Mass Production Method of Virus-Free Strawberry Plants Through Meristem Callus

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Strawberry virus diseases already attracted the attention of European and American scientists as early as the 1920s as an important injury for the growing and variety improvement.

Attention was first focused on this disease in Japan when Nishi introduced the American findings on it and indicated its seriousness in 1957[7]. Abe and Yamakawa (1959)[1] successively reported that Japanese strawberry varieties had already been affected by this disease.

The seriousness of this disease is now well known since Takai (1966)[12] examined the strawberry cultivars collected from all over Japan and reported that almost all the Japanese strawberry varieties were tainted with virus.

Method to get virus-free plants

Studies on virus disease have been under way for many years in the field of plant pathology. Numerous attempts have been made to prevent the spread of the disease and several methods have been tested to secure virus-free plants for example; physical (heat treatment), chemical (anti viral chemicals) and surgical methods (meristem culture).

As for anti-viral chemicals, it has not yet become practical. But the heat retreatment and meristem culture have already been put to practical use.

Mori et al.[5] succeeded in producing the virus-free plant of strawberry 11 other species after experimenting with more than 20 series of crop plants such as sweet potato, Irish or white potato and lily bulb for 10 years from 1957 to 1967.

Subsequently, meristem culture study was developed and applied practically all over Japan.

But no more than just one plantlet can be obtained from each meristem by means of this culture method. Furthermore, each plantlet must be checked for its virus infection so high practicability cannot be expected under this method. Under such circumstance, it was urgently necessary to conduct research to find a more efficient mass production method of virus-free plants.

Mass production method of virus-free plants

The authors and their collaborators have studied tissue culture, including embryo-, anther- and meristem-culture for growing of vegetables and have established the method of callus formation from anther tissue and of shoot formation through callus in the course of studying anther culture of strawberry[8].

By means of these technics, the callus formation of meristem which has been proved as a virus-free tissue was obtained and the formation of numerous shoots from this callus was successful with higher security on the elimination of virus compared with the meristem-culture method[9],[10].

1) Materials and methods

"Hokowase" was the strawberry cultivar used for this experiment.

A piece of runner tip, cut off in the length of 1 to 2 cm, was washed in cresoli saponate solution and after sterilization by immersing it into the supernatant solution of chlorinated lime (10 g of chlorinated lime and about 60% added to 140 cc of water) for 10 to 15 minutes, the meristem tissue was cut into small pieces of 0.1–0.5 mm (average 0.2–0.3 mm)* under a dissecting microscope and then these small pieces were put on the medium.

The medium was prepared by adding IAA, NAA, 2.4-D and benzyl adenine (BA) to the base of Linsmaier & Skoog[4]. (Table 1)

Table 1. Composition of the medium (mg/l) (Linsmaier & Skoog 1965)

$CaCl_2 \cdot 2H_2O$	440	$MnSO_4 4H_2O$	22.3
KH_2PO_4	170	$Na_2M_0O_4 \cdot 2H_2O$	0.25
KNO_3	1900	$ZnSO_4 \cdot H_2O$	8.6
$MgSO_4 \cdot 7H_2O$	370		
$NH_4 \cdot NO_3$	1650	$FeSO_4 \cdot 7H_2O$	27.8
		NaEDTA	37.3
$CoCl \cdot 6H_2O$	0.025		
$CuSO_4 \cdot 5H_2O$	0.025	Thiamine-HCl	0.4
H_3BO_3	6.2	Sucrose	30,000
KI	0.83	Agar*	7,000

* The original Linsmaier & Skoog medium has 10,000 g of Agar

Table 2. Effect of BA and Auxin concentrations on callus and shoot formation from meristem tissue

