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A Comparative Study of Some Phenotypic Features Amongst Three Variants of *Canna indica L.* Showing Varying Degrees of Sterility

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Abstract: *Canna indica L.* variants exhibit sterility in varying degrees, the criterion for sterility being based on seedlessness.

Failure to set seed is supported by the results of percentage germination of pollen and rate of pollen tube growth. Sterility in *Canna indica* variants studied appear to be associated with changes in morphological features of leaves, flowers, pollen size, general physiology and cytology.

1. Introduction

Canna which is confined to the warmer regions of the world belongs to the order Zingiberales, and family Cannaceae which has one known genus *Canna* Linn.[10,11,18]

Canna indica holds a unique place in the history of botanical nomenclature, it being the first named species in Linnaeus' Species Plantarum (1753). Here reference is made to a *Canna indica* specimen collected from Sri Lanka.[14] In Sri Lanka, *Canna indica* is widely distributed. It is considered to have been originally introduced to the island from the West Indies but is fully naturalized.

Canna species are herbaceous perennials and are popular ornamental plants. The general characters of *Canna indica* have been published by a number of authors and will therefore not be repeated.[3,6,10,15,17,18]

In the cooler hilly areas of Sri Lanka, a wild variant of *C. indica* with fertile seed grows naturally, (referred to as *Canna indica* variant I in this report). Several other cultivated variants however are found distributed in every part of the island in spite of climatic variations. These are ornamental and have been obtained by hybridising, crossing and re-crossing.

With the production of new and attractive horticultural variants. sterility has also been introduced and propagation is largely by vegetative means. Though viable seeds are produced in *some* variants, cultivation by vegetative means is quicker and easier.

There are at present several hybrid variants but very little work appears to have been done on the classification of these *C. indica* variants. In this study, for the purpose of distinguishing the three variants studied, from each other, different symbols have been assigned to the different variants investigated, taking into consideration their floral morphology.

C. indica *var. I - wild: Staminodia red in colour, narrow - maximum width less than 1.1 cm.

*var. II-cultivated: Staminodia red and yellow in colour, maximum width 3.0 to 3.5 cm.

*var. III-cultivated: Staminodia red in colour, broad - width more than 6.0 cm. (see also Table 1)

The objective of this report is to compare the variation in phenotype of the three *C. indica* variants I, II and III.

TABLE 1. Differences in seed production and morphology of the flower.

Variant	Colour of flower	Maximum width of staminodia (cm)	Degree of sterility
I	Red	Less than 1.1	Highly fertile (Numerous seeds)
II	Red and yellow	3.0 to 3.5	Low fertility (Few seeds)
III	Red	More than 6.0	Fully sterile (No seeds)

2. Materials and Methods

Plant source

Experimental plants of the variants (I, II and III) were obtained from single clones of each of the variants.

The three variants were planted in isolated plots, under similar environmental conditions. Sampling of material viz. pollen, ovules, seeds, flowers, leaves and petioles was carried out from plants from these three plots in order to reduce variability when replicating experiments.

*Note: Var. I, II, III represent the three *Canna indica* variants investigated in this study.

Measurements

Measurement of plant size and organs was done using standard methods. The maximum dimensions of fully expanded leaves were measured with a centimeter rule, while stomatal frequency and stomatal dimensions were measured from leaf epidermal peels taken from either side of the mid-rib, at leaf maturity. All microscopic measurements were made using a graticule.

Water potential determination

The water potential of the petiolar tissues of the three variants was determined by standard gravimetric and tissue tension methods.

Collection of pollen

In the expansion process of the fully formed bud (in which outer and inner whorls of perianth parts and petaloid staminodia are in the folded position), the following stages were recognised.

1. Outer whorl of perianth parts expanded, but inner whorl of perianth parts, and petaloid staminodia closed.
2. Outer and inner whorls of perianth parts expanded but petaloid staminodia closed.
3. All perianth parts (outer and inner) and petaloid staminodia fully expanded.

It was found that best results for pollen germination in variant I was obtained when pollen was collected from a flower in stage 3 of the expansion process described earlier. For var. II and III, stage 2 of the expansion process gave best results for pollen germination.

When replicating the experiments, samples of pollen were collected at the same time of day under similar environmental conditions, from flowers in the appropriate stage in the expansion process.

Pollen germination

The nutrient medium (sucrose-agar) for pollen germination was prepared as follows : [19]

Sucrose	10.0 g
Agar	0.5 g
Distilled water	100.0 cm^3

The sucrose concentration was varied from 10.0g to 40.0 g per 100 cm^3 distilled water in an attempt to determine the sucrose concentration most suitable for pollen germination in *C. indica* variants I, II and III.

Since addition of boric acid is said to improve pollen germination and pollen tube growth,[19] media with boric acid and a control set without boric acid were prepared. The concentration of boric acid was also varied from 0.005g to 0.02 g per 100 cm^3 medium.[19]

Pollen grains were spread on a drop of each of these media on a glass slide at room temperature (30°C), and maintained in a moist chamber to prevent drying. Observations were made at 15-20 min intervals under a microscope (10 x 10).

Formation of pollen tubes was considered to be a positive sign of viability. For each germination test random counts of 300-400 grains were made at a magnification of 10 x 10 to determine the percentage germination.

By scanning the slide under the microscope the length of the pollen tube (after a given length of time) in each medium was also observed. The rates of pollen tube elongation and percentage viability were compared.

The dimensions and general morphology of the pollen grains were also studied.

Formation of seeds

The number of ovules per ovary and the number of seeds per mature fruit were noted. Other observations made included the number of days taken for the ovules to reach maturity, and the weight of a single seed/ovule at maturity.

Storage and viability of seed

For viability tests fresh seeds were air dried and stored in sealed polythene bags and maintained at 5°C.

Viability tests by percentage germination were done at monthly intervals. Due to lack of sufficient seeds from var. II and III, viability tests with time were confined only to var. I. (var. II produced only a few seeds, while var. III was sterile).

To hasten seed germination the following treatments were employed with seeds of var. I and II.

(*a*) Fresh seeds were soaked for 65 hours in distilled water.

(*b*) Removed the seed coat as a mechanical means of breaking dormancy (scarification).[12]

(*c*) Seeds were soaked for 5 mn in a solution of concentrated sulphuric acid as a chemical means of breaking dormancy (scarification).[12;15]

After each treatment, to avoid fungal infection, seeds were surface sterilized by immersing in 0.1% mercuric chloride[1] for 3 min and then subject to repeated rinses in five changes of distilled water.

