Annals of Plant Sciences
ISSN: 2287-688X
www.annalsofplantsciences.com
OPEN ACCESS

Original Research Article

Role of some nutrients on *in vitro* pollen germination and tube development of *Luffa cylindrica* (L.) Roem.

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Received for publication: September 19, 2014; **Accepted:** September 23, 2014.

Abstract: *In vitro* pollen germination of Sponge Gourd, *Luffa cylindrica* (L.) Roem. of the family Cucurbitaceae has been carried out to determine the role of different nutrients like sucrose ($C_{12}H_{22}O_{11}$), boric acid (H_3BO_3), Calcium nitrate [$Ca(NO_3)_2$, $4H_2O$], and Magnesium sulphate ($MgSO_4$, $7H_2O$) and Potassium nitrate (KNO_3) in different concentration individually or in combinations on pollen germination as well as pollen tube elongation. Among the salts, Calcium nitrate showed 19% germination with 460±183.78 µm long pollen tube. Pollen germination with swelling and thickening of pollen tubes were recorded in sucrose solution but addition of boric acid facilitated an increased pollen germination as well as pollen tube elongation. Maximum 75% germination along with1230±294.58 µm long pollen tube was recorded in 30% sucrose solution supplemented with 50 µg/ml boric acid. The data were also analyzed and correlated statistically. Statistically significant pollen germination and pollen tube length was resulted due to addition of boric acid. The pollen grains collected immediately after anther dehiscence (05.15-06.00 hr) showed the best germination ability but gradually decreases with time and ultimately became almost ceased during the second day of flower anthesis. The results reveal that boric acid play vital role on *in vitro* pollen germination and pollen tube elongation.

Key Words: *Luffa cylindrica*, Pollen Germination, Pollen Tube Elongation, Anthesis.

Introduction

Luffa cylindrica (L.) Roem., commonly known as Sponge Gourd, is a profusely branched climber with yellow flowers (Fig. 1 A and B). Young fruits are widely used as vegetable due to its nutritional benefit. The plant has high medicinal value and mature dried fruits are used as body sponge. Plants are monoecious having unisexual flowers and their fruit set principally depends on dissemination of pollen grains, which are the units of male gametophyte and prerequisites for pollination, fertilization as well as fruit and seed set. But successful fertilization depends on the fertility and viability of the pollen grains. Pollen viability and stigma receptivity are critical factors for completion of successful post pollination events. Receptive stigmatic surface is the ideal place for germination of pollen grains because biochemical derivation of stigmatic tissues stimulates to germinate the pollen grains and guide to carry out fertilization through pollen tube elongation. But due to complexity involved of pistillate tissues in receptive surface of the stigma, biochemistry and physiology of pollen germination and pollen tube growth are rather difficult. Pollen viability in terms of germination ability largely comes from the *in vitro* studies, which may have important not only for fruit set but also for flower-flower and flower-pollinator interaction. In flowering plants, the pollen tube delivers sperm cells to the embryo sac. Pollen tube growth proceeds through tip extension and can be influenced by many factors including different nutrients. Thus primary goal is to determination of pollen viability, fertility and pollen tube elongation before going to an effective breeding programme. The present work is aimed to study the effect of sucrose, boric acid, Magnesium sulphate, Calcium nitrate and Potassium nitrate separately or in combination on *in vitro* pollen germination and pollen tube length of *Luffa cylindrica* (L.) Roem. belongs to the family Cucurbitaceae.

