Procedure for the efficient acquisition of progeny seeds from crossed potato plants grafted onto tomato

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Abstract  
Potato, *Solanum tuberosum* L. is an important crop. However, it is difficult to breed potato cultivars by applying conventional crossing methods because potato has a tetraploid genome and is vegetatively propagated. Flower formation and tuber development occur simultaneously. Many potato cultivars hardly produce any fruits after crossing and fail to produce seeds. We report an improved procedure for obtaining progeny seeds by grafting potatoes onto tomatoes. The rate of fruit formation was more than 19% when the grafted potatoes were used for the crossing experiments, whereas crossing using the ungrafted plants showed a rate of 1.1%. This result suggests that our procedure results in the easy acquisition of null-segregant progenies by crossing mutant lines. It is also expected to improve conventional potato breeding.

Key words: crossing, grafting, potato fruits, progeny seed, tomato.

Potato, *Solanum tuberosum* L., is one of the most important crops in the world due to its nutritional quality. The commercial cultivar of common potato has a tetraploid genome and propagates vegetatively. Thus, it is difficult to breed potato cultivars by applying conventional crossing methods.

In recent years, genome editing technology has been applied to potato to create various mutants (Dangol et al. 2019; Nadakuduti et al. 2018). Usually, the process of genome editing requires artificial nuclease genes such as TALEN and CRISPR/Cas9, and in most cases, these genes remain in the plant genomes. These artificial nuclease genes segregate and can be removed from the genomes of progenies by crossing mutant lines, resulting in null-segregant progeny lacking artificial nuclease genes.

In this study, we used mutant potato lines, named *gbss* and *sbe3*, that were established using *S. tuberosum* L. cv. Sayaka, in which a site-specific mutation was introduced in the *StGBSS1* and *StSBE3* genes by the CRISPR/Cas9 system, respectively (Kusano et al. 2018; Takeuchi et al. 2021). These genes encode the potato granule-bound starch synthase I and starch branching enzyme 3, respectively. Sayaka is a potato cultivar that is commonly used for transformation and genome editing. However, it is difficult to obtain seeds from the progeny of Sayaka because most pollinated flowers fall quickly and fruits hardly develop, although pollen fertility is observed.

Flower formation and tuber development occur simultaneously (Jansky and Thompson 1990). We presumed that a large amount of assimilation products would be transported to the underground tubers, and therefore insufficient amounts of substances would be supplied for the development of the fruits. Many potato cultivars hardly produce any fruits after crossing and fail to produce any seeds. In this paper, we report an improved procedure for obtaining progeny seeds by grafting potatoes onto tomatoes.

Potato plants were cultivated at 23°C under long-day conditions (16 h light, 200–400 µmol m⁻² s⁻¹, and 8 h dark). After 2–3 days of flowering, pollen was collected from the anthers of the flowers. The obtained pollen was stored in a microtube and desiccated with silica gel at 4°C. Then, the pistils of another line were prepared for pollination. The pistils were exposed after the stamens were removed from the flowers 2–3 days after flowering. The pistil was pollinated using pollen from the microtube. After pollination, plants were further cultivated under the same conditions. Tubers generated after flowering were removed as possible. Among the treated flowers, a small number of fruits were obtained, but many flowers fell within several days during cultivation. We obtained five fruits by crossing between two *gbss* mutant lines, whereas other crossing experiments resulted in no fruit generation (Table 1). As a control, flowers without pollination were maintained under the same conditions. All these flowers naturally fell without fruit formation.
TOMATO BELONGS TO THE SOLANACEAE FAMILY AND PRODUCES MANY FRUITS WITHOUT TUBERS. WE EXPECTED THAT GRAFTING POTATO ONTO TOMATO WOULD BE A GOOD PLATFORM FOR OBTAINING PROGENY SEEDS FROM POTATO CROSSES. TO MAKE A GRAFTED POTATO, THE MEDIUM-SIZED TOMATO CULTIVAR FRUTICA (TAKII & CO. LTD., KYOTO, JAPAN) WAS USED AS THE ROOTSTOCK. TOMATO SEEDS WERE SOWN IN A SMALL POT FILLED WITH GRANULAR CULTURE SOIL (NIPPON HIRYO, OSAKA, JAPAN) AND CULTURED UNDER LONG-DAY CONDITIONS OF 16 h LIGHT AND 8 h DARK IN A CULTURE ROOM. WHEN THE STEM OF THE TOMATO PLANT Grew TO 8–10 mm thick with several leaves, the stem was cut diagonally at a height of 3 cm above the ground using a sharp knife (Grafting guide cutter V FTG-10) (FEATHER SAFETY RAZOR CO. LTD., TOKYO, JAPAN). In parallel, a potato plantlet was grown to a similar size and cut diagonally with a sharp knife in a similar way. Potato sections were grafted onto tomato and fixed with a graft clip (Joinholder 2.4 mm, JH24-10) (SEEM CORP., KOKURA, KITAKYUSHU, JAPAN) (Figure 1A). The grafted plantlets were incubated for seven days under long-day conditions of 16 h light and 8 h dark with high humidity. When the connection between the plants was established, the graft clip was removed. The grafted plant was transplanted to a large pot and cultured under long-day conditions (Figure 1B–D).

