VARIATIONS IN HYDROPEROXIDASE ACTIVITIES IN TISSUE CULTURES OF HEMP

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Abstract: The level of catalase and peroxidase activities in the *in vitro* tissue cultures of hemp, initiated from explant detached from plants of different sexes, registered differences depending not on genotype (as *in vivo* experiments), but on the differentiation degree of culture and on hormonal composition of nutritive media. Significant differences in the soluble protein level were also registered.

INTRODUCTION

Catalase and peroxidase are oxidoreducing enzymes, with an important role in the degradation of toxic hydrogen peroxide and of some organic substrata and also in the cell respiration process. Concerning the hydrogen donor, these two enzymes use H_2O_2 (catalase) or organic substrata (phenols, aromatic amines, ascorbic acid, glutathione) (peroxidase). The biological role of peroxidase is still uncertain, although IAA-oxidase (peroxidase-indole acetic acid oxidase) it considered an important system for the control of plant growth and morphogenesis. IAA oxidation, mediated by peroxidase, is influenced by a lot of compounds, especially the polyhydrous phenols being inhibitors for this enzyme (Palmieri and Giovinazzi, 1982). Because of its intervention in the IAA regulation, the peroxidase has an indirect role in the sex determining mechanism in hemp, more exactly in stamenogenesis and carpellogenesis. When H_2O_2 is in smaller concentration, but other substrata are in greater quantities, the catalase acts as a peroxidase, reason for which the two enzymes are known as "hydroperoxidases". In cell, it seems that the peroxidase activity of catalase is favoured.

THE AIM OF INVESTIGATIONS

The aim of this work is to evidence the variations of some oxidoreducing enzymes, during callusogenesis and regeneration in the *in vitro* tissue cultures of hemp, depending on origin explant, differentiation degree and composition of nutritive media.

MATERIAL AND METHODS

The studied material (explants from leaves, stems, flowers) was collected from plants of hemp of different sexual phenotypes (female, male, monoic), randomly chosen from a population grown in the experimental field of Botanical Garden of University "Al. I. Cuza" Iaşi. The seeds were delivered by Agricultural Research Centre of Secuieni - Neamț. Also, from plantlets obtained by the aseptic germination of seeds harvested from monoic and female plants, we used cotyledons, hypocotyls, epicotyls to initiate *in vitro* cultures. Catalase activity was determined by iodometric method (Artenie, Tănase, 1981). The principle of this method is based on potassium iodide oxidation by undecomposed hydrogen peroxide, after an incubation interval with catalase, followed by titration of delivered iodine with sodium thiosulfate, in the presence of starch solution as indicator. The catalase activities were calculated knowing that one catalase unit is equivalent to the amount of enzyme which decomposes 1 μmol (0.034 mg) H_2O_2, during 1 minute. The results are expressed in mg H_2O_2 / g fresh matter. The peroxidase activity was quantified by photometric method, with o-dianisidine. The principle of this method is to determine the intensity of the colour of compound obtained by o-dianisidine oxidation, in the presence of H_2O_2, under peroxidase action. The values of extinctions were determined with a SPEKOL 20 spectrophotometer, at λ=540 nm. The specific activities for catalase and peroxidase were estimated by reporting the quantity of substratum consumed by enzyme to the concentration of soluble protein in tissue gram. They were expressed in mg H_2O_2 / mg protein.

For the determination of soluble protein, the Lowry method (LOWRY et al. 1951) was used. The enzymatic extract was treated with Folin - Ciocâlteu solution. The extinctions were registered with a SPEKOL

20 spectrophotometer, at λ=500 nm. The amount of soluble proteins are expressed in mg / g fresh matter. These values are required to estimate the specific activities of the two hydroperoxidases.

RESULTS AND DISCUSSIONS

In Table 1 and Table 2 are shown the registered values for the relative and specific peroxidase and catalase activities in the *in vitro* cultures of hemp.

