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QUALITATIVE & QUANTITATIVE ESTIMATION OF AMINO ACIDS IN FOUR LEGUMINOUS SEEDS, UNDER DIFFERENT STORAGE PERIOD

Anumapa Modi* **& A.K. Kandya**
Shri Umiya Kanya Mahavidyalay Rau, Indore

ABSTRACT

Biochemical changes to occur during seed storage and seed germination. Many food reserves in seeds interact during metabolism prior to germination. Proteolytic enzymes present in the endosperm and cotyledon, hydrolyze the reserve proteins into pet ides and finally into amino acids. The amino acids are then translocated into the growing embryo. Analysed amino acid in freshly collected seeds or A.precatorius, B. monsperima D.paniculata, P.marsupium by thin layer chromatography.

Keywords- *Biochemical, Germination, chromatography, Cotyledons, Fresh seeds & Reserve.*

I. INTRODUCTION

Seeds the embryo is large in relation to the seed as a whole. It fills the seed almost completely, and its body parts, particularly the cotyledons, store the food reserves inleguminous seeds with endosperm or perisperm are called albuminous. After attaining maturity, the seed is the means by which the new individual is dispersed. The success with which the new individualis dispersed. The success with which the new individual is established is largely determined by the physiological and biochemical features of the seed. The keys to this success are the seeds responses to the environment and, on a Biochemical level, its food reserves, which are available to sustain the young plant to the early stages of growth.

The details of chemical composition of seeds for cultivated species are available, information's on various chemical are biochemical constituents on seeds of several forest tree species are not available. With the increasing interest in finding alternative source of food and in improved genetic diversity within domesticated lines; the seeds of forest tree are now gaining more attention.

In addition to normal chemical constituents found in all plant tissues, seeds contain extra amount stored since source of food reserves support early growth of germinates. These are principally carbohydrates, fats, oil and proteins. The chemical composition of seed is determined ultimately by genetic factors and hence vary widely from species to species.

Biochemical components are stored in the endosperm/cotyledons. The principal storage carbohydrate is starch in the form of starch grains. Starch is combined in various proportions with proteins, oils and fats. Seeds during storage was taken to study its relative capacity, spoilage of quality and quantity of sugars in seed under different controlled conditions of temperature and relative humidity are recorded. The fungus reduced the quantity of sugars and qualitative spectrum of sugar was also found to be changed considerably. Infested seeds shows a depletion in non-reducing sugars, increase in reducing sugars and changes in qualitative spectrum of sugar, higher levels of relative humidity and temperature was observed to be more deleterious for seed health.

The present Endeavour was undertaken to determine quantitatively the amino acids in seeds of several age groups and related the changes to the suitability of a seed sample in the evaluation of their germinability.

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II. MATERIAL AND METHOD

Estimation of amino acids :

Analysis of amino acids, was done in two stages :

For the first experiment, freshly collected seeds of all four species were kept for germination test inside petridishes lines with 2 moistened filter papers. Those seeds which imbibed in 24 hrs., were regarded as readily germinable (non-dormant and those which did not absorb water but remained firm, were taken as dormant for the analysis of amino acids. imbibed seeds were air dried and powdered in a motor driven crusher; unimbibed seeds were also powdered separately.

For the second experiment, fresh one year, two year and three year old seeds of all the four species were powdered separately. There was no separation of dormant and non-dormant seeds in these samples. Detection of various amino acids was done by thin layer chromatography (TLC). Aqueous paste of one gram seed powder in 5ml of distilled and sterilized water prepared at room temperature was kept for 24 hrs. so that maximum free amino acids could leach out in the extract. For quantitative determination of amino acids standard solutions of 0.05%, 0.10%, 0.15%,).20%,).25% and 0.225% of known amino acid in 10% isopropanol were applied on whatman No. 1 paper and developed in n-Butenol:Acetic acid; water (B:A:W, 4:1:5 v/v). The chromatogram of unknown sample was also developed in the same manner. Both the chromotagrams were sprayed with 0.3% w/v ninhydrin and the developed spots were eluted with 5ml of 10% v/v isopropanol. The optical densities of known and unknown amino acids solutions were measured at maximum wave length 550nm by spectrophotometer model CL 24. Which was used to make the spot on the silica gel plate. Capillary of equal diameter was used for a fixed number (12 times) to make and concentrate the spot. The solvent system used was n-Butenol:Glacial Acetic Acid:Distilled water (4:1:1; v/v). The colour developing (Spraying) reagent used was 0.3% solution ninhydrin Rf valued and colours of different amino acids were matched with the standards (Pure amino acids of entrons, Bombay) run in the same solvent system, in the same conditions. Detection of amino acid-spots on the chromatogram was done in the background of UV light. Quantification of amino acid was done on the basis of size and colour intensity of the spot of a particular amino acids.

