FREDERICO ALCÂNTARA NOVELLI DIAS

ROOTSTOCK PERFORMANCE AND BUD DEVELOPMENT EVALUATION TO OPTIMIZE *Vitis vinifera* PRODUCTION

LAVRAS - MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

Orientador

Dr. Murillo de Albuquerque Regina

Coorientador

Dr. Nilton Nagib Jorge Chalfun

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APROVADA em 12 de fevereiro de 2015.

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ABSTRACT

Wine grapes are constrained to a narrow climatic range and consequently are especially sensitive to climate change, with potential effects on yield, quality and profitability. Researches to increase knowledge about reproductive development and new agriculture practices, and opening of new wine making areas are important to overcome climates limitations. In Brazilian southeast, a new management approach called double pruning allows the change of wine grape harvest season from wet summer to dry winter. Thus, the present thesis had as issue evaluation of different rootstocks on grapevine Syrah performance and wine quality in Brazilian southeast; and the validation of a new grapevine model, called Microvine, to winter bud development studies. A competition of ten rootstocks under Syrah was developed during two seasons in south of *Minas Gerais* state, Brazil. Rupestris du Lot and IAC 766 induced the highest pruning weight, while R110 and 161-49C showed the lowest vegetative development. The average yield per plant of two seasons identified Rupestris du Lot, IAC 766, 1045P and Kober 5BB as the most productive rootstocks. The more vigorous rootstocks did not affect negatively grape quality of Syrah under double pruning management. Syrah' wine from productivity and vigorous rootstocks, 'IAC 766' and 'Rupestris du Lot', showed satisfactory wine phenolic composition and alcohol/acidity balance. The development of microvine winter bud was also evaluated along the proleptic axis based on microscopy and X-ray micro-tomography image methods. Microscopy accuracy was higher to assess phytomers and inflorescence primordia initiation within winter buds. Lignified buds, exhibited a maximum of 6 phytomers and 2 inflorescences primordia, inserted on the distal phytomers (4 to 6), similarly to grapevine. Primary bud length was highly correlated with the number of inflorescences primordia and phytomers. Therefore, further studies, in Brazilian southeast, involving only vigorous and productive rootstocks and the traditionally rootstocks used should be continued to described vine performance and wine quality on aged plants. The framework of phenotyping bud development set up in this work could identify critical points on plant development to increase vine production under double pruning management.

Keywords: Double pruning. Rootstock. Vine performance. Wine quality. Microvine. Winter bud.

RESUMO

Uvas destinadas à elaboração de vinhos finos têm sua produção restrita a algumas faixas climáticas, e consequentemente estão mais vulneráveis aos efeitos das mudanças climáticas, em relação à produtividade, qualidade e viabilidade. Pesquisas que possam aprofundar o conhecimento sobre o desenvolvimento reprodutivo das videiras e novos manejos agrícolas, e a abertura de novas áreas são estratégias importantes para a superação dos efeitos das mudanças climáticas. No sudeste brasileiro, um novo manejo chamado dupla poda permite a mudança da época da colheita do verão quente e úmido para o inverno seco. Deste modo, este trabalho teve como objetivo a avaliação do desenvolvimento da variedade 'Syrah' sobre diferentes porta-enxertos e o efeito destes sobre a qualidade do vinho produzido no sudeste brasileiro; e a validação de uma nova planta modelo, chamada Microvine, para estudos do desenvolvimento da gema latente. Uma competição de dez porta-enxertos para a variedade Syrah foi avaliada durante dois ciclos no sul de Minas Gerais, Brasil. 'Rupestris du Lot' e 'IAC 766' conferiram o maior peso de poda, enquanto 'R110' and '161-49' induziram o menor desenvolvimento vegetativo. As médias de produção por planta de dois anos foram superiores para os porta-enxertos 'Rupestris du Lot', 'IAC 766', '1045P' and 'Kober 5BB'. A qualidade da uva, sob manejo da dupla poda, não foi afetada pelos porta-enxertos mais vigorosos. Os vinhos produzidos a partir dos tratamentos mais vigorosos e produtivos apresentaram qualidade fenólica e balanço álcool/acidez satisfatórios. O desenvolvimento da gema latente da microvine foi avaliado ao longo do ramo principal pelos métodos da microscopia e da microtomografia. A microscopia permitiu maior precisão na avaliação de novos entrenós e primórdios de inflorescência formados nas gemas latentes. As gemas latentes já lignificadas, apresentaram no máximo 6 entrenós e 2 primórdios de inflorescência, localizados na parte distal da gema, assim como observado para as videiras normais. O comprimento da gema primaria demonstrou alta relação com o número de entrenós e primórdios de inflorescência. Portanto, novos estudos no sudeste brasileiro envolvendo estes porta-enxertos mais vigorosos e produtivos e as variedades normalmente utilizadas na região devem ser conduzidos para validar este comportamento apresentado em plantas com estágio mais avançado de desenvolvimento. O modelo de análise do desenvolvimento da gema latente descrito neste trabalho pode ser utilizado para identificar pontos críticos para o aumento da produtividade das videiras submetidas ao manejo da dupla poda.

Palavras-chave: Dupla poda. Porta-enxerto. Desenvolvimento da videira. Qualidade do vinho. Microvine. Gema latente.

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SUMMARY

1 INTRODUCTION

Grapevines are sensitive to climate conditions and management, especially for yield and quality variations (JONES et al., 2005). Therefore, viticulture has developed very specific and codified relation with geographical spaces and technologies. The last decade several studies were made to better understand vegetative and reproductive development responses under different climate conditions and genetic background underlying the interactions between plants and environment (SHULTZ; STOLL, 2010; HOLLAND; SMIT, 2010). These studies are important to clarify physiological adaptation mechanisms of existing grapevine varieties and rootstocks helping the exploration of new viticulture areas for wine making.

In Brazilian southeast, a new management, the double pruning, allows the change of wine grape harvest season from wet summer to dry winter.

This method has allowed a high quality and sanity of Syrah grapes in a tropical country for wine making (FAVERO et al., 2011). Such management has been used in *Minas Gerais* and *São Paulo* states, which brings new viticultural perspectives in Brazilian southeast region. However, agricultural practices of double pruning management, as choosing rootstock, should be more effective to increase grape yield and competitive of this new Brazilian viticulture area.

The rootstocks enable cultivars to grow in different environmental conditions and some cultivar characteristics are modified by interaction with rootstock (BRANAS, 1974; GALET, 1988; JACKSON, 2000). One of the problems to choose the right scion rootstock combination is the anticipation of how the scion and rootstock will interact. The climatic and soil conditions can modify the expression of rootstock and scion traits, therefore their interaction may vary from year to year and from location to location (ZULINI et al., 2002). Only few results on rootstock and scion interaction are available in the Brazilian

southeast for grape wine growing under double pruning management (DIAS et al., 2012; SOUZA et al., 2015). For this winter harvest management, rootstock should induce vine vigor adequate to two vegetative growth cycles and avoiding the lack of yield and grape quality.

The grapevine yield is most related with the number of bunches per plant than the cluster weight or number of berry per cluster (VASCONCELOS et al., 2009). It is directly related with potential fruitfulness of latent bud (winter bud), which is the formation of inflorescence primordia from lateral meristem (anlagen) during previous season of reproductive development, before dormancy stage. The inflorescence formation and differentiation within latent bud depends of environment parameters and agriculture practices as choosing scion rootstock (DUNN, 2005).

Several studies have been made about bud development on traditional viticulture areas (BUTTROSE, 1974; PETRIE; CLINGELEFFER, 2005; SÁNCHEZ; DOKOOZLIAN, 2005). However, these works were assessed on field conditions, mostly due to physiological traits of grapevine as a perennial plant. The research of genes and the characteristics involved yield components is limited, since on field tests, the interaction of many environmental variables complicates a clear insight about latent bud formation and differentiation at molecular level (CARMONA et al., 2008).

A natural mutant of grapevine from layer L1 of ‘Pinot Meunier’, called microvine, has been applied on studies to infer temporal development pattern of berries and leaves along the shoot under abiotic stress in fully controlled environment (LUCHAIRE et al., 2013; RIENTH et al., 2014b). This is possible due to the reduced size of these plants, shorter reproductive cycle and continuous flowering along main shoot (CHAIB et al., 2010). Therefore, this plant could contribute to the progress of researches about bud development under abiotic and biotic stress in growth chambers.

In order to optimize grapevine production this thesis proposed two studies: a competition of Syrah grapevine onto different rootstocks, under double pruning management, to assess vegetative and reproductive development, and also, must and wine quality; and the validation of a plant model to further bud latent reproductive development studies under controlled growth environment.

2 BIBLIOGRAPHIC REVIEW

2.1 A new grapevine management

In the traditional viticultural regions of Brazil, the vegetative and reproductive development occurs during warmest and wettest seasons of the year, spring and summer. Thus, the grapevine shoots have a vigorous vegetative growth, which competes for carbohydrates accumulation with the clusters, preventing complete fruit maturation, particularly the phenolic maturation. Moreover, the vineyard becomes more susceptible to botrytis and other fungal diseases. The result is a light body and astringent red wine without potential of aging (FAVERO et al., 2011; JACKSON; LOMBARD, 1993). This situation happened mostly due to climatic conditions and traditional vineyard management adopted, with only one winter pruning in July-August and harvest spread over January to March.

Introduction into new areas for high quality winemaking followed two principles: quality wines are made during dry and sunny days, mild temperatures and high thermal amplitude; and the minimum temperature for grapevine vegetative development is 10°C (CHAMPAGNOL, 1984; TODA, 1991). In the Brazilian southeast, especially in the south of *Minas Gerais*, the ecological conditions of warm temperate climate (Cwa), such as low rainfall and high thermal amplitude are common during autumn-winter season and it promote sugar accumulation in berries and synthesis of phenolic compounds (AMORIM; FAVERO; REGINA, 2005; FAVERO et al., 2011).

The change of harvest period from wet summer to dry winter has become possible by double pruning management. This management system consists of a first pruning in August to promote shoot formation (winter bud development) eliminating the clusters and a second pruning in January to carry out the production cycle harvesting in July-August (FAVERO et al., 2011).

Such management has been used also in other regions of *Minas Gerais* and *São Paulo* state (REGINA et al., 2011). The summer cycles of these regions are characterized by maximum and minimum temperatures around 30ºC and 19.6°C and average precipitation during maturation and harvest over 800mm, whereas, in winter cycles, the thermal regime is around 28.0°C and 11.3°C and precipitation less than 200mm for the last four months of the cycle (MOTA et al., 2010; REGINA et al., 2011; TONIETTO; VIANELLO; REGINA, 2006). In this context, the insertion of the Southeast region in the scenario of Brazilian fine wines is becoming possible, particularly in the South of *Minas Gerais* and *São Paulo* state.

The Syrah cultivar on a vertical shoot position (north-south oriented) and pruned with two spurs has shown better performance under double pruning management (AMORIM; FAVERO; REGINA, 2005; MOTA et al., 2010). At winter harvest, Syrah grapevines showed longer growing season, greater berry sugar concentration and phenolic compounds, less organic acids degradation and smaller size of berries when compared with summer cycle (FAVERO et al., 2008; MOTA et al., 2010). Syrah presented better vegetative and reproductive development when grafted onto 1103Paulsen, as compared to SO4 and R110 (DIAS et al., 2012). On the other hand, vigorous and productive rootstocks improved vine performance of *Cabernet sauvignon* under double pruning in South of *Minas Gerais* not compromising berry quality (SOUZA et al., 2015). Therefore, further researches of grapevines under double pruning, grafted on different rootstocks should be developed to improve production and competitiveness of this new Brazilian viticultural area.

2.2 Rootstock effects on vine performance

The viticulture world is based primarily on grafting, where the scion is a cultivar of *Vitis vinifera* and the rootstock is either a North American *Vitis* species or an interspecific *Vitis* hybrid (HIDALGO, 2002). Some of the most common are *Vitis rupestris*, *V. riparia*, *V. berlandieri*, *V. champinii*.

There are many advantages for using rootstocks in viticulture. These include protection from the effects of soil-borne pests such as phylloxera (*Daktulosphaira vitifoliae*) and nematodes, and adaptation to problems such as drought and salinity. The rootstocks' use can also influence vine performance. It can have important implications for canopy light interception, bunch exposure, fruit composition and wine sensory characteristics (CLINGELEFFER, 1996; COOMBE; DRY, 1992; RŰHL; WALKER, 1990). These effects are consequences of interactions between environmental factors and the physiology of scion and rootstock cultivars.

The rootstocks are responsible for direct and indirect effects on grapevine development. The difference on root system development among rootstock species may affect water relations and nutrients uptake, which will affect shoot growth and modify grapevine physiology (JACKSON; LOMBARD, 1993). Moreover, it may cause canopy environment modifications, which could affect berries maturation and consequently wine quality (NUZZO; MATHEWS, 2006).

Rootstocks affect indirectly photosynthesis and stomatal conductance of scion cultivars by differences on root system development. The correct graft combination could increase carboxylation efficiency of scion leaves, which may help to improve drought resistance, by raising water use efficiency (DURING, 1994). However, the effects of a rootstocks/scion combination vary from area to area. Koblet, Keller e Candolfi-Vasconcelos (1996) demonstrated that the highest photosynthetic rates was found on ‘Pinot Noir’ grafted onto ‘Kober’ and

the lowest on ‘SO4’ in unfertilized vines, whereas ‘SO4’ could give the highest photosynthetic rates in the fertilized vines.

A rootstock found to be beneficial for one cultivar may not be universally advantageous for others, as the interaction of stock and scion influences the vine performance more than the stock or scion alone (HARTMANN; KESTER; DAVIES, 1993*).* In Pacific Coast of United States, the ‘Dog Ridge’ has been used over several varieties to overcome drought and salinity limitations; however in tropical and subtropical regions this rootstock induced high vegetative growth in Thompson Seedless which reduced bud fruitfulness (SATISHA et al., 2010).

Some authors suggested that yields are negatively correlated to vine vigor (PAREJO et al., 1995; WOLF; POOL, 1988). ‘IAC 766’ and ‘Rupestris du Lot’ have been always described as vigorous rootstocks (ALVARENGA et al., 2002; CHRISTENSEN, 2003). However, in Brazilian Southeast region these rootstocks were also described as vigorous, but it illustrated the higher and average production for ‘*Cabernet Sauvignon*’ (SOUZA et al., 2015). Moreover, other authors demonstrated that ‘Rupestris du Lot’ induced the lowest vegetative development of ‘*Cabernet Sauvignon*’ in California (WILLIAMS; SMITH, 1991).

