

Grafting of Potato Plants

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[Abstract] Grafting is a very useful technique for testing the transmission of long-distance signals in plants and is used in agriculture and gardening for different purposes. This protocol, based on a previously published one (Jackson *et al.*, 1998), describes a grafting method for potato plants, which has successfully been used to test the transmission of tuber-inducing signals (Martin *et al.*, 2009; González-Schain *et al.*, 2012). We describe first the procedures for plant growth and then the grafting procedure. Although this method has been used for grafting plants that were initially grown *in vitro*, it should work as well with plants grown from tubers in soil. This protocol probably works well for other plant species, with small modifications.

Materials and Reagents

1. Murashige & Skoog medium including vitamins (Duchefa Biochemie, catalog number: M0222)
2. Sucrose
3. MES (Sigma-Aldrich, catalog number: M8250)
4. ddH₂O
5. KOH
6. Gelrite™ (Duchefa Biochemie, catalog number: G1101.5000)
7. Soil composed of blocking compost (Plantaflor® Presstopfsubstrat) and sand in a 3:1 proportion
8. KNO₃
9. NH₄NO₃
10. K₂HPO₄
11. KH₂PO₄
12. Ca(NO₃)₂·4H₂O
13. MgSO₄·7H₂O
14. FeSO₄·7H₂O
15. Kelamix

16. Fertilization solution: Modified Hoagland's solution [Johnson *et al.*, 1957] diluted 1/60 (see Recipes)
17. 2MS medium (see Recipes)

Equipment

1. Plant growth chamber
2. Laminar flow hood
3. Sterile forceps
4. Glass jars, glass tubes or plastic containers suitable for *in vitro* growth
Note: Equipments 1-4 is not required if plants are grown from tubers in soil.
5. Sterile scalpels
6. Pots
7. Stakes
8. Plant twist ties (e.g. Garden Odyssey, catalog number: T001)
9. Beaker
10. Paper surgical tape (e.g. 3 M Micropore medical tape, catalog number: 1530-0)
11. Transparent plastic bags
12. Adhesive tape

Procedure

1. Micropropagate *in vitro* grown plants under sterile conditions in a flow hood, according to steps 2-4.
2. Carefully hold the plant stem with sterile forceps and use a sterile scalpel to cut a single-node stem piece.
Note: The forceps and scalpel can be surface sterilized by dipping them in ethanol and passing them through a flame.
3. Put the cutting in fresh 2MS medium.
4. Repeat steps 2 and 3 as many times as required.
Note: It is advisable to propagate 10% more cuttings than the number of plants required for grafting. This number will depend on the purpose of the grafting. For our tuberization experiments, in order to have at least 10 successful grafted plants per type, we made between 12 and 20 grafts. For example, to test the transmission of signals between 2 genotypes A and B, the following grafts will be required: A/B, B/A, A/A (as control) and B/B (as control), as well as intact A and B plants. To make 15 grafts of each type and leave 15

intact controls, you will need 45 A and 45 B plants. Then, propagate 50 cuttings per genotype.

5. Grow plants for approximately two weeks at 23 °C under long day conditions (LD: 16 hours light and 8 h darkness), until the roots are well developed.
6. Pull the plants out of the medium, taking care not to damage the stem or the roots.
7. Wash the roots by dipping into water and carefully remove the remaining medium.
8. Transfer the plants to soil, previously watered with fertilization solution.
9. Cover the plants with a plant propagator cover, an inverted transparent plastic bag or cling film to maintain the humidity high.
10. Grow the plants under appropriate conditions, watering them as needed with fertilization solution during the whole procedure.

*Note: We usually grow the plants at 23 °C under LD conditions in the greenhouse. Plants grown under LDs are more vigorous than plants grown under short days, at least for *Solanum tuberosum ssp. andigena*.*

11. After 3 days, allow the humidity to slowly decrease for 3-4 more days to gradually acclimate the plants from high humidity (*in vitro*) to low humidity (greenhouse). If you are using a propagator, open first one vent, one day later open another vent and 2-3 days later remove the cover. If you are using a plastic bag or cling film, make small holes (approximately 0.5 cm diameter, 40 holes/m²), gradually increase the size of the holes during the next 2-3 days and finally remove the cover.
12. Tie the plants to stakes as needed. Upright plants are grafted more easily than sprawling or bending plants.
13. Grow the plants for at least 3 weeks.

