International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Study on In *Vitro Pollen* Germination on *Jatropha integerrima* Jacq. Pollen

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Abstract: ***Jatropha integerrima Jacq. is known for its ornamental flower since long. But less is known about its pollen viability and in vitro pollen germination. The objective of this study was to assess the pollen viability and in vitro pollen germination in Jatropha integerrima Jacq. The pollens were collected from three flowering stages- pre anthesis, anthesis and post anthesisto evaluate the pollen viability, by TTC dye method. The flowering stage which showed maximum percentage pollen viability was taken into account for in vitro germination studies. Two culture mediums were used to assess in vitro pollen germination- Liquid medium (M1) and Semi solid medium (M2). In both the mediums maximum pollen germination was obtained in 20% sucrose, 0.04% Boric acid and 0.03% Calcium chloride. But in comparison to M1, M2 medium gave more percentage pollen germination in the respective concentrations.***

Keywords: *Jatropha integerrima, Invitro,* pollengermination, viability

1. Introduction

Pollen is a haploid cell of the plant which is used to transport male gamete to the female part of the flower and is a simple structure compared to other tissue and organ of the plants. Pollen grains play a vital role in various breeding programmes and there for it is important in fruit-set. In the flowering plants after the process of pollination, pollen germination takes place, a process which is important for the reproduction of plant. The germination of pollen grains begins by absorption of water and nutrients leading to formation of pollen tube. The pollen tube has a sperm cell and they fertilize ovule and develop embryo. There are two type of pollen germination: a) In Vivo (Pollen germination in environmental condition). b) In Vitro (Pollen germination in laboratory condition).

Identifying the viability helps in knowing whether the pollen is germinable or not in which stage of development. The process of in vitro pollen germination and pollination offers an opportunity for producing hybrid embryos amongst plants that cannot be crossed by conventional methods of plant breeding. New plant varieties of required traits can be developed by reducing the time in hybridization programme. In pollens that have short life span, in vitro pollen germination becomes helpful.

The genus *Jatropha* is native to tropical America with more than 200 species that are widely distributed in tropics with a promise for use as an oil crop for biodiesel. *Jatropha*is a large genus of diverse growth forms and are attractive monoecious or dioecious plants. These species are woody trees, shrubs and sub shrubs of disjunct distribution in the seasonally dry tropics of the old and New World. *Jatropha integerrima Jacq*. commonly known as peregrina or spicy jatrophais a species of flowering plant in euphorbiaceae family. *Jatropha integerrima* is native to the West Indies Cuba and Hispaniola. It is grown in sandy and semi-arid areas close to swampy zones and in distributed soil. It is a tall, woody shrub plant, having branches that are green-brown with prominent lenticels and isperennial.

From the review done, reports have been obtained regarding pollen viability and in vitro pollen germination in different plant species. Reports are also been obtained about pollen germination and viability of different *Jatropha* species like *J.curcas, J.ribidolia, J.gossypifolia* and alike. Also some information is available about pollen viability in *Jatropha integerrima* but not about its *invitro* pollen germination. So in this experiment an attempt has been made to observe *invitro* pollen germination in *J.integerrima* and also to standardize the media for proper pollen germination.

2. Material and Methods

Pollen collection:
In the *invitro* pollen germination experiment, only fresh dehisced anther was used. The flowers of *Jatropha integerrima Jacq*. were collected from the Microbiology Department of Gujarat University campus. Flowers were collected in morning time.

Pollen Viability:
In viability test, different stains can be used. In this experiment 2, 3, 5TriphenylTetrazolium chloride (TTC) stain was used to test the viability. For this flowers from three stages (Pre anthesis, anthesis, Post anthesis) were collected. Few drops of 1% TTC solution was taken in watch glass and pollens were dusted in TTC solution. The watch glass was kept in dark place for 2-4 hours, and then observed under microscope. The viable pollen gets stained in red color while the non-viable pollen remains unstained.

In Vitro Pollen Germination:
To evaluate pollen germination and select the best culture medium for the in vitro pollen germination of *Jatropha integerrima*, two culture mediums-one a liquid culture medium and second a semi solid medium were selected.

Medium 1 (M1): Medium 1 consisted of different concentrations of Sucrose, Boric acid and Calcium chloride.
Medium 2 (M2): Medium 2 consisted of different concentrations of Sucrose, Boric acid, Calcium chloride and 1% agar as an solidifying agent.

