SELF INCOMPATIBILITY MECHANISMS IN THE *CROCUS SATIVUS* AGGREGATE *(IRIDACEAE)*: A PRELIMINARY INVESTIGATION

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Abstract - Two molecular mechanisms responsible for SI (Self-Incompatibility) in dicotyledons were tested in the *C. sativus* L. aggregate. RNase and peroxidase activity assays were carried out on crude extract from un-, self- and cross-pollinated styles of *C. sativus* (male-sterile), *C. thomasii* Ten. (out-fertile) and *C. cartwrightianus* Herb (out-fertile). Results on RNase activity indicate that in the *Crocus* species studied the rejection mechanism of SI is not based on stylar RNase. Data on peroxidase activity indicate a relationship between pollen tube presence in the style and stylar peroxidase activity. Stylar peroxidase activity increase is related to pollen tube presence but does not stop tube growth. Compatible and incompatible pollen tubes grow along the style and their discrimination occurs in another region of the gynoecium.

Key words - Self incompatibility, *Crocus, C. sativus, C. thomasii, C. cartwrightianus*

Introduction

Self-incompatibility (SI) is one of the strategies that have evolved to prevent inbreeding and promote outcrossing in flowering plants. SI is often controlled by a single nuclear gene (the S-gene) with several alleles (Pandey, 1967; De Nettancourt, 1977). This gene prevents fertilisation by self pollen or by pollen bearing either of two S-alleles expressed in the style. The best-known model systems to elucidate SI molecular basis are the dicotyledon families *Solanaceae*, *Primulaceae*, *Scrophulariaceae*, *Rosaceae* and *Papaveraceae*. In several *Solanaceae* (Lee *et al*., 1994), *Scrophulariaceae* (Murfett *et al*., 1994) and *Rosaceae* (Tao *et al*., 1997) which posses gametophytic SI, the products of pistil S alleles (S-Rnase because of their ribonuclease activity) play an unequivocally established role in recognition and rejection of self pollen by the pistil. In poppy (*Papaveraceae*) SI alleles codify for a small stigmatic protein which is presumably recognised by a receptor on the pollen tube surface following self-pollination. This molecule is not internalised and it is thought that the binding of ligand to receptor generates a second message which stimulates the release of Ca^{2+} from internal stores. Increased levels of intracellular Ca^{2+} then inhibit pollen tube development (Campbell

and Lawrence, 1981). In *Primula acaulis* a link between incompatibility responses and peroxidase activity is suggested. In this model, the presence of apoplastic peroxidase activity in unpollinated styles and the increase in apoplastic peroxidase activity in the transmitting tissue after self-pollination have been related to predisposition of the style to the rejection of incompatible pollen tube growth (Carraro *et al*., 1985). In *Crocus*, a previous study on Ca^{2+} distribution along un-, self- and cross-pollinated pistils of *C. cartwrightianus* Herb. and *C. sativus* showed that Ca^{2+} is a factor influencing pollen germination and pollen tube growth. However, there was no evidence indicating that the calcium ion was involved in the mechanism of self-incompatibility in the *Crocus* species tested (Brandizzi and Grilli Caiola, 1996). *C. cartwrightianus* (diploid, out-fertile) and *C. thomasii* (diploid, out-fertile) belong to the *C. sativus* aggregate (triploid male-sterile) (Mathew, 1982), and seem to be the most likely ancestors of saffron (Mathew, 1982; Chichiriccò, 1989; Brandizzi and Grilli Caiola, 1998). A recent quantitative and qualitative evaluation of nuclear DNA by cytofluorimetric analysis, used to estimate genome size and base pairs composition, indicated that DNA of *C. sativus* was more similar to that of *C. cartwrightianus* than to that of *C. thomasii* (Brandizzi and Grilli Caiola, 1998). Seed set from all these species has been reported elsewhere (see Grilli Caiola *et al*., this volume). In this paper, RNase and peroxidase activity assay was carried out in un-, self- and cross-pollinated styles of *C. sativus*, *C. cartwrightianus* and *C. thomasii*, to explore the molecular mechanisms responsible for self-incompatibility in *Crocus* species.

