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Short Communication

Effect of accelerated aging of soybean seeds of different storage potential on the activity and isoenzymes of acid phosphatase

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Abstract

Effect of accelerated aging of soybean seeds of different storage potential *viz.*, Bragg, Hardee, Kalitur and T-49, was studied on the activity and isoenzymes of acid phosphatase in axis and cotyledons separately. Acid phosphatase activity in both axis and cotyledons, was siginificantly and positively correlated with germinability at $P \leq 0.05$ in all the varieties except in the case of axis of Kalitur where the correlation was non-significant. Aging caused alterations in the isoenzymic pattern, both with respect to Rf value and number of bands.

Key words: Soybean, cultivars, aging, germinability, acid phosphatase, activity, isoenzymes.

1. Introduction

Alterations in enzyme activities remains an important cause of seed deterioration[1-3]. A meaningful approach to assess the significance of an enzymatic change during deterioration is to compare both its activity and isoenzymic pattern in seeds of cultivars having different storage potential. Accordingly, the present investigations were carried out on four soybean cultivars *viz.*, Bragg, Hardee, Kalitur and T-49, known to differ in their ambient storage potential[4]. The enzyme chosen for the investigation was acid phosphatase (EC 3.1.3.2) which plays an important role in metabolic control of seed germination, by regulating the turnover of organic phosphates and on which no information is available in soybean. To the authors knowledge, this is the first report, where both activity and isoenzymic pattern of acid phosphatase was studied in any seed during induced aging.

2. Materials and methods

2.1 Accelerated aging

Seeds were subjected to accelerated aging (0,2,4,6 and 8 days) by placing in an incubator at $40 \pm 1°C$ and 94 per cent relative humidity[5].

2.2 Germination studies

Seeds were germinated in between moist paper towels kept vertically in an incubator at 28 ± 1 °C in dark. Germination counts were scored on fifth day taking visible emergence of the radicle as the criterion.

2.3 Enzyme extraction

Axes/cotyledons were hand homogenised in chilled glass mortar with the extraction medium suggested by Cooper and Beevers[6] at pH 7.6, using quartz as an abrasive. The extraction medium contained 0.1 M tris-HCl buffer, 0.4 M sucrose; 0.01 M KCl, 10 mM $MgCl_2$, 0.01 M mercaptoethanol and 2.5% PVP (polyvinyl pyrolidone). The homogenate was centrifuged through four layers of muslin cloth and centrifuged at 15,000 × g for 20 min at 0-4 ° C. The supernatant was used for further studies.

2.4 Enzyme assay

Acid phosphatase assayed according to Jones[7]. One unit was defined as the amount of enzyme that liberated one micromole of *p*-nitrophenol at 37 °C on 20 min. incubation.

2.5 Isoenzyme detection

Anionic system of disc acrylamide gel electrophoresis[8,9] was used for separating the isoenzymes of acid phosphatase, with 7.5 cm of 7.5% running gel and 0.5 cm of 2.5% spacer gel. 150 μg of soluble protein *i.e.* obtained from 15,000 Ω g supernatant was layered on each tube. Activity bands of acid phosphatase on gels were located using alpha-naphthyl phosphate as substrate and Fast Garnet GBC salt as coupler[10]. All the above experiments have been carried out in two replicates.

3. Results and discussion

Aging treatment considerably decreased acid phosphatase activity in axis and cotyledons of all the varieties (fig. 1). However, the loss of activity was less in the case of Kalitur which maintained a fairly high germination per cent even after 8 days of aging (fig. 2). Acid phosphatase activity in both axis and cotyledons was significantly and positively correlated with germinability at $P \leq 0.05$ in all the varieties except in the case of axis of Kalitur where the correlation was non-significant. Acid phosphatase is an unspecific enzyme acting on a wide range of phosphomonoesters, which constitute a major class of metabolites, participating in several aspects of cell structure and function. The enzyme helps in maintaining a balance between the organic and inorganic phosphates and regulation of the concentration of different forms of phosphate and determining the direction of several metabolic pathways. Hence its decreased activity in cotyledons and axis might affect many fundamental metabolic reactions leading to the onset of germination and early seedling growth, by lowering the turnover of various organic phosphates. It is well known that mobilisation and transformation of several organic phosphates precede and accompany the onset of seed germination and seedling growth[2,11-13]. Thus, decreased acid phosphatase activity may be responsible for decreased germinability and decreased and delayed growth of seedlings usually observed with aged seeds. Decreased acid phosphatase activity has hitherto been reported in crimson clover seeds of decreased viability by Ching[14]. Decreased acid phospha-

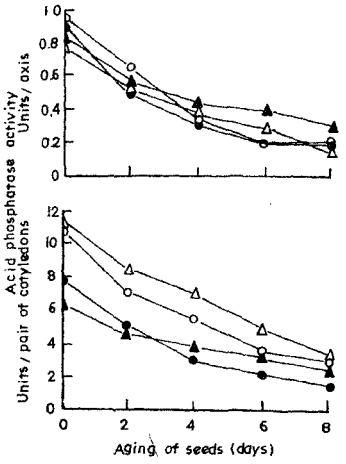

FIG. 1 Effect of accelerated aging of soybean seeds on the activity of acid phosphatase.
o - Bragg; ● - Hardee; Δ - T-49; ▲ - Kalitur.

