

Vitis 32, 223–228 (1993)

Identification of the *trans* isomers of abscisic acid and of abscisyl- β -D-glucopyranoside in latent buds of the grapevine and their evolution during the post-dormancy phase

by

M. BROQUEDIS and J. BOUARD

Laboratoire de Physiologie Végétale et des Sciences de la Vigne, Université de Bordeaux I, Talence, France

S u m m a r y : The *trans* isomers (*t*-ABA and *t*-ABA-GE) of abscisic acid (ABA) and of abscisyl- β -D-glucopyranoside (ABA-GE) have been identified in the latent buds of *Vitis vinifera* L., cv. Merlot, during the post-dormancy phase. During this phase, the amounts of the four forms of ABA in the buds became minimal. The quantities of ABA-GE and of *t*-ABA-GE, normally present in much greater quantities than free ABA, decreased strongly. This diminution appeared to be directly linked to the increase of water content of the buds. Although potential bud burst can be related to these phenomena, the role of the *trans* isomers remains obscure.

Key words : abscisic acid, abscisyl- β -D-glucopyranoside, bud, dormancy, *Vitis vinifera*.

Introduction

Abscisic acid (ABA) is an important growth regulator of grapevines. Its occurrence has been studied intensively in berries (BROQUEDIS 1987 b) but not in buds. However, it is known that a relationship exists between the quantity of free ABA in buds and bud burst, and that the probability of bud burst is greatest when the amount of free ABA is minimal (DÜRING and BACHMANN 1975; DÜRING and KISMALI 1975). As modern techniques allow to quantify the different forms of ABA, it was of interest to study the nature of the disappearance of free *cis* ABA in buds. Therefore, it seemed sufficient to measure ABA and abscisyl- β -D-glucopyranoside (ABA-GE). However, our research showed that the ABA present in the buds was not limited to these two most common forms and that it was necessary to take into account the *trans* isomers as well.

Material and methods

C o l l e c t i o n o f b u d s a m p l e s : The study was carried out on *Vitis vinifera* L., cv. Merlot, because the vegetation cycle of latent buds of this variety is particularly well known (POUGET 1963 and 1972). The buds were sampled every 8 d during the post-dormancy phase from vines bearing on average 12 buds. As soon as they were removed, the buds were plunged into liquid nitrogen and subsequently freeze dried.

M e t h a n o l i c e x t r a c t i o n : In order to avoid any risk of photo-isomerization of ABA, all stages of extraction were carried out avoiding sunlight (BROQUEDIS 1987 a and b). Freeze dried plant material (1–2 g) was crushed in methanol/water (80/20 v/v, 100 ml) containing an anti-oxidant (10 mg l⁻¹ of BHT; 2.6 di-*t*-butyl phenol). After 20 h of maceration under stirring at 4 °C, the methanolic solution was filtered and the methanol was completely evaporated under vacuum at 35 °C. The remaining aqueous phase contained ABA in its different forms.

Correspondence to: Dr. M. BROQUEDIS, Laboratoire de Physiologie Végétale et des Science de la Vigne, Université Bordeaux I, avenue des Facultés, F-33405 Talence Cedex, France

Dialysis: The aqueous extract (60 ml) was placed in a dialysis test-tube (length: 27 cm, diameter: 4 cm). The pH of the extract was adjusted to 2.5 using phosphoric acid (2.8 M) and then 120 ml of ethylic ether containing 10 mg l^{-1} of BHT was added. A dialysis tube (length: 23 cm, diameter: 2.38 cm) containing 30 ml of a basic solution of K_2HPO_4 (0.5 M) was placed in the ether phase so that it was completely immersed in the ether. The lower end of the dialysis tube was held 2 cm above the level of the lower acidified aqueous phase. Dialysis was carried out for 20 h under magnetic stirring. During the first hour, a small quantity of the basic solution passed through the membrane of the dialysis tube. Since the diffusion modified the pH of the lower acid phase containing the extract, the pH of this phase had to be reset to 2.7 after 1 h of extraction. After dialysis, pH was close to 3.0. The extraction method, as described above, comprised 3 separate continuous phases: the lower acidic aqueous phase (pH = 2.5) containing the different forms of ABA to be extracted, the intermediate ethereal phase, and the upper aqueous basic phase (pH = 9.0) in the dialysis tube. The free ether-soluble acids appearing in the lower aqueous phase were able to migrate and temporarily accumulate in the ethereal phase during extraction. The free acids then passed into the upper aqueous phase in which the acid function was kept by the basic pH.

