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EFFECT OF SUCROSE, BORON, CALCIUM, MAGNESIUM AND NITRATE DURING *IN VITRO* POLLEN GERMINATION IN *LUFFA AEGYPTICA* MILL.

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ABSTRACT

A study on *in vitro* pollen germination and pollen tube growth was made in *Luffa aegyptica* Mill. belonging to the family Cucurbitaceae. A modification of the basal medium of Brewbaker and Kwack was effected to get maximum percentage of pollen germination and tube growth in this taxon. Modified basal medium contains 10% sucrose, 50mg H_3BO_3, 200mg $MgSO_4.7H_2O$ and 350mg $Ca(NO_3)_2.H_2O$ in 1000 ml distilled water. The pH of the medium was adjusted to 6.0. The maximum percentage of pollen tube growth is 96.60% and bursting of pollen grain is 3.40%. Attempts were also made to study the effects of sucrose, boron, calcium, magnesium and nitrate during *in vitro* pollen germination.

Key words: *Luffa aegyptica, sucrose, boron, calcium, pollen germination.*

INTRODUCTION

Pollen grains are very specialized and complex plant cells. Pollen tube formation is a good and simple model of growth and development [1]. Thus pollen germination and growth of pollen tube are important research materials for morphological, physiology, biotechnological, ecological, evolutional, biochemical, molecular and biological studies [2]. *In vitro* pollen germination is an effective technique for understanding the basic [3,4] and applied aspects of pollen biology [5,6]. During *in vitro* pollen germination and tube growth not only the enzyme activity [7] but also the effect of Boron [8], calcium[9] , hormones[10] , light [11] and other factors were studied for different plants. Satisfactory pollen germination requires sugar, especially sucrose [12] , with other substances. It is generally known in pollen tube culture that externally supplied sugars are concerned in controlling osmotic pressure of the medium and at the same time serving carbohydrate for the growth of pollen tubes [13]. It is found that boric acid has pronounced stimulatory effect on germination and pollen tube growth [1,14]; Ca^{2+} plays a key role in the regulation of pollen tube growth[15]. The present study describes the development of a pollen germination media, and technique that provides high pollen germination level and improved pollen tube growth. The aim of the present study is firstly to find the suitable material for *in vitro* pollen germination tests for further biological studies about the effect of different kinds of chemicals such as Sucrose, Boron, Calcium and Magnesium on pollen germination and secondly to find out the concentration of sucrose, boron, calcium and magnesium required in the culture medium for optimum pollen germination.

MATERIAL AND METHODS

Luffa aegyptica Mill. belonging to family Cucurbitaceae is selected for standardization of culture medium for the optimum pollen germination and tube growth. For the present study, the dehisced flowers were collected in the morning from the plants growing in the botanical garden of the college.

In hanging drop and sitting drop culture methods , only a small volume of culture medium and a small quantity of pollen can be used; consequently, neither method is suitable for physiological and biochemical studies which require large amount of pollen suspension. The response of pollen in drop cultures is often erratic. So in present work suspension culture method is followed. In his method, pollen grains in a large number are cultured in 5 ml of culture medium in watch glasses. Pollen grains are germinated in media having different concentrations of sucrose i.e. 5,10,15,20, and 25% along with different quantities of boric acid (H_3BO_3), calcium nitrate ($Ca(NO_3)_2.H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) to find out maximum percentage of germination. Thus Brewbaker and Kwack's basal medium is modified for *in vitro* pollen germination and tube growth in *Luffa aegyptica*. The composition of the modified basal medium is given in Table 1.

Table - 1 Composition of Brewbaker & Kwack medium and Modified basal medium.

Contents	Composition of medium as per Brewbaker & Kwack (mg/l)	Composition of medium after modification (mg/l)
Sucrose	100,000	100,000
H_3BO_3	100	50
$Ca(NO_3)_2.H_2O$	300	350
$MgSO_4.7H_2O$	200	200
pH	7.3	6.8

Five replicates of each treatment were noted after every 15 minutes interval up to 120 min. Later on the number of germinated pollen grains, bursted pollen grains and pollen showing tube growth *i.e.* elongation of pollen tube, visible under microscopic field, are counted to calculate respective percentage. Table 2 clearly indicates that in the modified basal medium the percentage of pollen germination is more as compared to that of in the medium suggested by Brewbaker and Kwack (1963). Thus the composition of modified basal medium is used in the present study.

