

EVALUATION OF ONTOGENIC CHANGES ON YOUNG PLANTLET, IN MICRO PROPAGATION OF GRAPEVINE (*V. vinifera* ssp. *Sylvestris*)

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Abstract

Studied in this research was chlorophyll variability in young shoots of the grapevine (Paulsen & Kobber cultivars) in propagation "In Vitro". Two rootstocks of grapevine, inoculated for proliferation and rooting, in media MS, "In Vitro", with two different synthetic hormones in it, ANA and BAP. Search indicator received chlorophyll, in correlation with the percentage of sugar and hormones at three subcultures. The Initiation of callus from bud yeast started in culture containing 1 mg l⁻¹ and 2 mg l⁻¹ BAP and 1 mg l⁻¹ and 2 mg l⁻¹ ANA. Sucrose used in two doses 30 and 60 g / l. Based on observations of emission foliar (leafy) growth in length, significantly influenced the formation of callus was (1:32 LSD, q = 0.05) from the concentration of BAP and sucrose.

Best results observed with high doses of BAP and sucrose (78%). Concentration of 60 g / l sucrose improves the induction, differentiation and promotes regeneration of callus, better. Young shoots in three successive subculture have been foliar and root phenotype without changes (LSD 1.11, q = 0.05). Variance of variability like effect of vitrification was small; cv = 1.9 (coefficient of variation)

Keywords: In vitro, *Paulsen*, *Cobber*, saharoza, vitrification

Introduction

This work carried out about micro propagation of grapevine secured regeneration two rootstocks 1103Paulsen and Kober, through the application of mineral constituent and researching different formulations depending on the genotype. Study of two rootstocks , which constitute 85% of the material of propagate, with impact of different concentrations of citocinin and impact on the rate of multiplication intended to examine and find effect of cultures and show the superiority of BAP concentration in culture MS in relation other formulations tested.

Material and methods

Explants of two grapevine roots, 1103 P and Kober, inoculated nutrient soils MS with relevant additions to study the physiological changes, to explants in plantlets. Citocinin BAP applied in concentrations; (T1-1 mg l⁻¹ and T2 2 mg l⁻¹). Subculture carried out every 45 days of armpit buds, obtained. Explants and all cultures placed in a vegetative room (temperature 23 ° C ± 2 ° C, the intensity of the lighting 2000 luxury and photoperiod 16 hours), with white light with 30-35. Indicators of research : .i. Definition of regenerative potential, ii. Explants number, iii. Number of buds, iv. Length of new shoot for explants.

Results and discussions

Results of three years resulting from treatment expletive placed in culture MS1 with two concentrations of BAP, have proven positive impact on increasing the capacity proliferate seedlings, and buds all expletive of cultivars 1103P and Kobber. (Tab1 and 2)

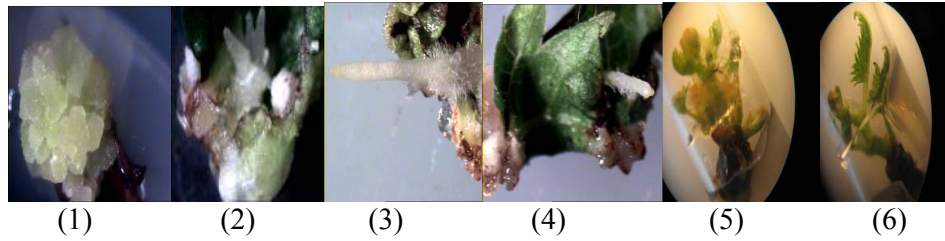


Photo (1,2,3,4) Differentiation Callus tissue.(5-6) proliferation in plantlets.

Results indicate that BAP at any concentration applied, has been important in cell division and increasing the length of the sprouts. Benzilaminopurina (BAP) significantly influenced the rapid proliferation of the cells and the highest dose, influenced better in increase in spur length. Evaluations performed about 20 days after inoculation represent increased by 5mm to 17mm but different in both cultivars. Sizes greater than bisques growths achieved when it is applied BAP concentrations $2 \text{ mg} / \text{l}^{-1}$. Using these nodal segments cut, to identical manner, improved multiply and after planting material and their development made possible interfering use for the realization of the recurrent cycle's initial propagation. In this way, is ensuring large number explants, in a relatively short period of 30-40 days. BA concentrations, influence on morphological characteristics of vegetable material. In table one and two, are three-year averages explants blown and differentiated leaves. Stage included the induction of meristematic centers of which are developed adventives organs. Shoot produced, used as the basis, for further breeding cycles, in which, they passed back to the culture (Subculture) to add to their number, and to prove their propagation coefficient.

