research article | International Journal of Plant Sciences, (July, 2010) Vol. 5 Issue 2 : 620-626

Reserve mobilization during germination of jackfruit (*Artocarpus heterophyllus* Lam) seeds

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Accepted : May, 2010

SUMMARY

Jackfruit seeds (*Artocarpus heterophyllus*) are recalcitrant and start germination immediately after harvesting as a continuum of development. Germination and reserve mobilization studies were conducted on fresh jackfruit seeds without storage up to 50 days of seedling growth. Starch was found abundant reserve stored in the cotyledons. Both ~ - and -amylases were very active in the ungerminated seeds and during germination there was continuous depletion of starch content as a result of the increased amylase activity. Metabolism of soluble carbohydrates during germination was unique in jackfruit seeds because loss of viability was under the control of soluble and insoluble carbohydrates status prevailing during germination associated metabolism of seeds which were categorized as recalcitrant.

Key words : Jackfruit seeds, Germination, Reserve mobilization, Amylase, Sugars, Starch

In recalcitrant seeds, development and germination are in continuum and immediately after shedding germination-associated metabolic changes occur. Berjak *et al*., (1989). Farrant *et al.* (1993) opined that it is difficult to identify the switch from reserve accumulation to the germination-associated reserve mobilization. Developmental aspects and desiccation sensitivity of recalcitrant seeds have been reviewed by Finch-Savage (1996) and Pammenter and Berjak (1999). Although germination potential is taken as an index or marker in the manifestation of desiccation sensitivity, storage behaviour and longevity of recalcitrant seeds, metabolism during germination and seedling development phase is not well documented in recalcitrant seeds.

Jackfruit seeds are recalcitrant (Chin *et al*., 1984; Fu *et al*., 1993; Chandel *et al*., 1995; Smith *et al*., 2001; Peran *et al*., 2004) and starch rich (Sheela, 2007). The main objective of this paper is to analyse the distribution of metabolites in Jackfruit seeds during germination. The other objective is to elucidate the hydrolysis and interconversion of metabolizable carbohydrates in the cotyledon and embryonic axis of Jackfruit seeds.

MATERIALS AND METHODS

Jackfruits (*Artocarpus heterophyllus* Lam.) for the present study were collected from a specific tree growing at Chathannur Village in Kollam District, Kerala state during 2004-2006. Fruits ripened on the mother plant were collected manually and brought to the laboratory. Fruits were cut open and seeds were collected, depulped and washed thoroughly in distilled water to remove any trace of perianth or aril. Washed seeds were wiped with clean towel and surface sterilised by wiping with a clean towel wetted with 80% ethyl alcohol and kept for germination studies. Sixty fresh seeds in duplicate were kept for germination in Petri plates lined with filter paper in darkness. Samples were collected at the interval of 2, 5, 10, 20, 30, 40 and 50 days after germination / seedling growth. Four seeds / seedlings each collected on sampling days were decoated, separated the cotyledons and axis. Samples of all biochemical analysis were taken from the pooled tissue. For dry weight determination of seedling parts, the samples were kept in hot air oven at 100^{0}C for one hour and then at 60^{0}C till weight became constant. Random sampling procedure was followed for each estimation. All experiments were repeated a minimum of 6 times using seeds of fruits collected from the same tree and during same period of two consecutive years for reproducibility of results.

Analysis of starch:

The method of Pucher *et al*. (1948) described by Whelan (1955) was used to extract and estimation of starch was done according to Montgomery (1957). The optical density of the solution was measured at 540 nm using Systronics Colorimeter. Soluble starch procured from Merck Chemical Company was used as standard.

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Analysis of sugars:

Known amount of tissue was homogenised in 80% ethyl alcohol by hot extraction method, and the samples extracted were used for HPLC studies. Glucose, fructose, rhamnose, maltose, sucrose, galactose, raffinose and stachyose procured from Merck Chemical Company were used as the standards. From the chromatogram of standards and samples comparison was made and from the peaks and area of each sample, the quantities of individual sugars were calculated.

