

## Changes in levels of soluble sugar, reducing sugar and lipid during germination of seeds of *Albizia procera*

Rupnarayan Sett

Division of Genetics and Plant Propagation, Tropical Forest Research Institute (ICFRE; MoEFCC), Mandla Road, P.O. RFRC, Jabalpur 482021, Madhya Pradesh, India  
ruppuran2001@yahoo.co.in, settrn@icfre.org

**Running Title:** *Sugar and lipid in seeds of Albizia procera*

### Abstract

The biochemical levels of total lipid, soluble sugar and reducing sugar in early germinating seeds collected from 3 trees of *Albizia procera* aged around 25 years were estimated from the 1<sup>st</sup> day of germination till the 15<sup>th</sup> day. With progression of germination, total lipid content decreased in all the 3 trees. No such continuous trend could be drawn regarding the levels of total soluble sugar and total reducing sugar when the results showed ups and downs in their concentrations at different days of germination. However, in both the cases, retaining a considerable level up to 12<sup>th</sup> day of germination was noticed. The recorded results might be beneficial for further studies on the metabolic activities of germinating seeds in *Albizia procera* or related tree species.

**Keywords:** *Albizia procera*, White Siris, Safed Siris, Karai, Seed, Seed germination, Soluble Sugar, Reducing Sugar, Lipid, Tree embryogenesis

### Introduction

During germination, the storage compounds like soluble sugars (mainly raffinose, stachyose, verbascose and sucrose) are degraded for use as energy-source for the projection of the radicles and promote development of seedlings; galactomannans appear in action after germination [1, 2].

The energy necessary for the *de novo* production of proteins during the initial significant stages of germination, is stored in the oleosomes of cells as triglycerides in the aleurone and embryos of dicotyledons. Upon water uptake, the fatty acids of the oleosomes are

liberated and proceed to glyoxysomes (specialized microbodies) where, by  $\beta$ -oxidation, the fatty acids are metabolized into acetyl-CoA. The mobilization of triacylglycerol in seed-tissue requires an intricate series of metabolic events which, following germination, is triggered and facilitates the conversion of oils to sugars [3, 4].

The genus *Albizia* comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa. *Albizia procera* (Roxb.) Benth, family Fabaceae, subfamily Mimosoideae, commonly called as Karai or White Siris or Safed Siris or Tall *Albizia*, is a large, fast growing tree species that occurs on many different sites, but prefers moist sites. This species provides wood for a variety of purpose, nutritious fodder for livestock and shade for tea plantation. It is an imported reforestation and agro-forestry species. It is widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and Northern Australia.

*Albizia procera*, a tree with an open canopy, is usually 60-70 cm in diameter and 25 - 30 meters in height; mature individuals are characterized by tall, clear, erect, sometimes curved trunk and large branches which form a thin, spreading crown. The bark is smooth, whitish to light greenish gray or light brown. It exfoliates in thin flakes with red under sides [5]. The natural regeneration follows the beginning of the rainy season; large numbers of seedling are common near mature trees. Seedlings, saplings and mature trees are formed from stumps and roots [6]. *Albizia procera* are often planted for shade or beautification along roads and are commonly used in traditional medicines [7]. The bark contains tannins and a reddish gum. Traditionally, parts of *Albizia procera* are used in anticancer, pain, convulsions, delirium, and septicemia. The protein-rich fodder of *Albizia procera* is eaten by cattle, buffaloes, goats, camels and elephants. Durable, strong and resistant to termites, the wood of *A. procera* is light to chocolate-brown in color with light and dark bands. The wood is used to produce wheels, carts, boats, furniture, flooring, posts, agriculture implements, boxes and carvings. This species is considered a promising source of pulp for high-quality paper [8].

The White Siris seeds are small, greenish-brown and elliptical to round, flat and have a hard, smooth seed coat; count 20,000-24,000 seeds per kilogram [9]. Fresh seed germinates rapidly without treatment [8]. Clean seeds can be stored at room temperature for 10 months with minimal loss of viability [9]. Seed that has been stored are treated before sowing, cut through the

seed-coat with a knife, or soak seeds in boiled water for 3 minutes; after any treatment, seeds are soaked in cold water for 12-24 hours and then sowed immediately [9].

The objective of the work was to determine changes in sugar and lipid level in early developmental stages in seeds of *Albizia procera*; no literature on this important issue could be made available within limited resources. The determination of the sugar and lipid levels revealed important scenario during embryological development of this important tree species.

## Materials and methods

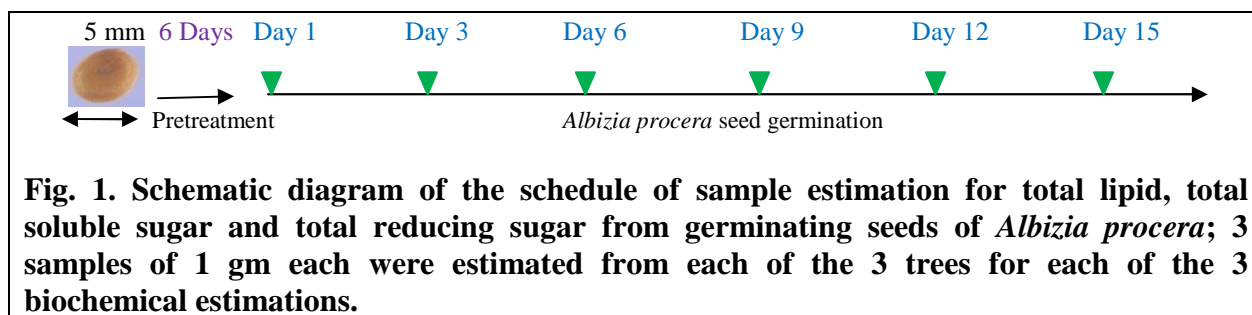
### Geological and climatic data of the experimental site

In Madhya Pradesh, India, varied type of vegetation occurs with a lot of variation. It is divided into 11 different agro-climatic regions on the basis of vegetations, rainfall and soil conditions. Jabalpur, a large district of Madhya Pradesh belongs to Kymore Plateau and Satpura Hills zones with an average rainfall 1100 mm in humid condition. Jabalpur is one of the central districts of Madhya Pradesh, India between 22°49' and 24°8' North Latitude, 78°21' and 80°58' East Longitude and 411m altitude. Tropic of cancer passes through the middle of the district and divides it in to nearly two equal halves. The mean annual rainfall, minimum and maximum temperatures are 1498.7mm, 18.5°C, 43.6°C respectively. Maximum rain occurs from July to October.

For the estimation of total lipid, total soluble sugar and total reducing sugar, 1 gram of germinated seeds was used of *Albizia procera* for every experiment; each experiment was done in 3 replicates. Such estimations were done from 3 different trees selected from TFRI campus (Jabalpur), GPS locations of which are shown in the Table 1.

**Table 1. GPS location of *Albizia procera* Trees selected from TFRI, Jabalpur**

Tree No.	GPS Location		
	N	E	Elevation (in feet)
1	23° 05' 58.27"	79 ° 59' 21. 80"	1353
2	23° 05' 58.57"	79 ° 59' 22. 26"	1353
3	23° 05' 49.65"	79 ° 59' 17. 65"	1355



**Fig. 1. Schematic diagram of the schedule of sample estimation for total lipid, total soluble sugar and total reducing sugar from germinating seeds of *Albizia procera*; 3 samples of 1 gm each were estimated from each of the 3 trees for each of the 3 biochemical estimations.**

### Design of experiment

Three trees were marked for each group of experiment, designated as Tree 1, Tree 2 and Tree 3. Seeds set in Petri-plates for germination with marking from each tree on wet blotting papers. Experiments were set for 1, 3, 6, 9, 12 and 15 days of germinating seeds. 3 replicates were taken for each day point. 3 samples were prepared from 1 gm of germinating seed material for each tree as marked, i.e. 9 samples were prepared for each estimation procedure. 3 estimations for lipid, soluble sugar and reducing sugar, i.e. 27 samples were tested for each day of development. Results were expressed in total Mean  $\pm$  SD. Each value in the tables and figures is expressed as mean of 3 replicates.

### Glass and plastic-ware

Different types of glass-ware made up of borosilicate were used (Borosil) such as test tube, measuring cylinder (10 ml, 50 ml, 100 ml, 500 ml and 1000 ml), conical flask (50 ml, 100 ml, 250 ml, 500 ml) and beaker (50 ml, 100 ml and 500 ml). Plastic-ware (Tarson) such as bottles, measuring cylinders, test tube stand etc. was used for the experiments.

