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BRIEF COMMUNICATION

Chlorophyll fluorescence as a tool for evaluation of viability in freeze-stressed grapevine buds

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Abstract

This study examined the utility of the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) to detect freezing injury on buds of two *Vitis vinifera* cultivars: Pinot noir and Pinot gris. Freezing treatments on buds caused a decrease both in F_v/F_m and percentage of budburst, more severely on Pinot gris than Pinot noir, specifically at the lower temperature (–20°C). F_v/F_m ratio showed a close correlation with percentage of budburst, and a threshold of the lethal F_v/F_m was proposed as an indicator of bud mortality.

Additional key words: bud break; frost damages; maximal photochemical efficiency of PSII; temperature; viability test; woody tissues.

Freezing temperatures can produce severe damage on grapevine, due to the wide distribution of this crop. Injury by freezing can reduce grape yield, damage vine parts, or kill the whole plant. During the dormant period, overwintering buds are more susceptible to freezing injury than are the canes or trunk (Quamme 1986, Miller *et al.* 1988, Jones *et al.* 1999). Injury evaluation after a freezing event requires a rapid test to determine damage prior to bud break, allowing for the growers to adjust pruning levels in accordance with bud injury (Fennel 2004). Use of chlorophyll (Chl) fluorescence yield (especially the ratio F_v/F_m) as a method of assessing woody tissue viability in grapevine was previously reported (Düring *et al.* 1990, Jiang *et al.* 1999, Jiang and Howell 2002). The objectives of our experiments were (*1*) to evaluate the effect of freezing on buds of two grapevine cultivars by means of the F_v/F_m ratio and the potential of budburst; and (*2*) to determine the efficacy of chlorophyll fluorescence method for evaluation of freezing damage on woody tissue.

The first experiment was conducted on buds of two different grapevine cultivars (*Vitis vinifera* L., cv. Pinot noir and cv. Pinot gris) in a 17-year-old vineyard trained to spur cordon and located at the Edmund Mach Foundation (Province of Trento, northeastern Italy). In the dormant period (early February), nodes 5 through 10 from the base of several canes were cut and sealed in plastic bags of 40 nodes each one. Cuttings were placed in freezing chambers at –15 and –20°C (75% R.H.) for 24, 48, and 72 hours and 7, 14, and 17 days; at the same times cuttings placed at +20°C were referred to as control. Both treated and control samples were kept in darkness during the whole time of the experiment. In each freezing time, nodes of both cultivars were extracted from freezing chambers for Chl fluorescence analysis (n = 20) and percentage of budburst evaluation (n = 20). In the second experiment nodes of both cultivars were cut every 3–4 days from 27 March till 21 April, (date of natural budburst: 19 and 21 April for Pinot gris and Pinot noir, respectively), and immediately used for Chl fluorescence yield and budburst analyses. Moreover, buds cut in the first ten days of April were also placed in freezing chambers at –12°C for 72 h. At the end of freezing treatment Chl fluorescence yield and percentage of budburst were assessed. Chl fluorescence yield was measured on dissected buds, placed in the clips, using a *PAM 2000* fluorometer (*Walz*, Effeltrich, Germany). Minimum fluorescence (F_o) was measured by switching on the modulated light 0.6 kHz; photosynthetic photon flux density (PPFD) was less than 0.1 μmol m^{-2} s^{-1} at the internal bud surface. Maximum fluorescence (F_m) was measured at 20 kHz with 1 pulse of

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Abbreviations: Chl – chlorophyll; F_o – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence of dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry.

