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CHANGES IN THE METABOLISM OF LIPIDS AND CARBOHYDRATES DURING GERMINATION OF SESAME (*SESAMUM INDICUM* L.) SEEDS

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SUMMARY

The levels of lipids, carbohydrates and enzymes concerned with their breakdown were determined in sesame (*Sesamum indicum* L.) seeds during an 8-days period of germination. Alkaline lipase and isocitrate lyase activities were at peak level when the lipid mobilization was highest. The content of total soluble carbohydrates, reducing and nonreducing sugars, increased during the initial 5-days period of germination followed by a reverse trend thereafter. An increase in α-amylase coincided with an increase in the content of reducing sugars in the cotyledons. Studies related to the distribution of enzymes in different organelles revealed that alkaline lipase and isocitrate lyase were restricted to the glyoxysomes. The development profiles of alkaline lipase and isocitrate lyase were followed in the sesame seedlings exposed to the inhibitors of protein synthesis. A substantial fall in enzyme activities was observed in the seedlings exposed to the inhibitors.

Key words: **Alkaline lipase, α- amylase, isocitrate lyase, protein synthesis inhibitors, sesame.**

INTRODUCTION

Sesame (*Sesamum indicum* L.) by virtue of its excellent quality oil is called 'the queen of the oilseed crops'. It is basically a crop of the tropics and subtropics. India ranks next to China in the production of this oil seed (Chandramony and Padmaja 1982). In Andhra Pradesh, sesame is grown both as a kharif (monsoon) and rabi (dry season) crop. Sesame seeds are of importance to human beings for their nutrient contents, particularly protein and oil. The oil is used directly in cooking. Sesemin has been shown to have hypocholesterolemic and anticarcinogenic effects and sesamolin has been shown to inhibit lipid peroxidation (Kang *et al*. 1998). Both these are effective synergists for pyrethrin, a potent insecticide. Sesamol is an antioxidant that gives excellent protection to vegetable oils against rancidity.

Degradation of lipids during germination of lipid rich seeds has been studied in peanuts (Wankhede *et al*. 1977), castor beans (Muto and Beevers 1974), sunflower (Bhatia *et al*. 1978), and cotton (Doman *et al.* 1982). Such studies are, however, lacking in sesame seeds. The present work is undertaken to provide information about mobilization of food reserves in sesame seeds during germination.

MATERIALS AND METHODS

Sesame seeds (var. Gauri) were obtained from Elamanchili, Andhra Pradesh, India and stored in glass jars at room temperature for 3 months. These seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 5 min and rinsed thoroughly with distilled water. Imbibition was carried out by soaking the seeds in

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distilled water for 6 h, then the seeds were spread on two layers of filter paper in a petri dish moistened with distilled water. Germination was carried out in diffuse light at 25±2°C for a period of 8 days. Additions of fresh water were made everyday to compensate for the water loss through evaporation. The seedlings were harvested at 24 h intervals and the cotyledons were used for analytical studies.

Lipid content of the dry seeds/cotyledons was gravimetrically estimated by the procedure of Folch *et al*. (1957). Total soluble carbohydrates (TSC) content of the alcoholic extract was estimated by the anthrone color reaction using glucose as a standard according to the procedure of Yemm and Wills (1954). The estimation of reducing sugars was done by the method of Nelson (1944) and Somogyi (1952). Non reducing sugar contents were calculated by subtracting the reducing sugars from total sugar content.

Enzyme extraction was done by homogenizing seeds/ cotylendons with 0.1 M Tris-HCl buffer, (pH 7.2) at 4°C using a sample to buffer ratio of 1:5 (w/v). The homogenate was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was used for the determination of various enzyme activities. Lipase activity was determined by a colorimetric method (Lin and Huang, 1986), based on the procedure developed by Nixon and Chan (1979) for quantifying free fatty acids. One unit of lipase activity was expressed as nmol fatty acid released per min under the experimental conditions. Specific activity of the enzyme was expressed as nmol fatty acid released/ min/ mg protein. Isocitrate lyase activity was determined by estimating the glyoxylate formed in the reaction mixture by the method of Friedemann and Haugen (1943). One unit of the enzyme activity was expressed as μmol glyoxylate formed per hour under the experimental conditions. Specific activity of the enzyme was expressed as μmol glyoxylate formed/ hour/ mg. protein. For controls, TCA was added prior to the addition of plant extract. Assay of α-amylase activity was done according to the procedure described by Bernfeld (1955). One unit of α-amylase activity was expressed as mg of maltose liberated in 10 min under the experimental conditions.