In the last 30 years, a significant attention has been given to the rootstocks' effects on the grape juice and wine quality (RÜHL; WALKER 1990). Usually, rootstocks affect these factors in an indirect manner, such as nutrient uptake, water status or vegetative growth. In warm irrigated Australian regions, the higher grape juice K content and high pH reduce wine color quality, longevity and sugar/acid balance. This problem was overcome by the rootstock use with lower K uptake (KODUR, 2011). The composition and sanity of cluster have been associated with vine vigor; scions grafted onto vigorous rootstocks demonstrated higher rot incidence on cluster and lower sugar accumulation (WHITING, 2002).

For a sustainable viticulture, it is important to know the interactions among rootstocks, different climate conditions and scion productivity (KELLER; KUMMER; VASCONCELOS, 2001). In the Brazilian southeast, the management double pruning is interested in rootstock varieties that could induce required vigor to overcome two double spur pruning maintaining sustainable production and high grape quality during autumn-winter time.

2.2.1 Rootstock varieties

The rootstocks can induce higher or lower vegetative vigor and yields depending on its origin species.

2.2.1.1 Rupestris du Lot (*Vitis rupestris*)

This rootstock is a vigorous and rustic plant with a long vegetative cycle, normally used for productive wine grapes and for early table grapes (GALLET, 1998). As it is a very vigorous rootstock, it promotes rapid fruit development and early plant exhaustion. This variety shows a good tolerance to phylloxera, but little resistance to nematodes.

It shows a good resistance to active limestone (up to 14%) and little adaptability to saline soils. This rootstock demonstrates a medium to high resistance to dry and poor soils, owing to its tap root system, deep and with a small angle geotropic, 20° (HIDALGO, 2002). However, it is sensitive to wet soils (GALLET, 1998). Moreover, in fertile soils this rootstock promotes flowers abortion due to vegetative and reproductive unbalanced development (LARREA, 1978). It is a vegetative material easily propagated by bench grafting.

2.2.1.2 IAC 766 ((*Vitis riparia* x *Vitis cordifolia*) x *Vitis caribea*)

It is a complex hybrid obtained by 'Agronomic Institute of Campinas', Brazil. This rootstock is normally used in North of *Paraná* state and in *São Paulo* state for table grapes, mainly 'Italia' and 'Niagara Rosada', where it induces medium to high vine production (CAMARGO, 1994; TECCHIO et al., 2014). It is a good combination for vigorous seedless varieties on *São Francisco* valley (CAMARGO, 1994). However, in this latter region, IAC 766 is described nematode susceptible (*Meloidogyne incognita*) (SOMAVILLA; GOMES; QUECINI, 2012).

This rootstock is vigorous and well adapted to deep soils. It shows a deep root system, which promotes a good drought tolerance (AGUIAR et al., 2006). It is an easily propagated vegetative material (CAMARGO, 1994).

2.2.1.3 101-14 Millardet et De Grasset (*Vitis riparia* x *Vitis rupestris*)

This rootstock is indicated for wine quality production, as it induces low vigor on scion and good grape maturation, with medium production. The '101-14' has a superficial semi-taproot system producing many thin roots, as *Vitis riparia* (HIDALGO, 2002). Therefore, this rootstock is more sensitive to drought, but it shows good development on wet soils, and it also develops well on clay soils with active limestone up to 9%.

The rootstock '101-14' is tolerant to *Meloidogyne incognita* but less tolerant to *M. arenaria*.

It still shows a high resistance to phylloxera and low resistance to *Fusarium*. It is a vegetative material easily propagated with its good grafting compatibility (GALET, 1998).

2.2.1.4 Teleki Selection Oppenheim n°. 4 (SO4) (*Vitis berlandieri* x *Vitis riparia*)

SO4 induces high vigor and vine production, but with medium quality. It induces late maturation of wine grapes, which is unfavorable to rainy seasons because it allows high acidity. This rootstock has a semi-taproot system producing many roots on angle geotropic around 60°, therefore it shows a low resistance to drought (HIDALGO, 2002). However, it demonstrates good tolerance to wet soils, and active limestone up to 17% (HIDALGO, 2002).

SO4 has medium resistance to phylloxera and high resistance to *Xiphinema* and *Meloidogyne*, but it is highly sensitive to Fusarium (HIDALGO, 2002). It shows medium potential to bench grafting and compatibility with scions.

2.2.1.5 Teleki 5BB Selection Kober (*Vitis berlandieri* x *Vitis riparia*)

This rootstock stands out for its resistance to active lime (up to 20%) and vigor. This rootstock also induces high productivity and medium quality (GALET, 1998). In fertile soils, this rootstock does not allow complete maturation of grapes, with low sugar accumulation and polyphenols. Its root system is semi-tap producing many roots on angle geotropic around 60°, therefore this rootstock shows a low resistance to drought. It is a rootstock resistant to soils with high moisture (HIDALGO, 2002).

Kober has medium resistance to phylloxera and high resistance to *Xiphinema* and *Meloidogyne*, but it is highly sensitive to Fusarium (HIDALGO, 2002). It shows medium potential to bench grafting and compatibility with scions.

2.2.1.6 161-49 Couderc (*Vitis Riparia x Vitis berlandieri*)

This crossing has *Vitis riparia* as mother plant. This rootstock induces good production and grape quality (GALET, 1998). As a *Vitis riparia*, it has a superficial

root system. This rootstock should be planted in fertile soils and well drained. It is very sensible to compact and humid soils (HIDALGO, 2002). It has a medium resistance to phylloxera, but it is susceptible to nematodes (GALET, 1998).

In the last 10 years a severe and unexplained decline was reported on plots established on '161-49' in France, Germany and Italy. A significant reduction in vigor after 3 or 4 years of planting accompanied by a decrease in production was observed for several scions grafted onto this rootstock (TORREGROSA et al., 2011).

2.2.1.7 1103 Paulsen (*Vitis berlandieri* x *Vitis rupestris*)

It has an early development, well suited to soils with high clay content and a good development in compacted soils. The most recommended rootstock to *Rio Grande do Sul* and *Santa Catarina* Brazilian states. It has a good compatibility with *Vitis vinifera* in soils with medium fertility, but with American varieties and hybrids it should be planted in high fertility soils (GIOVANNINI, 2008). This rootstock induces medium to high vegetative growth and productivity, and medium quality production.

This rootstock shows a high resistance to drought and limestone (up to 17%). It has a taproot system with thick roots (HIDALGO, 2002). It has a medium resistance to phylloxera and Fusarium, but it is resistant to nematodes (GALET, 1998).

2.2.1.8 1045 Paulsen (*Vitis berlandieri* x *Vitis rupestris*)

This rootstock induces medium to high vegetative growth and productivity, and medium quality production, as 1103 Paulsen. This rootstock also has the same traits of 1103 Paulsen root system, but medium resistant to dry and compact soils, and high resistance to active limestone at 14% (HIDALGO, 2002).

2.2.1.9 110 Richter (*Vitis berlandieri* x *Vitis rupestris*)

This is a vigorous rootstock, drought resistant and adaptable to compact soils (HIDALGO, 2002). As 1103 Paulsen, 110 Richter show a high resistance to limestone (up to 17%) (HIDALGO, 2002). In fertile soils, this rootstock delays cluster maturation due to high vegetative growth. It is a susceptible rootstock to Fusarium and nematodes. Moreover, this rootstock is very sensible to wet soils. Normally, it is used for table grapes and common wines in Brazil (NOGUEIRA, 1984).

This rootstock demonstrates some problems with rooting on bench grafting, and the grafts have a slow development in the early years (GALET, 1998).

2.2.1.10 99 Richter (*Vitis berlandieri* x *Vitis rupestris*)

The 99 Richter is a vigorous rootstock, but less vigorous and more sensible to dry soils when compared to 110 Richter (GALET, 1998). This is a rootstock sensible to saline soils. It has been applied to common wines in medium fertile soils in South of Brazil (GIOVANNINI, 2008). This rootstock also demonstrates some problems with rooting on bench grafting, as 110 Richter, and the grafts has a slow development in the early years (GALET, 1998).

2.3 Grapevine production

The reproductive development of grapevine is spread over two seasons and it is very influenced by environment conditions and viticultural practices. The clusters that make up the current season crop begin its formation on the preceding growing season within the winter buds. Therefore, the maximum number of clusters per vine (potential yield) is determined during the previous year.

The winter bud, formed on the previous season, is a compound bud, which developed on each node of grapevine shoot at the axil of lateral shoot

bract. Overall, inflorescence development within grapevine winter bud involves formation and differentiation of anlagen to form inflorescence primordia during first season and flowers organs differentiation after dormancy period during bud burst. This compound bud contains a primary bud (N+2), the primordial shoot apical meristem, which may have two axils buds on its bracts (secondary buds, N+3) (MAY, 2000). Normally, only primary bud forms nodes (phytomers) with leaf primordia and lateral meristem.

After a short vegetative development, i.e. three to four leaf primordium formation, the first anlage appears in the opposite position on shoot apical meristem. The bud apical meristem of *Vitis vinifera* keeps this vegetative development until dormancy following a three-node modular construction, i.e. a series of two consecutive phytomers containing opposed leaf and anlagen (P1 and P2) alternating with one phytomer bearing a solitary leaf primordium (P0) (CARBONNEAU, 1976). Thus, depending of grapevine cultivar and position on grapevine shoot the winter bud may reach 6 to 12 phytomers with 1 to 3 inflorescence primordia (VASCONCELOS et al., 2009).

The first crucial part of reproductive development happens on this first season (winter bud development), when the anlagen continue its development by repeated branching until dormancy. When the first two unequal parts are formed, the larger inner arm and the smaller outer arm, anlagen can develop into cluster or tendril pathway. Since, it is known that tendril and inflorescence are the same ontogeny origin.

The inflorescence or tendril formation depends of environment conditions (temperature and light), hormones balance (gibberellins and cytokinin) and cultural practices. Nutrition and vine water status are the two main cultural factors that influence the differentiation of cluster primordia (MORRISON, 1991). On the other hand, the rootstocks are main responsible for nutrient uptake and water relations of vine, since it replaces all root system of

scion varieties. Therefore, vine physiology is affected by rootstock traits, and consequently vine reproductive development.

2.3.1 Yield variation

The yield variation in grapevine is mostly related with number of clusters per plant, which explains more than 70%, while such variation is less sensitive to number of berries per cluster (~30%) and berry size (~10%) (VASCONCELOS et al., 2009). This variability depends on a range of factors, as climate conditions and agriculture practices, which mainly affects inflorescence primordia formation on first season, but also further stages of bud development after dormancy (CHLOUPEK; HRSTKOVA; SCHWEIGERT, 2004; JONES; LEE; WILSON, 2013; VASCONCELOS et al., 2009).

Temperature affects yield by altering the numbers of clusters per shoot and number of flowers per clusters. The differences of temperature among seasons during anlagen induction, anlagen differentiation (branching) and bud burst have been related with yield variations (DUNN; MARTIN, 2000; VASCONCELOS et al., 2009). The number of flowers per cluster is highly related to the number of primary branches formed per inflorescence, and the process of branching is directly dependent of thermal regime (WATT et al., 2008). Furthermore, the thermal regime before anlagen formation is described as an important factor on maximum number of inflorescence primordia formation (BUTTROSE, 1970, 1974; SRINIVASAN; MULLINS, 1981).

At first season, temperatures around 20°C was taken as optimum to anlagen initiation, although there are some differences between varieties from different origins (DUNN; MARTIN 2000; PETRIE; CLINGELEFFER, 2005). ‘Petit Manseng’ expresses the maximum number of inflorescence primordia when temperatures reach 24°C (DURQUETY; NAUDE; BLANCHARD, 1982), while Muscat of Alexandria requires a temperature around 25 to 28°C to

demonstrate the same behavior (BUTTROSE, 1970). On the other hand, temperatures around 12°C after dormancy produce higher number of flower per inflorescence but lower number of inflorescence per shoot when compared with temperatures around 25-28°C for ‘Merlot’, ‘Cabernet Sauvignon’, ‘Alicante Grenache’ and ‘Cardinal’ (EZZILI, 1993; POUGET, 1981).

In Australia, yield variations among seasons have been related to primary bud necrosis (PBN) (COLLINS et al., 2006). This physiological disorder hinders the primary bud development on the first season, then the secondary bud occurs to compensate. However, these secondary buds are less fruitful and smaller than primary buds. It causes a strong reduction on vine production due to decrease on clusters number per node and cluster weight (DRY, 2000). Some vineyard managements have been implemented to reduce PBN incidence, as choice of scion cultivars and rootstocks combinations (COX et al., 2012; DRY; COOMBE, 1994; DRY et al., 2003). This management aims to reduce high water deficit, canopy shading and excessive shoot growth, ensure better vine balance (vegetative and development competition) and increase the potential for bud fruitfulness.

The interactions among scion and rootstock genotypes, environment, and management practices allow this variability of vine production, which compromises wine industry sustainability. It causes a range of cluster architectures and asynchronous development of individual flowers within a bunch, individual clusters within a shoot, within a vine, and within a vineyard block, which reflect on fruit quality pattern and consequently on wine style.

2.4 A new plant model for bud latent studies

Evaluation of vegetative and reproductive development responses to interaction between climatic factors and agriculture practices requires long-term experiments, due to an extended reproductive development over two consecutive

years, succeeding an initial juvenile phase (CARMONA et al., 2008). Moreover, as a perennial crop, grapevine shows some limitation for studies under controlled environment due to its large size. In field conditions several climatic factors may vary, independently or not, interacting with crop management, and resulting in high season to season reproductive development variability (SOAR; COLLINS; SADRAS, 2009). In order to overcome these limitations several experiments under contrast vintage are made or simplified models have been proposed as fruiting cuttings (GENY; OLLAT; SOYER, 1998; SADRAS et al., 2012). Even so, the lack of plant response to individual climatic factors and huge differences between regular vine and the proposed model still limit these alternatives.

A natural mutant of grapevine was proposed to overcome these limitations in reproductive development studies, called Microvine (CHAIB et al., 2010). Microvine was first described as suitable for rapid forward and reverse genetic studies in small controlled environments (CHAIB et al., 2010), and recently appeared as a relevant material to study berry molecular responses to climate change (RIENTH et al., 2014a, 2014b).

