Tr. J. of Botany
23 (1999) 241-244

Research Article

The Effect of Uracil on the Germination and Growth of some leguminous Plants*

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Received: 27.11.1998
Accepted: 05.03.1999

Abstract: All known pyrimidine and pyrimidine-derived secondary producs originate from uracil or its precursor, uracil-6-carboxylic acid. The biosynthesis of these products has been suggested to be uracil detoxication mechanisms. The possible toxic effects of uracil on the germination and growh of *Pisum sativum* L. cultivar Meteor, *Lathyrus tingitanus* L. in which pyrimidine-derived secondary products occur naturally, and of *Phaseolus aureus* Roxb. and *Glycine max* (L.) Merr., in which these compouns do not occur, were examined. The results show that the germination and growth of the *P. aureus* and *G. max* seeds under investigation were considerably inhibited by exogenous uracil. The effect of uracil was obvious on the non-producer group of experimental plants, especially on *G. max.* However, there was not any noticeable effect of uracil either on *P. sativum,* or on *L. tingitanus* in the experimental periods of germination and growth. These results show that uracil accumulation is most probably toxic to plants and that the production of these pyrimidine-derived secondary compounds from uracil is therefore a detoxication mechanism.

Key Words: Uracil, detoxication, *Pisum sativum, Lathyrus tingtanus, Phaseolus aureus, Glycine max.*

Baklagillerden Bazı Bitkilerin Çimlenme ve Gelişmesinde Urasil'in Etkisi

Özet: Bilinen bütün pirimidin ve pirimidin türevi sekonder ürünler urasilden veya onun öncülü olan urasil-6-karboksilik asitten meydana gelirler. Bu sekonder maddelerin biyosentezi, urasilin detoksikasyon mekanizmaları olarak tahmin edilmektedir. Doğal olarak pirimidin türevi sekonder ürünleri sentezleyen *Pisum sativum* L.cv. Meteor, *Lathyrus tingitanus* L. ve bu ürünleri sentezlemeyen *Phaseolus aureus* Roxb. ve *Glycine max* (L.) Merr.'ın çimlenme ve gelişmesinde urasilin muhtemel toksik etkileri araştırılmıştır. Sonuçlar, *P. aureus* ve *G. max* tohumlarının çimlenme ve gelişiminin urasil tarafından oldukça fazla engellendiğini göstermiştir. Pirimidin türevi metabolitleri sentezlemeyen deney bitkilerinin, özellikle bunlardan *G. max*'in gelişimi üzerine urasilin etkisinin çok belirgin olduğu görülmüştür. Bununla birlikte, ne *P. sativum* ve ne de *L. tingitanus'un* çimlenme ve gelişiminde, urasilin göze çarpar bir etkisi görülmemiştir. Bu sonuçlar, urasil birikiminin bitkilerde büyük ihtimalle zehir etkisi yaptığını ve bu yüzden urasilden pirimidin türevi sekonder metabolitlerin sentezinin bir detoksikasyon mekanizması olduğunu göstermektedir.

Anahtar Sözcükler: Urasil, detoksikasyon, *Pisum sativum, Lathyrus tingitanus, Phaseolus aureus, Glycine max.*

Introduction

In recent years, the biosynthesis of a number of pyrimidine secondary products has been investigated from different plant sources, and it has been shown that all known pyrimidine and pyrimidine-derived secondary products originate from uracil or its precursor, uracil-6-carboxylic acid (orotic acid). These products include the isomeric non-protein amino acids willardiine and isowillardiine (1-3), the pyrimidine glucosides vicine and convicine (4), lathyrine (5, 6) and 5-ribosyluracil (7). Albizziine and subsequentially 2, 3-diaminopropanoic acid were also shown to be uracil-derived secondary products by our recent investigations (8, 9). These metabolic processes have been suggested (10) to be uracil detoxication mechanisms. The toxicity of exogenous uracil to microbial systems has also been reported (11, 12).

The present study, therefore, aimed to examine the possible toxic effects of uracil on the germination and growth of two groups of leguminous plants. There are several published reports that seedlings of *Pisum sativum* L. cultivar Meteor accumulate two unusual pyrimidine amino acids, willardiine and isowillardiine (1, 2, 13-15), and that seeds and seedlings of *Lathyrus tingitanus* L. accumulate another pyrimidine secondary product,

*This work was carried out at the University of Wales Swansea, UK.

