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EVALUATION OF SELF-(IN)COMPATIBILITY IN THE ALMOND (*PRUNUS AMYGDALUS* BATSCH) GENOTYPE POPULATION FROM THE SLANKAMEN HILL, SERBIA

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Abstract - Due to the importance of obtaining almond cultivars adapted to the agroecological conditions of Serbia, in the period 2005-2006 pollen viability and self-(in)compatibility in 19 almond genotypes selected from the seedling population on Slankamen hill, were studied. All analyzed almond genotypes had good (50-70%) or high (over 70%) pollen germination. The study of self-(in)compatibility was done by monitoring of the fruit set in the field and observing self-pollen growth by fluorescence microscopy. Self-incompatibility was confirmed in all the 19 genotypes by both methods. Pollen tube penetration was stopped mostly at the upper third of the style of all genotypes, with characteristic irregularities.

Key words: Almond, pollen tube growth, fluorescent microscopy

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INTRODUCTION

Self-incompatibility is a widely spread and heritable reproductive phenomenon in flowering plants, where self-fertilization is prevented by rejection of pollen from the same plant. This is an evolutionary advantage due to its effectiveness in avoiding inbreeding and the encouragement of outcrossing. *Prunus* species, thereby the almond as well, are characterized by a gametophytic type of self-incompatibility, which means there is no pollen germination on the stigma (Yamashita et al., 1987), or tube growth stops most often in the upper third of the style (Sanchez et al., 2004). Gagnard (1954, cited by Boskovic et al., 2003) was the first to explain that self-incompatibility in the almond (*Prunus amygdalus* Batsch) is controlled by a single multi-allelic *S*-locus. *S*-locus in the almond shows a high diversity with more than 37 *S* alleles established so far (Ortega et al., 2006; Halász et al., 2008; Kodad et al., 2008, 2010). These alleles encode specific ribonucleases in the style (Tao et al., 1997; Bošković et al., 2003), which are responsible for inactivating the growth of self-pollen tubes. Also, 25 cross-almond incompatibility groups have been established so far (Kodad and Socias i Company, 2009). In self-compatible genotypes S_f allele is present in the pistil (Bošković et al., 2003). However, the latest studies by Fernández i Martí et al. (2009) and Kodad et al. (2009) indicate that this mechanism of self-compatibility is much more complex.

The most frequently used method to determine self-(in)compatibility is the monitoring of the fruit set after the self-pollination of isolated flowers. Observation of pollen tube growth by fluorescence microscopy is also often used as a fast and reliable method. Similar to many plants, almond has pollen tubes that contain a considerable amount of callose that has the ability to absorb aniline blue and to produce fluorescence when illuminated with blue or UV light (Kho and Baër, 1968). This characteristic was used for observation of pollen tube growth in the style and ovary by fluorescence

microscopy in the almond (Ortega et al., 2002, 2006; Alonso and Socias I Company, 2005), sour cherry (Cerović, 1994), apricot (Milatović and Nikolić, 2007) and other species.

Self-(in)compatibility can be also identified by molecular methods based on the determination of ribonucleases (Certal et al., 2002; Bošković et al., 2003) and DNA amplification and identification by PCR analysis (López et al., 2004; Sánchez-Pérez et al., 2004; Ortega et al., 2005).

The objective of the study was to analyze pollen quality (germination) as a factor that directly affects fertilization as well as to establish the self-(in)compatibility of selected almond genotypes.

MATERIALS AND METHODS

Research was realized in the period 2005 – 2006. The applied method for pollen quality was *in vitro* germination on a substrate containing 15% sucrose (Eti et al., 1994) and 1% agar-agar. To collect the pollen, unopened flowers at late balloon stage were taken. Pollen germination was determined after incubation (24 h at room temperature). Approximately 250 pollen grains per genotype were analyzed. A germinated pollen grain was considered to be the one whose pollen tube was longer than the diameter of the pollen grain itself.

