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Effects of light and growth substances on senescence of barley leaf segments at different developmental stages

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Abstract. The acceleration of senescence by leaf detachment and incubation in the dark has been assayed with primary leaf segments of 6- to 16-day-old barley (*Hordeum vulgare* L. cv. Hassan), comprising young, mature and senescent leaves. The effect of senescence acceleration is more pronounced in old than in young leaves. The retardation of senescence by kinetin (when measured by protein loss) and near-UV light is more pronounced in old than in young leaf segments. Senescence acceleration by methyl jasmonate is stronger in young than in old leaves. In contrast, abscisic acid and ethylene promote senescence more in old than in young leaf segments. These results and others with red light interruptions and continuous lights, indicate that different effectors affect senescence by diverse mechanisms which depend quantitatively on the developmental stage of the leaves.

Key words: Abscisic acid; Ethylene; *Hordeum vulgare* L.; Kinetin; Methyl jasmonate; Phytochrome; Senescence; UV-light.

Introduction

Light and phytohormones control the senescence of leaves (Thimann, 1980; Thomas and Stoddart, 1980; Sabater, 1985). Usually, cytokinins retard and abscisic acid (ABA) and ethylene promote senescence. Sembdner and Gross (1986) revised other senescence promoters derived from cyclopentanone, methyl-jasmonate (Me-JA) being the most active (Satler and Thimann, 1981; Weidhase *et al.*, 1987). Visible light retards senescence, at least in part through phytochrome (Biswal and Biswal, 1984; Cuello *et al.*, 1984) which affects chloroplast protein synthesis (Cuello *et al.*, 1987). Quiles *et al.* (1988) reported an additional effect of near-UV light (320-390 nm) in retarding senescence.

One important question is whether different effectors of senescence act through a common primary action. We have investigated the effects of light of various qualities and growth regulators on the senescence of detached barley leaves of different ages, to distinguish possible differences in the action mechanisms of the effectors. Differences were judged on the basis of different responses of senescence to these effectors, depending on the ontogenic stage of the leaves.

Materials and Methods

Barley (*Hordeum vulgare* L. cv. Hassan) was grown under an 18-h photoperiod on vermiculite with Cron medium as described by Cuello and Sabater (1982). 0.5 g of 40 mm primary leaf apical segments (5 mm from tip) from barley grown during different

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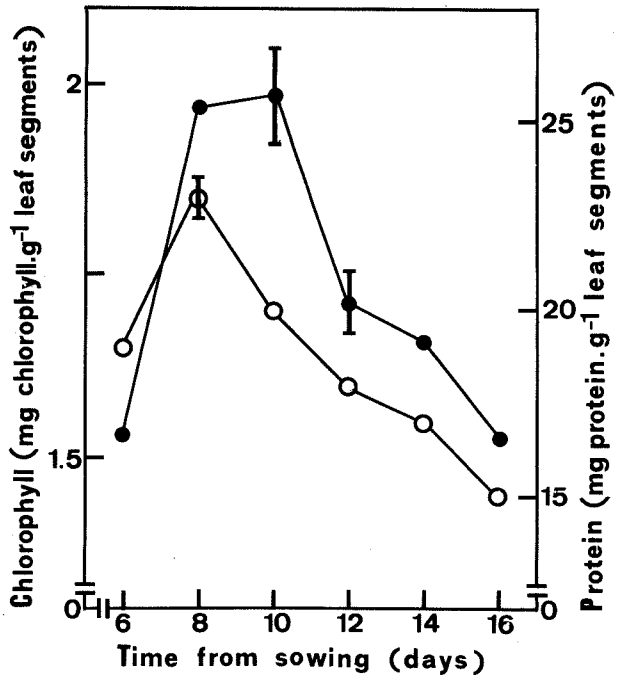

Fig. 1. Chlorophyll (●) and soluble protein (○) content of primary leaf segments of barley plants of different ages. Indicated values are the means of 3 independent experiments. Only SE≧2% are represented.

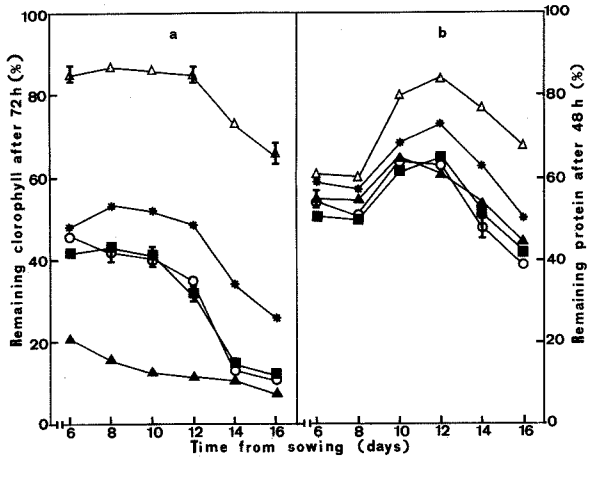

Fig. 2. Effects of 14 μM kinetin (△), 34 μM ABA (○), 70 μM ethylene (applied as ethrel) (■) and 45 μM Me-JA (▲) on the retention of chlorophyll (a) and protein (b) by primary leaf segments of barley of different ages. All the incubations were carried out in the dark. (*) is the control incubation of leaf segments in pure water. Values are means of 2-4 independent experiments (10-20 experiments for the controls). Only SE≧2% are represented.

times were incubated, in the dark or under specified light treatments, at 26°C in Petri dish with 35 ml pure water or different hormone solutions. Leaf segment senescence was measured after 72 h incubation, by chlorophyll depletion, and/or after 48 h incubation, by soluble protein depletion, by comparison with the values in freshly detached leaf segments.

