Pollen Fertility in Roses

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Staining ability and germination of pollen were examined in 32 wild species, 24 wild botanical varieties, 12 interspecific hybrids and 55 cultivars of roses. Medium contained 50 ppm boric acid is practically convenient for evaluating pollen germination at temperature of 20℃, 25℃ and 30℃. In two-hour incubation, no particular difference in germination was observed among temperature conditions tested. A regression equation derived from our experiments gave a good estimate of germination rate ($Y=4.642 \cdot 1.0297^{X}$, where Y is germination rate arcsine transformed, X is percentage of stained-pollen arcsine transformed), although germination rate was lower than the rate of pollen stained in most species and cultivars.

Key words : Rose, Pollen germination, Staining ability.

Introduction

Although the knowledge on the level of pollen fertility in plant materials is one of the important factors to advance a breeding program, there have been a few studies evaluating the pollen fertility in roses. Modern cultivars of roses have been developed through the path of complicated hybridization among the cultivars and wild species, however general performance on pollen fertility of modern cultivars is not yet known. In general, evaluation of pollen fertility by staining method is insufficient, thus, Pearson[1] proposed that so-called "absolute pollen viability", combined with feature of pollen shape, staining ability and pollen germination, is a better indicator to evaluate pollen viability than the staining method in roses.

Addition of boric acid to medium stimulates germination of pollen,[2,3] and diverse concentrations of sugar lead to increase rate of pollen germination.[2-5] Wholers *et al.*[5] recommended use of a complex medium for good pollen germination in roses.

Since rose species are widely distributed throughout the temperate and subtropical regions of the northern hemisphere, it is expected that the pollen of these roses has some ecological requirements for their native environmental conditions, such as, temperature, nutrition and so on. Flory[6] showed a tendency in the change of pollen fertility in relation to distribution of wild species.

In the present paper, we describe staining ability and germination of pollen in roses, and

discuss the variation of pollen fertility regarding to ecophysiological diversity of species and cultivars.

Materials and Methods

The pollen of 32 wild species, 24 wild botanical varieties, 12 interspecific hybrids (hybrids between two wild species) and 55 cultivars (including the selections of wild species and interspecific hybrids) of roses was examined. Flower buds just before anthesis were collected from the plants planted in Yachiyo research field of Keisei Rose Nursery, Inc. during April to July, 1985. The pollen was obtained from dehiscent anthers in opening flowers. The pollen packed in the paper bag was kept in a desiccator at about −28.0℃ until examination.

Germination of pollen was examined by using titration plate with 24 wells (Costar Co.). The pollen grains were scattered on 1% agar medium with 10% sucrose and 0, 50 or 100 ppm boric acid in each well (1.6 cm in diameter). The plate was incubated at 20℃, 25℃ and 30℃ for 2 hours. The pollen was stained with 1% acetocarmine solution. Germination and staining ability were evaluated on more than 500 pollen grains.

Results

1. Staining ability of pollen

Staining ability of rose pollen ranged from 2.2% (*Rosa* × *kochiana* Koehne) to 99.8% (*R. cinnamomea* L.) (Table 1). Cultivars and interspecific hybrids generally showed lower staining ability of pollen than wild species and wild botanical varieties (Table 1). Most wild species showed high staining ability of pollen, and some wild species lower staining abilities, i. e. *R. foetida* Herrm., *R. hugonis* Hemsl., *R. agrestis* Savi, *R. pisocarpa* A. Gray and *R. helenae* Rehd. & Wils. Two interspecific hybrids showed very low staining ability (*R.* × *alba* L. var. *suaveolens* Dieck and *R.* × *kochiana*), and other hybrids relatively high staining ability. In cultivars, there was a wide variation in staining ability of pollen, ranging from 2.6% (*R.* × *alba* cv. Semiplena) to 97.0% (*R. sericea* Lindl. cv. Heather Muir) (Table 1). Mean value of staining ability in wild species and wild botanical varieties was the highest, that in interspecific hybrids intermediate, and that in cultivars the lowest (Table 1).

