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Developmental Changes in Calcium, Magnesium and Potassium Homeostasis of Fool's Watercress Organs under Short-term Oxygen Deprivation

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ABSTRACT

Studying the traits that enable Fool's watercress (*Apium nodiflorum*) to survive oxygen deprivation in its wetland environment, we put forward the hypotheses that calcium, magnesium and potassium homeostasis may alter with age, and that their pools may fluctuate in size as a response to short-term hypoxic conditions at the whole plant level. Young and mature plants presented similar behaviour and allocation with regard to the examined nutrients. Under normoxia, calcium and magnesium homeostasis altered with age and calcium and magnesium levels found to be decreased drastically in the aged plants. In contrast, potassium homeostasis did not alter with age. Oxygen deprivation caused major alterations in the cases of calcium and magnesium homeostasis and minor fluctuations in that of potassium. In all organs of young and mature plants Ca concentration was decreased drastically after the hypoxic treatment. In contrast, Ca concentration in all organs of aged plants was increased drastically under hypoxia. Hypoxic duration of 3 and 4 h caused reduction of Mg concentration in all organs of young and mature plants, while in aged plants Mg concentration of no organ was influenced by the various hypoxic treatments. In contrast to calcium and magnesium, potassium oscillated at approximately the same level regardless of the age and hypoxic treatment. The changes of calcium concentration in the petioles of all ages, as well as the more rapid reduction of magnesium concentration in the petioles of young and mature plants (within one hour) compared to the leaves, the stems and the roots (within three hours), renders them suitable as organs of choice for diagnostic purposes.

Keywords: *Apium nodiflorum*, hypoxia, leaf, petiole, root, stem
Abbreviations: DW, dry weight

INTRODUCTION

In this report we have put forward the hypotheses that calcium, magnesium and potassium homeostasis may alter with age, and that their pools may fluctuate in size as a respond to short-term hypoxic conditions at the whole plant level. Thus, we examined the effect of oxygen deprivation on the calcium, magnesium and potassium pools of roots, stems, petioles and leaves of *A. nodiflorum* young, mature, and aged plants under various durations of oxygen shortage.

Higher plants are aerobes and depend upon a supply of molecular oxygen from their environment to support respiration and various life-sustaining oxidation and oxygenation reactions. Access to oxygen is often inhibited by environmental circumstances that restrict aeration of part or all of the plant. When this occurs, the resulting tissue oxygen deficiency inevitably suppresses oxygen-dependent pathways especially the energy-generating systems, disturbs functional relationships between organs such as roots and shoots, and suppresses both carbon assimilation and photosynthate utilization (Vartapetian and Jackson 1997). Oxygen deprivation most frequently affects the underground or submerged organs directly, because their environment is especially out of oxygen. Shoot systems are damaged indirectly by oxygen shortage, because the constraint causes losses in root functions upon which shoots depend (Otte 2001; Pezeshki 2001).

Root to shoot interaction in relation to mineral nutrition includes complex interactions and transfers between xylem vessels serving the leaves and those passing further up the stem and between xylem and phloem within the stem (Jeschke and Hartung 2000). Nutrient uptake needs to be regulated in response to shoot demand (Marschner 1995); thus, root and shoot co-operate in the assimilation of mineral nutrients into organic matter (Andrews *et al*. 1999). Under a condition of mineral imbalances sensed by the root, the partitioning of nutrients is altered in various cases. This communication between root and shoot may be direct or indirect, mediated by the nutrients themselves or by hormonal or other signals translocated in the long distance vessels to the developing tissues or organs (Jeschke and Hartung 2000).

Wetland plants possess various traits that enable them to survive oxygen deprivation in root environment. Under oxygen deprivation nutrition is influenced (Pezeshki 2001) and factors such as developmental and physiological status and flood-tolerance capabilities are important. Tolerance can vary from only a few hours to many days or weeks depending on species, the organs directly affected, stage of development, and external conditions (Vartapetian and Jackson 1997). Mechanisms that underlie tolerance to external oxygen deprivation have been reviewed (Kennedy *et al*. 1992; Perata and Alpi 1993; Ricard *et al*. 1994; Crawford and Braendle 1996; Morard and Silvestre 1996). For the submerged organs, these mechanisms include metabolic adaptations such as avoidance of self poisoning and cytoplasmic acidosis, maintenance of adequate supplies of energy and sugar, modifications to gene expression and metabolic acclimation to tissue anoxia by previous exposure to partial oxygen shortage. The aerial parts of the plant are often less susceptible to oxygen deficiency than the submerged ones. Systemic signaling between submerged and non-submerged plant parts, integrates root and shoot physiology (Vartapetian and Jackson 1997). In a wide variety of spe-

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cies, nutrient resorption from other, usually senescing, tissues minimizes nutrient loss effectively increasing nutrient-use efficiency. Nutrient resorption has been linked to nutrient availability at a site, and may be an adaptation to nutrient limitation (Andrews *et al*. 1999). Oxygen deprivation leads to inhibition of nutrient uptake.

