BIOLOGIA PLANTARUM (PRAHA)

31 (5) : 344–353, 1989

Changes in Transorgan Electric Potential in *Chenopodium rubrum* during the Course of Photoperiodic Flower Induction

L. ADAMEC* and J. KREKULE

Institute of Experimental Botany, Czechoslovak Academy of Sciences,
Ke dvoru 15, 166 30 Praha 6, Czechoslovakia

Abstract. Electrophysiological processes were investigated in reception organs of photoperiodism in a model short-day plant, *Chenopodium rubrum* L. (selection 374), within the inductive cycle for flowering. Transorgan (surface) electric potential (E_{tr}) was measured as a potential difference between the first leaf surface and the roots of an intact plant, and between the surface of an excised leaf and the petiole base. The time-course of E_{tr} in intact plants showed irregular, or partially regular, oscillations within both phases of the inductive cycle and under continuous light. The highest amplitudes were during the postinductive light period. E_{tr} in excised leaves behaved practically in the same way as in intact plants. The E_{tr} oscillations were localized in leaves. In general, no electrophysiological changes were found in the reception organs within the inductive cycle which could be correlated with the formation and transport of floral stimulus, or with the attainment of an induced state. The results indirectly support the idea that the floral stimulus is chemical in nature.

The nature of the floral stimulus poses a question crucial for understanding the regulation of photoperiodically induced flowering. Most indirect evidence supports the florigenic theory of flowering (VINCE-PRUE 1975), but predominantly negative results in the search for a chemical signal have led to several alternative suggestions, one of which concerns a possible biophysical (especially electric) nature of the stimulus (GREPPIN *et al.* 1978, PENEL *et al.* 1985). However, initiation of an electrophysiological signal in the receptor organs of photoperiodic induction has not yet been proved either in the long-day plant, spinach (MONTAVON 1984), or in the short-day plant, *Chenopodium rubrum* (ADAMEC and KREKULE 1989). The changes in electrophysiological parameters, such as membrane potentials (E_m), which occur in spinach plants during the induction phase apparently do not act as a floral stimulus (GREPPIN *et al.* 1973, GREPPIN and HORWITZ 1975, MONTAVON 1984). Such changes may result from photosynthetic electron flow, from alteration

Received November 27, 1988; accepted January 29, 1989

**Present address:* Czechoslovak Academy of Sciences, Institute of Botany Section of Plant Ecology, Dukelská 145, 379 82 Třeboň, Czechoslovakia.

of the ratio of one phytochrome form to the other, or from petiole electrical conductivity, *etc.*

Transorgan (surface) electric potentials have often been measured in relation to plant movement and development (*e.g.* PICKARD 1973, GREPPIN *et al.* 1973, JAFFE and NUCCITELLI 1977). The aim of the present study was to measure E_{tr} within the photoperiodic inductive cycle for flowering in intact short-day plants of *Chenopodium rubrum* or in their excised leaves.

MATERIALS AND METHODS

Cultivation of Plants

Qualitative short-day plants *Chenopodium rubrum* L. of selection 374 were used in all experiments. They were 14–17 days old. Germination and cultivation methods follow ULLMANN *et al.* (1985) and ADAMEC and KREKULE (1989). Selected plantlets were cultivated in small plastic vessels using half-strength Knop's solution, with or without perlite, under non-inductive conditions of continuous fluorescent illumination at 20 ± 1 °C. Small-volume growth chambers were employed.

Measurement of E_{tr}

Transorgan potentials were measured in two ways. First, by measuring E_m and E_{tr} in the same plant simultaneously (for details see ADAMEC 1989). The tip of the first leaf was fixed in a flow chamber (composition of the flowing solution was 1 mM KCl; 0.1 mM $CaCl_2$) *ca.* 3 h before measurement and the measuring calomel electrode was connected with the nutrient solution surrounding the roots. The characteristic property of such a connection is the opposite E_{tr} polarity as compared with the usual placement of the measuring electrode into the medium around the leaf (*cf.* HARTMANN 1975).

