# *Stem Propagation of Phalaenopsis Orchids*

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## Introduction

When Phalaenopsis stem sections containing a viable node are exposed to shoot growth promoting hormones, the dormant bud at the node will commence to grow, forming a new shoot. Prolonging the exposure will result in the shoot branching to form multiple new shoots.

If these new shoots are removed, separately and undamaged from the old stem section and exposed to root growth promoting hormones, roots will develop resulting in the production of plantlets. These plantlets can then be grown on to maturity in conventional solid orchid growing media.

Since the new plantlets produced are effectively ‘cuttings’ they will generally have all the characteristics of the parent plant although, rarely, ‘sports’ may be formed as with normal cuttings.

At the present state of the art, the growth hormones must be applied in the form of a gel and the hormone treatments must be carried out in sterile conditions to prevent mould growth leading to the death of the stem section.

## Equipment Required

**1.** A normal kitchen pressure cooker. This should provide a working pressure of 15psi. Fifteen minutes heating at this pressure is used to sterilise prepared bottles of the hormone treatment gels.

**2.** A methylated spirits or propane gas burner used for dry sterilising cutting and handling tools.

**3.** A sharp metal handled, short bladed knife for preparing stem sections. Ideally this should be constructed from one piece of stainless steel. A scalpel is the ideal form.

**4.** Stout metal tweezers or artery forceps, ideally constructed from stainless steel, for handling stem sections.

**5.** Scales for weighing dry chemicals.

**6.** Measures for use with the liquid chemicals.
(with items 5 and 6 above, precise measurement is not absolutely essential and there is considerable scope for individual innovation)

**7.** A number of wide necked glass jars with screw threads and metal lids in which to prepare and sterilise the treatment gels. Jars previously containing horseradish, tartare sauce or honey are suitable - total contents around 200ml/ 8oz.

**8.** Thin rubber household gloves.

**9.** Some form of enclosure in which to handle sterile stem sections and jars of sterile media is desirable. The ultimate is a laminar flow (sterile) cabinet but an open fronted cabinet with a transparent top is helpful in preventing contamination. Again there is scope for individual innovation.

**10.** A growing cabinet to hold the sterile jars containing the stem sections is helpful since this facilitates control of temperature and day length within the jars. This is a desirable but non-essential item.

**11.** A pottery or stainless steel dish about 4 - 6 inches diameter and 2 inches deep.

## Materials Required

**1.** Liquid bleach (sodium hypo chlorite solution). Basic ***thin*** liquid bleach is required ***not*** the "thickened" form that has tended to supersede it. It should be purchased from a supermarket where rapid turnover is likely since this material decomposes and loses its effectiveness quickly. When purchased, the bottle should be dated and it should be used within three months of purchase. This material is made by a limited number of manufacturers to a single standard and concentration but it is essential to use the above specified ***basic thin*** type.

**2.** Good quality detergent (washing up liquid).

**3.** Sigma Chemicals multiplication media - this contains the shoot growth promoting medium mentioned in the introduction.

**4.** Sigma Chemicals replating media - this contains the root promoting medium mentioned in the introduction.
(Both items 3 and 4 are supplied with expiry dates of 1 year from the date of purchase but this can be extended to 18 months without problems.)

**5.** Solid Agar - this is the gelling agent used in conjunction with the Sigma materials and is available from Health Food Stores.

**6.** Pure water of neutral pH. Distilled, de-ionised or reverse osmosis purified water is recommended by Sigma Chemical Company - clean rainwater is a satisfactory alternative.

## Preparation of the Jars of Sterile Media

During sterilisation of the jars and contents in the pressure cooker there are two alternative approaches.

**1.**Drill a small hole in the metal lid of the jar and place a small, tightly fitting, twist of cotton wool in the hole. This cotton wool acts as a filter, preventing mould spores entering the jar as pressure changes occur inside. Lids of this type can be tightly closed before and during sterilisation.

**2.** If undrilled lids are used and they are tightly closed before the sterilisation process, pressure build up can cause the jars to burst. Undrilled lids must be left very lightly closed - thread just engaged.

After sterilisation in the pressure cooker, the cooker and contents must be allowed to cool completely before it is opened. When it is opened, the lids on the jars must be fully tightened as they are removed.

For each jar being prepared, 50mls of media should be allowed initially. This quantity can be modified in the light of experience.

