Original Research Article

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 as collected from 3 trees of *Albizia procera* aged around 25 years were estimated with from the 1st day of germination till the 15th day. With progression of germination, total lipid content was found to be 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 results recorded might be of good use 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

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 Mimosaceae, 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 (Troup 1921).

Lateral roots are wide-spreading and the tap root is stout. The bipinnate leaves are reddish when juvenile and mature to length of 12-25 cm leaflets. Flowering time of this tree is June to September in India (Troup 1921). Flowers are borne on racemes 8 – 25 cm long near the end of a twig. Numerous greenish- yellow flowers form with whitish heads 20-24 mm in diameter. Individual flowers are 6-7 mm in length, have long white threadlike spreading stamens about 10mm long (Little and Wadsworth, 1964). The reddish brown flat pods, 10-20 cm long and 18-25 cm wide are produced in large numbers and ripen 3-5 month after flowering. The mature brown pods, each containing 6-12 seeds usually remain on the tree until the bearing the pods is shed (Troup 1921, Little and Wadsworth, 1964). 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.

Trees are often planted for shade or beautification along roads. *Albizia procera* is commonly used in traditional medicines (Venkataramany, 1968). The bark contains tannins and a reddish gum. Traditionally, parts of *Albizia procera* are used in anticancer, pain, convulsions, delirium, and septicemia. The decoction of bark is given for rheumatism and hemorrhage and is considered useful in treating problems of pregnancy and for stomach-ache, sinus. They are reported to exhibit various pharmacological activities such as CNS activity, cardio tonic activity, lipid-lowering activity, anti-oxidant activity, hepatic-protective activity, hypoglycemic activity, etc. Even through, traditionally, leaves of *Albizia procera* were extensively used for the treatment of variety of wounds. Seeds are powdered and used in amoebiasis. It cures urinary tract infections including glycosuria, hemorrhoids, fistula and worm infestation; it also suppresses skin diseases; fruits of *Albizia procera* act as astringent. The leaves are used to treat ulcers and have insecticidal properties (Parrotta 1987). 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 (Parrotta 1987).

The White Siris seeds are small, greenish-brown and elliptical to round, flat and have a hard, smooth seed coat; count 20,000-24000 seeds per kilogram (Roshetko, 1997). Fresh seed germinates rapidly without treatment (Parrotta, 1987). Clean seeds can be stored at room

temperature for 10 months with minimal loss of viability (Roshetko, 1997). 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 (Roshetko, 1997).

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 data of the experimental site

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.

Glass and plastic-ware

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

Equipments

For biochemical test, following equipments were used:

1) **Hot air oven**: Hot air oven (Sonar) for drying the glass wares.
2) **Pipettes**: 2ml, 10ml, 50ml pipettes were used (Borosil).
3) **Weighing balance**: Electronic digital weighing balance (Mettler) was used for weighing chemicals such as glucose, $HgCl_2$, NaOH, ammonium molybdate and fine chemicals.
4) **Water purification system**: (Millipore) water purification system was used for collection of water which was used for biochemical experiments and preparation of solutions.
5) **Micropipettes**: Micropipettes of different capacities (µl) were used for measurement of the volume of the different chemicals and other solutions.

6) **Centrifuge**: Centrifuge machine (Sigma 3-18K) and spin win were used for centrifugation purpose.
7) **Water bath**: 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.
8) **UV spectrophotometer**: 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 sample 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 were 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 they kept for germination in sterile Petri plates with double layered moistened filter paper. As pretreatment, the seeds were then immersed in hot water at 100^{o}C for 1 minute as described to be the best method by Azad *et al.* (2012). Germination started after 6 days; germination 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 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 growing seed.

Estimation of Soluble Sugar

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 phenol sulfuric acid (Dubois *et al.*, 1956) and Nelson-Somogyi (Somogyi, 1952) methods.

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

Materials

Phenol 5%: Redistilled (reagent grade) phenol (50g) dissolved in water and diluted to one liter. Sulphuric 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 5ml 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 (Nelson, 1944; Somogyi, 1952).

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), 2g Sodium bicarbonate and 20g Anhydrous Sodium Sulphate in about 80 ml of distilled water and the volume made up to 100ml. (b) To this 15 g of copper sulphate as a 10% (w /v) solution was added in a small volume of distilled water. One drop of Sulphuric acid was added and the volume was made up to 100ml. Both the solutions were mixed well before used (4ml of solution and 96ml 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 100mg glucose in 100 ml distilled 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 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

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

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

Results

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 dry humid condition.

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

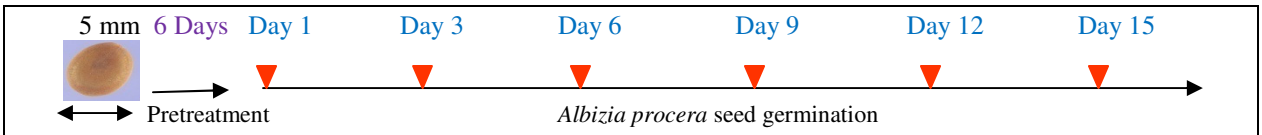


Fig. 1. Schematic diagram of the schedule of sample estimation for total lipid, total soluble sugar and total reducing sugar from germinating seeds of *Albizia procera*; 3 samples of 1 gm each were estimated from each of the 3 trees for each of the 3 biochemical estimations.

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.

Estimation of Lipid

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 milligram of total lipids per gram (mg/gm) of fresh seed tissue.

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

Table 2. Lipid content (mg/mg) in germinating seeds (mean value of 3 samples) 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			

