 °C until extraction. Extraction, purification, and isocratic HPLC (high performance liquid chromatography) analysis were performed according to the modified methods of Battal and Tileklioğlu (2001) and Kuraishi *et al.* (1991).

Frozen samples were powdered in liquid nitrogen. Then cold methanol was added and homogenized in an Ultra Tissue Lyses (Ultrasonic Processor Jenway LTD.) at 4 °C for 1 h. The homogenization process was continued at 4 °C for 24 h in the dark. The samples were filtered through filter paper (Whatman No. 1) and the supernatant was transferred in clean vials. The residues were reprocessed and combined with the former supernatant. The supernatants were filtered through PTFE filters (0.45 μm) and methanol was removed under reduced pressure. Then the extracts were redissolved in 0.1 M KH_2PO_4 (pH 8) buffer solutions and centrifuged at 10 000 rpm for 1 hour at 4 °C. The extracts were passed through PVPP and Sep-Pak C_{18} (Waters) cartridges. The hormone absorbed by the cartridge was eluted with 80% methanol–water (v/v) and the extract was collected in

Table 1. Nutrient solutions (mM).

Stock solutions	Cont.	+N	–N	+P	–P	+K	–K	+Ca	–Ca	+Mg	–Mg	+S	–S	+Fe	–Fe
$NH_4H_2PO_2$	1.60	2.40	–	12.10	–	1.60	1.60	1.60	3.60	1.60	1.60	1.60	1.60	1.60	1.60
NH_4NO_3	–	36.00	–		0.50	–	3.20	–	3.21	–	–	3.10	–	–	–
$Ca(NO_2)_3$	5.30	5.30	–	1.30	5.30	2.10	5.30	14.20	–	5.30	5.30	5.30	5.30	5.30	5.30
$CaCl_2$	1.30	1.30	5.46	2.30	3.30	3.20	1.30	6.20	–	1.30	1.30	1.30	1.30	1.30	1.30
$MgCl_2 \times 6H_2O$	–	–	–	–	–	5.21	–	–	1.00	4.00	–	–	1.00	–	–
$Mg(NO_3)_2Cl_2 \times 6H_2O$	–	–	–	–	–	–	–	–	–	3.40	–	–	–	–	–
$MgSO_4 \times 7H_2O$	2.00	2.00	2.00	2.00	2.00	–	2.00	2.00	2.00	8.00	–	8.00	–	2.00	2.00
KH_2PO_4	1.00	–	6.00	10.00	–	2.00	–	1.00	1.00	1.00	1.00	3.00	1.00	1.00	1.00
KNO_3	6.00	6.00	–	–	6.00	12.00	–	–	6.00	6.20	1.20	–	6.00	6.00	2.00
K_2SO_4	–	–	2.50	–	–	5.00	–	–	–	–	–	6.50	–	–	–
$FeCl_2$	–	–	–	–	–	–	–	–	–	–	–	–	–	2.59	–

– = Absence of the mineral compounds.
+ = Excessive concentration of mineral compounds.

Table 2. Morphological data from maize grown under different macronutrient concentrations at three developmental stages.