Abbreviations: CPSase: carbamoyl phosphate synthetase, ATCase: aspartate transcarbamoylase, DHUDHase: dihydrouracil dehydrogenase, UMP: uridine 5'-monophosphate.

lathyrine (16, 17). These species are, therefore, pyrimidine-secondary metabolite producers. It was shown in our earlier investigation that (18) *Phaseolus aureus* Roxb. and *Glycine max* (L.) Merr. do not synthesize and accumulate any pyrimidine-derived secondary products. These two plants, therefore, were used as'non-producers' for comparison with *P. sativum* and *L. tingitanus* in this investigation.

Material and Methods

Four plant species were used throughout the investigation, namely *Pisum sativum* L. cultivar Meteor, *Lathyrus tingitanus Phaseolus aureus* and *Glycine max* . Seeds of *P. sativum* were from Sharpes Intl. Seeds Ltd., Sleaford, Lincs., UK. *L. tingitanus* seeds were supplied by the University of Wales Swansea Botanic Garden and those of *P. aureus* and *G. max* seeds were purchased locally in Swansea, UK.

Uracil was purchased from Sigma (London) Chemical Company Ltd., Kingston-upon-Thames, Surrey, UK.

In all cases, dry seeds were well washed, and before separate sowing were allowed to imbibe for 15 hr in the dark in 5mM, 10mM, 20 mM and 30 mM solutions of uracil. As controls, seeds were separately soaked in water under the same conditions. After 15 hr the imbibed seeds were set to germinate in plastic trays 26 cm x 22 cm x 6 cm depth, containing pre-soaked vermiculite, obtained from Vitagrow Ltd., Stoneferry, Hull., UK. Each of the trays, which had drainage holes in the bottom, was watered daily with tap water or uracil solutions. Seedlings were grown in a constant temperature room at 25°C in a light cycle of 16 hr light (6 klx) and 8 hr dark. The term 'germination' was used to describe that period commencing with water uptake and ending with the penetration of seed coat by the developing radicle; subsequent development was described as 'growth'. The germination percentages were recorded on the 9^{th} day, and the growth of the seedlings was measured in cm on the 3^{rd}, 6^{th}, 9^{th}, 12^{nd} and 15^{th} days for all the experimantal plants.

The experiments were repeated 4 times.

Data obtained were evaluated with analysis of variance (19).

Result and Discussion

The results show that the germination of the *P. aureus* and *G. max* seeds under investigation was considerably inhibited by the effect of exogenous uracil. As seen in Table 1, the maximum preventive effect of most concentrated uracil is apparent in the non producer group of the experimental plants, especially on *G. max*. Retardative and preventive effects of uracil on the growth of the non-producer group were also observed (Table 2). However, there was no noticeable effect of even concentrated uracil either on *P. sativum,* or on *L. tingitanus* in the experimental periods of germination and growth (Tables 1 and 2).

The cause of excessive production of uracil in some groups of higher plants was previously investigated in our experiments, and it was shown that seedlings of *P. sativum* and *L. tingitanus,* which produce and accumulate pyrimidine-derived secondary products, have a greater capacity for uracil production than do seedlings of *P. aureus* and *G. max* (8). This is mainly attributable to greater relative activity of carbamoyl phosphate synthetase (CPSase; EC 2.7.2.5) and especially of aspartate transcarbamoylase (ATCase; EC 2.1.3.2), the rate-limiting enzyme in pyrimidine biosynthesis. The end product of the orotate pathway, UMP, is both the source of uracil for secondary product formation and the feedback inhibitor of ATCase. Thus, secondary product synthesis removes the main supressor of the orotate

Table 1. Germination rates of experimantal seeds after imbibition in different concentrations of uracil solution. Conditions under which the seeds were germinated are described in Material and Methods.

Conc. of uracil (mM)	Germination rate (%) of the seed (9^{th} day)			
	Pisum sativum	*Lathyrus tingitanus*	*Phaseolus aureus*	*Glycine max*
0 (Control)	97±1.1	95±2.8	96±4.1	92±3.4
5	97±2.3	93±2.3	93±6.1	89±4.7
10	98±1.9	94±1.7	85±3.8	78±3.1
20	95±2.6	96±2.2	70±3.2	63±2.6
30	96±1.8	95±2.0	52±2.7	41±3.5

pathway, and consequently further mobilises uracil production. Uracil accumulation would also be enhanced in pyrimidine secondary product-forming plants by their lower activity of dihydrouracil dehydrogenase (DHUDHase; EC 1.3.1.2), the key enzyme for the pyrimidine catabolic pathway. As uracil is the rate-limiting factor in the biosynthesis of pyrimidine amino acids like willardiine, isowillardiine and lathyrine (1, 7, 10), this explains the relatively large accumulations of these secondary compounds that can occur (1, 20).

