Association of Grain Shedding Habit with Polyploidy in Tartary Buckwheat (Fagopyrum tataricum) Strains

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Abstract: Sixty tartary buckwheat (Fagopyrum tataricum) strains were investigated for grain shedding habit, plant height, grain yield, dry weight of plant, weight of grain, days to flowering after sowing and DNA content of nuclei. The grain shedding habit was evaluated from the breaking strength of pedicel and the percentage of the grains dropped by threshing. DNA content of nuclei was detected by flow cytometry to classify the polyploidy level. The fluorescence intensity of the flow cytometry demonstrated no difference between common buckwheat (F. esculentum) and tartary buckwheat, but a clear difference was detected between the diploid and the autotetraploid strains. The survey of DNA content of the 60 tartary buckwheat strains revealed that most of the strains were diploids, but one strain was autotetraploid. The autotetraploid strain exhibited less grain shedding, and produced the largest grains among the strains tested. However, the other characters of the autotetraploid strain, such as plant height, grain yield, dry weight of a plant, and days to flowering, were similar to the mode of the traits of the diploid strains.

Key words: Buckwheat, Flow cytometry, Grain shedding habit, Grain size, Polyploidy, Tartary buckwheat.

Common buckwheat (Fagopyrum esculentum) is widely cultivated worldwide because it is a good source of dietary protein, starch, minerals, etc., while tartary buckwheat (F. tataricum) is cultivated only in China and Nepal. Tartary buckwheat flour has a bitter taste, but it has higher productivity, and about a hundred times higher rutin content than common buckwheat (Kitabayashi et al., 1995a, b). Tartary buckwheat is a self-pollinated plant, and therefore the genetic improvement and evaluation of this species is relatively easier than common buckwheat that is cross-pollinated.

The grain yield of common buckwheat is often reduced by its grain shedding habit (Aufhammer et al., 1994; Lee et al., 1996), and tartary buckwheat may suffer from more severe yield loss due to easier grain shedding than common buckwheat. Tartary buckwheat is an indeterminate flowering type plant, and its flower initiation is stimulated by short days, like most cultivars of common buckwheat. The shoots and inflorescence of tartary buckwheat grow indeterminately, and this makes it difficult to judge the harvesting time and the grain shattering habit, because the individual grains on a plant mature at different times. The measurement of breaking strength of pedicel is a useful technique for evaluating the grain shedding habit of buckwheat species. The breaking strength in tartary buckwheat tends to be weaker than that in common buckwheat (Oba et al., 1998), and autotetraploid buckwheat showed less grain shedding than diploid strains (Oba et al., 1999; Fujimura et al., 2001). In this study, we investigated the genetic variation of the grain shedding habit, morphological characters and polyploidy level in 60 tartary buckwheat strains, and discuss the relationship among these traits.

Materials and Methods

Sixty tartary buckwheat strains were provided by Dr. R. Ohsawa, Institute of Agriculture and Forestry, University of Tsukuba, Japan. Fifty-three strains were from Nepal, four from China, one from France, one from Japan, and one was unknown origin.

Tartary buckwheat strains were directly sown at the experiment field of Gifu University, on August 9, 2000. Seeds of each strain were scattered on a bed 50 cm wide and 3 m long, with a density of about 15 kg per 10 a. The space between beds was 40 cm. No fertilizer was applied to avoid lodging.

The days to flower (first flower) after sowing was recorded for each strain. When a grain ripened and became black, the grain was collected from the base of the pedicel. The breaking tensile strength of the grains was measured by the method of Oba et al. (1998) immediately, after collected from the plants. The pedicel of the grain was held on a tension gauge, and the grain was pulled in a direction parallel to the axis of the pedicel, until the grain was detached from the pedicel. The strength of pulling on the tension gauge at this point was recorded as the breaking tensile strength.

In the maturation period, plant height was measured for ten plants per strain, while grain yield per plant,
Oba et al. — Association of Grain Shedding Habit with Polyploidy

dry weight of a plant including leaves and grains, and dry weight of a grain for five plants. Furthermore, to simulate the grain shedding degree in the field and harvesting process, the percentage of grains shed by threshing was measured by a modification of the method of Hayashi (1992) with the plants in the maturation stage when most grains had become dark colored. Five plants per strain were banded, and were dropped onto a concrete floor from a one-meter height, repeatedly five times, to thresh the grains. The shed grains and the grains remaining on the plants were counted. Then, the averaged percentage of the shed grains per plants was calculated with three replications. For the measurement of the percentage of the grains shed by threshing, six strains were eliminated due to damage by frost.