Material and methods

Plants of *Crocus sativus* L., *C. thomasii* Ten. and *C. cartwrightianus* Herb. were cultivated at the University of Rome "Tor Vergata". Several flowers were emasculated two days before anthesis. At anthesis stigmas were self- or cross-pollinated. Unpollinated flowers were used as controls. At one and two days after pollination stylar crude extracts were prepared according to Jahnen *et al*. (1989) with some modifications. Several styles were ground to powder under liquid nitrogen using a mortar and pestle. The powder was extracted with buffer (2.5 ml per gram of styles, fresh weight; 0.1 M Tris HCl ph: 7.4, 0.7 NaCl containing 1% w/w insoluble polyvinyl pyrolidone). The homogenate was centrifuged (10000 g; 20 min; 4°C) and the supernatant was subjected to another centrifugation under the same conditions, to recover a clear crude extract. This crude extract was assayed for RNase (Brown and Ho, 1986) and peroxidase activity (Angelini *et al*., 1990). Self- or cross-pollinated styles were stained with Amido black to reveal pollen tubes within the style (Carney *et al*., 1994). Enzyme activity was compared from different samples, considering the time taken to obtain the same absorbance increase.

Results

Rnase Activity - Data on stylar RNase activity in styles of *C. sativus*, one day after self- or cross-pollination, are given in Table 1. They indicated very low levels of enzyme activity. No significant differences were detected between extracts from un-pollinated, self-pollinated and cross-pollinated styles of *C. thomasii* and *C. cartwrightianus*.

TABLE 1 - STYLAR RNASE ACTIVITY IN *C. SATIVUS* AT ONE DAY AFTER POLLINATION

	RNase activity (%)
Unpollinated	100
Self-pollinated	88
Cross-pollinated by *C. thomasii*	151
Cross-pollinated by *C. cartwrightianus*	220

Peroxidase Activity - One day after pollination: Stylar peroxidase activity increased in self-pollinated *C. sativus*, but it became four times higher after cross-pollination by *C. cartwrightianus* or *C. thomasii* (Fig 1). In *C. cartwrightianus* styles self- or cross-pollinated by *C. sativus*, peroxidase activity presented approximately the same value, but was higher than in unpollinated styles (Fig. 2). In addition, peroxidase activity was lower in styles of self-pollinated *C. thomasii*, compared to those cross-pollinated by *C. sativus* (Fig. 3).

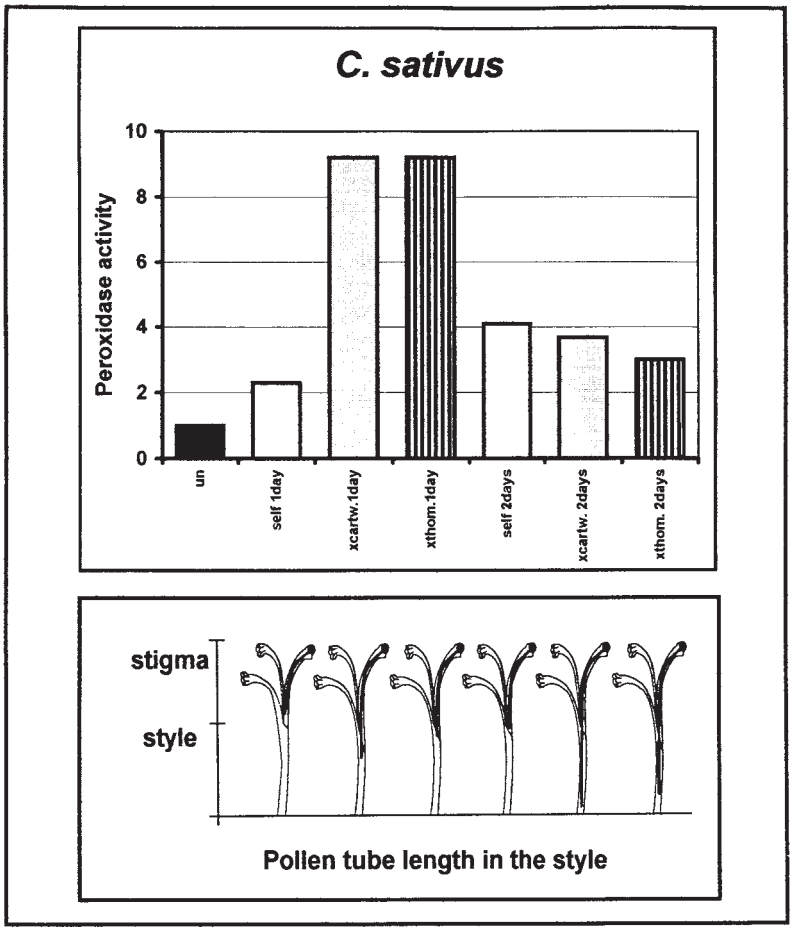

Figure 1 - Stylar peroxidase activity and pollen tube length in the style in *C. sativus* after self-or cross-pollination by *C. cartwrightianus* or *C. thomasii*.