Secondly, E_{tr} was measured in the more usual way by means of a salt bridge contact on the plant surface (HARTMANN 1975). The *C. rubrum* plants in their original cultivation vessel were placed in a plexiglass chamber before measurement. Air humidity was kept high in the chamber to prevent the electrolyte drying at the contact point. Temperature was kept at 20 ± 0.2 °C. E_{tr} was measured as a potential difference between the leaf surface and the nutrient solution around roots in which an earthed reference calomel electrode was placed. The contact of the salt bridge (a glass capillary filled with 50 mM KCl through which a thread was passed) with plant surface was ensured through a droplet of 50 mM KCl in 0.8 % agar gel. The droplet of agar was placed on the middle of the first leaf lamina, or on the hypocotyl stump of a decapitated plant. In some cases, E_{tr} was measured in an excised first leaf whose petiole base was immersed in the nutrient solution. All E_{tr} measurements were taken at least 3 h after completing the salt bridge contacts.

The measuring equipment (mV-meters, recorders) and the illumination used are described by ADAMEC (1989). The length of the inductive dark period was kept at 13 h, *i.e.* optimal for flowering (ULLMANN *et al.* 1985).

Measurement of Ion Leakage

To investigate the changes in cell membrane permeability in reception organs, ion leakage was measured during photoperiodic flower induction. The first leaf was excised either at the petiole or at its lamina base, and its cut surface was immersed in 4 ml of distilled water. This was contained in a small glass chamber, stirred magnetically and kept at 20 ± 0.2 °C. The conductance was measured both by means of two platinum electrodes and with a low-frequency conductometer, and

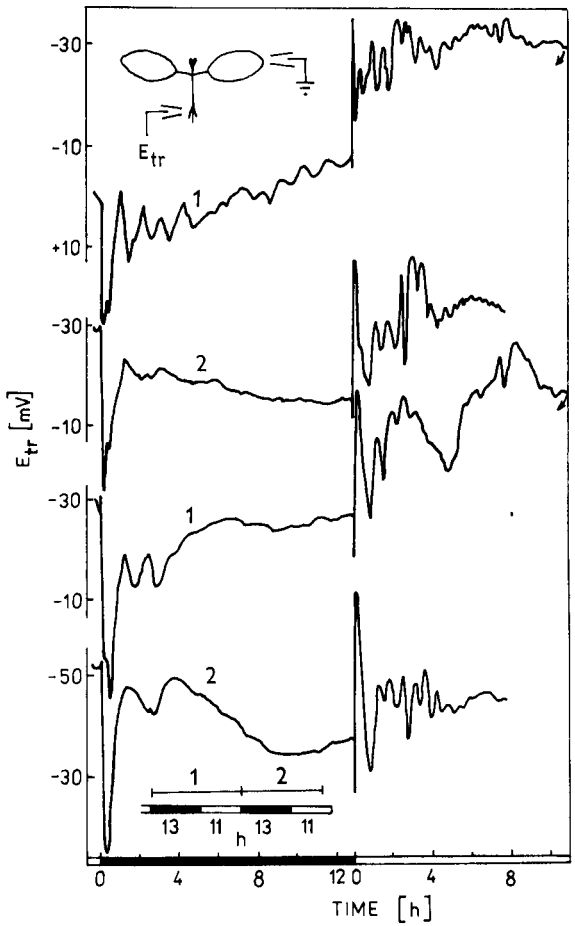

Fig. 1. Time-course of E_{tr} in intact *C. rubrum* plants during two inductive cycles. E_{tr} was measured between roots (measuring electrode) and first leaf (earthed). 1, first inductive cycle; 2, second inductive cycle. Full line – darkness; double line – light. Results of two measurements are presented.

outputs were automatically recorded. After the cut area of a leaf had been pre-rinsed. the leaf was inserted in the conductance chamber and the light was switched off simultaneously. Ion leakage was measured during 13 h of dark period and thereafter within even shorter periods of light and darkness. The illumination of the leaf before measurement, and during switching on the light, was the same as in E_{tr} measurements. Ion leakage from the leaf was expressed as net conductance of a KCl solution: mmol KCl kg^{-1} (fresh mass).