For the flow cytometry of tartary buckwheat strains, approximately 4 cm² of young leaves from each strain were chopped with a razor blade in 500µl of an ice-cold buffer containing 1.0 % (v/v) Triton X-100, 140 mM mercapto-ethanol, 50 mM NaHSO₄, and 10 mM Tris-HCl (pH 7.5). Crude samples were filtrated through a 35 µm stainless mesh and stained with 300 µl of 25 µg/ml propidium iodide (modified from Chaput et al., 1990). The relative fluorescence of total DNA was measured for each nucleus with a fluorescence activated cell sorter (FACSCalibur4S, Becton Dickinson) equipped with an argon laser. Each histogram was drawn after analyzing at least 1000 nuclei. For calibrating the scale of fluorescence, 2x and 4 x nuclei from F. tataricum and F. esculentum were used as control. The autotetraploid tartary buckwheat strain, AD27, which was used for the calibration of DNA content, was produced by colchicine treatment of a diploid strain by Fujimura et al., (2000). Common buckwheat cultivars used as the standard of DNA content were Shinsyuu-Oosoba (4X) and Shinano No. 1 (2X).

Results

Flow cytometry of a diploid and an autotetraploid strain of tartary buckwheat used as a control revealed that the fluorescence intensity of these ploidy levels corresponded to around 300 and 600 (in arbitrary units), respectively (Fig.1). The fluorescence intensity of the autotetraploid tartary buckwheat strain was twice that of the diploid strain (Fig. 1). In the flow cytometry of common buckwheat, diploid and autotetraploid strains also showed a peak at around 300 and 600, respectively. Thus, the flow cytometry proved to be a technique useful for distinguishing the difference of ploidy level in buckwheat.

Sixty strains of tartary buckwheat were analyzed by the flow cytometry. One Chinese strain, Putong-Kuqiao, revealed a fluorescence intensity of around 600, indicating that this strain was an autotetraploid. Microscopic observation of the root tip cells of Putong-Kuqiao supported this result. However, the flow cytometry of other strains revealed an intensity of around 300 as diploid.

Generally, autotetraploid plants exhibit a larger plant size, and later flowering time than diploid plants.
Thus, some characters of the autotetraploid strain, Putong-Kuqiao, were comparable to those of the other diploid strains. Most tartary buckwheat strains flowered around September 14 to September 24, about 35 to 45 days after sowing, but two strains in early November (Fig.2). The flowering time of autotetraploid strain, Putong-Kuqiao, was on September 15, which was not late compared with the diploid strains. The plant height of strains varied from 65 cm to 110 cm, except for one strain 40 cm, and the plant height of Putong-Kuqiao was 87 cm, which was comparable to the plant height in diploid strains. Yield per plant and dry weight of plant varied among 60 strains, and Putong-Kuqiao, the plant had a lighter dry weight than the others. The weight of a grain varied from 15 mg to 25 mg on most strains, but was over 27 mg in five strains (Fig.2). The strain with the largest grain was Putong-Kuqiao. The breaking strength of pedicel as the index of the grain-shedding habit was 100 to 450 mN in diploid strains, but that in Putong-Kuqiao was 728 mN, extremely larger compared with the other strains. The grain weight correlated with the breaking tensile strength among these strains at the 1% level, viz. correlation coefficient including Puton-Kuqiao was 0.763, and that excluding the extreme value of this autotetraploid strain was 0.580 (Fig.3).

The percentage of the grains shed by threshing when the plants were dropped repeatedly five times varied from 3 % to 84 % among strains (Fig.4). Putong-Kuqiao, an autotetraploid had the lowest percentage of the grains shed by threshing. The degree of grain shedding is associated with the strength of grain pedicels, and thus the percentage of grains shed by threshing showed a significant negative correlation with the breaking strength of pedicel at the 1 % level in all strains. However, when the strain the most resistant to grain shedding, Putong-Kuqiao, was eliminated from the evaluation, the correlation coefficient between the strength of grain pedicels and
the percentage of the grains shed by threshing was not significant.

