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Overcoming the barrier to interspecific hybridization of *Fagopyrum esculentum* with *Fagopyrum tataricum*

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Summary

Tartary buckwheat (*Fagopyrum tataricum*) was successfully hybridized with common buckwheat (*F. esculentum*), both diploid (2n = 16), using the latter as male parent during bud pollination. The barrier normally encountered in such hybridization was overcome by enhancing the cross-compatibility of the two species, which was accomplished by synthesizing a unique genotype of common buckwheat. This novel plant was produced by selecting common buckwheat plants that exhibited, at isozyme loci PGM, SKDH and ADH, alleles with similar mobility to those found in tartary and then transferring these alleles to a single plant through six generations of breeding. Ovule culture was used to rescue the 7–10 day old embryos. On the rescue-culture medium 41% of the hybrid embryos formed calli larger than 200 μm in diameter. Most ceased to grow before reaching 1500 μm, but four out of 263 cultured ovules continued to grow as callus. One of these differentiated and formed callus with buds and shoots from which cloned plants were produced. The remaining embryos either did not grow at all or formed very small calli. When tartary was crossed with the original genotype of common buckwheat, only 22% of the hybrid embryos formed small calli and none differentiated. Hybridity of the calli and the plantlets was determined by RAPD and isozyme analysis, respectively. Flowers produced by the hybrid plants were of the same type (homomorphic) and size as those of tartary, but with white sepals like common buckwheat.

Abbreviations: CTAB – cetyltrimethylammonium bromide; GA_3 – gibberellic acid; IAA – indoleacetic acid; IBA – indolebutyric acid; PVP – polyvinylpyrrolidone

Introduction

The seed (achene) of common buckwheat (*Fagopyrum esculentum* Moench) is highly nutritional, has important health benefits and possesses desirable flavor and milling quality (Marshall & Pomeranz, 1982; Koh et al., 1988; Choi et al., 1988; Cheng & Ni, 1992). However, the seed yield is relatively low and unstable. Common buckwheat is a dimorphic plant with short-styled and long-styled flower types on separate plants. Plants within a stylar type are self-incompatible as well as cross-incompatible (Marshall, 1969). Hence, seed production depends mostly on honeybees and other winged insects for cross pollination between stylar types. The low seed yield has been attributed to low seed set (Marshall, 1969; De Jong, 1972; Gubbels, 1978; Kreft, 1983; Guan & Adachi, 1992; Obendorf & Slawinska, 1993), seed shattering and lodging (Marshall, 1969; Elagin, 1977; Hayashi, 1992). Moderately high temperatures, low soil moisture levels, wilting during the day when other crops do not appear to be under stress and insufficient assimilates have been identified as the limiting factors in seed set.

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) on the other hand, has desirable traits such as high seed production, self fertility and resistance to environmental stresses but its seeds lack the desired flavor (Adachi, 1986; Ruszkowski, 1980; Baniya et al., 1992).

Conventional breeding techniques have not been successful in hybridizing common buckwheat with tartary (Morris, 1952; Ruszkowski, 1980; Adachi et al., 1989; Samimy, 1991). So far, application of protoplast fusion has also failed because the resulting calli did not regenerate (Lachman, 1991). Ujihara et al. (1990) have successfully hybridized tetraploid wild buckwheat (*Fagopyrum cymosum*, 2n = 32) with tetraploid common buckwheat using ovule culture. Back-crossing of the hybrids with common buckwheat resulted in fertilized ovules which had to be rescued for further growth.

Using conventional sexual hybridization, we have successfully hybridized diploid tartary with diploid common buckwheat (both diploid, 2n = 16). This hybridization was accomplished by synthesizing a unique genotype of common buckwheat which, based on isozyme markers, was genetically more similar to tartary than any other common buckwheat.

Materials and methods

Plant material

The source of diploid (2n = 16) common buckwheat (*Fagopyrum esculentum*) seed used was cultivars 'Mancan' and 'Manor' and an accession (PI 561670, USA) from Himachal Pradesh, India obtained from B.D. Joshi, Shimla, India (Indian NC67098). Tartary buckwheat (*Fagopyrum tataricum*) seed of a local landrace was obtained from a grower in Maine, USA (PI 632048, USA).

Isozyme analysis

Isozyme variability within tartary and common buckwheat was determined by analyzing one hundred seedlings of each species for enzymes shikimic acid dehydrogenase (SKDH; EC 1.1.1.25), phosphoglucomutase (PGM; EC 2.7.5.1) and alcohol dehydrogenase (ADH; EC 1.1.1.1). Due to considerable variability within the population of common buckwheat, each seedling was identified by a number which was subsequently used for crossing the selected plants carrying the desired alleles.

Enzyme systems 6-phosphogluconate dehydrogenase (6 PGD; EC 1.1.1.4.4), malate dehydrogenase (MDH; 1.1.1.37) and aconitase (ACO; EC 4.2.1.3) were used to determine hybridity of the seedlings that resulted from crossing tartary with common buckwheat.

