Lotte & Thomas Orchids

- seed germination
- node culture
- links
- guestbook
- mainpage
- pictures / books
- downloads & hints
- email us
- laminar flow hood
- seedling list

Hello and welcome on our website !

Cephalanthera rubra

ere we describe different techniques, which we have tried and modified, to propagate orchids esides seed sowing there are for example techniques to rise plants from sleeping buds (nodes)

austrian orchids (photos)

last update ovember at (seedling list)

Deutsche ersion

Seed germination

[The biology of orchid seed germination]

[How can I get some seeds ?]

[Packing and shipping seeds]

[germination on bark]

[asymbiotic germination]

Asymbiotic seed germination

lternative replating technique Deflasking protocorms on soil

f you are interested in seed exchange, please send us an email

back to seed germination

Maintaining sterile conditions

n symbiotic and asymbiotic germination it is vital that all seeds, flasks, instruments and media are kept sterile at every stage of the germination procedure f any fungi or bacteria get into the flasks they will grow much faster than our seeds or seedlings and will kill them soon

o prevent this contaminations, the flasks (inculding the media) have to be autoclaved or should be placed for minutes in the oven (C)

he seeds or plantlets (nodes,) must be sterili ed (e g with hydrogen peroxide) and transferred to the flasks without introducing extraneous bacteria or fungus

aminar flow hood

he laminar flow hood consists of a cabinet and a laminar air flow unit he laminar flow unit includes an very fine filter (hepa filters) which removes all bacteria and fungi he filtered (sterile) air flows out of the cabinet and produces a sterile area inside the cabinet

efore using the laminar flow hood you have to sterili e the inner surface of the cabinet with alcohol

Glove box

glove box consist of a glass box (e g an aquarium) which is closed on it s open side including two openings to put your hand trough efore you start working you have to place all necessary equipment, flasks, seeds and chemicals inside the glove box hen close the box and spray the area inside the box with desinfection solution (e g alcohol)

orking above boilding water

his is the cheapest way to propagate orchids in vitro t this technique you use the fact that steam is sterile he si e of the sterile area depends on the diameter of the used pot

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Necessary equipment

ools

Equipment	edia preparation	seed sowing and replating
beaker ml		
balance		
forceps (stainless)		
scalpel (stainless)		
replating tool		
glasfunnel		
spirit stove (collapsible cooker)		

alcohol burner (for flaming tools)		
oven		
cooking pot		
grill		
gloves		

articles of consumption

Equipment	edia preparation	seed sowing and replating
flasks (jars, test tubes,)		
labels		
paper towels		
distilled water		
Ethanol		
ydrogen peroxide ( ) to sterili e dry seeds		
bleach solution (e g Clorox) to sterili e green capsules		
gar gar powder (if it s not included in the media)		
media		
aluminium foil		

ources of supply

igma
Phytotechlab
Duchefa
issue uick Plant abs

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Media preparation

necessary tools

- beaker ml
- spirit stove (collapsible cooker)
- balance
- glasfunnel
- oven
- something to stir the cooking media (e g old spoon)

necessary articles of consumption

- flasks (jars, test tubes,)
- paper towels
- destilled water
- labels
- gar gar powder (if it s not included in the media)
- media
- aluminium foil

he sources of supply you can find under ecessary equipment

Culture vessels for sowing seeds (motherflasks)

e prever testtubes for seed sowing because of their length, they do do not get to hot while they are lying in the steril area (steam)

Culture vessels for replating

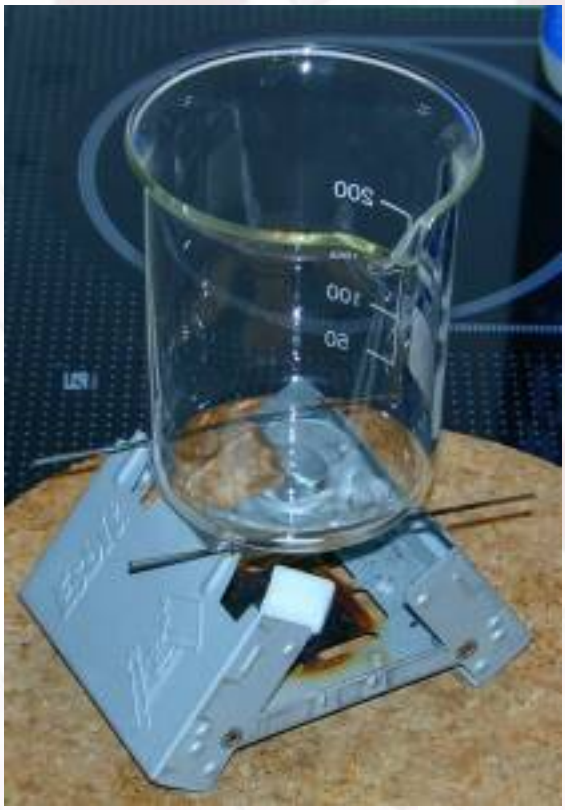
or replating we have to choose flasks which are about cm high with an opening smaller than cm f the si e of the opening is bigger than cm the risk of contaminations increases very fast abyfood jars are very good

Preparing the flasks

efore you can use food jars for in vitro culture you have to remove all rests of food and labels Check if the jars can resist C because we have to heat the flasks with this temperature for minutes to kill all fungi and bacteria

Preparing the spirit stove (only one time)

e prefer spirit stoves with solid fuel because they are easy to handle and they don t smell bad like others do n the picture below you can see how we have modified it



edia preparation

essure out the necessary quantity of media powder

f your media does not include a gelling agent (e g agar agar) you have to messure out the necessary quantity of agar agar or our medias we us g agar agar per liter media

urn on the baking oven (C)

Put about of required distilled water in your beaker and add the media powder tir the solution till the powder is completely dissolved

dd distilled water till you reach the final quantity of media and stir well again

easure the p and adjust it to , (use C to decrease p or a to increase p)

igma P media is adjusted, so measuring p is not necessary

ow you can place your beaker on the spirit stove to heat the media

s soon as the water starts boiling, stir the agar agar powder in the water

et the media boil for about minutes and keep stirring

Dispense the media into your culture vessels atch out that the media does not contact the opening of the flask because this can help fungi and bacteria to contaminate this flask glassfunnel is very helpful

crew the lids loosely on the flasks f you use test tubes you have to put a cottonplug into each test tube

ow you have to cover your flasks with aluminium foil f you use test tube we recommend to cover all tubes with an additional aluminium foil to make shure that the cottonplug stay in the tubes Place the vessels in the baking oven

fter minutes turn off the oven but don t open it et the flasks cool down in the closed baking oven e prepare our medias always after dinner, so nobody needs the oven and the flasks can stay in it overnight

ake the cold flasks out of the oven and let them rest for at least days to make shure that they are sterile

aking sterile distilled water

hen you want to flask dry seeds you should rinse them with sterile distilled water after sterili ation t is very easy to get sterile distilled water ill some distilled water in a screwable jar, screw the lid loosely on the flask and cover the flask with aluminium foil ow you can sterili e the water together with your flasks containing the medias in the oven

edia we use

seed germination	P (igma P)

replating	P (igma P without gar gar)
node culture	P (igma P)

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Sowing seeds from green capsules

he inside of an orchid capsule, if intact, is naturally sterile f you sterili e the outside of the capsule and open the capsule under sterile conditions the seeds should be sterile his method has the advantage that the seeds themselves do not need to be sterili ed (which can sometimes lead to damage) n addition, some seeds, if taken from capsules which are almost ripe, germinate quicker than those taken from mature capsules

necessary ools

- grill
- cooking pod
- alcohol burner
- gloves
- replating tool
- forceps
- scalpel

necessary articles of consumption

- flasks containing media
- kitchen paper
- ethanol
- Clorox (bleach solution)
- screwable flask (e g babyfood jar)

ou can find the sources of supply at Equipment

dvantages of green capsules

- easy to sterili e
- you don t have to wait till the capsules dehisces

Disadvantages of green capsules

- you can t be shure if the seeds in your capsule are ripe or not
- very often the seeds in the capsule are not completely dry so you can t store them in your fridge for further use

