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CHLOROPHYLL CLEARING IN DEVELOPING CANOLA SEED

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ABSTRACT

Chlorophyll clearing in developing canola seed occurs at a relatively fixed rate but is temporally influenced by environmental conditions. Swathing advances the time of chlorophyll clearing but does not affect the rate. Chlorophyll clearing is initiated relatively early in seed development at about the time the embryo expands to fill the seedcoat, which is well in advance of physiological maturity.

INTRODUCTION

At harvest, seed of canola may retain variable amounts of chlorophyll which can affect seed grade. As little as 3% distinctly green seed (>20 ppm chlorophyll) will reduce the value of the crop because chlorophyll is extracted with the oil and is then difficult to remove by conventional bleaching processes. Chlorophyll can inhibit the hydrogenation catalyst used for hardening in the manufacture of margarine. Oils from seed with elevated chlorophyll content are also less stable, their oxidation resulting in rancidity. The following studies were undertaken to elucidate the relationship between seed development and the chlorophyll clearing process.

EXPERIMENTAL

To study the affect of differing environmental conditions during seed development on chlorophyll clearing, four *Brassica napus* L. and and four *B. rapa* L. lines (including *B. rapa* L. strain 1559, data shown) selected for overlapping maturities were seeded in replicated randomized 3 m row plots at three dates, separated by approximately two week intervals, commencing the first week of June in 1988 and 1989. To study the affect of swathing, *B. napus* cv. Westar was seeded in 2 by 6 m plots with a 17 cm row spacing. Plots were swathed at ca. 5 day intervals. The time of initiation of chlorophyll clearing was also determined using seed the standing crop. For each study seed was harvested from the main raceme at 3 to 4 day intervals and moisture and chlorophyll content (Daun 1976) determined either on the seed or excised embryos.

RESULTS AND DISCUSSION

Chlorophyll clearing occurred at a relatively fixed rate despite differing environmental conditions imposed by varying the seeding date (Figure 1). However, varying seeding date altered the temporal relationship between moisture loss, a measure of the time and rate seed development, and chlorophyll clearing. Early seeding resulted in chlorophyll clearing occurring well in advance of moisture loss while later seeding, apparently due to cooler temperatures, resulted in chlorophyll clearing occurring in concert with moisture loss. Reduction in the temporal separation between chlorophyll clearing and moisture loss in the later seeding appeared to contribute to elevated residual chlorophyll content in mature seed. Analyses (data not shown) indicated that chlorophyll becomes entrapped when moisture content of developing seed dropped to <25%.

Swathing advanced the time of both moisture loss and chlorophyll clearing (Figure 2). While swathing altered the rate of moisture loss, from ca. 2% day^{-1} to 8-10% day^{-1}, it did not affect the rate of chlorophyll clearing. The rate of chlorophyll clearing in the swathed crop was comparable to that which would have subsequently occurred if the crop had been left standing. However, swathing did affect the time of initiation of chlorophyll clearing.

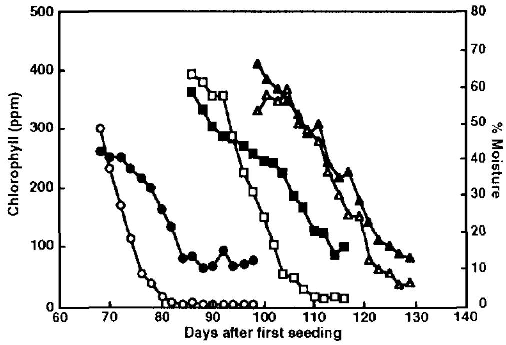

Figure 1. Chlorophyll content (open symbols) and moisture content (closed symbols) of developing *B. rapa* strain 1559 seed planted on June 1 (O), June 14 (□) and June 21 (Δ) 1988.