Table 1. Peroxidase activities in the *in vitro* cultures of hemp, obtained from explant with female, male, origin

Prove-nance	Nutri-tive media	Hormones (mg/l)	Culture	Px (mg H_2O_2 / g fresh matter)	Px (mg H_2O_2 /mg protein)	Soluble protein (mg/g fresh matter)
female	MS	0.1 2,4-D 0.5 KIN	beige-coloured, compact, unorganized callus	7.782	6.991	1.1129
female	MS	0.1 2,4-D 0.5 KIN	greenish-coloured, callus, great biomass accumulation	16.645	7.290	2.2832
female	MS	0.25 2,4-D 2.0 KIN	vitrified shoots	10.230	2.608	3.9217
female	MS	0.25 2,4-D 2.0 KIN	friable callus, without differentiations	17.707	5.567	3.1804
female	MS	1.0 2,4-D	beige-coloured, friable callus	19.713	10.762	1.8311
female	B 5	1.0 NAA	numerous neo-roots, with negative geotropism	24.132	4.904	4.9203
male	MS	0.25 2,4-D 2.0 KIN	abundant, greenish-coloured, hydrated callus	18.221	8.505	2.1423
male	MS	0.25 2,4-D 2.0 KIN	vitrified shoots	12.033	2.222	3.8115
monoic	MS	1.0 NAA	cream-coloured callus, strong rhizogenesis (vigorous roots with numerous absorptive hairs)	25.118	4.639	5.4141
monoic	B 5	0.2 NAA 1.0 BAP 1.0 KIN	callus with numerous shoot primordia	32.988	6.515	5.0633

Table 2. Catalase activities in the *in vitro* cultures of hemp, obtained from explant with female, male, origin

Prove-nance	Nutri-tive media	Hormones (mg/l)	Culture	CAT (mg H_2O_2 / g fresh matter)	CAT (mg H_2O_2 /mg protein)	Soluble protein (mg/g fresh matter)
female	MS	0.1 2,4-D 0.5 KIN	beige-coloured, compact, unorganized callus	17.008	15.282	1.1129

female	MS	0.1 2,4-D 0.5 KIN	greenish-coloured, callus, great biomass accumulation	19.323	8.463	2.2832
female	MS	0.25 2,4-D 2.0 KIN	vitrified shoots	16.557	4.221	3.9217
female	MS	0.25 2,4-D 2.0 KIN	friable callus, without differentiations	16.012	5.034	3.1804
female	MS	1.0 2,4-D	beige-coloured, friable callus	22.151	12.097	1.8311
female	B 5	1.0 NAA	numerous neo-roots, with negative geotropism	28.242	5.739	4.9203
male	MS	0.25 2,4-D 2.0 KIN	abundant, greenish-coloured, hydrated callus	18.563	8.664	2.1423
male	MS	0.25 2,4-D 2.0 KIN	vitrified shoots	14.716	3.860	3.8115
monoic	MS	1.0 NAA	cream-coloured callus, strong rhizogenesis (vigorous roots with numerous absorptive hairs)	28.132	5.196	5.4141
monoic	B 5	0.2 NAA 1.0 BAP 1.0 KIN	callus with numerous shoot primordia	33.371	6.590	5.0633

