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WA and AW developed
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ORIGINAL RESEARCH PAPER

The assessment of incompatibility and cross-compatibility in three populations of European globeflower (*Trollius europaeus* L.) by observing the pollen tubes growth

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Abstract

The European globeflower was shown to be highly self-incompatible by flower bagging experiments, however, a very small degree of selfing was observed in natural populations. The present study is an attempt to evaluate the level of self-incompatibility (SI) in three *Trollius europaeus* populations from different sites of Poland and to assess a degree of cross-compatibility (CC) between these populations. The SI and CC of the globeflower populations were evaluated based on the pollen germination index (PGI). The observations of pollen grains germination and pollen tubes penetration were made in pistils after self- and diallel cross-pollination of globeflower plants. The pollination combinations which had the PGI equal or higher than 2 were regarded as compatible. Generally, the PGI after self-pollination of all globeflower population was over 2, indicating that they are self-compatible. Also, in two globeflower populations after cross-pollination the PGI was higher than 2 showing that there was cross-compatibility between plants of these two populations. However, the third population, from Biedrusko, was fully cross-incompatible.

Keywords

globeflower; *Trollius europaeus*; self-incompatibility; incompatibility; pollen tubes

Introduction

According to East [1], the number of self-incompatible species among flowering plants is amounted to at least 3000. This number was also confirmed by Brewbaker [2]. Based on his observations concerning 600 genera, he concluded that self-incompatibility was present in 71 families from over 250 genera. The globeflower (*Trollius europaeus*) belongs to *Trollius* genus comprising about 30 plant species in the family Ranunculaceae. It is native species to the cool temperate regions of the Northern Hemisphere, with the greatest diversity of species in Asia. Some data existing in the literature [3–5] suggest that seeds of globeflower are set mostly by cross-pollination, but under some environmental conditions, e.g., long-lasting wet weather, the seeds can be formed also by self-pollination [6,7]. Gametophytic self-incompatibility system exists in the Ranunculaceae family and from data received by Lundqvist et al. [8] it is suggested that three or even four S-loci are governing self-incompatibility.

The objective of this work was to evaluate the level of self-incompatibility in three globeflower populations from different sites in Poland. The degree of cross-ability between populations was also investigated.



Fig. 1 Localization of the studied globeflower (*Trollius europaeus*) populations in Poland.

Tab. 1 The origin of *T. europaeus* populations used in this study.

Site	Region of Poland	Geographical coordinates	
		latitude	longitude
Uścikowo	Greater Poland	N 52°38'36"	E 16°45'20"
Biedrusko	Greater Poland	N 52°32'31"	E 16°56'39"
Łobżenica	Greater Poland	N 53°15'59"	E 17°15'10"

Material and methods

The plant material used for this study consisted of pistils after self- and diallel cross-pollination of globeflower (*Trollius europaeus* L. ssp. *europaeus*) plants from three populations located in Greater Poland, i.e., Biedrusko (B), Łobżenica (Ł), and Uścikowo (U) (Fig. 1, Tab. 1). According to the results obtained by Antkowiak [9], the globeflower plants from two localizations, Łobżenica and Uścikowo, showed interpopulation variability concerning 15 examined features. Particularly, significant differences the above-mentioned author observed with regard to the average number of follicles in multi-follicle, i.e., 43.1 and 54.4, respectively. The pollination was carried out in natural conditions. The pollen was placed on stigmas 2 days after emasculation, which was done at floral bud stage. Pistils for the microscopic observations were collected 6, 24, and 48 hours after pollination. They were fixed in the solution of ethyl alcohol : chloroform : acetic acid (6:3:1) for 24 h and stored in 70% ethanol in the refrigerator until use. Maceration was done in 1 N NaOH at 60°C for 2.5 h. The macerated pistils were stained in aniline blue according to Martin [10] with modification of Antkowiak and Wojciechowski [11]. For this purpose, pistils were rinsed three times with distilled water and submerged in a solution of 0.1% aniline blue for 24 h. To visualize the pollen tubes, each pistil was deposited on a glass slide with a drop of glycerin and covered with a glass cover slip. Ten pistils from five flowers out of each hybridization–time combination (Tab. 2) were examined. Pollen grain germination and pollen tube growth were observed using UV-light fluorescence microscope.

