

## Cabernet Sauvignon grapevine grafted onto rootstocks during the autumn-winter season in southeastern Brazilian

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**ABSTRACT:** The change of grape (*Vitis vinifera*) harvest from summer to winter through double pruning management has improved the fine wine quality in southern Brazil. High altitude, late cultivar and grafting combination all need to be investigated to optimize this new viticulture management. For this purpose, this study was carried out during the 2011 and 2012 growing seasons in a high altitude region of the state of Minas Gerais, Brazil, using eight grafting combinations for five year old Cabernet Sauvignon vines. The stem water potential, photosynthetic rate and stomatal conductance were not affected by rootstock type. The rootstocks IAC 766 and 101-14 induced, respectively, the highest and lowest vegetative vigor in Cabernet Sauvignon, as shown by leaf area and pruning weight. In the 2011 growing season, the leaf chlorophyll contents were increased in IAC 766, whereas vines grafted onto 101-14 accumulated more leaf starch, probably due to reduced vegetative and reproductive growth. In general, rootstocks K5BB, 1045P, SO4 and IAC 766 had the highest yield as compared to 1103P and 101-14. Berries from the grapevine with the highest yield did not differ in pH, total soluble solids and acidity. The rootstocks did not influence the anthocyanins and total phenols in both growing seasons. Quality parameters were better in the 2011 than in the 2012 growing season due to better climatic conditions, mainly less rainfall. The best performance of Cabernet Sauvignon was achieved when grafted onto K5BB, 1045P, SO4 and IAC 766 rootstocks.

**Keywords:** double pruning, leaf carbohydrates, vegetative vigor, grape composition

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### Introduction

The southeastern region of Brazil has shown potential for the production of high quality wines during the winter growing season through double pruning management. This technique has improved grape (*Vitis vinifera*) quality by changing the period of harvest from wet and warm summer to dry and mild autumn – winter (Regina et al., 2011). To harvest wine grapes during the autumn – winter, the first pruning takes place in August and the second in January (Favero et al., 2011).

The first studies carried out in the state of Minas Gerais showed that the ecological conditions of the autumn-winter season, such as low rainfall and high thermal amplitude, are favorable for improving Syrah grape composition from grapevines grown under warm temperate (Favero et al., 2011) and tropical (Favero et al., 2010) climates in the south and north of the state, respectively. However, double pruning management had not been validated either for high altitude, or for late maturing cultivars, such as Cabernet Sauvignon. Furthermore, the rootstock effects also needed to be investigated to optimize the effects of double pruning management.

In viticulture, rootstocks are widely used and make a significant contribution to scion performance under several cultivation conditions. Although the mechanism for grapevine scion vigor controlled by rootstocks is poorly understood, several authors have shown effects on water relations (Souza et al., 2009), gas exchange (Soar

et al., 2006), vegetative vigor (Keller et al., 2001), yield and grape quality (Nuzzo and Mathews, 2006). Therefore, the selection of an appropriate rootstock may provide a powerful tool for managing the growth and fruiting of grapevine scions subjected to the double pruning technique.

The first study on rootstock effects implemented under double pruning management showed that Syrah grafted onto 1103 Paulsen had a better vegetative and reproductive performance as compared to SO4 and 110 Richter rootstocks (Dias et al., 2012). However, there is no information about the effect on grapevine performance by rootstock in a high altitude region. Our purpose in this study was to investigate rootstock effects on vegetative growth, water relations, gas exchange, leaf starch and chlorophyll concentration, yield and grape composition of field grown Cabernet Sauvignon vines during the autumn-winter growing season conducted under double pruning management in southern Brazil.

### Materials and Methods

#### Location and plant material

The study was carried out during two years (2011 and 2012) in an experimental vineyard located in Caldas, in the south of the state of Minas Gerais, Brazil (22°55' S, 46°23' W, 1,100 m a.s.l.). Five year old 'Cabernet Sauvignon' grapevines grafted onto 1103 Paulsen (1103P, *Vitis berlandieri* × *Vitis rupestris*), 101-14 Mgt (101-14, *Vitis riparia* × *V. rupestris*), SO4 (*V. berlandieri* × *V. ri-*

*paria*), Rupestris du Lot (R. DU LOT, *Vitis rupestris*), 1045 Paulsen (1045P, *V. berlandieri* × *V. rupestris*), 110 Richter (R110, *V. berlandieri* × *V. rupestris*), Kobber 5BB (K 5BB, *V. berlandieri* × *V. riparia*) and IAC 766 (*Ripária do Traviú* × *Vitis caribaea*) were evaluated in a completely randomized design with four replicates represented by five vines per parcel.

