Changes in levels of hydrogen peroxide and phenolic compounds in grapevine latent buds during the annual cycle

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Abstract- The quantitative analysis of phenolic compounds and of hydrogen peroxide (H_2O_2) amounts in the latent buds of the vine (*Vitis vinifiera*. L) showed seasonal variations. These compounds change also as function of the vine development cycle. The total amounts of phenolic compounds are strongly accumulated in the buds in beginning of dormancy phase in mid-August. Nevertheless, the ending of dormancy phase in mid-November in the buds is associated with increasing of the hydrogen peroxide yield and a considerable drop in the content of the total amount of phenolic compounds. These variations appear to be strictly related to the climatic conditions. This is particularly visible in ending of dormancy phase. This is coinciding with the cold period where the temperature is below 10 ° C for at least 7 consecutive days.

Index Terms- *Vitis vinifera*- H_2O_2- dormancy- buds- phenolic compounds

I. Introduction

Perennial plants have an annual rate of activity apparently modeled on the rate of theseasons: growingin springand summer when the weather conditions are favorable, growths lowing followed by total arrest in autumn and winter when environmental conditions become increasingly difficult. The trees then become dormant, a particular physiological condition that allows them to withstand climatic adversities. Each year the vine grows depending on the season. It follows a growth cycle that consists of several steps (Pouget, 1963). Dormancy in the vineyard is triggered by the drop in temperature. However, the photoperiod decreaseis considered one of the biggest factors that can induce dormancy installation (Febvre, 1981; Olsen, 2006). The removal of dormancy is also controlled by environmental conditions. At the vine, the buds acquire the ability to clog under the effect of low temperatures (between 0 and 10 °C) for a variable period depending on species. Cold in temperate climates remains the most effective way to break dormancy in woody species (Nigond, 1967; Champagnant, 1983).To try to understand this resting phase of the plant, several authors have studied the main biochemical changes undergone by the buds. Some authors have mentioned in particular the involvement of hydrogen peroxide (Lavee and May, 1997) and phenolic compounds (Nagar 1996; Macheix *et al.*, 2005 and Kraiem *et al.*, 2011) in this phenomenon of dormancy. The vine is no exception to this type of study. Indeed, the main studies on latent vine buds focused on carbohydrates, fatty acids and some hormones (Koussa *et al.*, 1992). However, no study has been performed on the H_2O_2 content and phenolic compounds in grape buds during the annual cycle and especially during the phase of dormancy. The objective of this study was therefore twofold. At first it was to follow the variations of the levels of H_2O_2 and phenolic compounds in *Vitis vinifera* L. cv. Merlot. (black Merlot) latent buds during the annual cycle. In a second step, it aims to explain the physiological variations that may exist between these biochemical compounds and the different phases of development of the vine.

II. Material and Methods

II.1. Plant material

This study focused on a homogeneous plant material consists of a single vine variety of *Vitis vinifera* L. cv. Merlot. (black Merlot) . In this study, the sampling of plant material has taken place in region of Bordeaux. The shoots were sampled at the heart of the annual cycle, from the month of July until the month March (Pouget, 1963). It covered the first 10 latent buds from the base of the stems and the first three internodes merithallus type N1-N2, comprised between consecutive tendrils (Bouard, 1966). The samples were collected in order to obtain more homogeneous (random sampling in space). They were frozen in liquid nitrogen and then lyophilized.

II.2. Hydrogen peroxide content

Hydrogen peroxide was measured by using (Sergiev *et al.*, 1997) method. After extraction with TCA 0,1 % and centrifugation, the supernatant was added to potassium iodure 1mM and phosphate buffer 10 mM ph7. Then absorbance was read at 390 nm.

II.3. Extraction of polyphenols

The total phenolic compounds were extracted with a solution of ethanol/water/chloroform (1v/1v/2v) using an Ultra Turrax homogenizer following the protocol described by Darné *et al.* (1979). The water-ethanol phase containing phenolic compounds was separated from the organic phase containing lipids, chlorophylls and other pigments. The ethanol was removed from the aqueous phase using a rotavapor at low pressure at 35ºC.The aqueous solution containing the phenolic compounds was adjusted to a desired volume.

