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ORIGINAL ARTICLE

Effect of Sucrose and Boric acid on *in vitro* Pollen Germination of *Asparagus racemosus* Willd.

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ABSTRACT
During sexual reproduction of angiosperms, pollen grains play a vital role to transmit the male genetic materials to the egg cell. Thus, pollen germination and tube growth are crucial for the plant sexual reproduction and food production. Germination is the first critical morphogenetic event in the pollen towards fulfilling its ultimate function of discharge of male gametes in the embryo sac. Present investigation reveals the effect of sucrose and boric acid on in vitro pollen germination of Asparagus racemosus Willd. Belonging to the family Liliaceae. Pollen germination up to 86% along with 715 µm long pollen tube development was observed in 20% sucrose solution and 50ppm boric acid showed 24% germination along with 182 µm long pollen tubes but, maximum 98% pollen germination along with 780 µm long pollen tube development was observed in 20 % sucrose solution supplemented with 50 ppm boric acid. Pollen grains taken from flowers collected during anthesis (17:00 hrs.-18:30 hrs.) showed best germination.
***Key words**: Pollen germination, Pollen tube, Sucrose, Boric acid, Asparagus racemosus Willd.*

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INTRODUCTION

Asparagus racemosus Willd. is a highly important medicinal plant belonging to the family Liliaceae which distributed throughout India and used in Ayurveda, Unani, Siddha and Tibetan as well as in folk medicine. It is commonly known as Shatamuli, Shataavari, and Sataavar. The root extract is commonly used as refrigerant, demulcent, alterative, antidysentric, aphrodisiac [1] and exhibits antiallergic properties and root fed orally acted as immunomodulator [2]. The roots are also used in inflammation, diarrhoea, nephropathy, tumours, hyperdipsia, tuberculosis, leucorrhoea, leprosy, colic, hypertension, fatigue, abortion, agalactia and general debility [3,4]. The plant is propagated vegetatively as well as by means of seeds. For fruit and seed production, sexual reproduction is essential which needs fertile and viable pollen grains as they carry the male genetic materials to the stigma for fertilization. Delivering of sperm to egg is a little more complex phenomenon when the parents can't move around. Pollen grains are highly reduced male gametophytic structure and released in two or three celled condition [5]. Naturally, during fertilization pollen grains get deposited over the receptive stigmas and germinate to form pollen tube but, due to the complications involved in the pistilate tissues, studies on *in vivo* are cumbersome. 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. These complex system engaged a number of signalling events like cell-environment interaction, intercellular signalling etc. [6,7]. *In vitro* pollen germination and tube development in different nutrient medium was extensively studied in the past [6-10]. Pollen germination and growth of pollen tubes are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical and molecular biological studies [11] and it is also important to determine the importance of cytoskeleton in cell growth and differentiation [12]. So, not only the intensive study of physiology of male gametophyte, but also *in vitro* pollen germination in terms of pollen viability has huge applications in crop breeding and crop improvement programmes. Pollen

tubes are considered as the most rapidly growing cells in the plant world since they are capable of attaining considerable amount of length in a short duration under optimum conditions [13]. Thus, pollen grains, single celled structure provide a unique system for *in vitro* studies [14]. The present work is aimed to find out the effect of sucrose and boric acid on *in vitro* pollen germination of *Asparagus racemosus* Willd. of Liliaceae.

MATERIALS AND METHODS

Fresh flowers were collected in the evening (17:00 hrs.-18:30 hrs.) during anthesis and transferred to polythene bags. Fresh solutions of different concentrations of sucrose (1-50%) and boric acid (25-500ppm) were prepared which were then used as medium for *in vitro* germination. For this the fresh pollen samples were sown on several grooved slides containing sucrose and boric acid solution at different concentrations individually and in combinations. Slides were then kept in petridishes lined with moist filter paper at room temperature (25°C) and examined under Dewinter (ultima) microscope at different time intervals to know the germination percentage and pollen tube length following the method of Shivanna and Rangaswamy [15]. Pollen grains were considered as germinated when the tube length was twice greater than the diameter of the pollen grains [16].

RESULTS AND DISCUSSION

Initiation of pollen germination (4%) and pollen tube development (91 µm) was observed after 3 hours incubation in 2% sucrose solution and it reaches up to 86% pollen germination along with 715 µm long pollen tube in 20% sucrose solution (Table-1) while, 50ppm boric acid showed 24% germination along with 182 µm long pollen tubes (Table-2). However, maximum 98% germinating pollen along with 780µm long pollen tube development was found in 20% sucrose solution supplemented with 50 ppm boric acid (Table-3, Fig.1-3).

