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Extracellular Peroxidases as Indicators of Growth in Plant Cell Suspension Cultures

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ABSTRACT

Extracellular peroxidases were produced in plant cell suspension cultures in proportion to cell dry weight during exponential growth phase. This correlation was established for cell suspension cultures of *Artemesia annua, Coleus blumei, Pisum sativum* and *Salvia officinalis*. The correlation was maintained when growth kinetics were altered by variations in initial sucrose concentrations in cell cultures of *Artemesia annua*. This study indicated that extracellular perioxidases were readily measured indicators of growth in plant cell cultures.

Index Entries: Growth; peroxidase; dry weight; plant cells.

INTRODUCTION

Plants are important sources of chemicals used in medicine, food flavors, aromas and agrochemicals (*1–3*). Most of these chemicals are secondary metabolites extracted directly from plant tissues (*4*). However, it has been considered worthwhile to develop large-scale fermentation-type processes to produce secondary metabolites by plant cell culture (*5*).

Growth is an important parameter to be determined in developing processes for secondary metabolite synthesis in plant cell cultures. Growth is generally indicated in terms of fresh weight (*6*), dry weight (*7*), packed cell volume (*8*), peroxidase (*9*), or conductivity (*10*). Peroxidase has been determined to be correlated with dry weight in a few plant cell suspension cultures (*9*). With this correlation, a simple and rapid peroxidase

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assay can be used to monitor growth in plant cell suspension cultures. We have determined in this study that extracellular peroxidase activity can be used as an indicator of growth in cell suspension cultures of *Artemesia annua, Coleus blumei, Pisum sativum,* and *Salvia officinalis.*

MATERIALS AND METHODS

Cell Cultures and Conditions

Artemesia annua cells were obtained from Jim Linden of Colorado State University, Fort Collins. Seeds of *Coleus blumei* and *Salvia officinalis* were obtained from Ed Hume Co., Kent, WA. The seeds of *Pisum sativum* were obtained from Pure Line Seed Co., Moscow, ID. Callus cells of *Salvia officinalis* and *Pisum sativum* were developed from cotyledons and callus cells of *Coleus blumei* from stem tissue following standard procedures (*11*). The above callus cells growing in clumps were gently broken using a Waring blender under sterile conditions following the method of Mavituna et al., (*12*). The cell lines were maintained in shake flasks on an orbital shaker (125 rev/min) at 26°C in darkness. Subculturing was done at 7–8 d intervals using 20% (v/v) inoculum.

The basic medium used for the growth of plant cells (B5) was developed by Gamborg et al. (*13*). The medium also contained growth regulators 2,4-D and kinetin at concentrations of 1 mg/mL and 0.1 mg/mL, respectively. Two percent sucrose was used, except when effects of sucrose concentration on dry weight and peroxidase were determined for *A. annua.* The pH of the medium was 5.5. A total volume of 100 mL of culture, including inoculum, was maintained in 250-mL Erlenmeyer flasks. All sampling was done in triplicate with 10-mL samples. Every replicate treatment used at least five flasks to provide sufficient 10-mL samples.

Cell Dry Weight Determination

Ten mL of actively growing culture was removed every 2 d over the growth period. Cells were removed by filtration onto preweighed Whatman No. 1 filter paper, oven dried for 24–36 h at 70°C, and then reweighed to determine cell dry weight. Cell dry weight values were corrected to g/L of medium. The filtrate was retained and used for determination of peroxidase activity.