MATERIALS AND METHODS

Apium nodiflorum (L.) Lag. (Apiaceae, Fool's watercress) plants were collected from their natural habitat, transferred to the laboratory and used for all treatments immediately. Prior to treatment, plants were washed with tap water, NaOCl (2.5%, 5 min), and distilled water. All treatments were performed at 25°C, and a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (as commonly measured in their habitat) at water level. The various experiments were performed for two successive years with young, mature and aged plants 2, 6 and 10-month-old respectively, in three replicates.

In each experimental case, 100 g of plant biomass (fresh weight) were put in a 1-L glass vessel containing nutrient solution [KH_2PO_4 1.17 mM, KNO_3 0.83 mM, $Ca(NO_3)_2$ 0.83 mM, $MgSO_4$ 0.33 mM, NaCl 0.33 mM, NH_4Cl 0.3 mM, $(NH_4)_2SO_4$ 0.17 mM, $FeNaEDTA.3H_2O$ 2.3 μM, H_3BO_3 7.7 μM, $MnCl_2$ 1.47 μM, $ZnSO_4$ 117 nM, $CuCl_2$ 67 nM, $(NH_4)_6MoO_{24}$ 1.67 nM]. Plant biomass consisted of whole plants of almost the same size within each developmental stage, and the pot contained 15 young or 10 mature or 5 aged plants in each treatment and replicate. In any treatment care was taken to mimic the percent of submergence of the plant in its natural environment. To this end, in the case of young and mature plants roots-stems-petioles were submerged, while only roots-stems of aged plants were the submerged organs.

An Oxi320-WTW oxygen electrode was inserted at the geometrical centre of the vessel. In order to prevent oxygen diffusion from the atmosphere to the root environment, the solution surface was covered with a transparent plastic membrane. Each treatment lasted 4 h, an experimental duration dictated by the oxygenation rate of the hypoxic nutrient solution by the plants. In the normoxic treatment, air was bubbled into the medium until the dissolved oxygen concentration equaled to 6 ppm. In order to establish oxygen deficiency, pure N_2 was bubbled into the medium until dissolved oxygen concentration equalled to 0.2 ppm. Afterwards, N_2 applied at regular intervals to achieve the duration of the required oxygen deficiency in the various hypoxic treatments (**Table 1**). The dissolved oxygen concentration determined polarographically in the end of each treatment, ranged between 3 ppm (normoxic control) and 0.7 ppm (hypoxic control) for young and mature plants, or between 2.5 and 0.3 ppm for aged plants (**Table 2**). Distilled water was added to restore the initial volume at regular time intervals, and the added volume was recorded for the calculation of water losses due to guttation.

Table 1 Each of the examined treatments lasted 4 h (including the hypoxic and the subsequent post-hypoxic phase) and determinations were performed in the end of each treatment.

Treatment	Time (h)	
	Hypoxic phase	Post-hypoxic phase
0	normoxia for 4 hours	
0.5	0.5	3.5
1	1	3
2	2	2
3	3	1
4	hypoxia for 4 hours	

Table 2 The dissolved oxygen concentration determined polarographically in the end of each treatment.

Treatment	Dissolved oxygen concentration (ppm)	
	Young and mature plants	Aged plants
0	3.0	2.5
0.5	2.5	0.5
1	2.0	0.4
2	1.7	0.5
3	1.3	0.3
4	0.7	0.3