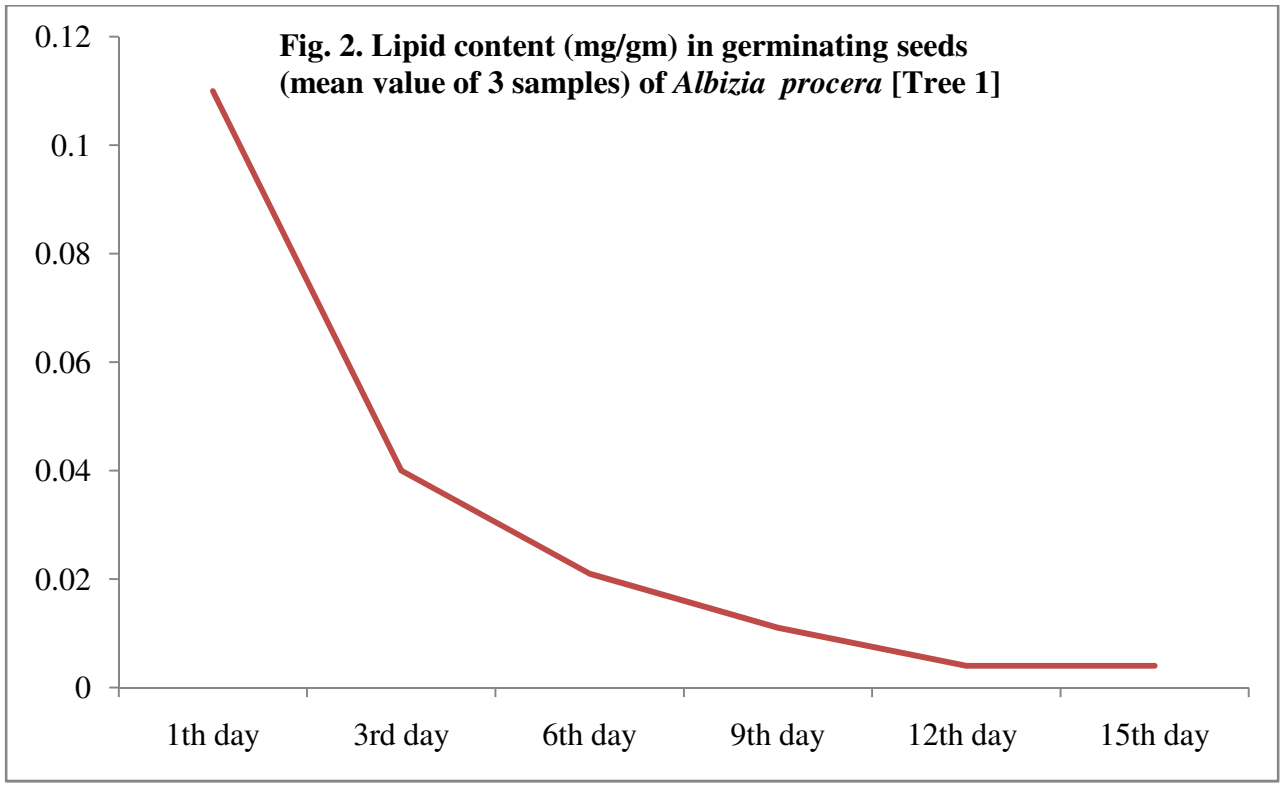


Fig. 2. Lipid content (mg/gm) in germinating seeds (mean value of 3 samples) of *Albizia procera* [Tree 1]

In Tree 2, the total the lipid content reduced from 0.030 mg/gm to 0.004mg/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 0.030mg/gm, on 3rd day 0.028mg/gm, on 6th day 0.020mg/gm, on 9th day 0.012mg/gm, on 12th day 0.010mg/gm and on 15th day 0.004mg/gm was observed. According to the table, from 0 day to 6th day result is found 0.026 ± 0.005mg/gm and from 9th day to 15th day of germination, the result was found 0.008 ± 0.004mg/gm (Average ± SD).

Table 3. Lipid content (mg/gm) in germinating seeds (mean value of 3 samples) 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			

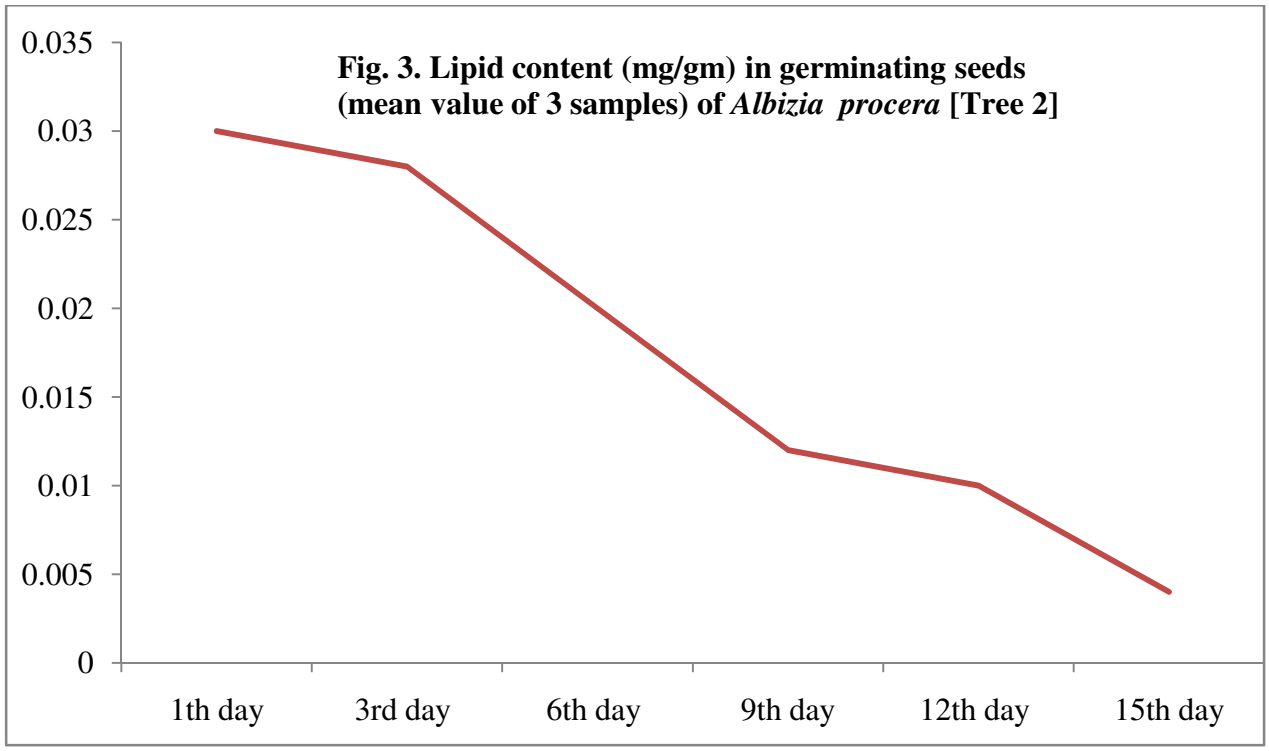


Fig. 3. Lipid content (mg/gm) in germinating seeds (mean value of 3 samples) of *Albizia procera* [Tree 2]

In Tree 3, the total lipid content reduced from 0.051mg/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, 0.051mg/gm, on 3rd day, 0.033mg/gm, on 6th day, 0.030mg/gm, on 9th day, 0.025mg/gm, on 12th day 0.024mg/gm and in 15th day 0.020mg/gm was observed. According to the table, from 0 day to 6th day result was found 0.038 ± 0.002mg/gm and 9th day to 15th day the result was found 0.023 ± 0.002mg/gm (Average ± SD).

Table 4. Lipid content (mg/gm) in germinating seeds (mean value of 3 samples) 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			

