On the Variation of Organ Formation in Cultured Tissues Derived from Carrot Roots*

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Synopsis. Variation of totipotency in cultured tissues originated from carrot roots was investigated. It was recognized, as has already been reported by several workers, that the totipotency in cultured tissues varies in the process of subculture. Moreover, it was also assumed that the totipotency in the original carrot roots from which the disks were excised varied hereditarily from root to root.

Variation in plant cells and tissues subcultured repeatedly in an artificial medium has been reported on many occasions. A well known case is the 'habituation' (GAUTHRET, 1955) of tissues which was caused by means of subculture in a medium containing a low concentration of auxin so that they became able to grow without the exogenous auxin which had originally been required. In recent years, through repeated subculture on the same nutrient medium, several kinds of clone have been obtained which differ from the original cultures in pigmentation, form, growth rate, and nutrient requirements (ARYA et al., 1962; FOX, 1963; STEWARD, 1963; BLAKELY et al., 1964; MURASHIGE et al., 1964). Also, the variation of totipotency in cultured tissues during the process of subculture has been reported. For example, a loss in the ability of the cultures to regenerate adventive buds and roots has been recognized (SKOOG et al., 1948; MURASHIGE et al., 1964). These kinds of variation in cultures are important problems that should be duly considered when the technique of tissue culture is being used.

This paper reports the nature of the variation of totipotency in cultured tissues originated from carrot roots. In this experiment, it is also confirmed that the potency of regeneration in cultures dwindles to zero in the process of subculture, and that, on the other hand, there are hereditary differences in the totipotency of individual carrot roots.

Materials and Methods

Several disks, each 2 mm thick, were made from cylindric tissues excised with a cork borer (5 mm in diameter) from the secondary phloem of carrot roots. These disks were put on White's agar medium (containing 2 % of sucrose) supplemented with yeast extracts (1000 ppm) and 2,4-D (1 ppm), and adjusted to pH 5.8–6.0 (this will be called 2,4-D medium henceforce).

Callus grew around the disks in 7–10 days. These callus tissues were transplanted to a new 2,4-D medium every 30 to 40 days. So long as the subculture was continued

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Fig. 1. Difference in totipotency among strains on agar medium. A,B,C,D-E, and F show the degree of organ (adventive buds and roots) formation, high, middle, low, slight, and none.

on the 2,4-D medium, adventive buds and roots were not usually observed to grow. On the other hand, when the cultured tissues were transplanted to the medium containing IAA instead of 2,4-D (this will be called IAA medium hereafter), organ formation was recognized depending on the strain of the cultures (Fig. 1). Therefore, the totipotency of the tissues cultured on the 2,4-D medium was assessed by transplanting it to the IAA agar medium or

the liquid medium (by means of a rotating method).

Experimental Results

I. The variation of organ formation recognized in the subcultured-tissues

Table 1 shows the results of a series of subcultured-tissue strains transplanted to the IAA medium. A-F shows the strains of cultured tissues originated from different

Table 1. Difference in totipotency among 6 strains

Strain of cultured tissues	Totipotency on		
	2,4-D agar medium (1 ppm)	IAA agar medium (1 ppm)	IAA liquid medium (1 ppm)
A	—	+	+
B	—	+	+
C	—	+	—
D	—	+	—
E	—	—	—
F	—	—	—

+ : Adventive buds and roots formed.

— : None of them formed.

disks, each of which was excised from a different carrot. On the IAA agar medium adventive buds and roots came up in the A, B, C, and D strains, but they did not sprout in the E and F strains. On the liquid medium, however, small seedlings as was already reported (STEWART, 1961) were observed only in two strains, A and B.

A positive difference, as mentioned above, in the potency of organ formation in cultured tissues was found among several strains. However, the fact that the nature of the tissue varies during the process of subculture has already been revealed by several workers. Then, variation of totipotency in the process of subculture was investigated in the A, B, C and D strains.