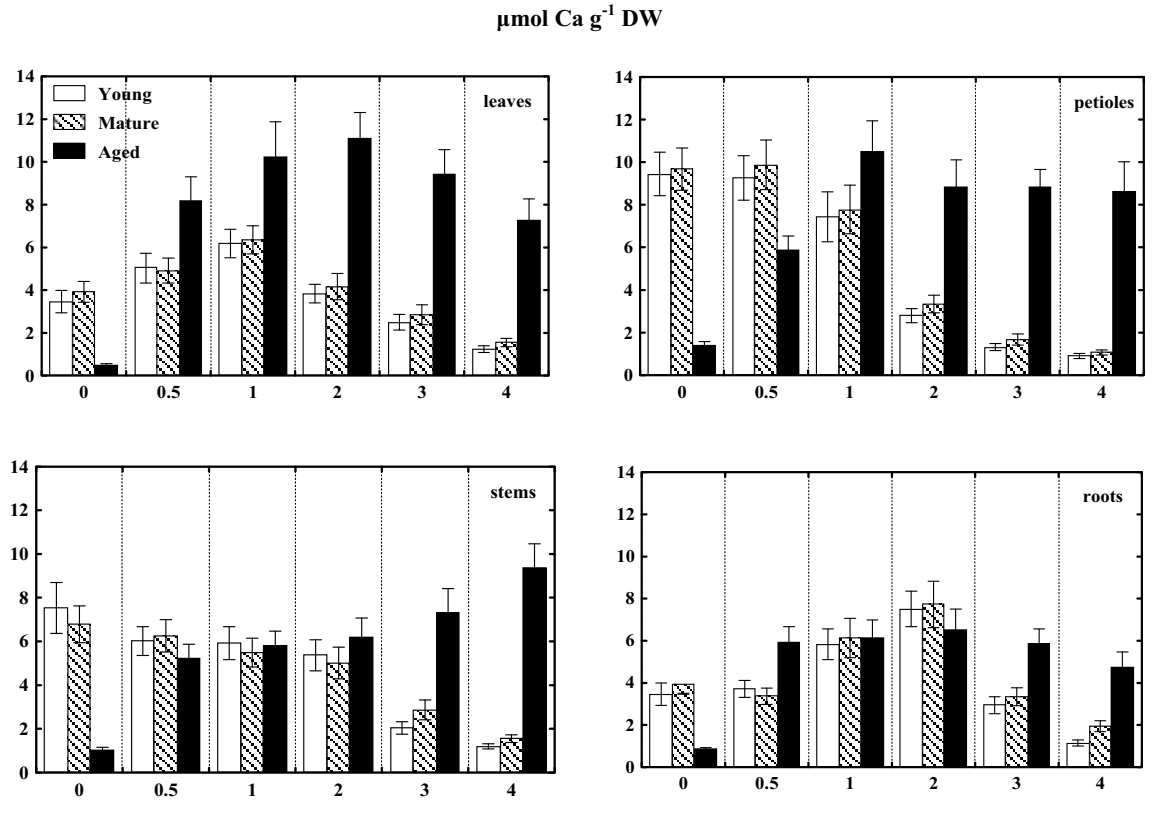

Fig. 1 The effect of short-term oxygen deprivation on calcium levels of young, mature and aged *A. nodiflorum* leaves, petioles, stems, and roots. Error bars represent SE. DW: dry weight.

Table 3 Within each cell the number represents the possibility that this case differs from the corresponding one of the next year in the concentration of the examined nutrient at p = 0.05 level by means of the Scheffe test.

Organ	Developmental stage	Ca	Mg	K
leaf	young	0.9458	0.9172	0.9231
leaf	mature	0.9928	0.9438	0.8273
leaf	aged	0.8628	0.8934	0.7971
petiole	young	0.7599	0.8453	0.9912
petiole	mature	0.6943	0.5934	0.6325
petiole	aged	0.8522	0.8952	0.8179
stem	young	0.6923	0.4923	0.5891
stem	mature	0.7152	0.5843	0.9436
stem	aged	0.5987	0.8239	0.3984
root	young	0.7293	0.3940	0.6379
root	mature	0.8692	0.4219	0.7738
root	aged	0.8428	0.6745	0.9827

An asterisk indicating statistically significant differences would appear only if a value was less than 0.05.

In the end of each treatment, plants were soaked in 0.1 N HCl solution for 10 min. After desorption, plants were washed with distilled water, blotted on towel and separated to roots, stems, petioles and leaves. Plant parts were dried at 60°C for 3 days, dried tissues were digested with $HNO_3/HClO_4$ and Ca, Mg and K concentrations were determined by the atomic absorption method using a GBC Avanta P atomic absorption spectrophotometer (Mills and Jones 1996).

In this way, the experimental design included 36 treatments (6 treatments per age and life cycle × 3 ages per life cycle × 2 life cycles) performed three times each. Post-hoc statistical comparison and plots were performed by means of the Statistica software package. Statistical analysis of the possibilities each organ of different age to differ in the concentrations of the examined nutrients between the two successive life cycles (**Table 3**), revealed no statistically significant variations (p=0.05) in all experimental cases.

