Vitis 6, 269—277 (1967)

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Effects of Certain Growth Substances and Boric Acid on Germination, Tube Growth and Storage of Grape Pollen (Vitis ssp.)

by

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This paper presents the results of experiments conducted on the fertility, germination, tube growth and storage of pollen grains of five grape cultivars. Attempts were made to explore the possibility of using various chemicals to stimulate the germination and tube growth of fresh and stored pollen grains. It is hoped that these studies would be useful in the grape hybridization programme now in progress at different grape research centres in the country.

Materials and Methods

Three cultivars of *Vitis vinifera* L. (Pearl of Csaba, Pusa Seedless and Bhokri) and two hybrids of *V. vinifera* × *V. labrusca* (Bangalore Blue and Golden Queen) were used for the present investigations. Four chemicals: boric acid, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellic acid (GA) were used to study their effect on pollen germination and pollen tube growth.

Acetocarmine staining test was used to determine pollen fertility. The plump and well stained pollen grains were classed as fertile and shrivelled unstained as sterile. Fertility percentage of the pollen grains was worked out of random fields in each slide. Germination test was conducted by hanging drop techniques using a wide range of sucrose solutions (10, 15, 20 and 30%). The highest pollen germination in different grape cultivars was in 20 per cent sucrose solution (Fig. 2 a), and further increase in sucrose concentration resulted in significant reduction in pollen germination. Thus optimum basal medium was found to be 20 per cent sucrose. Solutions of GA, IAA, IBA and boric acid were, therefore, prepared in 20 per cent sucrose solution. Germination tests were conducted at a temperature of 27—28° C with two replications. Preliminary observations, after various periods of incubation, showed that most of the pollen germinated after 12 hours at 27—28° C. Therefore, observations on germination were taken after 12 hours of incubation. The percentage of pollen germination was obtained from ten fields in each slide taking approximately 300 to 500 pollen grains. Fifty pollen tubes were measured, at random, by ocular micrometer and tube length recorded in microns.

Pollen grains were stored at three combinations of temperature and relative humidity (R. H.), viz., 25 to 29° C (room temperature), 4—5° C, —12 to —10° C and 0, 25, 50 per cent R. H. The humidity was maintained in desicators by a mixture of sulphuric acid and water (Wilson 1921). Anthers were collected in petri-dishes and allowed to dehisce in the laboratory. The whole pollen sample was divided equally into required number of small lots; transferred to small glass vials which were

