

Production of Virus-Free Plants by Means of Meristem Culture

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In Japan the damage increase from virus diseases in many plants has accelerated in recent years due to intensive land use and complication of cultivating forms. And as a practical control method to cure virus disease has not been established as yet, the emphasis on control has been passive control measures such as the removal and destruction of infected plant, control of insect vector and isolated cultivation. However, it is difficult to control the infection during growth; thus, the state of thing as of now is that many plants are subject to severe infection by virus diseases.

Fortunately, virus disease does not infect through seed in many plants such as rice and vegetables, but in many vegetative propagating plants such as potatoes, bulbs, fruit trees and other cuttings and separated roots because of their vegetative propagation, once a mother stock is infected by virus the infection is not confined to that plant alone but contaminates the descendants successively through seed tuber or root, bulb and scion, and with the help of insect vector the disease invades the whole variety and all plants become infected. Such a case is witnessed in many places.

The spreading of virus disease in such a way has a far reaching adverse impact on agriculture. It degenerated the variety and plants, caused the deterioration in growth, yield and quality and often resulted in utter destruction of superior variety. Notwithstanding such a situation in many cases farmers are not able to obtain virus-free seed and seedling, often reluctantly forced to

cultivate infected stock as it is.

So far practical chemical to effectively cure those virus diseases has not been discovered. The heat treatment has been found to be effective for several virus diseases, but the sphere of this control method is very much limited. The meristem culture requires a considerable time and labor but the findings by the writer et al. has clarified that the method is very promising as a practical control method to cure many plant virus diseases.

Historical background of meristem culture

White (1934)¹⁰⁾ has discovered that no virus has been observed in the tip portion of the tomato plant infected with aucuba mosaic virus, that the shoot apex of the TMV infected tobacco plant is very low in virus concentration and that virus is not uniformly distributed



Damage of virus

Lily plant



Potato (Healthy and mosaic)

throughout the plant body but is localized.

Then, Holmes (1948),¹⁾ Limasset (1949)²⁾ and Kassanis (1957)³⁾ have clarified that 'Imperfect distribution of virus in plant body and the nearer to the tip of plant the lower the content of virus'. Since then this fact has developed into production method of virus-free clone by grafting and cutting of the plant's tip by the Holmes' research (1948, 1955),¹⁾ and with this finding being connected by tissue culture method under the research finding of Morel (1952)⁴⁾ of France, ultimately resulted in the virus-free meristem culture which is a long-awaited breakthrough in the treatment of virus disease—the disease which repulsed the challenge of many researchers for so long.

Since then, meristem culture has been applied to dahlia, carnation and white potato by several researchers in France, Great Britain and Holland and succeeded in the elimination of virus in those plants.

The writer et al.^{5),6)} have since 1957 rendered their utmost effort to introduce this technique to Japan to control the virus from many plants which are violently infected by virus disease. And after groping in the dark they have succeeded for the time being in establishing the meristem culture in sweet and white potatoes, lily, dahlia, strawberry, petunia, carnation, sugar cane and many other plants and succeeded in producing many virus-free clones. Since then, many research institutions in Japan have launched upon the research for practical cultivation of virus-free clones by the meristem

culture and several institutions are in the stage of putting the culture into practical use.

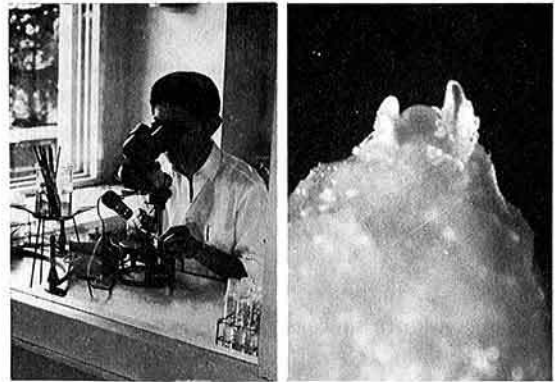
Meristem culture method

As stated above, this method utilizes the established fact that the virus is localized in the infected plant body, that is, either the virus does not exist in the apical meristem system or the virus concentration is low in a certain locality of the plant. A small portion of the apical meristem is cut off and virus-free cultivation is carried out in agar medium and a healthy virus-free plant is obtained from the infected plant.

As for the operation course, the isolation, culture and raising of the apical meristem system, and inspection of virus are standard procedures.