II.4. Determination of total phenolic compounds

Phenolic compounds were determined using Folin–Ciocalteu reagent method by reading the absorbance at 765 nm according to the method of Ainsworth and Gillespie. (2007). Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalent (GAE)/ g of fresh weight.

II.5. Statistical Analysis

All extracts of latent buds and internodes were analyzed in triplicate and the results are expressed as mean values ± standard deviations (SD). The data were subjected to statistical analysis using statistical program package SPPS and the differences between individual means were deemed significant at $\rho < 0.05$**.**

III.RESULTATS

II1.1. Case of Hydrogen peroxide (H_2O_2)

Hydrogen peroxide levels in the latent buds generally undergo variations according to the phase of development during the vegetative cycle (Figure1).These levels were higher with 1.16± 0,106 mg.g^{-1} dm in buds and internodes, respectively. These values were obtained during the pre-dormancy phase by the end of July. Then, H_2O_2 values decreased and reached (0.34± 0,030 mg.g^{-1} dm) by the end of October. In the post-dormancy the hydrogen peroxide levels were slightly increased and reached (0.58±0,054 mg.g^{-1} dm) on March. The dehydration phase of buds was observed at the end of the vegetative cycle, where the H_2O_2 concentration decreased.

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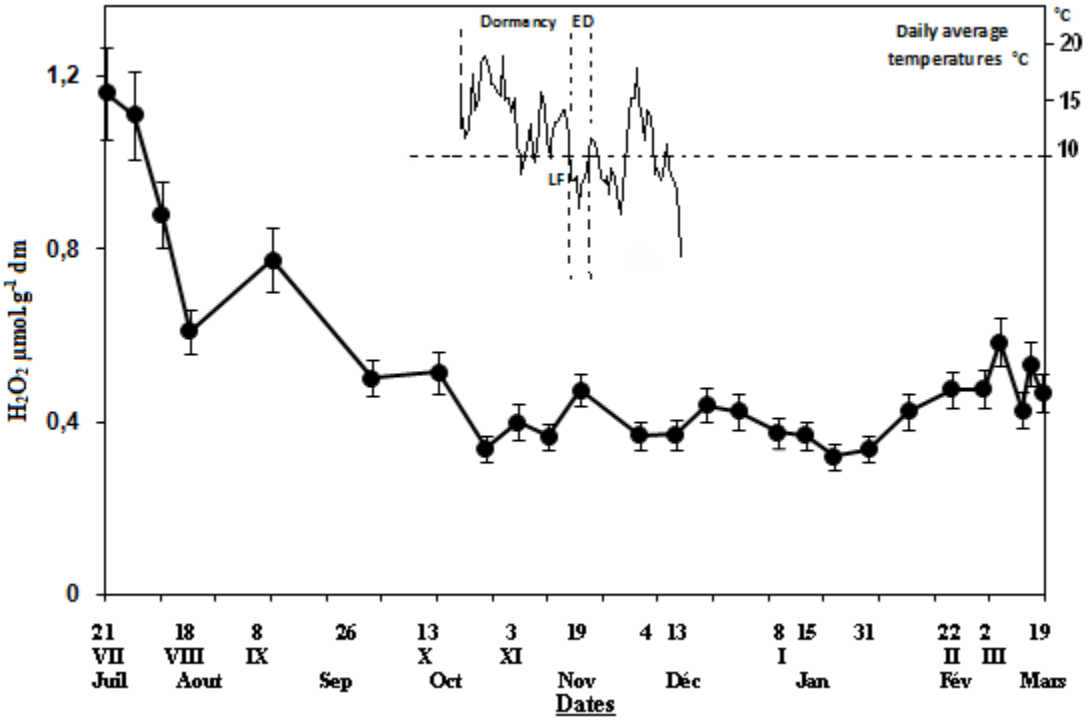

Figure1. Development of the hydrogen peroxide content of buds of Merlot variety of grapevine and the daily mean temperatures in the course of the annual cycle (—●—) Buds. Values represent the means (±SE) of three replicates**. CF:** leaf fall**; LD:** End of dormancy