The discovery of a L1 Pinot Meunier mutant plant (Microvine, ML1) has been described by Boss and Thomas (2002). The apical meristem of the grapevine shoot is organized into two distinct layers designated L1 (outermost) and L2 (THOMPSON; OLMO, 1963). Plants regenerated from L1 cell layers of ‘Pinot Menieur’ by passage through somatic embryogenesis, demonstrated semi-dwarf stature, a rapid cycling (no juvenile phase) and a continuous flowering along the axes (BOSS; THOMAS, 2002).

These plants have an altered gibberellins (GA) response, owing to a single mutated DNA base in the grapevine GA insensitive gene (VvGAI1). The natural mutant Vvgai1 allele confers important features for a plant model, small space requirements, short generation time, prolific flowering phenotypes, a semi-dominant

behavior manner, small diploid genome and it could be crossed to produce progeny carrying the Vvgai1 allele (BOSS; THOMAS, 2002; CHAIB et al., 2010).

This innovative material shares with grapevine key vegetative and reproductive developmental characteristics. Microvine opened new fields in grapevine studies, short term experiment can be designed under fully controlled environments in order to quantify the impact of abiotic and biotic stress on a variety of traits, specially yield and berry quality simultaneously (LUCHAIRE et al., 2013; RIENTH et al., 2014a, 2014b).

3 GENERAL CONCLUSIONS

The development of a new wine region needs research, investments, partnerships and clear objectives of wine production. The Research Team of EPAMIG 'NUTEV-*Núcleo Tecnológico Uva e Vinho'* has developed several works about wine production on Southeast Brazilian since 2001. These works range wine grapes performance at field level, vinification methods, wine quality, potential of aging in bottle, and others traits of wine production chain. Many public-private partnerships have been developed since 2001, this is very important to establish a way forward in the studies of this Research Team. The grower's difficulty and limitations are the questions of new hypothesis that should be tested.

Nowadays, this new region is able to produce high quality red wines and sparkling wines, from 800m to 1200m altitude. However to be competitive on wine market not only quality is necessary, but also a sustainable production, i.e. yield production and costs balanced. The main objective of this present work was generate knowledge about reproductive grapevine process and test new management system able to improve vine production keeping quality and reducing costs. Vigorous rootstocks induced high production maintaining the quality standards of grape wines, for vines until five years old. Thus, further studies, in the same location, involving only these vigorous and productive rootstocks and the traditionally rootstocks used in the region should be continued to described vine performance and influence on wine quality on aged plants.

The management double pruning changes harvest time and consequently the time of winter bud development, from 9 - 12 months on traditional management to 6 months. Therefore, effects on potential fruitfulness must exist. The knowledge about bud development and furthers studies about temperature and light effect on latent buds will contribute to new managements that could

improve bud fruitfulness, as leaves thinning or different timing and intensity of winter and summer pruning. Moreover, a single summer pruning to obtain autumn-winter harvest could be a good option to reduce costs of double pruning management, however no production is detected after application of single summer pruning. The framework of phenotyping bud development set up in this work could identify the critical points of this alternative management, and explain the phenomena of no production.

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CHAPTER 1: Rootstock effect on vine performance and wine quality of 'Syrah' under double pruning management

(Version submitted to Vitis-Journal of Grapevine Research)

Rootstock effect on vine performance and wine quality of 'Syrah' under double pruning management

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Summary

The studies about rootstock induction on scion vigor and production are incomplete for grapevine management under double pruning in Brazilian Southeast. Although the rootstock is defined as high, moderate or low vigor inductor, these mechanisms can vary for this new viticulture management. The present study evaluated the vegetative and reproductive development and wine quality of ‘Syrah’ grafted onto ten rootstocks and conducted during the autumn-winter in Brazilian southeast. Rupestris du Lot and IAC 766 induced the highest pruning weight, while R110 and 161-49C showed the lowest vegetative development. The average production of two seasons identified Rupestris du Lot, IAC 766, 1045P and Kober 5BB as the most productive rootstocks. The grape quality was mostly affected by plant development status of each season. The vigorous rootstocks did not affect negatively grape quality. ‘Syrah’ wine from productivity and vigorous rootstocks, ‘IAC 766’ and ‘Rupestris du Lot’, showed satisfactory wine phenolic composition and alcohol/acidity balance.

Key-words: Double pruning, Yield, Temperate warm climate, Rootstock, Berry quality.

Introduction

The original purpose of using rootstocks was the resistance to the american species and interspecifc hybrids of *Phylloxera vitifoliae.* However, nowadays, the grafting technique has been used also to control vegetative growth, yield, fruit composition, wine quality and to increase tolerance under environmental stress (Jackson and Lombard, 1993). The rootstock replaces all root system of the scion, which changes the water and mineral absorption affecting the physiological process and consequently vine vigor and yield equilibrium (Nuzzo and Mathews, 2006; Soar et al., 2006; Souza et al., 2015).

Although the rootstock is defined as high, moderate or low vigor inductor, these mechanisms can vary from different scions and climate conditions (Southey, 1992). The interaction of rootstock and scion influences the vine performance more than rootstock and scion alone (Hartmann et al., 1997). ‘Chardonnay’ vines showed different photosynthesis rate, yield, stomatal conductance and chlorophyll content when grafted onto ‘SO4’ and ‘1103 Paulsen’ rootstocks. On the other hand, the ‘Pinot Noir’ grafted onto the same rootstocks showed similar rates of assimilation, but when grafted onto ‘Kober 5BB’ the leaf area, stomatal conductance and transpiration presented a significant increase (Bica et al., 2000). The knowledge about rootstock-scion interaction is an important factor for the development of a new viticultural area. It provides the basis for selecting a range of grafting combination more adequate

for a particular environmental condition and vineyard management.

The Brazilian southeast has become a potential region for fine winemaking as a result of studies developed by public and private partnerships, coordinated by Núcleo Tecnológico EPAMIG Uva e Vinho (Carbonneau, 2010; Regina et al., 2011). The change of grapevine cycle timing through double pruning management (first pruning in August and second one in January) has opened up a new possibility of viticultural activity in this region (Favero et al., 2011). The ecological conditions (low rainfall and high thermal amplitude) of autumn-winter season are favorable to sugar accumulation and synthesis of phenolic compounds in berries as shown by several authors (Mota et al., 2010; Regina et al., 2011). Although, this new grapevine management appears very promising, other cultural practices, such as grafting combinations, need to be evaluated.

There are only two studies about rootstock recommendation for double pruning management. The first study with Syrah showed that 1103 Paulsen induced a better vegetative and reproductive balance as compared to SO4 and 110 Richter (Dias et al., 2012). However, with Cabernet Sauvignon a second study showed that the best performance was obtained with Kober 5BB, 1045P, SO4 and IAC 766 (Souza et al., 2015). Hence, the proposal of this study was to evaluate the effects of 10 rootstocks on vegetative vigor, yield, grape and wine composition of ‘Syrah’ managed under double pruning management in the south of Minas Gerais state, Brazil.

Material and Methods

Plant material and experimental design:

The experimental area was located on commercial vineyard in Andradas (22º04'S 46º34'W, altitude of 920m), Minas Gerais, Brazil, and was carried out during 2011 and 2012 seasons. The soil is Loamy acrisol, and the climate Cwa i.e. warm temperate according to Köppen classification (Tonietto et al., 2006). During the winter season (June to August), at least one month shows an average of precipitation lower than 60mm. The maximum and minimum annual average temperatures are 26.4°C and 12.1°C respectively (Tonietto et al., 2006).

The experimental area was installed in 2008 using *Vitis vinifera* L. variety Syrah, clone 174 ENTAV-INRA, grafted onto 10 rootstocks commonly used in tropical and subtropical climates: 1103 Paulsen (1103P), 1045 Paulsen (1045P), 99 Richter (R99), 110 Richter (R110) (*Vitis berlandieri* x *Vitis rupestris*); 101-14 Millardet et de Grasset (101-14MGT) (*Vitis riparia* x *Vitis rupestris*); SO4, Kober 5BB (Kober) (*Vitis berlandieri x Vitis riparia*); 161-49 Couderc (161-49C) (*Vitis riparia x Vitis berlandieri*); Rupestris du Lot (Rup) (*Vitis rupestris*); IAC 766 ((*Vitis riparia* x *Vitis cordifolia)* x *Vitis caribea*).

The double pruning management was applied as follows: the first pruning was done in August (vegetative cycle) for latent bud formation and the second pruning was done in January (reproductive cycle) for grape production

(Favero et al., 2011). During the vegetative cycle all clusters were removed at green pea stage of berries. Plants were trained in vertical shoot position with bilateral cordons and pruned in two-node spurs, for both pruning. Vines and rows were spaced 1.40m and 2.70m, respectively, totalizing 2,645 plants per hectare. The vineyard was not irrigated. The experimental design was completely randomized with three replicates for each rootstock, represented by five plants per plot, totalizing an experimental area of 150 plants.

Agronomical and physicochemical analyses:

Dry weight of pruned shoot and leaf area were evaluated as a measure of vine vigor. The pruned shoots were collected from each plant and dried in forced air oven at 60°C until constant dry weight was reached. This parameter was measured annually at winter season after grape harvest. Leaf area was estimated according to Regina et al. (2000) using one plant per plot at the beginning of *veraison,* after shoot trimming.

Chlorophyll concentration in leaves was assessed during flowering stage. Leaf disc was collected for each rootstock replicate and the chlorophyll was extracted with acetone solution (80%), the concentration was measured by spectrophotometry following standard method (Arnon, 1949).

Three Syrah vines on each plot were hand-harvested on July 20th 2011 and July 13th 2012. The average number of berries per bunch and the average

bunch weight were assessed from 30 bunches randomly collected from each treatment replicate. Berries weight were evaluated by 100 random berries collected for each replicate, and then analyzed for pH, titratable acidity (TA) and total soluble solids (TSS). Average vine production was estimated multiplying the number of bunches per plant by average bunch weight of plot. Yield was obtained multiplying the average vine production by the number of plants per hectare. The leaf area/fruit weight ratio (expressed as $m^2.kg^{-1}$) and the vine production/pruning-weight ratio (expressed as $kg.kg^{-1}$) were calculated to evaluate the balance between vegetative and reproductive development on autumn-winter cycle.

The phenolic quality of berries was also analyzed at harvest. Three randomized samples of 100 berries were collected for each treatment. A sample of 150 mg of skin was crushed on liquid nitrogen and homogenized in Ultra Turrax disperser T 18 basic (IKA, Wilmington, NC, USA) with extracting acidified methanol solution (1% HCl). The concentration of anthocyanins in berry skin was determined by the pH differential method (Giusti and Wrolstad, 2000). Total phenolics were determined by Folin‐Ciocalteau method based on standard gallic acid curve (Amerine and Ough, 1980).

Winemaking and Physicochemical quality of wine:

In 2012 season, harvested grapes were delivered at winery and stored at 4°C for 24 hours. For each treatment 10 kg of grape clusters were destemmed and crushed and the must with average total soluble solids of 20.7 °Brix, pH 3.56, titratable acidity of 6.98 g L^{-1} and density of 1.0932 for all treatments was placed in 13.25 L Pirex® glass carboy. The musts were inoculated with rehydrated wine yeast AWRI 1503 (*Saccharomyces cerevisiae x S. kudriavzevii,* Maurivim, Queensland, Australia) and added with 80 mg SO_2 kg^{-1}.

Wine density was determined daily during alcoholic fermentation at 21°C. When the density reached 990 mg L^{-1}, wines were transferred to 13.25 L Pirex® glass carboys for malolatic fermentation which was carried out at 21°C, without inoculation, until malic acid was not detected by paper chromatography method (Amerine and Ough, 1980). The wines were racked to remove lees, treated with potassium metabisulfite (35 mg SO_2 L^{-1}) and were kept at -3°C for 15 days to allow tartaric stabilization. The wines were allowed to age in 375 mL glass bottles at 15°C in a dark cell for 16 months. 'Kober' treatment was lost because its glass carboy was accidentally broken.

Physicochemical analyses consisted of alcohol, total acidity (g L^{-1} tartaric acid), volatile acidity (g L^{-1} acetic acid), pH, and ashes alkalinity (meq L^{-1}) (Brasil, 1986). Color intensity, color hue, polymerized pigments and total poliphenol indices (IPT 280nm) were evaluated by spectrophotometry (Curvelo-

Garcia, 1988; Ribéreau-Gayon et al., 2006). Total flavanoid content was evaluated by Bate-Smith reaction (Blouin, 1992). Anthocyanins and phenolics were measured by the pH differential method and Folin‑Ciocalteau method, respectively (Giusti and Wrolstad, 2000; Amerine and Ough, 1980).

Statistical analysis were performed using the SISVAR statistical package version 4.6 (Ferreira, 2008). Data from field evaluation and physicochemical composition of grape and wine were submitted to ANOVA. For field evaluation and physicochemical composition of grape a factorial analysis was applied for rootstocks and seasons. The mean values were compared using a Scott Knott test, at 5% probability. For wine data, the principal component analysis (PCA) was also carried out by R Program in order to ascertain trends or group formations of wine samples from different rootstocks (R Core Team, 2013).

Results

Vegetative and reproductive growth:

The vegetative vigor evaluated by leaf area and pruning weight of ‘Syrah’ was affected by rootstock and plant age in each season (Table 1). All treatments showed the lowest leaf area in 2011, except for ‘R110’, ‘161-49’, and ‘SO4’ which did not show increase on mean values between seasons. Moreover,

these latter rootstocks induced the lowest leaf area in 2012 growing season, when the plants reached four years old. The rootstocks ‘161-49’, ‘R110’ and ‘R99’ demonstrated the lowest values of pruning weight for 2011. The highest pruning weight was shown for ‘Syrah’ grafted onto ‘IAC 766’ and ‘Rup’, for both seasons. All treatments showed higher weight in 2012 season.

The mean values of chlorophyll concentration per m^2 of leaf were higher in 2012 for most rootstock combinations, as observed for mean values of leaf area and pruning weight (Table 1). In 2011, ‘Rup’ induced higher values of chlorophyll concentration in Syrah leaves. In 2012, ‘IAC 766’ induced high chlorophyll concentration as well as less vigorous treatments such as ‘161-49’, ‘101-14’ and ‘1045P’.

