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FLOWERING NEWSLETTER REVIEW

A molecular genetic perspective of reproductive development in grapevine

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Abstract

The grapevine reproductive cycle has a number of unique features. Inflorescences develop from lateral meristems (anlagen) in latent buds during spring and summer and enter a dormant state at a very immature stage before completing development and producing flowers and berries the following spring. Lateral meristems are unique structures derived from the shoot apical meristem and can either develop into an inflorescence or a tendril. How the grapevine plant controls these processes at the molecular level is not understood, but some progress has been made by isolating and studying the expression of flowering genes in wild-type and mutant grapevine plants. Interestingly, a number of flowering genes are also expressed during berry development. This paper reviews the current understanding of the genetic control of grapevine flowering and the impact of viticulture management treatments and environmental variables on yield. While the availability of the draft genome sequence of grapevine will greatly assist future molecular genetic studies, a number of issues are identified that need to be addressed—particularly rapid methods for confirming gene function and linking genes to biological processes and traits. Understanding the key interactions between environmental factors and genetic mechanisms controlling the induction and development of inflorescences, flowers, and berries is also an important area that requires increased emphasis, especially given the large seasonal fluctuations in yield experienced by the crop and the increasing concern about the effect of climate change on existing wine-producing regions.

Key words: Berry, climate, flower, meristem, grapevine, inflorescence, mutant, MADS-box, tendril, *Vitis*, yield.

Introduction

The three major uses for grapes are wine making, fresh fruit (table grapes), and dried fruit (raisins) production. Other products derived from grapes or wine-making waste include grape juice, jelly products, ethanol, vinegar, grape seed oil, tartaric acid, and fertilizer. There is also increasing interest in the health benefits of certain grape-derived anti-oxidant compounds (e.g. polyphenols, resveratrol) and these compounds are being investigated and used in the food additive, cosmetic, and pharmaceutical industries. Statistics from The International Organisation of Vine and Wine (OIV) (http://www.oiv.int/) estimated the worldwide surface area of vineyards to be nearly 8 million hectares in 2005. Total world grape production in 2005 was estimated to be 65.7% for wine production, 26.7% consumed as table grapes, and 7% dried for raisin production. Given the global size of the grape industry and its importance to the economy of many countries, it is often surprising to an outside observer that research literature on grape 'quality' and vineyard management techniques far exceeds genetic studies, including the genetic control of grapevine reproduction and yield. This is even more surprising when seasonal variations in yield usually vary by >15% and often >35% (e.g. Antcliff, 1965; Clingeleffer, 1984; Bramley and Hamilton, 2004;

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Keller *et al*., 2004; Clingeleffer, 2006). Part of the answer may be found in the nature of the dominant global wine industry that, mainly for marketing purposes, has increasingly concentrated on only a few major cultivars (This *et al*., 2006), restricting genetic solutions for yield improvement and leaving only plant management techniques as a limited solution. The other part of the answer is found in the biology of the grapevine plant that is a perennial with a reproductive developmental cycle over a year (Fig. 1) making it difficult to study, especially when the crucial early floral initiation and developmental stages are hidden within a latent bud.

The Vitaceae is a family of woody perennial deciduous plants within the basal eudicots (Judd, 1999). The basic vine growth habit and pattern of organ formation and development is distinct from those previously described for annual herbaceous plants or for woody polycarpic plants (Mullins *et al*., 1992; Boss *et al*., 2003; Carmona *et al*., 2007*b*). These differences make them interesting systems for the study of specific aspects of plant reproductive development. Indeed, in the Vitaceae family, flowering is initiated by the formation of lateral meristems, also historically called uncommitted primordia or anlagen (Tucker and Hoefert, 1968; Pratt, 1971; Gerrath and Posluszny, 1988*a*, *b*; Gerrath *et al*., 1998), which can differentiate, depending on several factors, into tendrils or inflorescences. Grape cultivars used for grape production have inflorescences with hermaphrodite flowers, although wild *Vitis vinifera* vines and American and Asian species are dioecious with either male or female flowers.

In studying grapevine flowering, it is useful to also consider it in an industry context and to understand how the industry views yield and grape 'quality'. For wine grapes and, to a much lesser extent, table and dried-fruit grapes, there is the complex and often confusing issue of 'vine balance' where a viticulturalist seeks to achieve a balance between carbon source (leaves) and carbon sinks (the critical one being berries) to achieve ripening of the berries to a desired sugar level and 'quality' (Howell, 2001). Unlike a commodity crop like wheat or maize where a higher yield (tons) per hectare is considered a desirable outcome, this is not necessarily true for a wine grape. There is a current perception in some parts of the wine industry that a low yield is desirable as it results in improved grape quality and subsequent wine quality. In some European countries such as France, there is 'Appellation d'Origine Contrôlée' that not only regulates what cultivar and viticulture techniques can be used in a particular region but also places a limit on the yield per hectare. Due to the vastly different wine styles within the wine industry, associated marketing, and differing consumer preferences it is not possible to quantify or qualify this subjective assessment of perceived 'quality' at an industry level except maybe by price paid per ton of grapes to the grower or price paid per bottle of wine to the winery. Regrettably this vague indefinable 'quality' term has also crept into some parts of the grape scientific literature. The term 'grape composition' is more appropriate for scientific studies and would be a valuable starting point to characterize wine grape 'quality' as the metabolite composition of grapes and wine can be measured and quantified. As a result of historical factors, environmental factors, and differing perceptions of grape quality, there now exists at a global level a plethora of management

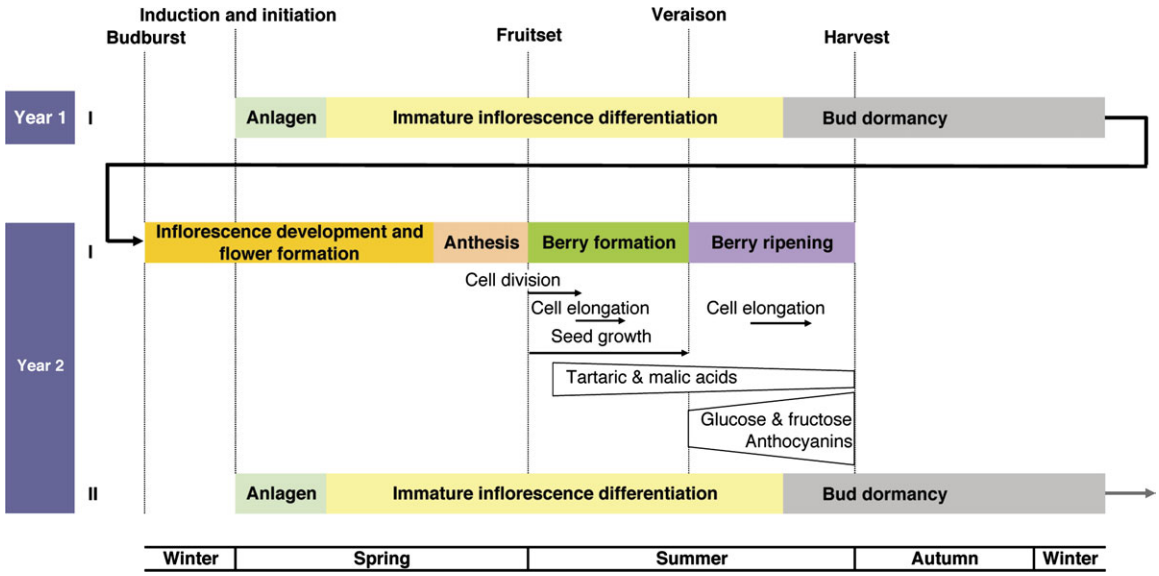

Fig. 1. An idealized 2 year grapevine reproductive developmental cycle showing the distinct stages of flowering and the two characteristic phases of grape berry development. The start and end of phenology stages and harvest can vary markedly depending on the region, seasonal climate, cultivar, and management treatments. The pre- and post-véraison berry stages are referred to as the berry formation stage and the berry ripening stage, respectively (after Coombe and Iland, 2004).

strategies to manipulate yield and berry composition with between 2 to over 200 bunches (clusters) per vine depending on the strategy adopted. The annual pruning regime is the major management method to control plant size and yield per plant in a vineyard (Fig. 2). It could be argued that more resources are devoted to the management of grapevines for manipulating yield and fruit composition than any other major crop, requiring a shift in thinking from simply genotype×environment interactions to genotype×environment×management interactions.