Amylase assay:

Dinitrosalicylic acid method as explained by Bernfeld (1955) was followed for amylase assay. The optimal conditions of the assay system were standardized. As per standardised optimal conditions, the assay system contained 0.1ml of enzyme, 0.6ml of 0.1M sodium acetate buffer (pH-5.3) or 0.1M sodium phosphate buffer (pH-8.0) and 0.3ml of 2% substrate (soluble starch) and the assay system was incubated for 30 minutes at 37^0C and the product formed was estimated. Unit activity of the enzyme was calculated as mg maltose formed during 30 minutes at 37^0C per g tissue (dry weight) was calculated.

Total protein:

The method of Lowry *et al*. (1951) was followed to estimate the total protein. The estimation was carried out using Folin-Ciocalteau reagent and the optical density was read at 700nm using Genesis 20 Spectrophotometer. Bovine Serum Albumin (BSA) fraction V powder procured from Merck Chemical Company was used as the standard.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Table 1 and 2

Tissue dry weight:

The tissue dry weight percentage of the seeds up to 50 days of germination was given in Table 1. On the second day of germination, the cotyledon tissue dry weight remained same as that of control seeds (Table 1). During subsequent days of germination, the dry weight percentage was decreased gradually. In the case of embryonic axis, the tissue dry weight remained unchanged up to 5^{th} day and thereafter it showed a sharp decline (Table 1).

Starch:

The cotyledons and embryonic axis of ungerminated seeds showed maximum starch content (Table 2). After

Table 1 : Tissue dry weight percentage distribution of jackfruit seeds during germination/seedling growth

Days of germination	Tissue dry weight %	
	Cotyledon	Axis
0	50.07±1.11	16.51±1.91
2	53.25±1.56	14.50±1.45
5	48.66±2.06	16.55±0.57
10	45.92±1.77	6.31±1.58
20	39.12±1.87	ND
30	34.50±1.61	ND
40	33.40±2.38	ND
50	30.80±2.15	ND

ND: Not done because after 10^{th} day of germination, the embryonic axis is highly heterogeneous tissue since plumule and radicle get differentiated

Table 2 : Distribution of metabolites in jackfruit seeds during germination/seedling growth mg g^{-1} dry tissue

Days of germination	Tissue	Starch	Protein	
			Total	Soluble
0	Cotyledon	730.30±20.48	104.92±3.78	101.03±2.18
	Axis	497.74±32.64	179.92±10.11	51.82±3.36
2	Cotyledon	565.74±27.16	96.66±3.24	80.23±1.97
	Axis	224.09±14.37	159.58±6.62	87.92±7.24
5	Cotyledon	536.25±14.65	127.83±4.27	97.74±4.56
	Axis	166.94±13.41	131.30±7.73	38.55±1.51
10	Cotyledon	566.66±21.08	103.85±6.03	76.28±1.72
	Axis	88.45±3.48	103.96±10.62	35.97±8.24
20	Cotyledon	612.85±29.40	87.29±6.80	79.60±5.83
30	Cotyledon	682.13±25.42	85.80±5.85	56.61±4.41
40	Cotyledon	355.12±33.17	82.54±5.03	78.89±6.02
50	Cotyledon	339.25±27.47	60.16±5.26	42.92±4.42

two days of germination, a significant reduction ($P<0.01$) of starch content was observed and more or less same amount was present in the cotyledon up to 30 days of seedling growth. But afterwards starch content was reduced drastically during 40-50 days. The starch content