The effects of rootstock and plant age on yield and its components are illustrated in Table 2. In general, the most vigorous rootstocks also induced the highest production and yield parameters whereas the less vigorous treatments induced the lowest values. Globally, ‘IAC 766’, ‘Rup’ and ‘Kober’ showed the highest vine production, yield, cluster number per vine and berry number per cluster, whereas ‘161-49’ showed the lowest values for these parameters. The grapevines grafted onto ‘IAC 766’, ‘Kober’, ‘Rup’ and ‘1045 P’ showed the heaviest clusters in both years, however only ‘IAC 766’, ‘Rup’ and ‘Kober’ reached yield around 10 $Mg.hectare^{-1}$ for four years-old plants. In 2012 all

treatments showed the highest values of yield and production parameters, as described for vigor traits.

Pruning weight was correlated positively with vine production, indicating that high production was induced by the largest vines. There was an increase on vine production until mean values of pruning weighed about 200 g plant^{-1}. After this limit, a tendency of reducing was detected suggesting that 'Syrah' grapevine under double pruning management demonstrated better vigor-production balance onto 'Kober' when compared to vigorous rootstocks 'IAC 766' and 'Rup' (Figure 1).

The leaf area:fruit weight ratio was also affected by rootstock (Figure 2). The rootstock '161-49' induced the highest ratio for both years, whereas 'Kober' presented the lowest values. In 2012, 'IAC 766', '1045 P', 'SO4' and 'Rup' expressed the lowest values as well as 'Kober', and the others rootstocks induced intermediate ratio values.

Grape composition:

Rootstock had weak influence on total soluble solids and titratable acidity concentration in must as compared crop load of each year (Table 3). However, 'R 110' and '161-49', less vigorous rootstocks, induced a decrease on titratable acidity in 2011. 'Kober' distinguished itself by the lowest must pH.

Rootstocks did not show an expressive contribution on anthocyanin and total phenolic accumulation in berries skins for each year. However, between seasons, a significant decrease in total phenolic content in berry skins of ‘R 110’, ‘R 99’ and ‘161-49’ was detected (Table 4).

Wine composition:

The characterization of ‘Syrah’ wines from different rootstocks after 16 months of aging in bottles was based on multivariate statistical analysis. Principal component analysis (PCA) was applied in order to ascertain trends or group formations of wine samples according to physicochemical properties as total titratable acidity, volatile acidity, pH, ashes alkalinity and alcoholic strength (Figure 3) and phenolic composition and color traits (Figure 4).

Physicochemical properties: The Comp1 explained 40.5% and Comp2 26% of variation among ‘Syrah’ wines for evaluated parameters (Figure 3). Wines from ‘Syrah’ grapevine grafted onto ‘Rup’ and ‘R 99’ rootstocks stood out with the highest alcoholic strength, followed by ‘IAC 766’. The Syrah grafted onto ‘1045 P’ showed higher titratable acidity content on wine than all other treatments, followed by ‘Rup’, ‘R99’ and ‘IAC 766’. On the other hand, ‘1103 P’ and ‘1045 P’ induced wines with highest volatile acidity. Moreover, pH from Syrah wine grafted onto ‘1045 P’ ‘SO4’, ‘161-49’ and ‘R 110’ was lower. Ashes alkalinity showed little relation with rootstock treatments. The moderate

vigor of ‘101-14’ induced intermediate behavior for most physicochemical wine parameters.

Phenolic composition and color traits:

The Comp1 and Comp2 were responsible for over 80% of variation among different wines and the first component was responsible for over 60% on aged wines (Figure 4). Therefore, three distinct groups were detected. ‘R99’ stood out by wine with the highest anthocyanin, total phenolics and flavanoid concentration; however the lowest polymerized pigments indices and one of the lower color intensity. An opposite behavior was observed to ‘161-49’. This latter rootstock tended to high color intensity. All others treatments were drawn together in the middle of the graph. ‘Rup’ and ‘R110’ had a tendency of high anthocyanins and flavanoid concentration. ‘IAC766’, ‘1103P’, ‘R110’ and ‘SO4’ wines presented high total phenolic content close to ‘R99’. Globally, these rootstocks induced intermediate characteristics of phenolic composition and color traits on ‘Syrah’ wines.

Relationship between wine quality and vine balance:

The relation of ‘Syrah’ wine quality and vine balance conferred by different rootstocks was based on multivariate statistical analysis. Principal Component Analysis (PCA) was applied in order to ascertain trends or group

formations of wine samples according to vigor (pruning weight), vine production and phenolic composition and color traits (Figure 5). The Comp1 and Comp2 were responsible for over 80% of variations. Pruning weight and vine production did not show negative relationship with phenolic composition and color intensity of ‘Syrah’ wines. However, the lower inducers of vigor and vine production (‘R110’, ‘R99’ and ‘161-49’) demonstrated an unbalanced behavior between phenolic composition and color traits of ‘Syrah’ wines. The other treatments were drawn together in the middle with a division in two groups: the vigorous productive rootstocks (IAC 766 and Rup) and less vigorous and productive rootstocks (‘1045P’, ‘1103P’, ‘SO4’ and ‘101-14’).

Discussion

Rootstock effects on vegetative and reproductive vigor:

The present study showed that some graft combinations can be more adequate to improve vegetative development and yield of ‘Syrah’ growing under double pruning-management. Pruning weight measurements were more efficient to detect differences among rootstocks than leaf area because that is a seasonally integrated measurement of vine vigor, and the pruning is carried on the shoots of the previous growing season (Smart and Robinson, 1991).

In both growing seasons, grapevines grafted onto ‘Rup’ and ‘IAC766’ showed the highest vegetative vigor (Table 1). The ‘161-49’, ‘R110’ and ‘R99’ induced smallest winter pruning weight on ‘Syrah’ in 2011. The highest vigorous rootstocks increased number of canes and buds at pruning, allowing a quick vine architecture formation. On tropical viticulture world, several studies have shown the increase of scion vigor by IAC 766 (Souza et al., 2015) and Rup (Satisha et al., 2010). In our study, the leaf chlorophyll content, which could be associated to N content and photosynthetic potential, did not vary between high and low vigor rootstocks. Souza et al. (2015) observed the same behavior for ‘Cabernet sauvignon’ grafted onto different rootstocks.

The smaller size of Syrah grafted onto ‘161-49’ was probably due to thin roots, superficial root system and lower power of soil penetration, typical characteristic from hybrids of *Vitis riparia* x *Vitis berlandieri* (Guillon, 1905; Walker and Clingeleffer, 2009). On the other hand, Bassoi et al (2007) showed that the larger root system of ‘IAC 572’, a hybrid also originated from *Vitis caribea*, as ‘IAC 766’, contributed to the highest vegetative vigor and yield of ‘Syrah’. The size, density and efficiency of the root system are involved in the regulation of shoot growth and biomass accumulation since the subterranean growth of the grapevines is in balance with its aerial vegetative growth (Smart et al., 2006; Southey, 1992).

All rootstocks induced a higher yield in 2012 season (Table 1). The lowest yield in 2011 was due to the age of vineyard with consequently lower shoot number per vine and consequently lower number of cluster per plant. In 2012 when the vines were four years old, IAC 766, Rup and Kober already induced yield over 10 $t.ha^{-1}$. Seven years old plants of Syrah showed similar production when grafted onto 1103P, SO4 and R110 under double pruning management (Dias et al, 2012). In 2012, the vine production induced by IAC 766, Rup, Kober and 1045P can be compared with adult plants of Syrah clone 100 grafted onto SO4 in France (Regina and Audeguin, 2005). This productivity on young plants is mostly explained by vigor induced by these rootstocks which allowed faster formation and numerous productive shoots.

In the present study, the vigor did not affect negatively the vine production. Furthermore, some authors have shown a positively link between vegetative growth and yield (Main et al., 2002). The most vigorous induced the highest yield due to increased number of clusters per vine and cluster weight (Table 2). However, the correlation of pruning weight and yield indicated that the rootstock vigor increased 'Syrah' yield up to a limit of 200 g $plant^{-1}$ of pruning weight material (Figure 1). Although there was a tendency to reduce the yield after this limit, the grapevines grafted onto 'Rup', 'IAC 766', Kober' and '1045P' still showed crop level mean values higher than 2.5 kg $vine^{-1}$.

In the currently concept, the fruit composition and wine quality are influenced by leaf area:fruit weight ratio. The leaf area required to ripen the grape of several *V. vinifera* varieties ranges between 0.8 and 1.4 m^2 per kg of fruit (Kliewer and Dokoozlian, 2005). The rootstock 161-49 induced high leaf area:fruit weight ratio for both seasons (Figure 1), mostly due lower vine production. Kober showed the expected leaf area:fruit weight ratio for both years, whereas this ratio was only observed in 2012 for IAC 766, SO4, Rup and 1045P.

Rootstock effects on grape composition:

Rootstock had a weakly influence on total soluble solids and titratable acidity concentration in must as compared to plant age (Table 2). However, in 2011, R110 and 161-49 induced a low titratable acidity. The low vigor of these plants as shown by pruning weight below 70 g $plant^{-1}$ may have contributed to a greater exposure of clusters to sun light and consequently higher acidity degradation. Spayd et al. (2002) observed that the exposed clusters showed lower acidity than shaded ones due to an increase in berries temperature. Kober contributed to lower pH in both seasons. pH and K are important factors that affect the quality of grape juice and the microbiological and physicochemical stability of the wine (Kodur, 2011).

Lower TSS and higher pH were observed in 2012 when the berries had lower mass (Table 3). Such characteristic can be explained by the greater

number of clusters per plant that influenced source-sink balance (Table 2). Moreover, the increase in vegetative growth on four year old plants (2012 season) could have allowed a higher rootstock system development and consequently higher nutrient uptake, which probably contributed to pH elevation (Table 1). Normally, the highest TSS values have been considered as a possible consequence of berry volume loss, and smaller berries have been correlated with lower pH values (Rogiers et al., 2004). Roby et al. (2004) observed that even though amounts of sugars, skin tannin, seed tannin and anthocyanins may be related to berry size at maturity, the sources of variation of berry size such as yield, light exposure, water deficit, are more important in determining its composition than size itself.

Vigorous and productivity rootstock normally delays vegetative growth and berry sugar accumulation (Main et al., 2002). Nevertheless, in this study, all rootstocks induced similar range of berry sugar concentration. The means values of TSS for each season respectively, 21.9°Brix and 20.5°Brix, were similar to common values found in others areas of Minas Gerais (Dias et al., 2012). These values are still close of Syrah vines grown in warm climate, e.g. Spain (Ortega‑Regules et al., 2008).

Berry mass, from all treatments for both seasons, was less than 2.0 g (Table 3), and considered small (Rizzon and Miele, 2004). This trait is important for wine quality, once small berries have greater solute:solvent ratio, i.e. higher

probability of phenolic compounds extraction from skin during maceration process (Conde et al., 2007).

As for sugars and acids content, phenolic composition of berry was poorly affected by rootstocks (Table 4). Other authors also described low differences on berry composition for ‘Cabernet Sauvignon’ onto different rootstocks (Koundouras et al., 2009). Moreover, the values of anthocyanin and total phenolic for all seasons and rootstocks remained in the expected range for quality wine, i.e. 30-750 mg of anthocyanin and 260-900 mg of phenolic per 100 g of berries (Mazza and Miniati, 1993). In southern Italy, leaf removal is a common practice to improve anthocyanin standard of Sicilian wines, since Nero d’Avila is a cultivar with considerable vegetative vigor (Cravero et al., 2012). In our experimental conditions, the higher vegetative vigor of some rootstocks did not affect negatively the phenolic maturation.

Although few differences was detected between rootstocks, reduction in total phenolics of berry skin in between seasons was observed in less vigorous rootstocks ‘R110’, ‘R99’ and ‘161-49’ (Table 4). These rootstocks showed almost two fold increase in pruning weight from 2011 to 2012 (Table 1). The high increase in plant vigor may have decreased the skin proanthocyanidin mean degree of polymerization in shaded fruits (Cortell et al., 2005). These treatments also showed reduction of 1.8 to 1.9 °Brix in 2012 season, higher than the other rootstocks (Table 3).

Rootstock effects on wine composition:

Physicochemical properties: Wine quality is influenced by grape quality factors such as total soluble solids, acidity, pH, phenolics and anthocyanins eventually. Rootstocks usually alter these factors in an indirect manner, by balance of vigor and production (Jackson and Lombard, 1993). The productive and vigorous rootstocks ‘Rup’ and ‘IAC766’ induced Syrah wines with high alcoholic strength as well as less vigorous ‘R99’ rootstock (over 12.0% vol; Supplementary Table A). Furthermore, all treatments presented wines with alcoholic strength within the limit defined by the International Code of Oenological Practices without the need of chaptalization practice (OIV, 2014) (Supplementary Table 1).

Two of the less vigorous rootstocks ‘R110’ and ‘161-49’ were grouped together with the lowest acidity, pH and alcoholic strength (Figure 3). On the other hand, higher acidity was observed in the productive rootstocks ‘Rup’, ‘IAC 766’, ‘1045P’ and the less productive rootstock ‘R99’. Therefore, ‘IAC 766’, ‘Rup’ and ‘R99’ promoted a balanced relationship between alcohol and total acidity in Syrah wines. The balance between alcohol and total acidity is important for wine sensorial quality especially for crisp and fresh taste (Rühl, 2000).

Phenolic composition and color:

The phenolic maturation of grapes is strongly linked with wine quality. Productive and vigorous rootstocks ‘IAC 766’ and ‘Rup’ did not differ from less vigorous rootstocks ‘SO4’, ‘1103P’, ‘101-14’ and ‘1045P’ on pigmented polymers indices, anthocyanins and flavanoids. However, treatments with pruning weight below 160 g plant^{-1} presented higher color intensity (‘1045P’, ‘R110’, ‘101-14’ and ‘161-49’) (Supplementary table B; Table 1). The highest color intensity and polymerized pigments indices was observed in the least vigorous rootstock ‘161-49’, which means the occurrence of anthocyanins copigmentation reactions. These bounded anthocyanins are less suitable for breakdown reactions favored by pH, SO_2, temperature, ketones, light or oxygen (Cortell et al., 2005; Ribéreau-Gayon et al., 2006).