Quantitative estimation of amino Acid :

By spectrophotometric method the quantitative determination of amino acids. Standard solutions of 0.05%, 0.10%,.15%, 0.20%, 0.25% and .225%, of known amino acid in 10% isopropanol were applied on what man No. 1 paper and developed in n-Butenol : Acetic acid : water (B:A:W, 4:1:5v/v). The chromatogram of unknown mixture of amino acids was also developed in the same conditions. Both the chromatograms were sprayed with 0.3% w/v ninhydrin and the developed spots were eluted with 5ml of 10% v/v 150 propanol. The optical densities of known and unknown amino acid solutions were measured at maximum wave length 550nm by spectrophotometer model CL 24.

III. RESULTS AND DISCUSSION

Qualitative estimation of amino acids :

Presence of different amino acids in variously aged seeds of different species are recorded in Table-1. through 4 spot intensities were classified as dense (+++). Medium (++) and Trace (+). In Abruspracatorius in all 5 amino acids were indentified in the sample of fresh seeds lot spot intensity was dense (+++) for cytosine, lysine and tyrosine, and medium (++) for valine and proline. In the seeds of one year old lot it was dense for tyrosine and medium for others. In the two year old seed lot tyrosine showed medium, cystine, lysine, proline and valine the spot intensity were so low that they classified under the spot intensity class-traces (+). With growing age of the seed lot, qualitatively, the amino acid concentration dwindled. It is conjectured that the quantity of different amino acids is likely to decline with increasing age of seeds.

From the samples of Buteamonosperma seven amino acids valine, cystine, lysine, glycine, L-roline, L-leucine and one unidentified amino acid could be detected (Table-2). Here also there was a gradual decline in the spot intensities, and some of them vanishings in the three year old seeds lot (lysine, glycine, and serine).

In the samples of Dalbergiapaniculata six amino acids namely valune, glycine, histidine and one unidentified amino acid were scored. It was noted that with ageing there is a gruadual dimunition in the spot intensities of all the amino acids except that of cystine, (Table-3).

From the seed samples of Pterocarpusmarsupium only four amino acids viz. aspartic acid, cystine, serine and valine were scored (Table-4). In this species also againg concentration as observed in other species.

Quantitative estimation :

The results of quantitative estimation of amino acids are recorded in table number 5-8. In A.precatorius the amino acids cystine, valine, proline, lysine and tyrosine show a decreasing tendency with increasing age (Table-5). in the 3 year old seed lot the quantity of cystine, lysine and proline could not be determined since they occurred in traces. The quantity of lysine decreased drastically from 6.03% in fresh seed lot to).044% in the two year old seed lot. The quantity of valine showed a decline with increasing age. The difference in quantity of valine in the fresh seed lot and that of 2 year old (5.565 and 4.815%) was not very marked, but in the samples of three year old lot its quantity fell to 1.403%.

From the seeds of Buteamonosperma (Table-8) eight amino acids scored. In the samples of different age group seeds lots the quantity of these amino acids decreased with increasing age. Lysine, glysine, proline and serine were not detected, quantitatively, in the 3 year old seeds lot.

The amino acid profile of the seeds of Dalbergiapaniculata are presented in the table-7. Six amino acids valine, cystine, lysine, glysine, histidine and one unindentified amino acid were scored. In these seed lots also there is a general trend of decreasing quantity of different amino acids with increasing age of the seed lots. Out of these histidine diminished drastically from 6.329% in the fresh seed lot to 1.840% in the two year old seed, finally leaving no traces in the 3 year old seed lot. Of the remaining four amino acids, though present in the three year old seed lot, the quantity was very less (See Table-7).

