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Beta-amylase Activity in Artificially Aged Soybean Seeds

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Abstract. Four soybean cultivars differing in storage potential were subjected to accelerated aging for different periods (2, 4, 6 and 8 days) and tested for germinability and beta-amylase activity. Aging caused a considerable and continuous increase in enzyme activity in both cotyledons and axes of all the four cultivars.

Soybean has gained wide importance because of its high protein and oil content. But non-availability of quality seeds at sowing time has been a serious problem in places where high moisture and high temperature greatly accelerate the aging phenomenon. Under Indian climatic conditions cultivars Bragg and Hardee are known to lose viability much faster than cultivars Kalitur and T49 (Gupta 1976). Soluble carbohydrates are an important source of energy during early germination and growth. Soybean cotyledons lose 70% of their total soluble carbohydrates during the first 3 days of growth (Wahab and Burris 1971). Increase in leaching of free sugars from aged seeds has been attributed to increase membrane damage (Villiers 1973, Parrish and Leopold 1978). The increase in leaching of free sugars may also be due to the increased formation of free sugars as a result of amylolytic activity in aged seeds. Hence, beta-amylase which is known to be present in a preexisting form in seeds (Kruger 1979) was estimated in cultivars of soybean of different storage potentials after subjection to accelerated aging for different periods.

MATERIAL AND METHODS

Seed Material

Seeds of four cultivars of soybean (*Glycine max* (L.) Merr.) *viz.*, Bragg, Hardee, T49 and Kalitur (Black Toar) were selected for the present investigation.

Accelerated Aging

Seeds were subjected to aging (2, 4, 6 and 8 days) by placing the seeds in an incubator at 40 ± 1 °C and 94% relative humidity. Seed samples

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were collected at desired intervals for germinability and beta-amylase activity.

Germination Studies

The initial moisture content of the seeds on fresh weight basis was 7.5, 5.5, 6.5 and 5.4 per cent for the cultivars Bragg, Hardee, Kalitur and T49, respectively. Seeds kept in the aging chamber absorbed water hygroscopically

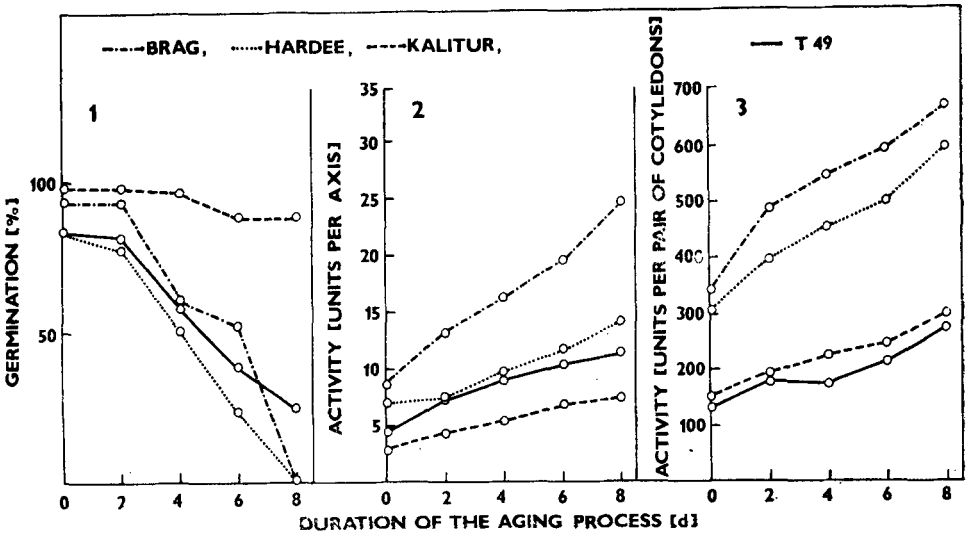

Fig. 1. Effect of accelerated aging on germination.
Fig. 2. Effect of accelerated aging on beta-amylase activity in axes. Each point represents the mean of two replicates.
Fig. 3. Effect of accelerated aging on beta-amylase activity in cotyledons. Each point represents the mean of two replicates.

and the maximum moisture content obtained after aging was 44.6, 59.8, 49.7 and 52.0 for Bragg, Hardee, Kalitur and T49, respectively. Moisture content was determined after keeping the seed samples at 105 °C for 6 h. Seeds after subjection to the required aging period were dried to their original weight at 28 ± 1 °C and germinated in between moist paper towels kept vertically in an incubator at 28 ± 1 °C in the dark, in 4 replicates (25 seeds per replicate). Germination counts were made on the fifth day taking visible emergence of the radicle as criteria.

