Tecoma stans L.(ノウゼンカズラ科)花粉の人工発芽

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In vitro pollen germination studies in *Tecoma stans* L.

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Introduction

Studies on pollen germination and tube growth in a large number of plants have received considerable attention (Martin, 1972; Vasil, 1974; Johri and Shivanna, 1977; Shivanna *et al.*, 1979). Pollen germination and tube elongation both require some organic as well as inorganic substances (Linskens and Kroh, 1970; Vasil, 1974; Mascarenhas, 1975). Besides, pollen germination and tube growth are influenced by a large number of external environmental factors apart from the initial nutritional status of the plant. The present investigation has, therefore, been undertaken to study the effect of temperature and inorganic as well as organic substances on *in vitro* germination in *Tecoma stans* an ornamental tree of *Bignoniaceae* exhibiting seasonally transient sterility.

Materials and Methods

Brewbaker and Kwack's (1963) hanging drop culture technique was used to study the effect of temperature on germination percentage and tube length. Fresh pollen samples were collected in the morning at regular intervals throughout the course of the investigation i.e. 1979-1983. In order to avoid discrepancies, the pollens collected at the same time every day from different anthers of the same flower were mixed thoroughly. Germination percentage and tube growth was also observed in 10, 15, and 20 per cent sucrose solution. The effect of boric acid, calcium nitrate, Cycocel (2-chloroethyl trimethylammoniumchloride), 2,4-D (2,4-dichlorophenoxy acetic acid), GA_3 (gibberellic acid), MH (maleic hydrazide or 1,2-dichloropyridiazene, 3-6-dione), IAA (indole-3-acetic acid), and NAA (naphthalene acetic acid) was

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studied by supplementing sucrose solutions with 100, 150 and 200 mg/*l* of these substances. These studies were made under approximately similar environmental conditions. The cultures were stored at room temperature in diffused laboratory light. All the cultures were run in duplicate and random counts of 100 pollen grains were made to determine the percentage of germination. The length of 10 pollen tubes, randomly selected was measured.

Results and Discussion

Effect of Temperature :

The pollens cultured in Brewbaker and Kwack' s (1963) medium during different seasons of the year exhibited interesting results (Table 1). Based on the percentage of pollen germination, pollen types during different months were grouped into four classes, namely : (i) normal type during the months of December, January and February exhibited 75-95 % germination. Temperature during this period ranged between 16-23°C, (ii) semi-functional-a type in the months of March, September, October and November, when 50-74 per cent germination was observed. The temperature was 28-34°C. (iii) semi-functional-b type during the months of April and August exhibited only 5-49 % germination and temperature recorded was 32-37° C and (iv) nonfunctional type during months of May, June and July with temperature reaching its peak i.e. 40-45°C, the germination of pollens was completely inhibited.

However, some deviations in the extent of pollen germination percentage were also recorded. During December, 1982 and January, 1983, the temperature decreased considerably (4-10°C) and germination percentage was severely inhibited. On the oher hand, significantly high germiantion percentage was recorded during April and May, 1983 when the temperature declined to 15-20°C due to unseasonal rains.

Stanley and Kieby (1973) have enumerated several external environmental conditions and the initial nutritional status of the plants which, according to them, may influence the genetic control of pollen development. Temperature and photoperiod are probably the most critical environmental factors influencing pollen viability. They are of the opinion that either low or high temperatures at meiosis and during wall formation can block further pollen development resulting into partial or complete sterility.

Thus, it is clear from the above mentioned facts, that germination of pollen grains is largely influenced by temperature and any significunt rise or fall in temperature caused pollen sterility in the presently studied species.

Effect of media :

Table 2 and 3 show the mean percentage of pollen germination and the mean tube length in several different culture media. Table 4 shows the results of the analysis of variance for effects of different concentrations of boric acid and calcium nitrate in different concentrations of sucrose on the mean percentage of pollen germination and tube length. It shows that there are significant differences in the concentrations of sucrose and chemicals for both pollen germination and tube length, and in the kind of chemicals for the latter.

