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HISTOLOGY OF ORGANOGENESIS FROM CALLUS CULTURES OF BLACK PEPPER *(PIPER NIGRUM* L.)

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Abstract: A histological study of the indirect organogenesis from leaf cultures of black pepper was carried out. The undifferentiated cells of callus were found to differentiate into vascular nodules called meristemoids, which then develop into xylem elements, especially tracheids. On culturing in the shooting medium, these nodules differentiated into shoot apical meristem. This adventitious origin provides chances for variability.

Key words: Black pepper, histology, organogenesis, somaclonal variation

INTRODUCTION

Black pepper *(Piper nigrum* L., Piperaceae) is one of the most economically important spice crops of India. The dried berries as a whole or in ground form is a favorite spice throughout the world and hence the name ' King of Spices'. The oleoresin and oil extracts are also of immense value. The intrinsic medicinal properties find uses such as an aromatic stimulant and antipyretic even in malarial fever. Several reports are there about the antimicrobial and insecticidal property of the powdered and extracted forms of black pepper.

Successful *in vitro* techniques for micropropagation of black pepper have been reported using mature shoot tip explants (Nazeem *et al.*, 1992; Philip *et al.*, 1992; Babu *et al.*, 1993; Joseph *et al.*, 1993). *In vitro* culture system could be well exploited for induction of variability in this vegetatively propagated species. Protocol for plant regeneration from leaf callus cultures has been reported by Babu *et al.* (1993) and Nazeem *et al.* (1993). The present study is a histological analysis of the growth and morphogenesis in the leaf calli of P. *nigrum.*

MATERIALS AND METHODS

Leaf explants were surface sterilized with 0.1 per cent mercuric chloride, washed free of the sterilant and cultured in modified MS medium (Murashige and Skoog, 1962) supplemented with IAA and BAP (1.0 ppm each). Creamy white to pale green friable calli with 10 to 40 shoot initials were obtained by the third subculture. Addition of silver nitrate 5-15 ppm in the medium increased the shoot initiation. The calli at different stages of growth after initiation (15, 30, 45, 60, 90 and 120 days) were selected for histological studies. Thin sections were taken, stained with safranin, mounted on glass slides and covered with a coverglass for microscopic observations. Photographs were taken under bright field with Nikon Optihot-2 microscope.

RESULTS AND DISCUSSION

It was found that during the early stages of callus formation, the parenchyma cells of the mesophyll tissue near the vascular bundles in the leaf explant resumed meristematic activity due to the wounding and produced an undifferentiated mass of cells which is called primary callus (Plate 1.1). The division and growth of callus cells continued for some time resulting in the enlargement of primary callus. According to Esau (1977), in a root or shoot apex, where active cell division occurs, certain cells of the meristems undergo divisions in such a way that, one product of a division becomes a new body cell, called derivative and the other remains in the meristem, called initials. A similar pattern of meristematic activity was observed in black pepper callus. Simultaneous with the increase in volume of the primary callus, the derivatives started differentiation into large parenchymatous cells while the initials appeared as darkly stained clumps (Plate 1.2).

The vascular tissues were found initiated in the form of procambium, the cells of which assume a rather narrow elongated form because of the predominance of longitudinal division. The procambium then develops into distinct vascular elements, especially tracheids, scattered in the basal callus tissue (Plate 1.3). According to Chen and Galston (1967), callus cultures contain vascular elements and parenchymatous cells, together called vascular nodules. The proportion of nodules formed depends especially upon the kind of sugar and growth regulators added to the medium and also on the species from which the culture originated. Cassells (1979) also reported

that callus differentiation begins when peripheral meristematic activity is replaced or supplemented by the formation of centres of cell division deeper in the tissue. These meristematic regions, which are characterized with small cells with densely stained nuclei, first produce a meristemoid. He also reported that the meristematic regions in the form of nodules in the callus may produce further expanded and undifferentiated cells (so contributing to callus expansion) or