RESULTS

Calcium

In the young and mature plants under normoxia Ca concentration of leaves, petioles, stems and roots was 4, 10, 7, and 4 μmol.g^{-1} dry weight (DW) respectively, while the aged plants under normoxia presented Ca concentration of leaves, petioles, stems and roots of 0.5, 1.5, 1, and 1 μmol.g^{-1} DW respectively (**Fig. 1**). After hypoxia of 0.5-1 h Ca concentration in the leaves of young and mature plants increased to 6 μmol.g^{-1} DW. The rest of the hypoxic treatments caused progressive reduction of Ca concentration to 1 μmol.g^{-1} DW. In aged leaves, the hypoxic treatment of 0.5 h and 1 h initially caused an enormous increase of concentration to 8-11 μmol.g^{-1} DW, while the hypoxic treatments of 3-4 h decreased Ca concentration to 7 μmol.g^{-1} DW. In the petioles of young and mature plants all hypoxic treatments caused reduction of Ca concentration, and as a result Ca concentration in the end of 4 h reached 1 μmol.g^{-1} DW. In the same organs of the aged plants an increase in the corresponding level of Ca concentration was observed after 0.5-1 h of hypoxic treatment, which reached the level of 10 μmol.g^{-1} DW. In the remainder hypoxic treatments the Ca concentration in the petioles of aged plants remained constant in the level of 10 μmol.g^{-1} DW. In the stems of young and mature plants hypoxia caused appreciable reduction of Ca concentration, which in the end of the fourth hour reached 1 μmol.g^{-1} DW. In contrast, an important increase of Ca concentration was caused at the same time interval in aged plants that reached 10 μmol.g^{-1} DW. In the roots of young and mature plants hypoxia up to 2 h caused the progressive increase of Ca concentration to 8 μmol.g^{-1} DW, while hypoxia of 3 and 4 h had as result the abrupt reduction of concentration of Ca content to 3 and 1 μmol.g^{-1} DW respectively. In the roots of aged plants the imposition of 0.5 h hypoxia increased Ca content to 6 μmol.g^{-1} DW, while the rest of the hypoxic treatments caused light reduction of Ca concentration to 5 μmol.g^{-1} DW.

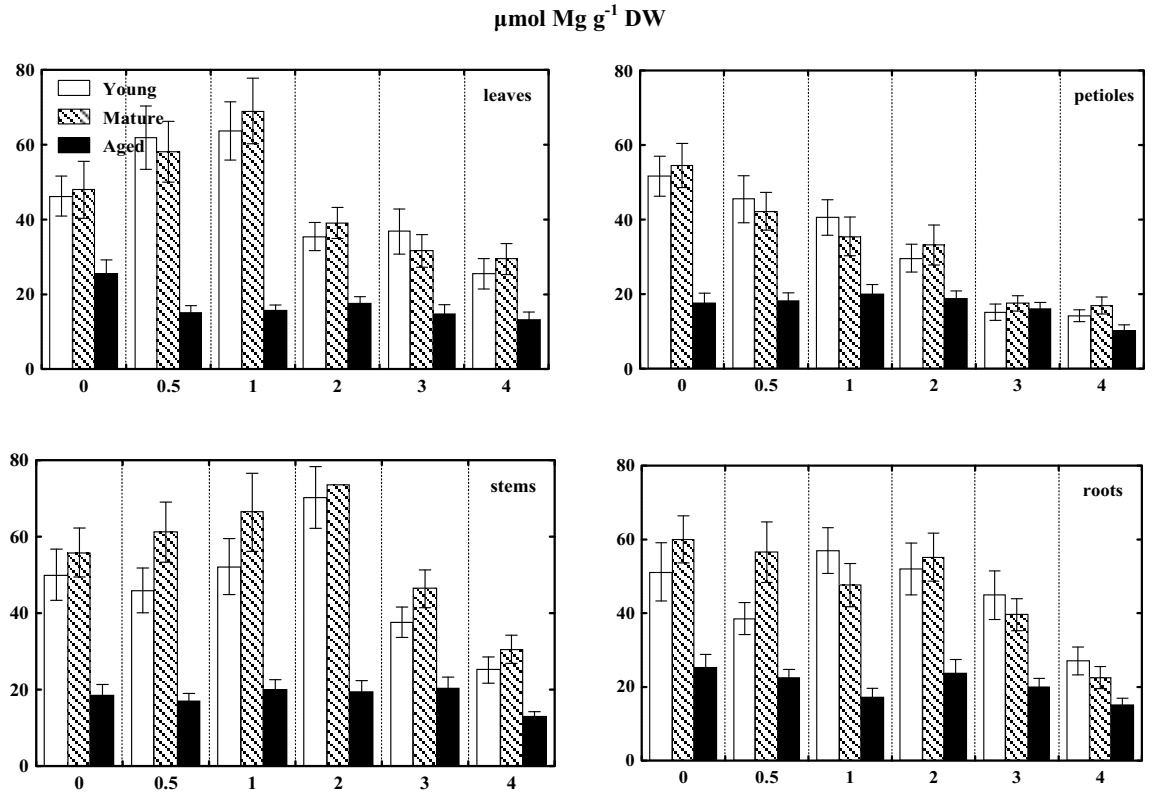

Fig. 2 The effect of short-term oxygen deprivation on magnesium levels of young, mature and aged *A. nodiflorum* leaves, petioles, stems, and roots. Error bars represent SE. DW: dry weight.

