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

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 1st day of germination till the 15th 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 12th 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 β-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 from a thin, spreading crown. The bark is smooth, whitish to light greenish gray or light brown. It exfoliates in thin flanks 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	23º 05’ 58.27”	79 º 59’ 21. 80”	1353
2	23º 05’ 58.57”	79 º 59’ 22. 26”	1353
3	23º 05’ 49.65”	79 º 59’ 17. 65”	1355

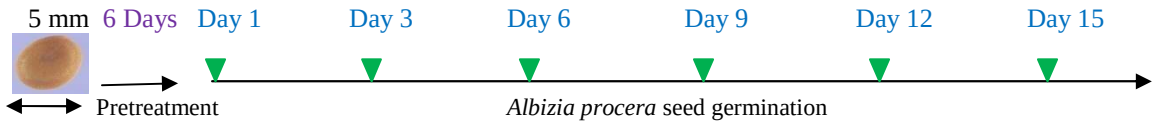


Fig. 1. Schematic diagram of the schedule of sample estimation for total lipid, total soluble sugar and total reducing sugar from germinating seeds of *Albizia procera*; 3 samples of 1 gm each were estimated from each of the 3 trees for each of the 3 biochemical estimations.

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 ± 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, $HgCl_2$, 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 (µl) 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% $HgCl_2$ 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^oC for 1 minute as described to be the best method by [10]. Germination started after 6 days, and was carried out at 30°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°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^{st} day of

germination to 15th 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 0.2milligram of seed tissue per 1ml 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 - 100mg in 100ml of water. Working standard - 10ml of stock diluted to 100ml 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 shacked well for 10 minutes. Then placed in boiling water bath at 25-30^{o}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

100 ml sample solution contain = $\frac{X}{0.1}$ x 100mg of glucose

= % of total carbohydrates

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 37^0C.

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 10ml 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/gm to 0.004 mg/gm from 0 day to 15^{th} day of germination (Table 2; Fig. 2). Approximately 95% reduction in lipid content was observed by the end 15^{th} day of germination. In 0-15 days, the concentration of sample was, on 0 day 0.11 mg/gm, on 3 day 0.040 mg/gm, on 6^{th} day 0.021 mg/gm, on 9^{th} day 0.011 mg/gm, on 12^{th} day 0.004 mg/gm and on 15^{th} day 0.004 mg/gm was observed. According to the table, from 0 day to 6 day result was found 0.057 ± 0.046 mg/gm and 9 day to 15 day result was found 0.064± 0.004 mg/gm (Average ± SD).

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

Day of germination	Lipid (mg/gm)			
	Mean	Average	SD	Result
1st	0.110	0.057	0.04687	0.057 ± 0.046
3rd	0.040			
6th	0.021			
9th	0.011	0.006333	0.00404	0.006 ± 0.004
12th	0.004			
15th	0.004			

