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BRIEF COMMUNICATION

Variation in the amino acid concentration during development of *Canavalia ensiformes*

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Abstract

The distribution of amino acids in distinct tissues of *Canavalia ensiformes* was determined during the life cycle of the plant. Glycine was shown to be the main amino acid in mature seeds, while the nonprotein amino acid canavanine exhibited a high concentration in 7-d-old seedlings. Canavanine was lower in the seeds when compared to other tissues analyzed. This does not support the nitrogen-storage function of canavanine, however, it suggests that it is involved in the translocation of amines during the early stages of the development.

Additional key words: nitrogen, protein, legume.

Amino acids can be essentially incorporated into proteins, but can also play major roles in the general metabolism in the soluble form (Rai 2002, Nayyar and Walia 2003, Tsai *et al*. 2003). Nonprotein amino acids have significant roles in medicine, nutrition and agriculture in general, and in some cases are toxic to humans and domestic animals (Bell 2003, Kirakosyan *et al*. 2003). L-Canavanine, a L-arginine analogue, is a nonprotein amino acid that has been shown to be the predominant soluble amino acid in some plant species (Rosenthal 1992). A high accumulation of canavanine in the seeds is primarily used during germination, which facilitates the translocation of nitrogen from cotyledons to the growing parts of the seedling, thus confirming the hypothesis that canavanine functions as a nitrogen storage compound (Rosenthal *et al*. 1988). In *Canavalia ensiformes*, canavanine accumulates in seeds and is present in vegetative organs, while in *Canavalia lineata* it is abundant in leaves accumulating in the vacuole (Hwang *et al*. 1996a). In tissue culture of *C. lineata*, canavanine was detected almost exclusively in the leaf tissue (Hwang *et al*. 1996b). In leguminous plant species, canavanine is utilized as a storage form of nitrogen and is metabolized to ammonia and canaline, with the latter being toxic and rapidly degraded to form homoserine, which is also involved in the aspartate metabolic pathway (Azevedo and Lea 2001, Azevedo 2002) and ammonia (Rosenthal 1992). In this study, we monitored the presence of canavanine in the tissues of *C. ensiformes* during the life cycle of the plant, in order to correlate its synthesis, storage, and remobilization.

Commercial seeds of *Canavalia ensiformes* (L.) were supplied by Piraí Sementes (Piracicaba, Brazil). Seeds were germinated on moist filter paper and then transferred to a glasshouse maintained at 25 - 30 ºC with 16-h photoperiod and maximum irradiance reaching 2050 µmol m^{-2} s^{-1}. The plants were grown for 100 d in Hoagland's nutrient solution containing 15 mM nitrate.

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Abbreviations: S0 - 7-d-old seedlings; S1 - vegetative stage; S2 - early flowering stage; S3 - fruiting stage.

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Three replicates were used for each developmental stage analyzed (7-d-old seedlings [S0], vegetative [50-d, S1], early flowering [75-80-d, S2] and fruiting [100-d, S3]), with roots, leaves and shoots harvested directly into liquid N_2 and kept at -80 ºC for further analysis. The extraction of amino acids was carried out as described by Bieleski and Turner (1966). The *o*-phthaldialdehyde (OPA) derivatives of the soluble amino acids were then separated and analyzed by reverse-phase high-performance liquid chromatography (HPLC) as described by Azevedo *et al.* (2003). The experiments had four replicates and appropriate statistical analyses of variance and averages were applied (Tukey test).

The contents of soluble amino acids in the tissues analyzed varied during the plant development with the roots exhibiting the highest concentrations in S1 and S3, whereas the leaves exhibited the highest concentrations in S0 and S2. In mature seeds, the total soluble amino acid concentration [4.86 µmol g^{-1}(FM)] was in the same range as in cotyledons and stems in S0 stage (Table 1). Glycine was shown to be the predominant soluble amino acid in mature seeds of *C. ensiformes* corresponding to 92.9 % of the total pool of amino acids, whereas glutamate, canavanine and valine represented 2.74, 0.95, and 3.41 %, respectively (Table 2). However, when amino acids incorporated into proteins were analyzed, the seeds of *C. ensiformes* exhibited high concentrations of glutamate + glutamine, arginine and serine (23, 15.7, and 13 %, respectively) (Table 2). Other amino acids such as histidine (10.29 %) and aspartate + asparagine (7.12 %) also exhibited significant concentrations. The high concentrations of the aspartate derived amino acids lysine (7.07 %) and threonine (7.61 %) also observed in the seeds (Table 2), indicate that a reduced rate of catabolism of these amino acids, particularly lysine, occurs in this plant species, as observed for other legume seeds (Azevedo *et al.* 1997, Cunha-Lima *et al.* 2003a,b).