II1.1. Case of total polyphenols

The amounts of polyphenols in latent buds (show seasonal variations (Figure 2). Those amounts change also as function of the development cycle (Pouget, 1963). The total amounts of phenolic compounds are increased in the beginning of sampling in July with a maximum of 1,65±0,10 mg.g^{-1} dm. By the end of this phase their amounts start to decrease, coinciding with the starting of dormancy phase. During this phase the amounts of phenolic compounds are decreasing gradually. After this period the phenolic compounds amounts in latent buds are dramatically decreased with a minimum of 0,55±0,06 mg.g^{-1} dm. This decreasing occurs in a short period of time that coincides with the ending of dormancy phase where temperature is lower than 10°C during at least 7 successive days, this is the colder phase. Afterward, their total amounts start to increase slightly until reaching the value of 0,76±0,08 mg.g^{-1} dm the remain almost stable, with slight variations during budburst . At the end of each cycle a decrease is detected. This is corresponding to the dehydration phase of buds.

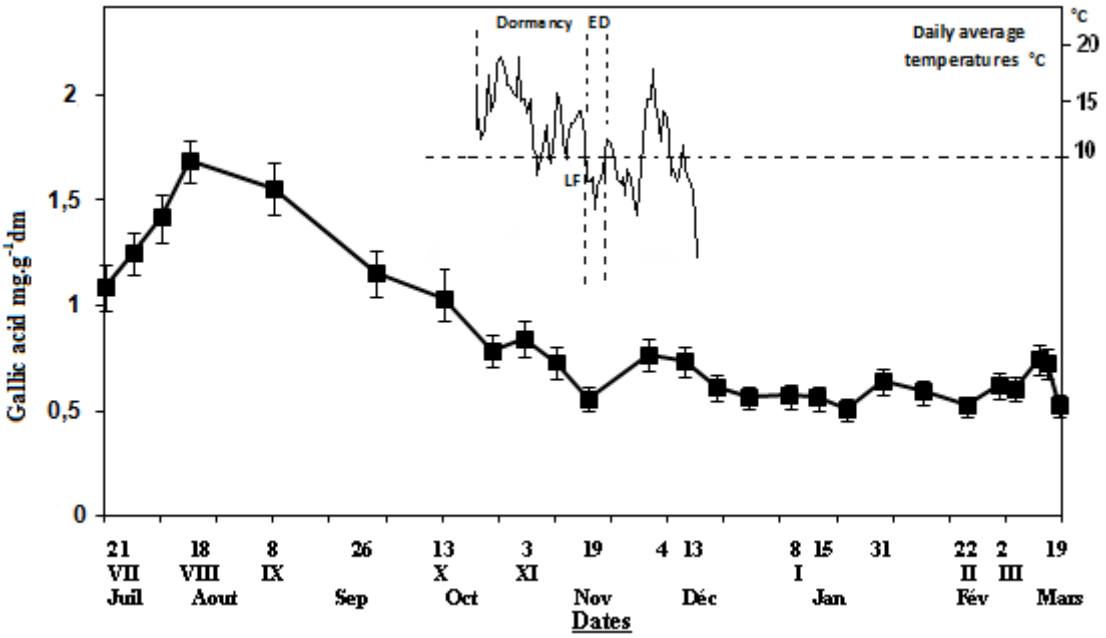

Figure1. Development of the total polyphenols content of buds of Merlot variety of grapevine and the daily mean temperatures in the course of the annual cycle (—■—) Buds. Values represent the means (±SE) of three replicates**. CF:** leaf fall**; LD:** End of dormancy

