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芍藥 葯培養에 있어서 小胞子의 初期分裂

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Initial Divisions of Microspores in *In Vitro* Cultured Anthers of Cultivated *Paeonia albiflora*

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ABSTRACT

Anthers of cultivated *Paeonia albiflora* were cultured on media supplemented with various combinations of growth regulators. Although the number of anthers with emerged calluses were very few, in the sectioned anthers were found many multinucleate, 2-celled, or multicellular microspores, the one-celled multinucleate microspores being most abundant in number, and the multicellular ones the least. In 2-celled or 3-celled microspores two kinds were observed: one is ordinary one with single nucleus in each cell, and the other is multinucleate one. Majority of the 2-celled microspores was found to be of equational-division irrespective of whether they were multinucleate microspores or ordinary non-multinucleate ones.

INTRODUCTION

It has been reported that the first mitotic division of microspore in *in vitro* cultured anther is unequational and the embryoid or callus develops from the large vegetative nucleus while the generative nucleus for the most part remains quiescent in most of the plant species investigated so far (Sunderland and Wicks, 1969; Clapham, 1971; Iyer and Raina, 1972) except for the *Tritcum aestivum* where the first division is reportedly equational (Wang *et al.*, 1973).

This is in remarkable contrast to the course of events *in vivo* where the product of the generative nucleus plays the major role in fertilization while the vegetative nucleus degenerates. The way of first division of microspore may also draw interest if the occurrence of albino frequently observed in anther culture of certain species (Wang *et al.*, 1973) has any particular relation with the development of generative cell.

This paper reports the changes of microspore during anther culture of *Paeonia albiflora*, with special reference to the initial divisions of microspore.

MATERIALS AND METHODS

Anthers at uninucleate microspore stage were taken out of sterilized floral bud of cultivated *Paeonia albiflora* (varietal name unknown), and cultured in the Murashige and Skoog's medium supplemented with combinations of 0.2—2.0 mg/*l* 2.4-D, NAA and kinetin. 3g/*l* of active carbon was also added to some of the media for the purpose of removing toxic substances. The pH of the medium was adjusted to 5.8. Observation of the change of microspore during culture was made by examining the paraffin-sectioned anthers 60

days after culture.

RESULTS AND DISCUSSION

Out of 7157 anthers cultured for about 50 days, calluses appeared from only six anthers. All the calluses but one develpoed from the media active carbon was additionally supplied. The mode of emergence of callus from the anther locule rupturing the anther wall(Fig. 1a) and the easy detachability of the callus from anther even by slight touch indicated that the calluses were of microspore-origin(Niizeki and Oono, 1968; Harn, 1969, 1972a,b; Iyer and Raina, 1972).

Although the number of anthers with emerged callus was very few, out of 569 anthers examined 118 contained changed microspores of various kinds such as multinucleate, 2-celled, or multicellular microspores in their locules(Fig. 1b-h). It seemed that after a few initial divisions further growth of the microspores were arrested, the callus seldom reaching the size big enough as to emerge out of anther lucule. The causes of growth stop at an early stage of microspore change were not certain. Probably the unbalanced combinations of growth regulators of unusually small amount of auxins as compared with that of kinetin supplemented to the basic media might be one of the reasons.

Among the changed microspores the one-celled free-nuclear multinucleate microspores (Fig. 1b) were most abundant in number, and the least were the multicellular ones(Table 1, Fig. 1h). In 2-celled microspores there were two kinds: one is ordinary one with single nucleus in each cell(Fig. 1c, d), and the other is multinucleate 2-celled microspore(Fig. 1e, e'). In the latter further divisions of nucleus were not followed by the laydown of partition walls. Exactly the same phenomenon as was observed in 2-celled microspore occurred in the 3-celled microspore.

When uninucleate microspore divided to become 2-celled one, two ways of cell division, i.e., equational(Fig. 1c) and unequational(Fig. 1d) division took place, the former being much more frequent than the latter(Table 2). In equational division the two daughter nuclei were equal in their size, but in unequational division the nucleus of the smaller cell was smaller as in the pollen. When unequationally divided microspores became multinucleate the unclei in the smaller cell were also smaller than those in the larger cell(Fig. 1e, e'). In some microspores after the initial unequational division the smaller cell divided further while the larger one became 2-nucleate(Fig. 1f,f').

The further fate of the free-nuclear microspores could not be traced, and it was not certain which

Table 1. Changes of microspores during culture of anthers of cultivated *Paeonia altiflora*

No. of microspores examined	No. of multinucleate microspores		No. of multicellular, nonmultinucleate microspores	
	One-celled	2-celled	2-celled	Multicelled*
7867	137	92	203	26

(3 paraffin sections per anther were observed)
* 3-celled microspores included.

Table 2. Frequency of equational or unequational division of 2-celled microspores in the anther culture of *Paeonia albiflora*

	Equational division	Unequational division
Multinucleate microspores	89	3
Non-multinucleate microspores	187	16
Total	276	19

one of the changed microspores developed into callus, but the equationally divided 2-celled microspore seem to be the ones forming calluses (Fig. 2) in view of their abundance in number and occasional occurrence of the non-multinucleate ordinary 3-celled microspores (Fig. 1g, g').

