REVISTA BRASILEIRA DE FRUTICULTURA
Published by the Brazilian Society of Fruit Crops
ISSN 0100-2945

Propagation - Original Article - Edited by: Virginia Silva Carvalho /Alexandre Pio Viana

In vitro callus co-cultivation strategy for prospecting growth regulators to induce *in vivo* grapevine graft union

Daniel Santos Grohs[1], Henrique Pessoa dos Santos[1], Paulo Vitor Dutra de Souza[2], Mikael Marcio Benati[3]

[1] Embrapa Uva e Vinho, Bento Gonçalves – RS, Brasil.
[2] Universidade Federal do Rio Grande do Sul, Porto Alegre – RS, Brasil.
[3] Instituto Federal do Rio Grande do Sul, Bento Gonçalves – RS, Brasil.
*Corresponding author: *daniel.grohs@embrapa.br*

Abstract: The use of growth regulators (GRs) in a mechanized bench grafting system is essential for the final quality of grafted grapevine plants. In Brazil, the GRs used (commercial waxes) are imported, increasing the production cost for nurseries. This work aimed to test the *in vitro* callus co-cultivation technique as a strategy for prospecting effective doses and mixtures of GRs for *in vivo* graft union on grapevines. Two genotypes were used as scions ('Bordo' and 'Riesling Italico') and two as rootstocks ('SO4' and 'P1103'). Callus from different scion/rootstock combinations were co-cultivated, varying the doses (0.25 to 20 mg l^{-1}) of auxins (DBA, IAA and IBA) and cytokinin (BAP), following the Doehlert design. The best *in vitro* treatments were validated *in vivo*, in plants grafted with an 'omega' cutter machine. Multivariate and non-parametric methods were applied to integrate the results. *In vitro* prospecting combined with the *in vivo* validation strategy allowed us to select effectively the IAA(2.50 mg l^{-1}) plus BAP(1.00 mg l^{-1}) formulation with the potential to induce graft callus union similar to commercial waxes. The induction intensity varied among the genetic combinations but was similar in the *in vitro* and *in vivo* conditions.

Index Terms: *Vitis* spp., micropropagation, grafting, phytohormones, nurseries.

Cocultivo de calos *in vitro* como estratégia na prospecção de reguladores de crescimento para indução da enxertia *in vivo* da videira

Resumo – A utilização de reguladores de crescimento (RC) no sistema de enxertia de mesa da videira são fundamentais para a qualidade final das mudas. No Brasil, os RC utilizados (ceras comerciais) são importados, aumentando o custo de produção para os viveiristas. Este trabalho teve como objetivo testar a técnica do cocultivo de calos *in vitro* na prospecção de doses e misturas de RC para indução da enxertia *in vivo* da videira. Foram utilizados dois genótipos como copas ('Bordo' e 'Riesling Italico') e dois como porta-enxertos ('SO4' e 'P1103'). Calos das combinações copa/porta-enxerto foram cocultivados, variando-se as doses (0,25 a 20mg l^{-1}) de auxinas

Rev. Bras. Frutic., v.46, e-522 DOI: *https://dx.doi.org/10.1590/0100-29452024522*
Received 27 Feb, 2023 ● Accepted 18 Dec, 2023 ● Published Mar/Apr, 2024. Jaboticabal - SP - Brazil.

(DBA, IAA e IBA) e citocinina (BAP), seguindo o delineamento Doehlert. Os melhores tratamentos *in vitro* foram validados *in vivo*, em plantas enxertadas com máquina de enxertia tipo 'omega'. Métodos multivariados e não paramétricos foram aplicados na integração dos resultados. A prospecção *in vitro* combinada com a validação *in vivo* foi eficaz na seleção da formulação IAA (2,50mg l^{-1}) mais BAP (1,00mg l^{-1}) com potencial para induzir o calo da enxertia de forma semelhante à cera comercial. A intensidade dessa indução variou entre as combinações genéticas de forma similar tanto na condição *in vitro* como na *in vivo*.

Termos de Indexação: *Vitis* spp.; micropropagação; enxertia; fito-hormônios; viveiros.

Introduction

In Brazil, vitiviniculture is distributed over an area of 74.826 ha (MELLO; MACHADO, 2021), ranking 19th in cultivated areas worldwide and 14th in grape production (MELLO, 2016). Despite being small compared to traditional grape-producing countries, Brazilian products have gradually been recognized by domestic and foreign markets (MELLO; MACHADO, 2021). Aiming at the sustainable growth of this sector, several practices are being improved, such as the production of grafted grapevine plants. Traditionally, in the preparation of grafted plants, grape growers used cuttings of scion cultivars and rootstocks collected directly from commercial vineyards (NACHTIGAL; SCHNEIDER, 2007). However, recently, nurseries have intensified the offer of commercially grafted grapevines, using cuttings collected from certified mother plants, with identity and sanitary control (GROHS et al., 2017). The number of grafted plants produced in commercial nurseries in Rio Grande do Sul, the largest grape-producing state in Brazil, grew by 22% between 2008 and 2015 (MELLO; MACHADO, 2017). This scenario is associated with both social aspects, due to the reduction of workers in the field, and technical aspects, such as the early death of plants due to fungal diseases when grafting is conducted in the field. Despite this advance, the grafting systems are still far from ideal in Brazilian nurseries, as they primarily adopt hand bench grafting. In contrast, in most traditional wine-producing countries, the mechanized 'omega' bench grafting system has been the most widespread and used since the 1960s (GROHS et al., 2017). Compared to hand bench grafting, the mechanical system results in fewer labor requirements, and higher yield and percentage of grafts with a uniform morphological pattern, thus improving the final quality of the grafted plants (WAITE et al., 2015).

To be considered functional, the graft union requires a vascular connection between the scion and rootstock, initialized from the formation of the callus bridge (JONARD et al., 1990). The callus is defined as an irregular mass of parenchyma cells at different stages of lignification (PINA et al., 2009). Callus formation (callogenesis) is determined especially by the balance of endogenous levels of phytohormones between the scion and rootstock. Among phytohormones, the relationship between auxin and cytokinin is the most important, since any imbalance between these two phytohormones induces an incomplete callus bridge, restricting the vascular connections of the xylem and phloem (NANDA; MELNYK, 2018; BARON et al., 2019). For this reason, in the mechanized bench grafting system, the use of paraffin with waxes rich in growth regulators has been recommended to modulate the balance between auxins and cytokinin (WAITE et al., 2015; ZHOU et al., 2020).

In general, these commercial waxes are produced in Europe and are mainly enriched with an auxinic growth regulator (DBA, 2,5-dichlorobenzoic acid). In addition to being expensive, the grafting quality of these imported commercial waxes exhibits variable results in Brazil due to the different genotype combinations used as a scion (S) and rootstock (R). As an example, using the omega cutter machine and commercial waxes enriched with growth regulators, Regina et al. (2012) obtained grafted plants productivity ranging from 8 to 85% for the European cultivar Chardonnay, grafted on thirteen different rootstock varieties. These

indexes tend to vary even more when considering the diversity of scion cultivars that are grafted in Brazil, involving genotypes of *Vitis vinifera*, *Vitis labrusca*, and hybrids. This diversity is also observed among rootstocks, as there are genotypes with high rooting capacity and grafting affinity to recalcitrant genotypes in both processes (MAYER et al., 2006). Similar results were also reported in Bulgaria, where Iliev et al. (2014) used waxes enriched with auxins from France and Spain and obtained a viable grafted plant yield between 90% and 66%, respectively, with local grape cultivars. Therefore, these results show that the benefits of formulations with growth regulators depend on the scion/rootstock combinations of each location and must be adjusted to achieve maximum efficiency in the production of grafted plants with mechanized grafting.

Despite the practical importance of these formulations for the grafting quality and yield of quality grafted plants, it is noteworthy that tests for prospecting types, doses and mixtures of growth regulators are very complex and costly regarding time, space and resources. This fact is evident in the design of *in vivo* tests based on complete factorials, which demand costly experiments, with many interactions (GABRIELSSON et al., 2002). To circumvent this problem, *in vitro* tests (tissue culture) have been performed in other cultures and presented as an effective strategy for prospecting chemical compounds with high analytical performance while demanding less space and time (MATOS, 2009). Among the *in vitro* techniques, the callus co-culture between species or cultivars has been used successfully in anatomical (PINA et al., 2009) and molecular studies (LIU et al., 2018). Co-cultivated callus consists of non-differentiated agglomerated tissues, obtained from vegetative explants, with physiological uniformity and closer to the pure genetic effect (JONARD et al., 1990). Traditionally, co-culture trials are important tools in studies for predicting and characterizing graft incompatibility in fruit species (ERREA et al., 2001; PEDERSEN, 2006). In these studies, full callus fusion requires adjusting the culture medium, especially types and doses of growth regulators. This *in vitro* approach is innovative and there are no records in the world literature on the application of this technique for adjusting the management of grapevine grafting. Therefore, this work aims to adjust and test the viability of the *in vitro* callus co-culture technique in the preliminary prospection of effective formulations with growth regulators (GRs) for mechanized grapevine grafting systems.