Mineral nutrients	Stem height			Leaf length			Dry weight (%)		
	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
Control	45.20 ± 4.21	173.01 ± 14.05	173.21 ± 15.69	55.23 ± 6.81	63.21 ± 4.21	63.04 ± 6.54	10.21 ± 3.21	13.21 ± 3.12	15.41 ± 3.68
+N	46.81 ± 3.85	176.20 ± 13.81	179.05 ± 12.38	56.21 ± 5.13	65.48 ± 5.98	64.89 ± 3.57	11.36 ± 2.65	16.23 ± 3.86	17.21 ± 3.98
–N	19.35 ± 2.14	23.54 ± 2.82	21.45 ± 1.27	22.18 ± 2.51	22.41 ± 3.85	22.51 ± 3.22	9.21 ± 1.200	10.01 ± 2.99	11.41 ± 2.15
+P	40.21 ± 5.47	174.50 ± 13.33	176.5 ± 15.43	54.29 ± 6.87	61.38 ± 4.77	63.93 ± 7.20	10.51 ± 3.58	13.24 ± 3.84	14.81 ± 3.64
–P	20.23 ± 2.23	25.42 ± 3.68	25.36 ± 2.85	24.58 ± 1.31	28.69 ± 1.38	27.89 ± 1.67	8.72 ± 1.32	10.53 ± 1.68	10.01 ± 2.98
+S	48.24 ± 6.27	174.21 ± 15.70	177.20 ± 11.01	54.26 ± 5.14	64.32 ± 6.74	63.24 ± 6.77	11.10 ± 2.56	14.21 ± 4.12	14.89 ± 2.86
–S	20.16 ± 3.80	21.18 ± 1.22	22.13 ± 1.33	20.25 ± 1.01	21.14 ± 1.89	21.36 ± 2.43	9.50 ± 1.67	9.95 ± 2.87	11.21 ± 2.44
+K	43.24 ± 5.07	169.57 ± 16.22	170.01 ± 10.00	56.12 ± 4.86	62.25 ± 4.71	62.81 ± 6.49	10.81 ± 2.10	12.69 ± 1.28	14.81 ± 2.74
–K	41.23 ± 4.18	148.62 ± 10.81	152.23 ± 9.87	43.24 ± 4.21	49.73 ± 6.11	50.46 ± 5.21	9.58 ± 1.95	11.49 ± 3.67	13.41 ± 2.55
+Ca	46.28 ± 3.89	168.20 ± 9.94	169.10 ± 11.91	54.21 ± 7.44	60.41 ± 6.94	60.15 ± 6.83	11.21 ± 3.19	12.86 ± 3.14	13.22 ± 1.28
–Ca	44.23 ± 2.50	160.21 ± 11.05	160.11 ± 8.12	50.32 ± 4.23	58.21 ± 3.05	59.32 ± 6.52	10.00 ± 1.35	12.11 ± 2.98	12.18 ± 2.01
+Mg	44.81 ± 4.21	172.45 ± 14.28	172.21 ± 10.08	55.42 ± 6.66	60.21 ± 7.43	59.95 ± 6.17	10.93 ± 2.78	12.93 ± 2.30	12.12 ± 1.12
–Mg	43.21 ± 3.00	170.25 ± 10.77	170.24 ± 12.55	51.24 ± 3.60	57.39 ± 4.06	59.21 ± 4.20	10.66 ± 2.98	12.19 ± 1.08	12.84 ± 1.33
+Fe	44.28 ± 2.11	169.25 ± 12.24	168.21 ± 12.22	54.45 ± 7.21	62.41 ± 5.70	61.97 ± 3.14	11.23 ± 3.27	12.86 ± 3.81	12.46 ± 2.25
–Fe	43.86 ± 4.02	170.45 ± 11.47	170.25 ± 6.85	50.27 ± 3.85	56.28 ± 2.37	57.65 ± 2.00	9.10 ± 1.03	10.11 ± 2.77	10.13 ± 1.00

a vial. ABA was separated by isocratic HPLC-system (Waters 6000A pomp, Unicam UV detector, mBondapack column).

Results

Deficiency of N, P, K and S inhibited flower and fruit emergence and retarded stem and leaf growth. Primary leaves turned yellow at the first stage and shed at the second and third stages. Intensive necrotic zones were observed at the margin of other leaves. While control plants reached 13–15 leaves at the end of the experimental stage, the number of leaves did not exceed 4–6 and the size of the leaves was affected dramatically in N, P, and S limitation, whereas, the total dry weight and the number and size of leaves were not affected very much by K inhibition in comparison with N, P, S limitation (Table 2). Plants were shriveled, however, under K limitation due to low turgor pressure. Mg, Fe and Ca deficiency led to severe inhibition of kernel size and number. The number of kernel per corncob was 7- to 10-fold less than that of control plants. Moreover, necrosis started at the leaf margins at the end of the second stage. Suppressed stem growth was observed in all stages of the experiment in response to N, P, K, S, Mg, Fe and Ca deficiency.

In N-, P-, K-, S-limited plants, the synthesis and the accumulation of ABA in root, leaf, stem and flower were increased as compared with the control.

ABA levels in plant organs were affected differently when plants were exposed to excessive and deficient concentrations of macronutrient. ABA concentration was slightly increased in root, stem, leaf and flower of N-, P-, K-, S- and Fe-limited plants. The highest increase was observed in the leaf of S-limited plants at the second stage (159%). ABA concentration was increased in all organs of N-, P-, K-, S-, Ca-, Mg-, and Fe-deficient and Ca-, Mg- and Fe-excessive plants at the third stage. The increase of ABA concentration was between 90% and 118% in the flower of Ca-, Mg-deficient and Ca-excessive plants at the second stage.

ABA concentration decreased in all organs of N-, P-, K- and S-excessive plants at all