### Equipments

For biochemical test, hot air oven (Sonar) was used for drying the glass wares. Pipettes measuring 2 ml, 10 ml, 50 ml pipettes were used (Borosil). Electronic digital weighing balance (Mettler) was used for weighing chemicals such as glucose,  $\text{HgCl}_2$ , NaOH, ammonium molybdate and fine chemicals. The water purification system (Millipore) was used for collection of water which was used for biochemical experiments and preparation of solutions. Micropipettes of different capacities ( $\mu\text{l}$ ) were used for measurement of the volume of the different chemicals and other solutions. A Centrifuge machine (Sigma 3-18K) and spin win were used for centrifugation purpose. Water bath (Shivaki PID 702) was used for regulating the temperature of substances subjected to heat. Water bath was used to heat the sample solution.

UV-VIS spectrophotometer (Hitachi U-2900/U-2910) was used for measuring the absorbance of the sample solution.

### **Plant material**

3 trees, each 25 years old, were selected for seed collection from the campus of Tropical Forest Research Institute at Jabalpur, Madhya Pradesh.

### **Chemicals**

All chemicals used in this study were of analytical grade, and were purchased from local suppliers at Jabalpur.

### **Germination**

Healthy seeds were selected and thoroughly washed with running tap water until the outer covering of seed was removed. Then the seeds were rinsed in Tween 20 for 5 minutes followed by running tap water for 5 minutes and then with 0.1%  $\text{HgCl}_2$  for 5 minutes followed by sterile water. The seeds were soaked for 24 hours before keeping for germination in sterile Petri plates with double layered moistened filter paper. As pretreatment, the seeds were then immersed in hot water at  $100^\circ\text{C}$  for 1 minute as described to be the best method by [10]. Germination started after 6 days, and was carried out at  $30^\circ\text{C}$  with 95% humidity and a photoperiod of 16 hours light and 8 hours dark inside seed-germinator (Remi Elektrotechnik Limited). The germinated seeds (cotyledons) were used for biochemical analysis with an interval of 3 days (1, 3, 6, 9, 12 and 15).

### **Estimation of Lipid**

Estimation of lipids was done by the method as described by Becker *et al.* (1978) [3]. 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) were 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  $60^\circ\text{C}$ . The weight of the lipid was determined. The results were expressed in terms of weight in milligram of total lipids per gram (mg/gm) of fresh seed tissue.

### **Estimation of Soluble Sugar**

During the present study we estimated the soluble and reducing sugar in three different trees of *Albizia procera*. The sugar content in fresh seeds was estimated from 1<sup>st</sup> day of

germination to 15<sup>th</sup> day compared with the sugar standard with two different concentrations i.e., 0.1 mg/ml and 0.2 mg/ml respectively and to find any variation in the concentration of sugars. For soluble and reducing sugars, they are expressed in 0.1 mg of seed tissue per 1 ml of the reactant solution and 0.2 mg of seed tissue per 1 ml of the reactant solution, and thereby the percentage of the respective sugar was calculated.

One gram of germinating seeds or cotyledons were ground in mortar and pestle in 5 ml of 80% ethanol (v/v) and then the mixture was boiled for 10 min and centrifuged at 2000 rpm for 10 min, the supernatant was collected and the pellet was re-extracted 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 Dubois *et al.* [11] and Nelson-Somogyi [12, 13] methods.

### **Determination of Soluble Sugars by Dubois Method (Phenol-sulfuric acid method)**

#### **Materials**

Phenol 5%: Redistilled (reagent grade) phenol (50 g) dissolved in water and diluted to one liter. Sulfuric acid: 96% reagent grade.

Standard Glucose solution: Stock - 100 mg in 100ml of water. Working standard - 10ml of stock diluted to 100 ml with distilled water.

#### **Procedure**

From the sample, a known volume of aliquot was pipette out and was made up to 1.0 ml using distilled water. To this solution, 1.0 ml 5% aqueous phenol and 5 ml concentrated chilled 96% sulfuric acid was added and shaken well for 10 minutes. Then placed in boiling water bath at 25-30°C for 20 minutes. The intensity of color was measured after proper dilution at 490 nm using UV-VIS spectrophotometer (Hitachi U-2900/U-2910). D-Glucose was used as the standard.

#### **Calculation**

From the standard curve find out the concentration of phenol in the test sample and express as % of total sugar present in plant material.

Absorbance corresponds to 0.1 ml of the test tube = x mg of glucose

$$\begin{aligned} 100 \text{ ml sample solution contain} &= \frac{x}{0.1} \times 100 \text{ mg of glucose} \\ &= \% \text{ of total carbohydrates} \end{aligned}$$

### **Estimation of Reducing Sugars**

An aliquot from the extract prepared for the estimation of total soluble sugar was used for the estimation of total reducing sugars according to the Nelson-Somogyi method [12, 13].

### **Determination of Reducing Sugar by Nelson- Somogyi Method**

#### **Materials**

##### **Preparation of Somogyi's Copper Reagent:**

This reagent was prepared by (a) dissolving 2.5 g of anhydrous sodium carbonate and 2.5 g of sodium-potassium tartrate (Rochelle salt), 2 g Sodium bicarbonate and 20 g Anhydrous Sodium Sulphate in about 80 ml of distilled water and the volume made up to 100 ml. (b) To this, 15 g of copper sulphate as a 10% (w/v) solution was added. One drop of Sulphuric acid was added and the volume was made up to 100 ml. Both the solutions were mixed well before used (4 ml of solution a and 96 ml of solution b).

##### **Preparation of Nelson's Arseno-molybdate Reagent:**

Nelson's Arseno-molybdate reagent was prepared by dissolving 2.5 g of Ammonium Molybdate in 45 ml of water. Then 2.5 ml of sulphuric acid was added and mixed well. To the mixture 0.3 g of disodium hydrogen arsenate dissolved in 25 ml of distilled water was added. The solution was mixed well and incubated for 24-48 hours at 37°C.

**Standard Glucose:** Stock: dissolved 100 mg glucose in 100 ml distilled water.

Working standard: 10 ml of stock diluted to 100 ml with distilled water.

#### **Procedure**

From the sample, a known volume of aliquot was pipette out and was made up to 2.0 ml using distilled water. To this, 1.0 ml of Somogyi's copper reagent was added. The mixture was then placed in a bath of boiling water and heated for 10 minutes. After cooling under tap water 1.0 ml of Nelson's Arseno-molybdate reagent was added with immediate mixing and the volume was made up to 10 ml with distilled water. The intensity of color was measured after proper dilution at 620 nm using a UV-VIS spectrophotometer (Hitachi U-2900/U-2910). D-Glucose was used as the standard.

#### **Calculation**

From the standard curve find out the concentration of copper in the test sample and express as % of total reducing sugar present in plant material.

#### **Formula used**

Absorbance corresponds to 0.1 ml of test = X mg of glucose

$$10 \text{ ml contains} = \frac{X}{0.1} \times 10 \text{ mg of glucose}$$

$$= \% \text{ of reducing sugar}$$

## Results

The experiments revealed the biochemical content of total lipid, total soluble sugar and total reducing sugar during seed germination and their molecular evolution with embryonic metabolism in *Albizia procera* seeds from the onset (Day 1) till the Day 15 with an interval of 2 days (Day 1, 3, 6, 9, 12 and 15) (Fig. 1). For these biochemical tests, we followed 3 different well-established methodologies, viz. for total lipid estimation Becker *et al.* (1978) [3] method, for total soluble sugar Dubois *et al.* (1956) [11] method and for total reducing sugar Nelson (1944) [12] and Somogyi (1952) [13] method. The absorbance in UV-VIS spectrophotometer for soluble sugar absorbance was read at 490 nm and for reducing sugar at 620 nm. For this biochemical estimation, three different trees of *Albizia procera* species from different locations were used (Table 1).

Biochemical estimation for total lipid, soluble sugar and reducing sugar was done in 3 different trees, all aged around 25 years. With progression of germination, the total lipid content was found to be decreased in all the 3 trees during a time period of 15 days starting from the onset. In Tree 1 the total lipid content was found to be reduced to approximately for 95%, in Tree 2 for 80% and in Tree 3 for 75% (Tables 2-4; Figures 2-4). High amount of reduction of lipid in the seeds indicated their higher level of mobilization and faster utilization by the embryonic axes.

