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ASPERGILLUS FLAVUS INDUCED ALTERATIONS IN THE ACTIVITY OF ENZYMES RELATED WITH AMINO ACID IN URD BEAN SEED

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The activity of proteolytic enzyme, non-oxidative deaminase and amino acid oxidase of DL-valine, DL- phenylalanine, Iso-leucine and L-serine were stimulated in the seeds of urd bean due to their storage with *Aspergillus flavus* while that of alanine- ammonia transferase, glutamic - alanine transaminase and glutamic - aspartate transaminase was noticed in inoculated seeds only. Similarly, amino acid decarboxylase activity of DL - valine, DL-phenylalanine and L-serine was observed in the inoculated seedlot only while that of Iso- leucine was found in inoculated and control seedlot both. The activity of all the said enzymes was rapid with increasing RH level of storage from 60 to 80%.

Keywords: Amino acid; *Aspergillus flavus;* Enzymes; RH; Storage; Urd bean seed.

Introduction

Quantitative and qualitative alteration in amino acids of the seeds due to storage fungi have been worked out earlier[1-4]. The authors have noticed the increase of some amino acids, decrease of others and appearance of new ones due to association of the storage fungi with the seeds. *De novo* synthesis of amino acids in plants involving transamination and transferase has been reported besides decrease due to decarboxylation, deamination and oxidation[5,6]. Glutamic-aspartate transaminase by *Neurospora crassa*[7], glutamic alpha - keto acid transaminase and D-amino acid oxidase by *Aspergillus niger, Penicillium chrysogenum, P. notatum* and a few others have also been reported[8]. Observing the source of above changes in amino acids, the present paper aims at reporting the enzymic activity related with increase, decrease and interconversion of amino acids resulting in some new ones in urd bean seeds stored with the storage fungus *Aspergillus flavus* at high RH levels congenial for luxuriant growth of the fungi with the seeds.

Materials and Methods

Twenty five g of urd bean(*Phaseolus mungo* L .) local variety of seedlot possessing 100% germinability was surface sterilized with freshly prepared 2.0% sodium hypochlorite solution for 2 min and washed three times with sterilized distilled water and inoculated with 0.4ml spore suspension (1 x 10^4 spores/ml) of *Aspergillus flavus* Link ex Fries in 10% Tween 20 growing on potato dextrose agar medium for 7 days at 28±1°C. The inoculated seedlot was thoroughly agitated taking in sterilized conical flasks of 50ml capacity. These seedlots were stored over glycerol solution to maintain 60,70 and 80 % RH in sealed desiccators at 30±1°C[9] besides the separate control without fungus. The seedlots were incubated for 30 days and enzyme activities noted below were assayed in them.

Proteolytic enzyme (PE): The activity was assayed[10] extracting 2g of stored seedlot with a mixture of 5ml each of water and 0.5 N HCl at 5°C and estimating the activity on dissolving casein to make 0.2 % solution as substrate in 0.1 M phosphate buffer pH7. Amino acid released (mg / min / ml) extract was calculated with the help of calibration curve using serial dilution of 0.1% glutamic acid.

Alanine - ammonia transferase (AAT): The activity was measured[5] based on the transamination between L-alanine and alpha- ketoglutaric acid to produce pyruvic acid and glutamic acid. The former which forms hydrazone with 2,4-dinitrophenyl hydrazine was read at 530nm[5] 2g of seed was extracted with 10ml of 0.2M Tris buffer pH 8.7. The result was expressed as mg pyruvic acid released/ml extract/ 30min with the help of calibration curve using serial dilution of 0.1% pyruvic acid.

Glutamic - alanine transaminase (GAlT) : 2g seedlot was extracted in 10ml of 0.17M Tris buffer pH 8.9 and the activity was assayed measuring the colour developed by 2,4- dinitrophenyl hydrazine at 550 nm[6]. The amount of pyruvic acid in mg formed was calculated with the help of calibration curve using serial dilution of 0.1% pyruvic acid.

Glutamic - aspartate transaminase (GAsT): 2g seedlot was extracted in 10 ml of 0.2 M Triethanolamine buffer pH7.4 and the activity was measured at 340 nm[5] at an

Table 1. Activity of Proteolytic enzyme, Alanine - ammonia transferase, Glutamic alanine transaminase and Glutamic - aspartate transaminase in Urd bean seeds stored with *A. flavus* at 60, 70 and 80% RH.

Enzymes		RH (%) 60	70	80
Proteolytic enzyme	I	0.052	0.083	0.181
(mg amino acid released / min / ml extract)	C	0.038	0.056	0.083
Alanine - ammonia transferase	I	0.287	0.421	0.628
(mg pyruvic acid released / 30 min / ml extract)	C	–	–	–
Glutamic - alanine transaminase	I	0.215	0.362	0.507
(mg pyruvic acid released / 30 min/ml extract)	C	–	–	–
	Time in sec			
Glutamic - aspartate transaminase	I 60	0.22	0.33	0.52
(0.D / 60 second)	*120*	*0.26*	*0.37*	*0.58*
	180	*0.29*	*0.40*	*0.63*
	C 60	–	–	–
	120	–	–	–
	180	–	–	–

I = Inoculated, C = Control, – = No activity

interval of 30 sec. The result was expressed as change in 0.D/60 sec for a period of 180 sec.

