CORRELATION OF SOME ENZYME ACTIVITIES WITH AXIS GROWTH IN SEEDLINGS RAISED FROM AGED SOYBEAN SEEDS

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ABSTRACT

Activities of ten enzymes were monitored separately in axes and cotyledons of 5-day old seedlings raised from 0, 2, 4 and 6-day aged seeds of Bragg, Hardee and T-49 cultivars of soybean (*Glycine max* L. Merill). In general, irrespective of the basis of expression of enzyme activity and cultivar, the activities of acid phosphatase, ribonuclease, isocitrate lyase and malate synthetase were significantly and positively correlated with axis growth. Possible role of these enzymes in axis growth is discussed.

INTRODUCTION

SEED deterioration is manifested in several crops by a reduced growth of seedlings[1-3]. Alteration of enzyme activities remains an important cause of seed deterioration[4-9]. The present investigation is a preliminary attempt to identify key enzyme(s) involved in the regulation of growth of seedlings raised from aged seeds. Once these enzymes are identified, study of their regulation may provide an answer for the manipulation of growth of seedlings raised from deteriorated seeds. The approach, to identify the enzymes involved in the axis growth, was as follows:

Ten enzymes, involved in the process of germination, mobilisation of food reserves and/or implicated in seed deterioration have been selected and their activities have been monitored separately in axes and cotyledons of 5-day old seedlings raised from 0, 2, 4 and 6-day aged seeds of different cultivars of soybean (*Glycine max* L. Merill) *viz* Bragg, Hardee and T-49. The activity of each enzyme has been expressed in three ways *viz* in relation to unit soluble protein (specific activity), unit dry weight and on organ basis, and in each case the activity was correlated with the axis growth (dry weight). Thus, in all, 18 correlations have been worked out for every enzyme (6 for each variety). The enzyme, which had the maximum number of significant correlations, has been regarded as the enzyme likely to play a major role in the control of axis growth. The possible manner by which these enzymes may contribute to axis growth has also been discussed.

MATERIALS AND METHODS

Seeds of Bragg, Hardee and T-49 were subjected to accelerated aging (0, 2, 4 and 6 days) by placing in an incubator at 40 ± 1°C and 94% relative humidity. Seeds were germinated between moist paper towels kept in an incubator at 28 ± 1°C, in the dark.

For preparing the enzyme extract, ten cotyledons or five or ten axes were separately homogenised in a chilled glass mortar, with the extraction medium suggested by Cooper and Beevers[10] at pH 7.6 using quartz as an abrasive. The extraction medium contained: 0.01M Tris-HCl buffer; 0.4M sucrose; 0.01 M KCl; 10 mM $MgCl_2$; 0.01 M EDTA (ethylenediamine tetraacetic acid); 0.01 M mercaptoethanol and 2.5% PVP (polyvinyl-pyrrolidone). The homogenate was filtered through four layers of muslin cloth and centrifuged at 15,000 g for 20 min at 0–4°C. The supernatant, after removing the fatty layer, was used for enzyme assays. Two independent extractions, each of axes and cotyledons were carried out.

Protease (EC 3.4.4.4) was assayed using α-N-benzyl DL-arginine *p*-nitroanilide hydrochloride according to Kakade *et al*[11]. One unit of the enzyme was defined as the amount of enzyme which caused a change in absorbance of 0.1 under the experimental conditions.

β-amylase (EC 3.2.1.2) was assayed as per Bernfeld[12]. A unit of enzyme activity was defined as the amount of enzyme which liberated 1 μmol of reducing groups calculated as maltose under the specified conditions.

The method of Jones[13] was employed for the assay of acid phosphatase (EC 3.1.3.2). One unit was defined as the amount of enzyme that liberated 1 μ mol of *p*-nitrophenol from *p*-nitrophenyl phosphate under the experimental conditions.

Lipase (EC 3.1.1.3) activity was determined by the colorimetric estimation of 2-naphthol liberated from 2-naphthyl laurate[14]. One unit of enzyme was taken as the amount of enzyme required to liberate 1 μg of 2-naphthol under the specified conditions.

Ribonuclease (EC 2.7.7.16) and deoxyribonuclease (EC 3.1.4.5) were assayed by the method of Wilson[15], using yeast RNA and calf thymus DNA as substrates. One enzyme unit was defined as the amount, which caused an absorbance change of 0.1 under the specified conditions.

Catalase (EC 1.11.1.6) activity was measured by recording the reduction of potassium dichromate by hydrogen peroxide[16]. A unit was defined as the amount of enzyme that caused a decrease in absorbance equal to 0.1 under the experimental conditions.