The rootstocks were chosen mainly on the basis of differences in vigor and genetic origin. The grapevines were spaced 1.5 m between vines and 2.5 m between rows, trained on a vertical shoot position (VSP) and were north-south oriented. All vines were spur pruned with two spur nodes, twice a year: the first pruning was carried out in Aug (2010 and 2011) and the second in Jan (2011 and 2012). The winter pruning (Aug) was carried out only to allow for vegetative growth, because all clusters were removed from the shoots. Although double pruning may reduce the carbohydrate reserves, the Aug pruning is really necessary to avoid bud infertility during the autumn-winter growing season. Ecophysiological, biochemical and agronomical measurements were performed after the summer pruning (Jan) and during the autumn-winter growing season.

#### Ecophysiological and biochemical measurements

During the ripening period, leaf gas exchange, air temperature and photosynthetic active radiation measurements were conducted on four fully mature and well-exposed leaves positioned in the middle of the main shoots (one leaf per treatment from one vine per parcel) with an infrared gas analyzer during the late morning. Stem leaf water potential ( $\Psi_{\text{stem}}$ ) was also measured in four leaves per treatment (one leaf per vine) from 10h00 to 11h00 using the pressure chamber method (Scholander et al., 1965).  $\Psi_{\text{stem}}$  was measured on non-transpiring leaves that had been bagged with both plastic sheet and aluminum foil for at least 1 h before measurements.

The leaf water potential was considered to be equal to the stem water potential when leaf transpiration was prevented (Choné et al., 2001). These measurements were taken during the ripening stage because it is a critical period for grapevines and the rootstock differences could be more evident during the stressful weather conditions of the winter season.

Leaf soluble (glucose, fructose and sucrose) and insoluble (starch) carbohydrate concentration were assessed on dried and powered leaf samples taken from four grapevines per treatment (one vine per parcel) during the flowering period. The carbohydrates were extracted from 100 mg samples with 80 % (v/v) ethanol (80 °C, 20 min) and centrifuged (9,160 × g, 15 min). The pellet was extracted twice and the combined supernatants (10 mL) used for the analyses of soluble sugars by the enzymatic method (Bergmeyer, 1974).

Glucose and fructose were measured by enzyme complex hexokinase/glucose-6P-dehydrogenase/phosphoglucosomerase, following the reduction of NADP

at 340 nm. The sucrose concentration was measured through quantification of resultant glucose from invertase-catalyzed hydrolysis of sucrose, after 1 h of incubation at 45 °C. The leaf starch was quantified in the pellet after hydrolysis with Alpha-amylase 120 L and Amyloglucosidase 300 L. Pellets were dried overnight at room temperature and 2 mL of Alpha-amylase (diluted 1:500 in water) were added followed by incubation at 75 °C for 1 h. After incubation with Alpha-amylase, 2 mL of Amyloglucosidase (28 unit mL<sup>-1</sup>, in sodium acetate buffer, pH 4.8) were added and kept at 50 °C for 1 h. The released glucose was quantified colorimetrically at 450 nm using glucose oxidase, peroxidase, and ABTS assays (Bergmeyer, 1974). Starch content was calculated as glucose × 0.9.

Leaf chlorophyll content was also determined during the flowering period. The fresh leaf discs of 3.14 cm<sup>2</sup> (one leaf disc per treatment) were collected and the chlorophyll pigments extracted with 80 % acetone and the concentration quantified spectrophotometrically following the method described in Arnon (1949). The flowering period was chosen for determining leaf starch and chlorophyll contents because it is at this phenological phase that the nitrogen and carbohydrates supply is known to be crucial for fruit set.

#### Agronomical measurements and berry quality

The effects of rootstock on vegetative vigor were evaluated by leaf area and pruning weight using eight vines per treatment (two vines per parcel). During the flowering phenological stage, the single leaf area was estimated using the equation  $y = 13.7904 - 1.4342x + 0.4145x^2$ , in which  $y$  is the estimated single leaf area and  $x$  is the sum of the lengths of the two main lateral leaf veins. The average shoot leaf area was calculated by multiplying the total number of leaves per shoot by the average single leaf area.

Average single leaf area was obtained from eight to ten leaves per shoot and the average shoot leaf area from four shoots per vine. The total leaf area per vine was calculated by multiplying the average shoot leaf area by the total number of shoots per vine. The pruning weight per vine was obtained at the end of the winter. All shoots per vine were pruned and dried in a forced air oven at 60 °C until constant weight was reached.

At harvest, the number and weight of clusters and the yield per vine were measured. Chemical analyses (soluble solids, pH and titratable acidity) were performed on the juice of pressed berries (four replicates of 100 berries per treatment) collected, at harvest, from all vines and representative of all cluster positions within the canopy and of all positions within the cluster. Soluble solids (°Brix) were determined using a handheld temperature compensated refractometer.

