LECTIN-LIKE COMPONENTS OF POLLEN AND COMPLEMENTARY SACCHARIDE MOIETY OF THE PISTIL ARE INVOLVED IN SELF-INCOMPATIBILITY RECOGNITION

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ABSTRACT

Using an *in vitro* assay in which self incompatible pollen grains are selectively inhibited, experiments were conducted to understand the biochemical basis of self-incompatibility recognition in *Petunia hybrida*. The results indicate the involvement of lectin-like components of the pollen and complementary sugar moiety of the pistil in pollen recognition.

INTRODUCTION

MUTUAL recognition of the gametes is an essential feature of sexual reproduction. In a majority of animals and cryptogams the interacting gametes come in direct contact with each other and establish recognition. Gamete recognition has been shown to be the result of complementation involving a lectin and specific saccharides present on the surfaces of the sperm and the egg, respectively. The interacting molecules have been characterized in a few systems[1-5]. Recognition results in establishment of membrane contact and eventual fusion of the two gametes. In the absence of complementation, gametes cannot establish membrane contact and thus are unable to fuse.

In flowering plants gametes recognition is complicated as it involves a haploid (pollen) and a diploid (pistil) tissue. Further, the cells of both the interacting partners are covered by a cell wall that prevents direct membrane contact. Pollen grains which carry male gametes become deposited on the stigma of the pistil and initiate a chain of sequential interactions during which they become recognized by the pistil. If the pollen grain is compatible, all post-pollination events proceed normally. The pollen tube succeeds in discharging the male gametes in the vicinity of the egg and fertilization is effected. The incompatible pollen tube, however, is effectively inhibited from reaching the female gamete[6].

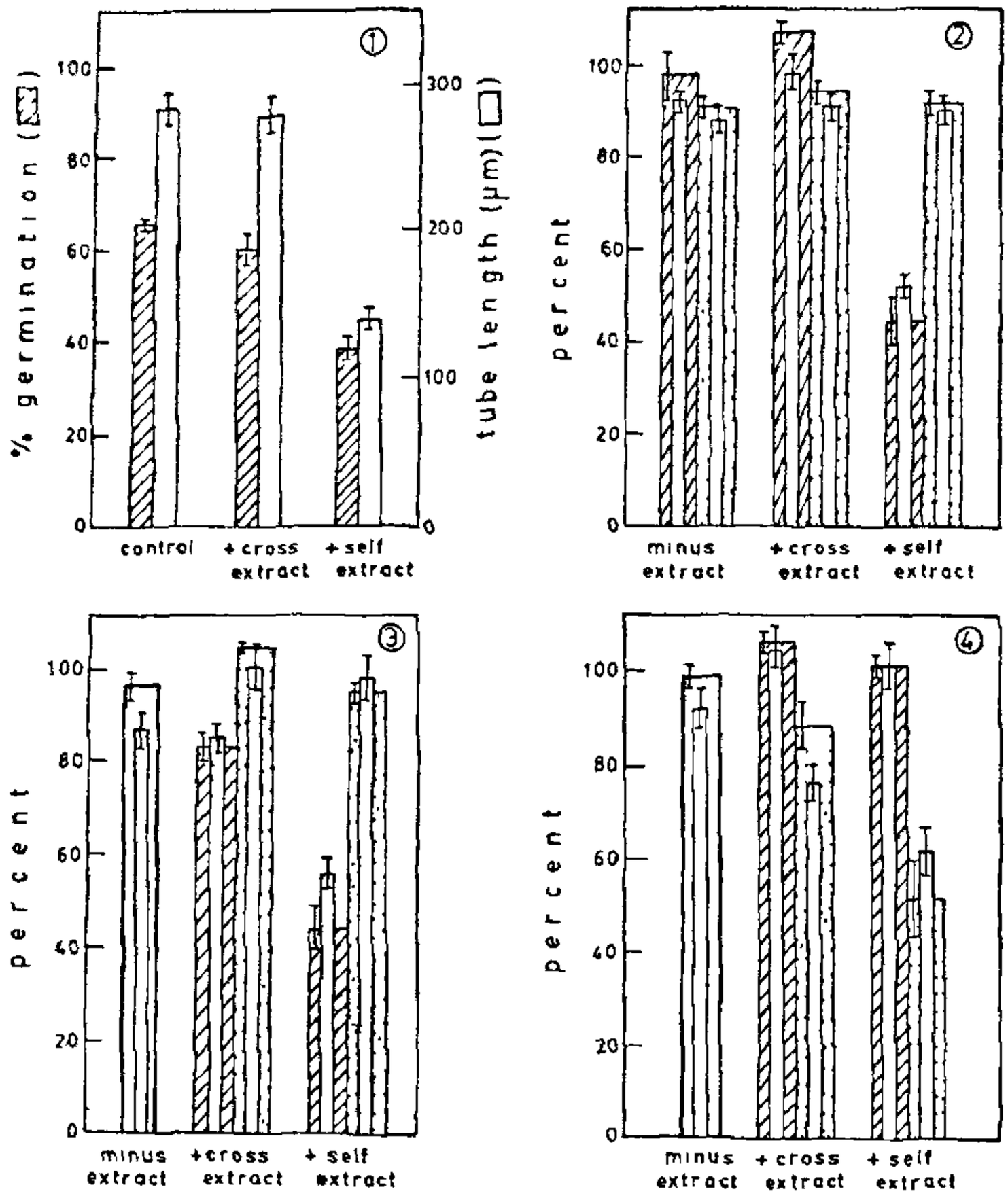
Incompatibility may be interspecific or intraspecific. The former is highly variable and our knowledge about its manifestation is still limited. Self-incompatibility is widespread in flowering plants and is an adaptation for preventing inbreeding. It is generally controlled by multiple alleles (termed S-alleles) at one or a few loci. Pollen grains carrying an S-allele identical to the one present in the pistil are inhibited[7].

