Identification of Polymeric Dye-Tolerant Oregano (*Origanum vulgare*) Clonal Lines by Quantifying Total Phenolics and Peroxidase Activity

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High phenolic-containing oregano clonal lines have been targeted as potential plants for developing pollutant-tolerant rhizospheres that could accelerate microbial degradation of pollutants such as polymeric dyes and related aromatic compounds. Seven tissue culture-generated oregano clonal lines were found to grow on hormone-free medium containing 0.01% of Poly R-478 or Poly S-119. The results showed that dye tolerance was associated with changes in total phenolics and peroxidase activity in these clonal lines. The total phenolics of most clonal lines decreased in response to polymeric dyes, whereas the peroxidase activity increased. Clonal line O-5 pretreated with *Pseudomonas* exhibited a higher level of peroxidase activity compared to untreated O-5 in response to the dyes. These observations suggested that the peroxidase activity was inducible. Stereomicroscopic observations revealed that the polymeric dyes were sequestered within the growing axis of the roots. This process may also enhance the polymerization of such dyes onto the cell wall.

Keywords: *Origanum vulgare; phenolics; peroxidase; polymeric dye tolerance; Poly R-478; Poly S-119*

INTRODUCTION

Changes in agricultural production practices to meet the demands of the growing global population along with activities associated with industrialization have resulted in the inevitable contamination of the environment with several types of pollutants. It is estimated that over the next 30 years, 750 billion U.S. dollars will have to be spent in the United States alone to remediate contaminated sites to current legal standards (Cunningham et al., 1996). Various physical, chemical, and biological processes are already being used to remediate contaminated soil and water (Cunningham et al., 1995; Caplan, 1993). Among them is phyto- or green plant-based remediation. It is defined as the use of plants to remove, contain, or render harmless environmental contaminants. This definition applies to all plant-influenced biological, chemical, and physical processes that aid in remediation of contaminated substrates (Cunningham et al., 1996). Phytoremediation, though not a new concept, is a relatively new technology. The use of plants in waste treatment originated over 300 years ago (Cunningham et al., 1993, 1995). Plants are regarded as solar-driven pumping and filtering systems that have measurable loading and degrading capacities, and their roots are described as exploratory extractors that can find, alter, and/or translocate elements and compounds from the liquid phase (Cunningham et al., 1995). Plants can also be cost-effective alternatives to physical remediation systems (Cunningham et al., 1995).

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Among different contaminants that pose environmental problems in both soil and water are various synthetic dyes including azo, anthraquinone, and triarylmethane dyes (Rafii et al., 1990). Many physical, chemical, and biological methods have been used in the remediation of synthetic dye-contaminated soil and water (Yeh, 1995; Churchley, 1994; Nigam et al., 1996). Several fungal isolates from soil environments were found to be able to decolorize polymeric dye Poly R-478 or Poly S-119 to various degrees (Wunch et al., 1997; Zheng et al., 1998). Several papers reported that the peroxidases from fungi and plants play a critical role in the degradation of azo dyes and aromatic pollutants (Ollikka et al., 1993; Spadaro and Renganathan, 1994; Klibanov and Morris, 1981). The cross-tolerance and ability to degrade polymeric dyes by fungi are also indicators of a common potential to degrade other aromatic pollutants such as polycyclic aromatic hydrocarbons (Field et al., 1992).

Peroxidases are a ubiquitous class of enzymes present in various tissues and cell components in plants, and they are involved in numerous processes, such as lignification, wound healing, antipathogen defense, and stiffening (Djiana et al., 1996). Kwak et al. (1996) indicated that peroxidase activity in sweet potato was enhanced by stress-related chemicals. A direct correlation was observed between the variation in peroxidase activity and the resistance to plant pathogen infection in *Hibiscus esculentus* and *Vigna sinensis* cultivars (Leina et al., 1996). The stimulation of peroxidase activity, as a part of the plant defense response to various stresses, is often accompanied by the increased accumulation of phenolics (Van-Loon, 1986). It was observed that the maximum of peroxidase activity coincided with an enhancement in the content of free phenolic acids in alfalfa (Cvikrova et al., 1996).

Phenolics or phenolic acids, intermediates in phenyl-

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propanoid metabolism, play many important roles in plant cells, tissues, and organs. They are precursors for the synthesis of lignin (Lewis and Yamamoto, 1990), and their deposition into the cell walls after pathogen infection is an important defense mechanism (Bolwell et al., 1985). Phenolics are also involved in the processes of differentiation and organogenesis (Mato et al., 1988). Their level is affected by a wide spectrum of external and internal factors such as phytohormones and growth-regulating substances (Zaprometoy, 1989).