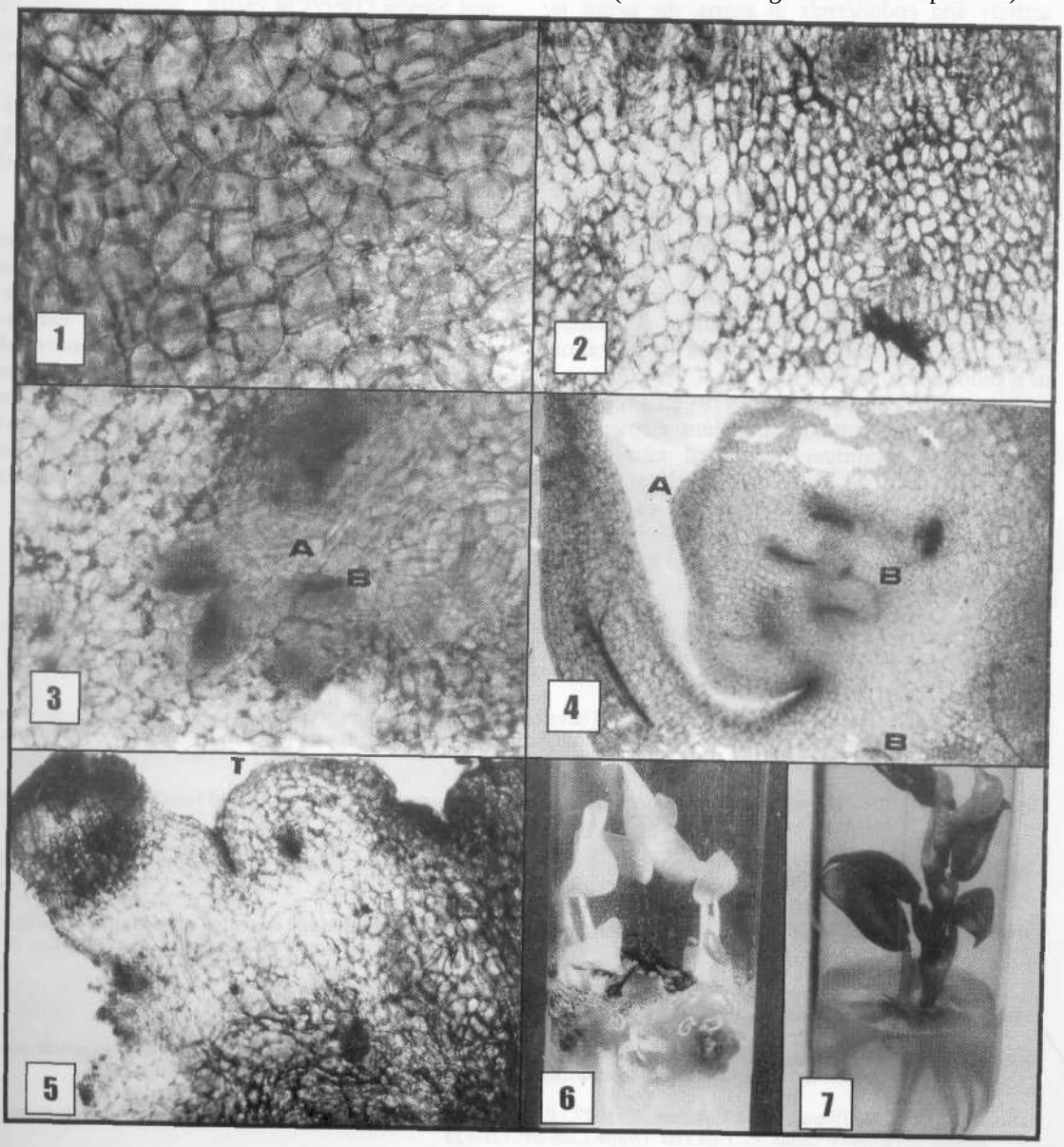

Plate 1. Stages of callus differentiation into shoot primordia

[1] Primary callus with undifferentiated cells; 2. Callus with derivatives (large cells) and initials (darkly stained); 3. Callus with **procambial** cells (A) and vascular nodules (B); 4. Origin of shoot primordia from the peripheral layer (A) and inner tissue (B); 5. Tunica corpus organization of shoot primordia from callus (T); 6. Multiple shoots from black pepper callus; 7. Callus regen-

cells which differentiate into xylem or phloem elements. The scattered nature of vascular bundles is a distinguishing feature of the genus *Piper*. Even though this is usually found in monocotyledons, due to the presence of cambial activity and endodermis in stems, the genus is grouped under dicotyledons (Metcalfe and Chalk, 1950; Rahiman and Nair, 1983).

Formation of vascular nodules in callus cultures may represent or be associated with an early stage of the development of shoot meristems (Chen and Galston, 1967). In black pepper, we observed the origin of shoot primordia to occur both at the superficial layer and embedded in the inner tissues of organogenic calli (Plate 1.4). The callus with numerous vascular nodules, on culturing in the shooting medium developed small buds with the tunica corpus organization of a shoot apical meristem (Plate 1.5). Chen and Galston (1967) and Cassels (1979) also reported that the nodules containing xylem elements in callus of *Pelargonium* developed into shoots when moved to an auxin free medium. Such nodules that protrude out from the callus and leaf buttresses, later on developed into distinct shoot or leaf primordium. Further growth of the shoot apical meristem in black pepper was similar to that of a normal shoot apex (Plate 1.6), a histological account of which is provided by Xia and Steves (1999) in carrot. Similar pattern of indirect organogenesis was reported in carnation (Kallak *et al.*, 1997), *Eucalyptus globulus* (Azmi *et al.*, 1997) and watercress (Claxton *et al.*, 1998). Histological abnormalities were not observed among the callus regenerants in black pepper and were successfully rooted and planted out with more than 80% success (Plate 1.7).

The detailed histological analysis shows that the shoots regenerated from the leaf derived callus of black pepper have no organized cellular connection with the original explant tissue, indicating an adventitious origin and hence, chances of genetic variability among the regenerants. Thus indirect organogenesis could be exploited in black pepper for the induction of somaclonal variation. These variants could be screened for desirable traits for which natural variability is quite narrow.

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