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ACCUMULATION OF VICINE AND CONVICINE IN *VICIA FABA* AND *V. NARBONENSIS*

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Key Word Index—*Vicia faba*; *Vicia narbonensis*; Fabaceae; faba bean; distribution; pyrimidone glucopyranosides; vicine; convicine.

Abstract—The pyrimidone glucopyranosides vicine and convicine were found in the roots of *V. faba* and *V. narbonensis*. The total amount present in the roots per plant at flowering exceeded that in one mature seed of *V. narbonensis*, indicating net synthesis. In *V. faba* the amount of pyrimidone glucopyranosides delivered per seed could account for the amount in the roots, but only if inter-conversion of the two compounds occurred. Stems contained very low levels of these compounds, and only in the first harvest of *V. faba*, whereas leaf laminae, pod walls and funicles lacked detectable amounts. A transitory peak was found in testae, which declined as accumulation took place in the cotyledons. In both testae and cotyledons the accumulation of pyrimidone glucopyranosides took place early in the growth of such tissues. The amounts accumulating in seeds, and particularly in testae and cotyledons, were much greater in *V. faba* than in *V. narbonensis*. Grafting between a low pyrimidone glucopyranoside mutant and its parent line demonstrated that the roots do not influence the concentration in the seeds. The probable site of synthesis for the developing fruits is the testa, with some additional synthesis in vegetative tissues leading to accumulation in the roots, at least in *V. narbonensis*.

INTRODUCTION

Given the current interest in reducing or eliminating legume anti-nutritional factors by genetic means, it is important to understand their biology. Information on their location within the plant can lead to insights into their role. Sites and timing of accumulation may indicate which tissues could gain protection from their presence, and hence lead to an assessment of the risks involved in their removal by genetic means. Patterns of accumulation and dispersal may also point to potential metabolic roles, for example as sources of metabolizable nitrogen. Such studies may also lead to the identification of sites of synthesis, an essential first step towards a molecular understanding of biosynthesis.

Among the N-rich secondary compounds in the Fabaceae, each has its own pattern of synthesis, redistribution and accumulation. The quinolidizine alkaloids in lupins, for example, are synthesized and accumulated in vegetative tissue and mobilized to developing seeds [1] without significant levels in root tissue [2]. In faba beans, the non-protein amino acid L-3,4-dihydroxyphenylalanine is almost absent from the seeds and is rapidly synthesized in seedlings [3]. The neurotoxic non-protein amino acid 3-*N*-oxalyl-L-2,3-diaminopropanoic acid in *Lathyrus* species, the causative agent [5], accumulating to high levels (up to 2.5 g $100\,g^{-1}$) in the dry seed [6]. Low vicine (2,6 diamino-5(β-D-glucopyranosyloxy)-4-pyrimidone) and convicine (6 amino-2-hydroxy-5 (β-D-glucopyranosyloxy)-4-pyrimidone) (see Fig. 1 for structures) lines of *V. faba* L. have been found [7] or induced through mutagenesis [8, 9]. The reduction of these compounds in this major crop will have benefits for human nutrition through the elimination of favism, a haemolytic anaemia known to be induced by these compounds [10], and for the use of faba beans in animal feed, particularly laying hens, where deleterious effects are well documented [11]. These compounds are known to accumulate early in seed development, to a maximum of 4 g $100\,g^{-1}$ dry matter [12]. They may be capable of reducing larval penetration and survival of one storage bruchid species [13], and have been reported to have anti-fungal activity [14]. Pod tissues have been identified as a site of biosynthesis [15], but a comprehensive study of the distribution and accumulation of these compounds in *V. faba* plants has not been reported.

RESULTS AND DISCUSSION

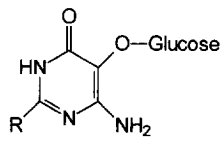

Fig. 1. The structures of the pyrimidone glucopyranosides vicine ($R = NH_2$) and convicine ($R = OH$).

6.0 mg pyrimidone glucopyranosides in the resting seed (Table 2). This reduced to *ca* 0.2 mg during flowering and fruiting. In contrast, the roots held *ca* 2 mg of each pyrimidone glucopyranoside during this period, much in excess of that held in the radicle of the resting seed. As the root system grew, the amount of vicine did not change significantly, indicating that no net synthesis occurred. The stem and petiole samples contained more pyrimidone glucopyranosides at flowering (growth stage 1) than was found in the plumule of a resting seed, but at later growth stages the levels fell below the limit of detection. No pyrimidone glucopyranosides were detected in any sample of leaf laminae. The total amount of vicine in the vegetative parts of the growing plant was less than that in a resting seed, but the vegetative parts held more convicine than that delivered by a resting seed. However, when both compounds are considered together the total amount of pyrimidone glucopyranosides in the vegetative parts of a growing plant was below 5 mg, less than the 6 mg of the resting seed. Consequently, there are two possible explanations for the observed amounts of vicine and convicine in vegetative tissues of *V. faba* plants. The first is that vicine and convicine are remobilized from cotyledons to the tissues of young plants and that inter-conversion takes place. Such inter-conversion requires one transamination. The second explanation is that loss of vicine and new net synthesis of convicine takes place. On the evidence presented here, no distinction can be made between these alternatives.

