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Correlations Between Changes in the Amount of Endogenous Phytohormones and Flowering in the Safflower (*Carthamus tinctorius* L.)

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Abstract: Changes in the amounts of endogenous gibberellic acid (GA_3), indole-3-acedic acid (IAA) and abscisic acid (ABA) during different growth periods of the safflower were studied by high-performance liquid chromatography (HPLC).

A correlation was confirmed between low levels of GA_3, as well as high levels of ABA, and the initiation of flowering. IAA reached its maximum level during bud formation, indicating that IAA might play an active role in the differentiation of bud formation.

Changes in the amount of endogenous phytohormones in flower buds located in different parts of the plant indicate that ABA in particular plays an important role in the formation of flowering order in safflower.

Key Words: *Carthamus tinctorius* L., flowering buds, phytohormones, HPLC

Aspir Bitkisinde (*Carthamus tinctorius* L.) İçsel Hormon Düzeyindeki Değişimler ile Çiçeklenme Arasındaki İlişkiler

Özet: Aspir bitkisinin farklı büyüme dönemlerinde içsel gibberellik asit (GA_3), indol-3-asetik asit (IAA) ve absisik asit (ABA) değişimleri yüksek basınçlı sıvı kromatografisi (HPLC) ile incelenmiştir.

GA_3'in düşük ve ABA'in yüksek düzeyleri ile çiçeklenmenin uyarılması arasında yakın bir ilişkisinin varlığı saptanmıştır. En yüksek IAA düzeylerine tomurcuklanma döneminde ulaşılmıştır. Bu sonuç, aspir bitkisinin çiçek tomurcuğu farklılaşmasında, IAA'in etkin bir rol oynadığını göstermiştir.

Aspir bitkisinin konumları farklı çiçek tomurcuklarındaki içsel hormon değişimleri, çiçeklenme düzenin oluşumunda özellikle ABA'in etkin rol oynadığını ortaya koymuştur.

Anahtar Sözcükler: *Carthamus tinctorius* L., çiçeklenme, fitohormonlar, yüksek basınçlı sıvı kromatografisi

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Introduction

Phytohormones in plants are found in minute concentrations. For this reason, special techniques are needed to measure them accurately. High-performance liquidchromatography (HPLC) is a progressive analytical and preparative method which permits highly effective separation, isolation, identification and measurement of substances found in biological material. In particular, the development of HPLC provides an accurate physicochemical method of detecting and separating cytokinin, gibberellins, IAA and ABA (1-6).

Endogenous and exogenous plant growth substances have been correlated with both the promotive and inhibitory aspects of flowering in many plants. However, litlle information is available concerning the distribution of these substances in relation to flowering in the safflower (7-10).

The safflower (*Carthamus tinctorius* L.), a member of *Compositeae,* is a valuable cultivated oil plants. It has a regular flowering order, beginning on the main stem head and continuing on the primary, secondary and tertiary stem heads, respectively, as a result of ordered dominance breaks. Nevertheless, many significant agronomic characters show considerable fluctuation during the flowering period, which can last as long as 3-4 weeks. For example, the oil content of the first flowering head seeds was 43.9%, while it was only 14.5% of the last (10). Furthermore, prolongation of the flowering process leads to heterous maturity, making combine harvesting difficult. If the flowering physiology of the safflower is accurately illuminated, these problems may be solved.

The aim of the present study was to obtain information on changes in the amount of natural hormones in safflower during the flowering process.

Materials and Methods

Plant material

Hormone analysis was conducted on safflower plants (*C. tinctorius* L. line E-10) grown on 70x20 cm field plots in the Field Crops Department at Akdeniz University from April to July, 1996. GA_3, IAA and ABA were analysed in the leaf at the rosette stage; in the leaf and stem at the bolting stages; and in the leaf, stem and flower buds (or heads) at the budding, first and full blooming stages.

