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Variation of Pollen Viability and Storability in Asparagus (*Asparagus officinalis* L.) Cultivars

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The optimal culture condition for evaluating pollen viability of asparagus was studied. Sucrose was an effective constituent of the media for raising pollen germination rate. The medium containing 30% sucrose, 0.01% borate and 3% agar was found to be optimum for assessing pollen viability. Effects of temperature and light conditions during *in vitro* culture on pollen germination rate were not recognized in the range of 20–30 °C in this investigation. Varietal difference of pollen viability and storability was also investigated. Pollen germination rate was varied individually within each diploid cultivar, whereas it was uniformly low in triploid cultivar 'Hiroshima Green'. Average percentage of pollen germination in each diploid cultivar was approximately the same, and it was higher than in triploid 'Hiroshima Green'. Pollen viability declined to 0% after storage for three months at 25 °C. Optimum storage temperature, at which pollen viability could be maintained after 12 months storage, was either –20 or –40 °C.

INTRODUCTION

Asparagus (*Asparagus officinalis* L.), a dioecious perennial species native to Europe and Eastern Asia, is an economically important vegetable crop cultivated for its medicinal and food value. The general aims of our breeding program are, i.e. earliness, uniform firm spears, low anthocyanin content, low fiber content, high vigor and resistance to diseases, e.g. *Phomopsis asparagii, Fusarium oxysporum* and *F. moniliforme*.

The preservation of viable pollen grains is valuable in many crops when specific crosses are desired between the plants of which the flowering times are not the same. Since male plants always bloom earlier than the females in asparagus, an effective pollen storage technique is useful for hand pollination. The viability of pollen depends on the conditions of storage; particularly on temperature and relative humidity. Marcellán and Camadro (1996) reported that pollen staining was not an appropriate technique to determine the viability of pollen because it only detects the presence or absence of living protoplasm and also recommended to apply *in vitro* pollen germination as the estimation method of pollen viability. Although there are a few reports describing the pollen storage in asparagus (Marcellán and Camadro, 1996; Snope and Ellison, 1963), further study is needed to determine whether or not the pollen viability depends on genotype before and during storage and on temperature conditions.

This work was carried out to (1) establish a suitable method for *in vitro* pollen germination to evaluate pollen viability, (2) investigate the effect of genotype on pollen viability within and among cultivars, and (3) demonstrate the effect of temperature during long–term storage on pollen viability.

MATERIALS AND METHODS

Conditions of *in vitro* pollen germination

Pollen grains were collected from a male plant of 'Mary Washington 500W' in May 1996, and they were placed in 90 mm diameter plastic petri dishes containing 20 ml culture medium. They were then sealed with Parafilm® and incubated until observation in all experiments. For investigating optimum condition of *in vitro* pollen germination, three experiments were carried out as follows; (1) To examine the time course of pollen germination rate on artificial medium, pollen grains were sown on a medium containing 30% sucrose, 0.01% borate and 3% agar. Then they were incubated at 25°C in light (45.79 μmoles m^2 s^{-1}), and germination was recorded at 0, 2, 4, 8, 16 and 24 hr after sowing. (2) Pollen grains were cultured on 3% agar medium supplemented with various concentrations of sucrose (0, 15, 30 and 45%) and borate (0, 0.01 and 0.02%) for determining optimum culture medium. (3) Pollen grains sown on the optimum medium determined in (2) were incubated at 20, 25 or 30°C in light or dark conditions to investigate the effect of culture temperature and light conditions on *in vitro* pollen germination.

Varietal difference

Pollen grains were collected from four diploid cultivars; 'Franklim', 'Geynlim', 'Hokkai 100' and 'UC157F_1', and one triploid cultivar; 'Hiroshima Green' from May to June in 1996. Pollen germination rate of each individual plant from the five cultivars was determined under the optimum condition for *in vitro* pollen germination as identified in the previous experiment. Number of investigated plants with different genotypes was from six to 19 in each diploid cultivar, and three in triploid 'Hiroshima Green'.

Storage temperature

Pollen grains were collected from four cultivars; 'Geynlim', 'Franklim', 'Hokkai 100' and 'Mary Washington 500W' from May to June in 1996. They were then stored at 25, 5, −20 or −40°C in steel containers with silica gel to maintain low humidity. Pollen grains of 'Hiroshima Green' were also stored at −40°C. Pollen germination rate was recorded after 0, 1, 2, 3, 6 and 12 months storage for each cultivar.

