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Pollen biology of some members of Euphorbiaceae family

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ABSTRACT

Pollen biology in which in vitro germination supplemented with different modified concentrations of nutrient media, study of pollen tube growth, pollen morphology and pollen viability using various stains were studied in some members of Euphorbiaceae family viz. Acalypha indica L., Chrozophora prostrata Dalz & Gibs., Jatropha curcas L., Pedilanthus tithymaloides (L) Poir., Ricinus communis L., and Tragia plukenetii L. Results were analyzed and would be help in plant identifications.

Keywords: Pollen biology, Pollen germination and tube growth, Pollen viability, Euphorbiaceae.

INTRODUCTION

Euphorbiaceae is a complex heterogeneous family consisting of about 322 genera and 8900 species in the world. In India, this family is represented by 73 genera and 410 species. The family is essentially tropical and occurs in diverse habitats from arid regions to humid tropics [1]. Pollen germination and pollen tube growth are prerequisite for fertilization and seed development. In vitro techniques have therefore been used extensively on a variety of pollen system. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth [2,3,4]. The sitting drop method [5] was easy and generally used to determine the rate of pollen germination. Generally it requires water or sugar sources (like sucrose of different concentration) for pollen germination and tube growth. To study different aspects of pollen development and fertility or to determine the optimal time for pollination, *in vitro* techniques need to be improved so that pollen quality can be determined reliably. Two basically different approaches can be taken to estimate pollen viability: staining pollen with dyes and *in vitro* germination assay. Staining techniques aim to determine pollen enzymatic activity and membrane integrity. *In vitro* germination assay determine the actual germination ability of pollen under suitable conditions [6]. The viability, tube growth and germination related to pollen quality are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists, and growers.

Pollen characters have been extensively employed by Wendeld [7] for generic separation in his studies on the Primulaceae. Pollens may be successfully used for identification of annual *Ranunculus* in the absence of fruits.

The *in vitro* germination is the most widely used method of testing pollen viability in breeding programs [8]. The main components of the culture medium for pollen germination have been different sugars types and concentrations [9]. The sugar used in the culture medium aim to provide a balance between the osmotic solution and pollen germination and provide energy to assist the process of development of pollen tubes [10].

The aim of this work is to assess pollen viability using the staining technique and *in vitro* pollen germination and its tube length with different concentrations of sucrose in some members of Euphorbiaceae family with references to its some morphological features contributing to the knowledge of the reproductive biology and subsidizing their conservation, management and utilization.

MATERIALS AND METHODS

The flowers of selected materials from Euphorbiaceae family viz. *Acalypha indica* L., *Chrozophora prostrata* Dalz & Gibs., *Jatropha curcas* L., *Pedilanthus tithymaloides* (L) Poir., *Ricinus communis* L.,and *Tragia plukenetii* L. from different localities of Nagpur like University Campus, LIT Campus, Futala, Ravinagar etc. (Plate: I)

Pollen germination and pollen tube length:
It was carried out in different concentration of sucrose like (5% sucrose, 10% sucrose) alone, in combination of 6% sucrose with 0.01% boric acid in the surface and also in Brewbaker and Kwack's medium [11,2]. Pollen grains which had germinated and produced pollen tubes in the medium were recorded. Length of pollen tube in different concentration was recorded 15 mins after the commencement of germination with the help of stage and ocular and percentage of pollen germination calculated and the average length of pollen tube was recorded. Pollen grains were observed through an optical microscope (light microscope with 45X and 100 X magnifications) and were subsequently classified as fertile or not fertile.

Pollen viability:
Three different stains were used for pollen viability i.e., saffranin, anilin blue, and acetocarmine. A drop of saffranin, anilin blue, and acetocarmine was taken on a different micro slide. Small amount of pollen grains were suspended and slide was covered with a cover glass. Micro slides were incubated in dark for 5 minutes. After incubation period, pollen grains were observed under optical microscope (light microscope with 45X and 100 X magnifications) and were subsequently classified as fertile or not fertile as pollen grains, which turned red, were counted as viable.

Pollen Morphology:
For pollen morphological studies, the acetolysis method for pollen analysis [12] was followed. In which the pollen structures were observed through Carl Zeiss fluorescence microscope with 100 X magnification.

RESULTS AND DISCUSSION

The palynological study reveals that the characters of the pollen are useful in the phylogenetic study of the plants as these characters are reliable and fixed reproductive characters.