Two days after pollination: In *C. sativus,* no differences were observed in peroxidase activity between self- and cross-pollinated styles; the different extracts showed very similar activity levels (Fig. 1). However stylar peroxidase activity in *C. cartwrightianus* cross-pollinated by *C. sativus* was twice as high as that found in self-pollinated styles (Fig. 2).

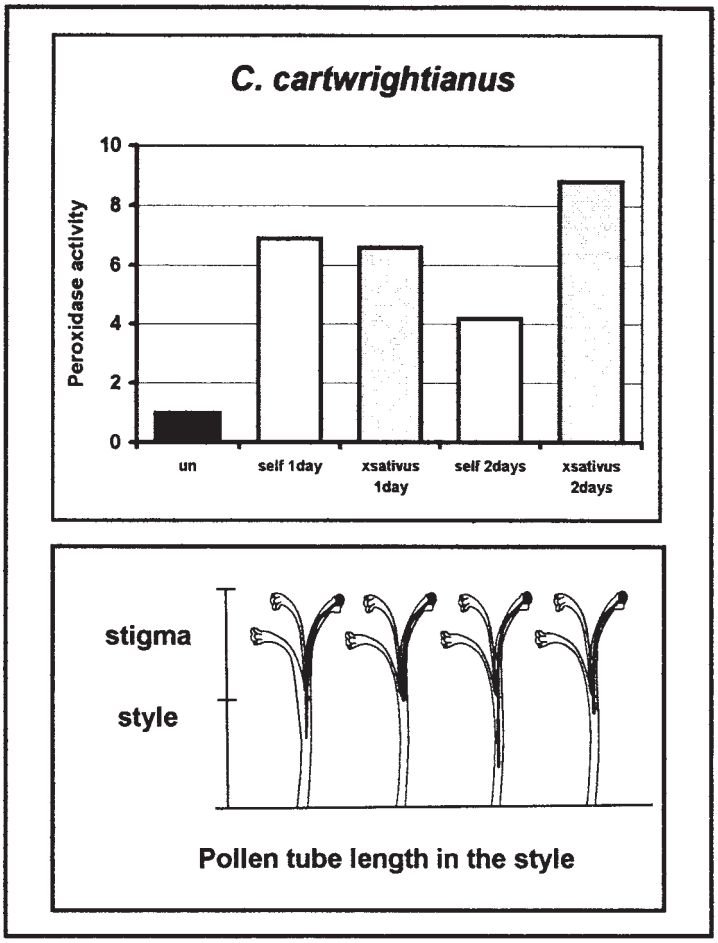

Figure 2 - Stylar peroxidase activity and pollen tube length in the style of *C. cartwrightianus* after self- or cross- pollination by *C. sativus*.

Pollen tube growth through style - C. sativus: At one day after pollination, in self-pollinated styles pollen tubes were detected in the upper part, while in styles cross-pollinated by *C. cartwrightianus* or *C. thomasii* they reached the median part. Twenty-four hours later, self-pollinated styles had still pollen tubes only in the upper part, whereas in styles cross-pollinated by *C. cartwrightianus* or *C. thomasii* pollen tubes occurred in the lowest region (Fig. 1).

