ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 6, pp. 11985-11989, June, 2016

International Journal of Recent Scientific Research

Research Article

STUDIES ON *IN VITRO* POLLEN GERMINATION AND TUBE DEVELOPMENT OF *BORASSUS FLABELLIFER* L.

Hemanta Saha[1], Subrata Mondal[2]* and Sudhendu Mandal[2]

[1]Suri Vidyasagar College, Suri-731101, Birbhum, West Bengal, India
[2]Department of Botany, Visva-Bharati, Santiniketan-731235, West Bengal, India

ARTICLE INFO

Article History:

Received 05th March, 2016
Received in revised form 08th April, 2016
Accepted 10th May, 2016
Published online 28st June, 2016

Key Words:

Pollen germination, sucrose, boric acid, salts, *Borassus flabellifer* L.

ABSTRACT

Present investigation deals with the effect of different nutrients like Sucrose, Boric acid (H_3BO_3) and salts like Calcium nitrate [$Ca(NO_3)_2$], Magnesium sulphate ($MgSO_4$) and Potassium nitrate (KNO_3) on *in vitro* pollen germination of *Borassus flabellifer* L.(Arecaceae), a plant with immense economic importance. It flowers during March to May which open in early morning (05:30hrs - 06:30hrs).Considerable pollen germination (65%) and tube development (990µm) occurred in 8% Sucrose solution while 100 ppm Boric acid showed 55% germination along with 394µm long pollen tube. However, maximum germinating pollen (80%) along with pollen tube development (1350µm) was observed in 8% Sucrose solution supplemented with 100 ppm Boric acid. Among the salts, maximum 50% pollen germination along with 270µm long pollen tube developed in 200 ppm Magnesium sulphate solution following 41% pollen germination along with 198 µm pollen tube in 200 ppm Calcium nitrate and 32% pollen germination along with 126 µm long pollen tube in 100 ppm Potassium nitrate solution.

INTRODUCTION

Pollen grains carry the male genetic material to the female part of a flower, thus plays a vital role in reproduction and help in successful fruit-set. Pollen fertility and viability have a paramount importance in hybridization programme as high crop yield generally depends on viable pollen grains.

Though, stigma provides suitable site for pollen germination, but studies on *in vivo* are not easily feasible due to the complications in the pistillate tissues. However, it is possible to germinate pollen grains of a number of taxa using rather a simple nutrient medium and to achieve a reasonable length of tube growth. The knowledge on physiology and biochemistry of pollen germination and tube growth comes largely from *in vitro* studies.

Pollen germination and pollen tube growth are essential processes that ensure the productivity of flowering plants. The complex processes involve a number of signaling events; including cell-environment interaction, intercellular communication and intracellular signaling (Taylor and Hepler, 1997; Franklin-Tong, 1999). Physiological and molecular mechanisms of regulation of pollen germination and pollen tube growth have been extensively studied in the past (Heslop Harrison, 1989; Taylor and Heplar, 1997; Franklin –Tong, 1999).

In vitro pollen germination is an effective technique for understanding the basic (Heslop Harrsion, 1989; Mascarenhas, 1993) and applied aspects of pollen biology (Herreo, 1991; Kristen and Kappler, 1990). Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth (Johri and Vasil, 1961, Shivanna and Johri, 1989; Heslop Harison, 1989). Pollen germination studies involve assessment of several nutrients which are stimulants for pollen germination and pollen tube growth (Brewbaker and Kwack, 1963; Imani *et al*., 2011) suggested that, the culture medium should contain in addition to Carbohydrates germination-stimulating substances such as Boric acid, Calcium nitrate, Potassium nitrate, Magnesium sulphate etc. So, *in-vitro* pollen germination assay was conducted to determine the effect of different nutrients like Sucrose, Boric acid, Calcium nitrate, Magnesium sulphate and Potassium nitrate at various concentrations on pollen germination of *Borassus flabellifer* L.

Borassus flabellifer L. is a plant of various economic importance and is used to produce beverage, sweet sap, gur etc. which are mainly from the sweet sap obtained by tapping or cutting the tip of the inflorescence. The naturally fermented sweet sap is used as a beverage. Fruit is stomachic, aphrodisiac, tonic, laxative, alexiteric; improves taste and allays thirst. Milky juice from immature fruit checks hiccup sickness. Pulp from the immature fruit is diuretic, demulcent and nutritive.