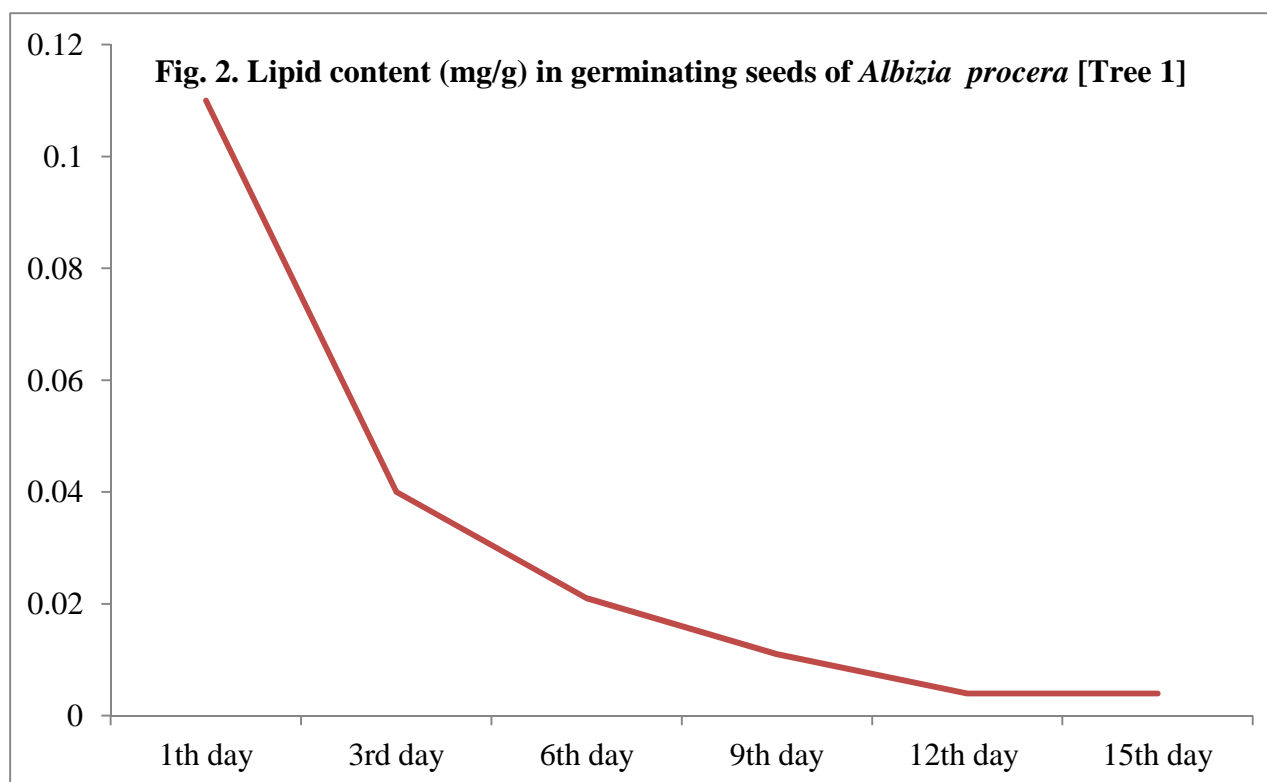
## Estimation of Lipid

The total lipid content decreased during seed germination in *Albizia procera*. In Tree 1, total lipid content reduced from 0.11 mg/g to 0.004 mg/g from 0 day to 15<sup>th</sup> day of germination (Table 2; Fig. 2). Approximately 95% reduction in lipid content was observed by the end 15<sup>th</sup> day of germination. In 0-15 days, the concentration of sample was, on 0 day 0.11 mg/g, on 3 day 0.040 mg/g, on 6<sup>th</sup> day 0.021 mg/g, on 9<sup>th</sup> day 0.011 mg/g, on 12<sup>th</sup> day 0.004 mg/g and on 15<sup>th</sup> day 0.004 mg/g was observed. According to the table, from 0 day to 6 day result was found  $0.057 \pm 0.046$  mg/g and 9 day to 15 day result was found  $0.064 \pm 0.004$  mg/g (Average  $\pm$  SD).



**Table 2. Lipid content (mg/g) in germinating seeds of *Albizia procera* [Tree 1]**

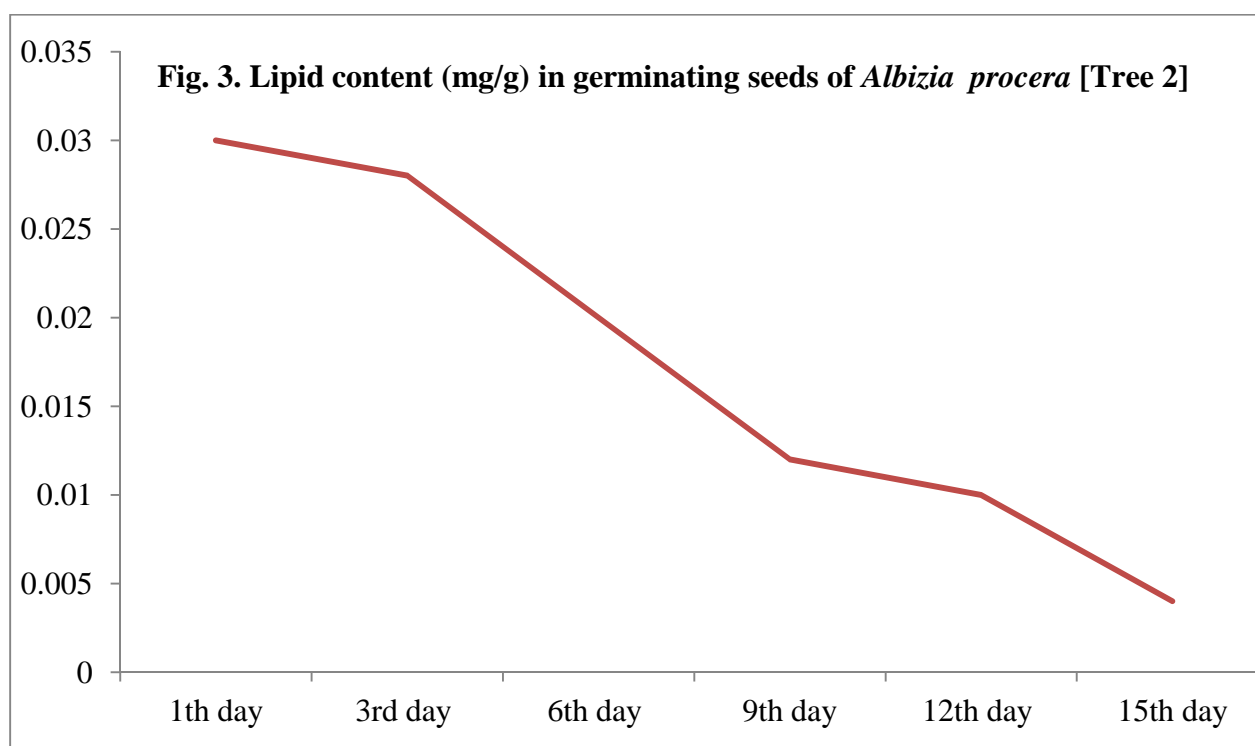
Day of germination	Lipid (mg/g)			
	Mean	Average	SD	Result
1 <sup>st</sup>	0.110	0.057	0.04687	$0.057 \pm 0.046$
3 <sup>rd</sup>	0.040			
6 <sup>th</sup>	0.021			
9 <sup>th</sup>	0.011	0.006333	0.00404	$0.006 \pm 0.004$
12 <sup>th</sup>	0.004			
15 <sup>th</sup>	0.004			



In Tree 2, the total the lipid content reduced from 0.030 mg/g to 0.004 mg/g from 1<sup>st</sup> day to 15<sup>th</sup> day of germination (Table 3; Fig. 3). Approximately 92% reduction in lipid content was observed by the end 15<sup>th</sup> day of germination. In 0-15 days, the concentration of sample is, on 0 day 0.030 mg/g, on 3<sup>rd</sup> day 0.028 mg/g, on 6<sup>th</sup> day 0.020 mg/g, on 9<sup>th</sup> day 0.012 mg/g, on 12<sup>th</sup> day 0.010 mg/g and on 15<sup>th</sup> day 0.004 mg/g was observed. According to the table, from 0 day to 6<sup>th</sup> day result is found  $0.026 \pm 0.005$  mg/g and from 9<sup>th</sup> day to 15<sup>th</sup> day of germination, the result was found  $0.008 \pm 0.004$  mg/g (Average  $\pm$  SD).

