ACTA AGROBOTANICA
Vol. 59, z. 1 – 2006
s. 91–97

Ocena samozgodności i zgodności krzyżowej w rodzaju *Pyrus* na podstawie obserwacji wzrostu łagiewek pyłkowych

WOJCIECH ANTKOWIAK[1], ANDRZEJ WOJCIECHOWSKI[2]

[1)] Department of Botany, Agricultural University, 60 625 Poznań, Wojska Polskiego 71c
[2)] Department of Genetics and Plant Breeding, Agricultural University,
60 625 Poznań, Wojska Polskiego 71c

The evaluation of self-compatibility and crossability in the genus *Pyrus* based on the observation of pollen tubes growth

(Received: 12.05.2005)

S u m m a r y

Interspecific compatibility and self-compatibility in *Pyrus communis, P. pyraster* and *P. salicifolia* were evaluated. Degree of compatibility was determined by means of fluorescence microscope. Self-incompatibility evaluated on the basis of the pollen tubes growth showed that two cultivars of *P. communis* (Bera Hardy and Lukasówka) were self-compatible and the other three cultivars were self-incompatible. All ecotypes of *P. pyraster* are self-incompatible. In interspecific crosses full inter-incompatibility or unilateral self-incompatibility was observed.

Key words: *Pyrus*, self incompatibility, pollen tube growth

INTRODUCTION

Self-incompatibility (SI) is a system in flowering plants that prevents self-fertilization. In the angiosperm plants exist two types of SI: sporophytic and gametophytic. In the gametophytic SI system, found in Solanaceae, Scrophulariaceae and Rosaceae, the pollen/pistil interaction is genetically controlled by the haploid genome of each pollen grain and the diploid genome of the pistil tissue. There are possible cases where at least part of rejection mechanism is probably taking place in the embryo sac, which could point to haplo-haplo incompatibility system (N e t t a n c o u r t, 1972). In this SI system pollen grain germination and pollen tube growth are controlled by a multi-allelic locus (S). According to McClure et al. (1989) the S-locus contains, among others genes, an RNase encoding gene, which is expressed in the style prior to anthesis. Most fruit species, including pear (*Pyrus)* exhibit gametophytic SI system and, when planning an orchard, at least two cross-compatible cultivars are needed to ensure successful pollination and eventually good yield (Zuccherelli et al., 2002).

Cross-compatibility between European cultivars of *Pyrus communis* has been studied mainly through field observations and biochemical analyses (Bellini, 1993, Zuccherelli et al., 2002, Zisovich et al., 2004). The present study was undertaken to determine compatibility and inter-compatibility among chosen European cultivars of *P. communis* and inter-compatibility between *P. communis* and five wild ecotypes of *P. pyraster* and *P. salicifolia* via a fluorescence light based method.

MATERIAL AND METHODS

The material used for the study consisted of three species of genus *Pyrus* i.e.: five cultivars of *Pyrus communis* (Packhams Triumph, Bera Hardy, Lukasówka, Konferencja and General Leclerc), five ecotypes of *Pyrus pyraster* (all wild ecotypes were collected from 5 different localisations not far from Poznań, named as 1, 2, 3, 4 and 5), *Pyrus salicifolia* (from the Botanical Garden of A. Mickiewicz University in Poznań). Observations of pollen grain germination and pollen tube growth were made after self- and cross pollination of five cultivars of *P. communis* and five ecotypes of *P. pyraster*, and after cross-pollination of two cultivars of *P. communis* (Packhams Triumph, Bera Hardy) with two ecotypes of *P. pyraster* (1 and 2) in full diallel cross combinations, and after cross-pollination of two cultivars *P. communis* (Packhams Triumph and Lukasówka, used as maternal form) with *P. salicifolia*. All pollination combinations are given in table 1. The pollination was carried out on cut branches in laboratory conditions. The pollen of particular pollinators was placed on the stigma of flowers which were emasculated at bud stage. Pollinated pistils were collected 24 and 48 hours after pollination and than fixed and stained with aniline blue according to Martin (1959) with modification of Wojciechowski (1985). Pollen grain germination and pollen tube growth were observed using UV-light fluorescence microscope. Ten pistils were used in each test.

Self-incompatibility (SI) or crossability (CC – cross compatibility, CI – cross incompatibility) were evaluated on the basis of the pollen grain germination index (PGI) according to Matsuzawa (1983): PGI = (b + 2c + 3d + 4e)/(a + b + c + d + e), where: a – number of pistils with pollen grains, b – number of pistils in which pollen grains do not germinate, c – number of pistils in which pollen grains germinate on the stigma, d – number of pistils in which pollen tubes enter the style tissue, e – number of pistils in which pollen tubes penetrate close to or enter the ovules. In case of PGI = or higher than 2 it was concluded that there was compatibility.