IV. Discussion

In our study, Levels of H_2O_2 in latent buds were reduced during pre dormancy and dormancy phases, which had been related to daily decreases in temperatures and photoperiod. (Figure1). These findings were in accordance with those of Verslues *et al.* (2007), who observed a decrease of photoperiod is accompanied with decreases of H_2O_2 amount with increasing of following enzymes: superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Moreover, the slight increase of H2O2 level during dormancy phase was also reported by Wanping *et al.* (2009) who argued that cold winter could be the cause of this high level of H_2O_2. On the other hand, Or *et al.* (2000) suggested that the antioxidant mechanism is induced to cope with increased levels of H_2O_2, in the buds during natural dormancy release. Accumulation of H_2O_2 observed during the budbreak phase is in accordance with previous results obtained by Lavee and May (1997). Moreover, they reported that the rising dormancy phase of bud mechanism is related to the overproduction of H_2O_2. Bajji and coll (2007), they explained this accumulation by inhibiting the activity of CAT, which converts hydrogen peroxide into oxygen and water molecule.

The evolution of phenolic compounds in the latent buds of the vine during the vegetative cycle.seemto be probably associated with different development phases (Pouget, 1963) and abiotic stress applied by the environment. Indeed, the accumulation of these compounds during the phase of the dormancy could be linked to the decrease in temperature and length of daytime. Such results were also suggested by many authors (Macheix *et al.*, 2005; Wahid and Ghazanfar, 2006 and Wahid, 2007) that have shown that certain phenolic also play a role in the tolerance in the plant to abiotic stress and have considered that the Temperature as a phenolic metabolism expression regulator, induces an accumulutaion of anthocyanins and that regulation could intervene at the level of activity Phenylalanine (PAL) (Macheix *et al.*, 1990).

Accumulation of phenolic compounds seems to be related to low temperatures, characterizing the phase of break dormancy (Pouget, 1963) (less than 10 ° C for 10 days in this study). It is paradoxical that the same treatment diet of temperature that induces dormancy in some species of plants,may also control its break dormancy (Olsen, 2006).Such a hypothesis seems in agreement with the findings of Pennycooke *et al.* (2005) who showed that the cold caused an accumulation of phenolic compounds in *Betuniahybrida* leaves. (Wróbel *et al.*, 2005) also have shown that the cold stimulates some phenolic compounds (free gallic acid and cathéchine) in the seeds of Vitisriparia. Moreover, vernalization is accompanied by modifications in levels of various substances: decreased levels of amino acids and increased levels of phenolics. Indeed, levels of these phenolic compounds fall strongly. According to these authors, these phenolic compounds are metabolized and transferred to other parts of the plant to meet their need ofdevelopment. Such results had been suggested by Kraiem *et al.* (2011) who reported that levels of quercetin increases during the phase of dormancy when it decreases during the phase of bud break in the bud vine var. Carignan.

The evolution of levels in phenolic compounds of latent buds during their development cycle seems to be related to that of hydrogen peroxide. Indeed, these results lead us to suppose that when the H_2O_2 levels are high, there would be an oxidation of phenolic compounds, whereas when these levels are reduced, it would promote the accumulation of phenolic compounds. This hypothesis is supported by Qian Li *et al.* (2011) which reported

that the cold treatment leads to higher CAT and APX activities in the leaves, but the combination of pretreatment of a cinnamic acid and cold treatment further enhances the antioxidant activities. These authors showed that in association with cinnamic acid, cold Reduces H_2O_2 levels. This result seems in agreement with several studies dealing with the involvement of the H_2O_2 in response to attacks of the abiotic environment (Ashraf and Harris, 2004). Indeed, the slight increase of the H_2O_2 observed in the breaking of dormancy phase seems to play a role as a mediator used for signal transduction of stress (Moussa, 2006). It would act as a messenger triggering a modification of ionic flux or a production of second messengers such as salicylic acid.

V.CONCLUSION

The present study, allowed to follow up the evolution of hydrogen peroxide (H_2O_2) and total amount phenolic compounds during the annual cycle of the Merlot variety for the vines.

The hydrogen peroxide (H_2O_2) and the phenolic compounds accumulate in dormant buds in order to contribute in the resistance against the environmental conditions during the winter season. However, this accumulation decreases during the budburst phase. After the dormancy phase the buds proved to involve an increase in the production of hydrogen peroxide inside cells. Indeed, the variation of these compounds had a close relation with different phases of bud development of the vine

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