Plant Biotechnology 26, 189–196 (2009)

Invited Review

Breeding of Japanese butterbur (*Petasites japonicus*) by using flowerhead culture

Yuzuri Iwamoto*

Faculty of Agriculture, Niigata University, Niigata, Niigata 950-2035, Japan
* E-mail: iwamoto@agr.niigata-u.ac.jp Tel: +81-25-260-1320 Fax: +81-25-260-1633

Received January 16, 2009; accepted February 18, 2009 (Edited by C. Matsukura)

Abstract Japanese butterbur (*Petasites japonicus*) is a perennial herbaceous plant belonging to the Compositae. The petioles are used mainly as a human food source, and 'Aichi-Wase-Fuki,' the most widely grown cultivar, is triploid and propagated vegetatively. Growth problems have been caused by three types of wide-spreading virus, arabis mosaic virus, butterbur mosaic virus and cucumber mosaic virus in Osaka Prefecture. To establish efficient mass propagation of virus-free plants, adventitious buds were regenerated directly from immature flowerheads of Japanese butterbur. Osaka native lines of Japanese butterbur were collected from the production field, and the highest yielding clonal line was selected by comparison cultivation using virus-free plants regenerated from the collections. To induce somaclonal variation related to plant yield, adventitious buds were regenerated directly from immature flowerheads of Japanese butterbur. The total yield of the highest-yielding variant induced by somaclonal variation was 20.89 t/ha, which was 129.8% that of 'Aichi-Wase-Fuki'. Subsequently, the highest yielding variant was registered in 2002 as 'Osaka-Nougi-Ikusei No.1.' Currently, all farmers in Osaka Prefecture cultivate this high-yield cultivar 'Osaka-Nougi-Ikusei No.1.'

Key words: Flowerhead culture, *Petasites japonicus*, somaclonal variation, virus-free.

Japanese butterbur (*Petasites japonicus*), a perennial plant of the Compositae, grows wild over a wide area of Japan, reaching from Hokkaido to Kyushu (Imazu and Fujishita 1962; Takagi 1994). Alongside *Angelica keisukei*, *Aralia cordata*, *Oenanthe javanica*, *Cryptotaenia japonica* and a few others, Japanese butterbur is one of the few examples of original produce. Japanese butterbur has a long history of consumption, with the earliest recorded mention of the plant appearing on mokkan (wooden tablets used as shipping tags) excavated from the mansion of Prince Nagaya (684–729) in the Heijo Palace of the Nara Era (Nara National Research Institute for Cultural Properties 1993) (Figure 1). Since then, the plant has been valued for its characteristic fragrance, bittersweet flavor and rapid growth, and is indispensable at festive occasions. It is a custom in Japan to eat Japanese butterbur on New Year's Day and on the Girls' (dolls') Festival on March 3 as part of a prayer for the growth of the family. As its flower buds appear in early spring, the vegetable is much esteemed as a harbinger of the spring season.

Japanese butterbur has long been cultivated in Osaka Prefecture, as far back as the Muromachi Era (1336–1573). Existing records show that in 1911 the plant was cultivated over a 17.2 ha area in Osaka City; this area had expanded to 27.5 ha by 1915 (Masui 1984). The plant, now cultivated principally in the Senshu region in the south of Osaka Prefecture, was introduced in the form of 'Kawachi-Fuki' ('Aojiku-Mizufuki') to what was then Kishima Village (now Kaizuka City) in 1910. The cultivar now under cultivation, 'Aichi-Wase-Fuki', was introduced to Kishima Village from Aichi Prefecture around 1926 (Masui 1984; Tanaka 1993). At that time, cultivation was restricted to a select number of producers, and the plant did not leave the region until around 1940, when it began to spread steadily on account of its profitability (Tanaka 1993). According to the publication entitled 'The Horticulture of Osaka' of the Osaka Statistics Information Office of the Kinki Regional Agricultural Administration Office, the production of Japanese butterbur in Osaka Prefecture was the highest in Japan from 1960 to 1973. After 1973 however, the production of Japanese butterbur in Osaka Prefecture has been falling steadily due to conversion of land for residential use, ageing of Japanese butterbur producers and declining demand. But, even at present, Osaka Prefecture is one of the four major producing areas in Japan alongside Aichi, Gunma, and Tokushima

Abbreviations: AlMV, alfalfa mosaic virus; ArMV, arabis mosaic virus; BA, 6-benzyladenine; BuMV, butterbur mosaic virus; CMV, cucumber mosaic virus; MS, Murashige and Skoog; NAA, α-naphthaleneacetic acid
This article can be found at http://www.jspcmb.jp/

