

Biochemical Changes During Seed Germination of *Sterculia urens* Roxb.

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Abstract

The present study describes biochemical changes taking place during seed germination of *Sterculia urens*. The levels of proteins, total amino acids, reducing sugars, total soluble sugars and lipids were studied during various stages of seed germination (0-15 days). Total protein content was decreased in cotyledons during seed germination while free amino acid content increased to its maximum extent by 9th day of germination and reverse trend thereafter. The levels of reducing sugars and total soluble sugars increased till 6th day of germination and decreased thereafter. The lipid content was high at initial stages of germination (0-6 days) but gradually decreased by 15th day of germination.

Keywords: *Sterculia urens*, seed germination, proteins, carbohydrates, lipids

Introduction

Seeds are excellent dispersal units which have emerged in the course of plant evolution. The chemical energy produced during photosynthesis accumulates as seed reserves in multiple forms including carbohydrates, lipids and proteins. In general mobilization of seed reserves following germination is essential for the embryo to complete seedling establishment and, also signals the start of a new life cycle. These seed storage reserves are used directly as a source of nutrition for animals and humans (Khattak *et al.*, 2003; Rao *et al.*, 1998).

Gum karaya (*Sterculia urens*) is one of the commercially important tree belongs to the family of *Sterculiaceae*. The tree yields a gum known as gum karaya and this gum has numerous applications in pharmaceutical, dairy and other industries. The natural propagation of *Sterculia urens* is through seeds. However the seed viability decreases as the time progress. Furthermore its propagation could be done by stem cutting which poses some difficulties. The seeds of *Sterculia urens* are rich in proteins (35%), oil (26%) and carbohydrates (28%). The seed oil is suitable for edible purposes and soap manufacturing (The wealth of India, 1952).

Good amount of work was done and reported on karaya gum of *Sterculia urens*. But no reports on biochemical changes during seed germination. The main objective of the present study was an attempt to understand the role of proteins, carbohydrates, lipids and amino acids during various stages of seed germination in *Sterculia urens*. This study certainly helps in understanding the seed metabolism in *Sterculia urens*.

Materials and methods

Seed source

Seeds were supplied by Kovala foundation a Non Governmental Organization (NGO), Sagarnagar, Visakhapatnam, AP, India. All chemicals used in this study were of analytical grade, and were purchased from Sd Fine Chemicals limited, India.

Germination

Healthy seeds were selected and were thoroughly washed with running tap water until the outer covering of seed was removed. Then the seeds were rinsed in Tween for 5 minutes followed by running tap water for 5 minutes and then with 0.1% HgCl₂ for 5 minutes followed by sterile water. The seeds were soaked for 24 hours before they kept for germination (Le Page-Degivry and Garelo, 1973) in sterile petri plates with double layered moistened filter paper. The germination was carried out at 30°C 16 hours light and 8 hours dark (Plummer and Bell, 1995). The number of seeds germinated was counted every day up to 15 days and the Laboratory Germination Count (LGC) was calculated. Radicle emergence of 1cm was used as a reference to consider seed germination. The germinated seeds (cotyledons) were used for biochemical analysis with an interval of 3 days (0, 3, 6, 9, 12 and 15).

Biochemical studies

Determination of proteins, amino acids, lipids, total soluble sugars, reducing sugars

Each time (0, 3, 9, 12, 15 days) one gram of germinating seeds (cotyledons) was used to carry out biochemical

analysis. The sample was macerated in motor and pestle with 5 mL of phosphate buffer. Homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant was collected and extraction was repeated 4-5 times. All the supernatants were pooled and made the volume to 50 mL with phosphate buffer. One mL of above extract was taken and 1 mL of ice cold 20% TCA was added (Rajaram and Janardhanan, 1990). The pellet was washed twice with acetone and again centrifuged at 8000 rpm. Supernatant was discarded and pellet was dissolved in 5 mL of 0.1 N NaOH. This was used for protein estimation. Total proteins were estimated by the method of Lowry *et al.* (1951) using BSA as standard. Each experiment was repeated thrice.

For soluble sugars and free amino acid determinations one gram of germinating seeds (cotyledons) were ground in a mortar and pestle in 5 mL of 80% ethanol (v/v) and the mixture was boiled for 10 min and centrifuged at 2000 rpm for 10 min, the supernatant was collected and the pellet was reextracted in 5 mL of hot 80% ethanol. Supernatants from both extractions were combined, and total soluble sugar and reducing sugars were then determined by the phenol sulfuric acid (Dubois *et al.*, 1956) and Nelson-Somogyi (Somogyi, 1952) methods, respectively, using glucose as standard. Free amino acids were determined in the final supernatant by ninhydrin method (Moore and Stein, 1954) using leucine as standard.

