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Induction and Multiplication of Thornless *Rubus Glaucus* Callogenesis from Leaves

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Abstract: The genus Rubus has the highest number of representatives within the Rosaceae family and is one of the most diverse in the Plantae kingdom, an economically important crop in the Andean region. In Colombia, the Castilla blackberry (Rubus glaucus Benth.) stands out among the cultivated species because of its various fruit size, color, and quality. This research aimed to conduct a callogenesis induction and then pro-embryogenic callus induction, where blackberry vitroplants were taken without stings in the multiplication stage; auxins such as 2,4-D and Picloram were used as growth regulators in six concentrations, with and without the presence of light. For callus multiplication, embryogenic calluses obtained during the induction phase were selected. These calluses were divided into portions approximately 0.5 cm in diameter with an initial approximate weight of 1.2 g. The analysis of variance yielded that statistically significant differences exist among the different treatments ($p < 0.05$), where callus formation with the Picloram hormone at different concentrations, with the presence of light and darkness, indicated that the most callus formation was verified with the 1.0 mg/l concentration and under darkness, (4.92 calluses on average). For embryogenic callus multiplication, analysis of variance indicated that the highest weight averages corresponded to the 3.0 mg/l treatment of 2,4-D combined with 3.0 mg/l of BAP for a mean of 3.5 under light conditions and 2.79 under darkness.

Keywords: callogenesis, hormones, embryogenesis, vitroplants.

葉無刺懸鉤子癒傷組織的誘導與增殖

摘要：懸鉤子屬是薔薇科中代表數量最多的屬，也是植物王國中最多樣化的屬之一，是安地斯地區重要的經濟作物。在哥倫比亞，卡斯蒂利亞黑莓(懸鉤子)因其不同的果實大小、顏色和品質而在栽培品種中脫穎而出。本研究的目的是進行癒傷組織誘導，然後進行促胚癒傷組織誘導，其中黑莓體外植物在繁殖階段沒有被刺。生長素如2,4D和毒莠定在有光和無光的情況下以六種濃度用作生長調節劑。對於癒傷組織增殖，選擇在誘導階段獲得的胚性癒傷組織。這些癒傷組織被分成直徑約0.5公分的部分，初始重量約為1.2克。變異數分析表明，不同處理之間存在統計上的顯著差異（p‹0.05），其中不同濃度的毒莠定激素在光和暗的存在下癒傷組織的形成表明，1.0驗證了最多的癒傷組織形成。毫克/公升濃度和黑暗條件下（平均4.

92個癒傷組織）。對於胚性癒傷組織增殖，變異數分析顯示最高的平均重量對應於3.0毫克/公升的2,4D與3.0毫克/公升的苯丙胺組合的處理，在光照條件下平均值為3.5，在黑暗條件下平均值為2.79。

关键词：癒傷組織發生、荷爾蒙、胚胎髮生、體外植物。

1. Introduction

The genus *Rubus* has the highest number of representatives within the Rosaceae family and is one of the most diverse in the Plantae kingdom. *Rubus* has been divided into 12 subgenera, of which only a few species have been domesticated. The subgenus *Idaeobatus* contains blackberries, the subgenus *Rubus* includes species found in Europe, Asia, and North America, and the subgenus *Orobatus* is exclusive to South America. The genus *Rubus* is distributed around the highlands of the tropics, usually above 800 mass, representing 25% of the entire global production, concentrated in Mexico, Central America, and South America, and is a crop of economic importance in these regions [1, 2].

In Colombia, Castilla blackberry (*Rubus glaucus* Benth.) stands out among the species cultivated by variability in fruit size, color, and quality and is considered an ancient selection derived from wild plants. It is accepted as a hybrid because it combines characteristics from the subgenera *Idaeobatus* and *Rubus and* is also a fertile amphidiploid.

Due to natural crosses in *Rubus*, up to 500 species can be identified. In areas where species of this genus are cultured, wild individuals grow in areas of secondary growth, on forest margins, and along roadsides. Wild and cultured plants have the potential to interact in many ways. Cultured materials can influence the genetic diversity of natural populations through gene loss and pollen transfer. Wild species can also serve as host plants for pests and their natural predators [1, 2].