RESULTS

In three of five cultivars of *P. communis* i.e. Packhams Triumph, Konferencja and General Leclerc the germination of pollen grains after their self-pollination was very weak and there was lack of germination (Fig. 1) or the pollen tubes were visible only on the stigmas and PGI ranged from 0.4 – 1.8 (Table 1, Fig. 2). Two remain cultivars, Bera Hardy and Lukasówka shoved better germination of pollen grains and pollen tubes growth (PGI = 2.1, Fig. 3). After cross-pollination of cultivar Packham Triumph with pollen grain of cultivars Lukasówka and General Leclerc the intensity of pollen tubes growth was low and PGI was a little over 1.0 (Fig. 4). The same intensity of pollen tubes growth was observed after cross-pollination of ecotype 1 of *P. pyraster* with ecotypes 3 and 5 (PGI= 0.8 and 1.0, respectively).

In the case of crossing of two *P. communis* cultivars – Packhams Triumph and Bera Hardy with the pollen of five ecotypes of *P. pyraster* the pollen germinated well

Table 1

Pollen germination index (PGI) after self and cross pollination of three species of genus *Pyrus.*

Combination of pollination	PGI after 48 hours	Combination of pollination	PGI after 48 hours
P. communis –		*P. communis*, Packhams Triumph x	
self-pollination		ecotype 1	1.2
Packhams Triumph	0.6	2	2.6
Bera Hardy	2.1	3	1.7
Lukasówka	2.1	4	1.2
Konferencja	1.8	5	0.8
General Leclerc	0.4	Bera Hardy x	
cross-pollination		ecotype 1	0.8
Packhams Triumph x		2	2.4
Lukasówka	1.0	3	1.8
General Leclerc	1.2	4	2.5
P. pyraster-		5	2.4
self-pollination		*P. pyraster*, ecotype 1 x	
ecotype 1	0.6	Packhams Triumph	2.8
2	0.6	Bera Hardy	1.7
3	0.6	Lukasówka	1.8
4	0.6	Konferencja	1.4
5	0.6	General Leclerc	2.6
cross-pollination		ecotype 2 x	
ecotype 1 x		Packhams Triumph	1.2
ecotype 3	1.0	Bera Hardy	1.2
ecotype 5	0.8	Lukasówka	0.8
		Konferencja	1.2
		General Leclerc	2.4
		P. communis x *P. salicifolia*	1.1

