GLYCOSYLATION OF FREE trans-trans ABSCISIC ACID AS A CONTRIBUTING FACTOR IN BUD DORMANCY BREAK

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Summary: By means of gas-liquid chromatography determination it was found that progress of dormancy break of almond buds is a function of relative proportions of free and glycoside-bound abscisic acid. Successive stages of bud break manifest a marked increase of bound abscisic acid accompanied by a parallel decrease in endogenous levels of the free form. Of the two stereoisomers involved it was found that while the cis-trans form maintains a more or less stable level throughout, changes were detected primarily in the trans-trans form. It is thus postulated that the binding of free hormone as well as its total content are of major physiological importance in the process of bud dormancy break.

INTRODUCTION

The role of ABA in the process of dormancy break in trees has been reviewed by Wareing and Phillips (8). It is postulated the decrease of endogenous levels of the naturally occurring cis-trans stereoisomer may be an important factor in emergence of buds from the dormant state, and conversely that the induction of dormancy may be due to accumulation of ABA. Since at least in several systems such as induction of α amylase synthesis in barley endosperm ABA acts as an antagonist to the phytohormone GA (1), it is furthermore believed that the degree of dormancy may amongst other factors be the reflection of an equilibrium between endogenous levels of ABA and GA. While the cis-trans isomer has been found to be of wide occurrence and is possibly more active biologically, Nitsch (7) has shown marked biological activity of the trans-trans stereoisomer which inter alia is obtained upon UV irradiation of the cis-trans form (3).

Evidence for the occurrence of both free and glycosidic-

Abbreviations: ABA - abscisic acid, GA - gibberellic acid, GLC - gas-liquid chromatography.

bound ABA has been presented (4, 6) but as yet information is sparse concerning the state of ABA-bound or free - during the various stages of dormancy break. Employing GLC this research has attempted to determine relative amounts of either glycosidic-bound or free ABA during progressive stages of bud break and furthermore to ascertain changes, if any, in endogenous levels of each of two abovementioned stereoisomers.

MATERIALS AND METHODS

Almond (Prunus amygdalus cv. Pariche) buds were picked 5 times at 3 week intervals commencing 1 Dec. The 5 samples obtained represent progressive stages of bud-break. After picking buds were immediately frozen.

ABA extraction and assay: ABA was extracted employing the procedure outlined by Mizrachi et al (5). 10g fresh weight bud tissue was pulverized in 150 ml cold 80% methanol using a Vortex homogenizer for 1 min at 100 rpm. The mixture was stirred at 4°C for 24 hrs and every 4 hours the methanol was removed and a like volume of fresh methanol applied. All methanol fractions were collected, filtered to remove coarse material and subsequently centrifuged at 10,000 g for 20 min. The supernatant was evaporated in a Buchi Rotary Evaporizer until only the water residue remained. (ca. 10% of initial volume). Distilled water was added to bring volume to 100 ml and pH adjusted to 8.5 with 5% $NaHCO_3$. This was then extracted three times with 100 ml ethyl acetate. The watery phase was collected and brought to pH 2.7 with 1M HCl and again extracted with a like volume of ethyl acetate. This was repeated five times and the combined ethyl acetate layers which contain the free ABA was evaporated to dryness for subsequent GLC determinations.

The remaining acid watery layer containing the glycosidic-bound ABA was brought to pH 12 with NaOH and allowed to stand for 1 hr. at 60°C thus causing hydrolysis of the glycoside-ABA complex. The pH was then again brought to 2.7 with 5N HCl and shaken twice with ethyl acetate employing 100 ml each time. The ethyl acetate layers now containing ABA which previously was bound but now free as a result of the hydrolysis, was brought to dryness as above. To obtain further purification the dried extracts - both free and 'bound' ABA - were dissolved in 1 ml/g fresh bud weight methanol and 0.5 ml aliquots

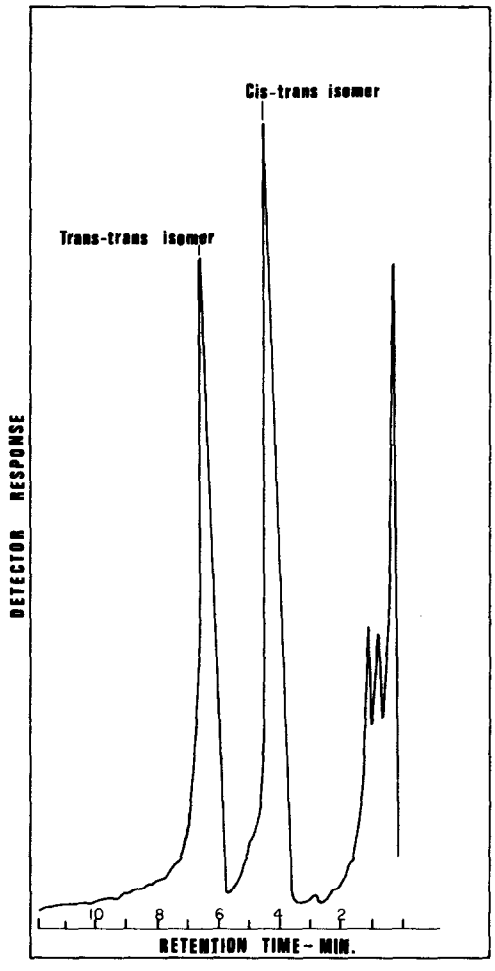

Fig. 1: GLC chromatogram of marker mixed isomer ABA.