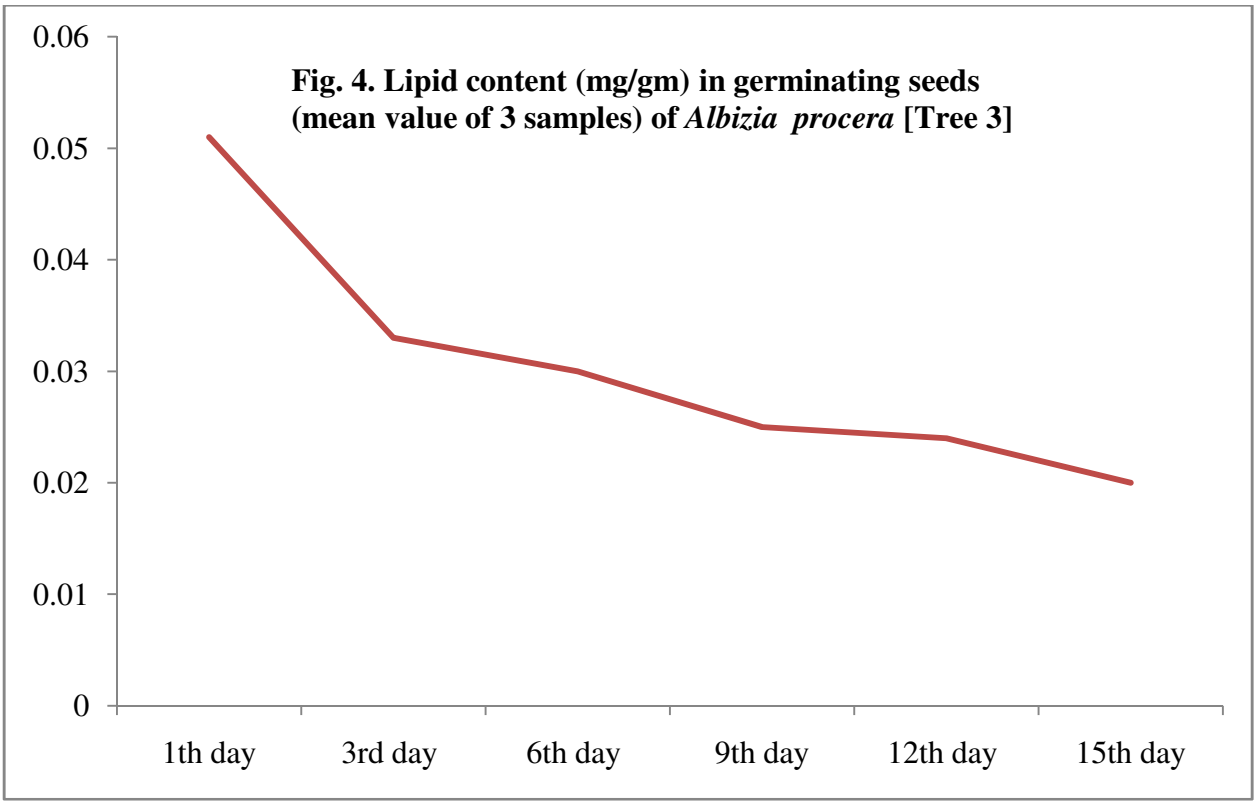


Fig. 4. Lipid content (mg/gm) in germinating seeds (mean value of 3 samples) of *Albizia procera* [Tree 3]

Sugar estimation

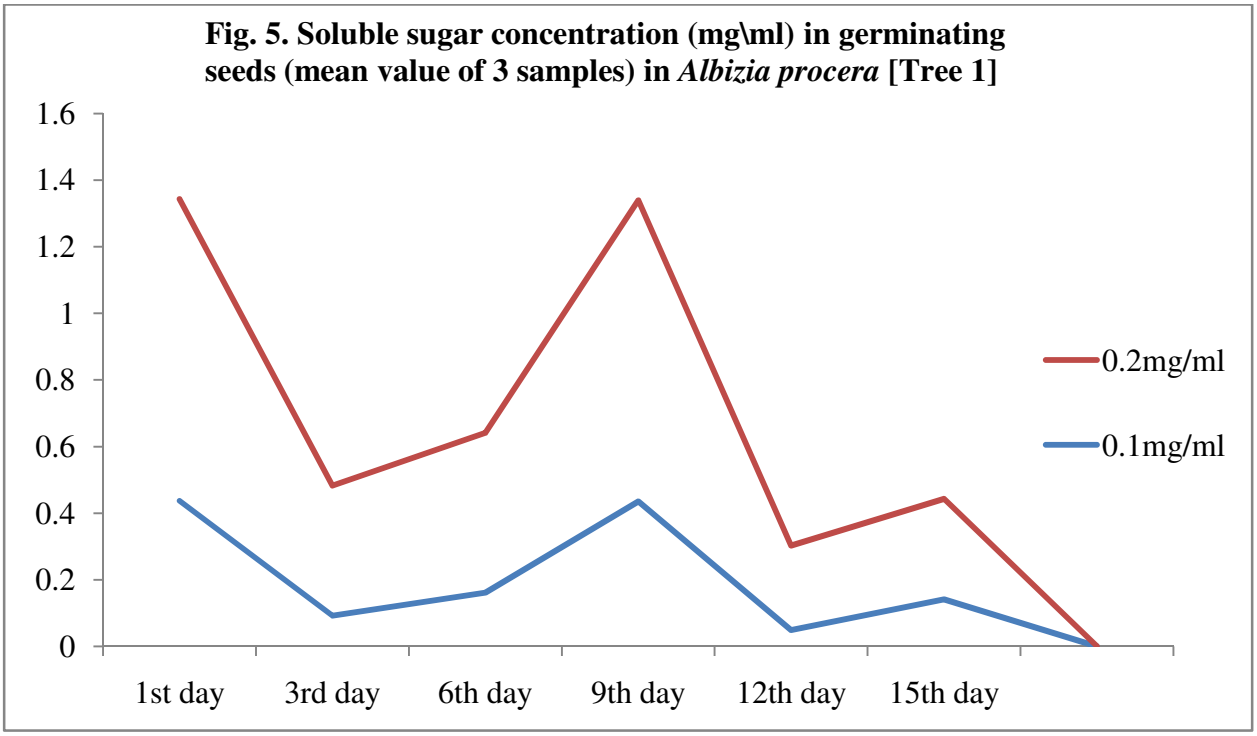
During the present study we estimated the soluble and reducing sugar in three different trees of *Albizia procera.* The sugar content in fresh seeds was estimated from 1st day of germination to 15th day compared with the sugar standard with two different concentrations i.e., 0.1mg/ml and 0.2mg/ml respectively and to find any variation in the concentration of sugars. For soluble and reducing sugars, they are expressed in 0.1milligram of seed tissue per 1ml 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.

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

Table 5. Soluble sugar content (mg/ml) in germinating seeds [mean value of 3 samples] 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 (mean value of 3 samples) in *Albizia procera* [Tree 1]