*Corresponding author: **Subrata Mondal***
Department of Botany, Visva-Bharati, Santiniketan-731235, West Bengal, India

The juice of the young leaves mixed with water is given in cases of dysentery. Root is cooling and restorative and useful in leprosy (Chopra *et al.,* 1956; Karuppusamy *et al*., 2011)

MATERIALS AND METHODS

For the study of *in vitro* pollen germination, fresh flowers of *Borassus flabellifer* L. were collected in the morning (06:00 hrs -07:00 hrs). Standard solutions of different concentrations of Sucrose (1% -25%), Boric acid (50ppm- 500ppm) and salts solution (50-500ppm) of Calcium nitrate, Potassium nitrate and Magnesium sulphate were prepared. The pollen samples were sown on several grooved slides containing solution of Sucrose, Boric acid, Calcium nitrate, Potassium nitrate and Magnesium sulphate at different concentrations. Slides were then kept in petridishes lined with moist filter paper and examined under an Olympus microscope at different time intervals to record the germination percentage and pollen tube length following standard method (Shivanna and Rangaswamy, 1992). A pollen grain was considered as germinated, if pollen tube length at least becomes twice the diameter of the pollen grain (Gupta *et al.,* 1989).

RESULTS AND DISCUSSION

Studies on *in vitro* pollen germination after anthesis revealed that 65% germinating pollen along with 990 µm long pollen tube development occurred in 8% Sucrose solution (Table-1).

Table-1 Effect of Sucrose on *in vitro* pollen germination of *Borassus flabellifer* L.

Conc. (%)	After 1 hr.		After 3 hrs.		After 6 hrs.	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
Distilled water	-	-	-	-	-	-
2%	12	70	18	130	22	160
5%	21	108	43	270	53	432
8%	***20***	***270***	***41***	***594***	***65***	***990***
10%	10	90	23	126	45	270
15%	5	54	11	72	24	180
20%	4	36	7	54	15	90

Table -2 Effect of Boric acid on *in vitro* pollen germination of *Borassus flabellifer* L.

Conc. (ppm)	After 1 hr.		After 3 hrs.		After 6 hrs.	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
50	12	54	21	121	31	171
100	***21***	***108***	***35***	***270***	***55***	***394***
200	13	72	31	180	45	245
300	15	54	22	144	34	180

Individually, 100ppm Boric acid showed 55% germination along with 394µm long pollen tube (Table-2). Maximum 80% germinating pollen along with 1350 µm long pollen tube developed in 8% Sucrose solution supplemented with 100 ppm Boric acid (Table-3, Figure-1).

Among the salts, maximum 50% pollen germination along with 270 µm pollen tube development was observed in 200 ppm Magnesium sulphate solution following 41% pollen germination along with 198 µm pollen tube in 200 ppm Calcium nitrate and 32% pollen germination along with 126 µm long pollen tube in 100ppm Potassium nitrate (Table-4). Both Magnesium and Calcium showed good results, however Magnesium sulphate was the most effective.

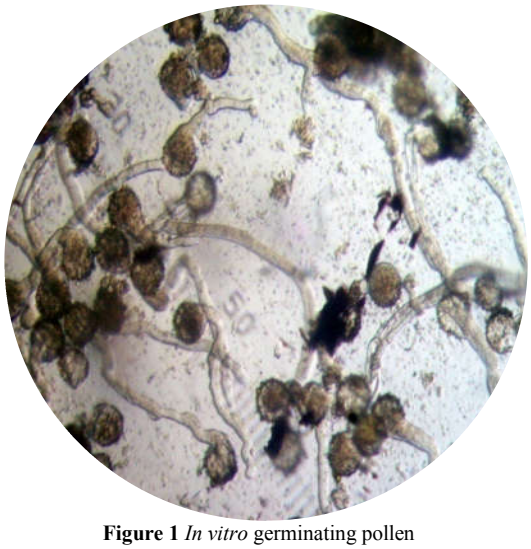

Figure 1 *In vitro* germinating pollen

Pollen grains are known to be packed with biochemicals like sugar, starch, lipids and phytic acid. These storage products get metabolized upon pollen germination and elongation of pollen tube. Thus, they play an important role in germination and in initial stages of pollen tube growth. Intake of the culture medium by the pollen grains initiates mobilization of the stored substances resulting into germination of pollen grains (Bertin, 1988; Wetzel and Jensen,1992; Stephenson *et al*.,1994). Energy required for the germination of pollen grains, formation of cell wall components and callose in angiosperms is provided by the nutrient reserves like starch, sugar and lipids stored in pollen grains (Baker and Baker, 1979).