**Table 3. Lipid content (mg/g) in germinating seeds of *Albizia procera* [Tree 2]**

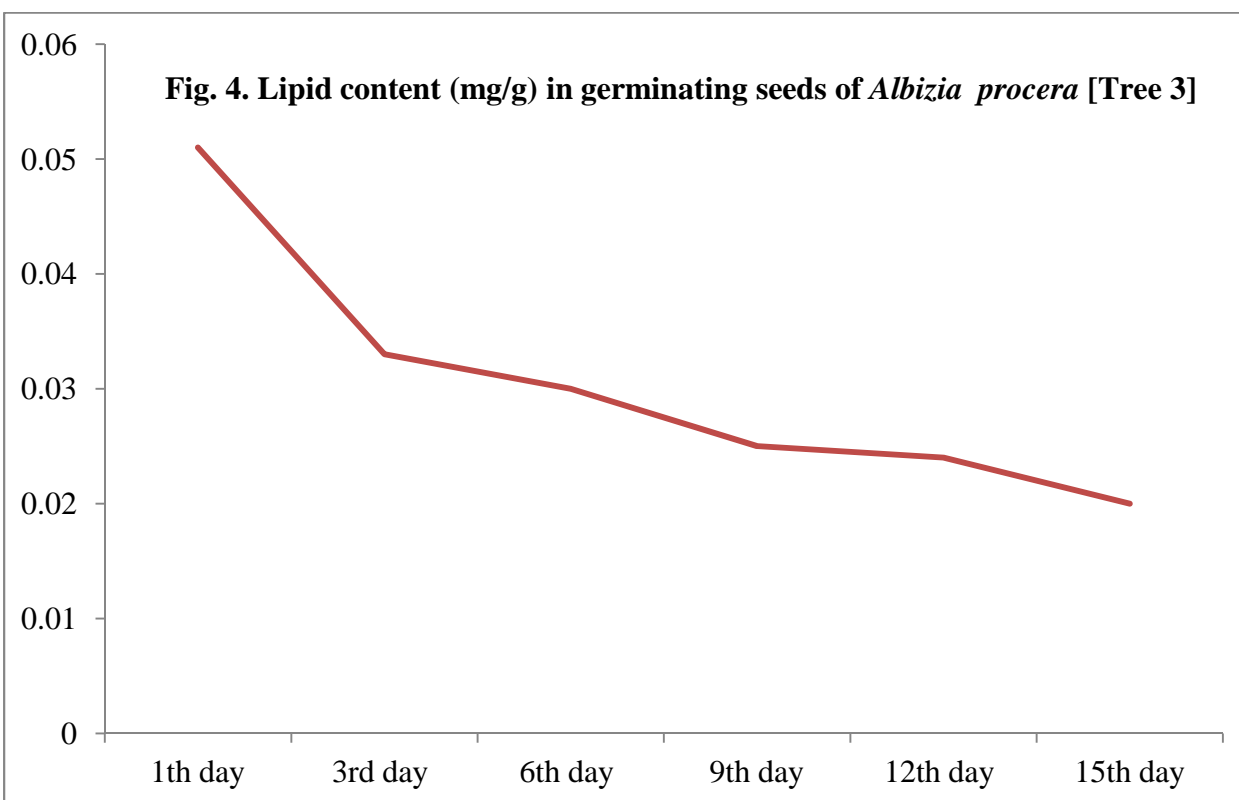
Day of germination	Lipid (mg/g)			
	Mean	Average	SD	Result
1 <sup>st</sup>	0.030	0.026	0.00529	$0.026 \pm 0.005$
3 <sup>rd</sup>	0.028			
6 <sup>th</sup>	0.020			
9 <sup>th</sup>	0.012	0.00867	0.00416	$0.008 \pm 0.004$
12 <sup>th</sup>	0.010			
15 <sup>th</sup>	0.004			



In Tree 3, the total lipid content reduced from 0.051 mg/g to 0.020 mg/g from 1<sup>st</sup> day to 15<sup>th</sup> day of germination (Table 4; Fig. 4). Approximately 55% reduction in lipid content was observed by the end 15<sup>th</sup> day of germination. In 0-15 days, the concentrations of samples were, on 0 day, 0.051 mg/g, on 3<sup>rd</sup> day, 0.033 mg/g, on 6<sup>th</sup> day, 0.030 mg/g, on 9<sup>th</sup> day, 0.025 mg/g, on 12<sup>th</sup> day 0.024 mg/g and in 15<sup>th</sup> day 0.020 mg/g was observed. According to the table, from 0 day to 6<sup>th</sup> day result was found  $0.038 \pm 0.002$  mg/g and 9<sup>th</sup> day to 15<sup>th</sup> day the result was found  $0.023 \pm 0.002$  mg/g (Average  $\pm$  SD).

**Table 4. Lipid content (mg/g) in germinating seeds of *Albizia procera* [Tree 3]**

Day of germination	Lipid (mg/g)			
Days	Mean	Average	SD	Result
1 <sup>st</sup>	0.051	0.038	0.01136	$0.038 \pm 0.002$
3 <sup>rd</sup>	0.033			
6 <sup>th</sup>	0.030			
9 <sup>th</sup>	0.025	0.023	0.00265	$0.023 \pm 0.002$
12 <sup>th</sup>	0.024			
15 <sup>th</sup>	0.020			

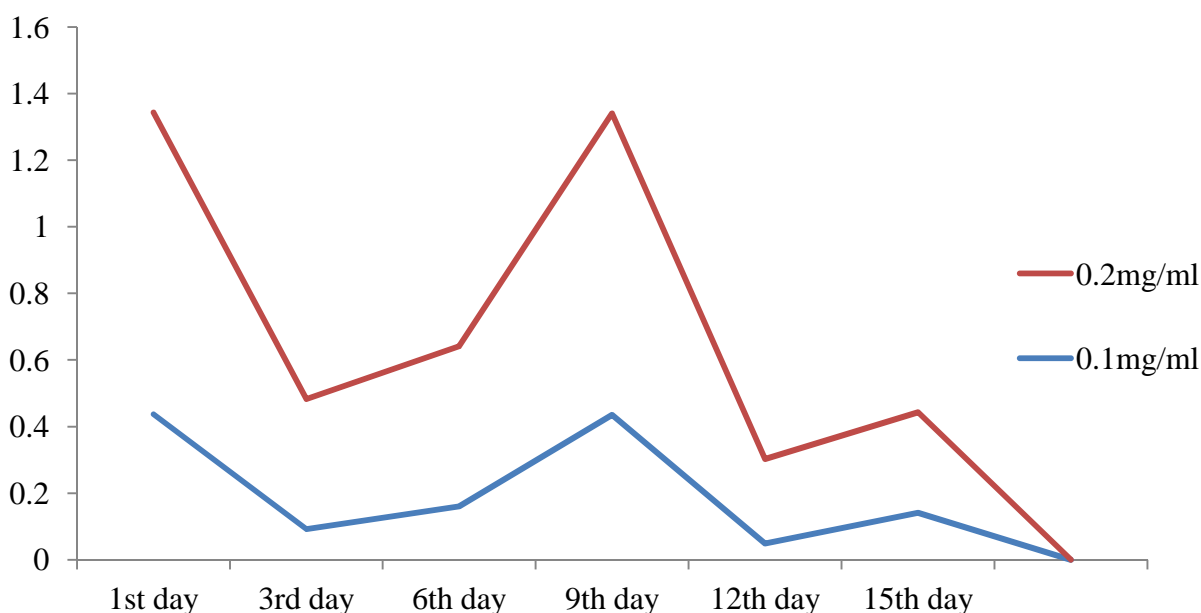


### Sugar estimation

In Tree 1, the soluble sugar concentration was found to be 0.436 mg/ml on 1<sup>st</sup> day of germination. On the 3<sup>rd</sup> day of germination, soluble sugar concentration decreased i.e., 0.092 mg/ml. On the 6<sup>th</sup> day to 9<sup>th</sup> day of germination, soluble sugar concentration again increased (0.160 mg/ml to 0.435 mg/ml); on the 12<sup>th</sup> day of germination, sugar concentration decreased (0.048 mg/ml) followed by the increase on 15<sup>th</sup> day of germination (0.141 mg/ml) (Table 5; Fig. 5).

