ALUMINIUM EFFECTS ON PYRIDINE NUCLEOTIDE REDOX STATE IN ROOTS OF SCOTS PINE

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ABSTRACT

After prolonged (3-9 weeks) hydroponic treatment of Scots pine seedlings with different concentrations (0.5-4.0 mM) of Al ($\text{Al}(\text{NO}_3)_3$), the levels of pyridine nucleotides were determined in root homogenates. After 3 weeks of Al stress, a significant decrease of the anabolic reduction charge (ARC: $\text{NADPH}/(\text{NADP}^+ + \text{NADPH})$) and an increase of the redox status ($\text{NAD(P)H}/\text{NAD(P)}^+$), catabolic reduction charge (CRC: $\text{NADH}/(\text{NAD}^+ + \text{NADH})$) and phosphorylation capacity expressed as $\text{NADP}^+/\text{NAD}^+$ ratio was found in the 4.0 mM Al treatment. After 6 weeks, Al at concentrations of 0.5 and 1.0 mM induced an enhancement of the NADH level and a reduction of NADPH level, but the redox ratios were not changed significantly. After 9 weeks treatment with Al concentrations ranging from 0.5 to 4.0 mM, decreases of the relative level of NADP^+ , NADPH and NADH and increases of NAD^+ were found. Consequently, the CRC, $\text{NAD(P)H}/\text{NAD(P)}^+$ and $\text{NADP}^+/\text{NAD}^+$ ratios reached a minimum and ARC a maximum as compared to previous measurements.

KEY WORDS: Al, *Pinus sylvestris*, pyridine nucleotides, redox ratios, roots

INTRODUCTION

The toxic effect of aluminium ions (Al) on plants has been frequently investigated. Numerous papers published on the subject deal primarily with changes in the growth and in the metabolism of roots caused by Al (Foy et al. 1978, Rengel 1992). The common responses of roots to Al action are: an inhibition of root cell mitosis (Morimura & Matsumoto 1978, Matsumoto & Morimura 1980) and root elongation (McQuattie & Schier 1990, Ryan et al. 1993); a reduction in nutrient uptake, accumulation and translocation (Sverdrup et al. 1992, Rengel & Elliott 1992, Huang et al. 1993, Nichol 1993); and alterations of the cell-wall Donnan free space (Cronan 1991, Jentschke et al. 1991), the plasma membrane (see Meharg 1993 for a review) and membrane transport proteins (Suhayda & Huang 1986, Tu & Brouillette 1987, Caldwell 1989). At the molecular level, Al interferes with calmodulin-dependent enzymes (Siegel & Haug 1983, Haug 1984).

One of the calmodulin-dependent enzymes is NAD-kinase, which may control glycolysis and the pentose-phosphate pathway via regulation of NADP synthesis (Ślaski 1989, 1990). However, little attention has been paid to the changes in pyridine nucleotide levels as a response to Al stress. Ślaski (1989) reported that a 10 h incubation of wheat roots in 0.74 mM Al, in contrast to a 24 h incubation, changed the NADP/NAD ratio as a result of an increase in NADP content in the total pool of pyridine nucleotides. The experiments reported here, give a more detailed analysis of the levels of the oxidized and reduced coenzymes in roots of Scots pine after long-term exposures to inorganic aluminium present in the

nutrient solution. These studies suggest that Al action leads to a change of the metabolic state with regard to the maintenance of the redox state in root tissue.

MATERIALS AND METHODS

Plant material and culture

One-year-old nursery-grown seedlings of Scots pine (*Pinus sylvestris* L.) were used. In early May, the seedlings were washed free of soil and grown hydroponically in a glass house under conditions previously described (Karolewski & Giertych, 1994) with some modifications. The composition of the nutrient solution (pH 4.2) was as follows (mg x dcm⁻³ of solution): nitrogen (N)-140, phosphorus (P_2O_5)-25, potassium (K_2O)-110, calcium (CaO)-55, magnesium (MgO)-20, sulphur (SO_4)-5, iron (Fe)-0.145, manganese (Mn)-0.02, boron (B)-0.015, zinc (Zn)-0.01, copper (Cu)-0.01 and molybdenum (Mo)-0.005. After a conditioning period of 7 days, the seedlings were transferred to the same nutrient solutions with or without Al added in the form of $\text{Al}(\text{NO}_3)_3$ (concentrations as indicated in the legends to the figures). The solutions were renewed every 7 days. Following 3, 6 or 9 weeks of Al treatment, randomly selected seedlings were harvested, the roots were rinsed with distilled water, gently blotted between filter paper, weighed and frozen in liquid nitrogen to stop metabolic reactions.