In our laboratory, we have found that several oregano clonal lines were tolerant to polymeric dyes Poly R-478 and Poly S-119 present in their growing environment. Poly R-478 is a polyanthraquinone dye, and Poly S-119 is an azochromophoric dye. These two polymeric dyes are representative of the majority of synthetic dyes. The aim of this study was to obtain information about the possible correlation between the level of total phenolics/peroxidase activity and tolerance response to polymeric dyes in oregano clonal lines. This could be a very important step in understanding the biochemical mechanisms associated with phytoremediation of synthetic dyes and aromatic pollutants. Polymeric dye tolerance of plant clonal lines could be used to develop a good plant rhizosphere environment in soil, in constructed wetland, or in contaminant-containing soil for degradation of similar pollutants by microorganisms.

MATERIALS AND METHODS

Polymeric Dyes. Poly R-478, a polyanthraquinone dye (polyvinylamine sulfonated backbone with anthrapyridone chromophore, violet color), and Poly S-119, an azochromophoric dye (polyvininylamine backbone with azochromophore, orange color), were purchased from Sigma Chemical Co.

Shoot Culture of Oregano. Seven shoot-based oregano clonal lines were each generated from individual heterozygous seedlings following germination of a heterogeneous seed population (Shetty et al., 1995; Eguchi et al., 1996). They were subcultured and maintained on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). The MS medium contained 1 mg of 6-benzylaminopurine (BAP), 30 g of sucrose, 1 mL of Nitsch and Nitsch (1955) vitamin solution (diluted from a 1000× stock, Sigma), and 3 g of phytogel (Sigma) per liter with an adjusted pH of 5.8, before being autoclaved at 121 °C for 15 min. Each Petri plate contained seven apices, and each apex had four lateral leaves below it. These Petri dishes were incubated at 20 °C under continuous light of 40 μmol·m^{-2}·s^{-1}. The shoot apex explants generated several more apices through axillary bud proliferation during incubation. All oregano clonal lines were maintained by subculturing the shoots at 30-day intervals.

Transferring the shoot apices to half-MS hormone (BAP)-free medium resulted in their rooting. To test their tolerance ability to polymeric dyes, oregano clonal lines were transferred and cultured on half-MS hormone-free medium supplemented with 0.01% of Poly R-478 or Poly S-119. The half-MS hormone-free medium without polymeric dyes was used as control. The total phenolics and peroxidase activity were measured on days 15, 30, and 45 following subculture.

Preinoculated O-5 Clone. The shoots of new apices of oregano clonal line O-5 cultured on MS medium for 30 days were inoculated by gently treating the stem end with *Pseudomonas* sp. and transferred to MS medium (Shetty et al., 1995). After several subculture generations, the surviving preinoculated O-5 shoot clones were used for the dye tolerance test. The rationale for preinoculation was based on previous unpublished results from our laboratory, which showed that O-5 treated with *Pseudomonas* stimulated phenolics and potentially the lignification response. Those *Pseudomonas*-treated shoot clones that survived many subcultures had enhanced tolerance to polymeric dyes due to, perhaps, prior stimulation of phenolics and peroxidase-linked lignification.

Total Phenolics Assay. The total phenolics of oregano tissue were determined by an assay modified from that of Chandler and Dodds (1983) and described by Shetty et al. (1995). Approximately 50 mg of shoot tips was placed in 2.5 mL of 95% ethanol and held at 0 °C for 48 h. Each sample was then homogenized with a Tissue Tearor (Biospec Products, Inc., Racine, WI) and centrifuged at 13000*g* for 10 min. One milliliter of the supernatant was transferred to a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample was added 0.5 mL of 50% Folin−Ciocalteu reagent. After 5 min, 1 mL of 5% Na_2CO_3 was added and the reaction mixture was allowed to stand for 60 min. The absorbance was read at 725 nm using a Genesys spectrophotometer (Milton Roy, Inc., Rochester, NY). Standard curves were established for each experiment using various concentrations of gallic acid in 95% ethanol. Absorbance values were converted to milligrams of phenolics per gram of tissue fresh mass. Each value reported in this study was an average of three replicate assays of three separate samples.