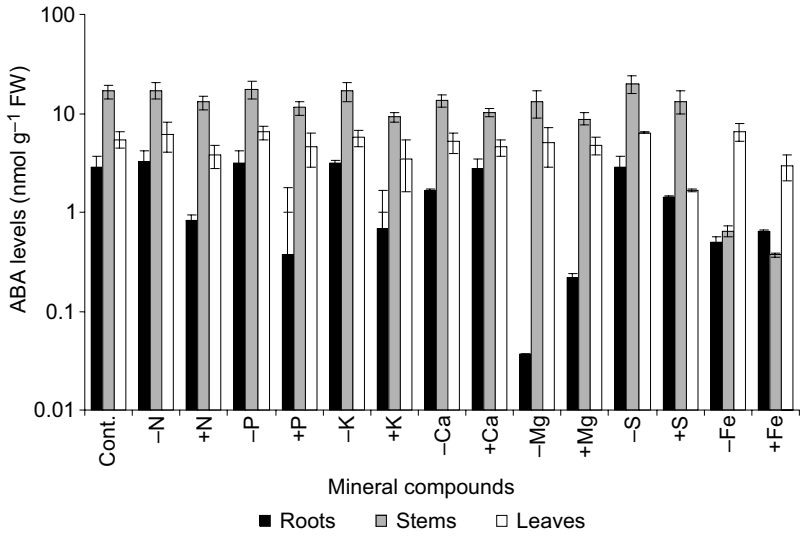

Fig. 1. Abscisic acid levels in the roots, stems and leaves of plants at the vegetative stage.

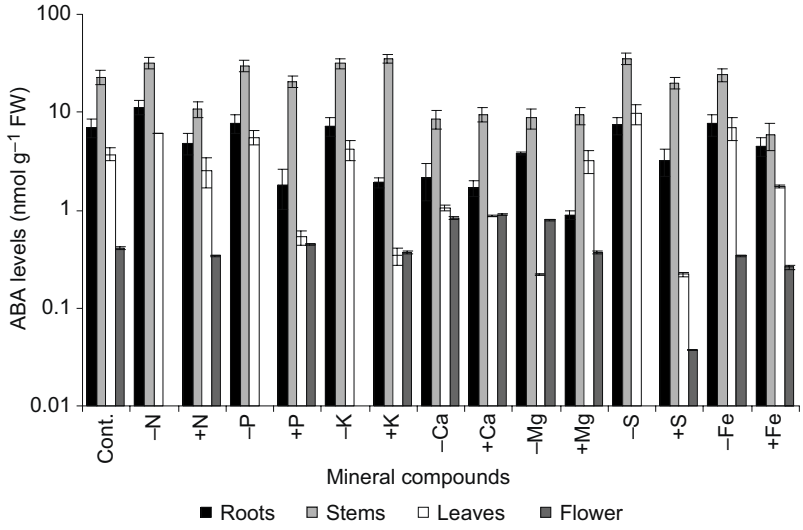

Fig. 2. Abscisic acid levels in the roots, stems, leaves and flowers at the flowering stage.

stages, in all organs of Fe-excessive plants at first and second stages, in all organs of Ca- and Mg-deficient and in all organs except flower of Ca-excessive plants at the first and second stage. ABA concentration was increased, however, in the root and stem of K-excessive plants at the second stage. The dramatic drop of ABA concentration was observed in the root and stem of Mg-, Fe-limited and Mg-, Fe-excessive plants at the first stage, in the root of Mg-excessive plants, in the leaf of K- and S-excessive plants and Mg-deficient plants at the second stage and in the root of P- and S-excessive plants at the third stage (Figs. 1–3).

Discussion

The present study was carried out to test the relationships among different macronutrient concentrations, growth rate, and ABA levels. Significant effects of excessive and deficient mineral concentrations on the levels of ABA were found in all parts of maize plants tested at three developmental stages. Limited nutrient supply correlates with reduced dry weight of the maize plant. This is in accordance with the finding of Peuke *et al.* (2002) that the fresh weight of the whole plant of *Ricinus communis* was lowered when potassium concentration in the nutrient solution was

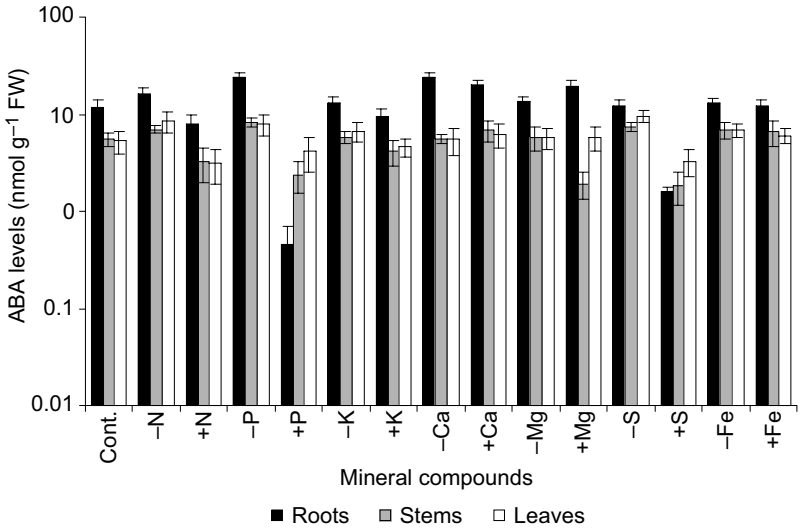

Fig. 3. Abscisic acid levels in the roots, stems and leaves of plants at the fruiting stage.