After dialysis, the basic solution was acidified (pH = 3.0) for ether extraction over 20 h using a low-boiling solvent extractor (BROQUEDIS 1987 a). The ethereal fraction was evaporated to dryness in an anhydrous nitrogen flow. The dry extract was then solved in 300 μl of methanol, prior to chromatography. At the end of dialysis the lower acidic aqueous fraction contained free ABA no longer, but the total amount of ABA-GE was still present. The fraction was then hydrolyzed in a basic environment (pH = 11.0) over 20 h at 40 °C in order to separate ABA from glucose. According to BARTHE (1983) this method of hydrolysis gives the best yield and does not affect the 1'-O-*abscisic acid*- β -D-glucopyranoside (ABA-GS), which was the other major conjugate form of ABA, identified by LOVEYS and MILBORROW (1981). The hydrolyzed solution with its pH adjusted to 3.0, was clarified with 5 g of PVPP before further dialysis to extract the released ABA. Extraction was then continued in the same manner to extract the free ABA, as previously described.

Analysis methods: The determination of ABA was carried out by HPLC, using a SpectraPhysics system consisting of 8100 chromatograph, a 8400 wavelength monitor (254 nm) and a 4100 recording integrator. Samples were introduced from a 10 μl fixed volume injection loop and separated at 35 °C on a Lichrosorb 5 μl particle size, 250 x 4.6 mm C18 reverse common phase column. The mobile phase, consisting of water, methanol and acetic acid (70/25/5 ml), had a flow rate of 1.5 ml min^{-1} . The different forms of ABA were detected at 254 nm.

Identification of *trans* isomers: Combined GC-MS allowed in particular the identification of the *trans* isomers and of the different forms of ABA studied in the extracts obtained from the buds, which had been previously methylated by diazomethane (SCHLENK and GELLERMAN 1960).

Conditions for chromatography: Chromatograph: Intersmat IGC 121M; silica capillary column, CP SIL 8 CB (25 m x 0.22 mm); programmed oven temperature: from 170 to 300 °C (4 °C min^{-1}); split injector temperature: 240 °C; split value: 1/50; volume injected: 5 μl ; carrier gas: helium, U^* (pressure: 1 bar). Under these conditions, the retention times of the methyl esters of ABA and of *t*-ABA were 6.33 and 7.52 min, respectively.

Conditions for spectrometry: Mass spectrometer: V.G. Micromass 16F type; temperature of the interface: 170-200 °C; ionisation conditions: source temperature of 140 °C for an energy of 70 eV and an intensity of 100 mA; magnetic scanning of masses of 600 m/z to 20 m/z at a speed of 0.5 s per decade.

Results

Evidence for the presence of *trans* forms of ABA in latent buds: At all sampling dates the chromatograms provided evidence for the presence of the

4 forms of ABA in buds, which may be characterised by the following abbreviations (BROQUEDIS 1987 b): free *cis* ABA (ABA), its glucose ester (ABA-GE) or abscisyl- β -D-glucopyranoside, the free *trans* ABA (*t*-ABA) and the *trans* abscisyl- β -D-glucopyranoside (*t*-ABA-GE). The mass spectra, corresponding to the methyl esters of the *trans* isomers, proved that these two isomers were indeed present in the buds studied (Fig. 1). It has been shown elsewhere (MILBORROW 1970)