Preparing the flasking area

e us the steam above a pot with boilding water to provide sterile conditions o minimi e the risk of contaminations you should reduce draft in your room as much as possible Close all windows and doors while you are flasking n the picture below you can see our preferred arrangement of tools (for right handed person)

ext steps

pen the bottle with ethanol and place you forceps or repaltingtool into it ill about cm water in your pot and turn on your oven he temperature of the boilding water must be high enough to produce a steady flow of steam s soon as the water starts to boil, take a kitchen paper, soak it with ethanol and use it to clean the grill hen you finished cleaning place the grill on the pod

terili ation of green capsules

Carefully remove dead flower parts off the capsule to reduce the risk of contaminations

capsule ready for sterili ation

 inse your screwable flask with ethanol and fill the flask with Clorox Put the capsule into the flask and make shure that the complete capsule is immersed

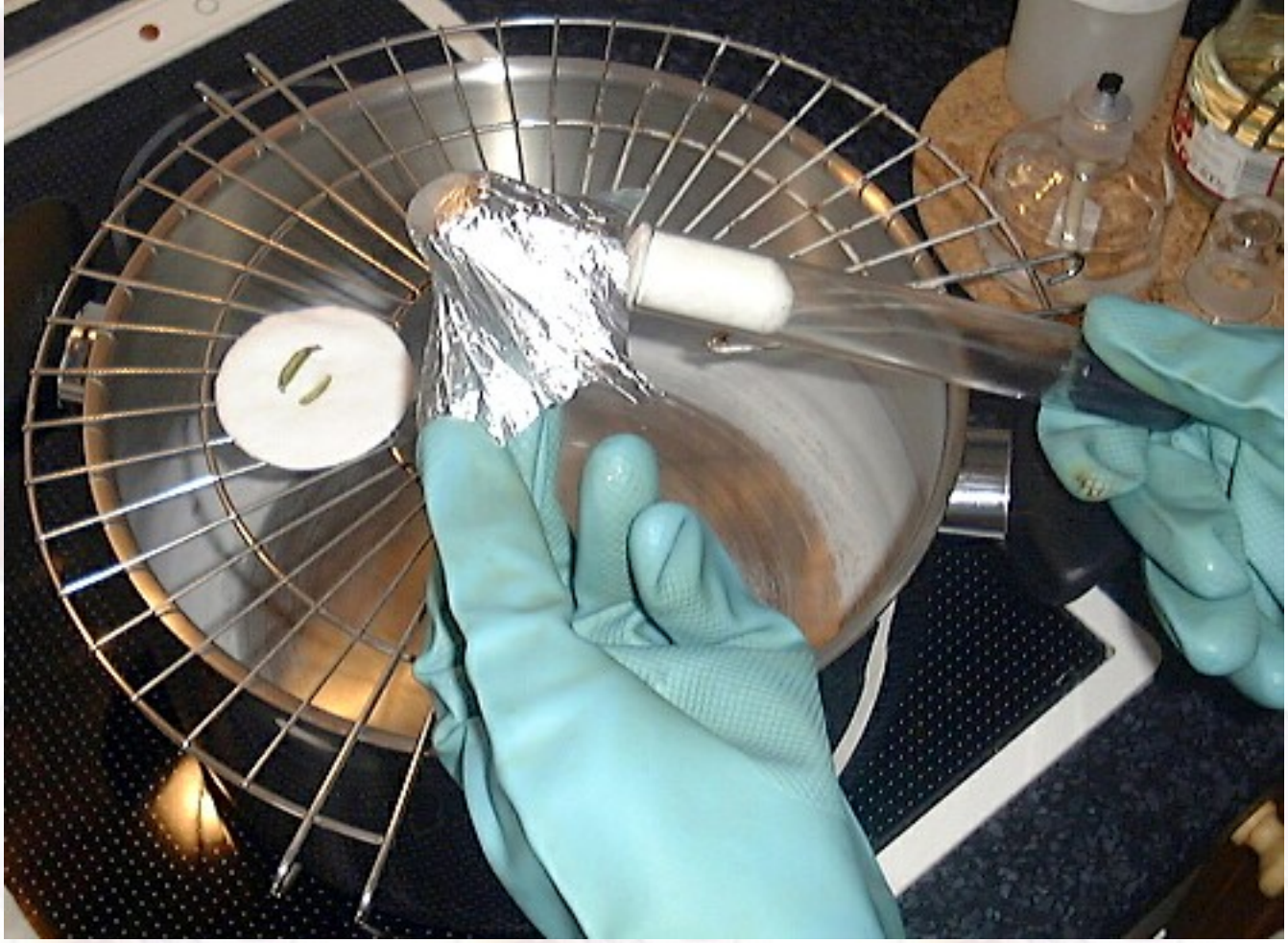
 eed sowing

he following steps must be done in the sterile area (steam) pen test tubes and their cotton plugs have to stay in the steam till the test tube is closed again

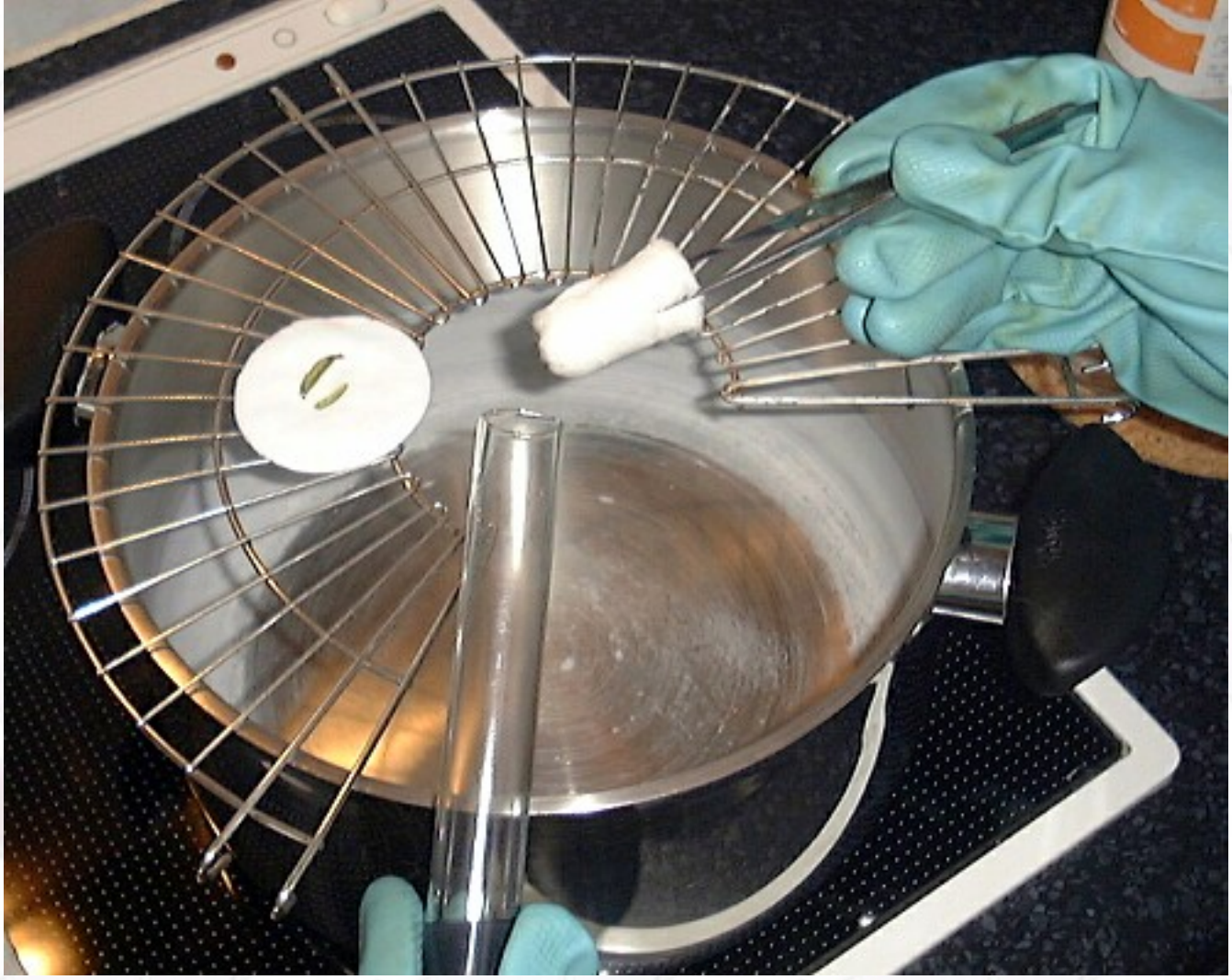
 fter sterili ing the capsule for minuten you can start to open the capsule in the sterile area (steam) Put on your gloves, soak a piece of kitchen paper with ethanol and put it down on the grill ake the flask containing the capsule and open it in the steam Place the lid of the flask somewhere on the table and transfer the capsule with a flamed forceps to the kitchen paper which is lying on the grill old your forceps for a short moment in the boiling water and bring it back into ethanol

pen the capsule with a flamed scalpel and forceps fter doing that, hold your forceps and the scalpel for a short moment in the boiling water and bring it back into ethanol

