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RESPONSES OF VARIOUS ORGANS OF *SOLANUM MELONGENA* TO DIFFERENT NUTRIENTS IN CALLUS FORMATION

A. S. ISLAM* and K. MARUYAMA

Laboratory of Plant Breeding, University of Tokyo, Tokyo 113.

Abstract

The anthers of the two varieties of *Solanum melongena* were found to require different media and different supplements for callus formation. The different parts i.e. fruit, filament, style and stigma of the same variety could be induced to callus but by different media and supplements. The probable reasons for such differential responses to nutrient media are discussed.

Introduction

It has been a well known fact that the requirement for callus formation may vary within the varieties of the same species (Nitsch 1969). One of the recent reports illustrating this point is by Kameya and Hinata (1970). Of the three varieties of 18-chromosome *Brassica oleracea* group used in their experiments on regeneration of plants from anther culture, two namely, Kawasaki Kanran and Murasaki Kanran callused and produced regenerants in the basic medium (Nitsch) supplemented by 2, 4-D plus kinetin and coconut milk. The third one, Yoshin Kanran, did not produce callus in either of the supplements. In order to see whether such differences exist in the varieties of egg plants in respect of their nutritional requirement for callusing and organ formation and also whether the different parts of the same plant form callus in the same or different media experiments were carried out in the laboratory of Plant Breeding, University of Tokyo. The present paper reports the results of these experiments.

Materials and Methods

The following two varieties of *Solanum melongena* L. var. *esculentum* were used : Oka 11 of Purple and Bandung Sho of White variety.

The flower buds were collected one day before their opening. They were washed in distilled water and then kept immersed in 5 per cent sodium hypochlorite solution for 10-15 min and again washed in sterile distilled water at least three times. For transfer of anthers into the medium, the most convenient method found was to cut across the bud at its extreme tip and then to squeeze out the anthers from the bud by holding it between the thumb and the forefinger and applying gentle pressure. The squeezed out anthers were then transferred

* Present address : Botany Department, Dacca University, East Pakistan.

into the medium aseptically by means of a pair of fine forceps. White's (WM) and Nitsch's media (N) were prepared according to the standard method. The percentage of agar and sucrose used in all media was 1 and 2 respectively. Coconut milk (CM) was filtered through Buchner's funnel. Tobacco revised medium (TRM) was made according to the formula suggested by Niizeki (1968) and its pH adjusted to 5.8. Excepting CM all other supplements, namely, indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy acetic acid (2, 4-D), benzyladenine (BA), kinetin in different concentrations were added before autoclaving. In each flask or test tube of 100 ml, 20 ml of the medium were used.

To induce callus formation from the fruit, only those below 5 cm of length were chosen. After washing the fruit with soap water, the surface of the fruit was sterilised by sodium hypochlorite solution in the same way as was done for the flower bud. By means of a sterile cork borer (1 cm dia), pieces of tissue were drilled out from the end of the fruit which remains covered underneath the persistent calyx. Since this portion did not contain any seed, this was the region found more suitable for tissue culture. Out of the cut pieces thin slices about 1 mm thick were made and aseptically transferred to the surface of agar medium.

Buds of different sizes were selected and transferred to the medium after surface sterilisation with soap water and sodium hypochlorite solution. For style and stigma culture no separate experiment was set up. In the same flask or the test tube containing anthers, the style together with the stigma was also transferred. The cultures were grown at 30/25 C day/night temperature at an artificial illumination of 6 Klux during the light period, 12 hrs per day.

As is shown in Table 1, in the Purple variety, Oka 11, 60 per cent of the anthers callused only in Nitsch's when supplemented by 1.5 ppm of kinetin and 1 ppm of 2, 4-D. The response was so specific that the callus formation was not observed when the concentration of kinetin was reduced to 1 ppm or when WM was used in place of Nitsch's as basal medium or in other supplements. Another significant observation was that the anthers burst indicating the formation of callus in less than one week.

The medium found suitable for the Purple variety failed to initiate any callus formation in the White variety. 83 per cent of the anthers of the latter variety callused in TRM, the first sign of callus formation being noted within a week from the start of the experiment—perhaps in the shortest possible time so far reported in literature. Tobacco revised medium contained 0.4 ppm of NAA and 10 per cent by volume of CM and while these two supplements proved essential for the callusing of the White variety, these were practically ineffective in the Purple

variety. Only one anther of the latter variety callused probably because of its having undergone mutation at an early stage of its ontogeny. However, its filament whenever attached to the anther was found to callus in TRM while the anther remained unchanged in this medium.

In a separate set of experiments pollen grains from freshly plucked flowers were cultured in Nitsch's medium supplemented by 10 per cent CM using the same technique as suggested by Kameya and Hinata (1970). In four weeks a number of cell divisions, different in different pollen grains, were observed under phase contrast microscope but no pollen grain was found to have burst at the end of 8 weeks as a result of cell divisions. However, tissue formation from isolated pollen grains is possible as indicated by a small mass of tissue observed on the surface of the culture medium in a test tube where some pollen got accidentally landed as a result of injury caused to the anther during its transfer into the tube.

Style and stigma culture : Although the anthers of the Purple variety did not callus in TRM, its style did so in two weeks. The stigma attached to the callusing style dried up. On the other hand, in WM fortified by 1.5 ppm of kinetin and 1 ppm of 2, 4-D, the stigmatic lobes proliferated as if in preparation for callus formation, but the style did not. In WM with 0.5 ppm of BA and 0.1 ppm of 2, 4-D, both style and stigma were found to callus. Another noteworthy observation was that the stigma along with the style in some young buds of the White variety was found to remain green and fresh in WM with 1.5 ppm of kinetin and 1 ppm of 2, 4-D. No enlargement of either the style or stigma was observed.

