Single-node cuttings for budbreak phenotyping in grapevine

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Abstract. Budbreak is a critical stagein terms of vulnerability tospring frost, a major issue accentuated by the advancement of grapevine development due to global warming. Recognizing the determinants of budbreak regulation is essential to allow the selection, or generation, of genotypes better suited to dealwith a changing climate. Due to the practical difficulties and inherent variability in observing complexphenomena such as spring phenology in field, standardized alternatives are desirable. For these reasons, the potential of characterizingdormancy release using single-node cuttings was tested using cultivars Chardonnay and Cabernet Sauvignon, known for their different budburst timing in the field. Visual phenotyping was employed, in concert with Differential Thermal Analysis (DTA), to evaluate differences in deacclimation and dormancy release. The results confirmed that in field differences remain noticeable in single-node cuttings, corroboratedby macroscopic differences and different deacclimation kinetics. In conclusion, single-node cuttings candelineate inter-varietal differences in bud burst timing under standardized conditions and can be considered for use in screening activities.

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1 Introduction

Early bud deacclimation and premature loss of cold hardiness can be counted among the effects of global warming on viticulture [1,2]. As a consequence, buds become more susceptible to late frost exposure athighly vulnerable developmental stages [3], thus increasing the risk of damage. Despite these considerations, the regulation of bud dormancy, including its release and responses to temperature changes, remains poorly understood andfocused studies are neededto define the molecular regulators of these aspects. Clearer insights into dormancy regulation will allow the selection, or generation, of genotypes better adapted to new climatic conditions[4].

Monitoring complex traits spanning long periods such as dormancy and cold deacclimation remains, however, challenging. These phenomena are influenced by multiple environmental factors such as temperature and hydration,implying a critical role inseasonal variability [5]. Furthermore, fieldphenotyping is a taxing task due to time-consuming observations to call each developmental stage when 50% of the buds reach it [6,7].

Based these premises, reducing field variability and increasing standardization are desirable goals. The use of bud cuttings represents a valuable option to increase the number of replicates while reducing the amount of space required for observation. Better control of environmental conditions could also be achieved. To test this possibility, single-node cuttings of Cabernet Sauvignon and Chardonnaygrapevine cultivars, late- and early-budbreak varieties respectively [8], were tested under two forcing conditions, and evaluated using visual phenotyping and differential thermal analysis (DTA).Consistency with their expected physiological behaviour is discussed.

2 Materials and methods

2.1 Plant material and experimental setup

One-year canes of the late-budbreak cv. Cabernet Sauvignon and the early-budbreak cv. Chardonnay were kindly provided by Vivai Cooperativi Rauscedo (VCR),collected in the field (45°41′N 13°24′E, Grado, Italy) in January 2023 and kept at 4°C until April 2023 to ensure chilling requirement fulfilment and improve budburst uniformity. For each variety, forty-five single-node cuttings were collectedfrom position 3 to 12,counted from the base of canes of similar calibre, and placed in water or hydrated perlite at forcing conditions of 21°C ± 1°C and 16/8 h of light-dark photoperiod.Stable hydration of perlite was maintained through water additions every two days.Sampling times (day of experiment, DOE) were set according to the phenological progression of early-budbreak Chardonnay.

Three biological replicates of 5 buds each were simultaneously sampled for differential thermal analysis (DTA). Buds' phenological development was monitored visually and classified according to the BBCH scale [6] (Table 1).Before each forcing experiment, canes were immersed in water overnight to standardize their hydration conditions.

Table 1. BBCH classification of bud-related developmental stages considered in the experiment[6].

Stage	Description
BBCH 00	Winter bud
BBCH 01	Beginning of bud swelling
BBCH 03	End of bud swelling
BBCH 05	Wool stage
BBCH 07	Green tips visible
BBCH 09	Budburst

2.2 Differential Thermal Analysis

Cold hardiness determination was performed with DTA using thermoelectric modules (TEM) and temperature probes placed in a T700BXPRO temperature-controlled freezing chamber (FDM, Rome, Italy). Temperature was brought to 7°C and subsequently lowered to -25°C at a rate of -2.5°Ch^{-1}. Data were recorded using CR1000 data-logger (Campbell Scientific, Logan, UT, USA). Temperature and voltage signals were analysed using RStudio software (R Core Team, 2021). Cold hardiness was assimilated to low temperature exotherms (LTEs), corresponding to peaks of intracellular water freezing events.