Three replicates of more than 300 pollen grains were counted for each experiment with an optical microscope to determine the pollen germination rate. Pollen grains were classified as germinated when the pollen tubes were longer than the diameter of the pollen.

RESULTS

Condition of *in vitro* pollen germination

Figure 1 shows the time course of pollen germination. The rate increased significantly from 0 to 4 hours, then gradually increased thereafter. It fluctuated more 8 and 16 hours after sowing than the rest of the times after sowing.

The effect of sucrose and borate concentrations in the media on pollen germination is

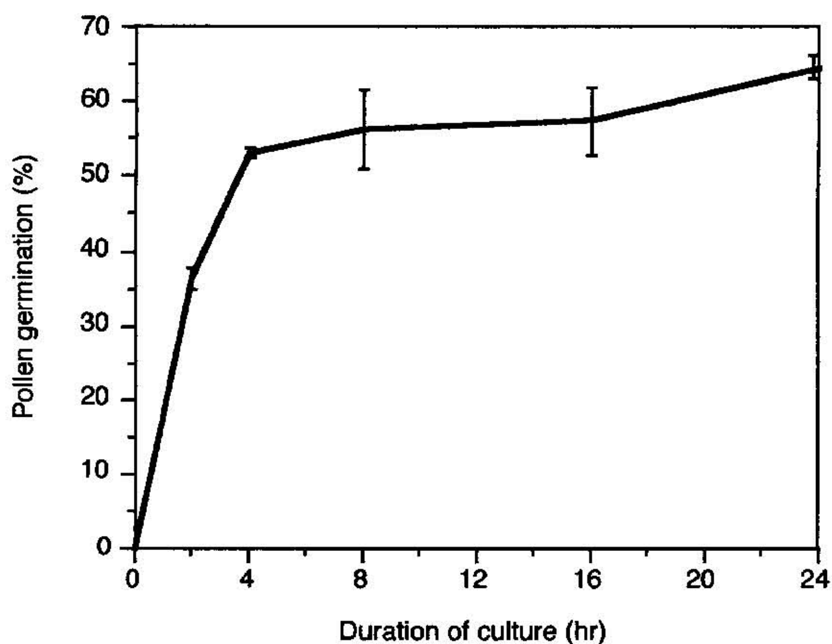

Fig. 1. Time course of *in vitro* pollen germination in asparagus 'Mary Washington 500 W'. Vertical bars are standard errors.

Table 1. Effect of sucrose and borate concentrations on *in vitro* pollen germination in asparagus 'Mary Washington 500 W'.

Sucrose (%)	Borate (%)	Pollen germination (%)
0	0	0.4 d[z]
	0.01	1.1 d
	0.02	0.7 d
15	0	54.4 b
	0.01	55.7 b
	0.02	63.6 ab
30	0	62.2 ab
	0.01	74.5 a
	0.02	64.2 ab
45	0	1.0 d
	0.01	0.3 d
	0.02	12.6 c

[z] Mean separation within a column by Duncan's multiple range test, 5% level.

summarized in Table 1. Relatively high germination rates were observed on media containing 15 and 30% sucrose, whereas germination was low in media containing 0 and 45% sucrose. Borate had little effect on accelerating or retarding the germination rate except in the medium containing 45% sucrose where germination rate increased with increasing concentration of borate. The medium containing 30% sucrose and 0.01% borate was evaluated to optimize the pollen germination rate.

Table 2 shows the effect of temperature and light conditions on *in vitro* pollen germi-

Table 2. Effect of temperature and light conditions on *in vitro* pollen germination in asparagus 'Mary Washington 500 W'.

Temperature (°C)	Light condition	Pollen germination (%)
20	Light	59.9 ab[z]
	Dark	60.2 ab
25	Light	64.6 a
	Dark	54.9 b
30	Light	59.3 ab
	Dark	64.8 a

[z] Mean separation within a column by Duncan's multiple range test, 5% level.