1. Pollen Germination:
Germination of pollen grains treated with the different media viz. 5% sucrose, 10 % sucrose, Brewbaker & Kwack's and boric & sucrose media. From this, it is concluded that *Chrozophora prostrata* showed highest germination in 5% sucrose solution media and in BK media, *Chrozhopora prostrata* and *Jatropha curcas* showed highest germination in 10% sucrose solution media while *Ricinus communis* and *Jatropha curcas* showed highest germination in Boric-Sucrose medium. (Table No. 1; Graph: 1, Plate II)

Table No.1: Pollen Germination in different media

Sr. No	Name of Plants	Total no. of pollen grains				Pollen grain germinated				% of pollen germinated			
		5%	10%	BK	B-S	5%	10%	BK	B-S	5%	10%	BK	B-S
1	*Acalypha indica*	520	640	488	458	440	516	155	279	84.6	80.6	31.76	60.91
2	*Chrozophora prostrata*	180	120	800	312	180	120	764	272	100	100	95.5	87.1
3	*Jatropa curcas*	592	120	96	68	396	112	94	68	81.0	93.3	97.91	100
4	*Pedilanthus tithymaloides*	400	120	84	192	224	100	64	120	56	83.3	76.1	62.5
5	*Ricinus communis*	392	440	1200	400	360	392	1112	384	91.8	89.0	92.6	96
6	*Tragia plukenetii*	400	280	104	80	364	248	84	72	91	88.5	80.7	90

***Note**: BK- Brewbaker and Kwack's medium, B-S: Boric- Sucrose Concentration*

2. Pollen Tube Growth:
In pollen tube growth, *Chrozophora prostrata* showed maximum growth in medium with 5% of sucrose. The pollen grains of *Jatropha curcas* showed well growth in medium with 10% of sucrose, Boric-Sucrose medium and in BK medium. (Table No. 2; Graph: 2, Plate II).

3. Pollen Viability Test:
Many workers have been worked out on the viability exercises with the help of different staining media. Lyra et al., [13] worked out pollen viability for various purpose with the help of different colourant agents such as 1% Acetocarmine to detect cytoplasmic content; 0.05% aniline blue (synonym: cotton blue) to detect callose in pollen walls and 2% acetic orcein, a micronuclei stain. With reference to this work, pollen viability was carried out with the help of different colouring agents viz. aniline blue, safrannin and 1% acetocarmine stain. Only 1% acetocarmine

stain gave clear results. Amongst the all plants the pollen grains of *Chrozophora prostrata* recorded as the highest percentage of viability while *Ricinus communis* as the lowest. Remaining showed average response. (Table No. 3)

Table No. 2: Pollen tube Growth in Various Media

Sr No.	Name of Plants	Pollen tube length in Various Media (in µm)			
		5%	10%	B.K	B-S
1	*Acalypha indica*	16.25	33.3	37.12	57.12
2	*Chrozophora prostrata*	52.35	38.07	38.07	38.07
3	*Jatropa curcas*	71.4	65.6	114.24	71.4
4	*Pedilanthus tithymaloides*	32.84	57.12	42.84	50.65
5	*Ricinus communis*	47.12	43.67	59.49	37.12
6	*Tragia plukenetii*	61.8	52.56	65.98	53.45

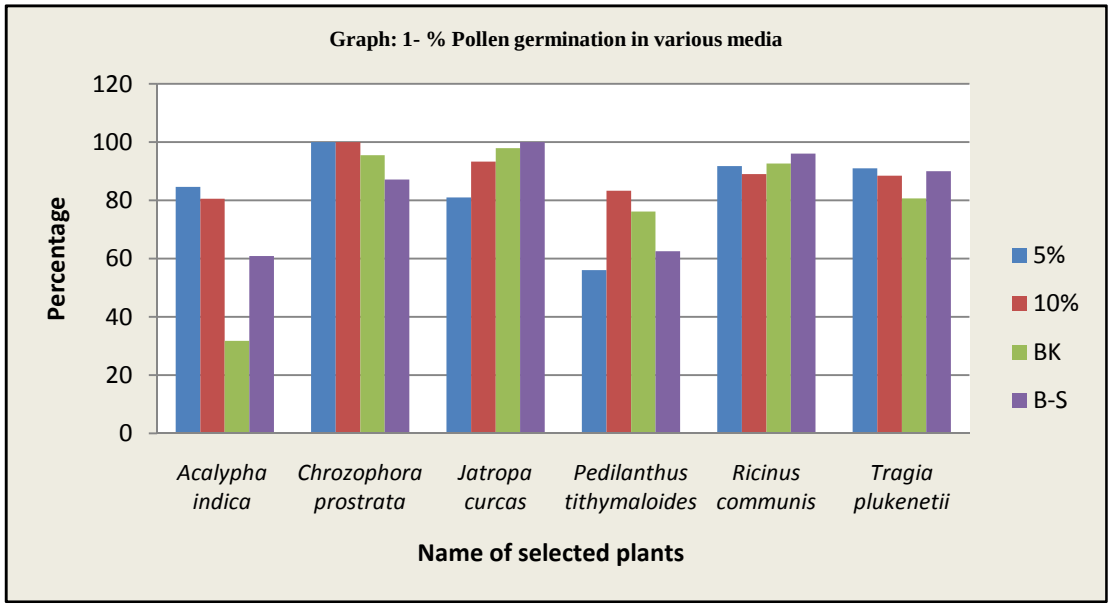
***Note**: BK- Brewbaker and Kwack's medium, B-S: Boric- Sucrose Concentration*