Thornless blackberry is a perennial plant with an arbustus semi-erect demeanor and creeping or semi-erect stems that form clusters. It has morphological similarities with Castilla blackberry (*Rubus glaucus*). The main characteristic of thornless blackberry is the total lack of thorns throughout the plant, in which vestiges are evident in small bumps perceptible to touch and accentuated mainly on the petioles of the leaves. In thorny blackberries, these are distributed throughout the entire plant, which, in addition to causing difficulties in handling, constitutes a point susceptible to anthracnose or black prickly pear. In turn, in thornless blackberries, there are isolated villi or thin hairs that are difficult to detect with the naked eye absent in thorny blackberries. Thornless blackberry produces clustering 15%–20% superior to thorny blackberry, with approximately 98% productive branches, which makes the material nearly 30% more productive than thorny blackberry. The fruit size of this material reaches lengths up to 3.5 cm and diameters up to 2.3 cm; fruit weight ranges from 6.1 to 7.8 g, similar to or slightly larger than that of thorny blackberry. The fruits are mainly conical. The seed is wedge-shaped, with a reticulate surface, and measures between 4 and 6 mm long by 2 mm wide. In thornless blackberries, 134–150 drupes per fruit were found, yielding up to 15 t/ha [3].

Somatic embryogenesis and organogenesis are two morphogenic processes that are quite frequent in *the in vitro* culture of plant species. Somatic embryogenesis is the process through which a structure similar to the zygotic embryo is obtained without the mediation of gamete fertilization, whereas stems, roots, or flowers can be obtained through organogenesis. These organs are induced from a cell or from a group of cells that, according to culture conditions, have the property of staying in active division [4–7].

From *the in vitro* sowing of different relatively large explants and under adequate conditions, the formation of new organs can be directly induced without callus formation. If the formation is of sprouts, roots, or flowers, it is denominated as direct organogenesis. If the formation of somatic embryos is induced, this process will be called direct embryogenesis. If, on the contrary, from the *in vitro* sowing of an explant, the proliferation of cells is observed in a disordered manner and without any predetermined function, the production of calluses or cell suspensions will initiate. Previous meristemoid formation conditioned differentiation of organs from calluses or indirect morphogenesis [4–7].

Traditionally, Castilla blackberry (*Rubus glaucus*) propagates vegetatively by layering and cutting; this type of propagation implies laborious systems until the final transplant of plants in the field is achieved. Moreover, seed reproduction, with very low and delayed regeneration percentages, generates genetically heterogeneous populations that limit the availability of materials but are phenotypically uniform and kelpful for the establishment of commercial plantations. Currently, *in vitro* culture techniques are of more application in the biotech industry and are an

instrument of great importance for propagating massively high-quality plants under controlled conditions.

Some investigations established that additional factors such as light, temperature, humidity, and the gelling agent also influence regeneration. Consequently, this work evaluated the induction process of calluses from thornless blackberry vitroplants *in vitro* in the multiplication stage using growth regulators, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and Picloram, at different concentrations and combinations in darkness and with light, and at the same time evaluated their multiplication.

2. Materials and Methods

2.1. Induction of Callogenesis

For callus induction, the study took thornless blackberry vitroplants, *Rubus glaucus*, in the multiplication stage, using a basal medium (MS) [8], supplemented with saccharose 30 g/l, 100 mg/l of Ascorbic acid, 100 mg/l of Myo-inositol, 2 mg/l of Thiamine, using auxins as growth regulators, like 2,4-D and Picloram (SIGMA) in six concentrations, with and without presence of light (light – darkness) (Fig. 1).

- 2,4- D: 0.5 – 1.0 – 2.0 – 3.0 – 4.0 – 5.0 mg/l - light – darkness
- Picloram: 0.5 – 1.0 – 2.0 – 3.0 – 4.0 – 5.0 mg/l - light – darkness

The pH was adjusted to 5.8, and gel Rite 2.8 g/l was used as the gelling agent. Media were dispensed into compote jars (Gerber jars) and autoclaved at 121°C at 15 lbs. of pressure for 20 min; within each jar, five explants were planted and 12 jars per treatment were evaluated for 288 jars with 1440 leaf explants. Each treatment was evaluated for six months, subculturing in the same medium every four weeks. The material was kept at 24±1°C (Fig. 1).

