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TISSUE SPECIFIC VARIATION OF *C*-GLYCOSYLFLAVONE PATTERNS IN OAT LEAVES AS INFLUENCED BY THE ENVIRONMENT

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Key Word Index—*Avena sativa*; Gramineae; primary leaf development; phytotron; field conditions; flavone localization; epidermis; mesophyll.

Abstract—A comparison is made between the flavone patterns accumulating in epidermal tissues and in the mesophyll of oat primary leaves grown in a phytotron and under field conditions. In developing leaves cultivated under standard conditions, varying patterns of two vitexin-derived *O*-rhamnosides and of one isovitexin *O*-arabinoside are produced in the basal region as the result of basal meristem activity. These patterns are tissue specific and differ quantitatively in the epidermis and the mesophyll. During the course of subsequent growth and differentiation, this pattern is constant as the compounds are moved upwards due to basipetal leaf growth. In comparison, the flavone patterns generated in the basal section of leaves grown in the field do not vary significantly. There is the additional accumulation of isoorientin *O*-arabinoside. Again flavone patterns are tissue specific, but in contrast to standard growth they are modified characteristically in those leaf tissues which are already morphologically differentiated. It is possible that the isovitexin moiety of the *O*-arabinoside is oxidized to the corresponding isoorientin derivative in the mesophyll. Moreover, field-grown leaves show a two-fold increase in flavone content in each leaf epidermis and a six- fold increase in the mesophyll when compared to the corresponding tissues of phytotron-grown leaves.

INTRODUCTION

Primary leaves of oat seedlings grown under standard conditions in a phytotron accumulate three main *C*-glycosylflavone *O*-glycosides of the apigenin type, two vitexin-derived 2″-rhamnosides (F_1, F_3) and one isovitexin 2″-arabinoside (F_2) [1,2]. Characteristic dynamics of flavone synthesis have been observed during the differentiation of leaf tissues. Although the total amounts of flavone accumulated by the various leaf tissues are similar, analyses of isolated epidermis and mesophyll tissues reveal that different tissues accumulate specific amounts of the three compounds [3].

Compared to standard growth, natural field conditions result in a decreased rate of leaf growth as well as in qualitative and quantitative changes in flavone patterns. In addition to the flavones F_1–F_3, a fourth major *C*-glycosylflavone *O*-glycoside of the luteolin type, isoorientin 2″-arabinoside (F_4), is accumulated up to a maximum level exceeding the combined amount of the other three compounds [2, 4].

In the present paper, flavone accumulation of primary leaves grown in the field has been analysed at the tissue level. Flavone patterns of various growing zones or leaf sections and their tissues, epidermal layers and mesophyll, are compared with corresponding data obtained with primary leaves grown in a phytotron.

RESULTS AND DISCUSSION

Field conditions

Field-grown primary leaves which exhibited developmental stages and a high rate of flavone accumulation comparable to those obtained under standard growth conditions were selected for use in these experiments (Figs. 1 and 2) [4]. A first analysis was carried out on 3 -4 cm long leaves obtained from 14-day-old field-grown seedlings. These are comparable to 4-day-old leaves from plants grown in the phytotron. Figure 1 (left) depicts the distribution of flavone in the upper and lower leaf epidermis and mesophyll isolated from the top section of the leaf (*t*) and from the lower section near the leaf base (*b*). As can be seen, flavones F_1, F_2 and F_3 are present in similar quantities in both epidermal preparations; F_4 is not detectable. In leaf mesophyll, on the other hand, qualitative as well as quantitative differences in flavone content are observed: F_2 is the major component, exceeding each of the other flavones in quantity by a factor of three to four. F_4 can be detected in the mesophyll, where its concentration is highest in the top section of the leaf. Leaf mesophyll accumulates a total of two to three times as much flavone as each epidermis.

