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Pigmentation and Soluble Peroxidase Isozyme Patterns of Leaves of *Pedilanthus tithymaloides* L. *variegatus* as a Result of Daily Temperature Differences[1]

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ABSTRACT

In a constant environment with a narrow (less than 8°C) daily temperature difference (*δt*), leaves of *Pedilanthus tithymaloides* L. *variegatus* usually appeared green. After at least two days of *δt* > 10°C, new leaves had become green-white, and a red pigmentation appeared and increased if high *δt* conditions were maintained. If plants were returned to a narrow *δt*, new green leaves reappeared. Electrophoretic patterns of soluble peroxidase isozymes changed during the color changes. Three groups of electrophoretic bands occurred, and each was related to a characteristic tissue. The development of red color was correlated with the appearance of the group with the fastest electrophoretic mobility and the highest peroxidase activity.

Many marked changes in pigmentations of plants have been related to various changes of environment (9, 10). Enzymes comprise the bulk of the soluble proteins involved in plant metabolism, and many changes in protein or isozyme patterns have been recorded during development (6, 17). Soluble peroxidase isozymes, in particular, have been studied (4, 14); in many cases, they were shown to be tissue-specific or correlated with special physiological phenomena (8, 16, 18). Reported here are changes of pigmentation and of isoperoxidases activities in leaves of *Pedilanthus tithymaloides* L. *variegatus*.

MATERIALS AND METHODS

Pedilanthus tithymaloides L. *variegatus* (*Euphorbiaceae*) is a branching, succulent bush with green stems bearing pale green, highly variegated, red-tinged ovate leaves. The plant is likely to be a periclinal chimera, and chimeras often are used to study the ontogeny of leaves (3, 19). The plant is easily propagated by cutting and culturing stems in soil or nutrient solution. Plants were grown in controlled environmental chambers (temperatures varying from 15 to 50°C ± 0.2°C; RH varying from 95% to 30% ± 2%; photoperiods: 10 h, 12 h, 14 h; light sources: fluorescent lamps Mazda fluor lumière du jour TF 65 w L5), in greenhouse, or in fields. Results in Figure 2 summarize separate experiments conducted in sequence in the same chamber and different environments in greenhouse and in fields in which plants were grown simultaneously. Plants were grown at least 2 months in a definite condition, with at least 10 pots with three plants each (stems were at least 0.3 m in height with at least three leaves). According to *δt*[3] values, four environments could be distinguished (Fig. 2). Temperature and RH were recorded simultaneously with a hygrothermograph (J. Richard, Paris). Temperatures were recorded with a maximum-minimum thermometer (Brand, Paris) and a telethermometer (YSI, Yellow Springs, OH); RH was measured with a psychrometer (Prolabo, Paris).

For enzyme assays, samples of 0.1 g fresh tissue were collected, immediately washed (with distilled H_2O), wiped, weighed, transferred into a mortar (chilled at −18°C), to which was added 1.0 ml extraction buffer (0.1 M Tris-HCl, 5 mM $MgCl_2$, 50 mM KCl, 5 mM 2-mercaptoethanol, 0.25 M sucrose, pH 7.5), and hand ground thoroughly with a pestle. The homogenate brei was centrifuged for 10 min at 5000*g*; the supernatant was filtered on cotton and used as the enzyme. All operations were carried out at 4°C.

Peroxidase isozyme patterns were determined by vertical polyacrylamide gel electrophoresis. The gel column (50 × 4 mm) was 7.5% (w/v) polyacrylamide and 0.2% (w/v) bis acrylamide in 0.25 M Tris-HCl (pH 8.9). Polymerization was performed with 1.4 mg/ml persulfate; 0.1 ml filtered extract was layered directly on gel under 5 mM Tris-glycin buffer (pH 8.3). The concentration of extract used per gel equalled 1.0 mg dry weight tissue. The temperature of the gel column was kept at 4°C. A 0.5-mamp current per gel during 20 min, then a 2.0-mamp current per gel during 60 min, was applied. Front migrations were marked with bromophenol blue. Removed gels were longitudinally sliced; one slice was incubated for peroxidase activity, and the other was stained for proteins (7). For preparative electrophoresis, the removed gels were frozen at −8°C, sliced into sections, and ground with 0.1 M citric acid-sodium phosphate buffer (pH 4.3) per 5 mm long gel.

