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The Microvine: A Versatile Plant Model to Boost Grapevine Studies in Physiology and Genetics

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Abstract

The microvine is a grapevine somatic variant. The *Vvgai1* mutation results in a miniaturization of the vegetative organs of the plant keeping fruit size intact and a systematic conversion of tendrils into inflorescences. The physiological characterization of the vegetative and reproductive development of the microvine makes it possible to infer kinetic data from spatial phenotypes. This biological model allows experiments on vine and grape development in tightly controlled conditions, which greatly accelerate physiology, molecular biology, as well as genetic studies. After introducing the main biological properties of the microvine, main results from various research programs performed with the microvine model will be presented.

Keywords: research tools, microvine, grapevine model, physiology, genetics

1. Introduction

As a perennial fruit crop, the grapevine (*Vitis vinifera*) needs a long juvenile period before the reproductive cycle starts. Even vine cuttings from adult plants allow the production of fruits only from the second year. Moreover, during the adult phase, common cultivars produce reproductive organs only once per growing cycle (generally once per year) and per proleptic axis. These biological features, together with the large size of an adult vine, represent major drawbacks for precise physiological, ecophysiological, and omics experiments on the plant and fruit development under well-controlled conditions. Furthermore, those characteristics of normal vines slow down advances in genetics and breeding.

The microvine ML1 is a somatic variant obtained through somatic embryogenesis from Pinot Meunier cultivar. This phenotype results from a somatic mutation in the *Vvgai1* gene involved in gibberellin signaling. The mutation is originally present at the heterozygous state in the epidermal cells of Pinot Meunier, being responsible for its well-known hairy phenotype. However, the introduction of the mutation in all cell layers resulted in a miniaturization of all vegetative organs and in a conversion of tendrils into inflorescences, which leads to a continuous flowering and fruiting along vegetative axes.

The small size of the microvine renders this grapevine model very convenient for experiments in usual growth chambers, where a tight control of environmental factors (radiation, vapor pressure deficit (VPD), temperature, water and nutrient

supplies) is possible, in contrast with experiments under vineyard conditions. Indeed, it is possible to grow the vines up to densities of 15–30 plants/m² and to limit their height to 1.2 m. Under such conditions, the most advanced fruits are mature 5–6 months after plantation of cuttings or seedlings, and the vegetative axis displays all developmental stages from young inflorescences (distal phytomers) to flowering, berry growth, and ripening (proximal phytomers). Under stable controlled conditions, the spatial gradients of vegetative and reproductive development of the microvine mimic well the temporal development of each phytomer, which allows to infer kinetic data from one-off spatial information along the proleptic axis.

In controlled conditions, microvine allows to experiment on berry development all year long, which greatly accelerates studies on physiology and molecular biology. Furthermore, by reducing the time lag between two generations and by increasing the precision of phenotyping, genetic approaches are much more efficient than the ones generally performed with macrovines. In the first section of the paper, we describe the genetic and molecular mechanisms underlying the phenotypes of the microvine and derived lines. Then, we review typical experimental designs that can be designed with the microvine. In the last section, we review recent project using this model to study grapevine development and fruit physiology and to identify quantitative trait loci (QTLs) of agronomic traits.

2. Biological origin of the microvine

2.1 Tissue chimerism and phenotypic consequences

The meristem of higher plants is organized in several cell layers. The outermost, which corresponds to epidermal cells, results from anticlinal divisions (i.e., following a plane of division perpendicular to the surface). This tissue which covers all the organs of the shoot system develops as a single cell layer [1]. Underneath, a multicellular zone, called L2 cell layer, is at the origin of all subepidermal tissues, following multidirectional divisions (i.e., primary structures but also lateral meristems, vascular cambium, phellogen, and their derivative tissues). No further, deeper cell layer (L3 cell layer), which forms in some species the core of shoot organs (pith), has been clearly identified in the grapevine yet [2].

In general, these cell lines that derive from initial cells located at the tip of the apical dome do not mix, unless there is an accident during cells multiplication. The organization in L1 and L2 cell layers is found in the various organs that derive from the shoot apical meristem (SAM) and in particular in the axillary meristems at the origin of caulinar organs. Because a somatic mutation is initially a single cellular event, it leads to the setting of chimeric tissues or organs, i.e., composed of cells of different genotypes and potentially displaying some phenotypic diversity [2]. When a somatic mutation appears laterally to a meristem, changes can only be distributed in the sector of the mutated organ. If the mutation occurs in an initial cell of a meristem, it can spread to all the tissues derived from the mutated cell. The resulting structure is a chimeric and periclinal genotype, i.e., including cell layers that are not all genetically identical. Periclinal chimeras can be stabilized by vegetative propagation, i.e., by cuttings or by grafting.

A somatic mutation can invade all the cell layers and spread uniformly to all derivative tissues, provided that the three following conditions are fulfilled: (i) the mutation is not lethal for the plant, (ii) the mutation appears in an initial cell within a meristem, and (iii) the mutation is established, by cell substitution in both L1 and L2 cell layers [2]. The probability of simultaneous occurrence of these three

conditions being very low, most of the mutations therefore develop sectorially or periclinally and give rise to chimeric tissues and organs.

In the 1990s, thanks to the use of codominant genetic markers (microsatellites, RFLP), the existence of genetic chimerism has been demonstrated in several vine varieties. As such, Franks et al. [3] showed that Pinot Meunier can display up to three alleles for some loci, whereas a vine, having a diploid genome, can theoretically only show one allelic form per homozygous locus and two allelic forms for a heterozygous locus. Boss and Thomas [4] were able to de-chimerise Pinot Meunier by somatic embryogenesis. They characterized the resulting L1 and L2 genotypes and studied the associated phenotypes. This work showed that Pinot Meunier carries a mutation in *VvGAI1* gene in the L1 layer which confers the hairy phenotype to the variety (**Figure 1**).

Plants regenerated from L1 or L2 cells exhibited very different phenotypes. The plants obtained from the deepest cell layer (L2) no longer had a mutation at *VvGAI1* locus and presented phenotypic traits very close to Pinot Noir. Conversely, the plants derived from L1 cells that retained a mutated version of *Vvgai1* associated with a wild-type allele *VvGAI1* were dwarf and hairy and displayed a full conversion of all tendrils into inflorescences (**Figure 2**). This phenotype has been called microvine, due to the small size of the mutant.

Thus, the microvine has the *Vvgai1* mutation present in both cell layers that confers a very different phenotype from the Pinot Meunier from which it derives and which only bears the mutation in the L1 cell layer. Another interesting feature is related to the genetic status of the mutation in the microvine. Although it is present in both cell layers, the *VvGAI* locus is heterozygous, i.e., each cell is carrying a mutated allele *Vvgai1* is associated with a wild-type allele *VvGAI1*. Because *Vvgai1* is

