Barrier to interspecific crossing of *Fagopyrum esculentum* with *Fagopyrum tataricum*: I. Site of pollen-tube arrest. II. Organogenesis from immature embryos of *F. tataricum*

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**Summary**

Following bud pollination of tartary buckwheat (*Fagopyrum tataricum*) with pollens from common buckwheat (*F. esculentum*), cv. 'Mancan', 31% of the ovaries began to grow but all turned brown and withered after 6 to 14 days. Fluorescence microscopy of the growing ovaries showed the pollen-tube entering the embryo-sac. The ovules from the growing ovaries failed to produce any embryo in the culture medium in which the immature embryos from the self-pollinated (compatible) tartary flowers were able to mature and germinate. No embryo development was observed after cross-pollination. The interspecific incompatibility is attributed to the failure of gametes to fuse.

**Abbreviations:** BAP – benzylaminopurine; IAA – indoleacetic acid

**Introduction**

The low seed yield of the common buckwheat (*Fagopyrum esculentum* Moench.) has been attributed to low seed set (excessive flower shedding and high incidence of abortion) and shattering (Marshall, 1969; Elagin, 1977). Although each plant, grown in the field at the usual seedling rate, may produce up to 2000 flowers, the average number of seeds produced has been reported to be between 31–53 (Marshall, 1969; Morton, 1966). In a population of buckwheat short-styled and long-styled flowers are found on separate plants. Plants within a stylar type are self-incompatible, as well as cross-incompatible (Marshall, 1969). One approach which may lead to increased seed yield is to cross the common buckwheat with tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) which is self-compatible. It has been reported that the leaf area, rate of photosynthesis and the root system of tartary do not differ from those of the common buckwheat, and that tartary seed yield is three times higher than common buckwheat (Adachi, 1986; Ruszkowski, 1980). Conventional interspecific hybridization of common buckwheat with tartary has not been successful (Adachi et al., 1989). Previous reports have shown that the growth of the pollen tubes of common buckwheat was inhibited at the base of tartary styles (Morris, 1952; Ruszkowski, 1980). The study reported here shows that following bud pollination the pollen-tube of common buckwheat enters the embryo-sac of tartary.
Limited number of studies have been conducted on tissue culture of common buckwheat (Bohanec, 1986). Neskovic et al. (1987) were able to produce buds and somatic embryos from the immature embryos of tetraploid buckwheat. The immature embryos used consisted of a short axis and tightly folded cotyledons. In the following study shoot regeneration from the immature embryos of tartary, only a few days after self pollination, is reported for the first time. This in vitro technique was used to rescue the ovules obtained following hybridization of the common buckwheat with tartary.

Materials and methods

The common buckwheat (Fagopyrum esculentum) cv. ‘Mancan’ and tartary buckwheat (Fagopyrum tataricum) were grown in pots in a growth chamber with a 12 h light at 22° C and a dark temperature of 18° C.

Bud pollination technique was used to cross ‘Mancan’ with tartary, using the latter as the female parent. Both species were diploid (2n = 16) (Quisenberry, 1927). About two days 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 ‘Mancan’ were transferred on to the stigma of tartary. The pollinated flowers were isolated by removing other buds, opened flowers and developing seeds.

To determine the site of the pollen-tube arrest, the pistils of tartary were softened in 1M NaOH at room temperature for 1 h, rinsed and squashed in a solution of 0.1% aniline blue in 0.07M KH₂PO₄. Aniline blue-induced fluorescence of the pollen-tube callose was observed using Bausch and Lomb microscope equipped with 50-watt HBO mercury lamp and exciter filter (transmission between 425 and 450 nm), a dichroic filter with transmission above 450 nm and a barrier at 500 nm.

To observe embryo development, ovules of tartary buckwheat which were either self-pollinated or cross-pollinated with common buckwheat were isolated 5 days after pollination, softened in 1M NaOH for 1 h and examined under the microscope without applying any pressure to the cover glass. Ten ovules from each cross were observed.

Ovules of tartary used for culture were 1.8–2.0 mm long 4 to 7 days after being self-pollinated or cross-pollinated with common buckwheat. The ovaries were surface sterilized in 10% Clorox for 10 min and washed with sterile water. Under a stereo microscope the ovules were removed and plated in Murashige & Skoog’s (1962) medium supplemented with 5% sucrose, 2 mg/l BAP, 0.2 mg/l IAA and 2000 mg/l casein hydrolysate and solili-