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Qualitative analysis of amino acids and sugars from fungal infested seeds of *Cyamopsis tetragonoloba* L. (Taub.) in storage

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Changes in the qualitative spectrum of amino acids and sugars in seeds of guar *(Cyamopsis tetragonoloba)* infested by six different fungi were assessed after different storage periods. In control seeds fifteen amino acids (both free and bound) were present upto 180 days of storage periods, while those artificially infested particularly with *Aspergillus flavus, Penicillium citrinum* and *Trichoderma viride* showed pronounced changes in amino acid contents. Similarly changes in sugar contents of seeds were observed in the fungal infested seeds upto 180 days storage. Seeds infested with *A. flavus, P. citrinum* and *T. viride* showed complete absence of sugars after 180 days of storage periods.

Key words : *Cyamopsis tetragonoloba*, Seeds, Seed-borne fungi, Qualitative analysis, Amino acid, Sugar.

INTRODUCTION

Cluster bean, *Cyamopsis tetragonoloba* L. (Taub.), commonly known as guar is a drought hardy crop of the Indian arid zone. In addition to its uses as vegetable, feed and foddar crop, guar gum mainly present in seed endosperm, provides a useful raw material for a wide range of industrial products. Annual production of guar seeds is around 1.11 million tons of which about 1/3 is used by the gum factories. Amino acids composition of various diseased plant tissues has been investigated (Chang *et al.*, 1959 ; Rudolph, 1963 ; Pillani and Shanta, 1965). Bhargava and Arya (1983) also observed that either some amino acids to disappear completely or their quality decreased considerably, while concentration of a few amino acids increased and some new ones appeared in seeds as a result of fungal infestation. The present paper aims to study the

qualitative analysis of amino acids and sugars of guar seeds due to fungal infestation during different storage periods.

MATERIALS AND METHODS

Cluster bean seeds cv. Pusa Naubahar, were used as the experimental material. Six dominant storage fungi isolated from guar seeds, viz. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium citrinnm*, *Rhizopus nigricans* and *Trichoderma viride*, were selected as the test fungi. These fungi grew frequently on seeds in culture plates during the experimental work. The pure cultures of the test fungi, maintained in agar slants, were transferred to Petri dishes containing PDA medium. Spore suspension of the ten day old culture of the each of the fungi were prepared separately. Concentration of the spores was adjusted to approximately $4x10^4$ spores ml with the help of a haemocytometer. Seeds (100 g) were taken for each treatment. The weighed samples were surface sterilized with 0.1%$HgCl_2$ solution and washed several times with sterile distilled water. The samples were soaked in folds of sterile blotting papers to remove excess of adhered water and then kept in an oven at 40°C for 24 h. After drying, the seed samples were transferred to 500 ml Earlen-Mayer flasks and subsequently inoculated with 5 ml of the spore suspension of the test fungi. The flasks were shaken vigorously for ascertaining uniform spore load over seeds. All the six fungi were tested separately. After inoculation each sample was kept in a sterilized cloth bag and stored in a desiccator at 96% relative humidity maintained with a solution of sulphuric acid (C.M.I., 1974). Another seed lot of the same quantity after surface sterilization was also kept in a similar cloth bag without fungal spore suspension for control in a desiccator at same relative humidity. Both the sets, control as well as treated ones were kept in the laboratory conditions for six months. Qualitatively the analysis of amino acids and sugars was done periodically for a period of six months.

Preparation of extracts

Alcoholic as well as acidic extracts of seed samples were prepared according to the methods suggested by Singh and Tondon (1970) and Sekhawat and Kothari (1971) respectively. Two g powdered seed sample was blended with 100 ml 80% alcohol and boiled on a water both till the volume was reduced to 25 to 30 ml. The solution was cooled down and filtered. The filtrate was evaporated to dryness in an evaporating disc and the residue left on disc was dissolved in 2 ml of 70% alcohol and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected in another clean test tube and was used for analysis of sugars and free amino acids. The residue left in the filter

paper was hydrolysed by adding 4 ml of 6N HCl and autoclaved for ten min. It was subsequently filtered and the filtrate was evaporated to dryness. The residue was taken in 2 ml of 70% alcohol and after centrifuging at 3000 rpm for 15 min, it was used for the analysis of bound amino acids.

