A PROBABALE PATHWAY OF UREIDE ASSIMILATION IN PIGEONPEA PODS

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ABSTRACT

Ureides (allantoin and allantoic acid) and the activities of glutamine synthetase, glutamate dehydrogenase, uricase. allantoinase and urease, the enzymes presumbly involved in the assimilation of ureides, were determined both in the vegetative organs and reproductive structures at 15-day interval starting from day 75 after sowing to complete maturity of the crop (day 120 after sowing). Based on the concentration of ureides and the distribution of the above enzymes in various plant parts, a probable pathway of ureide assimilation in pigeonpea pods has been proposed.

INTRODUCTION

UREIDES, chiefly allantoin and allantoic acid, constitute a predominant form of organic nitrogen transported out of the nodules into the aerial parts of a number of tropical legumes including pigeonpea[1-5]. Though it is known that ureides after transport are presumably stored in stems and leaves prior to utilisation in pods[1,6], the sequence of reactions by which these compounds are assimilated in developing pods is not clear. The present communication, an extension of the earlier work[3-5], suggests a probable mechanism of ureide assimilation in pigeonpea pods.

MATERIALS AND METHODS

Pigeonpea (*Cajanus cajan* L.) crop (Cv. UPAS-120) was raised in earthen pots under natural conditions of light and humidity as described earlier[3,4]. Starting from the commencement of flowering (75 days after sowing), the samples were taken at 15-day interval till complete maturity of the crop (120 days after sowing). The ureides and assays of glutamine synthetase (EC 6.3.1.2), glutamate dehydrogenase (EC 1.4.1.4). uricase (EC 1.7.3.3) and allantoinase (EC 3.5.2.5) was estimated as stated previously[4,5]. Urease (EC 3.5.2.5) was assayed by estimating the enzymically-produced NH^{+}_{4} from urea[7].

TABLE 1

Ureides and their assimilating enzymes in stems and leaves during reproductive phase

Days after sowing	Stems				Leaves			
	75	90	105	120	75	90	105	120
Parameter								
Glutamine synthetase (μ mol GMH[a] plant^{-1}h^{-1})	75.71 ± 7.14	52.85 ± 7.14	20.00 ± 2.86	ND[b]	196.36 ± 5.45	62.14 ± 2.14	183.75 ±15.00	30.00 ± 2.31
Glutamate dehydrogenase (μmol NAD^{+} plant^{-1} h^{-1})	508.20 ± 72.60	666.60 ± 13.20	199.80 ± 18.00	177.00 ± 4.80	786.00 ± 46.80	778.80 ± 1.80	476.40 ±63.60	127.20 ±19.20
Allantoinase (μg allantoic acid plant^{-1} h^{-1}	46.15 ± 7.69	66.64 ± 9.50	42.85 ± 6.28	35.71 ± 7.14	107.14 ± 4.55	40.50 ± 7.14	22.94 ± 6.25	ND
Urease (μmol ammonia plant^{-1} h^{-1}	69.04 ± 7.14	44.75 ± 0.95	39.27 ± 1.09	76.18 ± 7.14	23.18 ± 0.45	14.28 ± 2.86	10.93 ± 2.96	ND
Allantoin (μmol plant^{-1})	549.99 ±35.72	1571.43 ± 28.57	1128.57 ±100.01	ND	936.36 ± 9.09	407.14 ± 50.01	468.75 ± 6.25	ND
Allantoic acid (μmol plant^{-1})	400.00 ±45.46	1485.71 ±114.30	357.14 ± 71.44	ND	263.63 ± 45.46	121.42 ±35.71	331.25 ±18.75	ND

(Values are mean of 6 determinations Mean ± SD)
[a] = γ-glutamyl mono hydroxamate, [b] = not detectable

Results and discussion

Allantoin and allantoic acid concentration in stems increased till day 90 after sowing and declined thereafter at day 105 (table 1). However, in leaves, the level of both these compounds dropping at day 90, again increased at day 105. None of the ureides could be detected at day 120, both in stems and leaves. Except uricase, all other enzymes, *viz* glutamine synthetase (GS), glutamate dehydrogenase (GDH) allantoinase and urease were present both in stems and leaves. The activity of GS decreased continuously in stems until day 105, but in leaves, the activity decreasing first at 90 days again increased at day 105 followed by decline again at day 120. GDH and allantoinase activities after showing upward trend at day 90 decreased continuously till the end in stems, whereas, in leaves, the trend was almost similar for GDH, but allantoinase showed continuous decline until day 105 and was not present at all at day 120. Urease activity decreased continuously both in stems and leaves till day 105. At day 120, it showed maximum activity in stems but was undetectable in leaves.