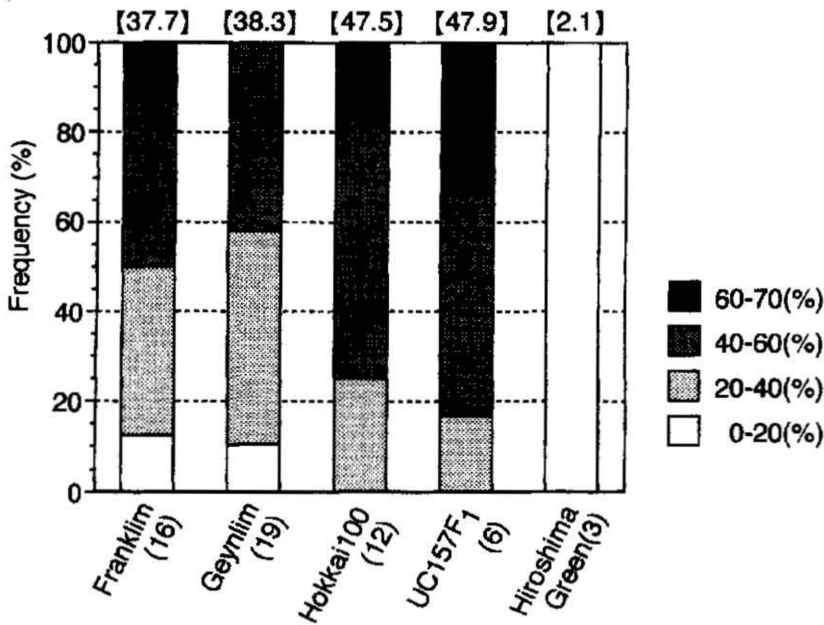

Fig. 2. Frequency distribution of individuals with indicated percentage ranges of *in vitro* pollen germination in asparagus cultivars.
( ) : No. of plants examined.
【 】 : Average percentage of pollen germination in each cultivar.

nation. There was little difference among the treatments. *In vitro* culture for evaluating pollen viability was, therefore, carried out at 25 °C under light thereafter.

Varietal difference

Figure 2 shows the variation of pollen viability measured by *in vitro* germination in five asparagus cultivars. There were no plants of which the pollen did not germinate at all. Pollen viability varied individually within each diploid cultivar. 'Franklim' showed the largest variation in pollen germination rate among individual plants from 3.0 to 66.5%. The rates in 'Hiroshima Green', a triploid cultivar, were uniformly low ranging from 0.8 to 4.2%.

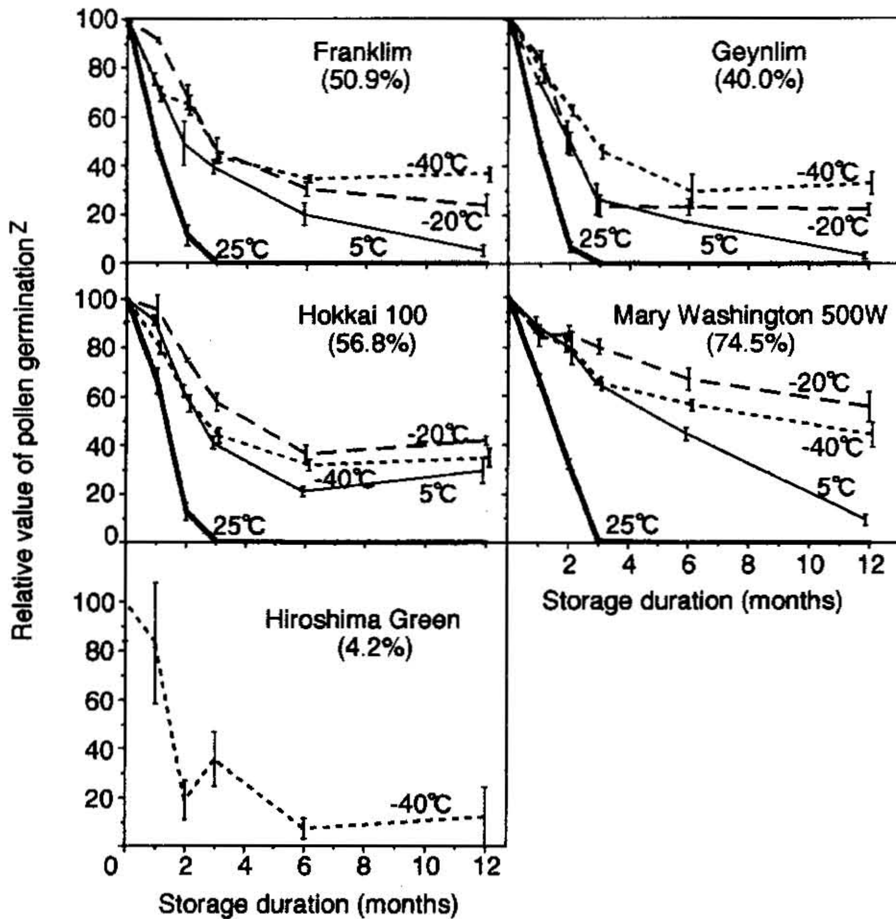

Fig. 3. Effect of storage temperature on *in vitro* pollen germination in asparagus cultivars. Values in parentheses indicate the percentage of pollen germination at the beginning of storage. Vertical bars are standard errors. [z] Value at the beginning of storage=100.