2.3 Statistical analysis

The statistical significance of DTA data was checked using one-way ANOVA and Tukey HSD as a *post hoc* test with SigmaPlot 14.0 software (www.systat.de).

3 Results

3.1 Morphological changes

The phenological progression of single-node cuttings was observed in water (Figure 1) and perlite (Figure 2). In both scenarios, the development of cv. Chardonnay progressed more rapidly than that of cv. Cabernet Sauvignon, with comparable speed between water and perlite. In detail, 80% of Chardonnay cuttings reached bud swelling stages (BBCH 03) at 3 DOE in water, compared to 50% in perlite.On the other hand, 35% Chardonnay cuttings developed up to wooly stages (BBCH 04-06) at 3 DOE in perlite whereas similar progression was seen for 20% of cuttings in water. Wooly buds' percentage further increased in perlite reaching 65% at 6 DOE, and 80% at 7 DOE in water. Stages from green tip (BBCH 07) to budbreak (BBCH 09) were reached simultaneously in the two forcing conditions at 10 DOE by 40% and 35% cuttings in

water and perlite, respectively. Twenty percent Chardonnay cuttings reached stages above BBCH 09 by 10 DOE in both conditions (Figure 1A, 2A). Consistent differences were observed between the two forcing methods for Cabernet Sauvignon. The entirety of buds reached swelling stages in water between 3 DOE and 4 DOE, whereas only 30% of buds appeared similarly developed in perlite up to 10 DOE. Eighty percent buds progressed to wooly stages in water at 10 DOE, while,in perlite, 5% and 10% of wooly buds were found at 12 DOE and 17 DOE, respectively. Ten percent of cuttings were found in BBCH 07-09 stages at 17 DOE in perlite (Figure 1B, 2B).

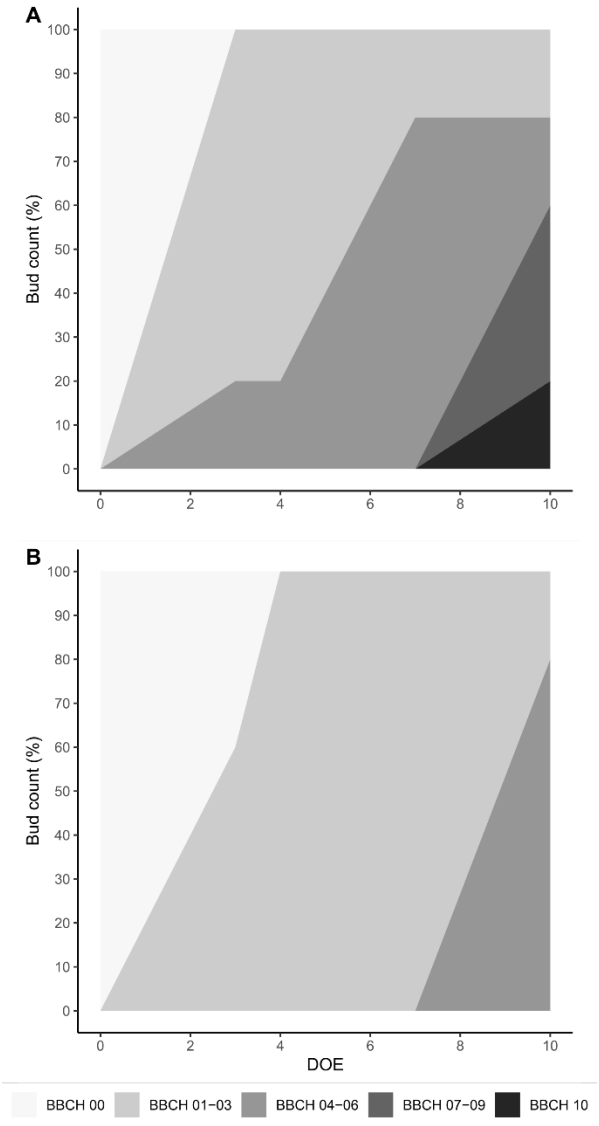

Fig. 1. Phenological progression of Chardonnay (**A**) and Cabernet Sauvignon (**B**) single-node cuttings under forcing conditions at 21°C ± 1°C in water. DOE = day of experiment.

