ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 7, pp 1062-1065, July 2016

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

STUDIES ON *IN-VITRO* POLLEN GERMINATION OF *HOLMSKIOLDIA SANGUINEA* RETZ.

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ARTICLE INFO

Article History:

Received 10th April, 2016
Received in revised form 29th May, 2016
Accepted 15th June, 2016
Published online 25th July, 2016

Key words:

In Vitro Pollen Germination, Tube Length, Sucrose, Boric Acid, Salts, *Holmskioldia Sanguinea*

ABSTRACT

Holmskioldia sanguinea Retz., a small garden tree under Verbenaceae, flowers during November to January. Flowers generally open in 07:00hrs-10:00hrs and anther dehiscence takes place simultaneously. The effect of different compounds on *in vitro* pollen germination of *Holmskioldia sanguinea* showed that, the pollen grains start to germinate in distilled water and 2% sucrose solution enhanced germination (97%) as well as tube length (624µm). Individually, 500ppm boric acid (H_3BO_3) showed 88% pollen germination with a pollen tube length of 555µm. It was also recorded that, maximum 98% pollen germination with tube length of 656µm developed in 2% sucrose solution supplemented with 500ppm boric acid. Among the salts, maximum 47%pollen germination occurred in 100ppm $Ca(NO_3)_2$ solution with 351µm pollen tube length while 44% pollen germination along with 195µm pollen tube length in 500ppm KNO_3 solution and 11% pollen germination with 208µm long tube developed in 200ppm solution of $MgSO_4$. The pollen grains of the flowers which were collected during anthesis showed the best result.

INTRODUCTION

Though, stigma is suitable place for pollen germination but studies on *in vivo* are not easily feasible due to complexity involved in pistillate tissues. However, it is possible to germinate pollen grains *in vitro* to achieve a reasonable tube length. Our knowledge regarding physiology and biochemistry of pollen germination and tube growth largely comes from *in vitro* studies. Therefore, it is important to know the physiological and biochemical characteristics of pollen germination. *In vitro* pollen germination is considered as the best indicator of pollen viability (Shivanna *et al*., 1991).

It is essential to know the determining factors for the formation of fruits and seeds of the plants. Male sexual reproductive materials are carried through the pollen grains and there is a need of viable pollen grains for fertilization as successful fruit-set and high crop yield generally depends on viable pollen grains. Pollen also plays an essential role in breeding programmes. Pollen germinates and with the formation of pollen tube, it discharges its genetic materials to the embryo sac to fulfil the fertilization. Pollen germination and growth of pollen tubes are important research materials for physiological, biochemical, biotechnological, ecological, evolutional and molecular biological studies (Ottavio *et al*., 1992). Elongation of pollen tube is a process in which it navigates and responds to female tissue to accomplish their mission to deliver the sperm cells for fertilization. In recent years, pollen germination and pollen tube development are used as materials for determination of cytoskeleton in cell growth and differentiation (Ma *et al*., 2000).The required environment for *in vitro* pollen germination is related to genetic composition and also the quality and quantity of nutrients reserves of pollen (Baker and Baker, 1979). The aim of this study is to find out the effect of different chemicals on *in vitro* pollen germination of *Holmskioldia sanguinea* Retz. (Fig. 1) which is a small tree planted in garden for its beautiful brilliant red inverted umbrella shaped flowers, also known as "Chinese umbrella". The plant has medicinal importance due to antioxidant and antimicrobial properties (Ajaib *et al*., 2013).

Figure 1 Flowering Plant

MATERIALS AND METHODS

In this study, flowers of *Holmskioldia sanguinea* were collected soon after anther dehiscence, which usually takes place in the late morning (07:00hrs -10:00hrs). The pollen grains collected immediately after anther dehiscence showed best germination. Solutions of sucrose (1%-50%), boric acid (50ppm-1000ppm) and salts (50ppm-600ppm) like Calcium

nitrate [$Ca(NO_3)_2$], Magnesium sulphate ($MgSO_4$) and Potassium nitrate (KNO_3) were prepared. The pollen grains were sown in different grooved slides containing solutions of different concentrations. The slides were then kept in petridishes lined with moist filter paper and examined under microscope (Olympus) at different intervals to determine the percentage of germination with the pollen tube length following the method of Shivanna and Rangaswamy (1993). A pollen grain was considered as germinated, if pollen tube length atleast became twice greater than the diameter of the pollen grain (Gupta *et al*., 1989). Photographs were taken with the help of Sony cyber shot camera.