Magnesium

Magnesium concentration in all organs of young and mature plants under normoxic conditions was about 50 μmol.g^{-1} DW. The corresponding Mg concentration of all organs of the aged plants was around 20 μmol.g^{-1} DW (**Fig. 2**). In the leaves of young and mature plants, hypoxic conditions of 0.5 and 1 h duration had as result the increase of Mg concentration to 70 μmol.g^{-1} DW. Hypoxic treatments of 2, 3 and 4 hours involved the reduction of Mg concentration in levels under those of the normoxic control, reaching 30 μmol.g^{-1} DW. In the aged plants, all hypoxic treatments only slightly influenced Mg concentration in leaves, which was limited around 15 μmol.g^{-1} DW. Mg concentration in the petioles of young and mature plants presented a continuous reduction with the increase of duration of hypoxia. After 4 h of hypoxic treatment Mg concentration had been decreased 3 times, reaching 20 μmol.g^{-1} DW. In contrast, hypoxic treatment of aged plants did not influence the Mg concentration of petioles. In the stems of young and mature plants a duration of hypoxic treatment of 0.5 up to 2 h involved a progressive increase of Mg concentration, the level of which reached 70 μmol.g^{-1} DW. Then, a hypoxic duration of 3 and 4 h drastically decreased Mg concentration to 30 μmol.g^{-1} DW. Stems of aged plants were not influenced by any hypoxic treatment and Mg concentration remained almost constant. Mg concentration in roots of young and mature plants oscillated round the level of the normoxic control up to 2 h of hypoxia. Subsequently, the concentration was decreased, finally reaching 30 μmol.g^{-1} DW after 4 h of hypoxia. In the roots of aged plants Mg concentration remained in the levels of the normoxic control in all hypoxic treatments.

Potassium

In the young and mature plants under normoxia K concentration of leaves, petioles, stems and roots was 500, 650, 350, and 80 μmol.g^{-1} DW respectively, while the aged plants under normoxia presented a K concentration of 550, 550, 400, and 100 μmol.g^{-1} DW in leaves, petioles, stems and roots respectively (**Fig. 3**). Potassium concentration in the leaves of young and mature plants after hypoxia of 0.5 h increased and reached 650 μmol.g^{-1} DW. In contrast, K content was not affected after hypoxia of longer duration. In aged plants all hypoxic treatments caused a short reduction of potassium content to 500 μmol.g^{-1} DW. In the petioles of young and mature plants hypoxia of 0.5 and 1 h reduced K concentration to 500 μmol.g^{-1} DW. In the same organs hypoxia for 2, 3 and 4 h caused an increase of the concentration to the levels of the normoxic control. In aged plants application of any hypoxic treatment did not affect K concentration in the petioles. In the stems of plants of all ages hypoxia for 0.5 and 1 h resulted in the gradual reduction of K concentration to 200 μmol.g^{-1} DW, while in the hypoxic treatments of 2, 3 and 4 h a tendency of rehabilitation of K concentration in the levels of the normoxic control was presented. In the roots of young and mature plants hypoxia of 0.5 and 1 h had as result the light increase of K concentration to 120 μmol.g^{-1} DW. In the aged plants, the corresponding hypoxic treatment had as result a small reduction of K concentration to 80 μmol.g^{-1} DW. A hypoxic treatment of 2-4 h had as consequence the tendency of rehabilitation of K concentration to the levels of the normoxic control in all three ages of the plant.

DISCUSSION

In many wetland plants an extensive oxygen transport system (aerenchyma tissue) may exist in roots, stems and leaves. This system allows a plant to transport the needed oxygen for maintaining aerobic respiration. The oxygen transport system has been considered as a major mechanism critical to plant's ability to cope with oxygen deficiency (Pezeshki 2001). Fool's watercress is a semi-aquatic plant and we found that young and mature plants presented similar behaviour and allocation with regard to the examined nutrients; practically we did not see alterations between their patterns. According to Chorianopoulou *et al*. (2001), young and mature plants elevated their substrate dissolved oxygen concentration when faced oxygen deprivation. Besides aerenchyma, in this plant the existence in petioles