Peroxidase Assay. The extraction and assay procedures for peroxidase were adapted from the methods described by Biles et al. (1997) and Laloue et al. (1997). One hundred milligrams of oregano tissue was ground at 4 °C for 2 min in the presence of 5 mL of extraction buffer using a pestle and mortar. The extraction buffer was 0.1 M potassium phosphate buffer (pH 7.5) containing 2 mM EDTA and 1% of poly(vinylpyrrolidone) to remove phenolics and polysaccharides. The extracts were centrifuged at 13000*g* for 15 min, and the supernatants were diluted to $\times 10^{-1}$ and used for enzyme assay. Peroxidase assays were conducted at 25 °C in a reaction mixture containing 50 mM potassium phosphate buffer (pH 6.5), 2 mM hydrogen peroxide, and 20 mM guiacol in a total volume of 2 mL. The reaction was initiated by the addition of 0.1 mL of enzyme extract. Spectrophotometric readings at 470 nm, which measure the oxidation product of guaiacol, were taken within 5 min after addition of the enzyme extract. One unit of peroxidase activity is defined as the micromoles of guaiacol oxidized per minute of reaction. The molar extinction coefficient for oxidized guaiacol of $\epsilon_{470} = 26.6$ mM^{-1} cm^{-1} was used to convert absorbance measurements to molar units (Laloue et al., 1997; Djiana et al., 1996). Enzyme activities were expressed as units per gram of fresh oregano tissue. Each value reported in this study was an average of three replicate assays of three separate extracts.

Stereomicroscopic Observation. The shoots and roots of oregano clonal lines grown on half-MS hormorne-free medium containing polymeric dye in Petri plates were observed under 25× magnification using a stereomicroscope (Olympus, Inc., Tokyo).

RESULTS AND DISCUSSION

The total phenolics and peroxidase activity of all selected oregano clonal lines grown on half-MS hormone-free medium supplemented with 0.01% of Poly R-478 or Poly S-119 for 15 days were measured and compared with the control (half-MS hormone-free medium without polymeric dyes). As shown in Figure 1, the average phenolic content of all oregano clonal lines except preinoculated O-5 decreased on polymeric dye-containing media. Statistically, the decrease in phenolic content of O-1 and O-24 in response to Poly R-478 and of O-24 and OM-8 in response to Poly S-119 were significant. In contrast, the average phenolic content of O-5 preinoculated with *Pseudomonas* increased in response to both Poly R-478 and Poly S-119, and the former increase was significant (Figure 1). Recently, inoculation of oregano shoot explants with a *Pseudomonas* sp. was reported to prevent hyperhydricity (Shetty et al., 1995, 1996). The results suggest that phenolic synthesis

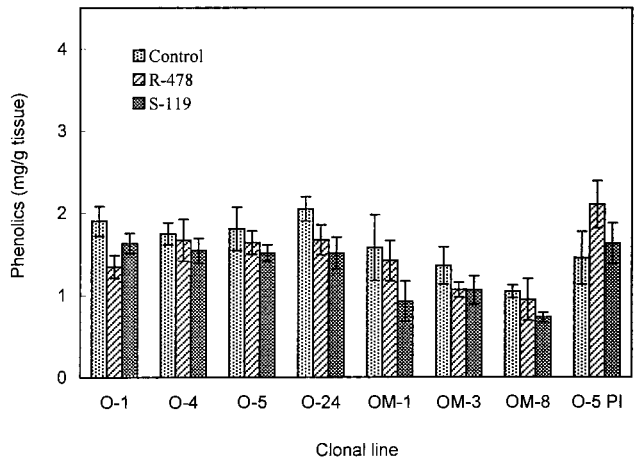

Figure 1. Phenolic content of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 15.

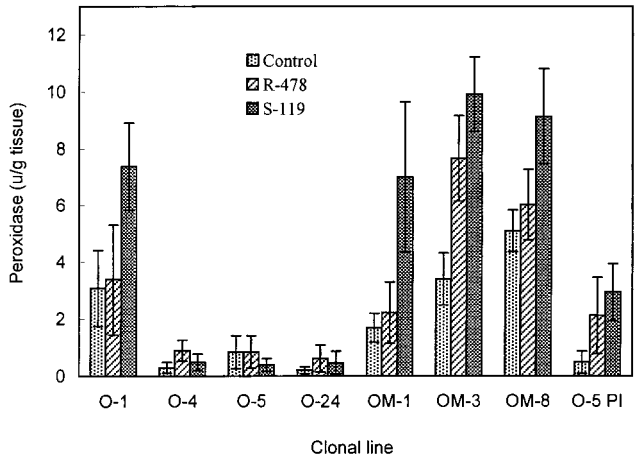

Figure 2. Peroxidase activity of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 15.

in the *Pseudomonas*-treated O-5 clonal line was further stimulated in response to polymeric dyes on day 15.

