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Research Note

Activities of polyamine oxidases and diaminopropane contents during flowering of *Vitis vinifera* L.

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K e y w o r d s : polyamine, diamine oxidases, polyamine oxidases, grapevine, spectrophotometric assay.

Introduction: Polyamines (PA) are the newest class of compounds considered to function as possible plant growth regulators. Within their biosynthetic pathways the first PA formed is the diamine putrescine (PUT) which may be converted into spermidine (SPD) and spermine (SPM) (MORRIS 1978, GALSTON 1983).

PAs appear to be good biochemical markers for organogenesis, especially with respect to the induction of flowering (MARTIN-TANGUY 1985). In Douglas fir trees, DAOUDI *et al.* (1994) observed that the PA levels are low during flowering, while arginine accumulates. This suggests the presence of diamine oxidase (DAO) and polyamine oxidase (PAO), which are responsible for the degradation of PAs.

In grapevines GÉNY *et al.* (1997) observed considerable variation in the PA composition after anthesis. In particular, diaminopropane (DAP), a product of PAOs, accumulated in young berries and was the major PA during the onset of fruit development. In view of the presumptive importance of these oxidases in berry development, we measured, for the first time, their activities in floral buds, flowers and berries of cvs Merlot and Cabernet-Sauvignon at different developmental stages.

The present paper describes the protein extraction to determine DAO and PAO activities and shows first results underlining the importance of PAO activities during flowering.

Material and Methods: At different stages of development flowers and fruits of Merlot and Cabernet-Sauvignon were collected in a vineyard at Léognan (Bordeaux, France). Each sample, consisting of 10 grapes, was frozen in liquid nitrogen and stored at -50 °C.

One gram fresh weight was suspended in 5 ml of 0.1 M Tris-HCl buffer, pH 7.5. The extraction medium contained 5.7 mM ascorbic acid, 0.1 % (w/v) Triton X-100 and 8 % (w/v) polyethylene glycol 4000 (Carbowax 4000) . The extracts were powdered in an ice-cold mortar. Polyvinylpolypyrrolidone 5 % (w/v) was added. The samples were then centrifuged at 8,500 x *g* for 30 min at 4 °C and the supernatants adjusted to pH 7.5 with a saturated solution of Tris, were used immediately for the PAO assays.

PAO activities were determined according to OKADA *et al.* (1983). The procedure involves the assessment of the rate of peroxidative oxidation of ortho-dianisidine by H_2O_2, released in enzyme assays containing SPD and SPM. The reaction mixture consisted of 8 ml of 0.1 M Tris-HCl buffer (pH 7.5), 0.3 ml each of 1 % ortho-dianisidine and 60 $U \cdot ml^{-1}$ of peroxidase (Horseradish Type II, Sigma). After preincubation of the components at 40 °C, the mixture was filtered; 0.4 ml of the crude enzyme extract and 0.1 ml of 10 mM SPD or SPM were added to 0.5 ml of this solution. The enzymatic activity was measured spectrophotometrically; absorbance at 460 nm was read after 30 min at 40 °C.

DAO activities were determined the same way, with 0.1 ml of 10 mM PUT in the assay instead of SPM or SPD. Non-specific reactions were determined in the absence of substrate.

Free, conjugated and wall-bound PA were extracted according to the method of FLORES and GALSTON (1982) and analysed by HPLC according to SMITH and DAVIES (1985), adapted for plant material by GÉNY *et al.* (1997).

Results and Discussion: In both varieties, PAO activities were determined in floral buds with a maximum in the inflorescences just before the onset of flowering (June, 2 (Merlot) and June, 6 (Cabernet-Sauvignon)). At the end of flowering and during fruit set, PAO activities decreased sharply and tended to zero (Figure, A).

After anthesis, in both varieties considerable variation in the PA composition and content was observed. In flowers DAP was present at low levels and accumulated (principally in its conjugated form) predominantly in young berries (Merlot: June, 15, Cabernet-Sauvignon: June, 23). Thereafter, DAP levels decreased and were very low in berries before and after veraison (Figure, B).