Emergence of pollen tube from pollen grain indicates pollen germination while elongation of tube indicates pollen tube growth. It may possible that all the germinated pollen grains do not show tube growth and hence, both the parameters are taken into the consideration in present work.

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RESULTS

Pollen grains of *Luffa aegyptica* Mill. are oblate, spherical, 3-colporate with smooth exine. During germination some time pollen shows polysiphonous condition (Fig. 3). Tubes mostly grow straight (Fig. 2). During initial period of germination there is a steep increase in the length of pollen tube which slows down as the time approaches. Pollen grain of 120 min. stage of germination shows 1287 µm length. (Fig. 1)

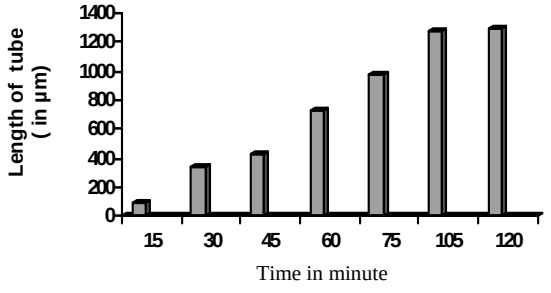

Fig. 1 Growth of pollen tube at various stages in *Luffa aegyptica*

Effect of Sucrose

In the basal medium containing 10% sucrose maximum percentage of germinated pollen grains is 94.50% and maximum percentage of pollen showing tube growth is also 94.50% at 60 min. stage. Whereas the percentage of bursting of pollen grain is 2.70%. After 60 min., there is a decrease in the percentage of germinating pollen and pollen showing tube growth with a simultaneous increase in the percentage of bursting pollen grains (Fig 4 -A).

Effect of Ca $(NO_3)_2$ (Calcium nitrate)

It is observed that if the amount of calcium nitrate is increased in the medium, the percentage of germination is also increased. Pollen grains were germinated by varying the quantity of calcium nitrate in 10% sucrose medium. Maximum germination was reported in 350mg/l calcium nitrate. At 60 min. the percentages of germinated pollen grains and pollen showing tube growth are maximum i.e. is 95.32 % and pollen bursting is minimum i.e. 3.70% Fig 4 -B). Later on as the time approaches percentage of germination decreases.

Fig. 2: Pollen showing tube growth

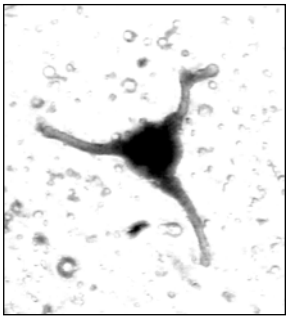

Fig. 3 Pollen showing three tubes

Effect of Boric acid (H_3BO_3):

Boric acid is used as a source of boron. The maximum number of pollen grains is germinated in the 10% sucrose medium containing 350mg $Ca(NO_3)_2$. H_2O and 50mg boric acid in 1000ml distilled water. At 60 min the percentages of germinated pollen grains are maximum (96.60%) and the percentage of bursted pollen grain is 3.40%. After 60 min., the percentage of germinated pollen grain decreases and bursting increases (Fig. 4 -C).

Effect of Magnesium Sulphate:

Maximum pollen grains were germinated in the medium containing 10% sucrose, 50mg boric acid, 350mg calcium nitrate and 200mg magnesium sulphate. Percentage of pollen bursting is 3.40% at 60 min. (Fig. 4 -D).