For fruit set monitoring shoots were randomly selected. All open flowers and closed buds were removed from the shoots. Emasculation and self-pollination was applied for a total of 100 flowers at the late balloon stage. To avoid possible open pollination, the shoots were bagged. Fruits were counted after 30 days and at ripening time.

To observe pollen tube growth the shoots with flowers at late balloon stage were taken and brought to the laboratory. Flowers were emasculated and self-pollinated after 48 h. Fixation of the pistils was done 72 h after pollination (Ortega et al., 2002) with FAA fixative (70% ethanol, glacial acetic acid and formaldehyde in a ratio 90:5:5). Just before microscopic observation, the pistils were stained for 24 h with 0.1N K_3PO_4 containing dissolved 0.1% aniline blue that react with callose from the pollen tube walls. To prepare the pistils for microscopic observation, the style was separated from the ovary and squashed, while the ovary was dissected longitudinally to detect the possible penetration of the pollen tube into the ovule (Cerović, 1994). Pollen tube growth was monitored on a "LEICA" microscope. At least 20-25 pistils were analyzed from each genotype.

Statistical analysis was performed with Statistica (StatSoft, Inc., Tulsa, Oklahoma, USA) program. Using a model of monofactorial experiment, where the year was taken as replication analysis of variance of random block system was done. The significance of the differences was tested by LSD-test at a probability of 1%.

RESULTS AND DISCUSSION

A knowledge of pollen functional ability is very important, because sterility and poor fertilization can be explained by pollen low viability. Pollen germination depends on a wide range of factors: species, cultivar, nutrition and environment factors. According to Imani and Talaie (2006), almond pollen fertility is positively affected by calcium content. Analyzing the effects of temperature Weinbaum et al. (1984) found that maximum pollen germination is achieved at 16^0C.

All analyzed almond genotypes had good (50-70%) or high (over 70%) pollen germination (Table 1), which is a prerequisite for successful fertilization. The lowest mean of pollen germination in the study period was found in the genotype 25/03 (57.9%) and the highest in genotype 17/03 (78.8%).

The results of the analysis of variance (data not shown) indicated statistically very significant differences in pollen germination between the study years, which are in agreement with the results obtained by Eti et al. (1994) who tested the pollen germination of four almond cultivars by a pendant drop method. A significant variation between years

Table 1. Pollen germinability and fruit set during the years 2005 and 2006

Genotype	2005	2006	Average	Initial fruit set (%)		Final number of fruits (%)	
				2005	2006	2005	2006
1/03	46.3	85.4	75.3	45.0	50.1	0	0
10/03	27.0	74.5	63.3	47.4	57.7	0	0
11/03	54.1	81.0	68.7	48.2	36.2	0	0
12/03	42.7	62.4	65.4	53.9	59.5	0	0
14/03	56.6	62.3	71.4	38.0	35.6	0	0
15/03	46.5	51.1	63.7	47.8	56.7	0	0
16/03	27.1	61.9	58.1	39.3	53.8	0	0
17/03	58.7	80.5	78.8	43.8	49.3	0	0
18/03	29.3	78.0	61.7	45.0	62.4	0	0
19/03	22.7	70.4	62.8	37.2	64.0	0	0
22/03	32.9	76.6	62.1	38.6	41.8	0	0
23/03	24.6	76.0	61.6	32.2	30.2	0	0
24/03	41.6	75.5	66.0	43.0	51.1	0	0
25/03	28.6	67.5	57.9	18.9	16.9	0	0
27/03	60.6	80.4	78.2	47.8	55.0	0	0
28/03	56.0	80.2	74.5	64.2	57.8	0	0
29/03	29.6	87.7	68.1	32.9	33.3	0	0
A/04	49.7	77.5	69.8	25.0	29.5	0	0
B/04	59.7	88.6	78.7	54.6	57.9	0	0
Average	41.8b	74.6a	67.7	42.3	47.3	0	0

in pollen germination was also observed for the sweet cherry (Radičević et al., 2008) and other pome and stone fruit species (Stösser et al., 1996). No significant differences in pollen germination were found between the studied genotypes.