Crossability of the globeflower populations was evaluated on the base of pollen germination index (PGI) according to Matsuzawa [12] with modification of Antkowiak and Wojciechowski [11]:

$$PGI = (b + 2c + 3d + 4e + 5f) / (a + b + c + d + e + f)$$

where: *a* – number of pistils without pollen grains; *b* – number of pistils on which pollen grains do not germinate; *c* – number of pistils in which pollen grains germinate on stigmas; *d* – number of pistils in which pollen tubes enter the style tissue; *e* – number of pistils in which pollen tubes penetrate ovary; *f* – number of pistils in which pollen tubes penetrate near to or enter the ovules. The pollination combinations which had the PGI equal to or higher than 2 were regarded as compatible.

Results

In three populations of globeflower (Biedrusko, Łobżenica, Uścikowo) germination of pollen grains on stigmas was already observed 6 h after pollination. Twenty-four hours after pollination, the pollen tubes reached the ovary in most combinations of pollination, and 48 h after pollination pollen tubes entering the ovules were observed (Fig. 2c,d). The only exception was the Biedrusko globeflower population. In this case, only after self-pollination pollen tubes were visible in the ovary 24 h after pollination, (Fig. 2b), and after 48 h they were entering the ovules (Fig. 2e). But in the remaining cross-combinations, pollen tubes reached the ovary 48 h after pollination and some pistils showed the lack of pollen grain germination on the stigma (Fig. 2a) or only short tubes on the stigma (Fig. 2f,g).

The PGI after self-pollination of all globeflower populations was greater than 2 (Tab. 2), pointing that they were self-compatible. Also, in two globeflower populations, Łobżenica and Uścikowo, after cross-pollination the PGI was higher than 2, showing

