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The Determination of Self-Compabilitiy Status of Thermopsis turcica Through Histological Analysis

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Abstract

Thermopsis turcica Kit Tan, Vural & Kucukoduk (*Fabaceae*) is a rare and endemic Turkish species grown around Aksehir Lake and Eber Lake of the southern areas of Central Anatolia. *T. turcica* has a gynoecium of 2-4 functional carpels. This characteristic of *T. turcica* is unique in the subfamily *Papilioideae* of *Fabaceae.* Although this genotype is valuable as female plants, to date there is no consideration given to the fertilization biology of *T. turcica*. In the present study, 2 populations of species *T. turcica* (Eber and Aksehir populations) were used. Selfing and reciprocal crosses between two populations of *T. turcica* were performed at Nezahat Gokyigit Botanical Garden during the pollination period between May and June 2012. Pistil samples were collected from the 1th to 10th day of the pollination without damaging the population. All pistils collected were fixed in FPA-70 solution and stored at +4 °C until microscopic observations. Pistil samples were stained with aniline blue. After staining, pistils were cut into two parts (stigma with style and ovary) and were further cut longitudinally, split into two parts. All samples were observed under a fluorescent microscope. In all third day-old samples of *T. turcica* (both of self- and reciprocal crossed samples), ovule fertilization was observed. Based on histological analysis's results, all samples of *T. turcica* have been clearly identified as self-compatible.

Keywords: Hybridization, pollen grain germination, self-compatible, *Thermopsis turcica*

INTRODUCTION

The *Fabaceae* is the third largest family with 750 genera and more than 19.000 species [1]**.** In Turkey, the family of *Fabaceae* with 28 genera and 400 endemic species ranks second in terms of the rate of endemism after that of *Astaraceae* (446 endemic species) [2, 3]. *Thermopsis turcica* belongs to family of *Fabaceae* and appears to be the only endemic deputative of the genus *Thermopsis* in Turkey. Although the vast majority of *Fabaceae* family has generally unicarpellate gynoecium, *T. turcica* is distinguished from all other species of the subfamily *Papilioideae* (=*Faboideae*) of *Fabaceae* by having a multicarpellate gynoecium, each with 3-4 completely formed pistils (Fig.1) [4-7].

According to a number of previous studies, it is known that the multicarpellary can be seen rarely in legumes [1, 8]. The first reports of multicarpellate gynoecia in legumes were published by Wolpers [9] and Baillon [10]. Multicarpellate gynoecium with 2-4 functional pistils per flower has often been found in the subfamily *Mimosoideae* of *Fabaceae* [11, 12]. Although *T. turcica* is unique in terms of its genetic characteristic, to date no consideration has been given to the biology of its fertilization according to a literature review.

In the present study, we analyze *T. turcica* with multicarpellate gynoecium with the aim of learning its fertilization biology. The study combines: (1) self-pollination of two populations of *T. turcica* (Eber and Aksehir) and (2) reciprocal crossing of Eber and Aksehir populations.

Figure 1. Multicarpellate gynoecium of *T. turcica*

MATERIALS AND METHODS

Plant material

In the present study, 2 populations (Eber and Aksehir populations) of *T. turcica* were used. For understanding whether gene transfer is possible between Eber and Aksehir populations of *T. turcica*, crosses were carried out with classical hybridization. For the crossing program, selfing and reciprocal crosses between Eber and Aksehir populations of *T. turcica* were implemented during the pollination period of May and June 2012 at Nezahat Gokyigit Botanical Garden (NGBB) of Istanbul. To protect the pollinated flowers from rainy weather and other contaminants, the 30 m^2 planted area was covered with a plastic material.

Pollinations

Pollination and histological analysis procedures were conducted as described by Eti [13]. To obtain fresh pollen, at least 60 flowers were collected immediately before anthesis from *T. turcica* of each population (Eber and Aksehir populations), and their petals and pistils were removed. The anthers were left to dehisce for 24h at room temperature of about 25°C. Fresh pollen was used for pollination. One day before anthesis, 200 flowers of each population of *T. turcica* were emasculated at the preanthesis stage, hand pollinated (100 flowers per treatment) with a paintbrush and covered with a cotton bag to prevent from any other pollen or damaging material.