Extraction

Ten grams of fresh tissue per sample was homogenized with 70% methanol and stirred overnight at 4°C. The extract was filtered through a Whatman filter and the methanol evaporated in *vacuo.* The aqueous phase was adjusted to pH 8.5 with a 0.1 M phosphate buffer and then partitioned three times with ethyl acetate. After removal of the ethyl acetate phase, the aqueous phase was adjusted to pH 2.5 with 1 N HC1. The solution was partitioned three times with diethyl ether, and then passed through waterless sodium sulphate. After the diethyl ether was evaporated *in vacuo,* the dry residue containing hormones was dissolved in methanol and stored in vials at 4°C (2, 3, 5).

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was used for separation and purification of the extracts dissolved in methanol (4). Extracts of 100 µl with a Hamilton syringe, and the plates were placed in a TLC tank containing a mixture of 21 ml methanol: 2 ml 25% amonium solution: 2 ml pure water. The relative fluidity (Rf) bands of hormones on the plates were studied by UV cabine at wavelengths 254nm and 365nm. IAA was found on the $Rf_{0.5}$, GA_3 on the Rf0.6. and ABA on the Rf0.7. The hormone extracts on the Rf bands were dissolved in grade methanol for use in HPLC analysis.

HPLC analysis

HPLC analysis was used to study growth hormones in extracts dissolved in 1ml grade methanol. Analyses of GA_3, IAA and ABA were performed on a Model Varian 9050 HPLC equipped with UV detector and Model Varian 9010 pumps enabling the use of a concentration gradient of the mobile phase. Separations and determinations were performed on a nukleosil C18 column (4.6 mm x 150 mm). The Mobile phase yielded results of 30% methanol (adjusted to pH 3.0 with 0.1 M H_3PO_4) for GA_3, 55% methanol (in 0.1 M acetic acid) for ABA and 35% methanol (in %1 acetic acid) for IAA. Wavelengths in the UV detector were 208nm, 265nm and 280nm for GA_3, ABA and IAA, respectively. Total run time for the separations was approximately 5 min at a flow rate of 1 ml/min (1-5).

Results and Discussion

At the rosette growth stage, with 5-8 true leaves and no stem, endogeneous ABA levels in the leaf were higher than other hormone levels. Levels of GA_3 in both the leaf and stem starded to increase during the bolting (stem elongation) stage, in contrast to decreases in ABA levels. No IAA was found during these growth periods (Figure 1). These results showed that stem elongation in the safflower was initiated and promoted according to increases in endogeneous GA_3 and decreases in endogenous ABA.

In a previous study, although external GA_3 application to the rosette leaf of the safflower was found to shorten the rosette stage and begin stem elongation rapidly, ABA application had little effect on plant growth (10). In the same study, GA_3 initiated flowering earlier, but inhibited the development of heads after flowering, causing low seed yield. In another study, GA_3 and the biosynthetic inhibitor of GA_3, paclobutrazol, were applied to the safflower plants. GA_3 increased epidermal cell size, internode length and cell number, causing stem elongation. Conversely, paclobutrazol reduced stem height, internode and cell size, cell number and overall shoot weight. Also, GA_3 increased total stem weight, but decreased leaf weight, flower bud number and seed yield (8). Thus, GA_3 promoted vegetative growth at the expense of reproductive ability in the safflower.

In the budding stage, the most prominent increases were in the total level of IAA (Figure 2), indicating that IAA could play an active role in initial bud formation in the safflower. Similarly, Chen (4, 5) reported that an increase in cytokinins is also associated with flower bud formation in mango (*Mangifera indica* L.) and lychee (*Litchi chinensis* Sonn.) plants.