Materials and Methods

In vitro pollen germination was conducted to determine the effect of different nutrients like sucrose ($C_{12}H_{22}O_{11}$); H_3BO_3; Ca $(NO_3)_2$, $4H_2O$; $MgSO_4$, $7H_2O$ and KNO_3 at various concentrations. Different grades of

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sucrose and salts solution were prepared and used individually or in combinations. A drop (50 µl) of each solution was kept into each groove of grooved slides individually or in combination. The fresh pollen samples were collected just after anther dehiscence at around 05.15-06.00 hr (Fig. 1C) and put into nutrient medium with the help of platinum needle, which in turns were kept in petridishes lined with moist filter paper and observed under a light binocular microscope (Olympus, Model no. CH20i B1MF) at low magnification (10x eye piece and 10x objective) after stipulated time of incubation. All experiments were performed in triplicate. Results were taken and tabulated following the method of Shivanna & Rangaswamy (1) and analyzed using standard statistical methods of Dutta (2). Pollen grains were considered to be germinated when the pollen tube length was greater than the diameter of the pollen grain (3).

Results

In vitro pollen germination study showed that 72% germinating pollen, with a mean of 675±187.45 µm long pollen tube in 30% sucrose (Table 1). Individually boric acid showed 45% pollen germination along with 500±149.07 µm tube length in 50 µg/ml solution (Table 2). The best pollen germination (75%) with a mean of 1230± 294.58 µm long pollen tube occurred after 8 hrs of incubation in 30% sucrose supplemented with 50 µg/ml boric acid solution (Table 3, Fig.1D). 80% pollen showed polysiphonous condition in sucrose (Fig. 1E). Swelling of the tip of pollen tube and its thickening (30±0.87 µm, Range 30-32, N=10) were resulted only in sucrose solution (Fig.1F). Narrow and thinner (10.40±0.51 µm, Range 10-11, N=10) pollen tubes were recorded in boric (Fig. 1G) and other nutrient salts. Calcium nitrate and Magnesium sulphate showed 19% and 10% pollen germination along with 460±183.78 µm and 245±134.26 µm pollen tube length (Table 4 and 5) in 100 µg/ml respectively, whereas Potassium nitrate showed poor pollen germination (6%) along with 180±33.73 µm (Table 6) tube length in 40 µg/ml. The pollen grains collected immediately after anther dehiscence showed the best germination ability but gradually decreases with time and ultimately becomes almost nil during the second day of flower anthesis.

Table 1: Effect of sucrose solution on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (%)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
1	-		-		-	
3	2	96±5.16 (Range 90-100, N=10)	5	105±10.80 (Range 90-100, N=10)	8	115±13.54 (Range 100-130, N=10)
7	4	104±5.16 (Range 100-110, N=10)	8	111±8.75 (Range 100-120, N=10)	12	131±20.78 (Range 100-150, N=10)
10	18	126±39.77 (Range 100-200, N=10)	22	148±44.91 (Range 110-200, N=10)	25	152±38.52 (Range 100-200, N=10)
15	21	162±53.49 (Range 100-220, N=10)	28	208±97.04 (Range 100-350, N=10)	32	282±128.30 (Range 100-500, N=10)
20	24	171±46.05 (Range 100-250, N=10)	40	253±122.38 (Range 100-400, N=10)	45	335±120 (Range 200-500, N=10)
25	30	189±36.65 (Range 100-220, N=10)	49	298±115.16 (Range 150-400, N=10)	55	420±147.57 (Range 200-600, N=10)
30	35	197±69.76 (Range 100-300, N=10)	60	405±101.24 (Range 200-500, N=10)	72	675±187.45 (Range 250-900, N=10)
40	30	124±40.87 (Range 100-200, N=10)	35	184±100.13 (Range 100-300, N=10)	42	315±81.81 (Range 200-400, N=10)
50	10	110±10.54 (Range 100 120-, N=10)	15	136±40.60 (Range 100-200, N=10)	25	240±56.76 (Range 150-300, N=10)
60	-		4	102±4.21 (Range 100-110, N=10)	10	108±9.18 (Range 100-120, N=10)
70	-		-		-	

