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CONSEQUENCES OF PURINE DEGRADATION INHIBITION INDUCED BY ALLOPURINOL ON GROWTH, NITROGENOUS COMPOUNDS AND CARBOHYDRATES IN YAM BEAN (*Pachyrhizus erosus Urban*)

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ABSTRACT

Allopurinol effects on Xanthine dehydrogenase (XDH) and uricase, the key enzymes of conversion of fixed nitrogen in ureides (allantoin and allantoate), exporting forms of ammonia from nodules to leaves, where investigated on growth, amino-compounds and carbohydrates in yam bean. Allopurinol decreased XDH and uricase activities in the nodules. As a consequence, ureides dropped down in nodules, tubers, stems and leaves. Amino-compounds declined in nodules and leaves but not in tubers or stems. Proteins were unaffected. Allopurinol also caused sucrose and starch accumulation in the nodules. In the tuber it did not affect sucrose level but decreased glucose, fructose and starch. The relationships between the enzyme activities, different metabolites and plant growth were discussed.

RESUME

EFFETS DE L'INHIBITION DE LA DEGRADATION DES PURINES, INDUITE PAR L'ALLOPURINOL, SUR LA CROISSANCE, LES COMPOSES AZOTES ET LES GLUCIDES CHEZ LE DOLIQUE TUBEREUX (*Pachyrhizus erosus Urban*).

Les effets de l'allopurinol sur la Xanthine déshydrogénase (XDH) et l'uricase, les enzymes clés de la transformation de l'ammoniac en uréides (allantoine et allantoate), forme de transport d'azote chez le dolique tubéreux, ont été étudiés sur la croissance, les composés aminés et les glucides. Allopurinol réduit les activités Xanthine déshydrogénase et uricase dans les nodosités. Il s'ensuit une chute des uréides dans les nodosités, tubercules, tiges et

feuilles. Les aminoacides baissent dans les nodosités et dans les feuilles mais pas dans les tubercules et dans les tiges. Les protéines restent inchangées. Allopurinol provoque l'accumulation de saccharose et de l'amidon dans les nodosités. Dans les tubercules il n'affecte pas le saccharose mais diminue le niveau du fructose et de l'amidon. Les relations entre les différents métabolites et la croissance des plantes sont discutées.

INTRODUCTION

Yam bean, *Pachyrhizus erosus Urban*, a tropical grain and tuberous legume covers its growth needs in nitrogen but also supplies the storage sinks (grains and tuber) in amino-compounds. As the other tropical legume of the tribe Phaseolae-Faboïdae, it transports ureides : allantoin and allantoic acid, (Sprent, 1980 ; Lamaze et al. 1985), derived from purine catabolism after the incorporation of ammonia into purines (Herridge et al. 1978, Streeter 1979, Rawsthorne et al. 1980).

It produces an edible tuber which can be eaten raw or cooked. Grains contain rotenone (Hansberry et al. 1945) and are used to propagate the species. Ureide formation takes place in the nodules (Matsumoto et al. 1977 ; Fujihara and Yamamoto, 1978). The key enzyme of the first step of purine catabolism into ureides is xanthine dehydrogenase (XDH) followed by uricase. Allopurinol is known as a specific inhibitor of XDH (Atkins et al.,1980). Triplett (1986) has used that effect to describe a rapid method of screening ureide and amide producing legumes. By soil drench application, allopurinol inhibited ureide formation in ureide producing plants but not in amide transporting plants. This paper reports the modifications of the growth, XDH and uricase activities, the consequent changes in amino-compound and carbohydrate compositions induced by allopurinol and nitrate in the different organs of yam bean (*Pachyrhizus erosus*) .

MATERIELS AND METHODS

1. Plant material

Seeds of *Pachyrhizus erosus* (cv tpe-1) were sown and plants grown in the greenhouse in pots of 1.5 l containing a mixture of ferrallitic soil and sand poor in nitrogen. Each pot received 5 g of potassium sulphate and 8 g of P2O5 brought as basic slag. The rhizobium, specific for yam bean, was present and efficient in the soil.

Four seeds were sown in each pot but thinned to 2 plants at the beginning of the treatments. Four weeks after sowing, the plants were treated according

to the method developed by Triplett (1986). Each pot received 50 ml of one of the following solutions as soil drench for 14 consecutive days : a) control : water ; b) 0.2 mM allopurinol ; c) 0.4 mM allopurinol. At the end of the treatments, samples consisted of 1 g for leaves, stems, nodules and of 5 g for tuber were drawn from 10 nodulated plants and stored at -32° C for the determination of enzyme activities, the analysis of nitrogenous compounds and carbohydrates. The remaining plants were divided in their different organs and dry matters determined.

2- Methods

Preparation of plant extracts

Extracts for the measurements of enzyme activities and nitrogenous compound level were prepared, by adding to 1 g tissue, 4 ml 0.1 M tris - HCL buffer (pH 7.5) containing 1 mM EDTA, 1mM dithiothreitol and 10 mM MgCl2. After grinding in a mortar and pestle at 4° C, the homogenate was centrifuged at 48 000 g for 30 min. The pellet was saved for the analysis of structural proteins. The resulting supernatant was separated by passage through a Sephades G-50 column into 3 fractions : Soluble proteins (total soluble proteins minus leghemoglobin), leghemoglobin, other metabolites.

