# Morphology and Physiology of Pollen in Indian Cork Tree (*Millingtonia hortensis L.*)

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Pollen grains of *Millingtonia hortensis* are oval in shape, about 45 μm in diameter and tricolpate. The best germination percentage and longest pollen tubes were recorded in Brewbaker and Kwack's medium and 15% sucrose supplemented with 100 mg/l borax solution. In several concentrations of sucrose solutions with calcim nitrate, IAA and chloral hydrate, tube elongation was promoted. However, NAA, GA , MH and 2,4-D were found to be inhibitory for pollen germination and tube growth.

**Key words:** Pollen germination, Pollen tube growth, Growth regulators, Indian cork tree.

## Intoroduction

Indian cork tree ( *Millingtonia hortensis* L.) a member of the family *Bignoniaceae* is native of Burma and Malaya. This tall erect handsome tree is planted in the gardens and avenues for its beautiful foliage and silvery sheen of flowers. In spite of normal flowering this plant remains seedless. Preliminary morphological studies have revealed that apparently viable pollen grains on landing the stigmatic surface fail to germinate. To overcome this problem growth substance have been used by several investigators [5,15,17]. Present investigation has, therefore, been undertaken to study the morphology and physiology of pollen in *Millingtonia hortensis* L.

## Materials and methods

Present investigation was carried out on *Millingtonia hortensis* plants growing at the Botanical Garden, Raja Balwant Singh College, Bichpuri, Agra and Delhi University, Delhi.

For morphological studies, the pollen grains were acetolysed after the procedure of Nair [12] and these were mounted in glycerine jelly. For scanning electron microscopic (SEM)studies fresh samples of pollen collected from Delhi were immersed in liquid nitrogen for 12 hours. These were freeze dried by sublimation of ice. This was brought about under vacuum for 6 hours in freeze drier containing dry ice and acetone. For SEM studies, the samples were mounted on a disc and coated with carbon and gold in vacuum. Microphotographs were taken on a A-II cambridge scanning electron microscope at Textile Division, Indian Institute of Technology, New Delhi.

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For physiological studies, the pollen samples were collected soon after the dehiscence of anthers which usually occur between 8–9 a.m. For each experiment, the samples were collected from all the five anthers of a single flower at the same time and under approximately similar conditions every day. The pollen grains thus collected were used after anthesis.

Viability of pollen was tested by their stainability after the method of Alexander [1] and by hanging drop technique using Brewbaker and Kwack's [2] medium.

Effects of boron (boric acid and borax), calcium nitrate, indole acetic acid (IAA), naphthalene acetic acid (NAA), gibberellic acid ($GA_3$), 2,4-dichlorophenoxy acetic acid (2,4-D), maleic hydrazide (MH) and chloral hydrate on germination percentage and tube growth were studied by supplementing various concentrations of these substances in 10, 15 and 20% sucrose solutions. The cultures were stored at room temperature (25 ± 2 °C) in diffused laboratory light. All the culture were run in duplicate and random counts of 100 pollen grains were made to determine the percentage of germination. The lengths of 10 randomly selected pollen tubes were measured after 24 hrs. Standard deviations were calculated.

## Results and Discussion

Pollen morphology:

The pollen grains of *M. hortensis* were oval in shape (Fig.1) and the diameter is 42–46μm. They had tricolpate and thick and operculate colpus membranes. Columellae circular varied in shape and arranged in a simplicolumellate and reticulate pattern (Fig.2). Lamella is reticulate and varies in shape. It was circular to ellipsoidal and 1–2μm in diametrer. The tectum was uniformly and finely reticulate.

Pollen morphology in several members of the family *Bignoniaceae* has been studied by Erdtman [6]. According to him these pollen grains have of various types but mostly of 3-colporoidate type.

Buurman [3] has described pollen dimorphism (in length of polar axis) related to staminal dimorphism in *M. hortensis*. Present study has, however, failed to show dimorphism in the pollen as well as in stamens of *M. hortensis* growing in different parts of Indian sub-continent. Buurman [3] has given a detailed account of pollen morphology in several members of the family *Bignoniaceae*. Description of the present authors on *M. hortensis* is similar to that of Buurman [3].

