

Cytological evidence for gametophytic self-incompatibility in the genus *Veronica*

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Abstract: The self-incompatibility (SI) of 3 Middle East *Veronica* species (*V. filiformis*, *V. gentianoides*, *V. teucrium*) is studied, for the first time, by pollination and pollen tube growth experiments. Cytological observations of the self-pollen tubes within the pistils permit a conclusion about the presence of a gametophytic SI system in the first 2 species. The investigated population of the third is self-compatible and confirms the facultative trait of its self-sterility. The presence of a gametophytic SI system in 2 of them suggests the putative conservation of this SI system within the genus *Veronica* (Plantaginaceae) and could be related to the well-known gametophytic SI system present in the other Plantaginaceae genus, *Antirrhinum*.

Key words: Pollen tube growth experiment, gametophytic, self-incompatibility, *Veronica*

1. Introduction

The mating system is one of the most fundamental characteristics of a plant species shaping population-level processes such as inbreeding effects, demography (Husband and Schemske, 1996; Morgan et al., 2005), and evolutionary trends (Takebayashi and Morrell, 2001; Ferrer and Good, 2012). However, information about the mode of plant reproduction beyond the dichotomous distinction between selfing and outcrossing is either indirectly inferred by population genetic analyses or based on pollination experiments. Such experiments have been common since the time of Darwin (Darwin, 1876) and started even before, with Kölreuter (1761) being credited for the first report of plants obligately requiring cross-pollination for seed set (Proctor et al., 1996). About 40%–60% of all species of flowering plants are thought to be self-incompatible (De Nettancourt, 2001; Iğic et al., 2008). However, many of the mating system studies have reported self-sterility rather than self-incompatibility since they often ignore factors such as pollen viability, stigma receptivity, and seed abortion (Wang et al., 2004; Chouteau et al., 2006 Yang and Guo, 2007) or forgot to investigate the type of self-incompatibility (Subaşı and Güvensen, 2011). Thus, to definitively demonstrate self-incompatibility, detailed and extensive crossing studies or studies of pollen tube growth after cross- and self-

pollination are required. Pollen tube growth investigations are important not just to determine self-incompatibility but also to differentiate the different types of self-incompatibility recognized by the place (stigma, style, or ovary) and the genetic control of the self-incompatible reaction. This method permits botanists to visualize rapidly in the female plant, by cytology, the different places where the self-incompatible reactions for the 3 types of self-incompatibility (sporophytic self-incompatibility (SSI), gametophytic self-incompatibility (GSI) and late-acting self-incompatibility (LSI)) take place. SSI systems can be differentiated from GSI or LSI systems by the fact that self-pollen tubes are not able to enter the female part of the plant and are stopped at the surface of the stigma, while this is not the case for GSI and LSI systems. Indeed, GSI reactions take place within the style before the entrance of the ovary, the opposite of LSI reactions happening within the fertilized ovules located in the ovary. Based on these criteria, botanists can differentiate easily among the 3 self-incompatibility systems for their studied species. The well-supported species-rich genus *Veronica* L. of approximately 450 species divided into 13 subgenera living in a large variety of habitats (e.g., alpine regions, aquatic habitats, dry steppes) and with a high diversity of life-forms (e.g., therophytes, tall herbs of 1–1.5 m, subshrubs, shrubs) is no exception to this absence of knowledge about self-

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incompatibility systems. Indeed, despite extensive crossing studies conducted in the first half of the 20th century, most studies were not extensive enough to permit conclusions about the mode of self-incompatibility (Lehmann, 1909, 1919, 1922, 1944; Härle, 1932; Beatus, 1935; Scheerer, 1937; Schmitz, 1946; Lehmann & Schmitz-Lohner, 1954). Nevertheless, studies by Lehmann and Filzer (Lehmann, 1919, 1922; Filzer, 1926) established *Veronica syriaca* Roem. & Schult. as just the second species of all the flowering plants to have a GSI system, while later adding *Veronica filiformis* Sm. as another species with that mating system (Lehmann, 1944). Recent pollen/ovule ratio investigations (Scalone et al., 2013) similarly permit conclusions about the range of mating systems varying from preanthesis selfing to self-incompatible and obligate outcrossing. However, no cytological evidence is available to confirm the specific type of self-incompatibility (SSI, GSI, or LSI) present in this genus. Our hypothesis is that pollen tube experiments can reveal, for the first time, the type of self-incompatibilities in *Veronica*, while our main objective is to confirm the precedent crossing results of Lehmann and Filzer cytologically. Here, we present such data to confirm GSI in the genus *Veronica* with a cytological approach using 3 different species, *V. filiformis* Vahl, *V. gentianoides*, and *V. teucrium* L. (Plantaginaceae sensu Angiosperm Phylogeny Group, 2009), belonging to 3 different subgenera (*Veronica* subgenus *Pocilla*, subgenus *Beccabunga*, and subgenus *Pentasepalae*, respectively) and previously studied by crossing studies.

