

REVIEW

Analysis of phenotypic responses of plants to changes in the environment in terms of stress and adaptation

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Key-words: life cycle, monocarpy, perception/transduction signal pathway, phenotypic plasticity, polycarpy.

INTRODUCTION

Plants respond to the environment in two ways. First, individual plants may respond by changes in morphological, physiological and biochemical characteristics. This phenomenon is often called phenotypic plasticity (Bradshaw 1965).

In addition to this short-term reaction, populations of plants may change in genetic composition; genotypes with a high fitness for the environment in question will be maintained, while less suitable genotypes will disappear. In a more or less specialized and predictable environment genetic differentiation may lead to maintenance of only a limited number of genotypes, with a high fitness for the environment in question. In a more variable and less predictable environment, a larger genetic variation will be maintained in the plant population.

The two responses of plants to the environment, genetic differentiation and phenotypic plasticity of individual plants, have been studied in many species and in much detail. This paper limits itself to the latter phenomenon, even though mutual interactions between the two responses cannot be excluded (see, for example, Bradshaw 1965).

DIRECT AND INDIRECT RESPONSES OF INDIVIDUAL PLANTS TO THE ENVIRONMENT

Plants represent organisms which are (relatively speaking) open to the environment. The open structure of most plants is already expressed in its numerous branching of elements

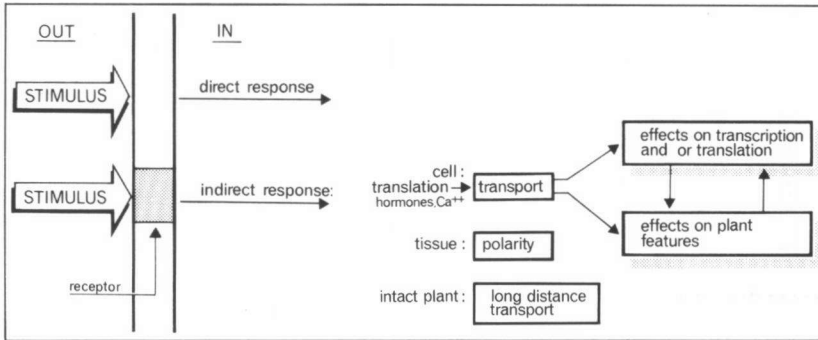


Fig. 1. Direct and indirect response of plants to the environment as part of phenotypic plasticity (adapted from Kuiper & Kuiper 1988).

in the shoot as well as in the root system. As autotrophic organisms, plants respond directly to light via the process of photosynthesis. Furthermore, another environmental factor, such as temperature, may exert direct effects on functioning of the individual plant, since temperature regulation by the plant is quite limited. Mechanical factors such as air velocity in the atmosphere and impedance by hard soil layers present further examples of direct environmental effects on plants.

Indirect effects of the environment on plants are defined as responses of the plant, in which the environmental signal is perceived by a receptor and consequently transduced to the genetic apparatus, eventually resulting in changes in genetic expression (Fig. 1). A pigment such as phytochrome and hormonal receptors may function in such perception-transduction pathways, in combination with secondary messenger systems such as the phosphatidyl inositol cascade and cytoplasmic calcium and proton concentrations.

Next to the expression of the environmental signal at the plant cell level, similar responses are evident at higher levels of organization, i.e. tissue, organ and the intact plant. Decreased availability of dissolved CO_2 in the aquatic environment will lead to a pH-polarity in the submerged aquatic *Potamogeton lucens*, with a light-induced alkalization of the water near the upper leaf surface and an acidification of the water near the lower surface; the latter leads to an increased availability of CO_2 , due to a shift in the $\text{HCO}_3^-/\text{CO}_2$ equilibrium (Elzenga & Prins 1989). Similar polarity phenomena may possibly be expected in mineral nutrient uptake and translocation processes occurring in plant roots.

