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NITRATE REDUCTASE AND UREASE ACTIVITY IN WHEAT SEEDLINGS DUE TO FAULTY STORAGE OF SEEDS

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Nitrate reductase and urease activities were found to be sluggish in the seedlings raised from *Aspergillus flavus* and *A. niger* infested and stored seeds. The amount of total free amino acid was also less in them besides scanty amount of protein in the grains. As the RH level and duration of storage of seeds increased, the value of the noted particulars decreased.

Key Words : Wheat seedlings, *Aspergillus flavus*, *A. niger*, Nitrate reductase, Urease, Total free amino acid, Protein.

The physiology and biochemistry of the seedlings raised from fungus stored seeds showed their slow growth, meagre amount of chlorophylls, sugar and total free amino acid in them besides stimulated activity of respiratory enzymes and those of amino acid degradation (Sao *et al.*, 1989; Singh, 1988, Pyare, 1991). This paper deals with the activity of nitrate reductase and urease besides determination of magnitude of total free amino acids in the seedling and total protein content in the grains of wheat plant raised from the seeds artificially deteriorating due to storage fungi at varying RH level for varying periods.

MATERIALS AND METHODS

Twenty g of wheat (*Triticum aestivum* L) var Sonalika 308 seed lots having 7.08% moisture level and possessing 100% germinability was infested (Sao *et al.*, 1989) each with storage fungi *Aspergillus flavus* Link ex Fries and *A. niger* V. Tieghem maintaining the control without fungus. Seed lots treated as above were stored each over 70, 80 and 90% RH at 30±1°C maintained with glycerine (Tuite, 1969) in sealed desiccators for 10,20 and 30 days. Five seeds at equidistance were sown in autoclaved garden soil taking in earthen pots (Base diameter - 15 cm, Top diameter - 25 cm and Depth - 30 cm) and seedlings were raised (Sao *et al.*, 1989) in ten replicates for each treatment in November, 1991. Nitrate reductase (NR) (Hewitt and Nicholas, 1964) and urease (UR) (Snell and Snell, 1971) activities were assayed besides estimating total free amino acid (TFAA) (Umbreit *et al.*, 1972) in the third leaf of randomly taken 21 days old seedlings. Seedlings were watered separately with 1% potassium nitrate and urea two days ahead of assaying of NR and UR respectively. They were allowed to grow to mature plants and grains were collected separately for each treatment. 5 g of the grains from each lot was dried at 80°C for 24 hr and cooled over fused $CaCl_2$ for next 24 hr and were powdered with mortar and pestle. Total protein was eastimated (Lowry *et al.*, 1951). Data were subjected to statistical analysis using ANOVA and Correlation coefficient.

RESULTS AND DISCUSSION

The activity of NR (Table 1) and UR (Table 2)

Table 1. Nitrate reductase activity in the leaf of wheat seedlings raised from the seeds stored with storage fungi at varying RH level for varying periods (Expressed as unit of enzyme/g green leaf)

Period of storage of seeds (in days)	Storage fungi Control	R H (%)			Correlation coefficient (r)
		70	80	90	
10	*A. flavus*	0.133	0.119	0.107	-0.999 VHS
	A. niger	0.146	0.135	0.125	-1.000 VHS
	Control	0.180	0.175	0.171	-0.998 HS
20	*A. flavus*	0.114	0.092	0.070	-1.000 VHS
	A. niger	0.124	0.107	0.090	-1.000 VHS
	Control	0.165	0.157	0.151	-0.997 HS
30	*A. flavus*	0.097	0.063	0.028	-1.000 VHS
	A. niger	0.105	0.077	0.051	-1.000 VHS
	Control	0.155	0.142	0.131	-0.999 VHS
'F' value for Fungi/control		10.0 S	16.0 S	24.0 HS	
'F' value for duration of storage		4.0 NS	8.0 S	12.0 HS	

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Table 2. Urease activity* in the leaf of wheat seedlings raised from the seeds stored with storage fungi at varying RH level for varying periods (Expressed as O.D.)