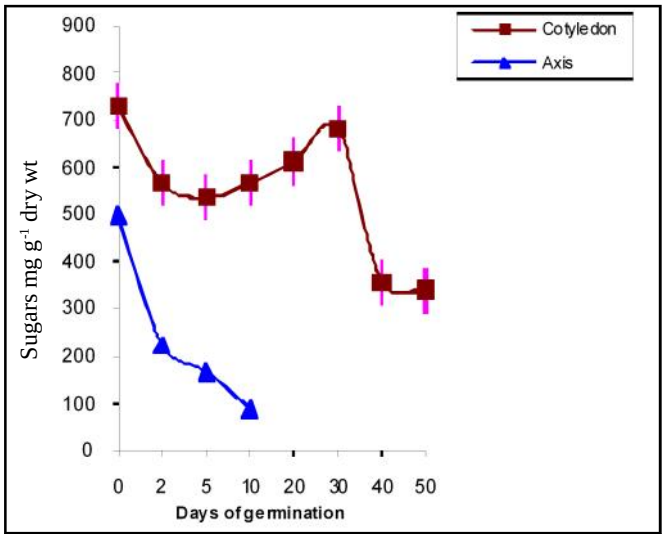

Fig. 1 : Distribution of starch in jackfruit seeds during germination

in embryonic axis showed a very sharp decline on the 2nd day of germination (Table 2, Fig. 1) and significant reduction was observed thereafter.

Sugars :

HPLC study on the sugars of control seeds showed that the embryonic axis contained only glucose, fructose, sucrose and maltose and these values were higher than that of cotyledon. Almost same amounts of glucose and fructose were present in the embryonic axis. In addition to glucose and fructose, cotyledon showed the presence of another monosaccharide, rhamnose. The sucrose and maltose showed about five times and eight times increase,

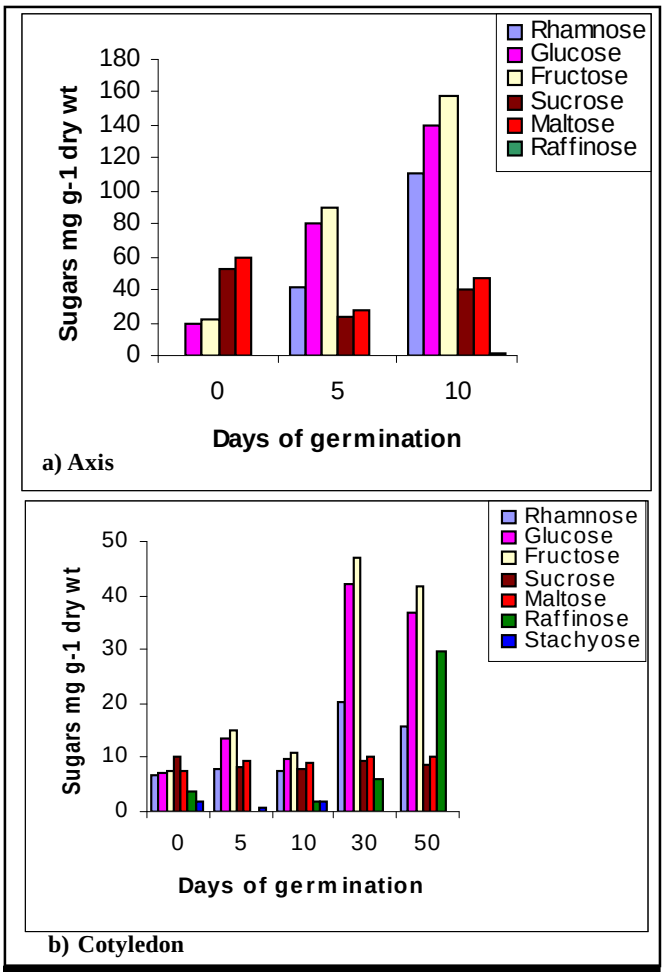

Fig. 2 : Distribution of sugars in jackfruit seeds during germination

respectively (Table 3, Fig.2a). The total sugar content of control axis was about 15.4% whereas cotyledons contained only 5.2%. Raffinose and stachyose were present in the cotyledons.

On 5th day of germination, the monosaccharide contents in axis were increased to about four times than

Table 3 : Distribution of sugars in jackfruit seeds during germination / seedling growth mg g^{-1} dry tissue