Horizontal starch gel electrophoresis technique in combination with isozyme assays were used to separate and detect the isozymes. In those assays where ADH activity was to be detected, half of the cotyledons from a 6-day old seedling was extracted in 100 μl of the buffer pH 8.0, containing 0.1 M tris-maleate, 10% glycerol (V/V), 10% soluble PVP-40, 0.5% Triton X-100 and 14 mM 2-mercaptoethanol. In other assays 10 mg of a growing leaf was extracted in 100 μl of the buffer. Samples were kept on ice and thoroughly crushed using a plexiglass rod as a pestle. Paper wicks (3e× 8 mm) were dipped into the crude extracts blotted on tissue paper to remove excess solution and inserted into a slit (the origin) across the 10% starch gel (Weeden & Emmo, 1985). A histidine buffer system at pH 6.5 was used to prepare the starch gel and run the electrophoresis (Cardy et al., 1980; Weeden, 1984; Stuber et al., 1988). After the gels were loaded with the wicks, they were placed in a refrigerator at 5° C and run at 30 mA for 10 min (Weeden & Emmo, 1985). The wicks were then removed and electrophoresis continued for about 4 h at 30 mA. As the current dropped below 30 mA during electrophoresis, voltage was increased until it reached 350 V. After electrophoresis the gels were sliced horizontally into 2-mm thick slabs and stained for the enzymes (Stuber et al., 1988; Weeden & Emmo, 1985; Weeden & Lamb, 1985).

Development of the new genotype

The new genotype of common buckwheat was developed by transferring PGM, SKDH, and ADH alleles found in different plants, but exhibiting similar electrophoretic mobility to those of tartary, into one plant. The PGM allele was found in a heterozygous form in a short-styled plant. To increase its frequency it had to be crossed with a long-styled plant which resulted in about 50% of the F_1 plants, consisting of both short-styled and long-styled flower types, carrying the desired PGM allele in a heterozygous form. Among those plants that carried the desired PGM allele, only a few short-styled flower type carried the desired SKDH allele. Consequently, approximately 25% of the F_2 population contained the desired PGM allele in a homozygous form amongst which plants with the desired SKDH allele were found. In the F_3 population each stylar type carried both of the desired alleles of PGM and SKDH in a homozygous form. These plants were selected and

used for seed increase. Subsequently, a common buckwheat accession from India was obtained which carried an ADH allele with similar mobility to the one found in tartary. Plants carrying the desired ADH allele were selected and crossed with those carrying the desired PGM and SKDH in a homozygous form. Continued selection and crossing resulted in plants that each carried the desired homozygous PGM, SKDH and ADH alleles.

Interspecific hybridization

Bud pollination technique was used to cross common buckwheat, cv. 'Manor' with tartary, using the latter as the female parent. Both species are diploid (2n = 16) (Quisenberry, 1927). One day before anthesis, flower buds of tartary were emasculated under a stereo microscope without damaging the plant, and then pollen from newly opened, long-styled flowers of common buckwheat were transferred on to the stigma of tartary. Accidental self pollination was kept to a minimum by removing opened flowers before pollination, and all surplus buds afterwards.

Embryo rescue

Enlarged ovaries resulting from the interspecific cross were removed 7 to 10 days after cross pollination, surface sterilized in 10% Clorox containing 0.04% Tween 20 for 10 min and washed with sterile water. Under a stereo microscope, the ovules were removed and plated in a medium containing modified Murashige & Skoog (MS) (1962) basal salt mixture (1/2 NH_4 NO_3 and 1/2 KNO_3), MS vitamins, 5% sucrose, 2 g l^{-1} casein hydrolysate, 0.2 mg l^{-1} IAA, 0.5 mg l^{-1} GA_3, 1.0 mg l^{-1} Zeatin and solidified with 0.25% Phytagel (Sigma). Ovules were cultured at 25° C in 16 h light/8 h dark. Those embryos that enlarged and emerged from the ovules and formed calli with buds and leaflets were subsequently transferred to Magenta (Sigma) boxes containing the MS salt mixture, MS vitamins, 3% sucrose, 2 g l^{-1} casein hydrolysate, 0.2 mg l^{-1} IAA, 2.0 mg l^{-1} Zeatin and 0.25% Phytagel. Shoots 1–2 cm in length were rooted on a medium containing the modified MS salt mixture, MS vitamins, 3% sucrose, 2 g l^{-1} casein hydrolysate, 0.5 mg l^{-1} IBA and solidified with 0.25% Phytagel. Not all shoots rooted on this medium. After about a month each plantlet was transferred to a Magenta box containing sterile peat-vermiculite growth medium (Boodley & Sheldrake, 1977) and covered with an inverted Magenta box perforated (11 holes, each 5.0 mm in diameter) at the top. The perforated area was coverd with a clear plastic and was punctured once two weeks after transferring. Thereafter puncturing continued for a week. Three weeks after transfer the plantlets were hardened, placed in pots, and grown at 20° C with 14 h light/10 h dark.

