RESEARCH ARTICLE

Micro Propagation of *Vitis vinifera*, Pinot Noir Genotype: Characteristics and Possibilities of Cultivation

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Abstract

The grapevine (*Vitis vinifera*) is considered one of the most important fruit crops in the world in terms of cultivated hectares and economic value, so is necessary obtain information about the viability of different genotypes to in vitro culture propagation. The objective of this study was to obtain mother plants of the Pinot Noir variety through in vitro culture, and callus for improvement. Nodal cuttings were split from these plants, disinfected and sown in 50% Murashige Skoog (MS) nutrient media y for callus 2,4-D added. After eight weeks of growth, explants establishment, rooting and number of leaves were recorded and statistically analyzed. Then, they were transplanted to a 1:1 mixture of perlite: sterile fertile soil, under appropriate humidity and temperature conditions and their ex-vitro behavior was analyzed for four weeks. The results showed that it is a viable genotype for in vitro cultivation, with mean values of 83% establishment and 57% rooting and production of 1,2 leaves per cutting. The percentage of acclimatized plants was 100% and survival too. Mass production of Pinot Noir mother plants in vitro was achieved, demonstrating that it is a genetic material with a high yield and health level and very good ex vitro acclimatization performance. The percentage of callus was 80 % representing good biological material for improvement.

Keywords: acclimatization; callus; clones; propagation; rooting

Introduction

The Pinot Noir grape variety has its origin in French Burgundy, it is also known as Burgunder, Pineau, Klevner, Plant Fin, Noirien, Pinoz, Dorada. It has small, very compact and uniform bunches, the berries are small to medium in size, with bluish-black epidermis, the pulp without anthocyanin pigmentation, soft and very juicy, very sensitive to cracking and rubbing. It is sensitive to climate change, adapting well to temperate climates. It has high nutritional requirements in magnesium, responds very well to phosphorus inputs and has low nitrogen and potassium requirements. The Pinot Noir grape variety produces a high-quality wine suitable for aging, fine, intense and complex, it produces musts with a lot of sugar if the grapes ripen properly, with medium acidity, suitable for cavas and champagne. Remember the aromas of raspberry and ripe strawberries. It loses part of its characteristics in very hot areas.

Plant tissue culture encompasses a variety of in vitro manipulations of plant cells, tissues, and organs that direct the dedifferentiation of parental cells into meristematic (or embryogenic) cells, which then divide and differentiate into plant organs and/or whole plants. The part of the mother plant used to start the culture (explant) is typically small and, in theory, each of its cells has the potential to produce a plant, the end result being the mass multiplication of the original genotype, known as micropropagation [1] (Watt, 2012). Growth of in vitro propagated plants is often more vigorous than that of in vivo propagated plants due to plant rejuvenation and pathogen free plants [2] (George et al., 2008). The use of in vitro culture for vegetative multiplication, called micropropagation, offers an important alternative to conventional plant propagation methods and is an important tool for initiating breeding programs. The use of efficient micropropagation protocols will result in the production of numerous plants that can be maintained under controlled conditions in a reduced space, until their transfer to the field for cultivation or grafting. In grapevine, virus infection is common and affects fruit yield and quality and thus can affect wine quality [3] (Melyan et al., 2015), In this context, the propagation of virus-free materials by micropropagation is of great interest because currently the propagation of the vine is done by cuttings. In vitro multiplication or cultivation is valuable in the application of techniques such as mutation and induced selection, exchange and conservation of germplasm, and physiological studies. The best organogenesis is achieved with a specific balance for the variety between auxins and cytokinin [4] (González, 2017). In vitro propagation of grapevine crops under standard culture conditions, its response is usually related to the genotype and the culture medium [5] (Morón, 2017) and plant growth regulators [6] (Nookaraju et al., 2008). There are stages that must be met to ensure the success of the in vitro culture, they are: selection and preparation of the mother plant, disinfection of the explants (uninodal cuttings), establishment of the crop, multiplication of shoots, rooting and acclimatization. The objective of this study was to analyze in vitro culture of this variety, from its establishment, growth, rooting and acclimatization, with minimal inputs, large production, and sanitized plants.

Material and Methods

Obtaining *ex Vitro* Mother Plants

It was started from rooted cuttings of V. vinifera Pinot Noir, which were planted in 500ml pots, in a substrate of fertile soil and perlite 1:1, watered and carried nursery for its development and growth.