Sugars	Days of germination/ seedling growth							
	0		5		10		30	50
	Axis	Coty.	Axis	Coty.	Axis	Coty.	Cotyledon	Cotyledon
Rhamnose	-	6.73	42.08	7.96	110.51	7.69	20.15	15.68
Glucose	19.68	7.02	80.06	13.36	140.41	9.80	42.17	37.00
Fructose	22.71	7.66	90.60	14.90	158.48	10.89	47.10	41.58
Sucrose	52.99	10.22	24.16	8.32	39.62	7.86	9.45	8.76
Maltose	59.05	7.67	27.18	9.25	47.54	8.93	10.14	10.00
Raffinose	-	3.83	-	-	-	1.96	5.94	29.86
Stachyose	-	2.04	-	0.58	-	1.85	-	-
Total	154.43	45.17	264.08	54.37	497.68	48.98	134.95	142.88

that of the control seeds and increased amount of rhamnose was also present (Table 3, Fig.2a). The sugar content of cotyledons on 5th day and 10th day of germination showed the same amount as that of control samples. On 10th day, the axis showed a marked increase in monosaccharides attaining the maximum amount of glucose and fructose which were about seven times than that of the control seeds. The total sugar content, monosaccharides and disaccharides of the axis was very high compared to all other samples.

On 30th day of seedling growth, the cotyledon showed a more than four times increase in glucose and fructose contents than the 30th day sample (Table 3, Fig. 2b) reaching the maximum quantity. The raffinose content was significantly increased. The total sugar content was increased to about 14.5%.

In the cotyledon on 50th day of seedling growth, sucrose and maltose were almost same as that of the previous stage and control seeds. The quantity of raffinose showed a significant increase which was maximum in comparison with all other samples.

Activity of amylases:

The embryonic axis of control seeds showed very high activity of ~- (pH 8.0) and -amylase (pH-5.3) (Table 4, Fig. 3a,b) and as germination started, on second day, the activity was very high attaining the peak values of the enzymes. But on 5th day, activity of -amylase was reduced to half and that of *á*- amylase was reduced to one fourth as that of the 2nd day. But on 10th day the activity was slightly decreased.

The cotyledon of showed the same ~-amylase activity on the 2nd day (Table 4, Fig. 3b). Peak activity

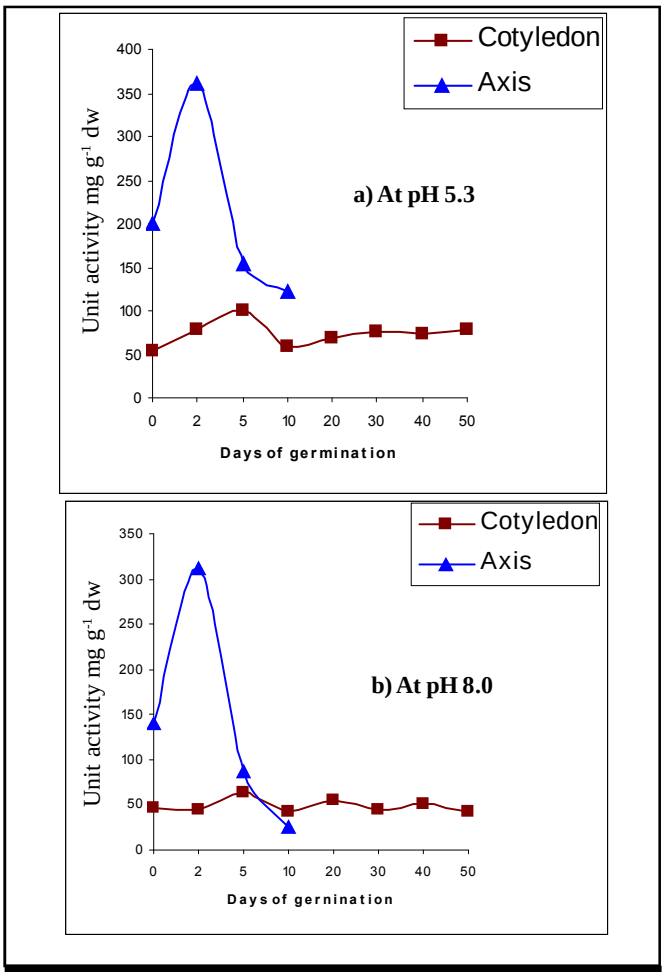

Fig. 3 : Pattern of amylase activity during germination of jackfruit seeds

Table 4 : Amylase activity in jackfruit seeds during germination/ seedling growth mg g^{-1} dry tissue