Experimental design: For in vitro germination studies two culture media were made and pollens were allowed to germinate in each media. One was a Liquid culture medium and second was a semi solid medium. Total six concentrations (5 to 30%) of sucrose were added in petriplates in replicates of three. The best concentration of sucrose was selected for further studies with boric acid and $CaCl_2.6H_2O$. Similarly, total five concentrations of boric acid (0.01 to 0.05%) and Calcium chloride (0.01 to 0.05%) were added in petriplates in replicates of three. In these way total three sets of petriplates were prepared- 1) six concentrations of sucrose 2) best concentration of sucrose and five concentrations of boric acid 3) best concentration of sucrose+ boric acid and five concentrations of $CaCl_2$.

Data Analysis
Since three replicates were taken into account, for the statistical analysis, standard deviation and standard error was calculated in case of pollen viability, pollen germination, and pollen tube length.

3. Result and Discussion

Pollen viability: The % viability differed according to the different stages of flower. The highest pollen were viable in anthesisstage of flower with 81.2±3.60% followed by post anthesis stage with 36.5±3.17% and pre anthesis stage showed the lowest percentage pollen viability with 29.6±0.93%.

Stages	Percentage (%) of pollen viability.
Pre anthesis.	29.62±0.93
Anthesis.	81.24±3.60
Post anthesis.	36.50±3.17

Pollen germination

Sucrose
The optimum concentration of sucrose is required for pollen germination and pollen tube growth. Two different mediums M 1 and M 2 were utilized as pollen germination media. In M 1 medium 25.63±2.6% germination was obtained after 96 hours which was highest than all other concentrations. In M 2 medium 77.37±1.95% of pollen germination was recorded after 72 hours. Thus the results obtained with M 1 and M 2 medium in different concentrations of sucrose showed maximum pollen germination in 20% Sucrose concentration. This result resembles the result obtained in *Jatrophacurcas* which also required 20% Sucrose concentration (F.Chen and S.Wang, 2010). This relates that there may be a relationship between the two species as they belong to the same genus. But different results were obtained in *J.ribifolia* and *J.mollisima* which required 10% Sucrose for pollen germination (Lyra *et al*., 2011). 20% sucrose concentration was also recorded in *Abelmoschusesculentus* L. (Baloch et al., 2004).

Boric acid
Pollen grains were germinated *in vitro* in various concentrations of Boric acid-0.01%, 0.02%, 0.03%, 0.04%, 0.05% in 20% sucrose as standardized before as maximum germination was obtained in 20%. In M 1 and M 2 medium maximum pollen germination was observed in 0.04% boric acid+20% sucrose. In M 1 medium 22.22±2.22% after 96 hours and in M 2 medium 72.58±2.80% germination was recorded which were highest in the particular mediums. Though same kinds of results were not obtained in any of the species, *Jatrophacurcas* showed good germination at a nearby concentration of 0.03% Boric acid (F.Chen and S.Wang, 2010).

Most of the work done with boric acid by various authors revealed 0.01% in *Brassica olaracea* (Ferrare and Wallace, 1975) and also for *Zea mays* (Pfahler, 1968, Pfahler and Linkens, 1973), 100-200 ppm for *Solanum* species (Ravindra and Chouhan, 1980), 100 ppm for *Pistaciavera* (K sarpkaya *et al.*, 2010), 0.025% for *Teasle gourd* (AbhishekhNaik *et al.*, 2016).

Calcium chloride
Pollen grains were germinated *in vitro* in different concentrations of calcium chloride-0.01%, 0.02%, 0.03%, 0.04%, 0.05% in 0.04% boric acid+20% sucrose concentrations as standardized before. The result obtained in M 1 and M 2 medium shows that maximum pollen germinate in 0.03% calcium chloride. In M 1 medium 49.04±3.91% and in M 2 medium 55.35±1.23% germination was obtained in this concentration.