Materials and Methods

This study adopted the coupled strategy: stage 1: *in vitro* prospection of GR candidate formulations in two experimental steps, and stage 2: *in vivo* validation of GR candidate formulations in one step. The study was conducted in Bento Gonçalves, Brazil, in 2018 and 2019. Each experimental step defined the treatments that should be carried on to the next step, up to the *in vivo* validation. The experimental steps conducted in each stage are described in the following sections.

Stage 1. *In vitro* trials

The callus used in the trial were obtained by the *in vitro* co-culture technique. First, the *in vitro* culture of isolated callus was obtained for each genotype, following a protocol adapted from Khan et al. (2015). Cuttings were collected from the mother plants of each cultivar in the dormancy phase (July) and rooted in pots until budburst (Figure 1A). Individual nodal segments were obtained from the shoot apical tips (≈10 mm) (Figure 1B) and placed in tubes (five segments per plant on average, Figure 1C) with 12 mL MS medium (Murashige and Skoog 1962), supplemented with 30 g L^{-1} sucrose and 2.0 mg l^{-1} 6-benzylaminopurine (BAP), and solidified with 0.6% agar (pH 5.8). The segments were kept in a growth chamber at 23 ± 2°C and 16-h photoperiod until the basal callus was formed (Figure 1D). After this period, the callus at the basal end was removed and stored in the same medium described above until the co-culture trial. For the trials, 3 to 5 mm tissues were removed from the internal part of the callus (Figure 1E) following the methodology described by Jonard et al. (1990).

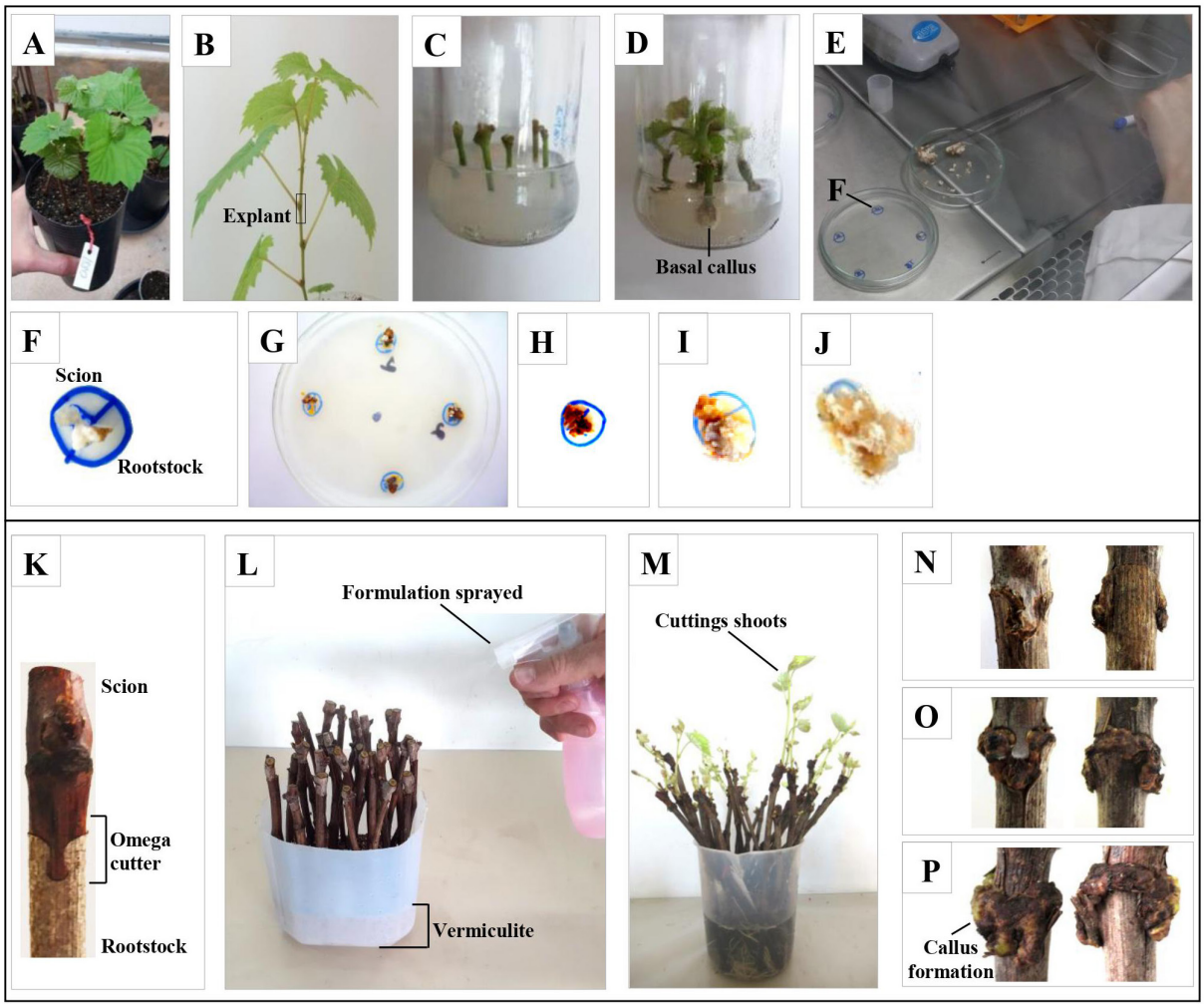

Figure 1. Details of *in vitro* callus co-cultivation for grafting compatibility tests with different scion/rootstock combinations and treatments with growth regulators: cuttings from mother plants for explant production (A); developmental stage for explant removal (B); *in vitro* establishment of explants in medium for basal callus induction (C); basal callus formation (D); removal of the basal callus from the scion (S) and rootstock (R), placing together in same plate with the specific medium for each treatment (growth regulator) (E); detail of scion/rootstock callus co-cultivation (F); plate with scion/rootstock callus fusion, recorded after 30 days of co-cultivation (G). Three levels of callus fusion compatibility: incompatible (H), partially compatible (I) and compatible (J). Details of *in vivo* grafting: omega cutter for scion/rootstock union (K); first application of candidate formulations in treatments not submitted to paraffinization (L). After 30 days of forcing (acclimatization period) (M), three callus formation rate around the graft union (frontal and lateral views) in treatmens not submitted to paraffinization: low (N), moderate (O), and high (P).

To start the co-culture, the callus pieces from the scion and rootstock were placed together in the same medium described above (but without BAP), with or without a GR according to treatment (Figure 1F-1G). The trials were carried out for 30 days in a growth chamber at 28 ± 1°C, without additional light. The growth conditions were based on the settings used as standard during forcing grafted cuttings (*in vivo*), seeking to emulate those in which the prospective formulations were validated. It is noteworthy that, in the following two steps, the experimental unit consisted of each callus scion/rootstock combination, with eight replications per treatment.

Step 1.1. Obtaining GR formulations

The trial was conducted on fusion callus obtained from the 'Riesling Italico' (*Vitis vinifera*) scion on 'SO4' (*Vitis berlandieri x Vitis riparia* hybrid) rootstock, abbreviated as R/S. This R/S combination is economically important in wine-producing countries, including

Brazil, while the grafting capacity and grafted plant yield have been historically classified between low and moderate. (VRŠIČA et al. 2015). As there are no reports of specific formulations of culture medium for fusion callus in the R/S, the GRs and reference doses were obtained from isolated callus induction works in different cultivars (CLOG, 1990; MII et al., 1991; SRNGH; BRAR, 1993; JIMÉNEZ; BANGERTH, 2000; AAZAMI, 2010; KHAN et al., 2015; KUMSA, 2017). From these protocols, the following list of GRs to be tested was established: IBA (indolebutyric acid), IAA (indoleacetic acid), DBA (2.5-dichlorobenzoic acid), BAP (6-benzylaminopurine). For the test of each GR, the Doehlert design (GABRIELSSON et al., 2002) was applied, starting from the reference dose and progressively increasing, considering the minimum and maximum limits of the most effective dose of the previous trial (details in Figure 2). Thus, the tests were progressively repeated in time, until reaching the adjusted dose of each GR (GR formulation), defined as the greatest callus fusion concerning the CONTROL (without the addition of GR in the culture medium).