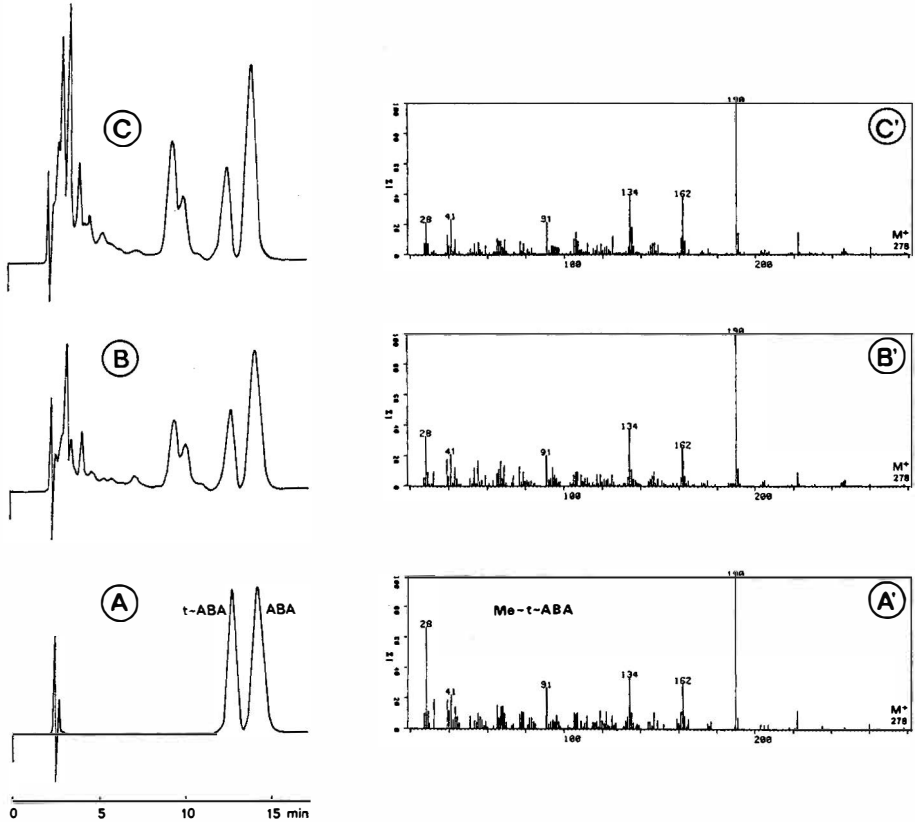


Fig. 1: **A** - Chromatogram obtained by HPLC with a standard methanolic solution of free *t*-ABA and ABA. Chromatograms B and C obtained by HPLC with the same extract of buds. **B** - represents the ABA and the *t*-ABA contained in a free status in this extract. **C** - represents the ABA and the *t*-ABA set free after hydrolysis of conjugated forms (ABA-GE and *t*-ABA-GE) contained in the same extract. This is an indirect analysis of conjugated forms because only the free forms given by these conjugated forms are analysed, that explains the similarity of chromatograms B and C. **A'** - Mass spectrum of methylated *t*-ABA contained in standard methanolic solution. **B'** and **C'** - Mass spectra of the peaks of chromatograms B and C which have the same retention times as the peak of standard (A) *t*-ABA. The identity of spectra B', C' and A' (reference) shows the presence of *t*-ABA and *t*-ABA-GE in the buds.

that the conjugate forms are derived from an esterification of the free forms and that the *t*-ABA is formed from ABA by isomerization. This latter reaction seems to be essentially light-induced and takes place without the intervention of isomerases. Although this is the first time that the existence of the *trans* isomers in the buds of the grapevine has been demonstrated, their presence does not seem to be surprising, because *trans* ABA does indeed exist under natural conditions and was found in substantial quantities in buds of peach and orange trees (LESHEM *et al.* 1974; JONES *et al.* 1976). Now it would be interesting to know whether these 4 forms of ABA are found throughout the vegetation cycle of the compound buds or if the *trans* forms are a characteristic of certain phases of their development.

Evolution of free ABA and of ABA-GE: The quantities of free ABA and ABA-GE in buds decreased from February 8 to March 22 (Fig. 2). But there were only small quantities of free ABA which diminished slightly (from 15 to 5 ng/bud), while the amount of ABA-GE was 7 times greater and diminished in a regular and distinct way from February 8 to March 8. The slight increase of ABA-GE which took place only during a short period, from March 8 to 15, was observed in all analyses. It seems that this increase was the result of an esterification of part of the free ABA. The quantity of ABA-GE then decreased again to reach its minimum value on March 22. The evolution of 'total' ABA (free ABA plus ABA-GE) was almost identical to that of ABA-GE, indicating the great predominance of this form (Fig. 2).

