INDUCTION OF FRUITING IN NON-BEARING AUSTRALIAN SPEICES OF CORCHORUS—C. PASCUORUM

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Introduction

Under the local climatic conditions fruiting in *C. pascuorum*, a natural tetraploid from Australia, is very poor or none at all. The failure of fruit formation may be due to the poor germination of pollen grains on the stigma of the selfed flower or may be due to the inability of the pollen tube to attain full length for bringing about fertilization or due to factors operating after fertilization.

To which of the above causes the failure was due was first determined by studying the behaviour of pollen grains *in vivo* and then attempts were made to induce fruiting by utilizing the results obtained *in vitro* germination.

Below are given the results of the above investigation.

Material and Method

Pollen grains of *C. pascuorum* Domin. were used. For *in vitro* germination different media were prepared in test tubes (cf. tables 1 & 2) and were sterilized in an autoclave at 15 atm. pressure for 15 minutes. It was stored in refrigerator at 5°C. Whenever needed the test tube containing the medium was dipped in boiling water to bring it into a semi-solid condition. A small drop of the medium was smeared on a slide. The slide was then dusted with the pollen and placed upside down inside a petri dish resting on glass rods of lengths which just fitted the inside diameter of the petri dish. Before placing the slide each petri dish was filled with 25 ml. of water. All the experiments were performed under natural day light conditions. Each experiment was repeated ten times and each time the calculation was based on 60 counts.

Germination *in vivo* was studied at different intervals after selfing the flowers with fresh pollen grains. The self-pollinated flowers were plucked at different time intervals and fixed in 1:3 acetic acid, alcohol for 8-10 hours after killing them in 2-5 minutes in hot water. The material was washed in 70% alcohol and then stained in cotton blue for 6-10 hours. The carpel was slit open lengthwise into two equal halves before mounting according to the methods used by Srinath and Kundu (1952) and Buchholz (1931).

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Experimental Results

The experiments were divided into four main parts which are dealt below separately.

Experiment I

In order to see the extent of pollen grain germination on the stigma and also the length to which the pollen tube grows inside the style the flowers were selfed. The selfing was repeated 150 times during a period of 3 months. Selfed flowers were fixed at different time intervals and studied for both pollen grain germination and pollen tube length down the style. The germination was poor in all the selfed flowers and where germination occurred, the pollen tube growth was found to be slow and not reaching the ovule (Fig. 1). The failure of fruit formation could therefore be attributable to the poor germination of pollen grains and also due to the inability of the pollen tube to reach the ovule for bringing about fertilization.

Experiment 2

At the next stage of investigation we wanted to know whether the failure of germination of pollen grains on the stigma was due to their inviability. To test this, the pollen grains were put for germination in vitro in different concentrations of sugar solution.

The maximum germination (60 per cent) was obtained in 25 per cent sucrose in 4 hours. The maximum pollen tube length (387 $\pm$ 115 μ) was also found in this concentration *i.e.*, 25 per cent and in 4 hours (table 1).

TABLE I

Selection of the best concentration of sucrose soln. for pollen grain germination and pollen tube growth; germination rate expressed in percentage and pollen tube length (PT) in microns.

Duration of experiments in hours	*Percentage of sucrose solution*									
	5	10	15		20		25		30	
	Germination	*Germination*	*Germination*	*PT*	*Germination*	*PT*	*Germination*	*PT*	*Germination*	*PT*
3	..	..	..	..	..	..	35	90$\pm$45	35	90$\pm$50
$3\frac{1}{2}$	..	..	..	..	..	..	45	252$\pm$42	40	250$\pm$73
4	..	..	10	90$\pm$45	20	91$\pm$47	60	387$\pm$115	55	299$\pm$69
$4\frac{1}{2}$	..	..	do	do	do	do	do	do	do	do

The effect of the addition in the medium of IAA, IBA and GA in various concentrations were studied separately in 25 per cent sugar solution since this concentration was found to be optimum (Table I). None of these growth promoting substances were found to increase, either the percentage of germination or pollen tube length (Table II).