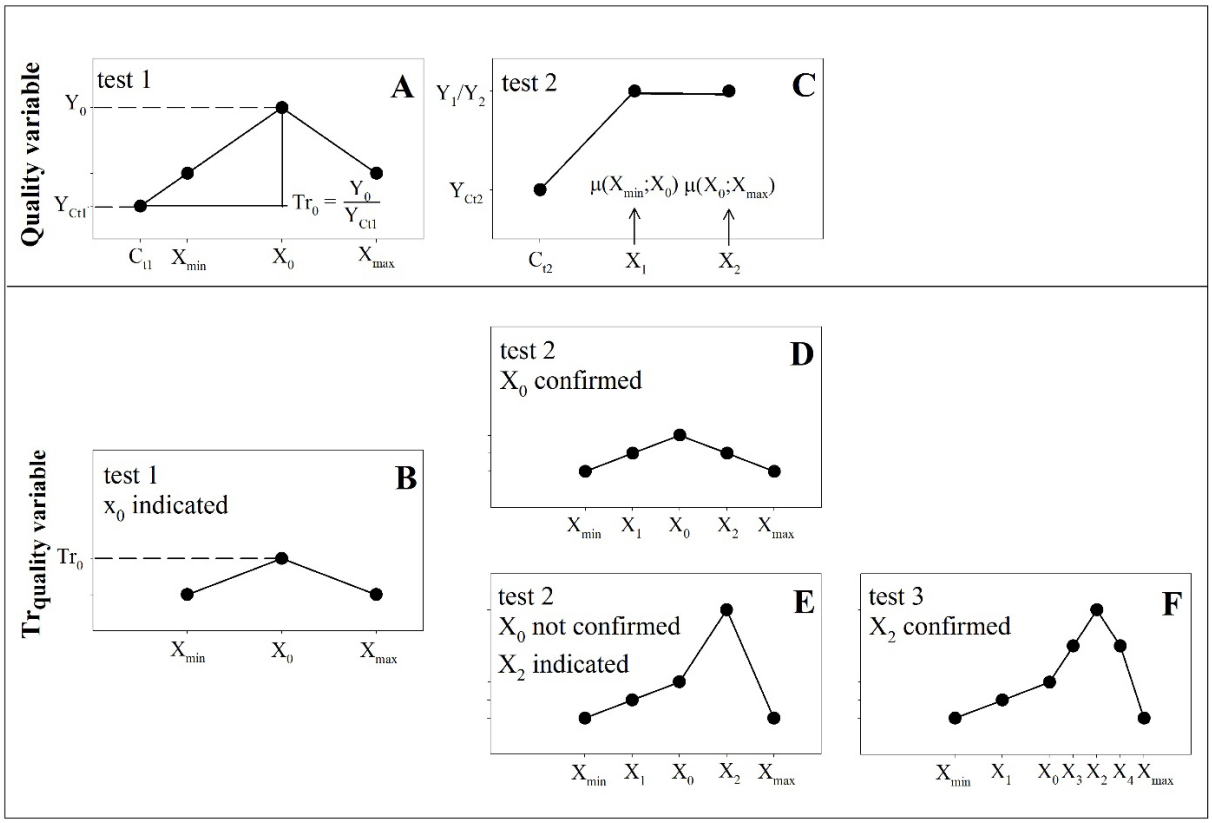

Figure 2. Example of sequential steps for analyzing the response of a given callus variable *in vitro* for the obtaing GR (growth regulator) formulation (dose adjustment), following Doehlert's design: first test - three initial doses (x) were used, which were selected from literature data: minimum dose (x_{min}), average dose (x_0) and maximum dose (x_{max}) plus CONTROL (C_{t1}) (without added GR in the culture medium) (A); $Tr_{quality\ variable}$ obtation (Tr_0), considering the responses of the different doses of the first test compared to the CONTROL, with x_0 being selected as the optimal dose (B); second test - analysis of two new doses close to x_0 (x_1 and x_2) (C) to confirm if x_0 remains the optimal dose (D) or if there is an indication of a new optimal dose (x_2) (E); third test - applying two more doses close to x_2 (x_3 and x_4), ending the test by confirming x_2 as the optimal dose (F). This is just a sequence of tests, as an example, and if the optimal dose is not clearly confirmed, new rounds are necessary, following the same test design.

Step 1.2. Obtaining GR candidate formulations

The GR formulation (obtained from the results of step 1.1) were mixed (auxin plus cytokinin), aiming to identify possible synergistic or additive effects in the R/S callus co-culture. In parallel, these formulations were also applied to other genetic scion/rootstock

combinations to verify the breadth use. The most promising composite formulations (dose and mixture) in all genetic combinations were named GR candidate formulation to validate in the stage 2.

The treatments were applied following a completely randomized design arranged as a factorial scheme, with factor 1 (formulation): a) isolated GR formulations and b) mixed GRs formulations; factor 2 (genetic combinations): a) R/S; b) 'Riesling Italico' (*Vitis vinifera*)/'P1103' (Paulsen 1103, *Vitis berlandieri* X *Vitis rupestris*), abbreviated as R/P; c) 'Bordo' (*Vitis labrusca*)/'SO4' (*Vitis berlandieri* X *Vitis riparia*), abbreviated as B/S and d) 'Bordo'/'P1103', abbreviated as B/P.

Stage 2. *In vivo* trials

The GR candidate formulations (obtained from the results of step 1.2) were validated in the cuttings grafting step (*in vivo*) to select the ideal formulation to improve the quality of the graft union. The mechanized grafting process, summarized below, was based on the protocol defined by Regina (2002) and Waite et al. (2015). Cuttings of dormant hardwood were collected in July, wrapped in plastic film, and stored in a cold chamber (3 ± 1ºC) for 60 days until grafting, using a semi-automated omega cutter machine (Figure 1K). Then, the grafted cuttings were placed in boxes with a 3cm layer of moistened vermiculite on the bottom (Figure 1L) and wrapped with plastic film to keep humidity above 85%. The boxes were placed in a forcing chamber for 30 days, without lighting, between 27 and 28°C. The grafted cuttings from the scion/rootstock combinations submitted to the STANDARD formulation were treated with commercial wax containing 0.01% oxyquinoline and 0.00175% DBA, which was previously melted (67°C) and applied right after grafting by immersing grafts in the wax (1 to 2s), followed by the same forcing conditions. The grafted cuttings treated with the GR formulations were not submitted to paraffinization. The doses of each GR formulations were previously dissolved in 1 M NaOH (for auxins) and 1 M HCl (for cytokinins), diluted in 1 L distilled water, and kept under refrigeration (± 3ºC). Starting on the grafting date (winter bud stage), each GR formulation was sprayed onto the grafted cuttings, in the boxes (Figure 1L), at three-day intervals throughout the forcing period.

The treatments were applied following a completely randomized design arranged as a factorial scheme, with factor 1 (formulations): a) GR candidate formulations, b) STANDARD and c) CONTROL (without candidates or STANDARD formulations); factor 2 (genetic combinations) (the same ones used in step 1.2). It is noteworthy that isolated and mixed formulations were validated only for the R/S genetic combination, while only those defined as candidates were validated for the all genetic combinations (R/S, R/P, B/S and B/P). Each genetic combination was represented by 30 replicates per treatment (grafted cutting was considered as the experimental unit.).

Assessment and statistical analysis

After 30 days of *in vitro* co-culture, callus quality was evaluated by the following five variables: (1) degree of callus fusion (JONARD et al., 1990), scored from 1 = non-fused callus (Figure 1H), 2 = incomplete fusion (Figure 1I) to 3 = complete fusion (fused) (Figure 1J); development of the (2) scion and (3) rootstock callus, scored from 1 = no development (Figure 1H), 2 = moderate (Figure 1I) to 3 = high (Figure 1J); (4) color (adapted from SRNGH; BRAR, 1993), determined by the percent without oxidation, where the high percentage values represent a more solid, white, and shiny mass of fused callus (Figure 1J); and, (5) mass of the co-fused callus (mg). After 30 days of *in vivo* forcing during the acclimatization period (five days) before planting (Figure 1M), the quality of the grafted plants was evaluated by five variables: percentage of plants with (1) visible graft callus union; (2) with developed shoots (budburst stage); (3) callus formation rate around the graft union (%) (CELIK, 2000), scored from low (below 25%) (Figure 1N), moderate (between 25%

and 75) (Figure 1O) to high (between 75 and 100%) (Figure 1P); (4) callus diameter (mm) at the graft union; and (5) phenological stage of shoots (EICHORN; LORENZ, 1977).

The statistical analysis was based on meta-analysis concepts. Thus, one indicator was generated to aggregate quality variables. The 'Tr indicator' (treatment response) was adapted from Lajeunesse (2011) and characterizes the performance of each formulation on the fusion quality of callus or grafted plants, compared to the CONTROL. First, '$Tr_{quality\ variable}$' was obtained (exemple in Figure 2A and 2B). The absolute value of a quality variable for a given formulation was divided by the absolute value in the CONTROL of the same genetic combination. Subsequently, the average of all '$Tr_{quality\ variable}$' was calculated to obtain the 'Tr indicator' for each formulation. 'Tr indicator' value above zero and close to 1 characterized the high capacity of the formulation to induce and fuse callus *in vitro* (called Tr_{vitro}) or to induce grafting callogenesis and union in plants (called Tr_{vivo}) of a scion/rootstock combination.

After calculating the Tr_{vitro}, a multivariate exploratory analysis was performed by applying the Principal Components Analysis (PCA) method. To adjust the Tr_{vitro} values to the PCA, these were mean-centered, integrating the scores of GR with the quality variable loadings. For the contrast of independent groups in the PCA spatialization, multiple standard deviation bars (± 2σ) were adjusted. The groups that did not overlap vertically or horizontally were considered distinct. The multivariate analysis was performed using the ChemoStat software (HELFER et al., 2015).

