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ACCELERATION OF HIGH TEMPERATURE-HIGH MOISTURE INDUCED SEED DETERIORATION BY CHEMICAL PRETREATMENTS

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Chemical replenishment of seeds, by imbibition of certain growth regulators and a respiratory substrate, accelerates, instead of retarding, the high temperature- high moisture induced deterioration as evidenced through decline in per cent germination and activities of certain enzymes.

Key words : Seed deterioration, Chemical pretreatment.

Seed viability deals with the retention of the capacity for germination, field emergence, seedling vigour etc., for varying periods during storage after harvest and its different aspects have been reviewed by several workers (Abdul-baki and Anderson, 1972; Barton, 1961; Harrington, 1972 and Roberts, 1972). Our earlier experiments (Agrawal, 1982; Vimala, 1984) confirmed the observations of other workers (Barton, 1966; San Pedro, 1936 and Toole and Toole, 1946) that high temperature-high moisture storage regime induces rapid seed deterioration. As, there are many report indicating reduction in the activities of certain enzymes (Anderson, 1970; Shamshery, 1978) and levels of several compounds as hormones (Arora, 1980; Juel, 1941) and metabolites (Abdul-Baki and Anderson, 1973) with seed ageing, effects of chemical replenishment through pretreatments with some growth regulators and a respiratory substrate on the high temperature-moisture induced seed deterioration were investigated. Alterations in germination percentage and activities of certain enzymes were used as indices of viability changes.

MATERIALS AND METHODS

For studies on the effects of chemical pretreatments in the artificially induced seed ageing system, and the patterns of changes in germination in the activities of certain enzymes accompanying decline in viability, freshly harvested *Phaseolus aureus* cv. MH 159 seeds were soaked in distilled water and 1×10^{4} M each of the hormones Indole-3-Acetic acid (IAA), Gibberelic acid (GA_3), Kinetin (KN), Abscisic acid (ABA) and a metabolite, Succinic acid (SA) for 4 h, air dried and stored at the temperature-moisture regime of 35°C-100% RH in desiccators, as standardized earlier (Agarwal, 1981; Vimala, 1982). Simultaneosuly, one parallel seed lot was kept dry at room temperature (28°C) for the sake of comparison. Such stored seeds were analysed for per cent germination just after treatment (0 day) and after 2, 25 and 50 days of storage. For this, about 30 seeds per set were used and analyzed at different days. The results are described on the basis of 2 experiments done in duplicate.

Enzymic Analysis : For enzymic studies 2 and 50 days of stored sets were selected. The crude enzyme extract was prepared by homogenizing 6 h imbibed seeds in a chilled common extraction-cum-assay buffer, i.e. 0.2 M KH_2PO_4- NaOH buffer of pH 7.2 developed and standardized earlier (Vimala, 1982), centrifuging at 1000xg for 5 min and collecting the supernatant. The supernatant was assayed for amylase (Filner and Varner, 1987), protease (Green and Neurath, 1954) and IAA-oxidase (Gordon and Weber, 1951) using the common assay buffer. The amount of protein was determined according to Biuret method and besides

Table 1: Effects of chemical pretreatments by pre-storage 4 h soaking followed by air drying on the induced loss of vaibility of *Phaseolus aureus* cv. MH 159 seeds stored at 35°C-100% RH.

Days of storage	Per cent Germination at 48 h Treatment+						
	Untreated dry seeds	Distilled water	IAA	GA_3	KN	ABA	SA
			1×10^{4} M each				
0	80.0	73.3	73.3	80.0	78.5	86.6	86.6
2	75.0	72.0	72.0	70.0	70.0	80.0	78.0
25	74.0	70.0	70.0	65.0	65.0	70.0	70.0
50	73.3	46.6	20.0	26.0	53.6	20.0	40.0

+ Mean of two experiments conducted in duplicate

Table 2: Changes in the activities of certain enzymes accompanying acceleration of induced loss of viability by certain chemical pretreatments.