Extracellular proteins and glycoproteins are present in the pollen grain wall and in the secretions of the stigma and transmittig tissue of the style. These substances are reported to be involved in self-incompatibility recognition[8-12]. However, the biochemical basis of recognititon and the consequent inhibition of incompatible pollen have so far remained elusive. A major difficulty in examining these aspects is the lack of a rapid *in vitro* assay system in which self-pollen grains can be selectively recognized and inhibited. We have recently established an assay using *P. hybrida*[13] for studying the biochemical details of recognition and inhibition. This paper presents evidence for the interaction of lectin-like components of the pollen and specific carbohydrate containing molecules in the pistil in self-incompatibility recognition.

MATERIALS AND METHODS

Plants of *P. hybrida* Vilm were raised under field conditions from seeds collected from the population used in our earlier studies[13]. Styles (along with the stigmas) from unpollinated flowers were homogenised in an ice-cold mortar in 0.015 M phosphate buffer (pH 5.9) with acid-washed sand (10 pistils ml^{-1}). The extract was centrifuged at 10,000 g at 4° C, and the supernatant was incorporated in the pollen culture medium (200 g l^{-1} sucrose and 0.2 g l^{-1} H_3BO_3 in 0.015 M phosphate buffer, pH 5.9). The final concentration of the extract was 2.5 pistils per ml of the medium. Pollen grains collected from freshly-dehisced anthers were germinated in 'sitting drop' cultures on microscope glass slides kept in petri plates lined with moist filter paper. Per cent pollen germination and tube growth were scored 10 hr after culture by which time pollen grains in the medium containing self (incompatible) extract showed marked difference in their response from those in cross (compatible) extract (figure 1). Sterilization of the medium was not necessary as there was no infection in a 10 hr period.

In one set of experiments pollen grains were treat



Figures 1-4 1. Effect of self-and cross-pistil extract on pollen germination and tube growth. Cross-extract had no effect while self-extract inhibited both germination and tube growth. **2-4.** Responses of pollen grains treated with glucose/lectins. Hatched/stippled bars represent germination and open bars tube growth respectively. **2.** Response of glucose treated pollen. Pollen grains were incubated either in control medium (hatched) or glucose-medium (stippled) for 30 min before culturing. Inhibition in the self-extract was fully overcome in glucose-treated pollen but not in pollen incubated in control medium. **3.** Responses of pollen grains to Con A (incorporated in the medium). Con A was able to overcome inhibition in plant B (stippled), but not in plant A (hatched). **4.** Pollen germination and tube growth in the medium containing PHA. Inhibition in self-extract was overcome in plant A (hatched) and not in plant D (stippled).