Corresponding controls were also set up.

All seeds were placed on Whatmann filter paper moistened with distilled water in partially covered petri dishes. The seeds in batches of 5 per dish were incubated at room temperature (30°C).

The emergence of the radicle through the seed coat was considered to indicate the beginning of germination.[12] Germination counts were made once in 3 days for 100 days (where necessary) for each batch of seeds. Survival was assessed at the three leaf stage.

Statistical analysis of data

Wherever possible the data obtained from the above experiments, were subject to statistical analysis. The validity of assuming certain ratios to be correct was tested with chi-square (X^2).

The confidential interval or the range in percentage of pollen germination, stomatal frequency, dimensions etc. obtained was statistically verified using the t-test formula.[5]

3. Results

General morphology

Initial observations of plant height showed an increase in size from variant I to variant III, though subsequent work showed that this character did not breed true through successive generations. (Unpublished data). Other characters like flower and leaf size, degree of sterility etc. bred true for successive generations (unpublished data) and are considered below.

With regard to flower size, an increase in dimensions was observed from variant I to variant III (Plate I A). This appeared to be associated with an increase in sterility from var. I to var. III. In fact var. III yielded no fertile seed at all. (Table 1)

Leaf morphology of the 3 variants also showed a corresponding variation (Table 2A, 2B). Leaf area increased in the 3 variants in the order var. I, II, III (Plate 1 B), though the mean length of the guard cells was found to be similar (10x3.2 μm). Stomatal frequency however was highest in var.II. (Table 3).

The number of flowers per inflorescence, ovules per ovary, seeds per fruit and average weight of an ovule/seed in the dehisced ovary varied in the 3 variants. These characters are listed in Table 4. The number of flowers per inflorescence was lowest in var. I but highest in variant II (Table 4), but the average weight per ovule and the percentage seed formation decreased from var. I to var. III. (Plate 2).

Time of flowering

Onset of flowering in these variants occured at the sixth, seventh or eighth leaf stage. After complete emergence of the floral axis opening of petaloid staminodia was first observed after 4, 6 and 8 days respectvely in var. I, II and III.

TABLE 2. (*a*) External morphology of the mature leaves

Variant	Texture	Colour	Surface	Thickness	Margin
I	Very smooth	Light green	Glabrous	Relatively thin	Not membranous
II	Smooth	Deep green	Not glabrous (Ash like fine powder on both surfaces)	Thick	Whitish and membranous
III	Very rough	Very deep green	Not glabrous (Ash like fine powder on both surfaces)	Very thick	Pigmented purplish in colour

TABLE 2. (*b*) Dimension of fully expanded leaves.

Variant	Maximum length (cm)	Maximum breadth (cm)	Length : Breadth
I	20.2±3.7	9.81±.1	2 : 1
II	34.5±7.7	11.6±2.1	3 : 1
III	40.4±3.6	19.4±1.2	2 : 1

TABLE 3. Stomatal frequency per 0.56 mm^2 surface area

Variant	Stomatal frequency	
	Upper epidermis	Lower epidermis
I	2.79±0.04	8.69±0.04
II	4.77±0.12	11.94±0.25
III	2.50±0.01	7.98±0.03

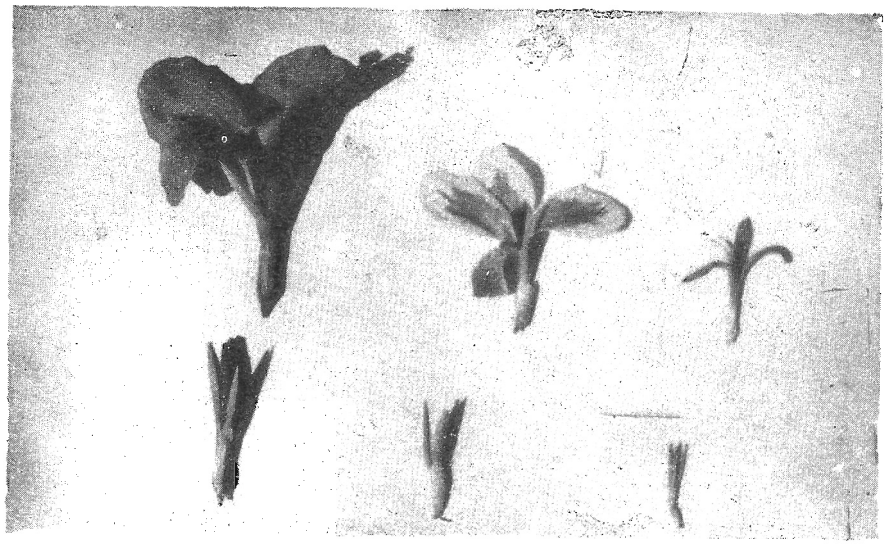


PLATE 1. A – Variation in size of *Canna indica* flowers (X 0. 25).

Right to left – Variants I, II and III respectively.

Top row – Fully expanded flowers. (See Collection of pollen stage 3).

Bottom row – flowers with three inner perianth parts expanded. (See Collection of pollen stage 2).

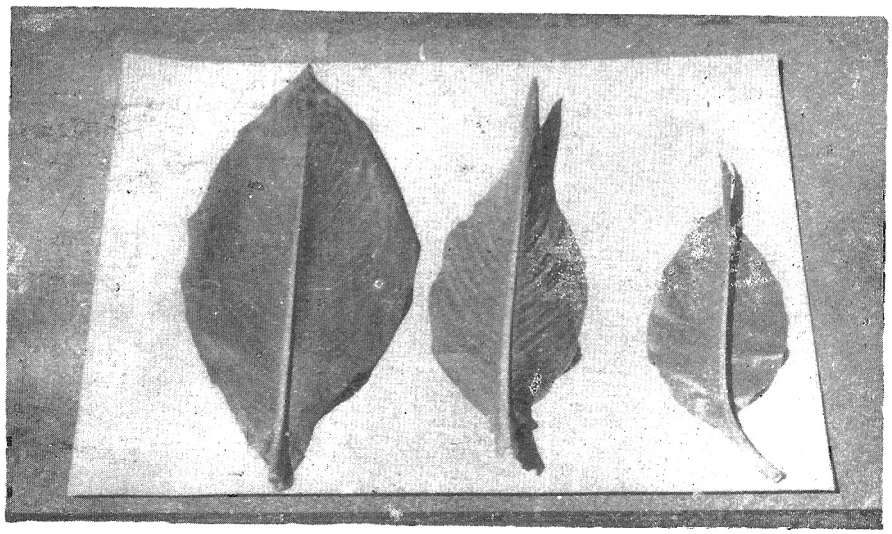


PLATE 1. B – Leaves of the three *Canna indica* variants showing variation in size (X 0. 2).
Right to left – Variants I, II and III respectively.

