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The Potential for Interbreeding between Different Species of Plants in the *Fagopyrum* Genus Using a Technique of Embryo Rescue

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[Introduction]

Breeding programs have improved the agronomic characteristics of buckwheat through selection, but there are still desirable traits that could be introduced through hybridization. Many attempts have been made to incorporate new traits into buckwheat through interbreeding with other species, but with limited success. In vitro techniques, along with conventional breeding methods, could be used to accelerate the breeding program and overall improvement of buckwheat. In this regard, embryo culture plays a crucial role as it can produce hybrid plants in interspecies hybridization. In this study, we have developed a new method that uses embryo rescue and in vitro ovule culture to overcome the breeding barriers in the *Fagopyrum* genus.

[Materials and Methods]

In this study, crosses were made between two species with different floral morphs (heterostylous) and two species with the same floral morph (homostylous), namely *Fagopyrum homotropicum* and *Fagopyrum tataricum*, as well as *Fagopyrum esculentum* and four other species within the *Fagopyrum* genus. Hand-pollination was performed in the morning when the flowers were fully open between *F. esculentum* and the related species. Fruits were harvested at 3 and 5 days after pollination, and ovules were excised and cultured on a medium containing Murashige and Skoog (MS, 1962) with 3% sucrose, 0.8% agar, 0.2 mg/L IAA, and 2 mg/L BA. Embryos that enlarged and formed calli with germinated seedlings were subsequently transferred to a hormone-free MS medium with 3% sucrose for further development.

[Results and Discussion]

Interspecies crosses were conducted among *Fagopyrum esculentum*, *F. cymosum*, and *F. homotropicum* in various combinations. The frequency of ovule production varied from 17% to 45% in different combinations, with an average frequency of 31% for all interspecies pollinations. The highest percentage of ovule formation (approximately 45%) was observed in the combination between *F. esculentum* (pin) and *F. cymosum*, while the lowest (17%) was in the combination between *F. esculentum* and *F. homotropicum*. A total of 255 ovules were excised, out of which 234 ovules did not survive or show any growth, and only 8% of cultured ovules survived and continued to grow. The influence of hormone concentration on ovule culture was examined, and it was found that MS medium without hormones resulted in a higher number of germinated hybrid embryos compared to MS medium supplemented with 0.2 mg/L IAA and 2 mg/L BA in ovule culture between *F. esculentum* and *F. cymosum*. The number of germinated embryos increased with the number of days after pollination, reaching the highest point at 5 days after pollination and then decreasing. Additionally, since fewer germinated embryos were obtained before 5 days after pollination, hybrid embryos should be developed to a later stage in order to rescue the embryos.

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