Table 3 : Pollen Viability Test

Sr. No	Name of Plants	Total no of pollen	No. of viable pollen present	% of viability
1.	*Acalypha indica*	96	60	62.5%
2.	*Chrozophora prostrate*	80	80	100%
3.	*Jatropa curcas*	68	48	70.58%
4.	*Pedilanthus tithmaloides*	256	216	84.37%
5.	*Ricinus communis L.*	1280	320	25%
6.	*Tragia plukenetti*	896	496	55.35%

4. Pollen Morphology:

The prominent pollen characters of the family Eubhorbiaceae are, pollen grains usually radially symmetrical, generally 3-colporate (upto 6-7), *Ricinus communis*, and *Pedilanthus tithymaloides* having shape more or less triangular. While *Jatropha curcas*, *Tragia plukenetii*, and *Acalypha indica*, showed spheroidal to oblate-spheriodal to suboblate in shape while *Chrozophora prostrata* having quadrangular shape. The pollen grains size varies from 15 x 12µm to 94.5 x 90µm in all taxa. In case of colpi, all the taxa range from 3-7 colporate.



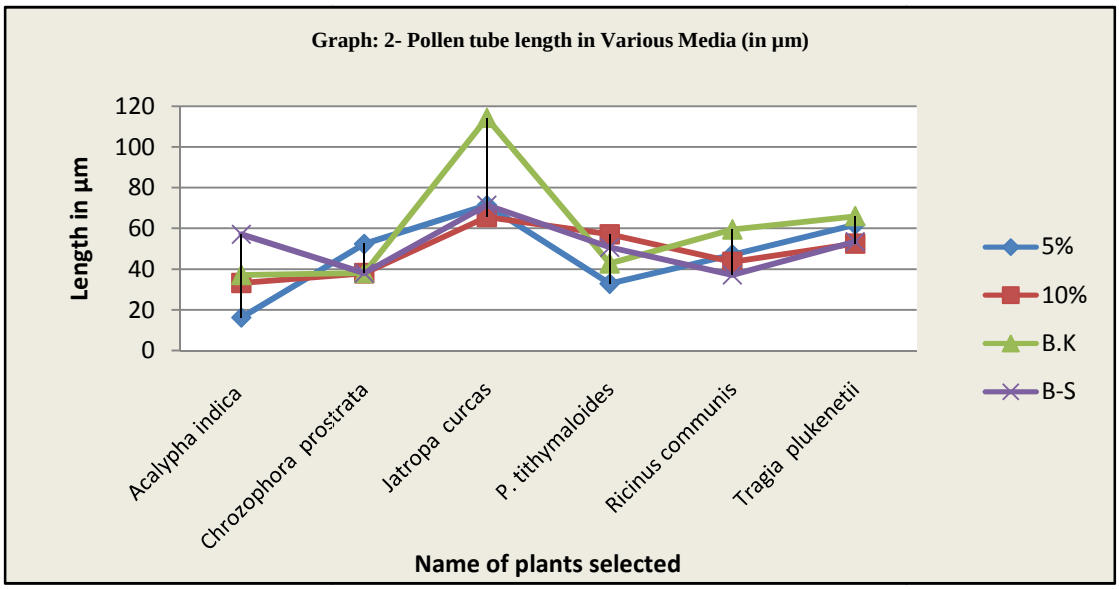
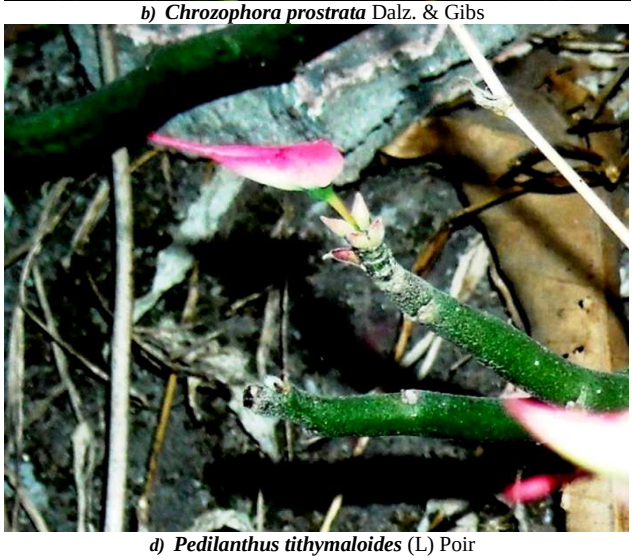