Pollen physiology:

I. Pollen viability: Pollen viability as checked by stainability method after Alexander [1] was 92%.

II. *In vitro* pollen germination:

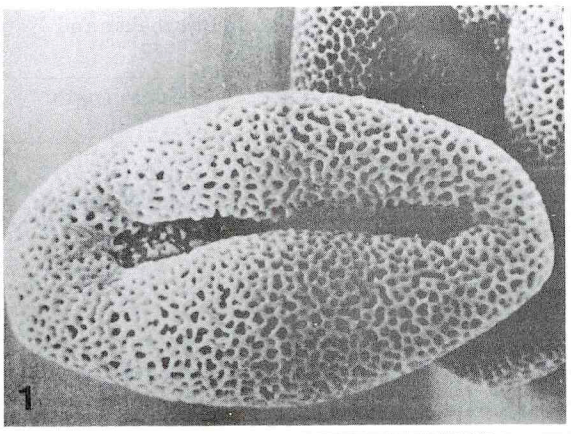
1. Effect of media: The percentage of the pollen germination and tube growth in Brewbaker and Kwack's medium [2] and in different concentrations of sucrose alone as well as in combination with different concentrations of various chemicals is described separately in Tables 1,2 and 3.

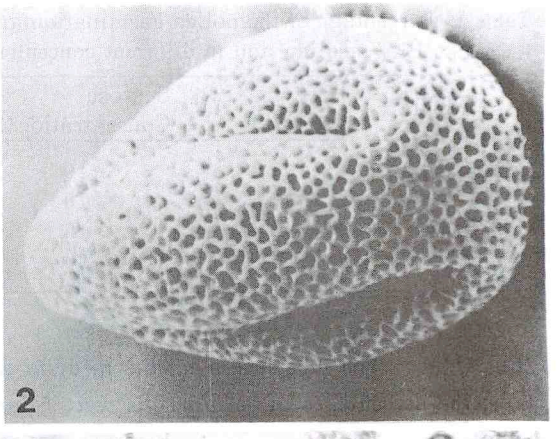
a. Brewbaker and Kwack's medium [2]:

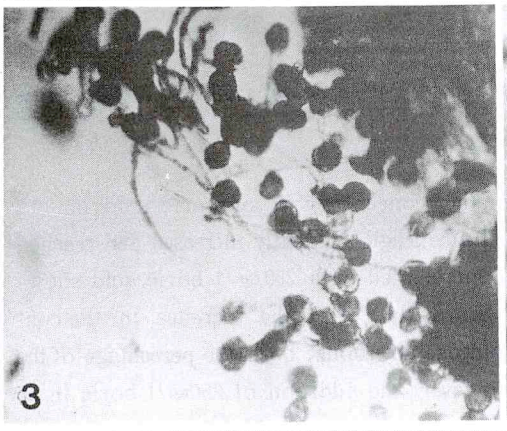
It is evident from Table 1 that the pollen germination in this medium was 80.5% and the average tube length was 1215μm (Fig.3).

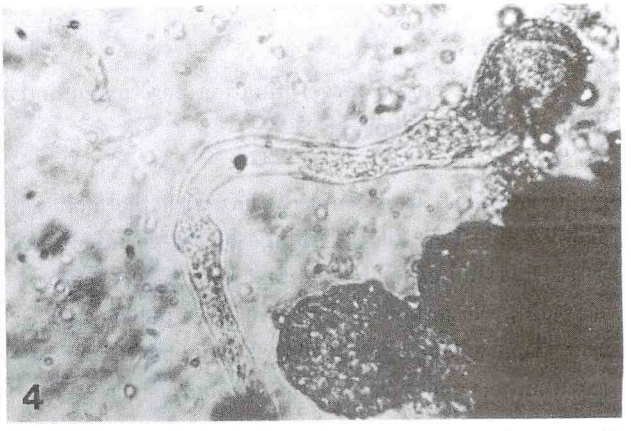
b. Sucrose:

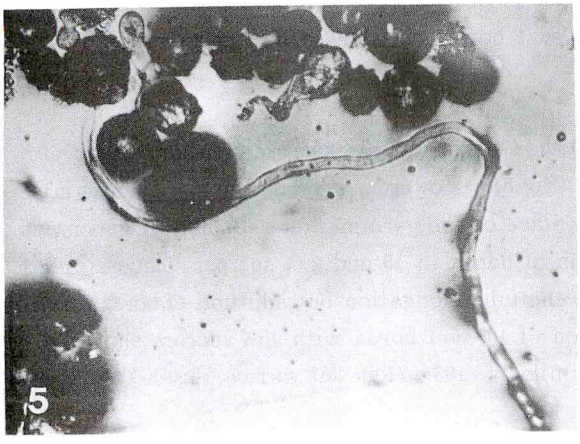
It is also clear from Table 1 that the highest germination (62%) showed in 10% sucrose solution