The implication of the present findings is that, uracil accumulation is most probably toxic to plants and that the production of these pyrimidine-derived secondary metabolites from uracil is therefore a detoxication mechanism. As yet there has been no report describing significant toxic effects of any pyrimidine-derived secondary product in the tissues of higher plants. It appears that *P. aureus* and *G. max,* which do not produce pyrimidine-derived secondary compounds, were poisoned in this experiment because of their lack of suitable detoxication mechanisms. However, *P. sativum* and *L. tingitanus,* which possess mechanisms for production of willardiine, isowillardiine and lathyrine are able to immediately handle a constant exogenous supply of uracil in concentrations approaching maximum solubility. These observations, therefore, emphasise the increased availability of uracil in the producer plants and their potential need for an alternative means of disposing of uracil.

There are few published reports concerning the toxicity of uracil to microbial systems (11, 12). The toxic effects of some uracil derivatives, like 5-aminouracil, 2-thiouracil and 5-bromouracil, on plants, animals and microorganisms have also been well documented (21,

Table 2. The exogenous uracil effect on the growth of the experimental seedlings. Conditions under which the seedlings were grown are described in Material and Methods.

Conc. of uracil (mM)	Age of seedlings (Day)	Growth of the seedlings (cm)			
		Pisum sativum	*Lathyrus tingitanus*	*Phaseolus aureus*	*Glycine max*
0 (Control)	3	1.7±0.2	5.2±0.8	3.2±0.7	2.5±0.9
	6	3.1±0.7	12.3±0.7	5.7±0.9	6.5±1.3
	9	4.6±0.3	19.5±1.1	8.4±0.9	9.8±1.5
	12	6.2±0.3	28.5±0.9	11.3±0.8	12.7±1.8
	15	7.0±0.5	35.7±1.0	13.5±0.9	15.4±1.9
5	3	1.8±0.3	5.0±0.4	3.0±0.8	1.7±0.5
	6	3.2±0.4	11.5±0.7	5.1±1.0	2.9±0.8
	9	4.5±0.3	19.1±0.6	7.3±1.1	4.8±0.9
	12	6.0±0.4	28.0±0.5	9.7±1.2	6.9±1.2
	15	6.8±0.7	35.0±0.8	11.6±1.1	8.7±1.5
10	3	1.6±0.4	5.4±0.9	2.6±1.0	0.9±0.2
	6	3.0±0.4	12.1±0.6	4.3±0.9	1.6±0.5
	9	4.5±0.2	19.7±0.8	6.6±1.0	2.6±0.7
	12	6.1±0.4	28.9±0.8	8.6±1.1	3.5±0.8
	15	6.7±0.6	35.5±1.0	10.9±1.3	4.7±0.9
20	3	1.9±0.5	4.9±0.5	1.7±1.0	0.5±0.1
	6	3.3±0.5	11.4±0.7	3.4±1.2	0.8±0.3
	9	4.8±0.4	19.0±0.6	5.2±1.2	1.6±0.5
	12	6.2±0.6	28.1±0.7	6.8±1.4	2.1±0.5
	15	6.8±0.7	35.1±0.7	8.5±1.3	2.7±0.6
30	3	1.7±0.4	5.2±0.9	1.1±1.2	0.0±0.0
	6	3.0±0.5	12.0±0.7	3.0±1.3	0.5±0.1
	9	4.6±0.5	19.3±0.8	4.4±1.5	0.8±0.1
	12	6.1±0.5	28.7±0.8	5.6±1.5	1.0±0.2
	15	6.8±0.6	35.9±0.9	7.1±1.7	1.5±0.3

22). However, no investigation has been published on the uracil toxication on the other groups of organisms. Thus, one of the most important results of this present experimental investigation is, for the first time, to show the toxicity of uracil on the germination and growth of higher plants, and also to confirm the diversion of excess uracil into pyrimidine-derived secondary products as a result of the action of plant detoxication processes.

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