Each treatment combination in the experiment was represented by 4-6 seedlings (replicates).

Measurements of pyridine nucleotide pools

An enzymatic cycling method described by Matsumura & Miyachi (1980) was used to measure pyridine nucleotides. The frozen roots were homogenized in a mortar with either 0.1 N NaOH (for reduced forms of the coenzymes) or 0.1 N HCl (for oxidized forms). To eliminate phenols from the extracts, 50 µl of a 1% polyvinylpyrrolidone-solution were added. The recovery of the oxidized and reduced coenzymes added externally to the homogenate was always between 81-98%.

Statistics

Statistical data analyses were performed using analysis of variance with the statistical package STATGRAPHICS (INTERSOFTLAND, USA).

RESULTS

The amounts of total pyridine nucleotides (PN) and non-phosphorylated ($\text{NAD}^+ + \text{NADH}$) PN in the roots of the control plants (not exposed to aluminium ions) decreased over the course of the experiment (Table 1). A decrease in phosphorylated PN and the ARC values was also observed in the control, but it was less pronounced (Table 1, Fig. 1A). In contrast, the CRC, NAD(P)H/NAD(P)^+ and $\text{NADP}^+/\text{NAD}^+$ ratios significantly increased throughout the 9 weeks of incubation of plants in the hydroponic culture (Fig. 1 B-D). The treatment of seedlings with Al significantly diminished the total PN contents in the roots. The reduction increased with an increase in the concentration of Al used and with the length of Al treatment (Table 1). Exposure to Al caused both quantitative and qualitative changes in the contents of particular coenzymes (Table 1).

TABLE 1. Effect of Al ($\text{Al(NO}_3)_3$) treatment for 3, 6 and 9 weeks on the pyridine nucleotide contents (nmol g⁻¹ fresh wt.; mean ± SD) of Scots pine roots.

	Al supply (mM)	NAD ⁺	NADH	ΣNAD(H)	NADP ⁺	NADPH	ΣNADP(H)	ΣPN
3 w e e k s	0.0	58.1±5.5 (50)	24.9±3.1 (22)	83.0±6.3 (72)	9.5±1.9 (8)	22.2±3.5 (19)	31.8±4.0 (27)	114.8±7.6 (100)
	0.5	46.7±5.6 (48)	21.9±3.3 (23)	68.7±6.5 (71)	9.8±1.9 (10)	18.2±4.1 (19)	28.0±4.6 (28)	96.7±8.0 (100)
	1.0	44.2±4.3 (49)	24.8±3.5 (28)	69.0±5.5 (77)	8.0±2.0 (9)	12.0±2.5 (15)	20.0±3.2 (23)	89.0±6.4 (100)
	2.0	29.1±3.1 (46)	17.9±2.7 (28)	47.0±4.1 (75)	6.5±1.3 (11)	9.0±2.5 (16)	15.6±2.5 (25)	62.7±4.8 (100)
	4.0	22.8±2.5 (38)	17.2±2.7 (29)	4.0±3.7 (67)	8.9±1.8 (15)	10.5±2.5 (18)	19.4±3.0 (33)	59.4±4.8 (100)
6 w e e k s	0.0	33.9±4.2 (39)	20.8±2.1 (24)	54.7±4.8 (62)	12.3±2.0 (14)	20.4±3.6 (23)	32.8±4.1 (37)	87.5±6.3 (100)
	0.5	29.0±2.9 (39)	22.8±2.8 (31)	51.9±3.5 (72)	9.12±1.3 (12)	13.1±2.0 (18)	22.2±2.4 (30)	74.2±4.2 (100)
	1.0	27.4±3.0 (39)	24.5±2.1 (31)	51.9±3.7 (72)	8.8±1.9 (13)	11.3±1.9 (17)	20.1±2.7 (29)	72.0±4.6 (100)
	2.0	21.5±1.7 (41)	10.1±1.6 (21)	31.7±2.3 (61)	8.1±2.4 (14)	12.1±1.5 (23)	20.2±2.8 (39)	51.8±3.7 (100)
	4.0	20.9±2.5 (42)	8.1±2.4 (16)	29.0±3.5 (59)	7.5±1.8 (15)	12.8±1.2 (26)	20.4±2.1 (41)	49.3±4.1 (100)
9 w e e k s	0.0	19.3±2.3 (31)	15.2±2.1 (22)	34.5±3.1 (54)	11.7±1.9 (18)	17.1±3.2 (27)	28.8±3.8 (45)	63.3±4.9 (100)
	0.5	18.6±1.8 (39)	12.0±1.8 (25)	30.6±2.6 (64)	6.7±0.9 (13)	10.8±1.2 (22)	17.4±1.6 (36)	48.0±2.98 (100)
	1.0	19.8±2.0 (56)	5.7±0.9 (16)	25.5±2.3 (72)	2.8±0.7 (8)	7.1±1.3 (20)	9.9±1.7 (28)	35.4±2.8 (100)
	2.0	15.5±1.4 (60)	3.8±0.7 (15)	19.3±1.5 (75)	1.6±0.4 (7.0)	4.9±0.7 (19)	6.6±0.9 (25)	25.9±1.8 (100)
	4.0	13.8±1.1 (65)	2.8±0.7 (13)	16.6±1.3 (77)	1.3±0.4 (6.0)	3.5±0.7 (16)	4.8±0.8 (22)	21.4±1.5 (100)

