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Research article

Expression of root glutamate dehydrogenase genes in tobacco plants subjected to boron deprivation

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ABSTRACT

Recently it has been reported that boron (B) deficiency increases the expression of *Nicotiana tabacum* asparagine synthetase (*AS*) gene in roots, and that AS might play a main role as a detoxifying mechanism to convert ammonium into asparagine. Interestingly, glutamate dehydrogenase (*GDH*) genes, *Ntgdh-NAD;A1* and *Ntgdh-NAD;B2*, were up-regulated when tobacco roots were subjected to B deprivation for 8 and 24 h. In addition, aminating and deaminating GDH (EC 1.4.1.2) activities were higher in B-deficient than in B-sufficient plants after 24 h of B deficiency. Ammonium concentrations were kept sufficiently low and with similar values in B-deficient roots when compared to control. Glucose and fructose contents decreased after 24 h of B deprivation. This drop in hexoses, which was corroborated by metabolomic analysis, correlated with higher *GDH* gene expression. Furthermore, metabolomic profiling showed that concentrations of several organic acids, phenolics, and amino acids increased after 24 h of B deficiency. Our results suggest that GDH enzyme plays an important role in metabolic acclimation of tobacco roots to B deprivation. A putative model to explain these results is proposed and discussed.

1. Introduction

Environmental changes can affect C and N assimilation by modifying their internal ratio of C to N [1]. To ensure homeostasis between these two macronutrients, plant must regulate the crosstalk between both elements. A crucial step in this complex coordination of C and N metabolism is performed by conversion of glutamate (Glu) to 2-oxoglutarate (2-OG), and vice versa, which can be catalyzed by glutamate dehydrogenase (GDH; EC 1.4.1.2). Certainly, GDH catalyzes the oxidative deamination of Glu to 2-OG as well as the reductive amination of 2-OG to Glu [2,3]. Hence, this enzyme can contribute in both carbon skeleton supply (2-OG) and ammonium assimilation in plants [4].

GDH is a hexameric protein consisting of two subunits, α and β, that hardly differ in molecular mass (about 43 kDa each) [5]. Several GDH isoenzymes are a result of the random assembly of these subunits [1]. In *Nicotiana tabacum* three cDNAs have been cloned: *Ntgdh-NAD;A1* encoding the α-subunit, and *Ntgdh-NAD;B1* and *Ntgdh-NAD;B2* encoding the β-subunit [6].

Many abiotic stresses such as drought, salinity, extreme temperatures, and metal toxicity influence GDH activity, and, interestingly, the deaminating role of GDH is enhanced under C-limiting conditions [1,3].

Boron (B) is an essential element for vascular plants with a structural role to form borate esters with apiose residues of rhamnogalacturonan II [7,8]. Furthermore, B is also involved in a great variety of physiological processes. Thus, B availability affects cytoskeleton and membranes [9], secondary metabolism and oxidative stress [10–12], nitrogen fixation [13], nitrate assimilation [14–16], and root development [17,18], among others. There is increasing evidence that B deficiency affects gene expression in processes such as oxidative stress [12], B uptake [19], and cell wall [20]. Recently, it has been reported that under B deprivation,

Abbreviations: 2-OG, 2-oxoglutarate; AS, asparagine synthetase; Asn, asparagine; Asp, aspartate; B, boron; CM, culture medium; GDH, glutamate dehydrogenase; Glu, glutamate; GS, glutamine synthetase; GOGAT, glutamate synthase.

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asparagine synthetase (*AS*) gene expression and asparagine (Asn) levels increased in tobacco roots and that AS might play a role as a detoxifying mechanism to convert ammonium into Asn [15,21].

The aim of this work was to ascertain whether *GDH* gene expression and GDH activity are affected by B deficiency given that this enzyme is involved in ammonium metabolism. For this purpose, the transcript levels of *Ntgdh-NAD;A1* and *Ntgdh-NAD;B2* genes, GDH activity, and several C and N metabolites were analyzed in roots of tobacco plants subjected to B deprivation.

2. Results and discussion

2.1. Root Ntgdh-NAD;A1 and Ntgdh-NAD;B2 gene expressions

Low and high boron (B) levels seem to play a role in gene expression regulation of several plant genes [12,20,22]. Recently, it has been reported that *AS* gene expression increased in roots when tobacco plants were subjected to B deficiency [21]. Interestingly, expression levels of *Ntgdh-NAD;A1* and *Ntgdh-NAD;B2* genes were higher in B-deficient than in B-sufficient roots at 8 and 24 h (Fig. 1A, B). The expression of *GDH* genes is also affected by salinity, cadmium toxicity, extreme temperatures [23], and hypoxia [24].