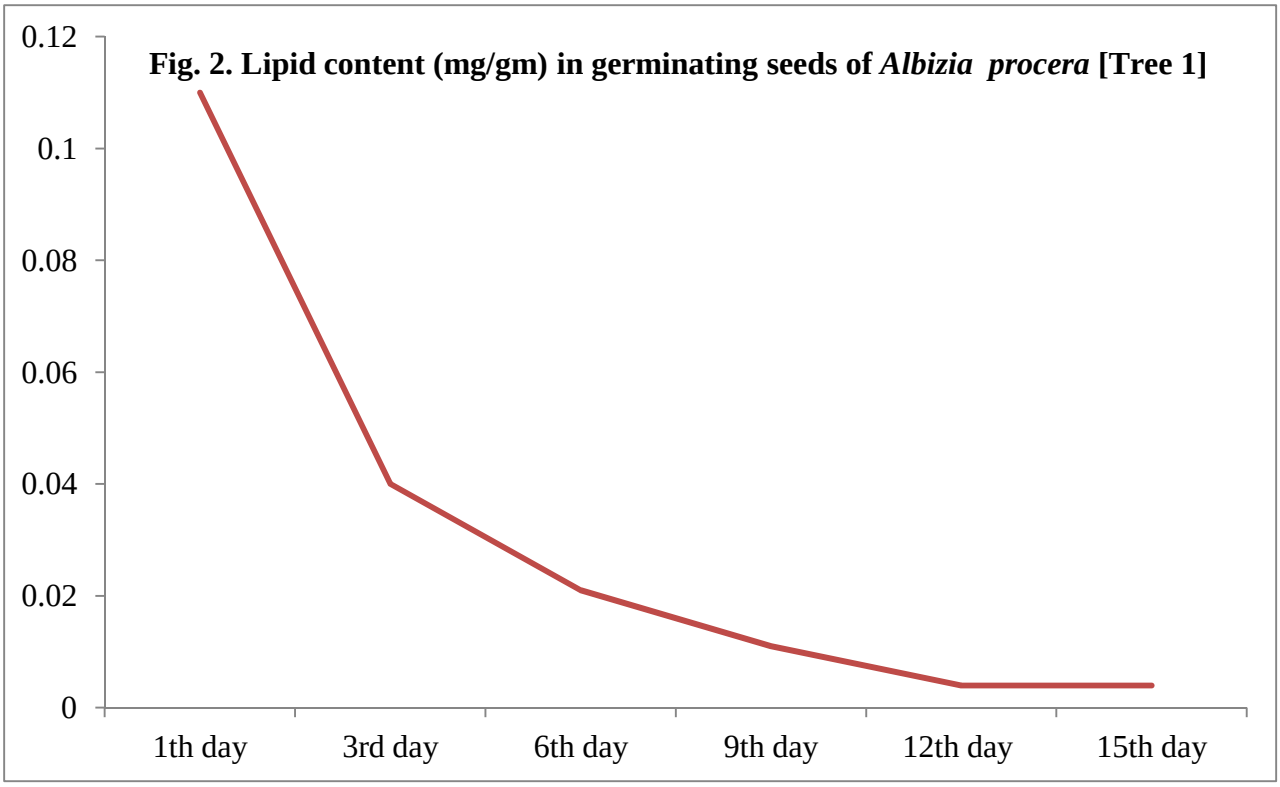
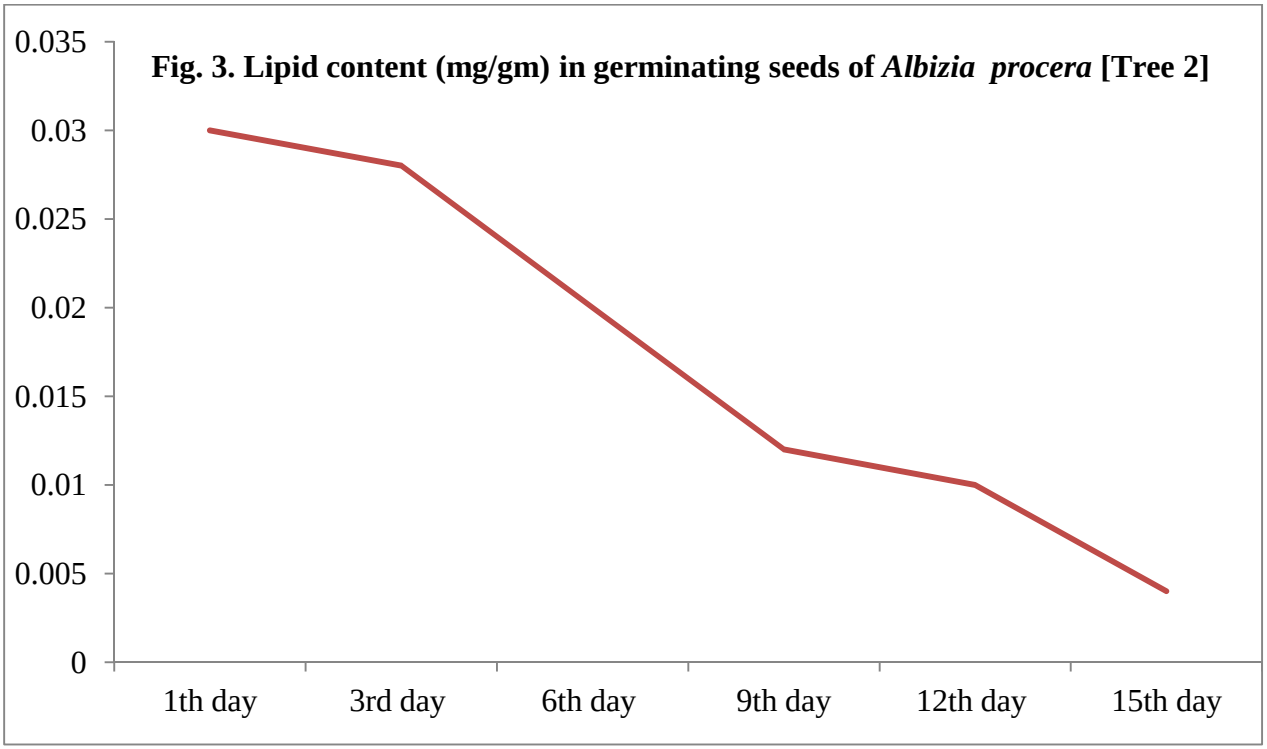


