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SHORT COMMUNICATION

ROLE OF CATIONIC AND ANIONIC PEROXIDASE IN BROWNING OF MULTIPLE SHOOTS OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) *IN VITRO*

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Multiple shoots were induced from nodal region of carnation (*Dianthus caryophyllus* L.) in Murashige and Skoog medium containing 1.5 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. Induction of the shoots was observed in three weeks, but good amount of tissue turned brown in sub-cultures. Cationic and anionic peroxidase activities were studied in green and brown multiple shoot culture of carnation. It was observed that anionic protein was more in brown tissue and specific anionic peroxidase activity was also higher. It is concluded that anionic peroxidase plays an important role in browning of the tissue.

***Key words*: Anionic peroxidase, carnation, cationic peroxidase, multiple shoots**

Plant peroxidases (POD) are monomeric, heme-containing proteins that are usually glycosylated. Peroxidases belong to a large family of enzymes that are ubiquitous in fungi, plants and vertebrates. POD produced by plants are present in multiple isozymic forms. These have various physiological roles in plant cells, some of them are excreted and participate in many reactions including lignification, cross-linking of cell wall polysaccharides, oxidation of indole-3-acetic acid, regulation of cell elongation, wound healing and phenol oxidation (Gasper *et al*. 1991).

It is a general observation that certain tissues turn brown in the culture media, even if fresh subculture is made. A number of studies that attempted to correlate pigment degradation with POD activities during senescence, led to the conclusion that POD associated with chlorophyll degradation appears to be synthesized *de novo* (Johnson-Flanagan and McLachlan 1990). However, no distinction for anionic or cationic POD is reported in the literature. In this experiment, changes in cationic and anionic POD activities were studied in multiple shoot generating tissues of carnation.

Carnation (*Dianthus caryophyllus* L.) obtained from the commercial market was cultured on Murashige and Skoog (1962) medium containing 1.5 mg l^{-1} BAP and 0.5 mg l^{-1} NAA. All medium components were mixed and adjusted to pH 5.0 prior to autoclaving at 121°C for 15 min. All these cultures were incubated at 25°C (±2) in a culture room under 16 h photoperiod and sub-cultured periodically. Multiple shoots were observed within 3 weeks and after that they were again transferred to the fresh medium. But it was noticed that growing green tissue turned brown. To investigate the reason of tissue browning, cationic and anionic POD was extracted from both green and brown tissues of multiple shoots.

The enzyme was extracted in 0.1M phosphate buffer, pH 6.4, centrifuged at 10,000 x g for 15 min. The supernatant was collected and precipitated with ammonium sulphate at 80 % saturation. The precipitates

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were re-suspended in phosphate buffer and anionic and cationic POD was fractioned using carboxy methyl cellulose (CM- cellulose) and diethyl aminoethyl cellulose (DEAE- cellulose), respectively. For anionic POD, CM-cellulose column (16x20 cm) was pre-equilibrated with phosphate buffer and loaded protein was eluted with buffer till the fraction showed zero reading at 280 nm. Similarly, DEAE- cellulose column pre-equilibrated with 0.1 M Tris-HCL buffer pH 8.7, used to fractionate cationic POD. Each fraction was assayed in triplicate for POD as described by Mahesh and Thaker (2004). The protein content of the enzyme extract was estimated according to Bradford (1976) using coomasie brilliant blue dye binding method.

It was observed that in a green tissue (Fig. 1a) there was no clear difference between cationic and anionic protein content. In contrast, in brown tissue (Fig. 1b) anionic protein was more as compared to cationic protein. Enzyme in green tissue showed higher activity in cationic fraction than in anionic (Fig. 1c). In contrast, in brown tissue anionic POD activity remained higher as compared to cationic POD activity, in almost all the fractions (Fig. 1d).

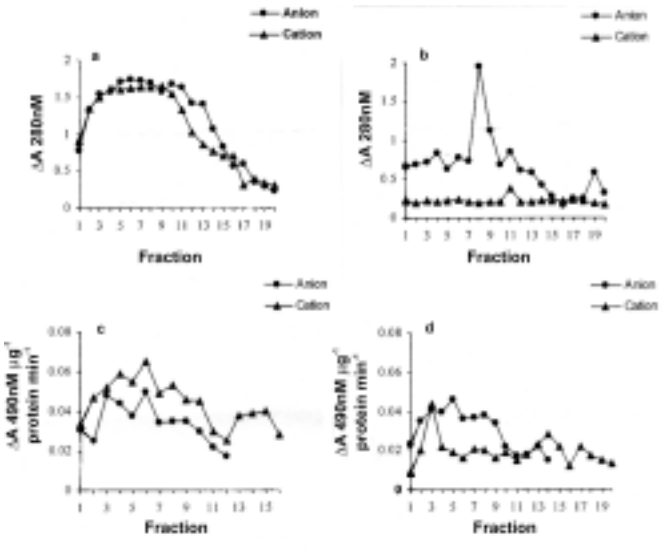

Fig. 1. Analysis of green and brown tissues of multiple shoots of carnation. (a) Changes in protein value (green tissue) of fractions eluted with CM cellulose and DEAE cellulose, (b) Changes in protein value (brown tissue) of fractions eluted with CM cellulose and DEAE cellulose, (c) Changes in peroxidase enzyme activity µg^{-1} protein min^{-1} (green tissue) of fractions eluted with CM cellulose and DEAE cellulose, (d) Changes in peroxidase enzyme activity µg^{-1} protein min^{-1} (brown tissue) of fractions eluted with CM cellulose and DEAE cellulose.

Numerous studies have examined the role of POD in chlorophyll catabolism (Kato and Shimizu 1987, Abeles *et al*. 1988). *In vitro* studies on higher plants, suggest that POD can degrade chlorophyll (Huff 1982). Kato and Shimizu (1987) demonstrated a correlation between POD activity and rapid pigment bleaching during senescence in tobacco leaves. A significant difference in both green and brown culture cells was observed in the present study when POD activity expressed on protein basis. In green cells cationic POD was higher (Fig. 1c) and difference was less significant ($P \leq 0.05$) statistically. In contrast, in brown cells, anionic POD was more (Fig. 1d) than cationic POD in almost all fractions and significant difference was observed ($P \leq 0.001$). Along with protein values, anionic POD activities also remained higher in brown tissue (Fig. 1b, d). A number of studies that attempted to correlate pigment degradation with POD activities during senescence led to a conclusion that POD associated with chlorophyll degradation appears to be synthesized *de novo* (Johnson-Flanagan and McLachlan 1990). ANOVA between green and brown tissues for cationic and anionic proteins, enzyme and enzyme activity is shown in Table. 1. In green tissue, cationic POD activity was higher than anionic POD. In contrast to this, in brown tissue both

Table 1. ANOVA between anionic and cationic proteins and peroxidase enzyme activity of green and brown tissues. Value in parenthesis indicates degree of freedom.

		Mean	F
Protein from green tissue	Anion	1.18635	0.35889*
	Cation	1.08640	(39)
Protein from brown tissue	Anion	0.65560	25.12169***
	Cation	0.21720	(39)
Peroxidase enzyme activity from green tissue	Anion	0.03408	5.67232**
	Cation	0.04400	(27)
Peroxidase enzyme activity from brown tissue	Anion	0.03029	12.81808***
	Cation	0.01953	(33)

Statistically significant P levels: 0.1*, 0.01**, 0.001***

were higher; anionic protein and enzyme activity. This suggests a significant role of anionic POD in tissue browning of carnation multiple shoot culture.

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