Separation of perception of the environmental signal and its genetic expression is evident in the daylength response of many plant species: perception takes place via phytochrome in the leaf and transduction occurs via an as yet unknown chemical or physical messenger to the apical meristem. A similar example is the regulation of shoot growth via signals in the root environment (e.g. a limiting mineral nutrient supply, see later).

Many studies on the response of physiological processes of plants to an environmental factor start with the assumption that either direct or indirect effects alone will be responsible for the observed response. Temperature responses are considered to be direct, even though indirect responses are evident in dormancy and vernalization; in the latter case a receptor is possibly formed by low temperature-induced aggregation of proteins (Levitt 1962, 1980). Responses of plants to mineral nutrition are supposed to be determined by setpoint regulation of cytoplasmic ion levels (Clarkson 1985), even though Itai

and Vaadia (1965) have suggested that cytokinins may be important in salt-induced shoot growth reduction. In studies of the plant response to pollutants, direct effects are automatically assumed to be responsible for the observed effects: nobody will reflect on the possibility that ozone may modulate the plant response via a receptor/transduction pathway! In my opinion, a careful consideration of the possibilities of both direct and indirect responses should precede each experiment in this field.

EVALUATION OF THE RESPONSE OF PLANTS TO THE ENVIRONMENT

In theory, responses of plants to the environment can be positive, neutral or negative (Kuiper 1985).

Positive: the response of the plant has adaptive value.

Neutral: the reaction of the plant indicates a change in metabolism, without further significance for the fitness of the plant.

Negative: the reaction of the plant indicates one or several degradative processes in the metabolism of the plant. This reaction is often called stress.

In practice, the choice between the three possibilities mentioned above can sometimes be quite difficult and suspect to justified criticism. Larcher (1987) defines adaptation as follows: stress conditions lead to an initial destabilization of functions, followed by normalization and improved tolerance. If the limit of tolerance is exceeded and the capacity of the plant is overtaxed, permanent damage will occur. In order to elucidate the various obstacles involved in a correct interpretation of the observed plant responses, the following topics will be dealt with:

1. the experimental design for analysis of plant responses to the environment;
2. the effect of the life cycle on phenotypic plasticity; differences between monocarpic (mainly annuals and biannuals) and polycarpic plants; and
3. further evaluation of the importance of the perception/transduction signal pathway in phenotypic plasticity.

Experimental design

1. In general a series of genotypes, inbred lines, or varieties have to be screened for their response to an environmental change. If possible, the selected series should cover a wide range of sensitivity for the studied environmental change. Studied parameters for the response are the degree of response, and the amount of time required for the response. Figure 2 gives an example of the response of the shoot to root ratio of four inbred lines of *Plantago major* to changes in the level of mineral nutrition, from high to low, and from low to high level of nutrition (Kuiper 1984; Kuiper & Kuiper 1988), showing no response (inbred line A), a response only during the pre-treatment (not shown in figure), when the plants were very young (line B), a partial response (line C) and a complete response to the level of the control plants which were kept permanently to the mineral nutrient level of the new condition (line D).

2. A second possibility is to test two genotypes under several (more than two) conditions in order to get a more quantitative analysis of the response to the environment, than the two conditions given in Fig. 2. This is especially important when the classical minimum–optimum–maximum response, a stimulation at low concentration and an inhibition at higher concentration, may be expected. Halophytes form a good example for such