2.2. GDH activities

Root GDH activity was significantly higher in B-deficient than in B-sufficient plants after 24 h of B deprivation (Fig. 2). Consistent with other authors [25], in vitro aminating reaction was quantitatively more significant than the deaminating one (Fig. 2A, B). Nonetheless, the in vitro aminating/deaminating GDH activities do not reflect the in vivo activities and, indeed, studies support that oxidative deamination of GDH is the major activity in vivo [2,25,26]. Recently the deaminating role of the distinct GDH isoenzymes has been established by overexpression of either α or β subunits of GDH [25,27]. Interestingly, *Ntgdh-NAD;A1* and *Ntgdh-NAD;B2* genes, which encode α and β subunits of GDH respectively [6], were up-regulated under B deficiency (Fig. 1A, B), which suggests that the in vivo deaminating reaction of GDH becomes important under this nutritional stress.

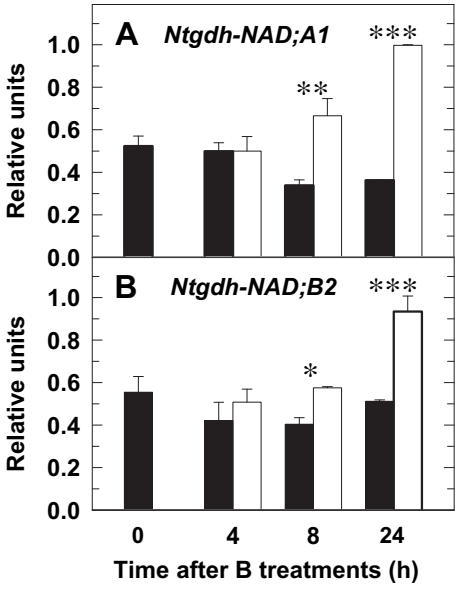

Fig. 1. Quantitative real-time PCR analyses of root transcript levels for *Ntgdh-NAD;A1* (A) and *Ntgdh-NAD;B2* (B) genes of tobacco. Plants were subjected (open bars) or not (filled bars) to B deprivation for a 24-h period. For more details see Material and methods. The results are given as means ± SD ($n = 5$ separate plants). Asterisks indicate statistically significant differences between plants treated or not with B (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$).

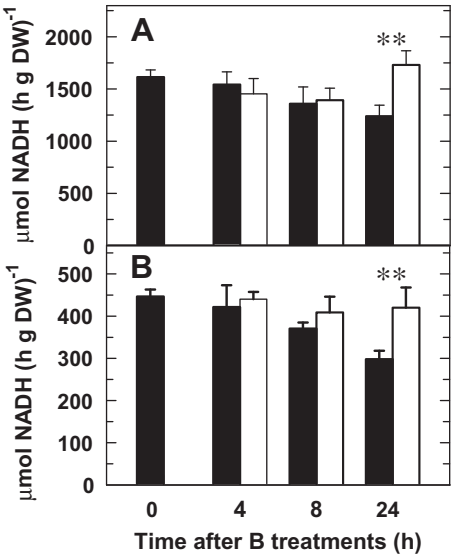

Fig. 2. Effects of B deprivation on root GDH activities in tobacco plants. Aminating (A) and deaminating (B) GDH activities were determined in roots from B-sufficient (filled bars) and B-deficient (open bars) plants corresponding to Fig. 1. The results are given as means ± SD ($n = 5$ separate plants). Asterisks indicate statistically significant differences between plants treated or not with B (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$).

2.3. Metabolite analyses

After 24 h of B deprivation, unlike sucrose (Fig. 3E), concentrations of glucose and fructose were significantly lower in B-deficient plants (Fig. 3C, D). These results were corroborated by metabolomic analysis (Table 1). This decrease could be explained because hexoses are converted into erythrose-4-phosphate, via oxidative pentose phosphate route, and phosphoenolpyruvate, via glycolysis, which serve as precursors of the shikimic acid pathway in the synthesis of phenolic compounds. In agreement with this explanation 3-phosphoglycerate, shikimate, and phenolics increased after 24 h of B deficiency (Table 1).

As B deprivation leads to a decrease in root concentrations of glucose and fructose this result correlates with the increased concentration of citrate measured after 24 h of B deprivation (Table 1 and Fig. 3C, D). In addition, the higher levels of *Ntgdh-NAD;A1* and *Ntgdh-NAD;B2* transcripts and of deaminating GDH activity would facilitate the supply of 2-OG into the Krebs cycle under B deficiency (Figs. 1A, B and 2B). Indeed, it has been extensively reported that the 2-OG produced by GDH enters the Krebs cycle as an alternative C source during C-limiting conditions [1,3,4].

Metabolite profiling showed that asparagine (Asn) concentration ratio of B-deficient to B-sufficient plants was 6.75-fold after 24 h of B deprivation (Table 1). Increased root Asn content under B deprivation is consistent with results previously reported in tobacco plants [15], and also with the C-limiting conditions (Table 1 and Fig. 3C, D) [28]. Other mineral deficiencies such as potassium, sulfate, and phosphate can increase Asn concentrations [29,30]. Furthermore, higher levels of other amino acids were found in B-deficient roots after 24 h of B treatment (Table 1).

Increased levels of osmoregulators, such as amino acids, might correct the osmotic imbalance caused by lower concentration of soluble carbohydrates under B deficiency (Fig. 3F). Interestingly, B-deficient roots had a higher Asp content (Table 1), which has