Peroxidase activity was a more appropriate measurement reflecting the tolerance response of oregano clonal lines to polymeric dyes. The changes in peroxidase activity of most clonal lines were much more prominent compared to the changes in total phenolics in response to polymeric dyes (Figure 2). Peroxidase activity in O-1, OM-1, OM-3, OM-8, and preinoculated O-5 clones significantly increased in response to Poly S-119 compared to that of control, whereas only OM-3 showed significant increase in peroxidase activity in response to Poly R-478. Peroxidase activity in O-4, O-5, and O-24 clones remained at a relatively low level and did not show an elevated response to polymeric dyes (Figure 2).

Peroxidases are known to be involved in numerous processes, such as lignification, wound healing, antipathogen defense, and stiffening (Djiana et al., 1996). There is evidence which shows that the peroxidase activities in plants were enhanced by stress-related chemicals and pathogens (Kwak et al., 1996; Leina et al., 1996). On the other hand, the stimulation of peroxidase activity, as a part of the plant defense response to various stresses, is often accompanied by an increased accumulation of phenolics (Van-Loon, 1986; Cvikrova et al., 1996). This is similar to our observations of oregano clonal line O-5 preinoculated with *Pseudomonas*. For many other clonal lines, however, the peroxidase activity increased substantially in response to polymeric dyes while the total phenolics were decreased. It is known that peroxidase is an enzyme responsible for the cross-linking of phenolic moieties during the biosynthesis of lignins in the plant cell wall (Morales and Barcelo, 1997). The polymeric dyes strongly stimulated peroxidase activity in some oregano clonal lines. As a result, the rate of polymerization of free phenolics to lignins and their precursors by peroxidase in the oregano tissues likely increased. This is an important defense reaction by plants to protect themselves from unfavorable environments.

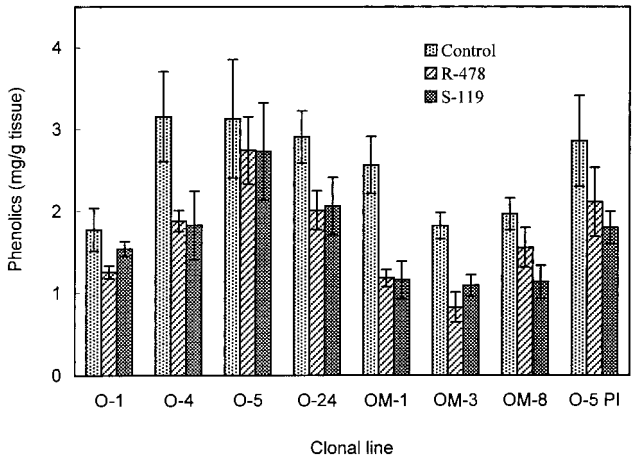

Figure 3. Phenolic content of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 30.

When compared to the results on day 15, the total phenolics of all oregano clonal lines, except O-1, significantly increased on day 30 for the untreated controls, but the total phenolics of many of those treated with polymeric dyes were significantly lower than their controls. Among all oregano clonal lines, O-4, O-24, OM-1, and OM-3 exhibited significant decrease in total phenolics in response to both Poly R-478 and Poly S-119 (Figure 3). Phenolics are intermediates in phenylpropanoid metabolism, and they are precursors for the synthesis of lignin (Lewis and Yamamoto, 1990). The deposition of free phenolics in plant tissues into the cell walls after stress-related infection or stimulation is an important defense mechanism (Bolwell et al., 1985). Their level is affected by many external and internal factors (Zaprometoy, 1989). On this basis, our observations suggested that polymeric dyes may have stimulated the synthesis of lignins by converting free phenolics into polymerized compounds through the action of peroxidases in oregano clonal lines. Concurrently, it is possible that polymeric dyes are also linked to the cell wall by peroxidases (Figure 4a).

