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Efficient selection of a high-yield line by using somaclonal variation in Japanese butterbur (*Petasites japonicus*)

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Abstract To induce somaclonal variations related to plant yield, adventitious buds were directly regenerated from immature flowerheads of a selected line A of the 'Aichi Wase Fuki' of Japanese butterbur (*Petasites japonicus*). Among the 280 independent regenerants obtained from 300 immature flowerheads, about 2/3 were regenerated within 90 d of culture. The total yield of the randomly selected 50 lines among the early regenerants ranged from 13.8 to 21.6 $kg\,m^{-2}$ (average: 18.2 $kg\,m^{-2}$) in the field, and a high correlation ($r=-0.869$) was observed between the total yield and the period required for the third leaf initiation in flowerhead culture. A high correlation ($r=0.923$) was also observed between the yield in the field and the fresh weight of rhizomes at 3 months of culture in the greenhouse. Since the yield of the original line A was 16.4 $kg\,m^{-2}$, direct adventitious bud regeneration is a useful technique for inducing high yielding variants in Japanese butterbur, which might be efficiently selected by monitoring the period required for third leaf initiation and the weight of rhizomes.

Key words: Flowerhead culture, *in vitro* selection, *Petasites japonicus*, somaclonal variation.

Japanese butterbur (*Petasites japonicus*) is a perennial herbaceous plant belonging to the Compositae. The plant is widely distributed in temperate regions of East Asia. The petioles are mainly used as food materials in Japan. The wild plants contain both sterile triploid and fertile diploid plants, but the cultivated ones are almost sterile triploid plants, including 'Aichi Wase Fuki' and 'Mizu fuki', being propagated vegetatively by division of rhizomes (Imazu and Fujishita 1962b), and the sizes are bigger than those of the wild ones (Takagi 1994). Among the cultivars, 'Aichi Wase Fuki', which was selected from a wild population of Japanese butterbur (Imazu and Fujishita 1962a), is the most widely grown cultivar in Japan (Takagi 1994). Only this cultivar is cultivated in Osaka prefecture. Since the main cultivars are sterile triploid, it is difficult to apply cross-breeding techniques in the breeding program of triploid Japanese butterbur cultivars.

Use of somaclonal variations is one possible strategy to breed vegetatively propagated crops (Heinz and Mee 1971). Selection of somaclonal variants has successfully been used to generate cultivars in a number of plants, including apple (Donovan et al. 1994), banana (Cote et al. 1993), celery (Heath-Pagliuso et al. 1988), cucumber (Burza and Malepszy 1995; Filipecki et al. 2005), garlic (Novak et al. 1982), lettuce (Engler and Grogan 1984), peach (Hammerschlag 1990), strawberry (Swarts et al. 1981; Toyoda et al. 1991; Takahashi et al. 1992; Hammerschlag et al. 2006), and tomato (Evans and Sharp 1983; Barden et al. 1986).

In Japanese butterbur, plant regeneration has been reported to occur from apical meristems (Matsubara and Masuda 1980), shoot primordia (Murakami et al. 1988), indirect adventitious buds via calli derived from flower stalk, petiole (Morishita et al. 1980), leaf (Yabe et al. 1986b), and protoplasts (Yabe et al. 1986a), direct adventitious buds of flowerhead (Iwamoto and Kagi 1995), and axillary buds (Iwamoto and Kagi 1995). Morishita and Yamada (1981) examined plants that were regenerated from adventitious buds via calli and reported a high frequency (77.3%) of morphological variations in the regenerated plants. Seventeen of 22 variants were inferior to the original line, and all of the variants showed undesirable variations. Therefore, this technique has not been used for the propagation of commercial Japanese butterbur.

Here, we report the selection and field performance of somaclonal variants obtained through direct adventitious

Abbreviations: ArMV, arabis mosaic virus; BA, 6-benzyladenine; BuMV, butterbur mosaic virus; CMV, cucumber mosaic virus; MS, Murashige and Skoog; NAA, α-naphthaleneacetic acid.
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bud organogenesis from immature flowerhead explants as a strategy to develop a breeding program for Japanese butterbur. We also discuss the usefulness of direct adventitious organogenesis compared to indirect adventitious organogenesis from calli in this crop.