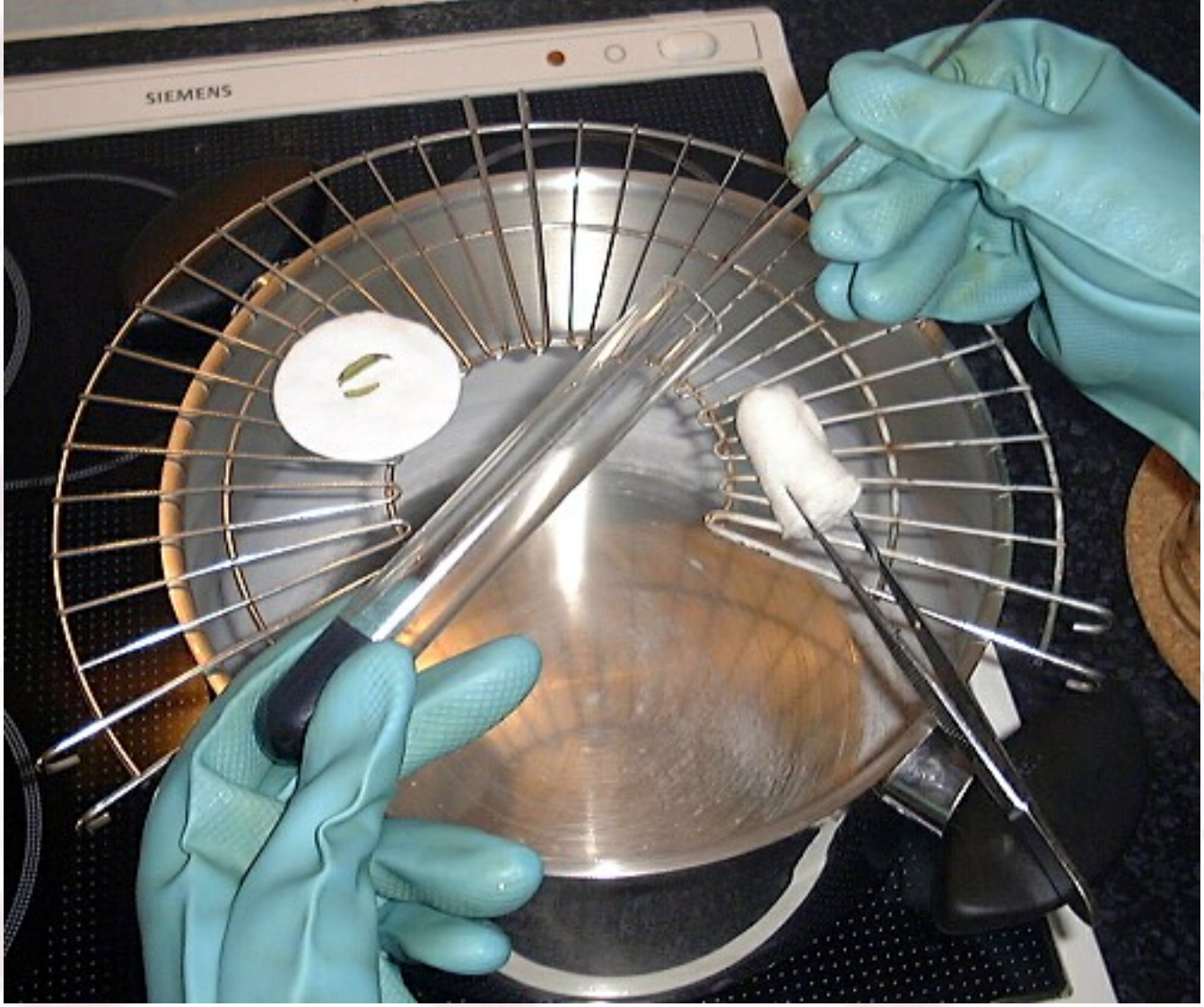
ake a test tube and remove the aluminium foil cap Place the foil cap close to the pod on a kitchen paper which is soaked with ethanol



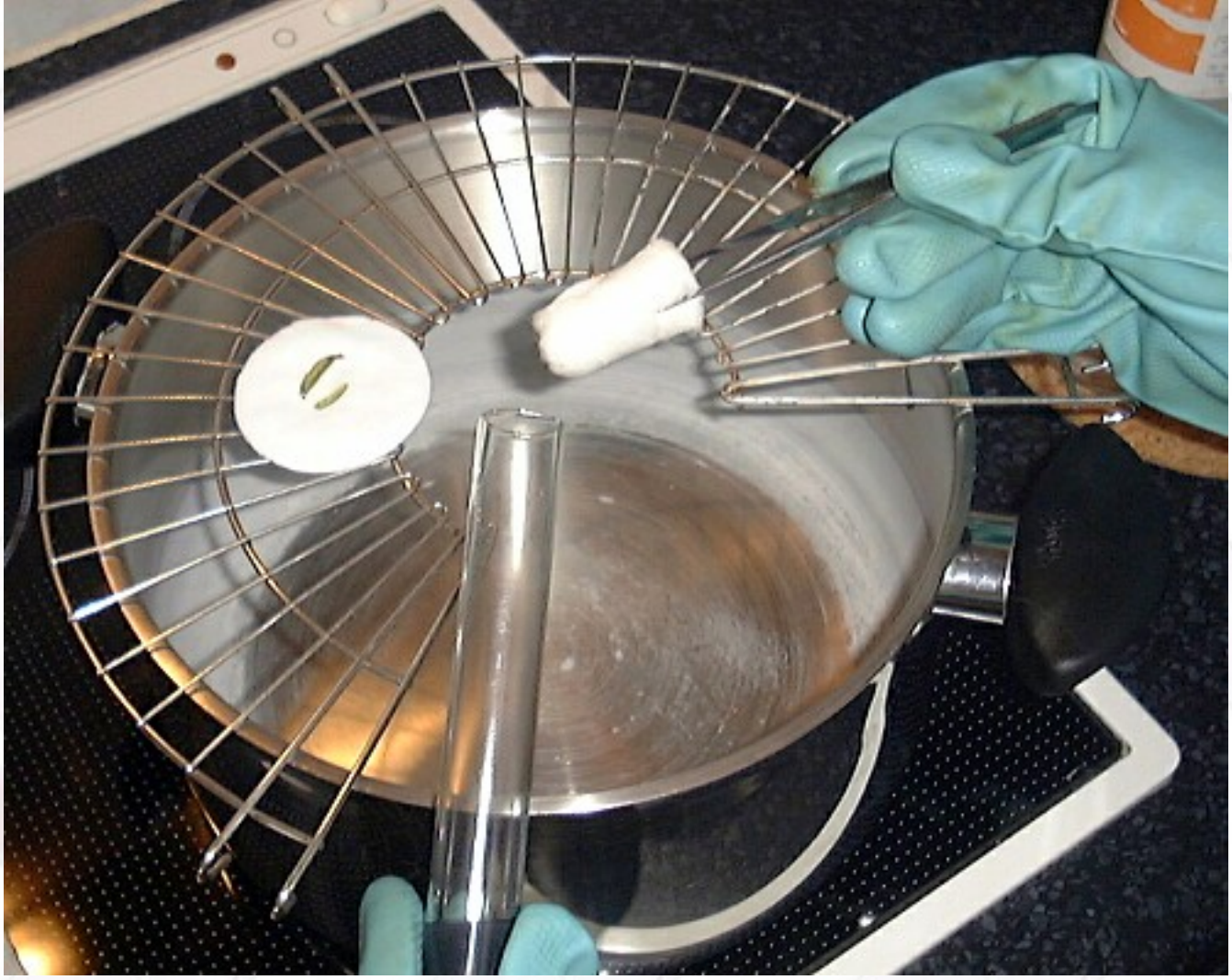
ake the forceps out of the ethanol and flame it emove the cotton plug with the flamed forceps and place the plug on the grill



lame your replating tool and pick up some seeds with it ransfer the seeds directly into the test tube on the media fter bringing the seeds on the media hold the replating tool for a short moment in the boiling water and bring it back into ethanol