The pH of undiluted juice of each sample was determined using a pH meter and titratable acidity was determined by titration with 0.1 M NaOH to a phenolphthalein end point and expressed as g L<sup>-1</sup>. The antho-

cyanins and total phenolics in the berries were analyzed as described by Mota et al. (2011) using four replicates of 300 berries per treatment. Approximately 0.5 g of frozen powder skin was mixed for 1 min with an extraction solution of acidified methanol (HCl 1 %, v/v). The samples were kept overnight in the extraction solution in the dark at 4 °C. Then, extracts were separated by centrifugation) for 10 min at 7,441 g

The pellet was washed with the extraction solution until complete removal of the color. The supernatants were brought together in a 100 mL calibrated flask. Total monomeric anthocyanins were measured using the pH-differential method (Giusti and Wrolstad, 2001), using molar absorptivity = 28000 and molecular weight = 529. (Amerine and Ough, 1980). Total phenolics were determined in the same extract by the Folin-Ciocalteu assay, with absorbance readings at 760 nm, and total phenolic content was expressed in terms of gallic acid equivalents in milligrams per gram of fresh grape berry (Amerine and Ough, 1980).

### Statistical analyses

A completely randomized design was used and all data sets were subjected to analyses of variance (ANOVA). For leaf chlorophyll content, leaf area, pruning weight, yield and berry quality variables, a two way ANOVA was used with rootstock and date as the main factors. For leaf starch determinations a two way ANOVA per growing season was used with rootstock and time as the main factors and for soluble sugar evaluations one way ANOVA was used. For stem water potential and gas exchange variables a two way ANOVA per growing season was used with the rootstock and date as the main factors. Tukey HSD tests were carried out to determine differences between treatment means.

## Results and Discussion

During both growing seasons, the vegetative vigor of Cabernet Sauvignon was affected by rootstocks as shown by values of leaf area and pruning weight (Figure 1). The leaf area was reduced for rootstock 101-14 as compared to vines grafted onto IAC 766 (Figure 1A) whereas there was no difference between other rootstocks. On the other hand, the highest vegetative vigor shown by dormant pruning weight was observed for vines grafted onto IAC 766 and R. DU LOT as compared to 101-14, R 110 and 1103P in both years (Figure 1B).

It was possible to detect more differences between rootstocks by dormant pruning weight since this measurement is a seasonally integrated measurement of vine vigor and is proportional to leaf area carried on the shoots from the previous growing season (Smart and Robinson, 1991). These results were expected since IAC 766 and R. DU LOT are highly vigorous rootstocks (Alvarenga et al., 2002; Christensen, 2003) whereas 101-14 is frequently associated with low vigor scions (Christensen, 2003).

Although the way rootstocks interact with scions is still unclear, the different root anatomy, morphology, development and distribution among rootstock species may affect water and mineral absorption which can eventually affect shoot growth and modify grapevine physiology (Jackson and Lombard, 1993). On most of the sampled dates, the leaf water status was not affected by rootstock as shown by stem water potential ( $\Psi_{\text{stem}}$ ) data (Figure 2 A, B). However, in the 2012 growing season, on June 15<sup>th</sup> the  $\Psi_{\text{stem}}$  was higher on vines grafted onto IAC 766 as compared to K5BB, R110 and 1045P, whereas there were no differences between other rootstocks (Figure 2B). Despite this difference, in all sampled dates the values of  $\Psi_{\text{stem}}$

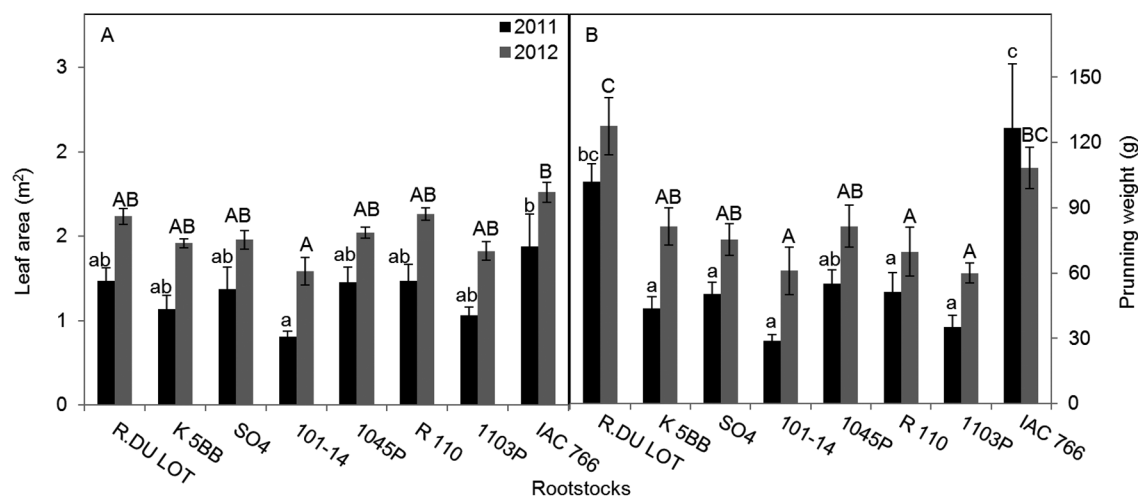


Figure 1 – Leaf area (A) and pruning weight (B) of field grown Cabernet Sauvignon grafted onto eight rootstocks during the winter growing seasons of 2011 and 2012 in Caldas, Minas Gerais, Brazil. Each point is the mean  $\pm$  standard error of eight replicates. Same lowercase (2011) and uppercase (2012) letter do not differ (Tukey test,  $p < 0.05$ ).