Peroxidase Activity

Peroxidase activity was determined and correlated to cell dry weight. 4-Amino antipyrine was used as the hydrogen donor in the peroxidase assay (*14*). The procedure involved premixing 1.4 mL of phenol/antipyrine solution with 1.5 mL of 0.0017*M* hydrogen peroxide, and incubating for 1–2 min to achieve equilibrium and establish the background reaction

Table 1
Peroxidase (PE) vs Cell Dry Weight (X),
using Model PE = (Slope) (X)
(only for exponential growth phase)

Cell line	R^2	Slope
Artemesia annua	0.992	0.0162
Coleus blumei	0.997	0.0202
Pisum sativum	0.986	0.0219
Salvia officinalis	0.983	0.0237
A. annua 1.5% sucrose	0.980	0.0164
A. annua 3.0% sucrose	0.978	0.0159
A. annua 4.5% sucrose	0.969	0.0141
A. annua 6.0% sucrose	0.983	0.0187

rate. Then 0.1 mL of extracellular filtrate (enzyme) was added, and the increase in absorbance at 510 nm was followed for 4–5 min. Activity was expressed as ΔA_{510}/min.

Results and Discussion

Growth measured as cell dry weight followed a typical growth pattern of little or no lag phase, approximately 14 d of exponential growth, followed by a stationary phase (Fig. 1; only exponential growth phase shown).). Extracellular peroxidases had a strong correlation to cell dry weight in all cell lines (Fig. 2) in the exponential growth phase, and in all cases the correlation coefficient (R^2) for peroxidase vs cell dry weight was >0.95 (Table 1), strongly indicating that extracellular peroxidase activity can reasonably be used as an indicator of growth in plant cell suspension cultures. Also, the growth pattern of *Artemesia annua* under variations in sucrose concentration showed typical growth characteristics and correlation to peroxidase activity (Figs. 3–5). With the strong correlation between peroxidase and dry weight, the point of maximum peroxidase activity could be used as an indicator to time the addition of microbial elicitors to enhance secondary metabolite synthesis (*15*).

Unique peroxidases are excreted into the growth medium in plant cell cultures (*16*). The activities of extracellular peroxidases have been shown to follow growth kinetics of plant cell suspensions (*9,16,17*). Our current study further confirms the parallel between extracellular peroxidase activity and growth. All of these studies indicate that peroxidases have an active role during the growth cycle and are found in proportion to plant biomass. The reason for accumulation of extracellular peroxidases in relation to biomass increases could be the active role peroxidases have in coupling cell wall components (*18*). The cell wall components cellulose and lignin develop as the cells grow, probably in proportion to cell number.