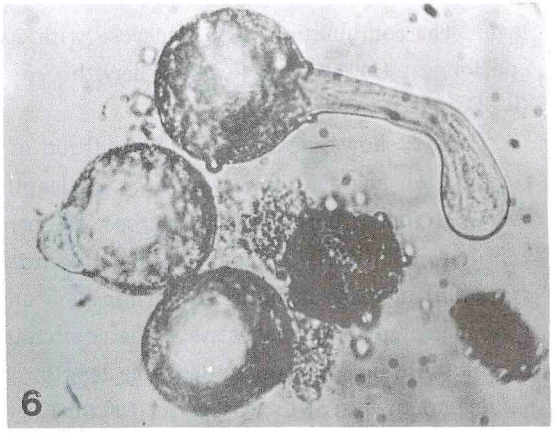



**Figs.1－2** SEM photographs of a pollen grain of *Millingtonia hortensis* L. 14000×
**Fig. 3** Germination pollen grains in Brewbaker and Kwack's medium. 132 ×
**Fig. 4** Single long pollen tube in 10% sucrose solution. 532 ×
**Fig. 5** A long pollen tube in 10% sucrose solution with 150 ㎎/l borax. 532 ×
**Fig. 6** A germinated pollen grain in 100 ㎎/l 2, 4－D and 10% sucrose solution. 532 ×

**Table 1.** Percentage of the pollen germination and tube length of *M. hortensis* in Brewbaker and Kwack's (BK) medium and in different concentrations of sucrose solution.

| Media | Sucrose Concentration (%) | Pollen germination (%) | Tube length (μm) |
|---|---|---|---|
| BK medium | 10 | 80.5 | 1215 ±7.615 |
| Sucrose | 10 | 62 | 933 ±6.554 |
| Sucrose | 15 | 54 | 873 ±7.222 |
| Sucrose | 20 | 49 | 756 ±0.163 |

(Fig.4). The tube length in this medium was 933μm. However, in 15 and 20% sucrose solutions the percentages of germination were 54 and 49% with 873 and 756μm pollen tube length, respectively.

c. Boron:

Effects of boron on germination percentage and tube elongation were studied in the forms of boric acid and borax.

(i) Boric acid:

It is evident from Table 2 that with the increase in the concentration of boric acid in 10% sucrose solution, the percentage of the germination and tube length gradually increased and reached to the maximum in the combination of 10% sucrose supplemented with 250 mg/l boric acid showing 40% germination and 540μm long pollen tubes. Similarly, with the increase in the concentration of boric acid up to 200 mg/l in 15 and 20% sucrose solutions, both the percentage of the germination and tube growth gradually increased. However, the addition of 250mg/l boric in 15 or 20% sucrose solutions failed to enhance both the pollen tube growth and germination percentage. The combination of 15% sucrose with 200 mg/l boric acid showed best germination (45%) and longest (1400μm) pollen tube length.

(ii) Borax :

Table 2 shows clearly that the addition of 150mg/l borax in 10% sucrose solution caused 60% germination and the tubes were 1440 μm length (Fig.5). However, with the further increse in the concentration of borax in 10% sucrose, the percentage of the germination and tube elongation was inhibited. Similarly, increase in the concetration of borax in 15 and 20% sucrose solutions caused a gradual increase in both the germination and the tube elongation but addition of borax beyond 100mg/l proved to be ineffective. The combination of 100 mg/l borax with 15% sucrose showed 80% germination and 1350μm pollen tube length. Similarly, 100mg/l in 20% sucrose caused 50% germination but tube growth was poor (396 μm).

Thus it is clear from these observations that 10% sucrose medium is the best for the pollen germination and tube growth, but by addition of boric acid or borax to particular extent not only in 10% but in 15% sucrose solution, the extent of the pollen germination and tube elongation can be enhanced. However, boric acid or borax in 20% sucrose failed to enhance both the germination and tube growth.

d. Calcium nitrate :

The effect of various concentrations of calcium nitrate in combination with sucrose solutions of different concentrations is presented in Table 2.