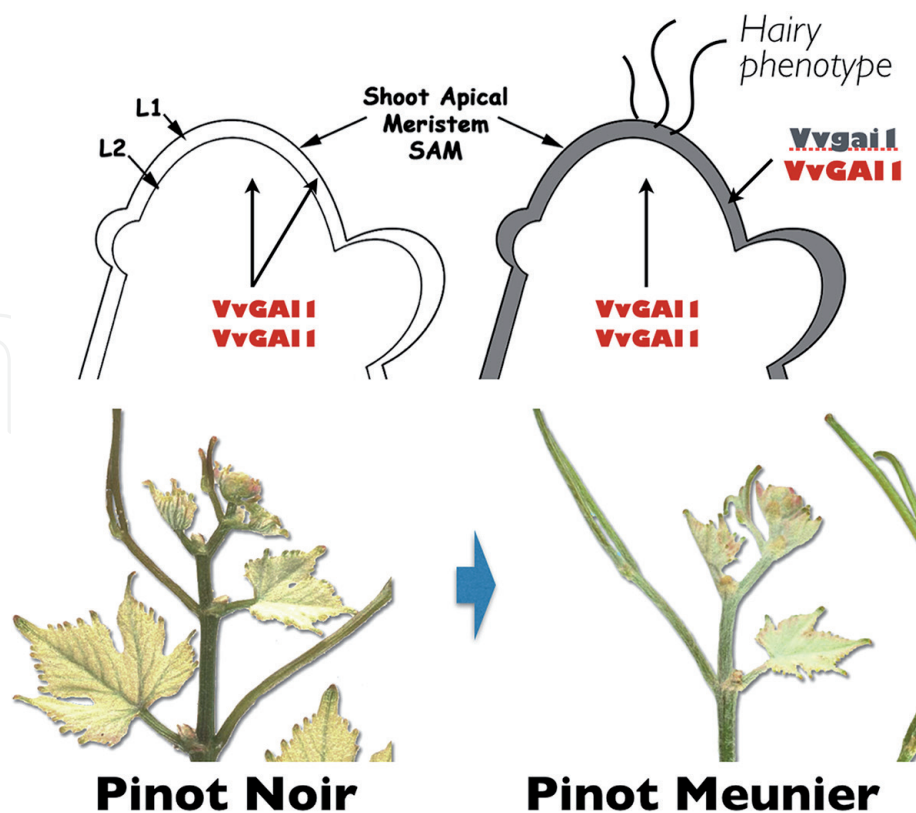


Figure 1. Genetic structures of pinot noir and pinot Meunier and their respective apex phenotypes. Pinot Meunier is a somatic variant of pinot noir, which carries the mutation (*Vvgai1*) at heterozygous status. Localized in the epidermal cells (L1 cell layer), the mutation exacerbates the hairiness of vegetative organs of this variety (<http://plantgrape.plantnet-project.org/en>), without any other significant phenotypic change.

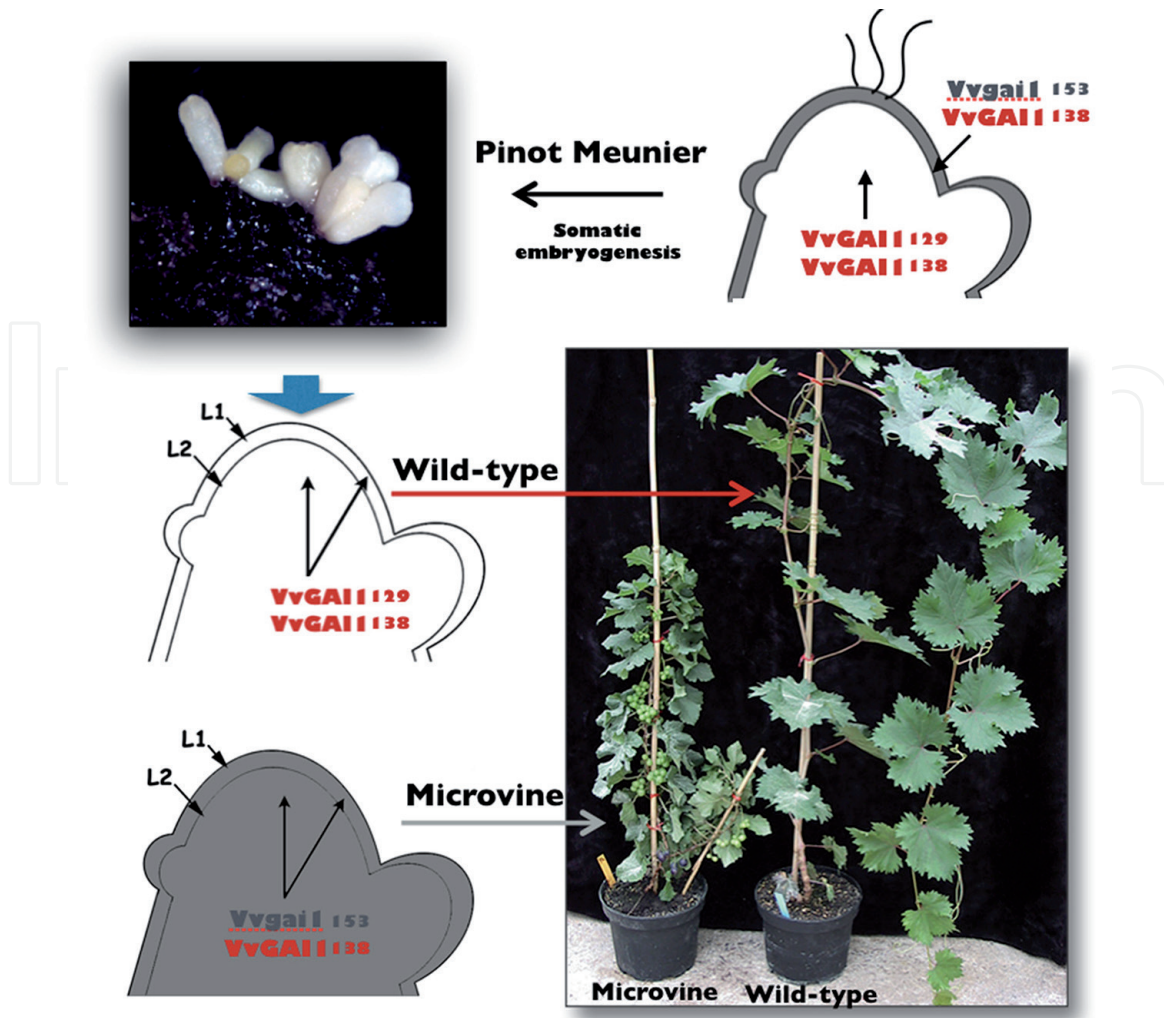


Figure 2.

By somatic embryogenesis from anthers of pinot Meunier, it is possible to obtain two types of plants. One, which no longer carries the mutation of *VvGAI* in the L1 and L2 cell layers, has a phenotype similar to pinot noir (large size, juvenility period, main production of clusters from proleptic axes, i.e., winter buds). The other, which carries the mutation of *VvGAI* in all the tissues, displays a miniaturized phenotype and extreme hairiness and produces inflorescences both in the winter buds and from the conversion of tendrils in inflorescences. In the figure, the numbers associated with *VvGAI* allele correspond to the nucleotide base length (bp) of the VVS2 microsatellite marker [4].

not a lethal mutation nor for the sporophyte or the gametophyte, this status can be rearranged by selfing in three genotypes:

- i. Homozygous *VvGAI1/VvGAI1*, which corresponds to a vine without any mutation at the locus. The phenotype associated with this genetic status is non-dwarf, similar to classical macrovine varieties.
- ii. Heterozygote *VvGAI1/Vvgai1*, which corresponds to the same genotype and (dwarf) phenotype than the original microvine ML1.
- iii. Homozygous *Vvgai1/Vvgai1*, which corresponds to plants carrying both alleles in a mutated version. The phenotype associated with this status, called picovine, corresponds to an extreme dwarfism, with plants displaying very miniaturized shoot organs [4] (**Figure 3**).