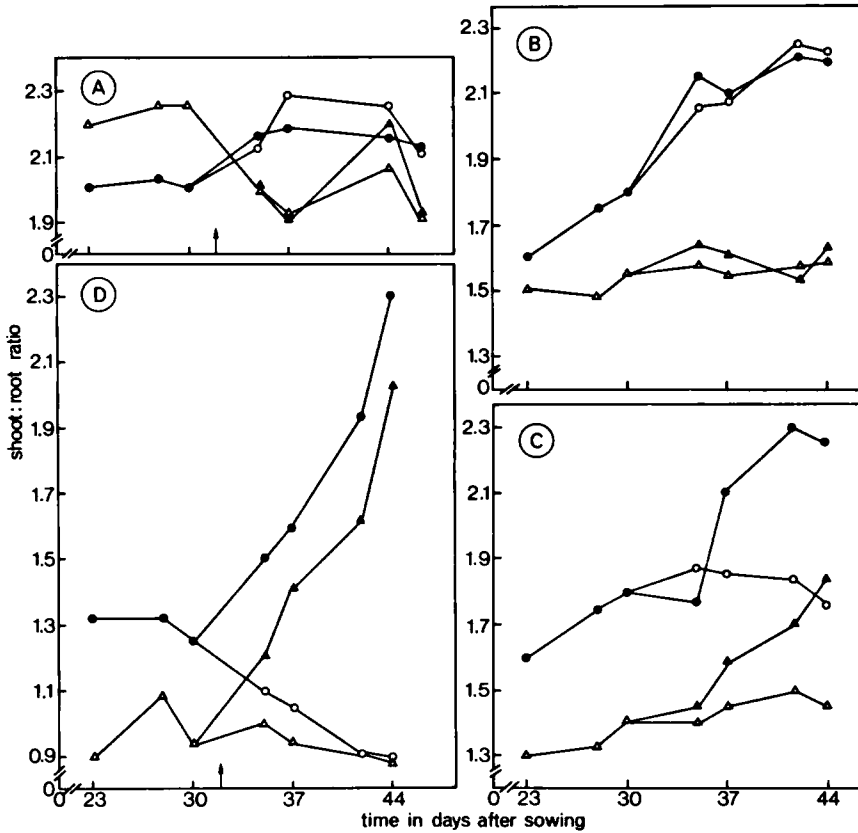


Fig. 2. Shoot to root ratio of four inbred lines of *Plantago major* as grown on a full nutrient solution (●) and a diluted nutrient solution (△). At day 32 part of the plants were transferred from a dilute to a full nutrient solution (▲) and others from a full nutrient solution to a diluted one (○). See text for further details. (After Kuiper 1984.)

behaviour: stimulation of growth at low salinity and growth reduction at high levels of salinity.

For an analysis of the response of plants to temperature, a large number of temperatures should also be investigated. The so-called 'broken curves' in Arrhenius plots of biochemical and biophysical reactions yield transition points, indicating physical changes in membrane properties, which in turn may yield information on genotype-dependent adaptive responses to the environment (Vigh *et al.* 1987). Phospholipids of the cold-sensitive wheat variety (Fig. 3b) show crystallization, indicating non-functioning in the membranes, at -6°C ; crystallization occurs at -16°C in the hardy variety (Fig. 3a).

A distinction between positive and neutral responses of two wheat varieties of contrasting frost hardiness to the effect of low temperature hardening (4 weeks at 1.5°C) was shown by Vigh *et al.* (1985). Hardening of the plants resulted in increased low temperature tolerance of light-stimulated proton uptake of isolated chloroplast thylakoids in the frost-resistant variety Miranovskaya but not in the frost-sensitive variety Penjamo. Neutral reactions, i.e. a similar response of both varieties to hardening, occurred for the following thylakoid lipid parameters: total phospholipids, phospholipid fatty chain lengthening to