But no significant difference in interactions among them. Boric acid and calcium nitrate affect in the same way for pollen germination but different for tube length. Calcium nitrate tends to make tubes long.

When GA_3 is included in the analysis, variance between chemicals shows significant difference. It

means that GA_3 acts different way compared with boric acid and calcium nitrate.

Effect of sucrose :

As is evident from Table 2 and 3, the highest percentage (57) was recorded in 10 % sucrose solution. The tube length in this solution was 590.31 μm. On the other hand, the percentage of germination was 52 and 34 in 15 and 20 % sucrose solution with 319.11 and 147.72 μm long tubes, respectively.

Effect of Boric Acid :

The highest germination percentage (62) and maximum tube elongation (790.12 μm) was recorded in 10 % sucrose with 100 mg/l boric acid. However, with an increase in the concentration of both sucrose and boric acid, the percentage as well as tube length declined. In 20 % sucrose with 200 mg/l boric acid, only 10 % germination was recorded with 43.34 μm long tubes (Table 2).

The stimulatory effect of boric acid in dilute concentrations may be caused by its increased absorption. Sugar-borate complex is known to increase oxygen uptake and it has also an important role in the synthesis of pectic material requir-

Table 1. Percentage of pollen germination of *Tecoma* plants in Brewbaker and Kwack's medium during different months of a year.

Pollen type	Pollen germination		Temp. (°C)
Normal	90-75	December January and Feb.	17-23
Semi-functional-a	74-50	March, Sept. Oct. and Nov.	28-34
Semi-functional-b	49- 5	April and August	30-37
Nonfunctional	4- 0	May, June and July	40-45

Table 2. Effect of sucrose, boric acid and calcium nitrate on pollen germination (%) and tube length (μm) in *Tecoma*.

		Sucrose concentration (%)					
		10		15		20	
	(mg/l)	PG*	TL*	PG	TL	PG	TL
Control		57	590.21±12.23	52	319.11±10.07	34	142.72±22.12
Boric acid	100	62	790.12±31.32	52	259.67±34.00	41	136.02±17.80
	150	43	427.34±17.06	24	90.72±10.24	22	87.11± 7.18
	200	39	307.71±21.04	12	58.12±15.03	10	43.34±10.70
Calcium	100	64	775.26±51.01	24	375.12± 7.81	9	179.81±18.12
Nitrate	150	31	512.62±16.35	28	310.85±17.12	7	11.26±11.26
	200	21	397.16±21.24	18	173.21±16.21	5	52.61± 7.02

* PG ; Pollen Germination (%)
TL ; Tube Length (μm)

Table 3. Effect of GA_3, 2, 4-D, Cycocel, MH, IAA and NAA on *in vitro* pollen germination in *Tecoma*.

		Sucrose concentration (%)					
		10		15		20	
	(mg/*l*)	PG*	TL*	PG	TL	PG	TL
GA_3	100	21	156.49±11.58	37	86.94±18.52	3	30.42± 0
	150	36	130.41±29.72	15	43.47± 0	7	104.33± 2.12
	200	14	78.25±18.52	24	130.41±18.22	8	86.94± 6.45
2, 4-D	100	11	60.76± 8.50	10	65.21± 9.07	0	
	150	10	73.89±10.34	0		0	
	200	6	99.38±12.50	0		0	
Cycocel		No germination					
MH		do.					
IAA		do.					
NAA		do.					

* Symbols are the same as Table 2.

Table 4. Analysis of variance for effect of the concentrations of boric acid and calcium nitrate in different concentrations of sucrose on mean percentage of pollen germination (PG) and tube length (TL) in *Tecoma*.