Days of germination	Tissue	Unit activity	
		pH- 5.3	pH-8.0
0	Cotyledon	53.28±4.73	45.83±4.09
	Axis	198.87±19.74	138.98±12.59
	Cotyledon	78.24±1.17	44.42±3.42
2	Axis	361.51±25.38	310.82±17.59
	Cotyledon	100.23±4.21	63.87±3.47
5	Axis	152.50±8.22	85.92±8.94
	Cotyledon	58.47±3.76	41.51±2.24
10	Axis	123.02±10.51	25.01±2.96
20	Cotyledon	69.48±2.15	55.14±3.58
30	Cotyledon	74.60±3.22	43.80±2.46
40	Cotyledon	72.54±3.92	51.59±2.87
50	Cotyledon	78.80±2.63	41.75±4.03

was shown by cotyledon on 5th day and significant reduction was observed on 10th day. A slight significant increase in activity was shown on 20th and 40th days, but a reduction was observed on 30th day.

The activity of *ß*-amylase in the cotyledon showed increase during the initial days of germination and the peak activity was observed on the 5th day (Table 4, Fig. 3a). On 5th day the activity was decreased and thereafter showed a slight increase.

Total protein:

The control seeds contained about 10% protein in the cotyledons (Table 2, Fig. 5a) and was slightly decreased insignificantly on the 2nd day of germination. But on 5th day about 20% increase in total protein content was observed. The seeds on 10th day showed reduction and after 10th day there was a sharp decline of total protein content in cotyledon and the same trend was continued up to 50 days.

The axis of control seeds showed about 18% of

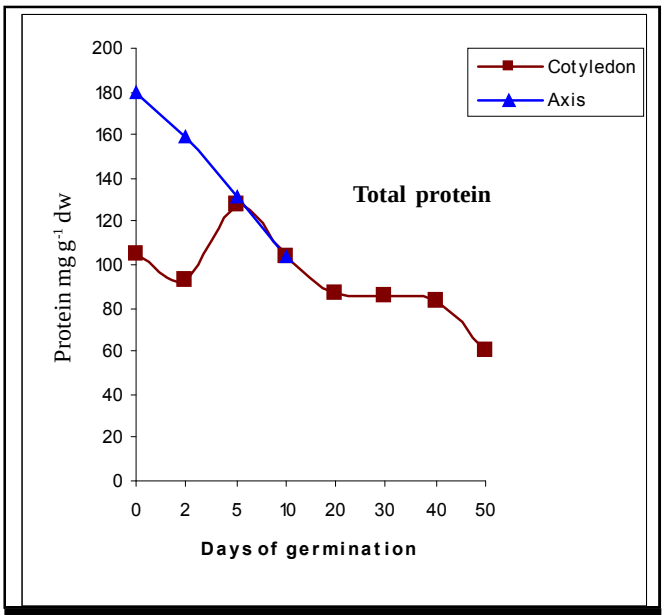

Fig. 5 : Distribution of protein content in jackfruit seeds during germination

protein content (Table 2, Fig. 5). Only a slight reduction occurred on 2nd and 5th days. On the 10th day of seedling growth, the total protein content was same in cotyledon and axis and both showed the same content as that of control cotyledon.

Fresh jackfruit seeds contain 73% and 49% starch in cotyledon and axis, respectively on dry weight basis. The metabolic changes during germination of jackfruit seeds particularly amylase activity (ß- and ~-amylases) is comparable with characteristics of germination-related reserve mobilization occurring in orthodox seeds (Khan, 1977; Bewley and Black, 1982, 1994; Mayer and Poljakoff-Mayber, 1989; Baskin and Baskin, 2001). But in orthodox seeds, significant starch hydrolysis occurs only after a few days of germination because *de novo* synthesis of amylase induced by gibberellic acid takes place only after germination and the duration varies from species to species (Mayer and Poljakoff-Mayber, 1989). In jackfruit seeds, both amylases are present as constitutive enzymes in the control seeds and 2nd day onwards, activity of these enzymes is increased revealing immediate germination-associated metabolism as a continuum of seed development which is characteristic of recalcitrant seeds (Pammenter and Berjak, 1999).