In spite of the difficulties in studying grapevine flowering, a number of excellent reviews and books already exist on grapevine flowering that together summarize the existing body of literature (Pratt, 1971; Srinivasan and Mullins, 1981; Gerrath, 1993; Boss and Thomas, 2000; May, 2000, 2004; Boss *et al*., 2003; Meneghetti *et al*., 2006; Lebon *et al*., 2008). Although fruit can be viewed as continued growth of the carpel after fertilization, fruit development and composition will not be covered in detail in this review and readers are referred to other sources (e.g. Coombe, 1992; Kanellis and Roubelakis-Angelakis, 1993; Ollat *et al*., 2002). Here an attempt will be made to highlight recent developments in understanding the genetic mechanisms involved in grapevine reproduction, the reproductive cycle in an industry context, areas for future research, issues to overcome, and the expected benefits to be derived from the research.

Grapevine reproductive biology

Classically, flowering and fruit development are major steps for plant reproduction. With the development of male and female gametes and the related meiotic recombination followed by fertilization, this part of the development cycle of plants is crucial to increase the genetic diversity of a species. For cultivated grapevine, this purpose has been supplanted by fruit production as the primary objective.

The reproductive biology of grapevine plants is slightly different in cultivated than in wild forms likely as an effect of domestication and viticulture culture conditions. Wild plants of *V. vinifera* (sometimes referred to as ssp. *sylvestris*) are still found in riverbank forests in temperate Eurasian regions. These plants originate from seeds germinating on the forest floor and using tendrils to climb up and over forest trees to reach the canopy, sometimes at heights of 20–30 m (Mullins *et al*., 1992). Wild plants are dioecious and flower once they reach the canopy top and are exposed to high light, producing a large number of small bunches of flowers. Berries produced by female plants are small and in small bunches. They are dark in colour and are sweet enough to attract birds, contributing to seed dissemination (This *et al*., 2006). Male, female, and hermaphroditic flowers are not visually attractive to insects as the flowers are small and the petals drop at anthesis (Fig. 3A–C). Unisexual flowers produced by *Vitis* species still possess rudimentary organs of the opposite sex (Fig. 3A, B). It is thought that, for wild dioecious plants, pollination is by either wind or pollinators; however, not much is known about the process, with pollen suggested to be the main attractant for insects (Branties, 1978; Kimura *et al*., 1998). Figure 3D, E shows bees at an Australian germplasm collection visiting both male and female flowers providing visual proof of insects acting as pollinators with scent appearing to be the main attractant. The female flower is characterized by reflex stamens and infertile pollen that does not germinate (Kimura *et al*., 1997; Caporali *et al*., 2003) while the male flower has an underdeveloped modified carpel (Fig. 3A, B). Commercial vineyards have plants with hermaphroditic flowers where autogamy (self-fertilization) is thought to be the major route for pollination. Pollen flow studies in Germany, using transgenic plants (M Harst *et al*., unpublished results), and in Australia, using protein and DNA markers (S Sykes *et al*., unpublished results), supports this view. Insect activity in commercial vineyards at anthesis is low (personal observations). When grown as a crop, plants of *V. vinifera* (sometimes referred to as ssp. *sativa*) are pruned to control plant size and bunch number. All major cultivars grown today have hermaphrodite flowers to maximize fruit production.

Fig. 2. Vineyards illustrating plant size differences. (A) Tempranillo trained as 'goblet' vines in the Rioja wine region of Spain. Each vine is pruned to produce ~18 bunches. (B) Cabernet Sauvignon grown on a multi-wire trellis system in the wine region of Willunga, Australia. Each vine is pruned to produce ~80 bunches.

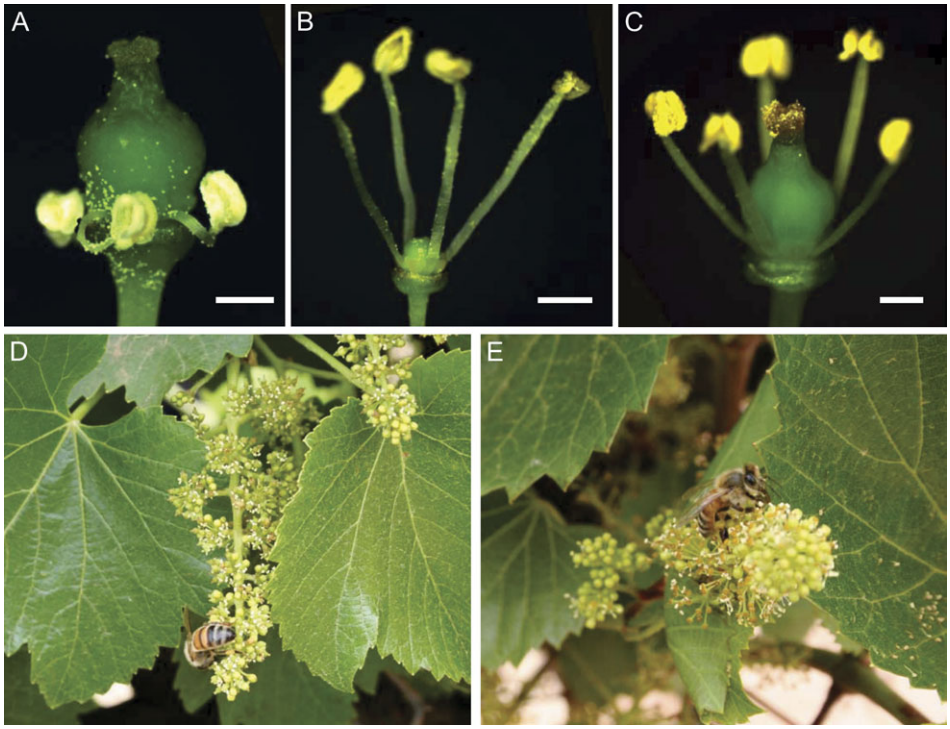

Fig. 3. Flower sex types in grapevine and bee-mediated pollination: (A) female flower with reflex stamens; (B) male flower; (C) hermaphrodite flower; (D) bee attracted to female flowers; (E) bee attracted to male flowers. Scale bar = 500 μm.

Grapevine cultivars are highly heterozygous (Thomas *et al*., 1993; Thomas and Scott, 1993) and show a large variation in inflorescence size, berry size, shape, and colour (Viala and Vermorel, 1901–1910; Galet, 1988–1990; This *et al*., 2006). To maintain varietal features, cultivars are vegetatively propagated. Therefore, their developmental pattern corresponds to that of the adult plants (Carmona *et al*., 2007*b*). Grapevine plants arising from seeds display a short juvenile phase when they produce 6–10 nodes that bear leaves in a spiral phyllotaxis. Most cultivars show leaf size and shape variation by the end of this phase. Leaf size and shape go from smaller round leaves to larger palmate leaves with different number and size of lobes depending on the genotype. Phase change not only affects leaf morphology but also phyllotaxis that changes from spiral to alternate and, more importantly, the production by the shoot apical meristem (SAM) of a characteristic sequence of leaves and lateral meristems, known also as uncommitted primordia or anlagen (Tucker and Hoefert, 1968; Pratt, 1971; Gerrath and Posluszny, 1988*a*; Gerrath *et al*., 1998). These lateral meristems alternate with leaf primordia in the SAM (see fig. 1 in Boss *et al*., 2003) but, due to unequal internode elongation, they become opposed to leaves in the expanded shoot and can differentiate either as tendrils or inflorescences. Generally, young plants do not initiate inflorescence differentiation until they are 2–5 years old under cultivation or until they reach the forest canopy in the wild. For some genotypes, light-exposed, well-watered and fertilized plants can produce inflorescences in their second year of life, highlighting the relevance of the nutritional state of the plant in the initiation of reproductive development.