Table 2: Effect of Boric acid on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (µg/ml)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
Distilled Water	-	-	-	-	-	-
5	1	97±4.84 (Range 90-100, N=10)	5	109±9.94 (Range 100-120, N=10)	8	144±48.80 (Range 100-200, N=10)
10	3	103±4.83 (Range 100-110, N=10)	8	133±40.29 (Range 100-200, N=10)	10	260±93.68 (Range 100-350, N=10)
20	10	137±39.73 (Range 100-200, N=10)	16	202±75.54 (Range 100-300, N=10)	20	305±125.72 (Range 100-400, N=10)
30	18	190±51.63 (Range 100-300, N=10)	30	245±121.22 (Range 100-400, N=10)	35	350±150.92 (Range 100-500, N=10)
40	20	240±124.27 (Range 100-400, N=10)	32	340±164.65 (Range 100-500, N=10)	38	415±145.39 (Range 200-600, N=10)
50	25	280±181.35 (Range 100-500, N=10)	40	440±157.76 (Range 200-600, N=10)	45	500±149.07 (Range 300-700, N=10)
100	22	250±143.37 (Range 100-400, N=10)	38	410±128.66 (Range 200-500, N=10)	42	460±157.76 (Range 200-600, N=10)
200	15	197±80.83 (Range 100-300, N=10)	20	310±110.05 (Range 100-400, N=10)	30	390±137.03 (Range 100-500, N=10)
300	10	157±53.13 (Range 100-250, N=10)	15	220±78.88 (Range 100-300, N=10)	20	245±155.36 (Range 50-400, N=10)
400	5	103±6.74 (Range 100-120, N=10)	8	167±44.73 (Range 100-200, N=10)	11	207±87.18 (Range 100-300, N=10)
500	3	102±4.21 (Range 100-110, N=10)	5	84±23.66 (Range 50-100, N=10)	8	147±48.08 (Range 100-200, N=10)
600	2	99±7.37 (Range 90-110, N=10)	3	105±8.49 (Range 100-120, N=10)	4	109±12.86 (Range 100-130, N=10)
700	-		-		-	

Table 3: Combine effect of Sucrose and Boric acid on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (%) + (µg/ml)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
30+5	30	450±135.40 (Range 300-700, N=10)	42	670±170.29 (Range 500-900, N=10)	50	690±179.19 (Range 500-1000, N=10)
30+20	36	560±134.98 (Range 400-800, N=10)	48	720±147.57 (Range 600-1000, N=10)	56	890±152.38 (Range 600-1000, N=10)
30+30	40	750±177.95 (Range 500-1000, N=10)	58	810±179.19 (Range 600-1000, N=10)	64	930±200.27 (Range 600-1200, N=10)
30+50	45	900±309.12 (Range 500-1400, N=10)	68	1140±245.85 (Range 700-1500, N=10)	75	1230± 294.58 (Range 500-1500, N=10)
30+100	42	780±285.96 (Range 300-1200, N=10)	52	970±258.41 (Range 500-1200, N=10)	60	1020±169.97 (Range 500-1300, N=10)
30+200	38	630±279.08 (Range 200-1000, N=10)	48	830±262.67 (Range 300-1000, N=10)	50	870±286.93 (Range 400-1200, N=10)
30+300	32	600±240.37 (Range 200-900, N=10)	42	740±337.30 (Range 200-1000, N=10)	43	790±251.44 (Range 300-1100, N=10)
30+400	30	540±254.73 (Range 100-800, N=10)	36	620±278.08 (Range 200-900, N=10)	40	770±226.32 (Range 300-1000, N=10)
30+500	22	510±223.35 (Range 100-700, N=10)	30	580±234.75 (Range 200-800, N=10)	36	750±206.82 (Range 300-900, N=10)
30+600	20	490±207.89 (Range 100-700, N=10)	27	590±233.09 (Range 200-800, N=10)	30	660±275.68 (Range 200-900, N=10)
30+700	15	450±171.59 (Range 100-600, N=10)	22	520±209.76 (Range 100-700, N=10)	25	540±211 (Range 200-800, N=10)
30+800	12	350±135.40 (Range 100-500, N=10)	18	410±191.19 (Range 100-600, N=10)	20	470±182.87 (Range 200-700, N=10)
30+900	8	310±117.37 (Range 100-450, N=10)	10	380±168.65 (Range 100-600, N=10)	15	410±144.91 (Range 200-600, N=10)
30+1000	5	260±69.92 (Range 100-300, N=10)	8	340±142.98 (Range 100-500, N=10)	12	360±134.98 (Range 100-500, N=10)