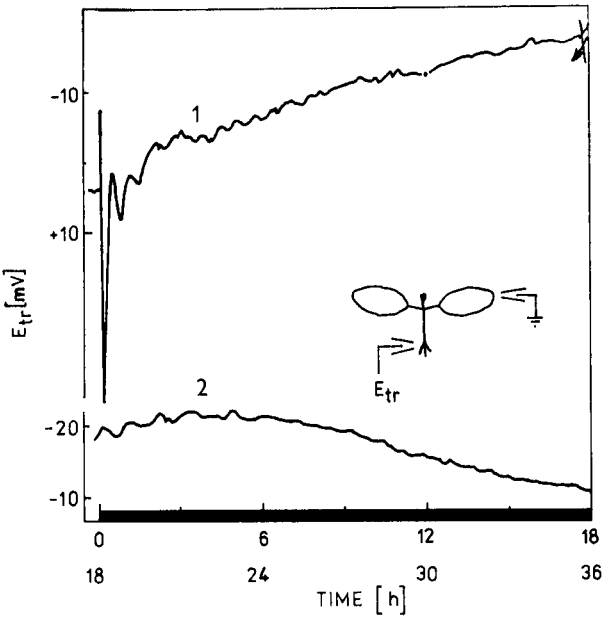

Fig. 2. Time-course of E_{tr} in intact *C. rubrum* plant during 36 h of dark period. 1, 0–18 h of darkness; 2, 18–36 h of darkness. For the other see Fig. 1.

RESULTS

The time-course of E_{tr} in intact plants measured in the flow chamber showed both regular and irregular oscillations within two inductive cycles (see Fig. 1). Changes in the light régime triggered marked transient E_{tr} changes, such as damped oscillations. Oscillations were usually more noticeable during the light period than during the dark one. Large individual differences were found in plants, and spontaneous oscillations were not always apparent. The second inductive cycle did not differ from the first one. No change in E_{tr} has been observed within the inductive cycle that would indicate a correlation with either induction or an induced state. The time-course of E_{tr} during 36 h of darkness was not related to the endogenous rhythmicity of flowering (Fig. 2). The oscillations of E_{tr} nearly disappeared after 25 h

of darkness. The time-course of E_{tr} in continuous illumination also showed a pattern of irregular oscillations (see Fig. 3). The amplitudes of E_{tr} oscillations were rather irregular during individual phases of the inductive cycle. There existed no obvious relationship between the period length of the oscillations and the course of induction.

A preliminary evaluation of the period length of fairly regular E_{tr} oscillations showed that the mean length was 31.2 min (n = 51 oscillations; S.E.M. = 2.0) in continuous light, 53.4 min (n = 69; S.E.M. = 2.0) during induction of the first

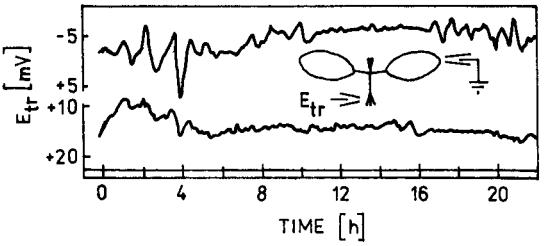

Fig. 3. Time-course of E_{tr} in intact *C. rubrum* plants during 22 h of continuous light. For the other see Fig. 1.