Monitoring of the fruit set 30 days after self-pollination showed that 17.8% to even 61.0% of the fruits were counted, depending on the genotype. Very significant statistical differences were established for the initial set fruits both between the studied genotypes and the study years, which is in accordance with previous reports by Kodad and Socias I Company (2008). However, at ripening time all fruits were dropped from the marked shoots. This indicates that the presence of pollen tubes in the style initiated auxin activity and ethylene synthesis which stimulated the ovary growth (Ketsa et al., 2006).

After pollen adhesion on the stigmatic surface the pollen grains germinate. During growth, the pollen tubes compete for space and nutrients and only a genetically compatible pollen grain can reach the base of the ovary and penetrate the ovule. In self-compatible cultivars pollen tubes reach the base of the style in 72 h (Dicenta et al., 2002; Ortega et al., 2002), while fertilization takes place seven days after pollination (Cousin and Maataoui, 1998).

Observation of pollen tube growth by fluorescence microscopy (Table 2) is shown only for the year 2006. Monitoring clearly showed germinated pollen grains on the stigma, as well as pollen tubes

Table 2. Pollen tube growth 72 hours after self-pollination

Genotype	Number of analyzed pistils	Reached level			
		Under stigma	Upper 1/4	Upper 1/3	Base of style
1/03	20	75	15	10	0
10/03	20	70	20	10	0
11/03	22	72	18	10	0
12/03	23	74	22	4	0
14/03	20	90	10	0	0
15/03	20	80	15	5	0
16/03	20	80	20	0	0
17/03	24	75	19	6	0
18/03	21	72	22	6	0
19/03	21	81	10	9	0
22/03	20	79	13	8	0
23/03	20	76	22	2	0
24/03	25	70	18	12	0
25/03	25	85	10	5	0
27/03	20	74	14	12	0
28/03	21	75	17	8	0
29/03	22	71	16	13	0
A/04	22	81	19	0	0
B/04	23	65	22	13	0
Average		76.1	16.9	7.0	0.0

in the different levels of style. A significant reduction in the number of pollen tubes from the stigma to the base of the style was noticeable in all genotypes. In most cases (70-90% of pistils) it was observed that the pollen tubes stopped growing on the stigma or just below it, which confirms the opinion of Pimienta et al. (1983) that the stigma plays an important role in the delay and reduction of pollen germination. In a considerably lower number of pistils (10-22%) tube growth stopped in the upper quarter of the style. The least number of pistils (0-13%) with pollen tubes reached the first third quarter of the style. No pollen tube was observed at the base of the style which confirms the findings reported by Pimienta et al. (1983) and Socias I Company and Alonso (2004) for the self-pollination of Nonpareil and Ferragnes and Ferralise, respectively. In accordance with the gametophytic type of self-incompatibility in the almond (Sanchez et al., 2004) for most studied genotypes loops, a swelling of the tips and twisting of pollen tubes were observed (Fig. 1)

Studies of self-(in)compatibility in the almond genotypes selected from the population on Slankamen hill by monitoring pollen tube growth by fluorescence microscopy, as well as by self-pollination under the field conditions, confirmed the self-incompatibility of all genotypes. Successful growing of these genotypes requires studies of both cross and compatibility with commercially grown almond cultivars.