Histological Analysis

Six pistils from each of self- and reciprocal- pollinated species were collected from the first to tenth day after pollination (DAP) to perform pollination possibilities. All pistils collected were fixed in FPA-70 (900 ml ethanol 70%, 50 ml formaldehyde, 50 ml pyropyonicacid) solution and stored at +4 °C until microscopic observations [13]. In addition, to determine embryo formation, paraffin block analysis was implemented. During the histological analysis of paraffin step, all samples were stained with hematoxylin dye and then observed under a fluorescent microscope.

Fluorescence Microscopy Analysis

To observe pollen tube growth, pollinated pistils fixed in FPA-70 solution were washed with tap water for a night and then left in 8N NaOH for 3 hours. Pistils were washed to remove NaOH from the softened tissue overnight and stained with aniline blue (0.1% aniline blue in 0.1N K_3PO^4) [14-15]. After staining, pistils were cut into two parts (stigma with style and ovary) and were further cut longitudinally, splitting them into two parts. Pollen tube growth was monitored. All samples were observed under a fluorescent microscope (Olympus BX51-DP72). To identify embryo formation, paraffin block analysis was implemented [13, 16].

RESULTS AND DISCUSSION

To identify pollen tube growth, both self-pollinated and reciprocal crossed samples were analysed and monitored. As a result, in all samples of 1 DAP, pollen tubes reached the base of the style (Fig. 2).

In all 4 DAP samples of *T. turcica* (both of self pollinated and reciprocal crossed samples), pollen grain germination was observed (Fig. 3)

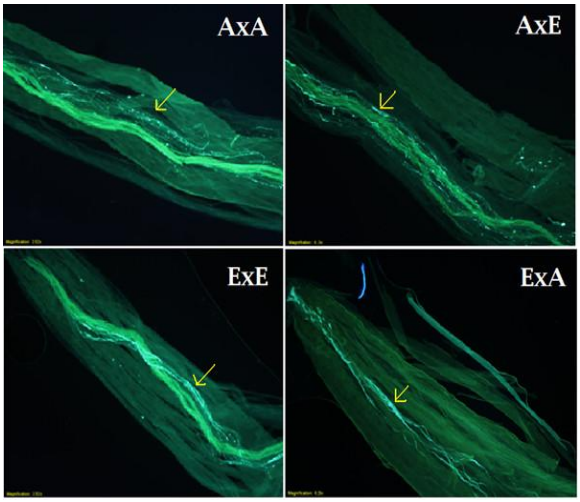

Figure 2. In all 1 DAP samples, pollen tube growth was observed (A: *T. turcica*-Aksehir population; E: *T. turcica*-Eber population; arrows: pollen tube) (Magnification: 6.3x)

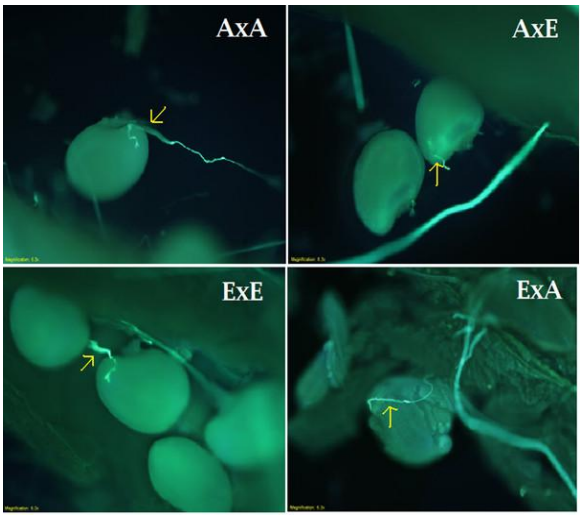

Figure 3. The status of pollen grain in all 4 DAP samples of hand-pollinated flowers (A: Aksehir population of *T. turcica*; E: Eber population of *T. turcica*; arrows: pollen grain) (Magnification: 6.3x)

When ovules of all hybridization combinations were dissected, globular embryo formation was captured in all samples (self pollinated and reciprocal crossed samples of *T. turcica*) at the eighth day of pollination (Fig.4).