Pick up the forceps which is holding the cotton plug and put the plug back into the test tube old the forceps for a short moment in the boiling water and bring it back into ethanol



lame the cotton plug

Put the aluminium foil cap on the test tube o make shure that the foil cap does not move around we put a rubber band around it

ome hints

- open flasks and their lids have to stay in the steam till they are closed again
- don t move around to fast while open flasks are lying in the steam
- don t speak while open flasks are lying on the grill

urther care

e place our flask at our windowsill like the picture below shows he temperature is about C t is very important to prevent direct sun because the seeds in the flasks will become to hot if they get direct sun f you have no windowsill available you can use a att fluorescent tubes e advice you to place the flasks at different places, so you can get a feeling which species needs which conditions to germinate

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Sowing dry seeds

f you want to flask dry seeds you have to wait till your capsule opens hen the capsule starts to dehisce cut the capsule and shake the dry seeds out on a sheet of paper efore you start sterili ing your seeds you should remove all contaminations (parts of the capsule, pollen tubes,)

necessary ools

- grill
- cooking pod
- alcohol burner
- gloves
- replating tool
- forceps
- scalpel

necessary articles of consumption

- flasks containing media
- kitchen paper
- ethanol
- hydrogen peroxide
- screwable flask (e g babyfood jar)
- sterile distilled water

ou can find the sources of supply at Equipment

dvantages of using dry seeds

- you can store a part of your seeds in the fridge for later use

Disadvantages of using dry seeds

- higher contamination risk because they are not as easy to sterili e as green pods are
- you have to wait till the capsule opens

Preparing the flasking area

e us the steam above a pot with boilding water to provide sterile conditions o minimi e the risk of contaminations you should reduce draft in your room as much as possible Close all windows and doors while you are flasking n the picture below you can see our preferred arrangement of tools (for right handed person)

aking a hydrogen peroxide desinfection solution

o reduce the concentration of the bought hydrogen peroxide (e g) to the required we have to add some distilled water to the high concentrated solution ith the formula below you can calculate how much distilled water you have to add to the high concentrated solution to get a solution

Example

anted quantity of hydrogen peroxide Concentration of the high concentrated solution	ml

ormula quantity high (total quantity low) high ( ) , ml

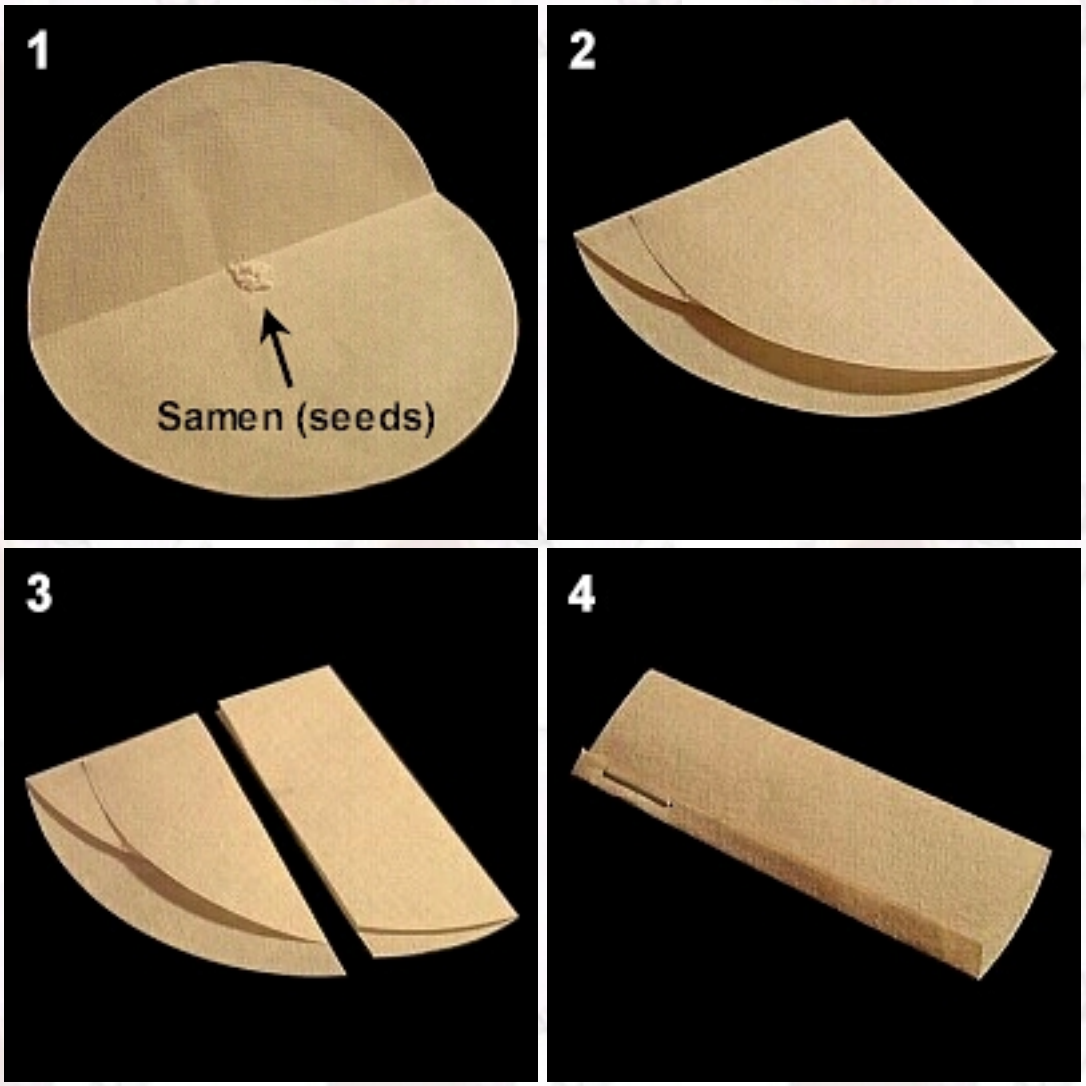
n this example you have to put , ml into a beaker and add distilled water till you reach ml total quantity his ml will have a concetration of

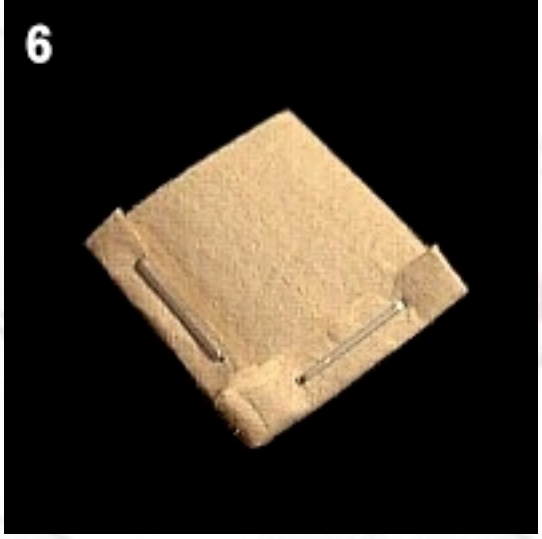
ext steps

pen the bottle with ethanol and place you forceps or repaltingtool into it ill about cm water in your pot and turn on your oven he temperature of the boilding water must be high enough to produce a steady flow of steam s soon as the water starts to boil, take a kitchen paper, soak it with ethanol and use it to clean the grill hen you finished cleaning place the grill on the pod

terili ation of dry seeds

Pack your seeds in a small filter paper envelope like the pictures below show





ill about cm hydrogen peroxide in a screwable flask and put the filter paper envelope into the flask crew down the lid and agitate the flask for minutes to make shure that the envelope has as much contact with the sterili ation solution as possible

