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Pollen morphology and viability in *Gliricidia sepium*

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Abstract
An investigation was carried out to study the pollen morphology and viability in *Gliricidia sepium.* The anthesis started around 6:30 a.m and the duration of the flowers on plant is approximately 8-10 hours. The pollen colour is yellow and pollen grains are circular in shape with three germpores. The size of the pollen grain is 0.40µm. The pollen grain of *Gliricidia sepium* showed maximum percentage of pollen viability by acetocarmine test at room temperature was found to be maximum (87.50%) in the first day of pollen collection and decreased to 18.50 % after 15 days of storage at room temperature. In *in-vitro* pollen germination the maximum germination 75.50 per cent was obtained in 20 per cent sucrose concentration. Within 20 per cent sucrose concentration the storage temperature of -18 ± 1˚C and 4 ± 1˚C gave germination percent of 59.50 per cent and 53.00 per cent after 90 days of storage. Pollen stored in room temperature lost viability after 60 days of storage.

Keywords: pollen grain, pollen viability, *in-vitro* pollen germination, *Gliricidia sepium*

Introduction
Pollen grains are small, male reproduction units (gametophytes) formed in the anthers of the higher flowering plants. The pollen is transferred onto the stigma of a flower (a process called pollination) by either wind, water or various animals (mostly insects), among which bees are the most important ones. Pollen morphological features such as symmetry, shape, apertural pattern and exine configuration are very conservative features for the taxonomic assessment of the plants (Keshavarzi *et al.*, 2012) [6]. Pollen represents the critical stage in the life cycle of plants, as viable pollen is crucial for efficient sexual plant reproduction. The quality of pollen is assessed on the basis of viability and vigour. Pollen viability refers to the ability of the pollen grains to perform their function of delivering the sperm cells to the embryo sac following compatible pollination and vigor can be considered as the time taken for pollen tube growth in the pistil (Shivanna *et al.*, 1991) [8]. The *in-vitro* pollen germination is most commonly used in breeding programs and also the most convenient method that is able to estimate the reserve's substances and membrane conditions as well as the reserve conversion for pollen germination (Marcellan and Camadro, 1996) [7].

Gliricidia sepium is a medium size, semi- deciduous leguminous tree belonging to family Fabaceae that typically grows 10m to 15m in height, with a broad canopy and has a medium crown and may be single or multistemmed. The species is native to Central America and possibly northern South America, its cultivation is now pantropical. It grows best in tropical, seasonally dry climates. It is fast-growing Nitogen-fixing tree used throughout the tropics for the many environmental services and products it provides. It is widely used to provide crop shade for coffee, cacao and other shade loving crops and other shade loving crops. Today it is used for many other purposes including live fencing, fodder for its high nutritional value, firewood, green manure, intercropping, rat poison and as an alternative energy source. This species stand out for its rapid growth, high regeneration capacity, drought resistance and ability to propagate sexually and asexually. It is commonly referred as *Gliricidia*.

The leaves and flowers are said to be eaten boiled or fried. The flowers attract honey bees (*Apis* spp.) hence it is important species for honey production (Katende, 1995) [3]. It is a good source of nectar. *G. sepium* leavea are rich in protein and highly digestible, and low in fibre and tannin. Crushed leaves are applied as poultice. It is also used as antihistaminic, antipyretic, expectorant and diuretic. The seeds and other parts are useful as a rodenticide. Tests of leaf and wood extracts have shown insecticidal and anti-microbial activity. *G. sepium* is suitable for ornamental use in residential and public landscaping, parking lot and along residential streets because of its modern size, clean appearance and colorful flowers.

Knowledge of pollen studies are pre requisite for advanced tree breeding and tree improvement programme. The objectives of this work were to deepen the understanding of the pollen morphology of *Gliricidia sepium* and were studied with the conservation perspective.

Methodology

Study site

Pollen grain observations of *Gliricidia sepium* tree have been carried out in the surroundings of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. Allahabad is situated at an elevation of 98 m above sea level at 25º 28' N latitude and 81º 55' E longitude. It is located in the south eastern part of Uttar Pradesh and has tropical to subtropical climate with extreme of summer and winter. There are three seasonal variations in the area: Winter (December to January), summer (April to June) and rainy (July to September). The temperature is lower during winter and drops down to as low as 5ºC in the month of December to January. Summer is very hot and humid with temperature reaching upto 48ºC.

Pollen studies

Flowers were collected early morning and filaments and anthers were excised in the butter paper and kept for shade drying until dehiscence. After dehiscence the pollens were collected and divided into two parts: one used for viability test and *in vitro* germination test. Pollen mixtures were obtained from 20-30 flowers from each tree. Three replications were carried for each condition. For viability test pollens were dusted on the slide and one to two drops of acetocarmine were added in the slide. Pollens were mixed using sterile needle and observed under a microscope. Stained pollens were counted as viable and unstained pollens were non–viable.