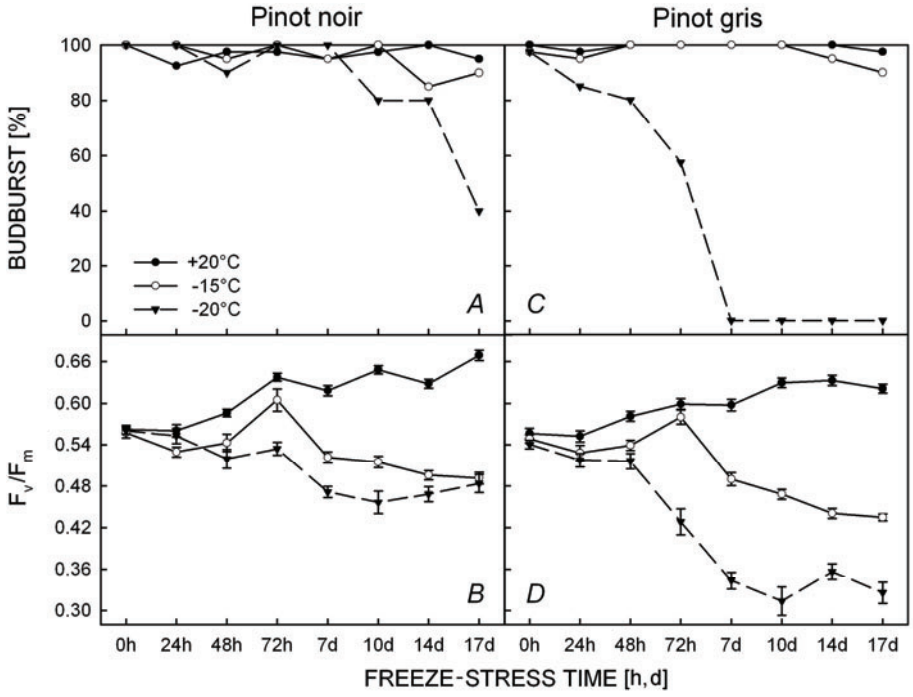

Fig. 1. Effect of freeze stress duration in darkness on buds of *V. vinifera* L. cv. Pinot noir (*A*) percentage of budburst, (*B*) F_v/F_m and of *V. vinifera* L. cv. Pinot gris (*C*) percentage of budburst, (*D*) F_v/F_m. (0 h: 2 February, 17 d: 19 February). Means ± SE, $n = 20$.

6,000 μmol m^{-2} s^{-1} of white light (duration of pulse 0.8 s). *PAM* measurements were performed in the darkness at a room temperature, one hour after removing the samples from freezing chambers. Percentage of budburst was assessed by planting the cuttings in substrate of rockwool Grodan® Macroplugs (*Grodan BV*, Roermond, The Netherlands) placed in a growth chamber at 25°C and 70% R.H., break of bud dormancy was checked. Data were subjected to one-way *ANOVA* using *SPSS 17* (*SPSS*, Chicago, USA). Pearson correlation coefficient was calculated between F_v/F_m ratio and the percentage of budburst.

The results of the first experiment revealed a higher sensitivity of Pinot gris than Pinot noir to the freezing treatment on dormant buds, specifically at the lower temperature (–20°C). In both Pinot noir and Pinot gris, the percentage of budburst was never lower than 85% at –15°C at all times of the trial (Fig. 1*A,C*). F_v/F_m measured on dissected buds was also slightly affected by this temperature (Fig. 1*B,D*), reaching the lower values at 17 days of treatment with 0.492 and 0.435 for Pinot noir and Pinot gris, respectively. The treatment at –20°C instead caused severe damages on buds of both cultivars even much more intensively on Pinot gris. This temperature caused a decrease in the percentage of budburst on buds of Pinot noir starting from 10 days of treatment (80% at 10 and 14 days, 40% at 17 days), whereas F_v/F_m shows a quite slightly decrease during the –20°C treatment (Fig. 1*A,B*) with the final point on the same value of –15°C treatment (0.484 and 0.492 at –20 and –15°C, respectively). Pinot gris buds were affected by the treatment at –20°C starting from 48 h as indicated by a significant decline of both budburst percentage and F_v/F_m. At the faster decrease of percentage of budburst (80% at 48, 60% at 72 h and 0% at remaining times) corresponds a similar decrease in the F_v/F_m ratio (Fig. 1*C,D*), and in correspondence of values 0 in the percentage of budburst, F_v/F_m values were 0.314 to 0.356. As also reported by Jang *et al.* (1999), when the temperature decreased after a certain freeze-stress temperature (–20°C in our work), F_v/F_m decreased greatly, indicating a decreased photochemical efficiency of photosystem II. Moreover, the absence of budburst from 7 days at –20°C treatment might indicate a high level of a damage in the woody tissues or their death status. Control samples show a slight increase of F_v/F_m ratio during the whole experiment. This is probably due to the control temperature of 20°C that was higher than the corresponding vineyard temperature in February.