The isolation and culture of meristem are carried out in a sterilized room quickly and by carefully avoiding miscellaneous fungus.

In the first place, about 10 mm long plant



Isolation of apical meristem

Meristem of potato



Cut off the meristem from shoot apex of plants.

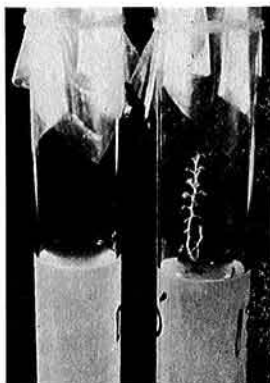
Table 1. Elements of culture medium (mg/l)

Authors Elements	White	Morel	Kassanis	Murashige	Nielsen	Jap. Cent. Agr. Exp. Sta.
CaCl ₂ ·2H ₂ O				440		
Ca(NO ₃) ₂ ·4H ₂ O	200	500	500		800	170
KCl	65					80
KH ₂ PO ₄		125	125	170	200	40
KNO ₃	80	125	125	1900	200	
MgSO ₄ ·7H ₂ O	360	125	125	370	200	240
NaH ₂ PO ₄ ·H ₂ O	16.5					
Na ₂ SO ₄	200					
NH ₄ NO ₃				1650		60
CoCl ₂ ·6H ₂ O				0.025		
CuSO ₄ ·5H ₂ O				0.025	0.08	0.05
Ferric citarte					5	5
FeSO ₄ ·7H ₂ O		Berthelot	Berthelot	27.8		
Fe ₂ (SO ₄) ₃	2.5	solution	solution			
H ₃ BO ₃	1.5	10 drops	10 drops	6.2	2.8	0.6
H ₂ M ₀ O·H ₂ O					0.02	0.02
KI	0.75			0.83		
MnCl ₂ ·H ₂ O					1.8	0.4
MnSO ₄ ·4H ₂ O	4.5			22.3		
Na ₂ MoO ₄ ·2H ₂ O				0.25		
Na ₂ -EDTA				37.3		
ZnSO ₄ ·4H ₂ O	1.5			8.6	0.2	0.05
adenine				5		5
biotin		0.01	0.01			0.01
Ca-panthothenate		10	10			10
cystin			10			10
glycine	2			2		
casein						1
inositol		0.1	0.1	100		0.1
nicotinic acid	0.5	1	1	0.5	5	1
pyridoxine	0.1	1	1	0.5	1	1
thiamine	0.1			0.1	1	1
kinetin				0.04~10		
IAA				1~30		
sucrose	20000	20000	20000	30000	30000	
glucose						10000
agar	10000	10000	10000		10000	7000

note: Berthelot solution—MnSO₄ 2.0 g, NiSO₄ 0.06 g, TiO₂ 0.04 g, CoSO₄ 0.04 g, ZnSO₄ 0.1 g, CuSO₄ 0.05 g, BeSO₄ 0.1 g, H₃BO₃ 0.05 g, Fe₂(SO₄)₃ 50.0 g, KI 0.5 g, H₂SO₄ (66 Baume) 1 ml, H₂O 1 l.

tip is cut off then disinfected by dipping into 20 times diluted antihormine (sodium hypochlorite) solution for 5 minutes and hold the same by the left hand, and after cutting the young leaf and young rudiment one after another by the right hand with a hand-made knife (attaching a safety-razor blade firmly to a bamboo stick) hold at right hand under the dissecting microscope and after exposing the center of the meristem, to the size of $200\ \mu$ is cut off from the tip. After washing this isolated system in distilled water, put it on agar medium in the test tube, then it is cultured in lighted incubator or in an illuminated constant temperature-fixed room (around 25°C).

There are several kinds of culture mediums as shown in Table 1 and although the use thereof differs by kind of plants the content is usually adopted at $\frac{1}{2}$ density of knop culture



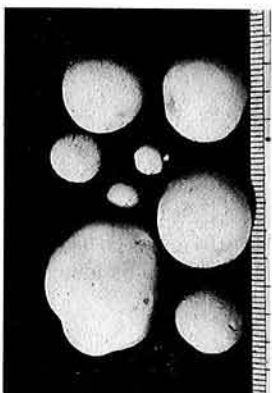
Meristem culture



Breeding



Plant to the soil



Virus freed potato

liquid to which various amino acids, trace element, vitamins, plant hormone, sugar and agar-agar are added in most cases. The addition of coconut milk (10%) is very effective in expediting the early growth of cultivation. And in the culture of carnation it is better to use filter paper instead of agar-agar.