Prefectures. Japanese butterbur from Osaka Prefecture has garnered much praise for its soft, juicy and crisp nature, with the ‘Osaka-Fuki’ brand traded at high prices. Furthermore, it can be shipped from November to June, and has therefore brought producers large profits. However, declining productivity and quality have been observed in various locations in the Senshu region from around 1980, and since the early 1990s many producers have expressed their wish to give up cultivation, citing inability to harvest good produce. The lack of desire to cultivate the Japanese butterbur has reduced producer numbers, and the problem has become increasingly serious.

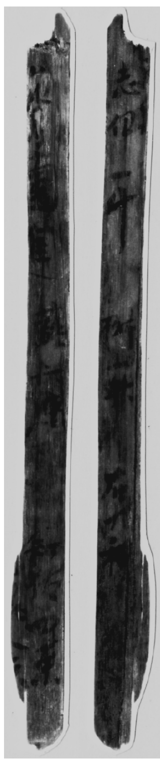

Figure 1. The *Mokkan* excavated from the mansion of Prince Nagaya (Possession of Nara National Research Institute for Cultural Properties). It was written with 6 bundles of Japanese butterburs and 4 bundles of cutting lettuces given from the Yamashiro vegetable garden.

The most commonly grown variety, ‘Aichi-Wase-Fuki’, was introduced from Aichi Prefecture in the 1920s and has now been cultivated for over 80 years. Throughout this time, selection and interchange of rhizomes has been conducted by individual producers in efforts to maintain productivity; however, production fields have become fixed with the introduction of protected cultivation, and repeated cultivation over several years in these fields has resulted in the aging of rhizomes. Furthermore, viral epidemics are appearing due to transmission by aphids and vegetative propagation by division of rhizomes. Complex infection of BuMV, CMV, and ArMV has been frequently found. Hence, these plants display mosaic symptoms immediately upon germination (Figure 2A). This results in decreased productivity and quality and has become an increasingly serious problem (Figure 2B). In the present review, the efficient production of virus-free plants and the breeding of a high-yield line by using somaclonal variation in Japanese butterbur are summarized.

Mass propagation of virus-free plants of Japanese butterbur using flowerhead culture

In a study of Japanese butterbur harvested from cultivation fields in Hiroshima, Ishikawa and Chiba prefectures (Tochihara and Tamura 1976), CMV was extracted from 84%, and BuMV was extracted from 77% of harvested plants, the majority of which were complex-infected with CMV and BuMV. The study reported that ArMV and AlMV had been extracted from 35% and 12%, respectively, of the harvested plants, and no virus-free plants were found. In a study of viral infection of Japanese butterbur in Osaka Prefecture conducted by Nakasone (unpublished data), CMV, ArMV, and BuMV were extracted from 60%, 80%, and 100%, respectively,

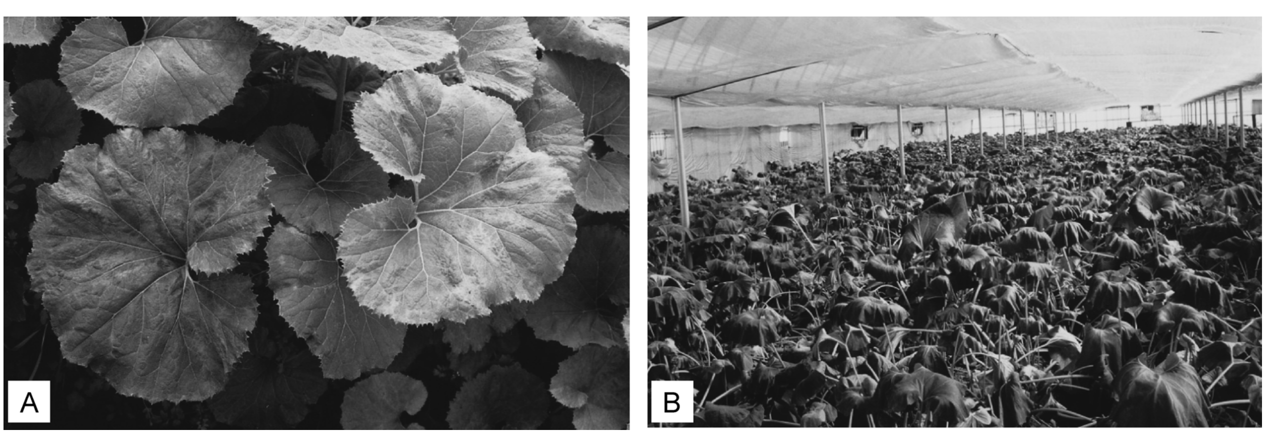

Figure 2. Stunting in vegetative growth by the effect of viral infection in Japanese butterbur. (A) Typical mosaic symptom by the complex infection of BuMV and CMV. (B) Wilting symptom by the complex infection of BuMV and CMV.