In *V. narbonensis* L. (Table 3), the total amount of pyrimidone glucopyranosides in the remaining cotyledons attached to the roots of flowering plants is less than that in resting seeds. Neither stem and petiole nor leaf lamina samples contained detectable pyrimidone glucopyranosides. The roots contained less vicine and convicine than those of *V. faba* but as the root system was smaller, the concentration (0.1 g $100\ g^{-1}$) was similar to *V. faba*. In *V. narbonensis ca* 1.3 mg of total pyrimidone glucopyranosides were found per flowering plant, exceeding the 0.14 mg

Table 1. Definitions of growth stages of *Vicia faba* cv Troy and *V. narbonensis* accession 557 plants

	Growth stage						
	1	2	3	4	5	6	7
Seed development	—	very early	early	mid	almost filled	filled	mature
Days–*V. faba*	42	—	73	79	—	94	108
Days–*V. narbonensis*	42	55	55	55	55	79	108

Two replicates of 5 plants each were used with the following two exceptions. Stem and leaf lamina samples were taken from a single replicate. Due to a lack of synchrony of fruit development of *V. narbonensis*, for growth stages 2–5 one collection of pods (17, 15, 18 and 11 pods respectively) was taken from 30 plants on day 55, sorted into growth stages and the data on vicine and convicine content adjusted to give the equivalent of 20 pods, to allow comparison with other harvests.

Table 2. The amount of pyrimidone glucopyranosides (mg $plant^{-1}$) in vegetative tissues of *V. faba* cv Troy

Compound / Tissue	Growth stage					
	Dry seed*	1	3	4	6	LSD
Vicine						
Sown cotyledon	4.63	0.22	0.34	0.20	0.12	ns
Root or radicle	0.26	1.87	2.29	2.01	2.44	ns
Stem and petioles or plumule	0.11	0.90	nd†	nd	nd	‡
Total	5.01	2.99	2.63	2.21	2.56	
Convicine						
Sown cotyledon	0.93	0.04	0.08	0.04	0.02	0.02
Root or radicle	0.01	1.87	2.16	1.64	1.96	ns
Stem and petioles or plumule	0.01	0.06	nd	nd	nd	‡
Total	0.95	1.97	2.24	1.68	1.98	

Table 3. Distribution of pyrimidone glucopyranosides in vegetative tissues of flowering *V. narbonensis* accession 557 compared to a resting seed

	Vicine (mg plant^{-1})		Convicine (mg plant^{-1})	
Tissue	Dry seed*	Flowering plant	Dry seed*	Flowering plant
Sown cotyledon	0.03	0.01	tr†	0.01
Root or radicle	0.09	0.68	<0.01	0.59
Stem and petiole or plumule	0.03	nd	<0.01	nd
Total	0.14	0.69	<0.01	0.60

*Data from ref. [17].
†Trace.

present in a resting seed about 10-fold. This rules out remobilization and inter-conversion as the source of pyrimidone glucopyranosides in the vegetative parts of the growing plant of *V. narbonensis* and demonstrates that net synthesis must take place.