Amounts of proteins were measured by the Lowry procedure (13). Levels of peroxidase activity were assayed by the colorimetric method of oxidation of benzidine (5) on a Beckman spectrophotometer at 610 nm at 25°C (pH 5.0). Standard protein was BSA, and standard peroxidase was horseradish (943 unit/mg, pH 6.0, at 25°C) from Calbiochem, prepared with extraction buffer. In each experiment, only tissues of a leaf were used for one sample assayed for enzyme activity or pigment content.

RESULTS

Many leaves were observed in greenhouse and in fields, and it appeared that leaves had green-, white-, and red-colored areas in

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[3] Abbreviation: *δt*, daily temperature difference (difference between maximum and minimum temperatures for the same day).

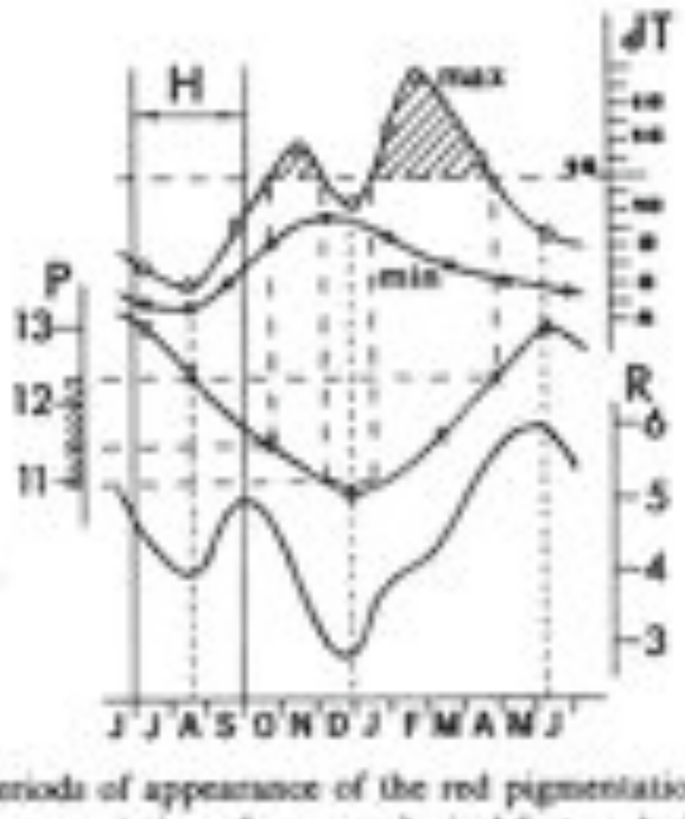

FIG. 1. Periods of appearance of the red pigmentation in field-grown plants and mean variation of some ecological factors during the year. The δt is given in °C; δt_a, δt for the day i of a station in a week of a year; δT, weekly averaged δt of a week, a station, a year $\delta T = \frac{\sum_{i=1}^{7} \delta t_i}{7}$, δT_{max}, δT of the station a (a = 1 to 6, Dakar, Cap-Vert, Sénégal) of the week b (b = 1 to 52) of the year c (c = 1 to 6, from 1975 to 1980); δT_{max} and δT_{min} are, respectively, the highest and lowest values of the observed δT in the week b; ○——○ *max* and ●——● *min* represent, respectively, the variations of δT_{max} and δT_{min} from week to week, over the entire year. R, Global radiation in 100 cal/cm²·d; *P*, photoperiod in H; H, rainy season (mean values of six stations during six years). During two periods of the year (cross-hatched areas), when *max* was ≥12°C, all leaves were green-white-red. ×——× P, Photoperiod mean values; —— R, global radiation mean values.