A normal pressure cooker will hold six jars and each batch of jars will therefore require 300mls of media.

Quantities required for 300mls of media

| | *Multiplication Media* | *Replate Media* |
|---|---|---|
| Dry Media | 7.5gms | 17gms |
| Solid Agar | 5gms | 5gms |

(If weighing these small quantities is an insurmountable problem, measure both quantities in the above proportions, mix dry and assume 1 level teaspoon is 5 gms.)

The weight of Agar is more critical than the weight of the dry media. Too much Agar will result in a stiff gel that is still useable. Too little will give a soft gel in which the stem sections will not stand upright and is therefore useless.

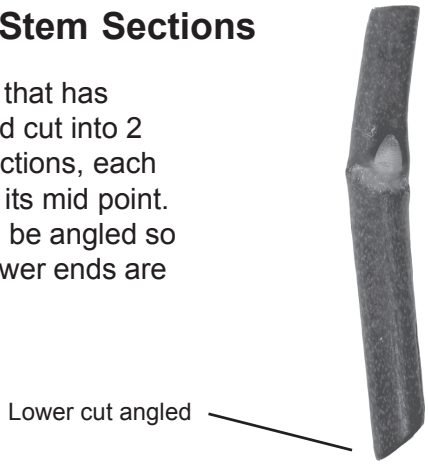
Mix the solid ingredients with the water and gently heat the mixture with constant stirring until the Agar is dissolved - ***do not boil.***

When the Agar has dissolved, pour the mixture into the empty jars to give a depth of half to three-quarters of an inch (13-20mm). Apply the lids to the jars, place them into the pressure cooker, heat to pressure, maintain pressure of 15psi for 15mins and then allow the cooker to fully cool before opening.

The jars are now ready to receive the stem sections.

## Preparation of Stem Sections

Select a flower stem that has finished flowering and cut into 2 inch (50mm) long sections, each containing a node at its mid point. The lower cut should be angled so that the upper and lower ends are easily identified.



## Treatment of Stem Sections

**1.** Prepare 500ml of 10% bleach (50mls bleach diluted to 500ml with tap water) add one drop of detergent. This is now ready for sterilising the stem sections. Part fill a suitable container with the bleach solution and immerse the stem sections at normal room temperature and leave immersed for at least 15mins with occasional agitation.

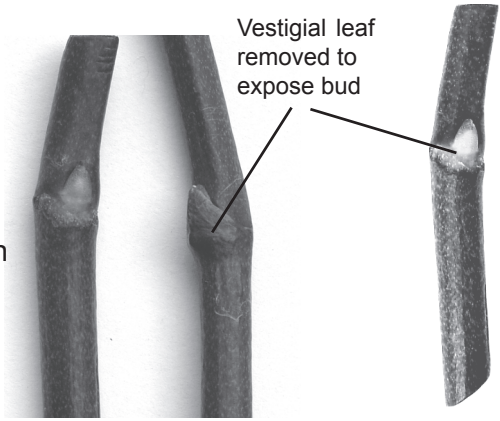
**2.** Now put on the household gloves and swab the outer surfaces of the gloves with bleach to sterilise them. Gloved hands are now used in all further handling procedures and swabbing with bleach should be repeated at convenient intervals to maintain sterilisation.

Immerse the short bladed knife and tweezers/artery forceps in bleach solution and leave for at least 15mins.

**3.** Remove one of the stem sections from the sterilising fluid using the sterilised tweezers. Carefully remove the vestigial leaf covering the bud, from the stem section using the short bladed knife. Take care not to damage or remove the underlying bud while carrying out this action.

**This is the most important part of the whole process.**

If any of the vestigial leaf remains on the stem section, this can trap mould spores and prevent complete sterilisation of the stem section. Examination with a 10x magnifier is useful at this stage



**4.** When the vestigial leaf has been fully removed (again reminding you, - without damaging the underling bud) immerse the stem section in a fresh batch of bleach solution and leave for a further 15mins.

Meanwhile re-immerse the tweezers and knife in bleach solution.

The stem section must not be allowed to come in contact with any un-sterilised surfaces or tools and all handling should be carried out within an enclosure (see 9 in Equipment Required).