The changes in peroxidase activity of most oregano clonal lines in response to polymeric dyes on day 30 were similar to those on day 15 (Figure 5). The results from both day 15 and day 30 indicated that the tolerance responses of nearly all oregano clonal lines to Poly S-119 were stronger than those to Poly R-478 in terms of the increases in guaiacol peroxidase activity (Figures 2 and 5). O-4, O-5, and O-24 lines, which had very little or no response to polymeric dyes in terms of the changes in their peroxidase activity on day 15, although remaining "insensitive" to polymeric dyes, showed a marginal increase in peroxidase activity on day 30. The differences among individual clones in the changes in total phenolics and peroxidase activity in response to poly-

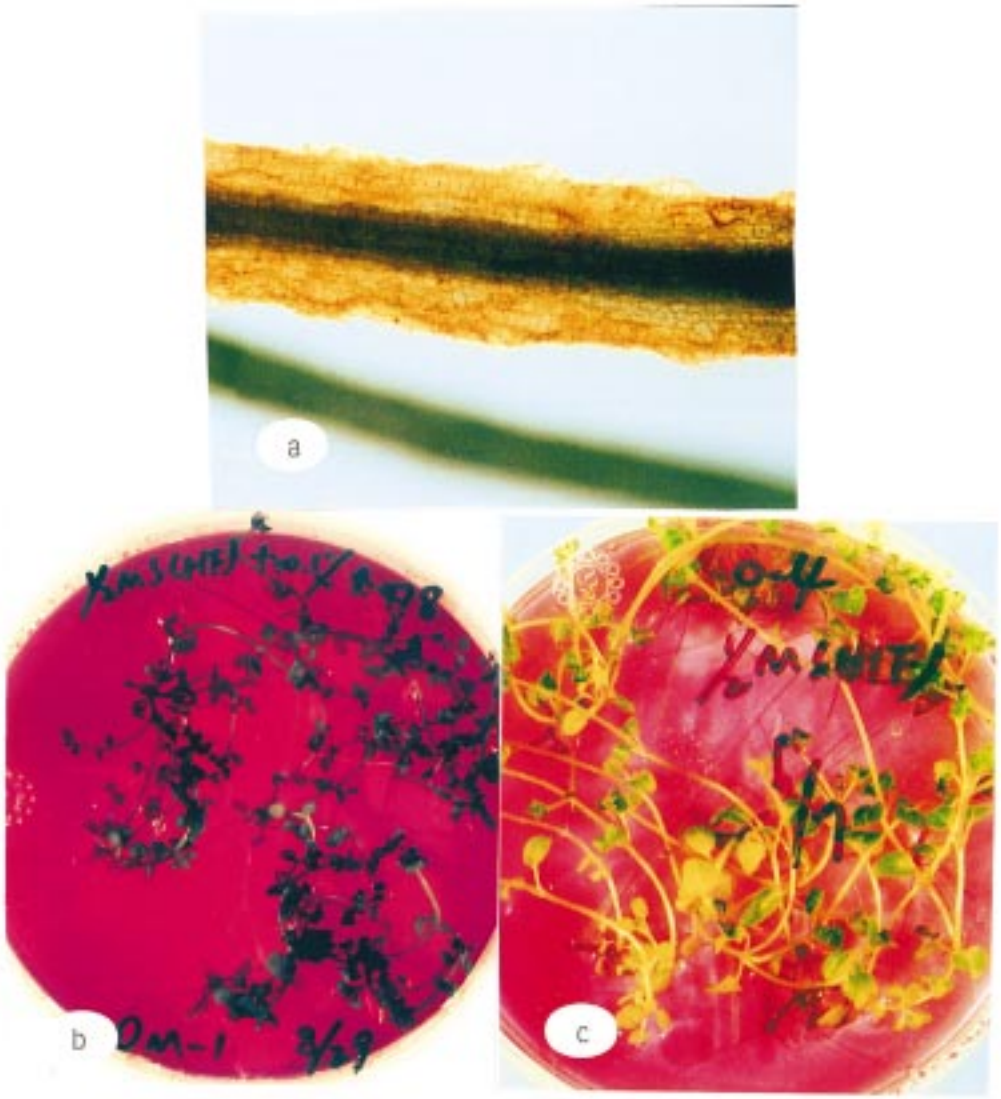

Figure 4. Observations of polymeric dye tolerance of oregano clonal lines: (a) stereomicroscopic view of Poly S-119 uptake by roots of oregano clonal line OM-8; (b) high peroxidase, OM-1 clonal line grown on half-MS hormone-free medium containing 0.1% of Poly R-478; (c) low peroxidase, O-4 clonal line grown on half-MS hormone-free medium containing 0.01% of Poly R-478.