Obtaining *in Vitro* Mother Plants

Culture Medium

50% [7] Murashige Skoog (MS) (1962) medium was prepared. The nutrient solution was prepared with distilled water, 2.2 g/l Murashige Skoog salts, 5 ml/l EDTA-Fe solution; 1 ml/l of vitamin mix (Thiamin, Nicotinic Ac. and Pyridoxine); 10 ml/l (10 g/l) myo-inositol; 10 ml/l (0.1 g/l) of Indole Acetic Acid (IAA) and 10 ml/l (8 g/l) of Adenine Sulfate, 30 g/l of sucrose and made up to 500 ml/l and the pH was 5.6 to 5.8. 8 g/l of agar-agar was dissolved in 500 ml of water and this solution was added to the nutrient solution, completing the liter of water, then transferred to glass jars and sterilized at 1 atm for 15 min at 121° C

Disinfection and Planting of Explants

Uninodal cuttings were used as explants, which were disinfected in water with two drops of detergent, 5 min; in 70% alcohol, 3 seg; 3 rinses 5 min; 15% sodium hypochlorite, 20 min; 3 rinses with sterile water, 5 min each. After disinfection, they were planted in the sterile culture medium.

Steps of Cultivation

Establishment: The percentage of establishment was evaluated for 8 weeks. An explant that showed aerial and root growth was considered established. Growth: the number of leaves over 8 weeks was used as growth parameter. Rooting: the percentage of seedlings rooted, in the same culture medium was evaluated. Acclimatization: the fully developed plants were transplanted ex vitro in a 1:1 mixed substrate of perlite: soil, previously sterilized. The explants were removed from the jars and the agar-agar was removed with the help of water. They were transplanted into pots, watered and covered with nylon bags with elastic bands, to generate a humid microclimate for 15 days and without polyethylene covers for 15 more days, conditioned in a growth chamber with a photoperiod of 16 h light / 8 h of darkness and 25º±2ºC.

Obtaining *in Vitro* Callus

Plant Material

The starting material for this study was obtained from the mother plants produced in vitro, Pinot Noir. Using disinfected scissors, shoots were cut, which were fragmented into uninodal cuttings, from which any plant material that was not necessary (tendrils, leaves, stem) was removed, leaving only the part of the node with its respective bud and the stem.

Culture Medium

For this experiment, the culture medium MS 50%, 10ml/l IAA (Indole Acetic Acid) and adenine sulfate 10 ml/l and 30 gL-1 of sucrose and 0.1; 0.5; 1 and 2 ppm of 2,4-D. Once the nutrient solution was dissolved, it was mixed with agar-agar (8 g/l), previously diluted in 500 ml of water. The pH was adjusted to 5.8 and it was distributed in bottles, which were sterilized.

Experimental Design and Statistical Analysis

Establishment: 20 jars and 40 explants (percentage explants with roots or leaves). Growth: 20 jars and 40 established plants (the number of leaves per explant) Rooting: 20 jars and 40 established plants (percentage). Acclimatization: 40 pots, with 40 ex vitro plants (percentage of acclimatized plants and the number of leaves per plant). Callus: 20 jars with 40 explants (percentage of callus).

With the establishment and rooting data, frequency tables were constructed. Normality tests [8] (Kolmogórov-Smirnov $p<0.05$) and homoscedasticity [9] (Levene's test $p<0.05$) were also performed. It was concluded that none of the assumptions were fulfilled, therefore, [10] Mann-Whitney and Kruskal-Wallis U test was used to analyze the correlation. For the descriptive analysis of the data on the number of leaves in the different weeks, the mean, median, and mode central tendency were calculated, along with measures of dispersion, standard deviation, and variance. The ANOVA test was also performed, comparing means of the number of established explants, percentage of rooted plants and number of leaves in the growth stage and in acclimatization ($p <0.05$).

Results

Establishment of Explants

This genotype had 83% establishment of explants from uninodal cuttings, leaving 17% of explants unestablished. In the first week, 37% of established cuttings were observed, increased to 70% in the second, 80% in the third and 83% in the fourth, thus remaining constant for the following four weeks (Figure 1a). There was a significant statistical difference in the increase of established explants between the 1st week and the rest of the weeks (Figure 1a).

Growth of Plants

Growth was determined by leaves produced by each cutting. Figure 1b shows the linear increase in the number of leaves with a mean of 1.2 in the eighth week. There was complete aerial development, behaving linearly until week six, then the average decreased, maintaining a plateau in the last two weeks.