In the seeds samples of Pterocarpusmarsupium (Table-8) four amino acids, namely aspartic acid, cystine, serine and valine were quantified. Quantitative analysis bore similar results, in so far as decreasing trend with increasing age is concerned, as recorded for the other three species under this study. Aspartic acid and serine did not register any quantity in the 2 and 3 year old seed lots, while cystine and valine did, though in diminished quantity (cystine from 9.200% in the fresh seed lot to 2.086; in the 3 year old lot; valine from 6.576% to 3.234%).

These observation of qualitative and quantitative analysis of the amino acids are in agreement with each other and also corroborate with the results of catalase quotient and excised embryo test.

Table-1 : Qualitative analysis of amino acids in variously aged seeds of Abrusprecatorius

S. No.	Spot Amino Acids	Visual colour assessment of concentration of amino acids developed			
		Fresh seeds	1 yr. old seeds	2 yr. old seeds	3 yr. old seeds
1.	Cystine	+++	++	+	+
2.	Valine	++	++	+	+
3.	Lysine	+++	++	+	+
4.	Proline	++	++	+	+
5.	Tynosone	+++	+++	++	+

Table-2 : Butea monosperma

1.	Valine	+++	++	+	+
2.	Cystine	++	++	+	+
3.	Lysine	+++	++	+	+
4.	Glycine	++	++	+	+
5.	L - proline	+++	+++	++	+

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6.	L-leucine	+	-	+	+
7.	Serine	+++	++	++	-
8.	Unidentified	+	+	+	+

+ = Trace, ++ = Medium, +++ = Dense

Table-3 : Qualitative analysis of amino acids in variously aged seeds of Dalbergia panciulata

S. No.	Spot Amino Acids	Visual colour assessment of concentration of amino acids developed			
		Fresh seeds	1 yr. old seeds	2 yr. old seeds	3 yr. old seeds
1.	Valine	+++	++	++	+
2.	Cystine	+++	+	++	+++
3.	Lysine	++	++	+	+
4.	Glycine	+++	++	+	+
5.	Histidine	++	++	+	++
6.	Unidentified	+	+	+	-

Table-4 : Pterocarpus marsupium

1.	Aspartic acid	++	+	-	-
2.	Cystine	+++	++	+	+
3.	Serine	++	++	-	-
4.	Valine	+++	+++	++	++

+ = Trace, ++ = Medium, +++ = Dense

Table - 5 : Quantitative estimation of amino acids in different individual plant species, under different storage period (A. Precatorius)

S.No.	Amino Acids		Fresh seeds	1 Yr. Old seeds	2 yr. old seeds	3 Yr. old seeds
1.	Cystine	A	0.060	0.040	0.030	-
		B	0.149	0.099	0.074	
		C	4.492	3.396	2.086	
2.	Valine	A	0.050	0.106	0.070	0.804
		B	0.124	0.263	0.174	0.200
		C	5.565	4.873	4.815	1.403
3.	Lysine	A	0.060	0.020	0.030	
		B	0.149	0.049	0.074	-
		C	6.034	2.199	0.044	
4.	Proline	A	0.205	0.050	0.060	-
		B	0.509	0.124	0.149	
		C	6.259	4.267	2.259	
5.	Tyrosine	A	0.050	0.070	0.050	0.030
		B	0.124	0.174	0.124	0.074
		C	5.267	4.815	3.432	2.779

A = Optical density, B= Concentration (%), C= Calculated (%)

Table - 6 : Quantitative estimation of amino acids in different individual plant species, under different storage period (B. monosperma)

S.No.	Amino Acids		Fresh seeds	1 Yr. Okd Seeds	2 Yr. old seeds	3 Yr. Old seeds
1.	Valine	A	0.090	0.401	0.050	0.106
		B	0.293	0.997	0.124	0.263
		C	6.161	7.108	3.426	3.234
2.	Cystine	A	0.070	0.090	0.104	0.030
		B	0.174	0.223	0.258	0.074
		C	8.254	7.827	3.189	3.143