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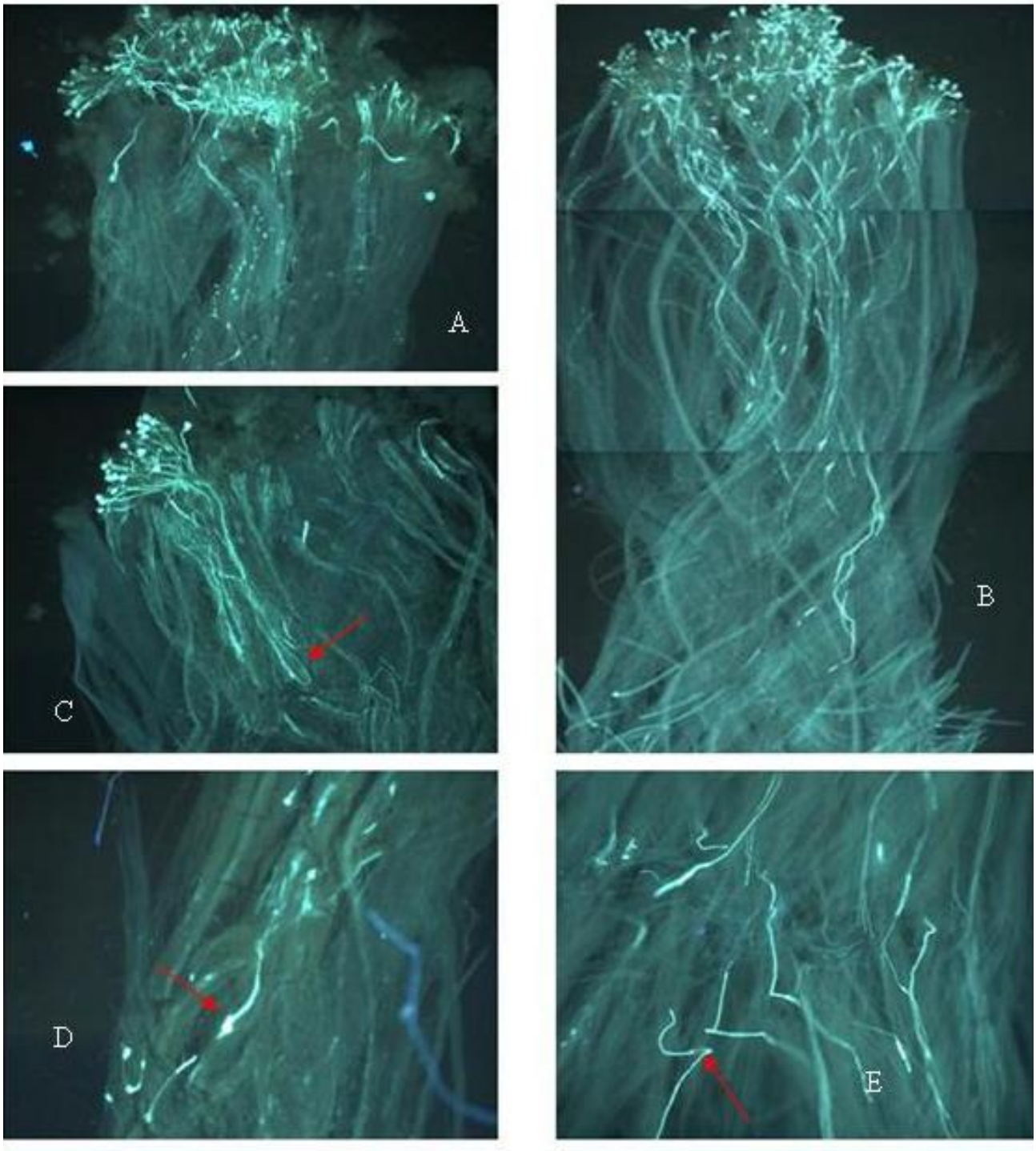

Fig 1. A – germination of pollen grains on stigma. B – pollen tube reached the upper third quarter of the pistil, 72 h after pollination. C – an incompatible pollen tube with loop; D – incompatible pollen tube with a broadened tip; E – twisting of an incompatible pollen tube.