Table 1. Means for oneway of Anova above average explants light and the number of leaves differentiated. (Each treatment = 16 explants)

| Treatment | Number Repetition | Mean Nr Leaf | Mean. Nr explants vivid |
|-----------|-------------------|--------------------|-------------------------|
| KO 1mlg/l | 4 | 31.2500 \pm 2.88 | 7.25 \pm 1.89 |
| KO 2mlg/l | 4 | 50.0000 \pm 2.43 | 10.00 \pm 0.81 |
| PA 1mlg/l | 4 | 44.2500 \pm 1.83 | 10.00 \pm 0.81 |
| PA 2mlg/l | 4 | 56.0000 \pm 1.12 | 10.50 \pm 0.57 |

Evaluation of morphological development has shown variability in starting capacity and growth in two cultivars of grapevine under the effect of different concentrations cytokinins. In general, the effect of BAP was powerful, because it has fostered a better rate and morphologic homogeneity propagation of seedlings, always interdependent concentration used in correlation with the features of genetic material.

Table 2. Means for one way ANOVA of the number of explants blown by subcultures Std Error Uses a pooled estimate of error variance

| Treatment | Number of Repetition | Mean of expl vivid | Number leaf vitrifikuar |
|-----------|----------------------|--------------------|-------------------------|
| Subcult-1 | 4 | 10.7 \pm 0.50 | 0 |
| Subcult-2 | 4 | 9.5 \pm 1.73 | 3 |
| Subcult-3 | 4 | 8.7 \pm 1.89 | 6 |

Using cytokinins gave large effects on the percentage of the opening and the formation of numerous seedlings from micro cleavage committed in aid buds, made in plants, cv.

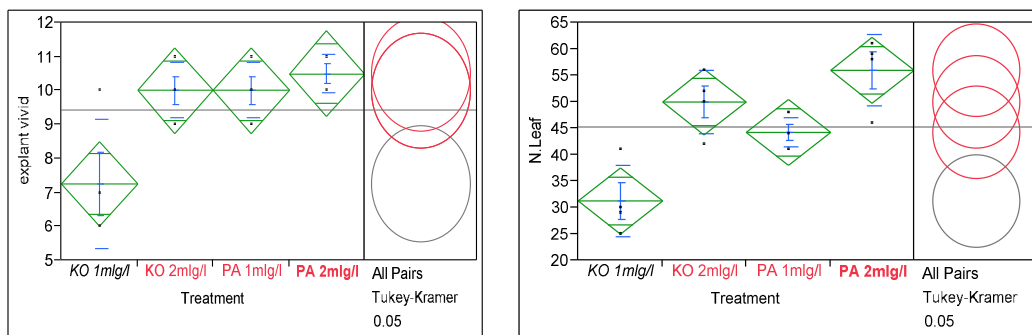
rootstocks 1103 Paulsen, Kobber. We analyzed two concentrations benzyl adenine (BA 1.0, 2.0 mg/l⁻¹), concentration 2 mg/l⁻¹, has given the best answer morphological. Good results have been above all concentrations, but particularly 2, mg / l⁻¹ have disposed flourishes with the highest percentage, and seedling multiplication. In which treatment is used BA 2mg / l⁻¹ of stitch inoculated are differentiated average from 10 -10.5 explants and the 50-56 leaves, for each explants planted. Results of all years for treatment averages, prove that 2 mg / l⁻¹ had the best indicators to the two types of proven and significant changes for HSD lsd.1.67, that it proves the effectiveness of use BAP dosage has therefore a maximum coefficient multiplication of buds and percentage of hatching.

Studies and the results for the capacity organogenic fragments stitch present interesting data for the development of explanted in the way of direct organogenesis. It is observe that immediately after the beginning of cultivation breeding ground, according Pierik; epidermal cells, show clear, differentiation cell characteristics, such as large diameter cores, nucleoli pyrophilic and cytoplasm currents visible.

Phase, defined as activation or meristematic. In 4-daylong explants, observed periclinal divisions in all epidermal cells, while 6-8 daylong explants, except appropriations periclinal, which increase in number, begins to appear anticline divisions in epidermal cells. This phase was similar for all the genetic material in the experiment. However, after 10 days, in explants appear primordial leaf strands, and it is differentiated in time and intensity influenced by genetic material. Remarkable for the opening speed 1103 Paulsen.

Percentage of explants, cv 1103 P fluctuated in accordance with the concentration of cytokinins. At a level, 66.1% obtained for auxiliary buds, with 2 mg / l⁻¹ BA, in cv.1103 P. For these cultivar acceptable results, 62 % obtained also in 1 mg / l⁻¹ BA

In the analysis of the ability proliferates the two cultivars, with weak results had cv. Kober, in terms of percentage explanative blown; bud and shoot multiplications coefficient (B.M.C) (S.M.C)



Graphic 1 and 2. Onaway Analysis of explant vivid By Treatment and Number of leaf by Tretment.

Cv.Kobber, has anatomical features with changes, in rootstock 1103P. Significant changes related to the size of large bone and reduced levels of wood tissue and flumes. Particularly cortexes tissue, which is the development, processes of reproduction and differentiation of seedlings and radical explants.

Variables replays average, each treatment, were within the limits of the standard deviation, without statistical differences, while the effect of applied concentrations of BAP has different effects on the development and growth of meristem. There are no major changes, in two moments of isolation, but in terms of dosage, 2 mg / l⁻¹ is provide, into others, the number, seedling and larger size.

