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In vitro pollen germination of selected important wild plants

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Abstract
Evaluate the functional quality of pollen aid appear in many approaches such as estimation the fertility of pollen or incompatibility, pollen vigor, pollen stigma interaction studies and for investigation of hybrid status. Main object of the work, to determine magnificent media for *in vitro* germination. *Senna occidentalis* L. (Fabaceae), *Argemone mexicana* L. (Papaveraceae) are wild plant. In *Senna occidentalis* L. after 60 min. highest germination and tube length reported are 89.38% and 73 % respectively in nectar of *Euphorbia tithymaloides* L., also superior result acquires in Brewbaker and Kwack (1963) media 88.56% germination and 71% tube length are reported and in Roberts *et al*. (1983) media 75% germination and 67% tube length are reported. *Argemone mexicana* L. (Papaveraceae) after 60 min. highest germination and tube length reported are 90.38% and 77% respectively in nectar *Euphorbia tithymaloides* L., also good result obtain in Brewbaker and Kwack (1963) media 92.55% germination and 76% tube length are reported and in Roberts *et al*.(1983) media 82% germination and 67% tube length are reported. In both species natural sugar of nectar performs as influencing factor.

Keywords: germination, pollen, approaches, factor

Introduction
Development of reliable methods for determining the functional quality of pollen it helps in monitoring pollen vigor during storage, genetics and pollen-stigma interaction studies, germination qualities crop improvement for breeding purpose, and incompatibility and fertility studies. Some of the pollen, which could not germinate usually, shows poor tube growth, is like ineffective in causing fertilization. *In vitro* germination tests have been used to indicate viability or germination efficiancy of pollen. In general case, there is a linear relationship between pollen viability and germination capability in many fruit species. High crop yield generally depends on viable pollen grains. Pollen fertility and viability have paramount importance in hybridization program. Pollen performance in terms of germinating ability may have the relative importance upon not only fruit-set, but also flower –flower and flower pollinators interaction. The basic needs for the improvements of plants before going to the breeding program are pollen quality, fertility, viability and its longevity. Aim of present investigation reveals the effect of different nutrients like; media Brewbaker and Kwack (1963), Robert's *et al*. (1983); natural substance such as nectar of *Euphorbia tithymaloides* L. on *in vitro* pollen germination and tube growth both *Senna occidentalis* L. (Fabaceae), and *Argemone Mexicana* L. (Papaveraceae).

Materials and Method
Pollen Grain Collection
In present work suspension culture method was followed. In this method, pollen grain in a large number are cultured in 2 to 10 ml of culture medium. Collection of flowers just after anthesis is primary requirement for the work. The flowers of *Senna occidentalis* L., (Fabaceae), *Argemone mexicana* L. (Papaveraceae) were collected from Omkar Vidhyalaya garden at Gandhinagar, India. Before the starting work, for desiccation pollen grain was dried under ambient condition (28 °C for 4 h). *In vitro* pollen germination have been required important factors for germination and tube growth, especially sucrose is essential, other factors are effects on tube length such as carbohydrates, boron, calcium, in some species magnesium sulfate, potassium nitrate, flavonols, enzymes, plant hormone, physical factors were analyzed.

Preparation of germination media
Under controlled laboratory conditions, previous testes were used to determine the best sucrose content (5, 10, 15, and 20%) during germination experiment. During the trials the result was establish that ideal sucrose concentration (10%) be used as experimental control media - M_1, it compares with two reliable media (1) Brewbaker and Kwack medium (1963)- M_2 and (2) Roberts *et al*. (1983) medium – M_3 were used for *in vitro* pollen germination. Compositions of M_2 and M_3 media are in given table no.1.1.

Table 1

Brewbaker and Kwack medium – M_2	
10%	sucrose
100 mg l^{-1}	boric acid
300 mg l^{-1}	calcium nitrate
200 mg l^{-1}	magnesium sulfate
100 mg l^{-1}	potassium nitrate
Roberts medium- M_3	
20%	sucrose
10 mg l^{-1}	boric acid
362 mg l^{-1}	calcium chloride
100 mg l^{-1}	potassium nitrate
60–130 mg l^{-1}	tris (buffer)

Another *euphorbia tithymaloides* L. flower nectar used as natural medium to examine influence of pollen germination and tube length. Flower collected from the Department of

Botany, Gujarat University at Ahmadabad. Fresh nectar direct collects from the young flower of *euphorbia. tithiymaloides* L. with the help of capillary glass tube and collect under watch glass.