Data in parentheses indicate percent of the total content of pyridine nucleotides (PN).

After 3 weeks of Al stress, a significant decline in NAD^+ and a reciprocal increase the relative levels of NADH and NADP^+ in the total PN pool was found only in the roots of seedlings treated with 4 mM Al (Table 1). After 6 weeks, the levels of oxidized PN did not undergo any significant changes (Table 1). On the other hand, the NADH level was enhanced and NADPH level was reduced during incubation in 0.5 and 1.0 mM of Al (Table 1). After 9 weeks, the decrease in the contribution of NADP^+ and reduced coenzymes to the total PN pool was accompanied by a rise in NAD^+ level (Table 1).

The modulation of pyridine pool-size levels by Al stress corresponds to the changes of the redox state of the pyridine nucleotides. After 3 weeks of the Al action, the redox state and the phosphorylation capacity was altered dependent on the Al concentration, and a significant increase in catabolism and a decrease in anabolism could be observed in the roots of seedlings incubated in 2.0 and 4.0 mM of Al (Fig. 1). On the contrary, the production and consumption of redox equivalents were relatively constant in the samples treated with Al in different concentrations after 6 weeks (Fig. 1A, B). After 9 weeks, both the catabolic and anabolic couples of PN showed a distinctive pattern in the Al stress conditions with a high anabolic and low catabolic reduction charge, redox status and phosphorylation capacity (Fig. 1).

DISCUSSION

The results of this study demonstrate that depending on the concentration of Al used (0.5-4.0 mM) and the length of exposure (3-9 weeks), Al causes various changes in the amount and the redox status of PN in Scots pine roots.

Pyridine nucleotides are located in different subcellular compartments where they are involved in many biochemical processes. Most intracellular NAD is present in the oxidized form, while NADP exists predominantly in the reduced form (Yamamoto 1963). Scots pine is no exception to this rule whether treated with different concentrations of Al or not (Table 1).

Nine-week incubation of Scots pine seedlings in the nutrient solution without or with Al significantly reduced the amount of the total PN, and probably as a consequence, resulting in the differences in absolute contents of the non-phosphorylated and phosphorylated PN observed (Table 1). However, the sum of any particular coenzymes plays a much less important role in the regulation of metabolic processes than the PN redox state, and the latter changed in a specific manner during Al exposure.

Roots of the control plants (not exposed to Al) as well as plants treated with Al for 3 weeks showed significant de-

Fig. 1. Effect of different concentrations of Al ($\text{Al}(\text{NO}_3)_3$) on A) the anabolic reduction charges (ARC: $\text{NADPH} / (\text{NADP}^+ + \text{NADPH})$); B) catabolic reduction charges (CRC: $\text{NADH} / (\text{NAD}^+ + \text{NADH})$); C) $\text{NAD(P)H} / \text{NAD(P)}^+$ and D) $\text{NADP}^+ / \text{NAD}$ ratios. Length of Al exposure: 3, 6 and 9 weeks. Values were significantly different from controls at 0.05* and 0.01** confidence levels. c*, c** – values significantly different between the controls at 0.05 and 0.01 confidence levels, respectively.

creases in the ARC (Fig. 1A) and increases in the redox state (Fig. 1 B,C) and phosphorylation capacity (Fig. 1D). This disturbance in metabolism could be result of enhanced senescence with increasing time of plant culture in the nutrient solution and Al-treatment. Moreover, such a modification of the ratio of PN could be also caused by a rise in metabolic turnover due to repair mechanisms (Hampp et al. 1990).