The response variables evaluated wereas follows:

- Callus formation
- Pro-embryogenic callus (with presence of embryos)
- Color
- Texture

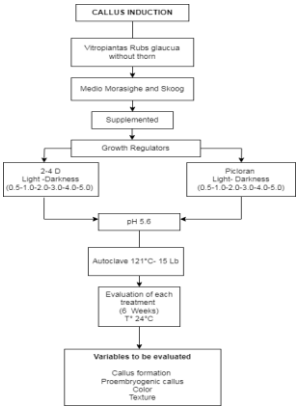

Fig. 1 Flowchart of the process of inducing *Rubus glaucus* callogenesis from leaves

2.2. Design and Statistical Analysis

A completely randomized 6x6x2 design was used, with three factors, where Factor A (concentration of 2,4-D) with six concentration levels: (0.5 – 1.0 – 2.0 – 3.0 – 4.0 – 5.0 mg/l); Factor B (Picloram concentration) with six concentration levels: (0.5 – 1.0 – 2.0 – 3.0 – 4.0 – 5.0 mg/l); and Factor C (presence of light) with two levels (with or without light), where each replicate of the experiment contained all the possible treatment combinations, that is, it contained the possible ABC treatments. The results were subjected to analysis of variance (ANOVA), and data related to the means of the treatments were subjected to the least significant difference (LSD) test with 0.05 significance level of 0.05. The data were processed using STATISTIX 7.0.

2.3. Callus Multiplication

For the multiplication of thornless *R. glaucus* Benth calluses from leaves, embryogenic calluses obtained during the induction phase were selected. These calluses were divided into portions approximately 0.5 cm in diameter with an approximate initial weight of 1.2 g and were sown in solid medium (MS), supplemented with 3.0 mg/l of 2,4-D, except for the control, and benzyl aminopurine (BAP) was added at four concentrations. One callus per jar was inoculated, with 10 calluses per treatment, placed in a 12-h photoperiod and in complete darkness at 25°C (Fig. 2).

The treatments used were:

- BAP: 0.5 mg/l – Light – Darkness
- BAP: 1.0 mg/l – Light - Darkness
- BAP: 3.0 mg/l – Light – Darkness
- Control without growth regulators (WGR): Light – Darkness

This phase evaluated auxin concentration and light – dark conditions on the growth of calluses in fresh weight (grams), in addition to color and sprouting (seedling formation) (Fig. 2).

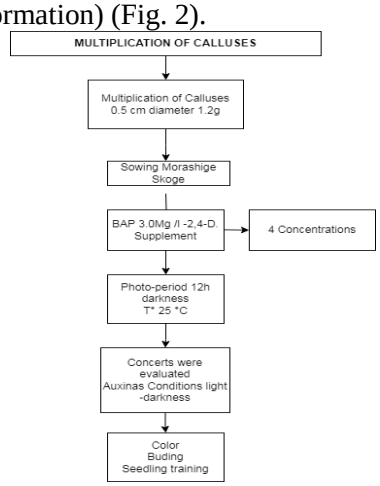

Fig. 2 Flowchart of callus multiplication in *Rubus glaucus* from leaves

2.4. Design and Statistical Analysis

A 4x2 factorial design was used, with two factors, where Factor A (BAP concentration), with four concentration levels: (0.0 – 0.5 – 1.0 – 3.0 mg/l); and Factor B (presence of light), with two levels (with or without light). The results were subjected to ANOVA, and the data related to the means of the treatments were subjected to the LSD test with 0.05 significance level processed with STATISTIX 7.0.

3. Results and Discussion

3.1. Induction of Callogenesis

The study showed that adding growth regulators (Picloram and 2,4-D) to MS medium at different concentrations induced various responses in leaf explants, where both growth regulators induced callus formation with different characteristics.