Peroxidase (EC 1.11.1.7) was measured by the method of Seevers *et al*[17] recording the change in absorbance at 470 nm due to the oxidation of *o*-dianisidine in the presence of hydrogen peroxide and enzyme. One unit was defined as the amount of enzyme which caused a change in absorbance equal to 0.1 min^{-1}.

The method of Dixon and Kornberg[18] was used for the assay of isocitrate lyase (EC 4.1.3.1) and malate synthetase (EC 4.1.3.2). For both the enzymes, one unit was defined as the amount of enzyme that caused an increase in the absorbance equal to 0.1 min^{-1} under the experimental conditions.

Protein in the 15,000 g supernatant was determined after TCA precipitation by Lowry *et al*[19].

RESULTS AND DISCUSSION

In general, irrespective of the basis of expression of enzyme activity and cultivar, the activities of acid phosphatase, ribonuclease, isocitrate lyase and malate synthetase in the cotyledons showed a significant and positive correlation ($P \leqslant 0.05$) with axis growth. Therefore, actual values have been given only for these enzyme activities (table 1). Hence it appears that the decreased activity of the above enzymes, might be responsible for the decreased growth of the axis in seedlings raised from aged seeds.

The manner in which the activities of these enzymes, influence the axis growth, however, remains a matter of conjecture. Inorganic phosphate liberated as a result of acid phosphatase activity is presumed to be translocated to the axis for its growth. Hence, decreased acid phosphatase activity might lower the supply of free inorganic phosphate to the axis thereby delaying and/or decreasing its growth. Decreased acid phosphatase activity and increased leaching of free inorganic phosphate has been reported in deteriorated seeds of crimson clover[20] and sorghum[7]. Decreased ribonuclease activity in cotyledons as a result of aging, may decrease the mobilisation of reserve RNA, required for the growth of the axis. In seeds rich in fat, sucrose formed from the fatty reserve forms a major source of reduced carbon for the growth of seedlings. The two key enzymes responsible for this conversion are isocitrate lyase and malate synthetase, which form a part of the glyoxylate cycle[21,22]. Hence, lower levels of these enzymes in the cotyledons of seedlings raised from aged seeds might be responsible for decreasing

Table 1 *Effect of aging of seeds on the activities of acid phosphatase, ribonuclease, isocitrate lyase and malate synthetase, axis growth, and germinability.*

	Units/pair of cotyledons				Units/pair of cotyledons			
	Aging of seeds (days)				Aging of seeds (days)			
Cultivar	0	2	4	6	0	2	4	6
	Acid phosphatase				Ribonuclease			
Bragg	190 (15.1)*	177 (14.5)	150 (8.8)	55 (2.9)	105 (8.3)	104 (8.5)	77 (4.5)	61 (3.1)
Hardee	157 (13.7)	115 (9.9)	66 (4.0)	58 (3.7)	93 (8.1)	81 (7.0)	38 (2.3)	36 (2.3)
T-49	124 (12.0)	115 (10.9)	90 (7.2)	42 (2.6)	107 (10.4)	104 (9.8)	89 (7.1)	37 (2.3)
	Isocitrate lyase				Malate synthetase			
Bragg	350 (27.7)	385 (31.6)	287 (16.9)	147 (7.6)	522 (41.3)	506 (41.6)	352 (20.7)	254 (13.2)
Hardee	202 (17.6)	195 (16.8)	137 (8.3)	142 (9.0)	246 (21.4)	224 (19.3)	96 (5.8)	107 (6.7)
T-49	227 (22.0)	221 (20.8)	182 (14.5)	62 (3.8)	306 (29.5)	332 (31.3)	254 (20.2)	109 (6.8)
	Axis dry wt. (mg/axis)				Germinability (%)**			
Bragg	31.2	29.8	16.5	7.7	94	94	61	53
Hardee	17.9	13.2	6.8	6.6	84	79	51	25
T-49	21.9	22.2	16.7	6.3	84	82	59	39

* Figures in brackets indicate specific activity (units/mg soluble protein).
** Germination counts were recorded on fifth day, taking visible emergence of the radicle as the criterion.

the supply of sucrose to the axis.

The correlation of other enzyme activities in cotyledons and of all enzyme activities in axes, with axis growth, was highly variable depending on the basis of expression of enzyme activity and cultivar (data not shown). Thus, these enzymes, apparently, are of lesser significance or reliability as an index, for axis growth. Alternatively, this situation may also be interpreted to mean, that in different cultivars, different enzyme activities may limit the axis growth, in which case, each cultivar has to be separately calibrated with respect to its enzyme(s) limiting axis growth.

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