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Evaluation of production explant or boneless blackberry *Rubus Glaucus* Benth in vegetative multiplication phase in a system of temporary immersion

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Abstract

In vitro cultivation of mulberry boneless Castile was performed using the temporary immersion system (SIT) to evaluate the dependence of the production of *Rubus glaucus* Benth in vegetative phase with respect to time of the cycle SIT were 12 , 15 , and 18 hours; and with respect to introducing culture medium which came the initial explants for SIT . As a result it was found that the best treatments for a good development of new shoots that will be obtained from the multiplication phase in the SIT are: cycle time of 12 hours and medium solid introduction; in addition to make the project economic analysis determined that multiplying the arrears boneless SIT increases in production compared to traditional propagation and consequent lower production costs , obtaining a profit ratio of 5 : 1

In vitro cultivation, mulberry boneless Castile, temporary immersion system, production costs.

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Introduction

In Ecuador, the demand for arrears of Castile boneless *Rubus glaucus* Benth to plus 3%, because this plant is related to a greater number of branches producing and tillering between 15% to 20% higher than the traditional default with spine (Sigarroa & Garcia, 2011). Ecuador is growing in the agricultural field; They are constantly developing new commercial productions, thanks to its growing conditions (Ramirez, 2009). The blackberry has productivity problems in Ecuador, by the use of traditional breeding. According to Monteiro (2004), the blackberry is propagated by vegetative methods such as cuttings and layering; these types of processes facilitate the spread of pests and diseases that affect the quality and quantity of production and consequently increase the economic loss to the producer; while by the in vitro method, a large number of outbreaks would be obtained from small amounts of tissue (Pati, Sharma, Sood, & Ahuja, 2004).

Being limited production boneless arrears of Castile for the reasons mentioned, it is looking for new strategies to increase the multiplication of *Rubus glaucus* Benth, and for this one of the techniques of vegetative propagation in vitro culture is used. Temporary Immersion System (TIS), which apart from cheaper production costs and overcome the difficulties of static cultures, they exhibit the ability to automate some stages of cultivation in vitro, allow ease of scale and increase the rates of multiplication is used , development and productivity of materials spread (Pérez, Ponce, Jiménez, & Agramonte, 1998).

Materials and methods

The mother plant blackberry castile boneless was collected in Sangolquí-Ecuador, located in the S 0`18`16.37``y O 78`27`06.92`` coordinates, at an altitude of 2.472 meters.

Collection of plant material

The sampled plants were selected in situ choosing explants with the best features: more leaves, free of disease; besides plants with an adequate number of buds and similar physiological conditions (Pierik, 1990).

Disinfection of plant material

twig washings vigorously with a solution of commercial detergent detergent, then immersed in a solution of Benlate® 2 g Tetracycline (5 mg), liquid soap (100 ml), iodine (5 ml) and citric acid (0.1 g), all volumetric 4 liters of distilled water for 45 minutes.

After disinfection, it was rinsed with distilled water three times for one minute each wash. The braces are cut into pieces of 10 cm, leaving the center of each cut one to two buds.The plant material was transferred to a laminar flow chamber, and placed in an 8% solution of commercial bleach for ten minutes, washed with distilled water three times for ten minutes.

Establishment of in vitro culture

Preparation of culture medium

Murashigue salts and 50% Skoog macro- and micronutrients were used additionally placed 15 gL -1 sucrose, 0.5 mL BAP [1500 mL L-1] and 0.1 mL AIA [1500 mL L-1]; was adjusted pH 5.6 culture medium, then 4 g of agar was added. 20 ml of medium was dispensed into pre-sterilized containers and then the containers were autoclaved at a pressure of 15 PSI at 121 ° C for 30 minutes.

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Planting in vitro explant

After disinfection, the explants were seeded in the culture media for establishing and transferred to growth room where they remained for a period of 20 days at constant light conditions (16 hours light and 8 hours dark), the average temperature 23 ° C.

Temporary immersion system

Preparation of liquid culture medium for multiplication

Murashigue salts and 50% Skoog macro- and micronutrients were used additionally placed 30 gL -1 sucrose, 1.3 ml BAP [1500 mL L-1] and 0.3 ml AIA [1500 mL L-1]; the pH of the culture medium was adjusted to 5.6. Means at a pressure of 15 PSI at 121 ° C were autoclaved for 30 minutes.

Planting in vitro explant

Temporary immersion system, formed by two glass flasks of 850 ml capacity, one for the growth of the explants and the other as a reservoir of culture medium was used. These bottles were connected together by a silicone hose 6 mm diameter.

The culture medium was circulated from a vial to another depending on the opening or closing according to the programmable timer to determine the frequency and duration of immersion. At the entrance of bottles hydrophobic filters (0.20 um,) was placed to ensure sterility air. The air pressure was regulated by a pressure gauge.

The explants used in the course of SIT were taken from the establishment phase, and those explants that had similar characteristics were considered: length (3 cm) and stem diameter (2 mm), and were free of contamination.