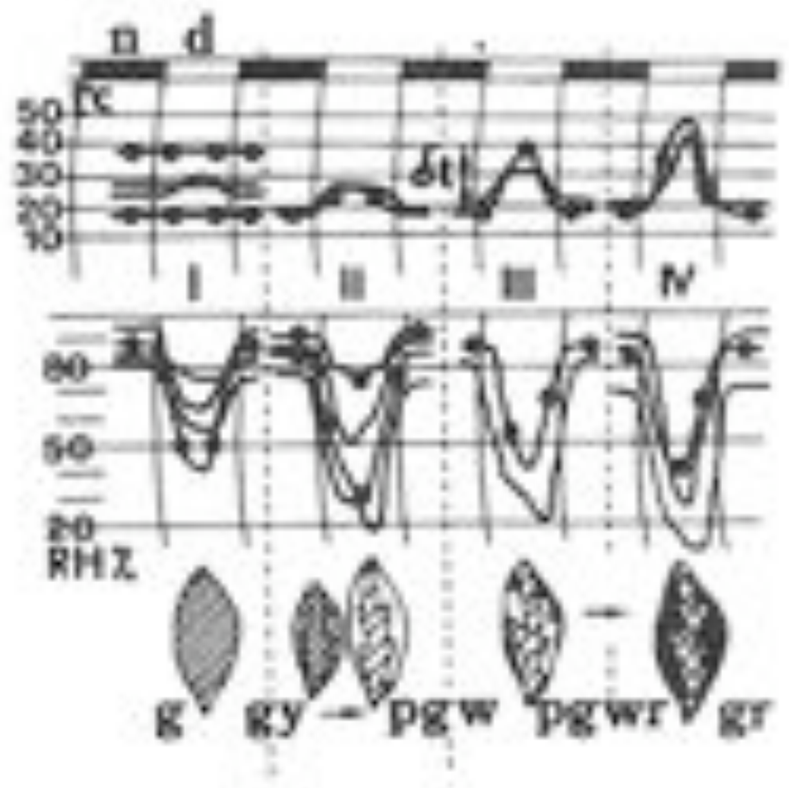

FIG. 2. Effect of environmental conditions on the realization of the types of leaves. Plants were grown in the following conditions: t, temperature in °C; δt in °C; RH, in %; n, night; d, day; photoperiod, 12 h ± 0.5 h. Pigmentation of leaves were: g, uniformly green; gy, green with yellow margin; gw, green with white margin; pgwr, pale green with bordered-red white margin; gr, green with whole margin red. δt Conditions were: I, δt below 8°C = low δt conditions (at least four separate experiments in growth rooms [●——●] and greenhouses [——], repeated three times at different periods of the year); II, δt between 8°C and 10°C = medium δt conditions (at least six separate experiments in growth rooms, greenhouses, and fields, in the same δt conditions, repeated three times at different periods of the year); III, δt greater than 10°C = high δt conditions (at least four separate experiments in growth rooms, greenhouses, and fields, repeated at least two times at different periods of the year); IV, highest δt conditions (at least four experiments in greenhouse with supplemental heating and fields—maximum δt observed was 52°C − 18°C = 34°C—during the period when δt was >12°C). Pigmentation changes (→): II, gy young leaves become mature gw (the color change progressed with time in the same environment during growth); III to IV, with transfer from III to IV red extended; the plants were moved from one condition to another to achieve the color changes. From I to II, no more new green leaves; from II to III, red appearance; from III to IV, red extension. Inversely, from IV to III, red extension failed; from III to II, new green-white leaves, no more red-white-green; from II to I, reappearance of green leaves.

either sunny or shady conditions; under various RHs and soil moistures, the main factor influencing color changes was found to be δt (Fig. 1). In controlled environmental chambers, when δt was below 8°C, often new leaves appeared only green; when δt was over 8°C, only leaves with white peripheral areas appeared; when δt increased to over 10°C, red pigmentation appeared (Fig. 2). When a high δt was not maintained for more than 2 d, red did not spread, and the subsequent new leaves appeared only green-white in medium δt conditions and often green in low δt conditions.

