Acta Bot. Croat. 45, 83—89, 1986.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 577.158.52:582.982:591.3 = 20

PEROXIDASES IN PUMPKIN EMBRYOGENIC CALLUS LINES

MARIJANA KRSNIK-RASOL, SIBILA JELASKA and *DRAŠKO ŠERMAN**

(Department of Botany, Faculty of Science and
*Department of Biology, Medical Faculty, University of Zagreb)

Received December 17, 1985

Peroxidase isozymes and their total activity have ben studied in pumpkin embryogenic callus lines DE and NA_3 in order to state how peroxidases are influenced by different kinds of exogenous growth regulators.

Peroxidase bands Rm 0.46 and 0.54—0.56 were present in both lines regardless of the growth regulator. NAA and adenine sulphate induced the enzyme activity at the position Rm 0.35 which disappeared if the tissue was growing on 2,4-D.

The changes in isoperoxidases pattern preceded any morphological change in the culture. The total peroxidase activity increased with time in line NA_3, however, it remained low in line DE. Protein content changed inversely with peroxidase activity.

Introduction

Pumpkin (*Cucurbita pepo* L.) established callus cultures display feasible organ regeneration and lasting potential for somatic embryogenesis (Jelaska 1980, 1985). Owing these characteristics, it represents an interesting system for the study of development and differentiation on different levels.

Peroxidases are concerned with several important events in plants and may be a suitable marker enzyme in numerous physiological processes. They have often served as parameters of metabolic activity du-

This paper is dedicated to prof. Z. Devidé on the occasion of his 65th birthday.

ring growth alterations and as probes of cell differentiation (Van Huystee and Cairns 1980, Kevers et al. 1981 a, b, Kraus et al. 1981, Moncousin and Gaspar 1983, Griffing and Fowke 1985). Peroxidases mediate the growth and the differentiation of the cells, among other mechanisms, also through their involvement in auxin catabolism and endogeneous auxin level changes in plant cells (Cairns et al. 1980, Gaspar et al. 1982, Grambow and Langenbeck-Schwich 1983). It has been suggested that peroxidase and indole acetic acid (IAA) oxidase are the same molecules. However, the specific arrangement and composition of the active site for IAA oxidase has not been fully described as yet (Lobarzewski and Wolski 1985).

Peroxidase isozymes and their total activity were preliminarily studied in pumpkin suspension culture (Krsnik-Rasol et al. 1982). The purpose of this paper was to compare two pumpkin callus lines: DE (growing on 2,4-D) and NA_3 (on NAA and adenine sulphate), and to state how their isoperoxidases pattern and total enzime activity were influenced by different kinds of exogenous growth regulators.

Materials and Methods

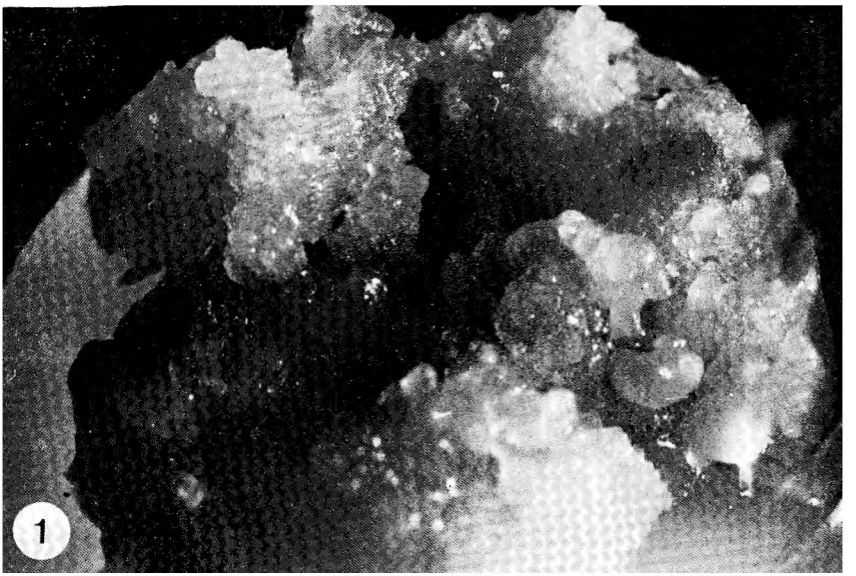
In the present experiment two callus lines, DE and NA_3, were used for analyses. The pumpkin callus lines were initiated and cultured on MS basal medium (Murashige and Skoog 1962) supplemented with 4.5 μM 2,4-D (line DE) or with 5.37 μM NAA 1 μM adenine sulphate (line NA_3) as described previously (Jelaska 1974).