Random amplified polymorphic DNA (RAPD) analysis

The RAPD technique was used to determine hybridity of the embryos that ceased to grow on the culture after reaching 200–1500 μm in diameter and of those that continued to grow and formed calli. To isolate DNA, 10–15 embryos from each cross were combined in a micro grinder and extracted in 100 μl of the extraction buffer. For embryos that continued to grow and formed calli, 50 mg from each individual callus was extracted separately in 250 μl of the buffer. For parental analysis, 50 mg of a leaf was extracted. The mixture was heated in a water bath at 60° C for 30 min. An equal volume of chloroform:octanol (24:1) was added and, after gentle mixing, centrifuged at 11000 rpm in Eppendorf 5415C for 5 min. The top phase was transferred to a new microfuge tube and 200–250 μl of 95% ethanol, pre cooled to 20° C, was added. After gentle mixing the solution was refrigerated (4–6° C) for 15–20 min and then centrifuged at 6000 rpm for 10 min. The precipitates were air dried and dissolved in 20 μl of TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0) by leaving for 1–2 hours at 4–6° C. DNA was quantified at A_{260} on Bausch & Lomb Spectronic 601. The extraction buffer was stored at 37° C and consisted of 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 2.0% (w/v) CTAB and 1.0% PVP-40. Just before using, 1 μl of 2-mercaptoethanol was added to 1 ml of the extraction buffer.

Each amplification reaction mixture consisted of 25 μl containing 10 mM Tris-HCl pH 8.3, 1.25 mM $MgCl_2$, 125 μM each of dATP, dGTP, dCTP and dTTP, 0.4 μM primer, 0.5 unit of Taq DNA polymerase (Promega) and 20 ng of genomic DNA. This reaction mixture was modified from Williams et al. (1990). The primers (5′ to 3′) used were CS 44 (ATTCGGCCGC) and BC302 (CGGCCCACGT) prepared by Cornell University Oligonucleotide Synthesis Facility, OPA-04 (AATCGGGCTG) and OPA-10 (GTGATCGCAG) prepared by Operon Technologies, Inc., Alameda, CA, USA. To prevent evaporation during amplification, each reaction mixture was overlaid with two drops of mineral oil. Amplification was performed in a PTC-

100 Programmable Thermal Controller (MJ Research Inc.) for 40 cycles of 1 min at 94° C, 2 min at 40° C and 2 min at 72° C. Amplification was ended by 8 min incubation at 72° C, followed by storage at 4° C until used.

From each reaction mixture 16 μl was placed on a 2.0% agarose gel for electrophoresis. The buffer system used to prepare the gels and run the electrophoresis was TBE (0.045 M Tris-borate, 0.001 M EDTA) pH 8.0. The gels were stained in ethidium bromide (1 μg ml^{-1}) for 20 min and destained in water for 5–7 min.

Results and discussion

In common buckwheat populations the isozyme phenotypes for PGM, SKDH and ADH varied considerably, whereas those in tartary were fixed (Figure 1A, B, C, D). The genetic variability in common buckwheat and lack of it in tartary is expected because the former is a cross-pollinated and the latter a self-pollinated plant. Some common buckwheat plants carried alleles that exhibited similar mobility to those found in tartary. Only 2% of common buckwheat plants carried a PGM allele similar in mobility to tartary in zone 2, but in a heterozygous form (Figure 1A). The remaining plants in this zone carried alleles with slower mobility than tartary. There was no variability among plants in zone 1. At the SKDH locus, 28% of the common buckwheat plants were homozygous for an allele with similar mobility to tartary (Figure 1B). This allele appeared as two bands, one intense and the other faint. The two bands never segregated in the progenies when parents with similar phenotypes were crossed. At ADH locus, common buckwheat, cv. 'Manor', exhibited a single homomorphic band with a faster mobility than that in tartary (Figure 1C). However, in 7% of the common buckwheat plants from India (PI 561670) a rare ADH allele with similar mobility to tartary was found in a heterozygous form (Figure 1D). A triple band associated with the heterozygous form is indicative of a dimeric enzyme system. The remaining plants exhibited a single fast band similar to the cultivar 'Manor' (Figure 1C). ADH isozymes were considerably more active in the cotyledons of the 5–6 day old seedlings than leaves from mature plants.