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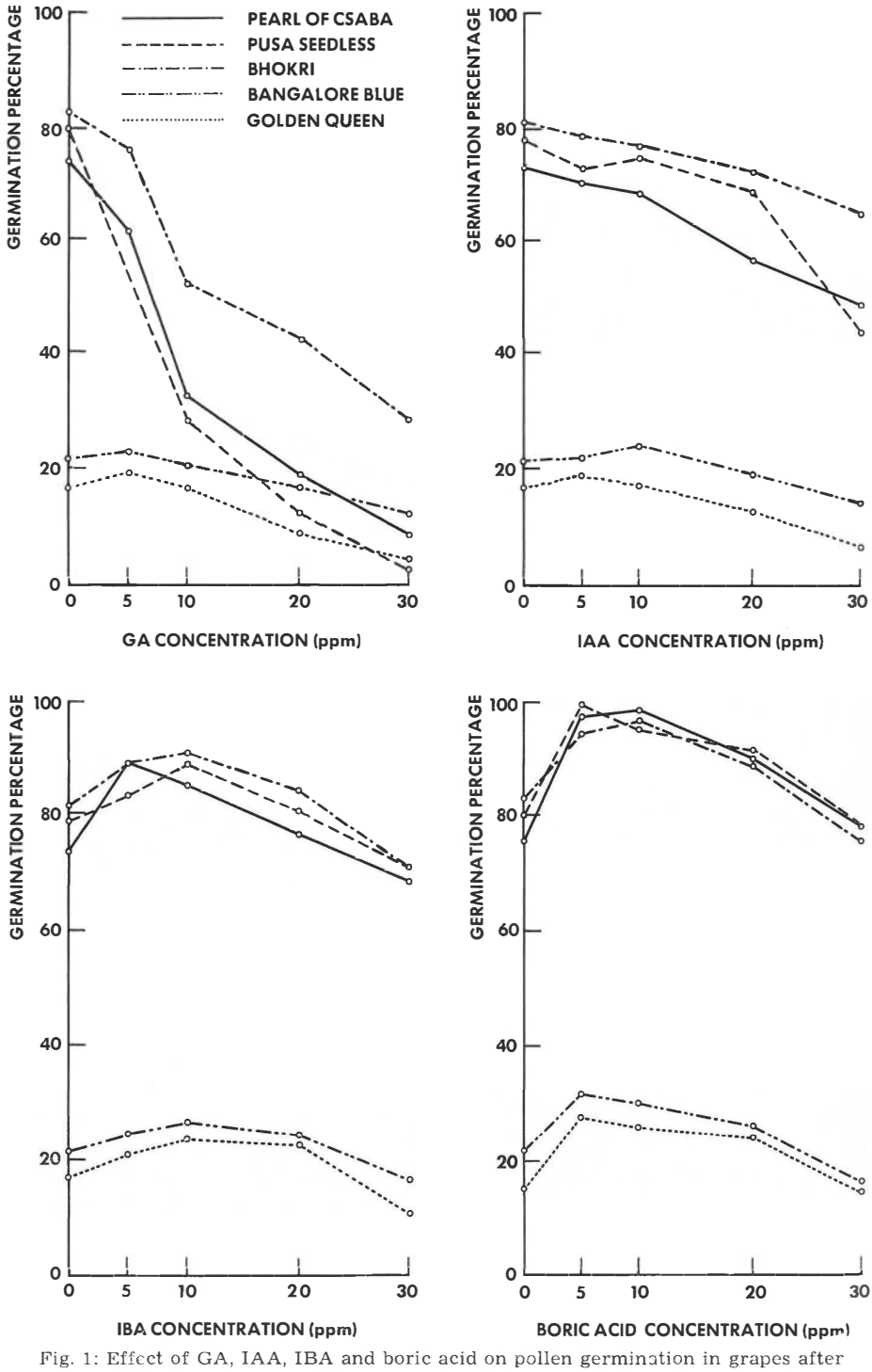

Fig. 1: Effect of GA, IAA, IBA and boric acid on pollen germination in grapes after 12 hours incubation at 27 to 28° C.

Table 1

Pollen fertility of grape cultivars as measured by acetocarmine stain test.

Variety	No. of grains observed	No. of fertile grains	% of fertile pollen grains
Pearl of Csaba	655	600	91.60
Pusa Seedless	603	583	96.68
Bhokri	729	693	95.05
Bangalore Blue	600	167	27.83
Golden Queen	304	86	28.28

loosely closed with cotton plugs and kept at the above mentioned temperature and relative humidity combinations.

The first viability test of the stored pollen was done after 15 days and later on at intervals of one, four, eight, and twelve months. Viability was tested by germinating the pollen grains *in vitro* in the standardized basal medium of 20 per cent sucrose solution. Since, boric acid, in the first experiment, stimulated germination and pollen tube growth considerably, it was decided to test the viability of stored pollen grain with boric acid (5, 10, 20 ppm) added to the basal medium.

Results

Pollen fertility

All the five grape cultivars used in this study had perfect flowers. The highest pollen fertility was in Pusa Seedless (96.68%) followed by Bhokri (95.05%). But, in Bangalore Blue and Golden Queen (*V. vinifera* × *V. labrusca*), majority of pollen grains were shrunken and the pollen fertility was considerably less than the other varieties (Table 1).