Besides, tests were applied to explore, contrast and relate the quality variables with Tr_{vitro} and Tr_{vivo}. Therefore, after checking data normality using the Kolmogorov-Smirnov ($p<0.05$), the non-parametric Chi-square test ($p<0.05$) was applied, followed by the Kruskal-Wallis of independent samples (for comparison of means), Q-Cochran of related samples (for comparison of frequencies) and Spearman (for correlation analysis). Statistical tests were performed using R software, Agricolae and Kendall packages (R CORE TEAM, 2023).

Results and Discussion

In general, the *in vitro* callus co-cultivation strategy was deemed an effective tool for a broad prospection of GR formulation that may be useful for the *in vivo* grafting of different grapevine genotypes. Until the writing of this work, the literature references on callus co-culture are found only for other species, such as *Citrus* spp. (JONARD et al., 1990) and *Prunus* spp. (ERREA et al., 2001; PEDERSEN, 2006; PINA et al., 2009), but not for grapevines. In addition, these references do not address the use of callus co-culture for prospecting GR types, doses, or mixtures to increase callus fusion and, consequently, the quality of callogenesis in plant grafting.

The co-cultivation assays (step 1.1) showed that the Doehlert design (Figure 2) provided a maximum response for the callus fusion values compared to the CONTROL with a small number of tests for each compound (from 10 to 12) (Figure 3). The Doehlert design has already been successfully applied for selecting new chemical drugs (MATOS, 2009) and this work showed that it can be an effective strategy in the prospection of GR formulations under *in vitro* conditions. The optimal doses of each GR were easily characterized with Tr quality variables (Tr_{mass}, Tr_{fused}, Tr_{color}, $Tr_{rootstock}$, Tr_{scion}) employing a multivariate principal component analysis (PCA) (Figure 3). This combined approach (design, meta-analysis indicator and multivariate method) avoids applying more costly full factorial experiments, whose data evaluation and interpretation involve a large number of interactions and complexity (GABRIELSSON et al., 2002).

The total variance of the *in vitro* results was explained by PC1 (58.87%) and PC3 (16.12%). According to their responses compared to CONTROL, three independent clusters were spatially constituted: low (negative scores on PC1), moderate (intermediate and positive scores on PC1), and high (positive and higher scores on PC1) effect (Figure 3).

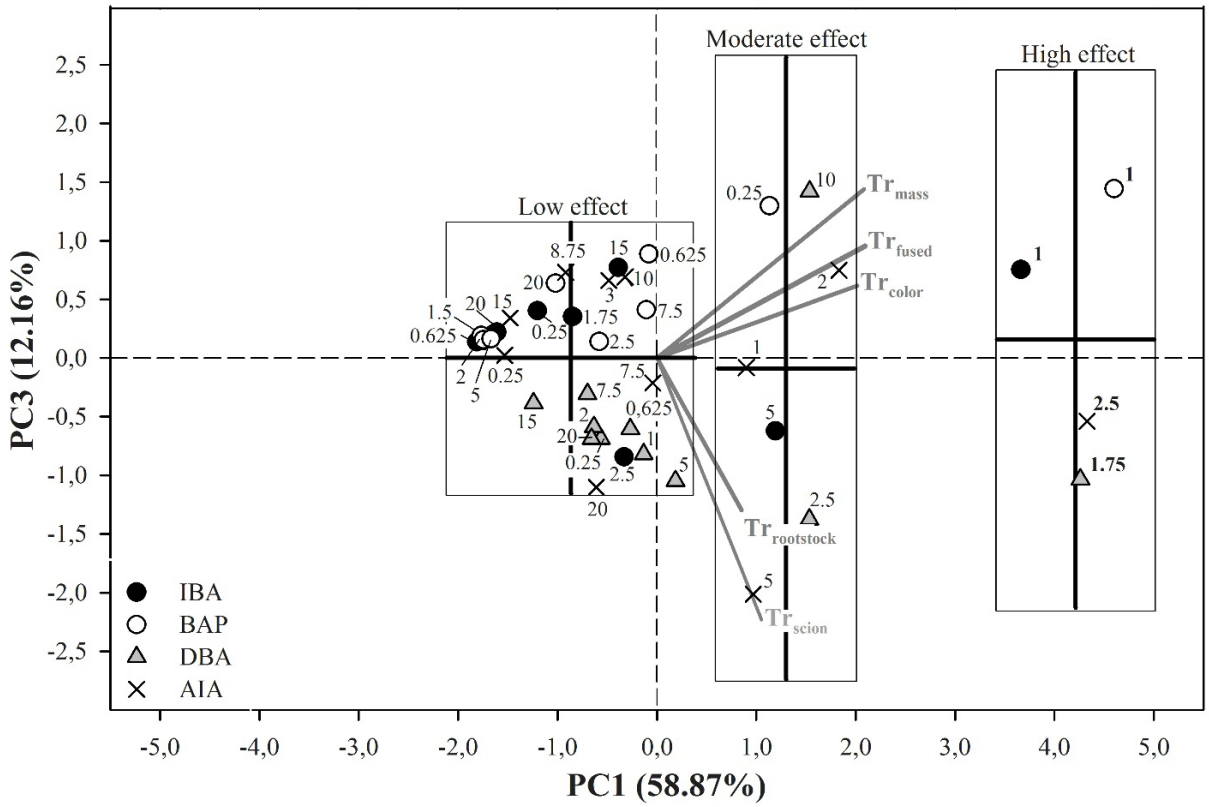

Figure 3. Principal component analysis (PCA) of $Tr_{quality\ variables}$: scion (Tr_{scion}) and rootstock ($Tr_{rootstock}$) development, color (Tr_{color}), fusion degree (Tr_{fusion}) and mass (Tr_{mass}) of 'Riesling Italico' and 'SO4' callus co-cultivated. Callus co-fusioned was treated with doses ranging from 0.25 to 20 mg l^{-1} of DBA, IAA, IBA and BAP. Non-overlapping multiple standard deviation bars (±2σ), indicate discrimination between doses with different effect levels in the fusion callus quality.

Can et al. (2014) reported that high concentrations of growth regulators in a culture medium can inhibit cell division and biochemical reactions, inducing explant death. This fact was observed in clusters with low and moderate effects, where callus fusion was limited (doses lesser than 1.00 mg l^{-1}) or inhibited by the phytotoxic effect (doses from 5.00 to 20.00 mg l^{-1}) of DBA, IAA, IBA and BAP. Therefore, the doses in the low and moderate effect groups were disregarded for the following steps, but the doses of IBA and BAP (1.00 mg l^{-1}), DBA (1.75 mg l^{-1}) and IAA (2.50 mg l^{-1}), grouped with better effect in step 1.1, were selected (GR formulations) for to step 1.2.

Detailing PC3, while BAP and IBA were associated with mass, degree of fusion and callus color, IAA and DBA were more related to the development of scion and rootstock callus (Figure 3). Indeed, these compounds have been previously reported to effectively induce isolated callus formation in *Vitis* spp. (MII et al., 1991; KHAN et al., 2015; KUMSA, 2017). Both auxins and cytokinin at adjusted doses trigger callus cell proliferation (JASKANI et al., 2008). Auxins containing culture medium change the gene expression of callus cell mass and the dose defines the continuity of globular growth or cell differentiation (JIMÉNEZ, 2001). Whereas cytokinin promotes the synthesis of RNA and proteins in the callus, increasing the enzymatic activity related to cell division and the loosening of the cell wall (KHAN et al., 2015). Highlighted, among the compounds used for *in vitro* tests, some GR traditionally used in tissue culture protocols were not tested in this study for being inadequate for *in vivo* use. The 2,4D was disregarded for being toxic to humans and the environment (JUNIOR et al., 2002). Likewise, despite not being an auxin traditionally used for *in vitro* callus cultivation, DBA was tested since it is commonly used as a component of the main commercial grafting waxes (WAITE et al., 2015; LIU et al., 2018).

In the selection of candidate GR formulations (step 1.2), approaching contrasts between isolated or mixtures of GR formulations, the mixed of IAA(2.50 mg l^{-1}) plus BAP(1.00 mg l^{-1}) (abbreviated IAA+BAP) and DBA(1.75 mg l^{-1}) plus BAP(1.00 mg l^{-1}) (abbreviated DBA+BAP) were observed to provide additive and superior effects over isolated applications of each compound (Figure 4A-D). The effects of these mixtures were more expressive on callus color (Tr_{color}) and callus development of the rootstock ($Tr_{rootstock}$), especially in grafting combinations on 'P1103' (Figure 4B and 4D). Moreover, to a lesser extent, the same trend can be observed with the 'SO4' combinations (Figures 4A and 4C).