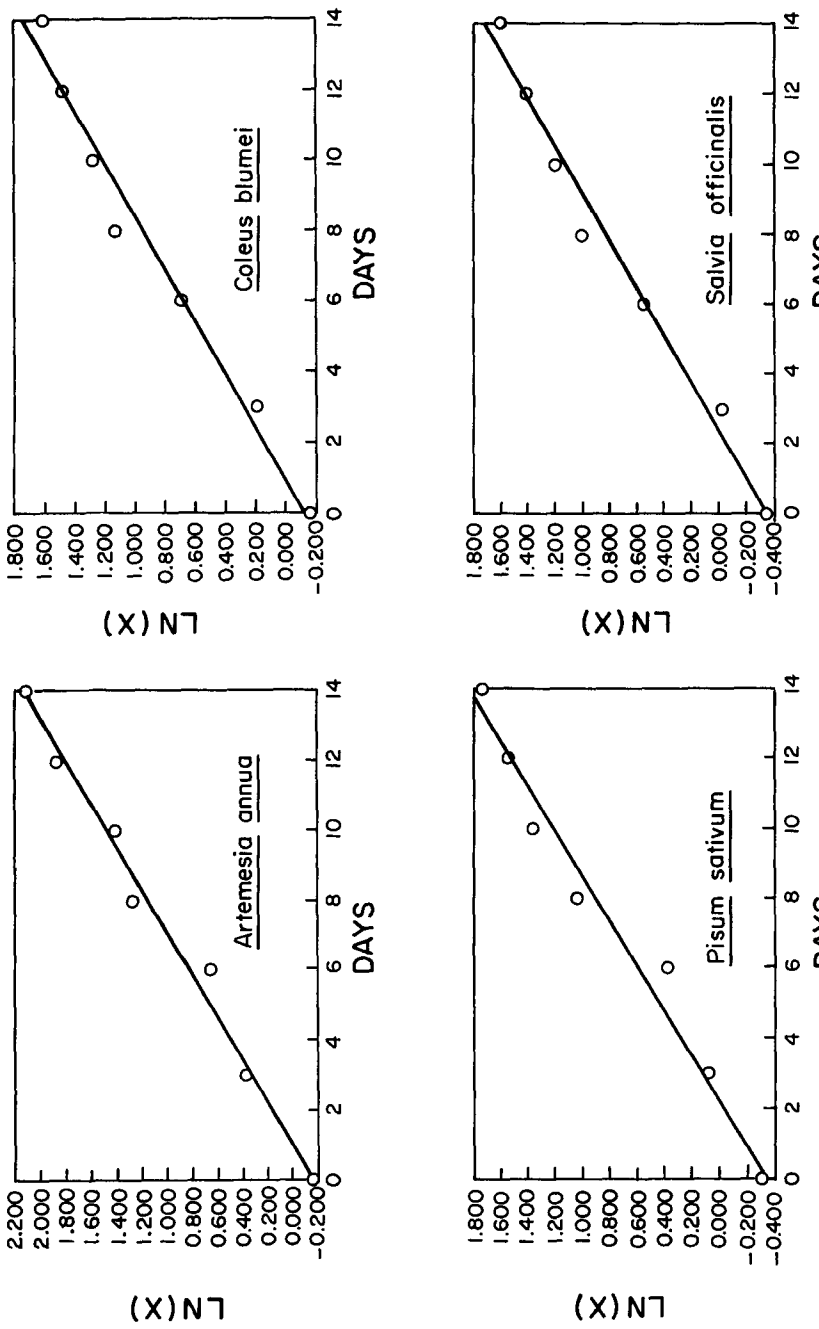

Fig. 1. Semilogarithmic growth plots of various plant cell suspension cultures used in this study. (The plots represent only the exponential growth phase.)