**Table 5. Soluble sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 1]**

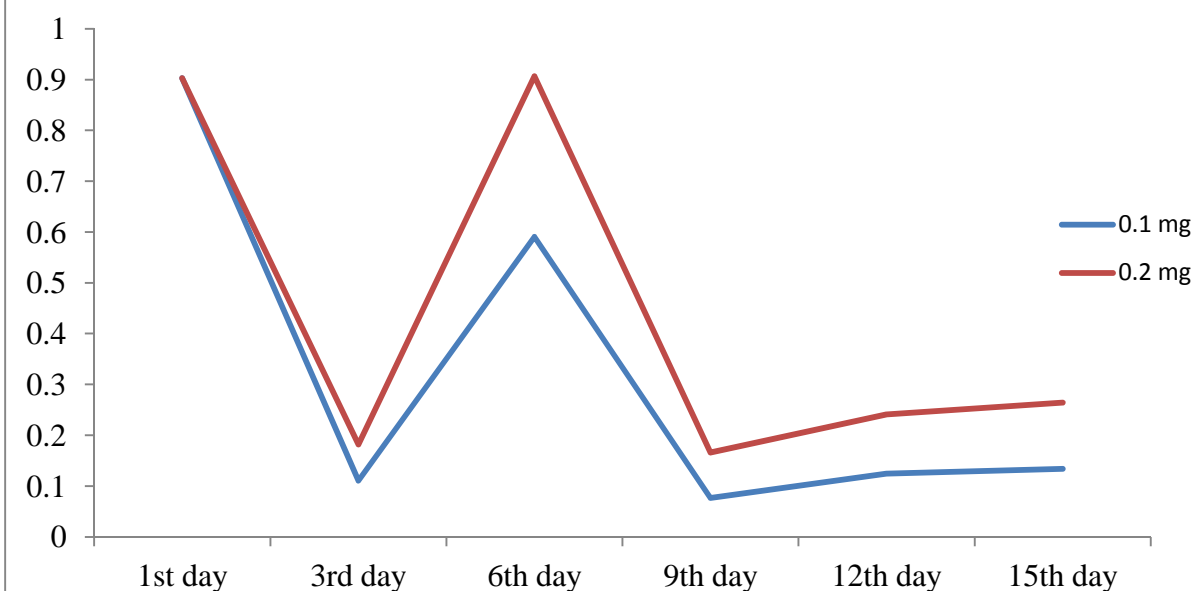
Days	0.1 mg/ml (Mean)	SD	% of Soluble sugar	0.2 mg/ml (Mean)	SD	% of Soluble sugar
1 <sup>st</sup>	0.436	0.17	11.74	0.906	0.29	11.73
3 <sup>rd</sup>	0.092		55.49	0.389		27.34
6 <sup>th</sup>	0.160		31.87	0.480		22.19
9 <sup>th</sup>	0.435		11.78	0.904		11.78
12 <sup>th</sup>	0.048		10.49	0.253		69.30
15 <sup>th</sup>	0.141		36.36	0.301		35.34
Average	0.219		26.28	0.539		29.61
Result			26.28 ± 0.17			29.61 ± 0.29

**Fig. 5. Soluble sugar concentration (mg/ml) in germinating seeds in *Albizia procera* [Tree 1]**

In the Tree 2, the soluble sugar concentration increased on 1<sup>st</sup> day to 3<sup>rd</sup> and 6<sup>th</sup> day of germination, i.e., 0.902 mg/ml to 0.110 mg/ml and 0.590 mg/ml respectively. On the 9<sup>th</sup> day of germination seed soluble sugar concentration decreased (0.076 mg/ml), on the 12<sup>th</sup> day of germination, the concentration increased (0.124 mg/ml) followed by the increase (0.134 mg/ml) on the 15<sup>th</sup> day of germination (Table 6; Fig. 6).

**Table 6. Soluble sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 2]**

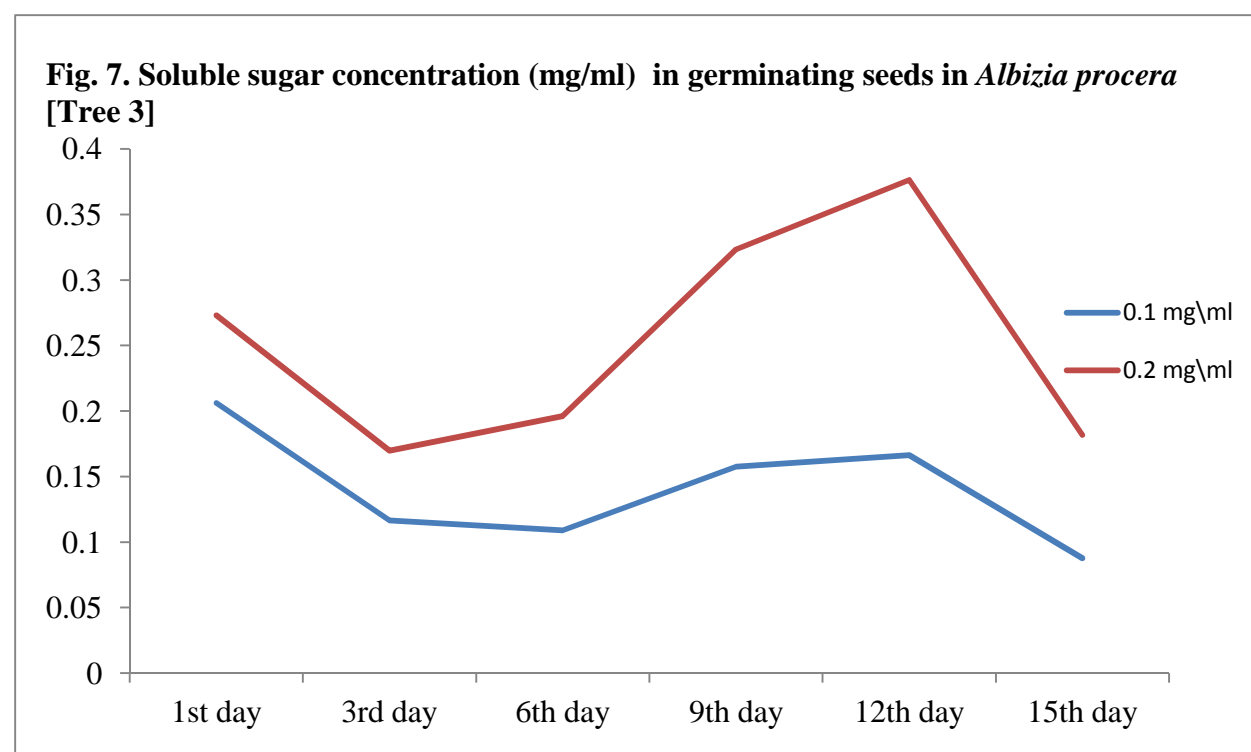
Days	0.1 mg/ml (Mean)	SD	% of Soluble sugar	0.2 mg/ml (Mean)	SD	% of Soluble sugar
1 <sup>st</sup>	0.902	0.34	11.81	0.903	0.35	11.80
3 <sup>rd</sup>	0.110		96.70	0.181		58.57
6 <sup>th</sup>	0.590		18.05	0.906		11.76
9 <sup>th</sup>	0.076		13.87	0.165		87.99
12 <sup>th</sup>	0.124		85.71	0.240		44.30
15 <sup>th</sup>	0.134		79.52	0.264		40.33
Average	0.323		50.94	0.443		42.45
Result			50.94 ± 0.34			42.45 ± 0.35

**Fig. 6. Soluble sugar concentration (mg/ml) in germinating seeds in *Albizia procera* [Tree 2]**

In Tree 3, the soluble sugar concentration was found to be on the 1<sup>st</sup> day of germination (0.206 mg/ml). On the 3<sup>rd</sup> to 6<sup>th</sup> day of germination, the soluble sugar concentration decreased i.e., 0.116 mg/ml to 0.108 mg/ml. On the 9<sup>th</sup> day of germination, the soluble sugar concentration again increased (0.157 mg/ml). On the 12<sup>th</sup> day of germination, the concentration increased (0.166 mg/ml) followed by the decrease on 15<sup>th</sup> day of germination (0.087 mg/ml) (Table 7; Fig. 7).