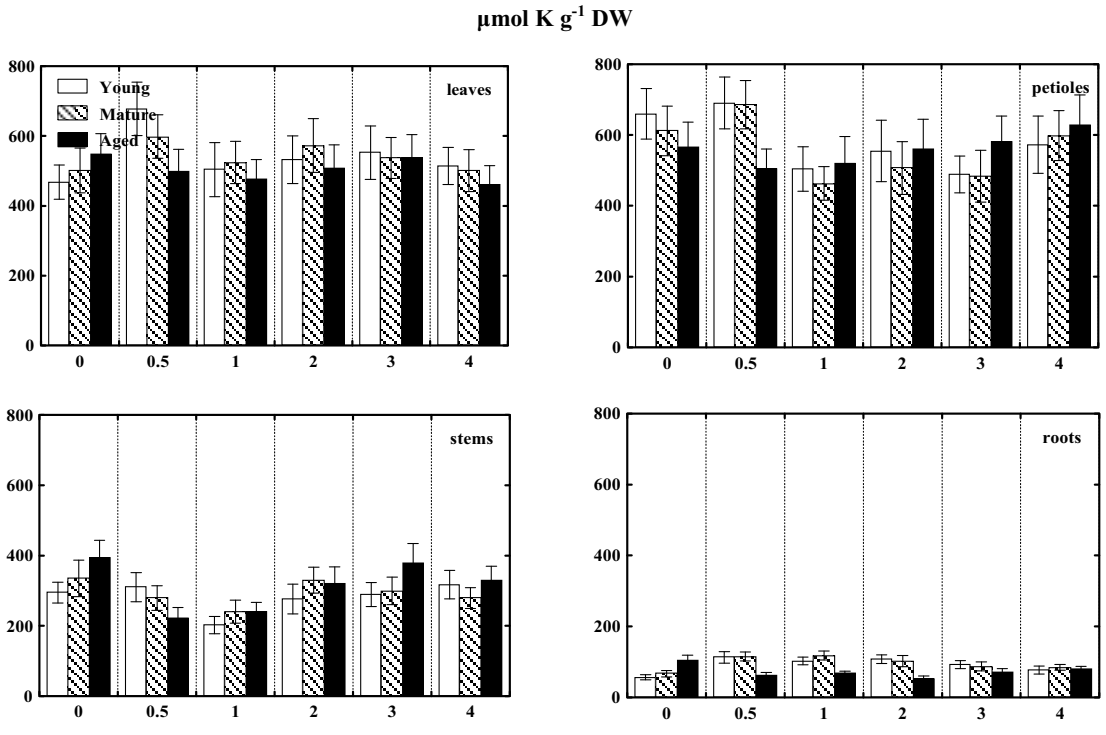

Fig. 3 The effect of short-term oxygen deprivation on potassium levels of young, mature and aged *A. nodiflorum* leaves, petioles, stems, and roots. Error bars represent SE. DW: dry weight.

Table 4 The probability each pair of organs of different age to differ in the concentrations of the examined nutrients at $p = 0.05$ level by means of Duncan's test, in both normoxic (**A**) and hypoxic (**B**) treatments. Asterisks indicate statistically significant differences.

A. Normoxia (treatment 0)						
	Ca		**Mg**		**K**	
leaves						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.9999	0.0146*	0.7167	0.0003*	0.4337	0.0839
mature	-	0.0029*	-	0.0002*	-	0.2812
petioles						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.9999	0.0001*	0.5360	0.0001*	0.2559	0.0410*
mature	-	0.0001*	-	0.0001*	-	0.2813
stems						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.9972	0.0001*	0.2660	0.0001*	0.3369	0.0318*
mature	-	0.0001*	-	0.0001*	-	0.1717
roots						
	mature	**aged**	**mature**	**aged**	**mature**	**Aged**
young	0.9999	0.0472*	0.0984	0.0001*	0.8086	0.2827
mature	-	0.0265*	-	0.0001*	-	0.3674
B. Hypoxia (treatment 4)						
	Ca		**Mg**		**K**	
leaves						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.5694	0.0001*	0.1319	0.0001*	0.7673	0.2465
mature	-	0.0001*	-	0.0001*	-	0.3477
petioles						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.7466	0.0001*	0.2841	0.1485	0.5550	0.2465
mature	-	0.0001*	-	0.0195*	-	0.5165
stems						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.5495	0.0001*	0.0494*	0.0001*	0.3781	0.7674
mature	-	0.0001*	-	0.0001*	-	0.2693
roots						
	mature	**aged**	**mature**	**aged**	**mature**	**aged**
young	0.5866	0.0001*	0.0923	0.0001*	0.8675	0.9528
mature	-	0.0001*	-	0.0054*	-	0.9057