Another interesting feature, linked to the heterozygous status *VvGAI/Vvgai1*, is the possibility to return to non-dwarf phenotype. Indeed, by crossing a microvine (*VvGAI1/Vvgai1*) with a classic grapevine variety, i.e., a macrovine (*VvGAI1/*

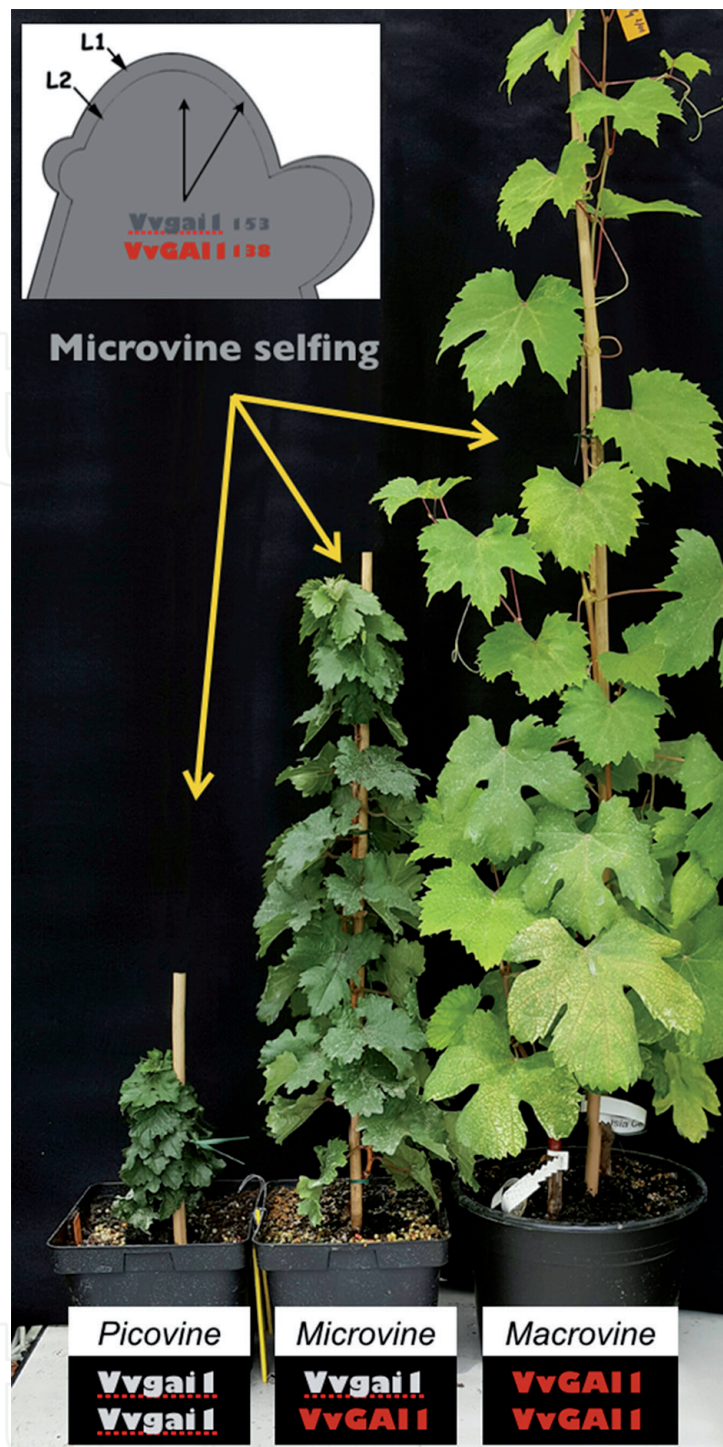


Figure 3.
The three genotypes/phenotypes that can be obtained by selfing from the microvine (*VvGAI1/Vvgai1*): left, extremely miniaturized vines that carries the homozygous locus *Vvgai1/Vvgai1*, called picovines; middle, individuals with the same phenotype as the microvine, heterozygous for the mutation (*VvGAI1/Vvgai1*); and right, normal-sized plants that no longer carry mutated alleles, homozygous for the non-mutated form of the gene (*VvGAI1/VvGAI1*).

VvGAI1), it is possible to recover 50% of individuals with a microvine phenotype and 50% of individuals with the characteristics of a non-dwarf grapevine.

2.2 Molecular mechanisms associated with the mutation *Vvgai1*

The comparison of the allelic *VvGAI* forms present in Pinot Meunier and the microvine [4, 5] showed that the mutation corresponds to a modification of a single nucleotide in the DELLA motif of the protein, which is important for gibberellin signaling.

After transient transformation of epidermal onion cells, green fluorescent protein (GFP) fusions to *VvGAI1* and *Vvgai1* sequences responded differently to gibberellin applications. The GFP signal of the GAI1::GFP fusion disappears rapidly from the nucleus under the effect of gibberellins, which indicates its degradation following the hormonal stimulus. On the contrary, the *gai1*::GFP translational protein fusion remains insensitive to hormonal signaling, which indicates that the mutation in the DELLA motif abolishes the property of the protein to be degraded when triggered by gibberellins [5].

The GAI gene is known to be an important regulator of vegetative growth and reproductive development [6]. In grapevine, gibberellins, produced under shade, stimulate growth and inhibit the formation of inflorescences [7]. This effect is mediated by the nuclear protein GAI1, which, in its mutated form *gai1*, no longer transmits the hormonal signaling [5]. Thus, vegetative growth and the inhibition of the conversion of tendrils into inflorescences are no longer maintained which explains the dwarf phenotype and the continuous fructification along the stems. The characterization of the expression profiles of different isoforms of *VvGAI* revealed that *Vvgai1* is mainly expressed in vegetative organs such as buds and young leaves, while other forms are expressed in reproductive organs (unpublished data). For instance, *Vvgai2*, which does not have any mutation in the DELLA protein motif, is expressed in reproductive organs from flowering to ripening [5]. This explains why *Vvgai1* mutation does not interfere directly with berry developmental program which is similar to non-dwarf varieties.

3. Application of the use of the microvine

3.1 Vegetative development

Several experiments have been conducted outdoor and in controlled environments to characterize the vegetative development of the proleptic axis of the microvine [8]. Different day/night temperature treatments were applied (22/12, 25/15, 30/15, 30/20, 30/25°C), while VPD was maintained constant (about 1 kPa). These experiments showed that the vegetative organogenesis rhythm of the microvine is similar to that of non-dwarf vines. Indeed, its phyllochron (leaf emission rate) is around 24°C, similarly to other varieties of *V. vinifera* such as Grenache [10], and it fluctuates only slightly with temperature and radiation variations between experiments (photosynthetically active radiation (PAR) has been experimented from 19 to 25 mol.m⁻²d⁻¹).

The duration of leaf and internode growth of the microvine is also similar to that of non-dwarf vines, lasting ca. 220°C (i.e., 20 days at 25/15°C) for leaves and ca. 150°C (i.e., 14 days under the same conditions) for internodes [9, 10]. The most significant phenotypic difference, induced by *Vvgai1*, is the size limitation of vegetative organs. The leaf area is reduced by half in the microvine compared to non-dwarf vines, and internodes are five times shorter. The dwarf phenotype is thus very valuable to conduct experiments under very well-controlled conditions in small growth chambers. Such property permits to study the impacts of single or combined abiotic factors (radiation, temperature, VPD, CO₂) on plant growth and development while minimizing uncontrolled biases arising from environmental fluctuation in field studies on perennial vines.

However, the shortening of the internodes increases leaf shading and promotes the development of fungal diseases as compared to non-dwarf vine. The control of powdery mildew (*Erysiphe necator*) on leaves and green berries or gray mold (*Botrytis*) on ripening fruits requires a strict phytosanitary management.

To improve the microclimate of the clusters, it is recommended to systematically remove the lateral branches to reduce the plant to a single proleptic axis and to systematically eliminate one leaf out of three, e.g., removing the leaves of all P0 phytomers which do not bear any inflorescence. Also, for the most fertile lines, it is necessary to control the number of ripening berries to avoid source/sink unbalance that could be prejudicial to the growth and the formation of new inflorescences as well as the accumulation of metabolites in the fruits. Because the microvine displays several levels of cluster at ripening stages, a good balance is achieved by limiting the number of ripening berries to 8–15 per cluster.