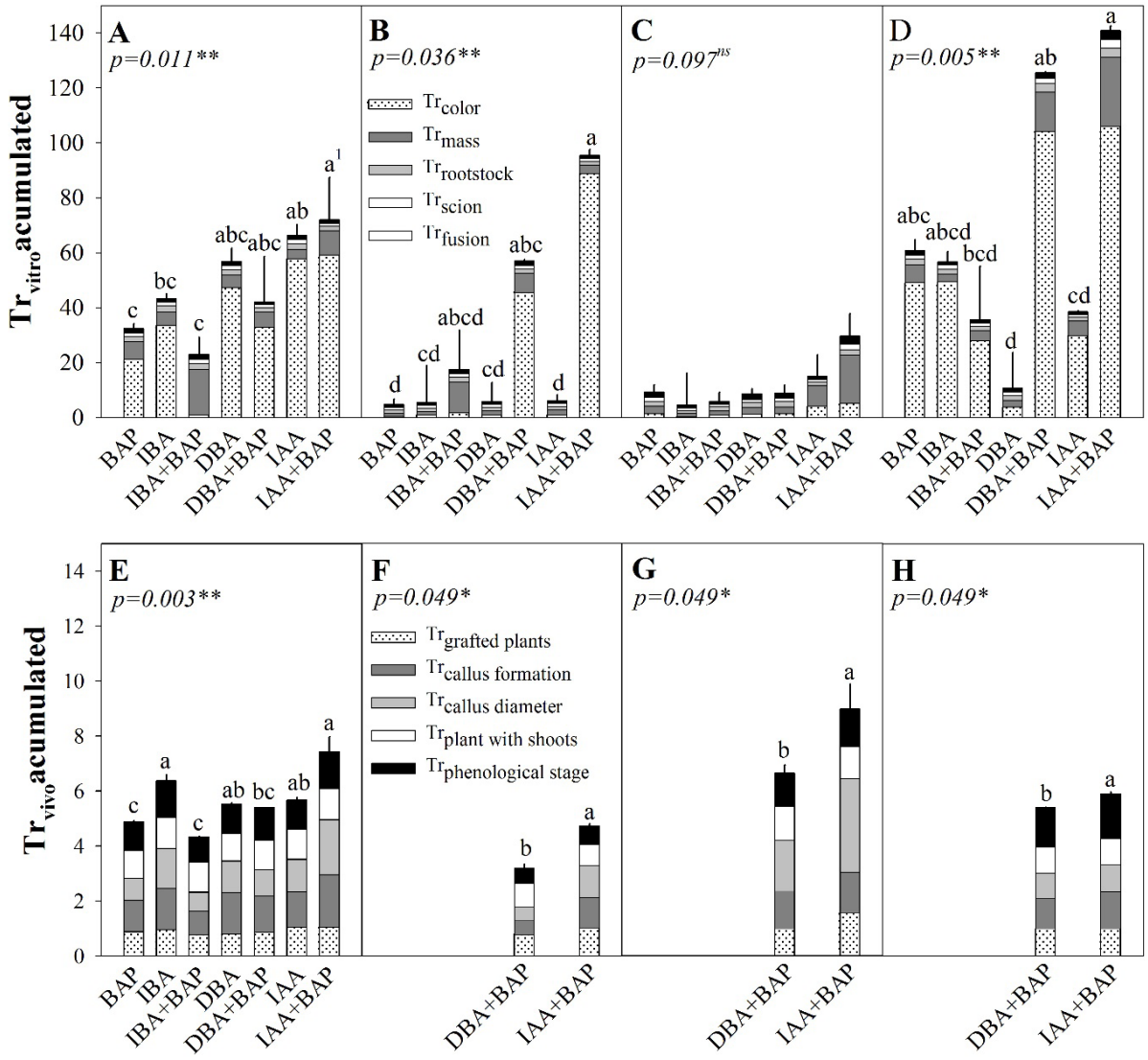

Figure 4. Tr_{vitro}accumulated for the five $Tr_{quality\ variables}$ (sum of $Tr_{quality\ variables}$) of callus fusion (A, B, C and D) and Tr_{vivo} accumulated for the five $Tr_{quality\ variables}$ of grafted plants (E, F, G and H), for growth regulators formulations (mg l^{-1}): BAP(1.00), IBA(1.00), IBA(1.00)+BAP(1.00), DBA(1.75), DBA(1.75)+BAP(1.00), IAA(2.50) and IAA(2.50)+BAP(1.00) in the following genetic combinations: (A, E) 'Riesling Italico'/'SO4'; (B, F) 'Riesling Italico'/'P1103'; (C, G) 'Bordo'/'SO4'; (D, H) 'Bordo'/'P1103'. [1]Same letters (± two standard error bars) do not differ statistically by the Kruskal-Wallis test ($p<0.05$). [ns]not significant. GR candidate formulations (F, G, H) selected in 1.2 step and validaded in stage 2.

In the metabolism of plant tissue damage repair, such as cuttings for grafting, auxin always acts in combination with cytokinin for the callogenesis process (NANDA; MELNIK, 2018), which corroborates this synergistic effect of the mixtures compared to isolated applications. Srngh and Brar (1993) also reported that in grapevine tissue culture, the isolated use of auxins favors high cell proliferation, but the callus produced are friable

and unstable over time. However, when associated with BAP, the resulting callus are more compact since this cytokinin favors lignification through the phenylpropanoid pathway (KAPARAKIS; ALDERSON, 2003). This combined and synergistic effect requires the right balance between the doses of both GR, with BAP generally being used in a lower proportion than auxin (KHAN et al., 2015; MASTUTI et al., 2017; LIU et al., 2018). At higher concentrations compared to auxin, BAP usually induces callus differentiation and leaf regeneration (KHAN et al., 2015; MASUTI et al., 2017).

The physiological responses of growth regulators are tissue-specific (ALONI, 1980; ENDER; STREIDER, 2015) so the candidate GR formulations obtained in stage 1, were specific to callus tissues. In the grafting, the development of callus (resulting from the multiplication of secondary xylem cells and phloem parenchyma cells close to the graft cut), is an initial and priority step for obtaining functional vascular connections (MIYASHIMA et al., 2013; COOKSON et al., 2013). Therefore, the *in vivo* validation becomes essential (stage 2). For this stage, the validated GR formulations are required to meet the following three requirements. Firstly, they must be effective not only to induce callus at the grafting point but also to favor scion budburst. Nanda and Melnyk (2018) reported that grapevine graft union is only considered effective and functional when the shooting and rooting processes are initially integrated by callogenesis, followed by active vascular connection. Second, the responses of each scion/rootstock combination to the validated GR formulation should be significant and advantageous over the CONTROL. The CONTROL treatment indicate the natural capacity of a scion/rootstock combination to form viable and quality grafted plants, due to the endogenous hormonal balance that these genotypes present. Third, the formulations selected and rated as promising must perform at least comparable to commercial formulation of grafting waxes (STANDARD treatment).

Analyzing the first requirement, the candidate GR formulations (IAA+BAP and DBA+BAP) improved the quality of grafting in combinations of 'Riesling Italic' and 'Bordo' with 'SO4' or 'P1103' (Figura 4E-H). However, the isolated application of BAP or mixed with IBA provided the lowest Tr_{vivo}acumulated in the 'Riesling'/'SO4' grafting (Figures 4E) as observed in the *in vitro* assays (Figure 4A). Highlighted it is, in all genetic combinations, IAA+BAP stood out compared to DBA+BAP because it induces a higher Tr_{vivo}acumulated (Figure 4E-H). Thus, the IAA+BAP was chosen as the validated GR formulation for the second requirement.

The effectiveness of this auxin/cytokinin mixture for inducing callogenesis and, consequently, obtaining grafted grapevine plants is in agreement with recent studies on the use of GR. The most significant effect of cytokinin mixed with auxins lies in its interaction with the auxin metabolic pathway (BARON et al., 2019). Cytokinin enhances the regulation and distribution of PIN proteins, favoring the transport of auxins between callus cells during the formation of vascular bundles. Bidabadi et al. (2018) obtained satisfactory quality in grafting callogenesis for a formulation based on ANA and BAP, with a simultaneous increase in the concentration of antioxidant enzymes (catalase, peroxidases, and ascorbate peroxidase) and less accumulation of H_2O_2 in the tissues of the graft union.

Analyzing the second requirement, the responses of each treated graft compared to the CONTROL shows that IAA+BAP induced a positive effect on different *in vivo* quality variables, especially for R/S, B/P and B/S combinations (Table 1). The benefit of IAA+BAP becomes more evident to the phenological stage of shoots (Table 1B), callus formation rate (Table 1D) and callus diameter (Table 1E). The contrast with the CONTROL was more expressive when using the 'SO4' rootstock. The CONTROL showed a high percentage of plants with shoots (Table A) and plants with visible callus at the grafting point (Table 1C), especially for R/P, B/P and

R/S combinations. The benefit of IAA+BAP becomes less evident in these variables, provided a superior effect in B/S combination. Thus, the IAA+BAP effects were more expressive in the genetic scion/rootstock combinations that present the lowest natural capacity for callogenesis in the grafting union. The Tr_{invivo} (Table 1F), indicated that IAA+BAP presented gain compared to the CONTROL in B/S (1.79), R/S (1.49) and B/P (1.18) combinations. In R/P, the gain about 1.0 (0.95), not justifying the use of formulation.

Table 1. Five quality grafted plants variables and Tr_{vivo} from the six combinations of 'Riesling Italico' and 'Bordo' scions on 'P1103' and 'SO4' rootstocks, treated with growth regulators formulations (IAA(2.50)+BAP(1.00); STANDARD) and CONTROL.