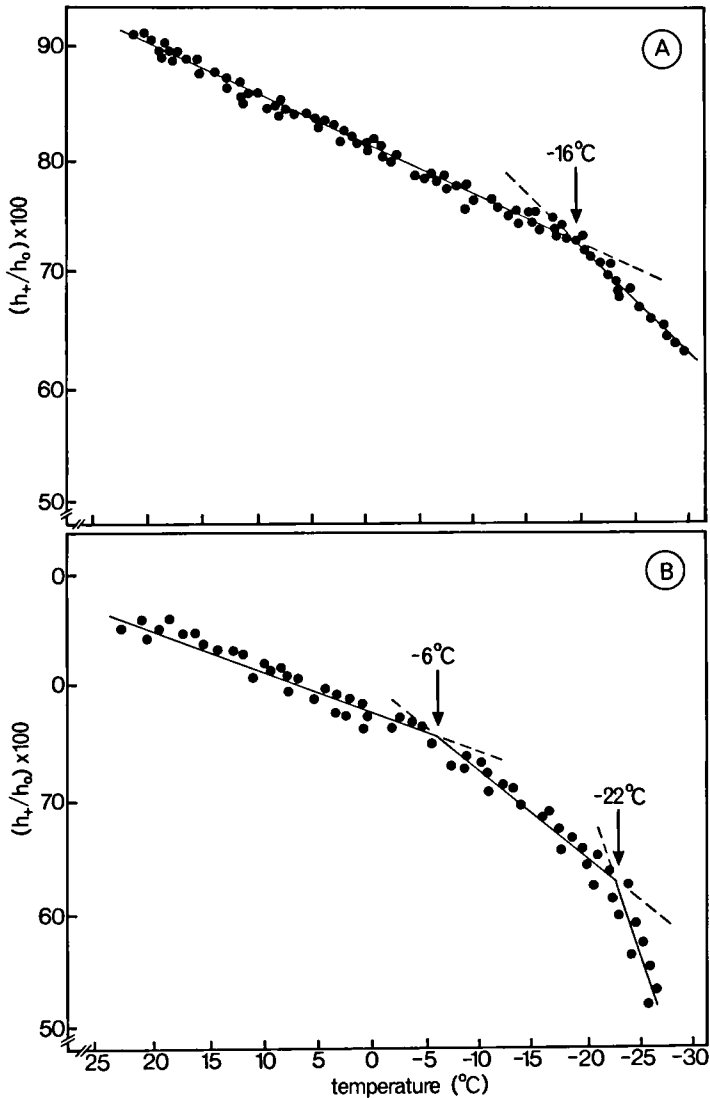


Fig. 3. Temperature profiles of spin-label motion, h_+/h_0 , measured in purified phospholipids obtained from leaves of hardened Miranovskaja (a) and sensitive Penjamo (b) wheat. (After Vigh *et al.* 1987.)

20 and 22 C-atoms, and increased phospholipid fatty acid desaturation, up to six double bonds (Table 1). A similar neutral reaction of fatty acid unsaturation upon low temperature hardening of wheat had already been observed by De la Roche *et al.* (1975).

Positive reactions, i.e. changes in thylakoid lipids upon hardening that only occurred in the hardy variety Miranovskaya, were observed for the following thylakoid glycolipid parameters: maintained glycolipid level, increased galactosylation of mono- into digalactosyl diglyceride, decreased mono-galactosyl diglyceride, increased sulpholipid fatty acid chain lengthening up to 20 and 22 C-atoms, and increased sulpholipid fatty acid unsaturation, up to four and six double bonds. All these factors may possibly contribute to low temperature tolerance of light-dependent proton uptake of chloroplast thylakoids.

Table 1. Neutral reactions (similar responses of both varieties to hardening) and positive reactions (differential response to hardening) of fatty acid conjugates of polar lipids (weight %) from thylakoids of hardened and unhardened wheat plants of Miranovskaja (resistant) and Penjamo (sensitive) varieties

Growth condition	Variety				Reaction
	Miranovskaja		Penjamo		
	Unhardened	Hardened	Unhardened	Hardened	
PC, 18:2	29	9	21	4	Neutral
PC, 18:3	20	4	28	4	Neutral
PC, 20:3	2	14	2	16	Neutral
PC, 20:4	5	21	6	17	Neutral
MGDG, 16:0	3	1	2	7	Positive
MGDG, 18:3	85	97	85	91	Positive
DGDG, 16:0	16	5	9	8	Positive
DGDG, 18:3	77	91	86	87	Positive
SL, 16:0	24	14	30	37	Positive
SL, 20:2	—	6	—	—	Positive
SL, 20:3	1	10	1	—	Positive
SL, 22:4	2	17	2	—	Positive

PC, phosphatidyl choline; MGDG, mono-galactosyl diglyceride; DGDG, di-galactosyl diglyceride; SL, sulpholipid; fatty acids are noted by number of C-atoms and double bonds respectively. After Vigh *et al.* (1985).