Fig. 2. Lipid content (mg/gm) in germinating seeds of *Albizia procera* [Tree 1]

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

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

Day of germination	Lipid (mg/gm)			
	Mean	Average	SD	Result
1st	0.030	0.026	0.00529	0.026 ± 0.005
3rd	0.028			
6th	0.020			
9th	0.012	0.00867	0.00416	0.008 ± 0.004
12th	0.010			
15th	0.004			



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

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

Day of germination	Lipid (mg/gm)			
Days	**Mean**	**Average**	**SD**	**Result**
1st	0.051	0.038	0.01136	0.038 ± 0.002
3rd	0.033			
6th	0.030			
9th	0.025	0.023	0.00265	0.023 ± 0.002
12th	0.024			
15th	0.020			

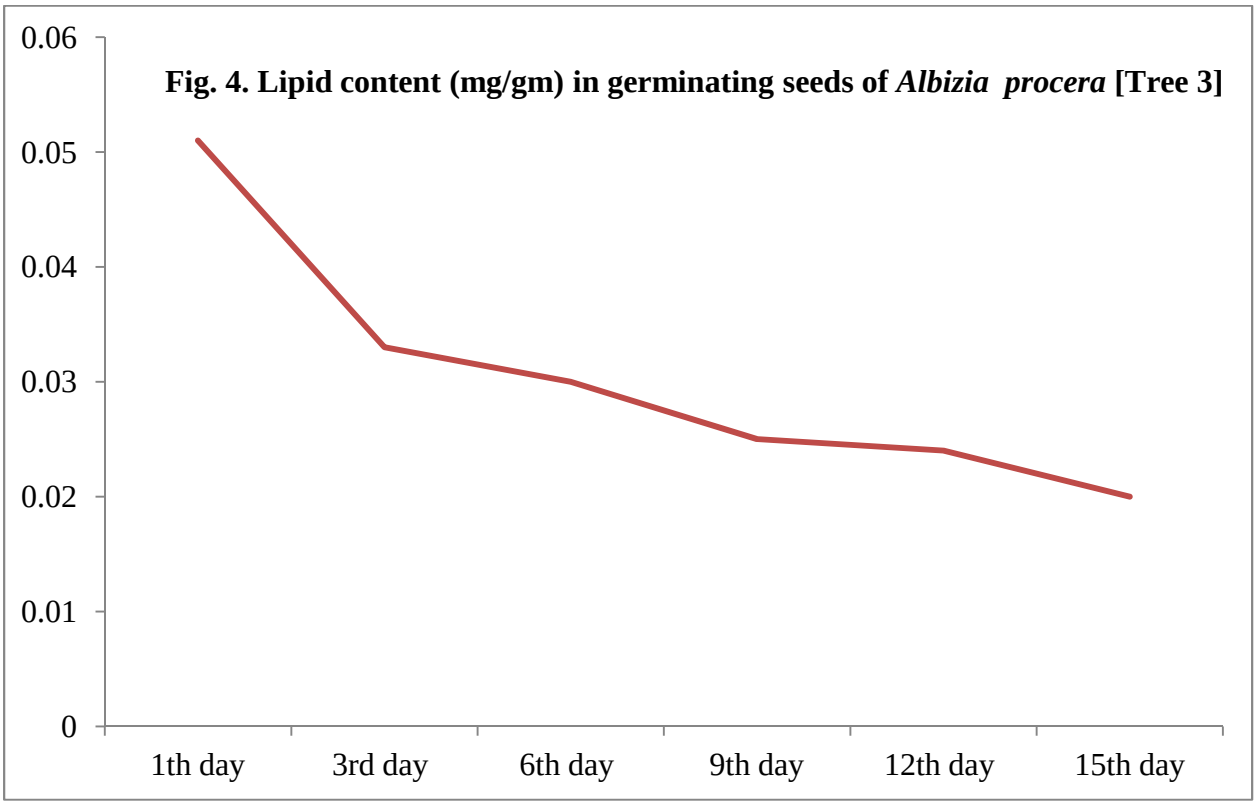


Fig. 4. Lipid content (mg/gm) in germinating seeds of *Albizia procera* [Tree 3]