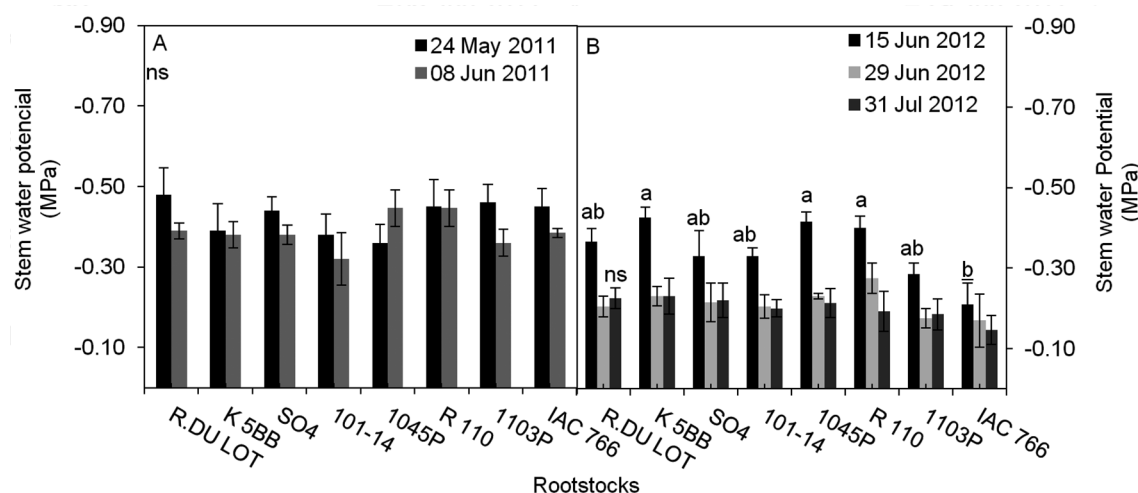


Figure 2 – Stem water potential of field grown Cabernet Sauvignon grafted onto eight rootstocks during the 2011 (A) and 2012 (B) winter growing seasons in Caldas, Minas Gerais, Brazil. Each point is the mean  $\pm$  standard error of four replicates. ns = no significant differences ( $p > 0.05$ ).

were higher than -0.5 MPa, indicating absence of water stress in all treatments (Leeuwen et al., 2009).

The photosynthetic rate and stomatal conductance were not affected by the rootstock during the ripening period in both growing seasons (Figure 3 A, B). The photosynthetic rates decreased in June 2011 and in July 2012, although there were also no solar radiation and diurnal temperature limitations (Figure 3 C, D). The reduction of the photosynthetic rates during the coldest months was probably due to the low night temperature in winter. The chilling stress during winter nights may have a

direct effect on carbon assimilation by impairing enzymatic activity of the Calvin cycle or inhibiting key enzymes in sucrose and starch biosynthesis (Flexas et al., 1999; Hendrickson et al., 2004).

The highest leaf chlorophyll content was induced only by IAC 766 in the 2011 growing season (Figure 4). The leaf chlorophyll may give an indirect estimation of the nitrogen status since considerable leaf nitrogen is incorporated in this pigment and, consequently, could optimize the photosynthetic and growth potential (Taiz and Zeiger, 1991). However, other factors may have contributed to increase the green mass of the Cabernet Sauvignon grafted onto IAC 766 since other rootstocks also induced high leaf area and pruning weight without differences in leaf chlorophyll and photosynthetic rate (Figure 3A).

Some differences in leaf starch levels induced by rootstock species in the 2011 growing season were detected (Figure 5 A, B). In general, there was a trend to increased starch accumulation in leaves of vines grafted onto the lowest vigorous rootstocks such as 101-14 and 1103P in both hours. However, there was interaction between rootstock and time, and only at the middle of the day was the amount of starch different in leaves of vines

grafted onto 101-14 as compared to other rootstocks (Figure 5A).

At the beginning of the morning, the largest differences were found between 1103P and SO4, IAC 766 and 1045P. The size, density and efficiency of the root system could also be involved in the regulation of shoot growth and starch metabolism since the subterranean growth of the grapevine is in balance with its aerial growth (Southey, 1992). Based on this relationship between rootstock rooting habit and its effect on scion growth, the low root density of the lowest vigorous grapevine rootstocks, such as 101-14 and 1103P, could be deemed to be inducing leaf starch accumulation in the scion (Southey, 1992; Christensen, 2003).