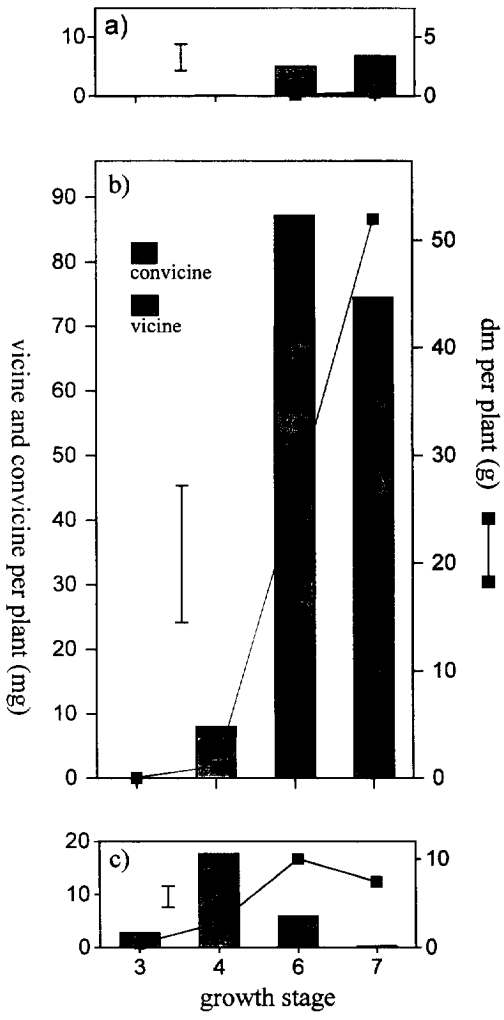

Fig. 2. The accumulation of pyrimidone glucopyranosides and total dry matter in seeds from early seed growth to maturity in

Pyrimidone glucopyranosides in fruiting tissues

Pyrimidone glucopyranosides were not detected in any pod wall or funicle sample. The amounts of vicine and convicine found in testae, cotyledons and seed axes of *V. faba* and *V. narbonensis* are shown in Figs. 2 and 3. There was a transitory peak in testae, present in both species but much more marked in *V. faba*, which declined to very low levels at maturity. The decline in the amounts in testae correlated with the major period of accumulation in cotyledons, but was not of a sufficient magnitude to account for the pyrimidone glucopyranosides in the cotyledon. Export from the testa and continuing synthesis must take place together. This transitory peak in the testa occurred before the maximum dry weight recorded at growth stage 6 for testae of both *V. faba* and *V. narbonensis*. The main period of accumulation in the cotyledons also occurred before the main period of dry matter accumulation, and was again particularly marked in *V. faba*.

The contrast in patterns of accumulation between *V. faba* and *V. narbonensis* is evident. In relation to the dry matter accumulated by the testae and the

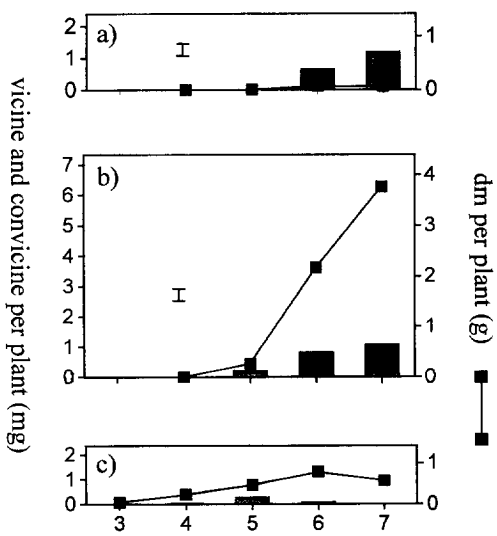

Fig. 3. The accumulation of pyrimidone glucopyranosides and

cotyledons, *V. narbonensis* synthesized much less pyrimidone glucopyranosides although with similar timing. However, the accumulation of high concentrations of pyrimidone glucopyranosides in the seed axes of *V. narbonensis* was very similar to that in *V. faba*, suggesting that these two species share a similar mechanism for accumulation in this tissue.

Genetic studies show that the levels of vicine and convicine in the mature seed are maternally determined [7–9]. Labelled orotate becomes incorporated into vicine at high rates when injected into the pod lumen of *V. faba* [15]. Together with the data presented here, this suggests that the testa, maternal tissue derived from the integuments of the ovule, may be the primary site of synthesis and export to the cotyledons and seed axis.

The ratio of vicine to convicine differed markedly between tissues. In testae of *V. faba* this ratio was 5:1, 6:1, 3:1 and 1.4:1 at growth stages 3, 4, 6 and 7 respectively. In axes it appeared to rise towards maturity (4:1, 18:1 and 25:1 at growth stages 4, 6 and 7), reflecting a large accumulation of vicine with a more constant low level of convicine. The cotyledons of *V. faba* maintained a ratio close to 4:1 throughout their development despite large changes in the total amount of pyrimidone glucopyranosides. A single codominant gene, *vcr*, controls vicine:convicine ratio in whole mature seeds of *V. faba* [9]. This gene also operates maternally. It is probable that the individual levels of vicine and convicine in the different tissues of the seed are a result of the interactions between differential synthesis or inter-conversion in the testa and differential transport between tissues.