Apical cut to plant in containers corresponding to the explants bioreactors. After seeding, the cultures were taken and remained in the growth chamber for a month in constant light conditions (16 hours light and 8 hours darkness) and average temperature of 23 ° C. after a month, we proceeded to the collection of the data.

Programming SIT

We worked with the constant immersion time two minutes, varying only the frequency and means of introducing the initial explants bioreactor.

Results and discussion

Selection of the optimal dose of BAP or BAP + PBZ in liquid culture medium partial immersion for the multiplication of blackberry *Rubus glaucus* Benth boneless.

Standardizing the culture medium for multiplication was performed by applying HIDE (paclobutrazol) at four different concentrations to homogenize the shoot growth.

Trat.	No. Outbreaks		Bud length (mm)		Bud diameter (mm)	
	Media		Media		Media	
T1	3.4	c	33.7	d	1.05	b
T2	3.3	b	3.9	c	1.10	c
T3	3.3	b	2.2	b	1.20	d
T4	0	a	0	a	0	a

Means with common letter are not significantly different ($p > 0.05$)

Table 1 Duncan Test. Variables analyzed in the partial immersion assay

In the variable number of outbreaks, the T1 (1.3 mg.L-1 BAP) had a higher number of sprouts (3.4 buds per explant); in the variable length of the outbreak, T1 (1.3 mg.L-1 BAP) had a longer length of shoots (33.7 mm).

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And for the variable diameter of the outbreak, have the T3 (1.3 mg.L-1 BAP; HIDE 1.25 mg.L-1) has the value of the average diameter higher outbreak (1.2 ± 0.01 mm) with respect to the other treatments (Table 1).

Positive results were obtained in treatments T2 (BAP: 1.3 mg.L-1; HIDE: 0.75 mg.L-1) and T3 (BAP: 1.3 mg.L-1; HIDE: 1.25 mg.L-1), the which showed homogeneous outbreaks, but decrease the number and length thereof; also thickened stem diameter, and these results are similar to those described by Rodriguez Aranguren & Farres (2005) in his research that works with concentrations HIDE 0.125 mg.L-1. This result was similar to that found by Avilán, Soto, Escalante, Rodriguez, & Ruiz (2003), who noted the effect of paclobutrazol in curtailing the size of outbreaks.

Using data shoot length depending on the concentration of HIDE, a polynomial regression, which was plotted (Figure 1) and the equation thereof was calculated was performed with reference to 35mm as ideal length for working in multiplication of delay in SIT. As a result the following equation is obtained: y = 1.786 + 0.006x2-0.253x, with R2 = 0.973. Where x is the length of the outbreak is expected to achieve (35 mm) and y is the concentration of HIDE to be found.

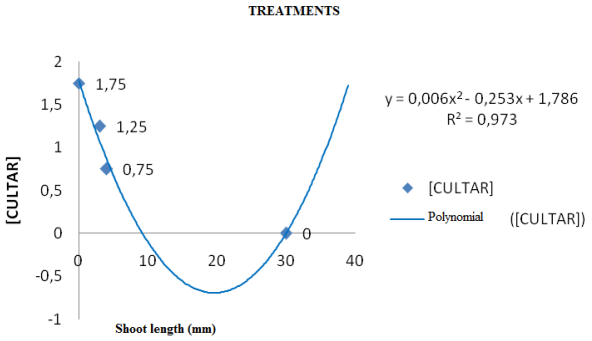

Figure 1 Linear regression polynomial concentration CULTURE.

Thus it was determined that the concentration of HIDE Ideally y = 0.281 mg.L-1. With this concentration, it can be obtained theoretically homogeneous shoots and bud expected length.

In addition, the T1 (1.3 mg.L-1 BAP) as the most suitable treatment to continue the multiplication phase delay in the Temporary Immersion System since according to Figure 1 shows is considered as low HIDE concentration, shoot length increases.

Evaluation of three days of temporary immersion cycle of production, for boneless explants blackberry *Rubus glaucus* Benth, in the multiplication phase of the SIT.