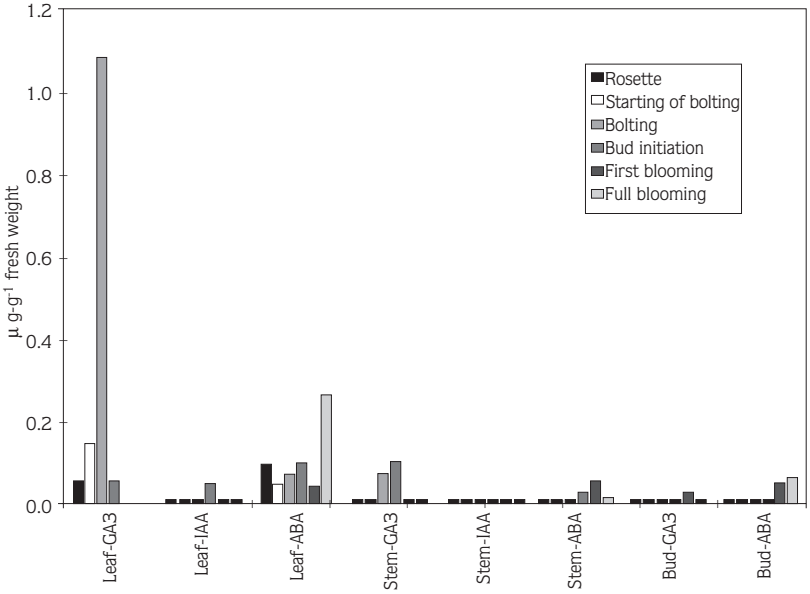

Figure 1. Endogenous phytohormones changes of safflower in the different growing stages

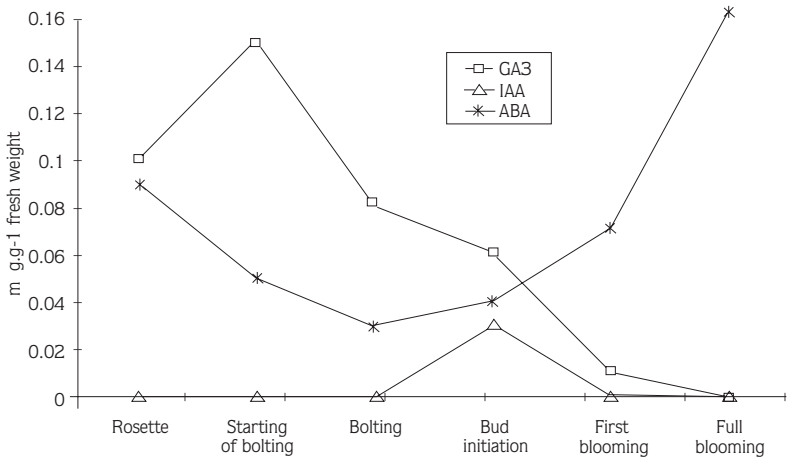

Figure 2. Changes of total GA3, IAA and ABA at the different growing stages in safflower.

ABA concentrations were relatively high at the rosette stage, decreased during bolting and later started to increase again during blooming, in contrast to the GA_3 changes. From first blooming to full blooming, GA_3 levels decreased, while those of ABA daramaticaly increased in the leaf and flower heads (Figure 1). These changes were also clearly observed during total plant analysis (Figure 2). Although external GA_3 applications accelerate transition to the blooming

stage (10), in the present study low levels of internal GA_3 were found during flowering. These results indicate that the incitement of GA_3 on floral initiation might have an indirect effect on stem elongation.

The safflower has a regular flowering order, from the outermost branch buds to the innermost branch buds (10). Changes of endogenous phytohormones in the flower buds located in different positions on the plant indicated that ABA had particularly important role in the formation of flowering order in safflower. Buds with a higher, ABA content flowered earlier than others, resulting in an ordered flowering cycle (data not shown).

The results suggest that low levels of endogeneous GA_3 and high levels of ABA were closely associated with floral initiation in the safflower. High levels of IAA were also associated with flower bud differentiation. Changes in ABA levels in the buds in different parts of the plant played an active role in the flowering order of the safflower.

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