REFERENCES

Bošković, R., Tobutt, K. R., Batlle, I., Duval, H., Martinez-Gomez, P. and *T. M. Gradziel* (2003). Stylar ribonucleases in almond: correlation with and prediction of incompatibility genotypes. *Plant Breeding*, **122**, 70-76.

Cerović, R. (1994). Histocitološki aspekti dinamike oplodnje kod višnje *(Prunus cerasus L.)*. Doktorska disertacija. Univerzitet u Beogradu, Biološki fakultet.

Cousin, M., and *M. El Maataoui* (1998). Female reproductive organs in self-compatible almond [*Prunus dulcis* (Miller) D.A. Webb] Laurannne and fertilization patterns. *Scientia Horticulturae*, **72**, 287-297.

Dicenta, F., Ortega, E., Cánovas, J. A. and *J. Egea* (2002). Self-pollination vs. cross-pollination in almond. Pollen tube growth, fruit set and fruit characteristics. *Plant Breeding*, **121**, 163-167.

Fernández I Martí, A., Hanada, T., Alonso, J. M., Yamane, H., Tao, R., and *R. Socias I Company* (2009). A modifier locus affecting the expression of the S-RNase gene could be the cause of breakdown of self-incompatibility in almond. *Sexual Plant Reproduction*, **22**, 179–186.

Halász, J., Fodor, Á., Hegedűs, A. and *A. Pedryc* (2008). Identification of a new self-incompatibility allele (S_{31}) in a Hungarian almond cultivar and its reliable detection. *Scientia Horticulturae*, **116**, 448-451.

Eti, S., Paydaş, S., Küden, A. B., Kaşka, N., Kurnaz, Ş. and *M. Ilgin* (1994). Investigations on the pollen viability, germination capability and the growth of pollen tubes on some selected almond types under Çukurova conditions. *Acta Horticulturae*, **373**, 225-233.

Imani, A. and *A. R. Talaie* (2006). A study on the relationship between pollen germinability and some of its elements in almond. *Acta Horticulturae*, **726**, 399-402.

Ketsa, S., Wisutiamonkul, A. and *W. G. Van Doorn* (2006). Auxin is required for pollination-induced ovary growth in *Dendrobium* orchids. *Functional Plant Biology*, **33**, 887-892.

Kho, Y. O. and J. Baër (1968). Observing pollen tubes by means of fluorescence. *Euphytica*, **17**, 298-302.

Kodad, O. and *R. Socias I Company* (2008). Fruit set evaluation for self-compatibility in almond. *Scientia Horticulturae*, **118**, 260-265.

Kodad, O., Sánchez, A., Saibo, N., Oliveira, M. M. and *R. Socias I Company* (2008). Identification and characterization of new S alleles associated with self-incompatibility in almond. *Plant Breeding*, **127**, 632–638.

Kodad, O., Socias I Company, R., Sánchez, A. and *M. M. Oliveira* (2009). The expression of self-compatibility in almond may not only be due to the presence of the S_f allele. *Journal of the American Society for Horticultural Science*, **134**, 221–227.

Kodad, O., Alonso, J. M., Fernández I Martí, A., Margarida Oliveira, M. and *R. Socias I Company* (2010). Molecular and physiological identification of new S-alleles associated with self-(in)compatibility in local Spanish almond cultivars. *Scientia Horticulturae*, **123**, 308-311.

López, M., Mnejja, M., Rovira, M., Collins G., Vargas, F. J., Arús, P. and *I. Batlle* (2004). Self-incompatibility genotypes in almond re-evaluated by PCR, stylar ribonucleases, sequencing analysis and controlled pollinations. *Theoretical & Applied Genetics*, **109**, 954-964.

Milatović, D. and *D. Nikolić* (2007). Analysis of self-(in)compatibility in apricot cultivars using fluorescence microscopy. *The Journal of Horticultural Science & Biotechnology*, **82**, 170-174.

Ortega, E., Egea, J., Cánovas, J. A. and *F. Dicenta* (2002). Pollen tube dynamics following half-and fully-compatible pollinations in self-compatible almond cultivars. *Sexual Plant Reproduction*, **15**, 47-51.