Table 4: Effect of Ca $(NO_3)_2$ on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (µg/ml)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
10	1	98±4.21 (Range 90-100, N=10)	2	149±39.84 (Range 100-200, N=10)	4	200±91.28 (Range 100-300, N=10)
20	2	130±25.81 (Range 100-150, N=10)	4	197±80.83 (Range 100-300, N=10)	6	265±137.53 (Range 100-400, N=10)
30	3	173±41.64 (Range 100-200, N=10)	6	247±95.34 (Range 100-350, N=10)	8	295±164.06 (Range 100-450, N=10)
40	5	203±60.37 (Range 100-250, N=10)	8	262±112.03 (Range 100-400, N=10)	10	360±155.99 (Range 150-500, N=10)
50	8	235±81.81 (Range 100-300, N=10)	10	297±158.04 (Range 100-500, N=10)	15	415±173.28 (Range 200-600, N=10)
100	10	305±89.59 (Range 200-400, N=10)	15	395±164.06 (Range 200-600, N=10)	19	460±183.78 (Range 300-800, N=10)
200	7	190±51.63 (Range 100-250, N=10)	9	270±103.27 (Range 100-400, N=10)	12	320±137.84 (Range 150-500, N=10)
300	3	140±21.08 (Range 100-150, N=10)	6	215±47.43 (Range 100-250, N=10)	10	265±100.13 (Range 100-400, N=10)
400	2	114±9.66 (Range 100-120, N=10)	4	142±37.94 (Range 100-180, N=10)	9	190±51.63 (Range 100-250, N=10)
500	1	103±4.83 (Range 100-110, N=10)	2	120±23.09 (Range 100-150, N=10)	6	175±35 (Range 100-200, N=10)
600	1	98±4.21 (Range 90-100, N=10)	1	109±9.94 (Range 100-120, N=10)	4	130±25.81 (Range 100-150, N=10)
700	-		1	102±4.21 (Range 100-110, N=10)	2	110±10.54 (Range 100-120, N=10)
800	-		-		1	95±5.27 (Range 90-100, N=10)

Table 5: Effect of $MgSO_4$ on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (µg/ml)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
10		94±5.16 (Range 90-100, N=10)	1	103±4.83 (Range 100-110, N=10)	3	121±14.49 (Range 100-130, N=10)
20	1	98±4.21 (Range 90-100, N=10)	2	110±10.54 (Range 100-120, N=10)	4	133±28.69 (Range 100-160, N=10)
30	2	114±15.05 (Range 100-130, N=10)	4	125±26.35 (Range 100-150, N=10)	6	170±42.16 (Range 100-200, N=10)
40	3	131±21.31 (Range 100-150, N=10)	5	145±49.72 (Range 100-200, N=10)	6	205±79.75 (Range 100-300, N=10)
50	3	136±29.13 (Range 100-180, N=10)	5	170±34.96 (Range 100-200, N=10)	8	220±91.89 (Range 100-300, N=10)
100	3	160±44.22 (Range 100-200, N=10)	7	205±49.72 (Range 100-250, N=10)	10	245±134.26 (Range 100-400, N=10)
200	2	130±20.54 (Range 100-150, N=10)	4	180±34.96 (Range 100-200, N=10)	7	210±69.92 (Range 100-300, N=10)
300	1	112±10.32 (Range 100-120, N=10)	3	125±26.35 (Range 100-150, N=10)	6	180±42.16 (Range 100-200, N=10)
400	1	96±5.16 (Range 90-100, N=10)	2	110±10.54 (Range 100-120, N=10)	4	125±26.35 (Range 100-150, N=10)
500	-		1	98±4.21 (Range 90-100, N=10)	2	118±15.49 (Range 100-130, N=10)
600	-		1	95±5.27 (Range 90-100, N=10)	2	107±4.83 (Range 100-110, N=10)