	Treatment	P1103		SO4	
		Riesling	Bordo	Riesling	Bordo
A. Plants with shoots (%)	IAA+BAP	75 ^{Bb}	94 ^{Ans}	96 ^{Ans}	92 ^{Ans}
	STANDARD	80 ^{Bb}	100 ^{A}	93 ^{A}	97 ^{A}
	CONTROL	100 ^{Aa}	100 ^{A}	84 ^{B}	79 ^{B}
	Mean	86	96	88	89
B. Phenological stage of shoots (Scale)	IAA+BAP	5.5 ^{Bb}	8.8 ^{Aa}	7.3 ^{Aa}	4.5 ^{Ba}
	STANDARD	5.1 ^{Ab}	6.0 ^{Ab}	6.4 ^{Aab}	5.1 ^{Aa}
	CONTROL	8.0 ^{Aa}	5.4 ^{Bb}	5.5 ^{Bb}	3.3 ^{Cb}
	Mean	6.0	7.3	6.4	3.9
C. Plants with visible callus at graft union (%)	IAA+BAP	100 ^{NSa}	100 ^{ns}	100 ^{a}	100 ^{a}
	STANDARD	100 ^{Aa}	100 ^{A}	100 ^{Aa}	100 ^{Aa}
	CONTROL	100 ^{Aa}	100 ^{A}	96 ^{Aa}	64 ^{Bb}
	Mean	92	100	93	76
D. Callus formation rate around graft union (%)	IAA+BAP	95 ^{Aa}	89 ^{Aab}	97 ^{Aa}	68 ^{Bb}
	STANDARD	94 ^{Aa}	96 ^{Aa}	100 ^{Aa}	93 ^{Aa}
	CONTROL	85 ^{Aa}	66 ^{Bb}	51 ^{Bb}	46 ^{Bc}
	Mean	75	76	72	59
E. Callus diameter at the graft union (mm)	IAA+BAP	4.9 ^{Ans}	3.5 ^{Bb}	4.8 ^{Aa}	3.0 ^{Bab}
	STANDARD	6.0 ^{A}	5.8 ^{Aa}	5.2 ^{Aa}	5.4 ^{Aa}
	CONTROL	4.2 ^{A}	3.6 ^{ABb}	2.4 ^{BCb}	0.9 ^{Cb}
	Mean	3.7	3.5	3.2	1.9
F. Tr_{vivo}	IAA+BAP	0.95 ^{Bns}	1.18 ^{ABns}	1.49 ^{Ans}	1.79 ^{Ab}
	STANDARD	1.00 ^{Bns}	1.23 ^{ABns}	1.48 ^{Ans}	2.47 ^{Aa}
	Mean	0.98	1.21	1.49	2.13

Values followed by the same uppercase (row) or lowercase (column) letter do not differ statistically ($p<0.05$) by the Q-Cochran (A, C, D) and Kruskal-Wallis (B, E, F) tests. $^{NS/ns}$not significant.

Several factors linked to the genetic origin of these scion/rootstock combinations, such as anatomical and biochemical contrasts, may be modulating the level of effect of each GR formulation on callogenesis and grafting quality. Among the anatomical factors, it is noteworthy that the variation in the vascularization pattern can occur even between cultivars of the same species, mainly in the xylem area and in the number of vessel elements (SANTAROSA et al., 2016). For example, the vascular alignment between the scion and rootstock interferes with chemical and molecular transport, affecting the functioning of the graft union (VAHDATI et al., 2010). Regarding the biochemical aspects of the scion/rootstock combinations, the contrasts that can occur in the accumulation of phenolic compounds (ASSUNÇÃO et al., 2019), in the isoenzyme activity (GÖKBAYRAK et al., 2007), or in the phytohormone (HE et al., 2018) are emphasized. Changes in the balance of these compounds or the accumulation of their phytotoxic forms can determine

the success of grafting or the incompatibility response. Possibly, these biochemical variations may also be present in the different combinations of American cultivars (e.g. 'Bordo') on hybrid rootstocks (e.g. 'P1103' or 'SO4'), typical in Brazil, which may increase callogenesis restrictions and grafting incompatibility without the use of GRs.

Analyzing the third requirement, the validate GR formulation must similiar the result obtained with the commercial waxes availables. According to Regina et al. (2012), the GR treatment is satisfactory only when in post-forcing the grafted plants present more than 90% of budburst/shoots and callogenesis. Furthermore, Hunter et al. (2013) classify post-forcing grafted plants as high quality if they present callus diameter at grafting union between 2.0 and 3.0 mm. Following these references, the IAA+BAP was similar to the STANDARD in the same parameters. Compared to STANDARD, IAA+BAP promoted higher or similar gains in the percentage of shoots (Table 1A), phenological stage (Table 1B), and plants with visible callus (Table 1C) to all genetic combinations. Callus diameter (Table 1D) and callus formation rate (Table 1E) were especially higher in the STANDARD to B/P and B/S combinations. However, the Tr_{vivo} (Table 1F) indicated that STANDARD and IAA+BAP formulations presented similar results compared to the CONTROL in R/P, B/P and R/S. Only in the B/S combination, the STANDARD presented higher Tr_{vivo} compared to IAA+BAP.

The commercial wax possibly offers the GR to the grafting region at a different rate compared to the direct application of the liquid formulation of IAA+BAP. The Tr_{vivo} result in B/S combination does not invalidate applying the liquid IAA+BAP formulation. The direct spraying of the IAA+BAP, during the forcing period, ensured adequate graft plant quality limits, thus becoming an alternative to commercial waxes. But, highlights the need for new adjustments of *in vivo* application in this genetic combination to reach maximize the IAA+BAP effects. This selection strategy and GR application method to produce grafted plants are highly important for the grape production sector, especially in countries that do not produce waxes. Although not evaluated in this work, these advances can provide direct benefits for nurseries, reducing the cost of grafted plant production.

This study demonstrated that callus induction and GR responses are determined by the genetic potential of the grafted cultivars. A joint analysis of Figure 4 and Table 1 allows us to correlate the indicators/variables (Table 2 and Figure 5) of the two experimental conditions (*in vitro* and *in vivo*). Both Tr_{vitro} and Tr_{vivo} increased similarly (p=0.89**, Table 2, Figure 5A) in R/S combination and are therefore suitable for characterizing and correlating the overall quality of cofused callus *in vitro* and grafting *in vivo.* Furthermore, both Tr_{vitro} and Tr_{vivo} were highly influenced and, consequently, correlated with the callus diameter evaluated *in vitro* (p=0.89**) and *in vivo* (p=0.99**) (Table 2, Figures 5B and 5C, respectively). This high correlation shows that the increase in callus in the grafting region *in vivo*, occurred proportionally to the increase in callus that were cofused *in vitro* and treated with the same GR formulation. Although tissue cutting for *in vivo* grafting activates a complex cascade of molecular reactions (ENDERS; STRADER, 2015; NANDA; MELNYK, 2018), these positive correlations show that the responses of the *in vitro* cofused callus interaction were similar, despite involving different physiological conditions. In addition, the association strength and tendency of such variables in callogenesis were modulated by the genetic combination. The Tr_{vitro} correlation with callus diameter (p=0.73*, Figure 5D) compared to the different genetic combinations, exposes the combination B/S with the lowest values of Tr_{vitro} and callus diameter, while R/P and B/P reached the highest values for the same variables. These results confirm the contrasts observed in Table 1F, indicating that the B/S combination tends to be responsive to GR.

Table 2. Matrix correlation analysis of callus fusion quality variables (fusion – F; development of the (– S) scion and (– R) rootstock; mass – M; color – C) and Tr_{vitro} with grafted plants quality variables (plants with shoots – PWS; phenological stage of shoots – PS; Plants with visible callus at graft union – GP; Callus formation rate around graft union - CF; Callus diameter at the graft union – CD) and Tr_{vivo} in the 'Riesling Italico'/'SO4' combination, treated with seven growth regulators formulations (mg l^{-1}): BAP(1.00), IBA(1.00), IBA(1.00)+BAP(1.00), DBA(1.75), DBA(1.75)+BAP(1.00), IAA(2.50) and IAA(2.50)+BAP(1.00).

	PWS (%)	PS (Scale)	GP (%)	CR (%)	CD (mm)	Tr_{vivo}	Tr_{vitro}
F (Scale)	-0.28	-0.94**	-0.28	-0.71	-0.55	-0.55	-0.38
S (Scale)	-0.41	-0.77**	-0.41	-0.26	-0.29	-0.29	0.46
R (Scale)	-0.51	-0.30	-0.15	-0.31	-0.12	-0,12	-0,09
M (mg)	-0.42	<0.0	-0.34	-0.18	-0.32	-0.32	-0.32
C (%)	-0.18	0.23	0.52	0.32	0.57	0.57	-0.43
Tr_{vivo}	0.53	0.69	0.79*	0.82*	0.99**	-	0.89**
Tr_{vitro}	0.29	0.71	0.74	0.79*	0.89**	0.89**	-

*/**Significant correlation by the Spermann test (*p<0.05* and *p<0.01*, respectively).