After desiccation treatment, Senna *occidentalis* L., *Argemone mexicana* L. plants pollen grains were placed on media M_1, M_2, M_3 and nectar containing different accurate watch glass. The watch glass was kept in petri dishes with filter paper moistened with water for incubation period, at 28 ºC. The tube length of pollen considered at when tube longer than twice its diameter (Sousa, Santos & Rego, 2013) [4].

Result and Discussion

After 15 min incubation time, watch glass was observed under the microscope and further reading was taken in every 15 minute for 2 hours. The germination rate was determined for 100 pollen grain from the total treated pollen grains. *Aregemone maxicana* is 36%in 10% sucrose solution and maximum tube bursting 18% saw in 20% of sucrose solutionafter the 2 hours' incubation time. In *senna occidentalis* 34%in 10% sucrose solution and maximum tube bursting 22% saw in 20% of sucrose solutionafter the 2 hours' incubation time. Highest germination and tube growth were shown in nectar of *euphorbia tithymalodis* L., in *Aregemone maxicana* 90.38%, tube growth 77% maximum germination shows than of *Senna occidentalis* 89%, tube growth 73%. Comparatively percentage of bursting is very low in nectar after 2 hours.In Brewbaker and Kwack (1963) media good resulted simultaneously *Aregemone maxicana* germination 92%, tube growth 76% and in *senna occidentalis* germination was 88%, tube growth 71% and percentage of bursting is negligible. In Roberts *et al*., 1983 media minor resulted in both, in *Aregemone maxicana* 82% germination, tube growth 67% and of *senna occidentalis* is75%, tube growth 67% after 2 hours.

Conclusion

An elementary and composite media may be vital and initiate to germination of pollen.

Presence of different sugars (sucrose, fructose, glucose) under natural blend -nectar of *Euphorbia tithymaloides* effect to the pollen which influence the germination rate at high level. The other reliable media was effects on germination rate but germination rate reduce than natural bland. The present study concludes that the, pollen was germinated in particular condition and presence of appropriate nutrient content. In process of pollen germination water and pH is enhance the various enzymatic chemical reaction in pollen wall, sucrose was providing the energy for chemical reaction, calcium provides the turgidity of pollen tube, boric acid necessary for strength of pollen tube. All the component plays important role in germination.

There for in which media has appropriate content of this elements that's media best for pollen germination. On the basis of present experiment results, the maximum pollen was germinating in Brewbaker and Kwack standard media and also used the nectar as media in experiments of pollen germination. The present study helps in time saving of experiment of hybridization techniques.

Figure and charts

Table 2

Argemone maxicana L.			
Germination Media	**Percentage of germination (after 60 min.)**	**Percentage of tube length showing (after 60 min.)**	**Percentage of bursting**
10 % sucrose solution	36%	0%	13%
Brewbaker & Kwack (1963)	92%	76%	8%
Roberts *et al*. (1983)	82%	67%	14%
Nectar of *Euphorbia tithymalodis*	90%	77%	4%