For *in-vitro* pollen germination; a few drops of sucrose solution was dropped on the cavity slide, the pollen grains were then dusted onto the slides and evenly mixed in the solution. The prepared slides were stored in moist condition and observed under the microscope after 24 hours. Pollen grains were considered as germinated when the length of the pollen tube exceeded its diameter. The media used for *in-vitro* pollen germination constituted of 5, 10, 15 20, 25 and 30 percent sucrose solution.

To construct viability curves, pollen was stored in individual vacuum vials to avoid the effects of successive openings for sampling on pollen viability. The vials were placed in zip lock bags and stored at room temperature, 4±1˚C and -18±1˚C. The pollens are stored and were tested for viability and *in-vitro* germinability after every fifteen days interval up to 90 days. The *in-vitro* germination tests of the stored pollen were conducted using the appropriate sucrose concentrations for different species. The sucrose concentration showing maximum germination in freshly collected pollens for a species was considered appropriate for that particular species and was utilized for the complete duration of the storage period.

Results

The pollen colour is yellow and Pollen grains are circular in shape with three germpores. The size of the pollen grain is 0.40µm. Observation carried under microscope revealed that pollen grain of *Gliricidia sepium* showed maximum percentage of pollen viability by acetocarmine test at room temperature was found to be maximum (87.50%) in the first day of pollen collection and decreased to 18.50 % after 15 days of storage at room temperature (Table 1). Kanthaswamy (2006) [5], Wani and Chauhan. 2009 [9] reported high viability in the first day of pollen collection and high rate of viability loss in the consequent days.

In the present study of *in vitro* pollen germination of *Gliricidia sepium* maximum germination was obtained in 20% (75.50%) sucrose concentration among different concentrations of 5%, 10%, 15%, 20%, 25% and 30% after 24 hours (Table 2). These findings are supported with the investigations of (Bhattacharya and Mandal, 2000) [1] in the case of *B. ceiba* and *M. oleifera* Wani *et. al.,* 2015 [10] in *Delonix regia* Wani *et. al.,* 2018 [11] in *B. variegata* where they observed maximum germination in 20% sucrose (71%) and 10% sucrose (82 ± 4.81%) respectively. Within 20% sucrose concentration the storage temperature of -18 ± 1˚C and 4 ± 1˚C gave germination per cent of 59.50% and 53% after 90 days of storage. Pollen stored in room temperature lost viability after 60 days of storage (Table 3).

Table 1: Pollen grain viability of five different trees in *Gliricidia sepium.*

Treatments	1st day	7th day	15th day
T_1	87.50	50.00	18.50
T_2	82.50	42.50	20.00
T_3	75.00	40.00	22.50
T_4	77.50	50.00	24.00
T_5	80.00	52.50	19.00
Mean	80.50	47.00	20.80
F- test	S	S	S
S. Ed. (±)	0.462	1.131	0.529
C. D at 5%	0.979	2.399	1.122

Table 2: Pollen grain germination percentage at room temperature (25ºC±5˚C) for different sucrose concentration after pollen collection.

Treatments	Different sucrose concentration (per cent)					
	5	10	15	20	25	30
T_1	27.50	50.00	57.50	75.00	65.00	45.00
T_2	30.00	47.50	57.50	77.50	62.50	47.50
T_3	22.50	47.50	52.50	72.50	67.50	47.50
T_4	27.50	50.00	52.50	80.00	60.00	50.00
T_5	35.00	47.50	57.50	72.50	62.50	52.50
Mean	28.50	48.50	55.50	75.50	63.50	48.50
F- test	S	S	S	S	S	S
S. Ed. (±)	2.309	0.163	1.826	2.380	2.236	2.160
C. D. (P = 0.05)	4.896	0.346	3.871	5.047	4.740	4.580

Table 3: Pollen grain germination percentage at 20% sucrose conc. at 3 different storage temperature.

Storage period	Storage Temperatures		
	Room temperature	4±1˚C	-18±1˚C
P_0 (0 days)	80.00	80.00	80.00
P_1 (7 days)	71.50	78.50	79.00
P_2 (15 days)	48.00	74.00	75.00
P_3 (30 days)	28.50	69.50	71.50
P_4 (45 days)	16.13	65.50	69.00
P_5 (60 days)	2.06	62.00	65.50
P_6 (75 days)	0.00	58.50	63.00
P_7 (90 days)	0.00	53.00	59.50
Mean	24.94	67.63	70.31
F- test	S	S	S
S. Ed. (±)	0.577	0.764	0.573
C. D. (P = 0.05)	1.224	1.619	1.214

