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Phyton (Austria)	Vol. 23	Fasc. 2	263—269	30. 9. 1983

Peroxidase Activity in *Arabidopsis* Mutant A-154

By

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With 1 Figure

Received September 19, 1982

Key words: *Arabidopsis*, peroxidase, polyphenol oxidase, catalase

Summary

Acedo G. N. 1983. Peroxidase activity in *Arabidopsis* mutant A-154. — Phyton (Austria) 23 (2): 263—269, with 1 figure. — English with German summary.

Peroxidase and other related oxidases were assayed in *Arabidopsis thaliana* to determine if there is a relationship in the activities of these enzymes to the phenotype of mutant A-154. Peroxidase and polyphenol oxidase activities are about 3- and 6-fold higher respectively in the mutant than in the wild type. Catalase activity, however, is lower in the mutant. The implications of the increased activities of peroxidase and polyphenol oxidase in relation to the expressed phenotype of the mutant are discussed.

Zusammenfassung

Acedo G. N. 1983. Peroxidase-Aktivität in der *Arabidopsis*-Mutante A-154. — Phyton (Austria) 23 (2): 263—269, mit 1 Abbildung. — Englisch mit deutscher Zusamenfassung.

Peroxidasen und andere verwandte Enzyme wurden auf ihre Beziehungen zur Ausbildung des Phänotyps der Mutante A-154 von *Arabidopsis thaliana* geprüft. Die Aktivitäten von Peroxidase und Polyphenoloxidase waren in der Mutante 3- bzw. 6mal so groß wie im Wildtyp. Die Beziehungen der erhöhten Aktivitäten von Peroxidase und Phenoloxidase zur Ausprägung des Phänotyps der Mutante werden erörtert.

(Editor transl.)

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Introduction

Mutant A-154 was obtained by EMS treatment and the mutation behaves as a single Mendelian factor (REDEI, personal communication). Mutant plants are characterized by reticular chlorosis, altered leaf index, and a slower growth than the wild type.

High peroxidase activity had been implicated in the morphological changes of some mutants. In hooded barley, peroxidase activity was higher in most tissues especially in the shoot meristem (GUPTA & STEBBINS 1969). High peroxidase and other oxidases were associated with the lanceolate mutants of tomato (MATHAN and COLE 1964), dwarf tomatoes (EVANS & ALLBRIDGE 1965), and with dwarf peas and corn (MCCUNE & GALSTON 1959). MÜLLER (1969) found high peroxidase activity in the shorter internodes of irradiated peas.

The effect of elevated peroxidase activity on the development of these mutants are questions that has yet to be answered. Peroxidase activity is related to many biochemical functions including oxidation of pyridine nucleotides and other biochemical compounds, utilization of H_2O_2, biosythesis of lignins, degradation of anthocyanins, and hormone metabolism.

Increased activity of peroxidase and other related oxidases could have a relationship to the phenotypic expression of mutant A-154 of *Arabidopsis thaliana*. The activites of peroxidase, polyphenol oxidase (PPO), and catalase were assayed to determine if there is any difference between the activities of these enzymes in the mutant compared to the wild type.

Materials and Methods

Liquid Culture of the Plants: The composition of the mineral media used for liquid culture is the same as the solid media (REDEI 1965) except for the omission of agar. 10 ml of the prepared media were dispensed in Erlenmeyer flask, plugged with cotton, and autoclaved at 121° C, 15 pounds pressure for 10 minutes. Seeds of *Arabidopsis* contained in small cloth bags, were sterilized in 5 percent calcium hypochlorite solution for 8 minutes, rinsed in several changes of sterile distilled water and dispersed into the autoclaved media. The seeded flask were put on a shaker with fluorescent lights above (1000 footcandle). After 15 days, growth was determined by drying the plants overnight in an oven at 85° C.

Pot Cultures: The plants used for the enzyme assays described below were grown in soil in pots at the greenhouse, and were assayed at the 2- and 6-leaf stages.

Enzyme Assays: Plants thoroughly washed with distilled water and blotted with paper towels were homogenized in 0.007 mM phos-

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phate buffer, pH 7.0, containing 0.02 M DTT (dithioreitol). Sucrose (0.5M) was added to the extraction media to prepare extracts for electrophoresis. The homogenate was centrifuged at 20,000 g for 10 minutes at 4° C, and the extracts used for peroxidase and catalase assays and for electrophoresis. Protein content of the extracts was estimated according to the procedure of LOWRY *et al.* (1951).