Table 1. Soluble amino acid (SAA) concentrations [µmol g^{-1} (FM)] and canavanine concentration [% of total soluble amino acid pool] determined during the development *C. ensiformes*. Values represent means of four replicates. Values for the same stage sharing the same letter did not differ significantly at 5 % (Tukey test). ND - not detectable.

Stage	Leaf SAA	canavanine	Cotyledon SAA	canavanine	Stem SAA	canavanine	Root SAA	canavanine
S0	2.15b	57.51 ± 0.03	4.44a	23.02 ± 0.02	5.19a	16.86 ± 0.02	2.81b	23.10 ± 0.01
S1	17.96b	ND			22.18ab	ND	27.90a	ND
S2	20.14a	15.96 ± 0.02			11.96b	1.14 ± 0.01	13.51b	ND
S3	9.93c	ND			12.96b	ND	25.36a	ND

Table 2. Soluble amino acids concentration [% of the total pool ± SD] and amino acids incorporated into proteins [% of total pool ± SD] in *C. ensiformes* dry mature seeds. Values represent means of four replicates. ND - not detectable.

Amino acid	Soluble amino acids	Amino acids in proteins
Aspartate + asparagine	ND	7.12 ± 0.02
Glutamate + glutamine	2.74 ± 0.01	23.04 ± 0.03
Serine	ND	13.01 ± 0.02
Histidine	ND	10.29 ± 0.01
Canavanine	0.95 ± 0.02	ND
Glycine	92.90 ± 0.03	2.02 ± 0.01
Threonine	ND	7.61 ± 0.02
Arginine	ND	15.78 ± 0.03
Alanine	ND	5.83 ± 0.01
Methionine	ND	0.34 ± 0.01
Valine	3.41 ±0 .02	0.22 ± 0.01
Phenylalanine	ND	0.42 ± 0.02
Isoleucine	ND	1.06 ± 0.01
Leucine	ND	0.42 ± 0.01
Lysine	ND	7.07 ± 0.02

The relatively low concentration of canavanine, when compared to the predominant amino acids in the seeds, suggested that in *C. ensiformes* the amino acids glycine, glutamate and glutamine are the main amino acids storing amino nitrogen. Although canavanine has been reported to be in high concentrations in *C. ensiformes* seeds, the concentration has been shown to vary depending on the genotype tested. Ramírez *et al.* (2000) observed variation between 0.79 and 3.65 % in *C. ensiformes*, whereas in *Medicago sativa* the variation was between 0.6 and 1.6 %, with canavanine being metabolized in the early stages of seedling development, thus increasing the concentrations of arginine and asparagine (Miersch *et al.* 1991).

Canavanine and asparagine were the dominant soluble amino acids in leaves, cotyledonary leaves, shoots and roots of 7-d-old seedlings with canavanine corresponding to 57.51, 23.02, 16.82, and 23.10 %, respectively, of the total soluble amino acids pool, and asparagine corresponding to 33.86, 44.43, 57.96, and 50.24 %, respectively, of the total soluble amino acids pool (Table 3). Glycine and glutamine concentrations were shown to be reduced (Table 3) when compared to the concentrations observed in mature seeds (Table 2).