Allantoin concentration was higher in flower buds followed by developing pods, seeds and podwalls, respectively (table 2). Though allantoic acid was present in flower buds and developing pods, it could not be detected in seeds and podwalls. Similarly, allantoin also could not be detected in matured seeds and podwalls, though it was present in both these organs at day 105, where the concentration was higher in seeds than in podwalls. Like vegetative parts, uricase again could not be detected in any of the reproductive structures. All other enzymes as above, were however, present both in flower buds and developing pods. Flower buds exhibited higher activities of allantoinase and urease, whereas developing pods had higher activites of GS and GDH. Seeds also contained all the enzymes except uricase. Podwalls showed the activity of GDH allantonase and urease. GS also could not be detected in podwalls. Activities of GDH, allantoinase and urease were higher in seeds compared to podwalls.

It is evident from the data included in table 1 that the ureides during vegetative growth are stored temporarily in leaves and stem, maximum accumulation being in stem. Stem in nodulated soybean[8,9] and cowpea[10] has also been shown to accumulate the maximum amount of ureides. During reproductive phase, these compounds are translocated to developing pods, where they may be utilised for the synthesis of seed proteins. The presence of allantoinase, urease, GS and GDH in vegetative organs as shown here indicates that ureides are partly metabolised in these organs also. Urea, one of the products of ureide metabolism, could be hydrolysed further by urease to ammonia and carbon dioxide, and glyoxylate could be transaminated to glycine. Ammonia formed as above could be assimilated as usual either by GS/GOGAT or GDH pathway[11]. These products of metabolism could either be transported to developing pods alongwith ureides or utilised for the synthesis of leaf proteins. The second

Table 2

Ureides and their assimilating enzymes in reproductive structures

Days after sowing	Flower buds	Developing pods	Seeds		Pod walls	
Parameter	75	90	105	120	105	120
Glutamine synthetase	0.28 ± 0.06	1.11 ± 0.06	0.30 ± 0.10	ND	ND	ND
Glutamate dehydrogenase	6.16 ± 0.53	8.54 ± 0.08	30.70 ± 0.20	25.00 ± 0.23	7.41 ± 0.15	1.19 ± 0.12
Allantoinase	0.56 ± 0.04	0.31 ± 0.01	1.02 ± 0.11	ND	0.13 ± 0.02	ND
Urease	16.86 ± 1.51	8.50 ± 1.38	870.00 ± 60.01	1076.92 ± 15.39	40.67 ± 5.75	73.96 ± 6.25
Allantoin	10.98 ± 0.68	7.90 ± 0.65	4.90 ± 0.31	ND	3.23 ± 0.13	ND
Allantoic acid	0.88 ± 0.09	3.72 ± 0.12	ND	ND	ND	ND

(Values are mean of 6 determination. Mean ± SD) ND = not detectable.

peak of ureides at day 105 in leaves indicate that during senescence of leaves, ureides in these organs could also arise either from the products of nucleic acid or protein degradation. In soybean cotyledons[12,13], stored nucleic acid were observed to be the source of ureides. Urea and glyoxylate produced as a result of arginine hydrolysis and glycine transamination, respectively could also condense to form allantoin[14,15]

Flower buds and developing pods has considerable amount of allantoin which decreased during maturity (table 2). Similar results have also been reported for soybean[6,8,16]. Higher amounts of ureides were found to be present in podwalls compared to seeds of soybean[16,17]. However, in the present investigation, seeds had higher amounts than podwalls. This difference could be mainly due to the reason that the time interval in the present case was more as compared to that of Thomas and Schrader[16], and at the time of estimation, the content in podwalls might have decreased, whereas, in seeds, it might be at the peak concentration. The activity of allantoinase was found to be greater in seeds than in pods of soybean[16] and cowpea[18]. In the present investigation also, the activity of allantoinase was about 8-fold higher in seeds than in podwalls and about 3-fold higher in developing pods. Keeping in view the above, a probable pathway (figure 1) by which ureides may enter seed proteins has been visualised as follows.