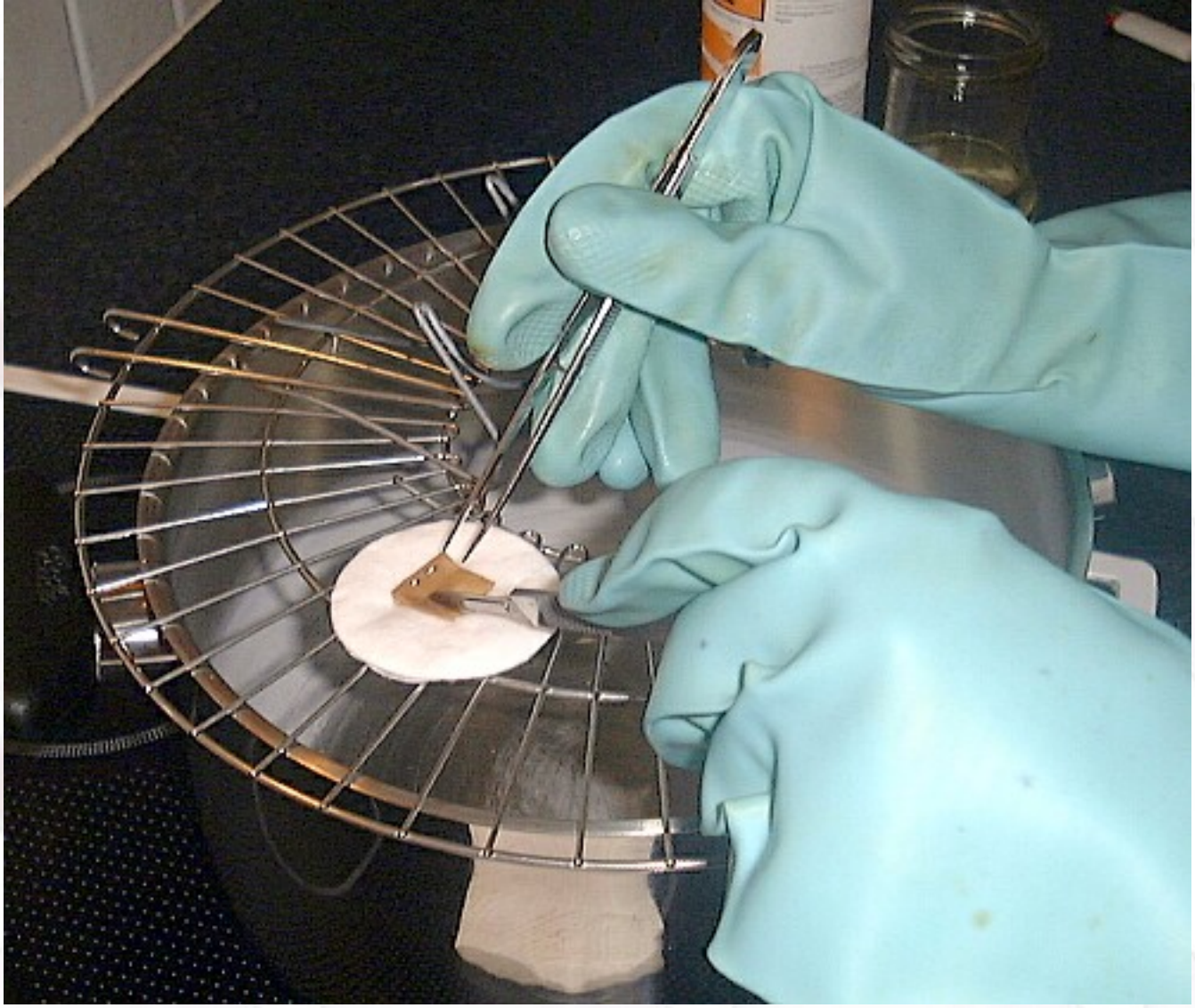
eed sowing

he following steps must be done in the sterile area (steam) pen test tubes and their cotton plugs have to stay in the steam till the test tube is closed again

fter sterili ing the seeds you can start to transfer them into your test tubes Put on your gloves, take the flask containing sterile distilled water and open it in the steam Put down the flask on the grill

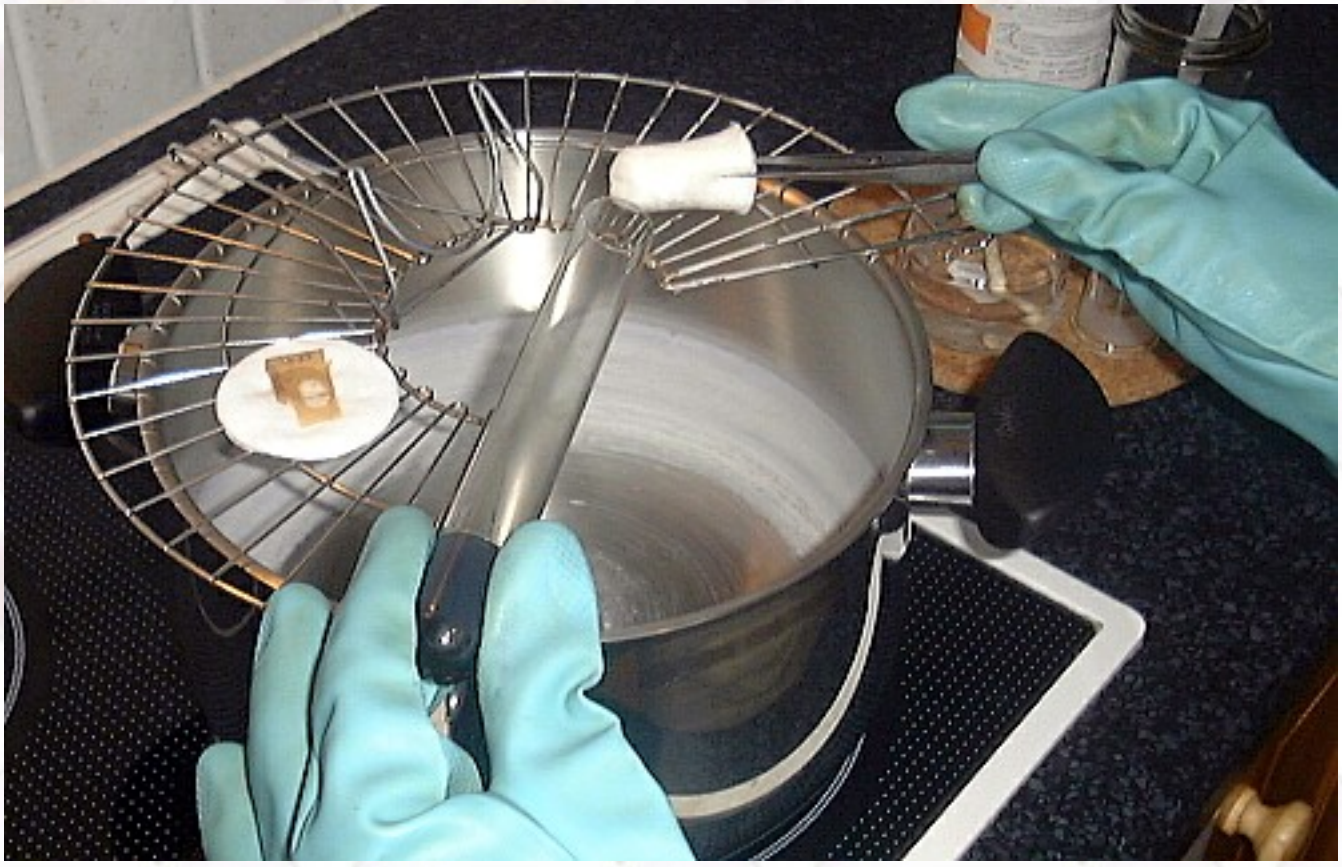
ow take the flask where the seed envelope is swimming in and open it in the steam lame your forceps and transfer the envelope to the sterile distilled water

fter rinsing the envelope for some seconds in sterile distilled water move the envelope with a flamed forceps to the ethanol soaked kitchen paper (on the grill)
lame a scalpel and open the envelope