Note: We have successfully grafted 3-5 week old plants, but plants of other ages could also be grafted. Grafting younger plants is more difficult as the stem is weaker and is more easily damaged during the grafting procedure.

14. Start with the plant that will be used as scion. Use a scalpel to cut the stem in a V shape below the third or fourth leaf from the apex.
15. Put the scion in water while the plant that will be used as stock is cut.
16. Use a scalpel to cut the stem of the stock plant in a V shape below the third or fourth leaf from the apex.

Note: Grafting works best when the diameters of the scion and stock are similar.

17. Add a drop of water to the top of the cut stock.

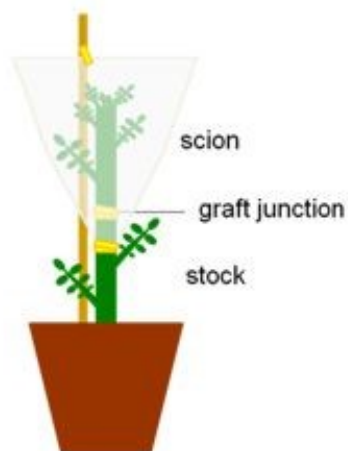
Note: Water prevents the stock and scion from drying out.

18. Slightly trim the end of the scion stem, maintaining the V-shape, immediately before inserting the scion into the stock.



19. Remove the water from the cut stock with a tissue paper, but do not dry it completely. Leave just enough water to keep the slit of the stock moist.
20. Immediately insert the scion into the top of the stock. If the scion and the stock do not fit well, slightly trim one of them to improve the fitting.
21. Wrap the graft junction with paper surgical tape to keep the scion in place.
22. Cover the scion with an inverted transparent plastic bag slightly bigger than the scion and use adhesive tape to close the bag around the stem, without tightening, below the graft junction. This maintains a high humidity, which helps the graft to succeed. Alternatively, use a big plastic bag to cover all the plants or groups of plants.

Note: Try to keep the bag loose enough to prevent it from clinging to the plant leaves. Holding a side of the bag to the stake using adhesive tape can help to keep the bag in place. Avoid moving the grafted plants to prevent the scion from detaching from the stock.



23. Grow the plants for 2 days in the LD greenhouse or under the same conditions used from step 10. Avoid direct sunlight. During this time - and until the graft heals - check the plants daily. If axillary branches grow in the stock, remove them.
24. Cut off a corner of the plastic bag to allow the humidity to slowly decrease.
25. One day later, cut off a second corner of the bag.
26. Two days later, remove the plastic bag.
27. Wait a few more days until the graft heals.
28. Your grafted plants are ready.

Note: It can take up to 7-10 days for the graft to heal. When the grafting is successful, the scion looks healthy and vigorous and resumes growth. Otherwise, scions wilt quickly.

Note: Grafting is easy and works very well in potato plants, with efficiencies as high as 90-100%. However, it is a good idea to practice before starting to graft your valuable plants. Experienced grafters usually obtain higher efficiencies than inexperienced ones.

Recipes

1. 2MS medium (1 L)
 - 4.40 g Murashige & Skoog medium including vitamins
 - 20 g Sucrose
 - 0.5 g MES
 - Add ddH₂O to approximately 800 ml
 - Adjust pH to 5.8 with 1 N KOH
 - Add ddH₂O to final volume
 - Add 2 g Gelrite™
 - Autoclave
 - Pour into appropriate sterile containers before the medium gelifies.
2. Fertilization solution (1 L)
 - 0.849 g KNO₃
 - 0.096 g NH₄NO₃
 - 0.209 g K₂HPO₄
 - 0.490 g KH₂PO₄
 - 0.590 g Ca(NO₃)₂·4H₂O
 - 0.173 g MgSO₄·7H₂O
 - 0.167 g SO₄Fe·7H₂O
 - 0.035 g Kelamix
 - 0.400 g microelements Extra AZ (Intraplex Agrichem)

Dissolve in 800 ml dH₂O

Adjust volume to 1 L

Acknowledgments

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