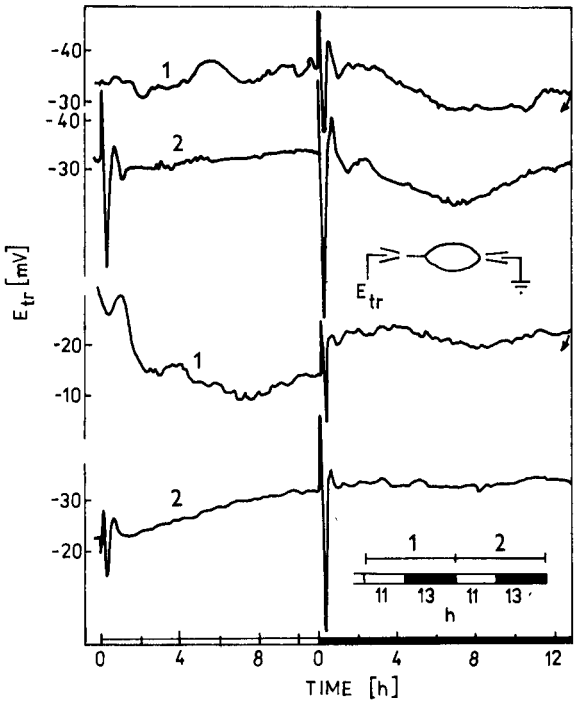

Fig. 4. Time-course of E_{tr} in excised first *C. rubrum* leaves during two inductive cycles. E_{tr} was measured between the cut off part of the petiole (measuring electrode) and the leaf tip (earthed). For the other see Fig. 1.

cycle, and 37.1 min (n = 58; S.E.M. = 1.0) during the postinductive light period. These values differ significantly one from another (t-test: P = 0.01). To study the processes occurring only in the reception organs, E_{tr} was measured in excised first leaves. The E_{tr} time-course in excised leaves during two inductive cycles was very similar to that in intact plants, but the oscillations were usually less distinct (Fig. 4). The pattern of E_{tr} in the excised first leaves with their tip cut off (*ca.* 1 mm) showed that the removal of a cuticular barrier did not affect oscillations (Fig. 5).

E_{tr} measurement with the salt bridge method confirmed the results obtained using the flow chamber. Again, irregular or partially regular oscillations in both intact plants and excised first leaves have been assessed (see Fig. 6). However, the hypocotyl stump was not affected by the changes in light régime or by a dark period. The distinct oscillatory pattern of E_{tr} has only seldom been obtained. No correlation was found between the E_{tr} fluctuation in an intact plant and the endogenous rhythm of flowering during 30 h of the dark period (Fig. 7).

The leakage of ions from an excised leaf was very low, and followed an approximately exponential course (Fig. 8). The ion leakage became nearly linear

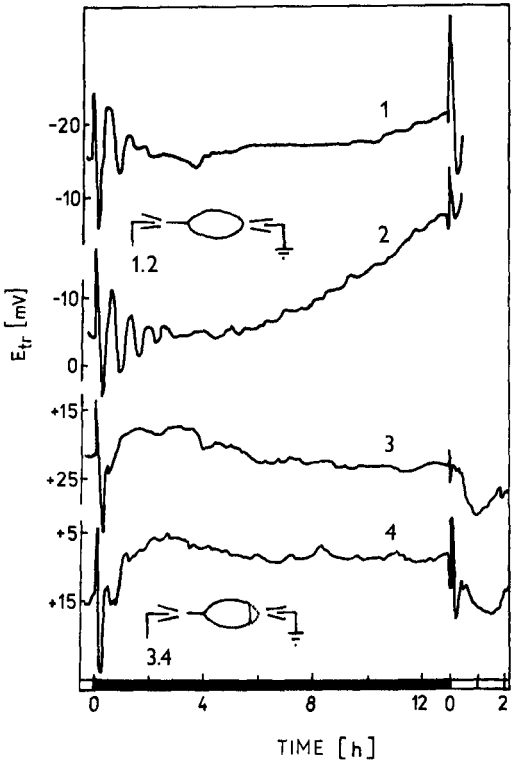

Fig. 5. Time-course E_{tr} in excised first *C. rubrum* leaves during inductive dark period. 1, 2, undamaged leaves; 3, 4, leaves with tip cut off. For the other see Figs. 1 and 4.