2. Pollen germination

There was a wide range of variation in pollen germination rate of materials examined in various combinations of temperature and boric acid concentration (Table 1). According to the analysis of variance for these data, there was a significant difference in pollen germination percentage as affected by boric acid concentration, but no difference at three temperature conditions (Table 2). Pollen germination was particularly different between 0 ppm and 50 ppm boric acid in the media, and both 50 ppm and 100 ppm boric acid led to a better germination of pollen (Tables 1 and 3). The pollen of wild species and wild botanical varieties germinated

Table 1. Staining ability and germination rate under the condition of various combination of temperatures and boric acid concentrations

	No.of spp.and cvs.	Staining ability (%)	Germination rate(%)								
			20℃			25℃			30℃		
			0ppm	boric acid 50ppm	100ppm	0ppm	boric acid 50ppm	100ppm	0ppm	boric acid 50ppm	100ppm
Wild species and varieties	56	79.7 (3.4-99.8)	18.6 (0-65.4)	39.1 (0-85.0)	39.7 (0-81.8)	16.5 (0-65.3)	44.4 (0-92.2)	44.7 (0-88.1)	15.3 (0-51.0)	43.5 (0-90.2)	44.5 (0.2-96.4)
Inter-specific hybrids	12	66.1 (2.2-96.6)	20.8 (0-63.5)	30.2 (0.4-69.8)	31.1 (0.2-70.9)	16.5 (0-44.9)	32.9 (3.6-75.7)	33.6 (1.8-69.2)	12.5 (0-42.2)	33.1 (2.4-77.3)	32.1 (1.8-82.2)
Cultivars	55	50.8 (2.6-97.0)	8.4 (0-81.2)	16.5 (0-82.5)	16.9 (0-87.7)	7.0 (0-42.6)	18.9 (0-89.0)	19.5 (0-90.4)	7.2 (0-66.4)	18.8 (0-88.3)	19.4 (0-90.8)

Figures in parenthesis indicate the range of mean values.

Table 2. Analysis of variance for pollen germination in roses

Source of variation	df	Mean squares
Temperature (T)	2	258.21
Concentration of boric acid (C)	2	22,043.40**
T×C interaction	4	267.93
Error	1098	337.93

** : Significant at the 1% level.

Table 3. Means of germination rate under various conditions

Items	Means (%)
Temperature	
20℃	17.8a[z]
25℃	19.9a
30℃	19.6a
Concentration of boric acid	
0ppm	8.5a
50ppm	25.1b
100ppm	26.0b

z : Means followed by the same letter are not significantly different at the 5% level.

well, and that of interspecific hybrids intermediately, and cultivars showed poor germination (Table 1).

3. The relation of staining ability and germination rate of pollen

Since staining ability and germination rate follow to a binomial distribution, these values were subjected to arcsine transformation, and then regression analysis was performed. Correlation between staining ability and germination rate in 50 ppm boric acid at 25℃ was significant at 1% level (r=0.691), and most species, interspecific hybrids and cultivars showed a lower rate

of germination than staining ability (Fig. 1). And the following regression equation was obtained ; $Y=4.642 \cdot 1.0297^{X}$, where Y is germination rate arcsine transformed, X is percentage of stained pollen arcsine transformed, 4.642 and 1.0297 are constant (Fig. 1).