Extraction and estimation of lipids was done by the method of Becker *et al.* (1978). One gram of germinated seeds was ground in mortar and pestle with chloroform-methanol mixture (2:1, v/v). For complete extraction the flask was kept at room temperature in the dark. Then chloroform and water (1:1, v/v) was added. The solution was subjected to centrifugation, three layers were observed. The methanol layer was discarded and lower organic layer was carefully collected and evaporated in water bath at 60°C. The weight of the lipid was determined. The results were expressed in terms of weight in mg of total lipids per gram of fresh tissue.

Statistical analysis

Each experiment has three replicates and the experiment was repeated thrice and the data was subjected to one way ANOVA using Minitab version 15. A significance level of 0.05 was used for all statistical tests.

Results and discussion

The total protein content decreased during seed germination in *Sterculia urens*. The total protein content at the beginning of germination (0 day) was 24.5 mg/gram tissue and decreased to 12.6 mg/gram tissue at the end of 15th day of germination (Tab. 1, Fig. 1). There was a reduction in the protein content from day 0 to day 15, with a rapid decrease between 6th to 12th day of germination. The protein depletion in *Sterculia urens* seeds was very slow within the first 3 days of germination and rapid during the 6th and 15th days of germination coinciding with the hypocotyls extension. A similar result was reported by Yoshida *et al.* (1997) in *Vigna mungo* cotyledons during germination. Considerable decreases in the protein content were observed in germinating *Lupinus luteus* and *L. angustifolius* (Olczak *et al.*, 1992), soybeans (Tan-Wilson *et al.*, 1996), Bambara groundnuts (*Voandzeia subterranea* L. Thouans) (Obizaba and Egbuna, 1992), fluted pumpkin (*Telfairia occidentalis* Hook) (Giami *et al.*, 1999), and sunflower seeds (*Helianthus annuus*) (Balasaraswathi and Sadasivam, 1997). The loss of proteins from the cotyledons could be due to the transport of amino acids to the growing axes or to respiratory loss, or it might result in the accumulation of free amino acids in the cotyledons. These reports are similar to results of Beevers and Spittoesser (1968) in germinating Peas and in the cotyledons of Mung Bean Seedlings (Kern and Chrispeels, 1978).

Total reducing sugar levels were increased from 0 day (1.90 mg/gram) up to 6th day (2.86 mg/gram) of germination and thereafter reverse trend was observed (Tab. 1, Fig. 1). This might be due to mobilization and hydrolysis of seed polysaccharides during seed germination. Polysaccharides can be further hydrolysed by amylases which might be responsible for the increasing total reducing sugar levels in cotyledons during initial stages of seed germination (Bemfeld, 1962). Similar results were reported by Khetarpaul and Chauhan (1990) in Pearl millet.

The total soluble sugars content varied from 1.70 mg/gram seeds to 0.8 mg/gram from 0 day to 15th day of germination (Tab. 1, Fig. 1). During germination, there was a decrease in storage carbohydrates and an increase in total soluble and reducing sugars up to 6th day of seed germination. This might be due to requirement of energy by grow-

Tab. 1. Biochemical studies during seed germination

Seed germination (days)	Protein content (mg/gram)	Reducing sugars (mg/gram)	Total soluble sugars (mg/gram)	Free aminoacids (mg/gram)	Lipids (mg/gram)
0	24.5±0.28	1.90±0.04	1.70±0.05	2.43±0.08	98.66±0.66
3	23.53±0.29	2.23±0.08	1.93±0.03	2.70±0.05	98.33±1.20
6	17.56±0.23	2.86±0.06	2.36±0.08	3.13±0.06	90±1.15
9	15.38±0.21	1.46±0.02	1.35±0.01	3.72±0.02	75±1.15
12	14.25±0.14	1.10±0.05	1.02±0.01	2.85±0.03	60.66±0.66
15	12.6±0.15	0.74±0.03	0.8±0.05	1.9±0.05	54±2.31

Note: The values represents the Mean±SE of three replicates

ing plant at initial stages of seed germination. These results agree well with the results of Jaya and Venkataraman (1981) in chickpea and greengram and also by in white beans Kon *et al.* (1973).

Total free amino acid content increased from 0 day (2.4 mg/gram) to 9th day (3.72 mg/gram) of germination and decreased thereafter (up to 15 days; Tab. 1, Fig. 1). Relatively low content of soluble amino acids were observed in dry seeds but increased levels during seed germination up to 9th day and decreased thereafter. The increase in amino acid content up to 9th day might be due to rapid hydrolysis of proteins, which results in release of free amino acids. The reduction in total amino acids thereafter shows rapid

utilization and rapid translocation of amino acids to the growing axes. Similar results were reported by Ali Al-Heal (1992) in *Cassia senna* seedlings.