Materials and methods

Plant materials and flowerhead culture

We used immature flowerheads (0.2–0.4 mm in diameter) of a special selected line A of the cultivar 'Aichi Wase Fuki', which has higher yield and quality than the original one (Iwamoto 1999) as the source of explants. Immature flowerheads were cultured on MS solid medium supplemented with 0.1 mg l^{-1} NAA, 1 mg l^{-1} BA, and 7 g l^{-1} Agar, pH 5.8, and subcultured on the same medium at 1 month intervals. Plant regeneration via adventitious buds was conducted according to the methods described by Iwamoto and Kagi (1995).

During flowerhead culture, the following characteristics of the regenerants were recorded: the periods required for adventitious bud initiation and third leaf initiation, the number of leaves per regenerant after 3 months of culture and the petiole length of the longest leaf in each regenerant after 3 months of culture.

Evaluation of early growth characteristics

Among the regenerants, 50 randomly selected lines were each propagated by axillary bud culture and the rooted plants were acclimatized according to the method of Iwamoto and Kagi (1995). All the 50 selected lines were checked for viral infection of ArMV, BuMV, and CMV, and confirmed to be virus-free (Iwamoto, unpublished data). Twenty plants per line were transplanted in a greenhouse on 25 March 1998 and the following characteristics were recorded after 3 months of culture: the number of leaves per plant, the petiole length of the longest leaf in each plant, and the fresh weight of the rhizomes per each plant.

Evaluation of the yield of regenerants

The yield of the regenerants was evaluated after producing mature rhizomes. To prepare the mature rhizomes, greenhouse-grown plants of the 50 selected lines and the original line A were transplanted to a field on 1 September 1998 and cultivated for 10 months. On 30 June 1999, the rhizomes of each line were harvested, washed, cut into segments of 15–20 cm long, and then stored at 2°C until September 1999.

To study the yield performance, the rhizome cuttings of the 50 selected lines and the original line A were transplanted to a plastic greenhouse on 15 September 1999, and the leaves (the petioles with the blades) were harvested three times (on 11 February, 8 April, and 22 May 2000). The total weight of the leaves for evaluating the yield, number of leaves per node of mother rhizome, diameter at the petiole midpoint, petiole length, petiole color, petiole trichome density, shape of transverse section at the petiole midpoint, degree of hollowness at the petiole midpoint, easiness of petiole peeling, size of the leaf blade, shape of the leaf blade, and color of the leaf blade were recorded at each harvest. Number of sprouts per mother rhizome, thickness of the rhizome, and internode length of the rhizome were recorded after 3rd harvest. The culture practices and fertilization treatments used were similar to those used in commercial Japanese butterbur production.

Results

After 3 months of culture, 5.2 adventitious buds per explant were directly regenerated from immature flowerheads (Figure 1A, B, C) and 280 independent regenerants were obtained from 300 immature flowerheads after 280 d of culture (Figure 2). The period required for adventitious bud initiation from flowerheads varied greatly from 21 to 280 d, although 214 flowerheads (76.4%) regenerated adventitious buds within 90 d of culture (Figure 2). According to microscopic observations, they were directly regenerated from flowerhead explants. Among the 214 early regenerants, 50 lines were randomly selected and used for the evaluation of grown characteristics in the greenhouse 3 months after transfer of *in vitro* propagated plants. These lines were finally evaluated for their yield by cultivating mature rhizomes, which were obtained after one season of cultivation.

The yield of the original line A (Iwamoto 1999) was 16.4 kg m^{-2}. In contrast, the yield of the 50 lines after cultivation of the mature rhizomes in the field varied greatly from 13.8 to 21.6 kg m^{-2}, and the mean yield of the 50 lines was 18.2 kg m^{-2} (Figure 3). The yield showed a significant negative correlation with the period required for adventitious bud initiation ($r=-0.492$) and the period required for third leaf initiation *in vitro* culture ($r=-0.869$; Figure 3) which varied greatly from 44 to 180 d. The yield showed a significant positive correlation with the number of leaves per regenerant present after 3 months of culture ($r=0.618$) and the petiole length of the longest leaf in each regenerant after 3 months of culture ($r=0.556$).

In the field experiment, variation was observed among the 50 lines and the original line in the following characteristics: diameter at the petiole midpoint, petiole length, petiole trichome density, size of the leaf blade, color of the leaf blade, number of leaves per node of mother rhizome, thickness of the rhizome, and internode length of rhizome. Although it was difficult to detect such variations during *in vitro* culture, none of the 50 lines that were regenerated from flowerheads had undesirable variations like red petioles, petioles with brown spots, and abnormal morphology. Further examination will be needed to evaluate these variations using large-scale field trials.

