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Stress tolerance in auxin heterotrophic and autotrophic tobacco tissue cultures

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ABSTRACT The natures of the stress tolerance of auxin autotrophic and heterotrophic tobacco calli were compared. 50 mM NaCl did not inhibit the growth of auxin autotrophic calli and they exhibited a lower level of peroxidase secretion into the media than that of heterotrophic cultures. The ascorbate peroxidase and catalase activities of these lines did not decrease in the presence of stressors. In the second half of the culturing period, the auxin autotrophic tissues expressed a higher GST activity than that of the heterotrophic lines.
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KEY WORDS

auxin heterotrophic calli
autotrophic tobacco calli
peroxidase secretion
ascorbate peroxidase
catalase
GST activity

Hormone autotrophy is a common phenomenon. In hormone heterotrophic cultures, hormone autotrophic lines regularly appear. Their auxin metabolism and sensitivity are changed. The auxin-habituated tissues proliferate without exogenous auxin; moreover, their growth is inhibited by it (Szabó 1996). There are several explanations for the origin of auxin autotrophy, one being stress adaptation to the *in vitro* conditions.

Materials and Methods

The callus cultures were initiated from protoplasts of *Nicotiana tabacum* SR1 plants and maintained on Murashige-Skoog medium (1962) containing 3 mg l^{-1}IAA, 0.04 mg l^{-1} 2,4-D and 1 mg l^{-1} kinetin. The auxin autotrophic lines were selected and cultured on auxin-free medium. GST activity was determined by the procedure of Habig et al. (1974), catalase and ascorbate peroxidase activities were measured according to De Gara et al. (2000), and the secretion of peroxidase was followed by the procedure of Gaspar et al. (1983).

Results and Discussion

The cell wall is one of the limiting factors in the growth of the plant cell. Peroxidase with cross-linked polymer leads to the irreversible formation of an elongated cell wall structure. Thus, peroxidase secreted to the cell wall is in correlation with the possibility of growth. In auxin heterotrophic and autotrophic calli, the peroxidase secretion maximum precedes the growth maximum in the one-month culturing period, but their growth kinetics are different.

In this work, we investigated the effects of some stress factors on the growth of these two tobacco tissue lines. We present here an account of the influence of NO_2^- and NaCl on the proliferation of auxin heterotrophic and autotrophic tissues. Treatments that decreased the growth rate of heterotrophic cultures did not exert any inhibitory effect on the autotrophic lines. In general, the autotrophic calli display a higher stress tolerance, this correlating with their peroxidase secretion.

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How do autotrophic tissues survive higher stress effects? We studied two possible mechanisms. The experiments of Hagége et al. (1996) with sugar beet calli suggested that the auxin autotrophic lines have an enhanced stress tolerance due to their higher activity of enzymes of free radical scavenger systems. Our data are basically similar, but nevertheless differ to a certain extent. The activity of ascorbate peroxidase in the heterotrophic cultures was decreased by treatment with a high concentration (100 mM) of NaCl; at the same time, the activities of auxin autotrophic tissues did not change even in this osmotic medum as compared with the untreated control. The activities of catalase in the two lines exhibited the same tendency.

Under stress conditions, among other functions the GSTs detoxify the cells from many toxic compounds, transporting them into the vacuole. In the second half of the culturing period, when the proliferation of heterotrophic calli has stopped, their GST activity displays a decreasing tendency. The auxin autotrophic cultures expressed the highest GST activity in this growth period.

Thus, the development of auxin autotrophic calli might involve both the function of free radical scavenger systems and detoxification processes by GST.

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