Table 6: Effect of KNO_3 on *in vitro* pollen germination of *Luffa cylindrica*

Concentration (µg/ml)	After 1 hr		After 4 hr		After 8 hr	
	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)	Pollen germination (%)	Pollen tube length (µm)
10	1	95±5.27 (Range 90-100, N=10)	1	102±4.21 (Range 100-110, N=10)	2	111±8.75 (Range 100-120, N=10)
20	1	96±5.16 (Range 90-100, N=10)	2	114±9.66 (Range 100-120, N=10)	2	122±12.29 (Range 100-130, N=10)
30	1	105±5.27 (Range 100-110, N=10)	2	121±11.97 (Range 100-130, N=10)	4	134±21.70 (Range 100-150, N=10)
40	2	115±8.49 (Range 100-120, N=10)	4	139±14.49 (Range 120-150, N=10)	6	180±33.73 (Range 100-180, N=10)
50	2	101±3.16 (Range 100-110, N=10)	3	118±11.35 (Range 100-130, N=10)	4	128±18.13 (Range 100-150, N=10)
100	1	97±4.83 (Range 90-100, N=10)	2	111±8.75 (Range 100-120, N=10)	2	119±11.00 (Range 100-130, N=10)
200	-		1	98±4.21 (Range 90-100, N=10)	1	105±8.49 (Range 100-120, N=10)

Table 7: Statistical analysis of *in vitro* pollen germination and pollen tube length of *Luffa cylindrica* in different nutrients

Substrate	N	Mean	SD	SE	t-value	df	$P_{0.01}$	Significant at 0.01 level of probability
***In vitro* pollen germination**								
G1	12	22.583	14.779	4.226	2.78	24	2.797	Not significant
G2	14	41.142	19.210	5.134				
G1	12	22.583	14.779	4.226	4.001	21	2.831	Significant
G3	11	5.272	2.533	0.76				
G1	12	22.583	14.779	4.226	4.54	17	2.898	Significant
G4	7	3	1.73	0.654				
G1	12	22.583	14.779	4.226	3.213	23	2.807	Significant
G5	13	8.153	5.161	1.431				
G2	14	41.142	19.210	5.134	6.91	23	2.807	Significant
G3	11	5.272	2.533	0.76				
G2	14	41.142	19.210	5.134	7.377	19	2.861	Significant
G4	7	3	1.73	0.654				
G2	14	41.142	19.210	5.134	6.195	25	2.787	Significant
G5	13	8.153	5.161	1.431				
G3	11	5.272	2.533	0.76	2.29	16	2.921	Not significant
G4	7	3	1.73	0.654				
G3	11	5.272	2.533	0.76	1.617	22	2.819	Not significant
G5	13	8.153	5.161	1.431				
G4	7	3	1.73	0.654	3.280	18	2.878	Significant
G5	13	8.153	5.161	1.431				
***In vitro* pollen tube length**								
T1	12	294.333	130.232	37.594	5.944	24	2.797	Significant
T2	14	741.428	243.779	65.152				
T1	12	294.333	130.232	37.594	3.164	21	2.831	Significant
T3	11	166.727	48.373	14.585				
T1	12	294.333	130.232	37.594	4.283	17	2.898	Significant
T4	7	128.428	24.744	9.352				
T1	12	294.333	130.232	37.594	0.850	23	2.807	Not significant
T5	13	252.307	115.643	32.073				
T2	14	741.428	243.779	65.152	8.607	23	2.807	Significant
T3	11	166.727	48.373	14.585				
T2	14	741.428	243.779	65.152	9.313	19	2.861	Significant
T4	7	128.428	24.744	9.352				
T2	14	741.428	243.779	65.152	6.736	25	2.787	Significant
T5	13	252.307	115.643	32.073				
T3	11	166.727	48.373	14.585	2.210	16	2.921	Not significant
T4	7	128.428	24.744	9.352				
T3	11	166.727	48.373	14.585	2.428	22	2.819	Not significant
T5	13	252.307	115.643	32.073				
T4	7	128.428	24.744	9.352	3.708	18	2.878	Significant
T5	13	252.307	115.643	32.073				

