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Use of DMACA to visualise flavan-3-ols in grape berry skins (*Vitis vinifera* L., cv Cabernet franc)

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Introduction : Phenolic compounds play a major role in the elaboration of red wines and during wine preservation. They contribute greatly to their organoleptic properties. Among them, flavonoids constitute the major group present in berry skins. The histological study of phenolics is based classically on the use of Toluidine blue O as a staining reagent. In this work, the DMACA – a specific reagent of flavan-3-ols – has been adapted to the study of the phenolic compounds in grape berry skins.

Materials and methods : Berries of Cabernet Franc cl 210 at the same stage of development were collected with their pedicels, from one month before veraison up to the end of veraison. A square portion of berry skin (0.25cm²) was taken off from the equator of the sun-exposed face and fixed immediately in a glutaraldehyde solution. There after, samples were dehydrated in a grade of ethanol series and embedded in a resin Technovit 7100 according to KROES et al. (1998). Sections (1-2µm) were cut on a Leica RM 2165 microtome.

The following staining solutions were used :

Toluidine blue O : 0.5% in distilled water, not specific of any type of phenolics ; DMACA (p-dimethylaminocinnamaldehyde) : 0.5 M sulphuric acid in 1-butanol (GUTMANN AND FEUCHT, 1991), specific of tannins.

Sample were examined under a BH2/RFC A Olympus microscope.

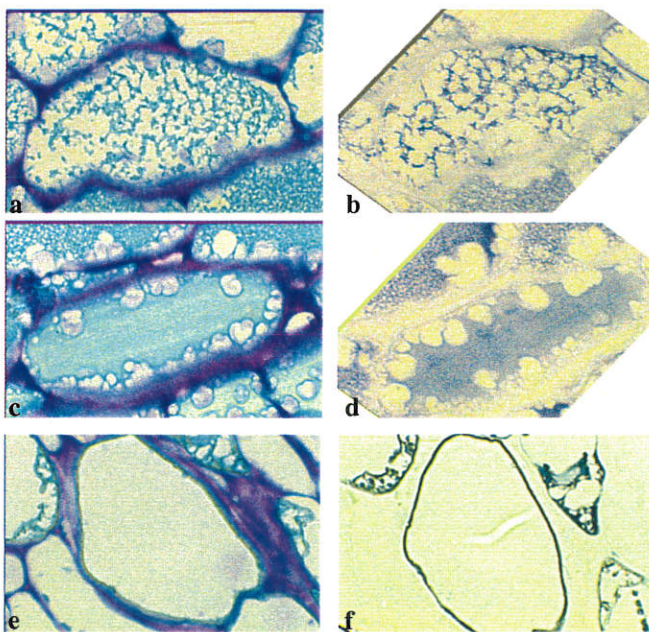


Figure 1 : Comparison of the toluidine blue O and DMACA coloration (x 2300)
a. granular aspect, coloured with toluidine blue O
b. granular aspect, coloured with DMACA
c. uniform aspect, coloured toluidine blue O
d. uniform aspect, coloured with DMACA
e. stuck to the tonoplast aspect, coloured with toluidine blue O
f. stuck to the tonoplast aspect, coloured with DMACA

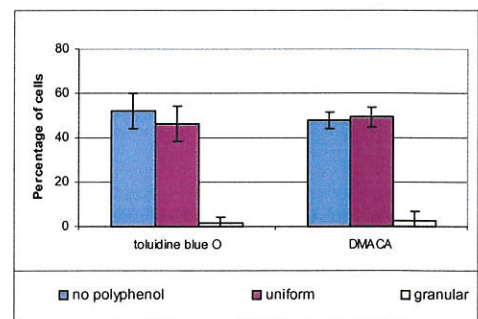


Fig. 2 : Comparison of Toluidine blue O and DMACA staining for the differentiation of phenolic types (23/07/2002, one month before veraison)

Results and Discussion : With Toluidine blue O, polyphenolic compounds have a characteristic blue-green colour where as cell-walls are blue purple and cytoplasm is green. With DMACA, flavan-3-ols are coloured in blue and cell-walls and cytoplasm are not stained.

Both staining solutions allowed to visualise 5 types of cells :

- without any precipitate or granulation into the vacuoles (not shown here)
- uniform aspect, the vacuole is completely coloured (fig. 1c, 1d)
- granular aspect, with an homogeneous distribution (fig. 1a, 1b)
- small spherical vacuolar inclusions, stuck to the tonoplast (not shown here)
- large spherical inclusions, free into the vacuole (not shown here).

The localisation of the flavan-3-ols coloured with DMACA coincided with that of phenolics coloured with Toluidine blue. Counting of cells gave the same results (fig.2). What is coloured with Toluidine blue may be a combination of different phenolics, including flavan-3-ols. The advantage of DMACA is that cell-walls or cytoplasm are not coloured. It is easier to distinguish the different types of cells.

The use of DMACA which is specific of tannins (flavan-3-ols), permitted to precise the localisation of these compounds without any risk of error ; it permitted also the visualisation of a new type of tannins, stuck to the tonoplast, which had not been identified with Toluidine blue O (fig.1f).