	Concentration substances added to the medium				No. of meristems (A)	Callus formation (B)[a]		Callus formation ratio (B/A)	No. of direct shoot formations	Soot formation through callus (C)[b]		Shoot formation ratio (C/A)
	BA	IAA	NAA	24—D		+	++			+	++	
	M	M	M	M					%			%
Expt. 1	0	0	0	0	10	0	0	0	8	0	0	0
	0	10^{-6}	0	0	10	4	0	40	0	0	0	0
	0	10^{-5}	0	0	10	4	4	80	0	0	0	0
	10^{-5}	0	0	0	10	1	9	100	0	3	5	80
	10^{-5}	10^{-6}	0	0	10	4	6	100	0	2	2	40
	10^{-5}	10^{-5}	0	0	10	4	6	100	0	2	2	20
Expt. 2	0	0	0	0	10	0	0	0	9	0	0	0
	0	0	10^{-6}	0	10	6	2	80	0	0	0	0
	0	0	10^{-5}	0	10	4	6	100	0	0	0	0
	10^{-5}	0	0	0	10	1	9	100	0	4	5	90
	10^{-5}	0	10^{-6}	0	10	0	10	100	0	3	2	50
	10^{-5}	0	10^{-5}	0	10	1	9	100	0	2	2	40
Expt. 3	0	0	0	0	15	0	0	0	13	0	0	0
	0	0	0	10^{-6}	14	6	7	93	0	0	0	0
	0	0	0	10^{-5}	15	2	13	100	0	0	0	0
	10^{-5}	0	0	0	12	2	10	100	0	3	7	83
	10^{-5}	0	0	10^{-6}	12	3	9	100	0	0	0	0
	10^{-5}	0	0	10^{-5}	15	0	15	100	0	0	0	0

a +: Number of test tubes containing the callus of which length is below 5 mm
a ++: Number of test tubes containing the callus of which length is over 5 mm
b +: Number of test tubes containing 1 to 5 shoots
b ++: Number of test tubes containing more than 5 shoots

* Mori et al (1969)[9] reported that all the plantlets obtained from the meristem piece of 0.2–0.3 mm were virus-free and about half of the plantlets grown up from the meristem piece of 1.0 mm were also virus-free

The prepared medium was adjusted to pH 6.0 and a 7 to 8 cc portion of it was poured into the 20 mm × 95 mm test tubes and autoclaved at a pressure of 1 kg/cm², for 10 minutes.

The culture was incubated at 25°C under 12 hours illumination (3,000 lux) with fluorescent lamps (plant lux) in a light-proof chamber.

2) Results

1) Callus and shoot formation from meristem tissue

Table 2 shows the results of experiments after eight to ten weeks from the beginning of culture. The effects of IAA, NAA and 2.4-D are shown in the columns of experiments 1, 2 and 3 respectively.

As to the culture with basic medium only, leaves developed and extended after two to four weeks from the commencement of culture, and plantlet grew up six to eight weeks later (Fig. 1) but no callus appeared.

In the medium culture which contained auxin or BA, callus formation was observed frequently especially in the case of 10^{-5}M BA and the callus formation was flawless without any exception.

The basic medium culture containing 10^{-5}M BA showed also the best shoot formation with the ratio of 80 to 90 per cent and it was acclaimed a success.

A fragile callus of yellowish white color appeared at the end of two or three weeks after the beginning of meristem culture with this medium (Fig. 2). Then green spots were formed on the callus during the four to five-week period and in the course of six to eight weeks many shoots were discovered on these green spots. (Fig. 3)

Auxin revealed inhibitory effect on the shoot formation.

IAA and NAA reduced the ratio of shoot formation to one-half of the 10^{-5}M BA and no