**Table 7. Soluble sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 3]**

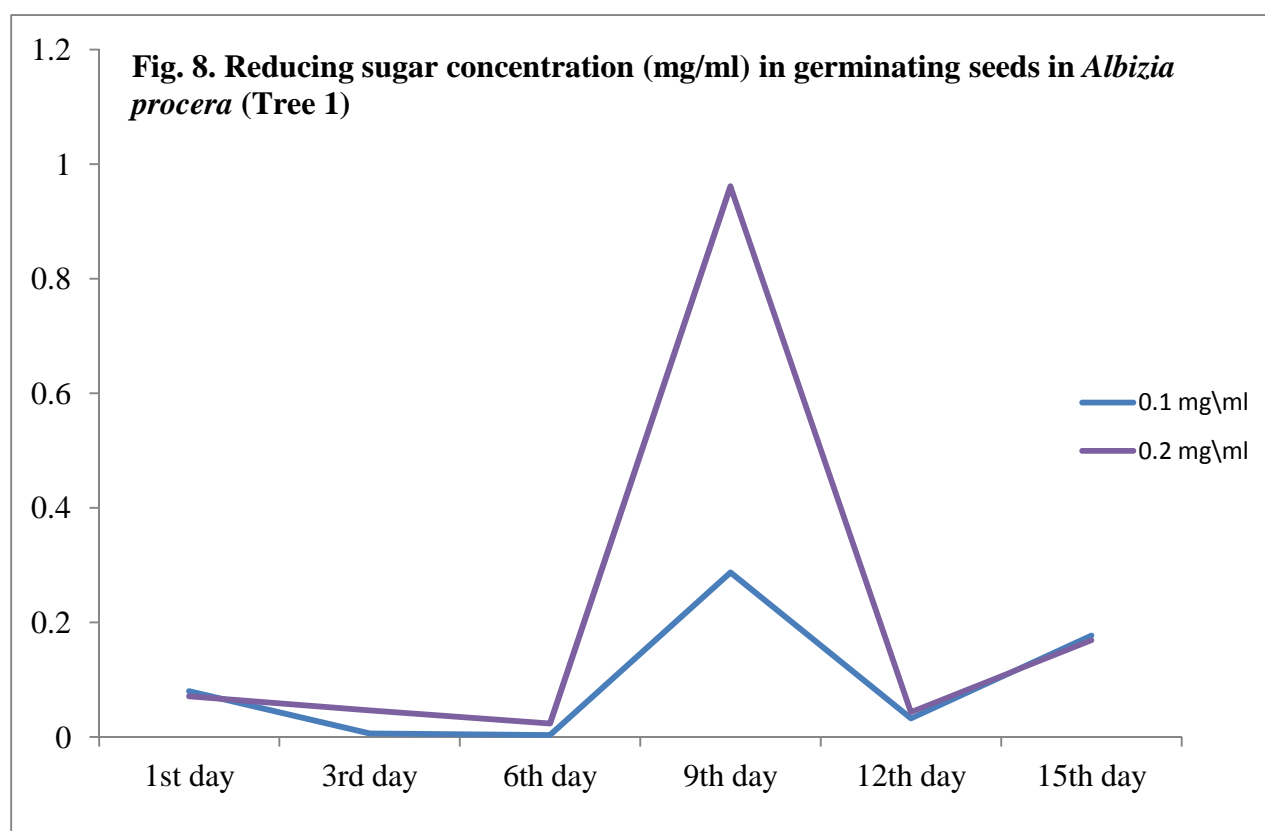
Days	0.1 mg\ml (Mean)	SD	% of Soluble sugar	0.2 mg\ml (Mean)	SD	% of Soluble sugar
1 <sup>st</sup>	0.206	0.04	51.71	0.273	0.08	39.03
3 <sup>rd</sup>	0.116		91.49	0.169		62.81
6 <sup>th</sup>	0.108		97.91	0.196		54.38
9 <sup>th</sup>	0.157		63.41	0.323		32.95
12 <sup>th</sup>	0.166		64.12	0.376		28.33
15 <sup>th</sup>	0.087		12.16	0.181		58.68
Average	0.140		65.81	0.253		47.43
Result			65.81 $\pm$ 0.04			47.43 $\pm$ 0.08



In Tree 1, the reducing sugar concentration was found to be 0.079 mg/ml on 1<sup>st</sup> day of germination. On the 3<sup>rd</sup> day to 6<sup>th</sup> day of germination, the reducing sugar concentration decreased (0.006 mg/ml to 0.003 mg/ml). On the 9<sup>th</sup> day of germination, the reducing sugar concentration again increased (0.287 mg/ml) and decreased on 12<sup>th</sup> day, followed by an increase on the 15<sup>th</sup> day (Table 8; Fig. 8).

**Table 8. Reducing sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 1]**

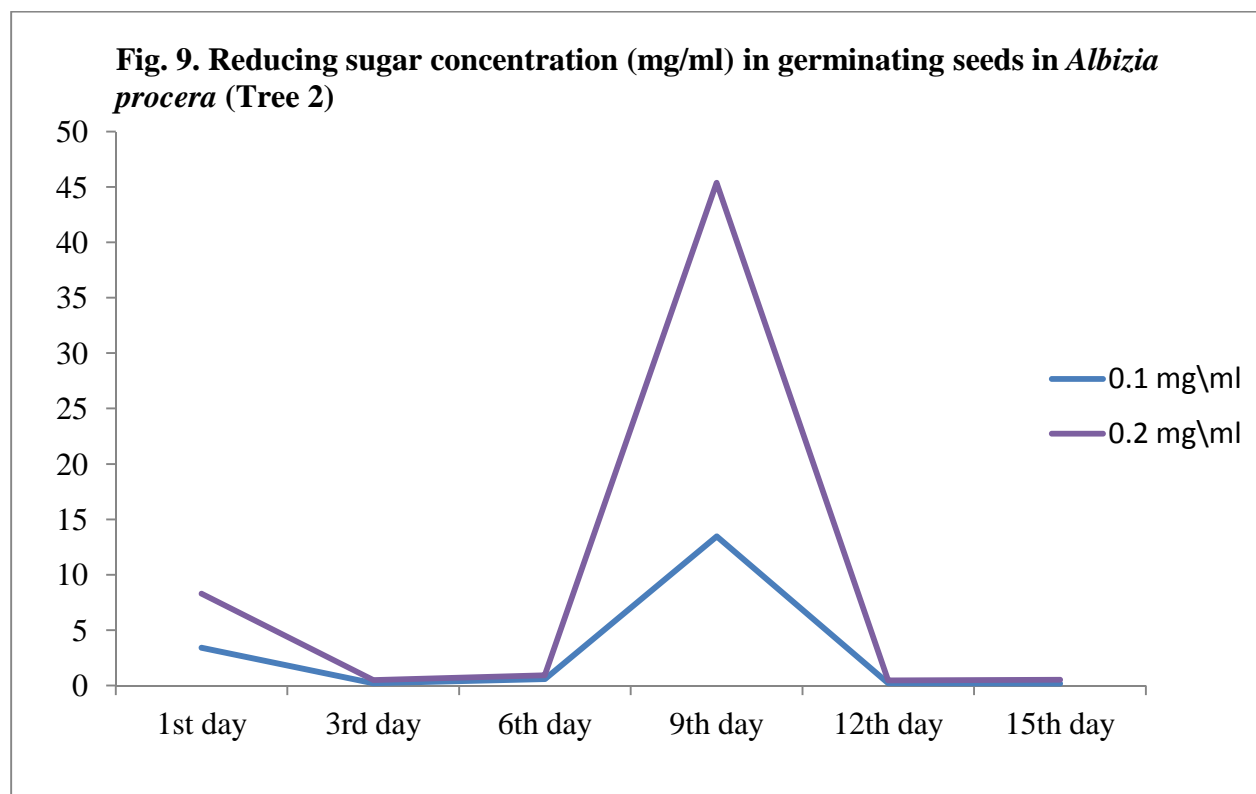
Days	0.1 mg/ml (Mean)	SD	% of Reducing Sugar	0.2 mg/ml (Mean)	SD	% of Reducing sugar
1 <sup>st</sup>	0.079	0.11	35.44	0.070	0.36	38.02
3 <sup>rd</sup>	0.006		43.57	0.046		58.13
6 <sup>th</sup>	0.003		76.92	0.023		11.36
9 <sup>th</sup>	0.287		99.70	0.961		28.06
12 <sup>th</sup>	0.032		87.71	0.043		61.72
15 <sup>th</sup>	0.177		16.12	0.168		15.94
Average	0.097		59.92	0.219		35.53
Result			59.92 ± 0.11			35.53 ± 0.36



In Tree 2, the reducing sugar concentration was found to be 3.397 mg/ml on 1<sup>st</sup> day of germination. On 3<sup>rd</sup> to 6<sup>th</sup> day of germination, the reducing sugar concentration decreased (0.203 mg/ml to 0.203 mg/ml). On the 9<sup>th</sup> day of germination, the reducing sugar concentration again increased (13.454 mg/ml). On the 12<sup>th</sup> day of germination, the concentration decreased (0.203 mg/ml) followed by a further decline (0.169 mg/ml) on 15<sup>th</sup> day of germination (Table 9; Fig. 9).