Environmental stimuli inducing floral initiation in grapevine are high temperature and high light intensity (Buttrose, 1974; Mullins *et al*., 1992) and are the same stimuli that the plant encounters when reaching the forest canopy top. Furthermore, as mentioned before, the developmental and/or nutritional state of the plant seems to be very relevant. Hormonal treatments have shown that gibberellins and cytokinins have antagonistic effects in the control of flower initiation. Cytokinins promote the development of inflorescences from anlagen (Srinivasan and Mullins, 1978, 1979, 1980), whereas gibberellins are required for anlagen initiation but inhibit their differentiation into inflorescences, favouring tendril development (Srinivasan and Mullins, 1980).

The reproductive developmental cycle

For grapevine plants grown in temperate regions their reproductive developmental cycle is completed over two consecutive growing seasons separated by a dormancy period between autumn and spring (Fig. 1). In the spring, every sprouting bud gives rise to a stem with alternate

leaves opposed to inflorescences in their basal part and to tendrils in the medium and apical part (Fig. 4A). Every leaf in the branch carries an axillary bud. The first-formed bud in the leaf axil produces a lateral shoot that develops during the season (Fig. 4B). In the axil of the prophyll of that lateral shoot, a compound latent bud will be formed in which the whole process of floral initiation and early stages of inflorescence development take place (Fig. 4C). This process has been characterized by scanning electron microscopy (Srinivasan and Mullins, 1976, 1981; Carmona *et al*., 2002, 2007*b*; Boss *et al*., 2003) and it is also described in the accompanying paper (Lebon *et al*., 2008). Briefly, the compound latent bud contains three separate latent buds (Fig. 4D) and the SAMs of the primary and secondary buds of the latent compound bud proliferate to reproduce the phases recognized in juvenile and adult plants. For the primary latent bud, the SAM produces first three to four leaf primordia before initiating the alternation of leaf primordia with lateral meristems. The first two to three lateral meristems have the potential to differentiate as inflorescences (Pratt, 1971; Srinivasan and Mullins, 1981) while the following lateral meristems produced will start differentiation as tendrils (Pratt, 1971; Srinivasan and Mullins, 1976, 1981). Inflorescence meristems proliferate to give rise to additional inflorescence branch meristems with a spiral phyllotaxis that will form an immature raceme structure (Fig. 4E). By the end of the summer these buds are dormant and the primary latent bud, if fruitful, contains a compressed shoot with inflorescence meristems and tendril and leaf primordia. Not all latent buds on a cane are fruitful (contain an immature inflorescence). For non-fruitful canes that originate from non-fruitful buds, no lateral structures (either inflorescences or tendrils) develop from the first two or three lateral meristem positions.

The following year, when the environmental conditions are permissive, bud growth resumes and the SAM produce further leaf and tendril primordia. The *V. vinifera* SAM for most genotypes produces two consecutive nodes containing leaf primordia and lateral meristems, which alternate with one node bearing a solitary leaf primordium. During initial stages of bud swelling, the inflorescence branch meristems can additionally branch to form further inflorescence branch meristems. Grapevine inflorescences are racemes formed by many branches that prefigure the conical shape of grape bunches. Then, each inflorescence meristem divides into a cluster of three or four flower meristems arranged as a dicasium. The terminal flower develops first, then the lateral ones and finally, the most basal. Flower development takes place when the bud swells and shoot internodes begin to elongate. Grapevine flowers are organized in four whorls

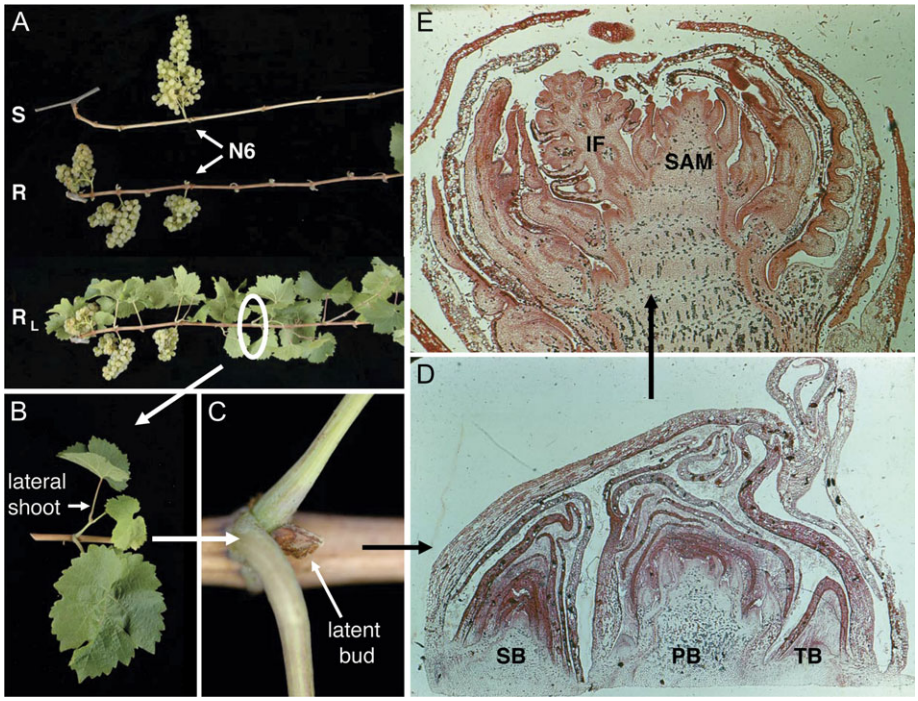

Fig. 4. Grapevine reproductive development: (A) position of bunches on Sultana and Riesling canes—the first Sultana bunch and last Riesling bunch are at the sixth node of a year 2 cane counting from the cane base; (B) latent bud at the base of the lateral shoot that developed from an axillary bud at the base of a leaf; (C) close-up of the latent bud; (D) longitudinal section of a Sultana latent bud showing primary, secondary, and tertiary latent buds (C Barnard, unpublished results, 1928); (E) transverse section of a Sultana primary latent bud showing an immature inflorescence on the left and a shoot apical meristem on the right (from Barnard and Thomas, 1933). IF, Inflorescence; N6, sixth node; PB, primary bud; R, Riesling cane; R_L, Riesling cane with leaves S, Sultana cane; SAM, shoot apical meristem; SB, secondary bud; TB, tertiary bud.

and the whorled pattern of flower development follows a basipetal direction. Flower meristems form, sequentially, sepal primordia, petals and stamens common primordia that soon divide to form separate primordia, and, finally, the innermost carpel primordia. Additional details on flower structure and development are provided in other reviews (Pratt, 1971; Srinivasan and Mullins, 1981; Gerrath, 1993; Boss and Thomas, 2000; May, 2000, 2004; Boss *et al*., 2003; Meneghetti *et al*., 2006; Lebon *et al*., 2008). The size and structure of the mature inflorescence and bunch are essentially determined by the time of anthesis (Shavrukov *et al*., 2004).

Berry development has previously been thoroughly described in grapevine (Coombe, 1992; Kanellis and Roubelakis-Angelakis, 1993; Hardie *et al*., 1996; Ollat *et al*., 2002). Briefly, the tissues of the berry derive directly from the ovary wall and comprise, from outside to inside, the outer epidermis or exocarp, the mesocarp with outer and inner parenchymal cells, and the inner epidermis or endocarp. The berry exocarp derives from the ovary exocarp and, at maturity, it is composed of the epidermis formed by a single layer of epidermic cells and the hypodermis composed by ~10 cell layers below the epidermic cells. Exocarp-differentiated cells accumulate polyphenols in their vacuoles which are important wine components. Furthermore, vacuoles of epidermal cells also contain anthocyanins responsible for colour in red or black berries. The fruit mesocarp develops as the result of multiple cell divisions followed by cell enlargement. It is possible to distinguish external and internal mesocarp characterized by different cell shapes. Cells in the mesocarp constitute the berry flesh and are specialized in the accumulation of sugars (mainly glucose and fructose), organic acids (chiefly tartaric and malic acids), and water representing most of the fruit tissue and volume. The endocarp is composed of cells layers around the carpel locules containing the seeds. As in the exocarp, it is also possible to distinguish an internal hypodermis formed by a few cellular layers as well as an internal epidermis. In mature berries the endocarp is difficult to distinguish from the rest of the flesh.