Besides accumulation of anthocyanins in the fruit during ripening, differences in cell structure, berry size and winemaking techniques influence the extractability of anthocyanins from fruit. Once anthocyanins have been released into the wine matrix, it rapidly begins undergoing reactions that can form pigmented polymers. Both anthocyanins and pigmented polymers contribute to wine color; however, as wine begins to age, the pigmented polymers play an increasingly important role in wine color (Cortell et al., 2007). Further studies on the evolution of anthocyanins and formation of pigmented polymers during winemaking and by vine balance may elucidate these questions.

Vine vigor did not affect negatively the red component of the wine color, expressed as the contribution of the component OD 520 in color intensity. On the other hand, low vigor rootstock ‘161-49’, however, contributed to keep the blue component (OD 620) (Supplementary table B).

Globally, vigorous and productive rootstocks induced an adequate balance of vegetative growth and reproductive vigor on Syrah grapevine under double pruning management in 2012, and consequently overall wine quality from these treatments did not vary from moderate and less productive rootstocks. Higher yielding rootstocks do not always have a negative impact on wine quality, as long as vegetative and reproductive growth of the vine is balanced (Dry and Coombe, 2005; Clingeleffer, 1996).

Conclusion

The rootstocks ‘IAC766’, ‘Rup’, ‘Kober’ and ‘1045P’ promote the highest production of ‘Syrah’ grapevines up to the fourth year of plant development. The vigorous rootstocks do not affect negatively the vine production and berry quality under double pruning management in warm temperate Brazilian region. ‘Kober’ shows a better balance of vigor and production since fists years of plant development. Grape quality for winemaking depends more on plant vegetative development level and crop load in the later

season than the rootstocks effects. The two most productive and vigorous rootstock, ‘Rup’ and ‘IAC766’, induce satisfactorily phenolic composition of ‘Syrah’ wine and alcohol acidity balance. The ‘161-49’ rootstock does not contribute to fruit set and vegetative development of ‘Syrah’ under double pruning management, however it keep color intensity in aged wines.

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Tables and Figures

Table 1. Author: DIAS, F. A. N. et al.

Rootstock	Leaf area ($m^2.plant^{-1}$)		Pruning weight ($g.plant^{-1}$)		Chlorophyll Concentration ($mg.m^{-2}$)	
	2011	2012	2011	2012	2011	2012
IAC766	2.55 ^{ns}B	4.55 aA	150.7 aB	291.0 aA	328.2 bB	454.8 aA
Rup	2.60 B	4.45 aA	167.7 aB	299.5 aA	403.1 aA	358.3 bA
Kober	2.42 B	4.07 aA	102.3 bB	176.6 bA	293.6 bB	387.1 bA
1103P	2.15 B	3.67 aA	103.0 bB	166.8 bA	268.9 bB	375.2 bA
1045P	2.35 B	4.02 aA	111.7 bB	159.0 bA	274.6 bB	421.5 aA
R110	2.21 A	3.17 bA	70.0 cB	139.3 bA	288.3 bA	299.6 bA
R99	1.96 B	3.91 aA	78.3 cB	168.8 bA	289.8 bA	355.9 bA
SO4	2.27 A	3.21 bA	111.7 bB	179.3 bA	265.5 bB	387.0 bA
101-14	2.36 B	3.79 aA	98.6 bB	158.2 bA	267.0 bB	410.7 aA
161-49	1.70 A	2.66 bA	62.0 cB	121.6 bA	300.1 bB	424.2 aA

(1) The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test. (ns = not significant).

Table 2. Author: DIAS, F. A. N. et al.

Rootstock	Vine production (kg)		Yield crop ($Mg.ha^{-1}$)		Number of cluster per plant		Weight of cluster (g)		Number of berry per cluster	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
IAC766	1.78 aB	4.13 aA	4.70 aB	10.93 aA	16 aB	29 aA	110.2 aB	142.2 aA	123 aA	115 aA
Rup	1.39 bB	3.88 aA	3.57 bB	10.25 aA	13 bB	28 aA	110.8 aB	137.3 aA	95 bA	114 aA
Kober	2.30 aB	3.85 aA	6.07 aB	10.19 aA	18 aB	28 aA	125.2 aA	136.0 aA	132 aA	107 aA
1103P	1.10 cB	2.13 cA	2.79 cB	5.64 cA	11 bB	19 bA	99.9 bA	111.1 bA	104 bA	100 bA
1045P	1.45 bB	3.40 bA	3.84 bB	8.99 bA	13 bB	28 aA	109.8 aA	122.8 aA	119 bA	102 bA
R110	1.11 cB	2.00 cA	2.93 cB	5.28 cA	12 bB	19 bA	95.2 bA	103.5 bA	93 bA	93 bA
R99	0.86 cB	2.13 cA	2.29 cB	5.64 cA	9 cB	20 bA	91.6 bA	105.3 bA	98 bA	97 bA
SO4	1.22 cB	3.00 bA	3.22 cB	7.93 bA	14 bB	23 bA	88.0 bB	133.1 aA	101 bA	90 bA
101-14	1.38 bB	2.44 bA	3.64 bB	6.46 cA	14 bB	24 bA	101.6 bA	103.0 bA	113 bA	96 bA
161-49	0.41 dA	1.12 dA	1.10 dA	2.96 dA	5 cB	13 cA	82.0 bA	76.1 cA	74 cA	79 cA

(1) The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test. (ns = not significant).

Table 3. Author: DIAS, F. A. N. et al.

Rootstock	Total soluble solids (°Brix)		pH		Titratable acidity (g L^{-1})		Weight of berry (g)	
	2011	2012	2011	2012	2011	2012	2011	2012
IAC766	21.8 ^{ns}A	20.6 ^{ns}B	3.41 ^{ns}A	3.51 aA	8.7 aA	8.13^{ns}A	1.37 ^{ns}A	1.32 ^{ns}A
Rup	22.1 A	20.7 B	3.44 B	3.54 aA	8.5 aA	7.9 A	1.48 A	1.28 B
Kober	21.4 A	20.4 B	3.38 A	3.40 bA	8.6 aA	8.2 A	1.43 A	1.26 B
1103P	21.9 A	20.4 B	3.43 B	3.57 aA	8.4 aA	7.3 B	1.31 A	1.20 A
1045P	21.3 A	20.4 A	3.40 B	3.51 aA	8.7 aA	7.7 A	1.36 A	1.21 B
R110	22.0 A	20.2 B	3.47 A	3.53 aA	7.4 bA	7.5 A	1.31 A	1.17 B
R99	22.0 A	20.1 B	3.45 B	3.59 aA	8.3 aA	7.2 B	1.32 A	1.16 B
SO4	22.2 A	20.6 B	3.42 B	3.53 aA	8.2 aA	7.7 A	1.39 A	1.22 B
101-14	21.8 A	20.5 B	3.43 B	3.55 aA	8.7 aA	7.7 B	1.33 A	1.17 B
161-49	22.4 A	20.6 B	3.42 A	3.51 aA	7.7 bA	7.3 A	1.40 A	1.21 B

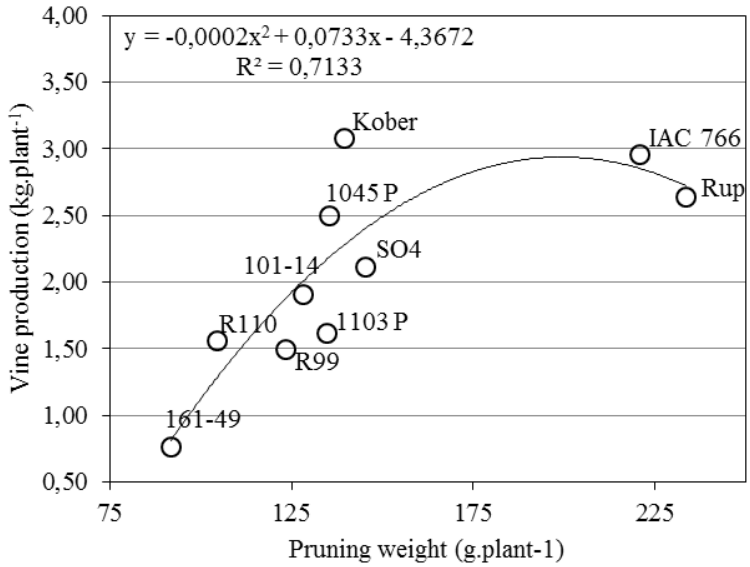
(1) The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test. (ns = not significant).

Table 4. Author: DIAS, F. A. N. et al.

Rootstock	Anthocyanins (mg. 100 g^{-1} of berry)		Total phenolic of berry skin (mg . 100 g^{-1} of berry)	
	2011	2012	2011	2012
IAC766	130.0 ^{ns}A	118.0 ^{ns}B	317.0 ^{ns}A	295.0 ^{ns}A
Rup	127.0 A	136.0 A	341.0 A	344.0 A
Kober	124.0 A	129.0 A	285.0 A	302.0 A
1103P	120.0 A	134.0 A	347.0 A	335.0 A
1045P	116.0 A	130.0 A	302.0 A	304.0 A
R110	123.0 A	121.0 A	366.0 A	294.0 B
R99	124.0 A	130.0 A	334.0 A	309.0 B
SO4	121.0 B	132.0 A	317.0 A	332.0 A
101-14	122.0 A	126.0 A	340.0 A	333.0A
161-49	126.0 A	103.0 B	333.0 A	263.0 B

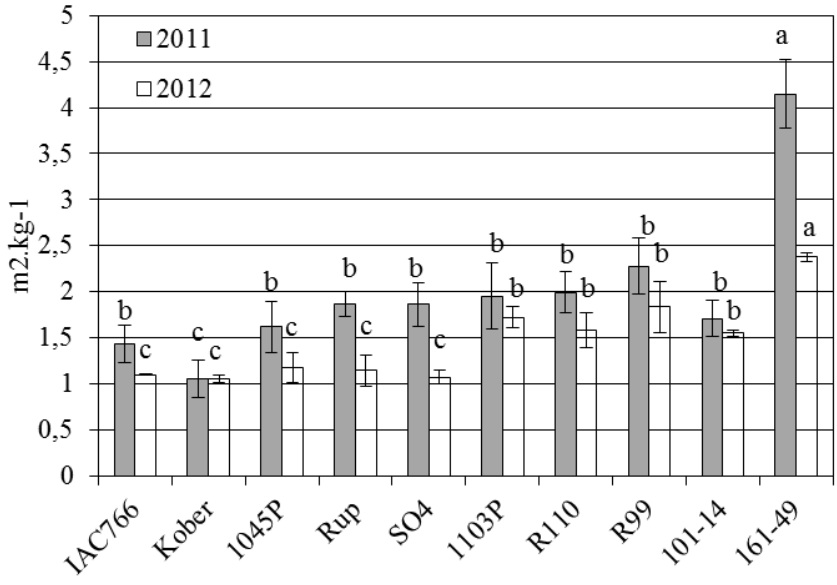
(1) The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test. (ns = not significant).

Figure 1. Author: DIAS, F. A. N. et al.



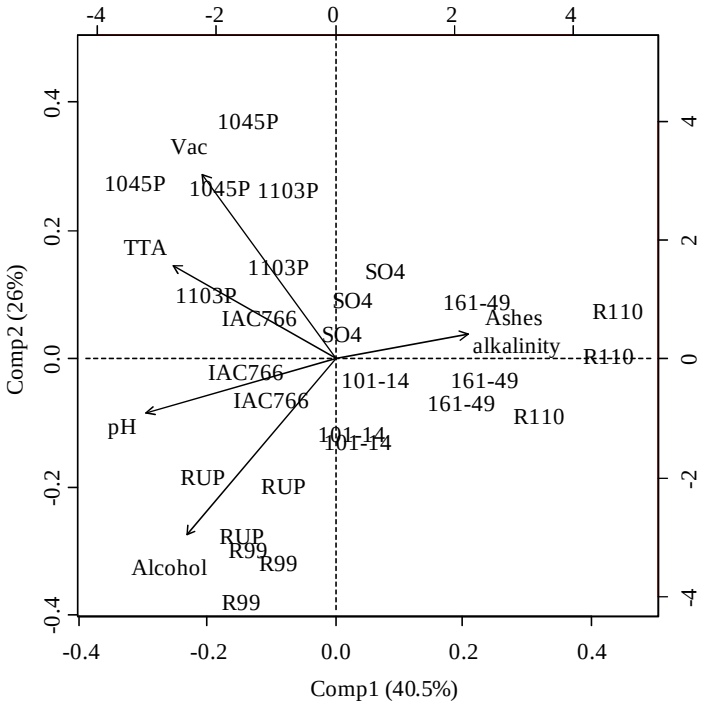
Each point was calculated by average of mean values of vine production and pruning weight of two years.

Figure 2. Author: DIAS, F. A. N. et al.



Means followed by the same letters, in each season, was not significantly different ($p<0.05$) by Scott Knott test. Values are means ±SE.

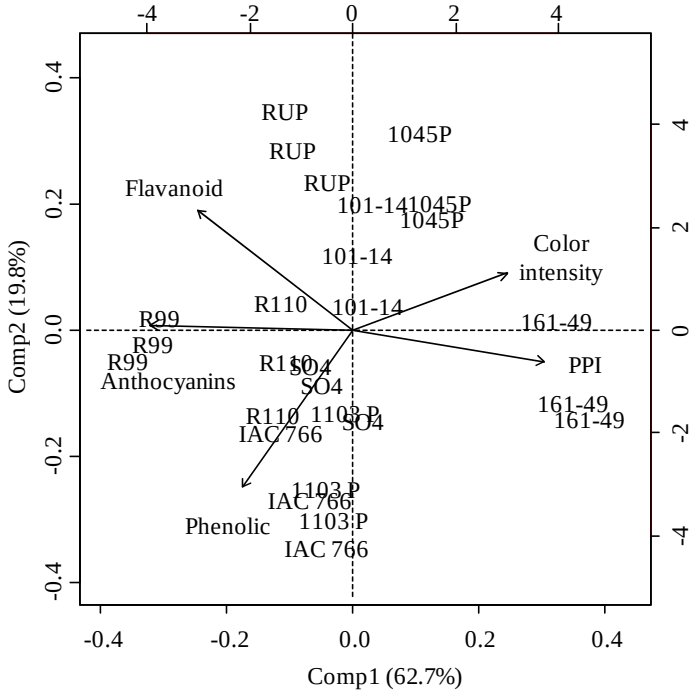
Figure 3. Author: DIAS, F. A. N. et al.



Each data represents one laboratorial replicate.

(Vac: Volatile acidity; TTA: Total acidity).