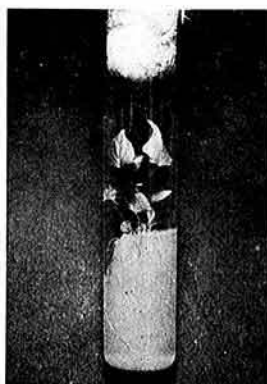
The culture of those tissues confronts various bottlenecks such as the outbreak of miscellaneous fungus, withering and drying-up of culture medium. The bottlenecks are further aggravated by a very slow growth of clone and only in few cases it is possible to obtain a full-grown, healthy individual plant without virus.

A young plant which has grown to the height of a few centimeters after an elapse of several months to two years is transplanted in disinfected soil saturated with water and carefully raised in a glass house giving attention as to avoid the re-infection by virus.

As for the grown plant the virus infection is tested by observing the disease symptom, through antiserum and by test plant and virus-free clones are propagated in an insect-proof-net room and isolated farm and practically used as foundation stock.

Result of virus-free clone by meristem culture

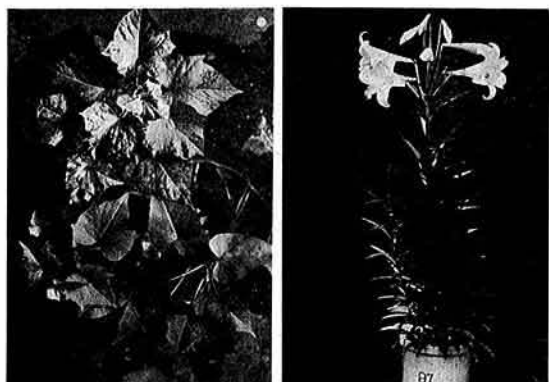
The plants tackled by the writer et al. to produce virus-free clone by meristem culture



Culture and virus freed sweet potato



Healthy plant produced by meristem culture



Sweet potato
Top: Mosaic
Bottom: Healthy

Lily

during the period of 10 years from 1957 to 1967 are white and sweet potatoes, lily, dahlia, carnation, petunia, chrysanthemum, strawberry, sugar cane, millet, orange, apple, cherry tree, paulownia and 20 other species violently attacked by virus infection.⁹⁾

Among the same the culture of clone in fruit tree has not been achieved but the num-

ber of virus-free plants reached 12 covering about 50 varieties. The result thereof has been compiled in Table 2. And those virus-free plants are distributed to related institutions as foundation stock and propagated under isolated cultivation.

The study of many kinds of plants and on various species of virus has revealed that the formation of the meristem varies by kinds of plant and that the isolation of meristem is easy in some plants and difficult in others. And the isolation and culture relatively differ by varieties.

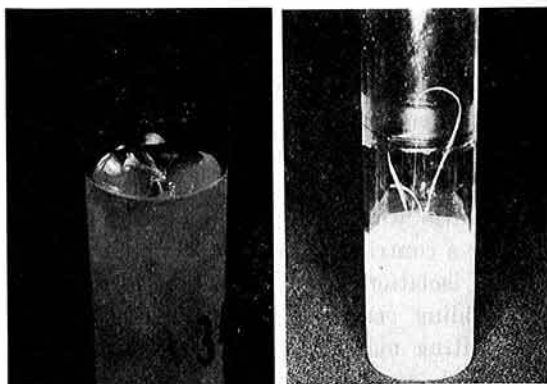
At the start of research, culture period was very long—requiring 1–2 years and culture yield ratio was very poor, as low as 1–2%. But by a contrivance such as the improvement of the isolation and disinfection method and by adding culture medium and growth stimulating material the efficiency in culture improved considerably, achieving favorable results in many plants.