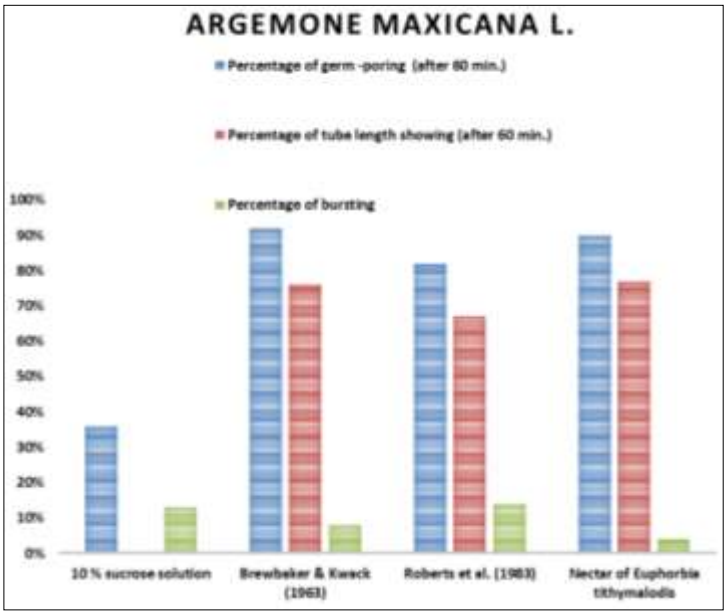

Fig 1

Table 3

Senna occidentalis L.			
Media	**Percentage of germ -poring (after 60 min.)**	**Percentage of tube length showing (after 60 min.)**	**Percentage of bursting**
10 % sucrose solution	34%	0%	11%
Brewbaker & Kwack (1963)	88%	71%	5%
Roberts *et al*. (1983)	75%	67%	19%
Nectar of *Euphorbia tithymalodis*	89%	73%	6%

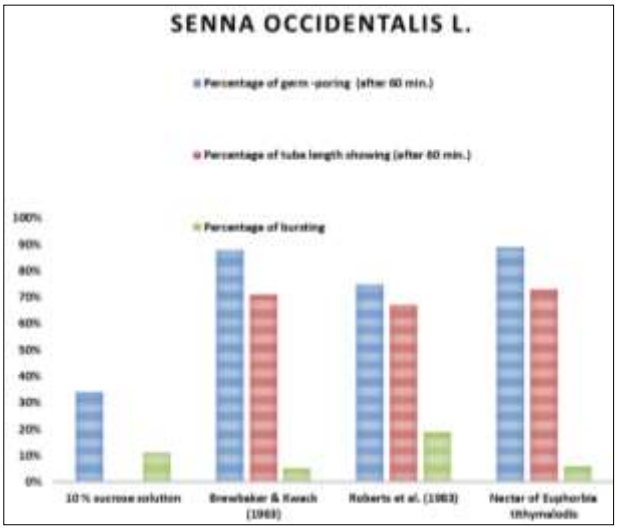

Fig 2

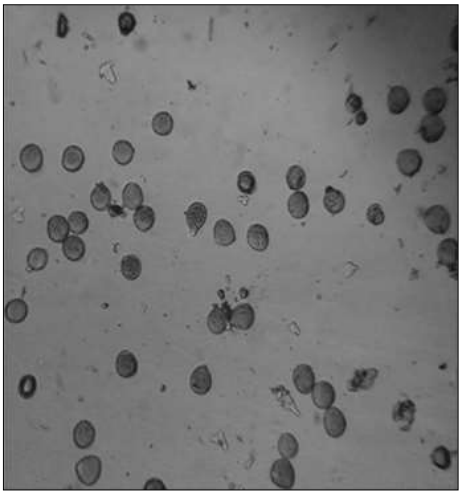

Fig 3: A. Mexicana in 10% sucrose solution

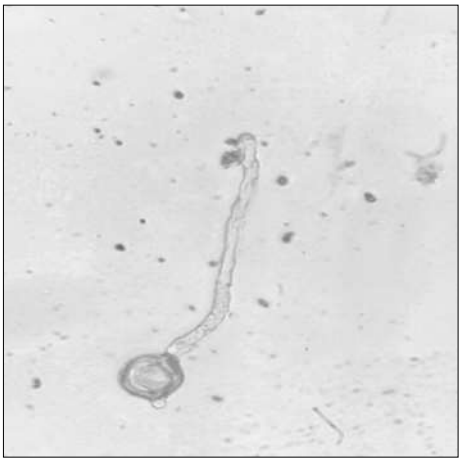

Fig 4: A. Mexicana in Brewbaker and Kwack media

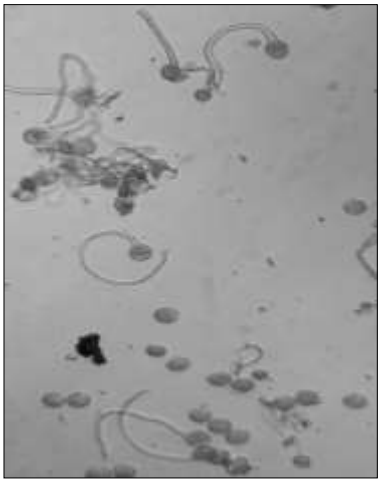

Fig 5: A mexicana in Roberts *et al*.