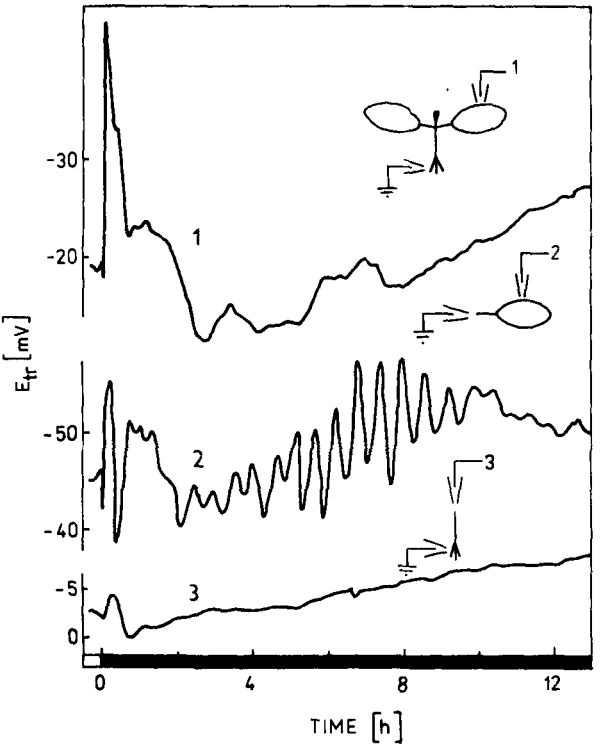

Fig. 6. Comparison of the E_{tr} time-course in intact *C. rubrum* plant (1), in excised first leaf (2) and hypocotyl stump (3). E_{tr} was measured by means of a salt bridge contact on the plant surface in a moist chamber. Full line – darkness; double line – light.

after only 3 h of the dark period. Alternation of light and darkness did not increase the ion leakage significantly. Cutting the leaf lamina which provoked an action potential of 20–70 mV (ADAMEC, unpublished results) did not affect the ion leakage either.

DISCUSSION

Both ways of measuring E_{tr} in intact plants integrate the bioelectric cell activity in roots, stem, petiole and leaf lamina. The leaf itself is responsible for the typical course of E_{tr} in an intact plant during the inductive cycle, as the hypocotyl stump exhibits only limited activity (*cf.* Figs. 1–6). Measurement values of E_{tr} by the flow chamber method were much more stable than those obtained using salt bridge contacts. In the latter method, frequent shifts of the salt bridge on the leaf surface occurred together with regular drying of the electrolyte at the point of contact. The results influenced by contact shifts were not taken into consideration. A shift of the

salt bridge on the leaf surface developed a potential difference of tens of mV. It was apparently localized on the cuticle and declined to a stable level within 3 h.

Both ways of E_{tr} measurement fully confirmed the conclusions drawn from the E_m measurements: that the photoperiodic induction and the induced state were not reflected in the electrophysiological changes observed (ADAMEC and KREKULE 1989). The phases of the inductive cycle did not differ in their patterns of E_{tr} fluctuation (*cf.* Figs. 1–4). The flow chamber measurements of E_{tr} showed a gradual mean hyperpolarization of 15 mV during 13 h of inductive dark period (ADAMEC 1989), but its development did not seem to be related to photoperiodic induction. As the same E_m hyperpolarization in leaf mesophyll cells occurred simultaneously (ADAMEC 1989), this change in E_{tr} cannot be explained as a change in the electrical polarity of leaf cells. The formation of electrical polarity in organs of higher plants occurs mainly with respect to tropic movements, and in the differentiation of some unicellular objects (JAFFE and NUCCITELLI 1977). It could theoretically represent a condition for the transport of the floral stimulus.

