STUDIES ON THE CHANGES IN THE FREE AMINO ACIDS AND THE EFFECT OF PRESOAKING ON THE GERMINATION OF JEQUIRITY SEEDS (ARBUS PRECATORIUS)

A. HAMEED KHAN, S. SHAHID HUSAIN, MASARRAT RIAZ AND KHADEJA ZAMIR

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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Changes in the non-protein nitrogen during three weeks of germination of jequirity seeds have been studied. After the first week, as paragine, proline and threonine were detected pesides those amino acids which were already present in the cotyledons. γ—Amino butyric acid was added to this list in the second week. Abrine did not appear to be metabolised by the plant and was detected throughout the seedling. Asparagine was present in greater amounts during the first week, but gradually decreased with the passage of time; at the same time the amount of asparatic acid increased. The effect of presoaking on the germination of Jequirity seeds has also been studied.

Introduction

It is the characteristic feature of the life cycle of many organisms that at one stage a spore or seed is formed which can persist in a viable state throughout prolonged periods without further development. When appropriate conditions are available, the spore or the seed is reanimated and from it a fully active, metabolising organism is established. The process that is involved in the reanimation of the quiescent but viable system is called germination.

As the seed germinates and grows into a seedling, marked changes in the nature and disposition of the nitrogen compounds of the seed occur. The study of these changes has occupied the attention of many workers, and has in general been carried out from the standpoint of analysis of the amounts of the several groups of nitrogen compounds in the tissues, rather than from the standpoint of the individual chemical reactions involved.

Most of the studies regarding the changes brought about in the seeds during germination have been carried out on peas, beans, cereals and so on. Although *Abrus precatorius* had been the centre of much attention, most of the work was carried out on the toxicity and haemagglutinating properties of these seeds.[1-5] To the authors' knowledge, the germination of *Abrus* seeds and the changes brought about in the free amino acids during the various stages of growth has not been reported so far. The present communication therefore, deals with (a) the changes in the non-proteinous nitrogen compounds, especially the free amino acids, (b) the various stages of the development of the seedling, (c) an attempt to study the metabolism of the amino acid, *Abrine*, and (d) the effect of pre-soaking on the germination of the seeds.

Experimental

MATERIALS AND METHODS

The scarlet variety of *Abrus precatorius* procured from the local market was used throughout this study.

Germination in Petri-dishes.—Since the seed coat is very thick in *Abrus precatorius*, its mechanical rupture and treatment with concentrated sulphuric acid at different intervals of time, prior to germination, were tried without much success. Ultimately the following procedure was adopted. The seeds were washed with water; dipped in a 5% mercuric chloride solution for 5-10 minutes; washed thoroughly in water; placed in petri-dishes (9 cm. diameter) having filter papers soaked in distilled water and allowed to germinate under the tungsten lamp light at a temperature of 20-25°C. Every day the seeds were cleaned gently with a brush to remove any fungal development on the embryo. Each petri-dish contained five seeds, which generally germinated within four days; those which failed to do so within this period were rejected. The germinated seeds were allowed to grow for three weeks only; thus the present study covers only this period of growth of the seedling. After the first week a batch of 300 or more seeds was removed for chemical analysis. Similar analyses were then carried out after the expiry of the second and the third week. Conventional A.O.A.C. methods were used for the determination of nitrogen and dry weight.[6] For the extraction of free amino acids, the seedlings were cut so as to separate the cotyledons, radicles, plumules and the leaves and the tips from each other. Each of these portions was partially dried in an oven and then extracted with 95, 80 and 60% hot alcohol. The combined extracts were evaporated *in vacuo* and then treated with 10% trichloroacetic acid to remove any dissolved protein. Two-dimensional paper chromatography, using the method of Levy and

Chung [7] was used for the identification of the various amino acids. The spots were made visible by spraying with 0.25% solution of ninhydrin and 0.2% solution of isatin in acetone. In extracts where the separation of various amino acids was not clear, the extract was passed through I.R. 120 and the adsorbed amino acids were eluted with N-ammonia solution.