Fruit culture : Sixty thin slices of the Purple variety were aseptically transferred into agar gelled Nitsch's medium, one third containing CM, another one third containing 2, 4-D and the remaining one third a combination of 0.5 ppm of BA and 2 ppm of IAA. Good callus was only observed in CM fortified Nitsch's medium while no callus formation took place in the other two supplements. In BA supplemented medium the tissue slices tended to turn green without any callus formation. Only a small number of tissue slices from the White variety were tried in different media. Coconut milk does not seem to favour callus formation in the White variety as it does in the Purple. It may be recalled here that in case of callus formation from anther the situation is different ; CM favours callus formation in the White and not in the Purple.

Flower bud culture : The size of the bud was found very important because no callus was formed from any bud either a little too long or too small. The bud of only right size was found to form callus in CM supplemented Nitsch's medium. The flower buds of the White variety got infected ; so the results on the Purple variety are only available.

Organ differentiation : The callus from various parts of the plant was transferred to WM, one lot containing only 1 ppm of kinetin and another containing a combination of 1 ppm of kinetin and 0.1 ppm of NAA. In less than a week three roots were observed in one bud culture containing kinetin only. Some bud primordia were also seen to be differentiated in fruit culture containing both kinetin and NAA. Only further observation will enable any conclusion to be drawn whether some of these primordia give rise to a fully differentiated plant.

Table 1

Effects of different media with or without supplements on the callus formation from anthers of Solanum melongena

Variety	Medium	Supplements in mg/l except where mentioned otherwise	No. of anthers cultured	Callus formation
Oka 11*	White's	—	20	—
,,	,,	K 1.5, 2,4-D 1	60	-
,,	,,	10%CM, 2,4-D 1	70	–
,,	,,	10%CM, IAA 1	20	—
,,	,,	10%CM, NAA 1	20	—
,,	Nitsch's	—	20	.
,,	,,	10%CM, 2,4-D 1	20	—
,,	,,	10%CM, IAA 1	20	–
,,	,,	10%CM, NAA 1	20	–
,,	,,	K 1.0, 2,4-D 1	20	– -
,,	,,	K 1.5, 2,4-D 1	20	12
,,	Tobacco Revised Medium**	—	40	1
Bandung Sho***	White's	—	20	—
,,	,,	K 2, 2,4-D 1	30	—
,,	,,	K 1.5, 2,4-D 1	40	—
,,	Nitsch's	—	20	—
,,	,,	K 1.5, 2,4-D 1	35	—
,,	Tobacco Revised Medium**	— -	145	119

*Fruit color is purple. **This medium contains 1 ppm NAA and 10%CM.
***Fruit color is white. K = kinetin. CM = coconut milk.

Discussion

The results reported here show that the two varieties of egg plant require different kinds of nutrient media and different growth supplements to callus from anthers under optimum temperature and light conditions. While 83 per cent of the anthers of the White variety callused in TRM within 1-3 weeks, almost none from the Purple variety showed any sign of it in this medium. On the other hand, 80 per cent of the anthers of the Purple variety showed callus formation in Nitsch's medium when supplemented by a mixture of 1.5 ppm of kinetin and 1 ppm of 2, 4-D but none from the White. If the experiment on callus formation was only tried with the anther, one would think that probably the White originated as a mutant from the Purple and as such in addition to kinetin and 2, 4-D, it may require the growth promoting substances present in CM. One may assume also that the total quantity of growth promoting substances i.e., endogenous and those supplied by CM proved not conducive for callus formation in the Purple variety. When the experiments to induce callus formation from other parts of these two varieties were carried out, it was clear that the above assumption could not have been correct because the same CM could induce callus in the Purple if the tissue selected for callusing was from the fruit or the flower bud. Not only that : the stigma and the style of the same flower responded differently to the same medium. For instance, the style of the Purple callused in TRM while the stigma withered. On the other hand, the stigma of the Purple proliferated in kinetin and 2, 4-D supplemented WM but the style did not. By choice of suitable growth substances, however, both style and stigma could be induced to callus (cf. results).

From earlier literature (Steward *et al*, 1958), one would get the impression that only a particular part of a plant organ may be induced to callus but from the recent experiments as well as from the results reported here, it is clear that any plant parts may be made to callus provided appropriate growth supplements are added to the growing medium. For example 2, 4-D has been found to be, by far, the most important chemical for callus formation but it may not prove good enough for all materials (Niizeki and Ono 1970). Whether or not a particular tissue calluses in a particular medium perhaps depends upon its physiological state i.e., its degree and kind of development. For instance, although the tissue constituting the anther, filament, style and stigma is largely parenchymateous, they must be physiologically and for that matter functionally different ; as such the levels of growth promoting substances in each of them may be different. On this assumption each plant part, in order to develop callus, will require that quantity of a substance or substances in which it is deficient for the purpose of callus formation. In other words, the tissue of a particular region responds to that particular medium which makes up for the deficiency in respect of callus formation.

This assumption seems to hold good also for organ differentiation in this species. In a separate set of experiments (unpublished) conducted by one of us (*), it was found that callus grown from seeds could be redifferentiated into root and shoot in just WM without any supplements. Perhaps the built-in reserve of growth promoting substances i.e., BA and 2, 4-D in the callus which it derived from the original medium (in which the seeds were sown) was responsible for organ formation without further addition of growth supplements.

Repetition of the type of study reported here with other material may lead to a better understanding of the problem of growth and differentiation.

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