The red color, which appears when plants are exposed to a high δt, was due to the synthesis of one anthocyanin pigment (Fig. 3), which was located only in the vacuoles of cells in upper or lower epidermis. In white areas, the palisade layer was lacking; only the spongy layer extended. The spongy tissue was histologically the same in green or in white sectors, with nearly the same thickness. In green sectors, the amount of greening was directly related to the thickness of the green palisade layer and to its number of green cell beds (Fig. 4). The white layer and the appearance and extension of red in epidermis were associated with qualitative and quantitative changes in isoperoxidase patterns (Fig. 5): three groups of bands were distinguished according to anodic migration (slow, medium, and fast migrating groups). The presence or absence of these bands, as well as increases or decreases in their intensities, was related with the δt conditions. With an increase of δt, the medium group decreased in the green layer and the slow-group was expressed, not only in the white layer but also in the green one a bit. With the appearance of red color, the fast group appeared in the red epidermis, and the slow group decreased in the white one. With a decrease of δt, the slow group decreased in the white layer, and the medium group often appeared in it and increased in the green layer simultaneously. The amounts of peroxidase activities were directly related to those of the migrating bands, and the changes in activities were tightly correlated with respective changes in electrophoretic patterns (Table I). Quantitative changes in the data for enzyme and pigment levels occurred

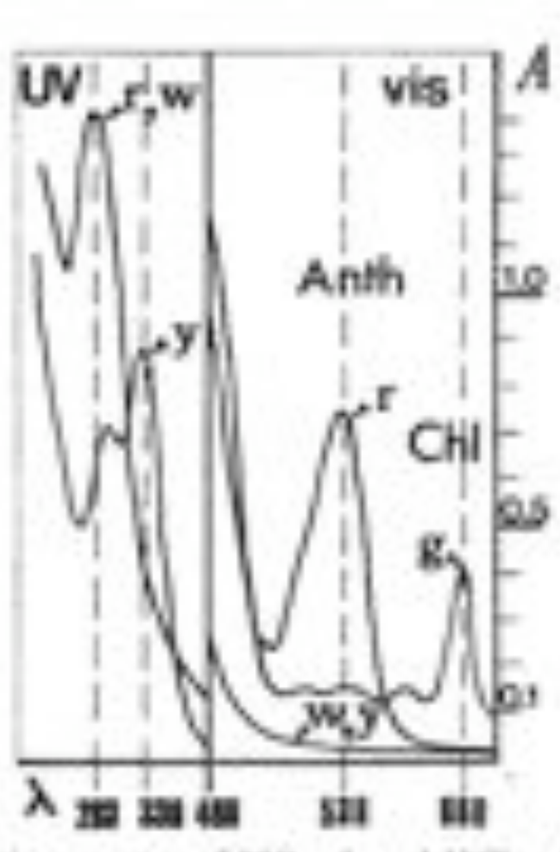

FIG. 3. Absorption spectra of 95% ethanol-HCl N 1% (v/v) extracts of uniformly colored areas of leaves. λ, Wavelength (in nm); UV, ultraviolet; vis, visible. Colored extracts: r, red; w, white; y, yellow; g, green. Pigment peaks: Chl, chlorophyll; Anth, anthocyanin. Recordings were made with a Beckman scanning 35 spectrophotometer.