Pollen germination

Significantly higher pollen germination was obtained with boric acid when compared to GA, IAA and IBA treatments and control. IAA and GA significantly reduced the pollen germination over the control except in Bangalore Blue and Golden Queen where 5 and 10 ppm IAA and GA showed slight increase in pollen

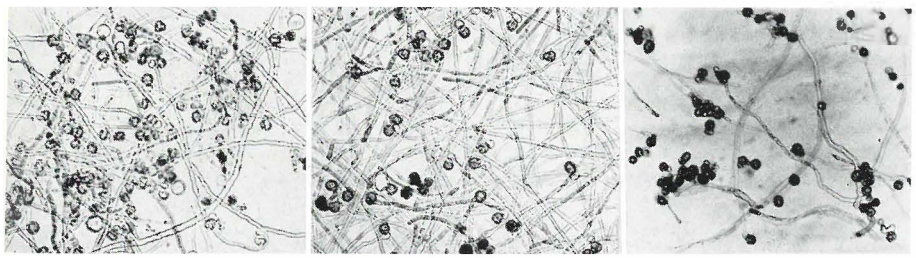

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Fig. 2: (a) Pollen germination in Pusa Seedless in 20 per cent surcrose solution (control), (b) pollen germination in Pusa Seedless treated with 10 ppm boric acid, showing the increased germination and (c) pollen germination in Pusa Seedless, treated with 5 ppm GA showing decreased germination but increased tube growth.

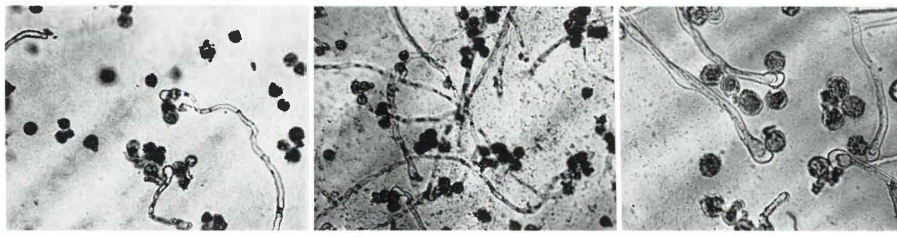

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Fig. 3: (a) Pollen germination in Pusa Seedless after 8 months storage at —12° to —10° C with 25 per cent R. H., without boric acid treatment (control), (b) pollen germination in Pusa Seedless after 12 months storage at —12° to —10° C with 25 per cent R. H. and treated with 10 ppm boric acid and (c) pollen germination in Pearl of Csaba after 4 months storage at —12° to —10° C with per cent R. H. and treated with 10 ppm boric acid.

germination over control. Bhokri exhibited the highest pollen germination followed by Pusa Seedless and Pearl of Csaba. The lowest pollen germination was observed in Golden Queen and Bangalore Blue. The response of grape cultivars to different concentrations of growth substances and boric acid varied considerably. But, in general, the lower concentrations (5 and 10 ppm) significantly increased the germination percentage (Figs. 1 and 2 b).

Table 2

Effect of growth substances on pollen tube growth (in microns).

Growth Substances	Concentration (ppm)	Varieties				
		Pearl of Csaba	Pusa Seedless	Bhokri	Bangalore Blue	Golden Queen
GA	5	882	1337	V.L.	422	462
	10	1091	V.L.	1176	487	543
	20	V.L.	1105	909	401	434
	30	538	411	361	406	354
IAA	5	753	983	787	339	411
	10	819	1007	871	372	406
	20	613	784	715	302	410
	30	318	543	464	205	280
IBA	5	572	1200	V.L.	378	491
	10	794	1266	V.L.	479	536
	20	964	1316	1275	424	549
	30	282	481	517	222	374
Boric acid	5	1083	V.L.	V.L.	567	593
	10	V.L.	V.L.	V.L.	562	644
	20	946	1181	1357	463	532
	30	402	537	702	310	435
Control	20% Sucrose	551	522	609	345	432

V. L. — very long.

Pollen tube growth

There was considerable influence of growth substances and boric acid on the pollen tube elongation. Although, GA and IAA retarded the pollen germination, tube growth was more than in control (Fig. 2 c). Boric acid produced very long pollen tubes at 5, 10 and 20 ppm which was followed by GA, IBA and IAA treatments. However, all the growth substances and boric acid at 30 ppm showed less pollen tube growth than control in all the grape cultivars, except Pusa Seedless where 30 ppm boric acid and IAA showed marginal increase over the control (Table 2).