G1- percentage of germination and T1-tube length in boric acid at 5-600 µg/ml; G2- percentage of germination and T2-tube length in sucrose (30%) and boric acid (5-1000 µg/ml); G3- percentage of germination and T3-tube length in magnesium sulphate at 10-600 µg/ml; G4- percentage of germination and T4-tube length in potassium nitrate at 10-200 µg/ml; G5- percentage of germination and T5-tube length in calcium nitrate at 10-600 µg/ml; N-number of observation; SD- Standard deviation in each group; SE- Standard error of difference of means; t-value-Student test value; df- Degree of freedom; $P_{0.01}$- Value of t for probability of 1% level.

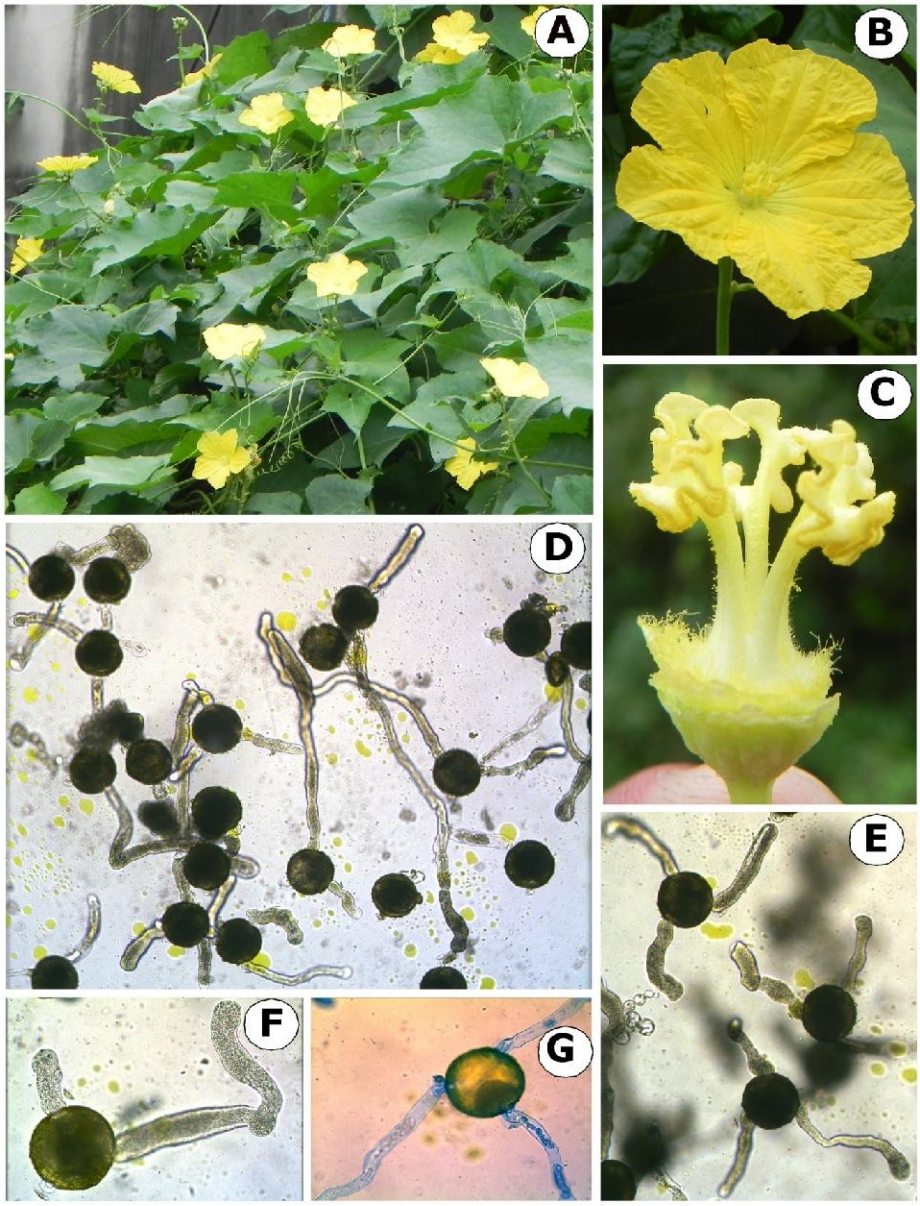

Figure 1: *In vitro* pollen germination of *Luffa cylindrica*: A. Plant habit, B. A flower, C. Dehisced anther lobe, D. Germinating pollen, E. Polysiphonous pollen tube, F. Development of thick pollen tube in sucrose solution and G. Development of thin pollen tube in sucrose solution supplemented with boric acid.

The t-value is insignificant in between the pollen germination of G1 and G2, G3 and G4 and G3 and G5, but significant in between G1 and G3, G1 and G4, G1 and G5, G2 and G3, G2 and G4, G2 and G5 and G4 and G5 (Table 7) at 0.01 level of probability respectively.

The t-value of pollen tube length in between T1 and T5, T3 and T4 and T3 and T5 is insignificant but significant in between T1 and T2, T1 and T3, T1 and T4, T2 and T3, T2 and T4, T2 and T5 and T4 and T5 (Table 7) at 0.01 level of probability respectively.

Discussions

The *in vitro* effect of sucrose suggests that sucrose has an increasing influence in pollen germination which is directly proportional to the concentrations of sucrose up to 30% beyond that the germination percentage decreases in *L. cylindrica*. The role of boron has also been confirmed in germination of pollen and growing pollen tubes (4). It was studied by Subramanyam (5) and Vasil (6) that pollen grains of apple, papaya, grape, guava and *Cucumis* germinate in boron. But, if boric acid is supplemented with sucrose, the germination of pollen and

