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Bangladesh J. Sci. Ind. Res. **55(2)**, 107-112, 2020

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

Role of amylase and protease in germinating *Sterculia urens* Roxb.

S. Botcha* and S. D. Prattipati

Department of Biochemistry, Andhra University, Visakhapatnam, India-530003

Received: 10 June 2019
Revised: 27 June 2019
Accepted: 01 July 2019

DOI: https://doi.org/10.3329/bjsir.v55i2.47631

Abstract

The present study explains the levels of proteins and enzymes like proteases and amylases associated with the breakdown of proteins and carbohydrates during various stages (0 day to 15th day) of seed germination of *Sterculia urens* Roxb.. Maximum protease activity (1.12 units/mg of protein) and amylase activity was observed on 12th day of seed germination (34 units/mg of protein) and decreased thereafter. Highest protein content was observed at initial stage of seed germination and decreased thereafter. Increased proteolytic activity and amylase activity proportionately increases free amino acid content and sugars that promotes the seedlings growth and development.

Keywords: *Sterculia urens* Roxb.; Seed germination; Protease; Amylase; Proteins

Introduction

Seed germination has been regarded as a series of steps which normally occur prior to the emergence of the radicle from the seed coat (Mayerand and Shain, 1974). During germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolytic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development. The remaining small amount of proteins represents enzymes concerned in metabolic processes during seed development and germination (Millerd and Thomson, 1975).

Seed germination studies are key tools in conservation programs because they can be used for management programs and species reintroduction (Ortega-base and Rojas-arechiga, 2007). Uniform and fast germinating seeds are of prime importance for agriculture.

Sterculia urens Roxb. (Botanical Fam.: *Sterculiaceae*) is one of the commercially important trees and commonly known as gum karaya. The gum has numerous applications in pharmaceutical, dairy and textile industries. The natural propagation of *S. urens* is through seeds. The seeds of *S. 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).

The major constraint in seed propagation is loss of viability with progression of time and seed becomes dormant (Subhashini *et al*., 2012). Maintaining optimum moisture content under proper storage conditions helps in retaining maximum germination capacity of the seed. Dormancy in *S. urens* seeds results due to hard seed coat, and poor growth of embryo. This might be due to poor nourishment of embryo or loss of viability of embryo due to decrease in moisture content. But this can be overcome by acid and mechanical scarification and by treating with gibberellic acid GA_3 (Subhashini *et al*., 2012). The present study has been aimed in order to understand the role of two important enzymes i.e., amylases and proteases during various stages of seed germination in *S. urens*.

Materials and methods

Seed source

Seeds were collected from Kovela foundation, an NGO Organization, Visakhapatnam, AP, India and stored in an air tight container. All chemicals used in this study were of analytical grade and were purchased from Sd Fine Chemicals Ltd., India.

*Corresponding author e-mail: satyabiochemau@gmail.com

Surface sterilization of seeds

Healthy seeds were selected and were thoroughly washed with running tap water until the outer waxy covering of seed was removed. Then the seeds were rinsed for 5 min in each of Teepol, running tap water and 0.1% $HgCl_2$ followed by sterile water. The seeds were soaked for 24 h in sterile distilled water; and the dead floating seeds were removed. Therefore, the seeds were allowed to germinate by placing them onto a layered filter paper placed in a Petri plate.

Preparation of enzyme extract

One gram of germinating seeds were collected each time at different intervals of growth period (0, 3rd, 6th, 9th, 12th and 15th d of germination) and weighed after removing the seed coat. The sample was then homogenised in a mortar with the help of pestle to a very fine paste by adding 10ml of ice cold phosphate buffer (0.1 M; pH 7.6). The buffer extract was filtered and centrifuged at 10,000 rpm and 4°C for 15min. Later the supernatant was saved and the pellet was discarded. This seed extract was used for the biochemical analysis. The above procedure was carried out from 0-15th d of germination with an interval of three days.

Assay of protease

Protease activity was assayed by the method of Reimerdes and Meyer (1976) using casein as substrate. The measurement was carried out by estimating the release of tyrosine calculated from the standard curve prepared with tyrosine. One unit of protease activity was defined as the amount of enzyme required for liberating 1 mg of tyrosine in 30 min at 45°C.

Assay of amylase

Amylase activity was assayed by the method of Jayaraman (1981). One percent buffered starch solution was used as substrate. The amylase activity was measured by estimating the amount of maltose released which was calculated from the standard curve of maltose. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 15 min at 37°C.

Total proteins

Total protein was estimated by the method of Lowry *et al*. (1951) with Bovine Serum Albumin as standard. One ml of 20%TCA was added to 1ml of extract. The pellet was washed twice with acetone and again centrifuged at 8000 rpm for 5 min and the pellet was dissolved in 5ml of 0.1 N NaOH. This was used for protein estimation. A standard graph was constructed by taking standard BSA (10µg-100µg/ml). To 1ml extract 5ml of alkaline copper sulphate was added, mixed thoroughly and incubated for 30 min at room temperature. Then 0.5ml of Folin-ceocalteau reagent was added. Contents were mixed and allowed to stand at room temperature for 30 min. Then the absorbance was measured in a colorimeter at 660 nm. The amount of protein in the extract was determined using standard graph. Each experiment has three replicates and the experiment was repeated thrice.