of the plants studied. All three viruses were found to exist simultaneously in 50% of plants, a complex infection of two viruses was found in 90%, and none of the plants were free of viral infection.

It has been reported that apical meristem culture has previously been conducted with the aim of producing virus-free plants. However, the frequency of microorganism contamination is very high due to the apical meristems present on the rhizomes in the soil (Matsubara and Masuda 1980, Morishita and Yamada 1979, Murakami et al. 1988). Morishita and Yamada (1979) have reported that some kinds of actinomycetes were found in cultured explants at a high level (70–87%), that growth was slow in those apical meristems that survived, and that shoot regeneration took at least 34 d, with slower plants requiring 10 months. Matsubara and Masuda (1980) have reported that tissues escaping microbial contamination often showed browning due to powerful sterilization. Furthermore, in some plants regenerated from apical meristem, it was not possible to remove the viruses. Hence, many problems remain with apical meristem culture of Japanese butterbur. Alternatively, Morishita et al. (1980) and Yabe et al. (1986) used petioles and leaf blades with low risk of microbial contamination as the explants for tissue culture. Plant regeneration was reported to occur from indirect adventitious buds via calli derived from the petioles and leaf blades. However, Yabe et al. (1986) has reported problems with this method; it is difficult to exclude ArMV from callus culture, and long-term culture of more than 1 year is necessary in order to remove BuMV. Morishita and Yamada (1981) have reported that such long-term culture reduces the regeneration rate of adventitious buds via calli and causes morphological variations in regenerated plants.

As another method, the author developed the flowerhead culture in order to efficiently produce virus-free plants that were regenerated directly from immature flowerheads (0.2–0.4 mm in diameter) without callus induction. The period required for plant regeneration from flowerheads is shorter than that obtainable using apical meristem. Furthermore, the plant regeneration rate is higher. In addition, difficult operations such as extraction of the apical meristem are not required and it is easy to extract aseptic flowerheads (Iwamoto and Kagi 1995). In addition, clonal propagules were rapidly obtained when 2 cm shoots were transferred to MS liquid medium (Murashige and Skoog 1962) supplemented with 0.01 $mg\,l^{-1}$ NAA, 3 $mg\,l^{-1}$ BA, and 30 $g\,l^{-1}$ sucrose (pH 5.8) and placed on a gyratory rotary shaker set at 100 rpm. After 8 weeks of culture, 80.9 shoots from axillary buds were obtained (Iwamoto and Kagi 1995). Although Japanese butterbur propagates vegetatively by division of rhizomes, the low reproductive rate of the rhizomes presents a problem for cultivation. The flowerhead culture system appears to be an efficient means of increasing reproductive rate by supplying virus-free seedlings to cultivation fields of Japanese butterbur.

Collection of clonal lines and selection of highest yielding clonal line using virus-free plants

Following an inspection of Japanese butterbur cultivation fields in Aichi Prefecture in 1926, Japanese butterbur producers Rihei Tanaka and Gon'ichi Tanaka brought back a box of rhizomes to their home in Kishima Village. At that time, 'Kawachi-Fuki' ('Aojiku-Mizufuki') was being cultivated in the region. However, the introduced rhizomes gave rise to high-yielding plants with faster germination and excellent petiole growth. These qualities earned the new clonal line a good reputation, which caused it to spread over the entire Senshu area. The clonal line was the 'Aichi-Wase-Fuki' cultivated in Osaka Prefecture (Tanaka 1993).