The analysis was extended using 6–7 cm long 19-day-old leaves (Fig. 1, middle). These are comparable to 6-day-old leaves grown in the phytotron. Using the 'ink-mark technique', it has been demonstrated that basal leaf tissues are steadily transferred upwards by division and elongation of cells derived from the basal meristem (cf. [5]). Thus, in addition to the top and basal sections, the middle section, *ms*, corresponding morphologically to the *b* section of the former (14 day) stage, was also investigated. As in the 14-day leaves, flavone F_2 dominates in the mesophyll, of all three leaf sections, and the flavone pattern found in the middle section *ms* resembles that of the lower section *b* of the 14-day leaves. In the top leaf section *t*, however, a striking increase in flavone F_4, chiefly in the

mesophyll, is observed; F_a also becomes clearly detectable in both the upper and lower epidermis. In contrast, F_a remains a minor component of the mesophyll in the lower sections *ms* and *b* and is not detectable in the epidermal layers. Evidently flavones, particularly compound F_a, accumulate predominantly in the top section of the leaf.

Leaves of a third stage, 23 days, corresponding to 7-day-old leaves from the phytotron, were also analysed. The concentration of flavone F_a is further increased in the mesophyll of the top section and now slightly exceeds that of the other major component F_2. Furthermore, F_a exists not only in the epidermal layers of the top section but is also clearly detectable in the tissues of the middle and basal leaf regions. Moreover, a distinct increase in flavone content is observed in the mesophyll of the middle region, whereas in the basal section, only a small amount is retained.

As shown with the analysis of whole primary leaves, flavone F_2 reaches its maximum level of accumulation after 21–24 days of growth, while the amount of F_a increases until the 28th–30th day [4]. However, flavone analysis at the tissue level is not feasible at this stage of growth, since the removal of epidermal layers from older leaves is incomplete or impossible.

Comparison with phytotron conditions

Cultivation of the same variety of oat leaves in the phytotron gives rise to qualitative as well as quantitative tissue specific variations in *C*-glycosylflavone patterns. Characteristic for phytotron-grown leaves (Fig. 2) is the high degree of accumulation of isovitexin *O*-arabinoside (F_2) in young (4-day stage) tissues. As the leaf ages (6–7 days), more and more of the vitexin derivatives F_1 and F_3 accumulate specifically in the epidermal layers, while F_2 remains the major component in the mesophyll of the top and middle section for several days. However, a characteristic change in flavone distribution is observed during development of the basal leaf tissues. Here the changing patterns of flavone distribution are established as a result of basal meristem activity. During the course of subsequent growth, these patterns remain constant as the compounds are displaced to upper leaf regions due to basipetal leaf growth (cf. [3, 5]). Thus, in the early differentiating top section of the leaf, as well as in the middle section, stable tissue-specific flavone patterns are attained. Furthermore, in studies on the incorporation of phenylalanine-[14C] into *C*-glycosylflavones of growing oat primary leaves it has been demonstrated that the compounds which accumulate are not turned over or interconverted [6].

In comparison, oat primary leaves from the natural field also show distinct patterns of flavone accumulation in leaf epidermal and mesophyll tissue, though these patterns are modified by environmental factors (Fig. 1). Furthermore, these modifications are expressed differently in the various leaf sections and their tissues. The most striking effect is the appearance of isoorientin *O*-arabinoside (F_a), which is not detectable in phytotron-grown leaves of all stages. In young 14-day-old field-grown leaves (corresponding to the 4-day stage in the phytotron), this component is localized exclusively in the mesophyll together with isovitexin *O*-arabinoside (F_2), the major flavone. During further leaf development up to 23 days, under field conditions, the amount of the isoorientin arabinoside increases, mainly in the mesophyll of the top section, continously, whereas the amount of the isovitexin derivative remains almost constant. In addition, isoorientin arabinoside accumulates slightly in both epidermal layers, beginning with the top section and proceeding downwards.