Rooting

Pinot Noir cuttings began to develop roots in the second week with 3% of plants rooted, in the third week 23%, in the fourth 53% and in the fifth 57% (Figure 1c). From the fourth week, the level of rooting was similar, without significant differences with respect to the rest of the subsequent weeks, but with respect to the 1st, 2nd and 3rd weeks.

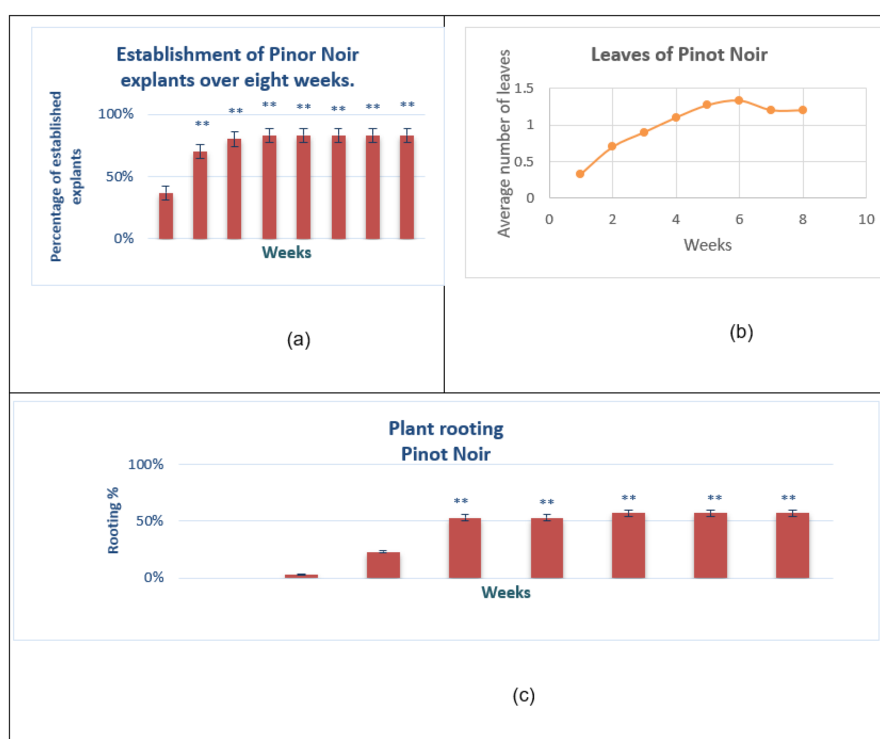

Figure 1: Step of micropropagation of Vitis vinifera Variety Pinot Noir; (a) Establishment of explants during eight weeks; (b) Number of leaves us measure of growth during eight weeks; (c) Percentage of rooting.

Acclimatization

Acclimatization is from in vitro to ex vitro culture, this is a drastic step for the plant, because the culture medium provides nutrients and water; when these plants are transplanting to the ground the roots must be adapted to the search nutrients elements Ex vitro, greenhouse, and field conditions are environments with lower relative humidity, higher light levels, and septic conditions that are stressful to micro propagated plants compared to in vitro conditions. The objective was to examine the acclimatization capacity of Pinot Noir plants obtained by micro propagation and to determine their ex-vitro survival. The

acclimatization was of 100% and was obtained 100 % of survival (Figure 2).

The analysis of the number of leaves produced in the acclimatization of 4 weeks was carried out. For the analysis of the central tendency and dispersion, Mean and Standard Deviation statistics were performed, respectively. All weeks are significant with each other, except the 1st and 2nd p=0.068 and the 3rd and 4th p=0.066. The rest of the pairs are statistically significant, their respective p values are: S1 and S3 p=0.043, S1 and S4 p=0.043, S2 and S3 p=0.039 and S2 and S4 p=0.042, according to Comparison test pairwise T-Student ($p \leq 0.05$). The Pinot Noir cuttings proved to be plants susceptible to micropropagation in vitro, with very good yields and capable of acclimatizing, thereby allowing them to tolerate ex vitro transplantation.