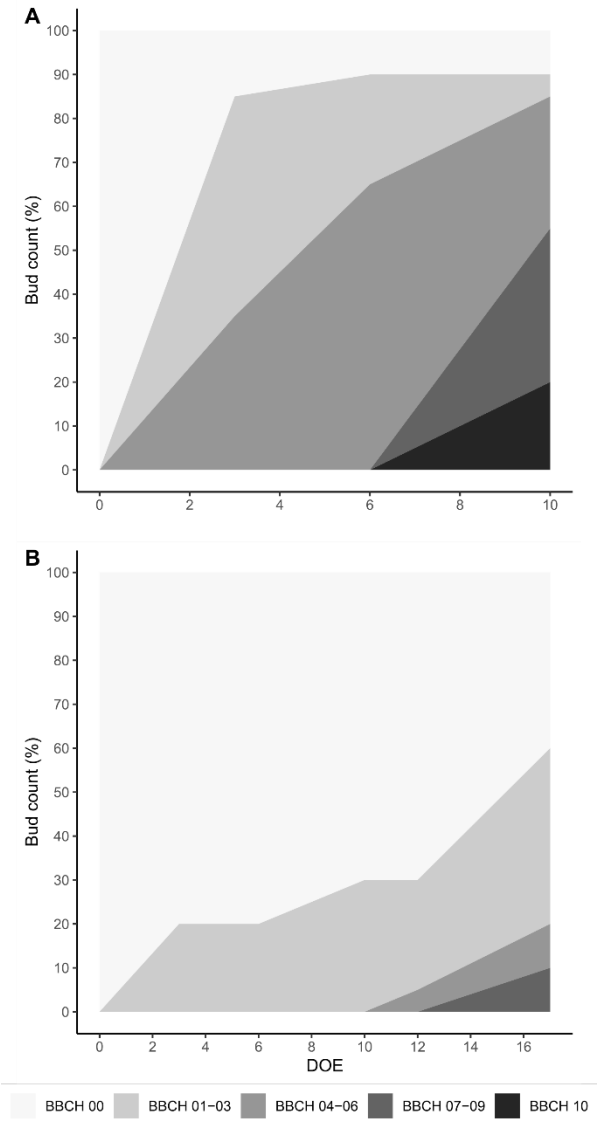

Fig. 2. Phenological progression of Chardonnay (**A**) and Cabernet Sauvignon (**B**) single-node cuttings under forcing conditions at 21°C ± 1°C in perlite. DOE = day of experiment.

3.2 Bud deacclimation dynamics

DTA successfully captured LTE dynamics in single-node cuttings under forcing conditions. Deacclimation levels in Chardonnay appeared comparable in both water and perlite, remaining stably between -5°C and -10°C to the end of observation. On the other hand, LTE dynamics varied substantially between water and perlite forcing in Cabernet Sauvignon. In detail, cold hardiness levels stood around -20°C at 0 DOE in both scenarios, following which water-forced cuttings showed a steeper deacclimation pattern starting from 4 DOE. Deacclimation appeared complete at 7 DOE. As for perlite-forced Cabernet Sauvignon cuttings, no LTEs were registered following 0 DOE up to 12 DOE, in which one LTE corresponding to -17.8°C was detected. Lastly, LTEs reached deacclimated levels at 17 DOE (Figure 3).

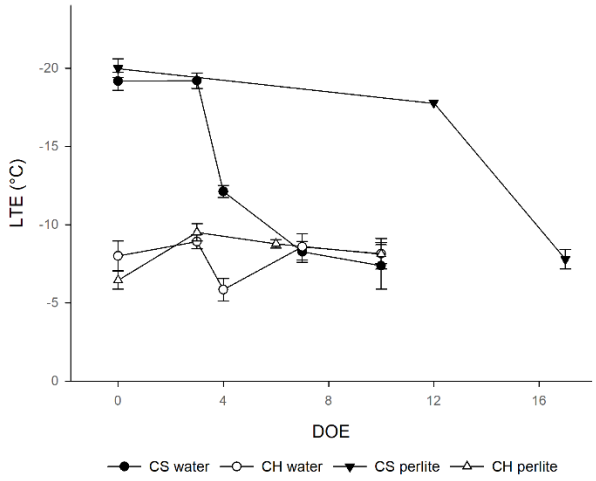

Fig. 3.LTE dynamics of single-node cuttings of cultivars Chardonnay (CH) and Cabernet Sauvignon (CS) forced in water and perlite.