In summary, oat primary leaves from field culture show several remarkable variations when compared to leaves grown under standard conditions: (1) Field-grown leaves have a slower rate of growth and accumulate an additional compound, isoorientin 2″-arabinoside. (2) Field-grown leaves produce considerable higher amounts of each single flavone, leading to higher concentrations in both epidermal layers and in the mesophyll. (3) When the total flavone content is considered as the sum of its components, this content is increased approximately two-fold, on the average, per unit of leaf length in the epidermal layers of field-grown leaves. In the mesophyll, the degree of enhancement is six-fold. (4) Leaves grown in the field generate in their basal regions similar, if not identical, flavone patterns. The distribution of the two *O*-arabinosides F_2 and F_a is altered only during the late phase of leaf development (cf. [4]). The highest concentrations are reached by isoorientin *O*-arabinoside (F_a) within the mesophyll of the top section at a stage at which this part of the leaf has already been completely morphologically differentiated for some time. In contrast, the flavone patterns of phytotron-grown leaves change only during the early differentiation of cells descending from the meristem.

With regard to the characteristic dynamics of isoorientin 2″-arabinoside accumulation in field-grown leaves, it is not known whether this compound is formed from isovitexin 2″-arabinoside (cf. [7]). An enzyme catalysing such an *o*-hydroxylation would be expected to be present primarily in the leaf mesophyll. So far, although a large amount of work on plant phenolases has been done, there is little information on the significance of this type of enzyme for flavonoid biosynthesis [8]. Thus, Wallace [9] has demonstrated that B-ring oxidation of the *C*-glycosylflavone vitexin leading to orientin does not occur in *Spirodela polyrhiza*. Therefore he concludes that substitution was determined prior to *C*-glycosylation possibly at the flavanone stage. From results obtained with *Fagopyrum esculentum*, Margna and Margna [10] suggested different enzyme complexes for the synthesis of 4′-OH-*C*-glycosylflavones (vitexin, isovitexin) and their 3′,4′-OH analogues (orientin, isoorientin), respectively. For oat leaves, appropriate experiments will be carried out to determine whether an *o*-hydroxylating activity converting F_2 to F_a is present and, if so, in which tissue(s) it is located.

Another, more physiological aspect should be stressed: in the literature, few data are available on the tissue localization of flavonoids other than anthocyanins in plant organs (for review see [11]). In *Allium porrum*-leaves [12], in bulb scales of *Allium cepa* [13, 14], in cotyledons of *Sinapis alba* [15] and in leaves of *Acer negundo* [16], flavonoids, especially flavonols, accumulate mainly in the epidermis, leaving little or no flavonoid in the mesophyll. However, as shown above, in leaves of *Avena sativa*, characteristic *C*-glycosylflavone levels are developed not only in epidermal tissues but also in the mesophyll. In a recent analysis of oat leaves cultivated in the phytotron, we have demonstrated the occurrence of vitexin rhamnosides F_1 and F_3 and of isovitexin arabinoside F_2 in photosynthetically active mesophyll protoplasts. In these protoplasts, flavone patterns represent whole mesophyll preparations [17]. Moreover, it is well known that several