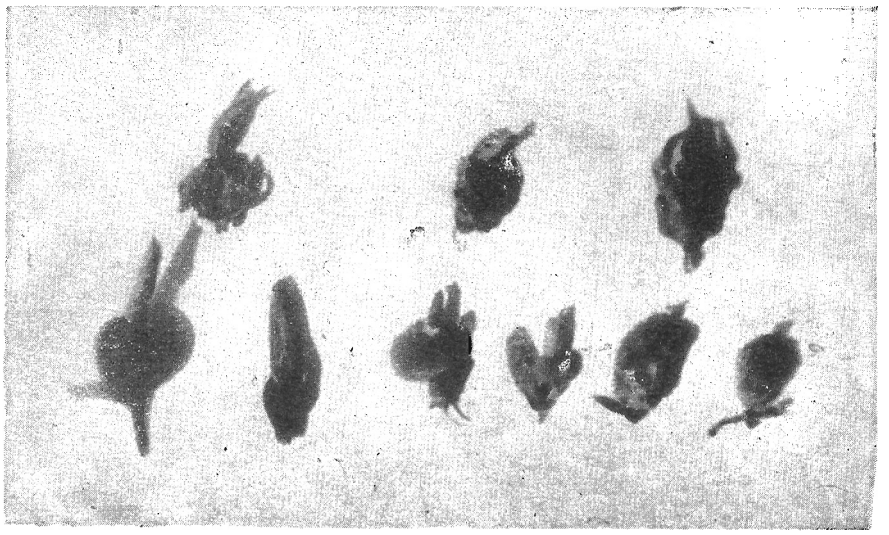


PLATE 2. Fruits of the three *Canna indica* variants (X 0.5).

Right to left - Variants I, II and III respectively.

Top row – Mature dry fruits opened out to show contents.

Bottom row – Pairs of entire fruits from each variant.

Left - Fruits from basal part of floral axis.

Right - Fruits from apical part of the floral axis.

Seed formation and maturation

In all 3 variants maturation of the ovary into a dry fruit (capsule) took 19-21 days from the time of full floral expansion.

In var. I all the flowers of an inflorescence yielded fruits with seeds, while in var. III all the flowers gave seedless fruits. Var. II gave fruits with few or no seeds.

Formation of viable seeds was as follows - maximum in variant I (91%), lesser in variant II (10%) and zero in variant III (0%). (Table 4).

TABLE 4. Some differences in ovule/seed production and flowers per inflorescence.

1– flowers per inflorescence,
2– ovules per ovary,
3– seeds per fruit { (*a*) towards the base, (*b*) towards the apex } of inflorescence,
4– weight in grams of a single ovule/seed in the ovary at maturity.

Variant	1	2	3 (*a*)	3 (*b*)	4	from ovules Mean Percentage seed formation
I	5.10± 1.07	22.5± 3.8	15.0±6.4	5.5± 3.0	0.20± 0.04	91.0*
II	21.90± 2.64	15.1± 1.8	1.5± 0.5	No seeds	0.12± 0.02	10.0*
III	11.20± 1.30	32.0± 4.1	No seeds	No seeds	0.003	

*Except these, other values are 95% accurate statistically.

Water potential

In all 3 variants, water potential determinations of the cell sap by tissue tension and gravimetric methods gave values approximately equivalent to that of a 25% sucrose solution. Assuming this to be isotonic with the general plant tissue all pollen grain studies were made in medium containing 25% sucrose.

Pollen and pollination

In all 3 variants, pollen was already shed upon the style when 2 or 3 inner perianth parts had opened (see collection of pollen, stage 2). More pollen was seen on the side of the style than on the upper stigmatic surface.

Microscopic examination of the pollen grains showed a remarkable variation in size even when the pollen was taken from a single anther. Pollen grains also showed variation in appearance. Sometimes pollen grains were seen in pairs, both members of a pair being either equal or unequal in size. (Fig. 1, Plate 3 A, B, C.) The number of pollen grain pairs increased in the order var. I, II and III (Tables 5 and 6). Occasionally, abnormal looking pollen grains were also observed.