Effect of Pre-Soaking on Germination.—Since the adsorption of water is the first condition of seed germination, one may expect that pre-soaking the seeds in pure water should accelerate germination. Within limits, this is correct, but the effect depends greatly on timing, on the soaking conditions and on the size and other properties of the seeds. Keeping this in view the effect of pre-soaking the *Abrus* seeds on their germination was studied. For this purpose, batches of 25 seeds were first treated with 5% mercuric chloride solution and then soaked in distilled water for 0, 8, 24, 48 and 72 hours. They were afterwards allowed to germinate in the manner described above.

Results and Discussion

Much work has been carried out on the germination of cereals and legumes, especially peas, but the germination of *Abrus* seeds in the laboratory has not been reported so far. Since in the majority of seeds, particularly after they have been stored for some months, germination follows when the system is exposed to water and air at a relatively high temperature, *Abrus* seeds were allowed to germinate at a temperature of 20-25°C. This resulted in rapid adsorption of water, which was followed after about three days by the emergence of the radicle (first week), which was then followed by the elongation of the hypocotyl and the appearance of leaves (second and third weeks). (Fig. 1).

Table 1 shows the total dry weight, total protein and the non-protein nitrogen, the last value being studied not only in the cotyledons, but also in the radicle, the plumule, the leaves and tips, during the various stages of the seedling development (Table 1).

It is evident that the total dry weight decreased rapidly, although the total protein did not change appreciably. Significant variations were, however, observed in the soluble nitrogen of the seedlings. In the cotyledons the soluble nitrogen has decreased, while in the plumule, the tip and leaves and the radicle it has increased from the first to the third week of germination, although the total non-protein nitrogen of the seedling as a whole has increased throughout the period of germination (Table 2).

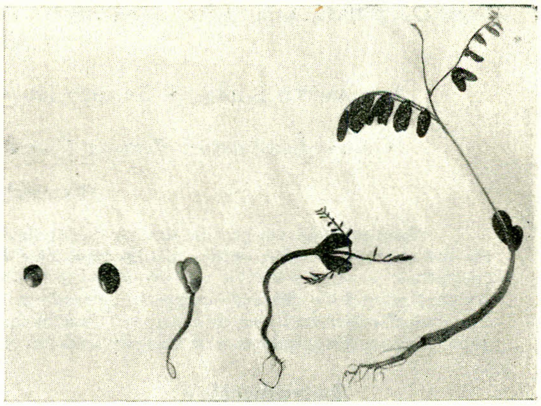

Fig. 1.—Showing various stages of germination of *Abrus percatorius*.

TABLE 1.—CONCENTRATION OF VARIOUS NITROGN COMPOUNDS FOUND IN SEEDLINGS OF *Abrus precatorius* DURING GERMINATION.

Compound	Amount per 100 g. original seeds			
	0 Days	7 Days	15 Days	22 Days
Total Dry Weight	100.00 g.	86.97 g.	72.10 g.	65.34 g.
Protein	22.33%	20.93%	18.36%	18.29%
Alcoholic extract of				
(i) Cotyledons	10.39	7.80	5.62	2.90
(ii) Plumule	—	—	0.75%	1.61%
(iii) Leaves and Tips	—	—	2.05%	5.03%
(iv) Radicle	—	2.95%	4.77%	5.56%

Paper chromatography of the alcoholic extract of the *Abrus* seed powder revealed the presence of abrine, alanine, aspartic acid, cysteine (traces), glutamine, glutamic acid, glycine, lysine, phenylalanine, serine, valine, an unidentified isatin sensitive, but ninhydrin-insensitive spot "U" and an unidentified ninhydrin sensitive spot "X". After the first week, asparagine, threonine and proline appeared in the cotyledons, proline at first appearing in traces, but gradually increasing in amount in the second and the third week. On the other hand asparagine decreased in quantity from the first to the third week with a corresponding increase in the aspartic acid content. In the plumule, the radicle, and the leaves,