Externally supplied sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism (Johri and Vasil, 1961; Shivanna and Johri, 1989). The effect of either sucrose or boric acid individually showed good results, but sucrose in combination with boric acid promoted pollen germination as well as tube development, because boron makes a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized

Table-3 Effect of Sucrose and Boric acid on *in vitro* pollen germination of *Borassus flabellifer* L.

Conc. of Sucrose (%) + Boric acid (ppm)	After 1 hr.		After 3 hrs.		After 6 hrs.	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
5+100	21	108	43	270	63	432
8+100	***30***	***450***	***55***	***684***	***80***	***1350***
10+100	20	270	41	594	65	990
15+100	10	90	23	126	45	270
20+100	5	54	11	72	24	108

sugar molecules (Gauch and Dugger, 1953; Vasil,1964; Sidhu and Malik, 1986).

pollen germination along with pollen tube development in *Morus indica* (Mondal *et al.,* 1997),

Table -4 Effect of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ on *in vitro* pollen germination of *Borassus flabellifer* L.

Salts	Conc. (ppm)	After 1 hr.		After 3 hrs.		After 6 hrs.	
		Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
$Ca(NO_3)_2$	50	-	-	-	-	-	-
	100	4	18	10	54	25	90
	200	***11`***	***36***	***21***	***126***	***41***	***198***
	300	2	11	5	36	10	54
KNO_3	50	-	-	-	-	-	-
	100	***10***	***54***	***21***	***90***	***32***	***126***
	200	6	18	11	54	21	90
	300	2	10	4	36	11	72
$MgSO_4$	50	-	-	-	-	-	-
	100	5	54	11	72	24	108
	200	***14***	***72***	***30***	***180***	***50***	***270***
	300	2	12	5	36	10	72

The role of boron has been established in pollen germination and pollen tube development in vascular plants (Lewis, 1980; Bhandal and Malik, 1985; Sidhu and Malik, 1986). Boron takes part in pollen tube development as it is directly involved in pectin synthesis and thus indirectly involved in development of pollen tube membrane (Stanley and Loewus, 1964) and it also exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism (Scott,1960). Naturally, water, sugar and amino acids are supplied by the stylar tissue for nourishing the growing pollen tube. Boron is also provided by stigmas and styles for facilitating sugar uptake and pectin production in the growing pollen tube (Richards, 1986). Boron in the form of boric acid is required at concentration of 100 ppm for most species for pollen germination and tube growth (Brewbaker and Majumder, 1961). Brewbaker and Kwack (1963) reported the induced role of Calcium and Boron on *in vitro* pollen germination. Flowering and fruiting process of *Pistachio* is enhanced by the use of boric acid (Brown *et al.*, 1994) and its deficiency results low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997). Thus, boron plays a vital role in pollen germination and pollen tube development for successful fertilization of flowering crops. Boron added in the form of boric acid, is very much important for the *in vitro* pollen germination from most species as elimination of boric acid from the culture medium often leads to tube bursting (Dabgar and Jain, 2001; Acar *et al*., 2010).Wang *et al.* (2003) studied the effect of boron on the localization of pectins and callose in the wall of pollen tubes in *Picea meyeri*. Acar *et al*. (2010) also reported the stimulatory effect of boron on *in vitro* pollen germination of *Pistacia vera*.

Salts like Calcium nitrate, Potassium nitrate and Magnesium sulphate were used to study their effects on *in vitro* pollen germination. The results indicated that Magnesium was the most effective to influence the pollen germination followed by Calcium and Potassium.