PLATE: I

a) ***Acalypha indica*** **L**

b) ***Chrozophora prostrata*** Dalz. & Gibs

c) ***Jatropa curcas*** **L**



d) ***Pedilanthus tithymaloides*** (L) Poir

e) Ricinus communis L

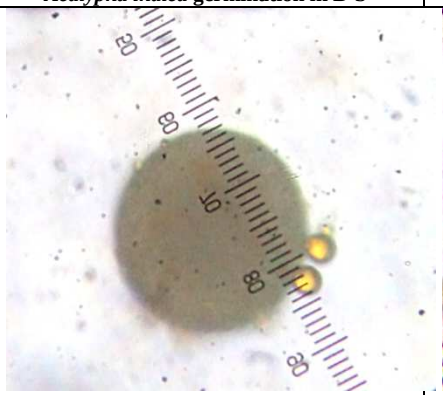
f) Tragia plukenetti L

PLATE: II

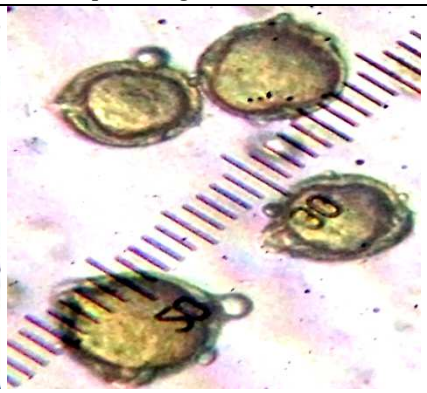
***Acalypha indica* germination in B-S**