loaded on 3 cm wide Whatman No. 3 paper chromatograms and developed with isopropanol:ammonium:H_2O (10:1:1 v/v). Marker Sigma mixed isomer ABA was run on parallel chromatograms and after a 25 cm run could be observed under UV light between the 0.6 - 0.8 Rf regions. Methanol eluates of these regions were thus employed for GLC comparisons of various stages of dormancy break. Elution was performed by gently shaking the pertinent Rf regions with 100 ml absolute methanol for 1 hr at 60°C. The methanol was removed and collected and the process repeated with fresh methanol. The combined methanol fractions were evaporated to dryness and subsequent esterfication of the ABA was performed employing diazomethane reagent (2). Excess reagent was removed by employing a warm air current. The ABA now in a methyl ester form was dissolved in

10 ml redistilled hexane and 10 µl aliquots injected into a Packard Model 873 Gas Chromatograph. Calibration was with 10 µl 4 x 10^{-7} mg/ml of methylated Sigma mixed isomer ABA dissolved in hexane. The Gas Chromatograph was equipped with a model 873 electron capture detector, the carrier gas being nitrogen and the flow rate 26-28 ml/hr. Temperature of detector and column was 200°C and inlet temperature 210°C. The electrometer range was 10 x 10^{-9} amp and power 23v.

RESULTS AND DISCUSSION

Figure 1 indicates a typical GLC chromatogram of marker mixed isomer ABA. In accordance with other reports (5, 6) it is noted that the cis-trans and trans-trans isomers are detected at retention times of 4.5 and 6.5 min. Like runs in quadruplicate were performed on free and 'bound' final ABA extracts of each of the five progressive stages of bud break, here designated 1, 2, 3, 4 and 5 - 1 being the most dormant stage and 5 prior to bud break.

Results presented in Figure 2 are peak areas as indicated on gas-liquid chromatograms as inferred from the peaks exactly corresponding to the retention times of each of the two isomers on the marker chromatogram. This figure clearly indicates that with progress of dormancy break there is a marked decrease in total free ABA (Figure 2-A) accompanied by a significant increase in bound ABA (Figure 2-B). Changes detected were primarily in the trans-trans isomer which appeared to be the more prevalent of the two. The cis-trans isomer manifests an essentially stable low endogenous level throughout.

Parallel bio-assay of ABA extracts in a medium containing embryoless barley endosperm halves and GA produced the typical ABA-induced reducing sugar production inhibition, thus furthermore indicating their ABA-like properties.

It thus appears that dormancy break of developing buds is a function of both the decrease in total ABA content and of relative proportions of free and bound ABA. It is therefore postulated that the glycosidic binding of the free trans-trans stereoisomer is a contributing mechanism to bud break. This conclusion does not overrule the possibility that the initially bound stereoisomer is the naturally

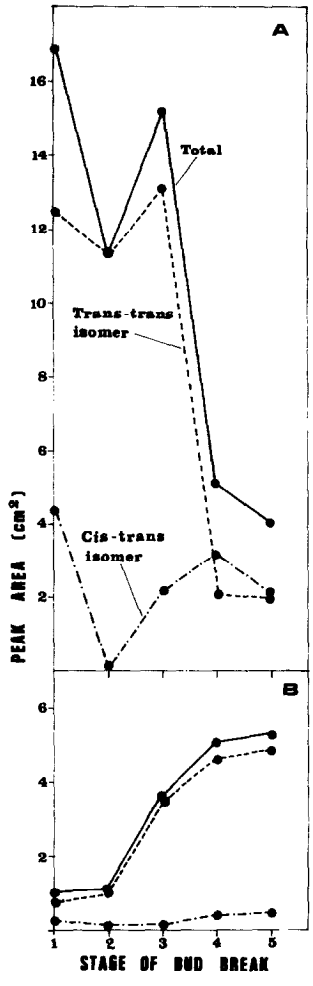

Fig. 2: Peak areas of cis-trans, and cis-cis ABA isomers during successive stages of bud break as determined by GLC and interpolated from identical peaks on the marker chromatogram. A. free ABA stereoisomers. B. bound stereoisomers.

occurring cis-trans form, which later due to lack of foliage protection over the naked buds in winter, is exposed to direct sunlight and the latter's UV component brings about conversion to the trans-trans form.

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