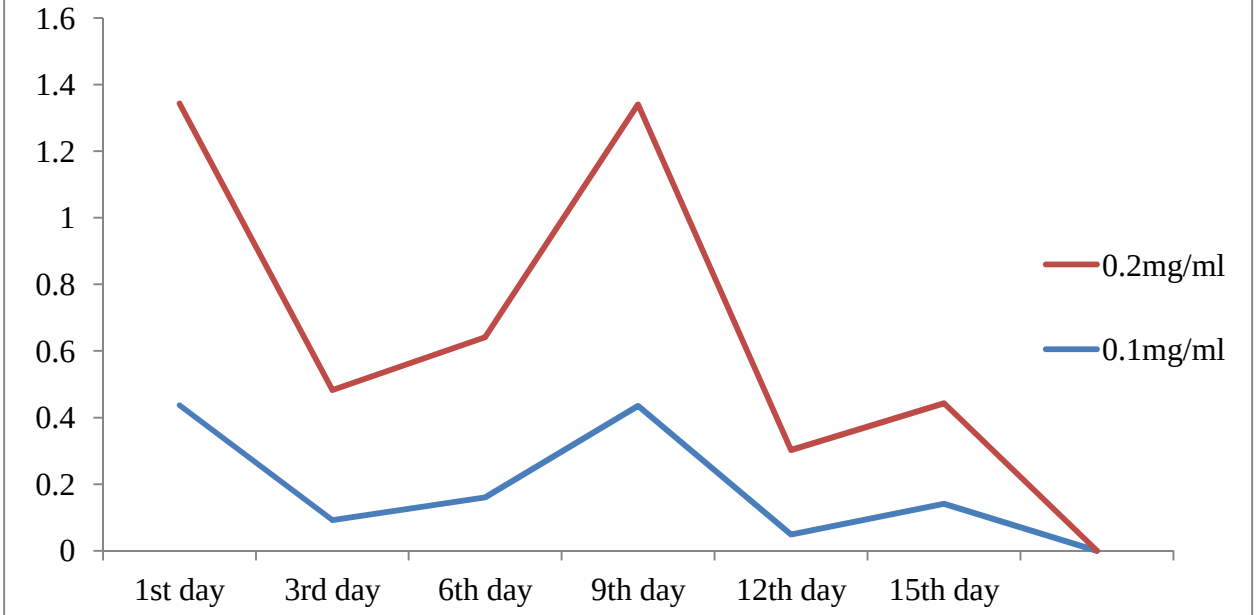
Sugar estimation

In Tree 1, the soluble sugar concentration was found to be 0.436763 mg/ml on 1st day of germination. On the 3rd day of germination, soluble sugar concentration decreased i.e., 0.092.415 mg/ml. On the 6th day to 9th day of germination, soluble sugar concentration again increased (0.160861 mg/ml to 0.435050 mg/ml); on the 12th day of germination, sugar concentration decreased (0.048843 mg/ml) followed by the increase on 15th day of germination (0.141005 mg/ml) (Table 5; Fig. 5).

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

Days	0.1mg/ml (Mean)	SD	% of Soluble sugar	0.2mg/ml (Mean)	SD	% of Soluble sugar
1st	0.436763	0.17	11.74	0.906388	0.29	11.73
3rd	0.092415		55.49	0.389890		27.34
6th	0.160861		31.87	0.480342		22.19
9th	0.435050		11.78	0.904661		11.78
12th	0.048843		10.49	0.253802		69.30
15th	0.141005		36.36	0.301614		35.34
Average	0.219150		26.28	0.539449		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]