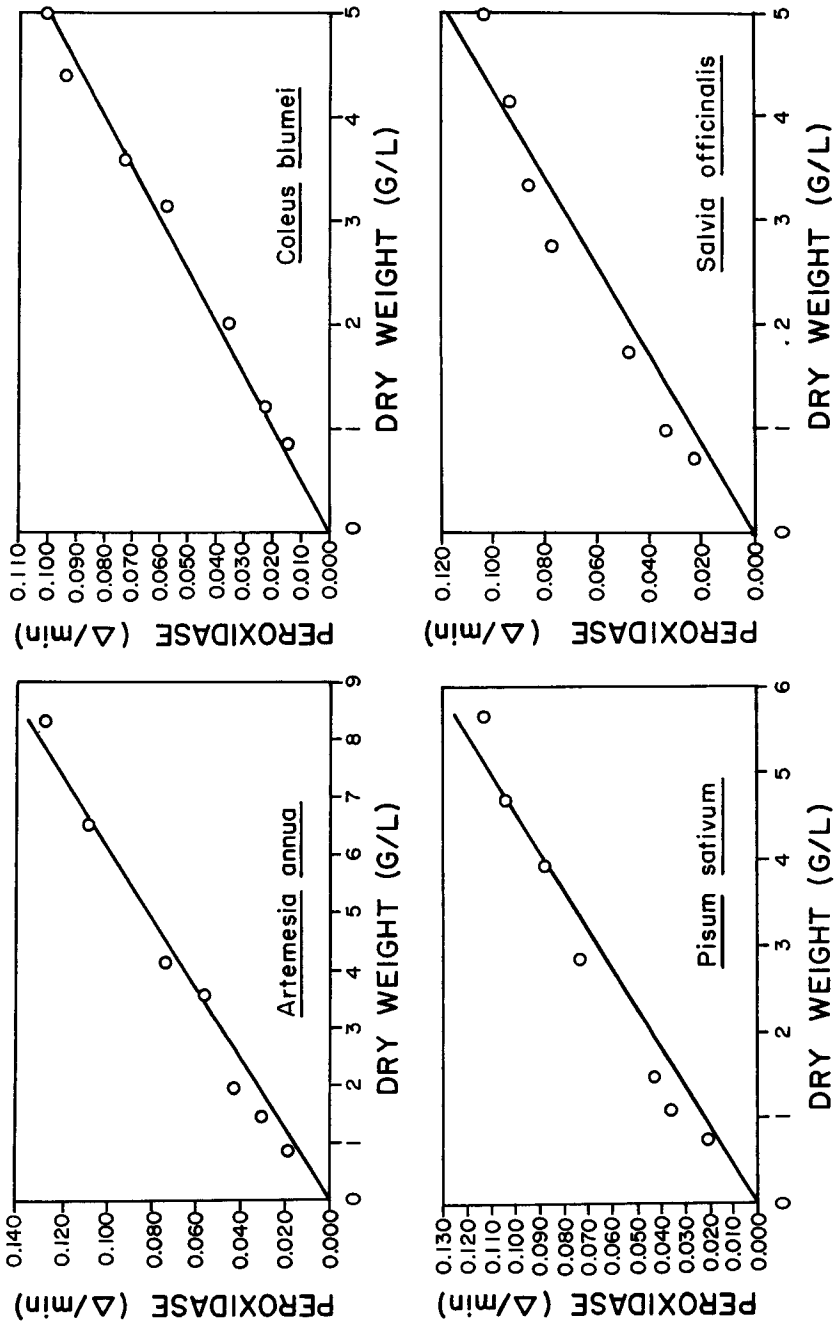

Fig. 2. Plots of peroxidase activity vs cell dry weight of various plant cell suspension cultures during the exponential growth phase.