Vertical lines on the bars represent standard error of the mean. As expected, in all treatments in which self-extract inhibited pollen germination and tube growth, the differences between self-and cross-extract were highly significant at $P \leq 0.05$; the differences were statistically insignificant in treatments which were effective in overcoming inhibitory effect of self-extract.

either with sugars such as D-glucose (Glaxo Laboratories) and D-mannose (Sigma) (50/100 mM) or with lectins such as concanavalin A (Con A, Sigma) and phytohemagglutinin M (PHA, CSIR Centre for Biochemicals, Delhi) (1 mg ml^{-1}) before culturing. These chemicals have been extensively used in studying recognition phenomenon in animal systems [1-5]. Sugars/lectins were incorporated in the germination medium and pollen grains were incubated for 30 min. Pollen grains were then separated by passing the sus-

pension through a millipore filter (pore size 0.45 μm), subsequently washed many times in control medium (minus lectins/sugars) and used for culture. In another set of experiments untreated pollen grains were cultured in the germination medium containing sugars/lectins plus pistil extract.

For each treatment pollen grains were cultured in two replicates and the experiment was conducted at least three times. In each replicate over 200 pollen grains were scored for germination and 50 tubes for tube length. Per cent germination and tube length obtained with untreated pollen in the control medium (figure 1, control) are taken as 100 and the values in figures 2–4 are expressed as percentages over this control. The data obtained as averages of germination percentage and tube length were subjected to *d* test, and *t* test respectively[14].

Results and Discussion

As mannose inhibited pollen germination even in the control medium, its effect on self-incompatibility recognition could not be studied. Treatment of pollen with glucose did not affect *in vitro* germination and tube growth (figure 2). The extract from cross-pistil had no effect on germination and tube growth of glucose-treated pollen as well as pollen incubated in the control medium. The extract from self-pistil significantly inhibited germination and tube growth of pollen incubated in control medium, but not in glucose medium. Apparently pollen components involved in self-incompatibility recognition are glucose-specific and treatment of pollen with this sugar effectively blocks recognition.

Although treatment of pollen with the lectins did not inhibit *in vitro* germinability and tube growth, it was not effective in overcoming inhibition caused by self-pistil extract. The response of the pollen treated with glucose or lectins was similar in all the plants tested.

Incorporation of lectins in the germination medium containing pistil extract was effective in overcoming the inhibition caused by self-pistil extract (figures 3,4). However, the response was variable from plant to plant. Amongst the four intercompatible plants tested (designated A, B, C and D), Con A was effective in overcoming inhibition of self-pollen in plant B, C and D but not in A (figure 3); in plant A it was marginally inhibitory even to cross-pollen. PHA was effective in plants A and B, but not in C and D (figure 4). Thus, pistil components involved in self-recognition are sugar-containing molecules. Blocking these sugars by specific lectins makes them ineffective in recognizing self-pollen. As the pistil components are active even after dialysis of the extract[13], they are macromolecules and appear to be glycoproteins[11,12]. Incorporation of glucose in the pistil extract did not affect self-recognition, indicating that sugar moieties of the pistil recognition components have stronger affinity for specific lectins than glucose molecules.

Present investigations have provided a strong evidence for the involvement of lectin-like components of the pollen and complementary sugar-containing macromolecules in the pistil, similar to that shown for many animals and lower plants, in self-incompatibility recognition. Glucose seems to be effective in binding to lectin-like recognition components of the pollen in all the plants tested. Apparently the recognition molecules of pollen show small, allele-specific structural variations that can discriminate complementary saccharide structures produced in the pistil, but not glucose. Similarly neither Con A nor PHA show S-allele specificity in their affinity to the sugar moiety present in the pistil, as both are effective in more than one intercompatible plants. Work is in progress to localize and further characterize the active components in the pollen and the pistil.