3.2 Reproductive development

The reproductive development of the microvine is divided into two distinct and simultaneously occurring patterns: (i) the fructification of proleptic shoots from preformed inflorescence primordia within winter buds and (ii) the continuous fruiting of proleptic and sylleptic axes resulting from the conversion of tendrils into inflorescences.

3.2.1 Fruiting from winter buds (two successive seasons)

In the grapevine, as for many other perennial fruit crops, fruit formation occurs during 2 consecutive years. The first step starts with the initiation and differentiation of inflorescence primordia in the winter buds prior to endo-dormancy until approximately the end of summer or beginning of autumn. During the subsequent cycle after the break of dormancy, approximately 2 weeks before budburst, the inflorescences resume their development and complete flower organogenesis and subsequently flowering in spring [6]. The level of differentiation of microvine winter buds (i.e., the number of preformed phytomers and inflorescence primordia) was analyzed during 80 days of growth under controlled environmental conditions (25/15°C day/night temperature, VPD 1 kPa, photoperiod 12 h). Two imaging methods were compared, the classic microscopy dissection and the noninvasive X-ray micro-tomography [11], with a resolution of 9 μm . These observations showed that winter buds of the microvine harbor a complex formed of primary, secondary, and tertiary buds of decreasing fertility, as non-dwarf vines [12]. The maximum fertility of the primary buds is two inflorescences in the microvine, whereas it can reach three or even four in some non-dwarf varieties. These inflorescences are inserted into phytomers n°4 to n°6 with an acropetal development as for macrovines [12, 13]. The lignification of the stem which develops from the vegetative axis base is concomitant with the slowdown of bud development and probably its entry into endo-dormancy, similarly as for non-dwarf vines [14].

3.2.2 Continuous flowering and fruiting (one single growing season)

The microvine has the particularity to develop inflorescences from tendrils along proleptic and sylleptic axes (**Figure 4**), which result in a continuous flowering and fruiting processes. A gradient of reproductive development stages is thus present simultaneously along the proleptic axis from the differentiation of inflorescences until maturity. This characteristic offers the opportunity to evaluate abiotic or biotic stress impacts on all reproductive stages of development along the proleptic axis simultaneously.

Top right, section of a winter bud analyzed by tomography. Bottom right, a longitudinal section of a winter bud exhibiting a lateral inflorescence primordium (IP) on the primary bud axis and a secondary preformed vegetative axis on the left side.



Figure 4.

Vegetative and reproductive development of the ML1 somaclone n°7, a microvine line regenerated from pinot Meunier cl. ENTAV 8 according to the method described by Torregrosa [15]. Top left, longitudinal section of an apex showing the preformation of 7–9 phytomers before emergence of caulinar organs. Upper middle, emergence of young inflorescences just below the apex. We note the very hairy appearance of the apex of the microvine ML1. On the middle, an 8-month-old ML1 microvine displaying all the sequences of the reproductive development from flowering to fruit ripening. Bottom left, a focus on the phytomers carrying bunches shifting from green to ripening stages and the concomitant lignification of the shoot (leaves have been removed for the clearness of the photograph). Top right, section of a winter bud analyzed by tomography. Bottom right, a longitudinal section of a winter bud exhibiting a lateral inflorescence primordium (IP) on the primary bud axis and a secondary preformed vegetative axis on the left side.

The synchronism between vegetative development and fruiting of the microvine also simplifies the study of their interactions compared to macrovines. The impact of contrasted source/sink balance on fruiting can be easily studied by manipulating shoot or fruit load (number of growing axes and/or number of leaves/inflorescence per axis). The continuous fruiting was found to be stable under standard environmental conditions (25/15°C day/night temperature, VPD 1 kPa, photoperiod 12 h) and when the leaf area to fruit fresh weight was less than 1 m².kg⁻¹. On the contrary, the capacity of flowering is strongly altered in the presence of abiotic or biotic stresses. High temperature (> 33°C), low radiation levels (PAR < 15 mol.m⁻².j⁻¹), or high VPD (>3 kPa) can induce inflorescences abortion and disrupt the continuity of the reproductive gradient along stem axes. The sensitivity of inflorescence development was found higher when the C reserves (starch) were reduced, in particular, in young plants. Thus, although it is possible to obtain fruiting organs from 5-month-old microvine cuttings, it is advisable to use 1-year-old or older plants that are much less susceptible to inflorescence abortion [16]. In experiments conducted in our lab, we obtained successive cycles of fruiting for at least 5 years without repotting.

The size of inflorescences of microvines is smaller (10–50 berries per cluster in average) than that of macrovines [17–19]. However, flowers and young fruits of the microvine do not display a very high abscission rate as observed in non-dwarf varieties. The development of flowers and berries is identical to non-dwarf vines. Flowering (50% of open flowers) occurs 320°C GDD (growing degree days) after the phytomer emission (i.e., 30 days at 25/15°C), which is comparable to the duration between budburst and flowering in the non-dwarf vines [18]. Ripening (onset of sugar loading) starts at ca. 500°C GDD (i.e., 47 days at 25/15°C) after flowering, and the physiological ripening (when metabolite loading stops) is reached at ca. 900°C GDD (i.e., 80 days at 25/15°C) after flowering or 30 days after the start of sugar loading. This behavior is similar in macrovines [18, 20]. Thus, berries of the

ML1 microvine reach a final individual size of 1.2 g, comparable to that of cv. Pinot meunier, from which this line derives. At physiological ripening, berries contain about 0.8 mmol berry⁻¹ of soluble sugars in non-limiting water supply conditions which is similar to other varieties of *V. vinifera* (Figure 5).

3.3 Genetics and genomics

3.3.1 Genetic mapping and pre-breeding

The microvine provides different advantages over non-dwarf vines to speed up or facilitate genetics. Since the mutation is transmissible by hybridization and has a codominant effect, it is possible to cross microvines (*VvGAI1/Vvgai1*) or picovines (*Vvgai1/Vvgai1*) with non-dwarf genotypes, i.e., without the mutation (*VvGAI1/VvGAI1*), to create microvine segregating populations. In the first case, 50% of individuals will display the microvine phenotype, while using picovines as parent, 100% of the progeny exhibit a dwarf behavior.

The *VvGAI1* gene is located on chromosome n°1, while the QTL determining grapevine flower sex is located on chromosome n°2. That means both loci segregate independently, and it is therefore possible to use female microvines or picovines, which facilitates crosses by avoiding the time-consuming emasculation and reducing the risk of selfing [19]. On the other hand, when a female microvine (f/f) is crossed with a hermaphrodite genotype (H/f, the most common genotype in *V. vinifera* varieties), the population will be composed of 50% of female plants and 50% of hermaphroditic plants. For instance, by crossing between the

