

Vitis 45 (2), 53–55 (2006)

Berry size variability in *Vitis vinifera* L.

L. FERNANDEZ, M. PRADAL, G. LOPEZ, F. BERUD, C. ROMIEU and L. TORREGROSA

UMR BEPC Campus Agro-M/INRA, Montpellier, France

Summary

In order to study the mechanisms of berry growth in *Vitis vinifera* L. we analysed cell division and pericarp enlargement of 6 genotypes showing high variability in berry size (range: 49–90 %. Both, cell number and cell volume were involved in the small-sized berries of the *flb* mutant producing wild-type-like berries. The differences of berry size observed in other small-sized clones were only due to cell expansion. The data suggest that the variability of berry size in *V. vinifera* cultivars predominantly results from modification of cell enlargement. Conversely, in wild-type-like berries, the variability of berry size could result from both, cell division and cell enlargement.

Key words: cell division, development, fruit size, diversity.

Introduction

The final size of an organ is determined by the cell number multiplied by the mean cell size (HO 1992, COWAN *et al.* 2001, RAPOPORT *et al.* 2004). Despite the fact that cell expansion may account for the main increase in volume of fleshy fruit, cell division is also an essential factor of fruit organogenesis (CONG *et al.* 2002). In grapevine, berry size is considered as a key determinant of harvest quality (CHAMPAGNOL 1998), but little is known about the mechanisms of berry growth. We studied the dynamics of cell division and pericarp enlargement of three *Vitis vinifera* L. cultivars: Ugni Blanc, Grenache and Mourvèdre and compared them with their respective small-sized berry clones.

Material and Methods

Three couples of *V. vinifera* genotypes were selected: Cv. Ugni Blanc (a control clone and the *flb* mutant, a sport recovered from the control clone, FERNANDEZ *et al.* 2006), cv. Grenache (cl. 70 as control and cl. SsbII) and cv. Mourvèdre (cl. 322 as control and cl. E271). Berries were harvested from Ugni Blanc plants grown in a greenhouse (ENSA.M-INRA, Montpellier, France), Grenache and Mourvèdre plants were cultivated in the field (ENTAV, Grau du Roi, France). To reduce artefacts due to berry position, samples were randomly taken from previously tagged inflorescences with synchronous flowering. Kinetics of growth were obtained from 60 berry samples for Ugni Blanc. For Grenache and Mour-

vèdre, kinetics of growth were obtained from 200 berry samples for early stages (anthesis to veraison) and from 80 berry samples during ripening. At maturity (90 d after anthesis), berry weight and seed number and seed weight of 100 berries of each genotype were determined. DNA was extracted from 20 seedless berries as described by OJEDA *et al.* (1999) for Ugni Blanc or using DNeasy Plant Mini Kit (Qiagen) for Grenache and Mourvèdre. For Ugni Blanc, DNA was quantified by fluorometry with 33 ng·ml⁻¹ DAPI (4,6-diamidino-2-phenylindol) in 0.01 M Tris-HCl (pH=8), 0.1 M NaCl and 0.01 M EDTA by reference to salmon sperm DNA standards, for Grenache and Mourvèdre with 100 ng·ml⁻¹ Hoechst 33258 in 0.1 M Tris-HCl (pH = 7.5), 1 M NaCl and 0.01 M EDTA by reference to Lambda phage DNA standards.