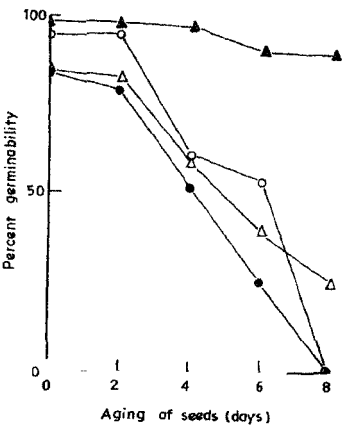

FIG. 2. Effect of accelerated aging of soybean seeds on germinability.
o - Bragg; ● - Hardee; Δ - T-49; ▲ - Kalitur.

tase activity in aged sorghum seeds has been attributed to increased protease activity[14]. In the present case also, above inference may hold true as enhanced proteolytic activity has been observed both in axis and cotyledons of all the varieties due to accelerated aging[15].

Isoenzymic pattern was similar in axis of seeds not aging, of all the varieties (fig. 3). Aging did not alter the number of bands, but altered the mobility of the two bands differently with respect to variety and aging treatments. Axis of Hardee and T-49 maintained a similar pattern in 2,4,6 and 8-day aged seeds. Rf values were found to be similar in axes of 6 and 8-day aged seeds of Bragg. In Kalitur, axis of 2-and 4-day and 6-and 8-day aged seeds had activity bands with similar Rf values, respectively. Unlike the axes, cotyledons of seeds not aging had three isoenzyme bands (fig. 4) out of which two bands seem to be common for all the varieties on the basis of their close Rf values. The number decreased to two as a result of aging in all the varieties except Kalitur. However, differential migration of the bands is observed in aged seeds. Aging the seeds for 2 days altered the mobility of the bands in cotyledons of all the varieties but further aging up to 6 days in the case of Hardee and Bragg and 8 day in Kalitur, appeared to have no appreciable effect on the enzyme bands with respect to Rf values. In T-49, Rf values of bands were similar in cotyledons of 2-, 4-, 6- and 8-day aging treatments, respectively.

From the above description of isoenzymic pattern, it is clear that induced aging alters only the mobility of isoenzymes in axis, while in cotyledons it also resulted in the loss of one

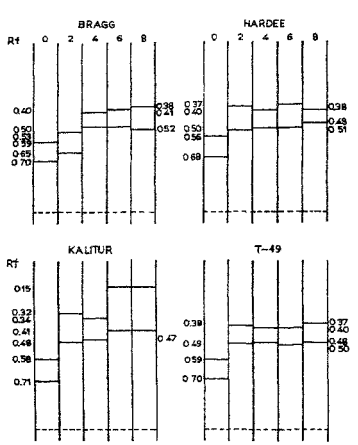

FIG. 3. Effect of accelerated aging of soybean seeds on the isoenzymic pattern of acid phosphatase in axis. (—) indicates position of the tracking dye (bromophenol blue) at the anode end.

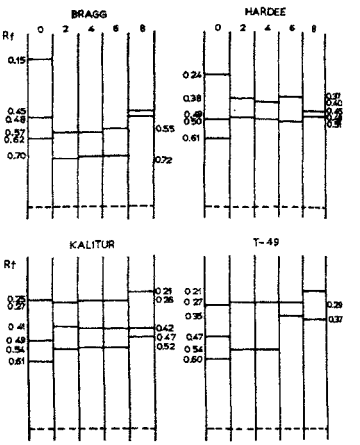

FIG. 4. Effect of accelerated aging of soybean seeds on the isoenzymic pattern of acid phosphatase in cotyledons. (—) indicates position of the tracking dye (bromophenol blue) at the anode end.

isoenzyme, except in the case of Kalitur. Hence, three factors may be responsible for the decrease in acid phosphatase activity in aged seeds *viz.*, loss of an isoenzyme, decrease in the amounts of individual isoenzymes and modification of the individual isoenzymes resulting in lowered activity and altered mobility. The first factor is apparently applicable only to cotyledons of Bragg, Hardee and T-49. The nature of modification(s) leading to altered mobility of the isoenzymes remains a speculation. Sub-unit association or dissociation presumably is not the reason for altered mobility, as it is evident from the genetic analysis of acid phosphatase isoenzymes in soybean[17] that the enzyme is a monomer and does not associate to form active dimers or oligomers. Mild proteolytic cleavage, attachment of sialyl residues and noncovalent association of substances (conformational isoenzymes) are some of the probable modifications, contributing to altered mobility of acid phosphatase isoenzymes[18,19].

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