of an outer air transport pathway was found, making *A. nodiflorum* plants capable of efficiently facing hypoxic conditions in their substrate. However, such a trait was absent in stems. In contrast to young and mature plants, the aged ones oxygenated their substrate poorly, due to the fact that in this developmental stage only their stems are submerged into the medium and not their petioles. Thus, the duration of the experiments was not an arbitrary choice. Within 4 hours, the hypoxic young and mature plants have reached a no more hypoxic state of equilibrium in dissolved oxygen concentration, but not the hypoxic aged plants. Thus, the experiment designed taking this trait into consideration, in order to be able to compare the situations from a physiological point of view. Examining if the hypotheses put forward in this report hold true for the micronutrients, we found that Fe and Mn levels in the organs of plants experiencing hypoxia did not shift from the normoxic levels. In contrast, Zn and Cu levels increased under oxygen deficiency, but each nutrient presented a different behavior. Copper increased in all organs of young and mature plants. Zinc increased only in leaves and roots of young and mature plants. Especially, the increase of Zn in roots was a tremendous one, 6 times above the normoxic levels. Such characteristic increases in Cu and Zn levels were not observed in aged leaves, and petioles. Zinc levels of aged roots almost doubled. Thus, there were alterations in the effect of oxygen deficiency on Zn and Cu homeostasis within *A. nodiflorum* organs, when the plants enter their reproductive stage (Chorianopoulou and Bouranis 2004).

Extending the hypotheses for the selected macronutrients, in this report we provide evidence that calcium and magnesium homeostasis alter with age, with calcium and magnesium levels decreasing drastically in aged plants. In contrast, potassium homeostasis does not alter with age (**Table 4**), thus providing a clear answer to our first hypothesis. With regard to the second one, oxygen deficiency caused major alterations in the cases of calcium and magnesium homeostasis and minor fluctuations in the case of potassium. It should be mentioned that Ca, Mg and K concentrations in plants of various ages under natural conditions were statistically (p=0.05) similar to that of treatment "0" (data not shown). It is known that the metabolism, growth and development of plants is exquisitely sensitive to modulation by environmental factors, however the alterations of nutrient pools by hypoxia is a poorly understood case of this interaction. Viewed in terms of causality, a directed change in the allocation of the examined nutrients between the various organs requires that their level is regulated by signals that affect the work of the appropriate transporters. We are still far from understanding how nutrient status is sensed, how the signals are transduced, and how information about different resources and their allocation is integrated to allow a flexible short-term response of the plant body to the applied hypoxia in terms of the physiological status of the plant (Stitt and Scheible 1998).

Calcium is shown to be involved as a second messenger in various stresses, including low oxygen. Subbaiah *et al*. (1994a, 1994b) presented evidence that an elevation of cytosolic Ca^{2+} triggers gene activation and tolerance to O_2 deprivation, while another work by Subbaiah *et al*. (1998) indicates that mitochondria act as a significant Ca^{2+} store contributing to this elevation. More recent work of Subbaiah *et al*. (2000) shows that anoxia-induced cell death initiation may depend on elevated cytosolic Ca^{2+} levels in the root tip cells. On the other hand, response to low oxygen in *Arabidopsis* might require both extracellular and organellar calcium (Dolferus *et al*. 1997). In this report the role of calcium was investigated at a whole organ level. Given the fact that cytoplasm and specific organelles are under complete homeostatic supervision, we understand that Ca, Mg and K pools are subject to fluctuations and this is what we finally measure under the examined conditions. Such pools seem to be mainly the apoplasm and the vacuole. Furthermore, nutrition is influenced by oxygen deprivation and many factors share important contribution to that, among them nutrient pools, plant developmental and physiological status, and flood-tolerance capability.

The co-ordinated behaviour of roots, stems, petioles and leaves is obvious. Short-term hypoxia affected the calcium and magnesium status of the whole plant. It is well known that plants display a high degree of physiological and developmental plasticity in response to changing conditions in the rhizosphere. Some responses are restricted to those roots directly exposed to the signal, while other responses are systemic and must involve the transmission of long-distance signals, usually between the root and the stem (Forde 2002). Besides, the uptake systems for various nutrients are regulated by demand from the stem. This feedback regulation also extends to enzymes involved in nutrient assimilation and the mobilization of nutrients from the rhizosphere. The role of long-distance signals from the stem in regulating gene expression in the root has been demonstrated for the responses to deficiencies in phosphorus, iron, and sulfur and a remarkable feature of these systemic responses is that each appears to be specific to the nutrient in question. Communication between root and stem may be direct or indirect, mediated by the nutrients themselves or by hormonal signals translocated in the long distance vessels to the other organs originating in the stem (Fox and Guerinot 1998; Jackson 2002).

Plants sometime exhibit a phenomenon referred to as root pressure or positive hydrostatic pressure in the xylem. Root pressure is most prominent in well-hydrated plants under high humidity where there is little transpiration. Plants that develop root pressure exhibit exudation of liquid from the leaves, a phenomenon known as guttation. Positive

Table 5 The effect of short-term oxygen deprivation on the guttation rates of young, mature and aged plants.