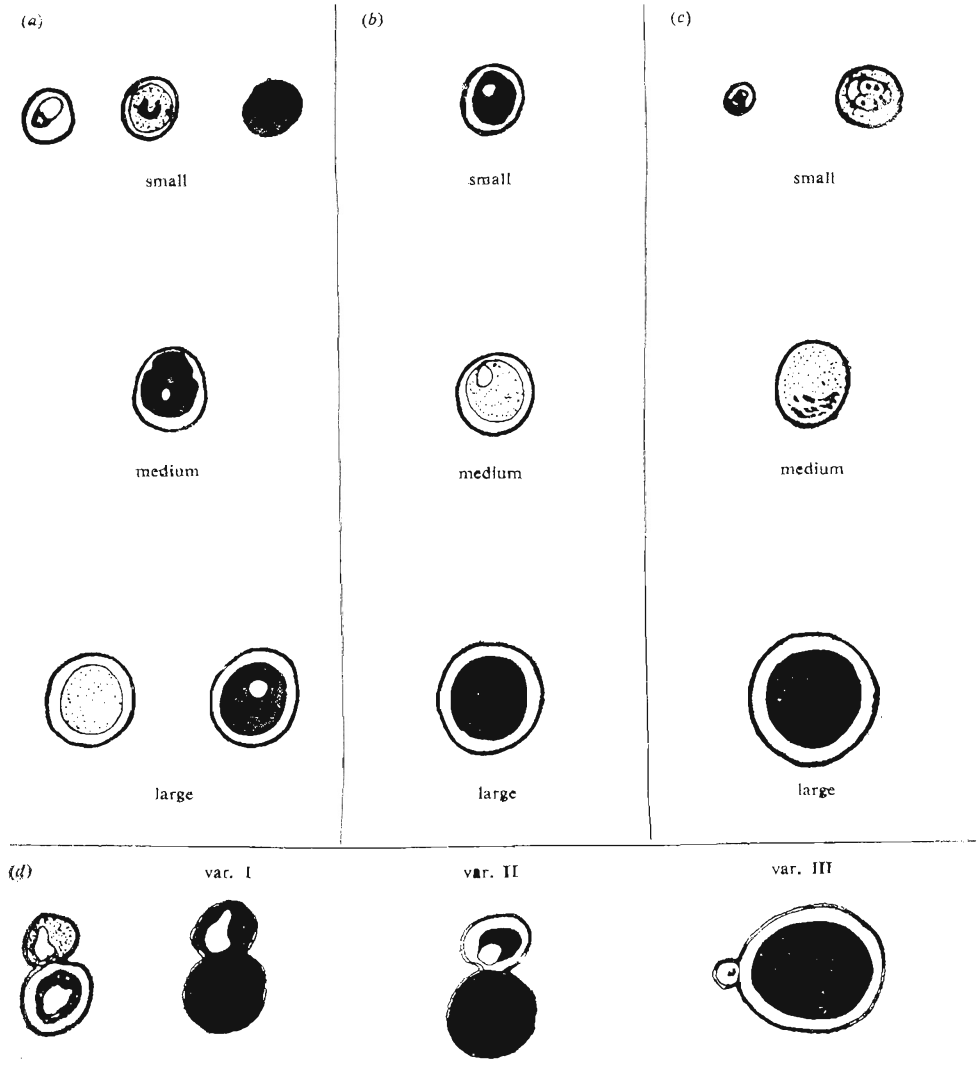


Figure 1. Camera lucida Drawings, of *Canna indica* pollen grains – small, medium and large
(*a*) Variant I
(*b*) Variant II
(*c*) Variant III.
(*d*) Pollen grain pairs. (The two grains of a pair unequal in size.)

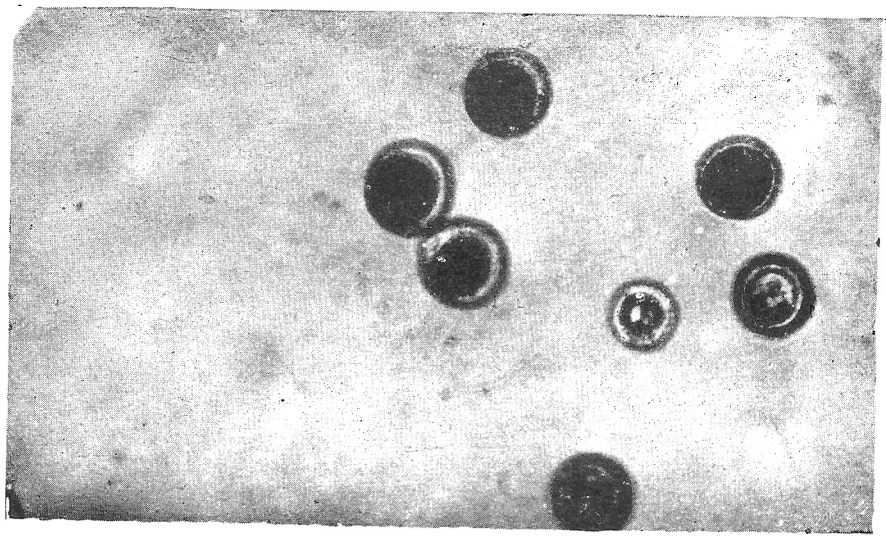
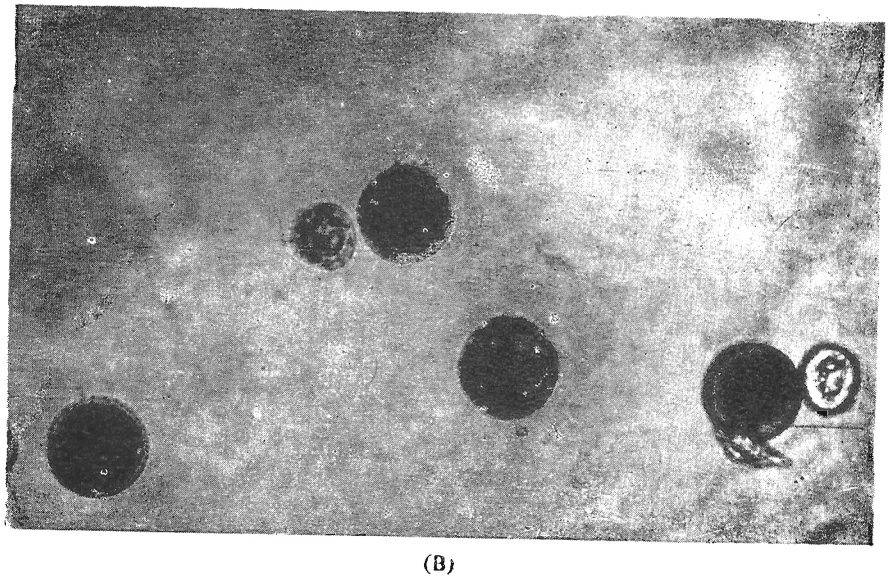


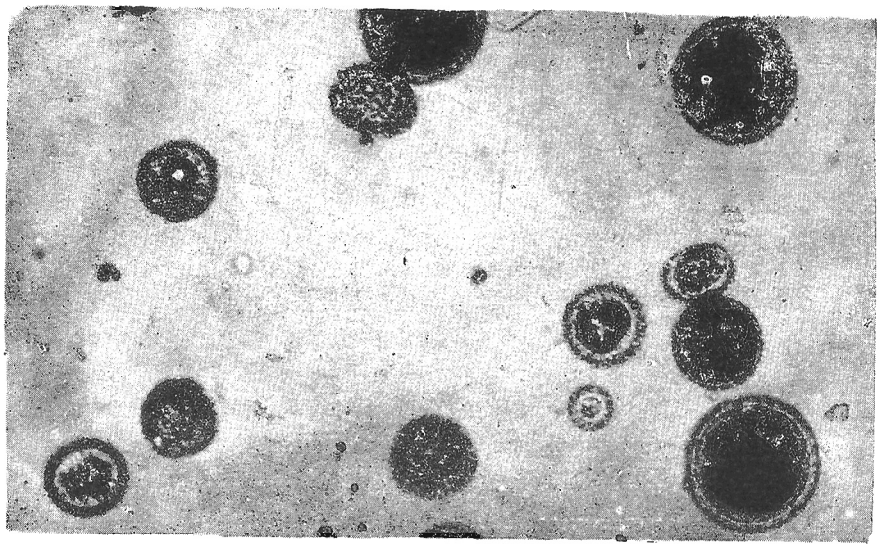
PLATE 3. Pollen grains of the three variants of *Canna indica* (X 400)

A – Variant I
B – Variant II
C – Variant III

Note the pollen grain pairs in A, B and C.