Grape-berry development follows a double sigmoid growth curve with two phases of active growth separated by a lag phase around véraison (Coombe and Hale, 1973). The first one, known as the green phase, is characterized by cell division and differentiation not only for the fruit itself but also for the seeds. Berry growth stops during the stationary phase which ends at véraison, a process characterized by the initiation of berry softening and colouration (in coloured berries) which conspicuously marks the large physiological and metabolic change taking place during berry ripening. The ripening phase is also characterized by exponential growth of the berry that in this case is mainly based on cell enlargement. Grape berries are considered non-climacteric fruits and little is known about the mechanisms regulating berry ripening.

Particular features of grapevine reproductive development

Grapevine reproductive development displays special developmental features when compared with herbaceous annual systems such as *Arabidopsis* or rice (Ausin *et al*., 2005; Carmona *et al*., 2007*b*) or polycarpic woody plants (Brunner and Nilsson, 2004). A major developmental difference when compared with other species analysed is the presence of tendrils, which in the Vitaceae could be considered as modified reproductive structures. The possible reproductive origin of the Vitaceae tendrils is based on the observations that both tendrils and inflorescences have a common ontogenetic origin, developing from the same meristematic structure, the anlage or lateral meristem. Intermediate hybrid structures; half tendril, half inflorescence are common in many genotypes (Fig. 5), reinforcing the concept of a common origin. In fact, intermediate organs can frequently be observed under field conditions (Pratt, 1971, 1974; Srinivasan and Mullins, 1981; Boss and Thomas, 2000; Boss *et al*., 2002) including occasions when the lateral meristem develops a SAM (see fig. 2 in Boss and Thomas, 2000).

The presence of tendrils as a climbing adaptation marks major differences in reproductive development between

Fig. 5. Intermediate floral structures on a Pinot Meunier shoot after budburst but prior to anthesis. The lower inflorescence has a tendril in place of an inflorescence arm (often called the outer arm or wing) while the upper anlagen has developed into a tendril with a few immature flowers present on the tip of one of the tendrils.

grapevine and other plant species. The grapevine phase transition from juvenile to the adult state is not only marked by phyllotactic and morphological changes of the leaves but also by the capability of the SAM to initiate lateral meristems (anlagen) that will give rise to modified shoots. The tendril requirement to climb results in seedlings having a very short juvenile phase encompassing eight to ten nodes as compared with other woody species. Thus, the flowering transition in grapevine involves two steps. The first step, the formation of the common anlagen takes place independently of flower-inducing stimuli and is more related with the developmental transition from juvenile to adult plants. The second step, the differentiation of the anlage as an inflorescence in place of a tendril is the result of floral induction. Under this model, tendrils could be considered as sterile reproductive organs, while a flowering-inducing stimulus would cause the initiation of reproductive meristems. As a consequence, the grapevine SAM continuously gives rise to vegetative and reproductive meristems within the same branch.

Environmental factors promoting flowering in grapevine do not correspond with the major factors inducing flowering in herbaceous plants such as photoperiod and vernalization for crucifer and cereal species. In this way, neither photoperiod nor vernalization is very relevant for flowering induction but short-term exposures to high temperature and high light intensity have been shown to promote grapevine flowering (Buttrose, 1974; Mullins *et al*., 1992). It should be noted that stimuli that promote the induction and differentiation of the lateral meristems as immature inflorescences in the latent bud will not have a major effect on flowering time, bunch size, or flower number in the following year, as these will be more modulated by the environmental conditions affecting bud burst time and growth rate. Finally, at the hormonal level, the flower-promoting effect of gibberellins observed in *Arabidopsis* and in other rosette species seems to have changed in grapevine where they inhibit inflorescence and flower initiation. This negative effect of gibberellins on the floral transition is commonly observed in other woody perennial angiosperms.

Viticulture management techniques and environmental factors affecting grapevine reproductive development

The general developmental cycle outlined in Fig. 1 and described above for grapevines grown in temperate regions can vary significantly, even for the same genotype, depending on the region in which the grapes are grown and the management system adopted (for general reading, see Winkler *et al*., 1974). There are regions in Europe where the vines require protection from the freezing conditions of winter and are buried during this period-necessitating pruning the vines close to the ground. By contrast, grapevines grown in subtropical and tropical regions are often managed to produce two crops in a year, especially for the commercial production of fresh fruit from table-grape cultivars. Because of the extreme differences in climate and management treatments that exist at a global level, it is difficult to be specific when discussing factors affecting yield; however, a general outline is represented in Fig. 6. Environment, genotype, and management treatments are major influences on the final yield (ton/hectare) from inflorescence initiation within the latent bud to final harvest of the berries (Fig. 6). The role of sugars in the processes outlined in Figs 1 and 6 has been discussed elsewhere (Lebon *et al*., 2008). For the

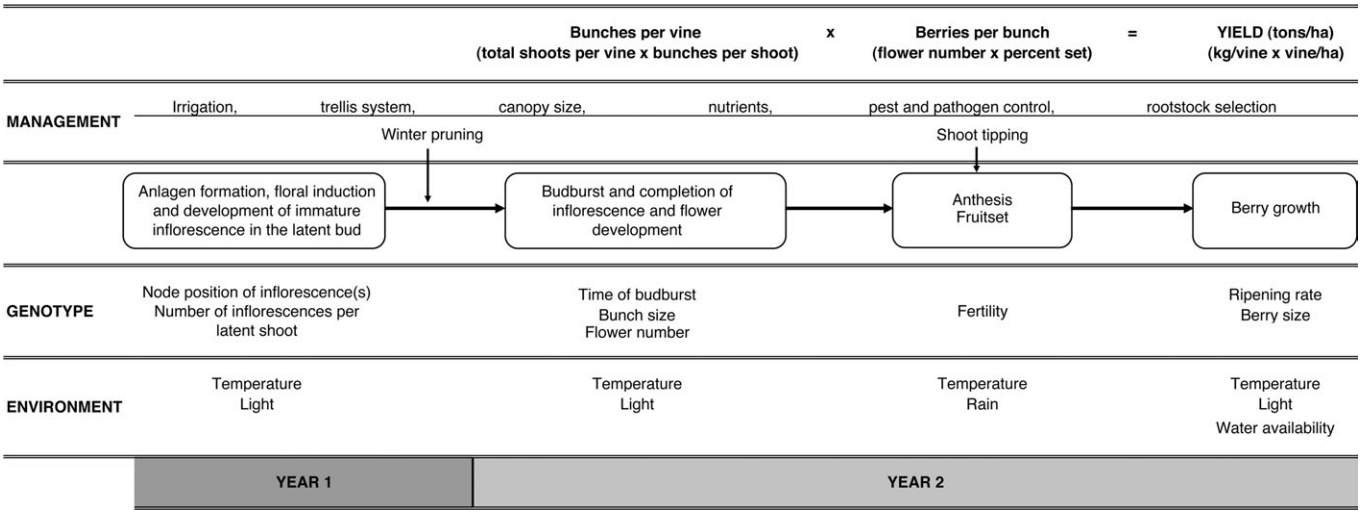

Fig. 6. A simplified schematic representation of genotype, environment, and management factors that determine the major components of yield for grapevine.

yield calculation shown in Fig. 6, it is now clear that it is bunch number per vine that is the major determinant of yield (Keller *et al*., 2004; Clingeleffer, 2006) and therefore, for industry, the critical stage of flowering is the induction and initiation stage of inflorescence development in the latent bud. Yield component analysis in one study showed that bunch number per vine explained 58–88% of seasonal variation, with bunch weight (suggested to represent berries per bunch) accounting for 11–38% (Clingeleffer, 2006). Genotype has a major effect on latent bud fruitfulness with cultivar differences observed for the number of fruitful canes, number of bunches per cane, and node position of the bunch on the cane. For example, Fig. 4 shows the differences between Sultana (Thompson Seedless) and Riesling grown under the same conditions with Sultana having only one bunch at node 6 and Riesling having three bunches at nodes 3, 4, and 6. Sultana also has low fruitfulness at basal nodes and this genotype difference determines the type of pruning carried out during winter. Riesling can be spur pruned to leave only the two or three basal nodes to provide the crop for the following season as the latent buds at these positions are fruitful and contain immature inflorescences. However, Sultana has to be cane pruned to leave ~14 basal nodes per cane to ensure fruitful buds remain on a plant to give an adequate crop in the following season.