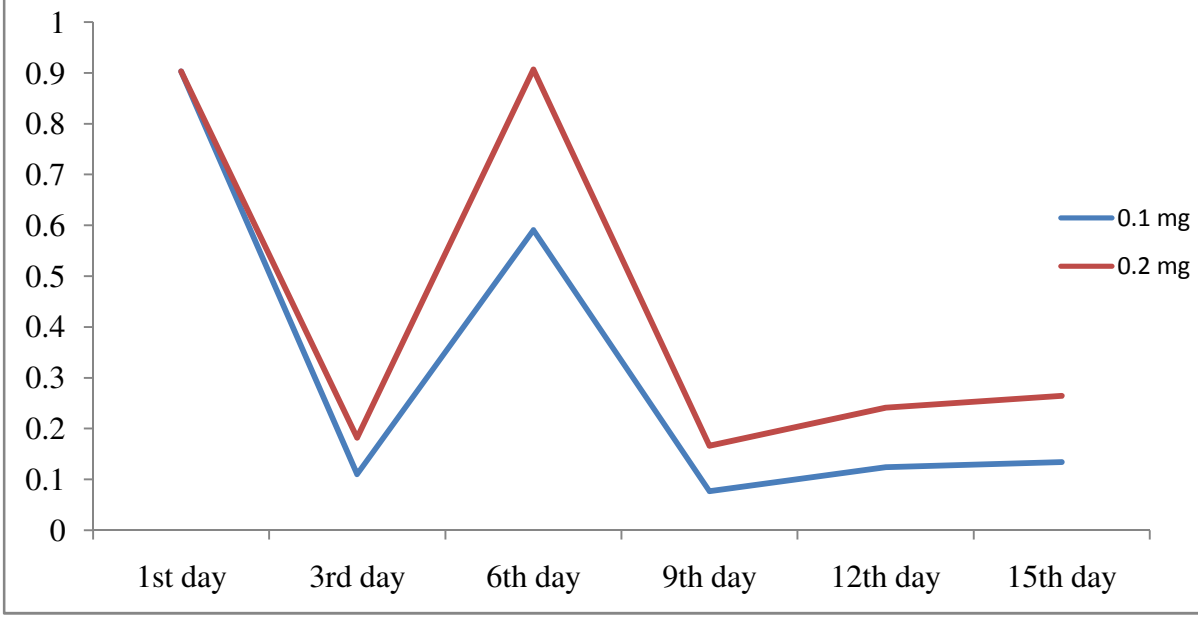


In the Tree 2, the soluble sugar concentration was found to be increased on 1st day to 3rd and 6th day of germination, i.e., 0.902721mg/ml to 0.110235mg/ml and 0.590649mg/ml respectively. On the 9th day of germination seed soluble sugar concentration was found to be decreased (0.076837 mg/ml), on the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

Table 6. Soluble sugar content (mg/ml) in germinating seeds [mean value of 3 samples] 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 (mean value of 3 samples) 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 was found to be decreased i.e., 0.116503mg/ml to 0.108871mg/ml. On the 9th day of germination, the soluble sugar concentration was again found to be increased (0.157438mg/ml). On the 12th day of germination, the concentration was found to be increased (0.166243mg/ml) followed by the decrease on 15th day of germination (0.087604mg/ml) (Table 7; Fig. 7).

Table 7. Soluble sugar content (mg/ml) in germinating seeds [mean value of 3 samples] 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

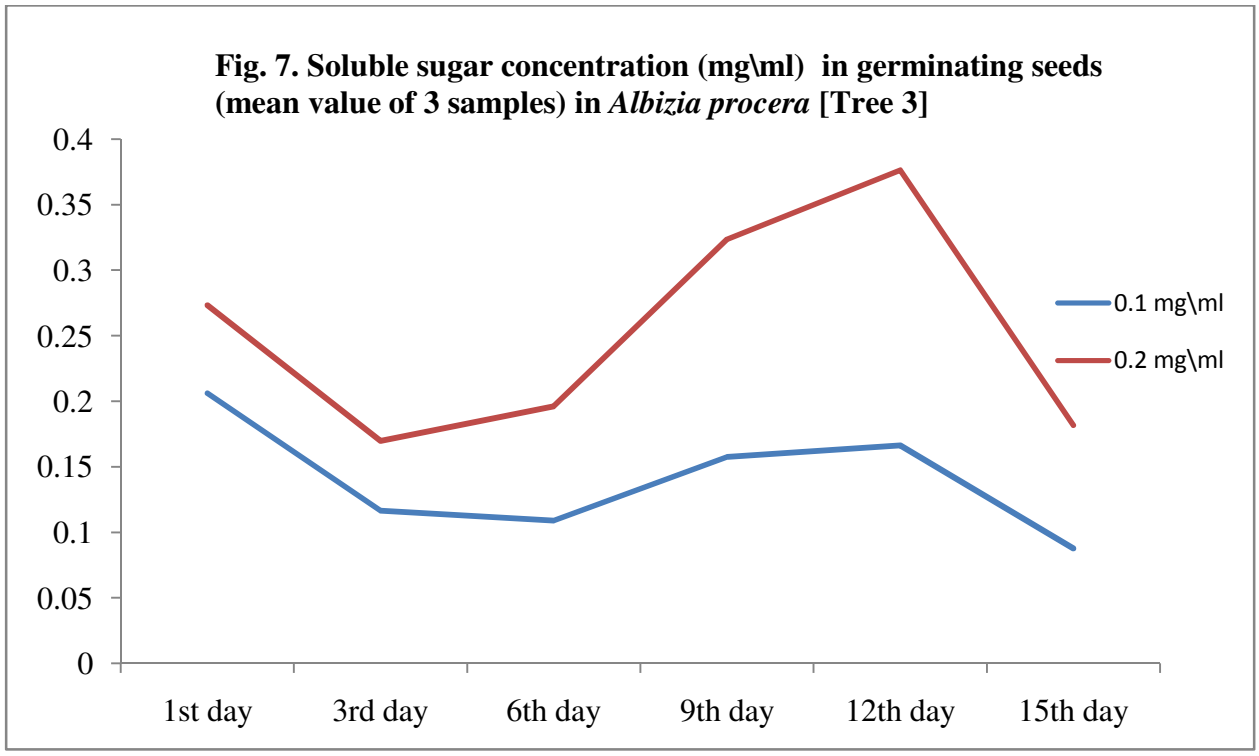


Fig. 7. Soluble sugar concentration (mg\ml) in germinating seeds (mean value of 3 samples) in *Albizia procera* [Tree 3]

In Tree 1, the reducing sugar concentration was found to be 0.079836mg/ml on 1st day of germination. On the 3rd day to 6th day of germination, the reducing sugar concentration was found to be decreased (0.006581mg/ml to 0.00372mg/ml). On the 9th day of germination, the reducing sugar concentration was again found to be increased (0.287008mg/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 [mean value of 3 samples] 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