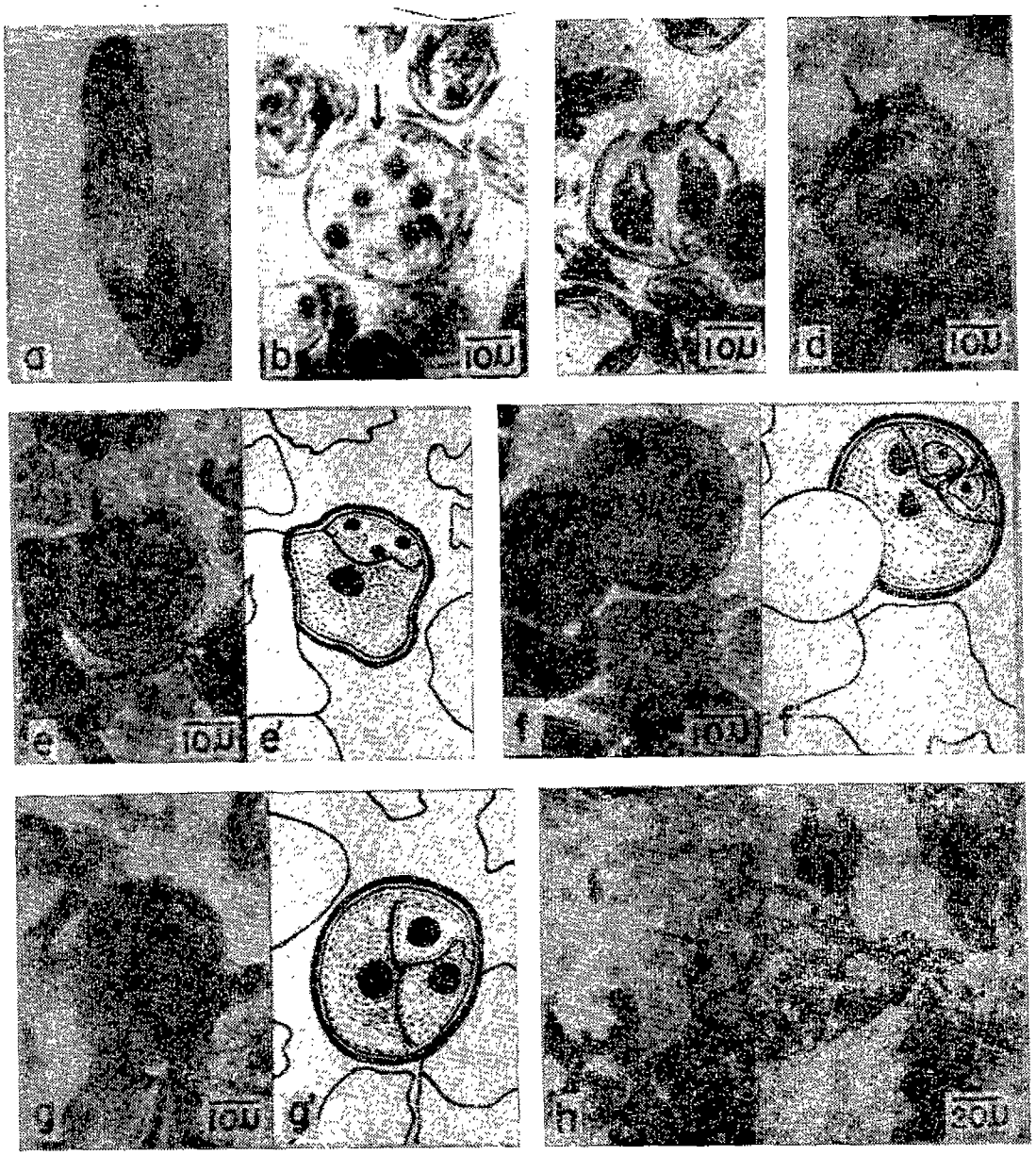

Fig. 1. Photomicrographs of callus and microspores from anther culture of *Paeonia albiflora*.

1a, Callus growing out of anther cavity in the anther culture of cultivated *P. albiflora*; b-h, Changes of microspores during *in vitro* culture of anther; b, One-celled multinucleate microspore; c, d, 2-celled microspores after equational (c) and unequational (d) division; e,e', Unequationally divided microspore with three smaller nuclei in the smaller daughter cell; f,f', After the initial unequational division, the smaller cell divided further while the larger one became 2-nucleate; g,g', 3-celled microspore of equational divisions; h, Multicellular body in the anther locule.

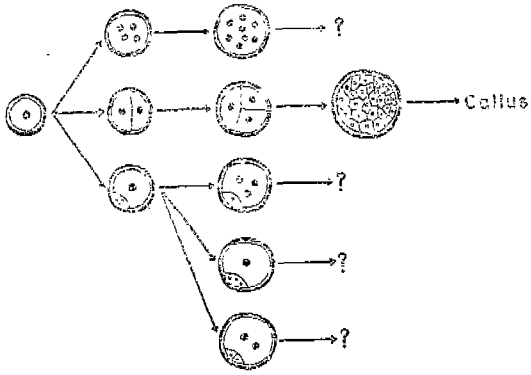

Fig. 2. Diagram showing changes of microspores into multicellular or multinucleate bodies during *in vitro* culture of anther of cultivated *Paeonia albiflora*.

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