The Isewan Typhoon of 1959 brought extensive damage to the Japanese butterbur-producing fields of Aichi Prefecture, with salt damage causing a large reduction in planting (Ishiguro 1981). It is said that at that time, producers from Kishima who had heard of the damage caused by the typhoon took dozens of trucks' worth of rhizomes to the region to repay the favor they had received many years previously (Tanaka 1993). Having returned to its original home, the 'Aichi-Wase-Fuki' gained a widespread reputation for its excellent qualities and was brought into Aichi Prefecture from then on, eventually supplanting even the clonal lines in regions that had not been severely affected by the typhoon (Ishiguro 1981). Furthermore, rhizomes which were left after transplanting were sold from Hokkaido to Kyushu through refrigeration companies, and the rhizomes were used to form several producing areas, including Tokushima (one of the four major producing areas), the medium-scale producing region Hyogo (Awaji, Ichinomiya), and Fukuoka.

The clonal line introduced to Kishima thus spread through the entire Senshu region of Osaka Prefecture, then on throughout Japan. Selection and interchange of rhizomes took place between producers, selection of the clonal line advanced significantly, and there was little difference between clonal lines. Given the historical background of the plant, the general consensus at the time was that, if the plants were made virus-free, they would be almost identical. However, the differences between the clonal lines had not been investigated sufficiently. Furthermore, all of the 'Aichi-Wase-Fuki' clonal lines in Osaka were infected with viruses, and no investigation of the differences between the clonal lines taking into account the effects of viral infection had been carried out. Hence, to select breeding materials for high-yield clonal lines, the author collected and carried out comparative cultivation of clonal lines and then carried out comparative cultivation of virus-free lines.

With the cooperation of the Fuki Production and Shipment Association of JA Osaka Senshu, 20 clonal lines were collected in Osaka Prefecture. A preliminary study of these allowed the author to narrow this number down to 5. Rhizomes from these 5 clonal lines were transplanted to a plastic greenhouse in Sen-nan. The culture practices and fertilization treatments used were similar to those used in commercial Japanese butterbur production. Furthermore, virus-free rhizomes were prepared from the 5 clonal lines using flowerhead culture and cultivated under the same conditions as the virus-infected clonal lines.

As a result, total yield for each of the five clonal lines was as follows: the largest yield was found with line A, at 17.4 kg m^{-2}, followed by lines B, C, and E. Line D had the lowest yield at 10.5 kg m^{-2}. The total yield of clonal line A was 1.66 times of that of clonal line D. Clonal line A had significantly higher yield and clonal line D had significantly lower yield than the other four (Iwamoto 1999). Following the production of virus-free plants from 5 clonal lines, all lines showed increased yield and the difference in yield between five lines decreased. However, there were differences between the lines even when using virus-free plants; virus-free line A had the highest yield at 18.3 kg m^{-2}, followed by virus-free lines B, C, and E. Virus-free line D had the lowest yield at 14.9 kg m^{-2}. The total yield of virus-free line A was 1.23 times that of D, and differed significantly (Iwamoto 1999).

In the test to compare yields between the 5 clonal lines and the virus-free plants produced from them, the tendency of differences in yield between the 5 lines was consistent. The results obtained when using virus-free plants that were free from damage caused by viral infection or from aging of the rhizomes indicate that there is wide genetic variation in the Japanese butterbur produced in Osaka Prefecture. In other words, bud mutation in Japanese butterbur occurs frequently in nature; this, coupled with producer-specific selection, is a potential cause of the unnoticed spread of differences between the clonal lines. Accordingly, the highest yielding clone (line A) was selected as the source of breeding material.

Efficient selection of a high-yield cultivar by using somaclonal variation

Plants can propagate vegetatively through cuttings, runners, suckering, and tubers, and produce clones by a means very common in nature. In the past, researchers have skillfully taken advantage of these propagation capabilities by isolating and culturing plant cells and protoplasts under sterile conditions, then successfully using them to regenerate complete plants (Nagata and Takebe 1971). The first researchers to work in this research field, believing regenerated plants to be genetically uniform clones, conducted considerable research using clonal propagation. After it became evident that several characteristic variations existed (Heinz and Mee 1971), research was actively conducted into breeding the variants.

Carlson (1973) has reported adding a toxic analog (methionine sulfoximine) of wildfire of tobacco (*Pseudomonas syringae* pv. *tabaci*) to the protoplast culture medium of a haploid tobacco plant, and regenerating methionine sulfoximine-resistant mutants of tobacco from the survivors. Building on this pioneering work, the plant cells were exposed to germ toxins of pathogenic microorganisms and various stressors, with the result that disease-resistant and salt-tolerant cells were obtained from many of the plant species tested. However, non-resistant plants began to appear among the plants regenerated from resistant cells, and the number of undesirable variations began to increase. As a result, researchers turned their attention to selection of the regenerated plants themselves. In other words, selection took place not at the cellular level, but on the basis of variations apparent in the regenerated plants (Larkin and Scowcroft 1981). This selection method is known as the somaclonal variation, and although there are limitations to the parameters of selection, the method does allow for reliable extraction of target breeding material. 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 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 in order 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, the tissue culture technique has not been considered useful in the commercial production of Japanese butterbur. In light of this, the author decided to use the flowerhead culture system that involves regeneration directly from immature flowerheads without callus induction (Iwamoto and Kagi 1995).