Source of variation	df	Mean Square	
		PG	TL
Sucrose (Su)	2	617.441 **	423316.5 ***
Chemical (Ch)	1	188.216	14406.0 *
Concentration (Co)	3	490.636 **	73421.1 ***
Su × Ch	2	32.716	7083.5
Su × Co	6	16.080	10232.6
Ch × Co	3	41.881	1833.1
Error	6	35.525	2504.9

*, **, *** ; Significant at 5%, 1% and 0.5% levels, respectively.

ed for tube wall (Vasil, 1974). However, boric acid in higher concentrations seems to be toxic. There are reports that boron is a direct inhibitor of certain enzymes (Stanley and Loewus, 1964). The higher concentrations of boric acid in the present experiment might have inhibited the certainity of these enzymes which play an essential role in tube elongation.

Effect of Calcium Nitrate :

Effects of various concentrations of calcium nitrate on pollen germination and tube length is shown in Table 2. It is clear that by addition of

lower amounts of calcium nitrate in sucrose solution, both percentage as well as tube elongation increase as compared to that shown by sucrose alone. The maximum germination (64 %) was observed in 10 % sucrose with 100 mg/*l* calcium nitrate. The same concentration also exhibited longest pollen tube (775.26 μm). However, with an increase in the concentration of calcium nitrate, both the percentage as well as tube elongation were inhibited. Calcium has been demonstrated to enhance the tube elongation and deficiency of these ions may lead to the inhibition of pollen tube growth (Brewbaker and Kwack, 1963 and Kwack, 1967). According to them , calcium overcomes the population effect, promotes germination and also enhances tube elongation.

Effect of Growth Substances :

The effect of Cycocel, 2, 4-D, MH, on pollen germination percentage and tube elongation has been shown in Table 3. As is evident, Cycocel and MH completely inhibit pollen germination. Similarly, 2, 4-D also showed inhibitory effects on pollen germination percentage and tube length. However, 10 % sucrose with 100 mg/*l* 2, 4-D showed highest germination percentage (10.7) and longest tube length (99.98 μm) was observed in 10 % sucrose with 200 mg/*l* 2, 4-D. These substances are well known to inhibit pollen germination and tube elongation (Mascarenhas, 1975 and Shivanna *et al.* 1979).

IAA and NAA in different concentrations on supplementing with sucrose also completely inhibited pollen germination. On the other hand, GA_3 showed less inhibitory effects. It is evident by the fact that 15 % sucrose with 100 mg/*l* showed 37 % germination with 86.94 μm long tubes, while 10 % sucrose with 100 mg/*l* GA_3 showed only 21 % germination but tube length was significantly promoted (159.49 μm). According to Vasil (1962), the pollen grains appear to contain adequate quantity of growth substances (except gibberellins) and therefore, their addition to the medium does not appreciably affect germination and tube growth.

Present results also indicate that only GA_3 helps in germination of pollen in *Tecoma* and that IAA and NAA inhibited pollen germination in this plant. Inhibitory effect of IAA on pollen germiantion indicate the presence of sufficient quantites of endogenous levels of IAA in pollen grains of *Tecoma*.

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要　　約

ノウゼンカズラ科（*Bignoniaceae*）の観賞植物である *Tecoma stans* (yellow bells)は種子不稔について季節変動がみられる．花粉の人工発芽試験を行ったところ，開花時期の気温と花粉機能の間に密接な関係がみられた．すなわち気温が 28°C以上に上昇すると花粉発芽率が低下するようになり，40°C以上ではほとんどの花粉が不発芽であった．

人工発芽床の蔗糖濃度は 10 %が最適であり，20 %の条件では花粉発芽率と花粉管伸長が共に抑制された．硼素あるいは硝酸カルシウムの添加に関しては，蔗糖濃度 10 %と各薬剤の 100 mg/*l* の組合せでもっとも発芽が良好であり，各薬剤の 200 mg/*l* の添加または蔗糖濃度 20 %と組合せた場合ではいずれも発芽が抑制された．生長調節物質を添加した場合には，Cycocel，MH，IAA あるいは NAA の添加で発芽が完全に抑制され，2，4-D や GA_3 の添加でも発芽がやや抑えられた．しかし GA_3 の添加は他の調節物質よりも影響が少なかった．