Externally supplied K+ ion is very important to enhance the rate of pollen germination as well as pollen tube growth in *Arabidopsis* (Fan *et al*.2001), while Mg^{++} and NO^{3-} also promoted the tube growth of *in vitro* germinating pollen of sugarcane (Moore and Jung, 1974). The stimulatory role of Magnesium in germinating pollen and tube development in *Nicotiana* (Loguercio, 2002) and *Gossypium* (Burke *et al*., 2004) has been established. Magnesium also plays a key role in *Luffa aegyptica* (Prajapati and Jain, 2010) and *Withania somnifera* (Ghanta and Mondal, 2013) like Calcium, Potassium and Nitrate.

Calcium is an inorganic substance with notable effect on pollen tube growth and the role of Calcium ions in growth of pollen tube has been established as Ca^{++} is an essential requirement of pollen tube growth and it also controls the permeability of pollen tube membrane. It is also known that, Calcium deficient medium results in loss of internal metabolites (Dickinson, 1967; Shivanna, 1979; Bednarska, 1989; Ge *et al.,* 2007). According to Brewbaker and Kwack (1963) Magnesium ions enhance the effect of Calcium ions resulting in vigorous growth of pollen tube.

Thus, the present experiment gets support from Pal *et al*. (1989), Mondal *et al.* (1991), Bhattacharya *el al*.(1997), Bhattacharya and Mandal (2004), Biswas *et al.* (2008), Mondal and Ghanta, (2012), Choudhury *et al*. (2012,2013), Dutta Mudi and Mondal (2014) and Ghanta and Mondal (2016).

Acknowledgement

Authors are thankful to Department of Botany (DST-FIST & UGC-SAP DRS), Visva-Bharati, for providing necessary laboratory facilities.

Reference

1. Acar, I., Ak, B.E. and Sarpkaya.K., (2010). Effect of boron and gibberellic acid on *in vitro* pollen germination of Pistachio (*Pistacia vera* L.). *African J. Biotechnol.,* 9 (32): 5126-5130.
2. Baker, H. B. and Baker, I., (1979). Starch in angiosperm pollen grains and its evolutionary significance. *Amer. J. Bot.,* 66 (5): 591-600.
3. Bednarska, E., (1989). The effect of exogenous Ca^{2+} ions on pollen grain germination and pollen tube growth – investigation with the use of Ca^{2+}.Verapamil, La^{3+} and ruthenium red. *Sexual Plant Reproduction*., 2: 53-58.
4. Bertin, R.I.,(1988). Paternity in plants. *In:* Plant reproductive ecology: Patterns and strategies (J. Lovett Doust and L. Lovett Doust Eds.), pp. 30-39.Oxford Univ Press, Oxford, New York.
5. Bhandal, L. S. and Malik, C.P., (1985). Effect of boric acid on some oxido reductase and hydrolase in

Crotalaria juncea pollen suspension culture. *In*: Recent advances in pollen research (T.M. Varghese Ed.), pp.75-81. Allied Publishers Private Ltd., New Delhi.