Pinot Noir had an excellent behavior in acclimatization where all the plants could be acclimatized. 100% survival of the plants was obtained, feasible to be transplanted to a nursery or field, in a very short period of time and there was no fungal contamination, which is common at this stage in vitro culture. In the Figure 2 the different steps of Pinot Noir micropropagation are observed, a- establishment of explants, b- plants growing, c- rooting- d- acclimatization

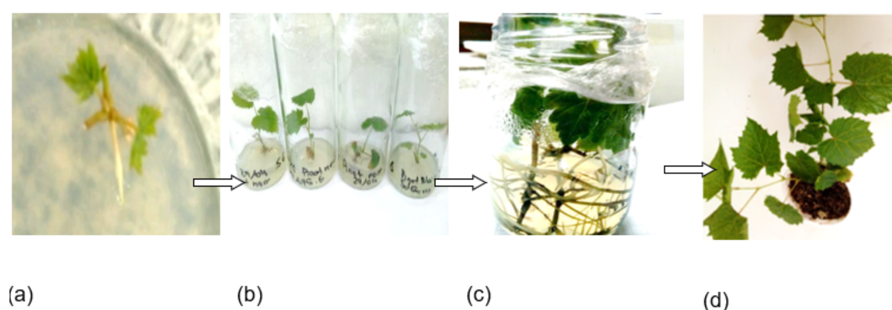

Figure 2: Images of micropropagation of Vitis vinifera variety Pinot Noir: (a) Induced establishment; (b) Production of leaves; (c) rooting (d) ex vitro acclimatization.

Callus Production

Fifteen days after planting the explants, it was observed that 60% of the Pinot Noir explants had produced callus, with a contamination level of 20%. At 30 days of the experiment, it was observed that Pinot Noir had increased its callus production to 80% and maintained 20% contamination. The concentration of 2,4-D (2 ppm) is adequate for callus formation, with the concentrations (0.1; 0.5; 1 ppm of 2,4-D) there was no callus formation.

Discussion

The percentage of establishment in this study, is considered similar to those reported by [11] (Cavazos-Galindo et al., 2018) in Cabernet Sauvignon of 80% in the cultivation of meristems and from 68 to 87% in the cultivation of axillary buds, 80% in the Muscat de Alejandría variety reported by [12] Abido et al., 2013 ; 88% for the Cabernet Franc variety [13] (García et al ., 2020a) and higher than 68 to 76% in meristems and 60 to 72% in axillary buds in the Merlot variety [11] (Cavazos-Galindo et al., 2018).

In this experience, the roots were developed, without specific protocols for rooting. [14] Martínez and Tizio (1989) micro propagated the Chardonnay, Pinot Noir, Pinot Blanc, Thompson Seedless, Cabernet Sauvignon, Chenin and Riesling varieties by budding uninodal cuttings in Murashige Skoog (1962) medium diluted by half and added with 0.5 mg/ l of gibberellic acid (AG3) and 1 mg/l of benzyl aminopurine (BAP) and the rooting of the shoots originated in a different medium described by [15] Martínez et al.,1986. In our experience, the medium used is not the one normally used for rooting, which suggests that they are cuttings with a high auxin content. [16] Martínez and Tizio (1990) concluded that the in vitro propagation efficiency is highly conditioned by the genotype. Numerous investigators have reported variability for micropropagation between varieties of Vitis vinifera, as well as variability in somatic embryogenesis and response to the addition of antibiotics [17] (Chée and Pool,

1983; [18] Gribaudo et al., 2004). Several species of the genus Vitis have been propagated by microcutting in simple media or in the presence of growth regulators [19] (Mhatre et al., 2000, [20] Pinto-Sintra, 2007). [21] Guiñazú et al., 2005, in studies carried out with Creole vines, reached the conclusion that the ideal medium for vine crops is solid MS with 50% salts, and for rooting they used auxinic hormones. In our work we show that a hormonal increase in auxin is not necessary to root this variety.

Different acclimatization methods have been reported for ex vitro culture of micropropagated vine plants of the following varieties Dogridge (Vitis champini), SO4 (V. riparia × V. berlandieri), H (V. vinifera × V. labrusca), 3309 C (V. riparia × V. rupestris), including the use of plastic pots with polyethylene covers or glass jars with polypropylene covers, obtaining an average acclimatization of 57.42% and 64.90% respectively, the use of glass jars with polypropylene lids was clearly a better strategy [22] (Alizadeh et al ., 2010). This result differs from that obtained in our study, where we used the method of plastic pots with polyethylene covers, obtaining 100% of acclimatized plants that arrived ex vitro with vigor and good physiological condition. It is evident that the physical conditions of the containers added to the environmental conditions of humidity and temperature would be influencing acclimatization, as well as the genetic vigor of the varieties [23] (Garcia et al., 2020b).

Conclusion

The results showed that micropropagation of Pinot Noir produced complete, healthy and vigorous plants, which allowed it to acclimatize to *ex vitro* conditions. It also produced calluses when it was induced for this purpose within a month, which makes it an excellent material to produce clones and to make genetic improvement

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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