Amino acid decarboxylase (AAD): 2g seedlot was extracted with 10 ml of 0.1M phosphate buffer pH 7 and as substrate 0.1 % DL - valine , DL- phenylalanine, Iso-leucine and L -serine were taken. CO_2 released from carboxyl group of amino acid was read with phenolphthalein as indicator at 550 nm[11]. The result was expressed as mg CO_2 released / 30 min/g seed using serial dilution of .1% of sodium bicarbonate solution

Non-oxidative deaminase (NOD): 2g seedlot was extracted with 10ml of 0.1M phosphate buffer pH6.8 and ammonia released from 0.1% DL- valine, DL - phenylalanine, Iso-leucine and L - serine was read with Nessler's reagent at 420nm[5]. The result was expressed as mg ammonia released / 30min / g seed using serial dilution of 0.1% ammonium chloride.

Amino acid oxidase (AAO): 2g seedlot was extracted with 10ml of 0.067 M phosphate buffer pH7 and the uptake of oxygen by those amino acids used for assaying the activity of NOD was measured with Warburg's manometer[6]. The result was expressed as µl oxygen uptake/ 15 min / ml extract.

Results and Discussion

The activity of PE which was more in the inoculated seed, increased due to rise in the level of RH of storage. The activity of AAT, GAlT and GAsT was observed in the inoculated seedlots only. Those, too, were augmented with rise in RH level. AAD activity of DL - valine, DL - phenylalanine and L - serine was observed in the inoculated seedlots only while that of Iso - leucine was observed in both types of seedlot, sluggish in the control one. The activity increased with rise in the RH. NOD and AAO activities of all the four amino acids were found in both types of seedlots but rapid in the inoculated and sluggish in the control lots. These were also observed stimulated at high RH.

Qualitative and quantitative alterations in amino acids in seeds due to the impact of storage fungi are the common features. It has been observed in coriander[12], finger millet[13], lablab bean[3] and other pulses seeds[2]. As the PE activity in the inoculated seedlot was observed to be higher than the control, it can be conjectured as the potential source of quantitative increase and qualitative change of amino acids in the seed. For decrease of a particular amino acid in pulses utilization by storage fungi has been held responsible[2] besides augmented respiration of the seeds[4,14]. The fungi such as *Neurospora crassa*[7], *Penicillium, Aspergillus* and others and many bacteria have been reported to possess glutamic alpha - keto acid transaminase and D - amino acid oxidase activities[8], disclosing that the fungi possessing noted enzyme activities related with amino acids metabolism may inflict qualitative alteration in the seed. Elaborating the above statements[2,4,14] stimulated NOD and AAO in the seed due to *A. flavus* appears one of the significant sources of reduction in the amount of amino acids and more at high RH suitable for luxuriant growth of the fungus on the seed. This has earlier been noticed[2,3]. The presence of DL - valine, DL - phenylalanine and L- serine decarboxylase

Table 2. Activity of amino acid decarboxylase, non-oxidative deaminase and amino acid oxidase in urd bean seeds stored with *A. flavus* at 60, 70 and 80% RH.

Enzymes		RH (%)		
		60	70	80
Amino acid decarboxylase (mg CO_2 released / 30 min / g seed)				
DL - valine	I	0.472	0.638	0.905
	C	-	-	-
DL - phenylalanine	I	0.516	0.763	0.986
	C	-	-	-
Iso - leucine	I	0.326	0.542	0.907
	C	0.042	0.065	0.082
L - serine	I	0.675	0.826	1.285
	C	-		-
Non - oxidative deaminase (mg ammonia released / 30min / g seed)				
DL - valine	I	0.096	0.177	0.293
	C	0.042	0.064	0.091
DL - phenylalanine	I	0.142	0.196	0.346
	C	0.047	0.068	0.098
Iso - leucine	I	0.123	0.262	0.513
	C	0.081	0.144	0.328
L - serine	I	0.212	0.376	0.586
	C	0.105	0.192	0.282
Amino acid oxidase(O_2 consumed / 15 min /ml extract)				
DL - valine	I	0.7	0.9	1.3
	C	0.3	0.5	0.7
DL - phenylalanine	I	0.6	0.8	1.1
	C	0.2	0.4	0.7
Iso - leucine	I	0.2	0.4	0.6
	C	0.1	0.2	0.3
L - serine	I	0.4	0.6	0.9
	C	0.1	0.2	0.3

I = Inoculated, C = Control, - = No activity

activity in inoculated seedlot only and absence in the control indicate that *A flavus* is the source of extracellular secretion of this enzyme in the seed and too, excited at high RH revealing more the growth of this fungus normally at high RH, more the activity and more the reduction of particular amino acid. The activity of AAT,GAlT and GAsT, the enzymes for interconversion of amino acids observed only in the inoculated seedlot denotes the secretion of these enzymes in the seed by *A. flavus* which may prove the source of appearing new amino acids as Bidwell[15] has reported transamination between glutamic acid and at least eighteen alpha-, beta- and gama- keto acids in plants.

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