Significant activity of α- and β-amylases and resultant starch reduction occurred in the cotyledon. The axis tissue showed drastic reduction (50%) on 2nd day and only about 10% was retained on 10th day. This behaviour of jackfruit seed embryonic axis was similar to the metabolism reported in embryonic axes of orthodox seeds (Murray, 1984). On 10th day, a marked reduction of starch content in the axis tissue (Table 2) was observed due to the activity of β-amylase (Fig. 1, 3) indicating the specific involvement of β-amylase in the hydrolysis of starch due to availability of oligomers formed as a result of β-amylase activity during 2-5 days of growth.

Unlike the orthodox seeds in which hydrolytic enzyme such as β-amylase, protease, phosphatase etc. are sequestered in organelles or vacuoles and are released or activated at appropriate time after germination (Bewley and Black, 1994), amylolytic activity was very high in embryonic axis and cotyledons of fresh jackfruit seeds (control). As germination proceeds, starch content of the axis was depleted rather exhausted, but that of cotyledon remained unchanged up to 30 days of seedling growth (Fig. 1, 3) and was reduced to about one half on 40th and 50th days without significant increase in β-amylase activity presumably due to activity of starch phosphorylase which is another enzyme involved in starch catabolism during seed germination (Bewley and Black, 1994)

HPLC studies of sugars in jackfruit seeds during germination and seedling growth showed that in the axis monosaccharides - glucose and fructose were present in considerable quantities and were increased due to high metabolism related to growth and differentiation and simultaneous reduction of sucrose in the cotyledon may be due to the translocation to the metabolically active growing axis. Absence of raffinose and other oligosaccharides in the embryonic axis tissue was found to be a characteristic feature of jackfruit seeds unlike the orthodox seeds, where raffinose family of oligosaccharides which were essentially formed during desiccaion are the first carbohydrates to be utilized by hydrolytic cleavage of α-galactosidic bond to yield sucrose and galactose (Bewley and Black, 1994). In orthodox seeds occurrence of raffinose and its metabolic role as an important reserve readily available on germination have already been reported (Keller and Pharr, 1996: Peterbauer and Richter, 2001). The absence of galactose in the axis and cotyledon also may be a feature of recalcitrant seeds during germination. Lack of raffinose and other oligosaccharides in the embryonic axis of jackfruit seeds can also be correlated with the absence of maturation drying in recalcitrant seeds.

The occurrence of raffinose family of oligosaccharides in the cotyledons of fresh seeds can be considered as a pre-requisite for imposing desiccation tolerance during post-harvest storage because raffinose family of oligosaccharides play vital role in the induction of desiccation tolerance in recalcitrant seeds is well established (Koster and Leopold, 1988, Bernal-Lugo and

Leopold, 1992, 1995; Horbowicz and Obendorf 1994). Total sugar content in the cotyledonary tissue which increased as growth advanced to 50 days, indicated retarded mobilization to the growing axis because the germination studies were conducted in the dark and during this prolonged period seedlings were not well developed or established rather seedlings were etiolated with impaired metabolism.

In orthodox seeds α- amylase hydrolysed mature starch grains and the activity increased during germination while β-amylase could not hydrolyse starch grains and hence it cleaved away successive maltose units from the reducing end of oligomers released by prior α- amylase acivity. But in jackfruit seeds α- and β-amylases were equally active in the control and during germination showing maximum activity of β-amylases on 2nd day (Fig. 3). These observations reveal that in addition to the abundant starch grains in the embryonic axis (Sheela, 2007) oligomers also occurred since the seeds exhibited developmental and germination behaviour as a continuum. Maltose was the most abundant sugar in the embryonic axis of control seeds and it was reduced gradually during germination where as in the cotyledon, maltose was very low due to comparatively low amylolytic activity (Table 3, Fig. 2). The utilization / hydrolysis of maltose by the enzyme ~-glucosidase (maltase) which was increased during germination (Bewley and Black, 1994) induced the amylase activity by avoiding the end product inhibition of the enzyme.

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