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Pear pollen selection using higher germination properties at low temperatures and the effect on the fruit set and quality of Japanese pear cultivars

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ABSTRACT

To determine the lowest temperature possible for pollen germination in pear, *in vitro* pollen germination was studied in 7 wild pear species and 16 pear cultivars at six temperatures (7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 °C) over two years. The extent of pollen germination was affected by the incubation temperature. Most cultivars did not germinate at a temperature below 12.5 °C; however, the cultivar *Pyrus communis* L. showed a higher percentage of germination than the other cultivars at 7.5 °C. The percentage of germination for La France and Le Lectier pears was >50% at 10.0 °C. We investigated the effects of cross pollination between the cultivars *P. communis* L. and 'Chojuro' and the Japanese pear cultivars 'Gold-Nijisseiki' and 'Hosui' pertaining to fruit set and quality at a low temperature (10.0 °C). The bearing rate of the cultivars 'Gold-Nijisseiki' and 'Hosui' depended on the type of pollen used for pollination. Pollination with European pear pollen resulted in a higher bearing rate (50%) in these cultivars. In contrast, the bearing rate of the cultivar 'Chojuro' was <20%. Fruit quality and number of seeds per fruit (seed yield) in the cultivars 'Gold-Nijisseiki' and 'Hosui' were not affected by the type of cultivar used for pollination. Methods involving the use European pear pollen combined the advantages of higher germinability and higher monocarpic under low temperature condition.

1. Introduction

Temperature is a major environmental factor that limits the geographical distribution of plant species (Saxe et al., 2001). Global climatic change (IPCC, 2001) resulting from the predicted absolute increase in temperature and frequency and amplitude of temperature variations could jeopardize plant cultivation in some areas. The consequences of global climate change are already visible in shifting species distributions and flowering times (Hedhly et al., 2005). Recently, significant increases in flowering time resulting from global warming have been reported for pear cultivation in Japan (Tamura et al., 2001). However, fruiting failure in Japanese pears has occurred since pollen germination has been inhibited by early flowering owing to frequent low temperatures.

In Japanese pear cultivars, artificial pollination is necessary because the Japanese pear has S-RNase-based self-incompatibility, and does not have the economic parthenocarpy found in the European pear (Nyéki et al., 1998); therefore, pollen germination and pollen tube development are important for fertilization. Extremely low pollen germination rates may cause fruit setting failure because of ovule degradation before the pollen tube reaches the ovary (Vasilakakis and Porlingis, 1985). Pollen germination is affected by multiple biotic and abiotic stresses, including low temperature (Ohnishi et al., 2010; Thakur et al., 2010). Low temperature generally has negative effects on pollen germination and tube growth in many species (Cohen et al., 1989; Rosell et al., 1999). The optimum temperature for pollen germination varies among species and cultivars of the same species, as shown in several woody

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Table 1
Germination percentages on pollen of *Pyrus* plants at different temperatures (2011).

	Registered name	Germination percentage (%)[z]					
		Incubation temperature					
		7.5	10.0	12.5	15.0	17.5	20.0
Wild pear	*P. betulaefolia*	0.0 c[y]	12.9 bc	12.6 bc	27.6 b	48.1 a	62.9 a
	P. calleryana (No.12)	0.0 c	0.0 c	3.0 bc	12.4 b	37.4 a	54.5 a
	P. communis L.	43.3 b	59.9 b	75.2 ab	78.1 ab	89.3 a	83.2 a
	P. dimorphophylla(No.5)	0.0 c	1.5 c	2.6 c	22.4 b	27.1 b	51.0 a
	P. hondoensis (Aonashi)	0.0 c	8.0 bc	2.5 c	23.8 b	26.8 b	61.0 a
	P. pyrifolia (Sawairiyamanashi)	0.0 d	0.1 cd	10.0 c	35.6 b	44.4 ab	63.3 a
	P. serotina	0.0 c	0.8 c	1.1 c	36.4 b	37.3 b	68.5 a
Chinese pear	'Yali'	0.0 b	0.0 b	17.1 b	18.6 b	45.5 ab	73.3 a
	'Pinggouli'	0.0 c	0.1 c	17.7 b	35.8 b	71.1 a	77.1 a
	'Hanheungli-Kou'	0.0 c	0.0 c	0.0 c	7.5 c	57.1 b	83.2 a
	'Hanheungli-Otsu'	0.0 b	0.2 b	0.0 b	17.1 b	46.2 a	66.4 a
Japanese pear	'Imamuraaki'	0.0 b	3.3 b	3.6 b	22.1 b	18.3 b	54.2 a
	'Chojuro'	0.0 c	9.8 c	13.1 c	46.5 b	59.8 a	73.4 a
	'Kosui'	0.0 b	1.3 b	2.1 b	4.0 b	39.4 a	50.5 a
	'Hosui'	0.0 c	1.3 c	2.0 c	8.6 c	41.6 a	52.4 a
	'Gold-Nijisseiki'	0.0 c	11.0 c	19.0 c	43.3 ab	32.4 b	75.7 a
	'Shinkou'	0.0 b	0.5 b	6.1 b	29.6 ab	45.0 a	53.5 a
European pear	'Doyenné du Comice'	0.0 c	3.1 c	36.5 b	39.9 b	78.5 a	66.1 a
	'Starkrimson pear'	0.0 c	4.2 c	23.7 b	43.8 b	76.4 a	62.5 a
	'General Leclerc'	0.0 c	16.4 b	14.6 b	28.3 b	35.7 b	52.8 a
	'Bartlett'	0.0 c	8.6 c	31.6 b	41.0 b	86.7 a	81.2 a
	'La France'	0.0 b	53.5 b	73.8 a	73.6 a	82.1 a	85.8 a
	'Le Lectier'	1.4 c	58.6 b	67.1 a	67.1 a	71.1 a	91.2 a