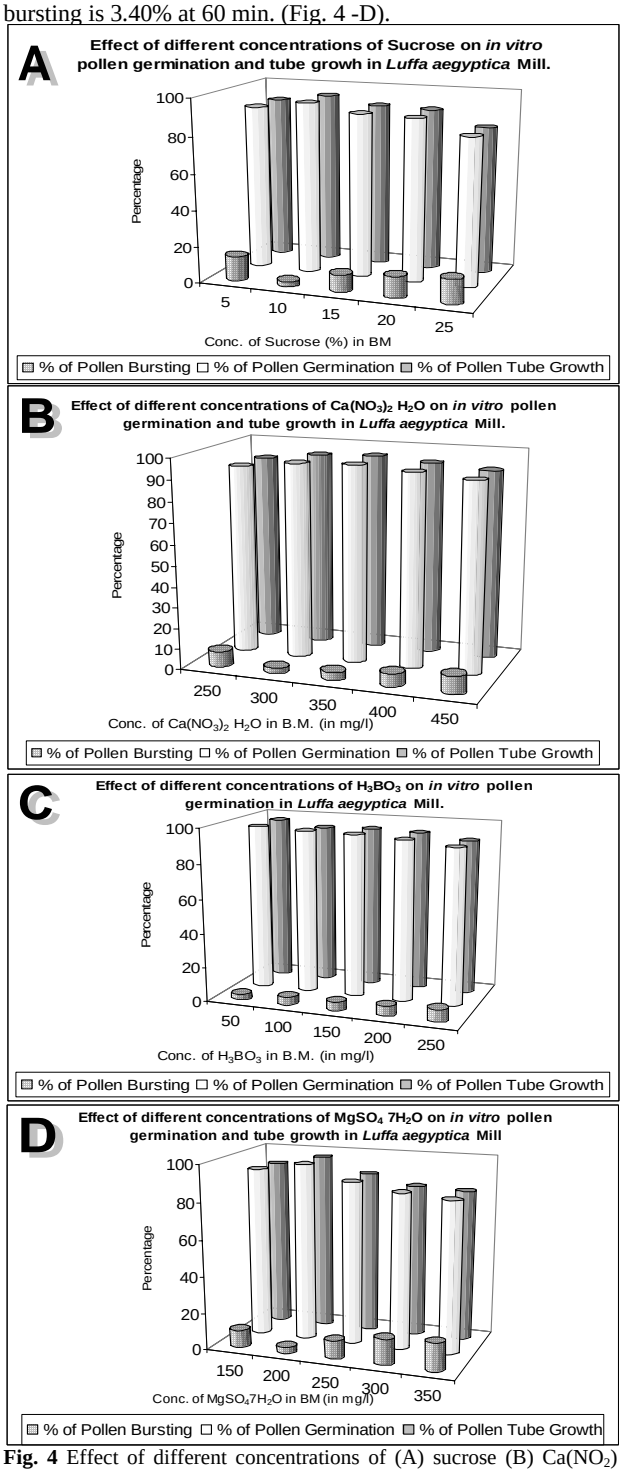

Fig. 4 Effect of different concentrations of (A) sucrose (B) $Ca(NO_2)$ H_2O (C) H_2BO_3 and (D) $MgSO_4$. $7H_2O$ on *in vitro* pollen germination and tube growth in *Luffa aegyptica* Mill. at 60 min. stage

Table - 2 *In vitro* pollen germination in Brewbaker and Kwack (1963) and Modified basal medium in *Luffa aegyptica* Mill.

Sr. No.	Time in Minutes	Brewbaker and Kwack (1963) basal Medium			Modified basal medium		
		Percentage of Germination	Percentage of Bursting	Percentage of tube Growth	Percentage of Germination	Percentage of Bursting	Percentage of tube Growth
1	15	59.10	0.00	49.46	85.84	1.80	74.52
2	30	81.44	1.03	72.16	90.00	2.70	90.00
3	45	83.30	1.90	82.40	92.56	3.30	92.56
4	**60**	**94.50**	**2.70**	**94.50**	**96.60**	**3.40**	**96.60**
5	75	93.30	4.80	93.30	94.80	5.20	94.80
6	90	91.90	5.10	91.90	93.23	5.30	93.23
7	105	88.76	7.80	88.76	91.50	6.60	91.50
8	120	86.27	8.82	86.27	90.56	8.50	90.56

DISCUSSION

Composition of culture medium for *in vitro* pollen germination as suggested by Brewbaker and Kwack[16] has not proved to be good for all plant materials and hence, in present work it has been modified for *Luffa aegyptica* Mill.