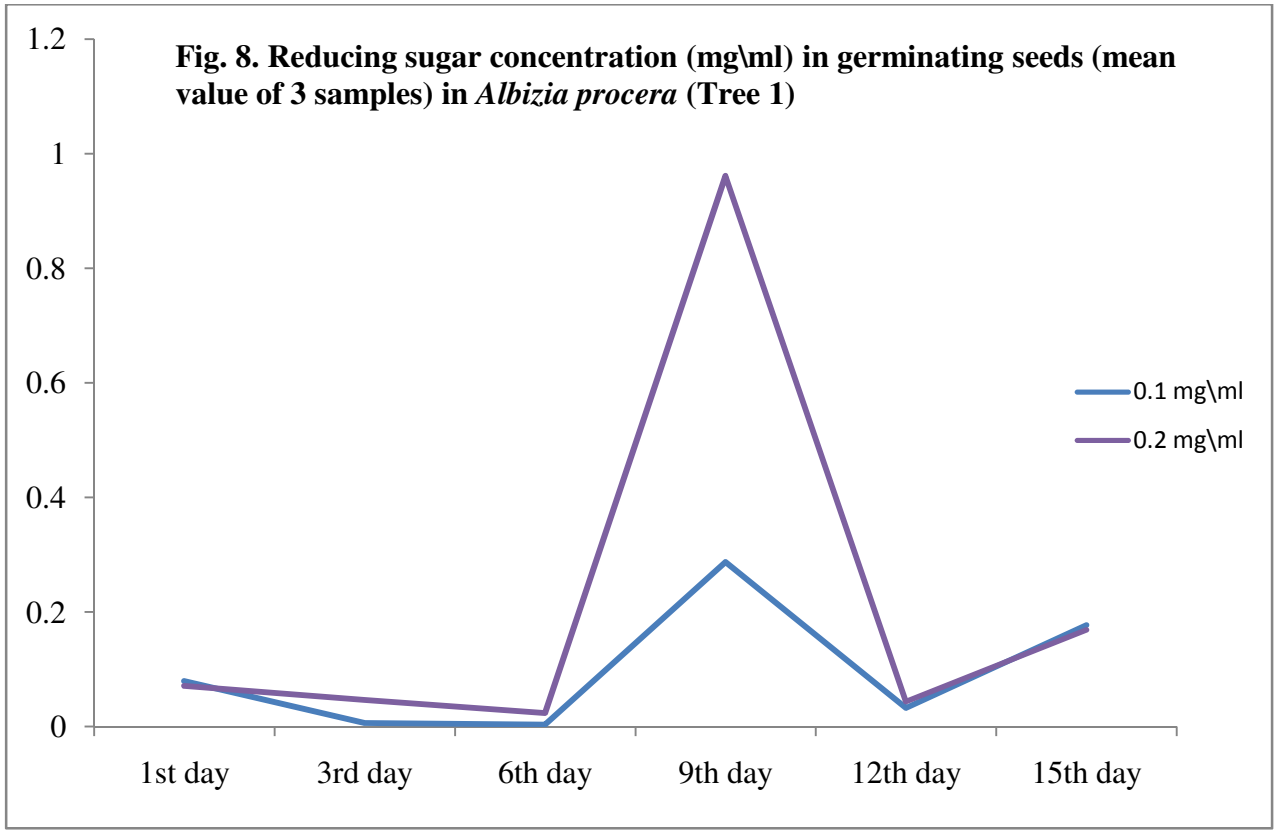


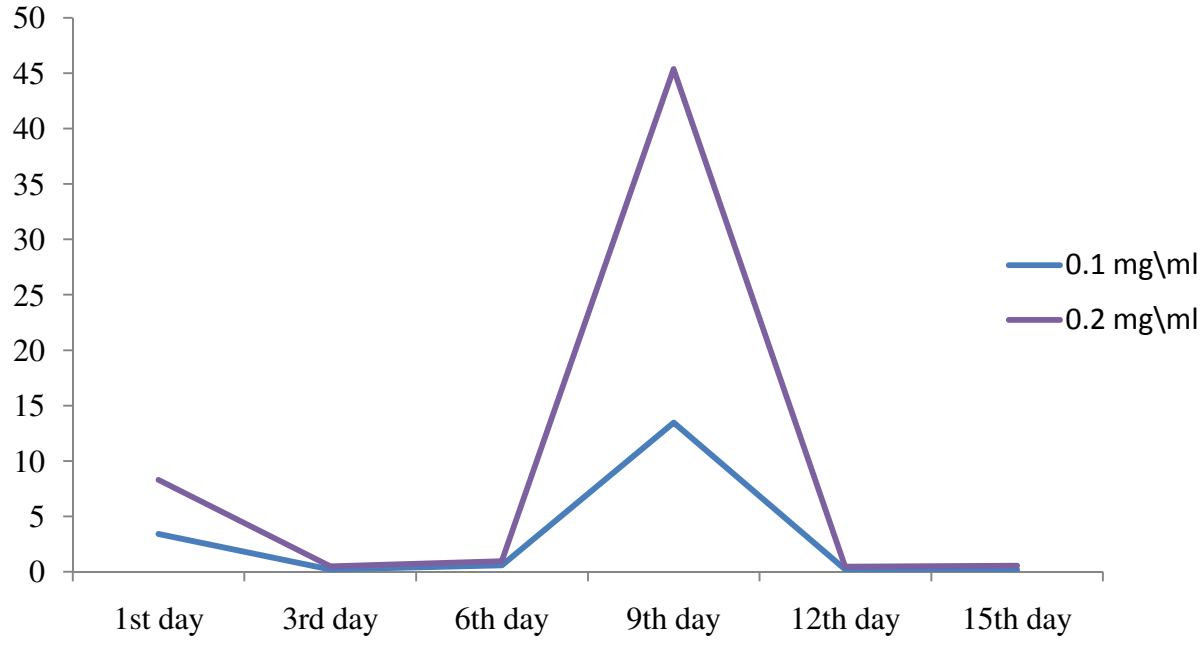
Fig. 8. Reducing sugar concentration (mg\ml) in germinating seeds (mean value of 3 samples) in *Albizia procera* (Tree 1)

In Tree 2, the reducing sugar concentration was found to be 3.39721mg/ml on 1st day of germination. On 3rd to 6th day of germination, the reducing sugar concentration was found to be decreased (0.20346mg/ml to 0.20346mg/ml). On the 9th day of germination, the reducing sugar concentration was again found to be increased (13.4545mg/ml). On the 12th day of germination, the concentration was found to be decreased (0.20346mg/ml) followed by a further decline (0.16986mg/ml) on 15th day of germination (Table 9; Fig. 9).

Table 9. Reducing sugar content (mg/ml) in germinating seeds [mean value of 3 samples] 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 (mean value of 3 samples) in *Albizia procera* (Tree 2)

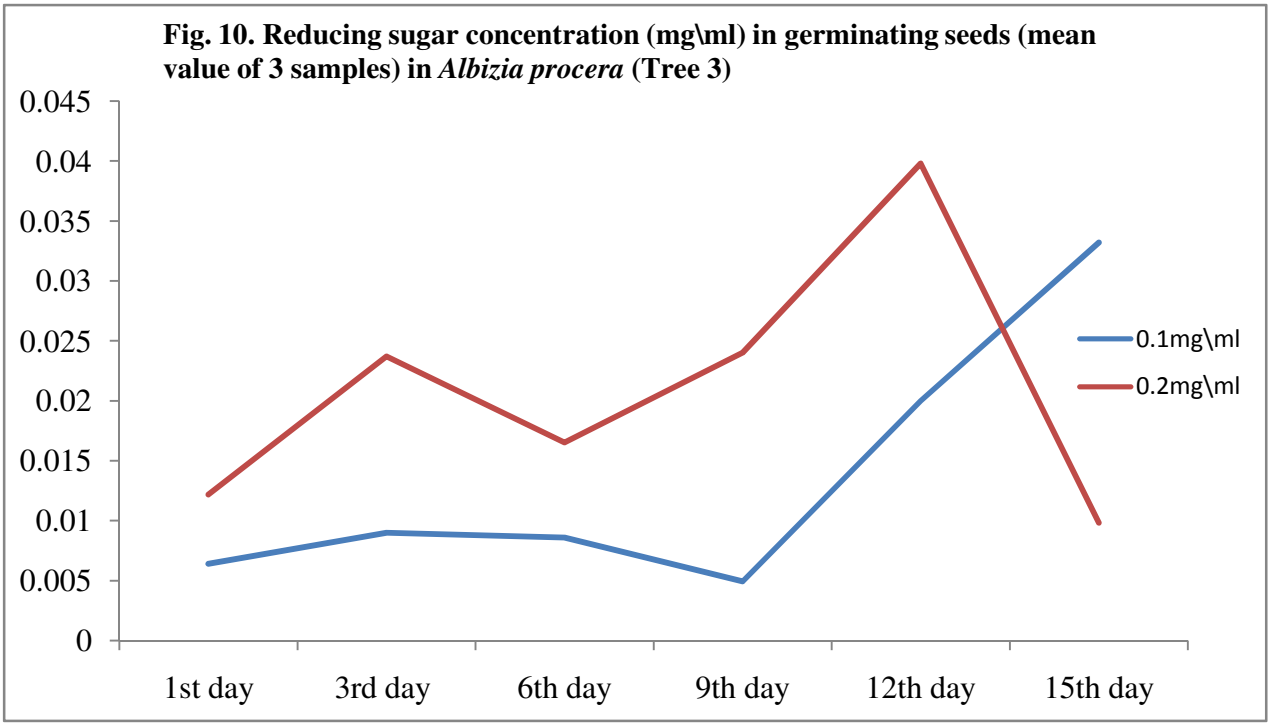


In Tree 3, reducing sugar concentration was found to be 0.006401mg/ml on 1st day of germination. On the 3rd day to 6th day of germination, the reducing sugar concentration was found to be decreased from 0.008993mg/ml to 0.008588mg/ml. On the 9th day of germination, reducing sugar concentration was again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