Glyoxysomes, proplastids and mitochondria were isolated from the cotyledons of 5-days old sesame seedlings by sucrose density gradient centrifugation following the method described by Theimer and Beevers, (1971) with slight modification. All the steps were carried out at 0-4^{0}C. For determination of protein content in glyoxysomal/mitochondrial/proplastid preparations, an aliquot of the respective fraction was treated with an equal volume of ice-cold 10% TCA and the precipitate was dissolved in 0.1 N NaOH. Protein was determined by the method of Lowry *et al.* (1951). Cytochrome-C-oxidase and succinic dehydrogenase activities were determined in the glyoxysomal/mitochondrial/proplastid preparations. Cytochrome-C- oxidase activity was assayed according to the method of Pearl *et al*. (1963). The enzyme activity was expressed as Δ A/min/mg protein. Succinic dehydrogenase activity was determined by the method of Bernath and Singer (1962). The activity was expressed as nmol of DCIP reduced /min/mg protein.

Sesame seeds were also treated with inhibitors of protein synthesis by soaking separately in aqueous solutions of different concentrations of inhibitors of protein synthesis, viz. azetidine-2-carboxylic acid, chloramphenicol, cycloheximide and streptomycin at 25-30^{0}C for 6 h. The seeds were then spread on wet filter paper and germinated at 25-30^{0}C. Inhibitor solution (2 mM, 15 ml) were sprayed daily on the seedlings. Seedling vigor was recorded at regular intervals of time.

Each value presented in figures and tables represent the mean of six independent measurements made unless otherwise stated. Statistical significance was calculated using the Student-t test.

RESULTS

The data on metabolic changes presented in this study have been expressed using the number of seeds/ cotyledons as the basis. Considering the small size of sesame seeds, values of the constituents were expressed taking 50 seeds/100 cotyledons as the basis. Lipid constituted about 85 mg per 100 cotyledons accounting for 48.2% in the seeds. TSC, which was present to the extent of 18 mg per 100 cotyledons constituted about 10.1% (Fig. 1). The changes in the lipids in cotyledons during germination of seeds showed steady decrease from day 0 to day 5 indicating that the maximum lipid

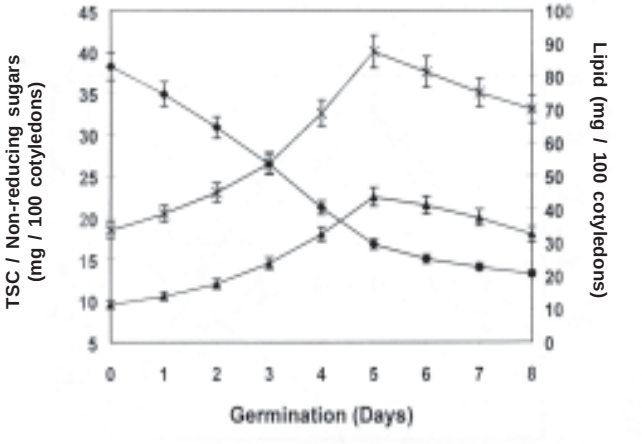

Fig. 1. Levels of lipid, total soluble carbohydrates and non-reducing sugars of the cotyledons during germination of seeds. TSC (-⋆-⋆-), lipid (-♦-♦-), non-reducing sugar (-▲-▲-). Values are mean ± SD and bars indicate standard deviations

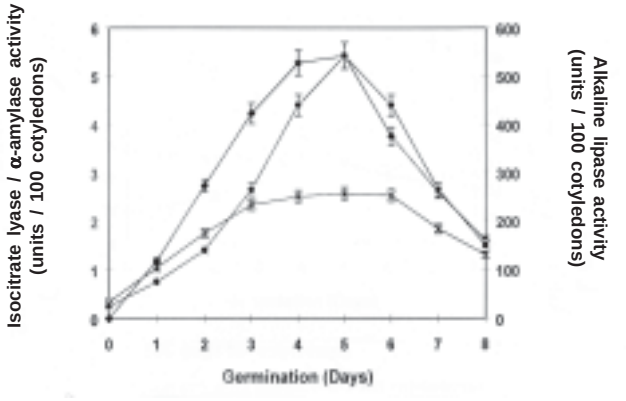

Fig. 2. Changes in enzyme activities in the cotyledons of germinating sesame seeds. Alkaline lipase activity (-●-●-), isocitrate lyase activity (-♦-♦-), α-amylase activity (-⋆-⋆-). Values are mean ± SD and bars indicate standard deviations

mobilization has taken place on day 5 followed by a slow decline thereafter. During germination of sesame seeds, there was a steady increase in the alkaline lipase activity reaching a maximum on day 5 followed by a decline from day 6 onwards (Fig. 2). A 20-fold increase in alkaline lipase activity observed on day 5 supports its active involvement in the mobilization of lipids. Isocitrate lyase activity in the cotyledons was detectable on day 1 and it was maximal on day 5 followed by a decline as germination progressed (Fig. 2). It should be noted that the enzyme activity was maximal at the time when lipid mobilization was rapid.