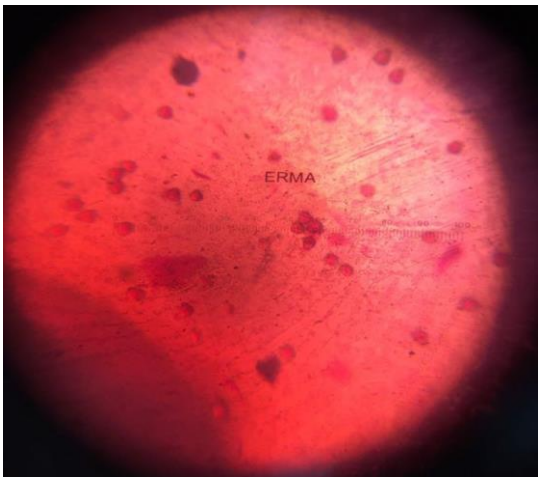

Fig a: Stain pollen grain in aceto carmine test of *Gliricidia sepium*

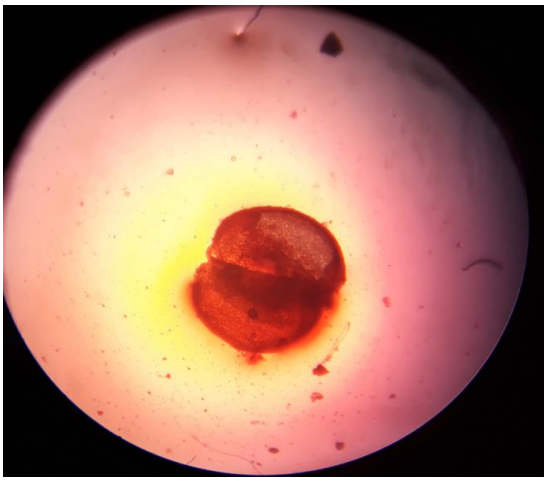

Fig b: Shape of viable pollen grain of *Gliricidia sepium*

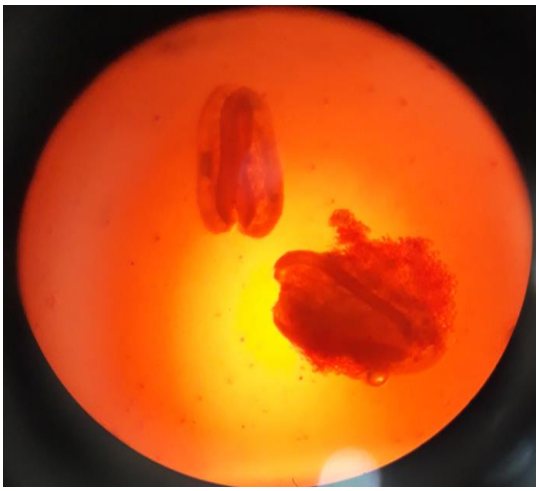

Fig c: Deformed shape of non-viable pollen in *Gliricidia sepium*

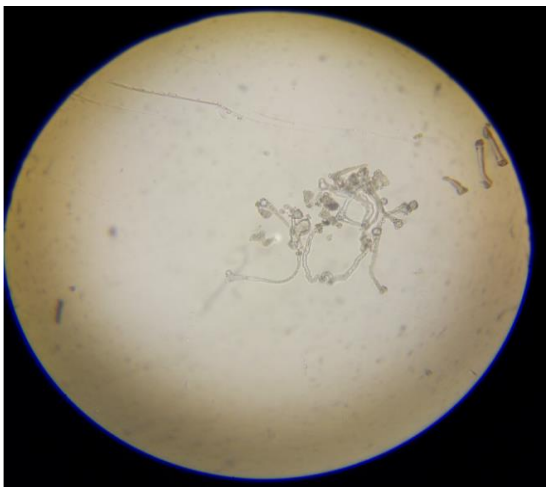

Fig d: Pollen grain germination in *Gliricidia sepium*

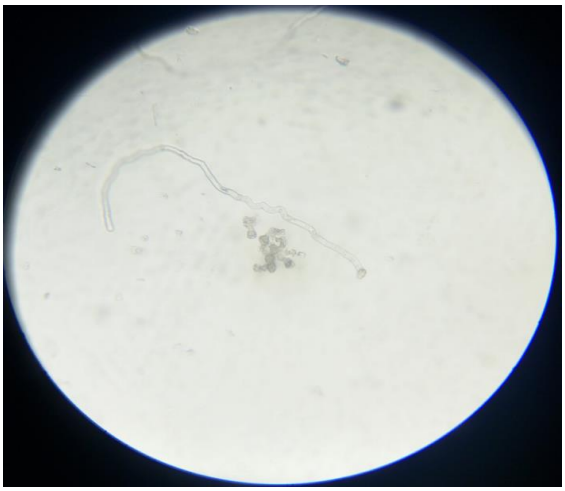

Fig e: Pollen tube growth at 20% sucrose solution in *Gliricidia sepium*

Conclusion

From our present study, it can be concluded that pollen grain of *Gliricidia sepium* showed maximum percentage of pollen viability by acetocarmine test at room temperature was found to be maximum (87.50 per cent) in the first day of pollen collection. *In-vitro* pollen germination was recorded highest under room temperature (25±5ºC) at 20 per cent sucrose solution on the first day of pollen collection. Germination decreased to 2.06 per cent under similar condition after 60 days and it was completely dead after 61 days. In 4±1ºC and -18 ± 1ºC germination was 53 per cent and 59.50 per cent respectively after 90 days.

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