RH (%)	Storage fungi/ control	Period of storage (in days)											
		10				20				30			
		Time of reading (in min)				Time of reading (in min)				Time of reading (in min)			
		1st	4th	7th	10th	1st	4th	7th	10th	1st	4th	7th	10th
70	*A. vlavus*	0.41	0.42	0.42	0.43	0.29	0.30	0.31	0.31	0.22	0.22	0.23	0.24
	A. niger	0.45	0.45	0.46	0.47	0.32	0.33	0.33	0.34	0.24	0.25	0.26	0.26
	control	0.50	0.51	0.52	0.52	0.37	0.38	0.38	0.39	0.30	0.31	0.32	0.32
80	*A. flavus*	0.33	0.34	0.35	0.35	0.26	0.27	0.27	0.28	0.18	0.18	0.19	0.20
	A. niger	0.36	0.37	0.37	0.38	0.28	0.29	0.30	0.30	0.19	0.20	0.20	0.21
	control	0.43	0.44	0.45	0.45	0.35	0.36	0.37	0.37	0.28	0.29	0.30	0.30
90	*A. flavus*	0.29	0.30	0.31	0.31	0.21	0.22	0.22	0.23	0.12	0.13	0.13	0.14
	A. niger	0.31	0.34	0.33	0.33	0.23	0.23	0.24	0.25	0.13	0.14	0.14	0.15
	control	0.40	0.41	0.42	0.42	0.33	0.34	0.34	0.35	0.25	0.26	0.27	0.27

*O.D. was observed per min but, it was recorded on the Ist, 4th, 7th and 10th min.

Table 3. Total free amino acid content in the leaf of wheat seedlings raised from the seeds stored with storage fungi at varying RH level for varying periods. (Expressed as mg of amino acid/g fresh weight of leaf).

Period of storage of seeds (in days)	Storage fungi/ Control	R H (%)			Correlation coefficient (r)
		70	80	90	
10	*A. flavus*	0.287	0.265	0.232	-0.993 HS
	A. niger	0.356	0.340	0.315	-0.992 HS
	Control	0.427	0.421	0.407	-0.974 S
20	*A. flavus*	0.238	0.207	0.162	-0.994 HS
	A. niger	0.274	0.252	0.215	-0.990 HS
	Control	0.375	0.360	0.334	-0.988 S
30	*A. flavus*	0.165	0.120	0.041	-0.988 S
	A. niger	0.195	0.157	0.101	-0.994 HS
	Control	0.300	0.275	0.240	-0.995 VHS
'F' value for fungi/control		58.0 HS	78.0 VHS	120.0 VHS	
'F' value for duration of storage		56.0 HS	76.0 VHS	104.0 VHS	

Table 4. Total protein content (%) in wheat grains collected from the plants raised from the seeds stored with storage fungi at varying RH level for varying periods.

Period of storage of seeds (in days)	Storage fungi/ Control	R H (%)			Correlation coefficient (r)
		70	80	90	
10	*A. flavus*	10.02	9.88	9.53	-0.971 S
	A. niger	10.28	10.15	9.92	-0.987 S
	Control	10.66	10.56	10.40	-0.991 HS
20	*A. flavus*	9.00	8.70	8.22	-0.991 HS
	A. niger	9.81	9.08	8.75	-0.994 HS
	Control	10.16	10.01	9.77	-0.991 HS
30	*A. flavus*	8.10	7.66	6.95	-0.990 HS
	A. niger	8.65	8.30	7.78	-0.992 HS
	Control	9.58	9.32	8.92	-0.992 HS
'F' values for fungi/control		4.75NS	4.375NS	5.070NS	
'F' value for duration of storage		9.18S	9.011S	9.981S	

was slower in the seedlings raised from the fungus stored seeds. As the RH level and the period of storage of seeds increased, the activity of two enzymes decreased besides meagre amount of TFAA (Table 3). Stimulated decarboxylase, deaminase and oxidase of amino acids (Sao *et al.*, 1989) might result in scantness of TFAA in the seedlings and protein in the grains but proportionate decrase in the activity of NR and UR in the seedlings, points out the deleterious effect of storage fungi of the seedlots. It is highly probable that the fungi under reference inflict hastened senescence of the seed in the present storage condition as described for high temperature of storage and moisture of the seed by Villieres (1980) moderating the enzymic activity. Dysfunction of cytoplasmic organelles and tardy enzymic activity of metabolic chains though not specified have been reported in the seeds stored in the comparable conditions mentioned here (Osborne, (1980). Also, Prasad *et al.* (1989) reported attenuation of NR and UR in coriander gall due to *Protomyces macrosporus*. This impels for storage of fungus free wheat seeds and also

under dehumified condition to discourage fungal growth to keep the seedlings healthy.

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