Results and Discussion

Cvs Ugni Blanc, Grenache and Mourvèdre were selected because their small-sized berry clones *flb* mutant, SsbII and E271 were available which exhibit the most distinct variability of berry size with similar genetic background in the *V. vinifera* collections of INRA-Montpellier and ENTAV (Fig. 1). At maturity, fruit weight of the small-sized berry clones was reduced by 49, 61 and 90 % for E271, SsbII and *flb* genotypes, respectively (Fig. 2 A). It is well known that seed number and berry growth are positively correlated (COOMBE 1960). Seeds of E271 and SsbII clones were shown to be lighter (up to 13 %) than controls, irrespective of the number of seeds per berry; they were lighter up to 34 % in the *flb* mutant (data not shown). Distribution of seed number per berry was almost the same in small-sized berry clones and in their respective controls, except for SsbII which produced more berries with only one seed than the control (Fig. 2 B). This berry type formed less pericarp than berries with multiple seeds (Fig. 2 C). Consequently, at the population level we can consider that the lower berry weight, partially results from the number of seeds. However, it is obvious that the reduction of pericarp weight is independent of the number of seeds per berry in the 3 small-sized berry clones (Fig. 2 C). Compared to E271 and SsbII clones, the pericarp weight of the *flb* mutant was distinctly lower, irrespective the number of seeds per berry (Fig. 2 C).

In grapevine, berry growth from the herbaceous phase I to the ripening phase III follows a double sigmoid pattern (STAUDT *et al.* 1986). Since the transition from phase I to phase II (usually distinguished by small growth rates) was not very pronounced in all curves, the onset of maturation

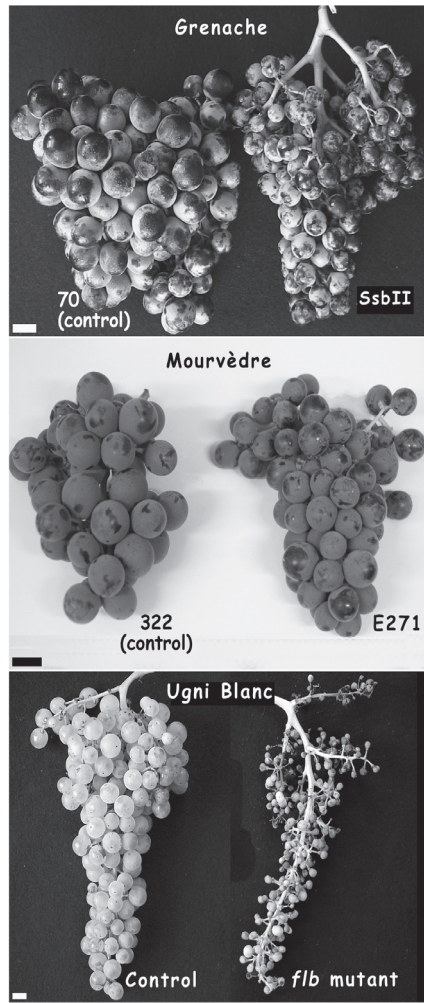


Fig. 1: Clusters of three small-sized berry clones and of their reference genotypes.

was marked by arrows in Fig. 2 A. Intense mitotic activity in the berry pericarp occurs during the first week after-anthesis, the last cell divisions were observed one week before the end of phase I (OJEDA *et al.* 1999). Both growth phases depend on cell enlargement but, during ripening, berry growth is exclusively due to cell enlargement. Fruit size of small-sized clones and controls started to be different shortly after anthesis (Ugni Blanc), and was obvious at the end of phase I (Grenache and Mourvèdre, Fig. 2 A). Whilst morphology of E271 and SsbII berries and seeds was found to be very similar compared to the control clones (data not shown), the shape and size of *flb* berries and seeds significantly diverged from the cultivated *V. vinifera* type, looking more like a wild-type species (FERNANDEZ *et al.* 2006).

The *flb* mutant clearly diverged from the two other small-sized berry clones by an extreme early slowdown of berry growth. Since early growth limitation may be due either to alterations in cell divisions or cell enlargement, DNA content was quantified to evaluate mitotic activity 10 d after anthesis and at veraison when the DNA content was stabilized (OJEDA *et al.* 1999). At veraison, SsbII and E271 clones had the same DNA contents as the controls, although DNA accumulation was found to differ at early