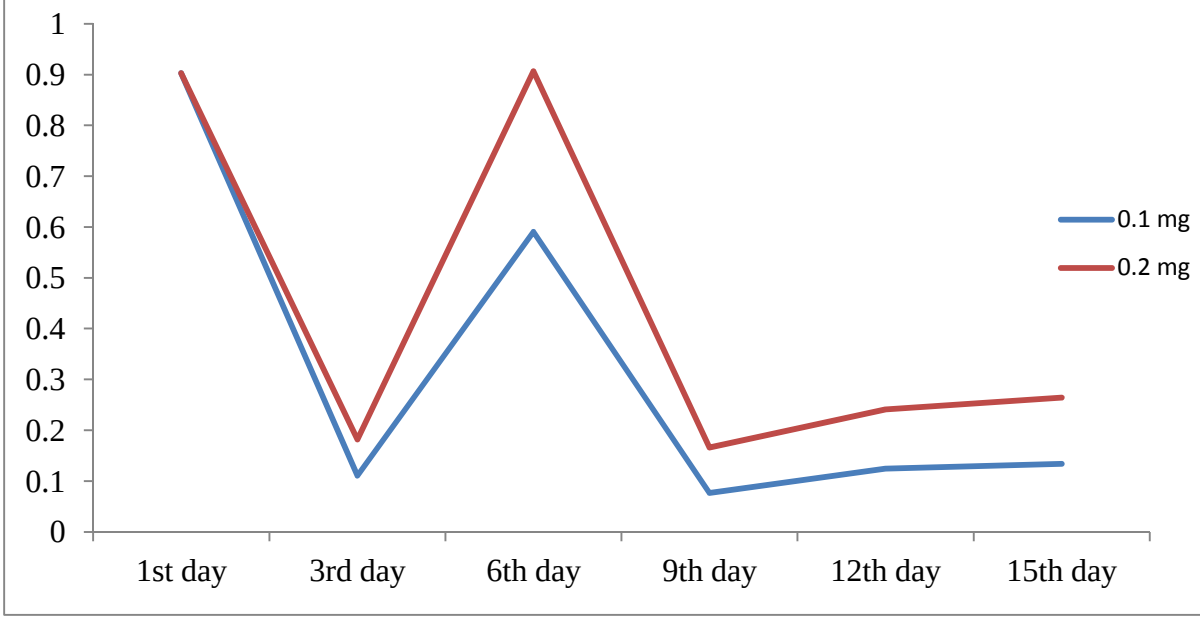


In the Tree 2, the soluble sugar concentration increased on 1st day to 3rd and 6th day of germination, i.e., 0.902721 mg/ml to 0.110235 mg/ml and 0.590649 mg/ml respectively. On the 9th day of germination seed soluble sugar concentration decreased (0.076837 mg/ml), on the 12th day of germination, the concentration increased (0.12436 mg/ml) followed by the increase (0.13406 mg/ml) on the 15th day of germination (Table 6; Fig. 6).

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

Days	0.1mg\ml (Mean)	SD	% of Soluble sugar	0.2mg\ml (Mean)	SD	% of Soluble sugar
1st	0.902721	0.34	11.81	0.903009	0.35	11.80
3rd	0.110235		96.70	0.181988		58.57
6th	0.590649		18.05	0.906505		11.76
9th	0.076837		13.87	0.165966		87.99
12th	0.124360		85.71	0.240628		44.30
15th	0.134060		79.52	0.264347		40.33
Average	0.323144		50.94	0.443741		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]

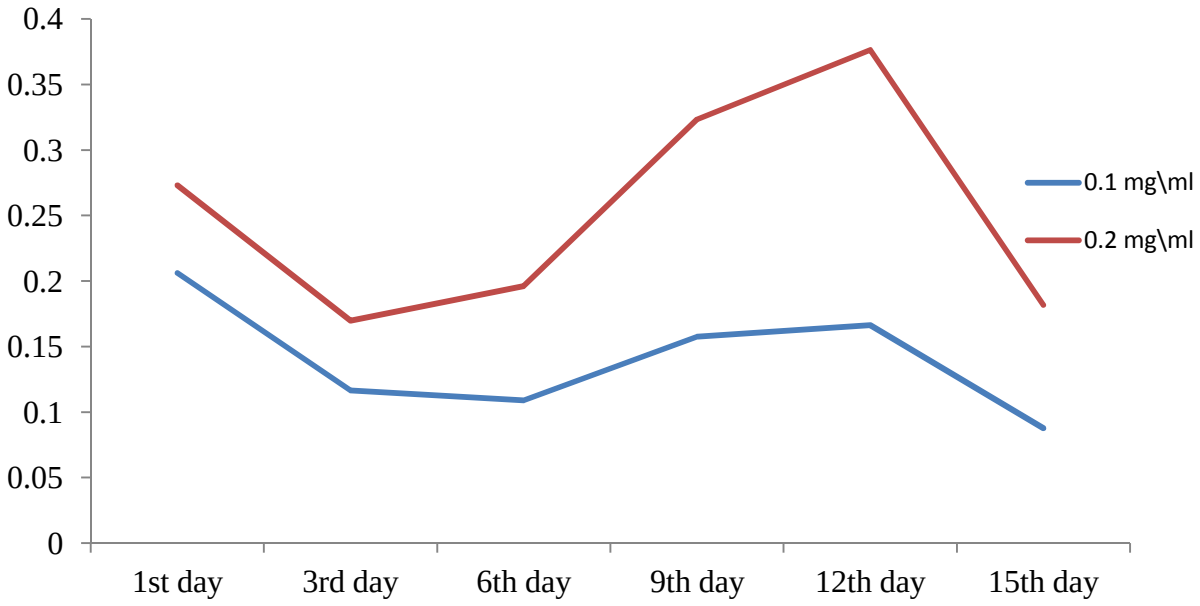


In Tree 3, the soluble sugar concentration was found to be on the 1st day of germination (0.206143 mg/ml). On the 3rd to 6th day of germination, the soluble sugar concentration decreased i.e., 0.116503 mg/ml to 0.108871 mg/ml. On the 9th day of germination, the soluble sugar concentration again increased (0.157438 mg/ml). On the 12th day of germination, the concentration increased (0.166243 mg/ml) followed by the decrease on 15th day of germination (0.087604 mg/ml) (Table 7; Fig. 7).