Winter pruning to control bunches per vine and shoot tipping at anthesis to increase flower set and berries per bunch (Fig. 6) are not the only management treatments employed to manipulate yield. Additional treatments to decrease yield can include shoot thinning (removal of whole shoots), bunch thinning (removal of whole bunches), bunch tipping (removal of part of the bunch), and berry thinning (removing some berries from a bunch). Shoot tipping at or near anthesis is thought to improve the number of flowers that set and produce fruit by removing the other main carbon sink on the cane competing with the inflorescence flowers (see May, 2004). The potential berry number for each grapevine inflorescence is usually far greater than the actually number of berries at harvest and an example of this was observed for three cultivars in a typical season where only 38–47% of flowers on an inflorescence successfully produced berries (Shavrukov *et al*., 2004). Severity of winter pruning also appears to have an effect on inflorescence size and flower number, possibly by changing the number of carbon sinks during the period after dormancy (Dunn and Martin, 2007). While physical management treatments are the major means of managing yield, there is also evidence that the rootstock chosen has an influence on yield (for examples, see May, 2004; Clingeleffer and Emmanuelli, 2006), suggesting a genotype–genotype interaction between rootstock and scion. However, whether this is a direct or indirect effect or both is unclear as some studies suggest that rootstock modification of vine (vegetative) vigour can cause shading of latent buds that indirectly affects fruitfulness (e.g. Sommer *et al*., 2000). Management treatments that modify the canopy either by pruning or trellis design also have a major role in the exposure of the latent bud to light and temperature differences and these microclimate differences have been suggested to have an effect on latent bud fruitfulness, including the occurrence of primary bud-axis necrosis (see Dry, 2000).

Despite active management at the plant level, the large seasonal variation in yield observed for grapevine is due to environmental conditions, and the major environmental influences for each stage are shown in Fig. 6. High temperature and high light appear to have a positive influence on the induction and development of the immature inflorescence in the latent bud in year 1 (for reviews, see Buttrose, 1974; Mullins *et al*., 1992; May, 2004). There is also evidence that lower temperatures increase inflorescence size and flower number after budburst (Petrie and Clingeleffer, 2005). Finally, adverse weather conditions involving rain are known to reduce successful pollination and fruit set (see May, 2004) with the resulting seed number per berry (one to four) also having an influence on berry size.

Molecular investigations of grapevine reproductive development

The analysis of the molecular regulatory network that controls the different stages of reproductive development in grapevine has been based on the identification and functional analysis of *V. vinifera* orthologues of the corresponding *Arabidopsis* genes (Boss *et al*., 2003; Carmona *et al*., 2007*b*). A significant number of genes has been isolated and related with specific processes or developmental stages on the basis of gene expression; however, information about their biological function in grapevine is still scarce. This section is a summary of the information currently available on the regulatory genes that could be involved in the flowering transition, flower development, and fruit development and ripening in grapevine.

Induction

As mentioned above, there is no evidence of the existence in grapevine of classical flowering regulatory pathways known in crucifers and cereal species such as the photoperiod or the vernalization pathways. However, genes homologous to the *Arabidopsis* or cereal genes involved in those pathways can generally be found in the grapevine genome and their function in flowering induction or other processes remains to be elucidated. So far, most molecular studies have focused on the identification of grapevine genes homologous to *Arabidopsis* flowering signal integrators and flower meristem identity

genes (Carmona *et al*., 2002; Calonje *et al*., 2004; Joly *et al*., 2004; Boss *et al*., 2006; Sreekantan and Thomas, 2006). In *Arabidopsis*, flowering signal integrators such as *SUPRESSOR OF CONSTANS1* (*SOC1*) and *FT*, further control the expression of genes specifying flower meristem identity (Boss *et al*., 2004; Ausin *et al*., 2005; Parcy, 2005; Sablowski, 2007). Among these flower meristem identity genes, *LEAFY* (*LFY*), *FRUITFULL* (*FUL*), *CAULIFLOWER* (*CAL*), and *APETALA1* (*AP1*) seem to play functionally redundant roles based on mutant analyses (Mandel *et al*., 1992; Weigel *et al*., 1992; Mandel and Yanofsky, 1995; Liljegren *et al*., 1999; Ratcliffe *et al*., 1999; Ferrandiz *et al*., 2000) and their expression patterns relate well with their proposed function. Regarding grapevine flowering signal integrators, three putative members of the *SOC1/AGL20* MADS-box gene subfamily of MADS transcription factors (Parenicova *et al*., 2003) are present in the grapevine genome based on EST and genome sequence data (MJ Carmona *et al*., unpublished results), although only one of them, *VvMADS8*, has been further characterized (Sreekantan and Thomas, 2006). Consistent with a regulatory role in flower initiation, the expression of *VvMADS8* is higher during very early stages of inflorescence development and decreases in later stages of flower development. Moreover, it is not detected in mature flowers and fruits, although it is slightly expressed during tendril development. Overexpression of *VvMADS8* in transgenic *Arabidopsis* plants accelerates flowering, supporting a function for this gene similar to that of the endogenous *Arabidopsis* ones. Although these results support the functional conservation of this MADS-box gene subfamily, further work is required to establish the role of each one of its members in grapevine and the signal pathways regulating their expression.

Genes homologous to the *Arabidopsis* flowering signal integrator *FT* have also been characterized in grapevine (Joly *et al*., 2004; Boss *et al*., 2006; Sreekantan and Thomas, 2006; Carmona *et al*., 2007*a*). In *Arabidopsis*, *FT* belongs to the small gene family (*FT/TFL1*) that encodes proteins with similarity to mammalian phosphatidylethanolamine-binding proteins with either positive or negative effects on flower initiation (Bradley *et al*., 1996). In the grapevine genome, there are six genes that could belong to the *FT/TFL1* gene family (www.genoscope.cns.fr) and five of them have recently been characterized (Carmona *et al*., 2007*a*). They can be grouped into three subfamilies (*FT*-like, *MFT*-like, and *TFL1*-like; Carmona *et al*., 2007*a*), as previously shown in other species (Carmel-Goren *et al*., 2003; Chardon and Damerval, 2005; Ahn *et al*., 2006). Among them, expression of the most likely *FT* orthologue, *VvFT*, is associated with seasonal floral induction in latent buds and with the development of inflorescences, flowers, and fruits (Sreekantan and Thomas, 2006; Carmona *et al*., 2007*a*). Furthermore, overexpression of *VvFT* in transgenic *Arabidopsis* (Sreekantan and Thomas, 2006; Carmona *et al*., 2007*a*) causes similar effects as the endogenous *FT* (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999) supporting their orthology. Three members of the grapevine *FT/TFL1* subfamily are related to *Arabidopsis TFL1* (Carmona *et al*., 2007*a*). They are expressed in latent buds and during the initial stages of inflorescence development, but are not detected during flower development in the following season. Overexpression of *VvTFL1* also named as *VvTFL1A*, the closest relative to *TFL1*, in transgenic *Arabidopsis* seems to delay flowering time and the initiation of flower meristems, yielding a phenotype of complex inflorescences with multiple co-florescences (Boss *et al*., 2006; Carmona *et al*., 2007*a*). These results support a role for this gene in maintaining meristem indeterminacy. Whether the additional grapevine *TFL1*-like genes are functionally redundant with *VvTFL1A* or have more specific roles in different meristems awaits further characterization. Overall, expression patterns of grapevine *FT/TFL1*-like genes are found associated with either meristem proliferation or determination processes (Carmona *et al*., 2007*a*), in agreement with the biological function proposed for these gene subfamilies in other species (Bradley *et al*., 1997; Pillitteri *et al*., 2004; Ahn *et al*., 2006; Lifschitz *et al*., 2006).