Total lipid content reduced from 100 mg/gram to 49 mg/gram from 0 day to 15th day of germination (Tab. 1, Fig. 2). Approximately 50% reduction in lipid content was observed by the end of 15th day of germination. Less amount of reduction of lipids and triacylglycerols in the cotyledons indicated their lower level of mobilization and slower utilization by the embryonic axes. The liberated fatty acids might be used for the formation of membrane lipids in the growing embryos. Similar results were reported by Munshi *et al.* (2007) in sunflower seeds.

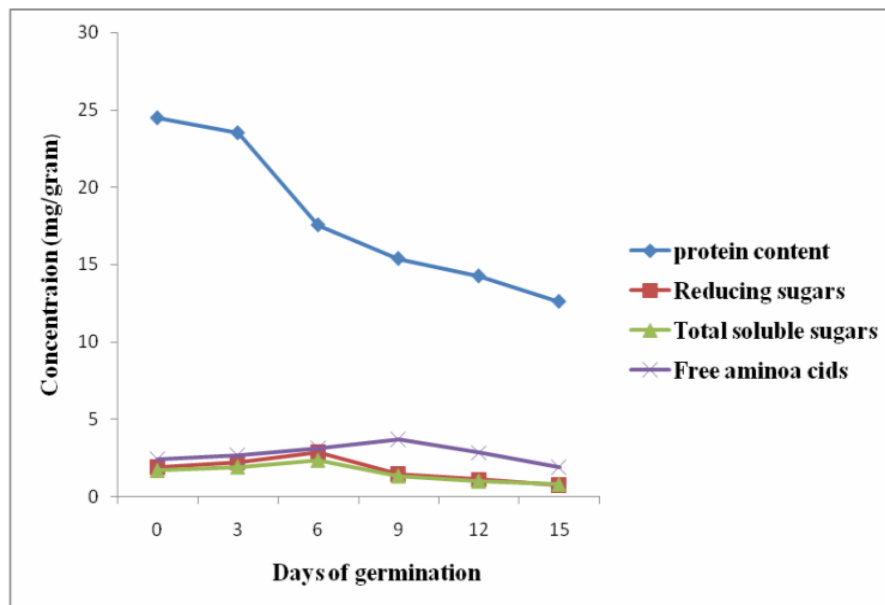


Fig. 1. Total proteins, amino acids, reducing and soluble sugar levels during seed germination (0-15 days)

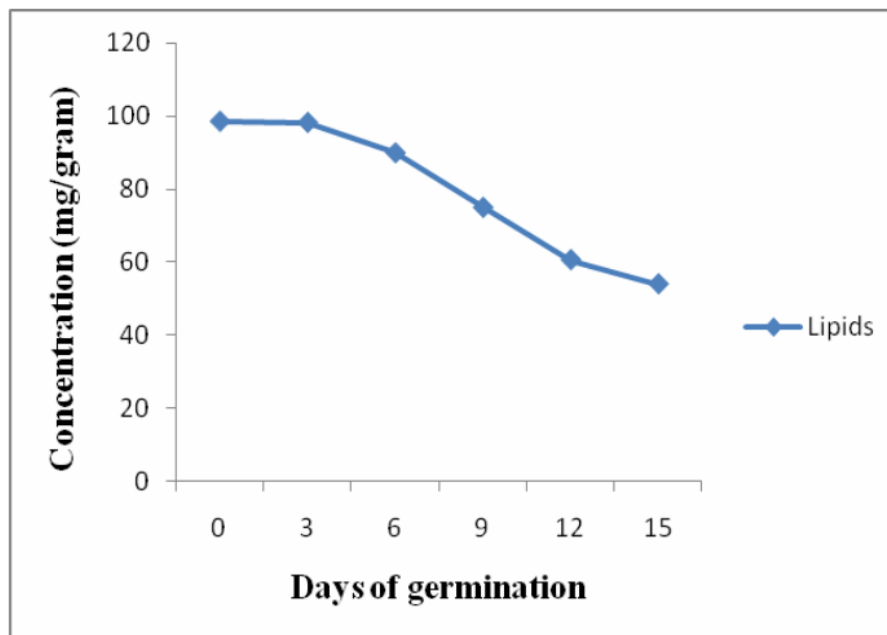


Fig. 2. Total lipid content during seed germination (0-15 days)

Conclusions

The seeds of *Sterculia urens* were rich in proteins, lipids as well as carbohydrates and their levels decreases as the germination progress, indicating their key role in the growth of embryonic axis. The work will be further continued to know the role different enzymes involved in germination of *Sterculia urens* seeds and the purification of enzymes by different methods.

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