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## **Rapid Generation Scheme in Asparagus (*Asparagus officinalis* L.) Breeding: Flowering Response and Pollen Germination in the Second Cycle Flower Induction with Carbamate Treatment**

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Flower induction treatment with a carbamate compound was studied for constructing the rapid generation scheme in asparagus breeding. Viable seeds were obtained by the crosses with carbamate-induced male flowers, and the hybridity and diploidy of the offspring were confirmed by isozyme and flow cytometric analyses.

Flowering response and *in vitro* pollen germination of the plants of the second generation induced with the carbamate treatment were also investigated. Germination rates of the first and second generation seeds induced with carbamate treatments were a little different in 'Geynlim' and similar in 'Mary Washington 500W'. Percentage of flowered seedlings was, however, higher in the second cycle treatment than in the first one in both cultivars. Although great variation in pollen germination was found even with or without carbamate treatments in each population, there was no distinct difference in average percentage of pollen germination among the populations.

These results suggest that flower induction with the carbamate compound can be applied to the rapid generation scheme in asparagus breeding, e.g., to produce homogenic cultivars including both sexes in a short term for genetic and systematical breeding purposes.

### INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a dioecious perennial crop, so that it has been difficult to develop systematical breeding as compared with other seed-propagated crops as dioecy makes selfing impossible. It requires a few years from seed sowing to flowering in open field cultivation; being one of the reasons for the difficulty of systematical breeding. Ellison (1985) stated that male and female plants flower 210–235 and 269–295 days after seed sowing in a greenhouse, respectively. There are reports that flowering of one-month-old asparagus seedlings was accelerated when the seeds were treated with atrazine, diuron and carbamate compounds (Abe and Kameya, 1986; Abe *et al.*, 1987; Yanosaka *et al.*, 1989), suggesting that the treatment might be applied to select commercially preferred males at an early growth stage.

Variations of flowering response through carbamate treatment in asparagus cultivars, and the possibility of obtaining fertile pollen grains from carbamate-treated one-month-old seedlings were previously reported (Ozaki *et al.*, 1999a). We also proposed that continuous back crossing to homogenic female parents with the treatment

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enables to produce uniform isogenic (homogenic except sex expression) cultivars including both sexes in a short term.

In the current study, we discuss the rapid generation scheme with flower induction treatment, i.e., seedling production by the crosses with carbamate-induced male flowers, flowering response and pollen germination in the plants of the second generation induced through carbamate treatment.

## MATERIALS AND METHODS

### **Artificial crossing with carbamate-induced male flowers**

Seeds of 'Geynlim' and 'Mary Washington 500 W' were placed on the two layers of filter paper in a plastic petri dish (90 mm in diameter and 15 mm in height) containing 50 mg/l *n*-propyl N-(3,4-dichlorophenyl) carbamate solution, and incubated at 25 °C for 12 days. Detailed procedures of the carbamate treatment were previously reported (Ozaki *et al.*, 1999a). The seeds/seedlings were transplanted to vermiculite in plastic trays in a greenhouse. Pollen grains collected from the induced male flowers were artificially pollinated to normally-flowered female plants, and fruit and seed sets were investigated. Pollen grains collected from normally-flowered male plants in each cultivar were also used as control pollination. The seeds obtained from the crosses (the second generation) were sown and grown up in the greenhouse, and the seedlings were provided for isozyme and flow cytometric analyses.

### **Isozyme analysis**

Immature cladophylls of the seedlings were homogenized in pre-cooled extraction buffer as described by Wendel and Parks (1982). Crude extracts were immediately used for the analysis of phosphoglucomutase (PGM; EC 5.4.2.2) isozymes with horizontal starch gel. Genetic basis and allozyme determination followed the previous study (Ozaki *et al.*, 2000).

### **Flow cytometric analysis**

A Partec PA Ploidy Analyzer (Germany) equipped with DAPI filter and Multicycle for MS-DOS software was used to analyze nuclear DNA content. Pieces of cladophyll of the offspring were chopped using a razor blade in High Resolution DNA Kit Type P (Partec, Germany). Haploid, diploid, triploid and tetraploid asparagus plants were also analyzed as standards. At least 7000 nuclei were counted for each sample, and ploidy level was determined according to the prominent peak in each sample.

### **Flowering response and pollen viability in the second cycle flower induction treatment**

Mature seeds obtained from the artificial crosses as described above were immediately prepared for second cycle flower induction treatment by the same manner as above. Mature seeds from the artificial crosses with normally-flowered male plants were also treated to induce flowering as control (the first treatment). Seed germination rate was measured after the treatment and percentage of transplantable and flowering plants were investigated 45 days after the treatments. Flowering rate was calculated by dividing

number of flowering plants by number of seeds/seedlings sown. *In vitro* germination of pollen grains collected from the male flowers induced by the carbamate treatment was evaluated in each treatment in the similar manner to the previous report (Ozaki *et al.* 1999b).