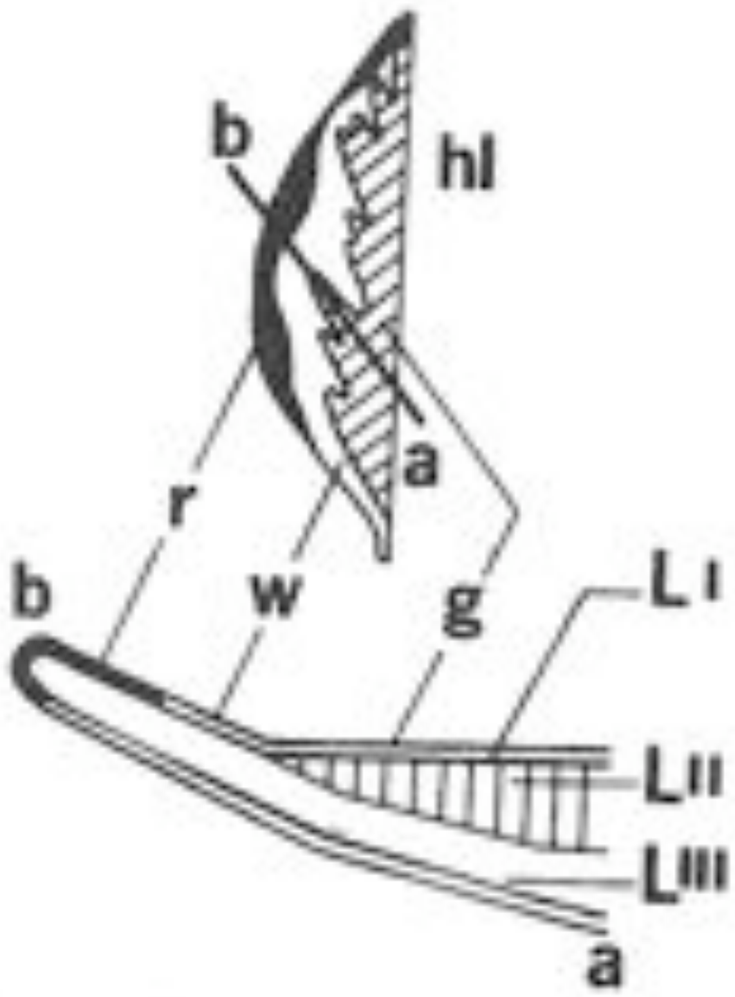

FIG. 4. Structure of leaves. hl, Half-leaf with green (g), white (w), and red (r) areas. ah, Cross-section of hl. L I, L II, and L III apical layers (19) produce epidermal layer, green palisade layer, and white spongy layer.