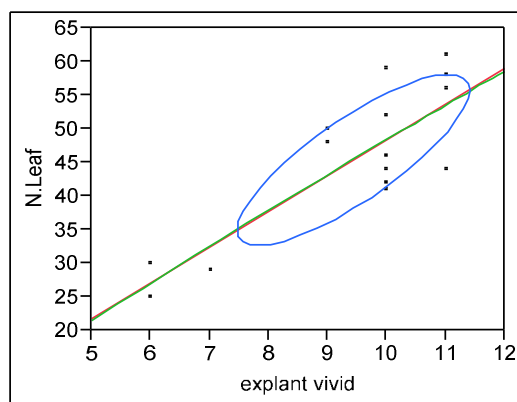
This result was identical in all subculture, analyzed in ANOVA testing for LSD 1.39, demonstrating that in vitro application, for micro propagation cv. Koober and Paulsen dose, 2 mg / l⁻¹ is economic, compared to the application of other concentrations in this cultivar.

From the analysis of the influence of cytokinins rootstocks, 1103P Kober, in 2 concentrations, results that improved gradually morphological indicators in parallel with the increase of BA concentration, but validated changes between them. Morphologic response, effect cytokinins concentrations, has been catalytic to 2 dose mg / l⁻¹ to two cultivars tested. In general, the two cultivars using BA, have reached 59.3% plantlet.

From the analysis of averages, TUKEY-Kramer HSD lsd - 1.39, demonstrates the significant changes the percentage opening of 1103 Paulsen, compared with Kober and for the effect of using cytokinins in this concentration in all subcultures.

The effect of statistical changes flowed from the application of concentration confirms dosage, 2 mg / l⁻¹ for economic effectively that brings together the maximum buds coefficient multiplying (B.M.C) and the coefficient multiplication of the shoots (S.M.C). Cultures stimulated the paces of opening have grown plantlets production from lateral buds, for each explants.

Modulate analysis of regression (graphic 3), expressed and confirmed that opening explants averages, at all times of isolation and regeneration; there are not changes and progress (in ascending). Graphical, demonstrates a linear slope line in climbing (R² = 0.87) (y = mx + b), which means that the treatments have had constant effect and affect the effective action.



Graphic 3. Bivariate Fit of N.Leaf By explant vivid

Hypothesis test in two concentrations of BAP analyzed for statistically et assessed value for $\alpha = 0.05$ has shown that hypothesis (Ho) that express the Treatment 2 mg / l⁻¹ confirmed, by correlative analysis showed that up to second sub culture multiplication cycle are not observed phenomena and material degeneration. In this way the effect morphologic of two concentrations cytokinins, 6-benzyl adenine purine (BA) has prompted the scale to better, breeding and morphologic homogeneity of the seedlings. Influence of concentrations of BA, induction growth and development of buds at the same time; number and leaf buds per explants went to increase in parallel with increasing concentrations up to 2 mg / l⁻¹.

Shoots proliferating have had different size, and caused by the impact of phytohormon whether in size and in the number of cells reproduced. The larger size of the seedlings are realized in the concentrations 2 mg / l⁻¹ (13.15 cm) compared with those obtained with BA in concentrations 1mg / l⁻¹ (1.75cm). Maximum axial buds developed per explants was 11 to two cultivars, and has fluctuated from 8.3 in cv Kober, 11 in 1103P cv.

Results followed by the effects of hormonal promoters' action proved that the assumptions have been acceptable. On analysis of the averages of each treatment through Testy TUKEY, and the resulting homogeneous groups after analysis confirmed the effects stemming from the use of cytokinins concentrations either in quantitative and qualitative morphological values. The all BAP concentrations cultivars have been influences in the same

way and without changes. Concentrations of BAP, have had results with statistical significant changes to the above morphological indicators, treatments I to II. Value of t ($H_0 > H_1$), $\alpha = 0.05$ resulted in all cultivars, is a very good test, because it has provided and the value of F . The effect of the performance indicators morphological of explants verified with regression analysis for hypothesis testing (graphic 3).

New leaflets have had the same phenotype, assessed in normal light index, and the shape and symmetry. We thus proved that the two cultivars suffered no deviations below applying sub - cultures. Large number of measurements has reduced the error, which observed in the absence of variability with no changes in any year from the average of four replays. $P = 0.0055$, that is significant because they have influenced the composition of explants to open and other morphological indicators.

Conclusions

- Cytokinins action, in organogenesis and the formation of new organs, induced by, chemical manipulation nourishing terrain.
- During organogenesis, explants followed a morphological process in conformity with general road, namely forming meristem, buds, shoots and adventives roots.
- Response of two varieties on the opening and the number of inter- nodes, the effect of the increase in length for each explants has shown that treatment containing 2mg/l^{-1} BA has been the best compared with other treatments.
- On the same premises of the two varieties of rootstocks, expressed differences on the effects that gives citocinin in proliferation explants.
- Cv.Kober expressed many changes through the two treatments applied, but cv. Paulsen has shown for the best effect all treatments cytokinins.
- Application of two cultivars in continuous regeneration in two sub - culture, no effects manifested in two grapevine cultivars genetic features.

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