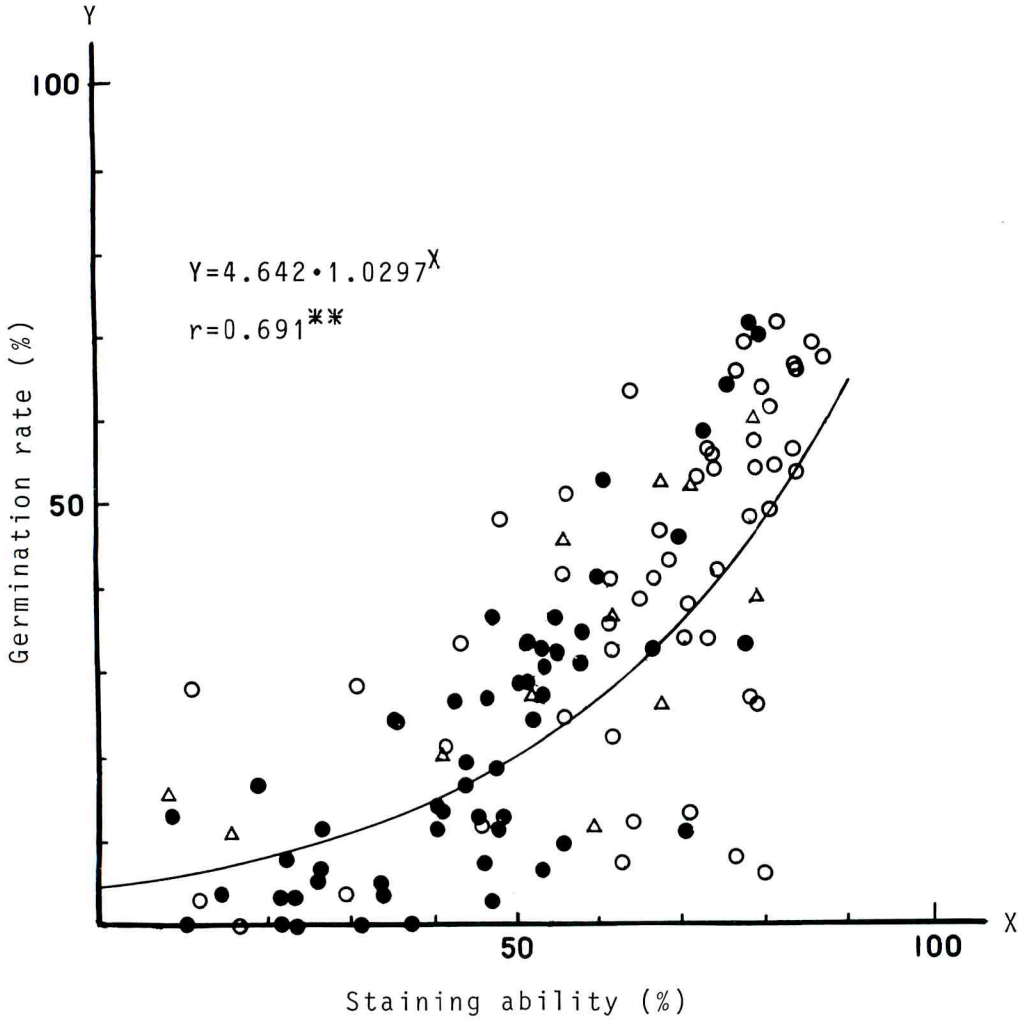

Fig. 1. The relationship between staining ability and germination rate of rose pollen. Calculation was made on arcsine transformed data of staining ability and germination rate. ○, wild species and wild varieties ; △, interspecific hybrids and ●, cultivars ; X, Germination rate arcsine transformed ; Y, Staining ability arcsine transformed and r, Correlation coefficient ; **, Significant at 1 % level.

Discussion

Effect of boric acid on pollen germination in roses is well documented. Ten ppm or 100 ppm boric acid stimulates germination,[3] and 40 ppm boric acid is recommended for sufficient

level so as to enable promotion of pollen germination.[2] Our experiments were done in order to confirm this point. Any particular difference in germination percentage between 50 ppm and 100 ppm boric acid could not be found out, so a 40–50 ppm level of boric acid may as well be considered to be optimum for pollen germination in roses. Then we discuss the following by using the data examined in the media containing 50 ppm boric acid.

Flory[6] showed that Western Asian and European native species generally have lower percentage of morphologically normal pollen than those of Eastern Asia, and that American species produce good pollen at a slightly higher level than the Eastern Asiatic species (Table 4). He also pointed out that the species native to the eastern part of every continents tended to increase in the amount of their normal pollen (Table 4). Though our examination was done with the limited number of European and American species, there has been found a similar tendency in Asian species that the normal pollen, which is germinated and stained, increases in its percentage as it comes nearer towards the eastward direction (Table 4). In general, the plant species show a migrating-distance dependent fitness, and Levin[7] pointed out that in *Phlox drumm-*

Table 4. Average rate of pollen germination and staining ability in the *Rosa* wild species and wild varieties with reference to their geographical distribution