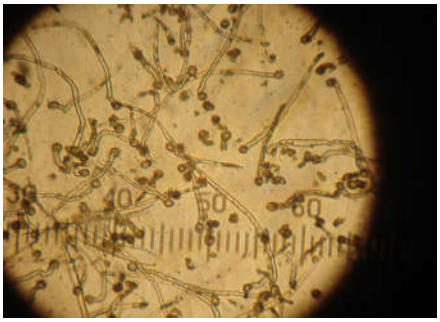

Figure 2 Germinated pollen (2% sucrose+ 100ppm boric acid)

RESULTS

In vitro pollen germination study showed that, 97% germinating pollen with a mean of 624 µm long pollen tube occurred in 2% sucrose solution (Table-1) and 88% germinating pollen with a mean of 555µm long pollen tube developed (Table-2) in 500ppm boric acid solution.

Maximum 98% pollen germination with a mean of 656µm long tube has been recorded in 2% sucrose solution supplemented with 500ppm boric acid (Table-3, Fig.-2). In case of salts, maximum 47% pollen germination along with 351µm pollen tube in 100ppm Calcium nitrate solution, 44% germination along with 195µm tube length in 500ppm

Table 1 Effect of sucrose on *in vitro* pollen germination of *Holmskioldia sanguinea* Retz.

	After 1 hour		After 2 hours		After 3 hours	
Concentration in %	Germination in %	Tube Length in µm	Germination in %	Tube length in µm	Germination in %	Tube length in µm
Dist. Water	17	180	25	220	30	250
1%	59	300	62	350	69	400
2%	80	480	89	510	97	624
3%	55	340	64	380	71	410
5%	49	290	58	300	59	310
8%	30	225	39	230	40	240
10%	09	110	11	115	12	122

Table 2 Effect of boric acid on *in vitro* pollen germination *Holmskioldia sanguinea* Retz.

	After 1 hour		After 2 hours		After 3 hours	
Concentration in %	Germination in %	Tube length in µm	Germination in %	Tube length in µm	Germination in %	Tube length in µm
50	10	125	12	132	15	135
100	15	185	18	135	20	141
200	30	198	32	195	35	207
300	40	198	45	205	48	215
400	70	267	72	286	73	299
500	80	520	85	545	88	555
600	75	395	80	351	82	442
700	65	225	68	252	70	275
1000	45	125	48	126	49	132

Table 3 Effect of sucrose and boric acid on *in vitro* pollen germination of *Holmskioldia sanguinea* Retz.

	After 1hour		After 2 hours		After 3 hours	
Concentration in (%+ppm)	Germination in %	Tube length in µm	Germination in %	Tube length in µm	Germination in %	Tube length in µm
1 + 500	65	420	70	425	75	450
2 + 500	90	625	95	630	98	656
3 + 500	80	490	83	495	84	505
5 + 500	56	365	58	370	59	380
10 +500	15	115	16	120	16	125

Table 4 Effect of $Ca(NO_3)_2$, $MgSO_4$ and KNO_3 on *in vitro* pollen germination of *Holmskioldia sanguinea* Retz.

Salt		After 1hour		After 2 hours		After 3 hours	
	Concentration in ppm	Germination in%	Tube length in µm	Germination in %	Tube length in µm	Germination in %	Tube length in µm
$Ca(NO_3)_2$	50	41	195	43	234	43	234
	100	39	260	42	325	47	351
	200	30	156	35	182	36	195
	300	16	130	18	143	18	156
	500	23	65	23.5	78	24	78
KNO_3	100	7	325	9	364	12	390
	200	15	338	18	370	20	390
	300	25	226	28	230	30	295
	500	42	117	46	156	44	195
	600	26	78	28	104	29	104
$MgSO_4$	50	2	95	4	98	5	100
	100	3	118	3	132	3	141
	200	8	129	10	182	11	208
	300	6	100	8	105	9	106
	500	4	98	4	95	5	105

Potassium nitrate solution and 11 % pollen germination along with 208µm long pollen tube in 200 ppm Magnesium sulphate solution were noticed (Table-4). Pollen grains were found to germinate even in distilled water (Table-1).