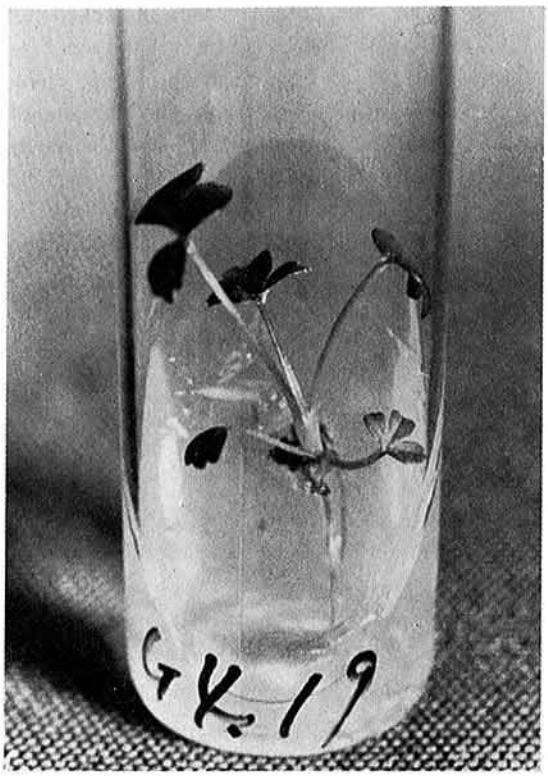

Fig. 1. Shoot formation by usual meristem culture

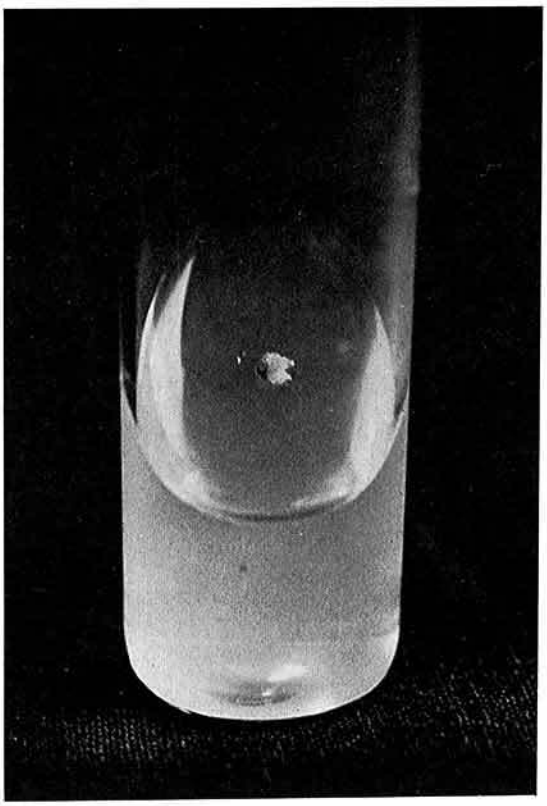

Fig. 2. Callus formation from a meristem tissue

shoot formation was noticed with the medium containing 10^{-5}M 2.4-D or 10^{-6}M 2.4-D.

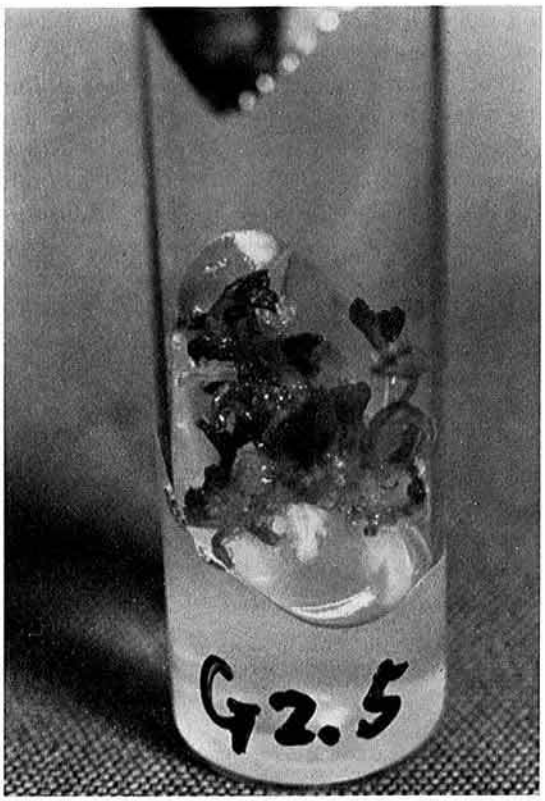

Fig. 3. Shoot formation through meristem callus

2) Mass production method of shoot through callus

Shoot formation through callus which had been cultured repeatedly with the basic medium containing 10^{-5}M BA developed progressively generation after generation as well as the case of the callus originated from anther tissue.

Subsequently, the newly formed shoots were separated individually and were planted on the basic medium to procure normal strawberry plantlets. (Fig. 4)

Thus, the mass production method of plantlets, more than 50 plantlets if needed, from only one meristem in terms of callus formation followed by shoot formation was found to be possible.