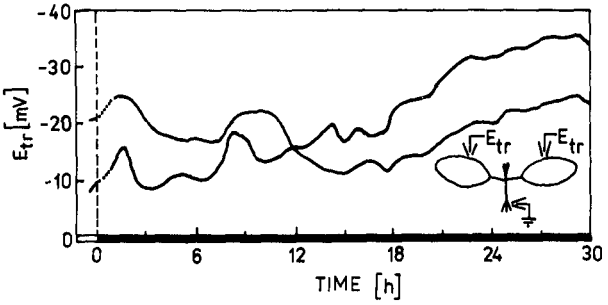

Fig. 7. Time-course of E_{tr} in intact *C. rubrum* plant during 30 h of dark period. E_{tr} was measured by means of a salt bridge contact on both first paired leaves. E_{tr} changes at the beginning of the dark period were omitted for clarity. For the other see Fig. 6.

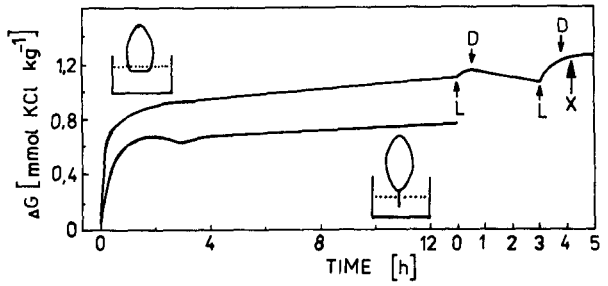

Fig. 8. Conductometric measurement of ion leakage from first *C. rubrum* leaf to distilled water during 13 h of inductive dark period and switching of light and darkness. Lower curve – leaf contacting water only through cut off area of the petiole; upper curve – leaf contacting water only through cut off area of the lamina. L, light; D, darkness; X, cutting to the apical part of leaf lamina (*ca.* 1.5 mm).

We considered the possibility that the processes of induction might be related to the high-frequency E_{tr} oscillations. The periods and especially the amplitudes of oscillations were rather irregular and not related to the course of dark period. However, the phases of the first inductive cycle significantly differed with respect to their oscillation periods. Thus, it seems that these changes in E_{tr} rhythmicity do not reflect photoperiodism of flowering but rather the effect of light and darkness alone. The oscillations of E_{tr} occurred in the excised leaves even after the contribution of a cuticular potential difference had been reduced by cutting off the leaf tip. This indicates that the oscillations of E_{tr} (and E_m) cannot be due to the rhythmicity in opening and closing of stomata (BOWLING *et al.* 1986) but rather to the rhythmicity in membrane ion fluxes.

No changes in membrane permeability for ions correlated with photoperiodic induction in excised first leaves (see Fig. 8). SEN GUPTA *et al.* (1981) obtained similar results with *Xanthium* leaf discs. They proved that phytochrome regulates membrane permeability for ions, too. The switching of light and darkness only led to negligible conductance changes (Fig. 8) mainly due to temperature shifts.

Thus, summarizing the methods used, electrophysiological changes related to induction of flowering have not been found in *C. rubrum*. The same conclusion was also drawn by MONTAVON (1984) for the long-day plant spinach. Therefore, it is unlikely that floral stimulus possesses biophysical character and the theory of its chemical nature is indirectly substantiated.

REFERENCES

ADAMEC, L.: The comparison between membrane and transorgan electric potentials in *Chenopodium rubrum*: **The methods – Biol. Plant. 31** : 327–335, 1989.

ADAMEC, L., KREKULE, J.: Changes in membrane potential in *Chenopodium rubrum* during the course of **photoperiodic flower induction. – Biol. Plant. 31** : 336–343, 1989.

BOWLING, D. J. F., EDWARDS, M. C., GOW, N. A. R.: Electrical currents at the leaf surface of *Commelina communis* and their relationship to stomatal activity. – J. exp. Bot. **37** : 876–882, 1986.

GREPPIN, H., AUDERSET, G., BONZON, M., PENEL, C.: Changement d'état membranaire et mécanisme de la floraison. – Saussurea **9** : 83–101, 1978.

GREPPIN, H., HORWITZ, B. A.: Floral induction and the effect of red and far-red preillumination on the light-stimulated bioelectric response of spinach leaves. – Z. Pflanzenphysiol. **75** : 243–249, 1975.