Table 3

Percentage viability of Pusa Seedless pollen stored at different temperatures and relative humidity combinations.

Temperature		−12 to −10° C			4 to 5° C			Rom temperature (27° C—28° C)		
Humidity		0%	25%	50%	0%	25%	50%	0%	25%	50%
Storage Period	Boric acid (ppm)									
15 days	0	59.92	60.47	52.26	57.11	60.10	41.06	7.61	4.70	0
	5	72.00	72.02	67.35	68.29	70.43	66.17	18.00	12.10	5.13
	10	72.56	75.21	63.81	70.47	74.14	66.79	15.10	11.26	5.92
	20	71.00	73.23	65.10	62.24	71.25	50.32	7.84	6.30	1.28
		Pollen fertility at 4° C to 8° C with 25% R. H.: 95.89%								
1 month	0	27.81	36.13	0	19.95	23.34	9.13	0	0	0
	5	64.24	64.53	22.00	47.73	63.15	28.90	0	0	0
	10	69.38	71.76	26.45	61.12	70.42	29.06	1.96	0	0
	20	43.10	48.95	8.77	34.16	52.29	17.11	0	0	0
		Pollen fertility at 4° C to 8° C with 25% R. H.: 95.02%								
4 months	0	15.26	20.44	0	11.73	17.00	1.89	0	0	0
	5	41.24	50.24	10.91	40.23	45.92	22.00	0	0	0
	10	56.17	66.29	15.36	49.62	60.10	22.14	0	0	0
	20	32.09	32.70	2.65	25.21	29.54	8.33	0	0	0
		Pollen fertility at 4° C to 8° C with 25% R. H.: 92.19%								
8 months	0	7.72	8.15	0	6.15	6.67	0	0	0	0
	5	33.85	36.60	3.57	31.19	33.28	9.25	0	0	0
	10	42.18	48.32	4.14	37.00	42.61	12.03	0	0	0
	20	26.12	29.46	0	17.37	18.19	2.79	0	0	0
		Pollen fertility at 4° C to 8° C with 25% R. H.: 92.00%								
12 months	0	0	0	0	0	0	0	0	0	0
	5	17.36	24.53	0	16.74	18.90	0	0	0	0
	10	22.00	30.68	0	19.35	7.87	0	0	0	0
	20	10.43	13.29	0	4.12	5.59	0	0	0	0
		Pollen fertility at 4° C to 8° C with 25% R. H.: 69.57%								

20 per cent sucrose solution was the basal medium for boric acid concentrations.

Pollen storage

Pollen grains of Pusa Seedless were stored on April 2, 1963 at different temperatures and relative humidity conditions. The viability tests were carried out after 15 days, 1 month, 4 months and 12 months, by *in vitro* germination test using 20 per cent sucrose solution as basal medium. Due to the limited material available in Pearl of Csaba, the pollen could be stored only for four months starting from March 23, 1964.

After 12 months storage at −12 to −10° C and 25 per cent R. H. Pusa Seedless pollen retained considerable viability (30.68%), (Fig. 3 b). However, at 4 to 5° C there was loss of viability after 12 months when compared to storage at −12 to −10° C at the same R. H. (25%). At room temperature, (25 to 29° C), pollen grains were viable only for a period of 6 to 7 days (Table 3). The addition of boric acid to the basal medium significantly increased pollen viability of Pusa Seedles and Pearl of Csaba throughout the storage period (Tables 3 and 4).