Each of the aforementioned three alleles was found in a different common buckwheat plant. All the three alleles were introduced into a single plant through selection and cross breeding for six generations. Hence, a new genotype of common buckwheat was synthesized that, based on isozyme markers, was genetically more similar to tartary than any other common buckwheat plant. An increase in the frequency of PGM and SKDH alleles, similar in mobility to tartary, is evident in the F_3 generation (Figure 2A, B; ADH is not shown). In determining phylogenetic relationship, based on isozyme analysis, Ohnishi (1983) concluded that *F. esculentum* and *F. tataricum* were derived from *F. cymosum* and that the three species differenti-

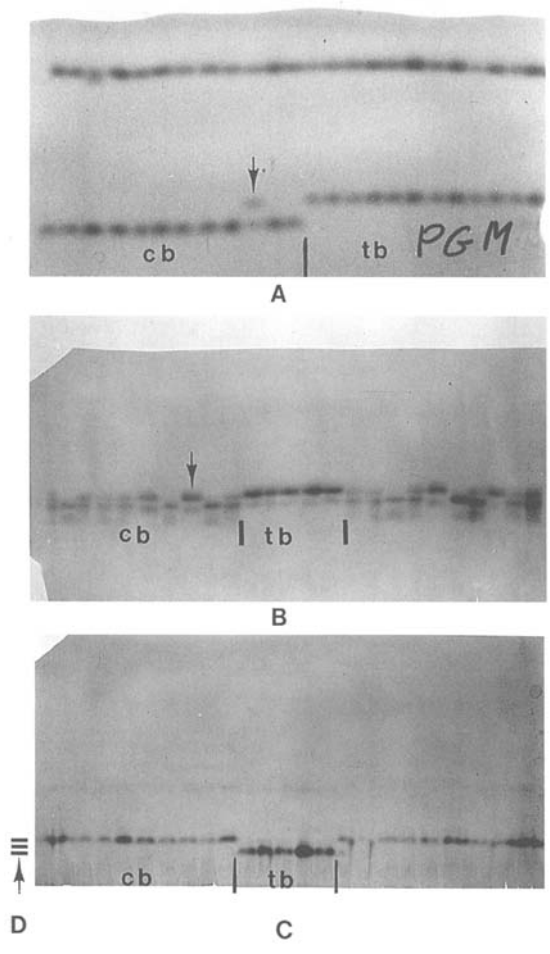

Figure 1. Isozyme phenotypes of A) PGM, B) SKDH and C, D) ADH exhibited by common buckwheat (cb) and tartary buckwheat (tb). Anode is at the top of figure. A) Arrow indicates a rare PGM allele in a heterozygous form with a mobility similar to tartary. B) Arrow indicates SKDH allele in a homozygous form with a mobility similar to tartary. A pair of two bands are associated with each allele. C) Common buckwheat cv. 'Manor' did not carry an ADH allele with a mobility similar to tartary. D) Arrow indicates a rare ADH allele in a heterozygous form found in common buckwheat from India which displayed similar mobility to tartary.

Table 1. Hybridization success of the new genotype and the original genotype of common buckwheat (cv. Manor) with tartary

	New genotype	Original genotype
No. buds pollinated	520	425
No. enlarged ovaries	322 (62%)	264 (62%)
No. ovules cultured	263	233
No. hybrid calli > 200 μm	108 (41%)	51 (22%)
No. hybrid calli > 1500 μm	4 (1.5%)	0 (0%)
No. surviving hybrids	1	0

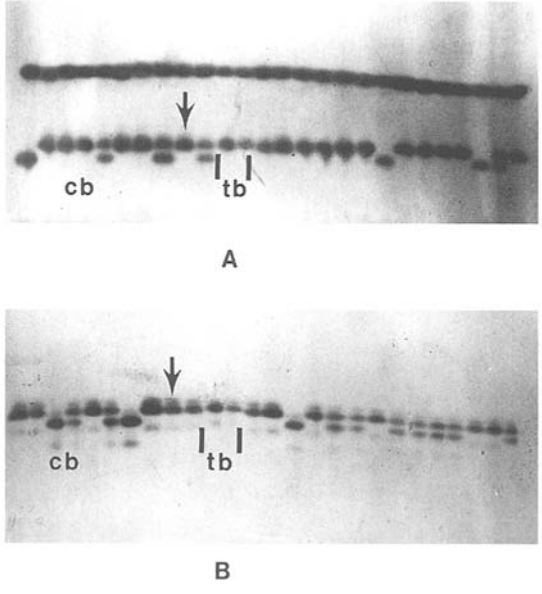

Figure 2. Isozyme phenotypes of A) PGM and B) SKDH exhibited by common buckwheat (cb) plants of F_3 generation and by tartary buckwheat (tb). Increase in the number of common buckwheat plants carrying the alleles with a mobility similar to tartary (arrows) is evident.

ated enough to be considered distinct species. To the best of our knowledge there has not been any natural introgression between common buckwheat and tartary.