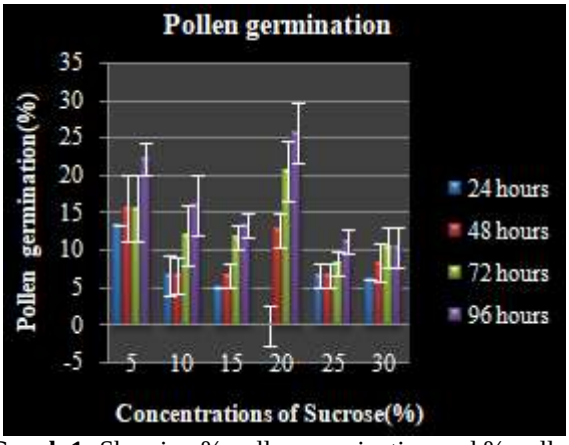
Jatrophacurcas required 0.04% calcium chloride (F.Chen and S.Wang., 2010). Similar result as obtained above was seen in *Plumeriaalba* which required 0.03% calcium chloride for best pollen germination (Shivanna and Johri, 1977). Some other reports obtained revealed 0.02% for *Crinum asiaticum* (Desai, 2008) and in *Solanumsurattense* (Patel, 2002), 0.04% in *Gossypiumhirsutum (*Rathod Zankhana, 2001).

Pollen tube length and pollen tube area
In 20% sucrose 31±2.08µm after 24 hours, 54±2.84µm after 48 hours and 107±1.52µm increase in length was observed. When Boric acid was added to 20% sucrose concentration, the length of pollen tube increased from 107±1.52µm to 128.16±1.58 µm after 72 hours. This reveals that boric acid at a concentration of 0.04% enhances pollen tube growth. When calcium chloride was added, the pollen tube length increased even more after 72 hours to 349.6±3.17µm. This fact establishes that concentration of calcium at 0.03% is beneficial for pollen tube growth.

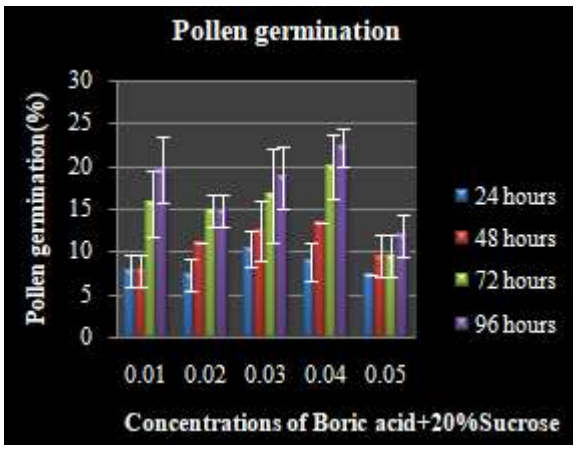
Camera Lucida was used for measurement of pollen tube area at 10X magnification. Using the camera Lucida pollen grains were drawn on the graph paper after an interval of every 24 hours. Photo plate-2 shows camera Lucida sketches of germinated pollen grains of *Jatropha integerrima Jacq*. at various stages.Subsequent increase in pollen tube area was observed from 24 to 96 hours. The area that was calculated was $10400\mu^2$, $12000\mu^2$, $14000\mu^2$, $15200\mu^2$ and $16400\mu^2$ respectively. The maximum area covered by the pollen tube was measured $16400\mu^2$ at 96 hours in *Jatropha integerrima Jacq*.

Comparison between two mediums (M1 and M2)
Two mediums were selected for pollen germination studies. Medium 1 was liquid culture medium and Medium 2 was a semi solid medium. Though in both the mediums the

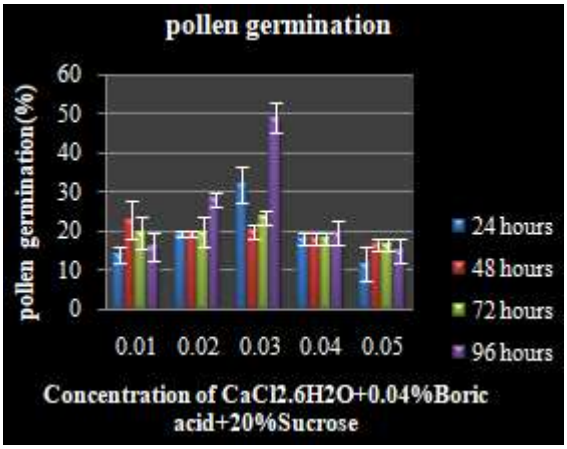
germination was obtained best in 20% sucrose, 0.04% boric acid and 0.03% calcium chloride, there was huge difference obtained between the germination rates. In 20% sucrose where maximum percentage of germination obtained was 77.37±1.95% after 72 hours in M2 medium, the maximum percentage obtained in M1 medium was 25.63±2.6%. Same kind of variations was observed with boric acid and calcium chloride in M1 and M2. This depicts that since M1 medium is a liquid culture medium, the pollen tube may be getting disturbed due to the movement of solution, or disturbance may be happening due to the touch of dropper during preparation of slides. Whereas M2 medium is semi solid medium where the pollen remains undisturbed and gets a solid base to grow upon. Also in M1 medium maximum bursting was observed, on the contrary in M2 medium not a single pollen had bursted. Pollen germination:



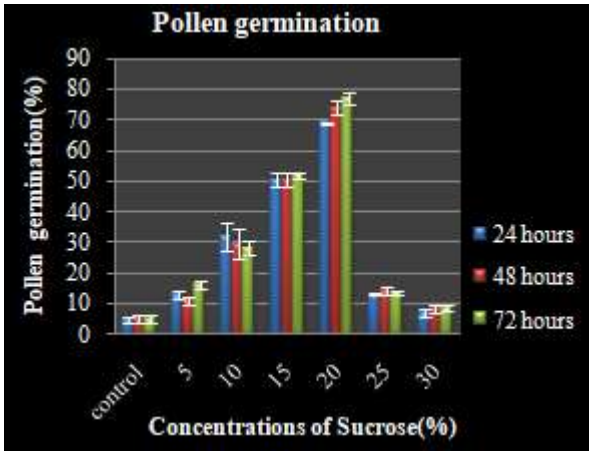
Graph 1: Showing % pollen germination and % pollen bursting under different concentration of Sucrose



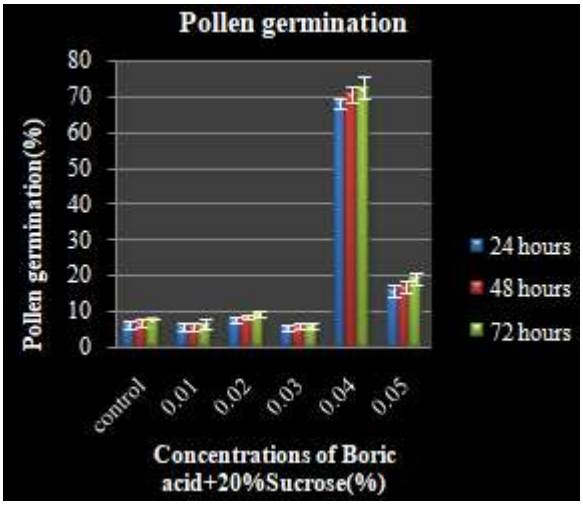
Graph 2: Showing % pollen germination and % pollen bursting under different concentration of Boric acid+20% Sucrose



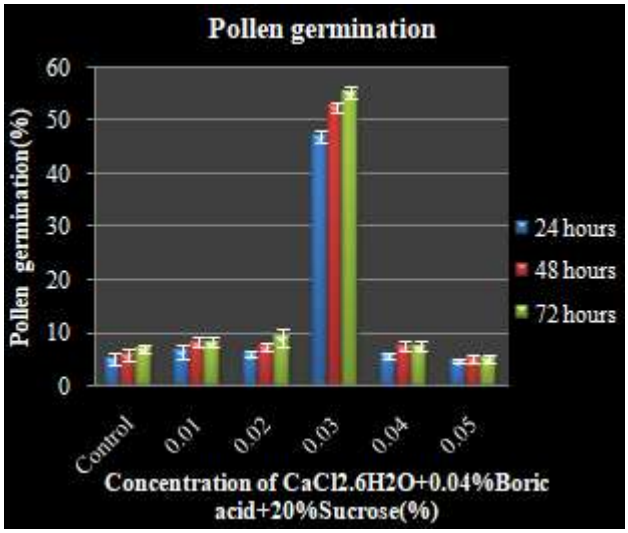
Graph 3: Showing % pollen germination and % pollen bursting under different concentrations of $CaCl_2.6H_2O$+0.04% Boric acid+20% Sucrose