In the case of plants treated with Al for 6 weeks, the dependence between production and consumption of redox equivalents and the $\text{NADP}^+/\text{NAD}^+$ ratio were not significantly different from the control plants (Fig. 1). The relative stability of the metabolic reactions could reflect the efficiency of the regulation mechanisms for the maintenance of the reduction charge, which in Scots pine roots still exists after 6 weeks of Al action.

Exposure of plants to Al for 9 weeks lead to a drastic decrease of the ratio of phosphorylated to non-phosphorylated PN (Fig. 1D). This could be a result of the inhibition of NAD-kinase activity (Ślaski 1989), and thus the transformation of NAD to NADP cannot take place. A significant reduction in the $\text{NAD(P)H}/\text{NAD(P)}^+$ ratio noted at the same time could also indicate a decrease in the total pool of reducing equivalents. (Fig. 1C).

After a period of 9 weeks of Al stress, the CRC values were significantly lowered by Al treatment (Fig. 1B). Considering that the dark respiration of roots was inhibited by Al treatment (J. Oleksyn, personal communication), the decrease of the NADH level in the PN pool could not have been caused by greater transfer of hydrogen to oxidative, energy-yielding processes. However, the reducing equivalents could take part in the detoxification of oxyradicals. Prolonged Al stress induces an enhancement of lipid peroxidation and causes formation of highly toxic oxygen free radicals (Cakmak & Horst 1991). An increase in activities of superoxide dismutase and peroxidase and a decrease of catalase activity indicates the presence of an antioxidant scavenging system in Al-treated roots (Cakmak & Horst 1991).

In contrast to the CRC, the reductive degree of phosphorylated PN was simultaneously stimulated by Al, resulting in a marked increase of the ARC (Fig. 1A). This might indicate an intensive potential of reducing power for synthetic reactions. However, a high redox state of NADP is characteristic for young, growing tissues (Yamamoto 1963) and not for advanced senescence, which was evident in control plants grown 9 weeks without Al. Therefore, a higher level of anabolic redox might reflect the acceleration of biosynthetic pathways to compensate for Al stress or that the compensating mechanism ceased to function.

Changes in the redox state of PN (Fig. 1) could be also result from disturbances in the reduction of Fe^{3+} to Fe^{2+} . In response to Al supply, Fe-deficiency chlorosis and an enhancement of Fe^{3+} reducing capacity were observed (Clark et al. 1981, Horst et al. 1992). Both reduced pyridine nucleotides NADH and NADPH were proposed as the electron donors for the enzymes involved in the reduction of Fe^{3+} to Fe^{2+} , (Craig & Crane 1981, Sijmons & Bienfait 1983, Sijmons et al. 1984, Qiu et al. 1985).

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WPLYW GLINU NA STATUS REDUKCYJNO-OKSYDACYJNY KORZENI SOSNY ZWYCZAJNEJ

STRESZCZENIE

Badano wpływ jonów glinowych (Al, Al(NO₃)₃) na poziom nukleotydów pirydynowych w korzeniach siewek sosny zwyczajnej traktowanych roztworami azotanu glinowego w stężeniach: 0.0 – kontrola, 0.5, 1.0, 2.0 i 4.0 mM (pH 4.2) przez 3, 6 i 9 tygodni. Stwierdzono, że zarówno kierunek jak i wielkość zmian w zawartości nukleotydów pirydynowych oraz w całkowitej puli ekwiwalentów redukcyjnych (NAD(P)H/NAD(P)⁺), katabolicznego ładunku redukcyjnego (NADH/(NAD⁺+ NADH)), anabolicznego ładunku redukcyjnego (NADPH/(NADP⁺+NADPH)) i pojemności fosforylacyjnej (NADP⁺/NAD⁺) zależały zarówno od wielkości zastosowanego stężenia Al jak i czasu oddziaływania. Jony glinowe powodowały zachwianie równowagi pomiędzy produkcją i konsumpcją ekwiwalentów redukcyjnych, prawdopodobnie w następstwie wzmożenia procesów metabolicznych prowadzących do uruchomienia mechanizmów kompensujących uszkodzenia wywołane przez Al i/lub przyspieszonego starzenia się korzeni.

SŁOWA KLUCZOWE: Al, korzenie, nukleotydy pirydynowe, *Pinus sylvestris*.