The cross-compatibility of the new genotype with tartary was compared with that of the original genotype (Manor), using bud pollination of tartary flowers (Table 1). The new genotype was used to pollinate 520 tartary buds, and the original genotype for 425 buds. Enlarged ovaries were obtained in 62% of the buds in each cross. Ovule culture was used to rescue 7–10 days old embryos from these ovaries. After three weeks on the embryo-rescue medium, 41% of the ovules from the cross with the new genotype formed globular calli

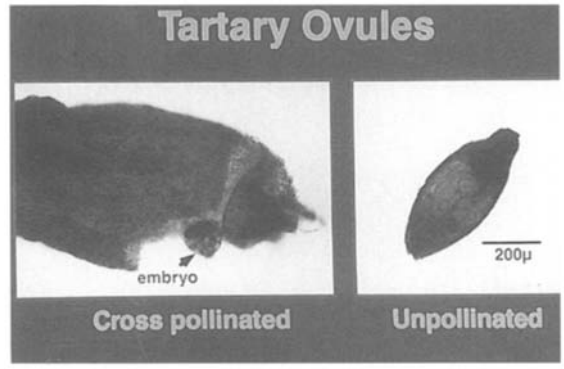

Figure 3. Tartary ovules after 3 weeks on the culture medium. Ovules from the unpollinated buds did not produce any type of embryonic growth. Ovules from the buds cross-pollinated with common buckwheat produced globular calli 200–1500 μm.

that were > 200 μm in diameter, whereas only 22% of the ovules from the original genotype formed such calli. Most of these calli stopped developing before reaching 1500 μm in diameter, but four of the 263 cultured ovules from the cross with the new genotype (1.5%) continued to develop beyond that size, whereas none of the ovules from the original genotype did so. Of the four hybrid calli, one survived and readily produced buds and shoots on the embryo-rescue culture medium. Shoots 1 to 2 cm in length were excised and placed on rooting medium for 25–30 days, and were then transferred to peat-vermiculite growing medium, and were grown at 20° C with 14 h light, 10 h dark. These shoots developed into plants which eventually flowered. Accidental self-pollination accounted for 1.5% of the embryos, which were easily identified by their rapid growth and RAPD.

Flowers produced by the hybrid plants were of the same type (homomorphic) and size as those of tartary with white sepals similar to common buckwheat. The hybrid plants developed leaves of different shape than those of the parents. The general leaf shape of the hybrid plant was ovate without any lobe at the base, whereas that of the parents was heart shape with base of the leaf extending into two either sharp (maternal) or blunt (paternal) lobes. The stem of the hybrid plant was solid, whereas the parents had hollow stems.

Ovules (7–10 days old) from the unpollinated tartary buds did not produce any type of embryonic growth on the embryo-rescue medium (Figure 3), but those from the self-pollinated buds germinated readily and produced buds and shoots. These results suggest that the small, globular calli that resulted from the hybridization and stopped developing before reaching

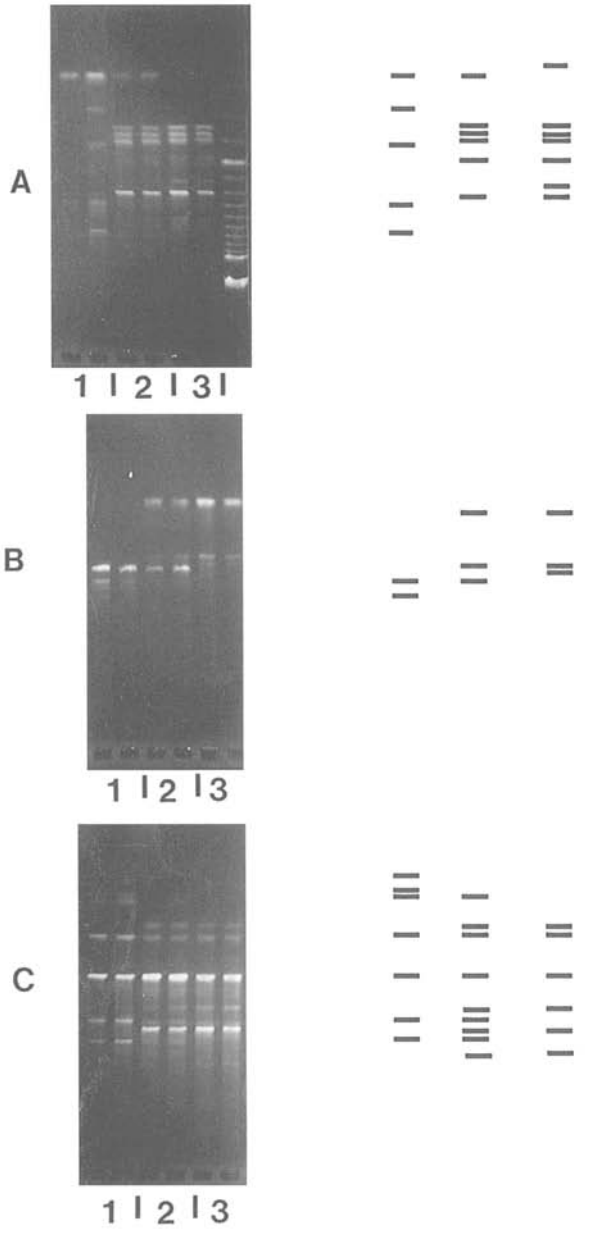

Figure 4. Stained gels and their schematics showing RAPD profiles of 1) the maternal parent (tartary), 2) the hybrid globular calli (200–1500 μm) formed on the culture medium and 3) the paternal parent (new genotype of common buckwheat). Primers used: A) OPA-04, B) OPA-10, C) BC302. On the right side of gel A is 100-bp DNA ladder.