There was a steady increase in the content of TSC during the first five days of germination followed by a fall from day 6 onwards. The content of TSC increased from 18.5 mg at day 0 to a maximum of 40 mg per 100 cotyledons at day 5. The content of non-reducing sugars in the cotyledons increased from 9.5 mg on day 0 to 22.5 mg per 100 cotyledons on day 5 followed by a fall from then onwards (Fig. 1). The changes in the content of reducing sugars paralleled with the changes in the contents of TSC and non-reducing sugars during germination of sesame seeds. The activity of α-amylase in the cotyledons of germinating seedlings increased from 0.34 units on day 0 to 2.57 units per 100 cotyledons on day 5, and this coincided with an increase in the content of reducing sugars in the cotyledons. The increase observed in the content of nonreducing sugars could possibly be due to active glyoxylate cycle operating on day 5 of germination.

An analysis of enzyme distribution in mitochondria and glyoxysomes showed that 60-64% of succinic dehydrogenase and cytochrome-C-oxidase of the crude particulate preparation were present in the mitochondrial fraction and these enzymes were undetectable in the proplastid and glyoxysomal fractions (Table 1). The results obtained suggest that the glyoxysomal preparation was pure and free from contaminating mitochondria. Compared to the mitochondrial preparations, the glyoxysomal preparations from sesame cotyledons were found to be rich in alkaline lipase and isocitrate lyase activities. Isocitrate lyase and alkaline lipase activities accounting for 55% and 39% of the crude preparations, respectively, were present in the glyoxysomes while their activities were minimal in the mitochondria and proplastids.

Of the four inhibitors of protein synthesis tested, cycloheximide (2 mM) maximally inhibited germination of sesame seeds while the other three inhibitors, chloramphenicol, azetidine-2-carboxylic acid and streptomycin, at the same concentration, partially inhibited the germination. The results obtained suggest that these inhibitors might have interfered with the synthesis of important structural and functional proteins and enzymes essential for seed germination. Cycloheximide and

Table 1. Distribution of enzymes in the different organelles isolated from cotyledons of 5 days old sesame seedlings.

Fraction	Enzyme Activity (Units / mg protein)			
	Succinic dehydrogenase	Cytochrome C oxidase	Isocitrate lyase	Alkaline lipase
Crude particulate	52±1.0	283±4.0	1.78±0.05	172±1.5
Gradient Supernatant	ND	ND	0.14±0.004	5.7±0.08
Mitochondria	31.2±0.5	181.2±2.0	0.09±0.002	6.6±0.01
Proplastids	ND	ND	0.11±0.003	ND
Glyoxysomes	ND	ND	0.98±0.04	67.3±0.8

Values are mean ± SD, ND - Not detected

chloramphenicol inhibit protein synthesis in the cytoplasm and mitochondria, respectively. Chloramphenicol is also known to inhibit the process of respiration. Activities of isocitrate lyase and alkaline lipase in the cotyledons of 5-days old seedlings decreased considerably by the inhibitors of protein synthesis in a dose dependent manner (Table 2). Cycloheximide, chloramphenicol, azetidine-2-carboxylic acid and streptomycin, each at 2 mM concentration, caused a decrease in isocitrate lyase activity by 58%, 53%, 48% and 38% and alkaline lipase activity by 69%, 66%, 50% and 46%, respectively, suggesting that these enzymes were *de novo* synthesized during germination of sesame seeds.

Table 2. Effect of inhibitors of protein synthesis on isocitrate lyase and alkaline lipase in cotyledons of 5 days old sesame seedlings.