**Table 9. Reducing sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 2]**

Days	0.1 mg\ml (Mean)	SD	% of Reducing sugar	0.2 mg\ml (Mean)	SD	% of Reducing sugar
1 <sup>st</sup>	3.397	5.27	54.94	8.278	17.91	35.19
3 <sup>rd</sup>	0.203		91.74	0.480		60.60
6 <sup>th</sup>	0.573		32.57	0.938		31.05
9 <sup>th</sup>	13.454		13.87	45.311		64.2
12 <sup>th</sup>	0.203		91.74	0.448		64.93
15 <sup>th</sup>	0.169		10.98	0.521		55.55
Average	3.000		48.18	9.329		55.26
Result			$48.18 \pm 5.27$			$55.26 \pm 17.91$

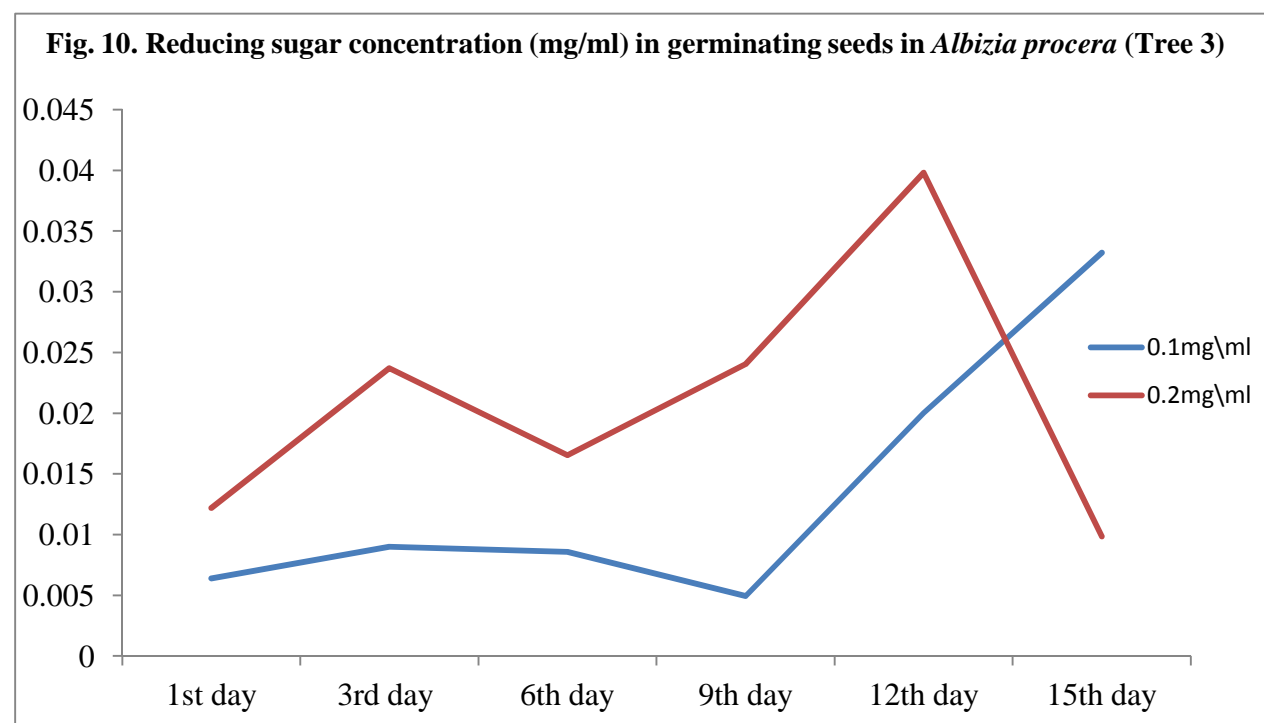


In Tree 3, reducing sugar concentration was found to be 0.006 mg/ml on 1<sup>st</sup> day of germination. On the 3<sup>rd</sup> day to 6<sup>th</sup> day of germination, the reducing sugar concentration decreased from 0.008 mg/ml to 0.008 mg/ml. On the 9<sup>th</sup> day of germination, reducing sugar concentration again decreased (0.004 mg/ml). On the 12<sup>th</sup> day of germination, the concentration increased (0.020 mg/ml) followed by a further increase on the 15<sup>th</sup> day (0.033 mg/ml) of germination (Table 10; Fig. 10).



**Table 10. Reducing sugar content (mg/ml) in germinating seeds of *Albizia procera* [Tree 3]**

Days	0.1 mg/ml (Mean)	SD	% of Reducing sugar	0.2 mg/ml (Mean)	SD	% of Reducing sugar
1 <sup>st</sup>	0.006	0.010	12.65	0.012	0.010	91.74
3 <sup>rd</sup>	0.008		90.09	0.023		47.16
6 <sup>th</sup>	0.008		94.33	0.016		67.56
9 <sup>th</sup>	0.004		16.39	0.024		16.51
12 <sup>th</sup>	0.020		40.48	0.039		28.08
15 <sup>th</sup>	0.033		24.39	0.009		11.36
Average	0.013		46.38	0.021		43.73
Result			46.38 ± 0.010			43.73 ± 0.010



## Discussion

The major biochemical components of endosperm are carbohydrate, lipid and triacylglycerols. With seedling growth, different enzymes are generated which mobilize these macromolecules [14, 15, 16]. In oleiferous seeds, the main source of energy during seed germination and seedling growth are the triacylglycerols [17]. During the metabolic procedures like germination and early seedling growth, which require high energy consumption, the oil-rich seeds lose almost all of their oil storage [18, 19, 20]. Soluble sugars protect from the damage of

desiccation in an anhydrous system which are extremely important bio-molecules involved in tolerance of water storage during seed storage and maturation. Next to sucrose, galactosyl-sucrose oligosaccharides, viz. raffinose, stachyose and verbascose are vital in the plant kingdom for viability and are accumulated during seed development and involved in various important physiological roles [15, 21].

Four new oleanane-type triterpene glycosides, proceraosides A–D (1–4) were purified from the seeds of *Albizia procera* by Yoshikawa *et al.* (1998) [22]. Compounds 1–3 comprised acacic acid as aglycon and a monoterpenic carboxylic acid linked to a monoterpene quinovoside as acyl moiety at C-21. The common oligosaccharide moiety linked to C-28 in 1–3 was determined as  $\alpha$ -l-arabinofuranosyl-(1 $\rightarrow$ 4)-[ $\beta$ -d-glucopyranosyl-(1 $\rightarrow$ 3)]- $\alpha$ -l-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -d-glucopyranosyl ester. Compound 4 was established as the 16-deoxy analogue of 1 [22]. The monosaccharide composition and physicochemical properties of *Albizia procera* gum was revealed by Pachua *et al.* (2012) [23] who found that Arabinose (44.94%), Galactose (30.17%), Rhamnose (0.22%) and Fructose (0.02%) were the main monosaccharides present in the exudates-gum.

In our findings, there showed a steady reduction in the level of total lipid concentration in case of all the 3 trees studied. This finding indicates the high metabolic activities inside the seed endosperm for the development of the embryonic axis, and this trend of reactions is well supported with the findings of other workers [18, 17, 19, 14; 15, 20].

Regarding the levels of total soluble sugar and total reducing sugar, no such continuous trend could be drawn clearly, when the results showed ups and downs in their concentrations at different days of germination (Tables 5-10; Figures 5-10). However, in both the cases, retaining a considerable level up to 12<sup>th</sup> day of germination was noticed in general. In *Sorghum bicolor*, Gill *et al.* (2003) [24] reported the effect of various stresses on germination rate, growth and soluble sugar content in seed embryos and endosperm during early germination; maximum total soluble sugar content was observed in embryo and endosperm under NaCl and PEG treatment. Gill and Singh (1985) [25] reported that germination, growth, respiration and other related processes could be affected in seeds that are subjected to environmental stresses; change in any one of these processes can affect other metabolic activities.

Colmenares and Bressani (1990) [26] reported the changes in chemical composition and in nutritive value during germination in Amaranth grain. For this study, three amaranth species

were used. Total soluble sugar increased with respect to germination time. During germination decreased in storage carbohydrates and an increase in total soluble sugar due to the energy needs of the growing plant. Total raffinose and stachyose content decreased quickly during the first 24 hours of germination and disappeared after 48 hours of the process.

The Amazonian tree *Himatanthus sucuuba* germinates in a brief period. Ferreira *et al.*, (2009) [27] reported that the concentrations of raffinose, stachyose, verbascose and sucrose get increased at the last part of seed maturation where they protect against desiccation process. Later on during germination, they mainly serve as carbon source in the metabolic processes, thereby functioning as dual function molecules.