Protein extracts were prepared with 6 volumes of extraction buffer (2 mM Na-HEPES, 2 mM Na_2-EDTA and 2 mM Na-isoascorbate, pH 7.6) by grinding in a mortar. The homogenate was filtered through 2 gauze layers and centrifuged for 5 min at 500 xg. Proteins were measured in the supernatant according to Lowry *et al.* (1951) after precipitation with 10% trichloroacetic acid. Absolute chlorophyll values in freshly detached leaf segments were measured according to Arnon (1949). Relative chlorophyll changes (after leaf segment incubation) were determined according to Schistad and Nissen (1984).

Visible light treatments, irradiances and filters were as described previously (Cuello *et al.*, 1987) except that irradiances (at the sample level) for continuous light treatments were 13.5 Wm^{-2} PAR without filters. Near UV light was provided by a 40 W tube (Black Light Lamp BLB, Sankyo Denki, Japan) which gave, at the sample level, 1.4 Wm^{-2} irradiance, peaking at 355 nm (half-band width 35 nm).

Results

Fig. 1 shows that, freshly detached primary leaf segments of barley reached the highest chlorophyll concentration between 8 and 10 days after sowing, after which it slowly decreased. Soluble protein content reached the highest value 8 days after sowing. Between days 8 and 10 after sowing, primary barley leaf apical segments seem to be at the mature stage of development under our culture conditions. They start natural senescence after 10 days, 1 to 2 days earlier than the whole leaf (Martîn *et al.*, 1986), which includes younger cells at the basal region. Before the 8th day, apical segments may be considered young.

Fig. 2 shows that chlorophyll loss was a more sensitive marker than protein loss for assaying the effect of different growth regulators on senescence. Kinetin retarded and Me-JA accelerated the loss of chlorophyll in incubated leaf segments. These effects were nearly similar for 6- to 16-day-old leaves. However, the ac-

celerating effect of ABA (abscisic acid) and ethylene on the loss of chlorophyll in leaf segments (relative to the control of incubation in water) increased with the age of the leaves. Based on either chlorophyll or protein loss, the acceleration of senescence by detachment and incubation in water in the dark was more pronounced in young and, particularly, old leaves than in 8 to 12 days old leaves. Although less pronounced than on chlorophyll loss, the effects (retarding or accelerating) of growth regulators on protein loss usually increased with leaf age.

Red light interruptions (10 min every 12 h) of the incubations in the dark of leaf segments retarded the loss of chlorophyll and protein (Fig. 3). The effect of red light interruptions is mediated by phytochrome, as it was suppressed by subsequent far-red illuminations (Fig. 3), and it slightly decreased as leaves became older. Near-UV light photoperiods (6 h) also retarded the loss of chlorophyll and protein. In contrast to red light interruptions, the retarding effect of near-UV light treatment slightly increased with the age of leaves.

Continuous illumination of incubated leaf segments with white, red or blue lights, but not with far -red light, retarded the loss of chlorophyll with respect

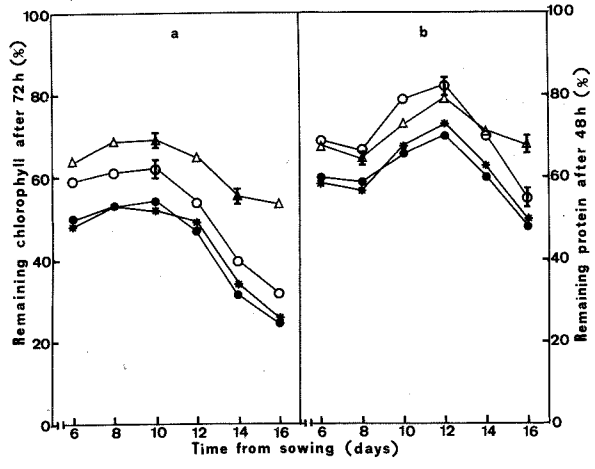

Fig. 3. Effects of 10 min red light (3.2 Wm^{-2}) interruptions (every 12 h) (○), 10 min red light interruptions followed 20 min far-red light (13.5 Wm^{-2}) (every 12 h) (●) and near-UV light 6 h photoperiod (1.4 Wm^{-2}) (△) on the retention of chlorophyll (a) and protein (b) by primary leaf segments of barley of different ages. (*) is the control incubation in continuous dark. Values are means of 2-4 independent experiments (10-20 experiments for the controls). Only SE≧2% are represented.