Table-1: Effect of sucrose on *in vitro* pollen germination of *Asparagus racemosus*.

Conc. of sucrose (%)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)
Distilled water	--	--	--	--	--	--
2	--	--	--	--	4	91
5	--	--	5	91	13	169
10	12	169	29	260	38	338
15	27	208	58	442	69	494
20	**39**	**260**	**78**	**689**	**86**	**715**
25	42	273	72	572	75	611
30	36	260	43	377	52	416
40	28	221	29	351	31	377
45	14	195	17	195	18	260

Table-2: Effect of boric acid on *in vitro* pollen germination of *Asparagus racemosus* .

Conc. of boric acid (ppm.)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)	Germination (%)	Pollen tube length (µm)
25	6	26	12	91	13	117
50	**16**	**104**	**21**	**169**	**24**	**182**
100	12	78	18	130	20	156
200	8	52	14	104	18	130

The pollen grains showed germination ability in both sucrose and boric acid solution (Table-1&2). However, sucrose supplemented with boric acid promoted both pollen germination as well as tube development (Table-3). Naturally, stylar tissues nourish the pollen after deposition, by supplying water, sugar, amino acids etc. To achieve this, a protuberance develops from pollen grains to invade the stigma through which the sperm cells are transported [17]. Boron is found in style and stigma which enhance sugar uptake and play a vital role in pectin synthesis in the growing pollen tubes [18]. Generally sucrose acts as respiratory substrate for pollen grains. Not only respiratory substrate but also it is very important to regulate the osmotic pressure. The conspicuous role of sucrose and boric acid on *in vitro* pollen

germination were reflected with the previous studies [19, 20]. Boron is an important micronutrient for the growth of higher plants [21]. In vascular plant, like sucrose, boron also play a vital role on pollen germination and pollen tube growth [22, 23] as it is directly involved in pectin synthesis to the development of pollen tube membrane [24]. Low concentration of boric acid enhanced pollen germination as well as pollen tube development whereas high concentration inhibited pollen germination and tube elongation. Similar findings were observed in *Eucalyptus* plant [25]. Not only pectin but also it help in the synthesis of callose during pollen tube development which was experimentally proved in *Picea meyeri* by [26] and important in sugar transport, cell wall synthesis, cell wall development, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism and membrane transport [27, 28]. Boron could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism [29]. Boron is crucial for pollen germination along with pollen tube development in most species [30], thus play an important role in fertilization of flowering plants towards the successful fruit and seed production. The important role of boron on *in vitro* pollen germination and tube development was studied in Pistachio [9, 10, 31]. The Boron deficiency affects the pollen viability, pollen germination, and pollen tube growth [32]. It is reported that tube bursting occurred due to elimination of boric acid from pollen culture medium [10, 33]. The deficiency of Boron in plants causes carbohydrate accumulation in chloroplasts, may slow the Krebs cycle and accelerates the action of the pentose phosphate cycle [34, 35]. Thus, the role of Sucrose and boric acid in pollen germination and pollen tube development was confirmed, however sucrose in combinations 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 sugar molecules [8, 23, 36] and involved in the synthesis of pectic substance required for the newly elongating pollen tube wall [19].

Table-3: Effect of sucrose and boric acid on *in vitro* pollen germination of *Asparagus racemosus*.

Conc. of sucrose (%) + boric acid (ppm.)	**After 1 hr.**		**After 2 hrs.**		**After 3 hrs.**	
	Germination (%)	**Pollen tube length(µm)**	**Germination (%)**	**Pollen tube length (µm)**	**Germination (%)**	**Pollen tube length(µm)**
20+ 25	58	195	72	546	79	650
20+ 50	**78**	**234**	**89**	**598**	**98**	**780**
20+ 100	73	221	82	572	87	728
20+ 200	54	182	69	494	74	624
20+ 300	49	156	62	416	69	546

Thus, the present investigation is corroborated with the previous studies [10, 33, 37-50] and it can be concluded that, sucrose and boric acid individually enhanced the pollen germination and tube development but sucrose in combination with boric acid promoted pollen germination and tube elongation of *Asparagus racemosus* Willd.

So, present experiment highlights the role of sucrose and boric acid on *in vitro* pollen germination as it is very important in maintaining tube membrane integrity and viability.

