AMINO ACID PATTERNS IN GERMINATING SEED OF *PHASEOLUS ACONITIFOLIUS* JACQ.

LAKSHMI KRISHNASWAMY(1) and B. D. DESHPANDE(2)

Abstract: Patterns of amino acids have been studied in different stages of germination of *Phaseolus aconitifolius*. A comparison of amino acids of freshly harvested seeds and stored seeds reveals that aspartic acid, glutamic acid, alanine and tyrosine are present in all stages of germination of freshly harvested seeds while aspartic acid is absent in stages I and II of stored seeds but appears in stage III. Arginine is present in stages I and III of fresh and stored seeds and absent in stage II of both. However in stage III of stored seeds arginine is lacking in plumule. This may be regarded as threshold of viability. Amino acids like proline, leucine and valine are lacking in plumule of both fresh and stored seeds. This would only mean that these amino acids have no role to play in initiation of leaf primordia.

INTRODUCTION

Some work has been done on the chemical composition of seeds, and the changes in composition taking place during germination in members of Leguminosae. Damodaran *et al.* (1946) reported a decrease in total nitrogen to 7% after 16 days of germination in *Phaseolus mungo*. Oota *et at.* (1953) worked on the metabolism of germinating beans, *Vigna sequipedalis* in which the cotyledons showed a loss of proteins on the one hand, while new proteins appeared in all the other organs. Yamamoto (1955) showed that asparagine present in the cotyledons of *Vigna sequipedalis* seeds disappears and later appears in the hypocotyl and plumule. Ambie and Shone (1959) reported a decrease in decarboxylase enzyme during germination of some legumes. Altschul *et al.* (1966) found that a major portion of globulin in *Arachis hypogaea*—conarchin was found to disappear during germination. Altston and Irwin (1961) worked out a comparative extent of free amino acids and other secondary substances among *Cassia* species. They used chromatographic techniques in their work. Boutler and Barber (1963) noted the disappearance of arginine during germination of *Vicia faba* seeds. Guardiola and Sutcliffe (1971) showed that protease activity was controlled by the shoot, and is closely related to the senescence of the cotyledons.

The present investigation has been undertaken to study the changes in free amino acids and sugars during seed germination of *Phaseolus aconitifolius*. The results obtained from stored and freshly harvested seeds have been compared.

MATERIALS AND METHODE

Both stored (duration of storage 6 months) and freshly harvested (put for germination after a week) seeds were taken for analysis. In each case, the seeds

(1) Department of Botany, University of Delhi, Delhi, INDIA.
(2) Department of Bio Sciences, Birla Institute of Technology and Science, Pilani, (RAJASTHAN) INDIA.

were germinated in petri-dishes. The stages considered for detection of amino acids and sugars were:

1. Dry seed.
2. Seed with radicle just emerging.
3. Seed with cotyledons just unfolding.

When the seedlings reached the appropriate stage, they were washed, and separated into the cotyledons, plumule and radicle. These were crushed, and squashes were made in cold absolute alcohol. For sugars, hot 80% ethanol was used. After centrifuging the extracts, the supernatant was used for spotting in chromatography. Both paper chromatography and TLC techniques were used.

Detection of amino acids:

Amino acids were detected by paper chromatography (using Whatman No. 1 paper), and by thin-layer chromatography. The former gave better results. The solvent systems used were:—

1. *For paper chromatography.*
   a. *n*-butanol—acetic acid—water (4:1:5 v/v/v)
   b. *n*-butanol—acetic acid—water (5:4:1 v/v/v)
2. *For TLC*
   n-butanol—acetic acid—water (4:1:1 v/v/v)

In case of paper chromatography, after spotting the paper was run for 9½ hrs. Descending chromatography was used.

Table I. Freshly Harvested Seeds

Stages and Plant Parts		Amino Acids: Arginine	Aspartic acid	Glutamic acid	Glycine	Alanine	Tyrosine	Proline	Methionine	Leucine	Isoleucine	Valine
Stage I	Dry seed	+	+	+	+	+	+	—	+	—	+	—
Stage II Seed with radicle just emerging	(a) Whole seed	—	+	+	+	+	+	—	+	—	+	—
	(b) Cotyledons	—	+	+	+	+	+	—	+	—	+	—
	(c) Radicle	—	+	+	+	+	+	—	+	—	+	—
	(d) Plumule	—	+	+	+	+	—	—	—	—	—	—
Stage III Seed with cotyledons just unfolding	(a) Whole seed	+	+	+	—	+	+	+	+	+	—	+
	(b) Cotyledons	+	+	+	—	+	+	+	+	+	—	+
	(c) Radicle	+	+	+	—	+	+	+	+	+	—	+
	(d) Plumule	+	+	+	—	+	+	—	—	—	—	—

