

Amino Acid Supplementation Effects on the Secondary Metabolites Profile in Nitrate Reductase Double-deficient *Arabidopsis thaliana*

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It was recently shown that nitrate reductase (NR) deficiency leads to a reduced synthesis of nitric oxide (NO), a key signal in plant defense responses. NR is an essential enzyme for nitrogen assimilation, and its deficiency leads to a decreased incorporation of nitrogen into amino acids and may affect the production of nitrogen-derived secondary metabolites, frequently associated with plant protection against environmental stresses. The aim of this work was to evaluate if amino acid supplementation of NR double-deficient mutant plants (*nia1 nia2*) could exert influence in the profile of secondary metabolites. NR-deficient plants were supplemented with 10 mM of glutamine or arginine and leaves and roots of 30 day-old wild-type and *nia1 nia2* plants were analyzed for amino acids and secondary compounds by reverse phase HPLC. Leaves of *nia1 nia2* presented lower levels of all individual amino acids than wild-type, but no differences occurred in roots. In *nia1 nia2* plants treated with glutamine or arginine, total amino acid content was recovered in leaves and reached levels higher than wild-type in roots. Outstanding differences in the secondary metabolite profile were found between wild-type and *nia1 nia2* plants. The lower levels of secondary compounds in NR-deficient plants were not recovered after amino acid treatment, while in roots a small increase occurred, but it remained lower than that of wild-type. In conclusion, amino acid supplementation was not able to recover the secondary metabolites profile in NR-deficient plants, supporting the proposition that NO is the signal for the synthesis of defense-related secondary metabolites.

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