Rosenthal (1992) reported that canavanine could store up to 13 % of the nitrogen in the seeds of *C. ensiformes*. The high concentrations of canavanine observed in the seedlings of the *C. ensiformes* genotype tested in this study, particularly in the shoots, suggest a role in the translocation of metabolized nitrogen, instead of a nitrogen storage compound in the seeds. Furthermore, the low content of canavanine observed in the seeds cannot account for that observed in seedlings, indicating that there is an elevated synthesis of canavanine in the early stages of development. On the other hand, in *M. sativa* the concentration of canavanine was reduced in the early stages of development (Miersch 1992). Hwang *et al.* (1996a) reported high concentrations of canavanine in vegetative organs of young seedlings of *Canavalia lineata*, possibly originated from the cotyledons and indicating an important role of canavanine as a metabolized nitrogen-storage compound. In this study, the high concentration of canavanine in the cotyledons of 7-d-old seedlings (Table 1) may be a result of a higher rate of canavanine synthesis in this tissue, which is then translocated to the other tissues, but not a remobilization of this compound.

The high concentration of soluble asparagine observed in 7-d-old seedlings (Table 3) in comparison to the lower concentration observed in the seeds (Table 2), suggests that this amino acid is synthesized in the early stages of development of *C. ensiformes*. In contrast, glutamine exhibited the opposite behavior. This variation in asparagine and glutamine concentrations during the plant development has been previously reported in *Linum usitatissmum* (Wanasundara *et al*. 1999). Such variations are expected depending on the plant species and during seasonal changes, with alterations in the synthesis and distribution of nitrogen storing compounds (Gloser 2002).

In this work, we observed that the soluble amino acid concentration varied considerably during plant development, with much reduced concentration in S0, but much higher concentrations in the other stages. Canavanine was shown to be mainly present in young seedlings, which does not agree with its function as a major nitrogen storing compound, but it clearly shows an important role in the translocation of metabolized nitrogen during the early stages of development until the early flowering stage (S2, Table 1). Moreover, the low concentrations of canavanine in the seeds and the trace concentration during the reproductive stage (S3, Table 1), also confirm that the role of this non-protein amino acid in the *C. ensiformes* genotype used in this study is limited to the translocation and partitioning of amino nitrogen during the early and vegetative growth stages.

Table 3. Soluble amino acids [% of total pool ± SD] in 7-d-old seedlings of *C. ensiformes*. Values represent means of four replicates. Values for the same stage sharing the same letter did not differ significantly at 5 % (Tukey test). ND - not detectable.

Amino acid	Leaf	Cotyledon	Stem	Root
Aspartate	2.69 ± 0.01a	1.45 ± 0.02b	1.38 ± 0.02b	0.20 ± 0.02c
Glutamate	3.19 ± 0.03b	4.85 ± 0.01a	0.40 ± 0.01c	0.13 ± 0.02c
Asparagine	33.86 ± 0.02c	44.43 ± 0.02b	57.96 ± 0.01^{a}	50.24 ± 0.01ab
Serine	ND	5.38 ± 0.02a	ND	2.69 ± 0.01b
Histidine	1.17 ± 0.02ab	1.81 ± 0.01a	0.96 ± 0.01b	0.49 ± 0.02c
Glutamine	1.19 ± 0.01c	3.01 ± 0.01b	1.83 ± 0.02c	4.72 ± 0.01a
Canavanine	57.51 ± 0.03a	23.02 ± 0.02b	16.86 ± 0.02c	23.10 ± 0.01b
Glycine	ND	3.21 ± 0.01c	7.40 ± 0.01b	10.27 ± 0.02a
Alanine	ND	ND	0.45 ± 0.02a	0.53 ± 0.03a
Tyrosine	ND	0.42 ± 0.01a	0.14 ± 0.02b	ND
Methionine	ND	5.32 ± 0.02a	5.25 ± 0.02a	0.17 ± 0.01b
Valine	0.38 ± 0.02bc	1.72 ± 0.02b	0.10 ± 0.01c	4.76 ± 0.03a
Phenylalanine	ND	0.06 ± 0.01c	2.40 ± 0.01a	0.29 ± 0.02b
Isoleucine	ND	3.49 ± 0.01a	2.97 ± 0.02b	2.39 ± 0.02b
Leucine	ND	1.80 ± 0.02a	1.88 ± 0.02a	ND

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