Crude enzymatic extracts were prepared from 500 mg fresh callus tissue in 3 ml 0.26 M phosphoric acid/Tris buffer (pH 6.9) with the addition of insoluble polyvinyl pyrrolidone. The macerates were centrifuged at 30000 g for 30 minutes. The supernatant was used for enzymatic determination. The guayacol peroxidase activity was determined photometrically by measuring the increase in absorbance at 470 nm. The test solution was prepared according to Siegel and Galstone (1967). The soluble protein was assayed by the method of Bradford (1976). Peroxidase isoenzyme patterns were determined by using the polycrylamide gel system after Davis (1964) and Ornstein (1964). The gels were stained in the test solution after Siegel and Galstone (1967). For scanning of the gels a Chromoscan MK II, Joyce-Loebl, was used.

Abbreviations: NAA — 1-naphthaleneacetic acid
2,4-D — 2,4-dichlorophenoxyacetic acid
IAA — indole-3-acetic acid

Results and Discussion

The long term callus lines DE and NA_3 which grow whit the addition of different growth regulators, differed in their morphology as well as in their patterns of electrophoretic peroxidase bands. The line DE grew as nodular tissue with high embryogenic capacity, but the embryoids were restricted to the globular stage (Fig. 1). A characteristic of the line NA_3 was very high embryogenic potential with all the developmental stages of embryoids including also plantlets and organ structures (Fig. 2). The 8-day old subcultures of both lines coincided in two peroxidase bands (Rm 0.46 and 0.56), while two more bands (Rm 0.35 and 0.68) were present in the extracts of line NA_3 (Fig. 3).



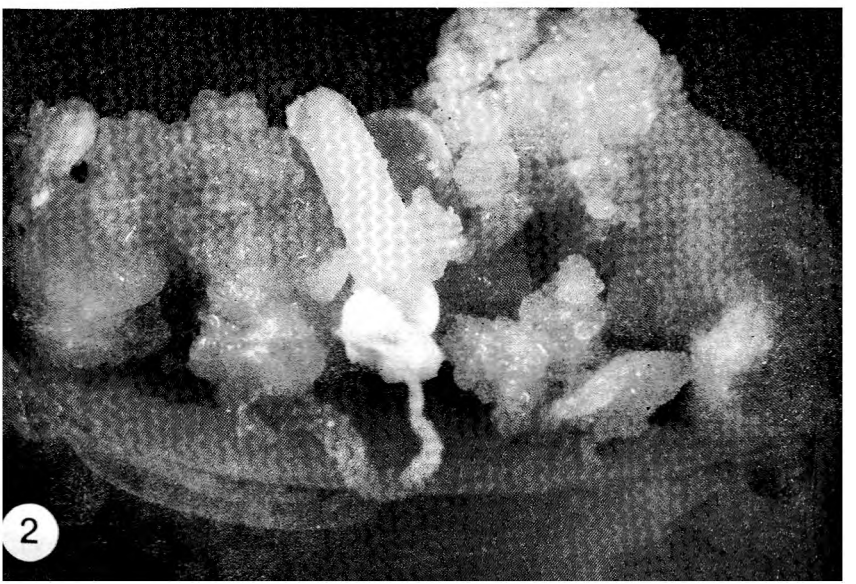


Fig. 1. Pumpkin callus culture — line DE growing on 2,4—D, morphological aspect.

Fig. 2. Pumpkin callus culture — line NA_3 growing on NAA and adenine sulphate, morphological aspect.

We transfered the pumpkin tissue of the callus line DE, which had been growing for years in the presence of 2,4-D, to the medium supplemented with NAA and adenine sulphate; on the other hand, the line NA_3 was transferred from the medium with NAA and adenine sulphate to one with 2,4-D. We stated that cultures of the same line, growing on different growth regulators, varied in peroxidase bands. The differences were detectable prior to evident morphological changes in the culture. The bands in the middle of the gels (Rm 0.46 and 0.54) were present regardless of the growth regulator added. The band Rm 0.35 appeared only in the case when the tissue was growing on the medium with NAA and adenine sulphate (Figs. 4 and 5). From these two figures it is evident that NAA and adenine sulphate, when present in medium, induce the appearance of the peroxidase Rm 0.35. As this peroxidase appears before it is possible to notice morphological changes in the culture, it may be supposed, that it is induced by auxin. Cytological, cytochemical and hystochemical analyses have not been done yet and therefore it is not possible to know whether this peroxidase is preceding differentiation, or whether it is the consequence of differentiation processes.