Detection of amino acids and sugars

For detection of free and bound amino acids and sugars, unidirectional paper chromatographic technique (Block *et al.*, 1955) was followed. An aliquot of 0.25 μl extract was loaded on the paper chromatogram parallel to the standard solution. The solvent system used for running the chromatogram was n-butenol : acetic acid : water (4 : 1 : 5, v/v). Ninhydrin 0.3% solution was applied for developing the spots of amino acids, while analine-pthalate solution was used for developing the spots of sugars. After the chromatogram was developed, it was dried under hood till the paper was free from solvent vapour. Thereafter the chromatogram was sprayed with ninhydrin for the amino acids and analine-pthalate reagent for the sugars and dried at room temperature for 15 min. It was then heated at 100°C for 5 to 8 min in an oven. The amino acids as well as the sugars gave various shades of spots. The identification of amino acids and sugars was done by comparing the Rf values of the spots with those of standard samples of each. The intensities of the spots were graded by visual observations in different categories and noted as low(+), medium (++), high (+++) and absent (—).

RESULTS AND DISCUSSION

Fifteen amino acids (both, free and bound) were present upto 180 days of storage, while change in amino acids in the infested seeds was not so well marked. The seeds treated with *A. flavus*, *P. citrinum* and *T. viride* showed pronounced change in amino acids. Moreover, maximum amino acids were present upto 120 days of storage either at the medium or low level but they were mostly absent after 180 days of storage. During the last storage periods (180 days), 8 amino acids disappeared in seeds infested with *A. flavus*, 7 in case of with *A. fumigatus*, 5 with *A. niger* 10 in *P. citrinum* and 2 in *R. nigricans* ; while 11 amino acids completely disappeared in caseof *T. viride*. Asparagine, serine, threonine and tryptophane were recorded only in bound from in all the treated samples including control (Table 1). Similarly changes in four sugars viz. glucose, fructose, maltose and sucrose due to infestation of these fungi were observed in the treated as well as the control seeds upto the last storage period of 180 days. All the sugars were present upto 180 days of storage period either at the high, medium or in low degrees. But

Table 1. Changes in amino acids in cluster bean seeds due to different fungi during different storage periods.

Amino acids	Storage period/days																								
	60							120							180										
	C	1	2	3	4	5	6	C	1	2	3	4	5	6	C	1	2	3	4	5	6			
Alanine *	3+	2+	2+	2+	2+	2+	2+	2+	+	+	+	—	2+	+	2+	+	—	+	—	+	—				
**	2+	+	+	2+	+	2+	+	2+	—	+	+	—	+	+	+	—	—	+	—	+	—				
Aspartic acid	3+	2+	3+	2+	2+	2+	2+	2+	+	2+	+	+	+	+	2+	+	+	+	—	+	—				
	2+	+	2+	+	+	2+	+	2+	—	+	+	+	+	+	+	—	+	+	—	+	—				
Asparagine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
	2+	+	2+	+	+	2+	+	+	—	+	+	+	+	+	+	—	—	+	+	+	—				
Cystine	2+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	—	+	—	—	+	+				
	2+	+	+	+	2+	+	+	+	—	+	+	+	+	+	+	—	—	+	—	+	+				
Glutamic acid	3+	2+	2+	2+	2+	2+	2+	2+	+	2+	+	+	2+	+	2+	—	+	—	—	+	+				
	2+	+	2+	+	+	2+	+	2+	—	+	+	+	+	—	+	—	+	+	+	+	—				
Glycine	2+	+	2+	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+	—	—	—				
	2+	+	+	+	+	+	+	+	—	+	+	—	+	+	+	—	—	+	—	—	—				
Isoleucine	2+	+	+	+	+	2+	+	+	—	+	+	—	+	—	+	—	+	+	—	+	—				
	3+	2+	2+	2+	2+	2+	2+	2+	+	+	+	2+	2+	+	2+	+	+	+	+	+	+				
Histidine	2+	+	+	+	+	+	+	+	—	+	—	—	+	+	+	—	+	—	—	+	—				
	2+	+	+	+	+	2+	+	+	—	+	+	—	+	+	+	—	+	—	—	+	+				
Methionine	2+	+	2+	+	+	2+	+	+	+	+	+	—	+	—	+	—	+	—	—	+	—				
	2+	+	2+	+	+	2+	+	+	+	+	+	+	+	—	+	—	+	—	+	+	—				
Proline	2+	+	2+	+	+	2+	+	+	+	+	—	+	+	—	+	+	—	—	+	—	—				
	2+	+	2+	+	+	+	+	+	+	+	—	+	+	—	+	—	—	—	—	—	—				
Serine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
	2+	+	2+	+	+	2+	+	+	+	+	+	—	+	+	+	+	+	—	—	+	—				
Threonine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
	2+	+	2+	+	+	2+	+	2+	+	+	+	+	+	+	2+	—	+	+	—	+	+				
Tryosine	+	+	+	+	+	+	+	+	+	—	—	—	+	—	+	—	—	—	—	+	—				
	2+	+	2+	+	+	2+	+	2+	+	+	+	+	+	+	+	—	—	+	—	—	—				
Tryptophane	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
	+	+	+	+	+	+	+	+	—	—	—	—	+	—	+	—	—	—	—	+	—				
Valine	2+	2+	+	+	+	2+	+	2+	+	+	+	+	+	+	+	—	—	+	—	+	—				
	2+	+	+	2+	+	2+	+	2+	—	+	+	+	+	+	+	—	—	+	—	+	—				