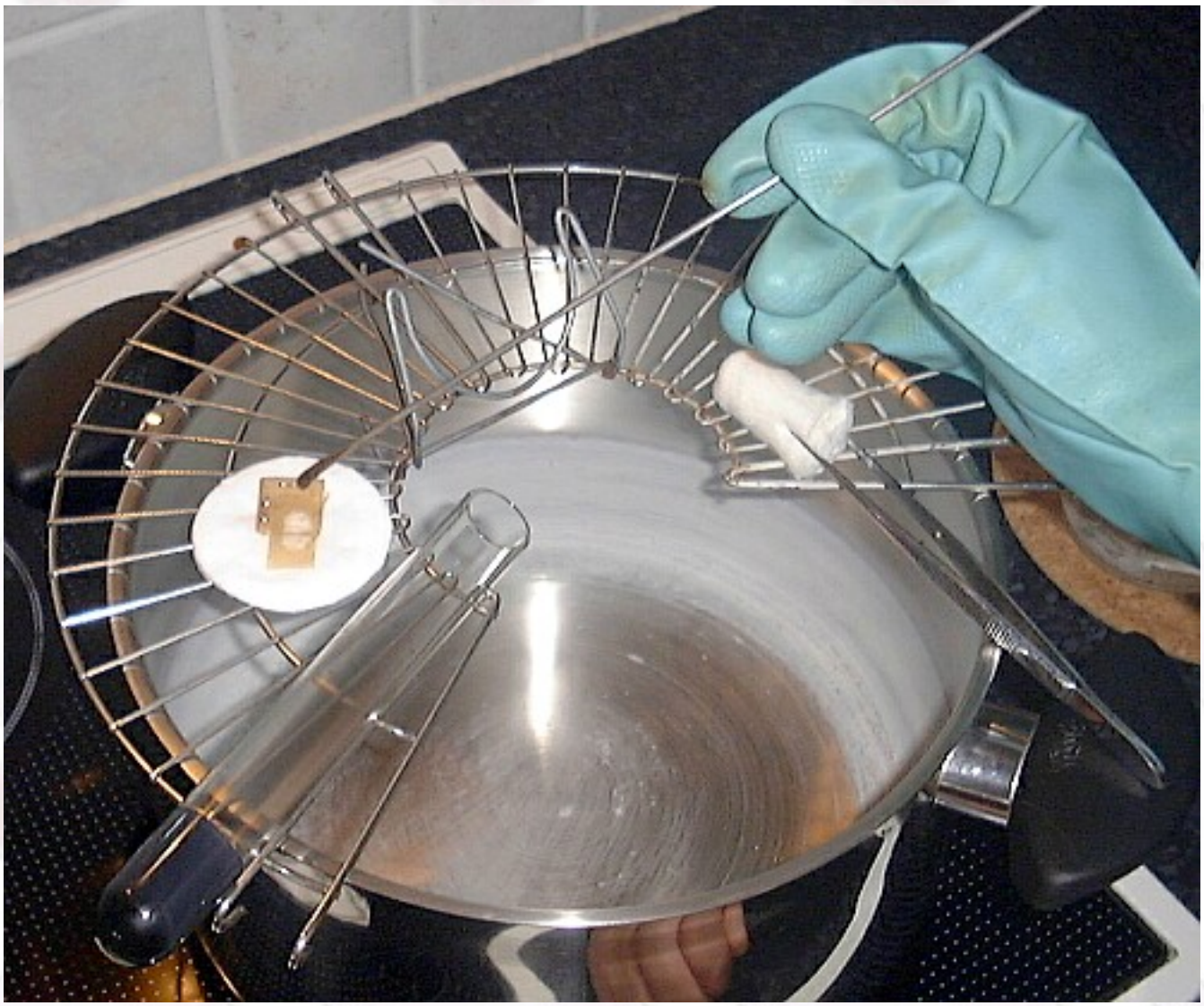
old your scalpel and the forceps for a short moment in the boiling water and bring them back into ethanol ake a test tube and remove the aluminium foil cap Place the cap close to the pod on a kitchen paper which is soaked with ethanol

ake the forceps out of the ethanol and flame it emove the cotton plug with the flamed forceps and place the plug on the grill



lame your replating tool and pick up some seeds with it ransfer the seeds directly into the test tube on the media fter bringing the seeds on the media

hold the replating tool for a short moment in the boiling water and bring it back into ethanol



Pick up the forceps which is holding the cotton plug and put the plug back into the test tube old the forceps for a short moment in the boiling water and bring it back into ethanol

lame the cotton plug

Put the aluminium foil cap on the test tube o make shure that the foil cap does not move around we put a rubber band around it

ome hints

- open flasks and their lids have to stay in the steam till they are closed again
- don t move around to fast while open flasks are lying in the steam
- don t speak while open flasks are lying on the grill

urther care

e place our flask at our windowsill like the picture below shows he temperature is about C t is very important to prevent direct sun because the seeds in the flasks will become to hot if they get direct sun f you have no windowsill available you can use a att fluorescent tubes e advice you to place the flasks on different places, so you can get a feeling which species needs which conditions to germinate

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Replating protocorms

necessary tools

- grill
- cooking pot
- candle
- gloves
- forceps

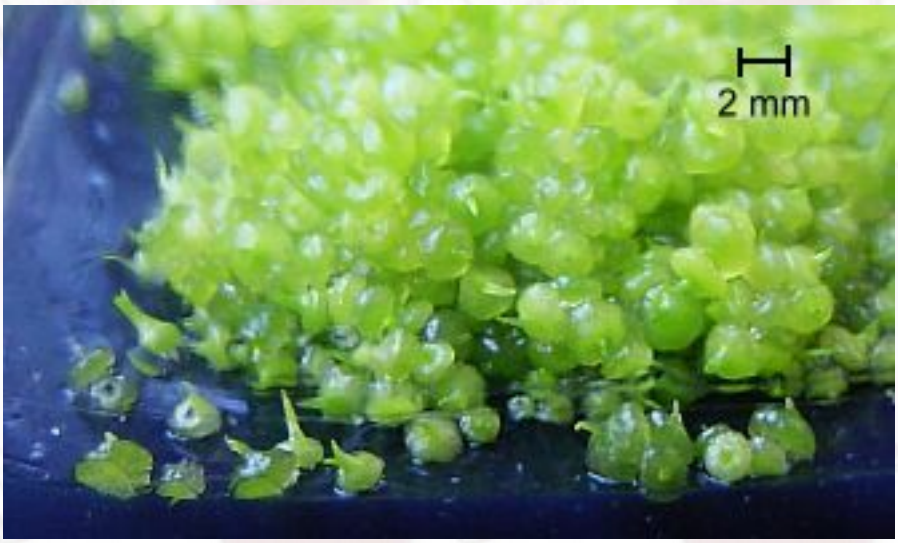
necessary articles of consumption

- flask with media
- kitchen paper
- ethanol

ou can find the sources of supply at Equipment

hen do have to replate my protocorms

et the protocorms grow on their media as long as they don t harm themself or till they start to build first roots s bigger and healthier they are as better they survive replating e sow very small quantities of seeds in flasks, so we can wait a little bit longer and the protocorms are stronger when we replate them