**Table 2.** Effect of boron(boric acid and borax) and calcium nitrate with the sucrose solution to the pollen germination in *M. hortensis in vitro*.

| Chemicals | concetration (mg/1) | Sucrose concentration(%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 | | 15 | | 20 | |
| | | GP | TL | GP | TL | GP | TL |
| Boric acid | 50 | 11 | 302± 8.21 | 20 | 495± 9.11 | 10 | 120± 6.21 |
| | 100 | 10 | 310± 9.67 | 35 | 605± 7.82 | 18 | 172± 5.81 |
| | 150 | 19 | 340± 7.24 | 40 | 760± 6.79 | 22 | 190± 8.12 |
| | 200 | 32 | 360± 10.71 | 45 | 1400± 11.20 | 30 | 326± 6.21 |
| | 250 | 40 | 540± 16.25 | 25 | 444± 6.21 | 25 | 250± 5.21 |
| Borax | 50 | 45 | 1200± 14.71 | 60 | 1150± 11.23 | 25 | 285±10.10 |
| | 100 | 50 | 1320± 16.81 | 80 | 1350± 10.61 | 50 | 396±12.17 |
| | 150 | 60 | 1440± 19.21 | 50 | 1260± 9.70 | 40 | 306± 8.13 |
| | 200 | 50 | 1250± 27.34 | 50 | 756± 8.80 | 35 | 232± 6.21 |
| | 250 | 40 | 1230± 21.22 | 45 | 600± 6.10 | 30 | 200± 3.40 |
| Calcium nitrate | 100 | 40 | 1146± 6.80 | 35 | 945± 8.09 | 30 | 606± 4.88 |
| | 150 | 32 | 729± 8.93 | 28 | 687± 9.82 | 22 | 564± 7.17 |
| | 200 | 22 | 576± 4.31 | 22 | 513± 5.93 | 18 | 291± 4.02 |

GP=Germination percentage TL=Tube length(μm)

Table 2 clearly shows that 10% sucrose containing 100mg/1 calcium nitrate was the best for both the germination percentage and tube growth. This combination exhibited 40% germination rate and 1146 μm pollen tube length. However, further increase in the concentration of both the constituents of the medium, both the germination percentage and tube growth failed to increase. The lowest germination (18.5%) and shortest tube length (291 μm) were recorded in 20% sucrose solution containing 200mg/1 calcium nitrate.

Thus,it is supposed that higher concentration of calcium nitrate inhibits the germination rate and tube growth.

III. Effects of plant regulators and chemicals :

a. Indole acetic acid (IAA) :

Table 3 shows the effect of different concentrations of IAA in combination with various concentrations of sucrose in the medium on the pollen germination and tube growth. It is evident from the results obtained that the percentage of germination decreased significantly in the media containing different concentrations of IAA as compared to that observed in different sucrose solutions alone. The decrease in percentage of the pollen germination was directly proportional to the increase in the concentration of IAA. It was, however, interesting to note that in spite of reduction in the germination rate, the tube growth in 15 and 20% sucrose solutions containing 100mg/1 IAA increased as compared to that shown by sucrose solutions alone. On the other hand, in 10% sucrose solution with 100mg/1 IAA a slight reduction was observed in tube length (773μm) as compared to 933μm in 10% sucrose alone. The combination of 20% sucrose with 100mg/1 although showed only 48% germination but the length of pollen tubes was 1119μm as compared to 756μm observed in 20% sucrose alone. However, with the increase in the concentrations of IAA

**Table 3.** Effect of IAA, NAA and $GA_3$ on the pollen germination in *M. hortensis in vitro*.

| Chemicals | concentration (mg/l) | Sucrose concentration (%) 10 GP | 10 TL | 15 GP | 15 TL | 20 GP | 20 TL |
|---|---|---|---|---|---|---|---|
| IAA | 100 | 34 | 777± 3.86 | 38 | 888 ± 7.52 | 48 | 1119± 9.93 |
| | 150 | 26 | 720± 7.28 | 26 | 597 ± 5.63 | 18 | 642± 5.84 |
| | 200 | 21 | 372± 2.93 | 18 | 435 ± 7.49 | 16 | 360± 2.36 |
| NAA | 100 | 05 | 81± 2.05 | 12 | 213 ± 4.95 | 15 | 327± 3.54 |
| | 150 | 11 | 282± 7.38 | 16 | 429 ± 5.85 | 20 | 570± 5.67 |
| | 200 | 28 | 825±12.21 | 18 | 478 ± 7.38 | 24 | 573± 9.60 |
| $GA_3$ | 100 | 32 | 561.0± 6.06 | 28 | 510 ± 6.06 | 27 | 462± 9.60 |
| | 150 | 40 | 586.5± 9.91 | 34 | 558 ± 6.46 | 31 | 636± 9.03 |
| | 200 | 58 | 729.0± 7.93 | 38 | 942 ± 10.30 | 48 | 699± 11.37 |

Symbols are the same as Table 2.