Pollen grains of Pearl of Csaba exhibited a similar trend as that of Pusa Seedless. Thus, after four months of storage at −12 to −10° C and 25 per cent R. H. the pollen viability was 40.52 per cent when 10 ppm boric acid was added to the basal medium (Table 4; Fig. 3 c).

Fertility, as measured by acetocarmine staining, did not show any difference even after 12 months storage. Fertility percentage recorded after 8 months of storage at 4 to 8° C was 92 per cent (Table 3). In Pearl of Csaba, the pollen fertility observed was 86.81 per cent after 4 months of storage (Table 4).

Table 4

Percentage viability of Pearl of Csaba stored at different temperatures and relative humidity combinations.

Tomperature		−12° C to −10° C		4° C to 8° C		Room Temperature	
Humidity		0%	25%	0%	25%	0%	25%
Storage Period	Boric acid (ppm)						
15 days	0	70.21	70.59	45.69	51.24	0	0
	5	72.14	75.15	63.73	71.10	9.38	9.00
	10	74.32	76.12	64.35	71.42	12.15	10.28
	20	68.48	73.00	59.26	67.60	5.00	0
	Pollen fertility at 4° C to 8° C with 25% R. H.: 91.00%						
1 month	0	61.27	62.02	20.89	27.22	0	0
	5	66.25	66.38	48.13	49.45	0	0
	10	65.83	69.47	50.29	53.96	0	0
	20	57.79	64.18	36.75	41.34	0	0
	Pollen fertility at 4° C to 8° C with 25% R. H.: 90.75%						
4 months	0	10.90	13.69	4.79	5.87	0	0
	5	29.10	29.77	18.84	22.27	0	0
	10	33.07	40.52	26.65	32.00	0	0
	20	24.15	28.23	11.47	12.59	0	0
	Pollen fertility at 4° C to 8° C with 25% R. H.: 86.81%						

20 per cent sucrose solution was the basal medium for boric acid concentrations

Discussion

The highest pollen fertility was observed in Pusa Seedless (96.68%) and Bhokri (95.05%) and the lowest in Bangalore Blue (27.85%), probably because of its hybrid nature. These findings are in accord with those of RANDHAWA and SHARMA (1960).

Boric acid has shown pronounced stimulatory effects on pollen germination and pollen tube growth. The highest pollen germination and longer pollen tubes were obtained at the lower concentrations of boric acid (5, 10 and 20 ppm) but it had inhibitory effects at 30 ppm. According to VISSER (1955), pollen grains of certain species require as much as 1200 ppm of boric acid for best pollen germination and tube growth.

Many theories have been postulated for the stimulatory effects of the boric acid on pollen germination and tube growth. GRAUCH and DUGGAR (1953) proposed that the borate ions are associated with the cellular membrances where they react chemically with the sugar molecule facilitating its passage as the ionized sugar-borate complex through the membrane and that the sugar is freed inside the cell by a second reaction. According to TUPY (1960), boron encourages sucrose absorption in proportion to the stimulation of pollen tube growth. It is, therefore, possible that the stimulatory effect of boron on growth is connected with carbohydrate metabolism. The possible use of the boron spray during the bloom period for increasing fruit set has been reported by THOMPSON and BATJER (1950) in Anjou pear and they attributed it partially due to the stimulatory effect of boron on pollen germination and tube growth. GÄRTEL (1952) indicated that fruit set in *Vitis* spp. is dependent upon the presence of adequate quantity of boron on the stigmatic surface which favours the pollen germination and tube growth. The present studies show the possibility of using boron sprays for improving fruit set in some of the grape varieties where the set is generally low.

The addition of IBA (5 and 10 ppm) to the basal medium also increased the pollen germination and tube growth in all the grape varieties. Pusa Seedless and Bhokri responded most to IBA as compared to other three varieties. The length of the pollen tube in Pusa Seedless increased approximately two and half times more than in control.