3. A third analytical problem concerns the fact that more than one environmental factor may contribute via hardening to a positive response of the plant to a change in the environment. Frost resistance of plants may be increased by a low temperature pre-treatment, a period of short days, drought and even salinity (Bervaes *et al.* 1978; Huitema *et al.* 1982). Drought may also contribute to low temperature tolerance in cucumber (Bulder *et al.* 1989). When more than a single environmental factor is involved, the experiment should include a multifactorial design. Such an experiment has been carried out for cold hardiness of pine and apple trees (Bervaes *et al.* 1978). Trees were grown at two temperatures, 0 and 20°C, and two daylengths, 9 and 14 h. All 16 possible combinations were investigated by transfer of the trees from the original condition to each of the other conditions. Understandably, more than two factors are, in practice, impossible to evaluate. In the above example it was clear that a distinction should be made for a single and a dual environmental change; for increased frost resistance a short-day treatment should precede a low temperature treatment in order to induce the signal system for frost resistance in a proper way, thus simulating natural processes (Till 1956; Larcher 1987). For further details see later.

The same reasoning also applies for negative responses: synergistic effects of frost and air pollution have been described. The same applies for combinations of air pollutants such as ozone and SO₂.

Finally, the adaptive value of a physiological response to an environmental factor can be studied by making crosses between two extreme genotypes, tolerant and sensitive to the specific environment. The plants of the F1 should show the same relationship between plant response and environment as that observed for the whole range of studied genotypes. The closer the fit the greater the genetic linkage between the studied plant

response and tolerance to the specific environment, assuming that major genes and gene modification do not play a decisive role in heritability.

The effect of the life cycle on phenotypic plasticity; differences between mono- and polycarpic plants

The four inbred lines of *P. major* in Figure 2 showed a large variation in phenotypic plasticity, from practically rigid to completely flexible. Populations of this species in the field were also characterized by differences in plasticity (Lotz 1989).

Differences in phenotypic plasticity, in degree and in response time, may be due to:

1. differences in number of receptors and sensitivity of receptors for the environmental signal. An induction time may be present, or the receptor may only be responsive within a limited range of the signal;
2. the transduction system differs between the genotypes, and may be, for example, expressed as differences in polarity or in long-distance transport; and
3. genetic expression may be different, leading to differences in physiological processes; e.g. more than a single copy of the gene may be involved in expression.

At the same time the mechanisms mentioned above are involved in developmental changes during the life cycle. Clearly the same genes may be involved in development as in plasticity, even though both processes may be regulated differently (Bagchi & Iyama 1983).

In several annual plants phenotypic plasticity is mainly restricted to the vegetative phase, it is practically absent in the reproductive phase. Plasticity during the vegetative phase enables the plant to perform as well as possible for reproduction (Horikawa 1980; Kataoka *et al.* 1981). The *P. major* genotype with the highest degree of plasticity (Fig. 2) had an annual life cycle; the more rigid genotypes were characterized by a perennial, longer life span (Kuiper 1984). In *Phleum arenarium*, plasticity is present in the reproductive phase; nutrient deficiency and drought may accelerate flowering in this annual with its shallow root system, a clear adaptive response (Sorensen 1954; Ernst 1983). A similar example has been given by Vasek (1977) for *Clarkia*. Desert annuals with an extremely short life cycle, 2–3 weeks, are probably programmed by a tight control between development and plasticity. It should be noted that the degradative processes, induced in the vegetative part of annual plants during seed development and ripening of the seeds have adaptive value; a part of the mineral resources of the vegetative part are saved in the seeds.

The situation in long-lived polycarpic plants is quite different. Phenotypic plasticity should be quite large; the longer the life span the greater the occurrence of unusual and unpredictable events in soil and climate and the greater the demand for phenotypic plasticity.