(B)



(C)

TABLE 5. Number of pollen grains falling into the different groups i. e. small, medium and large (per 200 grains approximately)

Variant	Diameter of pollen grains			Ratio S : M : L	Percentage medium size pollen grains
	Small (S) Less than 78.7 microns	Medium (M) 78.7 to 92.8 microns	Large (L) 92.9 microns and above		
I	26	158	26	1 : 6 : 1	75
II	27	78	106	1 : 3 : 4	37.5
III	131	27	60	5 : 1 : 2	12.5

TABLE 6. Summary of some important floral, pollen and seed characters

Floral and pollen characters	Variant I	Variant II	Variant III
Number of days taken for petaloid staminodia to open (expand) after complete emergence of the inflorescence axis	1½	2½	5
Maximum length of full petaloid staminodia (cm)	5.20±1.1	7.50±0.06	13.82±0.27
Percentage of pollen grain pairs	2.7±0.7	5.5±1.8	14.8±2.8
Percentage of medium size pollen grains	75	37.5	12.5
Percentage germination of pollen grains cultured under ideal conditions for 1.5 hours	70.7±3.3	31.0±3.8	3.2±3.0
Maximum length of pollen tube cultured under ideal conditions for 1.5 hours (microns)	2288.0±168.0	1573.0±123.0	643.5±51.0
Expected percentage germination of pollen grains on basis of size ratio (S: M: L)	75	37.5	12.5
Percentage number of ovules which develop into seeds	91	10	zero (No seeds)

Pollen grain dimensions

Depending on their diameter the pollen grains were broadly grouped into 3 categories—

Small : less than 78.7 μm (5.5 × 14.3 μm)
Medium : from 78.7 μm to 92.8 μm (6.5 × 14.3 μm)
Large : 92.9 μm and above

The number of pollen grains falling into the 3 categories - small, medium and large, varied when measured randomly, but the total number of medium sized pollen grains decreased in the order var. I, II and III respectively. (Table 5).

Pollen germination in vitro

The best medium for pollen germination was 25% sucrose with 0.005% boric acid for variant I, but for variants II and III 25% sucrose with 0.01% boric acid was most suitable. (Fig. 2)

Sucrose concentrations lower or higher than 25% proved to be inhibitory towards germination of pollen grains and pollen tube elongation.

The time taken for germination to commence was least in var. I, greater in var. II and most in var. III. (Fig. 3).

In all 3 variants the large and small sized pollen grains failed to germinate. Only the medium sized grains showed germination.

In the case of pollen pairs, if only one member of a pair was medium sized then only the medium sized grain germinated. Where both members of a pair were medium sized, then too, only one member germinated (Fig. 4a)

The maximum length of the pollen tube after 1.5 hours incubation under ideal conditions decreased in the order I, II, III respectively. (Table 6)

Initially, the rates of pollen germination and pollen tube elongation also varied widely in the three variants being highest in var. I and lowest in var. III (Fig. 5).

By germinating pollen in vitro, long slender pollen tubes, short stout pollen tubes and pollen with and without distinct nuclei were observed in all 3 variants (Fig 4b).

Germination and viability of seed

Scarification of the seed coat by mechanical means proved to be a quick method for the initiation of seed germination, but the survival of these plants was very low, possibly due to mechanical injury while removing the seed coat. Scarification of the seed coat by chemical means (acid treatment) was the quickest and easiest method, although the first few leaves of the plant had a scorched appearance. (Table 7).

Soaking seeds for 65 hours in water prior to germination proved to be a slow process, where survival too was high (Table 7). Percentage germination however was higher in var. I than in var. II (var. III had no seeds) (Table 8).

As stated earlier viability tests of the seeds with time was confined only to var. I as only this variant yielded sufficient seed for such a study. The change in viability with time for var. I is shown in Table 9. The fresher the seeds, the quicker the germination. With older seeds (1-2 months) however, a lag period was observed, followed by a rapid rise in percentage germination. A period of 3 months storage at 5°C resulted in loss of viability. (Table 9).