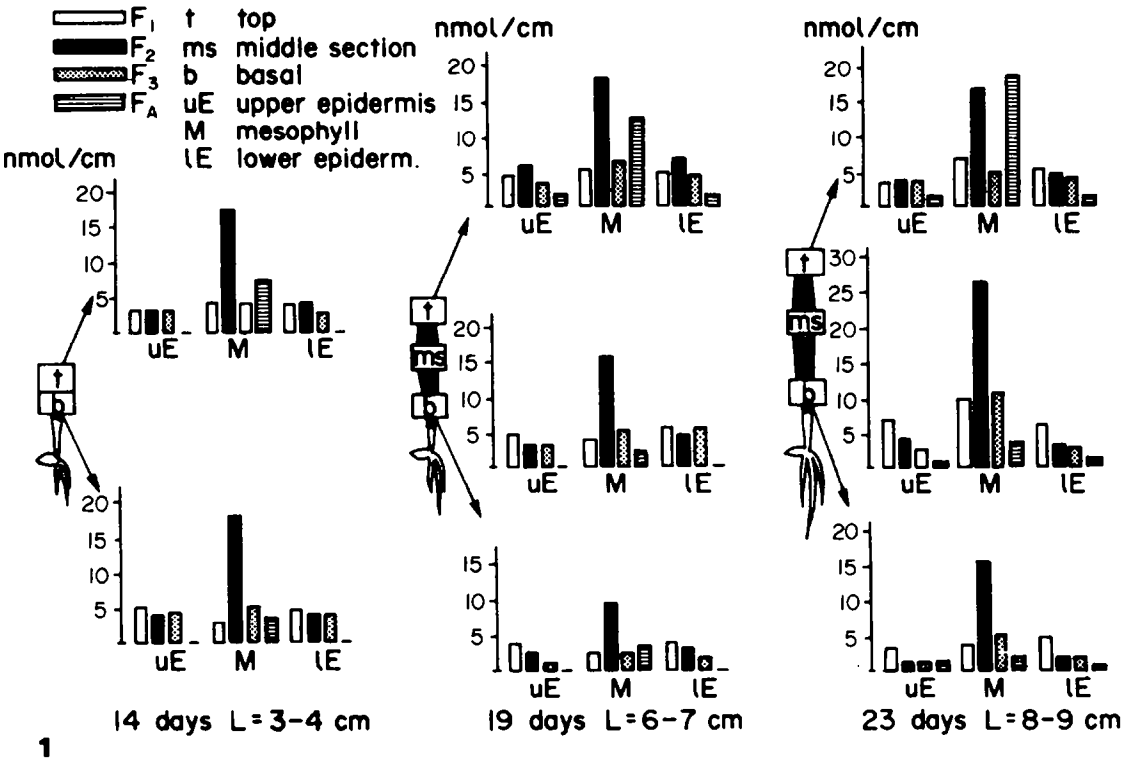

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Fig. 1. Distribution patterns of *C*-glycosylflavone *O*-glycosides F_1, F_2, F_3, F_A (see Introduction) in the upper and lower epidermis and the mesophyll of representative leaf segments (*t*, *ms*, *b* as indicated) of developing oat primary leaves. Flavone content in nmol per cm tissue of leaf sections. Leaf age in days after sowing; seedlings grown under natural field conditions. Representative data from two independent experimental series and harvests.