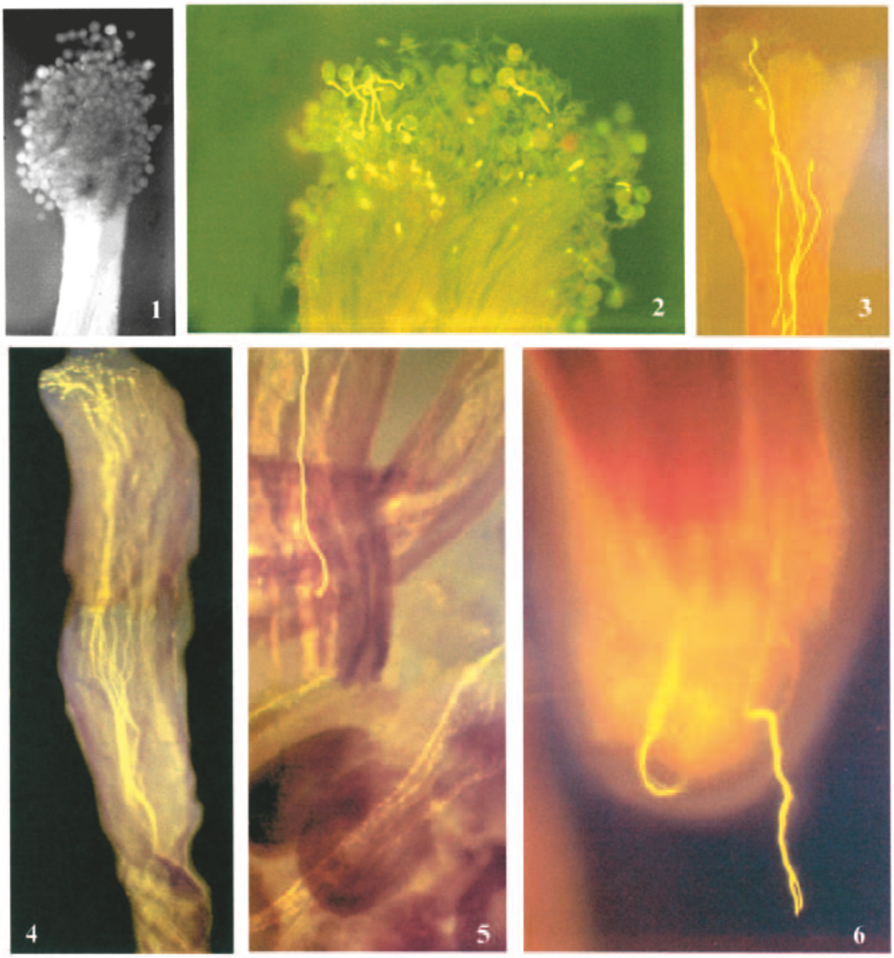

Fig. 1. The pistil of cultivar Packhams Triumph with a lot of not germinating pollen grains on the stigma. 48 h after self pollination.

Fig. 2. Pollen grains germinating on the stigma. Cultivar General Leclerc, 48 h after self pollina tion.

Fig. 3 4. Pollen tubes penetrating the style: fig. 3 – cultivar Lukasówka, 48 h after self pollination, fig. 4 – cross cultivar Packhams Triumph x cultivar General Leclerc.

Fig. 5 6. Pollen tubes in the ovary: fig. 5 – cultivar Packhams Triumph, 48 h after pollination with pollen of ecotype 2, fig. 6 – cultivar Bera Hardy, 48 h after pollination with pollen of ecotype 2.

on the stigmas but only after pollination with the pollen of ecotype 2 the pollen tubes reached ovary of Packhams Triumph and in some cases they were very close to the ovule (Fig. 5) (PGI = 2.6). In the case of cv. Bera Hardy the pollen grain of ecotypes 2, 4, and 5 germinated well and pollen tubes were visible in ovary (PGI > 2) (Fig. 6).

In combinations where ecotypes 1 and 2 of *P. pyraster* were pollinated with the pollen of five cultivars of *P. communis* the pollen tubes reached ovary only in three of ten cross combinations i.e. ecotype 1 x Packhams Triumph and General Leclark and ecotype 2 x General Leclark – PGI >2).

The reciprocal crosses of cultivars Packhams Triumph and Bera Hardy with the ecotypes 1 and 2 showed different intensity of pollen tubes growth and the value of PGI depended on which form was used as maternal. E.g. in the reciprocal crosses of cv. Packhams Triumph with the ecotype 2 there was better pollen tubes growth when it was used as maternal form (PGI = 2.6) than it was used as male parent (PGI = 1.2). Quite different situation was observed in the case of crosses with ecotype 1. In this case better pollen tubes growth was observed when Packhams Triumph constituted the paternal form (PGI = 2.8) than it was used as the maternal form (PGI = 1.2) (Table 1).

The crossing *P. communis* (cultivars Packhams Triumph and Laukasówka) x *P. salicifolia* showed that these two species do not suite to each other concerning their compatibility. The germination of pollen grains of *P. salicifolia* on the stigma of two cultivars of *P. communis* was rather weak and PGI = 1.1.

DISCUSSION

According to literature concerning interspecific crossability within four possible combinations of self-incompatible (SI) and compatible (SC) species pollen does not germinate only when SI constitutes the maternal form. In this case pollen is probably inhibited similarly as in self-incompatible pollination. In the remaining three combinations, i.e. SC x SI, SC x SC, SI x SI pollen germinates normally. Lewis and Crowe (1958) regard such pollen behaviour as normal and being rule. It has been impossible until now to characterise certain types of interspecific incompatibility through the use of such criteria as the stage of determination and the site of expression. The reaction of interspecific incompatibility in the majority of genera can be stigmatic or stylar. The rules for interspecific pollen-tubes incompatibility are the same in families with the gametophytic (e.g. Rosaceae, Solanaceae) and sporophytic (e.g. Brassicaceae) type of self-incompatibility. The most remarkable feature of interspecific incompatibility is that it usually occurs unilaterally (Sampson, 1962). The pollen tubes of certain self-compatible (SC) species are inhibited in the styles of self-incompatible (SI) species but the reciprocal crosses are compatible.