Fig. 2 shows the variation in the potency of redifferentiation in cultured tissues obtained by transplanting the cultures to the IAA medium from the 2,4-D medium. The A strain shows that the tissue still retains the potency of root formation when it is transplanted to IAA agar medium after 20 repetitions of subculture in the 2,4-D medium, while the potency of redifferentiation disappears after 9 repetitions of subculture in the B and C strains, and after only 5 repetitions in the D strain. In this experiment, it was recognized that the nature of tissues varied during the process of its subculture,

and also, that the number of repetitions of subculture required for loss of totipotency differed among strains.

From the fact that the pattern of variation in cultured tissues differed remarkably from strain to strain, it seemed that the difference of totipotency was existent in the nature of the individual carrot used as material. In order to clarify this point, the following experiments were conducted.

II. The variation of totipotency observed among individual carrots

Table 2 shows the totipotency of callus tissues which were transplanted to IAA medium from original disks. No subculture was performed in order to avoid completely a possible variation. In the case of tobacco cultures, organ formation was already known to be controlled by the balance between IAA and kinetin contained in the medium (SKOOG et al., 1957). In this experiment, too, various concentrations of IAA in the agar medium were prepared. In this table, it is recognized that adventive buds and roots came up in all strains

Fig. 2. Variation in totipotency in the process of subculture. Solid column : Both adventive buds and roots formed. Striped column : Only adventive roots formed. Open column : None of them formed.

Table 2. Difference in totipotency among strains on various medium

Group	Strain of cultured tissues	Totipotency on									
		IAA agar medium						IAA liquid medium	2,4-D agar medium		
		100 ppm	10	1	0.1	0.01	0.001	(1 ppm)	10 ppm	5	1
I	G	×	++	++	++	++	++	—	—		
	H	—	+++	+++	+++	+++	+++	—	—		
	I	—	+++	+++	+++	+++	+++	—	+		
II	J	±	+	++	++	++	++	—	—	—	+
	K	±	++	+++	++	+++	+++	—	+	+	+
	L	±	++	+++	+++	+++	+++	—	+	+	+

+++, ++, +, ±, — : Show the degree of organ (adventive buds and roots) formation ; high, middle, low, slight and none, corresponding for A,B,C,D-E, and F of Fig. 1.

× : Died.

G,H,I, and J,K,L on the agar medium, though they were a little poor in strains G and J. When put on 2,4-D agar medium, organ formation was recognized in every section of the K and L strains in which the density was 10, 5, and 1 ppm. In strain J, however, formation of adventive buds and roots was observed only in 1 ppm section, and not in the sections of 10 and 5 ppm.

As mentioned above, the totipotency in strains G and J was inferior to the other two strains H,I and K,L. But a dubious problem was included in the experiment.

Table 3. Comparison of adventive buds and roots formation among disks excised from the same root (strain M)

Disk No.	Buds and roots formation on						
	50 ppm	IAA agar medium				IAA liquid medium (1 ppm)	2,4-D agar medium (1 ppm)
		1	0.01	0.0001	0		
I (b) (r)	— ±	+	++	++	++	—	—
II (b) (r)	— ±	+	++	++	++	—	—
III (b) (r)	— ±	±	++	++	++	—	—
IV (b) (r)	— ±	+	++	++	++	—	—

(b), (r) : Bud formation, root formation.

++, ++, +, ±, — : Show the degree of buds or roots formation ; high, middle, low, slight, and none.

About 40 to 50 days had passed before the callus tissues were transplanted to IAA medium from the original disks. It was thought that a possible variation might have occurred during the above period in the nature of cultured tissues. So as a next step, 4 disks were taken from one original carrot root, transplanted the callus tissues which came from the above disks to IAA medium, and the potency of

organ formation within the same strain was compared. The results of this experiment are shown in the Table 3. No difference due to the different disks can be noticed.