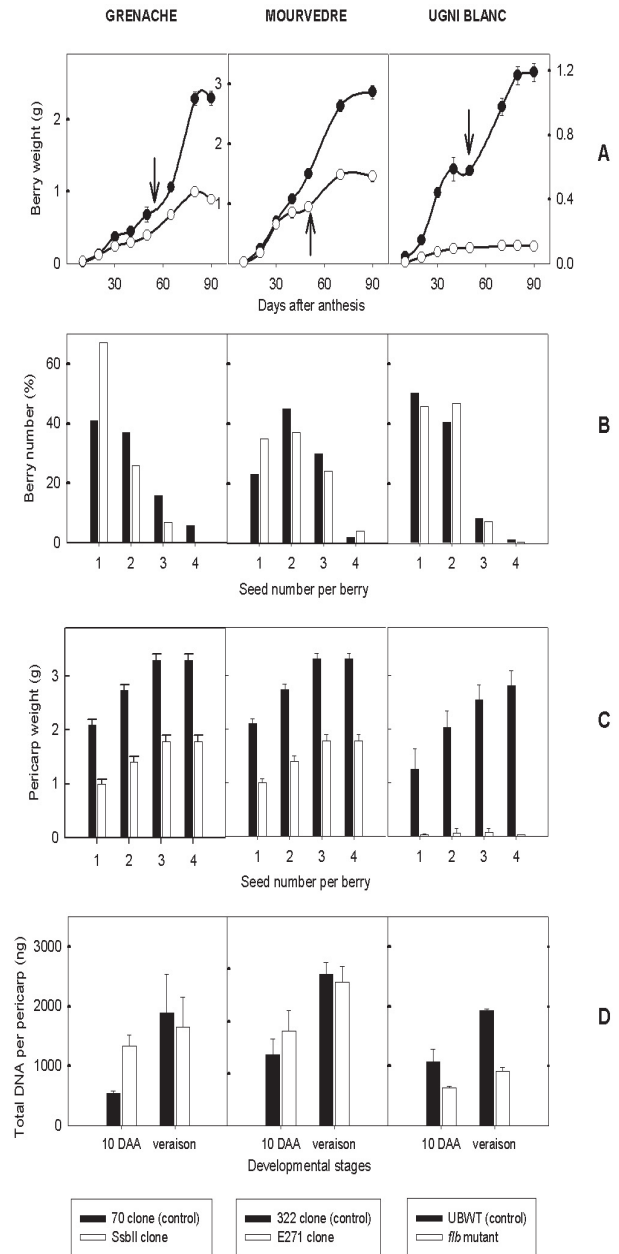


Fig. 2: Berry development. Total berry weight, arrows indicating véraison (A), frequency of seed number per berry (B), relationship between seed number and pericarp weight (C), total pericarp DNA content 10 d. after anthesis (DAA) and at véraison (D). Bars: confidence limits at $p = 95\%$ in (A, C) and SD in (D).

stages, possibly due to differences in the kinetic of cell division (Fig. 2 D). These data suggest that only alterations of cell enlargement caused differences of fruit size between SsbII and E271 clones and to their controls. In contrast, the *flb* mutant showed only half the DNA content of the control, suggesting a limitation in cell division activity. However, the difference in cell number (50 %) cannot explain the 90 % difference in berry weight, thus cell enlargement must also have occurred in berries of *flb*.

The three small-sized berry clones studied here exhibited two different berry growth patterns. The *flb* mutant producing wild-type berries and seeds showed a reduction of both, cell number and cell size in the pericarp

while E271 and SsbII clones that produced small berries but of the same shape as the controls, showed changes in cell enlargement only in the pericarp. Summarizing, these preliminary data suggest that variability for berry size in *V. vinifera* could result predominantly from modifications of cell enlargement while cell size variability of wild-type berries may be more complex, having a double origin, cell division and cell enlargement. Evidently, to validate this hypothesis, it would be interesting to extend this study to a larger number of *Vitis* genotypes including clones that represent near isogenic genotypes. More knowledge of the mechanisms regulating berry growth is essential for studies on genes controlling this developmental process.

Acknowledgements

The authors thanks J. L. GUIRAUD and S. SANTONI for excellent technical assistance and helpful discussions. This work was done in the frame of COST 858 action.

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Received September 27, 2005