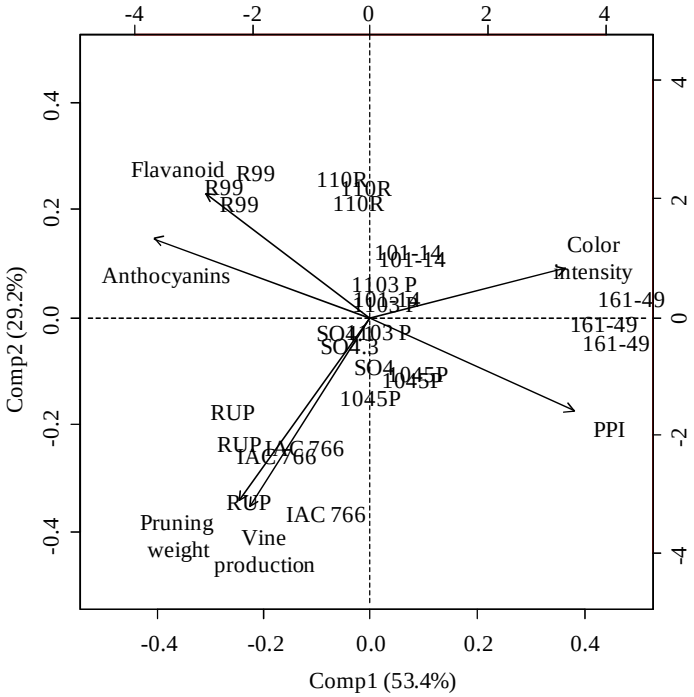
Figure 4. Author: DIAS, F. A. N. et al.



Each data represents one laboratorial replicate.

(PPI: Polymerized pigments indices).

Figure 5. Author: DIAS, F. A. N. et al.



Each data for wine qualities represents one laboratorial replicate and each data for vine traits represent field replicate.

(PPI: Polymerized pigments indices).

LEGEND

Table 1. Leaf area, dry matter of pruning weight and leaf chlorophyll concentration of Syrah grapevine grafted onto 10 different rootstocks in 2011 and 2012 seasons, Andradas, Minas Gerais state, Brazil[(1)].

Table 2. Production and yield parameters of Syrah grapevine grafted onto 10 different rootstocks in 2011 and 2012, Andradas, Minas Gerais state, Brazil[(1)].

Table 3. Total soluble solids (TSS), pH, total titratable acidity (TTA) and berry weight at harvest, from Syrah grapevine grafted onto 10 rootstocks in 2011 and 2012, Andradas, Minas Gerais, Brazil[(1)].

Table 4. Phenolic compounds on berry skin, at harvest, from Syrah grafted onto 10 rootstocks in 2011 and 2012, Andradas, Minas Gerais state, Brazil[(1)].

Figure 1. Relationship between mean vine production and shoot pruning weight of 'Syrah' grafted onto 10 different rootstocks in 2011 and 2012.

Figure 2. Leaf area:fruit weight ratio (m^2 kg^{-1}) produced by 'Syrah' onto 10 rootstocks in 2011 and 2012 growing seasons.

Figure 3. Principal components analysis for the physicochemical properties of wines from Syrah grafted onto different rootstocks in 2012, Andradas, Minas Gerais, Brazil.

Figure 4. Principal components analysis for phenolic composition and color traits of wines from Syrah grafted onto different rootstock in 2012, Andradas, Minas Gerais, Brazil.

Figure 5. Principal components analysis for phenolic composition, color traits, pruning weight and vine production of wine from Syrah grafted onto different rootstock in 2012, Andradas, Minas Gerais, Brazil.

SUPPLEMENTARY DATA

Supplementary Table A. Physical properties of Syrah wine, from vines grafted onto different rootstocks in 2012, Andradas, Minas Gerais, Brazil[(1)]

Rootstock	Alcohol	Total acidity	pH	Volatile acidity*	Ashes Alkalinity
IAC766	12.06b	5.6c	3.92c	0.71b	16.41^{ns}
Rup	12.25a	5.7b	3.96a	0.62c	21.67
1103P	11.86b	5.6c	3.96a	0.75a	20.28
1045P	11.94b	6.3a	3.91d	0.75a	21.05
R110	11.69c	5.4e	3.83g	0.55d	26.16
R99	12.37a	5.8b	3.95b	0.58d	22.45
SO4	11.93b	5.6c	3.89e	0.71b	24.61
101-14	11.94b	5.4e	3.94b	0.63c	22.91
161-49	11.97b	5.5d	3.84f	0.63c	19.58

(1) The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test for each parameter. Alcohol expressed in v/v; Total acidity expressed in g.L^{-1} of tartaric acid; Volatile acidity expressed in g .L^{-1} of acetic acid; ashes alkalinity expressed in meq L^{-1} (^{ns} no significant differences).

*Volatile acidity ratified for free SO_2, according to Brasil (1986).

Supplementary Table B. Phenolic composition and color traits of Syrah wine from vines grafted onto different rootstocks in 2012, Andradas, Minas Gerais state, Brazil [(1)].

Rootstock	Anthocyanins	Phenolics	Flavanoids	TPI 280nm**	PPI*
IAC766	140.92c	1.38b	1.12b	31.47f	74.74b
Rup	151.65b	1.06d	1.50a	38.60b	74.21b
1103P	142.75c	1.48a	1.26b	33.50e	75.39b
1045P	122.67d	0.99e	1.31a	36.87c	75.90b
R110	155.64b	1.43a	1.40a	36.20c	72.55c
R99	169.21a	1.49a	1.60a	40.17a	70.43c
SO4	138.30c	1.33b	1.36a	35.60d	76.76b
101-14	141.16c	1.25c	1.42a	35.17d	75.12b
161-49	99.04e	1.21c	1.06b	32.03f	81.88a
	Color intensity***	Color Hue***	OD 420%	OD 520%	OD 620%
IAC766	9.48d	0.80a	38.40^{ns}	47.71^{ns}	13.91b
Rup	10.45c	0.80a	38.31	47.90	13.80b
1103P	10.37c	0.78b	37.55	48.33	14.12b
1045P	10.72b	0.76c	36.67	48.21	15.12b
R110	10.85b	0.78b	37.43	48.03	14.54b
R99	9.53d	0.81a	38.40	47.39	14.21b
SO4	9.53d	0.77b	37.28	48.65	14.07b
101-14	11.27b	0.75c	36.45	48.81	14.74b
161-49	12.45a	0.75c	35.67	47.44	16.89a

[(1)] The means followed by the same letter, lowercase for column and uppercase for line, were not significantly different ($p \leq 0.05$) by Scott Knott test for each parameter. Phenolics and Flavonoids were expressed in $g.L^{-1}$; Anthocyanins was expressed in $mg.L^{-1}$; OD 420% yellow component; OD 520% red component; OD 620% blue component. (* Polymerized pigments indices (PPI) in percentage (%) ** total polyphenol indices (TPI) in percentage (%) ***sum of 420, 520 and 620 nm absorbances and ratio of 420/520 nm respectively).

CHAPTER 2: A novel analysis framework to study grapevine bud development and fruitfulness using the Microvine model (*Vitis vinifera* L.)

(Version submitted to Australian Journal of Grape and Wine Research)

A novel analysis framework to study grapevine bud development and fruitfulness using the Microvine model (*Vitis vinifera* L.)

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Abstract

Background and Aims: Microvine is a grapevine GA-insensitive mutant, characterized by a dwarf stature and a synchronism of vegetative and reproductive developments along the axes. Bud fruitfulness timing and sensitivity to climatic factors under controlled environment may thus be facilitated on microvine compared with grapevine. The present study aimed to develop an analysis framework of microvine primary bud development along the proleptic axis.

Methods and Results: The analysis framework was based on microscopy and X-ray micro-tomography methods. Microscopy accuracy was higher compared with X-ray micro-tomography to assess phytomers and inflorescence primordia initiation within winter buds. Lignified buds, exhibited a maximum of 6 phytomers and 2 inflorescences primordia, inserted on the distal phytomers (4 to 6), similarly to grapevine. The first and second anlagen were differentiated beyond the Plastochron Index (PI) 13 and 26, respectively, indeed 325 °Cd and 650 °Cd after bud initiation. Primary bud length was highly correlated with the number of inflorescences primordia and phytomers.

Conclusions: Microvine can thus be used as a grapevine model to study winter bud development. The bud length can be used as a macroscopic indicator of the potential fruitfulness.

Significance of the Study: The analysis framework developed provides a

relevant tool to further address bud fruitfulness and transcriptomic responses to biotic and/or abiotic stress.

Key-words: Bud fruitfulness, Microscopy, Microvine, Perennial plants, X-ray micro-tomography.

Introduction

In the perspective of climate changes, new insight into the developmental and molecular mechanisms controlling grapevine yield and quality, and their regulation by physical factors are major issues for wine industry. A better adaptation of existing grapevine varieties, together with the creation of new varieties are required to face the predicted elevated temperatures and water deficit (Ollat et al., 2011; Ollat et al., 2014). Final yield relies on the development of reproductive organs along the proleptic shoots over two vegetative cycle (Carmona et al., 2008; Pratt, 1971). The inflorescence primordia are first initiated and differentiated within the winter buds during the pre-dormancy period. After dormancy breaking and during the two weeks preceding budburst (i.e. nine to twelve months after inflorescence initiation), reproductive meristems form new inflorescences branches bearing dichasia of flowers (Fernandez et al., 2010). Up to 80% of season-to-season grapevine yield variations result from the variations in bunches numbers per vine (Vasconcelos

et al., 2009). Thus, bud fruitfulness potential, i.e. the number of inflorescence primordia differentiated in the winter buds, is critical for final yield. In commercial vineyards, according to bud fruitfulness potential, the pruning is implemented to control the number of winter buds and the resulting crop load (Vasconcelos et al., 2009).

Winter buds are complex structures made of a few preformed vegetative axis, each arising from single shoots apical meristems or SAM (Bernard, 1980; Carolus, 1970). Due to apical dominance, the main vegetative axis within bud, also called primary bud, displays both the highest probability of development at budburst and the highest fruitfulness. However, this axis is very susceptible to a range of abiotic and biotic stresses, as well as to various physiological disorders. When the primary axis is hampered or destroyed, secondary or tertiary meristems may ensure the development of new proleptic shoots, although less fruitful in *Vitis vinifera* cultivars. In the grapevine, the primary axis are made of five to nine preformed phytomers, depending on bud position on the supporting cane (Pratt, 1971). When winter buds are compared according to their position along shoots, the number of preformed phytomers and the resulting fruitfulness potential tend to be higher in median buds than for proximal and distal buds (Carolus, 1970; Huglin and Schneider, 1998).

Uncommitted primordia (anlagen) are differentiated oppositely to the leaf along the preformed primary axis, and they further develop into

inflorescences or tendrils (Srinivasan and Mullins, 1981). Generally, SAM initial development is strictly vegetative. For most *V. vinifera* cultivars, the first anlage is initiated on the third or fourth phytomer (Carolus, 1970; Vasconcelos et al., 2009). The timing of inflorescences initiations and differentiations, and their final numbers, depend on cultivar, bud position along the shoot and environment (Pratt, 1971; Srinivasan and Mullins, 1981). Along the cane, winter buds develop in an acropetal way, rather than simultaneously (Carolus, 1970). Anlagen initiation within proximal buds (i.e. winter buds located at the base of the shoot) start five weeks after shoot development under warm climate, for instance for Syrah in Montpellier (Cheema et al., 1996) or Merlot in Bordeaux (Carolus, 1970), and up to two weeks later for cv. Riesling and Aris under cool climate (Alleweldt and Ilter, 1969). Anlagen initiations in two successive winter buds within the same shoot is expected to be two days apart, or even less under warm temperatures (Swanepoel and Archer, 1988; Vasconcelos et al., 2009). In addition to temperature, light intensity, water and nitrogen supply during bud development are main factors conditioning bud fruitfulness (Buttrose, 1974; Guilpart et al., 2014; Petrie and Clingeleffer, 2005; Sánchez and Dokoozlian, 2005).

Grapevine bud fruitfulness responses to abiotic factors have been a key focus of many studies over the last decades. However, most of these studies were conducted under field conditions, where all climatic factors vary simultaneously for each different reproductive cycle. The asynchronous

vegetative and reproductive developments, and their differential susceptibility to physiological and microclimate factors, make it difficult to simply address the impact of climate changes on bud fruitfulness under field conditions. In addition, similarly to other perennial crops, the large size of grapevine plants hampers the possibility to compare current cultivars or new varieties responses to climate under fully controlled environments (Chaib et al., 2010).

Studies on bud fruitfulness may be facilitated by the use of the microvine, a natural grapevine mutant generated from the L1 cell layer of Pinot Meunier (Boss and Thomas, 2002). Indeed, the mutation confers to the plant a dwarf stature, a continuous flowering along the shoots and it shortens its juvenile phase (Chaib et al., 2010). Recent studies on microvine (Luchaire et al., 2013; Rienth et al., 2014a) have shown the possibility to infer temporal patterns of berry, leaf and internodes growths from spatial observations on the main shoot. Temporal morphological, biochemical and genetic adaptations of vegetative and reproductive organs to abiotic stress can thus be assessed from short-term experiments under controlled environments (Luchaire et al., 2013; Rienth et al., 2014b).

Bud development analyses generally rely on binocular microscope methodology (Cox et al., 2012; Dry, 2000; Jones et al., 2013; Sánchez and Dokkozlian, 2005). Although simple, this method is destructive and time-consuming. New approaches based on X-ray micro-tomography were recently

developed for non-destructive anatomy analyses of lignified organs. This technology can be used to produce high resolution 3D reconstruction of various organs. Notably, it is relevant to accurately describe wood anatomy and density (Fromm et al., 2001; Steppe et al., 2004; Stuppy et al., 2003), grapevine *in vivo* vessel network and even the internal organisation of the graft union (Brodersen et al., 2011; Milien et al., 2012). Thus, this technology appears very promising to characterize leaf and inflorescence primordium developments within winter buds.

The present study aimed to test the advantages of high-resolution computed X-ray micro-tomography, when compared with classical microscopy anatomy, for microvine primary winter bud phenotyping. These methods were used to set up an analysis framework of temporal bud development, based on spatial observations of buds morphogenesis along the proleptic shoot, and to identify an early and easily measured indicator of potential bud fruitfulness.