reduced to 8% of the control. For nitrogen and phosphorus deficiency the root to shoot ratio of *Ricinus communis* was increased due to reduced shoot and increased root growth (Peuke *et al*. 1994a, Jeschke *et al*. 1996). The deficiency of N, P, K and S caused chlorosis and necrosis in leaves; flower and buds did not develop and stalks were weakened. Similar findings were reported by Darral and Wareing (1981): low concentrations of mineral nutrients resulted in a rapid cessation of growth. K deficiency did not affect growth as significantly as N, P, and S deficiency. K limitation caused stomatal closure, which led to low osmotic potential. Thus pressure was lowered and stems were shriveled. Therefore, plant development was reduced at the second and third stages.

Endogenous ABA levels increased in all parts of maize plants at all stages when they were treated with the nutrient solution containing no N, P, K, S, and decreased with the treatment of excessive concentration of N, P, K and S. These results are in general agreement with Zdunek and Lips (2001), Peuke (2000), Lin *et al*. (1999) and Liya *et al*. (1998), who all found increased ABA levels associated with mineral deficiency. Our results disagree with those of Liu and Dickman (1992), Peuke *et al*. (1994b) and Chen *et al*. (1998), who reported increased ABA levels with increasing N and P concentrations. These discrepancies may be due to the fact that their N and P concentrations were higher than ours and that the studies were performed on different organisms. A slight decrease in ABA concentration has been found in K-deficient Scots pine and potato seedlings (Michniewicz & Stopińska 1980, Barghava *et al*. 1995). Jeschke *et al*. (1997b) reported that excess ABA was loaded into xylem vessels in P-deficient plants. The ABA was then translocated to the shoot where most of it was metabolized, and thus no ABA accumulation was observed.

N, P, K and S deficiency leads to senescence in leaves. ABA biosynthesis is normally associated with natural senescence of leaves (Mielke & Dennis 1978). This may point to extreme ABA synthesis and reduced stem growth in maize plants. ABA is synthesized in response to stress, including restricted growth (Jeschke *et al*. 2000). N, P, K and S are essential growth substances and their excessive concentrations have a fertilizing effect on plants. This may be the reason why excessive concentrations of them caused normal growth in plants.

Excessive Ca treatment reduced ABA levels in stem and root at the first and second stages. This finding is in full agreement with Zielenska's (2001) results in wheat. At the first and second stages Ca did not reach toxic levels; therefore, it did not influence plant growth, development, or biochemical processes. The high ABA concentration resultant from high Ca treatment might be due to mineral transportation suppression by Ca accumulation in plants at

the third stage (Galleago *et al*. 1991). Another explanation could be that the plant development was completed and the need for nutrients had decreased, thus Ca accumulation in plant tissues became toxic and this condition may have increased the ABA levels. The deficiency of Mg and Ca caused higher ABA levels in flowers at the second stage and in root, stem and leaves at the third stage. Fe deficiency resulted in increased ABA levels in all organs at the second and third stages, but in decreased ABA levels in only stem and root at the first stage. The reason for this may be that iron is necessary for RNA and chlorophyll synthesis and essential for many enzymes. Therefore, Fe deficiency led to increased ABA levels.

ABA levels increased in the case of excessive concentration of Ca, Mg and Fe at the third stage. This could be due to the antagonistic and toxic effect of mineral compounds accumulated in the soil and to the change of the osmotic potential, as was the case in salinity stress (Fosket 1994). These data suggested that mineral nutrients could act as signals that affect the ABA balance. Moreover, this accumulation can change the metabolic balance and cause some structural abnormality. Therefore, abnormal conditions might have resulted in increased ABA levels. This statement is in agreement with Dunlap and Binzel's (1996) findings in tomato plants. A similar idea suggesting the ability of the root to sense the soil environment and send signals to the shoot was proposed by Amzallag *et al*. (1992), and it was assumed that the signal was ABA.

At the first and second stages ABA concentrations were higher in stem than in root and leaf, but lower at the third stage. This was due to enhanced biosynthesis and deposition of ABA in young stem at the early stages of development in response to nutrient deficiency. Towards the end of development, ABA was recycled within the old part of the stem and transported back to the root via the phloem. The degradation of ABA was high in adult stem, thus ABA did not accumulate in stem as much as in root.

Our data and those in the literature show a close relationship between excess and deficiency of mineral nutrients and the increase or decrease of endogenous ABA concentrations. These data suggest that mineral nutrients, in addition to their nutritional role, participate in the process of regulating the growth-controlling hormonal balance of the plant. These data support the hypothesis that the effect on growth of various mineral concentrations results from their effect on endogenous ABA concentrations. However, it is not yet clear whether macroelements directly affect ABA metabolism, or whether ABA levels change as a secondary effect resulting from reduced growth.

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