# DYNAMICS OF LEAF PEROXIDASE ACTIVITY DURING ONTOGENY OF HEMP PLANTS, IN RELATION TO SEXUAL PHENOTYPE

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**Abstract**: During vegetation of female and male hemp plants (*Cannabis sativa* L.), five quantitative determinations of peroxidase activities were made (40 days, 55 days, 70 days, 85 days, 105 days). Peroxidase activity presented some differences in hemp plants, between females and males, during their vegetation cycle. In female plants, before anthesis were registered peaks of peroxidase activities. The blossoming of male plants was coincident with the increase of catalitic action of peroxidase. Generally, the male plants displayed greater levels of peroxidasic activity.

## INTRODUCTION

Plant peroxidases (EC 1.11.1.7) catalyse the oxidation of some organic substrata, in presence of $H_2O_2$, and are associated with growth, cell wall formation, fruit ripening, ethylene biosynthesis, resistance to pathogens, reactivity to stress (Frahry and Schopfer, 1998). This enzyme has more isoforms, each of them with an well-defined role in organism. The isoperoxidase pattern is complex, fact evidenced too for hemp (Truţă et al., 2002), just this complexity making difficult the understanding of specific functions of enzyme (Yun et al., 1998; Clemente, 1998). Peroxidase is placed in cytosol and cell wall, in several isoforms, genetically different. The cell wall, where there is 6-8% from total tissue peroxidasic activity, is considered the site of primary action of plant peroxidases (Fry, 1986). Generally, the idea of intervention of acid peroxidases in lignin biosynthesis is accepted, while basic peroxidases, placed in cytosol, are implied in IAA catabolism, by a decarboxylation step (Lamport et al., 1998, Limam et al., 1998). The chemical composition of hemp is very complex (flavonoids, fatty acids, phenolic spiroindans, dihydrostilbenes, nitrate substances, more than 70 cannabinoids). Although the hemp is a dioecious specis, as a consequence of intensive improvement, a lot of sexual phenotypes are cultivated, the most frequent being the monoecious forms. For hemp, it is not known a consistent study on differentiation between sexual phenotypes, regarding morphological, physiological or biochemical traits, in spite of some disparate data. For genus *Cannabis*, variable levels of cellular extract pH are cited, depending on sex. For these reasons, the objective of this study is to identify the existence of some enzymatic differences between the sexual phenotypes of hemp, to complete our previous data obtained from other similar studies.

## MATERIALS AND METHODS

We used leaves collected from female and male plants of hemp (*Cannabis sativa* L.), from a population grown in the experimental field of the Botanical Garden of University “Alexandru Ioan Cuza” Iaşi. The analyses were effectuated in dynamics, starting with plants 40 days old, until 105 days, at intervals of 2 weeks, excepting the last determination, determined by photometric method with o-dianisidine. The principle of this method is to establish the colour intensity of compound obtained by dianisidine oxidation, under the peroxidase activity, in the presence of $H_2O_2$. The values of extinctions were determined with a SPEKOL 20 spectrophotometer, at $\lambda$=540nm. The statistical analysis of the obtained data was performed realized after 3 weeks. Peroxidase activity was using the method described in RAICU et al. (1973). The arithmetical mean (x), the standard deviation (SD), the standard error of the mean (SE), the coefficient of variation (CV) and the standard error of the mean, expressed in % (SE %), were calculated.

## RESULTS AND DISSCUSIONS

As shown in Fig. 1, the enzymatic activity hasn’t a linear evolution , neither in females nor in males. Thus, if 40days old plants have enough close levels, although lightly more increase for males (4.84±0.50 UP/g, comparing with 4.30±0.58 UP/g, in females), until next quantification (55 days) it took place an important progressive increase of peroxidase activity: 6.30±0.45 UP/g, for females, 7.44±0.73 UP/g, for male plants. These values represent an increase with 46.51% for

plants with pistillate flowers and with 53.72% for males, comparatively with the previous determination. In the next two weeks interval, it was observed a diminution of peroxidase activity, more clear in females (5.09±0.55 UP/g), in comparison with plants with staminate flowers (6.83±0.42 UP/g) (70 days). Until 85 days, the values maintained over those registered at 70 days, for both sexual phenotypes: 8.01±0.63 UP/g, in female phenotypes, 8.12±1.31 UP/g, in male plants. Although these values are close, we ascertain, in comparison with former determination, a more marked increase of peroxidase activity in plants with pistillate flowers. The last quantification (105 days) registered a decline of peroxidase average values: 5.18±0.55 UP/g, in female plants, respectively 5.94±0.84 UP/g, in male plants.