pollen tube elongation are increased. The best 75% germination along with 1230± 294.58 µm pollen tube growths occurs in 30% sucrose supplemented with 50 µg/ml H_3BO_3 solution. This is attributed to the fact that sucrose acts as substrate for proper pollen nutrition and also functions as osmoregulator, but boron may enhance the sucrose uptake and stimulate germinating ability. This is due to formation of a sugar-borate complex, which acts as better traslocator than non borate complex (6). Pronounced effect of sucrose and boric acid on increasing tends of pollen germination might be reflected the views of Johri & Vasil (7). Statistical analysis in between G1 and G2 indicates that the t-value is insignificant at 0.01 level of probability, which indicating that the impact of boric acid alone and combine impact of boric acid and sucrose does not differ significantly on pollen germination but, significant t-value in between T1 and T2 indicating that boron influences pollen tube elongation. More over it was observed that boron deficiency causes morphological abnormalities of pollen tube which includes swelling of the tip as well as thickening of pollen tube, when grown in sucrose solution individually. Similar findings have been reported in several angiospermic species (8, 9, 10). The morphological effects of boron during pollen tube growth in angiosperms have also been investigated (8, 11, 12, 13). Biochemical investigation revealed that such swelling and thickening of pollen tube is due to accumulation of a polysaccharide components, callose and pectin. Boron may directly or indirectly influence the synthesis of callose and affecting its distribution (14), whereas pectin controls pollen tube elongation (15, 16). Stanley and Loewus (17) stated that boron is involved in pectin synthesis and thereby involved in development of pollen tube membrane. Acidic pectin may enhance tube strength and decrease extensibility of the pollen tube wall by accumulation of Ca^+ (18, 19).