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



336 **Table 11. Levels of lipid, soluble sugar and reducing sugar during seed germination in**
337 ***Albizia procera* [Tree 1]**

Day of seed germination	Total Lipid (mg/ml)	Total Soluble Sugar (mg/ml)	Total Reducing Sugar (mg/ml)
1st	0.110 ± 0.010	0.007 ± 11.73	1.82 ± 36.73
3rd	0.040 ± 0.020	19.900 ± 41.41	10.29 ± 50.85
6th	0.021 ± 0.002	6.844 ± 27.03	46.35 ± 44.14
9th	0.011 ± 0.008	0.077 ±11.78	50.65 ± 63.88
12th	0.004 ± 0.003	41.580 ± 39.89	18.37 ± 74.71
15th	0.004 ± 0.005	0.721 ± 35.85	0.12 ± 16.03

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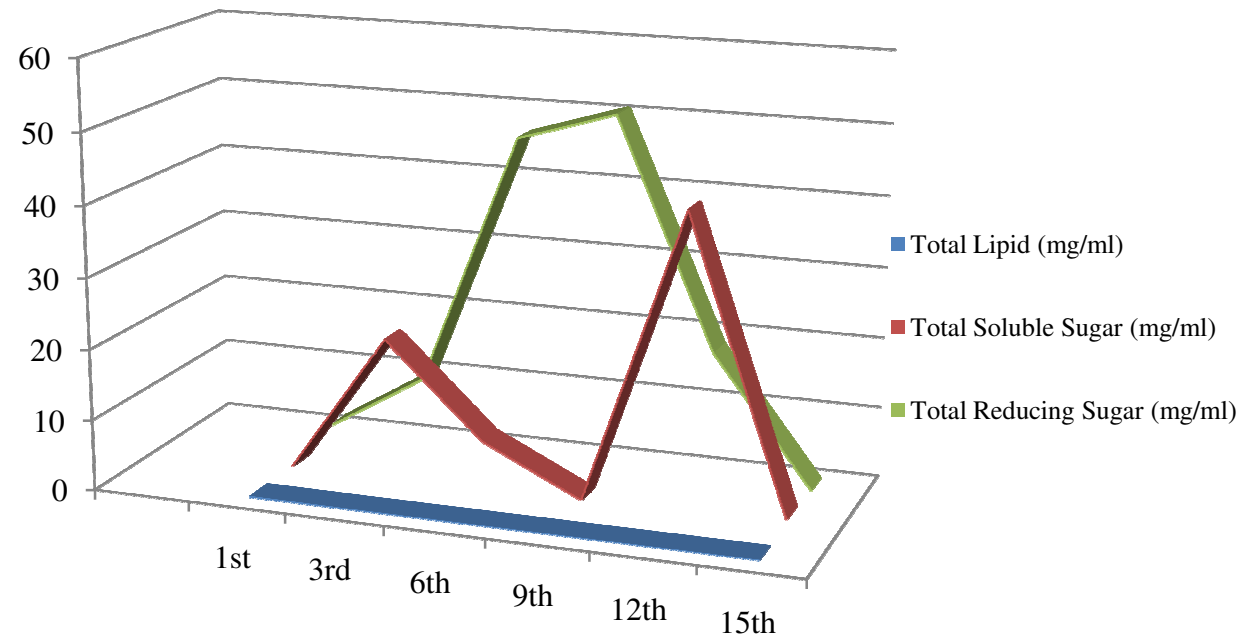
Fig. 11. Levels of lipid, soluble sugar and reducing sugar during seed germination in *Albizia procera* [Tree 1]



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344 **Table 12. Levels of lipid, soluble sugar and reducing sugar during seed germination in**
345 ***Albizia procera* [Tree 2]**

Day of seed germination	Total Lipid (mg/ml)	Total Soluble Sugar (mg/ml)	Total Reducing Sugar (mg/ml)
1st	0.030 ± 0.01	0.007 ± 11.80	13.96 ± 45.06
3rd	0.028 ± 0.02	26.960 ± 77.63	22.01 ± 76.17
6th	0.020 ± 0.02	4.447 ± 14.90	1.07 ± 31.89
9th	0.012 ± 0.40	52.410 ± 50.93	35.58 ± 39.03
12th	0.010 ± 0.06	29.281 ± 65.05	18.95 ± 78.33
15th	0.004 ± 0.02	27.710 ± 59.92	31.51 ± 33.26

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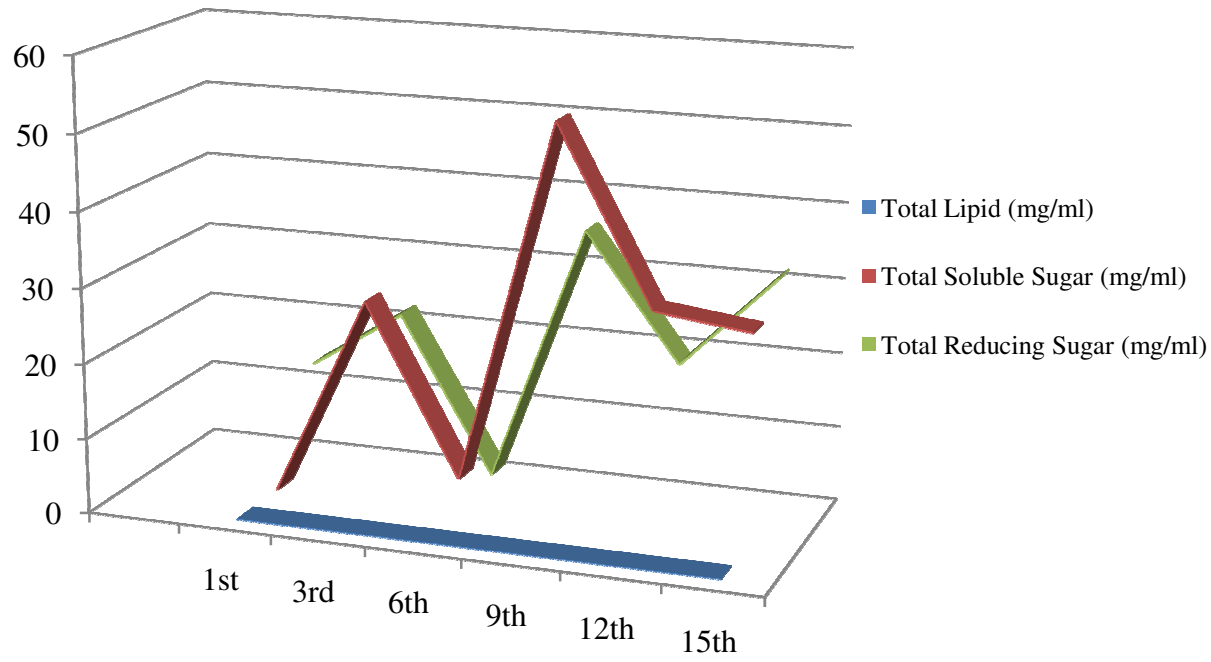
Fig. 12. Levels of lipid, soluble sugar and reducing sugar during seed germination in *Albizia procera* [Tree 2]