Fig. 3A shows callus presence as of the fourth week on the edges of the explant leaf in each of the treatments in light and darkness some with pro-embryogenic characteristics (Fig. 3B).

The analysis of variance yielded that statistically significant differences existed among the different treatments ($p < 0.05$), where callus formation with the Picloram hormone at different concentrations, with the presence of light and darkness, indicated their highest formation with a concentration of 1.0 mg/l and under darkness condition, (4.92 calluses on average), followed by a concentration of 4.0 mg/l (3.75 calluses on average).

Under light conditions, callus formation had the best results at concentrations of 3.0 and 4,0 mg/l with 3.33 callus formation on average (Fig. 4). Better performance was demonstrated with the Picloram growth regulator under darkness, in contrast with the performance of the 2,4-D growth regulator**,** whose best response occurred under light conditions (Fig. 4).

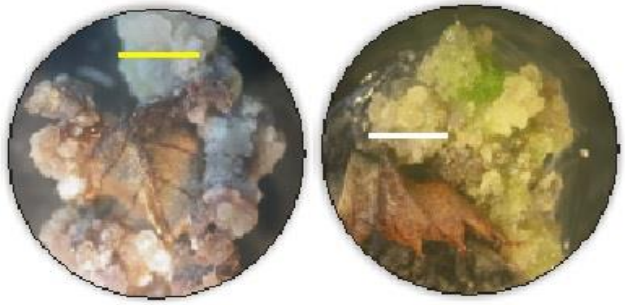

Fig. 3 Callus formation from *R. glaucus* leaves without stingers: left - callus formation, right - pro-embryogenic callus (Developed by the authors)

Growth regulators, mainly 2,4-D, have been indicated as promoters for callus formation and subsequent somatic embryogenesis; as seen in the analysis of variance, callus was obtained with each of the concentrations evaluated (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/l).

The same behavior was reported in [9] for thorny *R. glaucus* by adding auxin 2,4-D at a dosage of 1.0 mg/l in the culture medium.

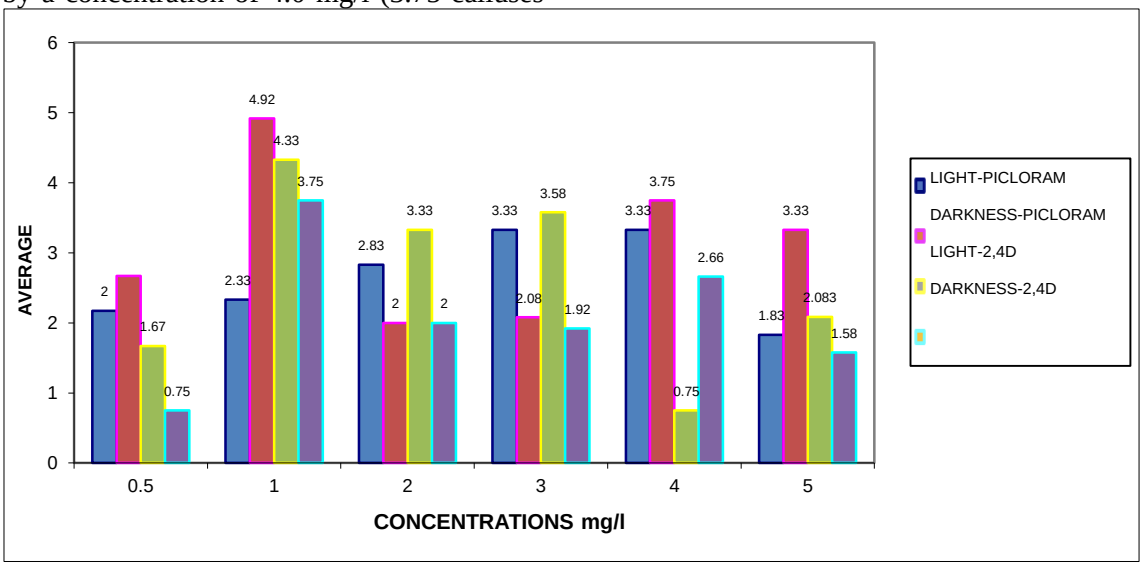

Fig. 4 Callus formation from *R. glaucus* leaf explants originating from light and darkness treated with Picloram and 2,4-D hormones (Developed by the authors)

[10] found that upon developing a protocol for callogenesis in *Viola odorata* L. (medicinal plant), they only produced cream-colored friable and translucid calluses, but on different days, where the maximum concentration of 2.5 mg l1 provoked the earliest induction of calluses.