The yield performance was also evaluated using the 21

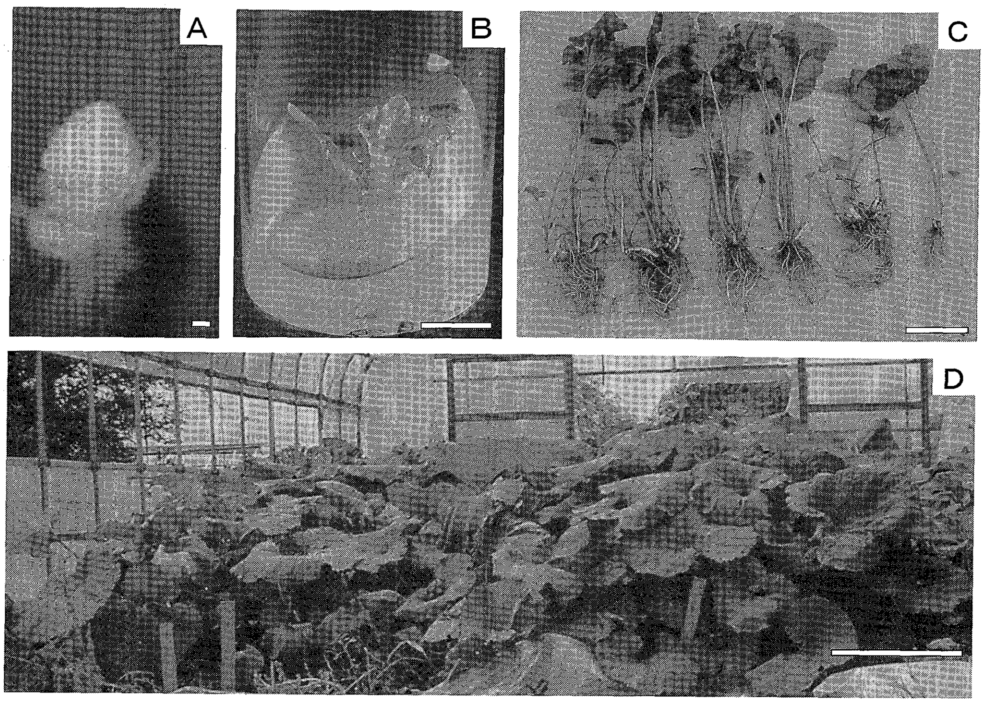

Figure 1. Selection of high-yield lines using somaclonal variation. (A) Immature flowerhead of Japanese butterbur. Bar=0.2 mm. (B) Adventitious bud regeneration from flowerhead after 70 d of culture. Bar=1 cm. (C) Rhizome proliferation after 3 months of cultivation in a greenhouse. Bar=10 cm. (D) Comparison of growth between the original line A (left) and the high-yield somaclonal variants (right). Bar=50 cm.

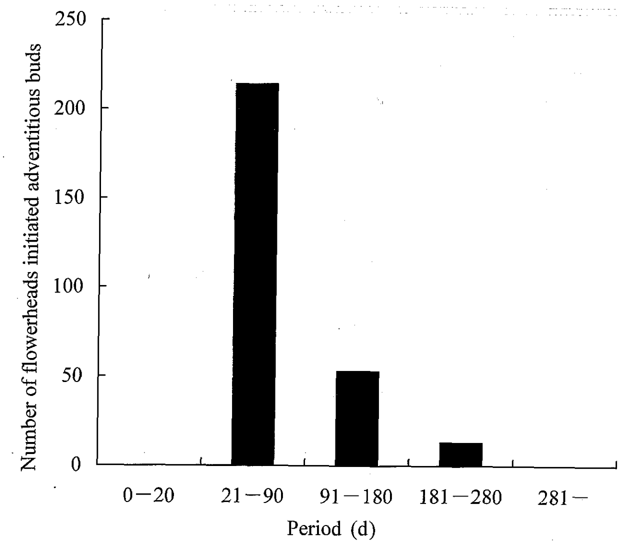

Figure 2. Frequency distribution of period required for adventitious bud initiation.