TABLE 2.—DISTRIBUTION OF FREE AMINO ACIDS IN THE SEEDLING OF ABRUS PRECATORIUS AT DIFFERENT STAGES OF GROWTH.***

Period of growth	Cotyledons	Plumules	Leaves and Tips	Radicles
0 Days	Cysteine (Traces) Glycine, Glutamine, Lysine, Spot "U" Spot "X"			
7 Days	Asparagine, Glycine, Glutamine, Lysine (traces), Proline (traces), Threonine (traces), Spot "U".			Asparagine, Proline.
15 Days	Asparagine, Glycine, Glutamine, Lysine (traces), Proline, Threonine, Tyrosine, γ-Aminobutyric acid. Spot "U".	Asparagine, Glutamine, Proline, γ-Amino-butyric acid, Two new spots near asparagine.	Asparagine, Proline, γ-Amino-butyric acid.	Asparagine, Proline, γ-Amino-butyric acid.
22 Days	Asparagine, Glutamine (traces) Glycine Proline, Threonine, Tyrosine, γ-Amino-butyric acid, Spot "U".	Asparagine, Glutamine (traces) Proline. γ-Amino butyric acid, Two spots near asparagine.	Asparagine, Glutamine (traces) Proline γ-Amino-butyric acid.	Asparagine, Proline, γ-Amino-butyric acid.

*** Exculnding those acids which were found to be present at all the four stages of growth, namely, abrine, alanine, aspartic acid, glutamic acid, phenylalanine, serine and valine.

tyrosine, glycine and threonine were not detected throughout the three weeks of study, although tyrosine was present in the cotyledons after the second week. γ-Amino butyric acid, however, could be recognised throughout the seedling during the second and the third week. The most significant observation was the presence of *abrine* not only in the cotyledons but also in the radicle, the plumule and the leaves and tips. This amino acid, therefore, does not appear to be metabolised by the plant (Table 3).

Kidd and West [8,9] found promotive effects of short pre-soaking periods on germination and seedling growth; however, as the presoaking period extended, these effects gave way to decreases in the germination percentages and the vigour of the seedlings. In the present study, therefore,

TABLE 3.—GERMINATION AND SEEDLING DEVELOPMENT IN *Abrus precatorius* AFTER DIFFERENT PERIODS OF SEED SOAKING.

Soaking hours	No. of seeds	After two days				After four days				After seven days				Seedling development (after 15 days)		
		A*	B*	C*	D*	A	B	C	D	A	B	C	D	Shoot length (cm.)	Root length (cm.)	Dry wt. of tops. (gm.)
0	25	6	17	2	Nil	4	14	7	Nil	4	14	7	Nil	4.73	1.65	0.1205
8	25	9	12	4	Nil	8	9	8	Nil	6	8	9	2	3.90	1.06	0.1118
24	25	5	13	7	Nil	5	11	9	Nil	4	12	9	Nil	5.71	1.34	0.2056
48	25	5	11	9	Nil	4	12	7	2	3	12	7	3	4.73	1.61	0.1004
72	25	3	17	5	Nil	3	16	6	Nil	1	14	5	5	2.88	1.16	0.0496

*A=Number of unsoaked seeds., B = Number of soaked but not germinated seeds., C=Number of seeds germinated., D=Number of seeds spoiled.

pre-soaking experiments on the seeds of *Abrus precatorius* were extended only upto 72 hours. Pre-soaking for 8 and 24 hours showed maximum number of germinated seeds but the differences between the various periods of soaking were not statistically significant. The seedlings in the 24 hours, presoaking batch looked much healthier than those soaked for 72 hours. Similar findings have also been reported on peas, beans and barley.[10]

Table 3 also shows the number of seeds that remained unsoaked in each batch. Even all the soaked seeds did not germinate, showing their non-viability which might be due to storage conditions. The family *Leguminosae*, to which *Abrus precatorius* belongs, is the one most commonly known to possess seeds with impermeable coats, but certain members of other families (Graminae, Malvaceae, Solanaceae and others) also produce such seeds. This character of impermeable seeds permits plants to be distributed in time instead of space and that may be an important factor in the continuance of the species. *Abrus precatorius* generally grows in draught ridden areas and the impermeability of some of its seeds might be useful in the survival of the species. Due to certain difficulties, long term studies were not carried out with these seeds, but it is expected that those seeds which remained impermeable to water during the three weeks of experimentation might become permeable to water in the course of time.

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