**Lag-Phase and Rate of Synthesis in Light-mediated Anthocyanin Synthesis**

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**Abstract.** Mustard (*Sinapis alba* L.) seedlings were irradiated with continuous far-red light either with or without a pretreatment with 3 or 6 h of the same far-red light, separated by a 15 h dark period. The pretreatment increases the initial rate of anthocyanin accumulation – as caused by the 2nd light treatment – at least 6-fold but leads to an earlier cessation of anthocyanin accumulation. Moreover, the pretreatment seems to shorten the apparent lag-phase of anthocyanin accumulation considerably but it does not eliminate the lag. If the pretreatment with far-red light is terminated before the seedling reaches competence (with regard to phytochrome and anthocyanin synthesis) the pretreatment has no effect on the apparent lag-phase even though the future capacity of anthocyanin biogenesis is considerably stimulated by the pretreatment. The time course of induction of anthocyanin and of phenylalanine ammonia-lyase (PAL) (Acton et al. 1980, Fig. 1) is in line with the concept that induction of PAL by light is a prerequisite for the onset of light-mediated anthocyanin synthesis.

**Key words:** Anthocyanin – Lag-phase (anthocyanin synthesis) – Phytochrome – *Sinapis*.

**Introduction**

Light-mediated synthesis of anthocyanin in seedlings of higher plants is preceded by an apparent lag-phase of the order of a few hours if the seedling was kept in complete darkness before the onset of the inductive irradiation ('initial lag-phase'). As an example, in the studies performed by Lange et al. (1967, 1971) on light-induced anthocyanin formation in the mustard seedling an initial lag-phase of the order of 2.5 h was observed. On the other hand, it was reported (Lange et al. 1967, 1971) that the lag-phase seems to disappear if the mustard seedling received a light pretreatment of several hours duration with the same kind of light as was used for the induction itself. In brief: a significant 'secondary lag-phase' was no longer detectable.

The specific role of a preirradiation in eliminating the lag-phase was challenged recently on experimental grounds by Acton et al. (1980) who worked out very detailed kinetics of phenylalanine ammonia-lyase (PAL) induction by light in the mustard seedling cotyledons and concluded that the apparent lag-phase of induction lasts between 30 and 60 min, both with and without light pretreatment. Moreover, the report by Acton et al. (1980) undermined the concept (supported by many data, see Mohr 1972) that the induction of PAL by light is a prerequisite for the onset of light-mediated anthocyanin synthesis. This view can obviously only be maintained if the lag-phase of anthocyanin induction is not shorter than the lag-phase of PAL induction in the same system. Under these circumstances a re-investigation of the kinetics of light-mediated anthocyanin synthesis in the mustard seedling cotyledons was demanded.

**Materials and Methods**

Seeds of white mustard (*Sinapis alba* L., harvest 1975) were purchased from Asgrow Company (Freiburg-Ebnet, FRG). The seeds were selected and seedlings were grown at 25±0.3°C according to the previously described procedure (Mohr 1966). The selected seed material is normally distributed with respect to seed weight and hypocotyl length (measured at 72 h after sowing).

Treatment with inductive far-red light (presumably operating exclusively through phytochrome, see Mohr 1972) was performed in the same standard far-red light field (fluence rate 3.5 W m⁻²) as used by Acton et al. (1980). Long wavelength far-red light (756 nm light, 5 W m⁻²) was produced with an AL-interference filter from Schott (Mainz, FRG).

For the determination of anthocyanin, the cotyledons of 20 seedlings were extracted in 10 ml extraction medium. Anthocyanin extraction and determination (A₅₂₅, 1 cm cuvette) were per-
formed as previously described (Lange et al. 1971), except that the vials were kept for 3 min in the boiling water bath and that centrifugation of the extract was performed for 20 min at 18,000 rev. min⁻¹ in a Sorvall centrifuge (rotor SS 34). All values presented are means of 8–26 parallels (4–13 independent experiments). Estimates of the standard errors (ranging from 4.0 to 1.5 per cent) are indicated in the figures by vertical bars if required for the argument.

Results and Discussion

Time Schedule. The onset of the inductive irradiation was chosen at 51 h after sowing even though the competence of the anthocyanin producing system has already begun to decrease at this stage of development (Wagner and Mohr 1966). The reason for the choice of time was that approximately 15 h of darkness were required after termination of a far-red light pretreatment before the accumulation rate of anthocyanin, due to the pretreatment, had dropped to zero (see Fig. 1 in Lange et al. 1967). For the sake of the highest possible precision of the kinetics of resumption of anthocyanin accumulation we had to wait until the effect of the pretreatment on the anthocyanin level had virtually ceased. Since the full competence of the anthocyanin producing cells was only reached 33 h after sowing and since a pretreatment with 3 h of far-red light was the minimum time period to overcome with certainty the initial lag-phase (see Fig. 1), the inductive light treatment could not be started before 51 h after sowing.

Initial Lag-Phase. The data in Fig. 1 suggest that the apparent lag-phase of anthocyanin formation in a so far dark grown seedling is of the order of 2 h (51 h after sowing, 25° C). This finding agrees with the data published previously for 48 h-old dark grown mustard seedlings (Lange et al. 1967).

Secondary Lag-Phase. A pretreatment with 3 h of far-red light was given between 33 and 36 h after sowing. This treatment causes considerable anthocyanin synthesis which is completed 15 h after termination of the far-red light. The 2nd treatment with far-red light commenced 51 h after sowing. It leads to a rapid increase in the amount of anthocyanin, reaching a plateau after 1.5 h (Fig. 2). The data suggest an apparent secondary lag-phase between 30 and 60 min, probably closer to 30 min (Fig. 2).

The Data Show: 1. The rate of increase of the anthocyanin content (presumably rate of synthesis) is much higher in the case of a secondary light treatment (in terms of absorbance, approximately 0.04 h⁻¹ vs. 0.007 h⁻¹). In fact, the initial rate in the case of a secondary light treatment is at least 6-fold as compared to the initial rate in the case of the first light treatment.

2. While the rate of anthocyanin accumulation is much higher in the case of secondary irradiation, accumulation ceases earlier than in the case of the first light treatment. Thus, not only the rate but also the kinetics of anthocyanin accumulation are strongly dependent on preirradiation.

3. The apparent lag-phase of anthocyanin induction seems to be considerably shortened in the case of a secondary light treatment but it is not close to zero as assumed previously (Lange et al. 1967, Fig. 1; 1971, Fig. 6). The previous conclusions were based on erroneous extrapolations of the experimen-