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

Days	0.1mg\ml (Mean)	SD	% of Soluble sugar	0.2mg\ml (Mean)	SD	% of Soluble sugar
1st	0.206143	0.04	51.71	0.273131	0.08	39.03
3rd	0.116503		91.49	0.169729		62.81
6th	0.108871		97.91	0.196016		54.38
9th	0.157438		63.41	0.323435		32.95
12th	0.166243		64.12	0.376245		28.33
15th	0.087604		12.16	0.181732		58.68
Average	0.140467		65.81	0.253381		47.43
Result			65.81 ± 0.04			47.43 ± 0.08

Fig. 7. Soluble sugar concentration (mg/ml) in germinating seeds in *Albizia procera* [Tree 3]



In Tree 1, the reducing sugar concentration was found to be 0.079836 mg/ml on 1st day of germination. On the 3rd day to 6th day of germination, the reducing sugar concentration decreased (0.006581 mg/ml to 0.00372 mg/ml). On the 9th day of germination, the reducing sugar concentration again increased (0.287008 mg/ml) and decreased on 12th day, followed by an increase on the 15th 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
1st	0.079836	0.11	35.44	0.070942	0.36	38.02
3rd	0.006581		43.57	0.046394		58.13
6th	0.003720		76.92	0.023737		11.36
9th	0.287008		99.70	0.961084		28.06
12th	0.032621		87.71	0.043698		61.72
15th	0.177413		16.12	0.168857		15.94
Average	0.097863		59.92	0.219118		35.53
Result			59.92 ± 0.11			35.53 ± 0.36

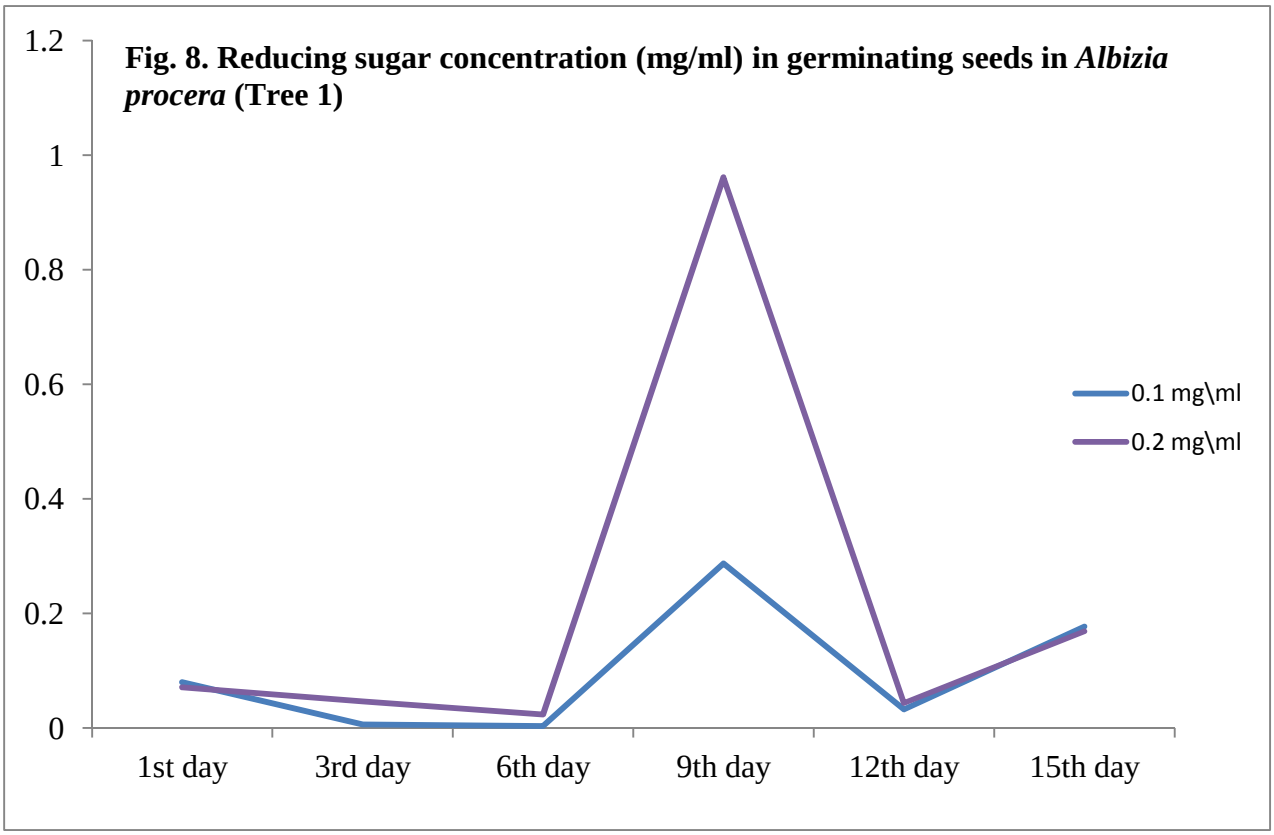


Fig. 8. Reducing sugar concentration (mg/ml) in germinating seeds in *Albizia procera* (Tree 1)

