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BIOCHEMICAL CHANGES DURING IMBIBITION AND GERMINATION OF SAFFLOWER SEEDS (*CARTHAMUS TINCTORIUS* var. *BHIMA*)

RUPAM KAPOOR
Department of Botany, University of Delhi, Delhi - 110 007, India.

During initial periods of imbibition of safflower seed (*Carthamus tinctorius* var. *Bhima*), the amount of lipids and total proteins did not change appreciably whereas, water-soluble proteins and water-soluble carbohydrates declined. Soluble starch, insoluble starch, α-amylase and β-amylase activity showed an increase. With the onset of germination, lipids and protein content decreased, while water-soluble carbohydrates, soluble and insoluble starch showed a slight increase. The amylase and protease activity was also enhanced.

Keywords : Biomolecules; Enzymes; Germination; Imbibition; Safflower.

Introduction

Seed germination involves activation of metabolism. The process is accompanied by a variety of significant biochemical changes. In a general way, these changes are (i) breakdown of seed reserves (carbohydrates, fats and proteins); (ii) a possible accumulation of resulting intermediates, some of which are themselves new and novel compounds, and (iii) the subsequent utilization of these intermediates for the synthesis of new plant mterials. Depending upon the nature of storage components, different metabolic pathways are associated in different seeds during their transition to seedling stage. The changes in metabolic pathways taking place during imbibition period which lead to root protusion, radicle emergence of visible germination are of much importance. It still remains an open question that what may be the interrelationship between different biomolecules that increase and decrease during germination and metabolism of specific tissues or whole seedlings. The present studies were undertaken to investigate corelations of metabolic pathways operative in safflower seeds during imbibition and germination on the basis of changes in concentration of various biomolecules.

Materials and Methods

Pure line seeds of Safflower (*Carthamus tinctorius* var. *Bhima*) were obtained from Punjab Agricultural University, Ludhiana (India). For imbibition studies, seeds were soaked in 50 ml of distilled water for varying time intervals viz. 4, 8, 12, 16, 20 and 24 hr. For germination studies, seeds were surface sterilized with 0.1% mercuric chloride for 2 min and rinsed thoroughly with distilled water. These were then placed equidistantly on moist filter paper kept on moist absorbant cotton in petri dishes and allowed to germinate at room temperature (26±1°C) in dark. The effect of various factors such as temperature and pH on the percent germination of seeds was also noted by germinating the seeds under respective conditions. The seedlings were taken out at intervals of two days starting from 2nd day up to 10th day.

Analytical Methods: 10% homogenate of seeds/seedlings was made in distilled water using pestle and mortar with little acid washed sand. It was then centrifuged at 2,000g for 10 min at room temperature. Supernatant was taken for the analysis of water soluble proteins, α-amylase, β-amylase and protease activity. Total proteins were extracted in 0.1N NaOH and lipids in petroleum ether. For total water-soluble carbohydrates, water-extract of seeds/seedlings was deproteinised with lead acetate[1] and hydrolysed with conc. HCl 68°C to get total sugars[2]. Soluble starch was extracted from water ex-

tract by precipitation with 80% ethanol and insoluble starch was extracted using 52% perchloric acid[3].

Total proteins and water-soluble proteins were calculated by method of Lowry *et al.*[4] using bovine serum albumin as standard. Lipids were estimated by colorimetric method suggested by Frings and Dunn[5] based on sulfophospho vanillin reaction. The intensity of pink coloured solution was read at 540 nm using double beam spectrophotometer (Shimadzu) with olive oil as standard. Total water-soluble carbohydrates, soluble starch and insoluble starch were determined by anthrone method employed by Yemm and Willis.[6]

α – Amylase activity was calculated by determining the concentration of unhydrolysed starch substrate in a specific time by the method of Bernfeld[7]. Enzyme activity was expressed in terms of decrease in starch content (μg) per min at room temperature (26°C). β–amylase activity was estimated according to procedure given by Bernfeld modified by Dure[8]. One unit of enzyme was taken as maltose (μg) liberated from starch substrate per min at 30°C. Protease activity was calculated by the method of Basha and Beevers[9]. Protease activity unit is defined as amino acid released from casein substrate (μg) per min at 37°C.