The effect of boric acid was also tested; the addition of boric acid did not improve the germination percentage and pollen tube length in this plant. It may be due to the fact that the natural level of boron in the pollen of this species was sufficient so that an additional supply was ineffective (see Table II).

TABLE II

Effect of various growth promoting substances and boric acid on germination and Pollen tube growth; in all experiments medium consisted of 25% sucrose.

Supplements in ppm		*Percentage of germination*	*Length of PT in microns*
I. Indole Acetic acid (*IAA*)			
	100	49	227±115
	200	55	,,
	300	60	,,
II. Indole Butyiic acid (*IBA*)			
	100	58	331±112
	200	60	300±122
	300	60	379±100
III. Gibberellic acid			
	100	45	221± 57
	200	48	330± 83
	300	59	,,
IV. Boric acid			
	250	49	289± 67
	500	58	299± 69
	1000	60	382±117
	2000	60	343±113

Experiment III

25 per cent sucrose solution having been found the best medium for maximum pollen germination and pollen tube length, this was applied on the stigma at the time of selfing the flower to see whether the use of 25 per cent of sucrose induces fruit setting as was expected from the results of *in vitro* experiments. There was not only good germination of pollen grains on the stigma flooded with sucrose but the pollen tubes grew their full length inside the style (Fig. 2) and affected

fertilization thereby causing fruit set. Out of 11 flowers selfed following the application of sucrose solution, 8 flowers developed into small fruits but all these were found to shed after 4-5 days from the date of pollination. In the control where the sugar solution was not applied at the stigma, shedding of flowers took place the next day.

Experiment IV

When the fruit of *Corchorus* species stays on to the plant for more than 3 to 4 days the shedding of immature fruit is usually attributable to the formation of abscission layer in the stalk of the pollinated flower. Since the use of auxins in lanolin on the flower stalk was found to prevent the formation of abscission layer in interspecific crosses of *Corchorus* species (Islam and Rashid, 1960), the use of IAA and IBA were considered worthwhile to try. Accordingly, 35 flowers were prepared to receive on their stigma, 25% sucrose solution plus 300 ppm IAA, and 31, the same concentration of sucrose plus 300 ppm IBA. Only flowers treated with IBA stayed on to the plant till their maturity and also the seeds in them were found to be fully developed. The application of IAA was not effective in preventing premature fruit drop.

Discussion

C. pascuorum grows wild in Queensland, Australia. When the plants of this species were grown here, they faced diurnal and seasonal variations different from those of its native habitat. As a result although they flowered profusely, none of the flowers developed into a fruit. Of the four *Corchorus* species brought from south hemisphere and grown here, two namely *C. asplenifolius*—a South African species and *C. walcottii*—another Australian species, did not produce any fruit. We investigated into the reasons of the failure of fruit formation and found this was due to (*i*) failure of pollen grains to germinate on the stigma of the selfed flower and (*ii*) short supply of auxins in its pedicel. We could not however determine whether the failure of germination was due to the incomplete development of the pollen grain or due to unreceptivity of the stigma or due to both.

C. pascuorum fruits abundantly in its native habitat. There must therefore be complete development of pollen grains there, full receptivity of the stigma and an adequate availability of auxin in the ovary as well as in the pedicel of the fertilized flower. When this species is grown here the pollen grains do not germinate on the stigma. When they are forced to germinate by sucrose solution they produce full length pollen tubes and bring about fertilization but they are unable to provide the auxin necessary for the development of fruit and prevention of abscission layer.

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Fig. 1. *In vivo* preparation of stigma and style of selfed flower of *C. pascuorum*. Note that the pollen grains have not germinated.