Enzyme	Days of storage	Enzyme activity ± S.E of 6 h imbibed seeds in untreated and following treated sets						
		Untreated dry seeds	Distilled water	IAA	GA_3	KN	ABA	Succinate
		Totaly activity (per seed)						
α-Amylase (mg starch degraded min^{-1})	2	0.99 ±0.10	1.00 ±0.18	1.00 ±0.12	1.20* ±0.18	0.96* ±0.10	0.63* ±0.15	0.80* ±0.12
	50	0.35 ±0.05	0.28* ±0.03	0.53* ±0.03	0.70** ±0.04	0.31 ±0.02	0.53* ±0.05	0.80** ±0.08
Protease (mg amino acid released h^{-1})	2	2.20 ±0.52	3.40* ±0.80	2.76 ±0.37	3.66* ±0.91	2.93 ±0.31	1.38** ±0.22	1.38** ±0.22
	50	0.48 ±0.09	0.48 ±0.07	0.46 ±0.05	0.21** ±0.02	0.21** ±0.03	0.14** ±0.01	0.14** ±0.01
IAA-oxidase (µg IAA degraded min^{-1})	2	4.00 ±0.85	4.00 ±0.80	5.00* ±0.78	2.00* ±0.34	3.00 ±0.70	3.00 ±0.35	3.00* ±0.30
	50	6.00 ±0.52	4.00* ±0.38	5.00 ±0.42	4.00* ±0.40	5.00 ±0.42	4.00* ±0.38	4.00* ±0.40
		Specific activity (Enzyme units mg $protein^{-1}$ x 100)						
α-Amylase	2	33.00 ±3.33	38.00 ±6.84	33.00 ±3.96	9.00 ±1.35	29.00 ±3.02	17.00 ±4.05	24.00 ±3.60
	50	11.00 ±1 .57	14.00 ±1.50	25.00 ±1.42	43.00 ±2.46	16.00 ±1.03	36.00 ±3.40	24.00 ±2.40
Protease	2	72.00 ±17.02	131.00 ±30.82	91.00 ±12.20	156.00 ±38.79	89.00 ±9.42	97.00 ±14.76	42.00 ±6.70
	50	15.00 ±2.81	25.00 ±3.65	22.00 ±2.39	13.00 ±1.23	11.00 ±1.57	6.00 ±0.86	6.00 ±0.43
IAA-oxidase	2	0.10 ±0.02	0.10 ±0.02	0.20 ±0.03	0.09 ±0.02	0.09 ±0.02	0.08 ±0.01	0.10 ±0.01
	50	0.20 ±0.02	0.20 ±0.02	0.20 ±0.02	0.20 ±0.02	0.30 ±0.03	0.20 ±0.02	0.20 ±0.02

* Significant at 5% level of significance.

** Significant at 1% level of significance.

total activity, specific activities of the enzymes were expressed in terms of Enzyme Units mg $protein^{-1}$ (1 EU= Amount of enzyme that brings about unit change in absorbance in one minute).

RESULTS AND DISCUSSION

Table 1 exhibits decline in vaibility in terms of per cent germination, in *Phaseolus aureus* cv. MH 159 seeds, pretreated and stored in the mentioned storage condition, with IAA, GA_3 and ABA inducing maximum acceleration of seed deterioration. Marked acceleration of deterioration was observed in the distilled water treated control set too, as compared to the untreated control. Kinetin partially shows some retention in the loss of viability. Table 2 shows the changes in the activities of amylase, protease and IAA oxidase, accompanying loss of germinability, 2 and 50 days after storage. The decline in total activities of amylase and protease, in all treated sets, including distilled water prestorage imbibition, was of greater extent than the untreated control. IAA-Oxidase did not exhibit considerable changes in total activity, although, slight increase in specific activity could be observed. Specific activities of amylase and protease paralleled changes in total activity as well as per cent germination.

In general, none of the growth regulators or metabolite replenished the lost amounts of these substaces during high temperature-moisture storage of seeds. Rather, interestingly enough, the results indicate acceleration instead of retardation of senescence.

This deterioration, thus, can be attributed to temperature dependent rise in respiration in the presence of hydrated activated enzymes and loss of membrane integrity leading to depletion, leakage of substrates during hydration-dehydration-rehydration procedure (Agrawal, 1981; Koostra and Harrington, 1969; Mc Kersie and Tomas, 1980). The repair processes during long term storage at high seed moisture content (Ellis and Roberts, 1980; Ibrahim *et al.*, 1983) were, however, not observed. The decline in the activity of hydrolytic enzymes accompanying loss of viability has been reported by several workers (Agrawal, 1982; Anderson, 1970; Shamshery and Banerji, 1979) and may be due to gradual inactivation of pre-existing enzymes and /or progressive inhibition of enzyme synthetic machinery. The present report indicates that decline in hormonal and metabolite levels during storage may not have causal relationship with loss of seed viability.

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