DISCUSSION

Fertilization in flowering plants requires remarkable co-ordination to carry sperm cells to the ovules through stylar tissues. *In vitro* pollen germination is rapid, reasonable, and simple and most commonly used method for assessing pollen viability. *In vitro* pollen germination tests have been used to determine the germination percentage of pollen and can also be used for assessing pollen vigor by monitoring the rate of germination over a period of time or the length of pollen tubes (Shivanna and Mohan Ram, 1993). It has been documented that internal, morphological and environmental factors play a role in determining the duration of pollen viability **(**Dafni and Firmage, 2000). Sucrose used for *in vitro* pollen germination is suitable carbohydrate source for pollen germination, maintains osmotic pressure of the medium and act as a substrate for pollen metabolism (Johri and Vasil, 1961; Shivanna and Johri,1985).The optimum uptake of sucrose solution varies from species to species (Bhattacharya and Mandal, 2000).

Though sucrose and boric acid individually (Table-1 and 2) showed good results but sucrose in combination with boric acid showed best result (Table-3). Sucrose is necessary for pollen nutrition, osmotic control and in combination with boric acid promoted pollen germination because boron makes a complex with sucrose and this sugar-borate complex is easily translocable rather than sucrose alone (Gauch and Dugger,1953). Boron may enhance the sucrose uptake and stimulate germinating ability. Boron has been determined as a mediator to play a role in the growth of the pollen tube and pollen germination (Lewis, 1980; Sidhu and Malik, 1986). Boron also provided by the stigmas and styles, facilitates sugar uptake and play a role in pectin production in the pollen tube (Richards, 1986).Boron which is essential for flowering and fruiting takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Boron is also known to be crucial for pollen germination and tube growth (Brewbaker and Majumder, 1961; Dickinson, 1978). Boron plays a role in flowering and fruiting process in pistachio (Brown *et al*, 1994) and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997).

Salts like Calcium nitrate, Potassium nitrate and Magnesium sulphate also have effective influence in pollen germination. The Calcium plays a critical role in pollen germination and growth of the pollen tube as it helps in permeability and integrity of cell membrane (Miller *et al*.,1992,Brewbaker and Kwack,1963,Brewbaker and Majumder,1961; Therios *et al*.,1985; Brown *et al*.,1994;Ak,*et al*.,1995; Shorrocks, 1997; Acar and Ak,1998). Calcium binding takes place in pectin of the pollen tube walls. This appeared to increase the wall rigidity and to regulate permeability of the pollen cells thereby enhancing pollen tube growth **(**Kwack, 1967). It has been suggested that inorganic ions like $Ca^{++}$and K^{+} transportation through plasma membrane of pollen and pollen tube regulates the pollen germination and growth of pollen tube (Feijo *et al*., 1995 and Taylor and Hepler, 1997).It has been proved that external supply of K^{+} ion enhanced the rate of pollen germination as well as pollen tube growth (Fan *et al*., 2001). Pollen tube growth also enhanced by the presence of $Mg^{++}$and No_3^{-}(Moore and Jung, 1974). Calcium, Magnesium and Nitrate play a key role in pollen tube growth and it has been proved by Prajapati and Jain (2010) on *Luffa aegyptica*. Stimulatory effect of sugar, boric acid and salts like Ca $(NO_3)_2$, KNO_3, $MgSO_4$ have also been established on *in vitro* pollen germination and tube growth of different taxa (Pal *et al*., 1989; Mondal *et al.,* 1991; Bhattacharya *et al*., 1997; Bhattacharya and Mandal, 2004; Biswas *et al*., 2008; Mondal and Ghanta, 2012; Choudhury *et al*., 2012 and 2013; Biswas and Mondal, 2014; Dutta Mudi and Mondal, 2014 and Ghanta and Mondal 2016).

So, the present study concludes that, sugar in combination with boric acid stimulate pollen germination and tube growth and salts like $Ca(NO_3)_2$, KNO_3,$MgSO_4$ also play key role in pollen germination as well as pollen tube development of *Holmskioldia sanguinea* Retz.

Acknowledgements

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

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