In general, as expected, leaf starch content was higher in the middle of the day than at the beginning of the morning. Our results corroborate those of Hunter et al. (1995) who reported that during the first part of the growth season (up to veraison) Cabernet Sauvignon leaves usually show an increase in starch accumulation from the morning to the afternoon. On the other hand, the soluble sugars quantified during the second growing season were not affected by rootstocks and only glucose and fructose showed the same diurnal pattern as leaf starch (Figure 5 C, D). In both periods sampled, the concentrations of leaf sucrose were higher than reducing sugars in all rootstocks. In the grapevine leaves, the most predominant sugar on a dry weight basis was sucrose (Grant et al., 2009), also considered the primary sugar transport via phloem to sink tissues (Taiz and Zeiger, 1991).

The components of yield were affected by rootstocks (Figure 6 A, B, C). The eight rootstocks used in this study could be classified into three groups based upon their productivity. In general, the lowest yield

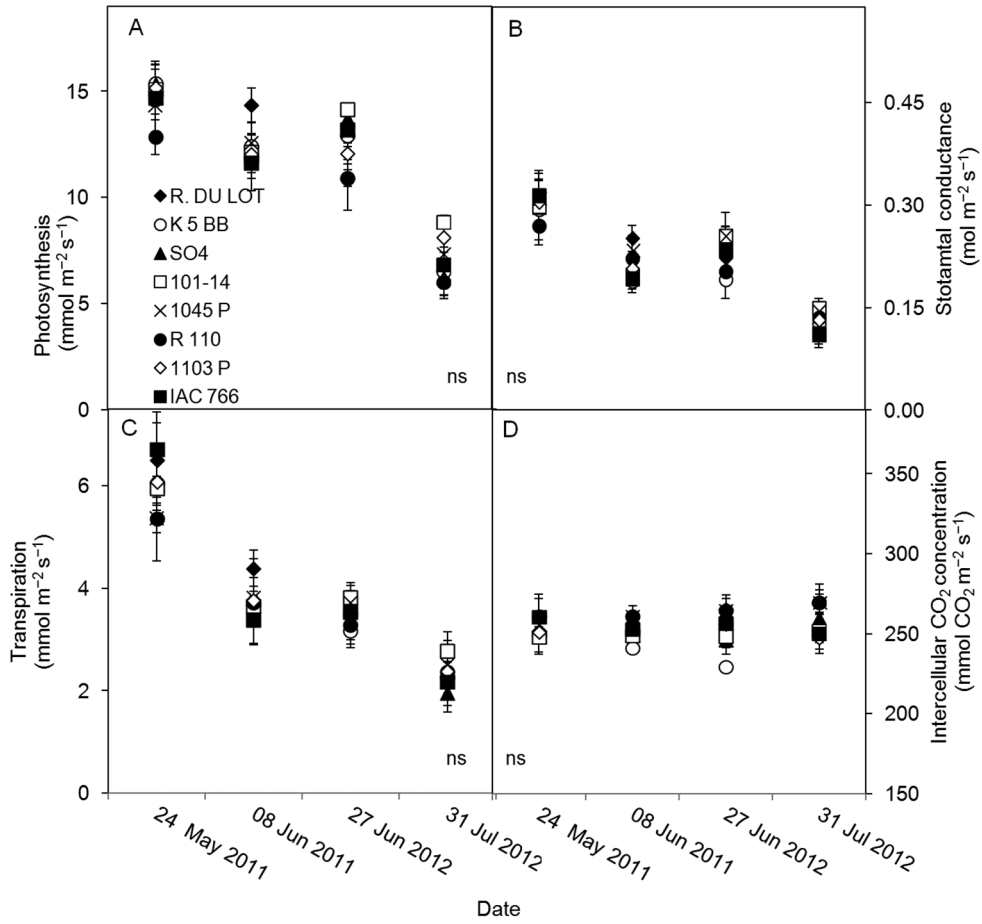


Figure 3 – Net photosynthesis (A), stomatal conductance to water vapor (B), photosynthetic active radiation (C) and air temperature (D) for field grown Cabernet Sauvignon grafted onto eight rootstocks during the ripening periods during the 2011 and 2012 winter growing seasons. Each point is the mean ± standard error of four replicates. ns = no significant differences (p > 0.05).