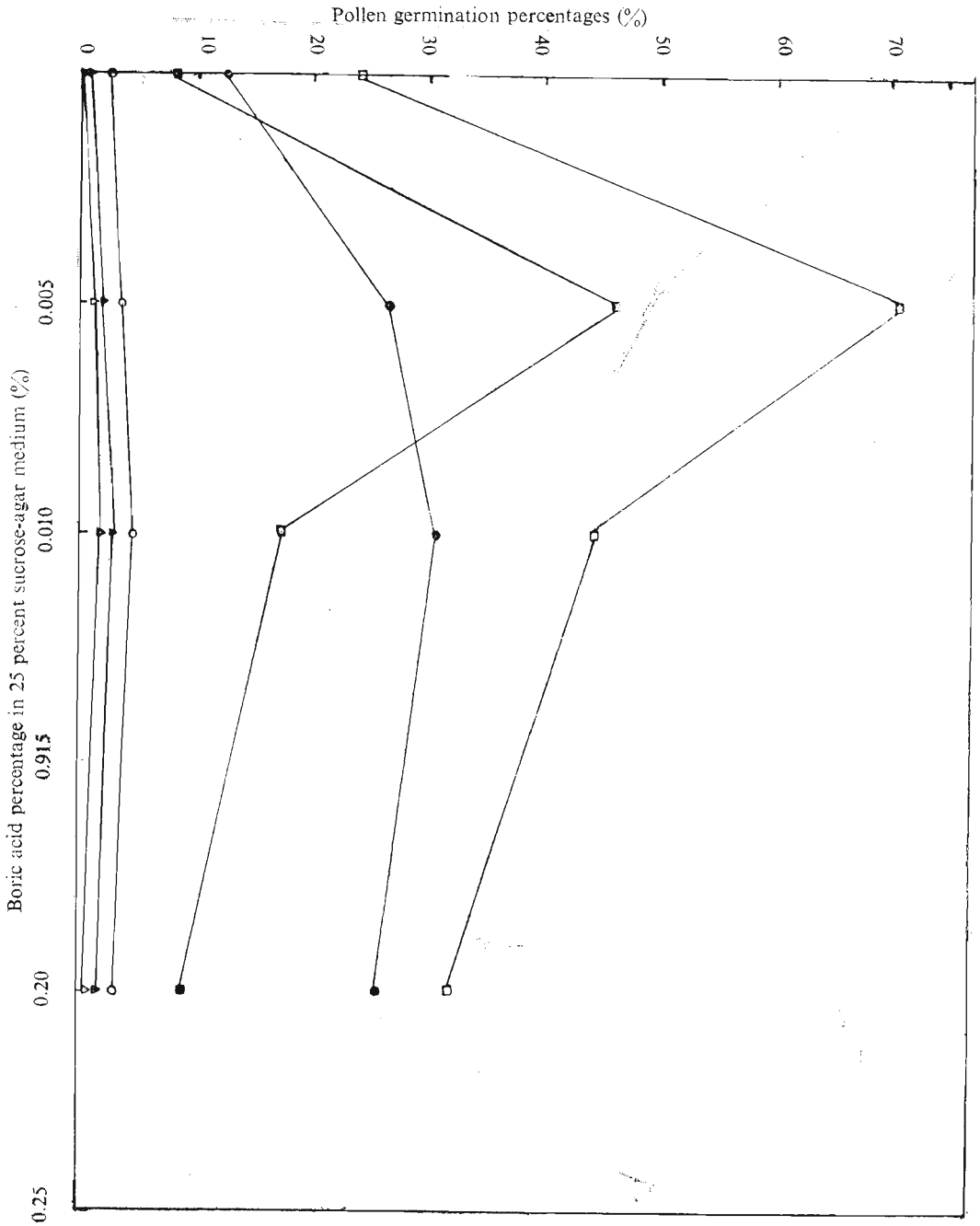


Figure 2. Percentage pollen germination of *Canna indica* variants I, II and III in 25 percent Sucrose–Agar media containing different Boric acid concentrations.

Variants I, II and III represented by squares (□, ■), circles (○, ●) and triangles (Δ, ▲) respectively. Symbols ■, ● and ▲ represent germination of pollen at stage two – see Collection of pollen (Materials and methods). Symbols □, ○ and Δ represent germination of pollen at stage three – Collection of pollen (Materials and methods).

All cultures incubated for 1.5 hours at room temperature (30° C).

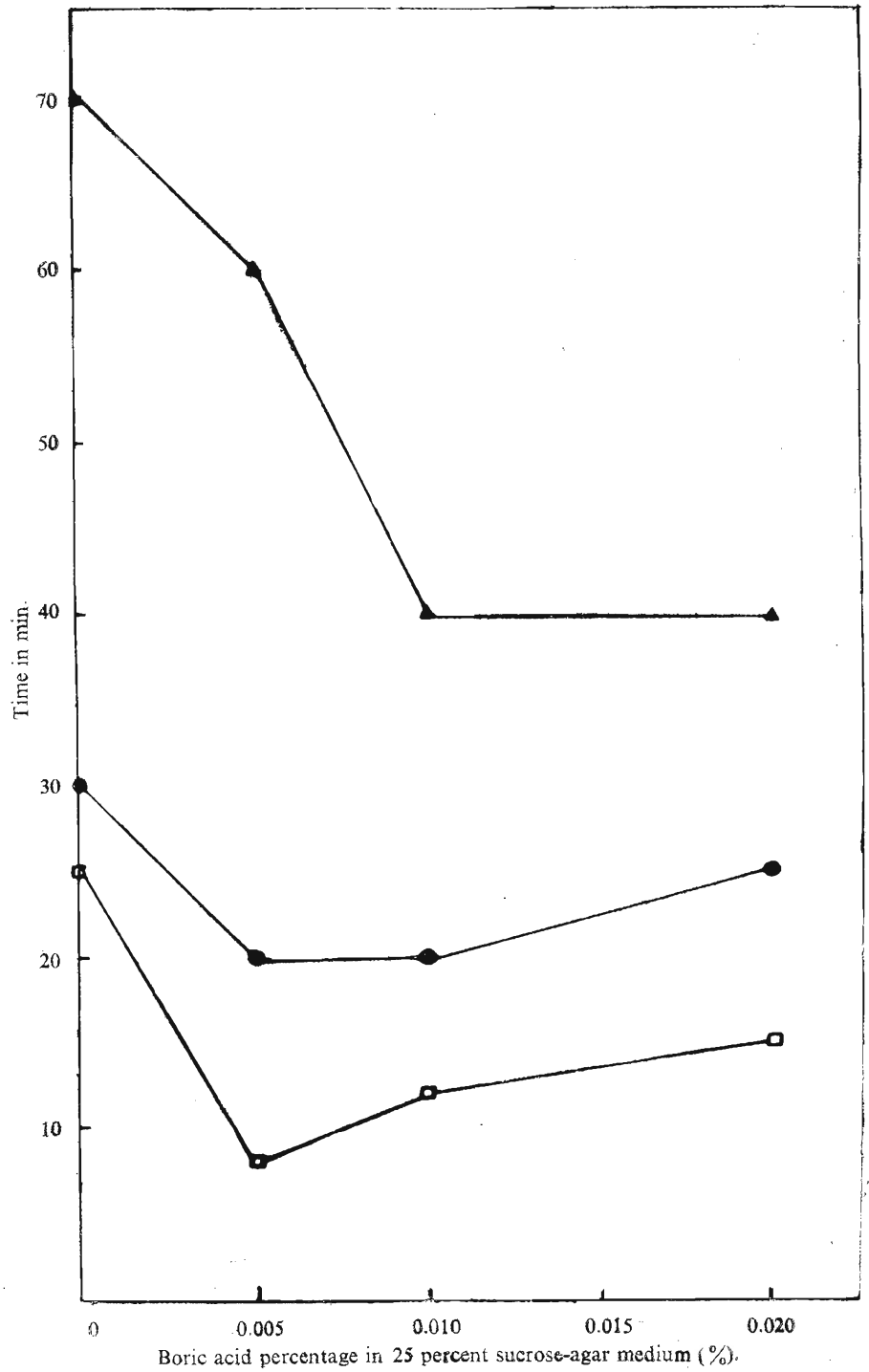


Figure 3. Comparison of times taken for initiation of pollen germination in the three variants of *C. indica*, in 25 percent Sucrose–Agar media containing different Boric acid concentrations.