Encyclia vespa Protokorme ready for replating

eplating technique

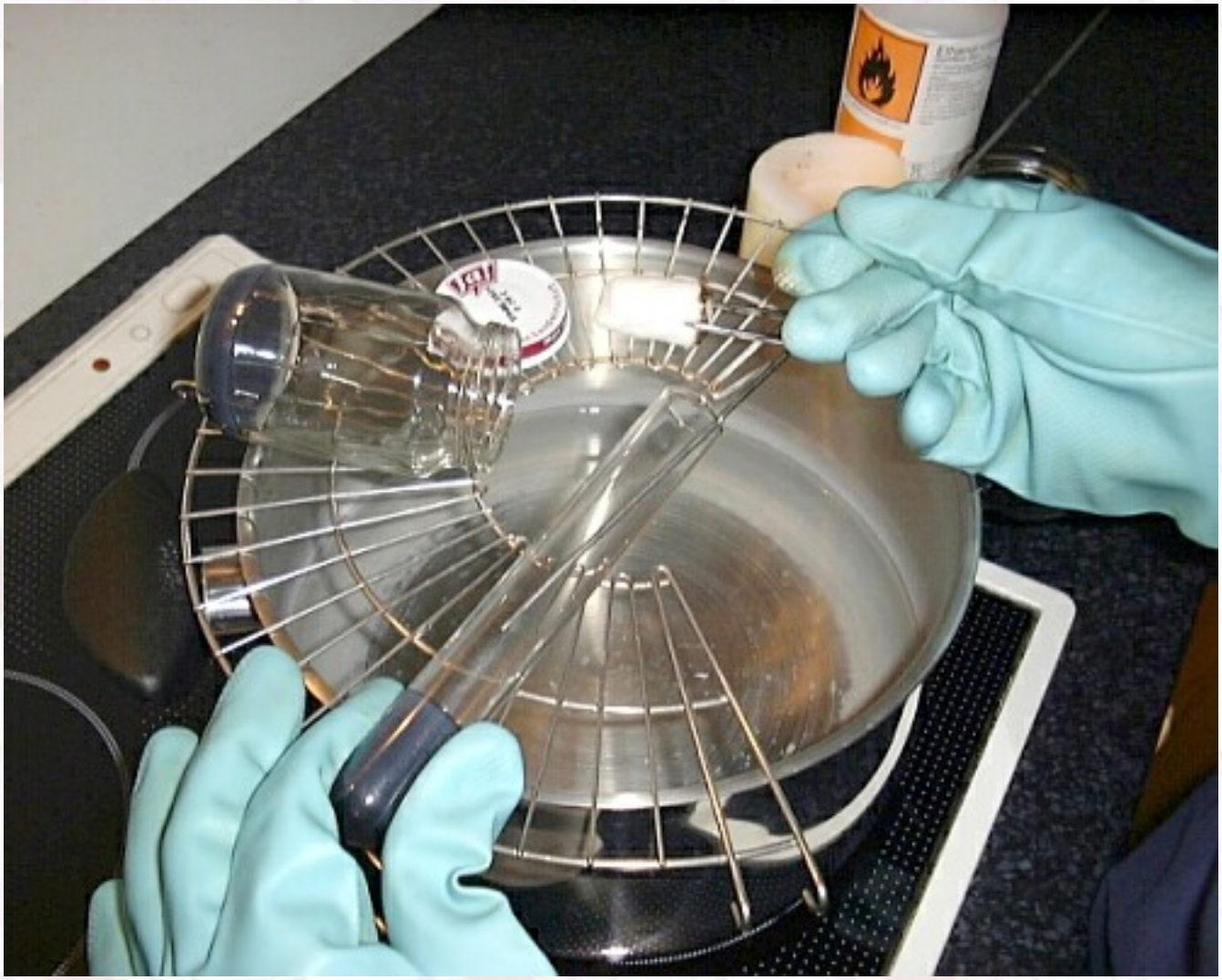
he following steps must be done in the sterile area (steam) pen test tubes and their cotton plugs have to stay in the steam till the test tube is closed again

Put on your gloves, take a test tube (mother flask) and remove the aluminium foil cap Place the cap close to the pod on a kitchen paper which is soaked with ethanol ake the forceps out of the ethanol and flame it emove the cotton plug with the flamed forceps and place the plug and the forceps on the grill

ow you can pick up a replating flask and remove the protecting aluminium foil in the sterile area (steam)

pen the flask and place the lid on the grill he replating flask can be put down on the grill too

ext take the replating tool out of the ethanol and pass the flame of your candle to sterili e it efore you pick up some protocorms you should dip the tool into the media, on which the protocorms are growing, to cool it fter cooling, take some protocorms and transfer them into the new flasks



ow dip your replating tool into the boilding water to clean it and place the clean tool in ethanol till you need it for your next flask Close the replating flask and place it on your desk for labeling (later)

ith the other replating flasks you can process the same way

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Deflasking seedlings

necessary tools

- forceps
- if you have glass bottles a chisel and a hammer

necessary articles of consumption

- seedling media
- kitchen paper
- styrofoam (for draining the community pots)
- disinfectant solution (e g , Chinosol)

ou can find the sources of supply at Equipment

hen should deflask my seedlings

et your seedlings grow on media as long as they don t harm themself and they grow well s bigger they are as easier they survive deflasking he best season for deflasking is spring

seedlings big enough for deflasking

mportant to know

rchid seedlings are raised under sterile conditions on media containing all necessary nutrients to reduce the in vitro time to a minimum hen we take the seedlings out of the sterile enviroment (the flask) they get in contact with a lot of stress causing things (fungi, bacteria,) he seedlings need some time to acclimati e to this harder conditions and we should try to do that as mild as possible efore you start deflasking you should find out how your orchids grows in wildlife and how you have to grow them

Plants without water storage tissues (e g asdevallias), which live in areas with constant humid conditions, require more humidity than succulent orchids (e g Cattleyas, aelias)

ost seedlings die because of to much water

efore you start to take the seedligns out of their flask you should prepare the seedling mix e prefer the following media

part pine tree bark
eil moss
eil charchoal
eil soil from the forest (e g soil made of beech leaves)

seedling mix

hen you finish media preparation you can start to moisten a piece of kitchen paper ext prepare the , disinfectant solution by dissolving a , g tablet in water ow you can open the flask and take the seedlings out without demaging their roots f you have a bottle with a thin neck it is best to cut off the bottom of the flask by using a thin chisel

fter taking the seedlings out of the flasks you should remove all the media where the seedlings are growing in arm water (about degrees celsius) helps you to remove small media pieces fter cleaning the seedling try to seperate them without demaging them f it is not possible, don t worry leave them together ext put the seedlings for minutes in your disinfectant solution hile the seedlings are swimming in the disinfectant solution you can prepare your community pots irst of all put about cm styrofoam pieces into the pot (drainage) ext put

seedling mix into the pot till the pot is filled for two thirds (about) hen the minutes of disinfection elapsed you can start to pot the seedlings with some additional seedling mix rchid babies want to be potted close together

community pot

urther care

n indoor green house with adjustable air supply is very useful for acclimati ation because you can increase the humidity step by step hen you use such an indoor green house make shure that no water remains in it and enough fresh air gets into it otherwise the seedlings will die soon f you are not shure to water them or not it s better wait one more day

indoor green house

ome links to other deflasking instructions on the internet

urleigh Park rchids
ob ynn ellenstein

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Contaminations

hat are contaminations

f fungi or bacteria start to grow on media we call it a contamination because fungi and bacteria grow much faster than orchid seeds and will kill them soon

Contaminations

ore photos of contaminations photo photo photo photo photo

hat are the sources of contaminations

here are many different causes why contaminations can appear

- sterili ation failed
- problems in your sterile technique
- leaky caps

ow do contaminations look like

any contaminations can be found or days after flasking seeds or plantlets (e g nodes) ere are some markings of contaminations

- fast growing discs on the media

- fast growing carpet (looks like thin hairs)
- edia turns white and becomes liquid

hat can do with contaminated flasks

t is very important to detect a contamination very soon because they can grow very fast f you have found a starting contamination you can try to replate clean seeds or protocorms into other flask hen you are culturing nodes you can sterili e the node with hydrogen peroxide ( ) for minutes again and then place it on new media

f one of your flasks is contaminated and you are not able to rescue it on the same day, you should transfer the flask to a darker and cool place because at this conditions the contamination grow a little bit slower

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Node culture

rom sleeping buds you can produce one or more plants (clones)

What are nodes ?

culture in soil

culture in vitro

What are growth regulators (hormones) ?

in vitro node culture

Preparing the nodes

he advantage of this technique is that the new plants are clones of their parents and look like they do e have used this technique to propagate *Phalaenopsis, Doritis pulcherima, Phaius tankervilleae* and *Chiloschista lunifera*

uitable are nodes, which you cut diagonal with cm below and above of the eye on the flower stalk t is very important to us a very sharp knife because otherwise the tissue will be hurt to much

Phalaenopsis flower stalk with bract

ext you have to remove the bract covering the node carefully

Phalaenopsis flower stalk (node) without bract

hich media should you use

o initiate the growth of the sleeping eye we have to use media which includes cytocinins (phytohormon) e use igma s P (Phytotechlab P)

Preparing the flasking area

ou can use the same equipment we discribed under eed sowing

lasking the nodes

Dip the trimed nodes for a few seconds in ethanol fter that put the nodes for minutes in , hydrogen peroxide ( ) ext put them for minutes in hen the minutes elapsed, place the sterili ed nodes (in the test tube) on the grill which is lying in the steam (sterile area) ow, pick up a flask and open it in the steam he cap should be placed on the ethanol soaked kitchen paper ake your forceps and pass the flame of your candle to sterili e it ransfer the forceps to the sterile area (steam) and catch one node which is swimming in the hydrogen peroxide solution and stick it with the end at the bottom of the node into the media

ext, dip the forceps into the boilding water to remove all rests of media and transfer the forceps into the bottle with ethanol Close the flask (in the steam) and placed it somewhere on you desk for labeling ith the next flask you can process in the same way

int o make your sterili ation solution more effective, add a drop of dish washing solution to your hydrogen peroxide