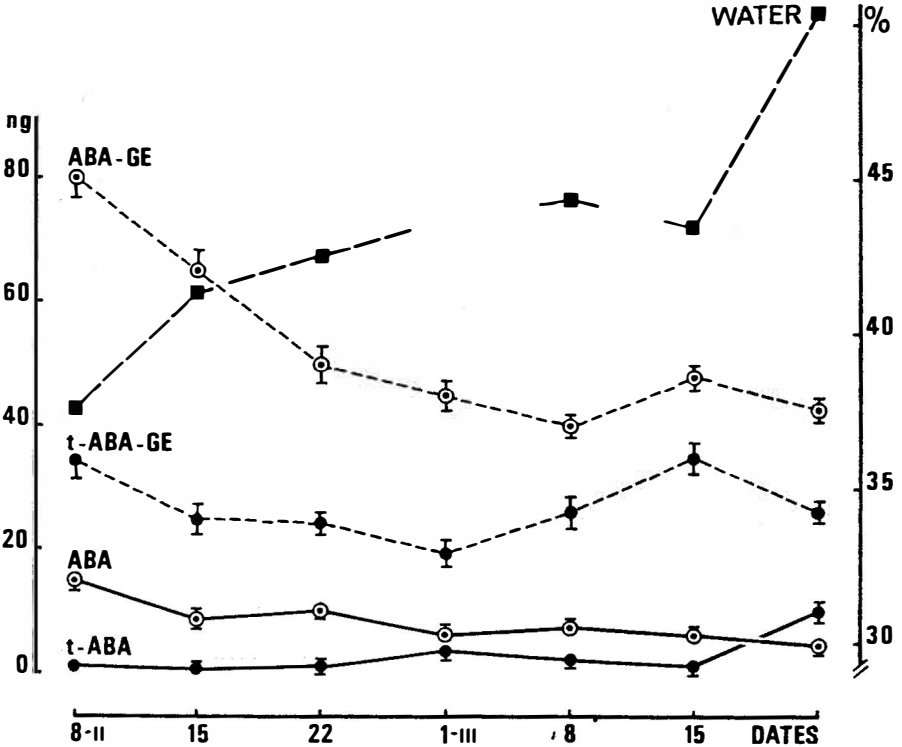


Fig. 2: Evolution of the amounts of ABA, *t*-ABA, ABA-GE and *t*-ABA-GE (ng/bud) and of the water content (in %) in the Merlot compound buds during their post-dormancy phase. Data are the means (\pm SD) of 5 replicate extractions.

Evolution of free *t*-ABA and *t*-ABA-GE: The quantities of the two *trans* forms were also very different, and it is again the conjugated form, the *t*-ABA-GE, which was by far more abundant. The quantity of free *t*-ABA changed little from February 8 to March 15. It increased during a brief period from March 15 to 22. The evolution in the amounts of *t*-ABA-GE was exactly inverse to that of *t*-ABA and the differences in quantity which exist between the two forms suggest that the *t*-ABA turned into *t*-ABA-GE. The 'total' *t*-ABA (free *t*-ABA and *t*-ABA-GE) evolves in parallel to the *t*-ABA-GE (the predominant form), and in an analogous manner to the 'total' free ABA.

If one compares the free and conjugate *cis* and *trans* forms, it is noticeable that the ABA-GE and the *t*-ABA-GE evolved in parallel and that the quantities of ABA-GE were always distinctly greater than those of *t*-ABA-GE. However, ABA and the *t*-ABA were inversely related and the amounts of ABA were always greater with an exception of those on March 22, the last sampling date.

The changes of the amounts of ABA-GE and *t*-ABA-GE appears to be closely linked to that of the water content of the buds. In effect, the two conjugate forms and the water content appeared to be inversely related, i.e. the percentage of water increased while the amounts of the conjugate forms decreased. Also during the short period (from March 8 to 15) when the water content decreased, the levels of ABA-GE and *t*-ABA-GE increased. Given the low quantity of free ABA, the same relationship was observed for the 'total' ABA. The increase of the amounts of glucose esters thus appears closely linked to changes in the water content.