The modified culture medium prepared in 1000 ml distilled water contains 10% sucrose, 50 mg boric acid, 350 mg calcium nitrate, and 200 mg magnesium sulphate. pH of the medium was adjusted to 6.0. The improved medium can be useful in fundamental and applied studies on the pollen biology of *Luffa aegyptica Mill.*.

Pollen grains are known to be packed with biochemicals like sugar, starch, lipids and phytic acid [17] . These storage products get metabolized upon germination and elongation of pollen tube. Thus, they play an important role in germination and in initial stages of pollen tube growth [18, 19] . Intake of the culture medium by the pollen grains initiates mobilization of the stored substances resulting into germination of pollen grains. According to Baker and Baker [20] the required energy for the germination of pollen grains, formation of cell wall components and callose in angiosperms is provided from the nutrient reserves like starch, sugar and lipids stored in pollen grains.

Optimum concentration of sucrose required for maximum pollen germination varies from species to species. For example 10% in *Najas marina* [21] and in *Tradescantia paludosa* [22] , 15% in *Zea mays* [23] and in *Avocado* cultivars[24] ; 11 to 15% in *Asclepiads syriaca* [25] ; 30% in *Catharanthus roseus* [12] and in *Abelmoschus esculents* [26]; 50% *Pistacia vera* [27]and 7.5 to 20% in some *sp*. of Cucurbitaceae family [28] . In the present work the optimum percentage of pollen germination is reported in 10 % sucrose concentration. In *Luffa aegyptica* 10% sucrose in the medium limits the diffusion rate of water into the pollen and thus prevents the bursting of pollen grains.

Boron is known to stimulate pollen tube growth in higher plants [29]. It reduces bursting of pollen tubes and enhances percentage of germination [30,31]. Vasil[28] also investigated the effects of sucrose and boric acid in *Cucumis melo* var. *utilissimus* from Cucurbitaceae, and in this species, optimum germination and tube length were obtained with 20% sucrose and 0.01% boric acid. He found that boric acid concentration higher than 0.02% was toxic for the species. In *Luffa aegyptica* (Cucurbitaceae) optimum germination and tube growth occurred with 10% sucrose and 50 mg/l boric acid. Requirement of higher amount of boric acid indicates that the pollen grains of *Luffa aegyptica* are in deficiency of boron which has to be supplied exogenously. Boric acid concentrations higher than 50 mg/l decreases pollen growth and increases bursting of pollen grain.

Calcium is another inorganic substance with notable effect on pollen tube growth. Ca^{2+} is an essential requirement of pollen tube growth [32]. It has been shown that calcium controls the permeability of pollen tube membrane [33]. Absence of calcium in the medium results in an increase in the membrane permeability leading to the loss of internal metabolites [34]. In the present work, the pollen of *Luffa* exhibited maximum pollen germination and tube growth at a higher concentration of calcium nitrate i.e. 350mg/l than that in the original Brewbaker and Kwack medium. It seems that pollen grains of *Luffa aegyptica* contain very small amount of calcium which may diffuse out of the pollen rapidly in the medium. Higher concentration of calcium in the medium prevents diffusion of the calcium from the pollen. Thus supplementation of calcium in the medium lead to development of straight and rigid pollen tube with vigorous growth. A positive correlation between speed of pollen tube growth and quality of the resulting progeny is also explained [35].

The role of magnesium in pollen germination and tube growth is not clear. According to Brewbaker and Kwack [16] magnesium ions enhance the effect of calcium ions resulting in vigorous growth of pollen tube. For *Luffa* pollen 200 mg/l of magnesium sulphate is better for optimum germination.

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