[z] Incubation of 5 h at each temperature.
[y] Mean separation within rows by Chi-square test at $P < 0.05$.

species, such as avocados (Sedgley and Annells, 1981), almonds and peaches (Weinbaum et al., 1984), walnuts (Luza et al., 1987), pistachios (Polito et al., 1988), apricots (Egea et al., 1992), and mangos (Sukhvibul et al., 2000). However, little is known about the optimum temperature for pollen germination among pear plants and the effect of fruit set of Japanese pear cultivars at low temperatures. It is important to select cultivars showing stable pollen germination rates at low temperatures for limited time periods to perform artificial pollination of Japanese pears.

In this study, we comparatively assessed pollen germination in pear plants and evaluated the extent of pollen germination in pear cultivars and wild pears to determine the lowest temperature possible for germination. To select an optimal pollenizer with a stabilizing influence on the fruit setting of Japanese pear cultivars at low temperatures, we investigated the effects of cross pollination between the pollination and Japanese pear cultivars 'Gold-Nijisseiki' and 'Hosui' on fruit setting and quality at a low temperature (10.0 °C).

2. Materials and methods

2.1. Pollen germination of Pyrus plants at different temperatures (Experiment 1)

We used pollens from 7 wild pear species and 16 pear cultivars grown in the orchards of Tottori University and Tottori Horticultural Experiment Station (35.5°N, 133.7°E). Pollens from each cultivar were collected in April in both 2011 and 2012. Pollens were extracted from the anthers of flowers in the balloon stage for germination assays. These anthers were dried on a piece of paper for 24 h at 24 °C. The dried pollens were sieved through a 0.25-mm mesh. The pollens were then immediately germinated in polystyrene Petri dishes (35 × 10 mm) at six temperatures (7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 °C). These pollens were scattered into the *in vitro* system, which had already been injected with a solidified germination medium consisting of 10.0% (w/v) sucrose, 1.0 mM boric acid, and 1.0% (w/v) agar. Pollen germination was arrested after 5 h. The percentage of pollen germination was then determined using a microscope (OLYMPUS CHB-233).

Pollen germination was defined as when the length of the pollen tube exceeded the diameter of the pollen grain. For each treatment, the percentage of pollen germination was determined three times, and fields in which the pollens had reached at least 100 were selected. Statistical analysis were performed using chi-squared tests. P values <0.05 were considered statistically significant.

2.2. Effect of fruit set by pollination treatment using pollen of different pear cultivars under the two temperature conditions (Experiment 2)

Experiments were performed using 5-years-old potted pear trees [(*P. pyrifolia* (Burm.f.) Nakai)] (pot volume: 50 L), and the cultivars 'Gold-Nijisseiki' and 'Hosui' were grafted onto *P. betulaefolia* B. seedlings planted in the field at Tottori University, Tottori, Japan (35.5° N, 134.2°E). *P. communis* L., 'La France', and 'Chojuro' were used as the pollen cultivars. Potted trees of the cultivars 'Gold-Nijisseiki' and 'Hosui' were placed in a climate chamber at 10.0 and 20.0 °C before anthesis.

The flowers were emasculated at the late balloon stage and hand pollination was performed using the pollens of the cultivars *P. communis* L., 'La France', and 'Chojuro' for approximately 20 clusters of 'Gold-Nijisseiki' and 'Hosui'. Trees that had been hand pollinated were kept in a climate-controlled chamber for 5 days. The procedures described above were all performed at 10.0 and 20.0 °C. After these procedures, the potted trees were returned to the outdoor environment. Fruit setting for all clusters was assessed four weeks after pollination. Statistical analysis were performed using the chi-squared test. P values <0.05 were considered statistically significant.