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348 **Table 13. Levels of lipid, soluble sugar and reducing sugar during seed germination in**
349 ***Albizia procera* [Tree 3]**

Day of seed germination	Total Lipid (mg/ml)	Total Soluble Sugar (mg/ml)	Total Reducing Sugar (mg/ml)
1st	0.051 ± 0.002	8.96 ± 45.37	55.92 ± 52.19
3rd	0.033 ± 0.224	20.27 ± 77.15	30.35 ± 68.62
6th	0.030 ± 0.021	30.78 ± 76.14	18.92 ± 80.94
9th	0.025 ± 0.001	21.53 ± 48.18	0.084 ± 16.45
12th	0.024 ± 0.002	25.30 ± 46.22	8.768 ± 34.28
15th	0.020 ± 0.021	32.89 ± 35.42	9.213 ± 17.87

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Fig. 13. Levels of lipid, soluble sugar and reducing sugar during seed germination in *Albizia procera* [Tree 3]

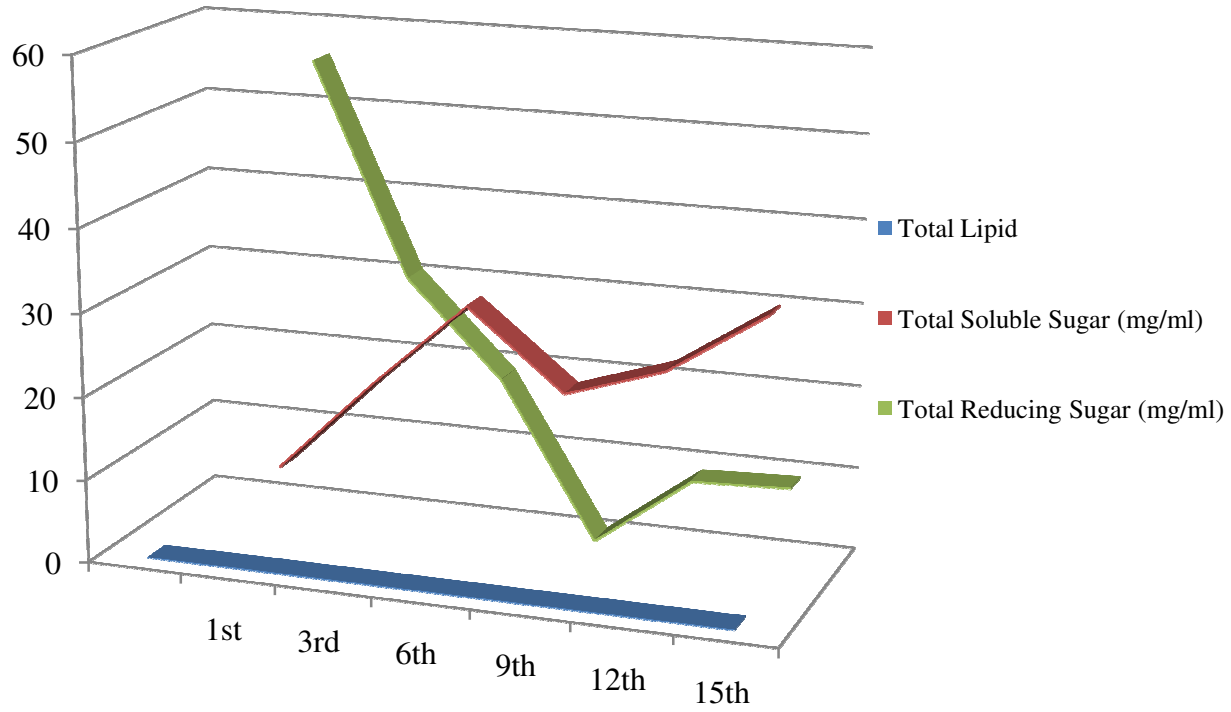

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Fig. 14. Total lipid content of germinating seeds of *A. procera* in Tree 1, 2 and 3 from Day 1 till 15

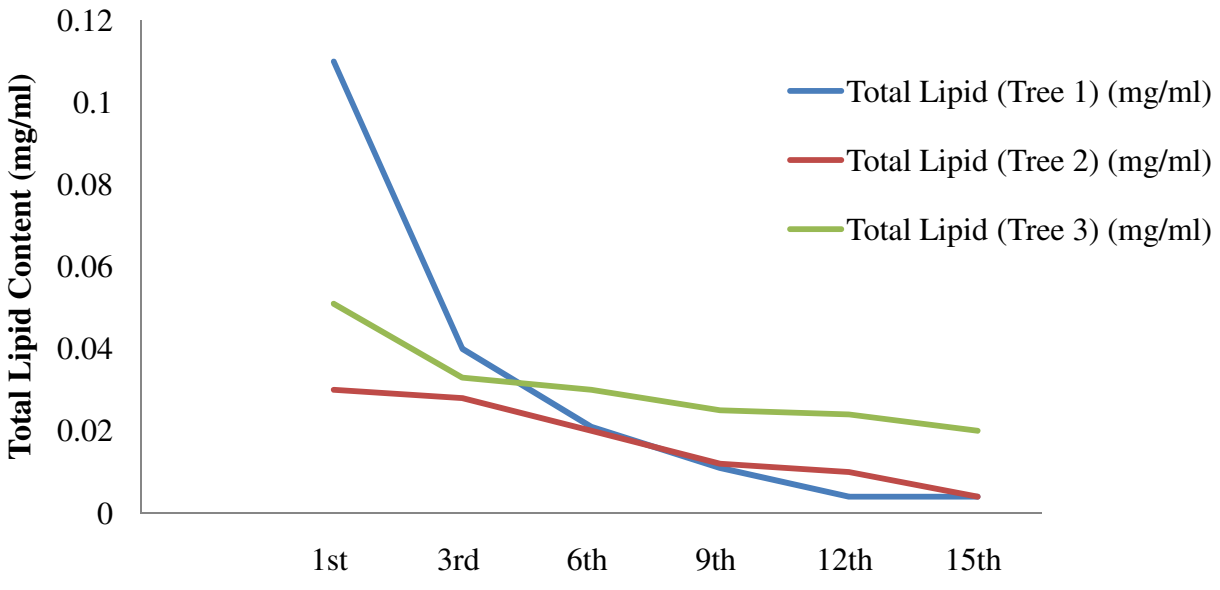


Fig. 15. Total soluble sugar content of germinating seeds of *A. procera* in Tree 1, 2 and 3 from Day 1 till 15