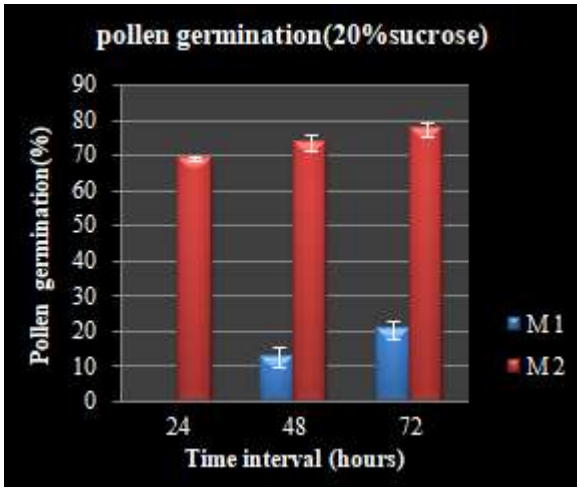
Graph 4: Showing percentage pollen germination (%G) under different concentrations of Sucrose (Semi solid agar medium)



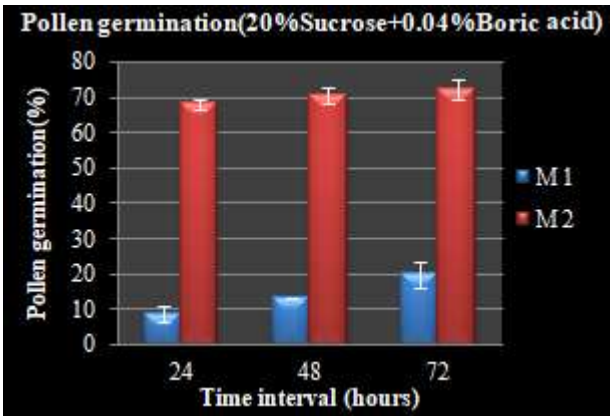
Graph 5: Showing percent pollen germination under different concentrations of Boric acid+20% Sucrose (Semi solid agar medium)



Graph 6: Showing percent pollen germination (%G) under different concentrations of CaCl2.6H2O+0.04% Boric acid+20% Sucrose (Semi solid agar medium)



Graph 7: Graph showing comparison of percentage germination between medium 1 and medium 2 in standardized Sucrose (20%) concentration



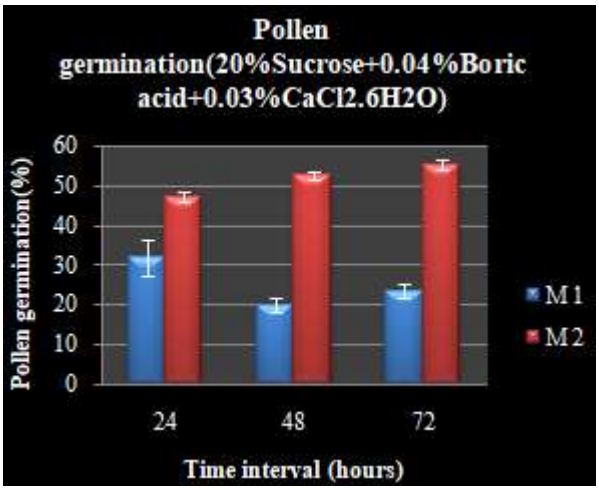
Graph 8: Graph showing comparison of percentage germination between medium 1 and medium 2 in standardized Sucrose (20%)+Boric acid (0.04%) concentration.

Table 2: Showing the Standardized medium for *Jatropha integerrima Jacq*. pollen grain

