BARRIER TO INTERSPECIFIC CROSSES IN *CICER*

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Attempts were made to cross *C. arietinum* and *C. soongaricum* using conventional as well as certain special methods of crossing. Although these techniques have been reported to be successful in other genera, none of these was found suitable for *Cicer*. Failure of pollen germination and penetration of pollen tubes into stylar tissues was observed in all cases, indicating the presence of a strong prefertilization barrier between *C. arietinum* and *C. soongaricum* which was active both on the stigma and in the style.

In vitro pollen germination experiments showed the presence of an inhibitory substance in the stigmatic and stylar tissues. This substance inhibited pollen germination as well as tube growth. The inhibitory substance was labile and most probably of low molecular weight.

INTRODUCTION

A successful interspecific hybridization programme can be an important means of introgressing desirable genes of wild species into the cultivated species. In addition, it is an important mechanism for the evolution of new species and provides a method to examine the taxonomic relationships of various species.

In practice, crossing of related species presents many complications like absence of pollen germination, abnormal development of the pollen tubes, etc. Even if fertilization takes place, hybrid seed development may be arrested due to several reasons. The present study was conducted to find out the cause for failure of interspecific crosses between two species of *Cicer*.

MATERIALS AND METHODS

Crossing techniques

Interspecific crosses were attempted between *C. arietinum* L., and *C. soongaricum* J. and S. Emasculations were done in the afternoon. Pollination was done the next morning between 8 and 11 A.M. (time of anthesis) using fresh pollen. The various crossing methods tried are given in Table I.

In vitro *pollen germination*

In vitro pollen germination and tube growth studies were carried out in a nutrient medium containing 0.5M sucrose and 100 ppm boric acid. Tube length was measured in microns by taking the average of ten longest tubes.

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To test the presence of an inhiitory substance in the pistil, 400 fresh styles with intact stigmas were collected in 1 ml of cold 0.05M tris maleate buffer (*p*H 7.00). These were ground with cold acid-washed sand and centrifuged at 1000 g for 15 min. The clear supernatant of the stylar and stigmatic extract was added to nutrient medium (final concentration: 0.5 M sucrose and 100 ppm boric acid) to test its effect on *in vitro* germination and tube growth. The stylar extract was found to lose activity even when stored frozen. Consequently, fresh extracts were prepared each time and used immediately.

Results and Discussion

The results of various methods tried to overcome the incompatibility barrier in the present case are summarised in Table I. The inability to obtain hybrid seeds between the two species of *Cicer* with the help of these methods suggested the presence of some inhibitory substance in the stigma and style. If such a substance was present, it should be possible to demonstrate its reaction *in vitro*. Tatebe (1961) found that the addition of crushed ovarian tissues to a standard sucrose-agar medium inhibited pollen germination in *Lythrum salicaria*. In the present studies extracts of crushed stylar and stigmatic material were used to test their effect on *in vitro* pollen germination.

When pollen grains were germinated in nutrient medium containing the stylar extracts of its own species, pollen germination and tube growth was normal as in the controls i.e. without any stylar extract (Table II). However, when pollen grains of one species were grown in nutrient medium containing stylar extract of the other species, the germination as well as tube growth of the pollen were inhibited. *In vitro* germination and pollen tube growth of *C. soongaricum* pollen was inhibited strongly by *C. arietinum* stylar extract (reduced to 9.67 per cent) while the germination of *C. arietinum* pollen in *C. soongaricum* stylar extract was inhibited comparatively to a lesser extent (reduced to 23.6 per cent). The *C. soongaricum* pollen also showed comparatively less germination (75.65 per cent) than *C. arietinum* pollen (87.07 per cent). When the pollen of one species were grown in nutrient medium containing half-diluted stylar extract of the other species, the inhibition was less as compared to the undiluted extract. The germination of *C. soongaricum* pollen was reduced to 39.75 per cent and that of *C. arietinum* to 51.3 per cent by the diluted stylar extracts of *C. arietinum* and *C. soongaricum* respectively.

Similarly pollen tube growth of *C. arietinum* was reduced from 11.46 to 4.35 μ when grown in the presence of *C. soongaricum* stylar extracts and that of *C. soongaricum* pollen was reduced from 10.95 to 3.66 μ when grown in the presence of *C. arietinum* extracts. The tube length in both cases increased when half diluted heterologous stylar extracts were used but was still significantly less than that in the controls.