Peroxidase was assayed according to BERGMEYER (1974). The reaction mixture consists of 0.05 ml of 20 mM aqueous guiacol solution; 2.5 ml phosphate buffer, pH 7.0; 0.05 ml of 12.3 mM H_2O_2; 0.01—0.2 ml enzyme extract, and water to make the total volume to 3 ml. The reaction was started with the addition of hydrogen peroxide and the increase in absorbance at 436 nm was recorded. Enzyme activity is expressed as units per mg protein per hour.

Gel electrophoresis was carried out by a modification of the method of SMITHIES (1962) using 7.5 percent gel at 4° C, 50 V potential for 10 hours. The gels were stained with a solution containing 100 mg o-dianisidine in 70 percent ethyl alcohol and 28 ml of 0.2 M acetate buffer (pH 5.0) and 0.2 ml of 30 percent H_2O_2 (BREWBAKER *et al.* 1968). The gels were later stained with naphtol blue-black and destained in 7.5 percent glacial acetic acid.

Polyphenol oxidase (PPO) were assayed according to the method of KOIVUNIEMI *et al.* (1980). The extraction medium for PPO was 20 mM glycylglycine buffer, pH 7.5, and the assay mixture consisted of 1.8 ml of 50 mM each of sodium phosphate-citrate buffer, pH 7.0; 1.0 ml of L-DOPA (3,4-dihydroxyphenylalanine, 0.04 g/10 ml buffer) and 0.2 ml enzyme extract. The increase in absorbance was monitored at 480 nm.

Catalase was assayed with a slight modification of the method of BERGMEYER (1974). The reaction mixture consisted of 1 ml 50 mM phosphate buffer, pH 7.0; 1.0 ml of 30 mM hydrogen peroxide; and 0.05 ml enzyme extract pipetted to the wall of the cuvette and covered with parafilm. The reaction, at 25° C, was started by rapidly tilting the cuvette and mixing the reagents. Enzyme activity was determined by measuring the decrease in absorbance at 240 nm for 60 seconds against a blank of phosphate buffer.

All experiments were repeated three times, each with 2 replications.

Results and Discussion

The mutant has a lower catalase activity than the wild type, but peroxidase and polyphenyl oxidase (PPO) are significantly higher than in the wild type (Table 1). The levels of peroxidase and PPO activities are about 3- and 6-fold higher, respectively, in the mutant than in the wild type. Peroxidase activity of the mutant at the seedling stage is about equal to the wild type, however the activity increases as the plants mature (Table 2).

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Table 1

The mean specific activity of 3 enzymes in *Arabidopsis* (expressed as enzyme units per mg protein per hour) with the standard error of the mean and the level of significance of the difference (P)

Enzymes	Wild Type	Mutant A-154	P
Peroxidase	3.25±0.20	8.43±0.30	0.001
Polyphenol oxidase	15.00±1.50	74.00±1.71	0.001
Catalase	33.00±3.20	21.00±2.20	0.05

Table 2

The activities of peroxidase and polyphenol oxidase (units per mg protein per hour) at the seeding stage and at maturity (growth = mg dry weight per plant)

Enzymes	Genotype	Seedling		Maturity	
		Growth	Specific Activity	Growth	Specific Activity
	+	0.42	0.24	3.43	3.04
Peroxidase	A-154	0.25	0.22	2.36	7.12
	+	0.42	0.48	3.43	12.20
Polyphenol oxidase	A-154	0.25	0.43	2.36	68.41

Peroxidase activity is related to a wide variety of biochemical functions. In Petunia, several structural and regulatory genes are involved in peroxidase action (VAN DEN BERG & WIJSMAN 1981). It appears that mutant A-154 has more intense peroxidase bands than the wild type. There is a higher peroxidase activity in the mutant than in the wild type plants in aggreement with the banding pattern and intensity observed.

Several biochemical functions, altered in the mutant, may be linked to the elevated peroxidase activity. The favourable effect of aeration on the growth of the mutant compared to the wild type (Figure 1) indicated differences in their oxygen metabolism.