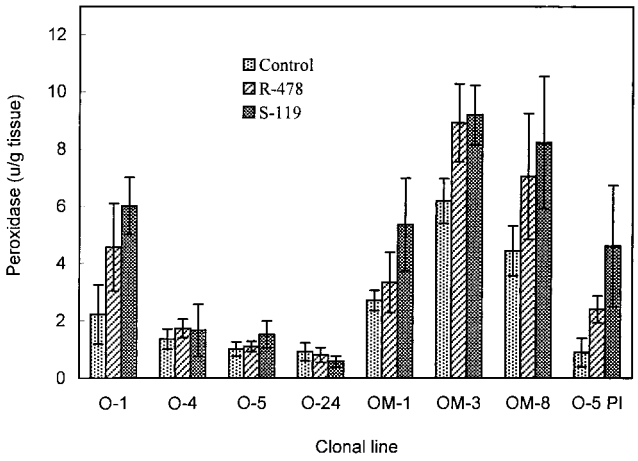

Figure 5. Peroxidase activity of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 30.

meric dyes could be due to the complexity of the defense systems in oregano tissues, and there may be other mechanisms regulating the phenolic synthesis, lignification, and dye polymerization processes. However, for most oregano clones, there is an inverse correlation between peroxidase activity and total phenolics. These observations are consistent with the idea that the increased peroxidase was used to convert more free phenolics to form lignin and other dye-polymerized products in oregano tissues.

After 45 days of growth on half-MS hormone-free medium, the total phenolics of all oregano clonal lines remained at a relatively higher level. The differences in phenolic content between the controls and polymeric dye-treated samples of clonal lines O-1, O-4, O-5, and O-24 decreased on day 45 compared to that on day 30, whereas the differences of OM-1, OM-3, OM-8, and preinoculated O-5 became much more prominent on day 45 (Figures 3 and 6). This is not surprising if the changes in peroxidase activity in those clonal lines are considered (Figure 7). On day 45, the differences in peroxidase activity of OM-1, OM-3, OM-8, and preinoculated O-5 clones remained high, which may have allowed the conversion of free phenolic compounds into lignins with the help of enhanced peroxidase activity in these oregano clonal lines. This may have also contributed to the polymerization of the dyes onto the cell wall.

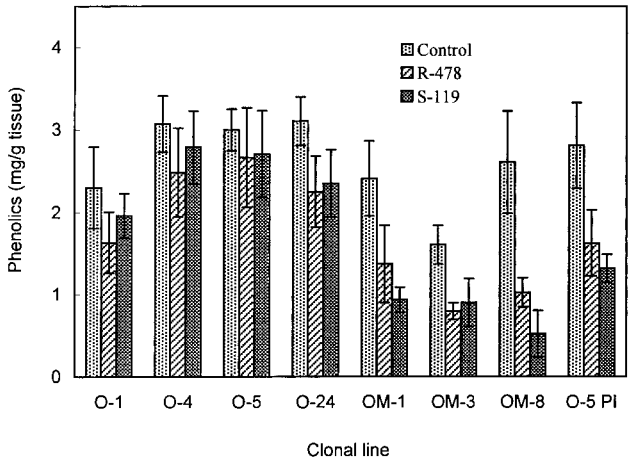

Figure 6. Phenolic content of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 45.

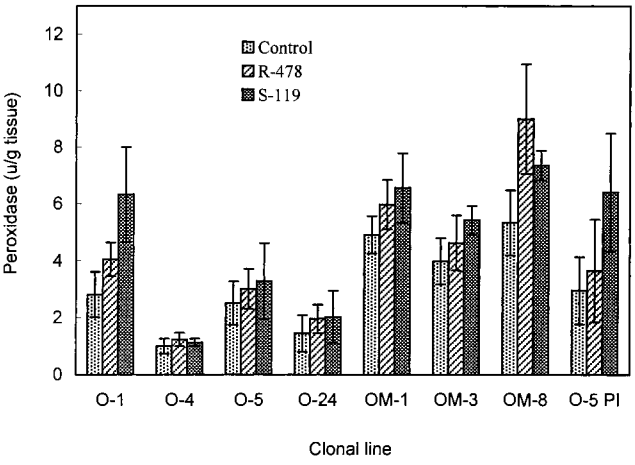

Figure 7. Peroxidase activity of oregano clonal lines in response to polymeric dyes Poly R-478 and Poly S-119 on day 45.

Although the peroxidase activity in oregano clonal lines O-4, O-5, and O-24 on day 45 increased to varying degrees compared to those on day 15 or on day 30, their overall levels were still low, and there were no significant changes in peroxidase activity in these three clonal lines at any one of the three time points. The low peroxidase clonal line, O-4, appeared bleached in the dye-containing medium (Figure 4c), whereas the high peroxidase-containing line, OM-1, had intense pigmentation (Figure 4b).