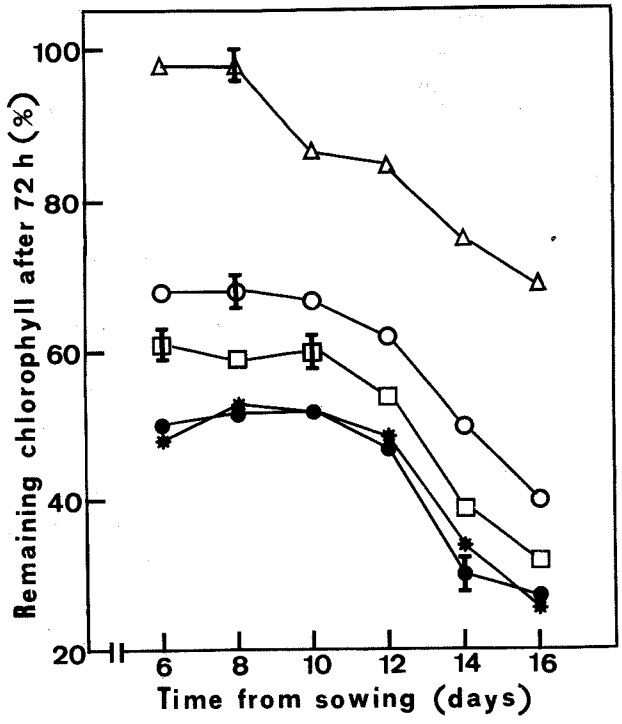

Fig. 4. Effects of continuous white (13.5 Wm^{-2} PAR) (△), red (600-685 nm, 0.8 Wm^{-2}) (○), blue (410-480 nm, 1.8 Wm^{-2}) (□)and far-red (>695 nm, 7 Wm^{-2}) (●) lights on the retention of chlorophyll by primary leaf segments of barley of different ages. (*) is the control incubation in continuous dark. Values are means of 3 independent experiments (10-20 for the controls). Only SE≧ 2% are represented.

to the control of incubation in the dark (Fig. 4). The retarding effect on senescence of continuous lights did not show a clear preference for young, mature or senescent leaves.

Discussion

Twelve days after sowing attached primary leaf apical segments are in natural senescence (Fig. 1). Significantly, the accelerating effect of leaf detachment and incubation in the dark on senescence strongly increases for leaves older than 12 days (Fig. 2). Consistently, Cuello *et al.* (1989) found a higher senescence rate of apical than basal segments when they are incubated in the dark.

The differential accelerating effects of Me-JA from ABA and ethylene on senescence, suggest that

they act through different mechanisms, Me-JA being more active than ABA and ethylene in young leaves. Similar considerations suggest different mechanisms for the retarding effects on senescence of red light interruptions, near-UV light treatment and continuous light treatment. One possibility is that kinetin and continuous light treatment retard senescence by similar mechanism. Clearly, the senescence response to several hormones and light depends on the developmental stage of the leaves in a way that is specific for each effector. Despite quantitative differences, qualitative senescence response to one effector do not vary during leaf ontogeny. The increased senescence accelerating effect of ABA with the age of leaves is at first surprising as ABA content of attached leaves increases during senescence (Sabater, 1985). However, this increase takes place at advanced stages of senescence, when most chlorophyll has been lost. Probably, barley leaves have still low ABA content in 16-day-old plants, thus responding to ABA addition.

Phytochrome, in the P_{fr} form, retards segment senescence at all the stages of development studied, the effect being more pronounced in young than in old leaves. As the retardation of senescence by near UV light is more pronounced in old than in young leaves (Fig. 3), this effect of near UV is, probably, not mediated by P_{fr}. In fact, far-red light applied subsequently to near-UV does not affect the retarding effect of the last (Quiles *et al.*, 1988). Continuous white light is highly effective in retarding senescence. Hurng *et al.* (1986) found that, in rice, continuous white light is more effective in old than in young leaves in retarding senescence. Very probably, the retardation of senescence by continuous light depends on additional mechanisms to phytochrome action (Thimann *et al.*, 1977; Hurng and Kao, 1988).

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光與生長素對不同發生期之大麥葉片之老化作用的影響

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利用 6 到 16 天之大麥第一葉片，以葉片切離和黑暗處理，探討促進年青葉片、成熟葉片及老化葉片老化過程之問題，發現老葉片比年青葉片更易老化。Kinetin和近紫外光延緩老葉片之葉片老化效果較明顯。以 methyl jasmonate 加速葉片老化過程時，發現對年青葉片的作用較強。反之，離層酸及乙烯促進老葉片之老化較明顯。以上結果以及利用紅光中斷與連續照光處理的結果，顯示不同影響因子對老化作用的影響是經由不同的控制機制，而這些機制又與葉片發展時期有程度上的關係。