Variant I (□) – Collection of pollen stage three. Variants II (●) and III (▲) – Collection of pollen stage two. (See Fig. 2)

All cultures incubated at room temperature (30° C).

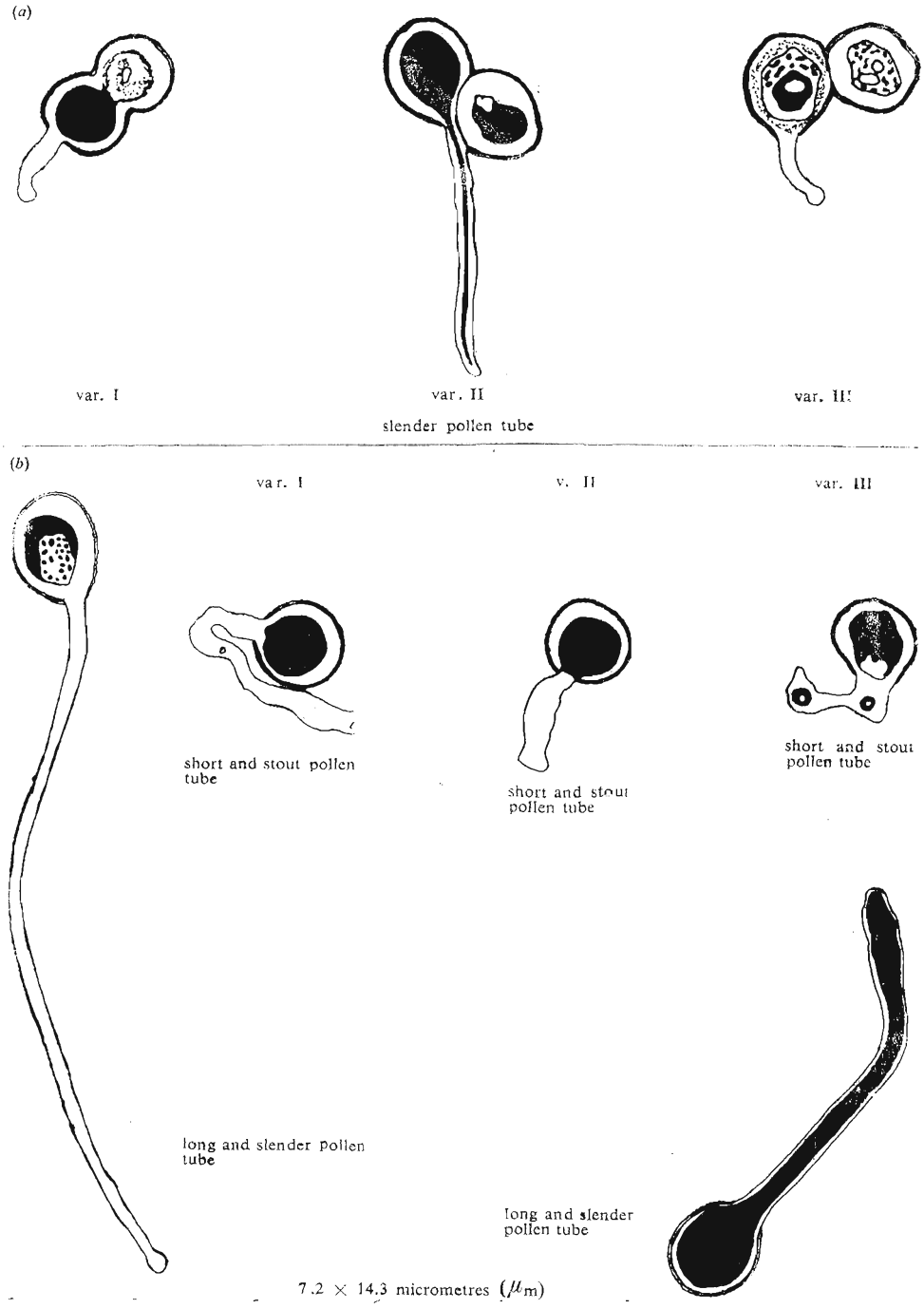


Figure 4. Camera Lucida drawings of germinating pollen grains —

(*a*) Even if both members of a pollen grain pair are medium sized, only one member of the pair germinates.

(*b*) Long slender pollen tubes and short stout pollen tubes.