6. Bhattacharya, A., Mondal, S. and Mandal, S., (1997). *In vitro* pollen germination of *Delonix regia* (Boj.) Raf. *Sci. and Cult*., 63 (5-6): 143-144.
7. Bhattacharya, A. and Mandal, S., (2004). Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk. *Grana*, 43: 48 – 56.
8. Biswas, K., Mondal, S. and Mandal, S., (2008). Studies on *in vitro* pollen germination of *Solanum surattense* Burm.f. and *Solanum nigrum* L. *Sci. and Cult.,* 74 (3-4): 149-152.
9. Brewbaker, J. L. and Kwack, B.H.,(1963). The essential role of Calcium ions in pollen germination and pollen tube growth. *Amer. J. Bot*., 50**:** 859-865.
10. Brewbaker, J. L. and Majumder, S.K., (1961). Cultural studies on the pollen population effect and the self-incompatibility inhibition. *Amer. J. Bot*., 48(6): 457-464.
11. Brown, P. H., Ferguson, L. and G. Picchioni, G., (1994). Boron nutrition of pistachio: Third year report. California Pistachio Industry, Annual Report- Crop Year 1992-1993 pp. 60-63.
12. Burke, J. J., Velten, J. and Oliver, M.J., (2004). *In vitro* Analysis of cotton pollen germination. *American society of Agronomy*, 96: 359-368.
13. Chopra, R.N., Nayar, S.L. and Chopra, I.C., (1956). Glossary of Indian Medicinal Plants. C.S.I.R., New Delhi.
14. Choudhury, S., Mondal, S. and S. Mandal, S., (2012). Studies on *in vitro* pollen germination of *Rauvolfia serpentina* (L.) Benth. Ex. Murz. *In*: Biology of Plans and Microbes. (D. Bose and S. Roy Eds.) pp. 156-161. Levant Books., Kolkata.
15. Choudhury, S., Mondal, S. and Mandal, S., (2013). Studies on *in vitro* pollen germination of *Carissa carandus* Linn, *Sci. and Cult*., 79 (1-2): 128-130.46.
16. Dabgar, Y. B. and Jain, B. K., (2001). Effect of sucrose, boron, calcium and magnesium during *in vitro* pollen germination and tube growth in *Abelmoschus esculentus* Moench. *J. Swamy Bot. Club*., 8: 25-29.
17. Dickinson, D. B. (1967). Permeability and respiratory properties of germinating pollen. *Plant Physiol*., 20**:**118-127.
18. Dutta Mudi, M. and Mondal,S., (2014). Studies on *in vitro* Pollen Germination of *Phyllanthus reticulates* Poir. *Indian Journal of Fundamental and Applied Life Sciences,* 4 (3):367-373.
19. Fan, L., Wang, Y., Wang, H. and Wu, W. (2001). *In vitro Arabbidopsis* pollen germination an characterization of inward potassium currents in *Arabidopsis* pollen grain protoplasts. *Journal of Experimental Botany*, 52 (361): 1603-1614.
20. Franklin –Tong, V. E., (1999). Signalling and the modulation of pollen tube growth. *The Plant Cell,* 11: 727 – 738.
21. Gauch, H.G. and Dugger, W.M., (1953). The role of boron in the translocation of Sucrose. *Plant Physiol*., 28: 457-466.
22. Ge, L. L., Tian, H.Q. and Russell, S.D., (2007). Calcium function and distribution during fertilization in angiosperms. *Amer. J. Bot*, 94 (6):1046-1060.
23. Ghanta, R. and Mondal, S., (2013). Effect of Some Nutrients on *in vitro* Pollen Germination of *Withania somnifera* (L.) Dunal. *Annals of Plant Sciences*, 02 (06): 182-187.
24. Ghanta, R. and Mondal, S., (2016). *In vitro* studies on pollen germination of *Aloe barbadensis* Mill. *Int. J Curr. Sci*., 19(2): E 146-153.
25. Gupta, S., Bhattacharya, K.N. and Chanda,S., (1989). *In vitro* pollen germination of *Solanum sisymbriifolium* Lamk. *Journal of Palynology*, 25:65-72.
26. Heslop Harrsion, J. (1989). Pollen germination and pollen tube growth. *Int. Rev. Cytol*., 107:1-78.
27. Herreo, M. (1991). Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific matings. *J. Cell Sci.,* 47**:** 365-383.
28. Imani, A., Kazem, B., Saeed, P. and Seiyed, H .M. (2011). Storage of Apple pollen and *in vitro* germination. *African Journal of Agricultural Research*, 6: 624-629.
29. Johri, B. M. and Vasil, J .K. (1961). Physiology of pollen. *Bot. Rev*., 27(3): 325- 381.
30. Jones, R.J.W. and Lunt, O.R., (1967). The function of calcium ions in plants. *Botanical Review*, 33: 407-426.
31. Karuppusamy, A., Shanmugam, S. and Thangaraj, P., (2011). Nutritional analysis and antioxidant activity of palmyrah (*Borassus flabellifer* L.) seed embryo for potential use as food source. *Food Science and Biotechnology*, 20(1):143-149.
32. Kristen, U. and R. Kappler. (1990). The pollen test system. Invittox, 55**:** 1-7.
33. Lewis, D. H., (1980). Boron lignification and the origin of vascular plants. A Unified hypothesis. *New Phytology*, 84: 209-229.
34. Loguercio, L., (2002).Pollen treatment in high osmotic potential: A simple tool for *in vitro* preservation and manipulation of viability in gametophytic populations. *Braz. J. Plant Physiology,* 14: 1-9.
35. Mascarenhas, J. P., (1993). Molecular mechanism of pollen tube and differentiation. *The plant cell*, **5**: 1303-1314.
36. Mondal, S., Bhattachaya, K. N. and Mandal, S.,(1991). Studies on *in vitro* pollen germination of *Holarrhena antidysenterica* Wall. *Indian Biologist*., 23(2) :33-35.
37. Mondal, S., Bhattachaya, K.N. and Mandal, S., (1997). *In vitro* pollen germination in *Morus indica* L. *Sericologia*, 37(2) 349-352.
38. Mondal, S and Ghanta, R., (2012). Studies on *in vitro* pollen germination of *Lawsonia inermis* Linn. *Advances in Bioresearch*, 3 (3): 63-66.
39. Moore, P. N. and Jung, W.L., (1974).Studies in sugarcane pollen *in vitro* germination of pollen. Phyton. *Rev. Int. Bot. Exp*., 32 (2): 147-158.
40. Nyomora, A. M. S. and Brown, P.H., (1997). Fall foliar-applied boron increases tissue boron concentration and nut set of almond. *J. Am. Soc. Hort. Sci.,* 122(3): 405-410.
41. Pal, J. K., Mandal, S. and Bhattacharya, G.N., (1989). Studies on the *in vitro* pollen germination of the two