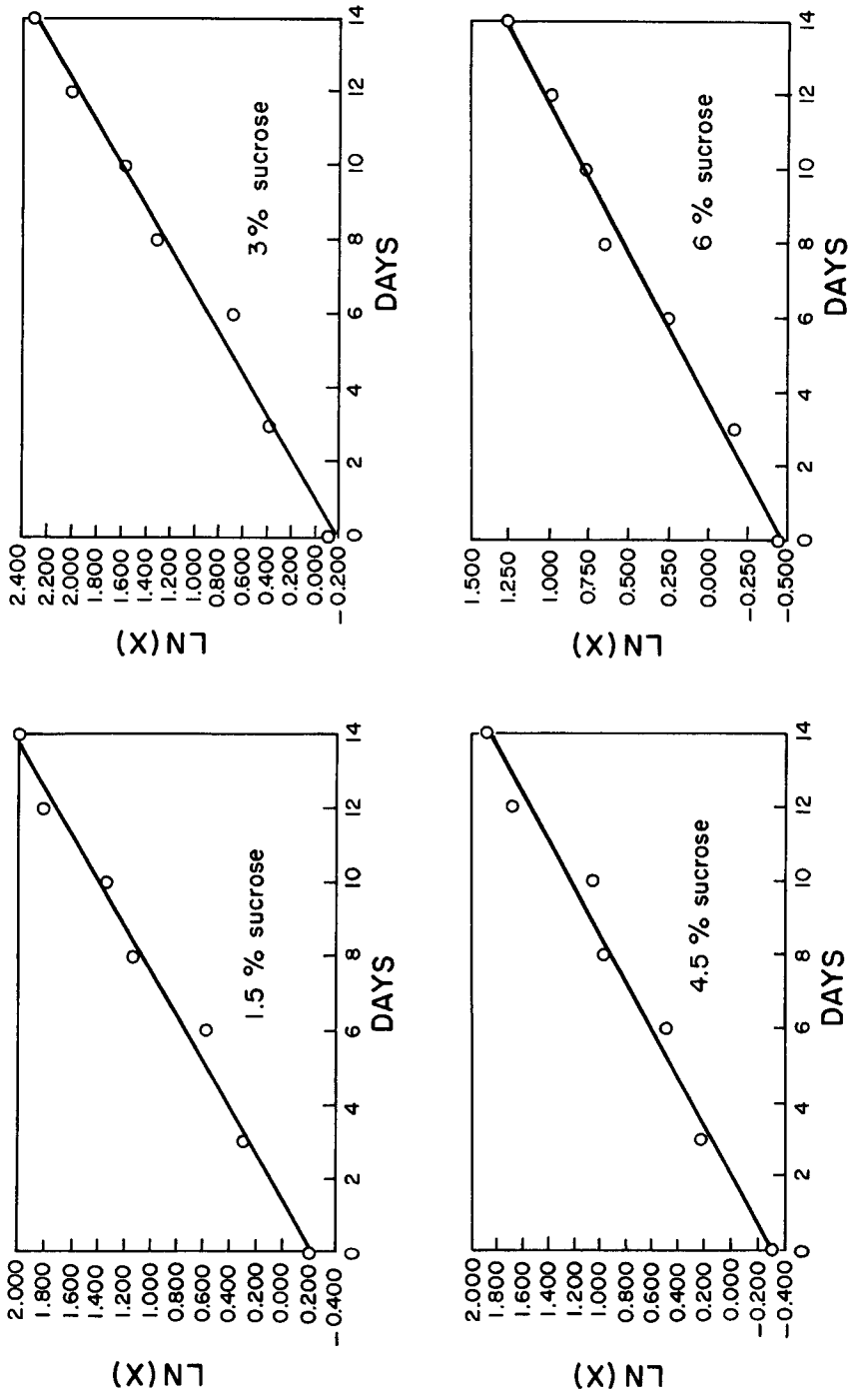

Fig. 3. Effect of sucrose concentrations on growth of *Artemesia annua* cell suspension cultures. (The plots represent only the exponential growth phase).

