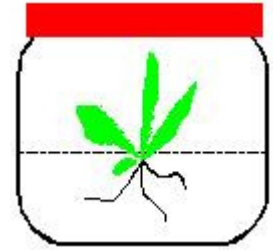


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NOTE: All protocols are intended to be used following the methods described in the KCK Manual: “Plant Tissue Culture for the Classroom and Home” or the online workshop handout.

Culture Immature Embryos of Polish Pea Cultivars

Adapted from Pniewski et al. 2003. Organogenesis and long term micropropagation of Polish pea cultivars. Acta Societatis Botanicorum Poloniae. Vol. 72 (4): 295-302.

MEDIA

Initiation Medium:

In a 1 liter container, combine the following:

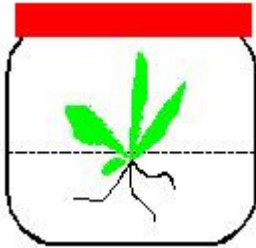
- _____ Distilled water - 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 2 tablespoons
- _____ BAP – 4.5 mg (use 4.5 ml of solution of 1 mg/ml)
- _____ NAA – 0.002 mg (1 drop of 1 mg/ml solution = 0.017 mg)

Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add 1 level “pinch” spoon of agar to each jar. **OPTIONAL: Dispense 2 tablespoons per baby food jar; add 1 level “smidgen” spoon of agar.** Sterilize via microwave or pressure cooker as described in the KCK manual.

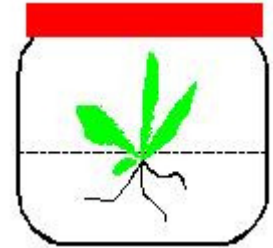
Multiplication Medium (DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

In a 1 liter jar, combine the following:

- _____ Distilled water - 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 2 tablespoons
- _____ BAP - 2 mg (use 2 ml solution of 1 mg/ml)



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Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add 1 level “pinch” spoon of agar to each jar. **OPTIONAL: Dispense 2 tablespoons per baby food jar; add 1 level “smidgen” spoon of agar.** Sterilize via microwave or pressure cooker as described in the KCK manual.

Stage III Medium – Pre-Conditioning (DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

In a 1 liter jar, combine the following:

- _____ Distilled water - 2 cups or about 500 ml
- _____ MS Basal Medium with vitamins (1 liter packet)
- _____ PPM - 1 ml
- _____ BAP – 0.02 mg (use 1 drop of 1 mg/ml solution)
- _____ Sucrose (table sugar) – 2 tablespoons

Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” spoon of agar per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

Stage IV Medium – Rooting (DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

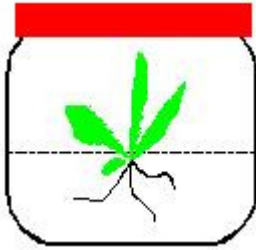
In a 1 liter jar, combine the following:

- _____ Distilled water - 2 cups or about 500 ml
- _____ ½ MS Basal Medium with vitamins (use 1 tsp from 1 liter packet)
- _____ PPM - 1 ml
- _____ Sucrose (table sugar) – 1 tablespoons
- _____ NAA – 1 mg (use 1 ml of 1 mg/ml)

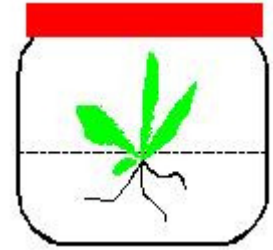
Optional:

- _____ Additional 166 mg of CaCl₂ is recommended
- _____ Additional B5 vitamins are recommended: Nicotinic Acid 0.5 mg; Pyridoxine Hydrochloride 0.5 mg; and Thiamine Hydrochloride 10 mg.

FURTHER RESEARCH IS NEEDED TO DETERMINE WHY THE AUTHORS RECOMMENDED THESE ADDITIONAL CHEMICALS.....cms



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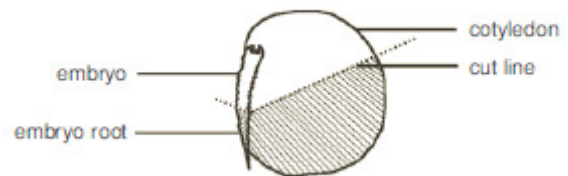
Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” spoon of agar per jar. Sterilize via microwave or pressure cooker as described in the KCK manual.

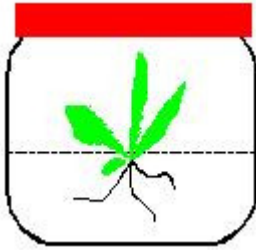
ISOLATION AND CULTURE OF EXPLANT

1. Harvest pods from mother plants. The immature seeds should be fully developed but not yet dry (*author says “just before drying”).
2. Rinse in soapy water for 5-10 minutes to remove debris.
3. Surface sterilize by immersing for 30 seconds in 70% ethanol (or isopropanol) followed by 20 minutes in 25% commercial bleach with a few drops of dish detergent. Stir occasionally to insure all plant material is in contact with the bleach solution.

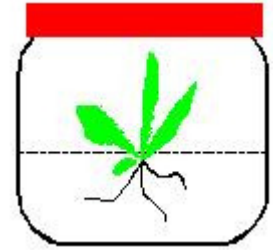
MOVE BLEACH SOLUTION/EXPLANTS TO CLEAN BOX

4. In the cleanbox, rinse in sterile water.
5. Spray a piece of paper toweling with 70% ethanol or isopropanol.
6. Place pod on toweling and cut longitudinally to open pod and remove seeds.
7. Remove seed coats and then embryo roots and the distal part of the cotyledons. See diagram at right (from page 196 of source article listed above).
8. Cut seeds along longer axis into two halves and each half is divided again producing 4 explants from one seed.
9. Transfer explants to **Initiation Medium** and incubate 3 days in darkness at room temperature.





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10. After 3 days the explants were transferred to **Multiplication Medium** and incubated in darkness (?) for 4-6 weeks to obtain callus. **Note: Authors did not indicate moving to light so it is assumed they continued to incubate the cultures in the dark.**
11. Shoots should develop within 2-3 weeks and will probably be from pre-existing meristems and tend to be “true-to-type” meaning they might be clones of the parent. Shoots that develop later will be from callus via organogenesis and not pre-existing meristems. These may exhibit other characteristics which are or are not desirable. Research “somaclonal variation” for more details.
 - a. Transfer callus to fresh **Multiplication Medium** every 4-6 weeks to induce more shoot production. Callus was described as “firm, lumpy green callus”. We assume this was cultured in 16 hr light.
 - b. Excise shoots from the callus and transfer shoots to **Pre-Conditioning Medium** to promote elongation and incubate in 16 hr light at room temperature.
 - c. Fully developed shoots are transferred to **Rooting Medium**.
 - d. Rooted plants are then transferred to soil/perlite mixture and acclimated to the outside environment. This can be accomplished by putting plastic bags over the pots or growing in a domed tray.

See original publication for more details. **Note: KCK has not tested this protocol.**

The following article needs to be consulted to answer many questions:

Griga, M., E. Tejklova, F.J. Novak and M. Kubalaková. 1986. In vitro clonal propagation of *Pisum sativum* L. *Plant Cell, Tissue and Organ Culture* 6: 95-104.