The method used and results obtained by us would pave the way for understanding the detailed mechanism of gamete recognition in flowering plants. It should be possible to overcome incompatibility barriers by treatment of pollen with specific sugars or the pistil with suitable lectins. Preliminary studies on these lines have given promising results.

Acknowledgements

We are grateful to Prof. H. Y. Mohan Ram for his keen interest and encouragement. NS thanks the CSIR, New Delhi, for a fellowship.

5 May 1983

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NITROGEN FIXATION DOES NOT LIMIT YIELD IN PULSES

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ABSTRACT

In this communication we report that tropical legumes pigeonpea (*Cajanus cajan*) and chickpea (*Cicer arietinum*) accumulate upto 301 kg N ha^{-1} but only 21 to 49% of it is utilised for grain development. Therefore, limitation in nitrogen fixation is not the cause of poor yield, but its mobilization for grain development is poor. A change in plant structure may be more important for yield improvement than efforts to increase nitrogen fixation.

PULSES (grain legumes) constitute a major source of proteins in the vegetarian diet in developing countries, but the yields of these crops are low[1]. It is often stated that poor nitrogen fixation limits the yield of various grain legumes including pulses[2] and soybean[3]. Enrichment with CO_2 or illumination of crop canopies in soybean led to the increased nitrogen fixation and grain yield[3,4]. However, several legumes such as cowpeas, mungbeans, pigeonpea, chickpea, etc experience water deficiency, salinity and other effects which adversely influence nitrogen fixation[5]. Therefore, there is considerable emphasis on nitrogen fixation studies in pulses and consequently worldwide programmes such as the International Biological Programme and Niftal are getting increasing attention. However, in most instances the Rhizobium inoculation experiments have given variable results on grain yield[6]. There are reports[2] suggesting that the tropical legumes fix nitrogen between 20 and 270 kg N ha^{-1}. Apparently there are very few quantified studies on nitrogen balance and most of the conclusions about nitrogen fixation are based on grain yield. Giri and De[7] have shown that substantial quantities of nitrogen are left behind in the soil after the harvest of grain legumes. Therefore, the total nitrogen content in the plant parts above the ground level was taken to represent the minimum amount of nitrogen fixation, after subtracting the quantity of nitrogen initially applied.

Seeds of determinate and indeterminate types of pigeonpea (*C. cajan*) cv. Prabhat were treated with an appropriate Rhizobial culture and planted on 7 July 1981 to obtain a population density of 20 plants m^{-2}. Fertilizer nitrogen, phosphorus and potash in the proportion of 20:40:40 kg ha^{-1} were applied. Irrigation and no irrigation constituted the two treatments. Irrigation was given after flowering. There were three replicates of each treatment, and each replicate consisted of 4 × 5 m size plot. At maturity, a crop area of 1 × 1 m was harvested. The leaves shed in a demarcated area of 1 × 1 m were collected and included in the harvested material.

Seeds of chickpea (*C. arietinum*) cv. JG-62 and its one mutant M 109, after treating with an appropriate Rhizobium culture, were planted on 5 November 1981. Irrigation and non-irrigation constituted the two treatments. There were three replicates and each replicate consisted of 4 × 5 m size plot. All the above ground parts from 1 × 1 m area were harvested at maturity. The leaves, stem, pod wall and seeds were separated and analysed for their nitrogen content. The different samples were digested according to the method of Novozamsky *et al*[8] and analyzed for nitrogen content using a Technicon Autoanalyser Model II, Industrial method No. 334-74W/B*. The total nitrogen was computed using the dry weight at harvest and nitrogen content of each part.

In pigeonpea (*C. cajan*) the total harvest of nitrogen ranged from 261.7 kg N ha^{-1} to 275.3 kg N ha^{-1} in the determinate and indeterminate types, respectively. Irrigation had no significant effect, probably because this crop was grown in the rainy season. However, the nitrogen harvest, essentially represented the dry mat-