## RESULTS

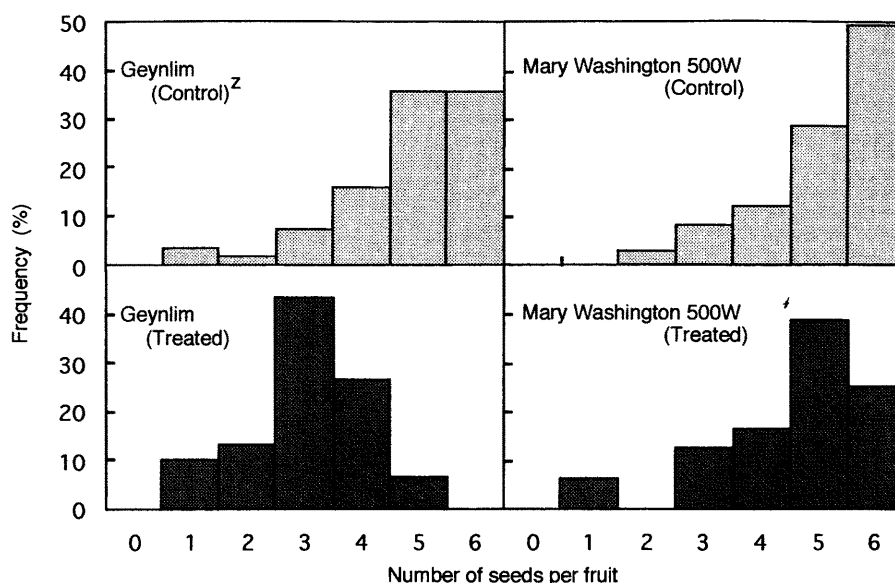
### Artificial crossing with carbamate-induced male flowers

Crosses with carbamate-induced flowers of 'Mary Washington 500W' resulted relatively high value (82.3%) of fruit set as the control crosses showed (96.6% in

**Table 1.** Results of crosses with carbamate-induced male flowers in asparagus cultivars.

Cultivar of pollen parent	Treatment	No. of flowers pollinated	No. of fruits harvested (%)	No. of seeds harvested	Average no. of seeds per fruit
Geynlim	Control	58	56 (96.6)	272	4.86
	Treated	48	30 (62.5)	92	3.07
MW500W <sup>z</sup>	Control	113	110 (97.3)	564	5.13
	Treated	96	79 (82.3)	362	4.58

<sup>z</sup> Mary Washington 500 W.

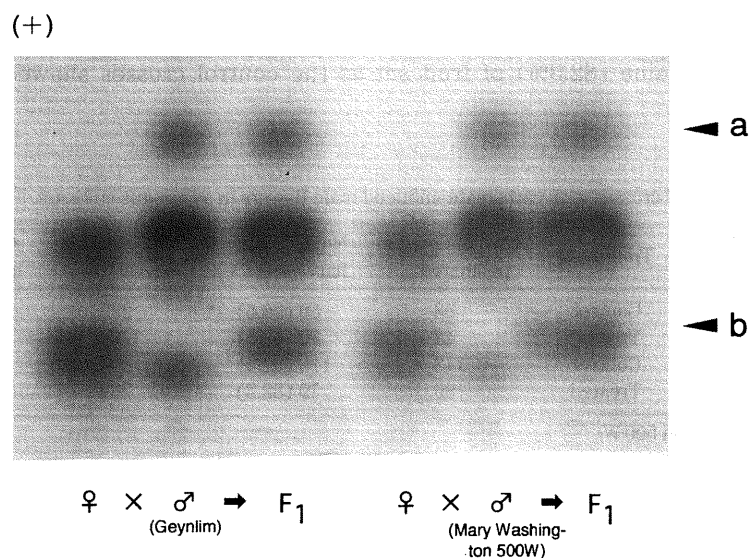


**Fig. 1.** Frequency distribution of fruits with indicated number of seeds in crosses with carbamate-induced flowers as pollen parents.

<sup>z</sup>Pollen parent cultivar and treatment.

'Geynlim' and 97.3% in 'Mary Washington 500 W'), while those of 'Geynlim' brought a little lower value (62.5%) (Table 1). Number of seeds per fruit obtained from the treated pollination was a little smaller than that from the control pollination in both cultivars (Fig. 1). 'Mary Washington 500 W' showed a little higher values in the number than 'Geynlim' in both treatments.