As a consequence of the results of the histological analysis, we report for the first time that all samples of *T. turcica* have been clearly identified as self-compatible. According to outcomes of this research, we can indicate that self pollen or non-self pollen obtained from other population of *T. turcica* were accepted by the pistil of this endemic species. Reciprocal crossess between two populations of T. *turcica* maintains the genetic inheritance within the species.

The research was conducted to understand the self compatibility properties of *T. turcica*. Although few studies have been conducted on *T. turcica*, to date no study on hybridization exists in understanding whether it is self compatible or self incompatible.

We know that the female parent in the crossing program is very important for the success of the cross [17]. To evaluate the effect of the male and female parent on the cross, each population (Eber and Aksehir populations of *T. turcica*) was used as a male donor and also a female recipient in the crossing program.

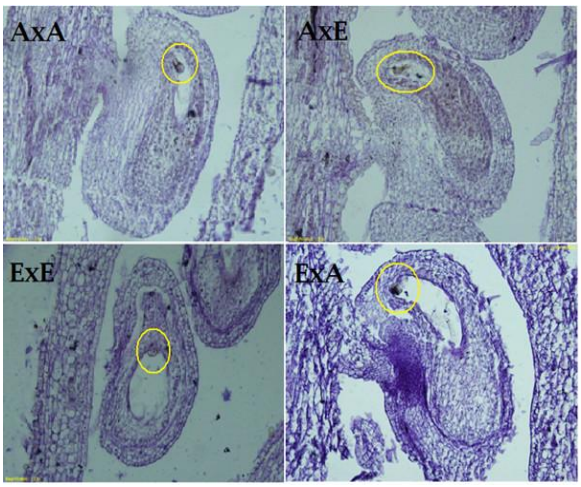

Figure 4. Globular embryo formation in self- and cross-pollinated samples of *T. turcica* (A: Aksehir population of *T. turcica*; E: Eber population of *T. turcica*; circulars: globular embryo) (Magnification: 12.6x)

According to pollen tube growth observations, a high difference between the reciprocal crosses and variations of the male-female or female-male combinations were not found. Variation on pollen performance depending on male-female interaction and genotype has been previously reported in other works [18-20].

In the present study, since flowers at balloon stage for hybridization were used in all combinations, stigma and style length were the same. The distance for pollen travelling was not significantly different. Thus, as a female receptor, neither Eber nor Aksehir effected pollen performance. Furthermore, as a male donor, flowers at balloon stage were selected in all combinations; thus, no variance in pollen viability was observed. Overall, comparisons of Eber and Aksehir as both female recipient and male donor, showed more or less the same features in the hybridization study. In some species (e.g. *Trifolium* spp.: Atwood [21]; *Butea* spp.: Tandon et al. [22]; *Erythrina* spp.; Etcheverry and Aleman [23]) after hybridization, a self-incompatibility reaction was observed.

To understand whether embryo formation occured in all combinations, paraffin block analysis was implemented; in all samples at the 8th day after pollination, embryos at an early age stage were observed. Thus, based on this result, crossing between the populations of *T. turcica* were successful and not affiliated with the genotype of *T. turcica*. In addition, this result confirmed that the late acting self-incompatibility of *T. turcica* was not possible due to embryo formation.

In our study, we aimed for a thorough understanding of the self compatibility status of *T. turcica* and did not obtain hybrids plants in limited attempts between the Eber and Aksehir plants.

CONCLUSION

Our results show that in the crossing program between the populations, there is no strong effect on male-female interaction. The present study has opened up avenues to cross this species with other members of *Fabaceae*. Intergeneric crosses of *T. turcica* may be a useful source for the introduction of genetic traits regarding with multicarpellate flowers for new cultivar development and crop improvement. In addition, further studies should be conducted to generate genetic diversity within *Fabaceae* by hybridization of *T. turcica* with other species within the family.

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