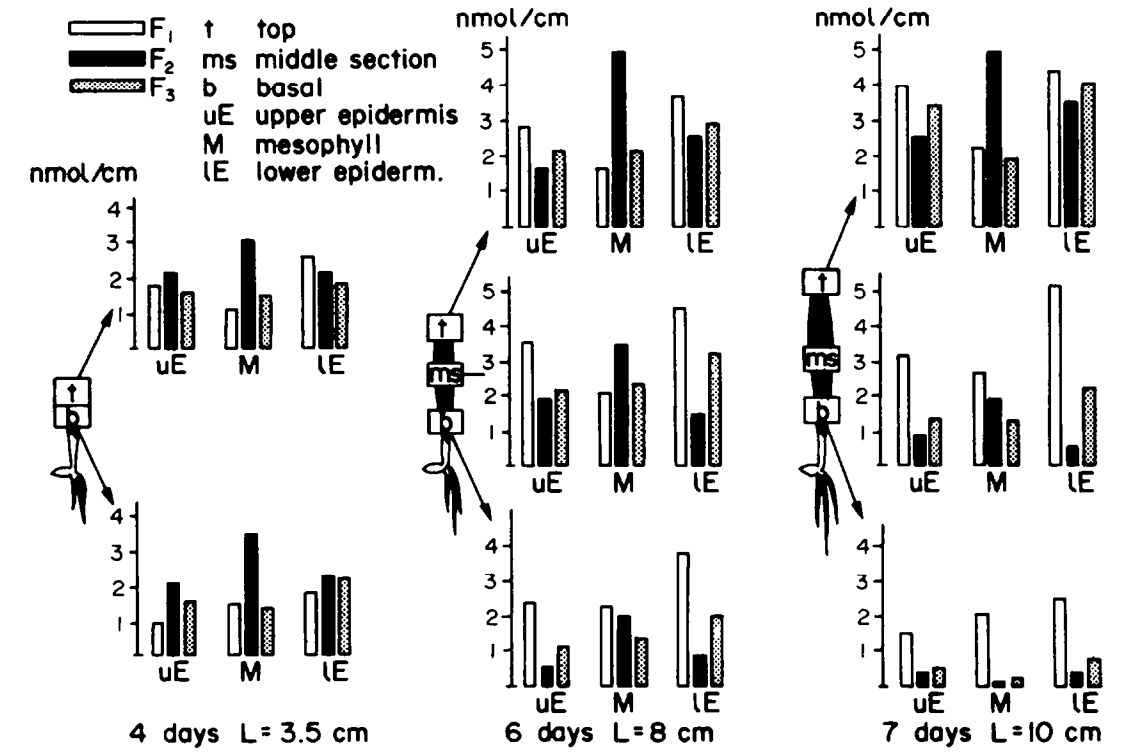

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Fig. 2. Distribution patterns of flavone compounds F_1, F_2, F_3 in epidermal tissues and mesophyll of representative leaf segments of developing oat primary leaves (cf. Fig. 1). Flavone content in nmol per cm tissue of leaf sections. Leaf age in days after sowing; seedlings of the same oat variety as in Fig. 1 but grown under standard conditions in a phytotron, where flavone F_A cannot be detected. Data after ref. [3].

environmental factors can stimulate flavonoid synthesis and accumulation [11]. With mustard cotyledons, Wellmann [15] obtained a correlation, at the tissue level, between phytochrome- mediated flavonoid accumulation and increase of corresponding enzyme activities, both of which have been localized nearly exclusively in the epidermis and not in the mesophyll. In addition, flavonoid synthesis can be increased by UV-light with a linear dose response [18-22] and, accordingly, the possible roles of flavonoids in absorbing UV-irradiation have been discussed (see also [11, 21]. Such adaptive functions for accumulated *C*-glycosylflavone(s) in field-grown oat leaves are not directly obvious especially as the additional flavone, in particular the isoorientin derivative F_a, produced in environments with high natural UV-(solar) irradiation, accumulate primarily within the leaf mesophyll. On the basis of these results, we cannot at present draw any conclusions regarding a possible physiological role for the flavones in oats.

EXPERIMENTAL

Plants and growth conditions. Avena sativa L., cv Gelbhafer-Flämingskrone, was grown alternatively under our standard conditions in a phytotron, photoperiod 13/11 hr [1], and under natural field conditions in the garden of the Botanical Institute from the end of April until May. Primary leaves were harvested from 4 to 7-day-old seedlings grown in the phytotron and from 14 to 23-day plants (comparable in their developmental stage) grown in the field (see Figs. 1 and 2). For a comparison of different growth rates and flavone accumulation in whole leaves see refs [1, 4, 5].

Tissues and flavone content. Representative 1-1.5 cm sections from different leaf regions were used as indicated in Figs. 1 and 2, and the lower as well as the upper epidermis was peeled manually with a forceps according to ref. [3]. Using this method, no microscopically visible part of the mesophyll contaminates the peeled epidermis. For further details and estimation of epidermal and mesophyll flavone see refs. [3, 5]. Flavone extraction was performed with epidermal layers and mesophyll obtained from at least 10 leaf sections *t*, *ms* and *b*, respectively, for each developmental stage. Two parallel series of experiments were carried out on field-grown leaves from separate harvests, as was carried out earlier with leaves grown in the phytotron [3].

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