4 Discussion

Cuttings have been conveniently used in the past to carry out experiments on a smaller scale, for example to test temperature and chemicals effects [9,10], as well as forcing studies for the generation of budbreak models and time-to-event studies [11,12]. In this study, the single-node cuttings model appeared effective under forcing conditions particularly in an experiment where the temporal shift between two grapevine cultivars was crucial.

Specifically, the expected behaviour of the two varieties,in terms of budbreak timing, was confirmed in cuttings under standardized conditions, and reasonable budbreak synchrony wad observed for single-node cuttings collected from node 3 to 12 on 1-year old canes. In detail, in both water and perlite, more than 50% of Chardonnay buds reached or surpassed the BBCH 07-09 stages by 10 DOE. Interestingly, Cabernet Sauvignon did not achievesimilar progress until four days thereafter (data not shown), and exclusively in water.This is consistent with natural budbreak timings observed in the field [8], accounting for the evidence that constant chilling temperature-treated cuttings appear to reach budbreak faster than field-collected ones [13].

The differences observed between the two forcing conditions in Cabernet Sauvignon cuttings indicate that the use of hydrated perlite significantly reduces the speed of development of this cultivar compared to water. This should be due to the reduced moisture availability in perlite which can slow down the physiological processes necessary for budbreak. While this might be beneficial to spread over time the observation of highly similar budbreak timing cultivars and better track their differences, it also appears to increase the unevenness of bud development in late-budbreak cultivars such as Cabernet Sauvignon. On the other hand, the use of water as a source of hydration to fuel budbreak is the most promising as bud development appears more uniform, despite resulting significantly fast. This suggests that perlite is suitable for the monitoring of early-budbreak cultivars, and that forcing conditions employing water might benefit lower temperatures to slow down development rates.

By measuring the thermal properties of buds, DTA can detect subtle changes in their physiological state as they transition through different phases of dormancy. Visual phenotyping is supported by DTA data, which clearly depict the different evolutions of cold hardy states in the two cultivars in response to forcing conditions and confirm the slower reactivity of late-budbreak Cabernet Sauvignon, as usually observed in field conditions. Interestingly, Chardonnay LTEs, which correspond to freezing events of intracellular water within buds, routinely assimilated to cold hardiness levels, remained unchanged throughout the time of observation. This may be due to a different sensitivity and perception of 4°C chilling temperatures between the two varieties. Moreover, temperatures that are adequately low to maintain buds in a cold-hardy state in one cultivar, may contribute to chilling accumulation and induce deacclimation in another [13].Since data collected through DTA correspond to intracellular water freezing events, the lack of detected LTEs from 3 DOE to 10 DOE in Cabernet Sauvignon buds forced in perlitecould be the result ofa higher hydration requirement compared to Chardonnay, inadequately fulfilled by perlite. The absence of detectable deacclimation peaks is also coherent with the visually detected lag of development.

In conclusion, these results show that single-node cuttings represent a viable option for budbreak phenotyping in standardized conditions. This method constitutes an effective approach for comparison among different grape varieties and the possibility of screening the spring phenology of entire populations. Moreover, our results demonstrate that the experimental setup is crucial, as the conditions under which the single-node cuttings are maintained directly influence the accuracy and reliability of the findings. Factors such as temperature and moisture must be carefully regulated based on the cultivar and the objectives of the analysis. On the other hand, it is also important to compare different cultivars under identical controlled conditions to ensure that observed differences in dormancy behaviour are due to inherent genetic traits rather than environmental variability. Therefore, meticulous experimental setup and comparative analysis of different cultivars are central to advancing our understanding of dormancy mechanisms and their applications in viticulture.

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