1500 μm in diameter were hybrids. Hybridity of these globular calli was also demonstrated by RAPD technique using four primers (Figures 4 and 5). The hybrid calli did not display all the RAPD markers present in the parents. This could be due to either incomplete

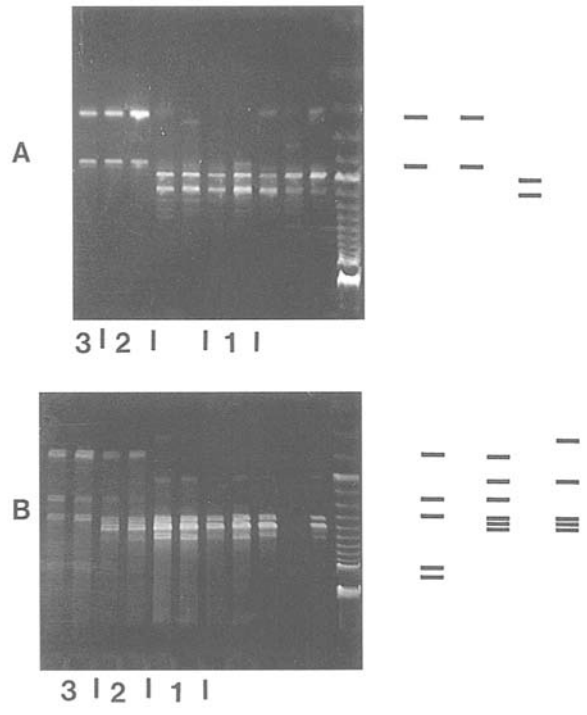

Figure 5. Stained gels and their schematics showing RAPD profiles of 1) the maternal parent (tartary), 2) the hybrid globular calli (200–1500 μm) formed on the culture medium and 3) the paternal parent (new genotype of common buckwheat). Primers used: A) OPA-10, B) CS44. On the right side of each gel is 100-bp DNA ladder.

inheritance of the parental nuclear DNA and/or faintness of the amplified products. The female parent contributed its cytoplasm. The hybrid plant that matured and produced flowers was shown to be a hybrid by displaying both parental isozyme bands at 6 PGD, ACO and MDH loci (Figure 6).

Sexual hybridization of diploid common buckwheat with tartary became a reality by synthesizing a new genotype based on isozyme markers. The isozymes serve as marker for regions of the chromosome that have common ancestry. While that will not be true for every isozyme allele, such linkage drag has been shown by molecular markers and marker gene to be common (Zeven et al. 1983; Young & Tanksley, 1989; Williams & St. Clair 1993). This novel approach may also be used on other cross pollinated species which display genetic variability within their population.

Modification of the genetic background of a species has been an effective approach in overcoming interspecific crossability barriers. Hogenboom (1972) was

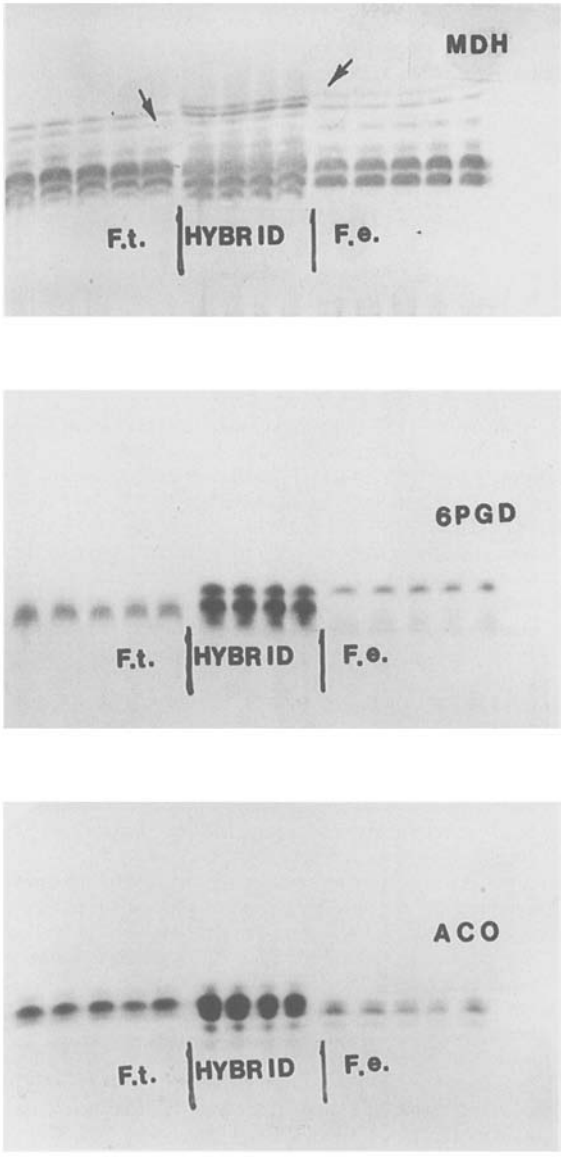

Figure 6. Isozyme phenotypes for MDH, 6-PDG and ACO loci displayed by the hybrid plant and its paternal (new genotype of common buckwheat), F.e.: *Fagopyrum esculentum*) and maternal (tartary, F.t.: *Fagopyrum tataricum*) parents. The hybrid displayed both parental bands. A fast and a slow MDH band displayed by the parents are shown by arrows.