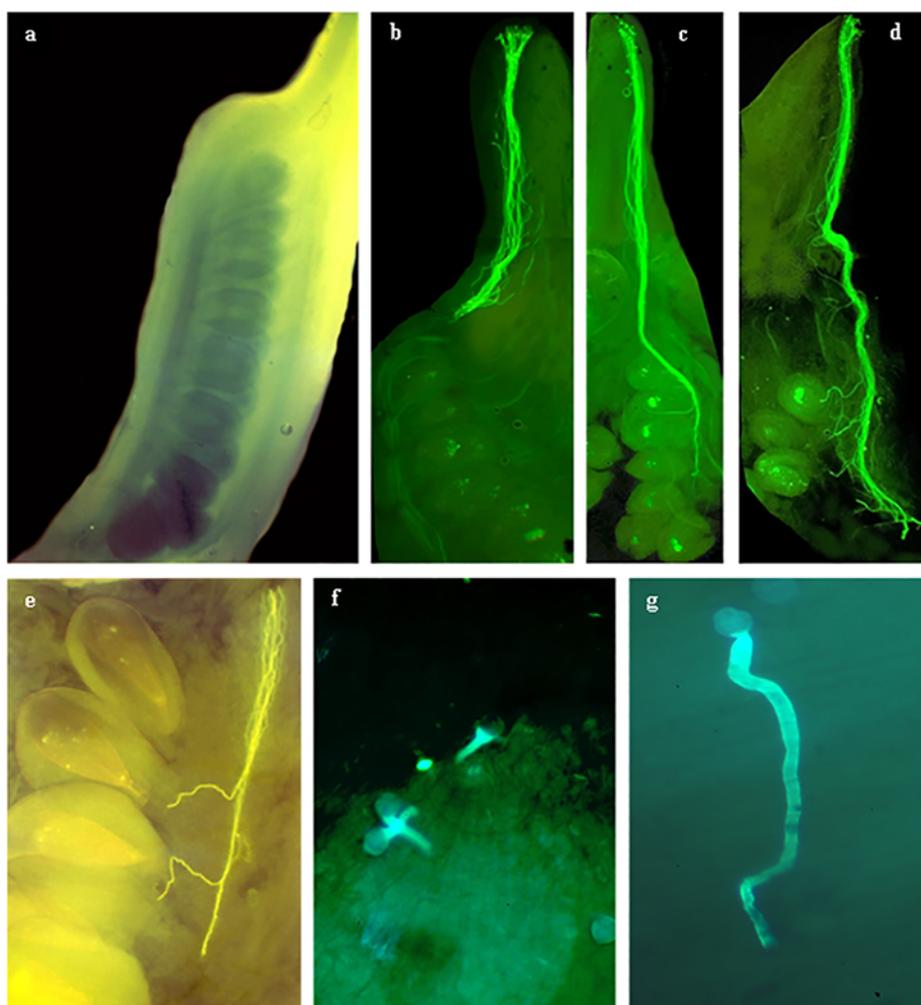


Fig. 2 The European globeflower (*T. europaeus*) pistils with ovules and pollen tubes penetrating different parts of pistil. **a** The globeflower pistil with 14 ovules visible in the ovary. **b–e** Germinating pollen tubes on the stigma and in the deeper parts of the pistil. **b** Pollen tubes on the stigma and in the style, close to ovary, 24 h after globeflower self-pollination in Biedrusko. **c** Pollen tubes in the ovary and entering into the ovule of a globeflower from Łobżenica, 48 h after self-pollination. **d** Pollen tubes in the ovary and entering into the ovule of a globeflower from Uścikowo, 48 h after cross-pollination with pollen of a globeflower from Biedrusko. **e** Ovary of a globeflower from Biedrusko with visible pollen tubes entering into ovules, 48 h after self-pollination. **f,g** short pollen tubes on the stigma of a globeflower from Biedrusko, 48 h after cross-pollination with pollen of globeflower from Uścikowo.

that there was cross-compatibility between plants of these two populations. The third population, Biedrusko, was fully cross-incompatible; the PGI ranged from 0.6 to 1.5 (Tab. 2).

Discussion

In many flowering plant species, flowers have close relationships with one or a few specific pollinating organisms. Some plants make use of multiple vectors, but many are highly specialized. The globose-shape flowers of *T. europaeus* are tightly closed and, according to the observations of Jager and Despres [5], the most effective pollinators for globeflower are six species of *Chiastocheta* (fly) and in this case plant–animal mutualism is observed [13]. In globeflower – globeflower fly interactions, pollination is achieved mainly passively [14], therefore both ovipositing females and males are effective pollinators [15,16]. These observations are confirmed by Denisow and Żuraw [17], who investigated four *Trollius* species and concluded that only *T. europaeus* flowers

Tab. 2 Pollen germination index (PGI) in diallel crosses of the European globeflower (*Trollius europaeus* L.) from three populations – Biedrusko, Łobżenica, Uścikowo and after their self- and cross-pollinations.

Combination		PGI
♀	♂	
Biedrusko	Self-pollination	2.5
Biedrusko	Cross-pollination	0.9
Biedrusko	Łobżenica	1.5
Biedrusko	Uścikowo	0.6
Łobżenica	Self-pollination	3.0
Łobżenica	Cross-pollination	2.9
Łobżenica	Biedrusko	3.2
Łobżenica	Uścikowo	3.0
Uścikowo	Self-pollination	2.5
Uścikowo	Cross-pollination	3.5
Uścikowo	Biedrusko	3.5
Uścikowo	Łobżenica	2.4

T. europaeus [5]. Within the genus *Ranunculus*, self-incompatibility has been reported for several European species, such as *R. acris* L. and *R. bulbosus* L. [8], but other European species may be self-compatible or apomictic [21]. According to recent literature data [22], four species in the group of *R. alpestris* are self-incompatible and obligate outcrossers, only *R. crenatus* and *R. magellensis* are partly self-fertile. It is known that some New Zealand species of *Ranunculus* appear to be fully self-compatible [23].