There was no significant difference within diploid cultivars in average percentage of pollen germination. Average pollen germination in the triploid cultivar, 'Hiroshima Green', was lower than that of all diploid cultivars.

Storage temperature

Pollen viability at each temperature diminished with storage in all cultivars (Figure 3).

In diploid cultivars, pollen viability declined to 0% after three months at 25 °C. It was also a sharp decline in pollen viability from zero to three months storage at other temperatures, thereafter, germination declined slowly from three to 12 months in all cultivars. Pollen viability throughout storage at 5 °C was relatively lower than at −20 and −40 °C. Optimum storage temperature for keeping pollen viability was found to be either −20 or −40 °C.

In the triploid cultivar 'Hiroshima Green' at −40 °C, pollen viability declined rapidly from zero to two months, and then more slowly. Some pollen grains of 'Hiroshima Green' were still viable after 12 months of storage.

DISCUSSION

The composition of a germination medium to obtain the optimal response has to be empirically formulated for each species. Only three constituents (sucrose, borate and calcium nitrate) are sufficient for many pollen germination systems. Although the optimal concentration of sucrose required depends on the species (approximately 5–20%), 100 mg/l borate and 300 mg/l calcium nitrate are optimal for most species studied (Shivanna and Rangaswamy, 1992; Stanley and Lichtenberg, 1963). Nevertheless, for effective *in vitro* germination it is necessary to incorporate the stigma, style, ovary and ovule in the germination medium in some species (Iwanami, 1980; Konar and Linskens, 1966). For example, pollen germination rate was improved when pistillate flowers were embedded on the basal medium in *Ficus carica* (Awamura *et al.*, 1995). The same phenomenon was observed when the stigma, style, ovary and ovule were added to media on which radish pollen was germinated (Matsubara and Miki, 1992). In asparagus, sucrose is an enough constituent for evaluating pollen viability from this experiment.

The optimum temperature for *in vitro* pollen tube growth differs with species, i.e., 30 °C for *Lilium speciosum* and *Erythrina variegata*, 10–25 °C for *Camellia japonica*, and 15–28 °C for *Impatiens balsamina* (Iwanami, 1980). Takamura *et al.* (1996) reported the effects of light and temperature on *in vitro* pollen germination in cyclamen. Light had little effect, whereas, temperature significantly affected pollen germination and pollen tube growth. Maestro and Alvarez (1988) also found temperature influenced pollen germination and pollen tube growth in muskmelon (*Cucumis melo* L.). In contrast, neither temperature nor light had much effect on *in vitro* asparagus pollen germination in the range of 20–30 °C in this study. It is considered that asparagus pollen can germinate over a relatively wide range of temperatures.

Pollen viability of triploid plants is generally lower than that of diploids because of the meiotic irregularity in many species. All the plants of the triploid cultivar 'Hiroshima Green' showed low pollen viability in this experiment. Although the diploid cultivars had

higher pollen viability than 'Hiroshima Green', some variation was observed in all the cultivars tested. Maeda *et al.* (1995) reported that the higher *in vitro* pollen germination, the more fruits and seeds were obtained in asparagus. Pollen viability, therefore, ought to be evaluated in selection of pollen parents of clonal hybrid cultivars for efficient seed production. The reason for the variation within the diploid cultivars cannot be determined from the present study. Further work is necessary to establish the relationship between meiotic behavior during microsporogenesis and pollen viability.

Snope and Ellison (1963) reported that pollen viability could be maintained for 60 weeks at −20°C. The results obtained in this study showed that pollen germination in 'Mary Washington 500W' was around 45% (relative value=60%) after three months storage at 5°C, and the relative value of pollen germination diminished to 20–60% after 12 months storage at −20 and −40°C. These results suggest that pollen storability was influenced by genotype within and/or between cultivars, and seems to be dependent on the pollen germination ability at the beginning of storage.

Pollen germination rate at one month after storage did not exhibit any difference at temperatures of 5 to −40°C in this experiment. In contrast, the pollen stored for more than two months showed lower germination rate at 5°C than at −20 and −40°C. Storage temperature under 5°C is, therefore, recommended for breeding use when the pollen is used within one month of storage. It is necessary to keep under −20°C when the pollen is to be stored for more than two months.

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