When the size of the meristem is larger at

Table 2. Elimination of viruses by means of meristem culture
(Jap. Cent. Agr. Exp. Sta. 1958~1967)

Plants	Virus	Length of cultured shoot apex to produce virus-free plants	Number of virus-free varieties
Sweet potato	Internal cork virus	~1.0~2.0 mm	6
	Rugose mosaic virus	~1.0~2.0	1
	Feathery mottle virus	0.3~1.0	2
Potato	Potato Y virus	~1.0~3.0	1
	Potato leaf roll virus	~1.0~3.0	3
	Patato X virus	0.2~0.5	7
	Potato G virus	0.2~0.3	1
	Potato S virus	0.1~0.2	5
Lily	CMV and lily mosaic	0.2~1.0	3
Strawberry	Complex of viruses	0.2~1.0	4
Carnation	Complex of viruses	0.2~0.8	5
Petunia	Tobacco mosaic virus	0.1~0.3	6
Dahlia	Dahlia mosaic virus	~0.6	1
Sugar cane	mosaic	~0.7~0.8	1
Iris	mosaic	0.2~0.5	1
Garlic	mosaic	0.3~1.0	1
Chrysanthemum	Complex of viruses	0.2~1.0	3
Horseradish	Turnip mosaic virus	~0.5	1

the start, the culture is easier in general and the growth is quicker. But the elimination ratio of virus is higher when the size is smaller. However, as the table indicates, it seems that the elimination limit differs considerably by kinds of plant and by species of virus so that it is not always necessary to isolate into 100–200 μ .



Petunia

Sugar cane

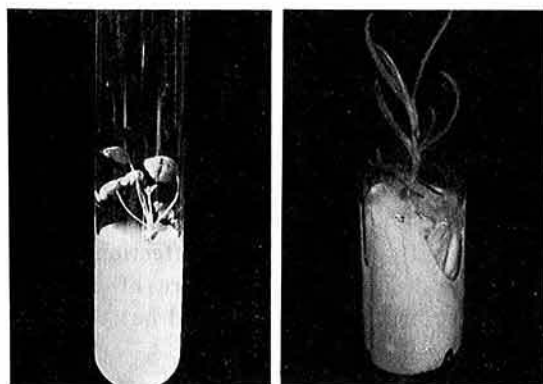


Petunia (Healthy plant and mosaic) Sugar cane (Healthy)

There are still so many points not clarified in the distribution and concentration of various viruses in the meristem of shoot apex and as to what are the reasons for the results obtained by the writer et al. here have not been clarified. The writer et al. hope to make clear those points by the fluorescence antibody method and electron microscope to further improve the culture efficiency.

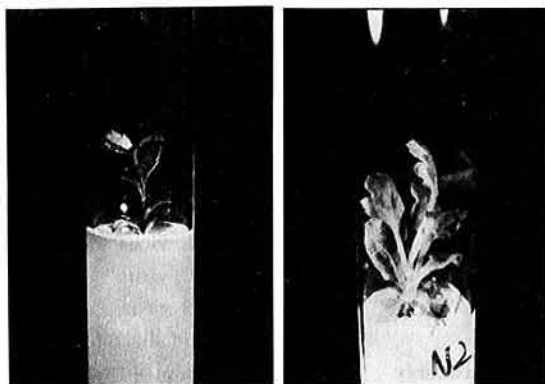
Future Problem

The above study has made possible to save many vegetative propagating plants from viruses by meristem culture. But if the culture efficiency can be improved by the improvement of culture technique under the combined use of improved culture medium and condition, anti-virus substance, antiserum and heat treatment, it will contribute immeasurably to agricultural production increase and plant cultivation.



Meristem culture of strawberry

Carnation



Dahlia

Chrysanthemum

One thing to be borne in mind is that those healthy plants cultivated under this method are not resistant nor immune to virus infection. Accordingly, if those plants are cultivated in upland or under installation-horticulture with large contagious sources the plants will

become infected within 2-3 years and return to virus infected plants.

And because thereof if this technique is to be widely extended to be put into practical use for agricultural production, not confined to the pursuit of study in plant pathology for the academic purpose and interest, it is absolutely necessary to establish such a system as foundation stock seed farm, that is, culture center and propagation distribution center of disease-free plants as witnessed in the production of white potato foundation stock seed farm by the Ministry of Agriculture and Forestry.

If such an institution ever becomes a reality it should not be limited to a certain region or for the whole country but it should be of world-wide scale under the international co-operation for the culture, propagation and distribution of virus-free clones. Because of the fact that coconut milk is very effective as growth stimulant in the meristem culture Southeast Asian region is deemed as a most ideal site for such an international institution.

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