Results and Discussion

Composition of Dry Seeds: The lipids are the major reserve material and amount to 38.50%. Total proteins and water-soluble proteins were found to be 24.68% and 19.84% respectively. Water soluble carbohydrates were 5.80% whereas, soluble and insoluble starch constituted 0.99% and 0.61% respectively. Activity of α–amylase was sufficiently high and corresponds to 24.08 μg/ml/min whereas β–amylase was negligible in dry seeds (0.48 μg/ml/min). Protease activity was calculated to be 7.28 μg/ml/min.

Biochemical changes during imbibition: In safflower, the metabolic changes are minimum durin 4-12h (Fig. 1). During this period, the amount of lipids and total proteins remained marginally same during early periods of imbibition. Lipids started depleting at 16h onwards, whereas total protein content increase slightly at 16h. However, water-soluble protein content decreased drastically at 16h period of imbibition (Fig. 1). Total water-soluble carbohydrates changed in a biphasic manner, showing decline during 4-12h and 16-24h with the maximum content at 16h. Our findings reveal that during early imbibition (4-12h), the embryo utilizes the available stored respiratory substrates (soluble sugars) present in the seeds, possibly to meet the demand of sugars during respiration[10]. After this period, the demand is met by the hydrolysis of lipids, proteins and carbohydrates as the amount of these biomolecules decreased during 16-24th period of imbibition.

The pattern of starch accumulation and utilization is somewhat unusual in imbibing seeds. Water-insoluble starch increased during 4-12h and depleted during 16-24h (Fig. 2). Soluble starch increased up to 8h. It may be that safflower seeds actively synthesize starch with onset of hydration and the newly formed starch is probably produced by gluconeogenesis using precursors from oil reserves[11,12]. There is appearance of α–amylase and β–amylase activity with increase in starch content (Fig. 3). It reflects that rate of starch synthesis is more than starch degradation by these two enzymes. So apparently, starch serves as a transient reserve material in safflower. The majority of amylase activity in the seed is clearly α–amylase whereas β-amylase appears during imbibition.

Biochemical changes during germination: Table 1 reveals that percent germination of safflower was maximum at 25°C and de-

Table 1. Percent germination of seeds at different temperatures.

Period of Germination (Days)	Temperature (°C)				
	10	25	40	50	60
2	-	58	44	23	10
4	10	66	58	33	10
6	15	87	71	39	10
8	15	94	89	54	10
10	15	94	94	63	10

Table 2. Percent germination of seeds at different pH values.

Period of Germination (Days)	pH values				
	2	4	6	8	10
2	23	56	58	58	42
4	25	67	67	67	53
6	36	88	87	86	53
8	45	93	94	94	53
10	45	94	95	94	53

creased with increase in temperature upto 60°C. At low temperature also (10°C), rate of germination was reduced. It has been found that dry seeds are frequently able to withstand a broad range of temperature, but after the germination process has been set in motion by imbibition of water, most of seeds appear to toerate a much narrower range of temperature.

Effect of pH indicates that seed germination was marginally same at pH 4, 6 and 8 (Table 2), while pH 2 and 10 were deterimental. The change in pH is known to disturb the nature of membrane potential by changing the protein motive forces across the plasma membrane. The germination in pH range of 4-8 indicates that the changes in membrane potential in safflower seed are favourable for the germination to occur.

There was continuous and steep decrease in lipid content during germination (Fig. 4). It has been found that during the first several days of germination, the fat reserves are used as respiratory substrates by conversion of sucrose and fatty acids. The developing embryonic plant is undergoing active cell division and relatively large amount of fatty acids are required for formation of new membranes[13]. These are obtained from breakdown of fat and are responsible for decrease in the amount of lipids in safflower as germination proceeds.