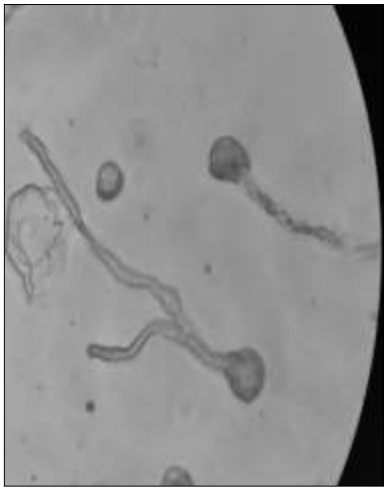

Fig 6: A. Mexicana in Necta

Fig 7: S. occdentalis in 10% sucrose solution

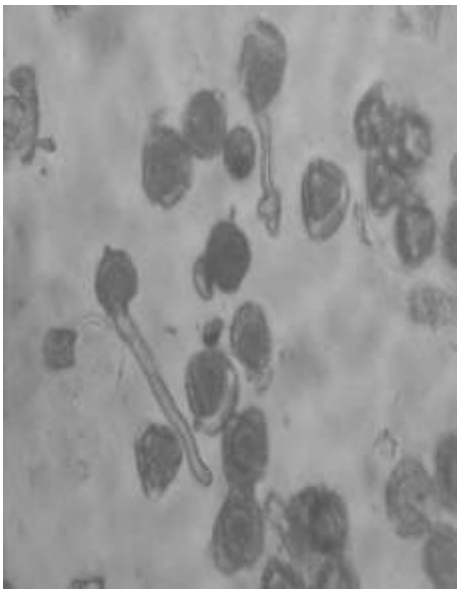

Fig 8: S occidentalis in Brewbaker and Kwack media

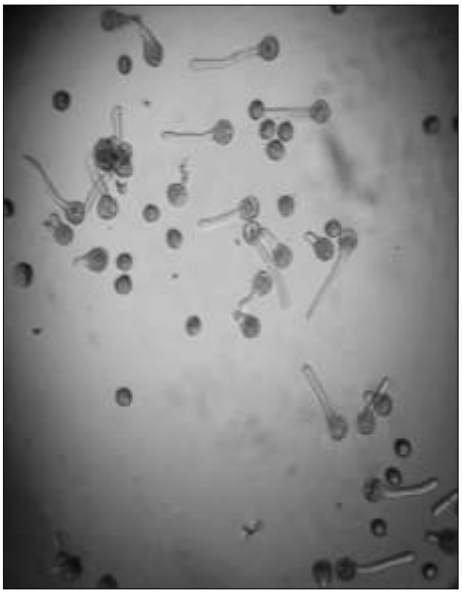

Fig 9: S. Occidentalis in Roberts, *et al*. Media

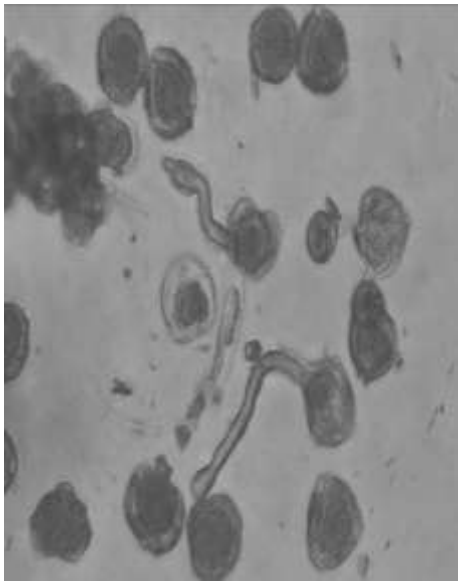

Fig 10: S. Occidentalis in Nectar

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