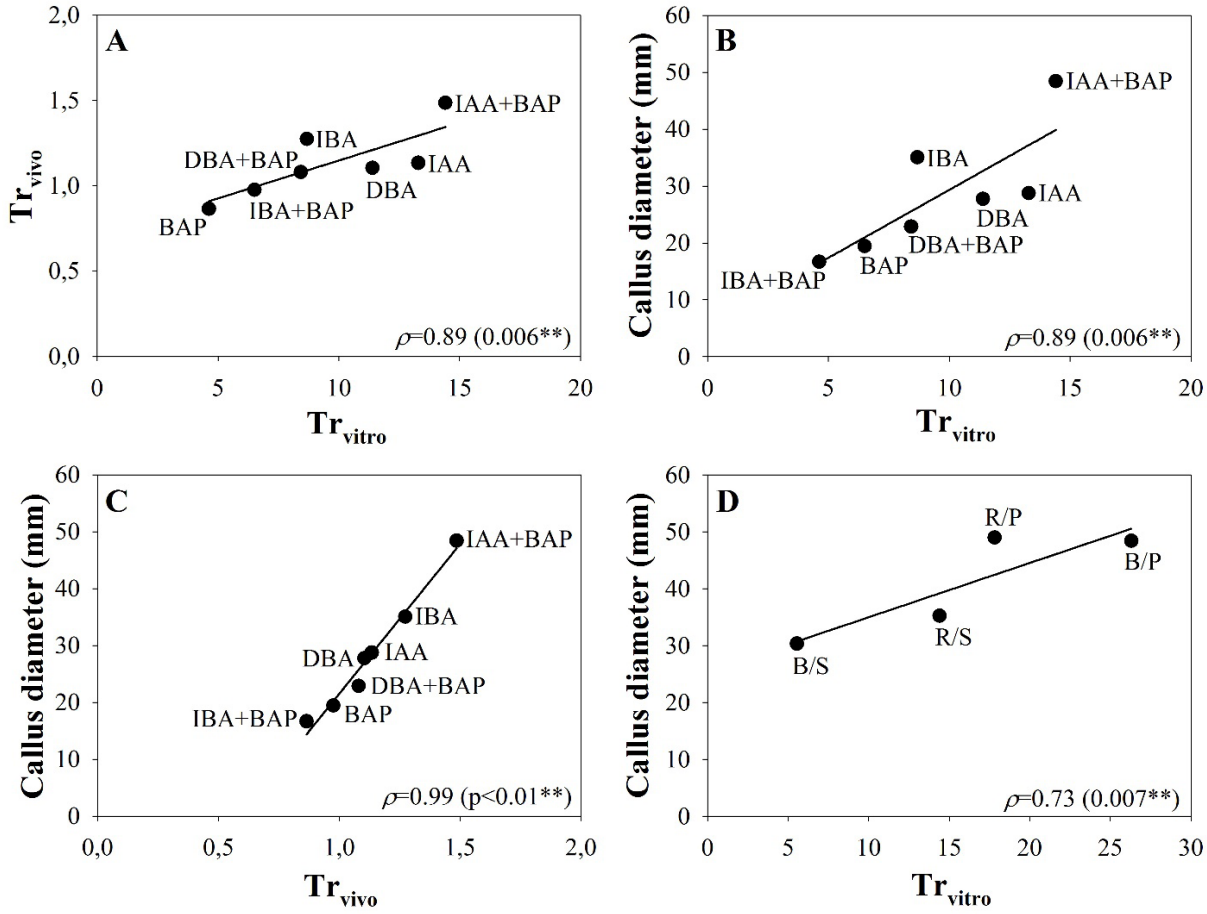

Figure 5. Correlation analysis of 'Tr indicator' of grafted plants (Tr_{vivo}) and fusion callus (Tr_{vitro}) (A), callus diameter at the graft union and Tr_{vivo} (B); callus diameter at the graft union and Tr_{vitro} (C) for seven growth regulators formulations (acronyms are described in the caption of Figure 4), just in the 'Riesling Italico'/'SO4' combination. Correlation analysis of callus diameter at the graft union and fusion callus indicator (Tr_{yitro}) for validate formulation IAA(2.50)+BAP(1.00) to following genetic combinations: 'Riesling Italico' or 'Bordo' as scions on 'SO4' or 'P1103' as rootstocks (D). **Significant correlation by the Spermann test (*p<0.01*).

Therefore, despite the contrasts in response intensity between the different genetic combinations, it is noteworthy that there was a direct response pattern between callus diameter and Tr_{vitro}. In general, the greater the fusion and growth of the callus by the *in vi-*

tro GR treatment, the greater the *in vivo* effect of this same treatment on the callus diameter and, consequently, the quality of the grafted plants. This association, between two direct visual analysis variables, proves to be an important tool for the *in vitro* prediction of future responses of genetic compatibility or grafting quality in grapevine plants. Until the writing of this work, only Aazami (2010) carried out a study with *Vitis* spp. correlating the developmental characteristics of grafted genotypes *in vitro* with the vigor responses of plants *in vivo*. However, the strategy of *in vitro* analysis to estimate graft compatibility has not been conducted in grapevines so far. In other perennial cultures, this approach has already been applied with predictive success. For example, with *Prunus* genotypes, Pina et al. (2009) were able to easily discriminate homografts from heterografts *in vivo*, through confocal microscopy analysis of the intercellular communication of callus that were co-cultured *in vitro*.

Conclusion

The *in vitro* prospecting coupled with the *in vivo* validation strategy was effective to select and validate GR formulation with the potential to induce graft callus union and assure the quality of grapevine graft plants. For the 'Bordo' or 'Riesling Italico' scions on 'P1103' or 'SO4' rootstocks, the formulation of IAA (2.50 mg l^{-1} indoleacetic acid) and BAP (1.00 mg l^{-1} 6-benzylaminopurine) was the most promising for the 'omega' mechanized bench grafting system. Furthermore, the intensity of grafting quality response to the validate GR formulation was more intense in grafts with 'SO4', especially with 'Bordo'. Finaly, the *in vitro* cofusion of callus in a medium containing the optimal formulation determined a direct and positive association with the callus diameter that was induced by the same formulation *in vivo* in the grafted plants.

References

AAZAMI, M. Effect of some growth regulators on "*in vitro*" culture of two *Vitis vinifera* L. cultivars. **Romanian Biotechnological Letters,** Bucharest, v.15, p.5229-32, 2010.

ALONI, R. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. **Planta,** Bonn, v.150, p.255-63, 1980.

ASSUNÇÃO, M.; PINHEIRO, J.; CRUZ, S.; BRAZÃO J.; QUEIROZ J.; DIAS J.E.E.; CANAS, S. Gallic acid, sinapic acid and catechin as potential chemical markers of *Vitis* graft success. **Scientia Horticulturae,** Amsterdam, v.246, p.129-35, 2019.

BARON, D.; AMARO, A.C.E.; PINA, A.; FERREIRA, G. An overview of grafting re-establishment in woody fruit species. **Scientia Horticulturae,** Amsterdam, v.243, p.84-91, 2019.

BIDABADI, S.S.; AFAZEL, M.; SABBATINI, P. Iranian grapevine rootstocks and hormonal effects on graft union, growth and antioxidant responses of Asgari seedless grape. **Horticultural Plant Journal,** Beijing, v.4, p.1-15, 2018.

CELIK, H. The effects of different grafting methods applied by manual grafting units on grafting success in grapevines. **Turkish Journal of Agriculture and Forestry,** Ankara, v.24, p.499-504, 2000.

CLOG, E.; BASS, P.; WALTER, B. Plant regeneration by organogen-esis in *Vitis* rootstock species. **Plant Cell Reports,** Weinheim, v.8, p.726-8, 1990.

COOKSON, S.J.; MORENO, M.J.C.; HEVIN, C.; MENDOME, L.Z.N.; DELROT, S.; TROSSAT-MAGNIN, C.; OLLAT, N. Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. **Journal of Experimental Botany,** Lancaster, v.64, n.10, p.2997-3008, 2013.

EICHORN, K.W.; LORENZ, D.H. Phaenologische entwicklungsstadien der rebe nachrichtembl astsch. **Pflanzenschutzdienstes**, Brauschweig, v.29, p.119-20, 1977.

ENDERS, T.A; STRADER, L.C. Auxin activity: past, present, and future. **American Journal Botanic,** Conecticut, v.102, n.2, p.180-96, 2015.

ERREA, P.; GARAY, L.; MARÍN, J.A. Early detection of graft incompatibility in apricot (*Prunus armeniaca*) using in vitro techniques. **Physiologia Plantarum,** Lund, v.112, n.1, p.135-41, 2001.

GABRIELSSON, J.; LINDBERG, N-O.; LUNDSTEDT, T. Multivariate methods in pharmaceutical applications. **Journal Chemometrics,** Amsterdam, v.16, p.141-60, 2002.

GÖKBAYRAK, Z.; AKKURT, M.; ÇELIK, H. Determination of grafting compatibility of grapevine with electrophoretic methods. **Scientia Horticulturae,** Amsterdam, n.113, p.343-52, 2007.

GROHS, D.S.; ALMANÇA, M.A.K.; FAJARDO, T.V.M.; HALLEEN, F.; MIELE, A. Advances in propagation of grapevine in the world. **Revista Brasileira de Fruticultura**, Jaboticabal, v.39, n.4, p.130-44, 2017.