Treatment	Guttation rate (mL H_2O . g^{-1} FW_{leaf}. h^{-1})		
	Young	Mature	Aged
0	1.22 ± 0.10*	0.85 ± 0.07 y*	0.37 ± 0.04 ym*
0.5	3.85 ± 0.35	3.20 ± 0.27	0.96 ± 0.06 ym
1	2.09 ± 0.15	1.68 ± 0.16	1.21 ± 0.10 ym
2	2.85 ± 0.30	2.77 ± 0.20	2.14 ± 0.16 y
3	5.68 ± 0.45	5.52 ± 0.38	2.82 ± 0.27 ym
4	3.24 ± 0.22	2.00 ± 0.19 y	3.92 ± 0.34 m

The asterisk in the normoxic values indicates that are statistically significant compared with any hypoxic value of the same column by means of t-test (p=0.05).
Different letters within a value of a row indicate statistically significant differences by means of t-test (p=0.05), when this value is compared with the corresponding value of young (y) or mature (m) plants.

xylem pressure causes exudation of xylem sap through hydathodes, structures that are located near terminal tracheides of the bundle ends around the margins of leaves. Guttation is most noticeable when transpiration is suppressed and the relative humidity is high (Taiz and Zeiger 2002). Root pressure and corresponding volume flow in the xylem are of particular importance for the long-distance transport of calcium into low-transpiring organs and for nutrient cycling in plants. The effect of transpiration rates on uptake and transport is usually absent or only low for potassium, nitrate and phosphate but may become significant for sodium or calcium. Frequently, a close positive correlation is observed between calcium distribution and the transpiration rates of shoot organs. The effect of transpiration on magnesium is much lower than its effect on calcium, and that on potassium is negligible. High root pressure, as indicated by the intensity of guttation, is closely correlated with an increased concentration of calcium in expanding leaves. Magnesium, which is highly phloem mobile, is only slightly affected by root pressure. Root pressure also strongly depends on root respiration and oxygen supply to the roots (Marschner 1995). Considering the fact that we studied a semi-aquatic plant, under the experimental conditions the measured water losses (**Table 5**) are mainly due to guttation, not to transpiration.

In all organs of young and mature plants Ca concentration was decreased drastically to about 1 μmol.g^{-1} DW after the hypoxic treatment of 4 h comparing to the corresponding normoxic Ca concentration. In contrast, Ca concentration was increased drastically to around 7 μmol.g^{-1} DW in all organs of aged plants after hypoxic treatment of 4 h concerning the corresponding normoxic. The pattern of changes between roots and leaves of all ages was similar, while similar was also the pattern between petioles and shoots of all ages. The drastic reduction of Ca concentration in all organs of young and mature plants may be due to increased guttation (**Table 5**) via hydathodes that are found in this plant, in connection with the fact that in the end of the treatment the whole plant was washed. The increased guttation rate may facilitate the fast transport of a signal from roots to aerial parts. On the other side, the drastic increase of Ca concentration in all organs of aged plants may be indicative of an emergency situation, where the plant facing oxygen deprivation in the growth medium absorbs calcium targeting to create inflorescences more rapidly (emergency flowering). It seems that aged plants do not allow calcium to be lost through hydathodes. Anyhow, the changes of calcium in the various organs point the petioles as organs with the highest changes, meaning that these organs could be useful as diagnostic indices of hypoxia.

Hypoxic duration of 3 and 4 h caused reduction of Mg concentration in all organs of young and mature plants. Thus, after 4 h of hypoxia the Mg concentration of all plant organs but petioles was around 30 μmol.g^{-1} DW. In petioles, the corresponding level was 20 μmol.g^{-1} DW. In aged plants Mg concentration of no organ was influenced by the various hypoxic treatments. The realised more rapid reduction of magnesium concentration in the petioles of young and mature plants (within one hour) compared to the leaves, the stems and the roots (within 3 hours) could be attributed to the higher sensitivity of petioles, a fact that renders them suitable as organs of choice for diagnostic purposes. This is justified by the finding that the path of oxygen transport to the growth medium is localized outside of that organ.

In contrast, our results showed that potassium oscillated at approximately the same level regardless of the age and hypoxic treatment. This means that the plant maintains the same levels of potassium in all cases, a fact pointing out that its contribution in plant functions remains constant and the plant continues checking its osmotic functions within this interval. Some minor changes of K concentration were observed only in the hypoxic treatments of 0.5 and 1 h. Consequently the K content cannot constitute diagnostic indicator of this constraint.

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