Total proteins increased throughout the period of germination except during 8th to 10th day (Fig. 4). Water-soluble proteins, however, showed continuous decrease (Fig. 5). It shows the high rate of protein turnover during germination period. In safflower active protein metabolism takes place in germinating seeds. Since the protein of the seedlings differ in amino acid composition from that of seed proteins, it is clear that degradation through proteolysis and synthesis must occur during germination.

The proteolysis of water-soluble proteins in safflower is concomitant with increase in protease activity (Fig. 6). It is suggested that peptidase activity is a pre-requisite for mas-

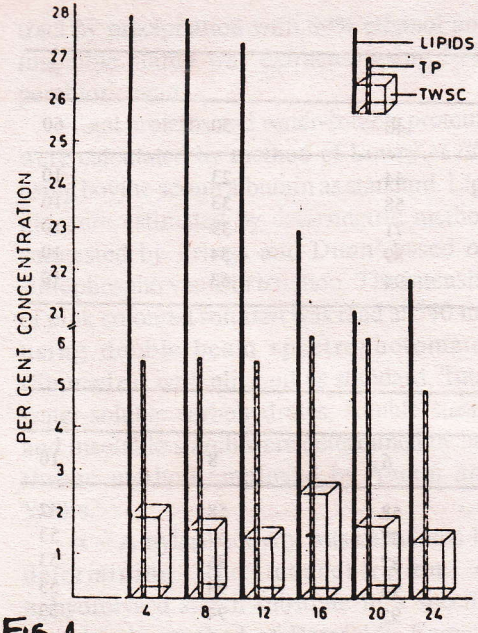

FIG. 1.

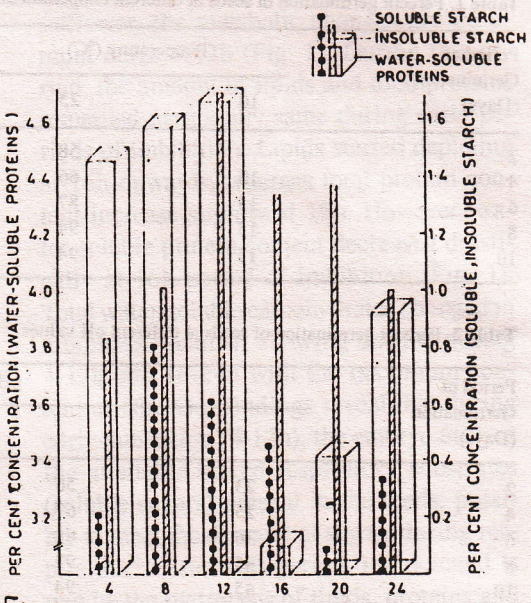

FIG. 2.

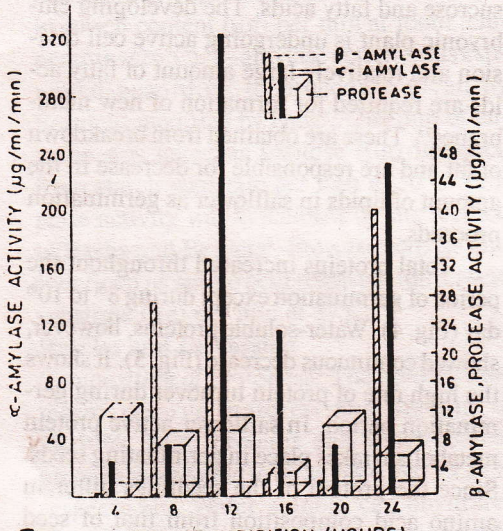

FIG. 3.