3) Effect of the elimination of virus

As for the virus infection of the plantlets formed from callus tissue, Svobodova (1964)[11] already reported that the plantlets raised separately through the callus which was formed from the potato tissue attacked by PVX, PVY, PVS were found virus-free.

Mori (1971)[6] investigated the virus vicissitudes of plants by means of the fluorescent antibody method and reported that the virus concentration of the infected callus derived from the plant tissue attacked by TMV is rather less than that of normal plants and

Fig. 4. Mass production of strawberry shoots through meristem callus

reveals unhomogeneous distribution in the tissue according to the growth of callus under subculture.

Furthermore, he reported that the shoots and roots formed from the infected callus were free from virus without any characteristic fluorescence of TMV.

The author (1970)[9] revealed that the plantlet from anther callus of strawberry showed no symptom of the virus disease which affected the mother plant and Abo・El-Nil et al.[2] made similar reports on the geranium plants.

Kondo (1968)[3] disclosed that the callus which forms green buds shows higher growth of virus so more detailed studies are necessary in the future.

However, the mass production method of virus-free strawberry plants through meristem callus shows, at least, higher efficiency in the elimination of virus compared with other conventional methods, therefore, the production of plantlets which needs no check up for their virus infection could be expected by the development of this method.

Summarry

The above mentioned procedure is summarized in Fig. 5.

Though only one plantlet had been obtained from one meristem by the conventional meristem culture, more than 50 plantlets can be secured in succession from one meristem by this meristem callus method. The virus disease can be entirely eliminated from the plantlets raised through the callus.

From these viewpoints, this method can be expected to serve as a useful technic for the establishment of the virus-free plant production and their distribution systems.

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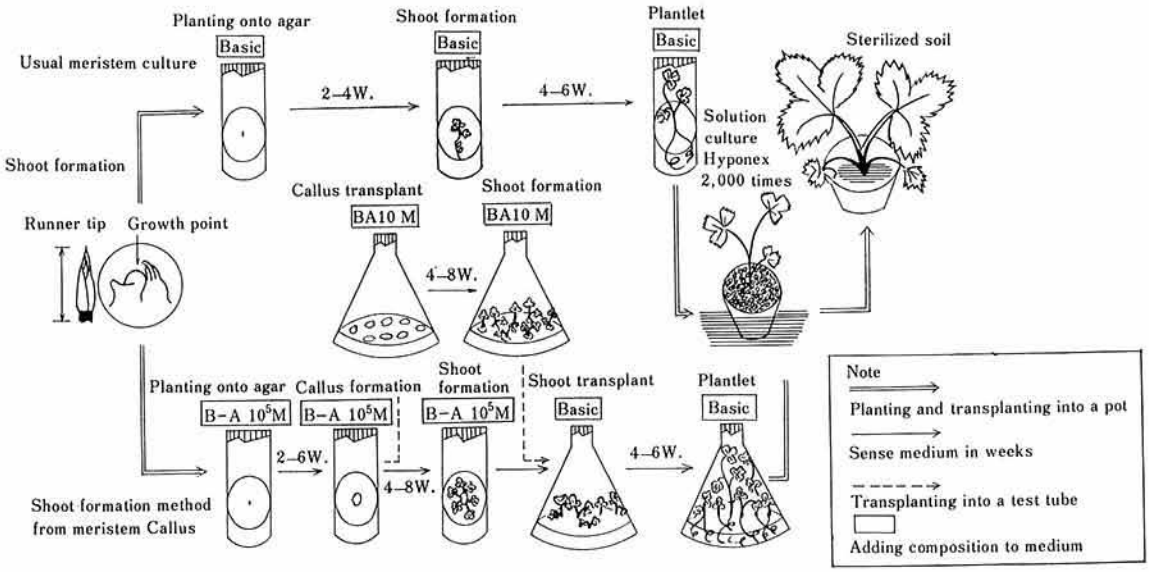

Fig. 5. Mass production method of virus-free strawberry plants from meristem callus

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