Flower meristem identity

Regarding grapevine flower meristem identity genes, a single orthologue of the *Arabidopsis LEAFY* (*LFY*) transcription factor (Weigel *et al*., 1992), known as *VFL*, has been found in grapevine (Carmona *et al*., 2002; Joly *et al*., 2004; Boss *et al*., 2006). *In situ* hybridization experiments showed that *VFL* expression is already detected in lateral meristems (anlagen) prior to any commitment, being down-regulated during tendril development and highly up-regulated in the developing inflorescence meristems of latent buds. Furthermore, *VFL* expression reaches the highest levels in the floral meristems that develop in bursting buds the following spring. *VFL* is also expressed in petal and stamen primordia, but expression declines as organs expand. This expression pattern suggests a central role for *VFL* in flower meristem initiation, and organization as has been suggested for most *LFY*-like genes analysed in other species (Maizel *et al*., 2005). Expression patterns spanning two growing seasons have also been described for the *LFY* orthologue in kiwifruit, another woody perennial with winter bud dormancy (Walton *et al*., 2001). In both species, the highest level of *LFY* expression corresponds to the time of flower meristem formation (first season in the case of kiwifruit and second season in the case of grapevine), supporting a role for *LFY* orthologous genes in the specification of flower meristem identity in these woody species. Interestingly, the expression of *VFL* in leaf primordia and the margins of developing leaves

suggests that *VFL* could be involved in maintaining cell proliferation in specific leaf areas, generating the palmate shape of the grapevine leaves. A similar role for *LFY*-like genes has been shown in pea where the *LFY* orthologue *UNIFOLIATA* is required to generate the wild-type compound leaves (Hofer *et al*., 1997) and in tomato where *falsiflora* mutants have leaves with fewer leaflets than wild-type plants (Molinero-Rosales *et al*., 1999).

Additionally, homologues of *Arabidopsis* flower meristem identity genes *APETALA1* (*AP1*) and *FRUITFULL* (*FUL*) MADS-box genes have also been characterized in grapevine under the names of *VAP1* and *VFUL-L* (Calonje *et al*., 2004). *FUL-L* is likely to be a paralogue of the *Arabidopsis FUL* (Litt and Irish, 2003). *VFUL-L* and *VAP1* are expressed very early in the uncommitted lateral meristems and maintain their expression in differentiating derived organs, either inflorescences or tendrils. Apart from their function as flower meristem identity genes, in *Arabidopsis AP1* and *FUL-L* also seem to play a role as flower organ identity genes at later stages of flower development. *AP1* was initially identified as a class A gene involved in sepal and petal identity (Irish and Sussex, 1990; Coen and Meyerowitz, 1991; Theißen, 2001), and *FUL* was shown to play a role in carpel and fruit development (Gu *et al*., 1998; Ferrandiz *et al*., 2000). As are their *Arabidopsis* homologues, *VAP1* and *VFUL-L* are expressed throughout flower development, suggesting that they could play a role in the specification of flower organ identity. *VFUL-L* transcripts become restricted to the prospective carpel-forming region of the flower meristem, which is consistent with its putative role in carpel and fruit development. *VAP1* is broadly expressed in the newly formed flower meristem but becomes excluded from the sepal-forming region soon after, and this is not consistent with a function in the specification of sepal identity. Similar observations have also been reported for the *Antirrhinum SQUA* gene (Huijser *et al*., 1992) and the *Gerbera hybrida AP1* (Yu *et al*., 1999), questioning the role of these genes in the specification of sepal identity and providing arguments to revise the concept of the A-function in flower organ identity (Litt and Irish, 2003). Moreover, the high expression of *VFUL-L* and *VAP1* in developing tendrils suggests that both genes could have been recruited for the regulation of tendril development in the Vitaceae. Alternatively, their expression throughout tendril development could be considered as a remnant expression related to the evolution of these climbing organs from inflorescences. Further functional analyses will be required to distinguish between these two hypotheses.

Flower organ identity

Flower organ identity genes that are preferentially expressed during flower and fruit development have also been characterized in grapevine. All belong to the MADS-box family of transcription factors and are homologues to B-, C-, D-, and E-function genes. B-function genes such as *APETALA3* (*AP3*) and *PISTILATA* (*PI*) of *Arabidopsis* are expressed in petals and stamens and are required to specify their organ identity. An ancient duplication in the *AP3* lineage, in the base of core eudicots, gave rise to the *euAP3* and the tomato MADS-box gene 6 (*TM6*) sub-lineages (Kramer and Irish, 2000). Functional characterization of *TM6* and *euAP3* in Solanaceae (de Martino *et al*., 2006; Rijpkema *et al*., 2006) indicates the existence of functional diversification between them, with *euAP3* playing a more direct role in petal and *TM6* in stamen development. In grapevine, three homologues of B-function genes have been characterized as *VvMADS9* (*VvPI)*, *VvAP3*, and *VvTM6* (Sreekantan *et al*., 2006; Poupin *et al*., 2007) and could represent all the members of this subfamily in the grapevine genome (www.genoscope.cns.fr). All three genes are expressed in petals and stamens, whereas *VvMADS9* expression is low or absent in leaves, roots, tendrils, latent buds, and the berry (Sreekantan *et al*., 2006). Furthermore, *VvTM6* is more broadly expressed in reproductive organs and has also been found in carpels, fruits, and seeds. As proposed by Poupin *et al*. (2007), this differential expression of *VvAP3* and *VvTM6* could suggest their possible sub-functionalization also in grapevine. Expression of *VvTM6* in the carpels, as shown for *TM6* in tomato (de Martino *et al*., 2006), as well as during berry development and ripening, suggests new roles for these B-function genes. The large developmental differences between dry, silique-type fruits and fleshy, berry-type fruits could be the basis of gene and gene-function differences related to the specific characteristic of each fruit type.

The C-function gene *AGAMOUS* (*AG*) is required in *Arabidopsis* to specify the identity of stamens and carpels. Additionally, *AG* together with D-function genes such as *SEED STICK* (*STK/AGL11*), *SHP1*, and *SHP2* are required to specify ovule identity. Those D-function genes are also involved in the regulation of fruit development (Pinyopich *et al*., 2003). C- and D-function genes form a monophyletic MADS-box clade, known as the *AG* subfamily of MADS-box genes. Several putative orthologues of the *AG* gene subfamily have been reported in grapevine. Among them, *VvMADS1* showed the closest sequence homology to *SHP1/2* (Boss *et al*., 2001), and was expressed in the two inner flower whorls as well as during berry development. Overexpression of this gene in grapevine is associated with altered sepal morphology (Boss *et al*., 2003). These results do not allow the classification of *VvMADS1* as either an *AGAMOUS* or *SHP1/2* orthologue. Another grapevine MADS-box gene, *VvMADS5*, also belongs to this subfamily (Boss *et al*., 2002). It shows homology with the *STK/AGL11* gene, and its expression in mature carpels, developing seeds and

pre- and post-véraison fruits, fit well with it being a possible orthologue of *STK/AGL11*.

The participation of E-function genes in flower development was discovered relatively late due to their high genetic redundancy. Four genes, *SEPALLATA1–4* (*SEP1–4*) have been reported in *Arabidopsis*, which are involved in floral meristem determinacy and organ identity in the four whorls (Pelaz *et al*., 2000, 2001*a*, *b*; Honma and Goto, 2001; Vandenbussche *et al*., 2003; Ditta *et al*., 2004). In grapevine, two genes *VvMADS2* and *4* (Boss *et al*., 2002), have so far been characterized and show homology to *SEPALLATA 1* and *2* (*SEP1/2*), respectively. *VvMADS2* and *4* are expressed early during inflorescence development until anthesis and can be detected in the three inner whorls of the flower. Additionally, *VvMADS4* is also expressed during fruit development.