Geographical source	No. of spp. and vars.	Germination rate[z] (%)	Staining ability (%)	Proportion of normal pollen[y] (%)
Europe				
Europe	2	58.7	86.1	38.7 (19)
Europe, N. Africa	2	38.6	61.5	69.0 (2)
E. Europe, W. Asia	5	51.8	90.8	49.3 (27)
Total	9	50.4	83.2	45.7 (48)
Asia				
Western	6	29.8	58.4	35.4 (10)
Central	17	44.2	78.8	50.9 (15)
Eastern	11	56.1	91.4	81.2 (17)
Total	34	45.5	79.3	59.5 (42)
N. America				
Western	5	47.3	67.8	78.9 (8)
Central, or all	2	11.6	74.2	84.0 (9)
Eastern	2	48.3	94.0	92.7 (7)
Total	9	39.6	75.1	84.8 (24)

z : Germination rate on medium contained 50 ppm boric acid at 25℃.

y : Data from Flory (1950). Regular appearing pollen grains filled with cytoplasm have been counted as normal.

Figures in parenthesis indicate the number of species and varieties examined.

ondii, the greater the distance from native place, the smaller the fitness of immigrant which is obtained from the seed germination, the plant survivorship and the seed production. Flory's evaluation and ours were done at the eastern edge of the continent, therefore a good germination in the eastern-continent native species may be verified by the above relationship of immigrant.

For example, *R. hugonis* showed very low pollen staining ability and germination rate, and shows very poor seed set in Japan as result of our observation. *R. hugonis*, which is native to Central China, seems to be sensitive to environmental conditions, and the dry and hot summer climate is favorable for the pollen development of this species.[4] Therefore, high humidity like in Japanese summer may be unfavorable for the pollen development.

Hurst[8] proposed septets theory in roses. According to this theory, septet means set of seven chromosomes and corresponds to genome. These septets are distinguished as A, B, C, D and E based on morphological and ecological characters. Each septet species is fitted for special ecological condition. The genus *Rosa* exihibits a polyploid series with the above mentioned chromosome number of seven, ranging from $2n=2x=14$ to $2n=8x=56$. Therefore, each species has various combinations of septets. A wide variation of staining ability and germination rate in roses may also reflect such constitution of chromosomes.

Traditional cultivars and modern cultivars showed lower pollen viability than the wild species (Table 1, Fig. 1). Such low pollen viability in cultivars might have resulted from the extensive hybridizations among many wild species and cultivars. Though interspecific hybrids and their hybrid-derivatives generally showed low pollen viability, the interspecific hybrids used in the experiment showed a higher pollen fertility than cultivars (Table 1, Fig. 1). This agrees to the suggestion given by Flory[6] that the hybrids obtained from the crosses between several wild species produce normal pollen in relatively high proportion. Since extensive hybridizations have been carried out over many generations in modern cultivars, it may safely be said that the pollen fertility might gradually decrease after the flower improvement of cultivars.

Although the pollen germination is affected by the distance from the individual native habitat, the history of its hybridization and the contents of medium, a good estimation of germination can be obtained by an evaluation of pollen stainability utilizing our equation, $Y=4.642 \cdot 1.0297^{X}$. As was indicated by Pearson,[1] the proportion of germinable pollen is usually lower than that of the pollen stained with acetocarmine (Fig. 1). However, it is reliable that our evaluation of germinable pollen by stainability gives a rough estimation of pollen fertility in rose as well as in plums.[9] A regression equation derived from our experiment also provide a good estimation of germinable pollen.

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バラの花粉稔性

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32種，24変種，12種間交雑種および55品種のバラの花粉を用いて，酢酸カーミンによる染色性および寒天培地での発芽率を調査した．発芽培地へのほう酸の添加は花粉の発芽を促進し，その濃度は50 ppmで充分であった．発芽率は温度条件によって顕著な違いがなかった．ほとんどの種および品種において，発芽率は染色率より低かったが，回帰式，$Y = 4.642 \cdot 1.0297^{X}$（ただし，Yは角変換後の花粉発芽率，Xは角変換後の花粉染色率）により，染色能力（%）から発芽率（%）がよく推定できる．