(150 and 200mg/1),both the germination rate and tube length decreased gradually.

b. Naphtalene acetic acid (NAA) :

From the Table 3, it is clear that both the pollen germination and tube length were significantly poor in the media containing NAA as compared to that recorded in sucrose alone. In the three grades of sucrose concentration, the percetage of the germination and tube growth increased gradually. The highest germination(28%) and longest pollen tube length(825 $\mu$m) were seen in 10% sucrose containing 200mg/1 NAA. On the other hand, there was only 5% germination and 81 $\mu$m pollen tube length in 10% sucrose supplemented with 100mg/1 NAA. However, with the increase in concentration of NAA, the germination percentage and tube length increased gradually but not to the extent as recorded in sucrose solution alone.

c. Gibberellic acid ($GA_3$):

The data presented in the Table 3 indicate that with the increase in the concentration of $GA_3$ in the medium both the germination rate and tube length increased gradually. However, this increase particularly the gemination rate was significantly poor as compared to that observed in sucrose alone. The highest germination(58%) was recorded in the medium containing 200mg/1 $GA_3$ in 10% sucrose solution and longest tube length(942 $\mu$m) were seen in 200mg/1 $GA_3$ in 15% sucrose solution as compared to 62% germination in 10% sucrose and 873 $\mu$m length of pollen tubes in 15% sucrose alone. The combination of 200mg/1 $GA_3$ and 20% sucrose showed 48% germination and 699 $\mu$m pollen tube length as compared to 48% germination and 756 $\mu$m length in 20% sucrose alone.

d. Maleic hydrazide( MH) :

Table 4 shows that maleic hydrazide(MH) was inhibitory for both the germination and tube growth and only 20% sucrose solution containing 200mg/1 MH showed maximum (22%) germination and longest tube length(486 $\mu$m). However, it was interesting to note that with the increase in the concentration of both sucrose and MH, the percentage of the germination and tube elongation increased but were considerably poor as compared to that shown sucrose solutions alone.

**Table 4.** Effect of MH, 2,4 −D and chloral hydrate on the pollen germination in *M. hortensis in vitro.*

| Chemicals | concentration (mg/1) | Sucrose concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 | | 15 | | 20 | |
| | | GP | TL | GP | TL | GP | TL |
| MH | 100 | 8 | 141 ± 1.85 | 10 | 171 ± 3.72 | 14 | 252 ± 4.11 |
| | 150 | 11 | 189 ± 3.13 | 16 | 321 ± 2.5 | 20 | 387 ± 2.92 |
| | 200 | 18 | 279 ± 7.39 | 18 | 354 ± 8.54 | 22 | 486 ± 6.85 |
| 2, 4 − D | 100 | 22 | 552 ± 7.65 | 18 | 438 ± 7.65 | 12 | 279 ± 7.11 |
| | 150 | 15 | 387 ± 6.65 | 14 | 552 ± 7.65 | 6 | 78 ± 2.56 |
| | 200 | 6 | 177 ± 2.68 | 8 | 192 ± 4.30 | 0 | — |
| Chloral hydrate | 75 | 58 | 1053 ± 11.82 | 38 | 705 ± 6.78 | 24 | 42 ± 4.96 |
| | 100 | 16 | 225 ± 2.82 | 10 | 108 ± 2.85 | 0 | — |
| | 150 | 0 | — | 0 | — | 0 | — |
| | 200 | 0 | — | 0 | — | 0 | — |

Symbols are the same as table 2.

e. 2,4−dichlorophenoxy acetic acid (2,4−D) :

The data presented in the Table 4 shows that with the increase in the concentration of both 2,4−D and sucrose, the germination rate and tube growth were inhibited. The lowest concentration of $GA_3$ (100mg/1) in 10% sucrose showed highest germination (22%) and 552 μm length of pollen tube(Fig.6). On the other hand, in the medium containing 20% sucrose and 200mg/1 2,4-D, the germination was completely inhibited.

f. Chloral hydrate :

Higher concentrations of chloral hydrate showed highly inhibitory for the pollen germination (Table 4). There was no germination in different concentrations of sucrose solution supplemented with 150 and 200 mg/1 chloral hydrate and similarly addition of 100 mg/1 chloral hydrate in 20% sucrose, the germination was completely inhibited. However, 75mg/1 chloral hydrate in 10% sucrose showed 58% germination as compared 62% shown by 10% sucrose alone. It was interesting to note that in this combination there were 1053 μm length of pollen tube as compared to 933 μm length observed in 10% sucrose alone.