Oxidation of phenolic compounds, the naturally occurring substrates in the plant cell, is one of the functions of peroxidases. The fact that the changes in total phenolics in oregano clonal lines were in the opposite direction from the changes in peroxidase activity was also supported by evidence of Mato et al. (1988), who found a similar correlation between changes in phenolics and accompanying changes in peroxidase activity during root formation in grapevine (*Vitis*) species.

With regard to azo dye degradation, Chivukula et al. (1995) reported that sulfonated azo dyes were oxidized by a lignin peroxidase from a white-rot basidiomycete fungus. The enhanced peroxidase activity in oregano clonal lines in response to polymeric dyes was one of the defense responses in oregano tissues, and the activated peroxidase was very critical for oregano in dealing with the "aromatic pollutants", polymeric dyes in the medium. Consequently, the polymeric dye tolerance mechanism was activated in the plant tissues. Therefore, understanding the polymeric dye tolerance responses in plants is a very important step in the development of phytoremediation systems for further development of effective rhizospheres for microbial degradation of similar and related aromatic pollutants.

An observation of the oregano clonal line OM-1 grown on half-MS hormone-free medium supplemented with 0.1% of Poly R-478 is shown in Figure 4b. The root growth in dye-containing medium was normal, and the dye was concentrated and sequestered along the growing root axis (Figure 4a). Although oregano clonal lines do not decolorize the polymeric dyes, their high tolerance to polymeric dyes can be potentially adopted for rhizosphere-based bioremediation using an appropriate plant clonal line−microbial system. One major advantage of the new phytoremediation system would be its potential to perform in a highly contaminated environment by providing a favorable rhizosphere zone for microbial degradation of dyes along with enhanced peroxidase-linked tolerance and detoxification pathways in plants. The conceptual insights provided by this study could be used to develop significant new phytoremediation systems based on manipulated peroxidase pathways in elite plant clonal systems tolerant to highly polluted environments.

LITERATURE CITED

Biles, C. L.; Kuehn, G. D.; Wall, M. M.; Bruton, B. D.; Wann, E. V. Characterization of chile pepper fruit peroxidases during ripening. *Plant Physiol. Biochem*. **1997**, *35*, 273−280.

Bolwell, G. P.; Robbins, M. P.; Dixon, R. A. Metabolic changes in elicitor-treated bean cells: enzymic responses associated with rapid changes in cell wall components. *Eur. J. Biochem*. **1985**, *148*, 571−578.

Caplan, J. A. The worldwide bioremediation industry: prospects for profit. *Trends Biotechnol*. **1993**, *11*, 320−323.

Chandler, S. F.; Dodds, J. H. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell Rep*. **1983**, *2*, 105.

Chivukula, M.; Spadaro, J. T.; Renganathan, V. Lignin peroxidase-catalyzed oxidation of sulfonated azo dyes generates novel sulfophenyl hydroperoxides. *Biochemistry* **1995**, *34*, 7765−7772.

Churchley, J. H. Removal of dyewaste color from sewage effluent-the use of a full scale ozone plant. *Wat. Sci. Technol*. **1994**, *30*, 275−284.

Cunningham, S. D.; Berti, W. R. Remediation of contaminated soils with green plants: an overview. *In Vitro Cell. Dev. Biol*. **1993**, *29*, 207−212.

Cunningham, S. D.; Berti, W. R.; Huang, J. W. Phytoremediation of contaminated soils. *Trends Biotechnol*. **1995**, *13*, 393−397.

Cunningham, S. D.; Anderson, T. A.; Schwab, P. A.; Hsu, F. C. Phytoremediation of soils contaminated with organic pollutants. *Adv. Agron*. **1996**, *56*, 55−114.

Cvikrova, M.; Hrubcova, M.; Eder, J.; Binarova, P. Changes in the levels of endogenous phenolics, aromatic monoamines, phenylalanine ammonia-lyase, peroxidase and auxin oxidase activities during initiation of alfalfa embryogenic and non-embryogenic calli. *Plant Physiol. Biochem*. **1996**, *34*, 853−861.

Djiana, R.; Berthe, T.; Burel, C.; Schaumann, A.; Bruyant, P.; Balange, A. P. Partial purification and characterization of basic peroxidases from flax cell-culture medium. *Plant Physiol. Biochem*. **1996**, *34*, 771−778.

Eguchi, Y.; Curtis, O. F.; Shetty, K. Interaction of hyperhydricity-preventing *Psudomonas* sp. with oregano (*Origanum*

vulgare) and selection of high phenolics and rosmarinic acid-producing clonal lines. *Food Biotechnol.* **1996**, *10*, 191−202.