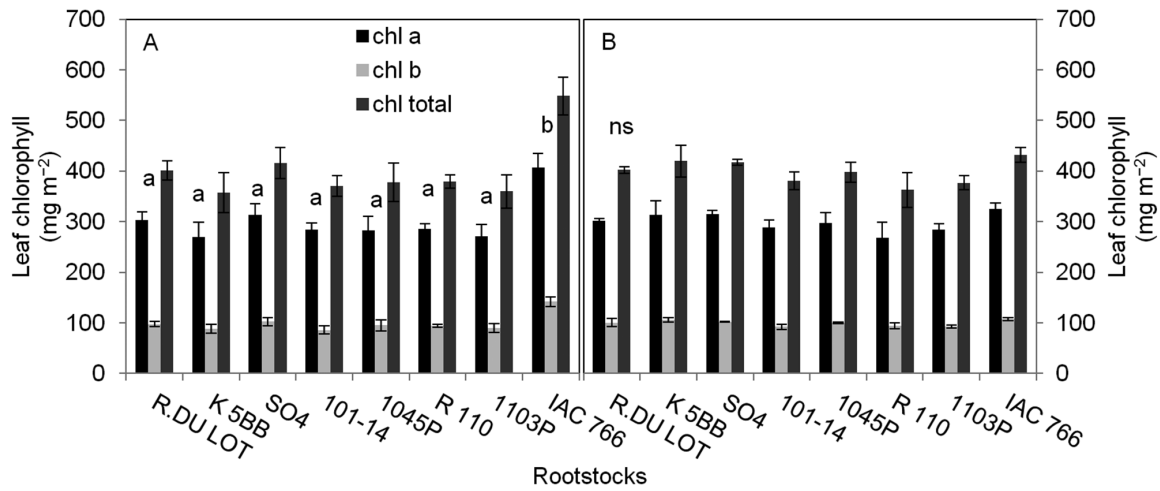


Figure 4 – Leaf chlorophyll content of field grown Cabernet Sauvignon grafted onto eight rootstocks during the winter growing seasons of 2011 (A) and 2012 (B) in Caldas, Minas Gerais, Brazil. Each point is the mean ± standard error of four replicates. Same lowercase (2011) and uppercase (2012) letter do not differ (Tukey test, p < 0.05). ns = no significant differences (p > 0.05).

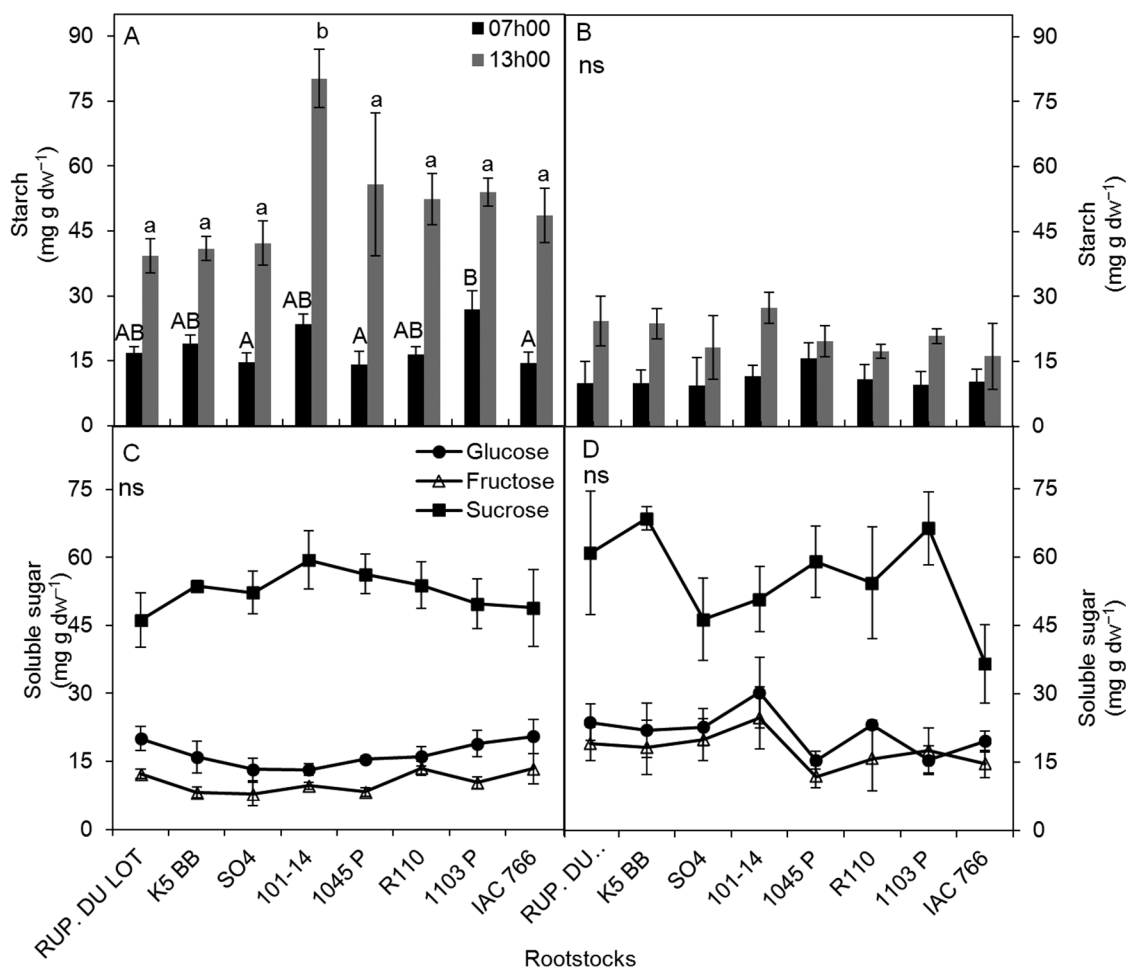


Figure 5 – Effects of rootstock on leaf starch concentration measured at 07h00 and 13h00 in the 2011 (A) and 2012 (B) winter growing seasons and leaf soluble sugars concentration at 07h00 (C) and 13h00 (D) during the 2012 winter growing season. Each point is the mean  $\pm$  standard error of four replicates. Same lowercase (13h00) and uppercase (7h00) letter do not differ (Tukey test,  $p < 0.05$ ). ns = no significant differences ( $p > 0.05$ ).