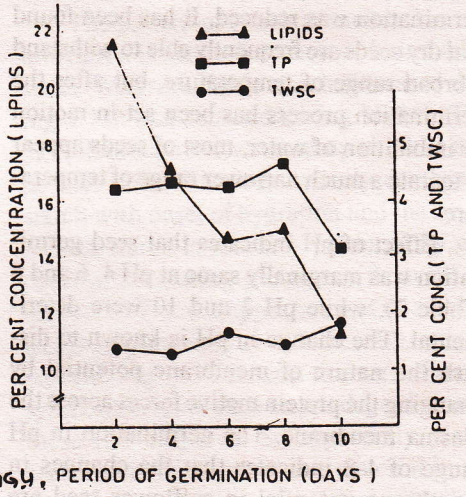

FIG. 4.

Fig. 1 Changes in lipids, total proteins and total water-soluble carbohydrates (%) during different periods of imbibition.

Fig. 2 Changes in water-soluble proteins, water-soluble starch and water-insoluble starch (%) during different periods of imbibition.

Fig. 3 Changes in α-amylase, β-amylase and protease activity (µg/ml/min) during different periods of imbibition.

Fig. 4 Changes in lipids, total proteins and total water-soluble carbohydrates (%) during differnet periods of germination.

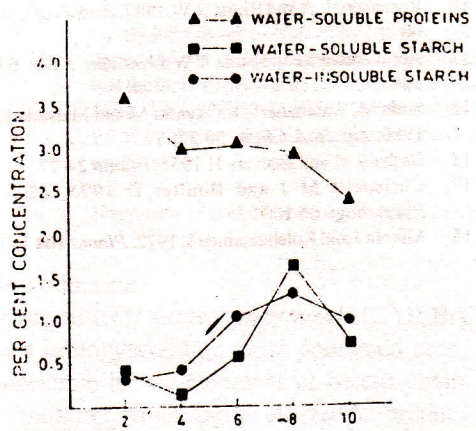

Fig. 5

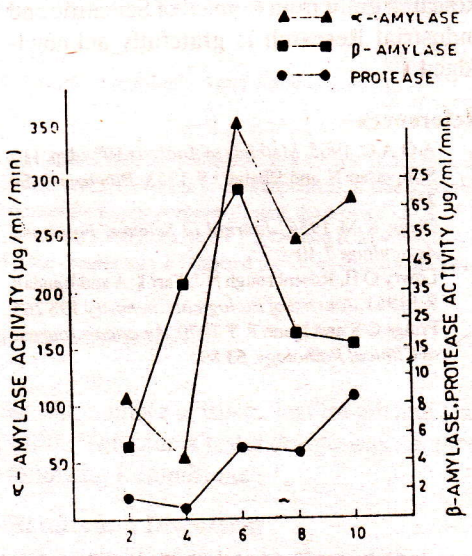

Fig. 6

Fig. 5 Changes in water-soluble proteins, water-soluble starch and water insoluble starch (%) during different periods of germination.

Fig. 6 Changes in α- amylase, β-amylase and protease activity (µ g/ml/min) during different periods of germiantion.

sive protien breakdown. Chrispeels and Boulter[14] followed breakdown of storage protein in mungbean and reported that the endopeptidase activity in cotyledons increase very sharply during germination. Mikola and Kolehemainen[15] reported that other peptidases are, also, of importance in germinating seeds.

Soluble sugars and starch level increased in cotyledons upto 8th day. Subsequent to this, their amount decreased on 10th day (Fig. 5). It indicates that with the initiation of visible germination, the rate of synthesis is more than their breakdown upto 8th day, whereas during later periods there is more utilisation or less formation of sugars. Synthesis of starch may utilize excess carbon derived from the breakdown and mobilization of lipid reserves. A rapid disappearance of lipid observed during this period supports this finding.

The α-and β–amylase activity increased upto 6th day in concordance with the increase in soluble and insoluble starch. The higher amount of starch upto 8th day indicates that these two enzymes are not participating in hydrolysing starch. It is also possible that the starch is present in the starch granules and is not accessible to the action of these enzymes. The rate of starch degradation is determined by both the amount of degradative enzyme activity and the accessibility of the particulate substrate to the enzymatic attack.

Acknowledgement

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