Discussion

The percentage of the grains shed by threshing may be used as an index of the grain shedding habit in the field and during the harvesting process, but it was influenced by the maturation period of individual grains on a plant. The breaking strength of pedicel is an index of grain shedding habit to eliminate the effect of different maturation period of grains within a plant, and it was reliable for evaluating the toughness of pedicel on the genetic difference of cultivars and strains. When the breaking strength of pedicel was compared with the percentage of grains shed by threshing, there was a significant correlation at the 5 \% level in 54 tartary buckwheat strains (Fig.2). When Putong-Kuqiao was eliminated from the evaluation, however, the correlation was not significant. The low correlation in diploid strains suggested that the difference in breaking strength lower than 500 mN was masked by the physiological difference of grains. However, Putong-Kuqiao, which showed the lowest percentage of the grains shed by threshing showed the strongest breaking strength of pedicel, indicating that the toughness of pedicel increases the resistance to grain shedding.

Genetic improvement of tartary buckwheat has been slow compared with common buckwheat, due to the limited use of tartary buckwheat. However, the development of autotetraploid is easier in tartary buckwheat than in common buckwheat, because the former is self-pollinated and the latter cross-pollinated. In the present study, we found one strain as autotetraploid among 57 strains collected in Nepal and China. Flow cytometry was a useful technique to search for an autotetraploid strain among many diploid strains. The strain, Putong-Kuqiao, was collected in China and conserved at the Hokuriku Research Center of National Agriculture Research Organization, Japan, but unfortunately the detailed information on the collection site is missing. This strain was comparable to the diploid strains in plant height, dry weight of plant, grain yield, and flowering time, but had the strongest breaking strength of pedicel and the largest grains among the 60 tested strains (Fig.2). It also showed the lowest percentage of grain shedding (Fig.4). We previously reported that the plant height and dry weight of the autotetraploid strain were comparable to those of the original strain of diploid, but the grain size and the breaking strength of pedicel of the former strain were significantly larger and stronger, respectively than those of the latter strain (Fujimura et al., 2001). The autotetraploid cultivars in common buckwheat also have larger grains and stronger breaking strength than the original cultivars of diploid (Oba et al., 1999). On rice (*Oryza sativa* L.), the breaking strength of the pedicel of non-shattering strains which were derived from the cross between Japonica and Japonica-Indica cultivars was over 500 mN, and that of most of the easy shattering strains was lower than 400 mN (Jin et al., 1982). Compared with the wide variation in the breaking strength of rice, the breaking strength of tartary buckwheat strains distributed within the variation of easy shattering rice strains. However, the breaking strength of Putong-Kuqiao was in the range of that of the non-shattering strains of rice. Thus, if the breaking strength of tartary buckwheat strains is stronger than that of the non-shattering rice strains or cultivars, we can judge the shattering habit of the tartary buckwheat to have improved drastically. Actually, Putong-Kuqiao, whose breaking strength was 728 mN, had the smallest yield loss by the grain shedding among the strains examined when they were evaluated by this threshing method.
The strains with larger grains tended to have a stronger breaking strength of pedicel (Fig.3). The strong breaking strength resulted from the thick pedicel, which was important to support the big grains (Oba et al., 1999). The autotetraploid buckwheat had larger grains than the diploid plants. Generally, autotetraploid plants are expected to have larger grains, leaves, shoots etc., which may increase the plant biomass and grain yield, compared with diploid plants. Actually, the autotetraploid tartary buckwheat strain had larger grains than the diploid strains, although plant height and leaf size were similar to the diploid strains. In addition to the grain size, the autotetraploid tartary buckwheat strain had the strongest breaking strength among the tested strains. These results suggest that the autotetraploid buckwheat is attractive for improvement of its grain shedding habit, as well as larger grain size. We observed that the grain size was related with the breaking strength in various common buckwheat cultivars and wild buckwheat species including ancestors of cultivated species (Fujimura et al., 2000). The grain shedding habit and the grain size were improved in the early stage of the domestication process from wild to cultivated forms. Thus, the relationship between the two traits is very interesting topic in crop evolution.

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