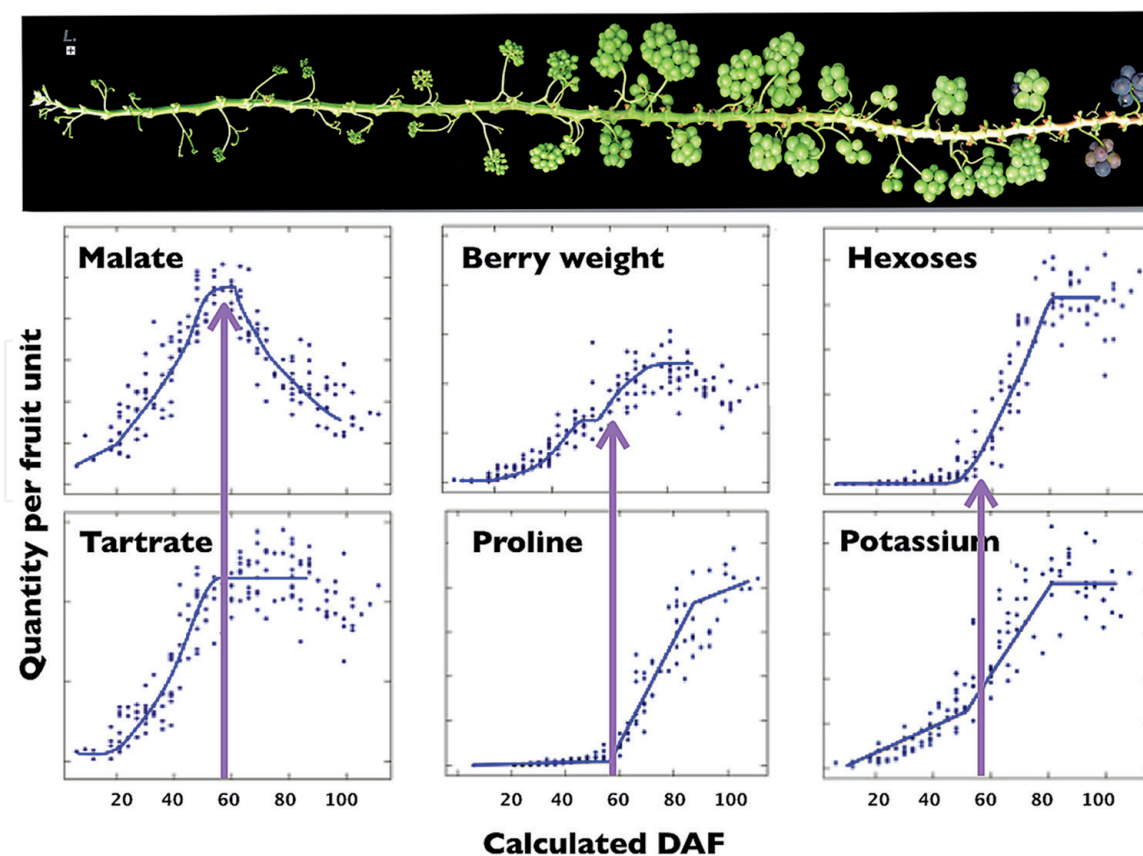


Figure 5. Spatiotemporal distribution of the reproductive developmental stages from flowering to ripening. On the abscissa, the calendar time in DAF (days after flowering) was recalculated for each phytomer converting the corresponding plastochron index in thermal time and inferred in calendar time with the phyllochron. Kinetics of fresh fruit weight and the contents of major primary metabolites and potassium are presented in quantity per fruit unit.

PV00C001V0008 [19] and the fleshless berry mutant of the ugni blanc [21], a range of genotypes and phenotypes can be obtained [5].

This progeny is composed of 100% microvines (since the female parent has a *Vvgai1/Vvgai1* genotype) and a very small proportion of individuals with both hermaphrodite flowers and pigmented berries. Indeed, these two characters are present at the homozygous recessive state in one parent (*f/f* and *n/n*) and in the heterozygous dominant state in the other (*H/f* and *N/n*). It should be noted that since ugni blanc is heterozygous at the sex locus (*H/f*), while the picovine is *f/f*, selecting hermaphrodite individuals leads to a segregation distortion in the progeny of the genetic traits determined on the chromosome n^2 .

As the microvine produces inflorescences as long as vegetative growth is maintained, it becomes possible to cross all year around without being hampered by seasonality. Under standard thermal and photoperiodic conditions (25/15°C day/night temperature, VPD 1 kPa, photoperiod 12 h), the microvine produces two to three new inflorescences per week, which enables to make hybridizations during long periods in repeating the crosses on the same plants. This also reduces the number of plants required for crosses and therefore experimental space while spreading the hybridization effort over selected and potentially long periods.

One to two months after a cross, it is possible to start harvesting seeds [22] to rescue zygotic embryos, which makes possible to establish a population maintained and amplifiable by micropropagation or microcuttings [23]. After a few micropropagation cycles, *in vitro* plants can be acclimatized to greenhouse conditions, and the first grapes are obtained within 12 months after the crosses. Thus, in less than a year, it is possible to start the study of the characteristics of the fruits and to proceed to new crossings to recover F2 populations. These speed up genetic mapping studies because it becomes possible to link a genotype and a phenotype in either F1 or F2 progenies in a few months instead of several years when using macrovines [23, 24].

Moreover, if a trait can be inherited through such crosses, it is possible to recover non-dwarf phenotypes (*GAI1/GAI1*) that can be directly proposed as breeding material. Indeed, 50% of the individuals from a cross between a microvine (*VvGAI1/Vvgai1*) and a macrovine (*VvGAI1/VvGAI1*) exhibit the same biological properties as conventional non-dwarf varieties. Thus, the microvine can be used both for the identification of QTLs of interest and also to combine or pyramid characters of interest in a pre-breeding perspective.

3.3.2 Functional genomics

The biological properties of the microvine are also of great interest for functional genomics [26]. Indeed, grapevine, as other perennial plants, is a difficult plant model to study the genes regulating the development of reproductive organs. The difficulty comes from its long juvenile period, its discontinuous fructification from winter buds, and the handling of large plants. The genetic transformation of classical varieties [28] requires several years to obtain adult plants and study the phenotypes linked to the ectopic expression of candidate genes.

With microvine, starting from embryogenic tissues compatible to *Agrobacterium tumefaciens*-mediated transformation (**Figure 6**), it is possible to recover transgenic fruiting plants in less than 1 year [19]. As for classical genetics, it is then easy to derive F2 lines to establish transgenic loci at homozygous state for further studies. In addition, the microvines have a very good aptitude for transformation by *Agrobacterium rhizogenes*, allowing to obtain transgenic organs stabilizable in axenic culture in a few weeks [25, 29, 30].