Hybridity of the seedlings was confirmed by the staining results for PGM isozymes



**Fig. 2.** PGM isozyme banding patterns of the offspring obtained from crosses with carbamate-induced male flowers. Arrows "a" and "b" indicate the bands derived from pollen and seed parents, respectively.

**Table 2.** Comparison of fluorescent intensity of the prominent peak in the offspring obtained from crosses with carbamate-induced male flowers.

Treatment of pollen parent	Pollen parent cultivar of seedlings	No. of seedlings	Fluorescent intensity	
			Average	Range
Control	(Pooled) <sup>a</sup>	10	104.5 ± 1.3	94.9 – 109.9
Treated	Geynlim	7	105.5 ± 1.3	98.8 – 108.6
	MW500W <sup>b</sup>	12	104.9 ± 1.1	96.4 – 109.7
Standard	Haploid	1	50.0	–
	Diploid	1	100.4	–
	Triploid	1	157.2	–
	Tetraploid	1	215.8	–

<sup>a</sup> Pooled data of seedlings obtained from control pollination with 'Geynlim' and 'Mary Washington 500 W'.

<sup>b</sup> Mary Washington 500 W.

because the offspring had both bands derived from the seed and pollen parents (Fig. 2).

Fluorescent intensity of the prominent peak was around 100 in diploid, and there was no difference among the populations (Table 2). The ranges of the values in the seedlings obtained from the treated pollination were also within the range of the values in the control.

### Flowering response and pollen viability in the second cycle flower induction treatment

No significant difference was observed in the rates of seed germination between the first and second cycle treatments in 'Mary Washington 500 W', whereas there was a little difference in 'Geynlim' (Table 3). There was no significant difference, too, in percentage of transplantable seedlings between the treatments in both cultivars evaluated from ANOVA. Seedlings from treated pollination with 'Geynlim' (second cycle treatment) revealed the highest flowering percentage, and that with 'Mary Washington 500 W' and control pollination with 'Geynlim' (first cycle treatment) followed in this order. Percentage of flowered seedlings was higher in males than in females in all the treatments.

All the flowered male seedlings except for only 10% of the control pollination with 'Mary Washington 500 W' produced viable pollen grains (Fig. 3). Variation in pollen germination was observed in each population. More than a half of the flowered male seedlings showed over 45% of pollen germination, and there was no distinct difference in the mean pollen fertility among the treatments.

## DISCUSSION

We previously clarified that the pollen grains collected from carbamate-induced male flowers of asparagus had a germinability *in vitro* (Ozaki *et al.*, 1999a) although Mizonobe *et al.* (1990) reported the pollen sterility in chemically induced flowers. In the present study, it was proven that fruit and seed sets were possible when the chemically-induced male flowers were used for artificial crossing. We have recognized that some chemically-induced flowered male seedlings presented low pollen germination in seven

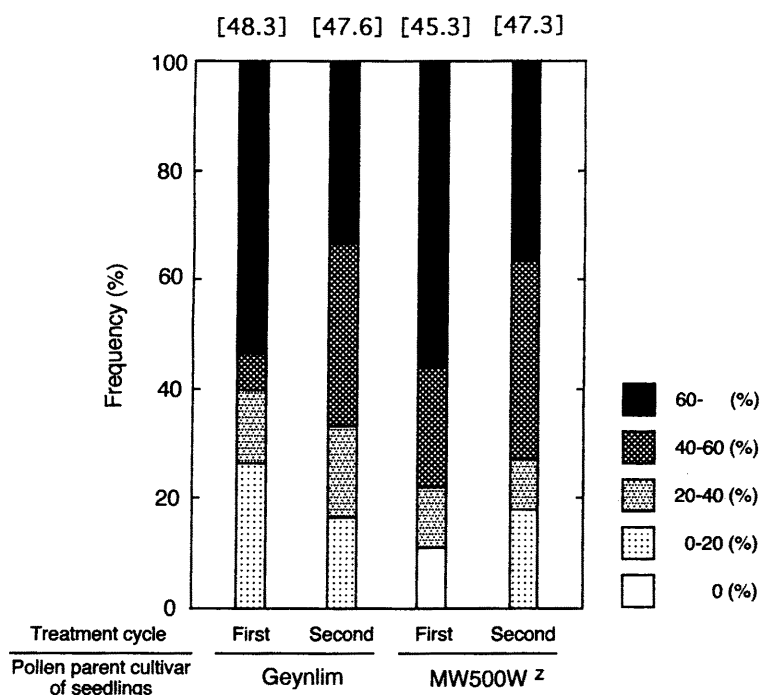
**Table 3.** Comparison of carbamate treatment cycle in flowering response of asparagus.