Conclusion

This study confirms that the amounts of free ABA in latent buds of the vine reached their lowest level during the post-dormancy phase (DÜRING and BACHMANN 1975; DÜRING and KISMALI 1975). It is not surprising to learn that free ABA can act as a growth inhibitor which has an influence on dormancy phenomena, and it can also be surmised that potential bud burst is partly linked to the low content of free ABA. This study revealed new facts on the presence of *trans* isomers which were identified for the first time in grapevine buds. The evolution of these *trans* forms during the period studied raises the question of their significance. The results presented in this paper do not prove a clear answer to this question. The presence of relatively large amounts of conjugate forms (ABA-GE and *t*-ABA-GE) has also newly come to light. In the absence of complementary data on the existence of these compounds during post-dormancy of the grapevine buds, it is impossible for the moment, to pinpoint their origin with any certainty. Their evolution does however appear to be linked to the changing water content of the buds. Some authors have noted that the intracellular pH of dormant tissues is always higher than that of non-dormant tissues (GENDRAUD 1981; GENDRAUD and LAFLEURIEL 1983; TORT *et al.* 1985). Thus one might imagine that such variations in pH are likely to happen in the case of buds in the post-dormancy phase. It is also known that the pH value influences the activity of enzymes such as the glucosyltransferase, an enzyme implicated in the formation of ABA-GE (LEHMANN and SCHÜTTE 1980). A further, more detailed study is currently evaluating these hypotheses that have been put forward and is also attempting to clarify the uncertainties with regard to the role of the *trans* isomers raised by these initial results.

References

- BARTHE, P.; 1983: Acide abscissique et dormance embryonnaire chez *Pyrus malus* L. Thèse Univ. Nice, France.
- BROQUEDIS, M.; 1987 a: Utilisation de la dialyse et de la chromatographie ultra-rapide pour le dosage de l'acide abscissique: application à la vigne. *Vitis* **26**, 19-26.
- ; 1987 b: L'acide abscissique et l'abscissate de β -D-glucopyranose dans le développement des baies de raisin, la germination des pépins et la formation des racines sur les boutures de vigne. Thèse Univ. Bordeaux, France.
- DÜRING, H.; BACHMANN, O.; 1975: Abscisic acid analysis in *Vitis vinifera* in the period of endogenous bud dormancy by high pressure liquid chromatography. *Physiol. Plant.* **34**, 201-203.
- ; KISMALI, I.; 1975: Die Rolle der Abscisinsäure bei der Knospenruhe di- und tetraploider Rebsorten. *Vitis* **14**, 89-96.
- GENDRAUD, M.; 1981: Etude de quelques propriétés de parenchymes de Topinambour cultivés *in vitro* en relation avec leurs potentialités morphogénétiques. *Physiol. Vég.* **19**, 473-481.
- ; LAFLEURIEL, J.; 1983: Caractéristiques de l'absorption du saccharose et du tétraphénylphosphonium par les parenchymes de Topinambour, dormants et non-dormants, cultivés *in vitro*. *Physiol. Vég.* **21**, 1125-1133.
- JONES, W. W.; COGGINS, CH. W.; EMBLETON, T. W.; 1976: Endogenous abscisic acid in relation to bud growth in alternate bearing 'Valencia' orange. *Plant Physiol.* **58**, 681-682.
- LEHMANN, H.; SCHÜTTE, H. R.; 1980: Purification and characterization of an abscisic acid glucosylating enzyme from cell suspension cultures of *Macleaya microcarpa*. *Z. Pflanzenphysiol.* **96**, 277-280.

- LESHEM, Y.; PHILOSOPH, S.; WURZBURGER, J.; 1974: Glycosylation of free trans abscisic acid as a contributing factor in bud dormancy break. *Biochem. Biophys. Res. Comm.* **57**, 526-532.
- LOVEYS, B. R.; MILBORROW, B. V.; 1981: Isolation and characterization of 1'-O-abscisic acid- β -D-glucopyranoside from vegetative tomato tissue. *Austral. J. Plant Physiol.* **8**, 571-589.
- MILBORROW, B. V.; 1970: The metabolism of abscisic acid. *J. Exp. Bot.* **21**, 17-19.
- ; 1978: The stability of conjugated abscisic acid during wilting. *J. Exp. Botany* **29**, 1059-1066.
- POUGET, R.; 1963: Recherches physiologiques sur le repos végétatif de la vigne (*Vitis vinifera* L.): la dormance des bourgeons et le mécanisme de sa disparition. Thèse Univ. Bordeaux, France.
- ; 1972: Considérations générales sur le rythme végétatif et la dormance des bourgeons de la vigne. *Vitis* **11**, 198-217.
- SCHLENK, H.; GELLERMAN, J. L.; 1960: Esterification of fatty acids with diazomethane on small scale. *Analyt. Chem.* **32**, 1412-1414.
- TORT, M.; GENDRAUD, M.; COURDURoux, J.-C.; 1985: Mechanisms of storage in dormant tubers: Correlative aspects, biochemical and ultrastructural approaches. *Physiol. Vég.* **23**, 289-299.

Received November 23, 1992