Material and methods

Plant material

The experiments were conducted on the microvine ML1 line, which exhibits a Dwarf stature and a Rapid and Continuous Flowering (DRCF) phenotype (Chaib et al., 2010; Luchaire et al., 2013). One year, own-rooted ML1 plants were grown in greenhouse at Montpellier SupAgro-INRA campus, France. Pots (3L) were filled with Neuhauss Humin-substrat N2 (Klasmann-Deilmann, Bourgoin

Jallieu, France). Osmocote standard fertilizer (15g, Everris, Limas, France) was added at bud burst. Irrigation supply was non-limiting, ranging 75 to 400 ml water per day, depending on leaf area. Plants were pruned to 2-3 winter buds. Shoots were thinned to maintain an unique proleptic axis per plant, after five leaves were unfolded. Sylleptics axis (lateral branches) emerging from axillary meristem were removed at a weekly time step to control crop load.

Climatic conditions in the greenhouse were set to a temperature range of 25°C-15°C during the day-night, a VPD of 1 KPa and a daily cumulated PAR of 20 $mol.m^{-2}$. Five ML1 microvines displaying homogeneous development were harvested among 20 plants when they reached 40 unfolded leaves, i.e. about 80 days or 1000°Cd after bud burst, considering a 25°Cd phyllochron (Luchaire et al., 2013). All lateral caulinary organs (leaves, flowers, fruits and tendrils) were removed (Figure 1) before the proleptic axes were stored in closed plastic bags at 4°C for further imagery analyzes (at the latest one week after harvest).

Bud development measurements

Bud development was assessed using either light microscopy or X-ray micro-tomography. All winter buds of the selected proleptic axes were analyzed with microscopy, while only four buds per axis sampled at different Plastochron Index (PI) levels were described with X-ray micro-tomography.

Primary buds development parameters

Three zones (distal, medium and proximal zones) were separated along the proleptic axis. Zone limits were based on the extent of bud and stem lignification, which are indicators of bud development and physiology (Vasconcelos et al., 2009). Winter buds were numbered from the top to the bottom of the proleptic axis, as follow:

- Zone 1 (PI-1 to PI-10): non-lignified buds (light brown to reddish external scale) developing on green nodes;
- Zone 2 (PI-10 to PI-25): partially lignified buds (brownish external scales) developing on dark green to yellowish nodes;
- Zone 3 (PI-25 to PI-40): lignified buds developing on brown nodes.

Six morphological parameters were measured on all primary buds, as illustrated in Figure 2. The length (height) of the bud (LB) corresponded to the distance between the bud basal part (i.e. main axis first phytomer) and distal part (i.e. most-external scale). The width at the base of the bud (WB) was the distance between the first and second basal leaves primordia insertion points. Bud main axis development parameters included the number of preformed nodes (nN), the total length of the primary axis (LA), the number of inflorescence primordia (nIP) and their position on the primary axis (pIP).

The probability for each primary axis phytomer to hold an inflorescence primordium of first or second rank ($p_{IP1}n$ and $p_{IP2}n$, respectively) was calculated,

as described below (equations 1 and 2):

$$p_{IP1}n = \frac{\sum_1^X IP1n}{X} \times 100 \qquad \text{eq. 1)}$$

$$p_{IP2}n = \frac{\sum_1^X IP2n}{X} \times 100 \qquad \text{eq. 2)}$$

where X was the total number of winter buds on the proleptic axis for the five plants and IP1n and IP2n were the number of first or second rank inflorescences primordia at the phytomer position n (i.e. 0 or 1 inflorescence).

Microscopy

Winter buds were longitudinally sectioned along the phyllotaxic plan of the primary bud (Figure 1). The two sides of the dissected buds were observed using a stereo-microscope (model Stemi 2000-C; Zeiss, Jena, Germany; Figure 1B and 2C) at magnification range 6.5x to 50x with a cold light source 15 V/150 W and no light screen (model KL 1500 compact; Schott, Mainz, Germany). The pictures were taken using a Spot Insight Color digital camera (model 3.2.0; Diagnostic Instruments, Sterling Heights, Michigan, USA). A microscale (10^{-1} mm graduation) was automatically included in the pictures. The parameters LB, WB and LA were measured using the ImageJ software (Rasband, 1997–2011), while the other parameters (nN, nIP, and pIP) were determined from direct observations under the microscope (Figure 2B).

X-ray micro-tomography

The X-ray micro-tomography was used to characterize the morphology of buds along the 3 zones of the proleptic axis (see above). High-resolution 2D images were taken with a micro-tomography SkyScan (model 1076, SkyScan, Kontich, Belgium; Figure 2A). The samples were scanned at the Montpellier RIO Imaging Center (France; http://www.mri.cnrs.fr/). The parameters were set for low-density objects (40 kV, 250µA and no filter). The resolution was 9 µm with a step rotation of 0.1 degree. 3D images were reconstituted from 2D images (16 bits) using the NRecon software (SkyScan, Kontich, Belgium), as described in Miliena et al. (2012).

When the 2D images were out of the phyllotaxic plan of primary bud, virtual cuts were made from 3D reconstructions using the Imaris software to orientate the bud in the right phyllotaxic plan (Bitplane, Zurich, Switzerland). For this purpose, 2D images were reduced to 8 bits using ImageJ software, and only the central region of bud was selected to reduce the image size. All the buds parameters (Figure 2A) were measured and/or visualized using ImageJ software from the 2D or 3D bud images reconstructions.

Statistics

Statistical analyses were performed using the SISVAR statistical package version 4.6 (University Federal of Lavras, Lavras, Brazil; Ferreira et al., 2008).

The effect of bud position along the proleptic axis (zone 1 to 3) on node number (nN) or inflorescences number (nIP) and on the probability that bud holds an inflorescence of first or second rank at each primary axis node position (p_{IP1n} and p_{IP2n}, respectively) were assessed from ANOVA analysis. A Tukey test was used for means comparison (α=0.05). Linear and no-linear regressions were fitted between buds morphological parameters to describe bud development along the proleptic axis and identify an early indicator of bud fruitfulness.

Results and Discussion

Comparison of X-ray micro-tomography and microscopy methods for bud morphology and fruitfulness assessments

The added value of X-ray micro-tomography for bud development analyzes, compared with microscopy, was assessed based on its level of handling, time requirement and image quality (Table 1).

The classical microscopy was faster than X-ray micro-tomography. The six buds phenotypic traits described above (i.e. the bud length and width, the primary axis length and nodes number, the inflorescence primordia number and their position on the primary axis) could be determined using this method. However, as it is a destructive method, buds samples were lost when they were dissected out of the phyllotaxic plan or when the protective hair removal damaged primary bud axis. In addition, bud storage over a one week period

made difficult the dissections, due to necrosis development and to tissues turgidity loss.

The X-ray micro-tomography is a non-destructive or minimally invasive imaging approach (Larabell and Nugent, 2010). This method may be recommended for rare plant material, or for those requiring special labor for additional histological traits observation (Smith et al., 2009). Although no bud dissection is required, the scanning and image reconstruction make the X-ray micro-tomography much more time-consuming than the microscopy (Table 1). When the bud phyllotaxic plan needed to be reconstructed from 3D images, the processing time for X-ray microtomography method was even higher (Table 1). This method provided an accurate assessment of most morphological traits, except the number of pre-formed phytomers of the primary axis and the position of inflorescences primordia. These last parameters could not be accurately determined, due to the lack of image contrast.

The micro-tomography image quality (contrast) results from the gradient of X-ray photons attenuation within tissues. Thus, it depends both on the X-ray radiation energy and on tissue thickness, density and biochemical composition. Variations in tissues water and cellulose contents impact their oxygen and carbon concentrations, and consequently their absorption, reflectance and radiation energy conductance (Milien et al., 2012). Leaf primordia (high water

content), contrasted on images with protective hair (low water content), which absorbed all photons (black background on image, Figure 2A).

The microscopy and X-Ray tomography provided same ranges of values for the number of phytomers and primordia inflorescence within buds along the cane (Figure 3). However, these traits were more easily and accurately measured on microscopy images, because of the higher contrast between bud nodes organs and tissues (Figures 1C and 2B). Grapevine stem vascular network studies with X-ray micro-tomography also showed that the resolution of the images obtained with classic histology techniques was superior to those obtained with X-ray tomography (Brodersen et al., 2011; Milien et al., 2012). In these studies, the resolution for the image analysis was not sufficient to observe individual vessels, in contrast with classic histology techniques.

Thus, in the conditions of our study, the classical microcopy appeared to be a more accurate and faster method to characterize the changes in winter bud development and fruitfulness along the cane. Indeed, the micro-tomography method was less effective, due to the insufficient scan resolution and the low tissues contrasts within grapevine winter bud organs. Nevertheless, emerging technologies could overcome these limitations. High-resolution X-ray micro-tomography and synchrotron radiation X-ray tomographic microscopy methods provide high-quality images with resolution at cell level (0.45 µm) (Dhondt et al., 2010; Smith et al., 2009). When combined to 3D image reconstruction, such

techniques may be promising for non-destructive analysis of the anatomy of anlagen initiation and differentiation.

Set-up an analysis framework of winter bud temporal development from spatial changes along the proleptic axis

The number of phytomers along the primary bud within winter buds gradually increased from the distal part to the proximal part of the proleptic cane (Figure 3A). Main axis held a maximum of 6 phytomers after the PI 25, which corresponded to the beginning of the 3rd cane zone characterized by lignified internodes and buds. From the middle zone of cane (zone 2, PI 25-10) toward the apex (zone 1, PI<10), the number of preformed phytomer progressively decreased, as expected for *V. vinifera* cultivars (Huglin, 1958).

No inflorescence primordium was observed in zone 1, indeed the zone with non-lignified stem and buds (Figure 3B). The first inflorescence primordium progressively differentiated along the partially lignified middle zone (zone 2). Zone 2 contained on average 4.5 phytomers per primary bud. Thus, the first anlagen were initiated beyond the third phytomer in microvine, similarly to other *V. vinifera* cultivars (Carolus, 1970; May, 1964; Vasconcelos et al., 2009). The second uncommitted primordia were not fully formed in zone 2 and they only showed a round shape. After PI 25, buds contained two ramified inflorescences primordia (Figure 3B). This result indicates that, within winter

buds, reproductive organ development follows an acropetal gradient. No new phytomer and inflorescence primordium development were noticed afterwards (PI>25), suggesting that the lignified buds of zone 3 had stopped their differentiation and they were probably entering the endo-dormancy phase.

Primary and secondary buds endo-dormancy phase is known to coincide with the time when shoot color turns from green to yellowish-brown. This color change is due to the activity of the cork cambium (phellogen), which isolates primary cortex and epidermis from the vascular tissues of the stem (Bernard, 1980). Depending on the cultivar, dormancy generally starts after 1 to 3 inflorescence primordia are initiated within winter buds. In our experiments on microvine, it corresponded to the transition between zones 2 and 3, indeed around PI 25.

Along the primary axis of microvine winter bud, the first inflorescence primordium was inserted on the fourth or fifth phytomer, while the sixth phytomer bore inflorescences of second order only (Figure 4). The fifth phytomer appeared to be the most fertile, with a probability of 92% to differentiate inflorescences primordia of first order (53%) or second order (39%) (Figure 4). Similarly to *V. vinifera* cultivars, microvine caulinar development followed a ternary rhythm with three types of phytomers being initiated: P0 developing no tendril or inflorescence and P1 and P2 bearing inflorescence or tendril (Carolus, 1970; Pratt, 1971). Along microvine primary bud, the 3th and

even the 4th phytomers for some buds were P0. They were then followed by P1 and P2 phytomers.

All plants and winter buds sampled in this study developed under same environmental conditions (25°C day and 15°C night), over a 80 days period. The first inflorescence primordium was differentiated on buds containing at least 4 pre-formed phytomers (PI 13), 26 days or 325°Cd after bud initiation (Figure 3), using a phyllochron of 25°Cd for microvine (Luchaire et al., 2013). Five phytomers and 2 inflorescence primordia were differentiated in buds on PI 26, 52 days or 650°Cd after their initiation (Figure 3). Thus, the 2 anlage initiations and ramifications were 26 days or 325°Cd apart. Similar winter bud development pattern was described for grapevine cultivars. May (1964) reported a short vegetative period of three to five leaf primordia formation before the first uncommitted primordium formation. For Chardonnay cv. in Australia, this vegetative growth period, for a winter bud located on the 4th phytomer of the cane, was 28 days (Watt et al., 2008). For Chenin blanc cv. grown in South Africa, 21 days were required between the initiation of the first and the second inflorescence primordium within winter buds (Swanepoel and Archer, 1988).

Ultimately, on microvine bearing about 40 buds on the cane (i.e. 1000°Cd after budburst), three winter bud main developmental stages were delimited (Table 2): i) pre-dormant buds in the cane proximal zone (PI 25-40, zone 3), which were at least 50 days-old or 625°Cd, displayed a complete

primary bud development with a maximum fruitfulness potential of two inflorescences and at least five preformed phytomers; ii) winter buds within the medium zone (PI 10-25, zone 2), which were 20-50 days-old or 250-625°Cd-old, showed an intermediate development with about four preformed phytomers, the first inflorescence primordia clearly distinguishable and in the most developed buds the initiation of second anlagen, and iii) buds in the cane distal zone (PI 1-10, zone 1), which were younger than 20 days or 250 °Cd, held a maximum of three preformed phytomers with no inflorescence.

The maximum of 6 pre-formed phytomers and 2 inflorescences along the primary axis in microvine winter bud, also observed by Chatbanyong et al. (2014), is lower than values generally reported for grapevine cultivars. Depending on cultivar and on growing conditions, 6 to 9 phytomers with up to 3 inflorescences primordia can be pre-formed in the primary axis of winter buds (Carolus, 1970; Huglin and Schneider, 1998; Willians, 2000). In the microvine, gibberellin signaling changes due to *gai* semi-dominant mutation are associated with a range of pleiotropic effects such as the shortening of juvenile phase and the dwarfism (Boss and Thomas, 2002). The insensitivity of vegetative organs to gibberellin or the changes in plant growth regulator balance may influence the development of primary axis in winter buds before dormancy.