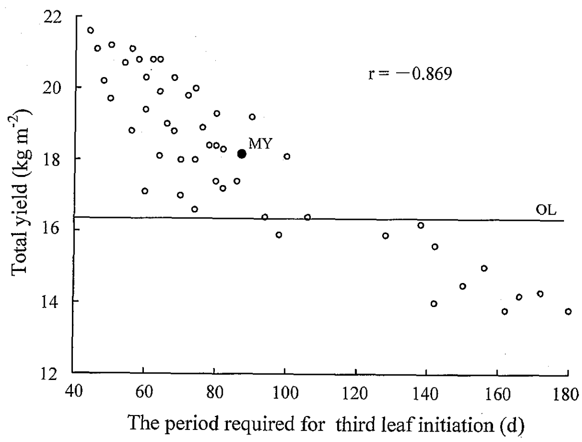

Figure 3. Relationships between the period required for third leaf initiation and the total yield. The black circle indicates the mean yield of all the lines (MY). The horizontal bar indicates the yield of the original line A (OL).

lines in which the third leaf initiated within 70 d in flowerhead culture. The yield was significantly and positively correlated with the number of leaves (r=0.661) and the petiole length of the longest leaf in each plant (r=0.627) after 3 months of cultivation in a greenhouse. The yield was also highly and positively correlated with the fresh weight of rhizomes (r=0.923; Figure 4) which varied from 96 to 198 g (Figure 1C, 4). Based on these results, we selected the line with the highest yield, which was 21.6 kg m^{-2}, as a candidate for the novel cultivar (Figure 1D, 4).

Discussion

The use of somaclonal variants is a possible strategy to breed vegetatively propagated crops (Heinz and Mee 1971). In Japanese butterbur, Morishita and Yamada (1981) observed variations in petiole length, petiole trichome density, petiole color, and yield in 22 plants that were regenerated indirectly from callus cultures. All of the variants had at least one of the undesirable variations, including red petioles, low yield, petioles with brown

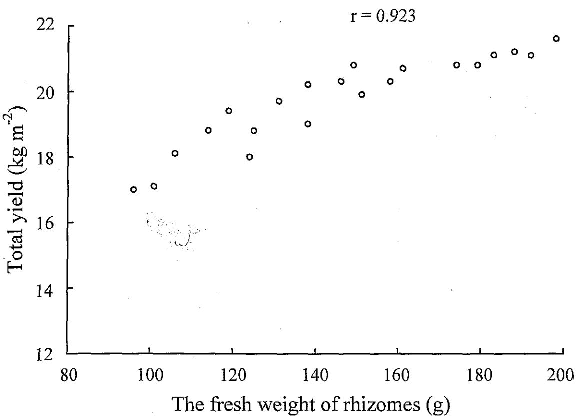

Figure 4. Relationship between the fresh weight of rhizome and the total yield. The fresh weight of rhizome was recorded after 3 months of cultivation of regenerants in the greenhouse.

spots, and abnormal rhizome morphology resembling that of horseradish (Morishita 1991). Moreover, 17 of the 22 variants were inferior to those of the original line (Morishita and Yamada 1981). Although it is important to use a regeneration method that results in a high frequency of somaclonal variation in the regenerated plants to efficiently select useful somaclonal variants (Ezura et al. 1995), in Japanese butterbur, it was difficult to control the high frequency occurrence of undesirable variation, which was observed in the regenerants from callus cultures (Morishita 1991).Thus, tissue culture technique has not been considered useful in the commercial production of Japanese butterbur.

In the present study, we obtained many high-yield lines (39 of 50 lines) compared with the original line A, that were regenerated directly from immature flowerheads without callus induction. These results indicate that direct adventitious bud regeneration induces moderate somaclonal variation compared to indirect regeneration from calli in Japanese butterbur, and that direct regeneration is superior to indirect regeneration for generating practically useful variants.

Both the number of leaves and petiole length are important factors that affect the yield. However, in the field test, neither of these factors nor the period required for adventitious bud initiation were strongly correlated with the yield (r=0.618, 0.556, and 0.492, respectively). In contrast, it is interesting that a strong correlation (r=0.869) was obtained between the yield and the period required for third leaf initiation after initiation of flowerhead culture (Figure 3). The yield also highly correlated to the fresh weight of rhizomes measured after 3 months of growth in the greenhouse among the lines which initiated the third leaf within 70 d of flowerhead culture (Figure 4).

The yield of standard 'Aichi Wase Fuki' cultivated in Osaka Prefecture is about 13.8 kg m^{-2}. The yield of the selected line A used in the present study as the original plant material was 16.4 kg m^{-2}, and the mean yield of the 50 regenerated lines was 18.2 kg m^{-2} (Figure 3). The yield of the highest yielding line was 21.6 kg m^{-2} (Figure 4). These results indicate that the selection method using flowerhead culture is a useful strategy to breed vegetatively propagated Japanese butterbur.

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