Short Communication

Effect of Photoperiod on Stomatal Opening in Vicia faba

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ABSTRACT

Stomatal apertures in darkness and subsequent average opening rates in light were measured in Vicia faba leaf discs throughout the nyctoperiods for plants grown on three light-dark cycles (8:16, 12:12, and 16:8). The time course of opening in darkness depended on the specific light-dark cycle with the maximum aperture always occurring at the time the lights normally went on. The light-induced opening rate was also maximum at the end of the nyctoperiod.

It was shown many years ago (2, 3) that the velocity of stomatal opening may differ depending on the time of day that the plants are illuminated. This phenomenon has been attributed to an endogenous rhythm affecting the stomatal opening rate (8) whereby the light-induced opening rate depends on the length of previous darkness. The width of the stomatal aperture in darkness is also influenced by an endogenous rhythm (9, 10, 14) with the commencement of dark opening occurring near dawn (8, 9). No positive correlation has been shown between the rhythms for opening rates and dark opening, nor have any experiments shown that the rhythms can be entrained to different photoperiods. Experiments with Vicia faba stomata reported here demonstrate entrainability and suggest a relationship between the aperture width in darkness and subsequent light-induced opening rate.

MATERIALS AND METHODS

Plant Material. Vicia faba L. var. Long Pod plants were grown in vermiculite in wooden flats (55 × 29 × 9 cm) (17 evenly spaced plants/flats), watered to field capacity daily and fertilized weekly with a solution of Hyponex plant food (Hydroponic Chemical Co., Inc., Copley, Ohio). The plants were grown in a growth chamber at 25 ± 0.5 C and 1.5 to 2 mw cm−2 white fluorescent light (YSI-Kettering model 65 radiometer).

Leaflets used for stomatal aperture determinations were within 1 day of reaching full expansion and from plants 3 to 5 weeks old. Preliminary experiments showed an age-dependent decline in the responsiveness of the stomata to light, thus making the above sampling procedure necessary.

Aperture and Opening Rate Determinations. Stomatal aperture widths and average light-induced opening rates were determined as previously described (6). Apertures in epidermal impressions were measured to the nearest 0.33 μm. Light-induced opening rates were determined using two groups of leaf discs. Impressions of one group were made just prior to a light treatment, whereas impressions of the other group were made subsequent to 20 min of 1.5 mw cm−2 white fluorescent light. The average of 120 aperture widths for each group was calculated and the difference between the average aperture of the groups was taken as the opening rate. The 20-min duration of illumination was about one-third of that required for the stomata to reach full opening under similar light and temperature conditions.

Photoperiod Experiments. Plants were grown under 8:16, 12:12, and 16:8 L:D cycles. At the end of the light period leaf discs were cut, floated abaxial side up on distilled H2O, and incubated in the dark in a second growth chamber at 28 ± 0.5 C. Apertures in darkness and opening rates were determined at intervals throughout the normal dark period and several hr into what normally would be the light period. Light-induced opening rates were determined at the same temperature used for dark incubation.

RESULTS AND DISCUSSION

The light-induced stomatal opening rates measured throughout the nyctoperiods for plants grown under 8:16, 12:12, and 16:8 L:D cycles are shown in Figure 1A. The course of recovery of the maximum opening rate is strongly influenced by the L:D cycle. For each cycle, the time at which the maximum light-induced opening rate occurs is at or near the time of peak light-illumination. Stomatal opening in darkness (Fig. 1B) responded in a similar manner with the largest aperture occurring at the end of the dark interval. The aperture width achieved in darkness was up to 30% of the maximum steady-state opening observed under white light.

The apparent dependency of the light-induced opening rate on the initial aperture is shown in Figure 2. Each point represents a single determination of the average initial aperture and subsequent opening rate taken from the data plotted in Figure 1. The relationship seems to be sigmoidal with an increase in the initial aperture width associated with a greatly increased potential for subsequent light-induced opening.

The dark opening represented in Figure 1B has previously been shown by Stalfelt (14) to be part of an endogenous rhythm. The increase in opening rate throughout the normal dark period (Fig. 1A) is assumed here to also be part of an endogenous rhythm similar to that found in several other species (7, 8). It is apparent from Figure 1 that V. faba stomata are strongly influenced by the L:D cycle such that they are adapted to open most rapidly at dawn. It was observed that individual leaves could be entrained to different L:D regimes after receiving three photoinductive cycles, thus indicating an ability of V. faba stomata to follow changes in photoperiod. This entrainability

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Abbreviation: L:D, light:dark.
A mechanism for the recovery of the opening rate during darkness might involve a progressive increase in the potential for photosynthesis during the nyctoperiod, thus causing either a greater rate of intercellular CO\(_2\) depletion or a lower absolute level of intercellular CO\(_2\) upon illumination. (Photosynthesis and CO\(_2\) compensation points have been shown to follow an endogenous rhythm in continuous light in several species [5, 11]). This explanation implies that the proposed CO\(_2\) depletion mechanism for steady-state stomatal opening (4, 13) is operative in controlling the opening rate also. This is probably not the case for *V. faba* because the CO\(_2\) compensation point (a measure of the minimum intercellular CO\(_2\) concentration) did not change when leaves were sampled throughout a 12-hr nyctoperiod (unpublished results) and the quantum requirement for saturation of the opening rate response is less than that for the light compensation point (6).

An alternative explanation proposes a dependency of the opening rate on the size of the initial aperture. Such dependency may be explained by a dark-associated metabolic change in the quality and quantity of osmotica and osmotica precursors. If malic acid generation is required for stomatal opening in the light (1, 12), the pool sizes of the more immediate malate precursors may increase during darkness. The dark opening may be attributed to a generation of certain precursor molecules which are osmotically active. Moreover, the rate of opening in the light would be greater for those stomata with the larger, more immediate precursor pools, and this rate would approach a maximum as the enzymes become substrate-saturated. This hypothesis must include a relatively rapid recycling of the light-generated osmotica back to distant precursors upon cessation of light because after only 2 hr of darkness, the stomata were “closed” and the opening rate was slow.

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