Samples of tubers or nodules were added to 40 ml of ethanol-water (80:20) and homogenized with an Ultra Turrax homogenizer for 30 s. The homogenate was centrifuged at 1200 g for 15 min. The supernatant was collected and the pellet was extracted again twice with 40 ml of the same aqueous ethanol. All the supernatants were put together, vacuum-concentrated and treated as previously described for soluble sugar analysis (Cerning-Beroard 1975). The starch of the peller, after extraction in dimethylsuldoxide, was hydrolysed into glucose with amyloglucosidase according to Boehringer method.

Measurement of enzyme activities

NAD-xanthine dehydrogenase (EC 1.2.1.37) was assayed according to Atkins et al. (1980). XDH activity was measured by the rate of NAD reduction followed at 340 nm. Uricase (EC 1.7.3.3) activity was assayed according to Tajima and Yamamoto (1975). Uricase activity was determined from the rate of disappearance of uric acid at 292 nm.

The enzyme activities were expressed in the basis of total soluble proteins.

Metabolite determinations

The ureides (allantoin and allantoic acid) were estimated as described by

Yound an Conway (1942). The structural proteins of the pellet were treated with HCl 6M at 120° C for 18 hours to hydrolyze them into amino-acids. Quantitative determination of amino-acids and amino-compounds was made at 570 nm using ninhydrin as reagent according to Yemm and Cocking (1955) and adapted to the Technicon autoanalyser (G. Vansuyt, Memoire DPE, ENSA, Montpellier, France). The proteins from the pellet are expressed in aspartic acid equivalents.

Soluble proteins were estimated according to Bradford (1976) using Coomassie dye (Brilliant blue G-250) as reagent.

Sucrose, fructose and glucose were estimated by the enzymatic methods of Bergmeyer (1979).

RESULTS

1- Allopurinol on plant growth, xanthine dehydrogenase and uricase activities

Allopurinol effect at the two concentrations, evidenced by comparing allopurinol treatments to control, reduced only leaf dry weight without affecting stem, root and nodule growth (table 1).

Xanthine dehydrogenase and uricase activities are shown in fig. 1. Allopurinol reduced the activity to about 60 % ot that in the control plants (122±25 vs 202±8 nKat (mg prot)-1 for XDH and 2.5±0.3 vs 3.9±0.4 nKat (mg prot)-1 for uricase). The difference between the two concentrations of Allopurinol is not significant.

2 - Effect of allupurinol on the ureides, amino-compounds and proteins in the different organs of the plant

Allopurinol drastically reduced ureide levels in stems and tuber (sites of storage), in nodules (site of formation) and to a lesser extent in the leaves (table 2). The decline was
about 70 % in the first three organs and 30 % in the leaves. The difference between the 2 doses of allopurinol is not significant.

The highest decreases in amino-compound concentration occurred with 0.4 mM allopurinol. They were about 50 % in the nodules, 70 % in the leaves and 30 % in the stems. Only tuber amino-compound levels remained unaffected (table 2).

The soluble proteins were reduced in the leaves by allopurinol. Soluble and

Table 2 Amino-compounds and ureides in the different organs of yam bean (expressed in mg per g of fresh weight) after 14 consecutive days of the following treatments : control , water; allop2, 0,2 mM allopurinol ; allop4 ; 0,4mM allopurinol. the averages are obtained from 4 determinations on organ samples collected from 4 different plants. Values without standard deviations are averages of 2 determinations.

	Amino-compounds			Ureide		
Treament	Control	Allop 2	Allop 4	Control	Allop 2	Allop 4
Leaves	1,01 0,12	0,41 0,05	0,27 0,24	0,22 0,02	015 0,03	0,16 0,01
Stems	0,61 0,04	0,33 0,06	0,42 0,24	1,25 0,20	0,07 0,01	0,06 0,01
Tuber	0,31 0,10	0,41 0,05	0,27 0,15	0,85 0,08	0,09 0,01	0,04 0,01
Nodules	1	0,49 0,02	0,51 0,02	0,58 0,07	0,09 0,02	0,07 0,01

Table 1 -Dry weights of organs of yam bean after 14 consecutives days of the following treatments : control : water, 0,4mM allopurinol. The values are averages of 10 plants and expressedin g per plant.