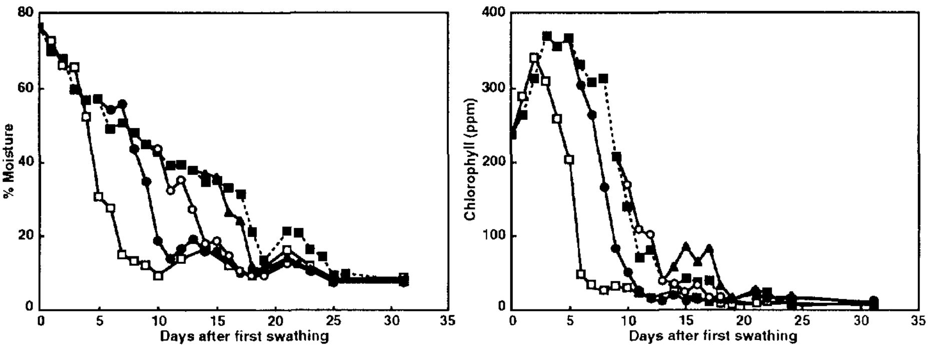

Figure 2. Moisture and chlorophyll content of developing seed of *B. napus* cv. Westar from standing crop (dashed line) and crop swathed at 4 day intervals (solid lines).

Both seeding date and swathing studies pointed to the timing of the initiation of chlorophyll clearing as a potentially important factor in determining the residual chlorophyll content of mature seed. Subsequent studies established that chlorophyll clearing is initiated at the time the embryo reaches maximum water content which is when the embryo is fully expanded (Figure 3).

Evidence is mounting which indicates that carbohydrates can modulate photosynthesis and in turn chlorophyll content through action on gene expression and subsequent enzyme activity (Stitt, 1991 and references therein). The fact that the rate of chlorophyll clearing is relatively fixed and only the timing of initiation varies may be consistent with reports that elevated carbohydrate affects photosynthetic anabolic pathways (Sheen, 1990). An inhibition of chlorophyll synthesis could lead to net degradation as the result of turnover.

Loss of chlorophyll is indicative of much more profound changes to the chloroplast. In addition to loss of thylakoid structure, soluble stomal proteins such as the Calvin cycle enzymes are lost (Krapp *et al.*, 1991, 1993, 1994) and the chloroplast reverts to a proplastid state (Fischer *et al.*, 1988). Recent studies by Krapp *et al.* (1994) have shown that elevating carbohydrate levels lead to a differential response between plastidic and cytosolic isozymes. While plastidic enzymes which are needed for photosynthesis or starch mobilization were lost, enzymes which are required for glycolysis were retained. Thus, carbohydrate induced changes to the plastids can be viewed as a redifferentiation rather than a dedifferentiation resulting in a change in function rather than a degradation. Presumably in developing seeds the plastids would continue to function normally as, for example, the site of fatty acid synthesis.

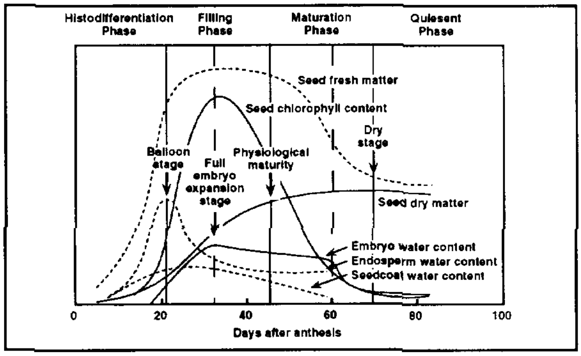

Figure 3. Schematic diagram showing the temporal relationship between seed chlorophyll content, embryo water content and other developmental paramters.

Control of the chlorophyll clearing process in developing seed may not be substantially different from that occuring at other times in development and in particular during terminal senescence when elevated carbohydrate concentration resulting from dehydration may initiate chloroplast redifferentiation. However, chlorophyll clearing and the related chloroplast redifferentiation during progressive senescence is likely initiated by a depletion of carbohydrates associated with nutrient drain (Kelly and Davies, 1988), a concept recently supported by transformation studies (Sonnewald *et al.*, 1994).

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