Tonguc *et al.* (2012) [28] reported change in seed reserve composition during germination and initial seedling development of safflower (*Carthamus tinctorius* L.). Two safflower cultivars were used as plant material study; Dincer 5-118 (high in linoleic acid) and Montola 2000 (high in oleic acid) is a cultivar, which used for the soluble sugar and reducing sugar estimation. The total soluble sugar content of Montola 2000 was higher than that Dincer 5-118. At 0 hour, the oil content was 54.7% in Dincer 5-118 and 57.4% in Montola 2000; at 72 h, the oil contents decreased to 49.5% and 45.1%, respectively. They concluded that the oil contents of both cultivars dropped steadily throughout the study period.

Huang and Moreau (1978) [29] reported on the lipases in the storage tissue of peanut and other oil seeds during germination; they experimented oil seeds from castor bean (*Ricinus communis* L.), peanut (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.), cucumber (*Cucumis sativus* L.), cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.) and tomato (*Lycopersicon esculentum* Mill.). The storage tissues of all these oil seeds except castor bean contained only alkaline lipase activity which increased highly during germination. In castor bean, the levels of acid and alkaline lipases did match with those in other oil seeds.

Koster and Leopold (1988) [30] reported the relationship between sugar content and the loss of desiccation tolerance in the axes of germinating soya bean (*Glycine max* L.), pea (*Pisum sativum* L.) and corn (*Zea mays* L.) axes. They analyzed the soluble sugar content of the axes throughout the transition from desiccation tolerance to intolerance. These analyses show the sucrose and larger oligosaccharides were consistently present during the tolerant stage and the desiccation tolerance disappeared as the oligosaccharides were lost. Smythe (1967) [31] reported

that many sugar and other organic compound tested, raffinose and stacchiose were the most effective inhibitors of sucrose crystal growth.

Bernal-Lugo and Leopold (1992) [1] correlated decreased levels of soluble sugar and starch with the accumulation of reducing sugar with seed deterioration in *Zea mays*, and predicted the event may occur due to less substrate being available for respiration, thereby reducing in seed germination and vigour.

Prado *et al* (2000) [32] recorded the effect of NaCl on germination, growth and soluble sugar content in seeds and seedling components during early germination in *Chenopodium quinoa* Willd.; maximum germination recorded in 12-14 hours in distilled water. In saline condition, the sugar content in both embryonic axes and cotyledons decreased notably the first 6 hours, then increased between 6 and 14 hours; total soluble sugar content increased in distilled water, peaking after 6 hours for both embryonic axes and cotyledons.

### **Conclusion**

In the present study, with progression of germination, the total lipid content was found to decrease, but no such continuous trend could be drawn regarding the contents of total soluble and total reducing sugars. The results recorded might be beneficial for further studies on the metabolic activities of germinating seeds in *Albizia procera* or related tree species.

### **Acknowledgement**

The author is obliged to the authority of Tropical Forest Research Institute (ICFRE), Jabalpur, for supporting the laboratory facilities with chemical and instruments, and to Mr. Arun Kumar Patel for assistance in the work.

## References

1. Bernal-Lugo I, Leopold AC. Changes in soluble carbohydrates during seed storage. *Plant Physiol.* 1992;98(3):1207-10.
2. Buckeridge MS, Santos HP, Tiné MAS. Mobilization of storage cell wall polysaccharides in seeds. *Plant Physiol. Biochem.* 2000;38(1/2):141-56.
3. Becker WM, Leaver CJ, Weir EM, Riezman H. Regulation of glyoxysomal enzymes during germination of cucumber. 1. Developmental changes in cotyledonary protein, RNA, and enzyme activities during germination. *Plant Physiol.* 1978;62(4):542-9.
4. Graham IA. Seed storage oil mobilization. *Ann. Rev. Plant Biol.* 2008;59:115-42.
5. Troup RS. *The Silviculture of Indian Trees*. London, UK. Oxford University Press. 1921. Page 1195.
6. Little EL, Wadsworth FH. *Common trees of Puerto Rico and the Virgin Islands*. Agriculture Handbook No. 249, U.S. Department of Agriculture, Forest Service. 1964.
7. Venkataramany. *Silviculture of genus Albizia and species*. *Silviculture of Indian trees*, No. 22. Government of India, Delhi, India. Page 54. 1968.
8. Parrotta JA. *Albizia procera* (Roxb.) Benth. *Silvics of Forest Trees of the American Tropics*. Rio Piedras, Puerto Rico USA: USDA Forest Service, International Institute of Tropical Forestry. Page 4. 1987.
9. Roshetko JM. Profiles of selected *Albizia* and *Paraserianthes* species. In: Zabala, N.Q. (Ed.), *International Workshop on Albizia and Paraserianthes Species*. Proceedings of workshop held in Bislig, Suriago del Sur, Philippines, 1994. *Farm, Forestry and Community Tree Research Reports Special Issue*. Winrock International. Pages 157-62. 1997.
10. Azad Md.S, Biswas RK, Matin Md.A. Seed germination of *Albizia procera* (Roxb.) Benth. in Bangladesh: A basis for seed source variation and pre-sowing treatment effect. *For. St. China.* 2012;14(2):124-30.
11. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956;28(3):350-6.
12. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 1944;153(2):375.
13. Somogyi M. Notes on sugar determination. *J. Biol. Chem.* 1952;195(1):19-23.

14. Mayer AM, Poljakoff-Mayber A. The Germination of Seeds. 4<sup>th</sup> edition. Pergamon Press, New York. 1989.
15. Bewley JD, Black M. Seeds: Physiology of Development and Germination. 2<sup>nd</sup> edition. Plenum Press. New York. 1994.
16. Linkies A, Graeber K, Knight C, Leubner-Metzger G. The evolution of seeds. New Phytol. 2010;186(4):817-31.
17. Anthony HCH, Robert AM. Lipases in the storage tissue of peanut and other oil seeds during germination. Planta. 1978;141(1):111-6.
18. Ashton FM. Mobilization of storage proteins of seeds. Ann. Rev. Pl. Physiol. 1976;27:95-117.
19. Sharma A, Sengupta UK. Changes in protease and amylase activity in germinating seeds of groundnuts. Ind. J. Pl. Physiol. 1987;30(3):176-82.
20. Ascencio J. Root secreted acid phosphatase kinetics as a physiological marker for phosphorus deficiency. J. Pl. Nutri. 1997;20(1):9-26.
21. McCleary BV, Charnock SJ, Rossiter PC, O'Shea MF, Power AM, Lloyd RM. Measurement of carbohydrates in grain, feed and food. J. Sc. Food Agricul. 2006;86(11):1648-61.
22. Yoshikawa K, Satou Y, Tokunaga Y, Tanaka M, Arihara S, Nigam SK. Four acylated triterpenoid saponins from *Albizia procera*. J. Nat. Prod. 1998;61(4):440-5.
23. Pachuau L, Lahlhenmawia H, Mazumdar B. Characteristics and composition of *Albizia procera* (Roxb.) Benth. gum. Ind. Crops Prod. 2012;40(1):90-5.
24. Gill PK, Sharma AD, Singh P, Bhullar SS. Changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses. Pl. Gr. Regul. 2003;40(2):157-62.
25. Gill KS, Singh OS. Effect of salinity on carbohydrate metabolism during paddy (*Oryza sativa* L.) seed germination under salt stress condition. J. Exp. Biol. 1985;23(8):384-6.
26. Colmenares DR, Bressani R. Effect of germination on the chemical composition and nutritive value of amaranth grain. Cereal Chem. 1990;67(6):519-22.
27. Ferreira CDS, Piedade MTF, Tiné MAS, Rossatto DR, Parolin P, Buckeridge MS. The role of carbohydrates in seed germination and seedling establishment of *Himatanthus*

- sucuuba*, an Amazonian tree with populations adapted to flooded and non-flooded conditions. Ann. Bot. 2009;104(6):1111-9.
28. Tonguc M, Elkoyunu R, Erbas S, Karakurt Y. Change in seed reserve composition during germination and initial seedling development of safflower (*Carthamus tinctorius* L.). Turk. J. Biol. 2012;36:107-12.
29. Huang AH, Moreau RA. Lipases in the storage tissue of peanut and other oil seeds during germination. Planta. 1978;141(1):111-6.
30. Koster KL, Leopold AC. Sugar and desiccation tolerance in seeds. Pl. Physiol. 1988;88(3):829-32.
31. Smythe BM. Sucrose crystal growth rate: II. Rate of crystal growth in presence of impurities. Aus. J. Chem. 1967;20(6):1097-114.
32. Prado FE, Boero C, Gallardo M, González JA. Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds. Bot. Bull. Acad. Sinica. 2000;41(1):27-34.