As the source of explants, 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 clonal lines cultivated in Osaka Prefecture (Iwamoto 1999). Immature flowerheads were cultured on MS solid medium (Murashige and Skoog 1962) 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. In addition, 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 were confirmed to be virus-free (Iwamoto, unpublished data). Twenty plants per line were transplanted to a greenhouse on March 25, 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 plant. In addition, 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 September 15, 1999, and the leaves (the petioles with the blades) were harvested three times (on February 11, April 8, and May 22, 2000). The total weight of the leaves for evaluating the yield was recorded at each harvest.

After 3 months of culture, 5.2 adventitious buds per explant were directly regenerated from immature flowerheads and 280 independent regenerants were obtained from 300 immature flowerheads after 280 d of culture. 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 (Iwamoto et al. 2007). Microscopic observations revealed they were directly regenerated from flowerhead explants. Among the 214 early regenerants, 50 lines were randomly selected and used for the evaluation of growth 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}. 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 ($r=-0.869$), which varied greatly from 44 to 180 d (Iwamoto et al. 2007). 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 ($r=0.556$).

The yield performance was also evaluated using the 21 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 ($r=0.923$), which varied from 96 to 198 g (Iwamoto et al. 2007). Based on these results, we selected the line with the highest yield, which was 21.6 kg m^{-2}, as a candidate for a novel cultivar.

Use of somaclonal variations is one possible strategy for breeding 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 (Swartz 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). Although it is important to use a regeneration method that results in a high frequency of somaclonal variation in the regenerated plants so as 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, the tissue culture technique has not been considered useful in the commercial production of Japanese butterbur. Therefore, 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 affecting the yield. However, in the field test, neither of these factors nor the period required for adventitious bud initiation was strongly correlated with the yield (r=0.618, 0.556, and 0.492, respectively). In contrast, it is interesting that a strong negative correlation (r=−0.869) was obtained between the yield and the period required for third leaf initiation after initiation of flowerhead culture. The yield was also highly correlated with 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.

Breeding method and main characteristics of a new variety of Japanese butterbur 'Osaka-Nougi-Ikusei No.1'

Based on the yield data in the production fields, we selected the highest-yielding variant in 1999, which was eventually recommended to Osaka Prefecture as a suitable Japanese butterbur variety and thereafter officially registered as 'Osaka-Nougi-Ikusei No.1' by the Ministry of Agriculture, Forestry and Fisheries of Japan. In 2002, 'Osaka-Nougi-Ikusei No.1' was assigned the variety registration No.10632.

Agronomic characteristics

To evaluate the agronomic characteristics of the new variety, mature rhizomes of 'Osaka-Nougi-Ikusei No.1' and 'Aichi-Wase-Fuki' were cultivated in a field at the Agricultural, Food and Environmental Sciences Research Center of Osaka Prefecture, Habikino, for 2 years (1998–1999). Preparation of mature rhizomes, culture practices, fertilization, and fungicide and insecticide treatments were similar to those used in the cultivation method applied for the characteristic test of Japanese butterbur.

The number of sprouts per mother rhizome of 'Osaka-Nougi-Ikusei No.1' was 5.7, which was 1.1 sprouts higher than that of 'Aichi-Wase-Fuki'. The number of leaves per node of a mother rhizome of 'Osaka-Nougi-Ikusei No.1' was 5.8, which was 0.7 leaves higher than that of 'Aichi-Wase-Fuki'. The petiole length was 71.0 cm, which was 10.7 cm longer than that of 'Aichi-Wase-Fuki'. The diameter at the midpoint of the petiole was 11.5 mm, which was 1.0 mm larger than that of 'Aichi-Wase-Fuki' (Figure 3). The trichome density on the petioles of 'Osaka-Nougi-Ikusei No.1' and 'Aichi-Wase-Fuki' was classified as slightly low and medium, respectively; the degree of green color of the leaf blades was classified as deep and medium, respectively; and the rhizome thickness was classified into thick and medium, respectively. The number of flower-stalk buds per mother rhizome of 'Osaka-Nougi-Ikusei No.1' was 4.2, which was 0.7 buds higher than that of 'Aichi-Wase-Fuki'. The timing of leaf sprouting of 'Osaka-Nougi-Ikusei No.1' occurred earlier and the heat tolerance was slightly higher than that of 'Aichi-Wase-Fuki' (Iwamoto and Nakasone 2007).