The results of our observation on pollen grain germination and penetration of pollen tubes into particular parts of the pistil showed that only two cultivars of *P. communis* are SC. The remain tested genotypes belonging to three different species are SI. It is interesting that crosses between SC cultivar Bera Hardy (*P. communis)* and SI ecotype 2 (*P. pyraster*) were compatible only when SC cultivar was used as maternal form. Reciprocal crosses (SI x SC) were incompatible. Such unilateral incomatibility was also observed in crosses between SI Packhams Triumph and SI ecotype 1. Cross-compatibility was observed only in these crosses where Packhams Triumph constitutes paternal form. These results are consistent with those obtained by Wojciechowski and Andrzejewski (1986) and Wojciechowski et al. (1996). Reciprocal crosses made by these authors between SC cultivars from families Solanaceae and Brassicaceae showed unilateral interspecific incompatibility. The data obtained from our observations are indicative of unilateral incompatibility also when two self-incompatible species are crossed.

CONCLUSION

1. The most of tested genotypes of genus *Pyrus* are self-incompatible. Only two cultivars of *Pyrus communis* (Bera Hardy and Lukasówka) are self-compatible.

2. Unilateral interspecific incompatibility occured in the crosses between two self-incompatible species, *P. communis* (Packhams Triumph – maternal form) and *P. pyraster* (ecotype 1), and between self– compatible Bera Hardy (paternal form) and self – incompatible ecotype 2.

REFERENCES

Bellini E., 1993. La cultivazione del pero. L'Informatore Agrario, Verona: 1 343.

Lewis D., Crove L. K., 1958. Unilateral interspecific incompatibility in flowering plants. Heredity, 34: 125 128.

Martin F., 1959. Staining and observing pollen tubes by means of fluorescencs. Stain Technology, 34: 125 128.

Matsuzawa Y., 1983. Studies of the interspecific hybridisation in genus *Brasssica.* II. Crossability in interspecific crosses, *B. oleracea* x *B. campestris*. Japan J. Breed. 33: 321 330.

McClure B. A., Haring V., Ebert P. R., Anderson M. A., Simpson R. J., Sakiyama F., Clarke A. E., 1989. Style self incompatibility gene products of *Nicotiana alata* are ribonuclease. Nature, 342: 955 957.

Nettancourt D., 1972. Self incompatibility in basic and applied researches with higher plants. Genetica Agraria (Pavia), 26: 163 226.

Sampson D. R., 1962. Intergeneric pollen stigma incompatibility in the Cruciferae. Can. J. Genetics and Cytology, 4: 38 49.

Wojciechowski A., 1985. Interspecific hybrids between *Brassica campestris* L. and *B. oleracea* L. I. Effectiveness of crossing, pollen tube growth, embriogenesis. Genetica Polonica, 26/4: 423 435.

Wojciechowski A., Andrzejewski R. P., 1986. Evaluation of crossability in intergeneric crosses *Lycopersicon esculentum* Mill. x *Capsicum annuum* L. based on the pollen tube growth. Bull. of the Polish Acad. of Sci. Biological Sci. 34/10: 219 225.

Wojciechowski A., Olejniczak J., Adamska E., 1996. Evaluation of crossability of *Cuphea lanceolata* and *C. viscosissima* based on pollen tube growth and seed set. J. Appl. Genet. 37/3: 277 284.

Zisovich A. H., Stern R. A., Sapir G., Shafir S., Goldway M., 2004. The RHV region of S Rnase in the European pear (*Pyrus communis)* is not required for the determi nation of specific pollen rejection. Sex Plant Reprod. 17: 151 156.

Zuccherelli L., Tassinari P., Broothaerts W., Tartarini S., Dondini L., Sansavini S., 2002. S allele characterization in self incompatible pear (*Pyrus communis* L.). Sex Plant Reprod. 15: 153 158.

Streszczenie

Na podstawie obserwacji łagiewek pyłkowych, wykonanych przy użyciu mikroskopu fluorescencyjnego, oceniono samozgodność oraz samoniezgodność u pięciu odmian sadowniczych gruszy pospolitej *Pyrus communis* ('Packhams Triumph', 'Bera Hardy', 'Lukasówka', 'Konferencja' i 'General Leclerc'), 5 drzew gruszy dzikiej *Pyrus pyraster* rosnących w okolicach Poznania oraz jednego drzewa gruszy wierzbolistnej *Pyrus salicifolia*.

Kwitnące pędy grusz zostały umieszczone w laboratorium i w tych warunkach przeprowadzono zapylanie. Słupki do obserwacji zbierano po 48 godzinach od momentu zapylenia. Obserwacje wzrostu łagiewek pyłkowych wykazały, że dwie odmiany *Pyrus communis* ('Bera Hardy', 'Lukasówka') były samozgodne a 3 inne były samoniezgodne. Wszystkie ekotypy *Pyrus pyraster* były samoniezgodne. W międzygatunkowych krzyżowaniach obserwowano pełną lub jednokierunkową niezgodność.