2. Materials and methods

A minimum of 5 different individuals for each of the 3 *Veronica* species (*V. filiformis*, *V. gentianoides*, and *V. teucrium*) present in the Botanical Garden of Mainz University were covered by tissue to conduct hand-controlled pollinations (more than 20 flowers). Emasculations and self- and cross- pollinations were conducted 24 h before pistil sampling and before anthesis and pollen dispersal. Pollinated (5 per self- and 5 per cross-pollination) and virgin (1 negative control) styles were fixed for 24 h in AFE (ethanol, formaldehyde, and acetic acid, 8:1:1). The AFE fixative was removed by pipetting and samples were washed 5 times with water. Afterwards, pistils were incubated in 5 N NaOH until they were transparent. More than 24 h later, the pistils were washed 15 times in water. Finally, the complex carbohydrate “callose” (β -1,3-glucan) produced by mature pollen tubes was stained by decolorized aniline blue overnight in a dark room. Afterwards, pistils were cleaned 5 times in water, put in glycerin on a slide, and slightly squashed under a covering slip. Preparations were observed with UV- or blue-excitation under a fluorescence microscope (Leitz Diapland & Leitz Wetzlar, Germany). The hand-pollinated

flowers, which were not collected for the pollen tube growth experiment (around 10 flowers), remained covered until the end of the flowering period in order to check their fruit production after self- or cross-pollinations.

3. Results

Three *Veronica* species from 3 different subgenera (*Veronica* subgenus *Pocilla*, subgenus *Beccabunga*, and subgenus *Pentasepalae*) were tested by crossing and pollen tube growth experiments (Figure 1). The morphology of *Veronica* pistils is relatively simple and structured by a capitate stigma with papillary cells at the top, a long style including 2 xylem tubes, and an ovary with 2 locules (Figure 1, negative control of *V. filiformis*). The positive controls (cross-pollination) demonstrate that the pollen grains (Figure 1) are producing pollen tubes growing into the style (yellow coloring in Figure 1) and further to the ovules in the ovary (24 h after pollination). At the entrance of the ovary, the pollen tubes split in 2 different directions corresponding to the 2 locules containing the ovules (Figure 1, positive control of *V. gentianoides*). This description is common to all 3 species tested. All controls produced normal seeds. Self-pollinated plants show pollen grains on the stigma (Figure 1), which also germinate to produce pollen tubes growing into the style (yellow coloring in Figure 1). However, the pollen tubes do not progress until the ovules (24 h after pollination) but are stopped before reaching the ovary in *V. filiformis* (Figure 1) and *V. gentianoides* (Figure 1). This is not the case for *V. teucrium* (Figure 1), where self-pollen tubes can enter the ovary. This latter species, *V. teucrium*, was also the single species of our 3 *Veronica* examples to produce capsules and normal seeds after self-pollination (Figure 2).

4. Discussion

The high conservation of the *Veronica* floral form characterized by 4 blue-lilac to pink or white petal lobes, 2 stamens, and only 1 style within the whole genus could be associated with a conservation of the genetic self-incompatibility system. Indeed, our crossings and pollen tube growth experiments gave cytological evidence for the gametophytic type of self-incompatibility in *Veronica gentianoides* (*Veronica* subg. *Beccabunga*; Figure 1) and in *Veronica filiformis* (*Veronica* subg. *Pocilla*; Figure 1). Our cytological observations confirmed, for the first time, the results of the crossing experiments conducted by Correns (1920) for *V. gentianoides* and by Lehmann (1944) for *V. filiformis*. Our results corroborated also the results by Scheerer (1937) about the fact that self-sterility in *V. teucrium* (*Veronica* subg. *Pentasepalae*; Figure 1) is facultative and only found in certain populations. Unfortunately, the population of *V. teucrium* investigated here is self-compatible. The presence of a GSI system in at least 2