Role of roots in the accumulation of fruit pyrimidone glucopyranosides

Although the amount of pyrimidone glucopyranosides found in the root system of growing plants did not change significantly between flowering and late pod development in *V. faba*, it was still possible that the roots may synthesize and export pyrimidone glucopyranosides to the fruits. In Table 4 the concentrations of vicine and convicine are given from mature seed from grafted plants. Reciprocal grafts and homografts of *V. faba* cv Troy and a low pyrimidone glucopyranoside mutant derived from this cultivar, MTG5, gave seeds with concentrations always corresponding to the scion. Similarly, a pea scion grafted on to a MTG5 stock gave seeds without detectable pyrimidone glucopyranosides. These results show that the pyrimidone glucopyranosides in the fruits are not transported from the roots, but synthesized in aerial tissues.

Sites of high concentration within the plant and conclusions

Changes in the amounts of pyrimidone glucopyranosides in different tissues may indicate patterns of synthesis, accumulation and dispersal, but concentration is more relevant to the possible roles of these compounds in defence. In *V. narbonensis* the only tissues investigated where concentrations of pyrimidone glucopyranosides exceeded 0.1 g 100 g^{-1} were the axes of seeds approaching or at maturity (3.0 g 100 g^{-1} at maturity, growth stage 7) and the roots of flowering plants (0.24 g 100 g^{-1}, growth stage 1). In *V. faba*, seed axes and plant roots have similarly high concentrations (5.5 g 100 g^{-1} for seed axes at maturity, 0.35 g 100 g^{-1} for roots at flowering). These results indicate that if these compounds have a defence role, the roots of young plants may be an important site for both species, particularly during establishment when the seedling may be vulnerable to soil pathogens.

V. faba has high levels of pyrimidone glucopyranosides in the cotyledons of mature seeds. This appears to give protection against the storage pest *Callosobruchus maculatus* [13]. However, developing seeds of *V. faba* are reported to have particularly high levels of pyrimidone glucopyranosides [12]. The peak concentrations in this study are similar to those previously recorded, and were found to be 3.1 g 100 g^{-1} in testae at growth stage 1 and 3.8 g 100 g^{-1} in cotyledons at growth stage 4. Such high concentrations could be expected to provide protection from the coleopteran and lepidopteran pests of seeds, most of which begin their life cycle early in the development of the seed.

The data presented here are consistent with the accumulation of a secondary metabolite in seeds and roots. There is no evidence of the use of pyrimidone glucopyranosides in further metabolism, although the events following germination need to be followed more closely. The pattern of accumulation in seeds and roots closely parallels that reported for the neurotoxic non-protein amino acid ODAP in *Lathyrus* [16]. This study confirms that the pyrimidone glucopyranosides are synthesized early in the development of the seed. They appear in the testa before the cotyledons and seed axis and are not transported from the roots. The testa is likely to be the major site of synthesis for the developing seeds and searches for enzymes involved in bio-

Table 4. The concentration (g 100 g^{-1}) of pyrimidone glucopyranosides in mature seeds from grafted plants of *V. faba* cv Troy, mutant MTG5 and *Pisum sativum* cv Orb

Scion	Stock	No. plants	Vicine (s.e.)	Convicine (s.e.)
Troy	Troy	2	0.53 (0.02)	0.10 (0.01)
MTG5	Troy	5	0.045 (0.002)	0.011 (0.001)

synthesis should begin in this tissue. The potential existence of a separate biosynthetic machinery for the roots prompts questions on the genetic control of root biosynthesis, including whether or not genotypes with low seed pyrimidone glucopyranosides also have low levels in the roots and consequently whether the roots in such genotypes could be at increased risk from pests or pathogens.

EXPERIMENTAL

Plant material. Seeds of *V. faba* cv Troy and *V. narbonensis* accession 557 were from the collection held at SCRI. The stocks used for this work were the same as those used previously [17]. Plants were grown in peat-based compost with slow release fertilizer at a constant 18° with 18 hr daylength supplied by 400 W sodium lamps giving 230 μEm^{-2} at canopy height. At the growth stages indicated in Table 1, plants were divided into roots, remaining cotyledons, stem with petioles, leaf laminae, and, where appropriate, pod walls, funicles, whole ovules or testae, cotyledons and seed axis. Tissues were immediately frozen in liquid N_2 then freeze-dried. Grafts were performed on seedlings within 2 days of emergence, and before the plumule hook straightened (G. Duc, pers. comm.), using a scalpel blade to make a straight cut which was bound by Nescofilm. A polythene bag covered the pot for 1 week following grafting and the plants then grew normally to maturity in a glasshouse.

Analysis. Samples were milled in a hammer mill with a 1 mm sieve and vicine and convicine determined following the method of ref. [18] with the modifications of ref. [17]. Confirmation of the identities of vicine and convicine was obtained for root and stem samples by utilising an alternative HPLC system [19] which reversed the order of elution. Peaks obtained for all tissues other than cotyledonary samples were confirmed as β-glucopyranosides by enzymatic cleavage as outlined previously [17].

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