Figure 3. Comparison of growth between 'Osaka-Nougi-Ikusei No.1' (left) and the original line A (right).

Yield and quality evaluation

To study yield performance, the rhizomes of 'Osaka-Nougi-Ikusei No.1' and 'Aichi-Wase-Fuki' were transplanted to a plastic greenhouse in Sen-nan on September 15, 1999, and the leaves (petiole with blades) were harvested three times (on February 11, April 8 and May 22, 2000). The culture practices and fertilization treatments were similar to those applied in commercial Japanese butterbur production using plastic greenhouses. The yield of the first, second and third harvests of 'Osaka-Nougi-Ikusei No.1' was 8.62, 8.06 and 4.21 t/ha, values which were 1.88, 1.99 and 0.93 t/ha higher than those of 'Aichi-Wase-Fuki', respectively (Table1, Figure 4). Therefore, the total yield was 20.89 t/ha, which was 129.8% that of 'Aichi-Wase-Fuki' (Iwamoto and Nakasone 2007).

Japanese butterbur harvests for market are classified into "high grade" and "middle grade" according to the market standard. The high-grade component rate of 'Osaka-Nougi-Ikusei No.1' was 91.3%, which was 5.8% higher than that of 'Aichi-Wase-Fuki' (Iwamoto and Nakasone 2007). A higher grade enhances the profit margin for farmers because the high-grade price is twice that of the middle grade. The moisture content of the

Figure 4. Field performance of 'Osaka-Nougi-Ikusei No.1' cultivated in the commercial plastic greenhouse.

Table 1. Yield and quality evaluation of 'Osaka-Nougi-Ikusei No.1' and 'Aichi-Wase-Fuki' (Iwamoto and Nakasone 2007).

Characteristics	Varieties	
	'Osaka-Nougi-Ikusei' No.1'	'Aichi-Wase-Fuki'
1st harvest (t/ha)	8.62 [a1]	6.74 [b]
2nd harvest (t/ha)	8.06 [a]	6.07 [b]
3rd harvest (t/ha)	4.21 [a]	3.28 [b]
Total yield (t /ha)	20.89 [a]	16.09 [b]
High-grade component rate (%)	91.3 [a]	85.5 [b]
Petiole moisture content (%)	95.23 [a]	94.40 [b]

[1] Within the same characteristics, means followed by different letters (a and b) indicate significant differences at 5% level by Tukey test.

petiole was 95.23%, which was 0.83% higher than that of 'Aichi-Wase-Fuki' (Table 1). In addition, the new variety displayed a good food performance, because the petiole is soft, juicy and crisp.

Conclusion

Since 1999, 'Osaka-Nougi-Ikusei No.1' has been distributed among Japanese butterbur producers in Osaka Prefecture by the Osaka seeds and seedlings supply industry. In 1999, the total volume of Osaka Prefecture-produced Japanese butterbur traded at the Osaka Prefectural Central Wholesale Market, Osaka Municipal Central Wholesale Market and Osaka Municipal East Wholesale Market was 580.2 t. As 'Osaka-Nougi-Ikusei No.1' spread, the total volume increased steadily, reaching 615.4 t in 2000 and 643.3 t in 2001. At present, all Osaka producers of Japanese butterbur cultivate the new cultivar 'Osaka-Nougi-Ikusei No.1'. Furthermore, the quality of 'Osaka-Nougi-Ikusei No.1' deteriorates little during transportation, and keeps for long periods of time. These characteristics make Japanese butterbur favorable as a foodstuff. Furthermore, the plant is pleasing to the bite, and not only is it delicious when used in cooking staple dishes, it is well suited to use in salads and on rice—new menu possibilities unthinkable until now. The leaf blade of Japanese butterbur is hard and thus has traditionally been used solely for boiling in soy sauce, with the majority being discarded unused. However, the leaf blades of 'Osaka-Nougi-Ikusei No.1' are suitable as a foodstuff, being soft and without the usual harsh taste. In addition, the dark green leaf blades contain large amounts of the strong antioxidant fukinolic acid, 3,4,5-tricaffeoylquinic acid and other phenolic constituents (Watanabe et al. 2007), as well as vitamins. The leaf veins of the plant are soft and have a texture when bitten similar to that of the leaves of the Japanese radish. The author is now pursuing further research into the use of the Japanese butterbur as a novel foodstuff.