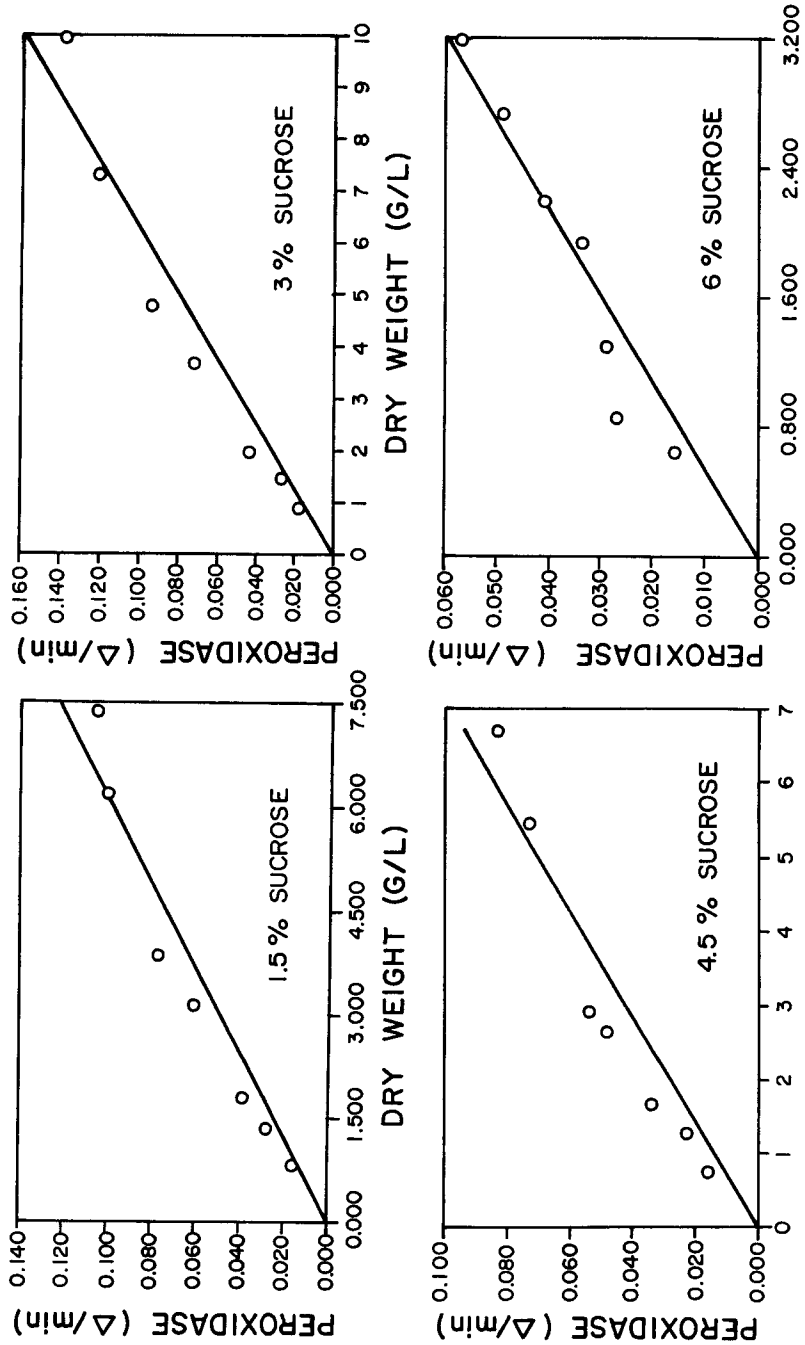

Fig. 4. Plots of peroxidase activity vs cell dry weight during the exponential growth phase of *Artemesia annua* at various sucrose concentrations.

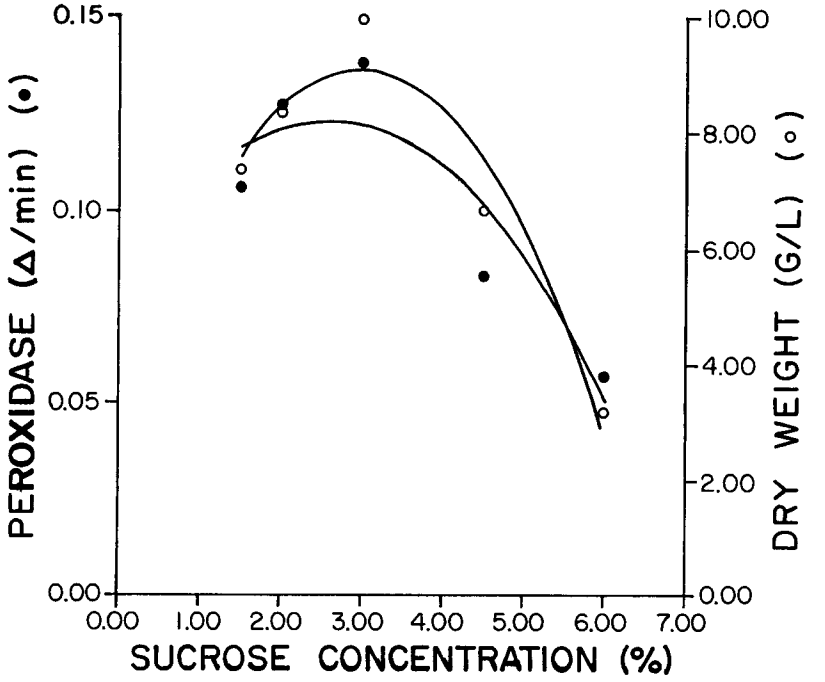

Fig. 5. Peroxidase activity and cell dry weight in response to sucrose concentrations. (All measurements were made at maximum peroxidase activity).

In summary, extracellular peroxidases can be used as simple and rapid indicators of growth in plant cell suspension cultures. In addition, the volume of extracellular medium required for the assay is very low (0.1 mL).

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