varieties of *Butea monosperma* (Lam.) Taub. *J. Palynol*., 25:113-120.
42. Prajapati, P. P. and Jain, B.K., (2010). Effect of sucrose, boron, calcium, magnesium and nitrate during *in vitro* pollen germination in *Luffa aegyptica* Mill. *Prajna*, 18: 5-8.
43. Richards, A. J., (1986). Plant Breeding Systems. George Allen Unwin, London, England.
44. Scott, E. G., (1960). Effect of supra optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus*. *Plant Physiology*, 35 (5) 53-661.
45. Sidhu, R. J. K. and Malik, C.P., (1986). Metabolic role of boron in germinating pollen and growing pollen tubes. *In:* Biotechnology and Ecology of Pollen (Mulcahy *et al.* Eds.), pp. 373-378. *Springer*, New York.
46. Shivanna, K. R.,(1979). Recognition and rejection phenomena during pollen pistil interaction. Proc. Ind. Acad. Sci*.,* 88 (B): 115-141.
47. Shivanna, K. R. and Johri, B.M., (1989).The Angiosperm Pollen Structure and Function*,* Willey Eastern Ltd., New Delhi.
48. Shivanna, K. R. and Rangaswamy, N.S., (1992). Pollen Biology: A Laboratory Manual*,* Springer, Berlin.
49. Stanley, R.G. and Loewus, F.A.,(1964). Boron and myo-inositol in pollen pectin biosynthesis. *In*: Pollen Physioilogy and Fertilisation (H.F. Linkens Ed.), pp-128-139 North-Holland Publishing Company, Amsterdam.
50. Stephenson, A.G., Erickson, C.W., Lau, T.C., Quesada, M.R. and Winsor, J.A., (1994). Effects of growing conditions on the male gametophyte. *In*: Pollen–pistil interactions and pollen tube growth. American Society of Plant Physiologists. (A.G. Stephenson and T. H. Kao Eds.) Series 12.pp 220-229. Rockville., MD. USA.
51. Taylor, L. P. and Hepler, P.K., (1997). Pollen germination and tube growth. Annual Review of Plant Physiology and Plant Molecular Biology, 48: 461-491.
52. Vasil, I.K., (1964). Effect of boron on pollen germination and pollen tube growth. *In*: Pollen Physiology and Fertilization (H. F. Linkens Ed.), North-Holland Publishing Company, Amsterdam, pp. 107-119.
53. Wang, Q., Lu, L., Wu, X., Li, Y., and Lin, J., (2003). Boron influences pollen germination and tube growth in *Picea meyeri. Tree Physiology*, 136: 3892-3904.
54. Wetzel, C. L. R. and Jensen, W.A., (1992). Studies of pollen maturation in cotton: the storage reserve accumulation phase. *Sexual Plant Reprod*., 5: 117-127.

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How to cite this article:

Hemanta Saha., Subrata Mondal and Sudhendhu Mandal.2016, Studies on in Vitro Pollen Germination and Tube Development of Borassus Flabellifer l. *Int J Recent Sci Res.* 7(6), pp. 11985-11989.