C = Control, 1 = *Aspergillus flavus*, 2 = *A. fumigatus*, 3 = *A. niger*, 4 = *Penicillium citrinum*,
5 = *Rhizopus nigricans*, 6 = *Trichoderma viride*, * = Free amino acids, ** = Bound amino acids,
+ = Present, — = Absent

in case of *A. flavus*, *P. citrinum* and *T. viride*, these sugars were completely absent in the treated sampling after 180 days of storage (Table 2).

Table 2. Changes in sugar contents of cluster bean seeds due to different fungi during different storage periods.

Fungi	Storage period/day											
	60				120				180			
	GL	FR	MA	SU	GL	FR	MA	SU	GL	FR	MA	SU
Aspergillus flavus	2+	2+	+	2+	+	—	—	+	—	—	—	—
A. fumigatus	2+	2+	2+	2+	2+	+	+	+	+	+	+	+
A. niger	3+	2+	2+	3+	2+	+	+	2+	2+	+	+	+
Penicillium citrinum	2+	+	+	2+	+	—	—	+	—	—	—	—
Rhizopus nigricans	3+	2+	2+	3+	2+	2+	2+	2+	2+	+	+	2+
Trichoderma viride	2+	+	+	2+	+	+	+	+	—	—	—	—
Control	3+	2+	2+	3+	3+	2+	2+	3+	2+	+	+	2+

GL=Glucose, FR=Fructose, MA=Maltose, SU=Sucrose, +=Present, —=Absent.

A perusal of Table 1 reveals that there are qualitative changes in the amino acids of seeds due to fungal infestation. Some amino acids disappeared at the end of incubation periods, while others decreased at different levels. The total disappearance of some amino acids in the infested seeds may be attributed to their preferential utilisation by fungi or degradation by their enzymatic activity. McComb and Winstead (1964) reported that considarable decrease in the level of several amino acids in the seeds was due to fungal invasion. Reiner and Zeigler (1970) reported that amino acids in the host, produced by the break-down of proteins, were readily utilised by the pathogens for the synthesis of fungal protein which naturally leads to a fall in amino acid level in diseased tissues. Similarly fall in sugar level varied with the fungal organisms associated with the seeds. In the present investigation all the sugars got reduced in their concentration in the infested seeds with increased storage periods, which may be due to their preferential utilisation by fungi. Preferential utilization of sugars by fungi has also been reported by Cochrane (1958).

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