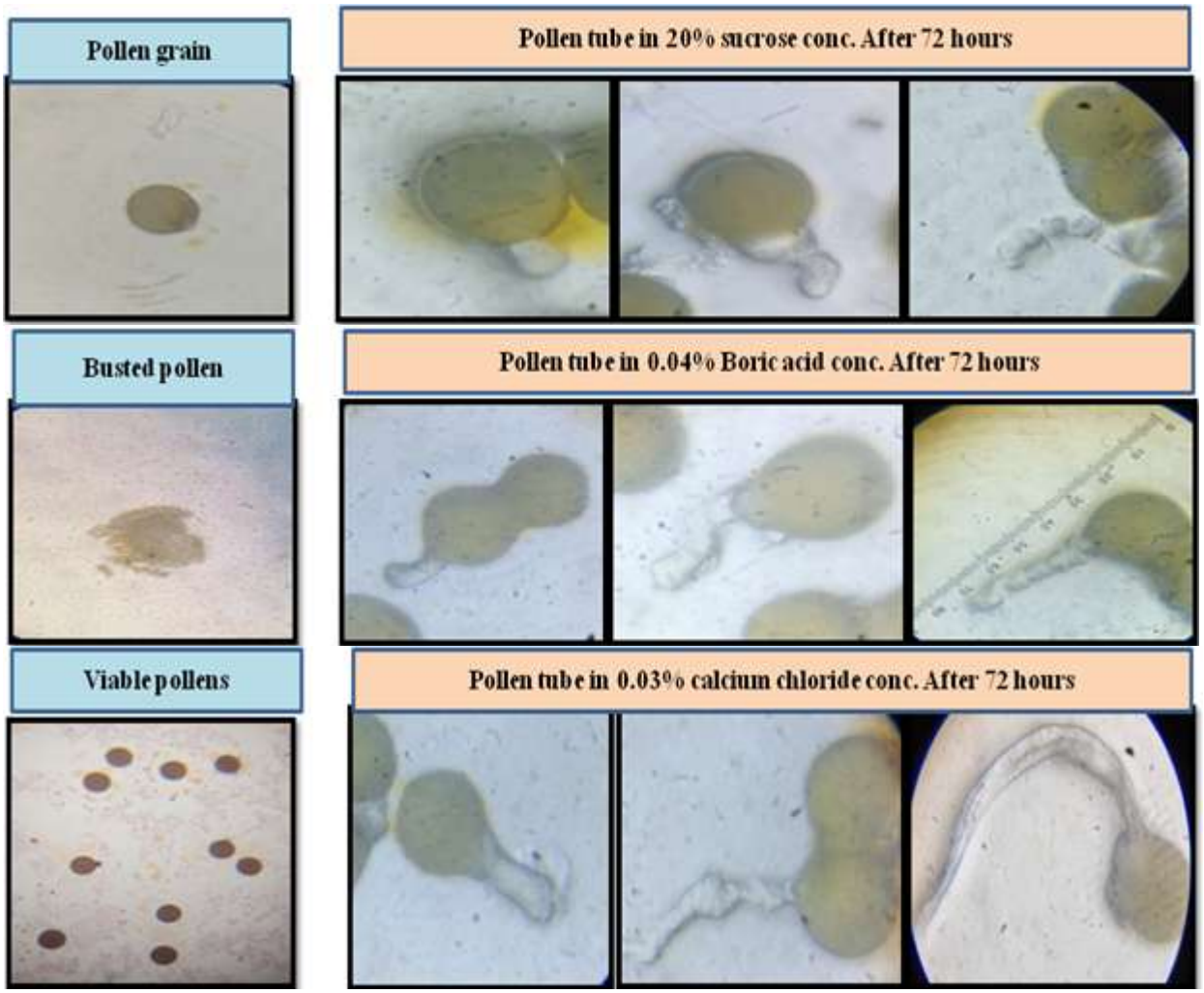
Sr. No.	Constituents	Concentration
1	Sucrose	20%
2	Boric acid	0.04%
3	Calcium chloride	0.03%

Table 3: Showing pollen tube length (µm) under the best concentrations of sucrose (20%), boric acid (0.04%) and calcium chloride (0.03%).

Concentrations	Pollen tube length (µm)		
	24 hours	48 hours	72 hours
20%Sucrose	31±2.08	54±2.84	107±1.52
20%Sucrose+0.04%Boric acid	49±0.57	86.6±0.60	128.16±1.58
20%Sucrose+0.04%Boric acid+0.03%$CaCl_2$	82.1±2.74	157.5±1.15	349.6±3.17



Graph 9: Graph showing comparison of percentage germination between medium 1 and medium 2 in standardized Sucrose (20%)+Boric acid (0.04%)+$CaCl_2.6H_2O$ (0.03%) concentration



4. Conclusion

1) For better results in *invitro* pollen germination experiments, the pollen must be collected during the anthesis stage of flower development as the pollens are maximum viable and in their best physiological conditions.
2) To obtain best *invitro* pollen germination, 20% sucrose, 0.04% boric acid and 0.03% Calcium chloride gives best results.
3) The M2 (Semi soild) medium gives better development of pollen tube as it remains undsiturbed and so can be recommended as the best method to assess the *invitro* pollen germination.

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