Table II. Stored Seeds

			Amino Acids										
Stages and Plant Parts			Arginine	Aspartic acid	Glutamic acid	Glycine	Alanine	Tyrosine	Proline	Methionine	Leucine	Isoleucine	Valine
Stage I		Dry seed	+	−	+	+	+	+	−	+	+	+	−
Stage II	Seed with radicle just emerging	Whole seed	−	−	+	+	+	+	−	+	+	+	−
		Cotyledons	−	−	+	+	+	+	−	+	+	+	−
		Radicle	−	−	+	+	+	+	−	−	−	+	−
		Plumule	−	−	+	+	+	+	−	−	−	+	−
Stage III	Seed with cotyledons just unfolding	Whole seed	+	+	−	−	+	+	+	−	+	−	+
		Cotyledons	+	+	−	−	+	+	+	−	+	−	−
		Radicle	+	+	−	−	+	+	+	−	−	−	+
		Plumule	−	+	−	−	+	−	−	−	−	−	−

For TLC, silica gel was used. A slurry of silica gel G in water (1:2 ratio) was made, and uniform plates were made. Plates were activated at 60°C, and samples were spotted, and plate run in the solvent system. Detection of amino acids was done using 0.25% ninhydrin solution (0.25gm of ninhydrin+90 ml butanol+10 ml distilled water). On spraying and warming at 60°C, the spots developed. Standard amino acids were also used.

Observations:

Observations are given in Tables I and II.

DISCUSSION

It is a well known phenomenon that seeds degenerate with age. The reason for the loss of vitality of seeds is probably not the depletion of stored foods, but is due to changes in the protein fractions. During storage, decrease in protein content, and increase in low molecular weight substances, such as free amino acids have been reported by Crocker and Barton (1957). Hence, due to such changes in reserve substances, the metabolic changes of freshly harvested seeds and stored seeds differ and this is what has been noted in the case of *Phaseolus aconitifolius* seeds.

It is significant to note that arginine, present in the dormant seed, (in both stored and freshly harvested seeds), disappears during germination (stage II) of *Phaseolus aconitifolius*. Similar disappearance of arginine was reported by Boutler and Barber (1963) in case of *Vicia faba* seeds. Whether the disappearance of arginine is due to increased synthesis of these proteins (arginine being incorporated into protamines and histones), or whether it is due to arginine degradation by reversal of ornithine cycle as suggested by Boutler and Barber (1963) from their studies on *Vicia faba*, needs further study. They suggested that the main storage products—arginine, histidine, aspartic acid and glutamic acid are broken down to provide the basic nitrogen compounds necessary for synthesis of new RNA templates, proteins and other prosthetic groups, needed by the germinating seeds. They also suggested a balance between degradation and synthesis of amino acids in *Vicia faba*. This is fairly well represented from the present observations on *Phaseolus aconitifolius*. Further arginine reappears in the stage III, *i.e.* at cotyledons unfolding stage of freshly harvested seeds as well as stored seeds. However in the stage III of stored seeds plumule lacks arginine. This may perhaps be the threshold of viability.

From the appearance of new amino acids, proline, valine and leucine in stage III, it seems that there is a rapid mobilisation of most amino acids. Their appearance may be due to breakdown of proteins (as the enzyme for this has been demonstrated in many seeds), or they may be synthesized from other amino acids.

Glutamic acid is present in all the stages, while proline appears in stage III (in freshly harvested seeds). There seems to be distinct relationship between glutamate and proline. α-ketoglutarate is the intermediate which gives rise to glutamic acid in presence of glutamic dehydrogenase, an enzyme widely distributed in living systems; either NADPH or NADH acting as coenzyme.

$$\alpha\text{-ketoglutarate}+NH_3 \underset{NADP^+}{\overset{NADPH+H^+}{\rightleftharpoons}} \text{L-glutamate}+H_2O$$

α-ketoglutarate also gives rise to L-glutamate in presence of pyridoxal phosphate enzyme. L-glutamate, in presence of NADPH or NADH, is reduced to glutamic-γ-semialdehyde, which in the presence of glutamic-γ-semialdehyde dehydrogenase, gives rise to proline. The overall pathway from glutamate to proline is reversible. Proline appears in the cotyledons and radicle in freshly harvested as well as stored seeds.