In Tree 2, the reducing sugar concentration was found to be 3.39721 mg/ml on 1st day of germination. On 3rd to 6th day of germination, the reducing sugar concentration decreased (0.20346 mg/ml to 0.20346 mg/ml). On the 9th day of germination, the reducing sugar concentration again increased (13.4545 mg/ml). On the 12th day of germination, the concentration decreased (0.20346 mg/ml) followed by a further decline (0.16986 mg/ml) on 15th 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	0.2mg\ml (Mean)	SD	% of Reducing sugar
1st	3.397211	5.27	54.94	8.278101	17.91	35.19
3rd	0.203465		91.74	0.480078		60.60
6th	0.573050		32.57	0.938241		31.05
9th	13.45453		13.87	45.311065		64.2
12th	0.203465		91.74	0.448473		64.93
15th	0.169860		10.98	0.521448		55.55
Average	3.000263		48.18	9.329567		55.26
Result			48.18 ± 5.27			55.26 ± 17.91

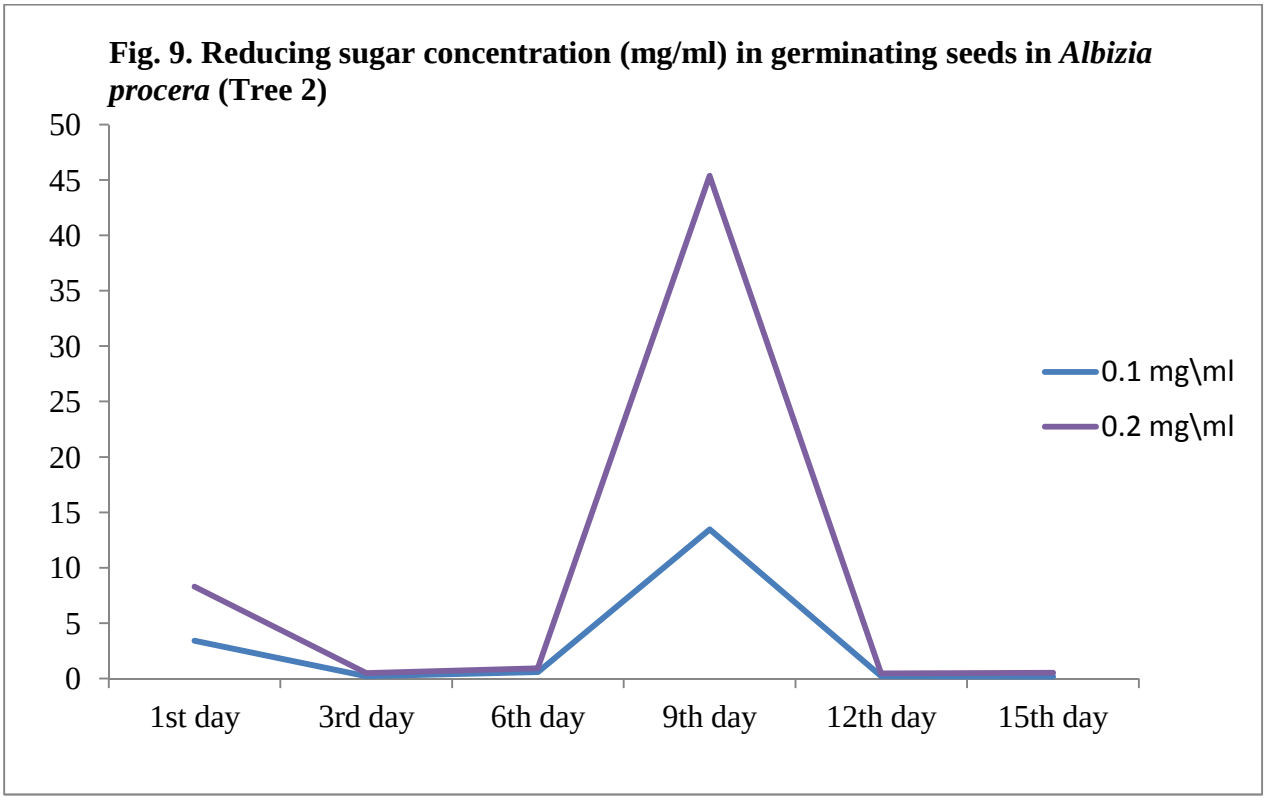


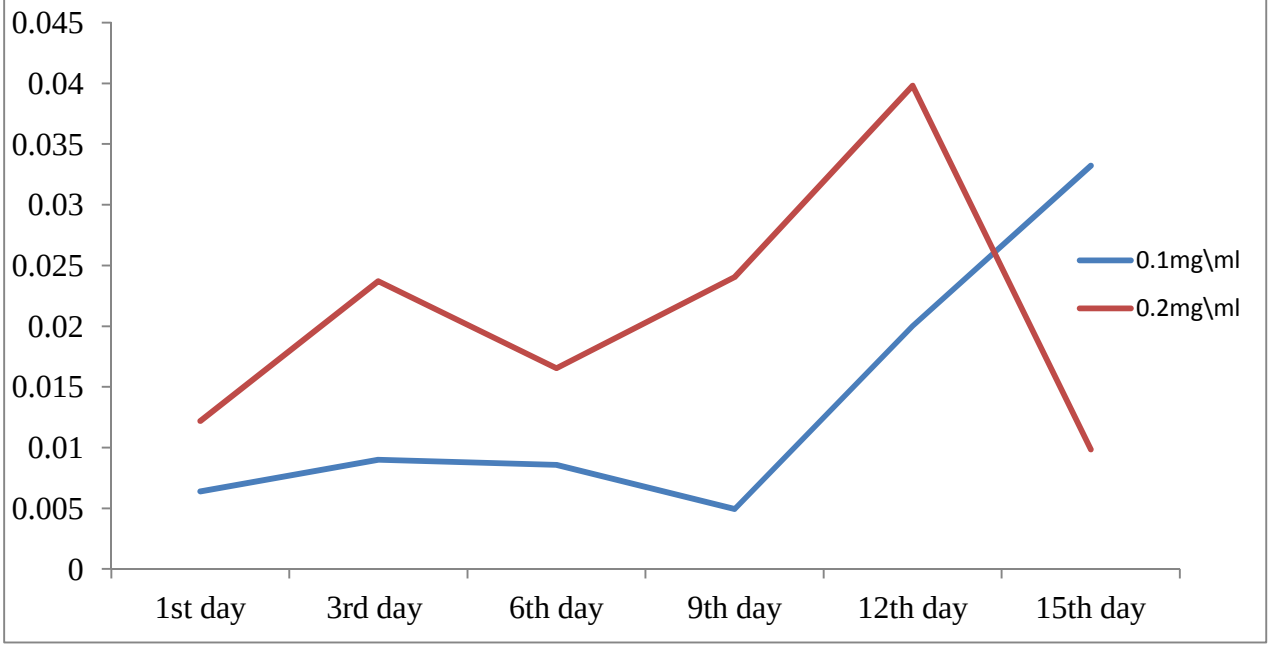
Fig. 9. Reducing sugar concentration (mg/ml) in germinating seeds in *Albizia procera* (Tree 2)

In Tree 3, reducing sugar concentration was found to be 0.006401 mg/ml on 1st day of germination. On the 3rd day to 6th day of germination, the reducing sugar concentration decreased from 0.008993 mg/ml to 0.008588 mg/ml. On the 9th day of germination, reducing sugar concentration again decreased (0.004942 mg/ml). On the 12th day of germination, the concentration increased (0.020012 mg/ml) followed by a further increase on the 15th day (0.033218 mg/ml) of germination (Table 10; Fig. 10).

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

Days	0.1mg\ml (Mean)	SD	% of Reducing sugar	0.2mg\ml (Mean)	SD	% of Reducing sugar
1st	0.006401	0.010	12.65	0.012185	0.010	91.74
3rd	0.008993		90.09	0.023699		47.16
6th	0.008588		94.33	0.016545		67.56
9th	0.004942		16.39	0.024035		16.51
12th	0.020012		40.48	0.039797		28.08
15th	0.033218		24.39	0.009838		11.36
Average	0.013692		46.388	0.021017		43.735
Result			46.388 ± 0.010			43.735 ± 0.010

Fig. 10. Reducing sugar concentration (mg/ml) in germinating seeds in *Albizia procera* (Tree 3)



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 α-l-arabinofuranosyl-(l→4)-[β-d-glucopyranosyl-(l→3)]-α-l-rhamnopyranosyl-(l→2)-β-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 Pachuau *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^{th} 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 stacchyose 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 stacchyose 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.

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.

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