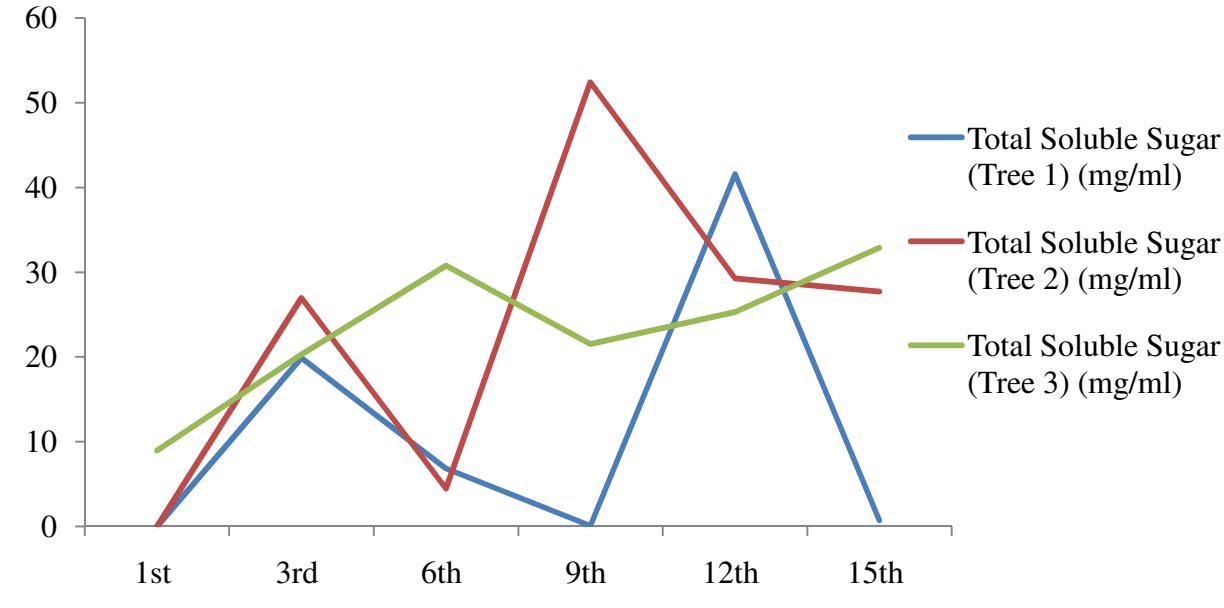
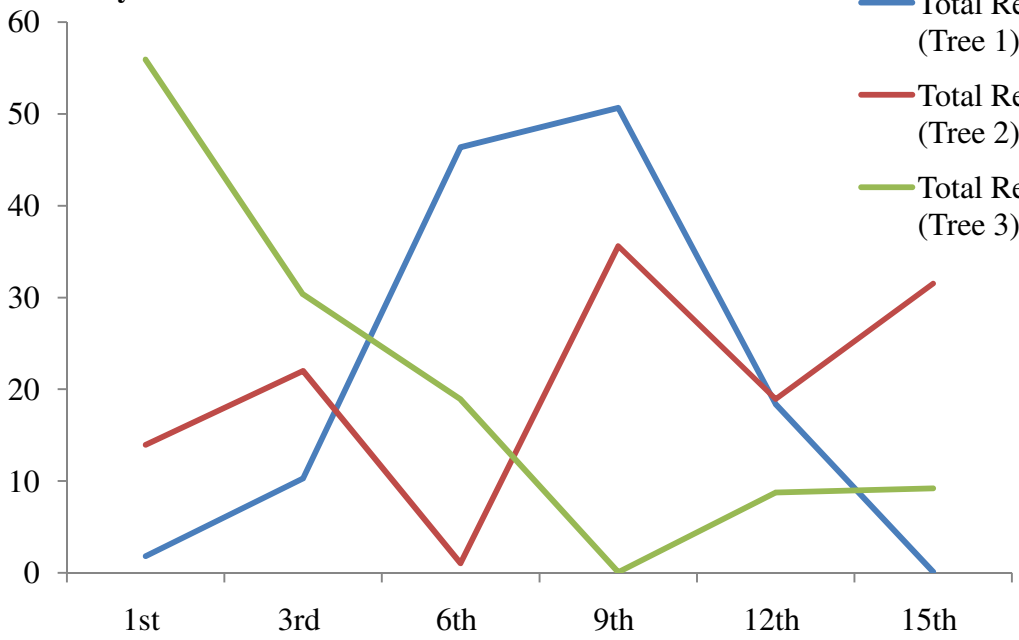


Fig. 16. Total reducing sugar content of germinating seeds of *A. procera* in Tree 1, 2 and 3 from Day 1 till 15



Discussion

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 this biochemical test we used 3 different well-established methodologies, viz. for total lipid estimation Becker *et al*. (1978) method, for total soluble sugar Dubois *et al*. (1956) method and for total reducing sugar Nelson-Somogyi (1952) method. The absorbance in UV-VIS spectrophotometer for soluble sugar absorbance was read at 490 nm and for reducing sugar at 620nm. For this biochemical estimation, used three different trees of *Albizia procera* species from different locations (Table 1).

Biochemical estimation for total lipid, soluble sugar and reducing sugar was done in 3 different trees of *Albizia procera*, designated as Tree 1, 2 and 3 for working convenience, 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; Fig.s 2-4). High amount of reduction of lipid in the seeds indicated their higher level of mobilization and faster utilization by the embryonic axes.

In general, seeds are structured by seed coat, endosperm tissue and embryo. The main function of the endosperm is to supply as the nutrient source for the germinating embryo (Linkies *et al.*, 2010). Being initiated by water uptake, seed germination is the ultimate outcome of different physiological processes. Imbibition of seeds also kick-starts some other very important physiological processes, like breakdown of reserves, mobilization and utilization of the broken-down products and growth and expansion of the embryo. Germination is considered to be completed when the radicle emerges from endosperm and seed coat (Bewley and Black, 1994).

The major biochemical components of endosperm are carbohydrate, lipid and triacylglycerols. With seedling growth, different enzymes are generated which mobilize these macromolecules (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994). In oleiferous seeds, the main source of energy during seed germination and seedling growth are the triacylglycerols (Anthony and Robert, 1978). 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 (Sharma and Sengupta, 1987; Ascencio, 1997; Ashton, 1976). 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 (McCleary *et al.*, 2006; Bewley and Black, 1994).

Four new oleanane-type triterpene glycosides, proceraosides A–D (1–4), were purified from the seeds of *Albizia procera* by Yoshikawa *et al.* (1998). 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 (Yoshikawa *et al.*, 1998). The monosaccharide composition and physicochemical properties of *Albizia procera* gum was revealed by Pachuau *et al.* (2012) who found that Arabinose (44.94%), Galactose

(30.17%), Rhamnose (0.22%) and Fructose (0.02%) were the main monosaccharides present in the exudate 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 (Ashton, 1976; Anthony and Robert, 1978; Sharma and Sengupta, 1987; Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994; Ascencio, 1997).

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 5-13; Fig.s 5-16). 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) 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) 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.

In Amaranth Grain, Colmenares and Bressani (1990) reported the changes in chemical composition and in nutritive value during germination. 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.

Tonguc *et al*. (2010) 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 h.

Koster *et al* (1988) reported the relationship between sugar content and the loss of desiccation tolerance in the axes of germinating soya bean (*Glycin max* L.), pea (*Pisum sativam*

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) 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) 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) 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 be decreased, but no such continuous trend could be drawn regarding the contents of total soluble and total reducing sugars. The results recorded might be of good use for further studies on the metabolic activities of germinating seeds in *Albizia procera* or related tree species.

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