Frost tolerance in trees like apple and pine seems to be regulated by a proper sequence of sets of environmental conditions which allow the tree species to survive in its habitat under the prevailing climatic conditions of temperature and daylength, which include irregularities and fluctuations (Bervaes *et al.* 1978). The proper sequence of daylengths and temperatures during the year is: decreasing daylength (late summer and early autumn), decreasing temperature (late autumn and early winter), low temperature and short days (rest of the winter), increasing daylength (early spring) and increasing temperature (late spring and early summer). In cases of 'wrong' seasonal changes (see Experimental design, section 3), the signal-transduction system was upset, resulting in a cold tolerance

which was inappropriate for the new climatic condition. As an example, short days and decreased temperature both increased frost tolerance of pine needles, while the combined change in daylength and temperature resulted in dehardening! Such a programming, according to the sequence of the natural seasons, was not only visible in frost resistance, but also in parameters such as lipid and fatty acid composition of bark (apple) and chloroplasts (pine; Bervae *et al.* 1987; Ketchie *et al.* 1987). Treatments of temperatures and daylengths, presenting the normal seasonal order, yielded changes in phospholipids and glycolipids which corresponded to data in literature for plant material collected all year round. Also in this case 'wrong' sequences of combinations of temperature and daylength yielded unusual and sometimes even unlikely phospho- and glycolipid compositions of the studied apple bark and pine needle chloroplasts. In conclusion, phenotypic plasticity in trees seems to be genetically controlled to a large extent by the sequence of the yearly seasons. Such a seasonal control also seems to be present for developmental processes, e.g. the temperature regimes needed for flower production in flower bulbs during storage (Hartsema 1961).

Further evaluation of the importance of the perception/transduction signal pathway in phenotypic plasticity

In several cases phenotypic plasticity of vegetative annual plants seems to be regulated by the perception/transduction pathway, while in older plants phenotypic plasticity has disappeared, the physiological functioning now being determined by environment-independent setpoint regulation. Kuiper (1984), in experiments with young plants of the *P. major* genotype with the highest phenotypic plasticity (line D of Fig. 2), showed that responses of growth to changes in mineral nutrition depended primarily on changes in endogenous cytokinin levels and not in internal ion concentrations; bringing the plants into a diluted mineral nutrient solution resulted in a growth reduction of the shoot within 24 h, while the N, P, and K content of the roots responded much slower (Kuiper 1988). Application of benzyladenine postponed the low-nutrient-induced growth reduction for a week (Kuiper & Staal 1987); this short-term growth reduction was accompanied by a reduction of internal cytokinin levels (Kuiper *et al.* 1987, 1989), nitrate being the most important factor. Clearly in this species, cytokinins are involved in growth regulation by mineral nutrition, with cytokinins acting on the perception/transduction pathway. In *P. major*, gibberellins may be involved in environment-independent genetic differences in growth between genotypes (Dijkstra & Kuiper 1989).

When the plasmalemma and tonoplast are involved in the plant's response to an environmental signal, the transduction pathway can be quite complicated. Patch-clamp studies on vacuoles of *Plantago maritima* (Maathuis & Prins 1989) indicated regulation of the Na-channel activity by external NaCl: 100 mM NaCl dramatically decreased channel activity, with a large decrease in frequency of bursts, resulting in a decreased probability of the ion channels being open. This reaction is adaptive, because NaCl accumulated in the vacuole will not easily leak to the cytoplasm. *P. maritima* contains cholesterol, the most effective sterol in the control of passive ion permeability; its level is increased upon exposure of *Plantago* to salinity, at the expense of sitosterol, a much less effective sterol in permeability control (Erdei *et al.* 1980). In another halophyte, *Suaeda maritima*, cholesterol is also present: it is almost exclusively found in the tonoplast (Flowers *et al.* 1989). Presumably, the NaCl signal leads to increased synthesis of cholesterol, which by a cholesterol-specific transport protein is exclusively transported to the tonoplast and incorporated into the latter, contributing to the reduced tonoplast ion channel activity.