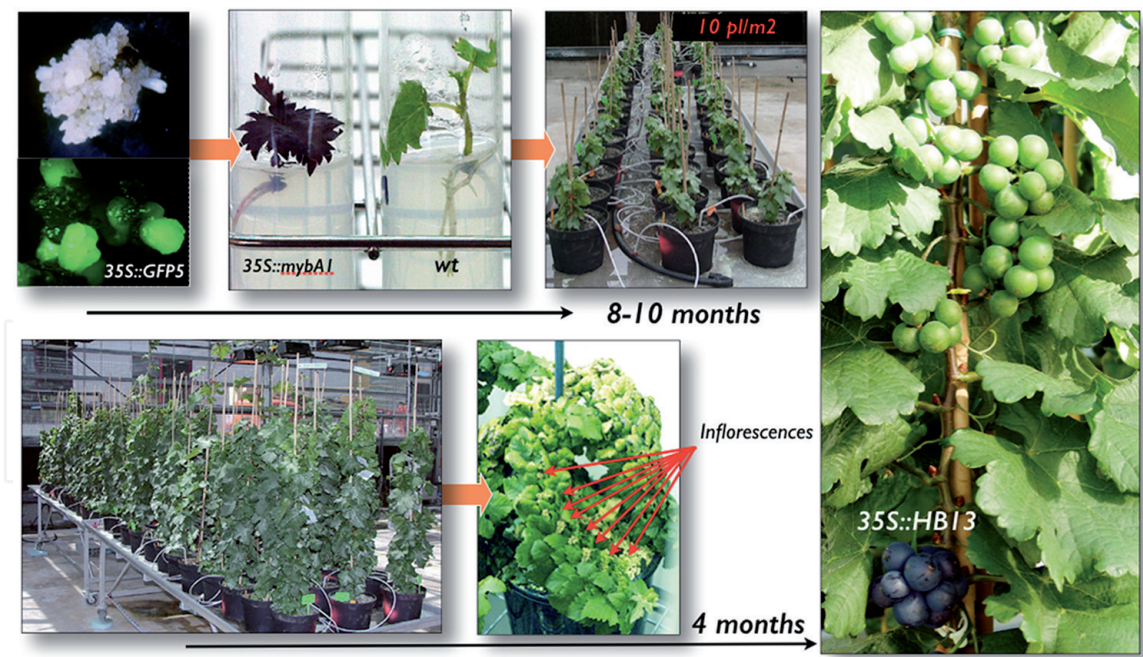


Figure 6. From competent embryogenic tissues (top left), it is possible to regenerate transgenic plants in a few months and obtain reproductive organs in less than a year. This allows the study of the regulation of flower and fruit development within shorter delays than with the non-dwarf vines. On the right, a microvine line V9 overexpressing the gene *VvHB* was identified as a major regulator of the development of the flesh in grapevine fruit [27]. Using genetically modified microvines, it is possible to segregate the transgenes in different genotypic configurations or combine them with various other transgenic traits or not.

4. Temporal inference of spatial observations obtained on the proleptic axis

We have tested the possibility of converting spatial observations (along the proleptic axis) into temporal dynamics at a given stage of vegetative or reproductive development.

4.1 Temporal conversion of spatial profiles

Under controlled and stable environment (25/15°C day/night temperature, VPD 1 kPa, photoperiod 12 h), the development of the proleptic axis of the microvine is stable. The phyllochron is constant reaching ca. 24°C. The growing dynamics of leaves (surface) and berries (volume) from continuous fructification was found to be constant at a given level of phytomer, regardless of the date of bud break [20]. The growth durations of leaves and berries (herbaceous phase) are ca. 220°C after the emission of the phytomer and 500°C after flowering, respectively, as mentioned in Section 2.2. The development of these organs is also spatially stable: the dynamics of leaf area and berry volumes (herbaceous phase) for all levels of phytomer are superimposed when they are represented as a function of cumulative thermal time after the emission of the corresponding phytomer.

Based on these outcomes, the conversion of spatial dynamics of leaf and berry development along the stems into time profiles was tested (Figure 7). For this purpose, the positions of the phytomers along the axis were converted into cumulated thermal time after their emission by multiplying their plastochron index (or rank position from the apex) by the phyllochron. The temporal profiles of leaf area and berry volume (green growth phase) resulting from this spatiotemporal conversion are similar to the real temporal profiles obtained at a given level of phytomer [8, 20, 31]. This property makes it possible to reconstruct temporal dynamics of

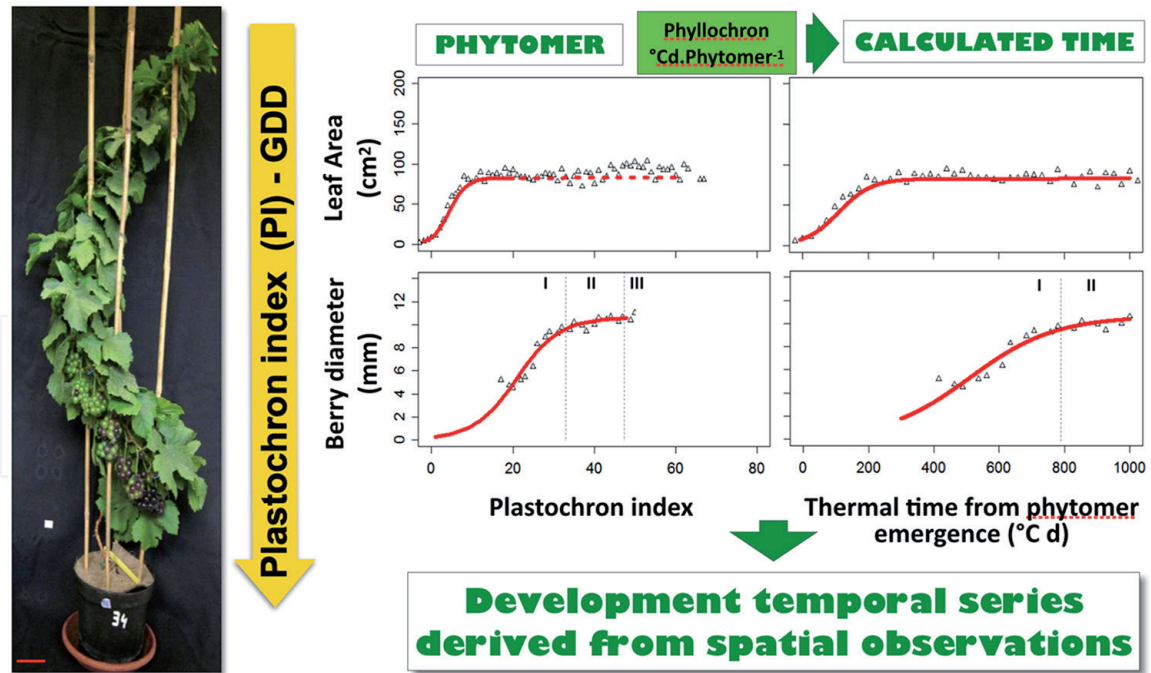


Figure 7. Conversion of leaf and young berry growth data collected from the position along the microvine main shoot (plastochron index) into cumulated thermal time after phytomer emergence.

development from a single spatial observation of the axis at a given stage. The flow of biomass or metabolites within the organs and their responses to environmental constraints were then addressed using those calculated temporal profiles (Section 5.1).

4.2 Dynamics of inflorescence differentiation within winter buds

The spatiotemporal conversion approach presented above can also be used to characterize the evolution of winter bud development along the proleptic axis of the microvine [12]. Bud development was analyzed on microvines grown under standard environmental conditions (25/15°C day/night temperature, VPD 1 kPa, photoperiod 12 h), as explained in Section 3.2.1. The number of preformed phytomers initiated by primary axes within buds increases linearly as a function of the plastochron index (PI) of the proleptic axis in the non-lignified zone (PI < 25). The temporal dynamics of bud development were calculated from the spatial profiles using the proleptic axis PI x phyllochron. The primary axis of the bud displayed a maximum of six phytomers from IP 25 (lignified zone), i.e., 625°C or 57 days after its initiation (phyllochron of 24°C). A maximum of two inflorescence primordia was observed in this zone. The primordia of the first and second inflorescences, located between the preformed phytomers n°4 and n°6 of the primary axis, were initiated from IP 13 and 26 of the proleptic axis, respectively, corresponding to 325°C (or 30 days) and 650°C (or 60 days) after bud initiation. The timing of inflorescence primordium development in winter buds in non-dwarf vines [32] is similar to our observations on microvines. This pattern of winter bud development parameterized for the microvine can be used to evaluate, and potentially predict, the environmental stress impacts during the season 1 on the fructification potential of the season 2.

4.3 Dynamics of fruit development deriving from neo-formed inflorescence

The primary characterization of fruit development along a microvine axis showed that the microvine berry displays the two classical growth phases as observed for berries of macrovines [32, 33]. Microvine berry growth and metabolite

accumulation were analyzed in details [34]. Ten microvines were grown under controlled conditions in a climatic room (30/22°C day/night temperature, photoperiod 14 h, VPD 1 kPa, PAR 400 mmol.m⁻² s⁻¹). Sampling was performed when proximal fruits attained physiological maturity and when maximum berry volume was reached. Sampling of the present reproductive organs from fruit set to maturity was performed at the same time for each plant. Analysis of the main berry compounds (malic acid, tartaric acid, glucose, fructose, proline) has been carried out. To normalize the stages of development between plants, the spatiotemporal conversion described above was applied using the individual phyllochron of each plant.