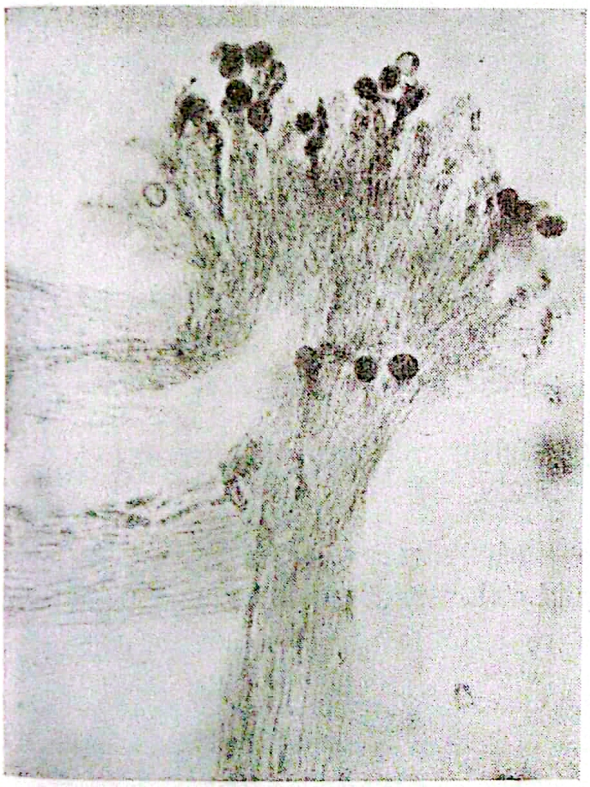


Fig. 2. Same with 25 per cent sucrose applied on the stigma showing pollen tube down the style.

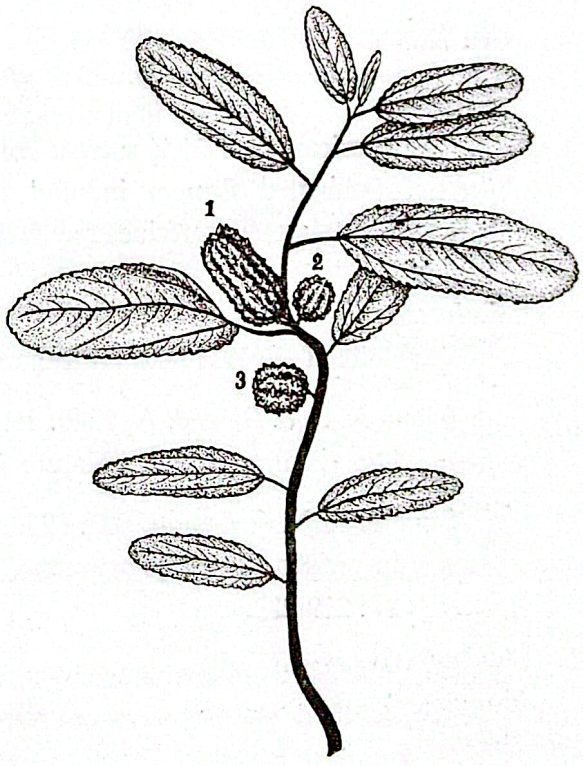


Fig. 3. Fruit developed from a flower treated with sucrose and IBA (1) while fruits (2) and (3) are from untreated flowers.

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As a result the fruits drop even after forced fertilization. It is only after the selfed flower were supplied in adequate quantity of IBA in addition to the sucrose soln. that the fruits stayed on until their full development.

We also found that the untreated open flowers and flower buds lying adjacent to the hormone treated flowers developed into mature fruits (Fig. 3). It is quite likely that IBA applied on the stigma of the selfed flower after being utilized in the ovary and pedicel of this flower travelled down the pedicels of the adjoining untreated flowers and caused them to develop into fruits. It is difficult to explain as to how the pollen grains germinated on the stigma of untreated flowers adjoining treated ones when the results of the *vivo* experiment show that the pollen grains do not germinate on the stigma of an untreated flower (see experiment I). May be sucrose soln. from a treated flower got translocated into the untreated ones helping germination of the pollen grain in the latter. Equally difficult it is to explain as to why of the two auxins only IBA prevented the formation of abscission layer.

It will be worthwhile to try the results of the present investigation as one of the means in overcoming the crossability barrier between species where incompatibility is due to the failure of germination of the pollen grain of one species on the stigma of other.

Summary

The application of 25% sucrose solution and 300 ppm IBA on the stigma of the self pollinated flowers induced fruiting in *C. pascuorum* which did not, before such treatment, produce any fruit under local climatic conditions.

Reference

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