Table 4 shows that a difference was recognized between the two strains originated from two separate carrot roots, but that no difference was observed between the two disks within the same strain. First, in comparing two strains, innumerable small seedlings (referred to before) were observed in strain N on the liquid medium, though nothing came up in the O strain. Also, concerning the agar medium, a clear difference was found between the sections of IAA 50 and 1 ppm. On the other hand, when comparison was confined to the same strain, no difference could be found between the two disks in either strain N or O. From this, it can be assumed that the difference found between the two strains is a distinctive one which exists naturally in the totipotency of the individual carrot root.

Table 4. Comparison of adventive buds and roots formation between strains and within the same strain

Strain of cultured tissue	Disk No.	Buds and roots formation on						
		50 ppm	IAA agar medium				IAA liquid medium (1 ppm)	2,4-D agar medium (1 ppm)
			1	0.01	0.0001	0		
N	I (b) (r)	+	++	++	++	++	++	+
	II (b) (r)	+	++	++	++	++	++	+
O	I (b) (r)	±	++	++	++	++	—	+
	II (b) (r)	—	++	++	++	++	—	+

++, ++, +, ±, — ; (b), (r) : cf. Table 3.

Discussion

In this experiment, as several investigators have already pointed out (Skoog et al.,

1948 : MURASHIGE et al., 1964), the totipotency of cultured tissues originated from carrot roots varied during the process of subculture. After a few repetitions of subculture, adventive shoots and roots were formed in the cultures, but only the roots were regenerated after several repetitions of subculture and eventually even these didn't appear. In the process of the above mentioned variation, the number of subculture repetitions was remarkably different, depending on the strain. This reveals the fact that a hereditary difference in totipotency exists among strains obtained from different carrot roots.

From this point of view, the second experiment was attempted. In this experiment, callus tissue formed on the original disk was used for the purpose of avoiding a possible variation in subculture, and a comparison was also made among several disks which were excised from the same carrot root. As for the result, there was no difference among the disks originated from the same carrot root, but a clear difference in organ formation could be recognized among the different carrots in response to auxin concentration in the medium.

LINSMAIER et al. (1965) have reported that the totipotency of tobacco tissue differed among several agricultural varieties. LA RUE (1947) pointed out that in maize endosperm culture some are able to grow easily on the nutrient medium, while others cannot, depending on the variety. Thus, it is well known that tissues respond differently to the same medium when they belong to different varieties. In this experiment, a difference was found among individuals of the same variety.

It is assumed that since each individual is so heterogeneous in the carrot plant, which is allogamous, a difference in totipotency among the individuals is found. As mentioned above, the potency of regeneration in the cultured tissues of carrot roots seems to be determined by two interwinding factors, one is the totipotency which is being kept naturally in the individual, and the other is the variation during the process of subculture.

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ニンジンの培養組織における形態形成能力の変異について

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継代培養中に培養組織の性質が変つてしまうことは、組織の色、形態、形態形成能力、生長速度、養分要求などについて、すでに報告されているが、このような培養組織におこる変異は、組織培養法を利用する場合に著しく障害となる点の一つであると考えられる。本報告は、そのような観点からニンジンの培養組織にみられる形態形成能力の変異について、検討したものである。

すでに、1・2 の研究者によつて報告されているように、継代培養をくり返えすと、培養組織の個体形成能力（芽や根を分化して個体に発育する能力）が低下し、ついにはその能力を全く失つてしまうことを、本実験でも明ら

かにすることができた。一方、そのような変異の速度は培養組織の系統によつて著しく異つていることがみられたので、さらに、その点を追究してみた。その結果、ニンジンの培養組織においては、培養組織の起原となつたニンジンの個体によつて、個体形成能力に明らかな差があることが確認できた。

したがつて、ニンジンでは、培養組織の起原となつた個体の遺伝的な形態形成能力と、継代培養中に起こる変異とが組合わさつて、著しい相異を培養組織の系統間に生じているものと考えられた。