Pollen parent cultivar of seeds	Treatment cycle	Germination (%)	Transplantable seedlings (%)	Flowering (%)		
				Total	Male	Female
Geynlim	First	91 a	55	23 ab	18	5
	Second	97 b	80	43 c	25	18
MW500W <sup>*</sup>	First	95 b	46	8 a	7	1
	Second	95 b	60	28 bc	17	11

Analysis of variance established significant differences among treatments in germination and flowering of total plants ( $P=0.05$ ).

Means followed by the same letters within columns are not significantly different ( $P=0.05$ ) as determined by Duncan's multiple range test.

<sup>\*</sup> Mary Washington 500 W.



**Fig. 3.** Frequency distribution of individuals with indicated percentage ranges of *in vitro* pollen germination through first and second cycle carbamate treatments in asparagus.

[ ] : Average percentage of pollen germination.

<sup>z</sup> Mary Washington 500 W.

cultivars investigated among which 15 and 26% of flowered male seedlings in 'Geynlim' and 'Mary Washington 500 W', respectively, showed less than 20% of *in vitro* pollen germination (Ozaki *et al.*, 1999a). Existence of these seedlings with low pollen germinability seemed to be one of the causes of the decline of fruit and seed sets in the treated pollination in comparison with the control in the current study.

When the flower induction through carbamate treatment would be applied for rapid generation scheme, hybridity and diploidy of offspring must be certified. Existence of males in the offspring gave evidence of the hybridity, because there must be no male (Mm) seedlings if the seedlings were only originated from gametophytic or sporophytic cells of pistillate (mm) parents. Hybridity was also confirmed by isozyme analysis, and this method had an advantage over investigating individual sexuality since the analysis could be carried out from early stages of seedlings (even 2- or 3-week-old seedlings). Flow cytometric data showed diploidy of the offspring, and the similar values of pollen germination between the first and second cycle flower inductions also suggested the diploidy of the offspring.

This study has clarified that pollen germination can remain effective in the second cycle carbamate-induced flowers as well as in the first cycle, and it was also recognized in this investigation that flower induction frequency of the second cycle treatment was higher than that of the first cycle. The sensitivity of flower induction to the treatment might become higher as the treatment cycle advanced, because only flowered seedlings by the treatment were selected as the pollen parents of the next generation.

From these results, it was proved that flower induction of seedlings with the carbamate treatment enables rapid generation scheme in asparagus breeding program, and it can produce homogenic cultivars including both sexes as described in the previous report (Ozaki *et al.*, 1999a). The homogenic cultivars are useful materials for investigating regulatory mechanism of sexual differentiation by analyzing the difference of gene expression between both sexes, because the genetic factors in both sexes are almost the same except sex determinant.

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### REFERENCES

- Abe, T. and T. Kameya 1986 Promotion of flower formation by atrazine and diuron in seedlings of *Asparagus*. *Planta*, **169**: 289–291
- Abe, T., R. Shimizu, H. Iwamura and T. Kameya 1987 Flower induction by atrazine analogues in seedling of *Asparagus officinalis*. *Physiol. Plant.*, **70**: 228–230
- Ellison, J. H. 1986 Asparagus breeding. In "Breeding Vegetable Crops", ed. by M. J. Bassett, AVI Publishing Co. Westport, pp. 523–569
- Mizonobe, G., K. Yamaguchi, T. Maeda, T. Harada, T. Yakuwa and H. Iwamura 1990 Effects of s-triazine and carbamate compounds on the flowering in asparagus seedlings. *Hokkaido Engeikenkyu Danwakaihou*, **23**: 24–25 (In Japanese. Tentatively translated into English by Y. Ozaki from the original Japanese title.)
- Ozaki, Y., T. Kurahashi, T. Tashiro and H. Okubo 1999a Carbamate-induced flowering in asparagus (*Asparagus officinalis* L.) seedlings: optimization of treatment and cultivar variation in flowering response and pollen germination. *Euphytica*, **110**: 77–83
- Ozaki, Y., T. Tashiro, T. Kurahashi and H. Okubo 1999b Variation of pollen viability and storability in asparagus (*Asparagus officinalis* L.) cultivars. *J. Fac. Agr., Kyushu Univ.*, **44**: 1–8
- Ozaki, Y., T. Tashiro and H. Okubo 2000 Use of allozyme variation for evaluating genetic purity in asparagus (*Asparagus officinalis* L.) cultivars. *J. Hort. Sci. & Biotech.*, **75**: 105–110
- Wendel, J. F. and C. R. Parks 1982 Genetic control of isozyme variation in *Camellia japonica* L. *J. Hered.*, **73**: 197–204
- Yanosaka, K., H. Iwamura and T. Fujita 1989 Flower induction in seedling of *Asparagus officinalis* L. by n-phenylcarbamates. *Z. Naturforsch.*, **44c**: 226–232