The data presented in Rienth et al. [35, 36] shows that microvine fruit accumulates malic acid during the green growth stage for about 40 days after fruit set, until it ceases when the lag phase (herbaceous plateau), which separates the two growth phases, is reached. At the end of the herbaceous phase, at the 24 hours lasting véraison phase, the degradation of malic acid is triggered simultaneously with the accumulation of sugars and proline, which is often used as an indicator of ripening. These processes proceed throughout the second growth or ripening phase. With regard to tartaric acid, we found that it is also accumulated only during the first growth phase as for macrovines and that its amount remains quasi-constant during the ripening phase. The slight decreases in tartaric observed during ripening might be attributed either to enhanced tartaric precipitations as shown by Rosti et al. [37] or variations of microenvironment depending on bunch rank. At the end of green growth stage, the two major organic acids represent approximately 500 mEq, which is comparable to the acidity of the fruit of macrovines. The accumulation of sugars, triggered from the *véraison*, continues until the moment when the phloem unloading is slowed down (maximum volume of the fruit). From this point, the amount of sugars per berry remains constant, but the concentration increases due the loss of berry volume during over-ripening.

5. Examples of studies performed with the microvine

5.1 Impacts of temperature on carbon fluxes and fruiting

The impact of elevated temperature on growth and carbon distribution between vegetative and reproductive organs was investigated. Two contrasting thermal regimes with a difference of 8° C (30/20°C vs. 22/12°C day/night temperature) were imposed during a period of 450°C GDD. The VPD was 1 kPa and the PAR 19 mol. m⁻².d⁻¹ for the two thermal regimes. The biomass, size, and carbon contents of the leaves, internodes, and berries were characterized from spatial observations at harvest and converted into temporal profiles according to the method described in Section 4. Only the organs that developed during heat treatments, i.e., vegetative phytomers younger than 450°C GDD at harvest and the reproductive phytomers, which were at pre-flowering stage at the beginning of experiments, were retained for analysis. Luchaire et al. [20, 36] have shown that high temperature accelerates the growth and the accumulation of biomass in vegetative organs (leaves and internodes) in thermal time, at the expense of the accumulation of sugars in internodes and the surface area to mass of the leaves (thinner leaves).

Under high temperature, the growth and accumulation of biomass of the fruit slowed down on a thermal time basis. Sugar loading of proximal phytomers (from the post-flowering stage to onset of heat treatment) was also delayed by ca. 400°C GDD at high temperatures. High temperatures increased inflorescence abortion rate (+ 20%) at pre-flowering stages, concomitantly with the beginning of sugar loading in the proximal clusters ripening [20, 36, 38]. These results suggest that

high temperature decouples vegetative and reproductive development, increasing the total biomass of vegetative organs while reducing the accumulation of carbon reserves and hampering continuous fruiting.

5.2 Circadian variations of the grape transcriptome

Transcriptomic studies are difficult to run with macrovines grown outdoor because of the seasonality of fruiting and the day-to-day environment fluctuations. Thus, while transcriptomics is a very common approach today to understand the genetic mechanisms regulating grape development, no work has attempted to describe the circadian evolution of the grape transcriptome. The results published by Rienth et al. [39] were the first for a perennial fleshy fruit that addressed this topic. For this experiment microvines were grown in climatic growth chambers [40] under controlled environments (30/20°C day/night temperature, photoperiod 14 h, VPD 1kPA) for 3 months to encompass a complete reproductive cycle from flowering to ripening. When most proximal grapes reached physiological maturity, berry samples from two green and two ripening developmental stages were collected at different periods of the photo and nyctiperiod, and a whole genome transcriptomic analysis was carried out by Nimblegen® Vitis 12x microarrays.

All genes modulated during the day also showed some variation of expression at night, with 1843 genes that are only regulated at night. The detection of this very large number of specifically regulated genes during the night emphasized the importance of the regulatory mechanisms associated with the nocturnal fruit development. The comparison of differentially modulated transcripts between day and night at different stages showed that circadian regulation was very specific to the stage of development with only nine commonly deregulated genes between day and night at all stages. With respect to activated or deactivated functional categories, genes related to photosynthesis appear strongly repressed at night, in particular in young green berry, and several functional categories related to secondary metabolism (phenylalanine) and abiotic stress have shown strong overexpression at night at all developmental stages.

5.3 Effect of temperature on grape development

Until recently, the studies on the effect of temperature on grape development have only been performed using non-dwarf varieties, with the experimental limits associated with this model. Rienth et al. [41, 42] were the first to perform temperature experiments using microvines grown under tightly controlled environmental factors (photoperiod, light intensity, temperature, VPD, water, and mineral supply). This study was carried out with the ML1 microvine applying temperature gradients ranging from 12 to 35°C during 2 h to 4 weeks.

A first series of experiments focused on short-term stress effects (2 h, 35°C) of microvine fruits at different stages between green growth and ripening sampled during day and night. Nimblegen® Vitis 12x microarray assays revealed that a large number of genes (5653) respond to the increase in temperature, at all stages of development (**Figure 8**). Temperature effect was time and mainly development stage specific, with berries close to *veraison* being the most reactive to temperature elevation, especially for some categories such as anthocyanin synthesis which was specifically heat repressed at this stage. Furthermore, various genes of secondary metabolism (phenylalanine, anthocyanins) are repressed at the *veraison*, by high temperature with a larger number of genes regulated during the nocturnal phase.

Long-term thermal stresses (> 30 days) were also experimented using various temperature charts to several stages of grape development, taking into account

growth and cellular respiration rate, malic acid respiration, as a supplemental energy source in the fruit, is not compulsory. In cool climate, the allocation of carbon to the fruit can support glycolysis, malate synthesis, and sugar accumulation into the vacuole. Conversely, under hot climate, cytoplasmic sugars could be limiting when the cell starts to accumulate sugar in the vacuole at the onset of ripening. Thus, the malate would be drained from the vacuole to supply energy through respiration and/or through H^+ /sugar exchange at the tonoplast.

5.4 Identification of QTLs of development stable under fluctuating environments

Air temperature elevation combined with the shift of all phenological stages to warmer period is causing critical changes on vine yield and grape composition. Plant breeding has the potential to offer new cultivars with stable yield and quality under warmer conditions, but this requires the identification of stable genetic traits. The investigation about the stability of developmental QTLs with regard to abiotic factors is complicated with the non-dwarf varieties, because of its biological properties (long juvenile period, big size of the plants). Most of previous studies were carried out outdoors, in uncontrolled environmental conditions and with a relatively low experimental flow.

Houel et al. [25] reported the first experiment performed with microvines, to identify QTLs of vegetative and reproductive development, testing their stability under fluctuating environments. A F1 mapping population consisting of 129 microvines derived from the PV00C001V0008 x ugni blanc fleshless berry mutant was genotyped using an Illumina[®] 18 K SNPs chip (single-nucleotide polymorphism). Forty-three vegetative and reproductive traits were phenotyped over four vegetative cycles in the field, and a subset of 22 characters were measured over two climatic chamber culture cycles under two contrasting temperature regimes. Ten stable QTLs were identified for the development and composition of the berry and the leaf area on the parental genetic maps. A new major QTL accounting for up to 44% of variance of the berry weight was identified on the chromosome 7 in the ugni blanc parent. This QTL co-locates with QTLs of number of seeds per berry (accounting for up to 76% of the total variance), QTLs of fruit acidity before maturation (up to 35% of explained variance), and yield components such as the number of clusters and berries per cluster (up to 25% explained variance). In addition, a minor leaf surface QTL was found on the chromosome 4 in the same parent. This study which combined the use of microvine population to boost and facilitate the phenotyping with high-throughput genotyping technologies was innovative in grapevine genetics and also for perennial fruit crops. It allowed the identification of 10 stable QTLs, including the first QTLs of *V. vinifera* berry acidity detected in an intraspecific cross.