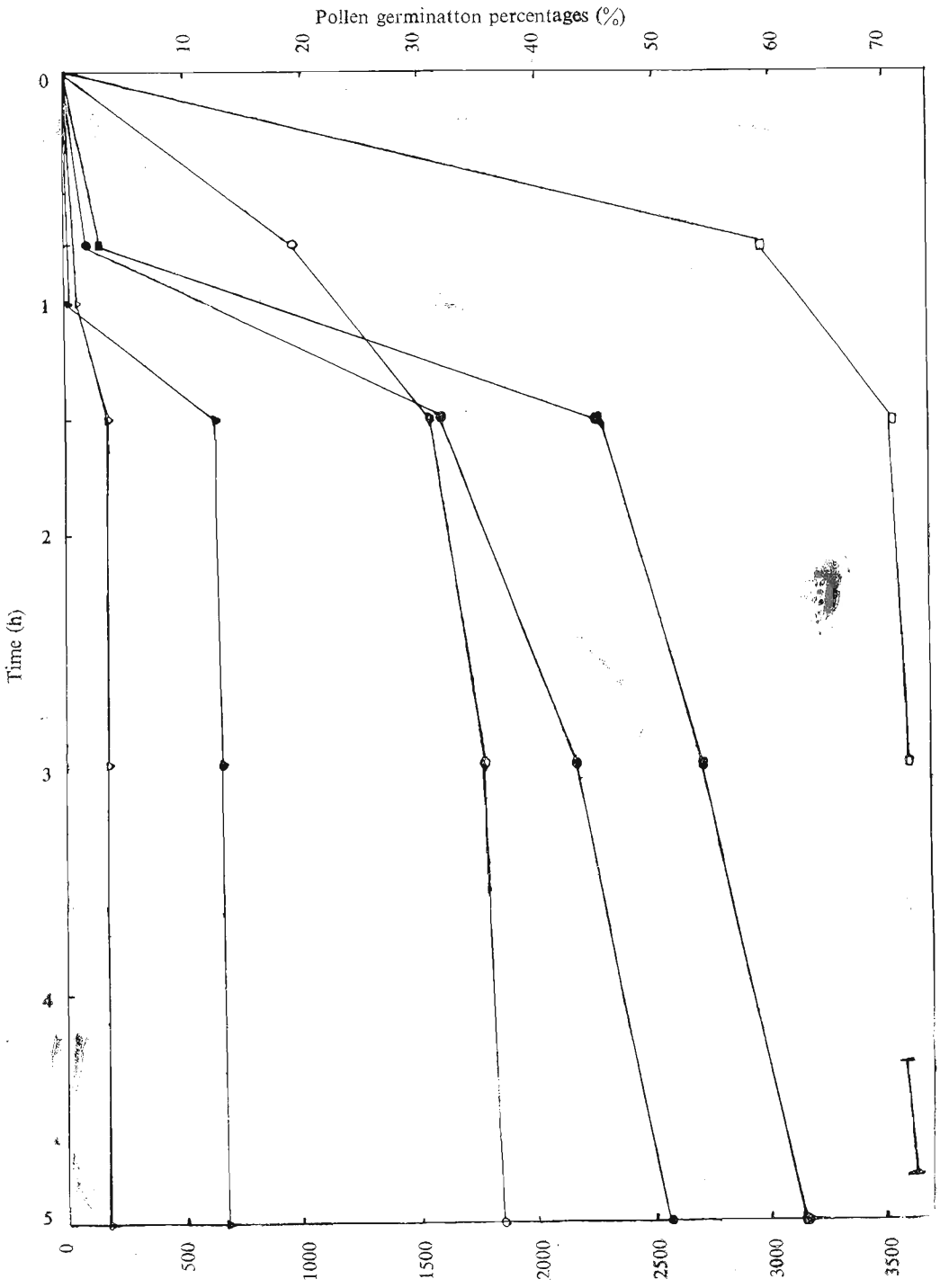


Figure 5. Percentage germination of pollen (variants I, II and III represented by □, ○ and △) and maximum length of pollen tube observed (variants I, II and III represented by ■, ● and ▲) versus time. (hours)

Note— Initilly the rates of both pollen germination and pollen tube elongation varied widely in the three variants of *Canna indica*.

TABLE 7. Effect of scarification (seeds being given a, b, c and d treatments, and incubated at room temperature) – Percentage germination of variant I seeds vs. time.

Treatment	Percentage seed germination – Variant I					
	Number of days					
	3	6	9	12	15	18
(*a*) Soaked in water 65 hr.	0	14	42	71	85	100
(*b*) Mechanical removal of seed coat	100	100	100	100	100	100
(*c*) Chemical treatment of seed with conc: H_2SO_4	20	20	100	100	100	100
(*d*) Control (Untreated)	0	0	0	0	0	0

(Note:– All seeds were derived from one inflorescence to avoid unwanted variability).

TABLE 8. Variation in the percentage germination of seeds with time in the different variants of *Canna indica*.

Variant	Percentage germination of seeds				
	Number of days				
	3	6	9	12	15
I	20	20	100	100	100
II	10	10	50	80	80
III	No seeds				

(Note: All seeds were pre-treated with conc. H_2SO_4 and incubated at room temperature).

TABLE 9. Change in viability of seed with storage var: I only.

Period of storage	Percentage germination of seeds				
	Number of days				
	3	6	9	12	15
Fresh	30	30	100	100	100
One month	—	50	100	100	100
Two months	—	—	20	100	100
Three months	—	—	—	—	—

(No germination. Seeds infected with fungus)

4. Discussion

Among the three variants of *C. indica* studied we see a variation in the degree of seed formation extending from high fertility in variant I to total sterility in variant III. Support for this also comes from a study of pollen size and germination. Pollen grains could be grouped into at least three different sizes in each variant (plant) investigated. Variation in size of the pollen grains within each variant (plant) itself, and the added presence of pollen grains in pairssuggests irregular meiosis and an uneven chromosome complement. The total number of viable pollen grains and pollen tube length decreased in the order var. I, II, III respectively (Tables 5 and 6). This follows the trend in the increase in sterility from var. I to var. III. It is possible that though some of the pollen grains of var. III showed germination that the pollen tubes were unable to reach the ovules due to genetically and/or physiologically controlled reasons.

With reference to seed germination and viability, var. I gave highest viability, var. II was next, while var. III was zero, having no seeds at all. (Table 8).

The presence of a hard seed coat does not appear to bear any relationship to the possible variation in the chromosome complement in the three variants. It is interesting to note however that soaking of seeds in concentrated sulphuric acid for 5 min gave comparable results to soaking in water for 65 hours. The process is clearly one of softening the seed coat.

From a study of morphological features like leaf size, floral characters, etc., it appears that var. II is intermediate in several characters between var. I and var. III. The stomatal frequency of epidermal peels of var. II is however significantly different from that of var. I and III (Table 3).

It appears that var. I is more of the wild type, while var. II and III may have arisen by hybridization and resulted in an increase in size of leaves, flowers, etc., in comparison to var. I (Table 1 and 4).

The commercial banana (*Musa sapientum L.*) which also belongs to the order *Zingiberales* is a triploid with a high degree of sterility but shows greater vigour and size than the diploid.[2,4,7,9,13] Reproduction in the banana too is entirely by vegetative means like in *C. indica*.

Usually polyploids are larger (larger habit, fruits, flowers and thicker leaves) and more vigorous than the naturally occurring diploids of the same group.[3]

Due to lack of data regarding chromosome number in the three *C. indica* variants studied one can only speculate that var. I could be close to a fertile diploid of *C. indica*, while variants II and III are polyploids bearing uneven ploidy levels. Supporting evidence comes from the relatively high fertility of var. I and reduction in fertility of the other two variants. To verify this however, investigation into the chromosome number and karyotype of the three variants is necessary.

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