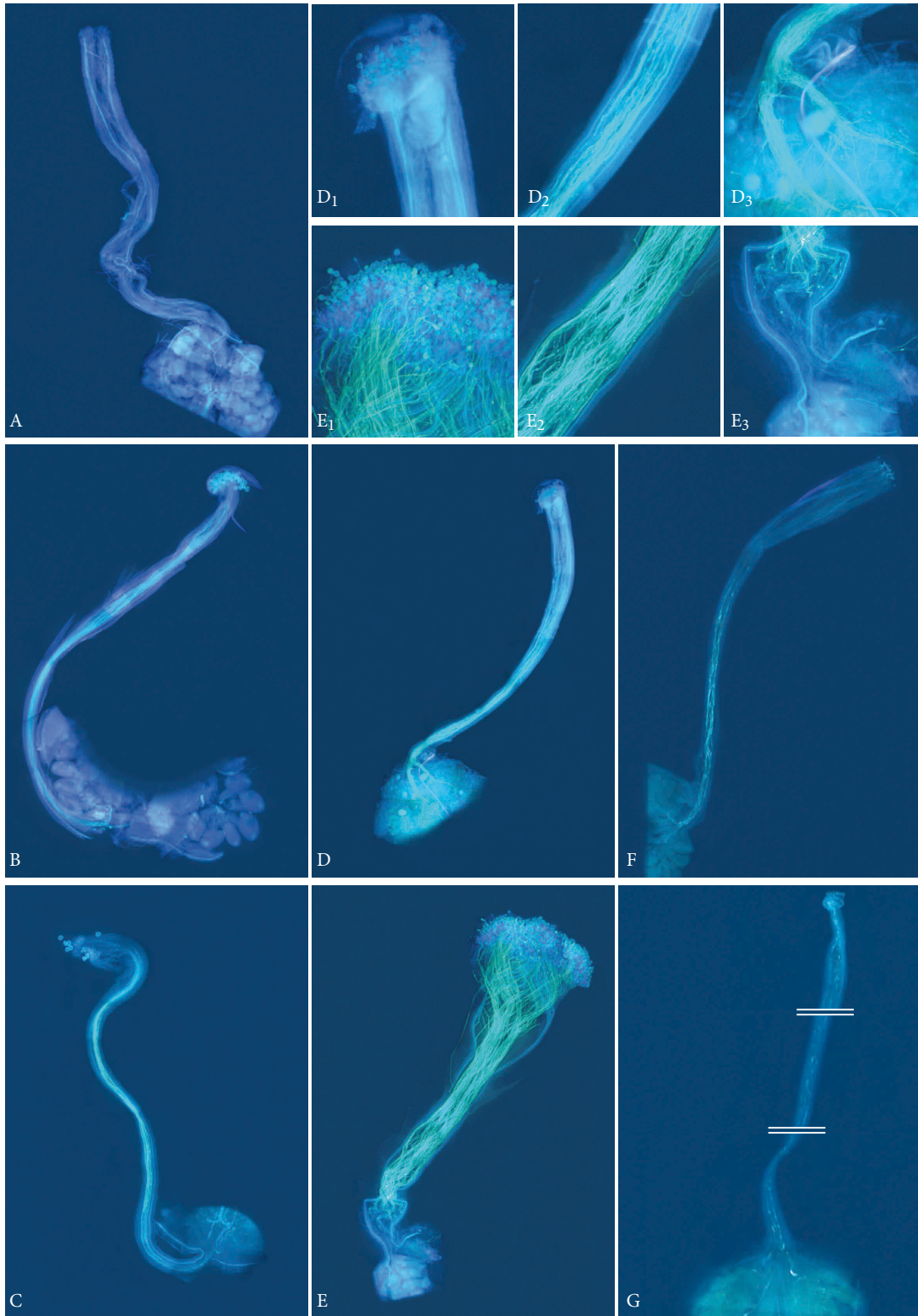


Figure 1. Results of the pollen tube growth experiments in *Veronica filiformis* (A, B, C), *V. gentianoides* (D, E), and *V. teucrium* (F, G). A- Virgin carpel of the negative control; B, D, and F- cross-pollinated carpels of *Veronica filiformis*, *V. gentianoides*, and *V. teucrium* as corresponding to positive controls; C, E, and G- self-pollinated carpels of *V. filiformis*, *V. gentianoides*, and *V. teucrium*, respectively; D1, D2, D3, E1, E2, and E3- enlargements of the pistils, the style, and the entrance of ovaries for the cross- and self-pollinated carpels of *V. gentianoides*.



Figure 2. Capsule production after self-pollination in *Veronica teucrium*. The black arrows indicate the position of capsules on the stem.

different subgenera (*Pocilla* and *Beccabunga*) of the genus *Veronica* could suggest that the GSI system is conserved within the genus. Moreover, the *Veronica* GSI system could be homologous to the GSI system of *Antirrhinum*, another Plantaginaceae genus, and possibly across higher eudicots (Vieira et al., 2008). More crossing experiments should investigate the extent of self-incompatibility in the genus and related genera. However, genetic analyses will be necessary to determine the molecular mechanisms of the *Veronica* GSI and to investigate whether *Veronica* shares the

same S-RNase-based self-incompatibility with *Antirrhinum* (Plantaginaceae), Solanaceae, or Rosaceae (Vieira et al., 2008).

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