This progeny was also included in a study addressing the diversity for fruit volume, main sugar, and organic acid amounts in *V. vinifera* [43]. A panel of 33 genotypes, including 12 grapevine varieties and 21 microvine offspring, were characterized. Fruit phenotyping focused on two critical stages of fruit development: the end of green growth phase when organic acidity reaches a maximum and the physiological ripe stage when sugar unloading and water uptake stop. To determine the date of sampling for each critical stage, fruit texture and growth were carefully monitored. Analyses at both stages revealed large phenotypic variation for malic and tartaric acids as well as for sugars and berry size. At ripe stage, fruit fresh weight ranged from 1.04 to 5.25 g and sugar concentration from 751 to 1353 mmol.L⁻¹. The content in organic acids varied both in quantity (from 80 to 361 meq.L⁻¹) and in composition, with malic to tartaric acid ratio ranging from 0.13 to 3.62. At the inter-genotypic level, data showed no link between berry growth

and osmoticum accumulation per fruit unit, suggesting that berry water uptake is not only dependent on fruit osmotic potential. The report showed that diversity for berry size, sugar accumulation, and malic to tartaric acid ratio could be exploited through crossbreeding.

These studies which (i) identified genotypes with contrasted fruit composition for compounds controlled by environmental factors and (ii) mapped QTLs of development, including for berry composition, provide interesting prospects to mitigate some adverse effects of climate warming on viticulture.

5.5 Identification of the genetic traits of aromatic character of cabernet sauvignon

Methoxypyrazines are a family of volatile compounds found in many fruits and vegetables and especially in grapes, providing herbaceous flavors (green capsicum aroma) to the wines of some varieties such as Cabernet Sauvignon or sauvignon blanc. While several methoxypyrazine biosynthetic pathways have been proposed, none of the metabolic steps have been genetically confirmed. Dunlevy et al. [24] used a F2 population derived from a F1 microvine obtained by crossing the Cabernet Sauvignon and a picovine. The Cabernet Sauvignon variety is capable of producing the molecule 3-isobutyl-2-methoxypyrazine (IBMP), the major compound associated with capsicum flavors, while the microvine that derives from Pinot Meunier produces very little amount of this compound. In F1 offspring, all individuals produced IBMP, suggesting a homozygote dominant genotypic status for this trait in Cabernet Sauvignon. The phenotyping of the F2 individuals identified 43 lines able to accumulate IBMP, while 21 individuals lacked this compound confirming the dominant homozygous genotype for Cabernet Sauvignon and the homozygous recessive genotype for picovine progenitor.

After genotyping and phenotyping, the entire F2 progeny, a 2.3 Mb locus determining IBMP accumulation in grape berries, was found on chromosome n°3. Of the 261 genes identified in the corresponding QTL, two candidate methyltransferase genes have been identified, *VvOMT3* and *VvOMT4*. Screening a collection of 91 grapevine genotypes differentially accumulating IBMP into the grapes indicated *VvOMT3* as the most likely candidate to explain the genetic determinism of the green capsicum trait in grapevine fruits. Moreover, the data suggested that the low level of methoxypyrazines found in most cultivated grape varieties resulted from human selection for mutations in methyltransferase. The markers identifying this locus are valuable tools for the selection of grape varieties that are aromatically typified by IBMP and recalling Cabernet wines.

5.6 Effect of application of exogenous stimulants of fruit metabolism

The microvine plant model which displays unique reproductive organ behavior offers new experimental options for grapevine fruit physiological studies, not only because of the size of the plants which facilitate experimental handling in greenhouse or growth cabinet but also because it is possible to study several developmental stages at once. Taking advantage of the biological properties of the microvine, two studies were recently performed to study the impact of exogenous compound application to the ML1 microvine grapes on the aroma accumulation during ripening. The first study was about the impact of vine-shoot aqueous extracts, which have been proposed as bio-stimulants to be sprayed to the canopy to modify wine aromatic profile. Sanchez-Gomez et al. [44] experimented the effect of vine-shoot extract foliar application on the composition of the grapes at 21 stages of development. The application was carried out from BBCH53 (detached inflorescences) to BBCH85 (berry softening) to reveal stage-specific responses of the accumulation of

glycosylated aroma precursors at BBCH89 (ripe stage). Fifty grape sampling time points spreading to 86 days were established and normalized using the cumulative growing degree days parameter. The results confirmed that vine-shoot extract treatment had a positive impact on the accumulation of glycosylated compounds [45], especially aglycones such as alcohols, terpenes, and C13-norisoprenoids, with a higher impact when the treatment was applied at grape ripening stage.

The same approach was carried out to characterize the behavior of glycosylated aroma precursors in microvine fruits after foliar application of guaiacol. Previous outdoor experiments have showed that spraying guaiacol on vines could modify the contents of aroma compounds in grape and grape-derived wines. It was shown that such treatments could increase guaiacol glycoconjugates in leaves, shoots, and fruits of Monastrell variety, where there was a release of aglycone compounds during wine processing. However, the effect of such application and its timing on glycosylated aroma precursor pool remained unstudied. Sanchez-Gomez [46] studied the effect of guaiacol sprays when applied at several fruit developmental stages on glycosylated compound accumulation. The applications were carried out from phenological stage BBCH71 (fruit set) to BBCH85 (berry softening), to reveal stage-specific responses of the accumulation of glycosylated aroma precursors at BBCH89 (ripe stage). Data confirmed that guaiacol is an elicitor of the accumulation of glycosylated aromatic compounds in the microvine fruit, with a higher efficiency of application during ripening stages of the fruits. Geraniol, a terpene compound, exhibited the higher increase increment with up to 50-fold high concentration after guaiacol spraying than in the control.

6. Conclusions

The studies summarized here have shown that at a given phytomer level, the development of the vegetative and reproductive organs of the microvine exhibits comparable kinetics to those of non-dwarf vines grown outdoor. Given its original biological properties (small size, continuous fructification, possibility of inferring temporal observations from spatial data), this model can be used in fundamental studies on vine response to abiotic constraints or on fruit physiology under well-controlled environments. Thus, the microvine has already been used as a model in several scientific experiments on the effect of temperature on the vegetative and reproductive development, on changes in gene expression in grapes, and their plasticity under high temperature. This model has also shown its potential to accelerate conventional and reverse genetic approaches, including the identification of genetic determinants of developmental traits stable under fluctuating thermal conditions or major loci controlling the composition of the grapes. Studies are underway to use this model to study the impact of physical factors (drought, CO₂ concentration, temperature, etc.) on the development of the vine and the quality of the grapes but also to develop tools (markers of QTLs, pre-breeding lines pyramiding several agronomic traits of interest) for the selection of new varieties displaying original properties, i.e., traits of adaptation to climate changes.

Acknowledgements

These studies were supported by fundings from the following agencies or institutions: National Research Agency—Genopole (DURAVITIS project ANR-2010-GENM-004-01), Montpellier SupAgro, the departments EA (Environment-Agronomy) and BAP (Plant Biology and Improvement) of INRA, the Poupelain

Foundation, the European Eurasia 2 thesis mobility programs, EulaLink, and the Brazilian CNPq scientific cooperation program. Special thanks to Mark Thomas, Pat Corena, Don MacKenzy, and Ian Dry from CSIRO Agriculture (Adelaide) for mentoring and helping during some important steps of these experiments.

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