Although, pollen germination was inhibited by IAA treatments, there was an appreciable increase in the length of pollen tubes in Pearl of Csaba, Pusa Seedless and Bhokri at 5, 10 and 20 ppm. KONAR (1958) cultured *Pinus roxburghii* pollen grains in IAA medium and obtained 80 per cent germination with longer pollen tubes. It is possible that IAA can stimulate the pollen germination and tube growth specifically of only certain species or varieties.

The percentage of pollen germination in all the grape cultivars gradually decreased with the increasing concentration of GA. Similar findings were reported by WEAVER and MCCUNE (1960) in grapes and SINGH and RANDHAWA (1961) in mandarins. But, DHURIA and RANDHAHA (1963) reported that GA stimulated pollen germination in Marsh Seeded grapefruit, whereas, in Sweet lime it had an inhibitory effect. KATO (1955) obtained increased pollen germination in *Lilium* and *Petunia* by GA treatments. This shows the specificity of GA action in different species with respect to pollen germination. Since GA stimulated pollen tube growth in several species it can be used to overcome certain physiological incompatibilities in certain clones of tree fruits.

Pollen grains of Pusa Seedless and Pearl of Csaba were stored under different combinations of temperature and humidity to find out the optimum combination for prolonging their viability. The pollen viability in Pusa Seedless after 12 months

storage at -12^0 to -10^0 C and 25 per cent relative humidity was 30.68 per cent. This can facilitate crosses between Pearl of Csaba (♀) with Pusa Seedless (♂) which otherwise is not possible because Pearl of Csaba flowers 15 days earlier than Pusa Seedless under Delhi conditions. After 15 days of storage at -12^0 to -10^0 C and 25 per cent R. H. pollen grains of Pearl of Csaba showed as much as 70.59 per cent viability as compared to 51.24 per cent at 4^0 to 8^0 C temperature and 25 per cent R. H. However, comparing the R. H. percentages, pollen viability at zero per cent was considerably higher than 50 per cent R. H. and not much different from those stored at 25 per cent. Thus it appears that with controlled temperature alone, it is possible to store pollen grains for a year or so. GÄRTEL *et al.* (1953) also found that grape pollen can be stored for one year by controlling the temperature alone. Further, NEBEL and RUTTLE (1937) and GOLLMICK (1942) could store grape pollen for one year at even a comparatively higher temperature of 8^0 to 10^0 C and 45 per cent R. H. However, in contrast to their findings in the present study, no viable pollens were found after 12 months storage with 50 per cent R. H. even at a lower temperature of 4^0 to 8^0 C (Table 3).

The present work has revealed that germination percentage of the stored pollen can be increased by boric acid treatment. This suggests that it is possible to increase the fruit set by boric acid sprays soon after crosses are made with stored pollen grains.

Summary

Investigations on the effects of growth substances (GA, IAA and IBA) and boric acid on grape pollen have shown that maximum germination and tube growth was obtained with boric acid at lower concentrations (5, 10 and 20 ppm) as compared to GA, IAA, IBA and control. Although GA showed lesser pollen germination, pollen tube growth was enhanced at 5, 10 and 20 ppm in the grape cultivars under study.

Pollen grains of Pusa Seedless were stored for 12 months and Pearl of Csaba for four months at different combinations of temperature and humidity. The pollen viability was highest when stored at -12^0 to -10^0 C with 25 per cent R. H. which was followed by 4^0 to 8^0 C and 25 per cent R. H. The pollen germination increased throughout the storage period by boric acid at 5, 10 and 20 ppm.

Acknowledgements

Authors are grateful to Dr. S. K. MUKHERJEE, Head of the Horticultural Division, Indian Agricultural Research Institute, for giving the facilities and for scrutiny of the manuscript. Thanks are also due to Dr. R. K. BAMMI and Mr. ELIAS CHACKO for going through the manuscript.

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Eingegangen am 9. 6. 1967

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