A general view on results evidenced a constant maintenance of average peroxidasic activity at a superior level for investigated males, during whole period of vegetation. The activity of this enzyme is correlated with the modifications that accompany the plant development.

In organogenesis, the role of peroxidase is often explained by the double function of this enzyme, involved both in the specific oxidizing of some substrata and in auxin catabolism (Legrand and Bouazza, 1991). By this second function, the peroxidase induce a modulation of morphogenesis, because of the influence on the endogenous hormonal balance.

Because of its intervention in regulation of IAA (indole-3-acetic acid) level, the peroxidase has an indirect role in sex-determining mechanism in hemp, more exactly in stamenogenesis and carpellogenesis. Hemp is one of the species in which a high level of IAA induces the female sex phenotypisation. In hemp, as well as in other monoecious or dioecious plants, the gibberellins, auxins, ethylene and cytokinins have an important contribution to sex expression.

These hormones generally intervene in the derepression of reglator genes which enables the synthesis of specific proteins that control the flower organogenesis. In other species (of the *Mercurialis* genus, for example) the situation is different: in males, auxin is in greater quantities than in females ones, fact in accordance with the peroxidasic profiles, emphasized for females, and attenuated in males.

Excepting the influence on male and female sexual organs, the peroxidase, by diminution or increase of auxin level, are notable effects on plant growth. At small auxinic levels, more or less significant inhibitions of plant growth appear. The idea of a strong peroxidase activity associated with an increased auxin catabolism is generally accepted. In female plants, during vegetation, smaller peroxidasic activities are registered, fact that maintains endogenous auxin at an increasing level and allows the phenotypisation of female sex. In individuals with staminate flowers, the greater peroxidasic activities reflects on diminution of endogenous auxinic phytohormone and in the intervention of some other factors that will induce the stamenogenesis. The results obtained by quantitative determinations of the level of peroxidase activities, in dynamics, in female and male individuals, expressed in greater values for staminate plants, must be analysed and correlated with the results obtained in our previous research (Truţă et al., 2002), that evidenced significant differences between the isoperoxidase pattern of the two sexual phenotypes. Thus, the female plants have four bands, and the males presented ten fractions, the genetic determinism of these isoforms being multigenic. For hemp, are still unexplained details about the genes coding these peroxidases.

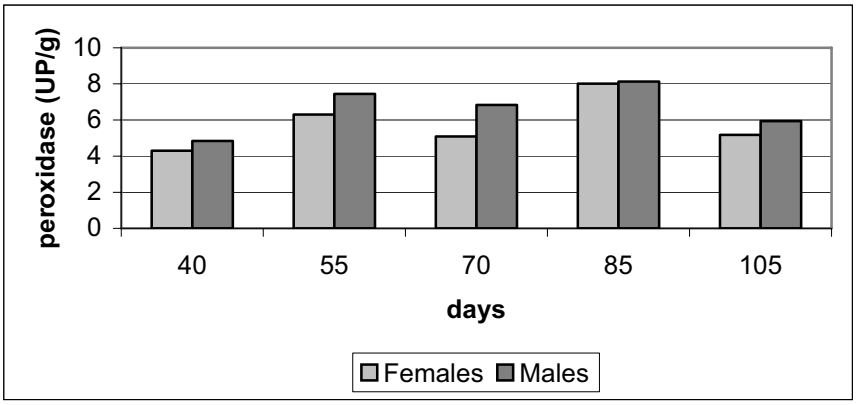


Fig. 1.Diagram of average values of peroxidase activities during ontogenesis of female and male plants of hemp

In investigated female plants we registered peaks of peroxidase activities before anthesis. The carpellogenesis was stimulated by IAA, that increases as result of diminution of peroxidase activity (at 70 days). The blossoming of male plants was early. This moment was coincident with the increase of catalitic action of peroxidase (7.44 UP/g). The other peak of peroxidase activity, at 85 days, could be discussed in relation with the fiber production and the intensification of lignification process. In a previous paper (Truță et al., 2002), significant differences we found, between the isoperoxidase patterns of staminate and pistillate hemp plants, both in the number and stain intensity of bands. The isoenzymatic spectrum was richer for male plants.

## CONCLUSIONS

During ontogeny of female and male hemp plants, the peroxidase activity was fluctuant, with periodic increases and decreases.

In female plants, before anthesis were registered peaks of peroxidase activities.

The blossoming of male plants was coincident with the increase of catalitic action of peroxidase in this group of sexual phenotypes.

During all vegetation cycle, the average values of peroxidase activities were greater in male plants than in female plants.

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