(cluster number and yield per vine) in both growing seasons were observed in vines grafted onto 1103P, 101-14 and R110. The rootstocks K5BB and 1045P had the highest yield whereas IAC 766, SO4 and R.DU LOT showed intermediate values (Figure 6 A). However, greater differences were found between the extremes. The major factors determining vine productivity in this study were cluster number per vine and, to a lesser extent, cluster weight (Figure 6 B, C).

Grapevines grafted onto K5BB, 1045P, SO4 and IAC766 had the highest cluster number (Figure 6 B), whereas only K5BB induced the most differences on cluster weight among grafting combinations (Figure 6 C). The effect of rootstocks was more pronounced on yield than on the fruit composition variables. In general, berries from grapevines with the highest yield showed no variation in pH, acidity and soluble solid sugars (Fig-

ure 6 D, E, F). The levels of these variables in the 2011 growing season are in the range usually found for Cabernet Sauvignon grapes harvested during the summer in the south of Brazil (Rizzon and Miele, 2002).

The highest berry acidity observed in the 2012 growing season was probably due to the highest rainfall (177 mm) that occurred during the ripening period as compared to the same period in 2011 (55 mm). Under excessive rainfall the ripening process is delayed or not completed owing to low solar radiation and high soil water availability affecting grape composition and wine quality (Jackson and Lombard, 1993; Deloire et al., 2004).

The rootstocks did not influence the concentration of grape phenolic compounds and the levels were higher in the 2011 than in the 2012 growing season due to the weather conditions (Figure 7 A, B). The concentration of anthocyanins and total phenols was similar