Finally, another previously described grapevine MADS-box gene, *VvMADS3* (Boss *et al*., 2002) shows homology to *Arabidopsis AGL6* and *AGL13* and has a similar expression pattern to *AGL6*. In *Arabidopsis*, *AGL6* seems to be involved in the development of both flowers and vegetative organs (Alvarez-Buylla *et al*., 2000).

Berry development and ripening

The results of transcriptional profiling are fragmentary and still limited to berry development and ripening (Deluc *et al*., 2007; Pilati *et al*., 2007). Concerning the regulation of grape ripening, using a first generation Affymetrix chip, 8.5%, 6.2%, and 4.4% of the genes showing differential expression during berry development and ripening encode transcription factors, proteins involved in signal transduction, or proteins involved in hormone metabolism and response, respectively (Pilati *et al*., 2007). Among the transcription factors, several MADS-box genes, mentioned above, are expressed during berry development. *VvMADS1* was the first to be shown to be highly expressed during berry development (Boss *et al*., 2001) similar to *VvMADS4* (Boss *et al*., 2002), with *VvMADS2* and *VvMADS5* having lower expression (Boss *et al*., 2002). Transcriptome analysis with arrays showed *VvMADS2* and *VvMADS5* to be highly expressed during early berry development. Furthermore, transcripts related to light responses (*CONSTANS*-like, *VvFT*, putative *EARLY FLOWERING 4* family, circadian clock-related proteins, etc.) seem to be also positively regulated, as well as factors involved in light and auxin signal cross-talk. *VvFT* but not *VvMADS8* was found to be expressed during berry development (Sreekantan and Thomas, 2006). Regarding ripening in this non-climacteric fruit, similar expression patterns of ethylene receptors were observed as those reported in other non-climacteric fruits such as strawberry. In these systems, ethylene increases slightly and transiently before ripening (Chervin *et al*., 2004) and the similar expression patterns suggest the existence of conserved mechanisms for action of this hormone in fruit ripening in both species (Deluc *et al*., 2007).

Genetic investigations of grapevine reproductive development

Grapevine transformation can be used for gene function determination but the methodology is complex and still restricted to relatively few research groups (Kikkert *et al*., 2001; Bouquet *et al*., 2006). Of the grapevine flowering genes that have been studied, only preliminary data on transgenic grapevines containing *VvMADS1* has been reported to date (Boss *et al*., 2003).

The use of natural genetic variation can be informative in establishing gene function, overcoming problems of genetic transformation (Koornneef *et al*., 2004). Natural genetic variation can be used in forward and reverse genetic approaches to support causal relationships between gene sequences and phenotypes. A large part of grapevine natural genetic variation is within the cultivated compartment and maintained in germplasm centres (This *et al*., 2006). In recent years, this genetic variation has started to be exploited to identify the genetics of disease resistance and quality traits in grape through inheritance studies and genetic mapping (Doligez *et al*., 2002; Fischer *et al*., 2004; Barker *et al*., 2005; Cabezas *et al*., 2006; Doligez *et al*., 2006). Among them, some traits related to grapevine reproductive development have been characterized. Sex determination was proposed by Negi and Olmo (1971) to be controlled by a single locus with three alleles with dominance relationships of male>hermaphrodite>female, and this locus was located on the currently known linkage group 2 (LG2), using interspecific crosses involving different *Vitis* species (Dalbo *et al*., 2000; Riaz *et al*., 2006). In grapevine, genetic variation has been observed for many traits related to flowering such as bunch number per shoot, flowering time, véraison time, ripening rate, as well as berry and bunch size. However, no results have yet been published on the genetics of these traits. Regarding berry weight, in grapevine, as in other fruit species, there is a positive correlation observed between seed number and berry size (Fernandez *et al*., 2006*b*). Berry size reduction is also observed as a result of stenospermocarpy seedlessness of Sultana or parthenocarpy of Corinth cultivars (Doligez *et al*., 2002). Stenospermocarpy of Sultana and related cultivars has been shown to be controlled by a dominant allele at a major QTL on LG18 (Doligez *et al*., 2002; Cabezas *et al*., 2006). Another QTL on LG4 explains part of the variation in seed number and concomitantly on berry weight (Fanizza *et al*., 2005; Cabezas *et al*., 2006). Finally, independently from seed number or seed development, natural variation for berry weight is also associated with at least four additional QTLs that could be involved in the

control of carpel growth and development (Fischer *et al*., 2004; Cabezas *et al*., 2006). Genes responsible for these QTLs have not been identified yet. However, identification of genes differentially expressed in inflorescences of seeded and seedless Sultana berries identified a chaperonin which silencing in transgenic tobacco and tomato fruits promoted seed abortion (Hanania *et al*., 2007). These results provide new approaches for the genetic engineering of this trait.

One interesting approach that has been recently exploited in grapevine is somatic variation, which appears spontaneously and can be maintained through vegetative propagation. Somatic variants are commonly periclinal chimeras that are heterozygous for spontaneous mutations in the L1 or L2 meristematic layers (Franks *et al*., 2002). Mutant plants can be recovered by hybridization when mutations are in the L2 or through somatic embryogenesis from either L1 or L2 cells. Somatic variants affecting berry traits such as colour, seedlessness, or aromas have been selected throughout the history of grapevine cultivation (This *et al*., 2006), and many others affecting leaf development or reproductive development have been maintained as curiosities. Among those affecting reproductive development, a dominant mutation in the *V. vinifera* homologue of the *Arabidopsis GIBBERELIC ACID INSENSITIVE* (*GAI*) gene causes hairy leaves when present in the L1, but a more drastic phenotype when mutant plants are regenerated from this cell layer (Boss and Thomas, 2002; Franks *et al*., 2002). These plants display a reduction in internode length and tendrils are transformed into inflorescences (Boss and Thomas, 2002; Franks *et al*., 2002). The phenotypes of these gibberellic acid (GA)-insensitive plants strongly support the hypotheses of the role of this hormone in the repression of flower initiation in grapevine (Boss and Thomas, 2002). Other somatic variants altered in reproductive development have recently been described (Chatelet *et al*., 2007). Among them, the Carignan *reiterated reproductive meristems* (*rrm*) shows defects in inflorescence and flower development caused by the reiterated production of inflorescence meristems, while others are more affected in the development of flower organs such as stamens and carpels (Chatelet *et al*., 2007). Expression analysis of MADS-box genes showed that the variant phenotypes are associated with alterations in the expression of genes in this family (Sreekantan *et al*., 2006; Chatelet *et al*., 2007), and the mutants could be useful to help understand the biological function of some of these genes in grapevine.

Somatic variants affecting berry size are also well known among widely cultivated varieties such as Grenache and Mourvèdre, with the best characterized being the *fleshless* somatic variant of the cultivar Ugni Blanc (Fernandez *et al*., 2006*b*). The mutation in this somatic variant affects carpel and berry development, giving rise to fruits lacking the berry flesh (Fernandez *et al*., 2006*c*). The mutation has been mapped to linkage group 18 of grapevine (Fernandez *et al*., 2006*a*) and represents an interesting model for the study of flesh development in the berry (Fernandez *et al*., 2007).

Future prospects

The recent releases of draft genome sequences of grapevine (Jaillon *et al*., 2007; Velasco *et al*., 2007), suggest that grapevine may have a simpler genome than other dicot species, like *Arabidopsis thaliana* and *Populus trichocarpa*, that have gone through additional polyploidization events in their evolutionary history (Jaillon *et al*., 2007; Velasco *et al*., 2007). This simpler genome structure could be useful in comparative studies to understand plant genome evolution in angiosperms better. Furthermore, genome sequence availability provides new genome-derived tools such as dense genetic maps and microarrays allowing grapevine reproductive biology to be approached from a genome perspective. It can be expected that extensive genomic and transcriptome analyses will allow identification of the complete gene set for each class of regulatory genes, the subsets of genes involved in every regulatory process, the related signal transduction systems, and the corresponding downstream metabolic networks, focusing the selection of candidate genes for the final analysis of biological function. However, there are still experimental bottlenecks, and new approaches need to be developed for gene function assignment in grapevine.