GREPPIN, H., HORWITZ, B. A., HORWITZ, L. P.: Light-stimulated bioelectric response of spinach leaves **and photoperiodic induction. – Z. Pflanzenphysiol. 68** : 336–345, 1973.

HARTMANN, E.: Influence of light on the bioelectric potential of the bean (*Phaseolus vulgaris*) hypocotyl hook. – Physiol. Plant. **33** : 266–275, 1975.

JAFFE, L. F., NUCCITELLI, R.: Electrical controls of development. – Annu.Rev. Biophys. Bioeng. **6** : 445–476, 1977.

MONTAVON, M.: Lumière et biopotentiels chez l'épinard (*Spinacia oleracea* L. cv. Nobel): phytochrome, communication interorganique et floraison. – PhD. Thesis, No. 2099. Univ. Genève 1984.

PENEL, C., GASPAR, T., GREPPIN, H.: Rapid interorgan communications in higher plants with special reference to flowering. – Biol. Plant. **27** : 334–338, 1985.

PICKARD, B. G.: Action potentials in higher plants. – Bot. Rev. **39** : 172–201, 1973.

SEN GUPTA, D. N., MOITRA, D., SEN, S. P.: Phytochrome controlled leaching of metabolites from *Xanthium* leaf discs during photoperiodic induction. – Plant. Sci. Lett. **23** : 321–325, 1981.

ULLMANN, J., SEIDLOVÁ, F., KREKULE, J., PAVLOVA, L.: *Chenopodium rubrum* as a model plant for testing the flowering effects of PGRs. – Biol. Plant. **27** : 367–372, 1985.

VINCE-PRUE, D.: Photoperiodism in Plants. – McGraw-Hill, London–New York 1975.

BOOK REVIEW

GUTSCHICK, V. P.: A FUNCTIONAL BIOLOGY OF CROP PLANTS. – Timber Press, Portland 1987. 230 pp., US $ 39.95.

Most of the recent advances in the plant physiological ecology are based on the research of wild plants which ensure the long-term survival of the species and the genus. The book reviewed expands the "functional biology" viewpoint to crop plants, integrating the physiology, morphology, phenology and ecology.

The matter is divided into five chapters. In the introductory one "Functional Biology and Plant Strategies" the author explains the principles of plant functional biology, summarizes the requirements of plants in their growth and functions, sums up adaptive strategies, calculates the costs and benefits, tries to quantify the risks and management, deals with effectiveness and limitations of strategic adaptations and enumerates additional strategic considerations for the biotic environment. The second chapter on "Mineral Nutrition" is devoted to essential elements in the ecosystem and their availability in soil, to physiological limitations of internal transport and use, to severe imbalances between nutrients, to toxicity of nonnutrients, mainly metals, to consequences of nutritional challenges and to costs and benefits of adaptive responses. The next chapter dealing with "Photosynthesis" briefly reviews the basic photosynthetic reactions, CO_2 and water exchange, CO_2 and photon transport in canopy, units and determinants of photosynthesis, with costs, benefits and challenges of energy and soil resources, consequences of challenges to photosynthesis, adaptive responses and their costs and benefits. The fourth chapter concerning "Water Relations" is again introduced by a short review of water in plant structure and function, continues with a brief review on water potential and terminology, water transport in soil, water uptake by roots, transport in plant, water consumption in photosynthesis and analyses of cost-benefit in water use. Drought, flooding and salinity stresses are dealt with from the viewpoint of challenges, consequences of challenges, costs and benefits of adaptive strategies. In the concluding chapter "Integrative Processes" new features in long-term coordination of all resource uses, plant reproduction, seed germination, morphogenesis and the control of biotic interactions are discussed. The volume is supplemented by lists of 479 references, 150 symbols and a voluminous (10 pages) Index.

The book is intended to advanced undergraduate and postgraduate students of plant physiology, genetics and breeding and to all those interested in the functional approach in general biology.

JARMILA SOLÁROVÁ (*Praha*)