Thus it is evident from the foregoing description and the data presented in the Tables 1— 4, that Brewbaker and Kwack's medium comprising sucrose, boric acid, calcium nitrate, magnesium sulphate and potassium nitrate is the best for the germination and tube growth. This medium has also proved to be the best for several other members of family *Bignoniaceae*[4,7,14]The pollen grains of many taxa germinate in distilled water, but generally require carbohydrate source and sucrose is the most commonly used one. It is presumed that sucrose has dual function in maintaining osmotic pressure as well as in acting as a substrate for metabolism of pollen[18]. According to Shivanna *et al.*[16], the optimum concentration of sucrose varies from species to species. Besides carbohydrates, boron and calcium are the other important substances required for the germination and tube growth. Boron is known to stimulate the pollen tube growth and is used in the form of boric acid. According to Portnoi and Horovitz[13], incorporation of boron to the medium

containing different concentrations of sucrose improve the pollen germination. The stimulatory effect of boric acid is largely due to its increased absorption of water. Sugar-borate complex is known to increase oxygen uptake and it has also been known to play an important role in the synthesis of pectic material for the pollen wall[20]. Pollens are known to be deficient in boron and, therefore, exogenous supply of boron enhance the pollen germination and tube growth[17,18]. Besides boron another important substance required for the tube elongation is calcium. The role of calcium in the tube elongation have been emphasized by Brewbaker and Kwack[2] and Kwack[10]. According to them calcium overcomes the population effect, promoters the germination and also enhances the tube elongation. Chauhan *et al*[4] have observed the similar effect of calcium nitrate in *Tecoma stans* another member of family *Bignoniaceae*.

The results of present study indicate that IAA and NAA have failed to enhance both the germination and tube growth. However, NAA was much inhibitory. On the other hand, addition of $GA_3$ into sucrose medium enhanced both the germination and tube elongation. According to Vasil[20], the pollen grains appear to contain adequate quantity of phytohormones (except gibberellins) and, therefore, the addition of either IAA or NAA to the medium failed to affect the germination and tube growth appreciably. However, Kumar *et al*[9] have shown 94.4 % germination of *Millingtonia hortensis* in the medium containing 10mg/l IAA. Comparatively less inhibitory effect of $GA_3$ has also been reported by Chauhan *et al*[4] in *Tecoma stans*. Addition of higher concentrations of 2,4-D and chloral hydrate was also inhibitory to both the germination and tube growth. Inhibitory effect of 2,4-D is well known[4,11,16]. However, lower concentrations of chloral hydrate proved to be stimulatory for both the germination and tube elongation. Johri and Shivanna[8] have studied the effect of chloral hydrate on the differential be haviour of two- and three-celled pollen. According to them chloral hydrat have failed to bring out any basic difference between two-celled pollen of the *Impatiens balsamia* and *Catharanthus raseus* in the medium containing chloral hydrate. Present observations have also indicated a similar inhibitory effect of chloral hydrate in *Millingtonia hortensis* having two-celled pollen. According to Chauhan *et al*.[5], best pollen germination and pollen tube growth were observed by pollinating non-pollinated receptive stigmas with the fresh pollen suspended in Brewbaker and Kwack's medium. And they also observed a large number of pollen tubes to penetrate to the stylar tissue. In addition to pollen suspension in Brewbaker and Kwack's medium, this study shows that there are possibility to apply the pollen suspension which shows good pollen elongation to the stigmas for obtaining seeds.

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抄録　　コルクノウゼン（*Millingtonia hortinsis* L.）の花粉の形態と生理

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コルクノウゼンの花粉は卵形三溝型でその直径は42－46$\mu$m である。最高の花粉発芽率および最長の花粉管伸長はBrewbakerとKwackの溶液および15％のショ糖液に100㎎/1の硼砂を加えた溶液で得られた。硝酸カルシウムやIAA, 抱水クロラールを含む液ではショ糖濃度によっては花粉管伸長を促進するものもあった。しかしながらNAAやGA$_3$, MH, 2, 4－Dを含むものではすべて阻害した。

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