Statistical analysis

Each experiment has three replicates and the experiment was repeated thrice. All 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

Germination

Radicle emergence occurred on 2nd day of germination. As the days progress, germination percentage increased significantly. Germination percentage of the *S. urens* was shown in the Table I and Figure 1.

The changes of amylase, protease and total protein content during different stages of seed germination of *S. urens* seeds was presented in the Table II. Protease activity in cotyledons varies from 0.02 - 1.12 units/mg of protein. A gradual increase of protease activity was observed with maximum activity (1.12 units/mg of protein) at 12th day of germination and reverse trend was observed thereafter. Protease activity was 100 times increased in 12th day of seed germination with respect to initial day of germination.

Table I. Germination percentage of *Sterculia urens* seeds

S. No	No. of seeds taken for germination	No. of seeds germinated	LGC*
1	50	46	93.33±3.06

*LGC: Laboratory Germination Count

Fig. 1. Seedlings of *Sterculia urens*

Table II: Activity of protease, amylase, proteins and total soluble sugar content in cotyledons during different stages of seed germination of *Sterculia urens* seeds

S. No	Days of germination	units/min/mg protein(±S.D)		mg/g(±S.D)
		Protease*	Amylase*	Proteins*
1.	0 day	0.02±0.01	0.38±0.03	37.66±1.52
2.	3rd day	0.16±0.02	1.61±0.11	18.67±3.06
3.	6th day	0.47±0.07	7.23±0.25	9.66±1.52
4.	9th day	0.65±0.06	7.97±0.54	8.03±0.76
5.	12th day	1.12±0.02	34.33±2.52	6.33±0.40
6.	15th day	0.61±0.06	11.56±0.86	2.85±0.35

*The values represent the means (±SD) of three independent experiments and the values were significant at p=0.05.

Amylase activity in cotyledons varies from 0.38 - 34.33 units/mg protein. Low level of amylase activity was observed at initial stages of seed germination (0 d) and maximum amylase activity was observed at 12th d of seed germination (34.43 units/mg of protein).

The soluble protein content was decreased during seed germination in *S. urens*. The total protein content at the beginning of germination (0 d) was 37.66 mg/g tissue and decreased to 6 mg/gram tissue at the end of 15th d of germination (Table II). There was a reduction in the protein content from day 0 - 15, with a rapid decrease between 0 and 6th d of germination.

The seeds of *S. 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). Mobilisation 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).

Higher protease activity was observed on 12th day of germination (1.12 units/mg of protein). Generally storage proteins are hydrolysed by proteolytic enzymes and provide nutrients for seedlings growth and development (Wang *et al*., 2007; Rahman *et al*., 2007). According to Mikola (1983), there will be three distinct stages in proteolysis of germinating seeds, where in the first stage there will be hydrolysis of proteins to amino acids for the purpose of synthesising enzymes which may be in turn degrade the insoluble reserves of the endosperm. In the second stage there will be hydrolysis of the main reserve protein which provides amino acids for the growing seedlings. In the third stage there will be senescence of the reserve depleted storage tissue which provides the last part of amino acids to the seedlings before the onset of autotrophic growth. The similar pattern of changes was observed during seed germination of *S. urens* where the proteolytic activity was increased slowly in the first two days and drastic increase thereafter followed by declining the activity. The decrease in proteolytic activity after 12th day of germination might be due to substrate depletion or auto digestion or an increase in the content of proteinase inhibitors. There is also probability that the loss of protein during germination was accompanied by either an activation of proenzymes or *de novo* synthesis of proteases. Similar findings were observed in germination of sesame seeds (Hemalatha and Siva Prasad, 2003), mungbean varieties (Rahman *et al*., 2007), castor beans (Alpi and Beevers, 1981), soybeans (Asano *et al*., 1999), *Vigna mungo* (Muntz, 1996; Toyooka, 2000), and winged beans (Usha and Singh, 1996).

Maximum amylase activity was observed on 12th day of seed germination (34 units/mg of protein). The increase in amylase activity was due to rapid hydrolysis of storage reserves and the products will be transported to the growing seedlings for their development (Rahman *et al*., 2007). The results were agreed with the findings of seed germination of mungbean seeds (Vijaylaxmi, 2013), sesame seeds (Hemalatha and Siva Prasad, 2005), cowpeas (Uriyo, 2001), which shows positive relationship between germination and amylase activity.

The rapid depletion of protein content in *S. urens* seeds during 0 day to 6th day of germination was coinciding with the hypocotyls extension. The result was agreed with the findings of Yoshida *et al*. (1997) in *V. mungo* cotyledons during germination. Considerable decreases in the protein content were observed in germinating *Lupinus luteus* and *L. angustifolius* (Olczak, 1992), fluted pumpkin (*Telfairia occidentalis* Hook; Giami, 1999), and sunflower seeds (Balasaraswathi and Sadasivam, 1997). The loss of proteins from cotyledons could also be due to the transport of amino acids to the growing axes or it might result in the accumulation of free amino acids in the cotyledons. Similar findings were observed by Beevers and Spittoesser (1968) in germinating Peas and in the cotyledons of Mung Bean Seedlings (Kern and Chrispeels 1978).

Conclusion

From the present study it is concluded that the seeds of *S. urens* were rich in proteins as well as carbohydrates and their levels decreases as the germination progress, indicating their key role in the growth of embryonic axis. Further studies are needed to have better understanding of biochemical and molecular events associated with seed germination.

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