Inhibitors of Protein synthesis	Concentration	Isocitrate lyase activity (Units / mg. protein)	Alkaline lipase activity (Units / mg. protein)
Control	-	1.77±0.04	173.0±1.5
Azetidine - 2 -	$2´10^{-5}$ M	1.41±0.02	129.8±1.2
carboxylic acid	$2´10^{-4}$ M	1.22±0.01	114.1±1.1
	$2´10^{-3}$ M	0.92±.0.01	86.5±0.8
Chloramphenicol	$2´10^{-5}$ M	1.38±0.02	100.2±1.0
	$2´10^{-4}$ M	1.11±0.01	79.5±0.7
	$2´10^{-3}$ M	0.83±0.01	58.7±0.5
Cycloheximide	$2´10^{-5}$ M	1.31±0.02	89.9±0.8
	$2´10^{-4}$ M	1.09±0.01	72.6±0.7
	$2´10^{-3}$ M	0.74±0.01	53.6±0.5
Streptomycin	$2´10^{-5}$ M	1.49±0.03	131.3±1.3
	$2´10^{-4}$ M	1.29±0.02	114.1±1.1
	$2´10^{-3}$ M	1.09±0.01	93.4±0.9

DISCUSSION

In the present investigation, biochemical analysis of dormant and germinating sesame seeds was undertaken. Lipid, the principal constituent, accounted for 48.2% in the seeds. This value falls in the range 44 -54% reported for six varieties of sesame (Weiss 1971) and is much lower than those reported (60-68%) for five other sesame varieties (Chandramony and Padmaja 1982). The content of TSC (10.1%) observed in the seeds of var. Gauri are much lower than that reported (15.9%) for other brown variety seeds (Weiss 1971).

During the germination of lipid rich seeds, there is commonly a conversion of lipids into carbohydrates particularly sucrose. Lipolysis is initiated by lipase followed by β-oxidation, glyoxylate cycle and reversal of glycolysis to produce sugars from fatty acids. The fall in the lipid content, which coincided with maximal alkaline lipase activity resulting in a depletion of about 65% of lipid by day 5, supports the active role of alkaline lipase in the lipolysis during germination of sesame seeds. Even

though a fall in lipid content associated with an increase in alkaline lipase activity is a common finding in germinating oil seeds such as peanuts (Wankhede *et al.* 1977), castor beans (Marriott and Northcote 1975), sunflower (Bhatia *et al.* 1978) and cotton (Doman *et al.* 1982), the rate of mobilization of lipid depends on the type of the seed as well as on the nature of the lipid present in the seeds. The degraded lipids supply energy, provide precursors for the synthesis of new tissues. Lipid derived substances apart from being supplied to the growing axis are also retained in the sesame cotyledons where they could be utilized for expansion and differentiation of the cotyledons into photosynthetic organs. In the case of cotton seedlings, lipid derived substances are also partitioned between the growing axis and the cotyledons (Doman *et al.* 1982).

Consistent with the conversion of lipid to carbohydrates, variation in the activity of isocitrate lyase was also observed in sesame cotyledons. The pattern of the changes in isocitrate lyase activity in sesame cotyledons is in close agreement with the enzymatic changes observed in the cotyledons of castor beans, pumpkin, cotton and peanuts (Carpenter and Beevers 1959, Marriott and Northcote 1975). The fall in the isocitrate lyase activity also occurred whether or not the cotyledons were allowed to become functional green leaves (Carpenter and Beevers 1959). It has been reported that during germination of peanuts, isocitrate lyase activity increased in the glyoxysomes. Unlike pumpkin and peanut seeds, the dormant sesame seeds had no isocitrate lyase activity.

An increase of 2.5-fold in the content of reducing and nonreducing sugars in the sesame cotyledons was observed when the isocitrate lyase activity was maximal. Such a pattern, although at different rates during germination was observed in germinating sunflower (Balasaraswathi and Sadasivam 1977), peanut (Wankhede *et al.* 1977) and bambara groundnut (*Voandzoia subterranea* L.Thouaes) (Eastmond *et al.* 2000) seeds. The levels of the sugars present in the cotyledons represent the sugars remaining in the tissue after their utilization for respiration, incorporation into cell walls and/or translocation to the growing embryo. The increase in α-amylase activity also coincided with an increase in reducing sugars in the sesame cotyledons. Similarly, it was reported that through the action of amylase, there was an increase in total sugars during the initial period of germination in soybeans (Salliyamoorthy and Vivekanandan 1990). Since, starch is absent in sesame seeds, α-amylase could act on available substrates, possibly soluble oligosaccharides to generate reducing sugars. Even though isocitrate lyase and α-amylase activities were undetectable in resting sesame seeds, the presence of reducing and nonreducing sugars in considerable amounts in the dormant seeds suggests that they might have been synthesized during the formation of the seeds by related enzymes. Dormant lima bean and soybean seeds were also found to contain small amounts of reducing sugars. From the results obtained, it is clear that the two inducible enzymes - alkaline lipase and isocitrate lyase, which were present in glyoxysomes, play a significant role in the degradation of lipids in sesame seeds during germination.

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