Acknowledgements

I thank Mr. Kusuhiko Dohgu for assistance during the field experiments. I also thank Mr. Norihiro Tanigawa, Mr. Kazuhiro Sarashi, Mr. Tetsuya Tuji, and Mr. Hajime Fujioka of the Osaka Prefectural Senshu Office for Agriculture Forestry Promotion and Nature Conservation for previous arrangements regarding the field experiment.

References

Barden KA, Smith SS, Murakishi HH (1986) Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. *Plant Sci* 45: 209–213

Burza W, Malepszy S (1995) Direct plant regeneration from leaf explants in cucumber (*Cucumis sativus* L.) is free of stable genetic variation. *Plant Breed* 114: 341–345

Carlson PS (1973) Methionine sulfoximine-resistant mutants of tobacco. *Science* 180: 1366–1368

Cote FX, Sandoval J, Marie P, Auboiron E (1993) Variations in micropropagated bananas and plantains: literature survey. *Fruits* 48: 15–23

Donovan AM, Morgan R, Valobra-Piagnani C, Ridout MS, James DJ, Garrett CM (1994) Assessment of somaclonal variation in apple. I. Resistance to the fire blight pathogen *Erwinia amylovora*. *J Hort Sci* 69: 105–113

Engler DE, Grogan RG (1984) Variation in lettuce plants regenerated from protoplasts *J Hered* 75: 426–430

Evans DA, Sharp WR (1983) Single gene mutations in tomato plants regenerated from tissue culture. *Science* 221: 949–951

Ezura H, Amagai H, Kikuta I, Kubota M, Oosawa K (1995) Selection of somaclonal variants with low-temperature germinability in melon (*Cucumis melo* L.). *Plant Cell Rep* 14: 684–688

Filipecki M, Wisniewska A, Yin Z, Malepszy S (2005) The heritable changes in metabolic profiles of plants regenerants in different types of *in vitro* culture. *Plant Cell Tiss Org Cult* 82: 349–356

Hammerschlag FA (1990) Resistance responses of plants regenerated from peach callus cultures to *Xanthomonas campestris* pv. *pruni*. *J Amer Soc Hort Sci* 115: 1034–1037

Hammerschlag FA, Garces S, Koch-Dean M, Ray S, Lewers K, Maas J, Smith BJ (2006) *In vitro* response of strawberry cultivars and regenerants to *Colletorichum acutatum*. *Plant Cell Tiss Org Cult* 84: 255–261

Heath-Pagliuso S, Pullman J, Rappaport L (1988) Somaclonal variation in celery: Screening for resistance to *Fusarium oxysporum* f. sp. *apii*, race 2. *Theor Appl Genet* 76: 976–981

Heinz DJ and Mee GWP (1971) Morphologic, cytogenetic, and enzymatic *variation* in *Saccharum* species hybrid clones derived from callus tissue. *Amer J Bot* 58: 257–262

Imazu T and Fujishita N (1962) Morphological, ecological, and cytological studies on cultivated and wild butterburs, *Petasites japonicus* Maxim. III On the pollen- and seed-fertilities. *J Japan Soc Hort Sci* 31: 23–29 (in Japanese with English summary)

Ishiguro K (1981) Japanese butterbur. In: Suzuki T (ed) *Aichi Prefecture Horticultural Development History*. Aichi Prefecture Horticultural Development History Compilation Association, Aichi (in Japanese)

Iwamoto Y (1999) Production of a favorable line of Japanese butterbur (*Petasites japonicus* Fr. Schmidt) by *in vitro* selection. *Kinki J Crop Sci Breed* 44: 73–76 (in Japanese with English summary)

Iwamoto Y, Kagi T (1995) Mass-propagation of virus-free plants of Japanese butterbur (*Petasites japonicus* Fr. Schmidt) by tissue culture. *J Japan Soc Hort Sci* 64: 103–111 (in Japanese with English summary)

Iwamoto Y, Nakasone W (2007) New Japanese butterbur variety with high yield and high quality, 'Osaka-Nougi-Ikusei No.1'. *Breed Sci* 57: 249–251