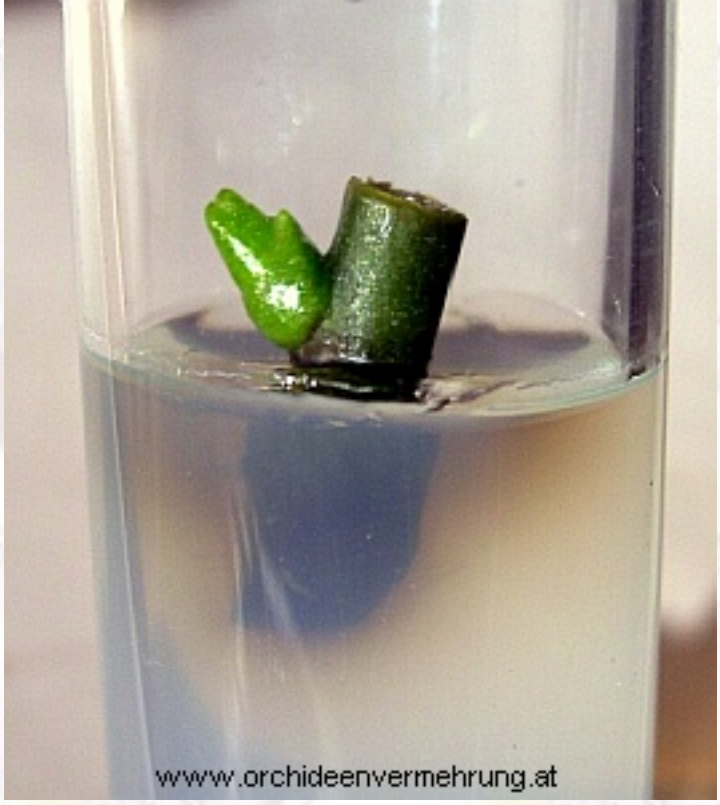
urther care

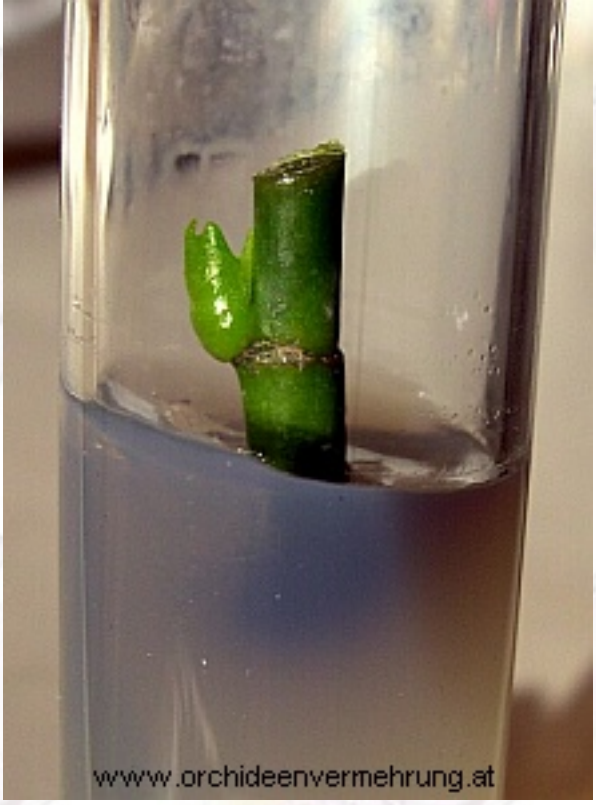
he place where you culture your nodes should be bright and warm (about C) Prevent direct sun because it will become to hot inside the flasks if they are standing in direct sun

growing Chiloschista lunifera bud

ecause of the si e and the structure of the nodes the contamination rate is higher than using asymb seed germination o, it s very important to check them in the first week every day if there are any contaminations f you find some fungi or bacteria you can try to sterili e them once again

any nodes exudate phenolic compounds into media which make the media black his phenolic exudations will kill your nodes if you don t replate them to new media any nodes stop exudating phenolic compounds after or replatings





phenolic exudations

s soon as the node has got or leaves you should replate it to media without hormones (e g igma P) to initiate root development

hat can do if want more than one plant

f you want to produce more than one clone you should cut the top of the node his will cause the node to put out up to a about do en shoots instead of one

Phaius tankervilleae node culture

Phalaenopsis equestris
young plant from a node

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Growth regulators (hormones)

What are growth regulators ?

Growth regulators are any organic or synthetic compounds that influence growth and multiplication hey are produced in plants (e g in growing buds) to control the growth

Auxins

 uxins influence cell enlargement, root initiation and adventitious bud formation hey suppress the initiation of lateral buds (which is the bud of choice for ensuring genetic stability) uxins are commonly used in tissue culture media, either combined with cytokinins during the multiplication stage or without cytokinins for the rooting stage

name	abbreviation
ndole cetic cid	
ndole utyric cid	
aphthalene citic cid	
Phenylacetic cid	P
Dichlorphenoxyacetic cid	, D
, , richlorphenoxyacetic cid	, ,
Picloram	
Dicamba	
p chlorophenoxyacetic cid	CP

Cytokinins

Cytokinins, formerly called kinins, are required in tissue culture media for cell division, shoot multiplication and axillary bud proliferation hey help delay senescence (aging), and they influence auxin transport f cultures are too spindly, increased cytokinin will help foster shorter, stouter stems

name	abbreviation
en yladenin	

en ylaminopurine	P
Pentyladenin	
Dimethylallyladenin	
inetin	
eatin	
eatinriboside	
sopentenyladenine	iP
sopentenyladenosine	iP
hidia uron	D

Gibberellins

Gibberellins are a group of naturally occuring substances that influence cell enlargement and stem elongation urasawa noted in that secretions from a fungus (*Gibberella fujikuroi*) resulted in abnormally rapid growth in rice seedlings he substance was gibberellic acid, which was later isolated in crystalline state from both fungi and higher plants

name	abbreviation
Gibberellic cid	G
Chlorcholinchlorid	CCC

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Node culture in soil

Preparing the nodes

e have tried the following technique with *Phaius tankervilleae* and it works very good uitable are nodes, which you cut with cm below and above of the eye on the flower stalk t is very important to us a very sharp knife because otherwise the tissue will be hurt to much ext you have to remove the bract covering the node carefully

Place the nodes in soil

Place the prepared node in soil hori ontal, the node should be on the highest point

oisten it well and close the box with plastic foil like the picture below shows

urther care

Place the box with the nodes on a bright warm place and prevent direct sun Check them every days if they are moisten enough

fresh nodes

weeks later

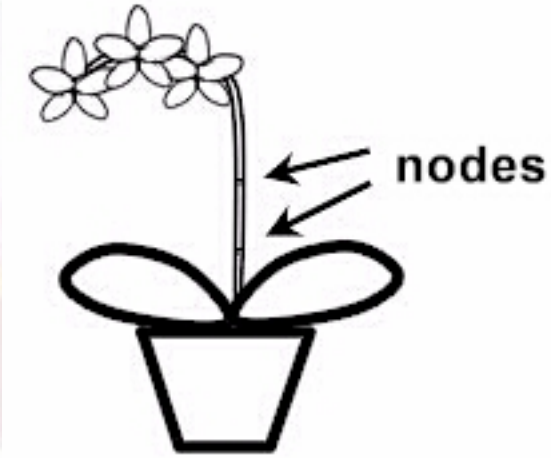
further weeks later

further weeks later

index

What are nodes ?

Plants build sleeping buds to make sure that it can survive if the apical bud dies (eaten by a pest,) s long as the apical bud is growing it produces a growth regulator (hormon) which suppresses the growth of the other buds on the stem f the apical bud dies, the growth regulator is missing and the sleeping buds start to grow



Phalaenopsis

Phalaenopsis node (detail view)

here can you find sleeping buds

odes can be found e g

- on the stalks of *Phalaenopsis, Doritis* and *Phaius*
- on bulbs of *Dendrobium*
- on bulbs of *Cattleya*

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