The first observation is that the level of *in vitro* peroxidase (Table 1) is much greater than that registered for intact plant (Truță et al., 2000, 2002). What is the explanation? The *in vitro* tissue cultures are considered to develop in conditions of high oxidative stress, this being one of the factors explaining the increased values of *in vitro* peroxidases. Besides, even detachment of explants from intact plant represents a stress which determines a greater H_2O_2 synthesis. Secondly, if *in vivo* peroxidase activity displayed differences depending on genotype, in these experiments we can not make a conclusion relative to the differential behaviour of peroxidase depending on explant origin (female, male, monoic). Rather, the differences are related to the differentiation degree of culture and the hormonal composition of nutritive media. The singular presence of 2,4-D (1 mg / ml) induced the increase of level of peroxidase activity (19.713 mg H_2O_2 / g fresh matter), especially regarding the specific activity (10.765 mg H_2O_2 / mg protein). It is known that peroxidase role on 2,4-D degradation in two compounds, one contributing to the neoforming roots growth (Lesney, 1990; Berthon et al., 1991). It is possible that the increase of peroxidase activity, when in culture 2,4-D was added, to be due to acid fraction, placed in great amount in cell wall (Limam et al., 1998). IBA and NAA are two other synthetic auxins used to induce *in vitro* rhizogenesis, they being more resistant to degradation than IAA. The accumulation of conjugation products following their metabolization is correlated with an increase of peroxidase activity. A high peroxidase level,

comparatively with the undifferentiated callus, was noted both on B 5 (a culture initiated from female plant) and MS (a culture started from a monoic plant), media containing NAA (1 mg / ml), with visible rhizogenesis (well developed roots, with a lot of absorptive hairs and, generally, with negative geotropism). Important increases of peroxidase level were cited in *Picea abies*, during root initiation (Jarvis and Shaheed, 1986). Greppin et al. (1986) concluded that the peroxidase may be used as marker for rhizogenesis. A great peroxidase level was also registered on media with a hormonal balance inducing organogenesis. Thus, on B 5 supplemented with a ratio 1 : 10 between auxins and cytokinins, on callus originated from monoic plant, numerous shoot primordia appeared, the peroxidase level being 32.988 mg H_2O_2 / g fresh matter. Caullogenesis was also correlated with the synthesis of a greater protein quantity. Therefore, the specific peroxidase activity was 6.515 mg H_2O_2 / mg protein. We can make a short commentary on the indirect role of peroxidase in morphogenesis and organogenesis, by the modification of hormonal balance. The increase of peroxidase activity leads to an increase auxin catabolism, to the diminution of auxin level and to the modification of the auxin / cytokinin ratio, in favour of cytokinin, fact resulting in bud and shoot appearance. Thus, a high peroxidase activity is "a budding promoter", by the modification of the endogenous auxin / cytokinin ratio (peroxidases having an auxin - oxidizing activity) (Gaspar et al., 1982).

For catalase (Table 2), greater values of relative activities were noted for the callus with differentiated roots (on NAA supplemented media), although the level of specific activity (approximately 5 mg H_2O_2 / mg protein) is not very high, fact explicable by the great quantity of soluble protein to which is made the estimation (4.9203 mg protein / g fresh matter, respectively 5.1414 mg protein / g fresh matter). A high catalase activity (33.371 mg H_2O_2 / g fresh matter - relative activity, 6.590 mg H_2O_2 / mg protein - specific activity), associated with an active peroxidase, was registered on B 5 supplemented with 0.2 mg / l NAA, 1.0 mg / l BAP and 1.o mg / l KIN, balance favourable to organogenesis. In vitrified shoots, the catalase activity was smaller (16.557 mg H_2O_2 / g fresh matter, for female originating culture, respectively 14.716 mg H_2O_2 / g fresh matter in the callus originated from male plant). In these two cases, the medium was the same (MS + 0.25 mg / l 2,4-D + 2.0 mg / l KIN). The specific catalase activities were, also, smaller.

CONCLUSIONS

In vitro, the level of relative peroxidase activity exceeded the values obtained for intact plants.

The behaviour of peroxidase and catalase depends not on explant origin (monoic, female, male), but on the differentiation degree of the culture and on hormonal composition of nutritive media.

On media supplemented with 2,4-D (1mg / l) and on those containing auxins and cytokinins in 1 : 10 ratio, peroxidase was greater.

In vitrified shoots, the peroxidase and catalase activities were lower.

Significant differences in the soluble protein level were also registered**.**

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