In the second experiment, buds were cut close to a natural bud break revealing 100% of percentage of budburst in each time (except once) for both cultivars (Table 1). Corresponding values of F_v/F_m are very close to 0.700 which would suggest that this value shows a status of complete reactivation of buds, also considering that in buds of both cultivars cut on 31 January and 27 February, the F_v/F_m values were about 0.530 and about 0.585, respectively (data not shown), with 100% of budburst (under forcing conditions). The low increase of F_v/F_m was probably caused by an increase of the day mean temperature and higher irradiance. A similar trend in F_v/F_m on grapevine woody tissues over the dormant season has been found by Jiang and Howell (2002). Freezing treatment (–12°C for 72 h), performed on buds cut during the first ten days of April, caused severe damages (Table 2) on both cultivars: none of the buds

Table 1. F_v/F_m and percentage of budburst measured on buds of *V. vinifera* L. cv. Pinot noir and cv. Pinot gris, close to the time of natural bud break (19 to 21 April). Means ± SE, $n = 20$.

Date	Pinot noir F_v/F_m	Budburst [%]	Pinot gris F_v/F_m	Budburst [%]
27/03	0.651 ± 0.004	100	0.681 ± 0.004	97
03/04	0.686 ± 0.005	100	0.699 ± 0.006	100
06/04	0.688 ± 0.004	100	0.680 ± 0.008	100
10/04	0.695 ± 0.005	100	0.693 ± 0.007	100
14/04	0.699 ± 0.002	100	0.700 ± 0.004	100
18/04	0.710 ± 0.003	100	0.708 ± 0.004	100
21/04	0.714 ± 0.005	100	0.717 ± 0.003	100

Table 2. Effect of freeze stress (–12°C for 72 h), on F_v/F_m and the percentage of the budburst, of *V. vinifera* L. cv. Pinot noir and cv. Pinot gris, applied close to the time of the natural bud break (19 to 21 April),. Values measured before the freeze treatment are reported in Table 1 at the corresponding dates. Means ± SE, $n = 20$.

Date	Pinot noir F_v/F_m	Budburst [%]	Pinot gris F_v/F_m	Budburst [%]
03/04	0.189 ± 0.013	0	0.161 ± 0.012	0
06/04	0.226 ± 0.018	0	0.176 ± 0.015	0
10/04	0.234 ± 0.016	0	0.212 ± 0.019	0

burst and F_v/F_m was 0.189 to 0.234 and 0.161 to 0.212 for Pinot noir and Pinot gris, respectively, confirming the high sensitivity to cold, near the bud-break time.

The correlation between F_v/F_m and percentage of the budburst was calculated using all the data and treatments from both two experiments. F_v/F_m ratio revealed a strong correlation with the percentage of the budburst and Pearson's correlation was 0.866 (p=0.000). If we consider that below the F_v/F_m value of 0.428 there was no budburst and the higher F_v/F_m value found in the complete damaged buds (no bud break) was 0.357, we hypothesized that the possible threshold for bud mortality is about 0.400.

In conclusion, our work demonstrates that under severe freeze treatment on buds cut in mid-winter, grapevine cultivar Pinot gris is more sensitive than Pinot noir in terms both of budburst and photochemical efficiency measured on woody tissues. On the other hand, freeze treatment of lower intensity causes severe damages on buds cut 2 weeks before the natural bud break in vineyard, and no differences were found among cultivars. The F_v/F_m ratio was shown to be an objective tool to determine mortality of buds due to frost injury. However, further works are needed to assess a more accurate threshold for tissues mortality.

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