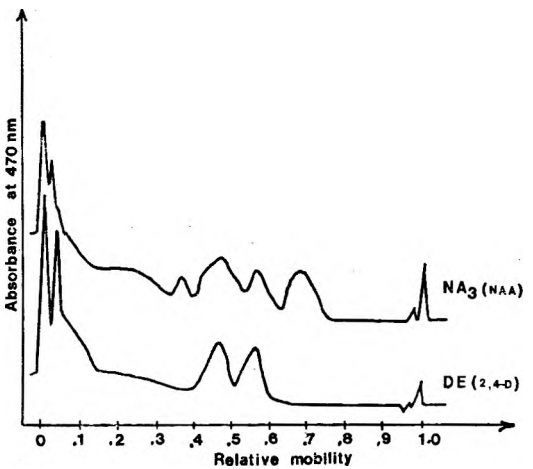


Fig. 3. Scans of electrophoretic gels of isoperoxidases in the 8-day old pumpkin callus lines DE and NA_3.

However, our experiments clearly show that Rm 0.35 appears in the presence of NAA and adenine sulphate, that it disappears on the medium with 2,4-D and that this disappearance is happening earlier than the 2,4-D-line (line DE) assumes the macroscopically perceivable morphological characteristics of the line NA_3.

The faster band Rm 0.68 was quantitatively better expressed in the presence of NAA and adenine sulphate. This indicates that the growth regulators may influence the pattern of isoperioxidases in the pumpkin callus tissue, although the pattern is also a reflection of the specific developmental stage in the culture (Krsnik-Rasol et al. 1982).

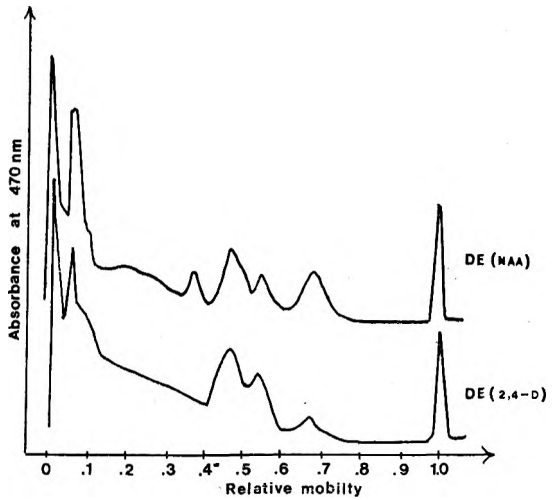


Fig. 4. Scans of electrophoretic gels of isoperoxidases in the 18-day old pumpkin callus line DE.
DE (NAA) — NAA and adenine sulphate in nutrient medium, DE (2,4—D) — 2,4—D in nutrient medium

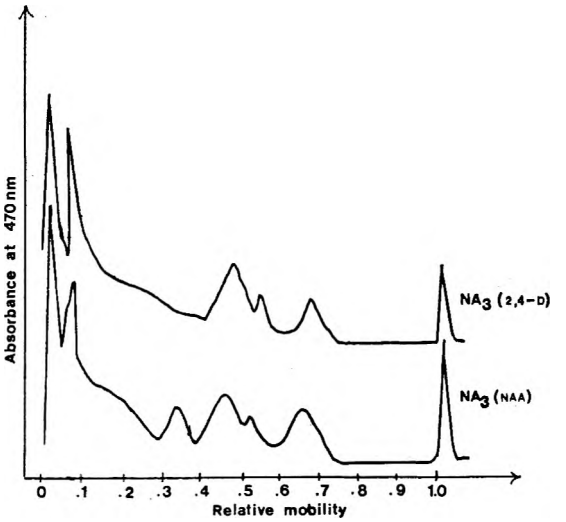


Fig. 5. Scans of electrophoretic gels of isoperoxidases of the 18 day old pumpkin callus line NA_3
NA_3 (2,4—D) — 2,4—D in nutrient medium
NA_3 (NAA) — NAA and adenine sulphate in nutrient medium.