Ortega, E., Sutherland, B. G., Dicenta, F., Bošković, R. and *K. R. Tobutt* (2005). Determination of incompatibility genotypes in almond using firs and second intron consensus primers. detection of new S alleles and correlation of reported S genotypes. *Plant Breeding*, **124**, 188-196.

Ortega, E., Bošković, R. I., Sargent, D. J. and *K. R. Tobutt* (2006). Analysis of S-RNase alleles of almond (*Prunus dulcis*). Characterization of new sequences, resolution of synonyms and evidence of intragenic recombination. *Molecular Genetics and Genomics*, **276**, 413-426.

Pimienta, E., Polito, V. S. and *D. E. Kester* (1983). Pollen tube growth in cross- and self-pollinated "Nonpareil" almond. *Journal of the American Society for Horticultural Science*, **108**, 643-647.

Radičević, S., Cerović, R., Đorđević, M. and *S. Marić* (2008). Ispitivanje fenofaze cvetanja i klijavosti polena novijih sorti trešnje. *Voćarstvo*, **42**, 89-95.

Sanchez, A. M., Bosch, M., Bots, M., Nieuwland, J., Feron, R. and *C. Mariani* (2004). Pistil factors controlling pollination. *Plant Cell*, **16**, 98–106.

Sánchez-Pérez, R., Dicenta, F. and *P. Martínez-Gómez* (2004). Identification of S-alleles in almond using multiplex PCR. *Euphytica*, **138**, 263-269.

Socias I Company, R. and *J. M. Alonso* (2004). Cross-incompatibility of “Ferragnèz”and “Ferralise” and pollination efficiency for self-compatibility transmission in almond. *Euphytica*, **135**, 333-338.

Stösser, R., Hartman, W. and *S. F. Anvari* (1996). General aspects of pollination and fertilization of pome and stone fruits. *Acta Horticulturae*, **423**, 15-22.

Tao, R., Yamane, H., Sassa, H., Mori, H., Gradziel, T. M., Dandekar, A. M. and *A. Suiguira* (1997). Identification of stylar RNases associated with gametophytic self-incompatibility in almond (*Prunus dulcis*). *Plant Cell Physiology*, **38**, 304-311.

Weinbaum, S. A., Parfitt, D. E. and *V. S. Polito* (1984). Differential cold sensitivity of pollen grain germination in two *Prunus* species. *Euphytica*, **33**, 419-426.

Yamashita, K., Gaude, T., Dumas, C., Graselly C. H. and *P. Crossa-Raynaud* (1987). Protein analysis on pistils and pollens of almonds with special reference to S_f, a self-fertile gene. *Journal of Japanese Society for Horticultural Science*, **56**, 300-305.

ИСПИТИВАЊЕ АУТО-(ИН)КОМПАТИБИЛНОСТИ ГЕНОТИПОВА БАДЕМА (*PRUNUS AMYGDALUS* BATSCH) СЕЛЕКЦИОНИСАНИХ НА СЛАНКАМЕНАЧКОМ БРЕГУ

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**Аутор за коресподенцију*

У циљу стварања сорти бадема прилагођених агроеколошким условима Србије у периоду 2005.-2006. године проучавана је клијавост полена и ауто (ин)компатибилност 19 генотипова бадема одабраних из популације сејанаца на Сланкаменачком брегу. Сви испитивани генотипови бадема имали су добру (50-70-%) или високу клијавост полена (преко 70%). Испитивања ауто (ин)компатибилности су обављена праћењем заметања у пољским условима и методом флуоресцентне микроскопије. Ауто инкомпатибилност је у оба случаја била изражена код свих 19 генотипова. Код свих генотипова поленове цевчице су заустављале раст најдаље у горњој трећини стубића уз карактеристичне петље, задебљања и кривудања.