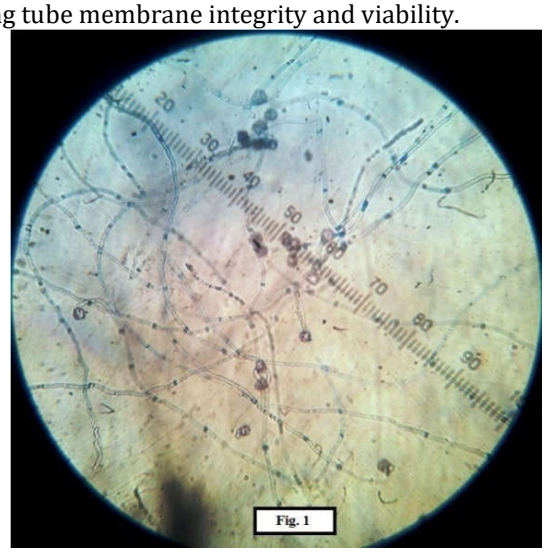
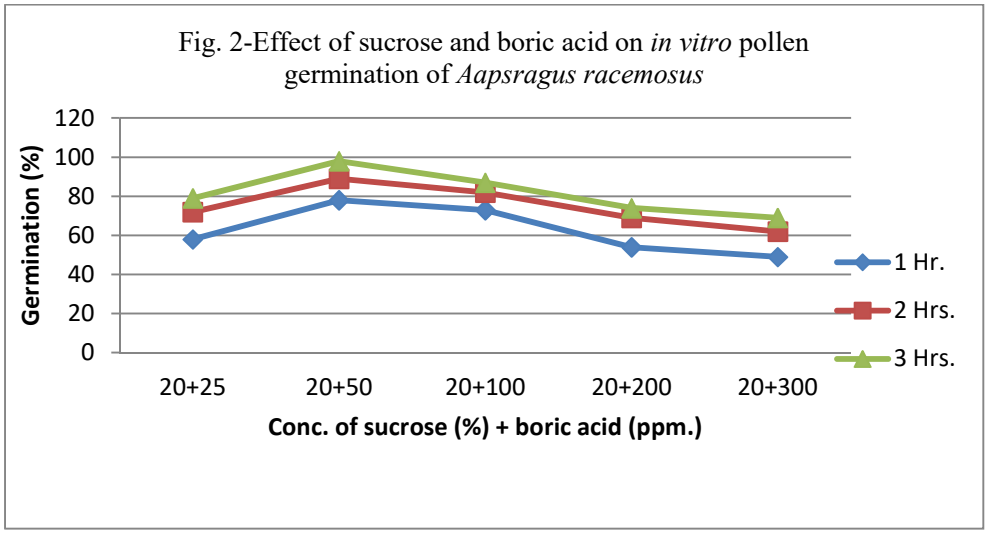
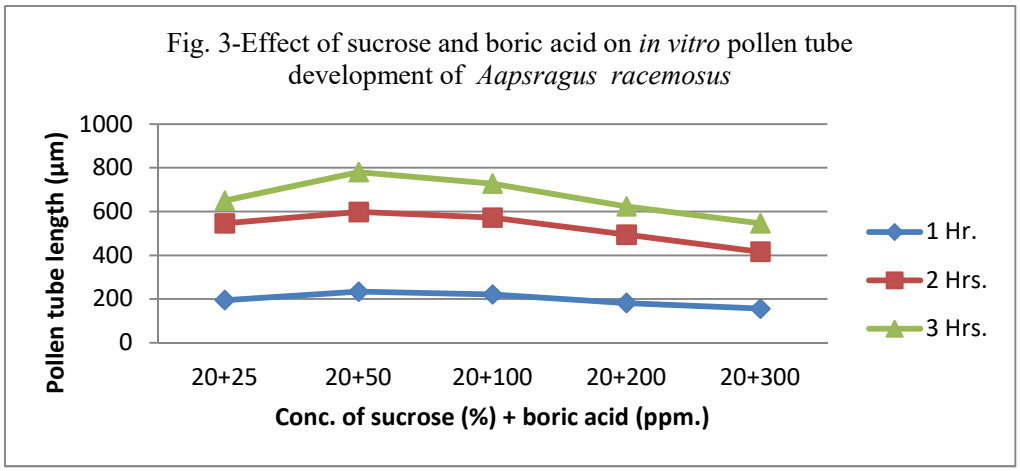


Fig. 1: *In vitro* germinating pollen of *Asparagus racemosus*.





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