able to overcome the inhibition of *Lycopersicon esculentum* pollen tube growth in the style of *L. peruvianum* by selfing the latter prior to hybridization and hence obtaining homozygous plants carrying recessive genes. Chetelat et al. (1989) crossed wild nightshade (*Solanum lycopersicoides*) with tomato (*Lycopersicon esculentum*), but the F_1 hybrid was male sterile and its style rejected the tomato pollen. They overcame this barrier by crossing the F_1 hybrid (ovule fertile) with pollen derived from another hybrid which was obtained by crossing tomato with *L. pennellii*. Hence, *L. pennellii*-derived hybrids acted as a bridge to circumvent the crossability barrier. Snape et al. (1979) found considerable differences in crossabilities of 18 varieties of bread wheat (*Triticum aestivum*) with *Hordeum bulbosum* and with rye (*Secale cereale*). The most crossable varieties were found to be Chinese Spring and Chinese Winter wheat. They were able to increase or decrease the crossability of Chinese Spring by substituting some of its chromosomes with those of the non-crossable variety 'Hope'. Chinese Spring has also been used in hybridization of wheat with barley (*Hordeum vulgare*) (Hart et al., 1980). In a broader use of finding genetic similarity, a third species can be used as a bridge between two dissimilar species. For example, to transfer disease-resistance genes from *Cucurbita martinezii* to *C. pepo*, butternut squash (*C. moschata*) was used as a bridge (Whitaker & Robinson, 1986). Rhodes (1959) used *C. lundelliana* as a bridge to transfer genes between various *Cucurbita* species.

So far, the hybrid buckwheat has not produced any seed. Its pollen and ovule viability, chromosome number and pairing have not been studied yet. However, this hybrid plant has a potential for transferring genes from tartary to common buckwheat by protoplast fusion technique if it were found to be sterile. Failure of tartary and common buckwheat fusion products to grow (Lachman, 1991) may indeed, be overcome by using the hybrid that we have developed because it is genetically closer to common buckwheat than tartary. The paternal parent of the hybrid can also be used to develop additional hybrid plants which may be fertile.

Acknowledgments

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References

Adachi, T., 1986. Is it possible to overcome the low yield of buckwheat by means of biotechnology? Proc 3rd Int Symp Buckwheat, Pulawy, Poland, pp. 108–116.

Adachi, T., A. Yamaguchi, Y. Miike & F. Hoffman, 1989. Plant regeneration from protoplasts of common buckwheat (*Fagopyrum esculentum*). Plant Cell Reports 8: 247–250.

Baniya, B.K., K.W. Riley, D.M.S. Dongol & K.K. Sherchan, 1992. Characterization and evaluation of Nepalese buckwheat (*Fagopyrum* ssp.) landraces. Proc 5th Int Symp Buckwheat, pp. 64–74.

Boodley, J.W. & R. Sheldrake Jr., 1977. Cornell Peat-Lite mixes for commercial plant growing. Cornell Univ Plant Sci Info Bull 43.

Cardy, B.J., C.W. Stuber & M.M. Goodman, 1980. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Dept of Statistics Mimeo Ser no 1317, North Carolina State Univ, Raleigh.

Cheng, C. & R. Ni, 1992. Preliminary study on the addition of buckwheat flour and reserve of nutrient elements in buckwheat health food. Proc 5th Int Symp Buckwheat, pp. 480–483.

Chetelat, R.T., C.M. Rick & J.W. DeVerna, 1989. Isozyme analysis, chromosome pairing, and fertility of *Lycopersicon esculentum* × *Solanum lycopersicoides* diploid backcross hybrids. Genome 32: 783–790.

Choi, M.G., E.T. Koh & J.S. Ju, 1988. Comparison of repeated feeding of buckwheat, soybean, and brown rice meal responses in non-insulin dependent diabetic subjects (NIDDS). Fed Amer Soc Exp Biol 2 (5): abstract 3080.

De Jong, H., 1972. Buckwheat. Field Crop Abstracts 25 (3): 389–396.

Elagin, I.N., 1977. Role of bees in increasing yield and improvement seed germination and crop qualities of hybrid buckwheat seed. In: R.B. Kozin (Ed.), Pollination of Entomophilous Agricultural Crops by Bees, pp. 31–36.