The amino acids, valine and leucine also appear in stage III, while isoleucine, present in stages I and II, disappears by the time the seedling enters the cotyledon unfolding stage. The routes of synthesis of leucine, valine and isoleucine follow closely-related pathways. The biosynthesis of leucine and valine could proceed through a common intermediate, α—ketoisovaleric acid, which by transamination gives rise to valine on one hand, and through a series of steps involving acetyl-CoA to leucine. This pathway accounts for the presence of both leucine and valine in stage III. The presence of both leucine and valine, in stage III, only in the cotyledons and radicle, shows that they have no role in the initiation of leaf primordia.

Isoleucine is present in the dormant seed and in the stage II—where the radicle just emerges out, but completely disappears in the stage III. This is seen in both stored and freshly harvested seeds. Since isoleucine and valine follow closely related pathways, isoleucine could have given rise to valine by the following sequence of reactions:—

$$\underset{\text{(isoleucine)}}{\alpha\text{-keto-}\beta\text{-methyl isovaleric acid}} \rightarrow \alpha\text{-ketoisovaleric acid} \rightarrow \text{valine}$$

This accounts for presence of valine in stage III.

Methionine is present in the dormant seeds, and in stages II and III in the cotyledons and radicle. The biosynthesis of methionine involves the intermediate, aspartic—β—semialdehyde. There seems to be some relation between methionine and aspartic acid. This intermediate, aspartic—β—semialdehyde is catalyzed by aspartyl-kinase to give β—aspartyl phosphate, which gives rise to aspartic acid. Aspartic acid is present in all stages of germination of freshly harvested seeds only stage III of stored seeds and forms a continuous pathway from the radicle to plumule and cotyledons. It is during these stages of germination that the pro-cambial strands of these three organs become continuous.

Thus all these results, regarding the changes in amino acids, go to show that there is an active breakdown of proteins during germination. Further, there is a rapid mobilisation of the amino acids during germination. These have been discussed in case of freshly harvested seeds. Storage has been found to affect the nitrogenous constituents of the seeds of *Phaseolus aconitifolius*.

According to Crocker and Barton (1957), during storage, proteins become less soluble and show an increase in free amino acids. In case of *Phaseolus aconitifolius*, this phenomenon is seen in case of the amino acid, leucine. While it is absent in the dormant seed and stage II in freshly harvested seeds, leucine is present in stored seeds in the dormant seed, and in stages II and III. This could be explained on the basis that in freshly harvested seeds, leucine may be present in a bound-form bound to proteins while during storage, this amino acid is liberated. In stored seeds it is found that aspartic acid is not present in the dormant seed and stage II, while in freshly harvested seeds, it is present in the dry or non-imbibed seeds, and in all stages of germination. This could be explained on the basis that during storage, aspartic acid is bound to proteins, or may get converted into some other amino acid.

Further, in stored seeds, methionine is restricted to the cotyledons, while in freshly harvested seeds, it is present in the cotyledons and radicle in stage II. This suggests that during storage, the amount of the amino acid could be becoming lesser as a result of which it is not sufficient enough to appear in the radicle. Or it could suggest that the enzymes (involved in breakdown of the proteins in which methionine is incorporated) are being affected by the storage conditions. Yet there is another possibility that in stored seeds, methionine moves from the radicle to the cotyledons to be stored.

The amino acid, isoleucine, also shows some differences in freshly harvested and stored seeds. It is present in all parts of the stored seeds in stages II, while it is present only in the cotyledons in stage II of freshly harvested seeds. This suggests that, during storage, the amount of isoleucine could increase due to breakdown of proteins. The proteins in the radicle and plumule could break down to give isoleucine. Isoleucine hence, could be in a bound form in freshly harvested seeds.

Proline and valine appear only in the stage III of germination. In freshly harvested seeds, both appear in the cotyledons and radicle, while in stored seeds proline appears in the cotyledons and radicle and valine only in radicle. This again suggests that proteins or amino acids, conld be migrating from radicle to the the cotyledons during storage.

Thus, all these results go to show that storage does affect the nitrogenous constituents of the seeds. It is significant to note that amino acids like proline, leucine and valine are conspicuously lacking in plumule of stored and freshly harvested seeds. This would only mean that these amino acids have no role to play in initiation of leaf primordia. Interestingly most of the changes observed in stored seeds are mainly in plumule and radicle which contain apical meristems.

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