The results of these experiments suggest the presence of an inhibitory substance in the stylar and stigmatic tissues which inhibits germination and tube growth of heterologous pollen. However, an almost complete inhibition of pollen germination as seen '*in vivo*' was not found '*in vitro*', nor were the growing tubes abnormal. One reason could be that the inhibitory substance was not fully extracted from the stylar and stigmatic tissues and its concentration in the solution was not sufficiently high to produce complete inhibition. Secondly, the inhibitory substance was found to be

TABLE I

Details of methods tried for interspecific crosses betewen C. arietinum *and* C. soongaricum *and their results*

Sl. No.	Method	No of reciprocal pollinations attempted	Result	Remarks
1.	Simple crossing	900 each way	No pollen germination and no seed set	Incompatibility barrier operating at the stimga
2.	Crossing after nutrient spray (0.5M sucrose containing 100 ppm boric acid)	600 ,,	No pollen germination and no seed set	Lack of sufficient nutrients for pollen germination is not the cause
3.	Crossing with germinated pollen (in 0.5M sucrose containing 100 ppm boric acid)	400 ,,	Penetration of pollen tube into stigma not affected and no seed set	Stigma not receptive even to germinated pollen
4.	Crossing after amputation of stigma	400 ,,	No pollen germination and no seed set	Cuticular barrier not operating
5.	Crossing after amputation & application of nutrient jelly (in 0.5M sucrose, 12.5% gelatin containing 100 ppm boric acid)	1300 ,,	Approx. 42% of pollen grains in *C. soongaricum* × *C. arietinum* cross & 51% in the raciprocal cross germinated and pollen tubes with average lengths of 5.6μ in former and 6.0 μ in latter were noted after 24 hr. No penetration of pollen tubes in Stylar tissue. Several pollen tube abnormalities were observed. Only 5% of pollen tubes were normal. No seed set	Indicates presence of a prefertilization barrier, some sort of inhibitory substance(s) which prevent(s) proper germination
6.	Crossing as in 5 and subsequent hormone treatment (40 ppm aqueous soln. of 3-indole acetic acid)	200 ,,	No seed set	Delayed flower abscission due to hormone treatment also not effective for penetration & fertilization by the normal pollen tubes noted in (5) above
7.	Pollination withl imited number of self (20–30) and then with an excess of cross pollen	200 ,,	No seed set	—
8.	Bud pollination	200 ,,	No seed set	Either stigma not receptive or inhibitory barrier is operative at bud stage also

TABLE II

Inhibition of pollen germination and tube growth by stylar and stigmatic extracts

Pollen parent	Experimental conditions	Germination (%)	Average tube length (μ)
C. arietinum	Nutrient medium (N)	87.07	10.73
	N + SE of *C. arietinum*	89.69	11.46
	N + ½ SE of *C. arietinum*	88.33	10·80
	N + SE of *C. soongaricum*	23.60	4.35
	N + ½ SE of *C. soongaricum*	51.30	7.10
C. soongaricum	Nutrient medium (N)	75.65	10.40
	N + SE of *C. soongaricum*	78.31	10.95
	N + ½ SE of *C. soongaricum*	78.03	8.10
	N + SE of *C. arietinum*	9.67	3.66
	N + ½ SE of *C. arietinum*	39.75	6.80

SE = Stylar and stigmatic extract
½ SE =Stylar and stigmatic extract diluted to half strength

unstable. Consequently, it might have lost a greater part of its inhibitory activity during the course of extraction.

These studies lead to the question about the nature of the inhibitory substance. In case of self-incompatibility, it has been found to be a protein. In the present study, the stylar extracts lost their activity on storage at 0°C. When the concentration of extracts was diluted to half, a corresponding decrease in the inhibitory reaction was noted in both species. Such results are expected from a low molecular weight inhibitory agent and it could be a protein. However, the exact nature of this inhibitory factor needs to be worked out further.

The failure to cross different species can be due to genetic, physiological or developmental factors. Some of the physiological factors known to inhibit pollen germination on the foreign stigma and arrest tube growth are, inability to dissolve the cuticular layer on the stigmatic surface or utilize nutrients (Thompson 1940; Tatebe 1947; Swaminathan 1955; Khanna and Singh 1956; and Linskens 1961). In certain instances, pollen tubes develop either abnormally or swell and burst and constitute barriers to crossability (Dione 1961; Muller 1961). Special techniques have been devised to overcome different types of crossability barriers. Irradiation of the pollen before using for pollination is one unexplored possibility as has been done in pears (Celestre 1946). Intra-ovarian pollination is another device suggested to overcome crossability barrier in *Argemone mexicana* × *A. ochroleuca* crosses (Maheshwari and Kanta 1961). In these cases, the ovary is big with a large ovarian cavity which can be filled with pollen suspension without inflicting undue damage to the flower. Somatic cell hybridization combined with tissue culture is another technique, which when perfected could be used to obtain interspecific hybrids in cases where conventional methods fail to produce successful results (Carlson, Smith and Dearing 1972).

The present studies thus indicate that the interspecific hybridization is rather difficult to achieve in *Cicer* due to pre-fertilization crossability barrier. The pollen grains of one species fail to germinate and penetrate the stigmatic and stylar tissue of the other species. The crossability barrier does not appear to be cuticle or some nutrient factor. The failure of germination is due to some inhibitory substance. The present studies provide a scope for further investigating the nature and properties of the inhibitory substance and for trying newer techniques to achieve successful hybrid formation between the various species of *Cicer*.

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