Characterization of Hormonal Complex in Pea Phenotypes Differing in Leaf Morphology

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Abstract—Two genotypes of the pea (Pisum sativum L.) with wild-type leaves (variety Orlovchanin, Af/Af genotype) and the “afila” morphotype (aphyllous variety Nord, aflafl genotype) were compared in terms of growth performance and hormonal characteristics of different leaf parts and the whole plant. The replacement of leaflets by tendrils in the afila variety led to a reduction in total dry weight and the area of photosynthesizing surfaces. The loss of leaflets was partly compensated for by rapid expansion of stipules at early stages of plant development and by the hypertrophy of tendrils at later stages. The excessive development of stipules in afila plants was paralleled by the increase in IAA and cytokinin level in their tissues. The hypertrophied development of tendrils and chlorophyll accumulation in tendrils of afila plants was correlated with a high IAA and cytokinin content at a low ABA background level. The elevated content of ABA in tissues of wild-type plants was associated with the preferential development of leaflets and a larger transpiratory surface compared with those in the afila form. It is assumed that this feature ensures the turgescence of wild-type plants. The possible involvement of phytohormones in growth and morphogenesis of pea mutants is discussed.

Key words: Pisum sativum - leaf morphotype - mutation - growth - morphogenesis - phytohormones

INTRODUCTION

The involvement of phytohormones in the control of growth and plant morphogenesis is one of the main postulates in hormone science. The use of morphological mutants became a common approach to studying specific actions of individual phytohormones. This approach yielded an explanation for the frequent lack of correlation between the hormone content and growth performance. The underlying causes for such discrepancies are disorders in biosynthesis, metabolism, transport, or in the perception of a particular hormone, as well as the altered content of other phytohormones [1–3]. Previous studies on the hormone action in plants benefited from the use of mutants showing variations from the wild type in quantitative or ontogenetic characteristics. However, the homeotic mutants are a no less interesting or effective means for studying numerous problems of hormonology. These mutants are characterized by (a) drastic and non-quantitative changes in organ morphology or (b) the development of a new organ in place of a lost one. Pea forms with genetically determined leaf-morphotype changes are classic examples of homeotic mutants in the plant world.

The wild-type pea leaf (Af/Af genotype) contains a pair of well-developed stipules in a proximal position and one or two pairs of leaflets situated along the central leaf axis. The leaf axis extends to moderately developed tendrils (Fig. 1).

Mutation of the Af gene (aflafl genotype) alters the normal morphogenesis in such a way that leaflets are replaced by compound branched tendrils that are hypertrophied and strongly twined. The leafletless “afila” forms with the tendril-type leaf were first obtained as spontaneous mutants in the 1950s by Kujala [4] and Solov’eva [5].

Despite profound distinctions in the morphology of the wild-type (normal) and afila pea forms, the relations between the endogenous phytohormone levels and the growth parameters of separate leaf components (parameters reflecting leaf performance in Af/Af and aflafl genotypes) escaped the attention of plant physiologists. Studying this issue was the main goal of our investigation. This work became a logical continuation of the studies pursued by R.Kh. Turetskaya in the laboratory headed by M.Kh. Chailakhyan.

MATERIALS AND METHODS

Two varieties of peas (Pisum sativum L.) with different leaf morphologies were used. One of these varieties (Orlovchanin, Af/Af genotype) was characterized by the wild-type leaf, and the other (Nord, aflafl genotype) represented the afila form (Fig. 1). The choice of these varieties with contrasting leaf morphotypes was dictated by the similarity of other traits, such as stem
height, the number of internodes, and the time of transition to reproductive development.

Pea plants were grown under natural illumination in the greenhouse at the Institute of Plant Physiology (Russian Academy of Sciences) in 5- to 6-l pots filled with soil.

Plant growth parameters were determined at the four-leaf stage and the stage with 8–9 fully expanded true leaves. The total dry weight of plant components (stipules, leaflets, tendrils, and stems) was determined after drying samples in a thermostat at 105°C to a constant weight. The term "tendrils" is used here to designate tendrils together with the central axis. The total surface area of each photosynthesizing component was measured by means of a photoplanimeter (Li-3100, Li-Cor, United States). The genuine area of cylindrical components (tendrils, stem) was calculated with the use of correction coefficient of 1.57 (1/2π). All measurements were performed individually for each plant. Measurements were made at least in 10–15 replicates on different plants.

For analyses of phytohormones—IAA, ABA, and cytokinins (zeatin derivatives)—plants were harvested at the stage with 7–8 fully expanded true leaves. This stage precedes the plant transition to floral morphogenesis and is most suitable for analysis, because the transition to reproductive development is paralleled by alterations in principal metabolic pathways. This could obscure the relationships between growth characteristics of different plant components and phytohormone concentration in their tissues. The phytohormone content was determined in portions of two subapical leaves, which almost completed their development, and in the internode situated between these leaves. The apical plant portions, subject to ongoing development, were not analyzed. Prior to analyses, the plant material was lyophilized.

Phytohormones were determined by immunoanalysis using suitable antibodies [6, 7] with some modifications. The essence of the modification was that the cytokinin-containing fraction was additionally purified on Sep-Pak C18 concentrating cartridges (Milford, United States). Phytohormone determinations were repeated at least 3–4 times in three replicates and 4–5 assays.

The data in the tables represent mean values and their standard errors. The bars in the figures correspond to standard deviations of the means.

RESULTS

By the time that the comparative analyses were performed, the pea plants reached the stage when several true leaves had completed or nearly completed their growth and development. Additionally, there were two scale leaves (embryonic leaves) at the stem basement and 2–3 actively growing true leaves at the apical part of the stem. It should be noted that the leaf morphotype became evident only after the appearance of the third or fourth metamere (true leaf), when the characteristic hook-like structures were formed in the terminal parts of tendrils. The components of the first (bottom) true leaves were small at this stage and insufficiently expressed.

Already at the stage of four fully expanded true leaves, the plants of AflAf and aflaf genotypes substantially differed in growth performance parameters, i.e., dry weight and the area of photosynthetic surface. At this stage, the aphyllous plant exceeded the normal one in total dry weight owing to the relatively high weight of its stipules, tendrils, and stem (Table 1). However, at a later stage, when 8–9 true leaves fully expanded, the afla mutant was underweight by a factor of 1.5 with respect to the wild-type plant (Table 1). These age-dependent differences seem to arise from the accelerated development of the remaining leaf components in the afla plant lacking the leaflets. Furthermore, the trait “leaf morphotype” became clearly expressed at later stages of plant development, i.e., at the period prior to flowering. Nevertheless, the contribution of stipules and tendrils to the total dry weight was higher for the afla plant than for the AflAf form, irrespective of the developmental stage (Fig. 2a). The contribution of stipules to the total dry weight reduced somewhat with plant age, whereas the contribution of tendrils increased.