Although use has been made of existing genetic diversity as described above, the available genetic material suitable for genetic dissection of traits and gene function determination is limiting for grapevine. All genetic mapping studies published to date are based on F_1 progeny populations, and transgenic studies have been restricted to only a few specific genotypes. For *V. vinifera* the use of cultivars with hermaphroditic flowers, the increased focus of global wine companies on only a few cultivars and the vegetative propagation of cultivars by cuttings have all contributed to a reduction in genetic diversity in commercial plantings. Vegetative propagation has meant that a Pinot noir plant in France is genetically identical to a Pinot noir plant grown in any other region of the world apart, from natural somatic mutations that may have randomly occurred in one of the cell layers (Franks *et al*., 2002; Hocquigny *et al*., 2004; Moncada *et al*., 2006; This *et al*., 2006). Induced mutagenesis has not been used for genetic analysis in grapevine due to the problems of managing highly heterozygous plants, the long generation time, and the need for experimental fields. However, the development of near-homozygous lines such as PN40024 (Jaillon *et al*., 2007) and rapid cycling lines such as the *Vvgai* mutant or microvines (Boss and Thomas, 2002; Franks *et al*., 2002) might provide useful

resources to generate mutagenized populations to increase phenotypic diversity for gene-function studies including the application of TILLING (McCallum *et al*., 2000) to link genes to phenotypes. In species with an efficient transformation system, the association of a phenotype with a genetic difference is usually confirmed by transgenic studies. As mentioned previously, current grapevine transformation procedures are restricted to a few groups, and for studies investigating reproductive biology or fruit development and composition there is the added need to wait a number of years before plants flower. New grapevine transformation methods that are easier, efficient, and reduce the time for trait evaluation are needed. Most of the published grapevine flowering studies have been done on field-grown plants with many uncontrollable variables impacting on treatments and resulting observations and conclusions. Experiments performed in environmentally controlled glasshouses or growth rooms may provide more robust conditions for dissecting the biology of grapevine flowering and gene×environment interactions. The study of the early stages of floral induction and inflorescence development is very difficult due to the processes occurring within a latent bud being hidden from view. Non-destructive methods to observe and study this process are required and the use of the microvine where floral development occurs outside the latent bud may be useful for some investigations. We believe that none of the above experimental bottlenecks and issues are insurmountable and it is expected that solutions and resources will become available in the near future that will greatly assist gene function analysis in grapevine.

The knowledge from *Arabidopsis* and other species represents a great resource to study flowering in grapevine to uncover similarities and differences. The expression of MADS-box genes (e.g. *VvMADS1*, *VvMADS4*) in the developing grape suggests that the representation of flower and fruit development as distinct separate processes, may be an artificial separation based on science compartmentalization rather than biological compartmentalization; a fruit is simply the continued growth of the carpel. Supporting the MADS-box expression evidence in grape is the finding that the fleshless berry phenotype (Fernandez *et al*., 2006*c*) appears to be due to a locus involved in ovary development (Fernandez *et al*., 2006*a*), and high levels of proanthocyanidins (condensed tannins important in mouth-feel and colour stability of wine) are present in grapevine flowers prior to anthesis (Bogs *et al*., 2005). Some challenging questions requiring answers to understand grapevine flowering better include:

(i) What are the genes and processes controlling the formation of the lateral meristem (anlage) at the SAM?

(ii) How does the plant manage the production of SAMs, tendrils, and inflorescence meristems on the same shoot?

(iii) What is the mechanism that determines whether a lateral meristem will be a tendril or inflorescence and what is preventing tendrils forming flowers?

(iv) What is the genetic difference between genotypes with variations in bud fruitfulness and node position of the fruitful bud?

(v) What are the important environment–gene interactions determining lateral meristem commitment, bud fruitfulness, and flower number per bunch?

It is expected that many of the answers to these questions will involve understanding the spatial and temporal expression of genes, gene products, and small RNAs as well as their movement and interactions. The *Arabidopsis* (Corbesier *et al*., 2007) and rice (Tamaki *et al*., 2007) model of FT protein transport in the phloem from leaves to the SAM for conversion into an inflorescence will be interesting to evaluate in grapevine as the plant produces both vegetative and inflorescence meristems on the same shoot at the same time.

Answers to the questions above are not only of scientific interest but will also be of considerable importance to crop improvement efforts to improve yield, ensure consistent yields from year to year, and address climate change issues. As mentioned above and in Fig. 6, there is a large management effort involved in manipulating yield and berry composition. Actually, a disproportionate amount of resources is devoted to this in grapevine compared with most other crops. These management input costs will continue to increase and are not likely to be sustainable over the long-term due to increasing labour and energy costs. To remain competitive, it is likely that genetic solutions will become increasingly important to an industry that has over the last 100 years favoured management solutions to problems due to a marketing focus on a relatively small number of cultivars. Illustrative of this is that the Sultana yield problem that Barnard and Thomas investigated on behalf of the Australian industry in the 1920–30s (Fig. 4D, E; Barnard and Thomas, 1933, 1938; Thomas and Barnard, 1937, 1938) still exists today, because the problem is related to genotype and is only partially managed by pruning to control plant size and leaving longer canes with more buds (cane pruning). Cane pruning is costly and labour intensive and in some countries the fresh fruit and raisin industries now prefer new cultivars that can, at a lower cost, be machine pruned to a shorter cane and still give high yields the following season. Machine pruning of wine grapes is increasingly common in many parts of the world due to high labour costs, and genotypes with fruitful buds on lower nodes are more suited to this type of pruning.

Other traits of interest to the table and raisin industry include seedless berries of different sizes. There is market subdivision in the raisin industry for dried berries of

different sizes depending on the final purpose and, for table-grapes, large seedless berries are preferred. Most table-grape cultivars require the spray application of GA during the early pre-véraison stage of berry development to increase berry size for increased market appeal. Research on the genetics of seedlessness and berry size may allow the development of new genotypes that do not require the application of GA sprays. Inflorescence and bunch architecture is important for controlling Botrytis bunch rot and for spray penetration to control other pathogens and pests a more open bunch is desirable. It has been shown that flower number per inflorescence does not have a major effect on final inflorescence length between a small tight bunch genotype (Riesling) and a large open bunch genotype (Exotic) (Shavrukov *et al*., 2004). Instead, the genetic control of bunch openness appears to be mainly due to internode length of the inflorescence rachis (Shavrukov *et al*., 2004). Control of bunch size is also of interest to the table-grape industry as large bunches require trimming by hand to fit packaging and seller requirements in some markets adding to input costs.

However, by far the most important and common problem of the three grape industries is the large seasonal variations in yield. Compared with 16 crops analysed over a 58–75 year period, grapevine was found to have by far the highest seasonal variation in yield (32.5%), nearly twice that of the next closest crop (Chloupek *et al*., 2004). For the wine industry the other dimension to this seasonal problem is accurate yield prediction prior to harvest, as the earlier this information is available the better a large wine company can plan and schedule harvests, transportation, and ferments. The seasonal variation is likely to be due to genotype×environment interactions and an understanding of this may allow the development of genotypes that are less responsive to environmental factors and have reduced seasonal variations in yield. The increased awareness of climate change and potential effects on existing wine regions and grape and wine composition (Jones *et al*., 2005; White *et al*., 2006) is very relevant because all aspects of flowering described in Figs 1 and 6, from budburst to harvest, are driven by climate. It appears that to maintain existing wine regions over the long-term a genetic solution will be necessary to address the concerns of yield, grape and wine composition, as well as abiotic and biotic stresses. Part of this solution will be the development and planting of better-adapted consistently high-yielding varieties that may also differ in date of bud burst and flowering and berry-ripening rates compared with currently existing traditional regional cultivars.

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