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FORMATION OF ARGININE FROM ORNITHINE AND CYANAMIDE IN BUSH BEANS (PHASEOLUS VULGARIS) AND RAPE (BRASSICA NAPUS)

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ABSTRACT

The uptake of cyanamide by plants results in an increase of basic free amino acids, especially arginine. The enhanced increase in arginine content by additional doses of ornithine to cyanamide nutrition suggests that the usual synthesis of arginine (along the course: ornithine --> citrulline --> arginino succinate) is shortened to ornithine --> arginine. Additionally, there is a reduced activity of arginase as a consequence of cyanamide application. Cyanamide is obviously metabolized by plants to lysine and histidine, and after supply of ornithine via the nutrient solution incorporated into arginine.

INTRODUCTION

In a previous study with small doses of cyanamide, we could point out a favoured synthesis of arginine (1). So, cyanamide was well metabolized by plants and supported

the synthesis of some amino acids e. g. arginine, compared with other nitrogen forms like nitrate, ammonium and urea. This behaviour suggests that cyanamide is an "active nitrogen form" which does not enter the "main-gate" of the nitrogen pool via the amides asparagine and especially glutamine (2). Other basic amino acids like ornithine, lysine and histidine were enriched, too. Ornithine as an intermediate in the arginine synthesis (Ornithine cycle; 3) could play a special role in the incorporation of cyanamide.

Ornithine like arginine belongs to the glutamate family and is only scarcely found in healthy plant parts (4, 5, 6). Increases of ornithine levels are in most cases due to disturbances of plant metabolism (7) like deficiencies (8), infections (9), or injuries (10). The free amino acid ornithine is hardly mentioned at all, e.g. in wheat kernels (11), oil seeds and peas (12), fruits (13), perennials (14), stems (15), seeds and leaves of orpine (16) and potatoes (17). In roots ornithine is scarcely found (7, 18, 19).

In spite of these facts we expected an increase of ornithine by cyanamide nutrition with respect to earlier results (20) because of the close relationship of ornithine and arginine formation. According to Lignowski and Splittstoesser (21) arginine is metabolized by Cucurbita seedlings to urea and ornithine. They also made evident the direct uptake of urea and ornithine. Some of the carbon from ornithine was found in arginine.

The following investigations concerns the rapid incorporation of cyanamide into the metabolism of plants. The role of ornithine in the inversion of the ornithine cycle (3, 22) was studied to prove the hypothesis, that plants may also catalyse the well known chemical reaction: the synthesis of arginine from ornithine and cyanamide (23).

MATERIAL AND METHODS

Rape plants (Brassica napus L.) were grown in a nutrient solution according to Brezeale (24) up to a height of

Table 1: Free amino acids after ornithine and cyanamide application in green rape

Plant part	Treatments	GAB	ORN	LYS	HIS	ARG	Arginase Activity abs.	Arginase Activity rel.
		µM amino acid/1 g DM						
Leaves	NO_3	8.6	tr.	1.2	tr.	0.8	300	100
	NO_3 + Orn	6.0	tr.	0.8	0.4	0.8	200	67
	Cy	3.0	tr.	8.6	5.4	6.6	180	60
	Cy + Orn	2.0	tr.	8.4	5.3	12.2	100	33
Stems	NO_3	21.6	tr.	0.5	tr.	0.3	72	100
	NO_3 + Orn	13.1	tr.	0.6	0.5	0.3	60	83
	Cy	6.1	tr.	1.5	1.6	0.9	40	56
	Cy + Orn	4.4	0.3	1.3	1.8	4.2	32	44
Roots	NO_3	66.3	0.5	2.5	1.0	1.5	60	100
	NO_3 + Orn	40.5	5.6	2.3	0.7	8.7	56	93
	Cy	33.6	tr.	4.5	2.0	1.5	32	53
	Cy + Orn	41.5	28.9	7.8	tr.	33.4	28	47

GAB = γ-amino butyric acid
tr = traces = reliable but not numerically assessable amounts (ca. 0.1 µmol per gramm dry matter)

25 - 30 cm. Thereafter they were transferred to a similar nutrient solution with 100 mg nitrogen per liter as nitrate or cyanamide respectively. Every treatment was divided into pots with and without ornithine (0.01 mol/1). The plants were harvested after 4 days of treatment.

Analytical procedures:

a) Qualitative chromatography according to Wünsch and Amberger (20)
b) Quantitative chromatography for free amino acids according to Schaller and Wünsch (25) and for arginase activity according to Hagan and Dallam (26)

RESULTS AND DISCUSSION

In roots and leaves of bush beans (these results are considered as preliminary and not recorded), an increase of arginine content by cyanamide could be recognized by qualitative chromatography. Additional doses of ornithine enhanced this effect.

In rape, ornithine was found in all parts of the plants. In roots traces of ornithine were detected after ornithine applications. However, when applied together with cyanamide the ornithine content in roots is raised fivefold. The striking increase of arginine just by ornithine application (1.5 -> 8.7 µmol) is raised even twentyfold (1.5 ->33.4 µmol) due to additional feeding of cyanamide. In stems and leaves the known effect of cyanamide on the arginine synthesis (20) is anew confirmed again, but in case of added ornithine, the synthesis of arginine is enhanced further. This supports our hypothesis stated above that cyanamide enhances arginine formation, being increased additionally by ornithine application The contents of other basic amino acids like histidine and lysine are also increased by 3 to 10 fold by cyanamide but not by either ornithine or cyanamide plus ornithine.

The γ-amino butyric acid (GAB) level is reduced in nearly all treatments. It is suggested that in the presence of ornithine cyanamide especially influences the synthesis pathway: glutamic acid ----> γ-amino butyric acid (catalysed by glutamic acid decarboxylase, 27).

The activity of arginase is reduced by ornithine as well as by cyanamide applications in the respective plant parts. When supplied together, this effect is enhanced. The striking increase in arginine synthesis in rape roots does not seem to be the result of enzyme inhibition (the arginase activity is only reduced by 10 %) rather than of a direct arginin formation (20 fold increase).

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