The grafted plants grew normally, similar to the ungrafted plants. They bloomed approximately 2 months after starting cultivation. Using a vibrating tip of the electrical toothbrush, pollen was collected from the flowers of the grafted plants, and pollination was performed by the method described above (Figure 2A). Among the pollinated flowers, a large proportion were retained without drop and formed mature fruits due to successful pollination (Figure 2B); however, all the flowers that were not pollinated fell within a few days without bearing any fruits. Many seeds were obtained from the potato fruits (Figure 2C). These results indicated that the crossing procedure using the grafted plants produced many progeny seeds because the loss of flowers was significantly reduced compared to that using ungrafted plants.

The obtained progeny seeds were soaked with 0.1 N HCl for 40 min and then washed with deionized water four times. They were immersed for 24 h in a 2,000 ppm gibberellin A₃ solution (as 95% solution, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). After this treatment, the seeds were sanitized by washing with 70% ethanol and 0.4% hypochlorous acid solution in turn. They were washed with deionized water five times, soaked with 0.1 N NaOH for 20 min, washed with deionized water five times, and finally allowed to stand for 24 h in fresh deionized water. The seeds were sown in trays filled with granular culture soil (NIPPON HIRYO, OSAKA, JAPAN) and cultured under long-day conditions. After 5 months, the potato fruits were harvested and individually stored in a refrigerator at 5°C. The progeny seeds were extracted from the potato fruits and then cleaned with deionized water to remove any residue. Additionally, the seeds were soaked with 0.1 N HCl for 40 min and then washed with deionized water four times. They were immersed for 24 h in a 2,000 ppm gibberellin A₃ solution (as 95% solution, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). After this treatment, the seeds were sanitized by washing with 70% ethanol and 0.4% hypochlorous acid solution in turn. They were washed with deionized water five times, soaked with 0.1 N NaOH for 20 min, washed with deionized water five times, and finally allowed to stand for 24 h in fresh deionized water. The seeds were sown in trays filled with granular culture soil (NIPPON HIRYO, OSAKA, JAPAN) and cultured under long-day conditions. After 5 months, the potato fruits were harvested and individually stored in a refrigerator at 5°C. The progeny seeds were extracted from the potato fruits and then cleaned with deionized water to remove any residue.
times and dipped in deionized water for 16 h. After these treatments, to increase the germination efficiency, potato seeds were scratched with a knife or a sandpaper #240. These seeds were placed onto MS medium plates (Murashige and Skoog 1962) supplemented with 0.5 ppm gibberellin A3, 3% sucrose, and 0.3% Gelrite (FujiFilm Wako). The plates were incubated at 23°C until the seeds germinated. Scratched potato seeds germinated faster than nonscratched seeds, which requires 3 months or more to germinate (Figure 3).

It is considered technically difficult to produce progenies of tetraploid potato cultivars by crossing. Sayaka has both pollen fertility and female fertility, but these fertility levels are weak. The fertility rate was very low when crossing was attempted using ungrafted plants. We attempted multiple crossing experiments, but the formation rate of fruits was 1.1% overall. Low efficiency on fruit generation was observed even when pollen from the wild-type plant was used (Table 1). On the other hand, when the grafted gbs3 and sbe3 mutant potatoes were used for the crossing experiments, the fruit formation rates were 35.3%, 19.0%, and 34.5%, respectively (Table 1). The efficiency of fruit formation was significantly higher than that of ungrafted plants ($p<0.01$). In this study, the crossing was performed on flowers and pollen that were derived from ungrafted plants, and those from the grafted plants. We considered that grafted plants resulted in no change of the pollen fertility and showed no significant difference from ungrafted plants. This procedure may enable anyone to achieve good results when crossing potato.

Genome-edited mutants are often required to remove internal artificial nuclease genes from plant genomes. Our procedure contributes to the easy acquisition of null-segregant progenies by crossing mutant lines and is also expected to improve conventional potato breeding.

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