Iwamoto Y, Nakasone W, Ezura H (2007) Efficient selection of a high-yield line by using somaclonal variation in Japanese butterbur (*Petasites japonicus*). *Plant Biotechnol* 24: 289–293

Larkin PJ, Scowcroft WR (1981) Somaclonal variation–a novel source of variability from cell cultures for plant improvement. *Theor Appl Genet* 60: 197–214

Masui S (1984) Production and cultivation technique of the vegetable. In: The Agricultural Council of Osaka Prefecture (ed) *Agricultural History of Osaka Prefecture* Osaka. Osaka Prefectural Goverment, Osaka, pp 298–334 (in Japanese)

Matsubara S, Masuda T (1980) Production of virus free plant of *Petasites japonicus* Fr. Schmidt (Compositae) by apex culture. *Sci Rep Fac Agr Okayama Univ* 56: 21–28 (in Japanese with English summary)

Morishita M (1991) Study of tissue culture and its practical application of butterbur. *PhD Thesis, Kobe University*, Kobe, pp 1–114 (in Japanese with English summary)

Morishita M, Kagi T, Yamada K (1980) Mass production of virus free butterbur (*Petasites japonicus* Miq) from callus derived from the pith tissue of the scane and petiole *in vitro*. *Bull Osaka Agr Res Cent* 17: 1–6 (in Japanese with English summary)

Morishita M, Yamada K (1979) Organic differentiation from shoot apex and callus of Japanese butterbur. *Kinki J Crop Sci Breed* 24: 25–30 (in Japanese)

Morishita M, Yamada K (1981) Variation of characters in the plant regenerated from callus of butterbur (*Petasites japonicus* Miq.) *Bull Osaka Agr Res Cent* 18: 9–18 (in Japanese with English summary)

Murakami A, Tanaka R, Taniguchi K (1988) Clonal mass-propagation of *Petasites japonicus* by using shoot primordium method. *Japan J Breed* 38 (Suppl 2): 86–87 (in Japanese)

Murashige T, Skoog F (1962) A revised medium for rapid growth bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473–497

Nagata T, Takebe I (1971) Planting of isolated tobacco mesophyll protoplasts on agar medium. *Planta* 99: 12–20

Nara National Research Institute for Cultural Properties (1993) The fourth report of the wooden tablets excavated from the mansion of Prince Nagaya. *The Preliminary Report of the Wooden Tablets from the Nara Palace Site* 27: 5 (in Japanese)

Novak FJ, Havel L, Dolezel J (1982) *In vitro* breeding system of *Allium*. In: Fujiwara A (ed) *Plant Tis Cult* 1982. Maruzen, Tokyo, pp 273–298

Swartz HJ, Galletta GJ, Zimmerman RH (1981) Field performance and phenotypic stability of tissue-culture-propagated strawberries. *J Amer Soc Hort Sci* 106: 667–673

Takagi H (1994) Japanese Butterbur, Fuki. In: Konishi K, Iwahori S, Kitagawa H, Yakuwa T (eds) *Horticult Jpn.* Asakura Publishing, Tokyo, pp 63–66

Takahashi H, Takai T, Matsumoto T (1992) Resistant plants to *Alternaria alternata* strawberry pathotype selected from calliclones of strawberry cultivar Morioka-16 and their characteristics. *J Japan Soc Hort Sci* 61: 323–329

Tanaka H (1993) *A private inquiry of my home town 'Kinoshima'*. Sekkasen-bunko, Kaizuka (in Japanese)

Tochihara H, Tamura M (1976) Viruses in Japanese butterbur (*Petasites japonicum* Miq.) *Ann Phytopath Soc Japan* 42: 533–539 (in Japanese with English summary)

Toyoda H, Horikoshi K, Yamano Y, Ouchi S (1991) Selection for *Fusarium* wilt disease resistance from regenerants derived from leaf callus of strawberry. *Plant Cell Rep* 10: 167–170

Yabe K, Sakurai Y, Iida T, Washida S (1986) Production of Japanese butterbur (*Petasites japonicus* Fr. Schmidt) virus free plants by leaf and petiole tissue culture. *Res Bull Aichi Agric Res Ctr* 18: 102–109 (in Japanese with English summary)

Watanabe S, Hashimoto K, Tazaki H, Iwamoto Y, Shinohara N, Satoh K, Sakagami H (2007) Radical scavenging activity and inhibition of macrophage NO production by fukinolic acid, a main phenolic constituent in Japanese butterbur (*Petasites japonicus*). *Food Sci Technol Res* 13: 366–371