Significant t-value in between G1 and G3, G1 and G4, T1 and T3 and T1 and T4 indicating that the impact of boric acid is significantly better than that of magnesium sulphate and potassium nitrate on pollen germination as well as pollen tube elongation in *L. cylindrica*. But significant t-value in between G1 and G5 and insignificant t-value in between T1 and T5 indicate that boric acid is significantly better on *in vitro* pollen germination than that of Calcium nitrate but, boric acid and Calcium nitrate has similar impact on *in vitro* pollen tube elongation.

Insignificant t-value in between G3 and G4, G3 and G5, T3 and T4 and T3 and T5 indicating that impact of magnesium sulphate is similar with that of potassium nitrate and calcium nitrate on pollen germination as well as pollen tube elongation in *L. cylindrica*. But significant t-value in between G4 and G5 and T4 and T5 indicate that calcium nitrate is better than that of potassium nitrate on pollen germination and pollen tube elongation of *L. cylindrica*, although, Shivanna & Johri (20) postulated the crowding effect of pollen grains, where better germination of pollen grain is due to calcium ions. It is assumed that calcium is required for maintenance of membrane integrity and permeability (21, 22, 23, 24, 25). Picton & Steer (26) and Miller *et al.,* (27) indicated that calcium concentration is helpful in pollen tube elongation. Moore & Jung (28) demonstrated that enhance growth of pollen tube is due to magnesium ions. However reduced pollen germination and pollen tube growth in KNO_3 is due to active transport of K^+ ion and NO_3 (29). Weisenseel & Jaffe (30) and Feijo *et al.,* (29) reported the requirement of K^+ ions in pollen germination as well as pollen tube elongation in sugarcane. Sawidis & Reiss (31) and Taylor & Hepler (32) reported that pollen germination and pollen tube growth may affected by many factors which includes temperature, availability of calcium, zinc, boron etc. Significant t-value in between G2 and G3, G2 and G4, G2 and G5, T2 and T3, T2 and T4 and T2 and T5 indicating that combine effect of sucrose and boric acid is significantly better than that of Magnesium sulphate, potassium nitrate and calcium nitrate on *in vitro* pollen germination and pollen tube length in *Luffa cylindrica*.

However, pollen germination of certain members of cucurbitaceae and *Luffa cynindrica* have been documented by Zaman (33), Khan & Perveen (34) and Prajapati & Jain (35) in Brewbaker and Kwack's media. But in the present investigation the role of sucrose, boric acid, Calcium nitrate, Magnesium sulfate and Potassium nitrate on pollen germination as well as pollen tube elongation was studied and the results were corroborated by the findings of Steer & Steer (36), Mondal *et al.,* (37), Demeke & Hughes (38), Kaliamoorthy *et al.,* (39) Bhattacharya

& Mandal (40), Biswas *et al.,* (41), Mondal & Ghanta (42), Choudhury *et al.,* (43) and Biswas & Mondal (44).

Conclusion

Pollen viability is crucial in plant breeding programme. The viable pollen is prerequisite for successful post pollination events, which ultimately results high crop yields. The knowledge about biochemistry and physiology of pollen viability largely comes from *in vitro* pollen germination. In *Luffa cylindrica*, significant pollen germination as well as pollen tube elongation was resulted in sucrose solution supplemented with boric acid, because the sugar borate complex is capable of better translocation which maintains osmoregulation, pectin synthesis and subsequent distribution for pollen tube elongation. Salts like Calcium nitrate, Magnesium sulphate and Potassium nitrate also play positive role on *in vitro* pollen germination and pollen tube elongation.

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Source of support: Nil
Conflict of interest: None Declared