Table 2. Critical nutrient concentration and luxury consumption of a fast- and a slow-growing *Taraxacum* microspecies (after Hommels *et al.* 1989a,b,c)

Species Growth Habitat	<i>T. sellandii</i> Fast Nutrient-rich			<i>T. nordstedtii</i> Slow Nutrient-poor		
	N	P	K	N	P	K
Critical nutrient concentration (mM)	404	18	87	380	14	40
Luxury consumption (%)	0	45	55	10	60	80

A similar response of the tonoplast may be involved in the growth response of *Taraxacum* microspecies to reduced mineral nutrition (Table 2; Hommels *et al.* 1989a,b,c). The critical nutrient concentration, i.e. the internal nutrient concentration, which just allows maximal growth of the plant, was consistently lower for a slow-growing (*T. nordstedtii*) than for a fast-growing microspecies (*T. sellandii*). Differences between the microspecies were particularly large for K; in the fast-growing microspecies a biphasic growth reduction upon K depletion was observed; the first phase, from 87 to 40 mM internal K, being characterized by a lack of visible deficiency symptoms, except reduced growth (Hommels *et al.* 1989b). Both microspecies showed visible signs of K deficiency below 40 mM internal K. Clearly, K played an important role in 'osmotic growth' of the fast-growing microspecies; *T. sellandii* also showed an increase in K use efficiency upon mild K deprivation (below the critical internal K concentration of 87 mM); K use efficiency (or mineral productivity: the ratio of relative growth rate to internal K concentration) increased to a threefold higher level than observed at the critical internal K concentration (Hommels *et al.* 1989c). Again the results indicate the importance of vacuolar K and its regulation by the tonoplast in the growth response of *Taraxacum* to K depletion. In line with these observations were the data on luxury consumption, the ion absorption in excess of the immediate growth requirement. Luxury consumption was negligible for N and large for K. Differences between microspecies were mainly determined by differences in critical internal nutrient concentration. A low growth potential will certainly prevent the occurrences of mineral deficiencies during growth at later stages of development and will contribute to an adaptation to a nutrient-poor environment (Hommels *et al.* 1989c).

CONCLUDING REMARKS

This paper presents a personal view on the topic of responses of plants to environmental changes; I did not intend to present a more extensive literature review. The discussed topics, experimental design, differences between mono- and polycarpic plants and the importance of perception/transduction pathways may be summarized and integrated as follows.

In the future, a proper experimental design may include genetic experiments for an analysis of whether an observed relationship between a plant response and an environmental factor has adaptive value or merely presents coincidental circumstances. Crosses between two extreme genotypes could be made and the F1 offspring should be analysed

for the above relationship. Alternatively, a population which has never been exposed to a specific environmental stress (e.g. an air pollution component) may be studied under stress: how many generations are required before clear and distinctive relations (correlations) between plant response and the stress factor appear in the population? Such studies seem particularly promising for an analysis of the nature of genetic control of adaptation of plants to environmental stress.

A second important question concerns phenotypic plasticity in mono- and polycarpic plants. Genetic control of phenotypic plasticity of polycarpic plants like trees and bulb flowers seems to be primarily based on the natural sequence of seasonal changes in temperature and daylength. This applies for species from the temperate zones and it will be interesting to investigate whether tropical polycarpic species still respond to the much smaller seasonal changes or that phenotypic plasticity contains more endogenous components.

Thirdly, analysis of the role of perception/transduction pathways in phenotypic plasticity seems to be a most promising goal. Undoubtedly in the coming years great progress in elucidation of such mechanisms may be expected. Molecular biological techniques and electrophysiological studies on plasma membranes and tonoplasts (patch-clamp technique) of cells from species sensitive and resistant to an environmental stress condition will certainly yield information on the molecular basis of adaptation of plants to changing environments.

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