***C. prostrata* germination in 5%**

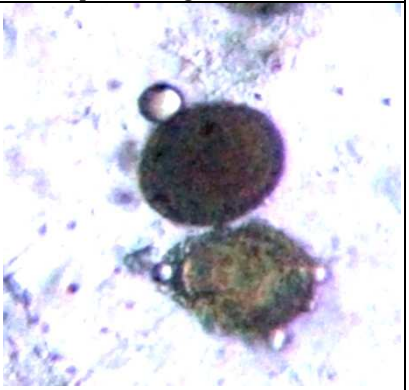
***Jatropha curcas* germination in 5%**



***Jatropha curcas* germination in B-S**

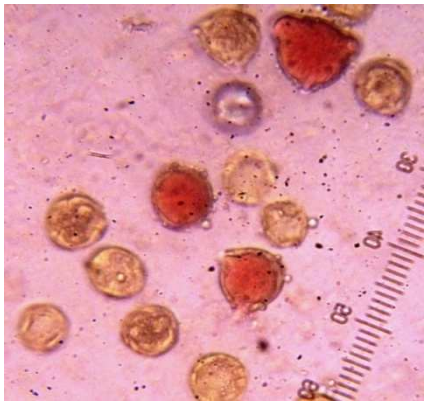


***Ricinus communis* germination in B-S**

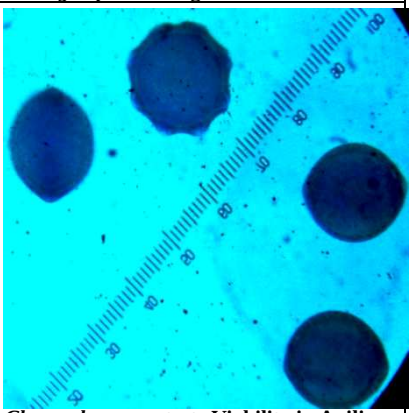


***Tragia plukenetii* germination in B-S**

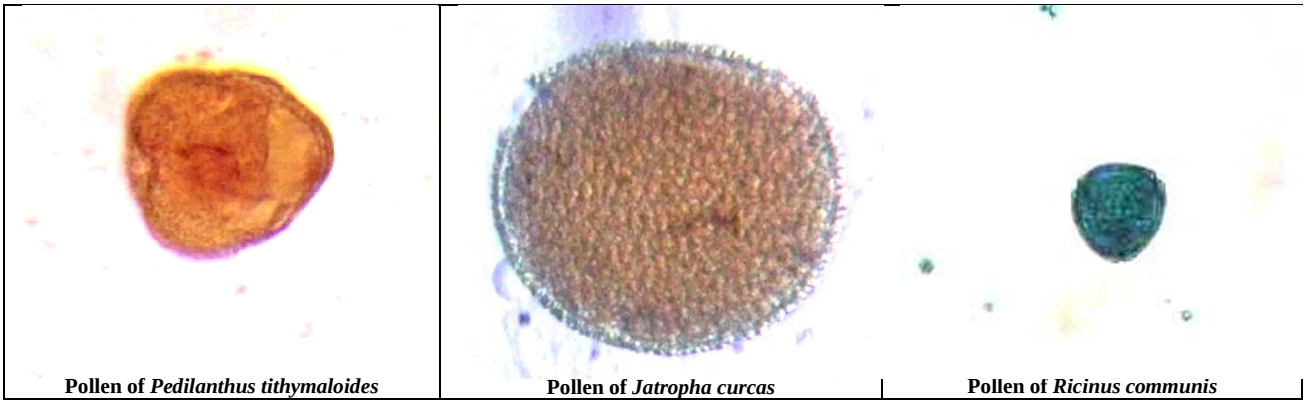
***Jatropha curcas* Viability in acetocarmine**



***Ricinus communis* Viability in acetocarmine**



***Chrozophora prostrata* Viability in Aniline**



Pollen of *Pedilanthus tithymaloides* | **Pollen of *Jatropha curcas*** | **Pollen of *Ricinus communis***

CONCLUSION

The palynological study reveals that the characters of pollens are useful in the phylogenetic study of the plants as these characters are reliable and fixed reproductive characters. From above pollen physiological observations, germination of pollen grains, it is concluded that *Acalypha indica, Chrozophora prostrata, and Tragia plukenetii showed* highest germination in 5% sucrose solution media, *Chrozophora prostrata, and Pedilanthus tithymaloides* showed highest germination in 10% sucrose solution media while *Ricinus communis and Jatropha curcas* showed highest germination in Boric-Sucrose medium. According to [13], in *Jatropha ribofolia* and *Jatropha mollissima* the rate of germination is higher in medium with 10% of sucrose for both the species because the trend is that the sucrose concentration increases the supply of carbon, changes the osmotic potential and inhibits the formation of pollen tube *in vitro* while from our observations in *Jatropha curcas* provided with the concentration of Boric and Sucrose it showed higher germination. In same way, [14] investigated *in vitro* maturation and germinations of *Jatropha curcas* microspores in China providing with different media concentration supplemented with the variations in BK and MS salts, with the effect of different factors viz. light, temperature, cold storage, boric acid concentrations, calcium ions, vitamin B_1, coconut water etc.

In case of pollen tube growth, the highest growth occurred in *Jatropha curcas* in BK medium than other selected plants. Lyra et al [13] investigated highest rate of pollen tube growth in *Jatropha ribofolia* and *Jatropha mollissima* in medium with 10% of sucrose. *Chrozophora prostrata* showed maximum growth in medium with 5% of sucrose. The pollen grains of *Pedilanthus tithymaloides* showed well growth in medium with 10% of sucrose. *Ricinus communis* along with *Tragia plukenetii* pollen grown in BK medium. While remaining pollen grains of *Acalypha indica* showed maximum tube growth in Boric-Sucrose medium.

From all these, this study enlightened the developmental aspects about the pollen grains of selected taxa of family Euphorbiaceae in the manner of pollen physiological aspects like pollen germination records in different medias, pollen tube growth and its analysis, the viability of pollen grains. It is very supportive in the context of the system of classification and in the identification of plants.

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