The increased activity of peroxidase in the mutant is reflected also in the low activity of glutamate synthase activity which requires NADPH as a cofactor. The glutamate synthase of the mutant becomes comparable to the wild type only on addition of NADPH to the reaction mixture (to be published elsewhere). The wasteful reoxidation of the pyridine nucleotides especially NADPH may activate the pentose phosphate pathway which sets the pace for other reductive events such as fatty acid reduction (BEEVERS 1974). An aberration in fatty acid metabolism may result in membrane defect. Indeed, electron micrographs of the mesophyll cells of mutant A-154 show poorly developed

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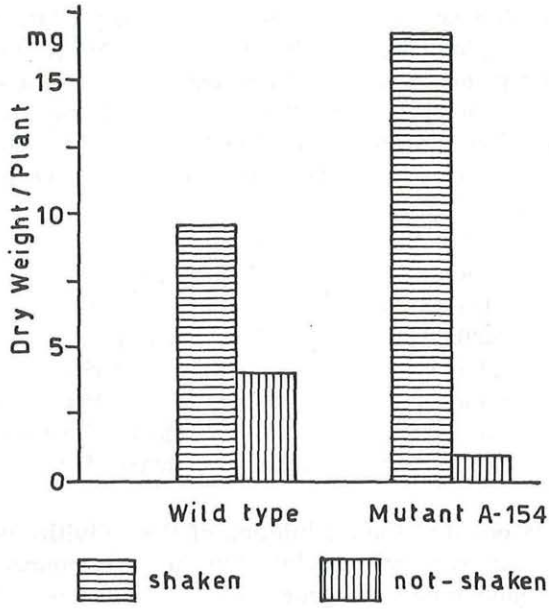

Fig. 1. The improved growth of Mutant A-154 in liquid, shaken culture

plastids due perhaps to the inablility of the fatty acids to aggregate to membranes. Peroxidase action also affects lignin synthesis and cell wall mutants in higher plants show altered activities of the oxidases (CATT 1981).

Like peroxidase, the mutant has a higher polyphenol oxidase (PPO) activity than the wild type (Table 1). High PPO activity inactivates other enzymes and proteins (MAYER & HAREL 1979). PPO activity is intimately related to that of peroxidase but mainly oxidizes monophenols, including the amino acids tyrosine, tryptophan, and phenylalanine. Supplementary phenylalanine, but not tyrosine and tryptophan, is actually beneficial to the growth of mutant A-154 (ACEDO 1976). An important aspect of PPO metabolism is that it may be involved with quinone in oxidation-reduction during electron transport (KOIVUNIEME *et al.* 1980). Phenols or quinones may react as photosynthetic cofactors, donors or acceptors in the light (MEYER & BIEHL 1982) and these reactions seem relevant to the metabolism of mutant A-154.

PPO is usually in the latent state, and is mainly located in the thylakoid membranes, however, the presence of free fatty acids, especially linolenic acid, activates the enzyme (GOLBECK & CAMMARATÀ 1980). In conjunction with peroxidase action, PPO activity may also affect the integrity of the membrane system. A defect in the membrane function of mutant A-154 is indicated by improved growths at low pH

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(ACEDO 1980). The activity of adenosine triphosphatase (ATP'ase) in the mutant is higher than in the wild type (ACEDO 1981). ATP'ase functions to keep the membranes in an energized state and to generate ATP. Since the role of these oxidases and ATP'ase are to regulate energy distribution and membrane functions, the high levels of these enzymes could contribute to a large extent to the abnormal phenotype of the mutant.

Catalase activity in the mutant is low in contrast to the high activities of peroxidase and PPO (Table 1). Catalase and peroxidase activity in bean leaves show an inverse relationship during development (BRABER 1980). The low catalase activity in the mutant may reduce the available oxygen in the respiratory chain. The improved growth of the mutant over the wild type in the liquid, shaken culture could result in more oxygen being available to the mutant. Catalase mainly acts in the cells as a detoxifying agent for excess hydrogen peroxide.

It is appearent that the modulation of the activities of peroxidase, PPO, and catalase is very important for the maintenance of the integritiy of the phenotype. The genes controlling the activities of these enzymes are obviously under the control of a common regulatory gene that is affected by the mutation in A-154. This system appears to be a good example of the complex pleiotropic effects of a single gene.

Acknowledgement

Thanks are due to Dr. G. P. REDEI for his careful, and critical reading of this paper, for helpful suggestions and comments, and for taking the photographs of the gels. This work was supported from funds of the Missouri Agricultural Experiment Station.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 1983

Band/Volume: 23_2

Autor(en)/Author(s): Acedo Gregoria N.

Artikel/Article: Peroxidase Activity in Arabidopsis Mutant A-154. 263-269