

Prevention of diseases

The description of the Base package does not take into consideration the fact that the plants, selected by the client for tissue culture, may be infected with pathogens such as viruses, viroids, phytoplasmas, fungi and bacteria. By testing the plants in tissue culture for latent bacteria the final reproduced plants will not be infected with the most common fungal and bacterial diseases, such as Xanthomonas, Erwinia, Argobacterium, Verticillium, Fusarium and many more. This is however not the case for phytoplasmas, viruses and viroids. If a client requires absolute assurance that these pathogens will not be present in the final reproduced plant, extra measures will have to be taken.

Select Package

The most simple measure to be taken is that the selected plants are analysed for the presence of pathogens before initiation of tissue culture. Only plants which show no trace of pathogens are then used for tissue culture. This will not guarantee that the plants are completely pathogen free. A primary infection with pathogens is often difficult to confirm with only one analysis. It is therefore necessary to repeat the analysis on every clone which goes to the glasshouses for analysis on trueness to type. The clone numbers that appear to be infected are removed from the laboratory.



Test Package

When it appears that all the plants received are diseased and the client has no means of selecting clean plants, the plants will have to be cleaned. This requires a special procedure called meristem(-tip) culture. It involves initiating the tissue culture by cutting the growing tips (meristem) of the plants very small. When this is achieved by as many meristems as possible, some of the reproduced plants will be free of the pathogens. The preparation of the meristem cultures is a time-consuming task. The likelihood of producing pathogen-tested plants from these cultures also depends on a number of factors: type of pathogens, pre-treatment of the plants, number of pathogens present and concentration of pathogens in the plants. For every clone number received in tissue culture, plants are again sent to the glasshouse to be examined for trueness to type and re-analysed for the pathogens that were present in the mother plant. At this stage the analysis is repeated a number of times to be certain that the plants are pathogen free. Clones which still appear to be diseased are removed from the laboratory.



Dahlia



Dahlia
virus diseased

Star Package

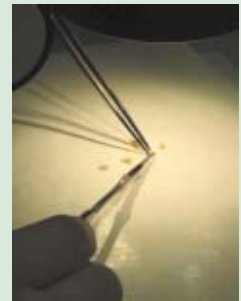
The Star package offers the best guarantee of pathogen tested plants propagated by tissue culture. The Star package includes the initiation process and also gives the guarantee of the Dutch inspection of Horticultural crops which means that the plants are delivered with a SEE-certificate. Within the Star package plants are not only tested for the most common occurring pathogens during the initiation of the tissue culture but every clone number is then tested for every pathogen known to occur in the crop. During this procedure the guidelines are followed which have been laid down by the Dutch Inspection Service of Horticultural crops, called the Naktuinbouw-Elite (R) classification.

Each analysis is performed in laboratories which are recognised by the Dutch Inspection Service of Horticultural crops, the Phyto sanitary office or the bulb inspection service. Within the compulsory analysis scheme research takes place on indicator plants to establish whether pathogens are present which have not yet been detected. The Dutch Inspection Service of Horticultural crops, reviews that the correct procedures are carried out. When plants which have been delivered with the Naktuinbouw Elite (R) certificate do not appear to be disease free, the Dutch Inspection Service of Horticultural crops will carry out an independent investigation.

schematic example of a meristem culture. left the top of a shoot, where a meristem is isolated in vitro. A plant will form from this meristem which will then be planted into soil.



Meristem preparation



Continuation

All the separate steps used in the packages can be seen in Table 1. All the separate steps used in the packages can be seen in Table 1. SBW International BV has ample experience in the initiation of tissue-culture propagation. Each year approximately 1500 varieties from more than 100 different crops are initiated in tissue cultures for more than 150 clients in Holland and abroad. Only when one of the packages has been successfully completed bulk production of trustworthy plants can commence.

Table 1: Activities per package for initiation

Activities	Base	Select	Assay	*	Star
Virus assays on mother plants		*	*		*
Initiation of a maximum of 40 tubes using single-node culture	*	*			o
Initiation of a maximum of 40 tubes using meristem-tip culture			*		o
Assay for bacterial contamination of regenerated plants in vitro	*	*	*		*
Propagation to 100 plants on clone number	*	*	*		*
Trial delivery of 50 plants (or more when needed) for true-to-type analysis	*	*	*		*
Germ-plasm conservation of 50 plants for the first half year after trial delivery	*	*	*		*
Virus assays on weaned plants in the greenhouse		*	*		*
Germ-plasm conservation of 40 plants (maximum 10 lines) for one year				*	*
Trial delivery of 50 plants per year from the conserved germ plasm for true-to-type analysis				*	*
Certification of the plants according to the NAKB-Elite, system each year					*
Quarterly progress reports	*	*	*		*
One invoice after starting the work according to offer	*	*	*		*
One invoice per year according to a fixed price set per year				*	*
Invoice of assaycost		*	*		

- * = Is included
- o = Is included depending on the crop and the circumstances
- * = Extension of the Base, Select and Assay Package

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