Toward an early indicator of winter buds potential fruitfulness in microvine

Microscopy and micro-tomography were shown to be relevant methods to assess winter bud phytomers and inflorescences primordia development. However, their use at large scale, e.g. to phenotype a segregating population or genetic resources (Chatbanyong et al., 2014), is laborious and requires specific technical skills. Whether winter bud development and potential fruitfulness can be predicted from simple bud morphological parameters was thus evaluated. Bud length can be easily measured, regardless of the phyllotaxic plan of the supporting proleptic axis. This parameter was found to be highly correlated with the number of pre-formed phytomers and inflorescence primordia (Figure 5).

The winter buds longer than 2 mm held 4 to 6 phytomers and until 2 inflorescences primordia (Figure 5A and 5B). Such buds were located beyond PI 13, thus corresponding to zones 2 and 3. Buds of PI 1-13, were shorter than 2 mm and contained less than 4 preformed phytomers and no inflorescence (zone 1) (Figure 4). Moreover, winter buds entering in pre-dormancy or in dormancy stage were longer than 2.70 mm and they exhibited a fruitfulness potential of 1 or 2 supported by 5 to 6 phytomers (Figure 4).

The number of phytomers and inflorescence primordia were also found to be strongly correlated (Figure 6), thus indicating that reproductive and vegetative developments are related during winter bud development as expected from previous studies in grapevine (Carolus, 1970; Huglin, 1958). In the

grapevine, the vigour which results in longer proleptic axes also induces a higher development of winter buds (Huglin and Schneider, 1998; Jones et al., 2013; Lebon et al., 2008). Different authors reported some relationships between vigour during the development and potentially fertility of winter buds at the previous cycle and the yield at the next season (Guilpart et al., 2014; Huglin and Schneider, 1998)

These results allow us to propose the length of winter bud (LB) as a potential estimator of primary axis development and potential fruitfulness of winter buds. This estimator which is simple to measure directly on the plant or from macroscopic imaging, without any dissection or sophisticated technology, is cheap, quick and preserve organ integrity.

Conclusions

The number of inflorescence primordia and preformed phytomers were shown to be important parameters to describe winter bud development. In this study, a temporal bud vegetative and reproductive development framework was parameterized from spatial buds observations on microvine proleptic axis. Bud developmental patterns were found to be similar to the grapevine. In the state-of-the-art, light microcopy was found more convenient and accurate for bud development characterization compared with micro-tomography, although emerging micro-tomography technologies appear promising for such

approaches. A simple non-destructive and early indicator of winter bud potential fruitfulness, based on external bud morphology is proposed.

Microvine can thus be used as a grapevine model to study winter bud development. Its dwarf stature and its short generation cycle are expected to provide new opportunities to study early reproductive organ development responses to abiotic stress under fully controlled environment in the same time of temporal studies of flower and berries development in the same proleptic axis (Rienth et al., 2014b). This possibility will allow studies about synchronization of latent bud development and vine reproductive cycle under different climate conditions.

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Figure legends

Figure 1. Microvine winter bud phyllotaxic plan and pre-formed axes (A) Lateral view of the winter bud; (B) Winter bud view, orthogonally to the phyllotaxic plan of supporting proleptic axis; (C) Longitudinal section of the winter bud along its phyllotaxic plan. Abbreviations: N+2: primary vegetative axis; N+3: Secondary vegetative axis; a: Proleptic axis plan of phyllotaxic; b: Winter bud plan of phyllotaxic.

Figure 2. Winter bud morphological parameters measured by X-ray micro-tomography (A) and light stereo-microscopy (B). Abbreviations: N+2: Primary vegetative axis; N+3: Secondary vegetative axis; sc: Bud scale; WB: Width of the primary bud; LA: Length of the primary vegetative axis; LB: Length of the winter bud; IP: Inflorescence primordium; N: Node; IN: Internode and lp: Leaf primordium.

Figure 3. Number of phytomers (A) and inflorescence primordia (B) within winter bud primary axis as a function of the cane plastochron index or cumulated thermal time after bud initiation, determined by stereo-microscopy and X-ray micro-tomography. Each point is the mean of 2 to 5 buds for microcopy and corresponds to one unique bud for X-ray micro-tomography. Bars indicate standard errors.

Figure 4. Probability for each phytomer of the winter bud main axis to hold an inflorescence primordium of first or second order. Bars indicate standard errors. Letters indicate significant difference for total probability of inflorescence primordium formation between phytomers ($P < 0.05$).

Figure 5. Number of pre-formed phytomers (A) and inflorescences primordia (B) as a function of the winter bud length (LB) measured by stereo-microscopy and X-ray micro-tomography.

Figure 6. Number of inflorescences primordia as a function of the pre-formed phytomers number measured by stereo-microscopy and X-ray micro-tomography.

TABLES

Table 1. Comparison of stereo-microscopy and x-ray micro-tomography methods for winter bud development study

Criteria	Microscopy	X-ray micro-tomography
Proceeding	Dissection + microscopy/camera + image analysis	Tomography + 2D & 3D reconstructions† + image analysis
Time per bud	0h25 = 0h05 + 0h10 + 0h10	03h20 = 01h50‡ + 1h20 + (0h25)† + 0h10
Bud pre-dissection requirement	Yes	No
Bud storage conditions	Less than 1 week at 4°C	Less than 3 weeks at room temperature
Bud phenotypic traits	LB, WB, LA, nIP, nN, pIP	LB, WB, LA, nIP
File size	20Mb per image, 2 images per bud	60Mb per image >500 images per bud
Application	Difficult	Easy

†optional phyllotaxic plan 3D reconstruction

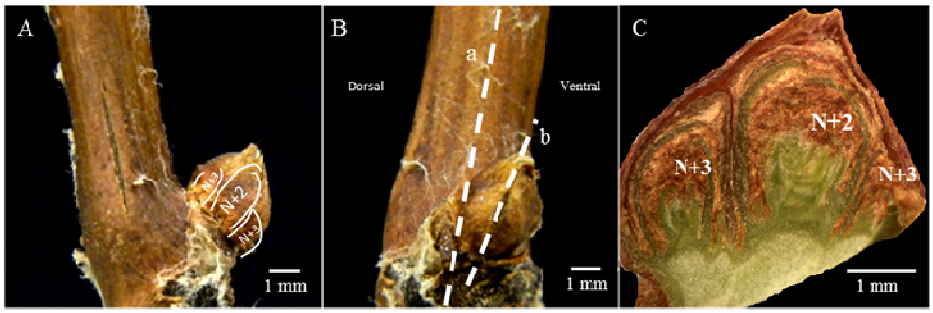
‡one to five buds simultaneously

Table 2. Number of preformed phytomers and inflorescence primordia within winter buds along the three zones of the proleptic axis.

Zone	1	2	3
Proleptic axis			
Number of pre-formed phytomer	2.7[c]	4.2[b]	5.3[a]
Number of inflorescence primordia	0.0[c]	1.1[b]	1.8[a]

Zone 1: Bud formation (vegetative primordia differentiation); Zone 2: bud differentiation (inflorescence and vegetative primordia differentiations); Zone 3: bud maturation (bud lignification and dormancy). Numbers with the same letter superscripts within parameters are not significantly different at $P < 0.05$.

FIGURE



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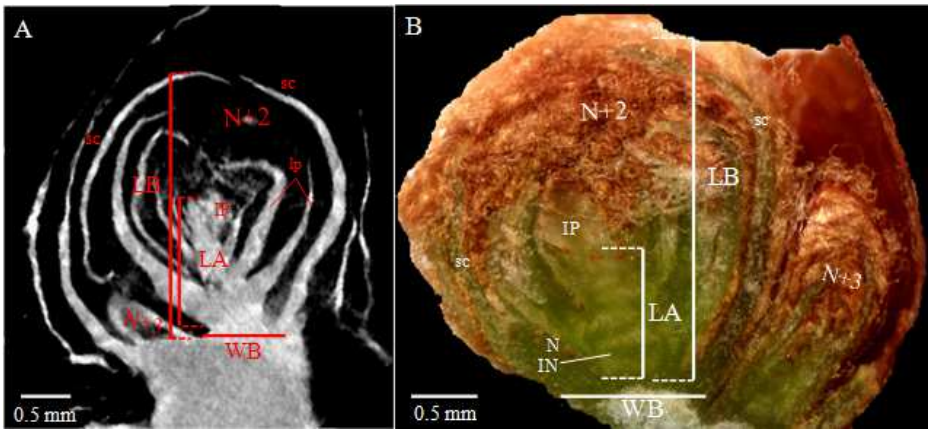

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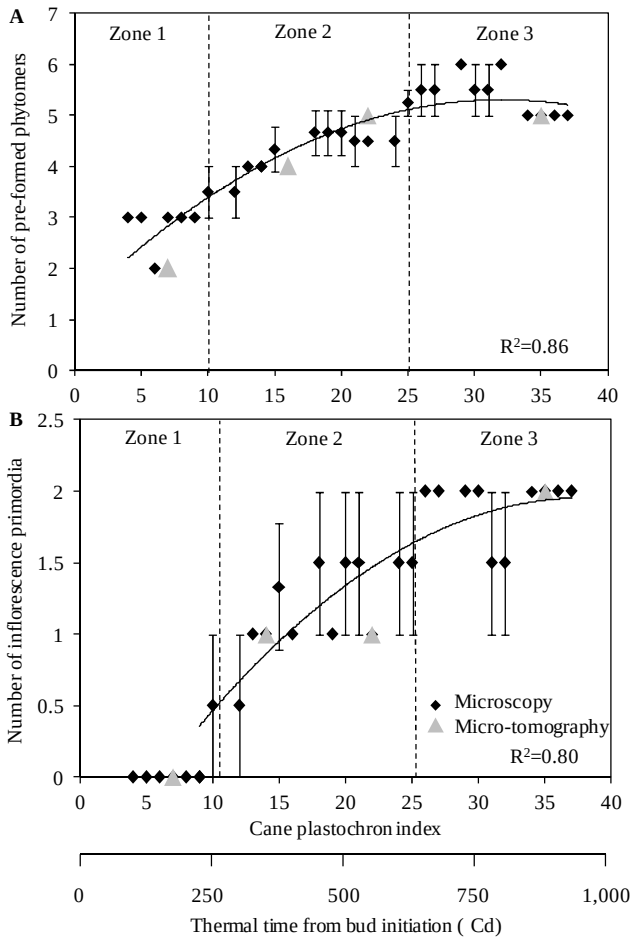

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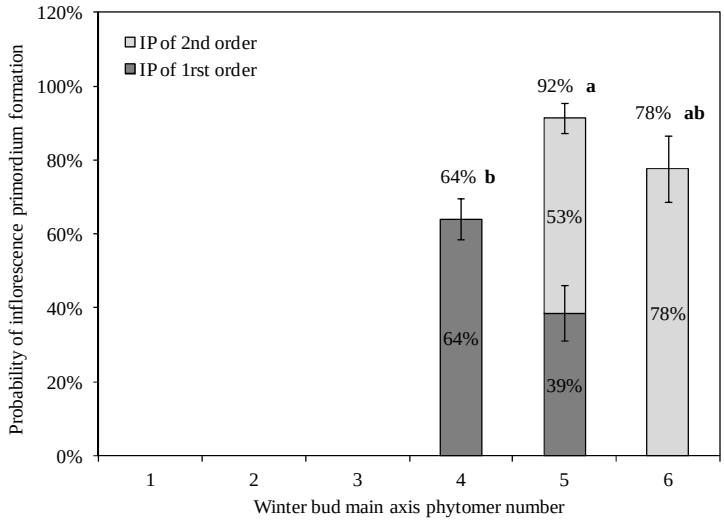

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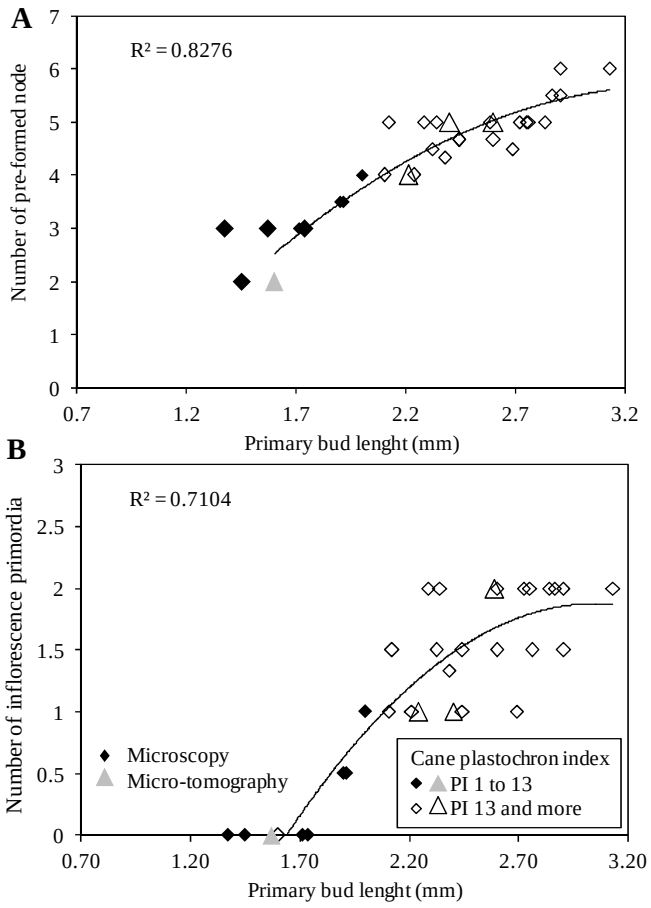

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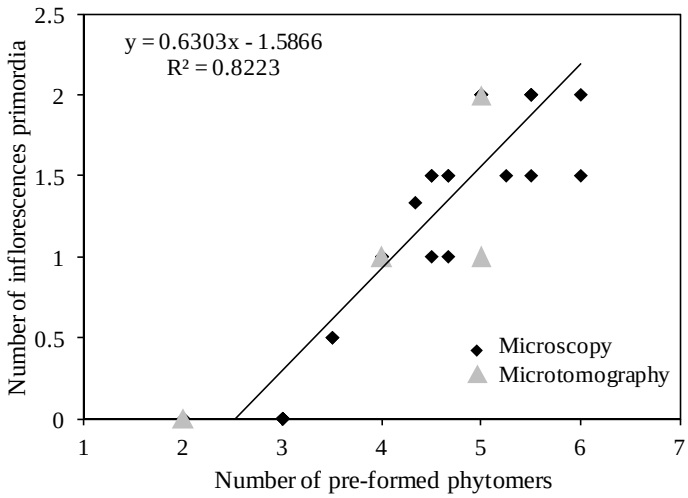

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