**5.** Prepare a work surface by swabbing with bleach and cover with a cloth wetted with bleach. Swab the outer neck portion of one of the jars of multiplication media with bleach. Remove the lid and place it open end down on the work surface, lay the open jar on its side on the work surface and cover both with the cloth wetted with bleach.

**6.** Heat the blade of the knife in the methylated spirit burner which will dry it and ensure complete sterility. Remove the stem section from the bleach solution using the sterilised tweezers/forceps and place it on the sterilised work surface.

Holding the sterile stem section on the sterile surface, re-trim both ends of the section with the sterile knife to remove previously cut surfaces which have had a lengthy exposure to bleach solution. Immediately transfer the section into the jar containing the sterile media, pushing the lower angled end into the gel media. The gel media should hold the section upright - immediately apply the lid to the jar.

This completes the first stage of the procedure.

## Storage of Jars containing Stem Sections

Ideally, the jars should be maintained at a temperature of about 70°F with a day length of 13-14 hours until the bud on the stem section has developed to become a shoot about 1 inch (25mm) long. Under the conditions given above this should take about 12 weeks. At lower temperatures and with shorter day lengths this period will obviously be prolonged.

## Treatment of Mature Shoots

When the shoots have reached a length of approximately 1 inch they can be treated in two alternative ways.

**1.** If the shoot is removed from the stem section and transferred to a fresh jar of multiplication media this will cause the shoot to divide, producing generally 3 or 4 shoots. If these are then divided and each inserted in a further jar of multiplication media, the process can be repeated ad infinitum to produce large quantities of shoots.

**2.** If the shoot is removed from the stem section and transferred to a fresh jar containing replate media it will develop roots and continue to grow to become a small plant. In time this can then be transferred to a small pot of normal seedling (i.e. fine) compost to grow on thus completing the propogation process.

Needless to say all the sterile handling processes already detailed must be followed throughout.

## Preparation of Shoots for Transfer to Replating Media

**1.** Prepare a sterile work surface and sterilise the short bladed knife and tweezers/forceps as described previously. Fill a pottery or stainless steel dish about 1 inch deep with dilute bleach solution.

**2.** Using sterile tweezers/forceps take a stem section with a shoot from the jar in which it has developed, transfer it to the dish of bleach and ensure it is fully immersed. Hold the stem section with the tweezers and using the sterile knife, separate the shoot from the stem section. At this stage the new shoot is still sterile and can be transferred directly to a fresh jar of sterile media. There is no need to trim the cut end of the shoot.
The above details cover transfer to either multiplication or replate media.

**3.** Shoots previously transferred to replate media which have already divided are transferred to a dish of bleach solution and divided into individual shoots as in 1 and 2 above. They are then ready for placement into fresh jars of the selected media.

## Storage of Jars containing Un-rooted Shoots

This is exactly the same as the treatment of jars containing the original stem sections and in due course these shoots will result in small plants. Root development is rapid and the plants with roots and $1^{1/2}$ -2 inch (30 -50mm) leaf span can develop in about 8 weeks after transfer to replate media.

With vigorous plants, optimum conditions for growth of the shoot and development of roots the flowering size can be reached in about two years from the original preparation of the stem sections.

## Trouble Shooting

**1.** *Stem sections do not stand upright in the sterile gel.*

Agar is a natural seaweed product and does vary a little from batch to batch and from source to source. If the gel is too weak - increase the concentration of Agar.
Exposure to high temperatures for extended periods in the pressure cooker will weaken the gel and must be avoided. Repeated sterilisation is not recommended.

**2.** *Bud on stem fails to develop.*

Almost invariably means that the bud was removed along with the vestigial leaf surrounding it.

**3.** *Mould growth develops in the prepared jars.*

Invariably occurs in the first jar of multiplication media and is due to incomplete sterilisation of the stem section caused by incomplete removal of vestigial leaf.

It is worth re-sterilising stem sections affected in this way if only a small amount of mould has developed.

Poor techniques allowing contamination during handling stem sections or ineffectual sterilisation of tools and surfaces is a lower possibility if the detailed directions given above are carefully followed.

Various stages of the process can be tested for contamination by using blanks. For example, using pieces of wooden toothpick instead of stem sections.

Sigma Chemicals website http://www.sigmaaldrich.com

Sigma products required:
**P6793** Phytamax Orchid Multiplication Medium
**P1056** Phytamax Orchid Medium

The above can only be ordered by a company registered with Sigma.



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