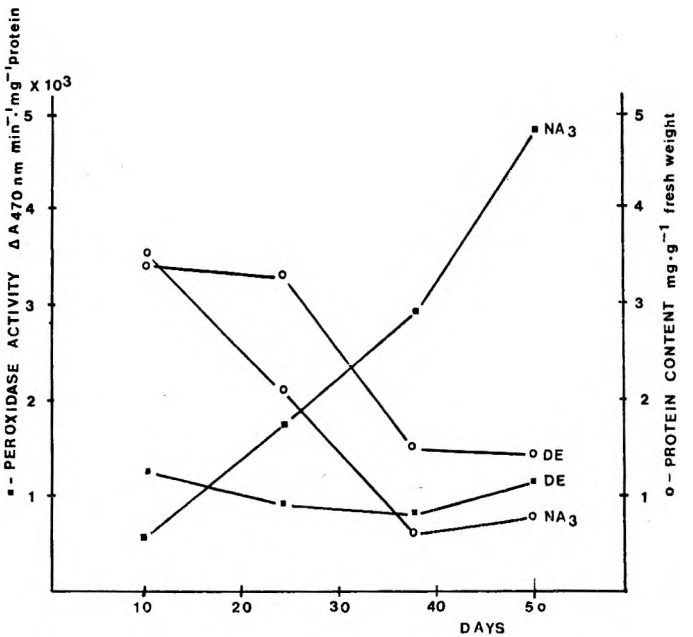


Fig. 6. Total peroxidase activity and protein content in callus line DE and NA_3.

Working with tobacco callus Mäder et al. (1975) stated that the isoperoxidases pattern is independent of the growth regulators responsible for the differentiation and that only the kind of differentiation itself determined the pattern.

Development of the peroxidase/IAA oxidase isoenzymes was found to be differentially associated with 2,4-D growth promotion or inhibition in many plant species (Gaspar et al. 1982). A hypothesis has been proposed that 2,4-D may affect plant growth by upsetting the auxin-peroxidase balance (Galston and Davis 1969). Peroxidase activity is inversely related to growth rate, perhaps as a consequence of IAA oxidase activity of the enzyme (Van Huystee and Cairns 1980). The enzyme activity increased gradually in line NA_3 with the aging of the culture. The protein content changed inversely to peroxidase activity. However, in line DE, on 2,4-D the activity remained low until the 40th day. The protein content was higher in line DE than in NA_3, which can be connected with juvenile growth characteristics of DE callus culture (Fig. 6).

Quantitative determinations of the isoenzymes present (which are in progres) should better explain the relations of Figs. 4 and 5 with Fig. 6. They should show which of the isoenzymes are taking part in the increase of isoenzyme activity and why there are differences between the lines NA_3 and DE.

The band Rm 0.68 has not been found in younger cultures of the line DE, while it is possible to detect it in older cultures, but it is

there of a weaker intensity than in the line NA_3. It is probably more the consequence of the differentiation level than of a direct effect of growth regulators.

Thus the band Rm 0.68 is not specific for the line NA_3 and is not induced exclusively by the presence of NAA and adenine sulphate in the medium, but appears also in older cultures of the line DE (grown on medium with 2,4-D) as a band of weaker intensity.

•

The research has been sponsored by the Science Reserach Council of SR Croatia (SIZ — IV, Grant IV-44-0119).

References

Bardford, M. M., 1976: A rapid and sensitive method for the quantities of protein utilizig the principle of protein-dye binding. Anal. Biochem. *72*, 248—254.

Cairns, E., R. B. Van Huystee, W. L. Cairns, 1980: Peanut and horse radish peroxidase isoenzymes. Physiol. Plant. *49*, 78—82.

Davis, B. J., 1964: Disc electrophoresis II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. *121 (2)*, 404—427.

Galston, A. W., P. J. Davies, 1969: Hormonal regulation in higher plants. Science *163*, 1288—1297.

Gaspar, T., C. Penel, T. Thorpe, 1982: Peroxidases. Université de Geneve Centre de Botanique, Geneve.

Griffing, L. R., L. C. Fowke, 1985: Cytochemical localization of peroxidase in soybean suspension culture cells and protoplasts: Intracellular vacuole differentiation and presence of peroxidase in coated vesicles and multivesicular bodies. Protoplasma *128*, 22—30.

Grambow, H. J., B. Langenbeck-Schwich, 1983: The relationship between oxidase activity, peroxidase activity, hydrogen peroxide, and phenolic compounds in the degradation of indole-3-acetic acid in vitro. Planta *157*, 131—137.

Jelaska, S., 1974: Embryogenesis and organogenesis in pumpkin explants. Physiol. Plant. *31*, 257—261.