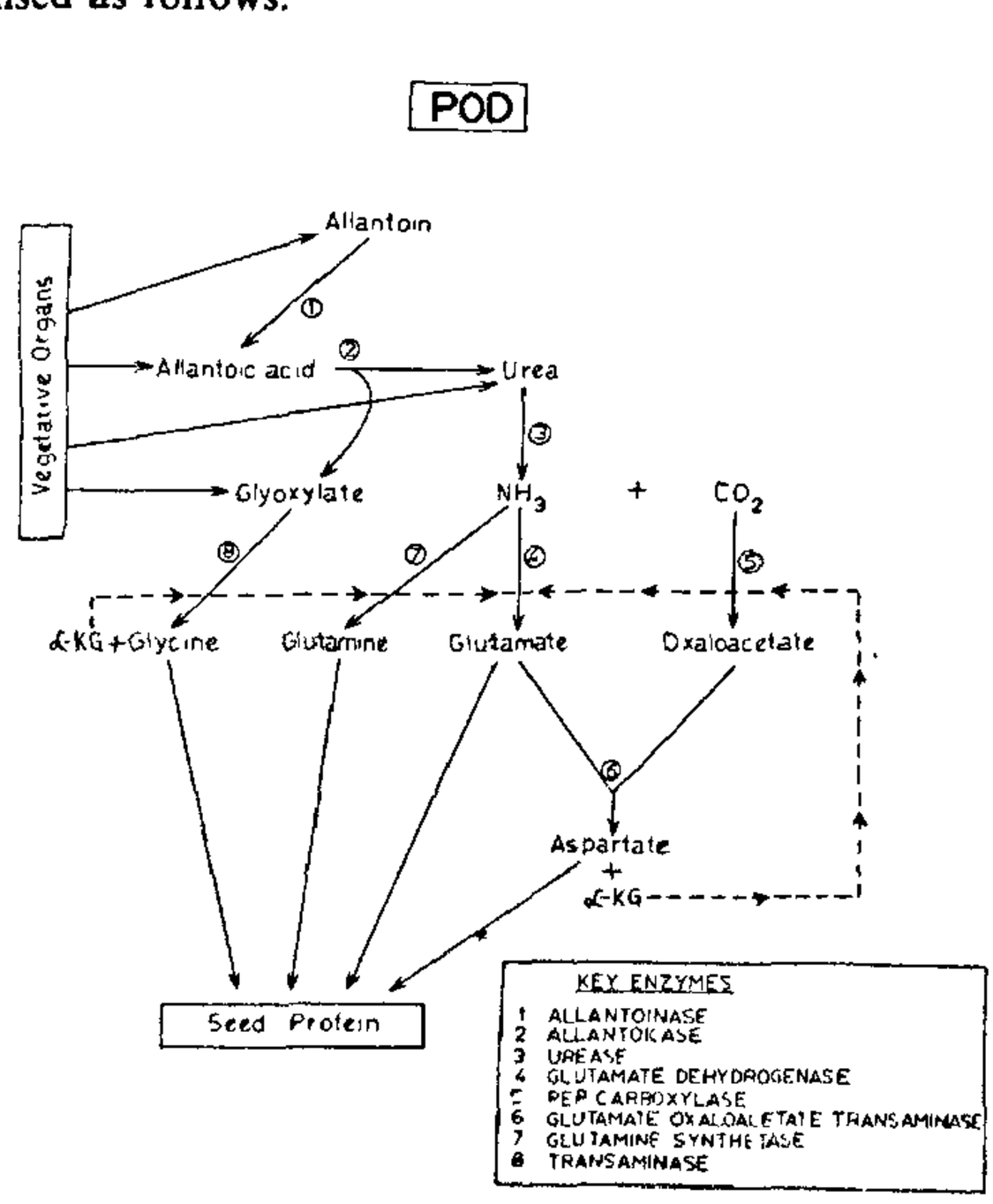

Figure 1. A probable pathway of ureide assimilation in pigeonpea pods.

Ureides present in vegetative parts are partly transported as such and partly converted to other nitrogenous forms mainly amino acids and amides before transportation to developing pods. The presence of GS, GDH, allantoinase and urease till 105 days in vegatative parts confirms the above view. In this case, amino acids/amides can directly be utilised for the synthesis of seed proteins. Ureides received by the developing pods are apparently metabolised to urea and glyoxylate. Urea is further hydrolysed by urease to ammonia and carbon dioxide. Substantial increase in allantoinase activity at 105 days in seeds and urease activity till 120 days in both podwalls and seeds provide a strong supporting evidence that the ureides are metabolised to urea and further to ammonia and carbon dioxide. The fact that allantoic acid could not be detected either in seeds or podwalls at 105 days after sowing suggests an extremely rapid conversion of allantoic acid to urea. The ammonia liberated in the urease reaction seems to be reassimilated by GDH rather than GS as evidenced by high GDH activity in seeds and podwalls. The carbon dioxide liberated, the other product of urease reaction, is either released to the atmosphere and/or recaptured by PEP carboxylase which is quite active in pods[19]. Oxaloacetate and glutamate liberated in PEP carboxylase and GDH reactions, respectively can give rise to aspartate and α-ketoglutarate by transamination reactions which are known to be widely occurring in plant tissues[20]. Similarly, glyoxylate formed can also be transaminated to glycine. α-ketoglutarate formed as above can sustain the GDH reaction.

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