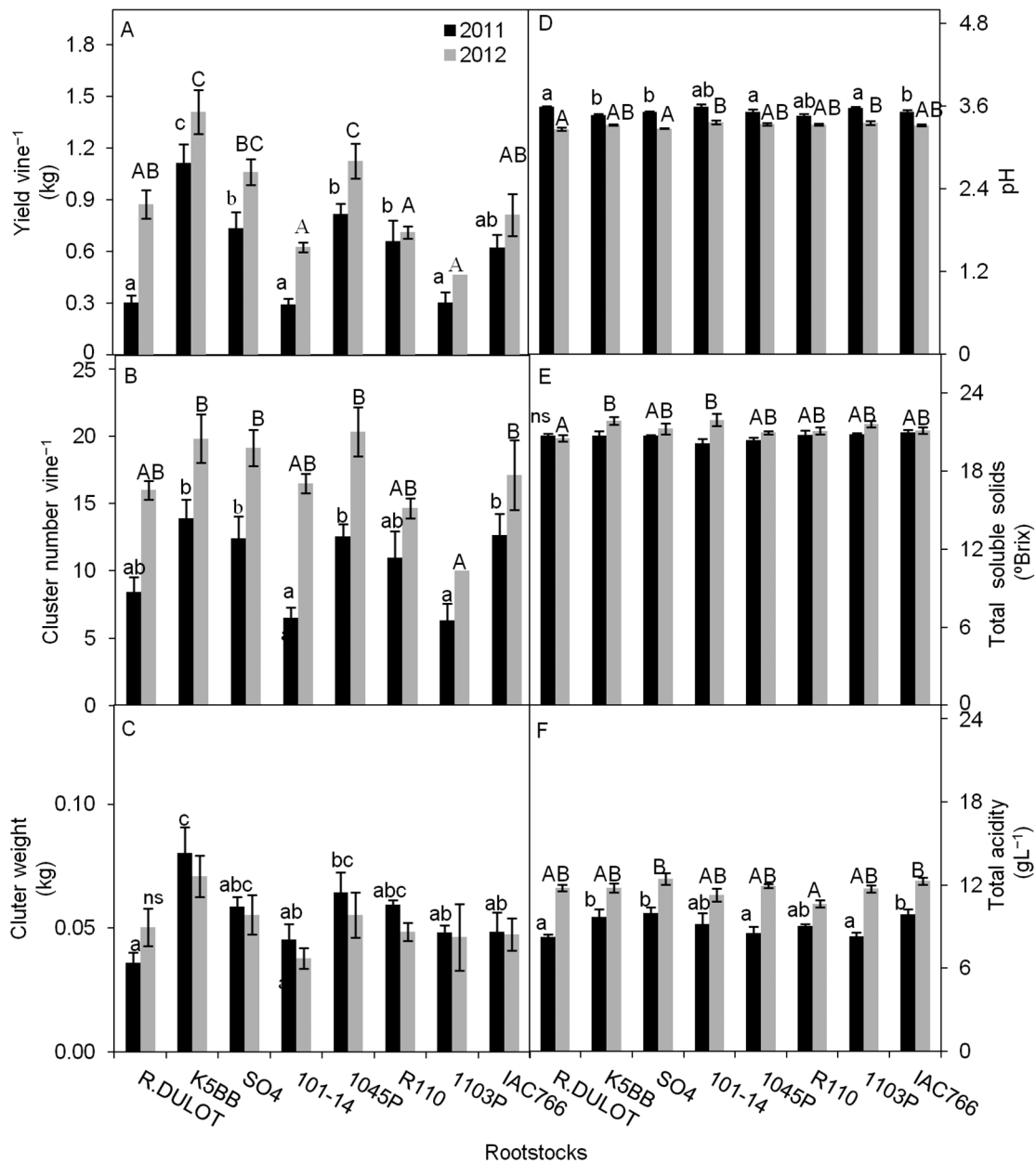


Figure 6 – Yield (A), cluster number (B) cluster weight (C), pH (D), total soluble solids (E) and total acidity (F) of field grown Cabernet Sauvignon grafted onto eight rootstocks during the 2011 and 2012 winter growing seasons, in Caldas, Minas Gerais, Brazil. Each point is the mean  $\pm$  standard error of four replicates. Same lowercase (13h00) and uppercase (7h00) letter do not differ (Tukey test,  $p < 0.05$ ). ns = no significant differences ( $p > 0.05$ ).

to the values observed for Syrah under double pruning management in a mild winter in Três Corações (21°41' S, 45°15' W), located in the south of the state of Minas Gerais (Dias et al., 2012).

Rootstock effects on grape composition and wine quality are not well known, but they are probably affected by rootstock vigor and, consequently, by its influence on growth and canopy exposure. Rootstocks that promote

low vegetative growth to the scion are desirable because low canopy vigor requires less labor inputs and increases light in the fruiting zone, leading to improvements in phenolics in red grape varieties (Jackson and Lombard, 1993). However, Cabernet Sauvignon has shown weak performance under double pruning management as compared to other cultivars such as Syrah and Sauvignon Blanc (Regina et al., 2011). Moreover, high vigor did not have a

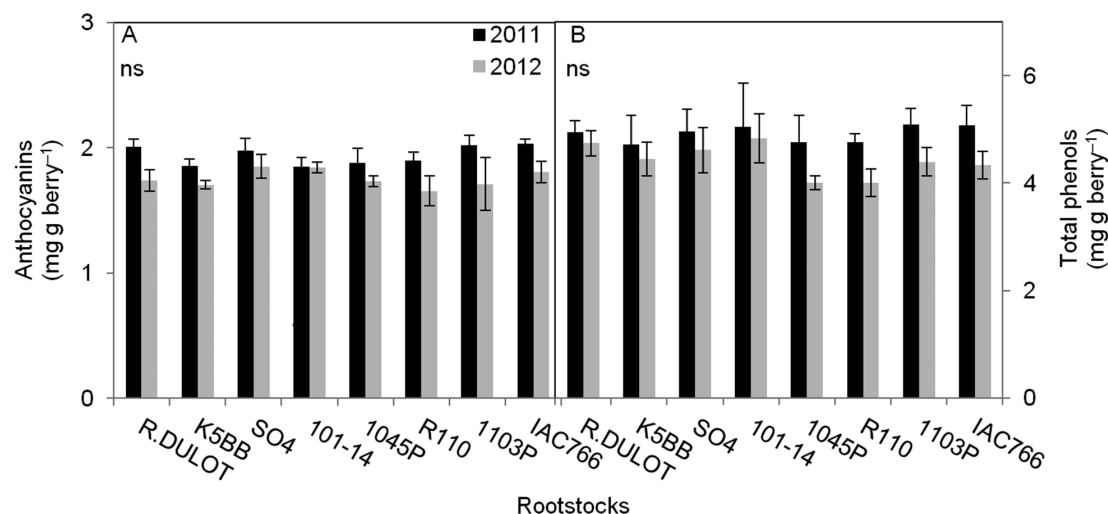


Figure 7 – Anthocyanins (A) and total phenols (B) in berries of field grown Cabernet Sauvignon grafted onto eight rootstocks, during the 2011 and 2012 growing seasons in Caldas, Minas Gerais, Brazil. Each point is the mean  $\pm$  standard error of four replicates. ns = no significant differences ( $p > 0.05$ ).

negative impact on grape quality in this study. From this perspective, the adoption of rootstock with the highest vegetative and reproductive vigor such as K5BB, 1045P, SO4, and IAC 766 can improve Cabernet Sauvignon performance under double pruning management for the harvesting of grapes during the winter. In general, as these rootstocks showed no significant differences in grape quality, their election should be based mainly on the yield in both growing seasons. We can therefore conclude that the use of the most suitable rootstocks could be an alternative for improving Cabernet Sauvignon performance during the autumn-winter cycle in the high altitude regions in the south of the state of Minas Gerais, Brazil.

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