3.	Lysine	A B C	0.101 0.251 6.935	0.040 0.099 3.712	0.106 0.263 3.251	-
4.	Glysine	A B C	`0.108 0.268 9.190	0.050 0.124 3.495	0.209 0.519 4.787	- -
5.	L-Proline	A B C	0.103 0.256 3.882	0.050 0.124 4.252	0.106 0.263 3.234	- -
6.	L-Leucine	A B C	0.108 0.268 4.611	- -	0.080 0.199 5.610	0.040 0.099 2.158
7.	Serine	A B C	0.080 0.199 8.453	0.206 0.512 6.296	- -	- -
8.	Unidentified	A B C				

A = Optical density, B = Concentration (%), C = Calculated (%)

Table - 7 : Quantitative estimation of amino acids in different individual plant species, under different storage period (Dalbergiapaniculata)

S.No.	Amino Acids		Fresh Seeds	1 Yr. Old seeds	2 Yr. Old Seeds	3 Yr. Old Seeds
1.	Valine	A	0.401	0.090	0.050	0.106
		B	0.997	0.293	0.124	0.263
		C	7.108	6.161	3.426	3.234
2.	Cystine	A	0.070	0.104	0.100	0.30
		B	0.174	0.258	0.248	0.074
		C	8.254	3.189	4.267	2.044
3.	Lycine	A	0.101	0.300	-	0.107
		B	0.251	0.746	-	0.266
		C	6.935	5.318	-	3.271
4.	Glycine	A	0.090	0.060	0.209	0.050
		B	0.233	0.149	0.519	0.124
		C	0.457	6.034	4.787	3.495
5.	Histidine	A	0.060	0.105	0.020	-
		B	0.149	0.261	0.049	-
		C	6.329	2.407	1.840	-

A = Optical density. B = Concentration (%), C = Calculated (%)

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Table - 8 : Quantitative estimation of amino acids in different individual plant species, under different storage period (P.marsupium)

S.No.	Amino Acids		Fresh Seeds	1 Yr. Old seeds	2 Yr. Old Seeds	3 Yr. Old Seeds
1.	Aspartics	A	0.104	0.300	-	-
		B	0.258	0.746	-	-
		C	7.129	5.318		
2.	Cystine	A	0.101	0.104	0.209	0.030
		B	0.251	0.258	0.519	0.074
		C	9.200	3.189	4.809	2.086
3.	SErine	A	0.060	0.030	-	0
		B	0.149	0.074	-	-
		C	4.117	2.044	-	3.271
4.	Valine	A	0.304	0.401	0.030	0.106
		B	0.756	0.997	0.074	0.263
		C	6.576	7.108	2.230	3.234

A = Optical density. B = Concentration (%), C = Calculated (%)

Amino acids play an important role in seed viability and longevity. There appears to be a dynamism in quality and quantity of amino acids during the storage of seeds. In B. monosperma seeds showed presence of more amino acids (8) as compared to other 3 forest tree species. In case of D. paniculata concentration of cystine is increased because some of the amino acids might have degenerated during seed storage.

In the present investigation the amino acid profile (quantitative) presents a decreasing tendency with increasing age of the seeds. All amino acids, except cystine, in D. paniculata of all the species decrease with prolonged storage. Cystine in D.paniculata showed a tendency to increase with increasing age of the seeds. Quantitative analysis of the amino acids in seeds stored for an unknown period should be helpful in determining the seed quality.

IV. CONCLUSION

A study of amino acids present in variously aged seeds (fresh, one year, two years and three years old) of four selected species revealed that the number of amino acids was 5 in A.precatorius seeds. Though in B.monosperma, the number os amino acids was 8. In case of D.paniculata, the number of amino acids was 6 and in case of P.marsupium, the number of amino acids was 4. Different storage conditions were also found to affect the qualitative and quantitative changes of amino acids in relation to storage duration. However, no significant pattern could be observed. Some of the amino acids remained unidentified because of their of value and/or colour could not be matched with the standards.

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