During flowering and fruit development changes in free and conjugated PA contents were similar to those reported for various other plants (MARTIN-TANGUY 1997). However, to our knowledge, this is the first report on the occurrence and possible implication of PAO activities and DAP in flowering and the pollination processes.

In flowers and young berries, the PAO activities and the DAP content were inversely correlated. This correlation was similar in both varieties. It was independent of the date but depended on the stage of development. The maximum PAO activities occurred before DAP accumulated, *i.e.* flowering was characterized by maximum PAO activities and pollination by maximum DAP contents. These compounds can be considered to be good indicators of these two developmental stages.

These data raise the question of the function of bound and conjugated PAs and especially the function of DAP. DAP was the major PA observed in young berries at the onset of fruit development (GÉNY *et al.* 1997). In plants, amine oxidases are involved in the regulation of PA concentrations and transport at the subcellular level, depending on the physiological stage of the tissues (ARIBAUD *et al.*

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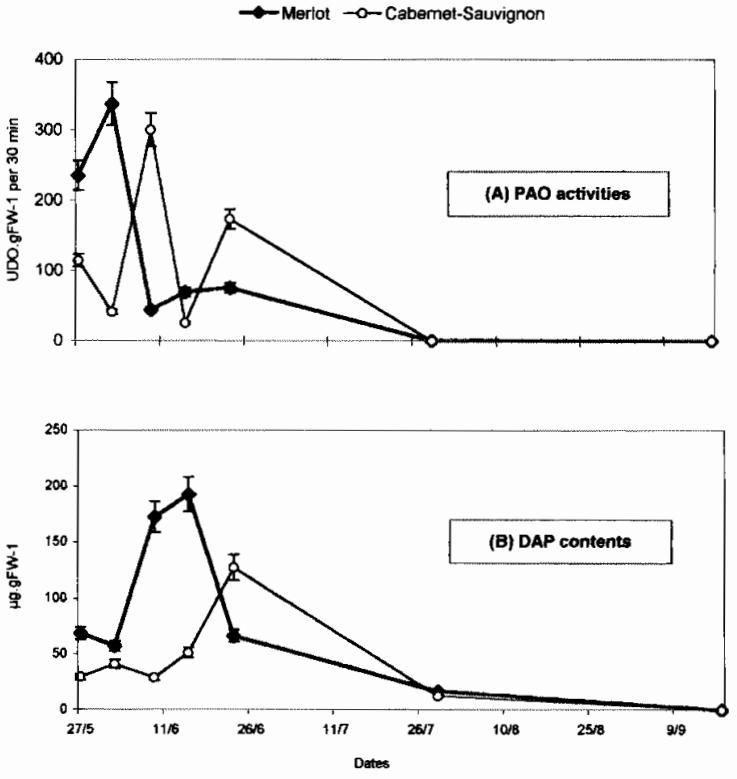

Figure: PAO activities (**A**) and DAP contents (**B**) in floral buds and in young berries of grapevine. Values are means ± SD of two determinations of samples consisting of 10 grapes each.

1994). Data presented by SHIH *et al.* (1982) demonstrate that DAP is a potent inhibitor of dark-induced senescence inhibiting ethylene production. DAP may bind polyribosomes and affect protein synthesis, which may account for the decrease of protease activity. The antagonism to Ca^{2+}, however, suggests that the initial step of the retardation of senescence by DAP probably involves its attachment to membranes (SHIH *et al.* 1982).

We believe that the study of the relationship between PA catabolism and pollination will lead to further experimental efforts. One possible approach would be to utilize specific precursors or inhibitors of PA synthesis and to examine the resulting physiological properties of the tissues. On the other hand, cloning of genes for amine oxidases in *Vitis vinifera* L. would allow to investigate the mechanisms controlling PA catabolism. This includes the knowledge of molecular mechanisms in which these PAs play a role in the processes of grapevine growth and development.

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