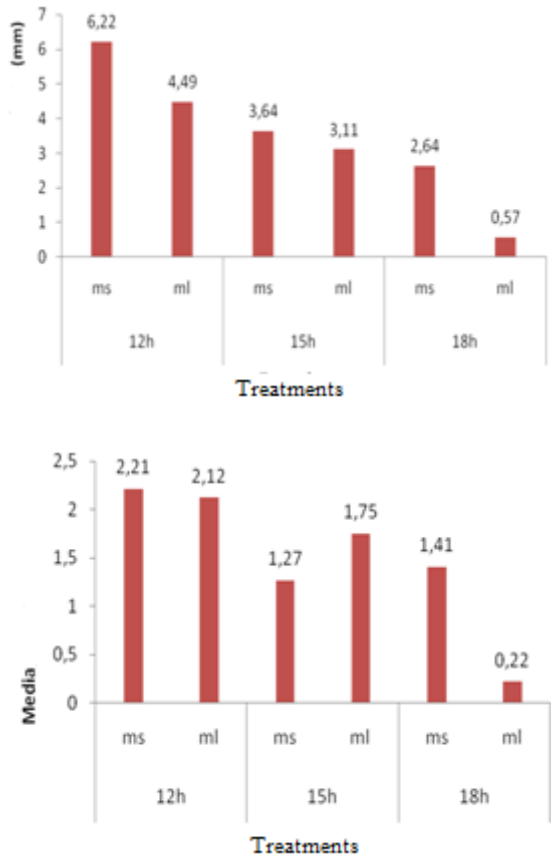

Figure 2 Mean (cycle-time interaction through introduction) of the variables fresh weight, shoot length, number of leaves per shoot and production rate

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The value of the highest average for the fresh weight was in the TR1 (12 hours, solid medium) (1 ± 0.04g); for shoot length was obtained TR1 (12 hours, solid medium) (6.22 ± 0.12 mm); for the variable number of leaves per shoot was the TR1 (12 hours, solid medium) (5.07 ±. 0,17 cm and the production rate corresponds to TR1 (12 hours, solid medium) (2.21 ± 0.23) compared to other treatments.

According Castro & Gonzales (2002), to achieve development in in vitro tissue culture, it is necessary to provide plants with essential nutrients sufficient, so they are not a limiting factor for multiplication in the plant growth factor. Therefore, the T5 (18 hours, liquid medium) and T6 (18 hours, solid medium) treatments reported no multiplication; According Escaletes and DOSBA, (1993), the phenolization is characterized by a change in the color of the tissue from green to brown, and this happens not to have a willingness bioregulators; as well as in the present work, where explants changed color from green to brown by the phenolization and also for dehydration they suffered when there are very long frequencies.

The results showed significant differences between treatments with the same cycle time but with the initial explants from different culture media introduction. Thus, T1, T3 and T5, despite being with T2, T4 and T6 respectively in the same cycle time, had higher production index (greater stem length, the greater number of leaves, fresh weight) per stem initial explants solid culture medium introduction.

These results also reflect according Debergh and Maene (1981) and Robert Smith (1990) and Preil and Hempfling (2002), Bautista, et al., (2004).

Who claim that the explants that are in liquid culture medium the surface of the explant come into direct contact with the environment which allows the most efficient uptake and release of nutrients which can accumulate in the tissue area that disperse quickly in liquid culture medium in the solid toxic metabolites.

It can also be attributed to a higher absorption of growth regulators in the culture medium changes from solid to liquid (Jambhale, 2000). Research has shown that in the liquid medium, the availability of water, minerals, and growth regulators, is greater when compared with semisolid and solid media culture, which promotes faster growth (Debergh, Harbaoui , & Lemeur, 1981).

In addition, the peculiarity that randomly explants, regardless of treatment had shoot formation took two forms: the shoots formed from an origin point, and throughout the explant, this can be attributed to a lack light distribution or arrangement in which the explants are inside the bioreactor by the agitation produced by the medium when in the immersion time, since the direction in which the sprouts are grown from explants are conditioned by the auxin production, and this tends to accumulate in the plant part that is shaded; therefore if the plant does not have shade, auxin drop from the apical part of the explant to the base, promoting the formation of buds from the same point, otherwise, they will go along the explant (Squeo & Cardemil, 2006).

Cost analysis

The comparative analysis of costs between multiplication of delay in SIT and conventional form showed that the increase in the SIT has lowered cost and more useful than conventional multiplication.

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In the SIT, in the best treatment production plants 170 in a month was obtained with PVP 7.48 USD per plant and a return of 62 months, while conventionally multiplying an output of 35 plants in one month is obtained with a PVP 32.05 USD per plant and a return of 296 months. Thus it is said that the production rate of blackberry *Rubus glaucus* Benth is 5-1 between SIT and multiplication in the conventional manner respectively.

Conclusions

For multiplication of delay in SIT, the T1 (1.3 mg.L-1 BAP 0 mg.L-1 HIDE) gave highest number of outbreaks (4 shoots per explant) and longer (35mm) to T2 (3 shoots per explant, shoot length 30mm), T3 (3 shoots per explant, shoot length 20mm) and T4. Paclobutrazol at concentrations of 0.75 mg.L-1 (T2) and 1.25 mg.L-1 (T3), homogenizes the length of outbreaks of blackberry *Rubus glaucus* Benth boneless without inhibiting their growth and development altogether but decreases number of shoots and length.

The treatment showed higher production of blackberry *Rubus glaucus* Benth boneless SIT in bioreactors are working 12 hours cycle time and their means of explants derived from solid introduction.

1 (SIT Multiplication: PVP = 7.48; conventional Multiplication: PVP = 32.05) with the use of SIT producing mulberry *Rubus glaucus* Benth because 5 is increased relative to the conventional method which reduces production costs and increases earnings.

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