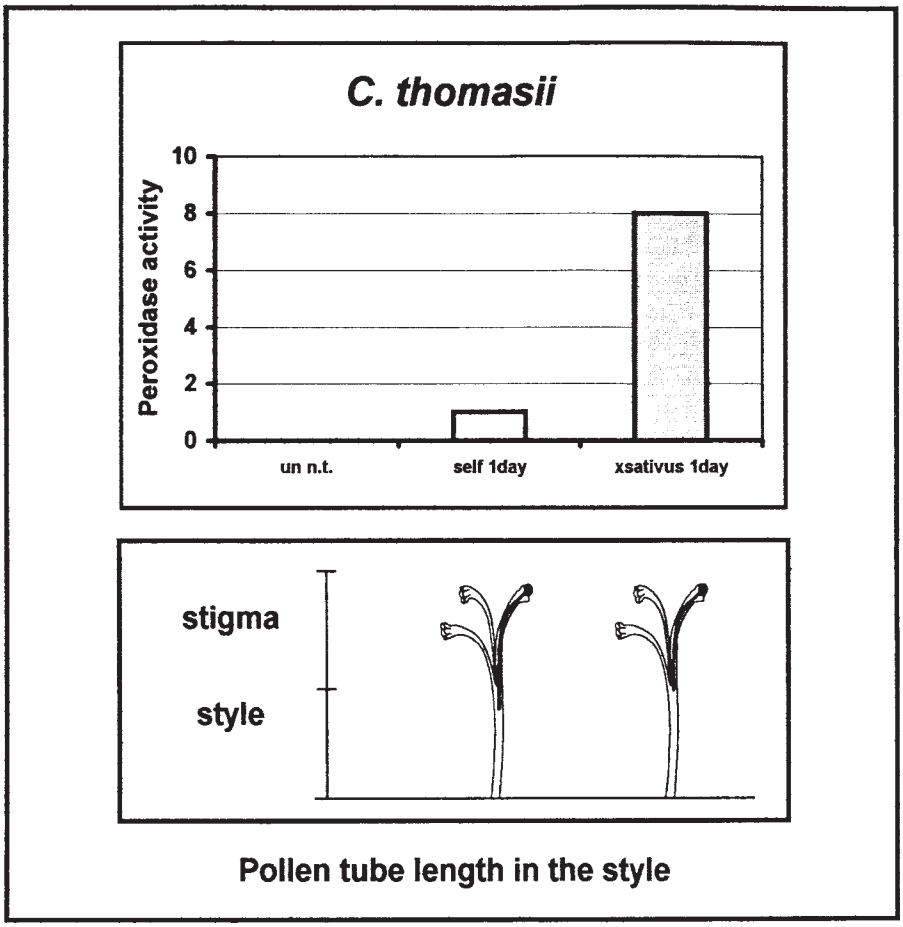

Figure 3 - Stylar peroxidase activity and pollen tube length in the style of *C. thomasii* after self- or cross-pollination by *C. sativus*.

C. cartwrightianus: One day after pollination, pollen tubes were present in the median parts of self-pollinated styles. Two days after pollination pollen tubes had reached the upper portion of the ovary. On the other hand, pollen tube growth was low in styles cross-pollinated by *C. sativus*: one day after pollination they were present half way down the style (Fig. 2).

C. thomasii: Results of pollen tube growth in *C. thomasii* were very similar to those in *C. cartwrightianus*. Pollen tube growth was faster in self-pollinated styles than in styles cross-pollinated by *C. sativus* (Fig. 3).

Discussion

The very low values of RNase activity in self- and cross-pollinated *C. sativus* styles indicate that in this species the rejection mechanism of incompatibility is not based on stylar RNase. This hypothesis was supported by the small differences in RNase activity obtained in crude extracts of *C. sativus* styles after self- or cross-pollination by *C. thomasii* or *C. cartwrightianus*. Data obtained in all the experiments were not comparable to those of *Nicotiana* (McClure *et al*., 1989), but were similar to those found in *Papaver rhoeas* (Franklin-Tong *et al*., 1991). *C. sativus* stigmas were receptive to pollen from either related species. In all crosses, pollen grains of related species germinated on *C. sativus* stigmas, and the resulting pollen tubes grew along the host style. Pollen tube elongation in self-pollinated styles of *C. sativus* is slower than after cross-pollination by *C. thomasii* or *C. cartwrightianus*. Both *C. thomasii* and *C. cartwrightianus* self-pollinated styles showed pollen tubes growing faster than those in styles cross-pollinated by *C. sativus*. Data indicated a relationship between stylar peroxidase activity and growth of pollen tubes through the style. In all species examined, although with some differences, stylar peroxidase activity increased when pollen tubes were in the style. Moreover in *C. sativus* (self-sterile but cross-fertile) and *C. thomasii* and *C. cartwrightianus* (both self-sterile but out-fertile), there was higher peroxidase activity in cross-pollinated styles than in self-pollinated ones. Peroxidase activity is therefore related to the presence of compatible or incompatible pollen tubes in the style. However, it is known that pollination and subsequent pollen tube growth produce in the style "metabolic changes coupled with alterations in the enzyme activities" (Roggen, 1967). Pollination and pollen tube growth cause an increase of total peroxidase activity and several peroxidase isoenzymes. Breedemeijer and Blaas (1975) showed by electrophoresis that only some isoenzymes could be involved in the rejection of incompatible pollen tubes. Our method of analysis does not allow us to distinguish between the various peroxidase isoenzymes involved in the rejection of pollen tube. However, in cross-pollinated styles of *C. sativus* and self-pollinated styles of *C. cartwrightianus,* two days after pollination pollen tubes had covered the whole style in spite of the high level of peroxidase activity already present. These results suggest that stylar RNase or peroxidase activity are not involved in self-incompatible systems in the species of *Crocus* examined. Self-incompatibility seems to be a more complicated process in *Crocus* than that reported in most dicotyledons. Among the species of *Crocus sativus* aggregate, incompatibility could be controlled by other rejection

system operating in pregamic or postgamic phases (Chichiriccò, 1996; Grilli Caiola and Chichiriccò, 1991; Grilli Caiola *et al*., this volume).

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