Similarly, based on our results obtained from observations of pollen tubes growth, all globeflower plants from three studied sites showed PGI higher than 2.0, which means that they are self-compatible. Cross-pollinations of sister plants from the same localization showed full cross-compatibility, but only in two localizations, i.e., Łobżenica and Uścikowo. Diallel cross-pollinations of globeflower plants from three analyzed localizations resulted in full cross-compatibility in most studied combinations. Quite opposite data were obtained in the case of Biedrusko population, where all plants were cross-incompatible, irrespective of whether the source of the pollen was the sister plants or plants from two other localizations.

According to the literature concerning crossability within four possible combinations of crosses between self-incompatible (SI) and compatible (SC) forms, pollen does not germinate only when SI constitutes the maternal form. In such crosses, pollen is probably inhibited similarly as in self-incompatible pollination. In the remaining three types of crosses, i.e., SC × SI, SC × SC, SI × SI pollen germinates normally. Lewis and Crowe [24] regarded such pollen behavior as normal and being a rule.

Inability to germinate pollen of self-compatible plants on stigmas of self-incompatible plants (SI × SC) is described as a unilateral interspecific incompatibility. Röbbelen [25], Sampson [26], and Wojciechowski [27], who studied crossability between species within Cruciferae obtained the results indicating that unilateral compatibility occurs also in the case of two self-incompatible forms (SI × SI). The results obtained in our work indicate a broader range of unilateral incompatibility, which cannot be confined to the cases given by the authors cited above. The globeflower plants used in the experiments were self-compatible (SC). Unilateral inhibition of the pollen tube growth in the cross Biedrusko globeflower (maternal form) × Łobżenica and Uścikowo globeflower proves that unilateral incompatibility exists also when two self-compatible plants (SC × SC) are crossed. Similar incompatibility was found by Wojciechowski and Andrzejewski

were sporadically visited by such insects like bumblebee and honeybee, which very often visited flowers of the three other observed species. These views on effective pollinators in globeflower are also confirmed by the data obtained by Suchan et al. [18], who observed the interactions between *Trollius* and *Chiastocheta*. These authors report that among groups of insects which visited the flowers of globeflower were *Chiastocheta* (54%), Staphylinidae (Omaliinae; 21%), Mordellidae (10%), Oedemeridae (*Oedemera* genus; 0.7%), and some Hymenoptera (together 4%). They also add that existing *Chiastocheta* had significant positive effect both on the seed set and net seed set, but negative effect on the seed germination rate.

Habitat patchiness and the variation of weather conditions are likely to induce variability in *Chiastocheta* activity. This could lead to selection of self-compatibility and strategies to attract other pollinators when *Chiastocheta* provide unpredictable or inadequate service [5]. Studies on other plant species suggest that a strong limitation of reproductive success due to poor or variable pollination activity can result in selection for self-compatibility [19,20] and self-pollination was reported in

[28], who crossed different SC cultivars of *Lycopersicon esculentum* with *Capsicum annuum*. It seems that differences between plant species with regard to pollination may result from environmental conditions under which the experiments were carried out. Numerous data from literature indicate that environmental factors and especially temperature can affect the level of incompatibility [29,30]. Determination whether unilateral incompatibility occurs in the crosses between SC globeflower plants, as can be concluded based on the observations of the pollen tube growth, will be possible to estimate after evaluating this trait based on the seed set. According to Hermsen and Ramanna [31], evaluation of the compatibility based of the pollen tubes growth intensity cannot always be confirmed by the number of developed seeds. This problem will be the subject of our further research.

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