Treatments	Leaves	Stems	Root	Tuber	Nodules
Control	16,1 0,1	2,8 0,4	1,1 0,2	2,8 0,5	0,8 0,1
Allopur.	4,1 0,7	2,1 0,3	1,4 0,2	2,2 0,4	0,9 0,2

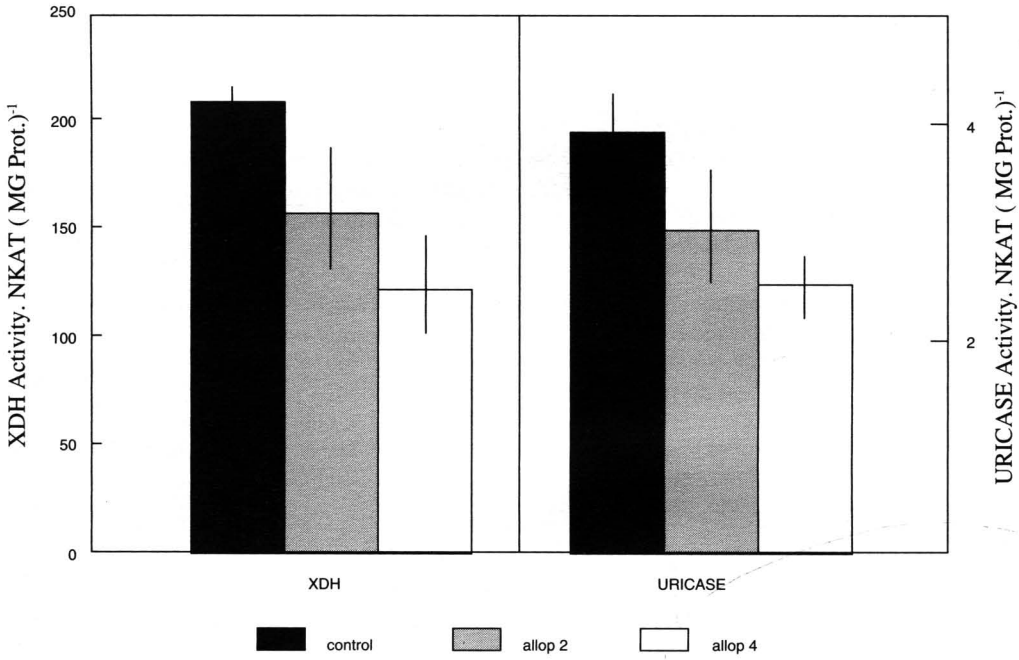
Table 3 : Soluble and structural proteins (expressed in mg per g of dry weight) in the different organs of yam bean after 14 consecutive days of soil drench treatments : control, water ; Allop4, 0,4mM allopurinol . The averages are obtained from 4 determinations on organ sample collected-from 4different plants.

	Soluble proteins		Structural proteins	
Treatments	Control	Allop 4	Control	Allop 4
Leaves	9,1 1,6	4,6 0,3	3,3 1,9	1,9 1,2
Stems	3,3 0,5	3,0 0,7	2,0 0,3	1,8 0,2
Tuber	1,7 0,3	1,4 0,4	1,2 0,4	1,1 0,4
Nodules	6,5 1,2	4,5 0,5	4,4 2,0	4,5 0,5

Table 4 - Carbohydrate content of nodules and tuber after 14 consecutive-days of soil drench treatments : Control, water ; Allopur, 0,4mM allopurinol. The values are expressed in mg per g of dry weight. The averages are obtained from 4 determinations of nodule or tuber sample collected from 4 plants.

	Nodules		Tuber	
	Control	Allopur	Control	Allopur
Glucose	3,5 0,2	2,8 0,3	68 3	36,2 4
Fructose	2,4 0,5	3,0 0,1	71 2	37 1
Sucrose	37 3	64 4	66 4	63 1
Starch	22,0 1,2	41,5 1	127 4	64,5 3

XANTHINE AND URICASE ACTIVITIES



structural proteins remained unchanged in the other organs (Table 3).

3 - Effects of allopurinol on carbohydrate composition of the nodules and the tuber

Allopurinol treatment did not change glucose and fructose levels in the nodules but sucrose and starch concentrations increased by about 70 % and 85 % respectively (table 4). As for the tuber the highest levels of each sugar were encountered with the control. The applications of allopurinol did not affect sucrose but reduced glucose, fructose and starch by about 45 %.

DISCUSSION AND CONCLUSION

Allopurinol decreased XDH and uricase activities in yam bean nodules. It is known as a specific inhibitor of XDH in vitro (Atkins et al. 1980) and in situ (Triplett, 1986).

The reduction in XDH and uricase activities generally caused decrease of ureide levels in nodules (site of formation), in stems and tuber (sites of storage) at the end of the different treatments. Our result is in agreement with those reported before for other legumes.

Allopurinol brought about sucrose and starch storage in the nodules. So the reduction of ureide formation caused a carbohydrate accumulation in the nodules which seemed to result in the lesser consumption of the sugars. Our result could be compared to that reported by Viands et al. (1979). They observed in the ineffective nodules of alfalta a higher concentration of starch than in the effective ones. Allopurinol, in that case, seemed not to have affected phloem sugar supply to the nodules. Sucrose in excess was transformed into starch.

In the tuber allopurinol reduced glucose, fructose, sucrose and starch amounts. The decrease under allopurinol treatment could be the consequence of a progressive starvation of ureide transported to the leaves, that limited shoot development, the size of photosynthetic apparatus and thus photosynthesis and carbohydrate synthesis. Under allopurinol treatment Triplett (1986) had reported a leaf chlorosis in ureide-producing legumes as soybean, vigna and lima bean.

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