Enzyme Extraction and Assay

Immediately after removing from the aging chamber seeds were transferred to cold distilled water for 30 min to facilitate the removal of seed coat. Ten cotyledons and 25 axes were separately hand homogenized in chilled pestle mortar with tris grinding medium (Cooper and Beevers 1969) at pH 7.6, using quartz as an abrasive. The homogenate was filtered through four layers of muslin cloth and centrifuged at 15 000 × g for 20 min. The supernatant was used for the assay of beta-amylase according to Bern-

FELD (1955) using 1% soluble starch as the substrate. A unit of activity is that liberating one micro-mole of reducing groups calculated as maltose per 5 min at 35 °C.

RESULTS AND DISCUSSION

Cv. Kalitur maintained high germination percentage even after 8 days of aging. Cultivars Bragg, Hardee and T49, showed a decline in germination percentage after 4 days of aging and by 8 days of aging cv. Bragg and cv. Hardee completely failed to germinate, but cv. T49 could maintain 25% germination (Fig. 1).

It follows from the data presented in Figs. 2 and 3, that cotyledons and axes of all the cultivars possess beta-amylase activity with the axes contributing much less. Axes and cotyledons of all the cultivars showed continuous and considerable increase in beta-amylase activity with increase in aging period, magnitude of increase being more in the case of the axes. Soluble carbohydrates are an important source of energy during early germination and growth. Beta-amylase in combination with maltase is responsible for the degradation of soluble oligosaccharides into glucose, which is then utilized in the cotyledons and also transported to the growing axis for energy purpose and for its interconversion into other utilizable metabolites. The increase in leaching of sugars in aged seeds has been attributed to membrane damage (VILLIERS 1973). Considerable increase of amylolytic activity during aging (Figs. 2 and 3) suggests that increase of free sugars in the seed as a result of amylolytic activity may in part be responsible for the increased leaching of sugars.

Cv. Kalitur maintained a consistently high germination percentage throughout the aging period. But cultivars Bragg, Hardee and T49 showed a decline in germination percentage after 4 days of aging and by 8 days of aging, while cultivars Bragg and Hardee completely failed to germinate, cv. T49 could maintain only 25% germination. This suggests that probably increase in free sugars as a result of increased amylolytic activity occurs in seeds of all the cultivars during aging, but these sugars are better metabolized in cultivars like Kalitur, which maintain a high germination percentage after aging. The work of WAHAB and BURRIS (1971) indicates that low and high quality soybean seeds have initially the same amount of soluble sugars, but high quality seeds were better able to mobilize and interconvert these sugars to utilizable metabolites which are essential for germination and early seedling growth.

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REFERENCES

BERNFELD, P.: Amylase alpha and beta. — In: COLOWICK, S. P., KAPLAN, N. O. (ed.): Methods in Enzymology. Vol. 1. Pp. 149—158. Academic Press, New York—London 1955.

COOPER, T. G., BEEVERS, H.: Beta-oxidation in glyoxysomes from castor bean endosperm. — J. biol. Chem. **13** : 3514—3520, 1969.

GUPTA, P. C.: Viability of stored soybean seeds in India. — Seed Res. **4** : 32—39, 1976.

KRUGER, J. E.: Modification of wheat beta-amylase by proteolytic enzymes. — Cereal Chem. **56** : 298—302, 1979.

PARRISH, D. J., LEOPOLD, A. C.: On the mechanism of ageing in soybean seeds. — Plant Physiol. **61** : 365—368, 1978.

VILLIERS, T. A.: Ageing and longevity of the seeds. — In: HEYDECKER, W. (ed.): Seed Ecology. Pp. 265—288. Pennsylvania State University Press, University Park 1973.

WAHAB, A. H., BURRIS, J. S.: Physiological and chemical differences in low and high quality soybean seeds. — Proc. Ass. Off. Seed Anal. **61** : 58—67, 1971.