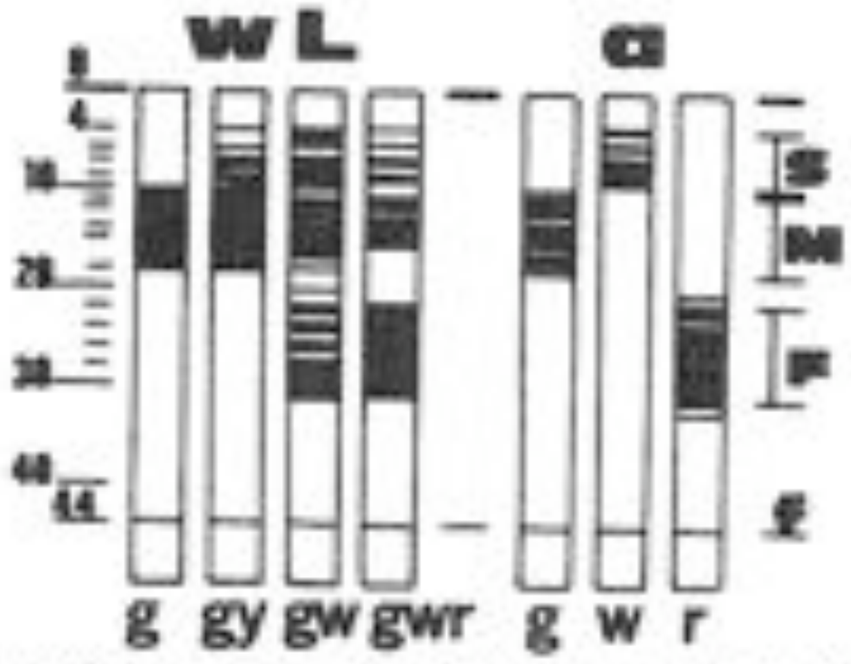

FIG. 5. Analytical electrophoresis:peroxidase patterns and *ôt* conditions. Whole leaves (wl): g, green (in low *ôt* conditions); gy, green with yellow margin (young), and gw, green with white margin (mature), in medium *ôt* conditions; gwr, green with white bordered-red margin (in high *ôt* conditions). Uniformly colored areas (a) are: g, green L II of green leaves; w, white L III of green-white ones; r, red L I of green-white-red ones, respectively, in low, medium, and high *ôt* conditions. Peroxidase groups are: S, slow-migrating group (two major and two minor bands); M, medium-migrating group (five major and two minor bands); F, fast-migrating group (five major and 5 minor bands); f, front (bromophenol blue) at 44 mm.

with the position of a leaf on the stem, its age, and the time of d. The chronobiology of these changes is actually studied.

DISCUSSION

Environmental factors often alter the color of plant organs (15). With *P. tithymaloides* L. *variegatus*, the increase in *ôt* causes the failure of extension of green palisade layers and the appearance of red pigmentation from the border to the middle of laminas. Lighting and photoperiod or rainfall patterns in fields (Fig. 1), even soil moisture, did not influence the appearance of the red pigmentation; these factors could influence the red spreading only. The main factor of the extension of the white areas and of the appearance of the red pigmentation appeared to be *ôt* (Figs. 1 and

Table I. *Relative Peroxidases Activities and ôt Conditions*

Tissues and Type of Peroxidase Isozymes	Relative Peroxidases Activities of Different Leaf Types		
	g[a]	gw[b]	gwr[c]
L I, fast-migrating group	0.0	0.5	2.0
L II, medium-migrating group	1.0[d]	0.4	0.1[e]
L III, slow-migrating group	0.1[e]	1.0	0.6

[a] g, Green leaves in narrow *ôt* conditions ($\delta t \leq 2$°C).
[b] gw, Green-white leaves in medium *ôt* conditions (8°C < *ôt* < 10°C).
[c] gwr, Green-white-red leaves in high *ôt* conditions ($\delta t \geq 12$°C).
[d] Reference (1.0 = 100 μM benzidine oxidized/min·mg protein at pH 5.0 at 25°C).
[e] Traces only during the hottest period of the day.
[f] Traces only during the coldest period of the day.

2); the same 10°C *ôt* had a common effect, regardless of whether it occurred between 15 and 25°C, 20 and 30°C, or 25 and 35°C. Moisture, light, and temperature can strongly change in tropical lands, where seasons are very different. In the wet season, water or sunlight are not limiting factors; temperature is high and relatively constant. In the dry season, water is a limiting factor. Daily moisture or temperature differences show progressive variations, day to day, during the end of the wet season and the start of the dry one (Fig. 1), periods which are especially important for vegetative growth and fruit development. We assume that daily moisture or temperature differences may be governing factors of plant development during this transition period, in which the main influence of sunlight may be elevation of the temperature of leaves. Temperature injuries may lead to increases in anthocyanin content, and anthocyanins have been reported to be correlated with hardiness (11, 12). Similar results were observed in response to daily temperature rises. The differential growth rates between L II and L III (Fig. 4) can reflect differential growth of genetically different lineages in response to the *ôt* stress (19). Since certain leaves may be green-white in one-half and, in the other, only white or only green, the action of temperature could be at the level of differentiation of the bud meristem (1, 3, 19). Changes in enzyme activities are common features of development, and peroxidase activities are frequently malleable with environmental factors (2). Here, the peroxidases of the fast group are correlated with the development of anthocyanin pigmentation. Fast group activity was the highest during anthocyanin increase. Slow, medium, and fast peroxidase groups may be used as physiological markers of each tissue. All three groups may be potentially present in each tissue, but the group to be expressed depends on the tissue and on the daily temperature differences.

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