Guan, L.M. & T. Adachi, 1992. Reproductive deterioration in buckwheat (*Fagopyrum esculentum*) under summer conditions. Plant Breeding 109: 304–312.

Gubbels, G.H., 1978. Yield, seed weight, hull percentage, and testa color of buckwheat at two soil moisture regimes. Can J Plant Sci 58: 881–883.

Hart, G.E., A.K.M.R. Islam & K.W. Shepherd, 1980. Use of isozymes as chromosome markers in the isolation and characterization of wheat-barley chromosome addition lines. Genet Res 36: 311–325.

Hayashi, H., 1992. Buckwheat production in a mountainous area of the central part of Japan. Proc 5th Int Symp Buckwheat, pp. 36–40.

Hogenboom, N.G., 1972. Breaking breeding barriers in *Lycopersicon*. 5. The inheritance of the unilateral incompatibility between *L. peruvianum* (L.) Mill. and *L. esculentum* Mill. and the genetics of its breakdown. Euphytica 21: 405–414.

Koh, E.T., M.K. Choi, J.S. Ju, C.S. Choi & Y.E. Park, 1988. Comparison of long-term effects of buckwheat, rice, and potato on glycemic indices in healthy subjects. Fed Amer Soc Exp Biol 2 (5): abstract 5259.

Kreft, I., 1983. Buckwheat breeding perspectives. Proc 2nd Int Symp Buckwheat, pp. 3–12.

Lachman, S., 1991. Plant cell and tissue culture in buckwheat: an approach towards genetic improvements by means of unconventional breeding techniques. In: T. Adachi (Ed.), Proc Int Colloquium on Overcoming Breeding Barriers by Means of Plant Biotechnology, pp. 145–154. Miyazaki, Japan.

Marshall, H.G., 1969. Description and culture of buckwheat. The Pennsylvania State University Bulletin 754.

Marshall, H.G. & Y. Pomeranz, 1982. Buckwheat: description, breeding, production, and utilization. Adv Cereal Sci Tech 5: 157–210.

Morris, M.R., 1952. Cytogenetic studies on buckwheat. J Heredity 42: 85–89.

Murashige, Y. & F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15: 473–497.

Obendorf, R.L. & J. Slawinska, 1993. Seed set in buckwheat altered by temperature and water deficit stress (abstract). Agronomy Abstracts: 152.

Ohnishi, O., 1983. Isozyme variation in common buckwheat, *Fagopyrum esculentum* Moench, and its related species. Proc 2^{nd} Int Symp Buckwheat, pp. 39–50.

Quisenberry, K., 1927. Chromosome number in buckwheat species. Bot Gaz 83: 85–89.

Rhodes, A.M., 1959. Species hybridization and interspecific gene transfer in the genus *Cucurbita*. Amer Soc Hort Sci 74: 546–551.

Ruszkowski, M., 1980. The possibility of changing the yielding of buckwheat by breeding the homostyle varieties. Symp on Buckwheat, Ljubljana, Yugoslavia, pp. 7–15.

Samimy, C., 1991. Barrier to interspecific crossing of *Fagopyrum esculentum* with *Fagopyrum tataricum*: I. Site of pollen-tube arrest. II. Organogenesis from immature embryos of *F. tataricum*. Euphytica 54: 215–219.

Snape, J.W., V. Chapman, J. Moss, C.E. Blanchard & T.E. Miller, 1979. The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity 42 (3): 291–298.

Stuber, C.W., J.F. Wendel, M.M. Goodman & J.S.C. Smith, 1988. Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Tech Bull no 286, North Carolina State Univ, Raleigh.

Ujihara, A., Y. Nakamura & M. Minami, 1990. Interspecific hybridization in genus *Fagopyrum* – properties of hybrids (*F. esculentum* Moench × *F. cymosum* Meissner) through ovule culture. pp. 45–51. Gamma Field Symp No 29, Inst Radiation Breeding, NIAR, MAFF, Japan.

Weeden, N.F., 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. Euphytica 33: 199–208.

Weeden, N.F. & R.C. Lamb, 1985. Identification of apple cultivars by isozyme phenotypes. J Amer Soc Hort Sci 110 (4): 509–515.

Weeden, N.F. & A.C. Emmo, 1985. Isozyme characterization of Kentucky bluegrass cultivars. Can J Plant Sci 65: 985–994.

Whitaker, T.W. & R.W. Robinson, 1986. Squash breeding. In: M.J. Bassett (Ed.), Breeding Vegetable Crops, pp. 209–240, AVI Publishing Co.

Williams, C.E. & D.A. St. Clair, 1989. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome 36: 619–630.

Williams, G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski & S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18 (22): 6531–6535.

Young, N.D. & S.D. Tanksley, 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. Theor Appl Genet 77: 353–359.

Zeven, A.C., D.R. Knott & R. Johnson, 1983. Investigation of linkage drag in near isogenic lines of wheat by testing for seedling reaction to races of stem rust, leaf rust and yellow rust. Euphytica 32: 319–327.