Field, J. A.; DeJong, E.; Feijoo-Costa, G.; DeBont, J. A. M. Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. *Appl. Environ. Microbiol*. **1992**, *58*, 2219−2226.

Klibanov, A. M.; Morris, E. D. Horseradish peroxidase for the removal of carcinogenic aromatic amines from water. *Enzyme Microb. Technol*. **1981**, *3*, 119−122.

Kwak, S. S.; Kim, S. K.; Park, I. H.; Liu, J. R. Enhancement of peroxidase activity by stress-related chemicals in sweet potato. *Phytochemistry* **1996**, *43*, 565−568.

Laloue, H.; Weber-Lotfi, F.; Lucau-Danila, A.; Guillemaut, P. Identification of ascorbate and guaiacol peroxidases in needle chloroplasts of spruce trees. *Plant Physiol. Biochem*. **1997**, *35*, 341−346.

Leina, M. J.; Tan, T. K.; Wong, S. M. Resistance of *Hibiscus esculentus* L. and *Vigna sinensis* (L.) Endl. to *Pseudocercospora* and plant peroxidase activity in relation to infection. *Ann. Appl. Biol*. **1996**, *129*, 197−206.

Lewis, N. G.; Yamamoto, E. Lignins: occurrence, biosynthesis and biodegradation. *Annu. Rev. Plant Physiol*. **1990**, *41*, 455−496.

Mato, M. C.; Rua, M. L.; Ferro, E. Changes in levels of peroxidases and phenolics during root formation in *Vitis* cultured *in vitro*. *Physiol. Plant*. **1988**, *72*, 84−88.

Morales, M.; Barcelo, A. R. A basic peroxidase isoenzyme from vacuoles and cell walls of *Vitis vinifera*. *Phytochemistry* **1997**, *45*, 229−232.

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

Nigam, P.; Banat, I. M.; Singh, D.; Marchant, R. Microbial process for the decolorization of textile effluent containing azo dye, diazo, and reactive dyes. *Proc. Biochem*. **1996**, *31*, 435−442.

Nitsch, J. P.; Nitsch, C. Action synergique des auxines et du jus de tomate sur la croissance des tissus vegetaux cultives *in vitro*. *Soc. Bot. Fr. Bull*. **1955**, *102*, 519−527.

Ollikka, P.; Alhonmaki, K.; Leppanen, V. M.; Glumoff, T.; Raijola, T.; Suominen, I. Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol*. **1993**, *59*, 4010−4016.

Rafii, F.; Freankalin, W.; Cerniglia, C. E. Azo-reductase activity of anaerobic bacteria isolated from human intestinal microflora. *Appl. Environ. Microbiol*. **1990**, *56*, 2146−2151.

Shetty, K.; Curtis, O. F.; Levin, R. E.; Witkowsky, R.; Ang, W. Prevention of vitrification associated with *in vitro* shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp. *J. Plant Physiol*. **1995**, *147*, 447−451.

Shetty, K.; Curtis, O. F.; Levin, R. E. Specific interaction of mucoid strains of *Pseudomonas* spp. with Oregano (*Origanum vulgare*) clones and the relationship to prevention of hyperhydricity in tissue culture. *J. Plant Physiol*. **1996**, *149*, 605−611.

Spadaro, J. T.; Renganathan, V. Peroxidase-catalyzed oxidation of azo dyes: mechanism of Disperse Yellow 3 degradation. *Arch. Biochem. Biophys*. **1994**, *312*, 301−307.

Van-Loon, L. C. The significance of changes on peroxidase in diseased plants. In *Molecular and Physiological Aspects of Plant Peroxidases*; Greppin, H., Penel, C., Gaspar, T., Eds.; Universite de Geneve: Geneve, 1992; pp 405−418.

Wunch, K. G.; Feibelman, T.; Bennett, J. W. Screening for fungi capable of removing benzo[*a*]pyrene in culture. *Appl. Microbiol. Biotechnol*. **1997**, *47*, 620−624.

Yeh, R. Y. L. Color difference measurement and color removal from dye wastewater using different adsorbents. *J. Chem. Technol. Biotechnol*. **1995**, *63*, 55−59.

Zaprometoy, M. N. The formation of phenolic compounds in plant cell and tissue cultures and the possibility of its regulation. *Adv. Cell Cult*. **1989**, *7*, 240−245.

Zheng, Z.; Levin, R. E.; Pinkham, J. L.; Shetty, K. Decolorization of polymeric dyes by a novel *Penicillium* isolate. *Proc. Biochem*. **1998**, in press.

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