HE, W.; WANG, Y.; CHEN, Q.; SUN, B.; TANG, H-R.; PAN, D-M.; WANG, X-R. Dissection of the mechanism for compatible and incompatible graft combinations of *Citrus grandis* (L.) Osbeck ('Hongmian Miyou'). **International Journal Molecular Science**, Basel, v.19, n.2, p.505, 2018.

HELFER, G.F.; BOCK, F.; MARDER, L.; FURTADO, J.C.; COSTA, A.B.; FERRÃO, M.F. Chemostat, um software gratuito para análise exploratória de dados multivariados. **Química Nova**, São Paulo, v.38, n.4, p.475-579, 2015.

HUNTER J.J.; VOLSCHENK, C.G.; FOUCHE, G.W. Graft union abnormality: some impacting factors. **Ciência e Técnica Vitivinícola**, Dois Portos, p.938-943, 2013.

ILIEV, A.; DIMITROVA, V.; PEYKOV, V.; PRODANOVA–MARINOVA, N. Technological Investigations for Improvement of Grapevine Propagation Material Production in Bulgaria. Part I. Results of affinity study and comparative testing of paraffins in the production of grafted rooted vines turkish. **Turkish Journal of Agricultural and Natural Sciences**, Amsterdam, v.1, p.1274-9, 2014.

JASKANI, M.; ABBAS, H.; SULTANA, R.; KHAN, M.M.; QASIM, M.; KHAN, I.A. Effect of growth hormones on micropropagation of *Vitis vinifera* L. CV. Perlette. **Pakistan Journal of Botany**, Karachi, v.40, p.105-9, 2008.

JIMÉNEZ, V.M.; BANGERTH, F. Relationship between endogenous hormone levels of grapevine callus cultures and their morphogenetic behavior. **Vitis**, Siebeldingen, v.39, n.4, p.151-7, 2000.

JIMÉNEZ, V.M. Regulation of *in vitro* somatic embryogenesis with emphasis on to the role of endogenous hormones. **Revista Brasileira de Fisiologia Vegetal**, Campinas, v.13, n.2, p.196-223, 2001.

JONARD, R.; LUKMAN, D.; SCHALL, F.; VILLEMUR, P. Early testing of graft incompatibilities in apricot and lemon trees using *in vitro* techniques. **Scientia Horticulturae**, Amsterdam, v.43, p.117-28, 1990.

JUNIOR, O.P.A.; SANTOS, T.C. R.; BRITO, N.M.; RIBEIRO, M.L. Revisão das Propriedades, Usos e Legislação do Ácido 2,4-Diclorofenoxiacético (2,4-D). **Cadernos de Pesquisa**, São Luís, v.13, n.1, p.60-70, 2002.

KAPARAKIS, G.; ALDERSON, P.G. Differential callus type formation by auxins and cytokinin in vitro cultures of pepper (*Capsicum annuum* L.). **Plant Biosystems**, Roma, v.137, n.3, p.275-80, 2003.

KHAN, N.; AHMED, M.; HAFIZ, I.; ABBASI, N.; EJAZ, S.; ANJUM, M. Optimizing the concentrations of plant growth regulators for in vitro shoot cultures, calos induction and shoot regeneration from caloses of grapes. **Journal International des Sciences de la Vigne et du Vin**, Villenave D'ornon, v.49, n.1, p.37-45, 2015.

KUMSA, F. Effect of growth regulators on indirect organogenesis of two grapevines (*Vitis vinifera* L.) cultivars. **African Journal of Biotechnology**, Abraka, v.16, n.16, p.852-9, 2017.

LAJEUNESSE, M. On the meta-analysis of response ratios for studies with correlated and multi-group designs. **Ecology,** Washington, v. 92. p.2049-55, 2011.

LIU, J.; HU, X.; QIN, P.; PRASAD, K.; HU, Y.; XU, L. 2018. The WOX11-LBD16 pathway promotes pluripotency acquisition in callus cells during de novo shoot regeneration in tissue culture. **Plant Cell and Physiology**, Oxford, v.59, n.4, p.734-43, 2018.

MASTUTI, R.; MUNAWARTI, A.; FIRDIANA, E.R. The combination effect of auxin and cytokinin on in vitro callus formation of *Physalis angulata* L. – A medicinal plant. *In*: INTERNATIONAL CONFERENCE ON GLOBAL RESOURCE CONSERVATION, 8, Malang, 2017. **Conference Proceedings [...]**. Malang: AIP, 2017. p.1-6.

MATOS, F.J.A. **Introdução a fitoquímica experimental**. 3.ed. Fortaleza: Edições UFC, 2009. 150 p.

MAYER, J.L.S.; BIASI, L.A.; BONA, C. Capacidade de enraizamento de estacas de quatro cultivares de *Vitis* L. (Vitaceae) relacionada com os aspectos anatômicos. **Acta Botanica Brasilica**, Brasilia, v. 20, n.3, p.563-8, 2006.

MELLO, L.M.R. de. **O Brasil no contexto do mercado vitivinícola mundial**: panorama 2015. Bento Gonçalves: Embrapa Uva e Vinho, 2016. 4p. (Comunicado Técnico, 192).

MELLO, L.M.R. de; MACHADO, C.A.E. **Cadastro Vitícola do Rio Grande do Sul**: 2013 a 2015. Brasília: Embrapa, 2017. 85p.

MELLO, L.M.R. de; MACHADO, C.A.E. **Vitivinicultura brasileira**: panorama 2020. Bento Gonçalves: Embrapa Uva e Vinho, 2021. 18p. (Comunicado Técnico, 223).

MII, M.; ZOU, Y-M.; SUGIYAMA,T.; YANAGIHARA, S.; IIZUKA, M. Frequency callus formation from protoplasts of *Vitis labruscana* Bailey and *Vitis thunbergii*. **Scientia Horticulturae**, Amsterdam, v.46, p.253-60, 1991.

MIYASHIMA, S.; SEBASTIAN, J.; LEE, J.; HELARIUTTA, Y. Stem cell function during plant vascular development. **The EMBO Journal**, Heidelberg, v.32, p.178-93, 2013.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, Copenhagen, v.15, p.473-97, 1962.

NACHTIGAL, J.C; SCHNEIDER, E.P. **Recomendações para produção de videiras em sistema de videiras em sistemas de base ecológica**. Bento Gonçalves: Embrapa Uva e Vinho, 2007. 68p. (Documentos, 65).

NANDA, A.; ·MELNYK, C.W. The role of plant hormones during grafting. **Journal of Plant Research**, Tohoku, v.131, n.1, p.49-58, 2018.

PEDERSEN, B.H. Determination of graft compatibility in sweet cherry by a co-culture method. **The Journal of Horticultural Science and Biotechnology**, Warwick, v.81, n.4, p.759-64, 2006.

PINA, A.; PILAR, E.; ALEXANDER, S.; MARTENS, H. Cell-to-cell transport through plasmodesmata in tree callus cultures. **Tree Physiology**, Edmonton, v.29, p.809-18, 2009.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2023.

REGINA, M.A. Produção e certificação de mudas de videira na França 1. Situação atual da produção. **Revista Brasileira de Fruticultura**, Jaboticabal, v.24, n.2, p.586-89, 2002.

REGINA, M.A.; SOUZA, C.R.; DIAS, F.A.N. Propagação de *Vitis* spp. pela enxertia de mesa utilizando diferentes porta-enxertos e auxinas. **Revista Brasileira de Fruticultura**, Jaboticabal, v.34, n.3, p.897-904, 2012.

SANTAROSA, E.; SOUZA, P.V.D. de.; MARIATH, J.E. de A.; LOUROSA, G.V. Alterações anatômicas do sistema vascular em porta enxertos de videira. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.51, n.4, p.320-9, 2016.

SRNGH, Z.; BRAR, S.J.S. *In vitro* plant regeneration in seedless grapes (*Vitis vinifera* L.). **Vitis**, Siebeldingen, v.32, p.229-32, 1993.

VAHDATI, K.; HASSANI, D.; REZAEE, R. Walnut grafting success as affected by different grafting methods, cultivars and forcing treatments. **Acta horticulturae**, Leuven, v.861, p.345-52, 2010.

VRŠIČA, S.; PULKOA, B.; KOCSISB, L. Factors influencing grafting success and compatibility of grape rootstocks. **Scientia Horticulturae**, Amsterdam, v.181, n.2, p.168-73, 2015.

WAITE, H.; WHITELAW-WECKERT, M.; TORLEY, P. Grapevine propagation: principles and methods for the production of high-quality grapevine planting material. **New Zealand Journal of Crop and Horticultural Science**, Wellington, v.43, n.2, p.144-61, 2015.

ZHOU, Q.; GAO, B.; LI, W-F.; MAO, J.; YANG, S-J.; LI, W.; MA, Z-H.; ZHAO, X.; CHEN, B-H.; Effects of exogenous growth regulators and bud picking on grafting of grapevine hard branches. **Scientia Horticulturae**, Amsterdam, v.264, p.109-86, 2020.