Jelaska, S., 1980: Growth and embryoid formation in Cucurbita pepo callus culture. In: Application de la culture in vitro de l'amélioration des plantes potagères. Réunion EUCARPIA, Soc. Legumes, Versailles, 16—18. IV, 172—178.

Jelaska, S., 1985: Cucurbits. In: Y. P. S. Bajaj (Ed.): Biotechnology of Plant Improvement. Vol. 2 pp 371—386, Springer Verlag, Heidelberg.

Kevers, C., M. Coumans, W. De Greef, M. Hofnger, T. Gaspar 1981 a: Habituation in sugarbeet callus: Auxin content, auxin protectors, peroxidase pattern and inhibitors. Physiol. Plant. *51*, 281—286.

Kevers, C., M. Coumans, W. De Greef, M. Jacobs, T. Gaspar, 1981 b. Organogenesis in habituated sugarbeet callus: auxin content and protectors, peroxidase pattern and inhibitors. Z. Pflanzenphysiol. *101*, 79—87.

Kraus, J. E., W. Handro, S. M. C. Dietrich, 1981: Growth, peroxidase activity, and protein content in stem pith and callus tissues from haploid and diploid *Nicotiana tabacum* plants. Physiol. Plant. *51*, 157—162.

Krsnik-Rasol, M., S. Jelaska, D. Šerman, 1982: Isoperoxidases — early indicators of somatice embryoid differentiation in pumpkin tissue. Acta Bot. Croat. *41*, 33—39.

Lobarzewski, J., T. Wolski, 1985: The function of free carboxyl groups in the action of peroxidase and indole-3-acetic acid oxidase. Phytochemistry *24 (10)* 2211—2313.

Mäder, M., P. Münch, M. Bopp, 1975: Regulation und Bedeutung der Peroxidase-Musteränderungen in sprossdifferenzierenden Kalluskulturen von *Nicotiana tabacum* L. Planta (Berl) *123*, 257—265.

Moncousin Ch., T. Gaspar, 1983: Peroxidase as a marker for rooting improvement of *Cynara scolymus* L. cultured in vitro. Biochem. Physiol. Pflanzen *178*, 263—271.

Murashige, T., F. Skoog, 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. *15*, 473—497.

Ornstein, L., 1964: Disc electrophoresis. I Background and theory. Ann. N. Y. Acad Sci. *121*, 321—349.

Siegel, B. Z., W. Galston, 1967: The peroxidase of *Pisum sativum*. Plant Physiol. *42*, 221—226.

Van Huystee, R. B., W. L. Cairns, 1980: Appraisal of studies on induction of peroxidase and associated porphyrin metabolism. Bot. Rev. *46*, 429—446.

SAŽETAK

PEROKSIDAZE U EMBRIOGENIM KALUSNIM LINIJAMA BUNDEVE

Marijana Krsnik-Rasol, Sibila Jelaska, Draško Šerman

(Botanički zavod Prirodoslovno-matematičkog fakulteta i Zavod za biologiju Medicinskog fakulteta Sveučilišta u Zagrebu)

Embriogene kalusne linije bundeve (*Cucurbita pepo* L.) koje rastu uz dodatak 2,4-D (DE) odnosno NAA i adenin sulfata (NA_3) uspoređene su s obzirom na elektroforetsku sliku peroksidaza i njihovu ukupnu aktivnost.

Peroksidazne pruge Rm 0,46 i 0,54—0,56 utvrđene su kod obje linije bez obzira na vrstu regulatora rastenja u hranidbenoj podlozi. NAA i adenin sulfat inducirali su enzimsku aktivnost u položaju Rm 0,35 koja je registrirana prije vanjskih morfoloških promjena u kulturi (embrioidi u kasnim razvojnim stadijima i biljčice). Peroksidazna aktivnost u položaju Rm 0,68 bila je naročito izražena uz dodatak NAA i adenin sulfata, a izostala je u mlađim kulturama linije DE na podlozi s 2,4-D.

Aktivnost peroksidaza naglo je rasla tijekom starenja kultura linije NA_3, dok je kod linije DE ostala niska. Sadržaj topivih proteina mijenjao se obrnuto proporcionalno peroksidaznoj aktivnosti.

Dr. M. Krsnik-Rasol
Dr. S. Jelaska
Botanički zavod IV, PMF
Rooseveltov trg 6/III P. p. 933
YU-41001 Zagreb

Prof. dr. D. Serman
Zavod za biologiju
Medicinski fakultet
Šalata 3
YU-41000 Zagreb