[4] found that the different types of hormones, at varying concentrations of 2,4-D, NAA, and BAP, have the potential to induce varying colors and textures in callus induction and proliferation in cultivars from Troyer and Feutrell's citrange leaf explants. For five combinations of 2,4-D, NAA, and BAP, the Troyer citrange had an efficient performance that showed 56.73% callus proliferation with 27.4 days for the start of calluses compared with Feutrell's Early, which exhibits callus proliferation (52.13%) in 29.46 days,

and no individual leaf explant of both cultivars showed necrotic symptoms in different compositions of 2,4-D, NAA, and BAP.

[11] reported that callus induction in an East African cooking banana cultivar "Nakitembe" (AAA-EA genome), from immature male flowers, varied dramatically with media composition, where the medium based on Morishige and Skoog (MS) produced 2.9% calluses, while fortifying this medium with L-glutamine, L-proline, and casein hydrolysate increased the response to 4.2%, concluding that although the MS medium is popular for *in vitro* culture, it is not always optimal for some plant culture responses.

[12] studied the sources of explants and culture conditions for *in vitro* callogenesis in *Eugenia uniflora* for the induction of embryogenesis. *E. uniflora* is an American tree species of ecological, agricultural, and medicinal importance, where the combination of 2,4-D (5 or 10 mM) and BAP (5 mM), besides using 10.0 mM of NAA alone, was also efficient for callus induction in leaf explants of *Eugenia involucrate*, promoting callogenesis in 75%–92.7% of the explants. Use of NAA + BAP (5.0 mM each) induced callus formation in cotyledons (74% of the explants) of *Ugni molinae* (Myrtaceae), whereas auxins from NAA only promoted higher callus induction in hypocotyls (94%) and leaves (80%) at 2.5 mM concentration.

[1] developed a callus induction protocol for the establishment of blackberry cell suspensions (*Rubus adenotrichius* Schltdl.) and their cell analysis through optical microscopy and transmission electron microscopy (TEM) for the potential production of phenolic compounds. To produce callogenesis, segments of blackberry leaves were disinfected and placed in different concentrations of 2,4-D and control media (0; 0.5; 1.0; 1.5; 2.0; 2.5; and 3.0 mg/l of 2,4-D); the largest calluses were obtained in the medium with 1.5 mg/l of 2,4-D.

[13] worked with *Guarianthe skinneri* (Bateman) Dressler and W.E. Higgins, which is a native orchid of Mexico and considered a threatened species in the NOM-ECOL-059-SEMARNAT-2010. Leaf and pseudobulb marrow explants cultivated in semisolid MS medium were evaluated by adding BAP, 2,4-D, Kin, a combination of BAP/2 and 4-D/Kin/Sad, and a control without any type of plant growth regulators. Statistical analysis showed that pseudobulb marrow explants were more adequate for *in vitro* callus induction compared with leaf explants because of a lower concentration percentage (18.8% in marrow and 73.2% in leaves). Likewise, pseudobulb marrow explants showed greater callus formation (10.8%) than leaf explants (7.6%). Regarding the phytoregulators used, BAP promoted higher callus formation (17%) than other phytoregulators (7%–10%).

Production of pro-embryogenic calluses of thornless *R. glaucus* (Figs. 5 and 6), larger cells and abundant cytoplasmic content were found to form pro-embryogenic masses. The analysis of variance yielded that statistically significant differences exist among the different treatments ($p < 0.05$), where pro-embryogenic callus formation with the Picloram hormone at 1% and darkness had the highest callus formation with 4.5, followed by auxin 2,4-D at 1%, with presence of light, with 4.25 pro-embryogenic calluses and the Picloram hormone at 1% concentration with light developed, on average, 3.58 calluses.

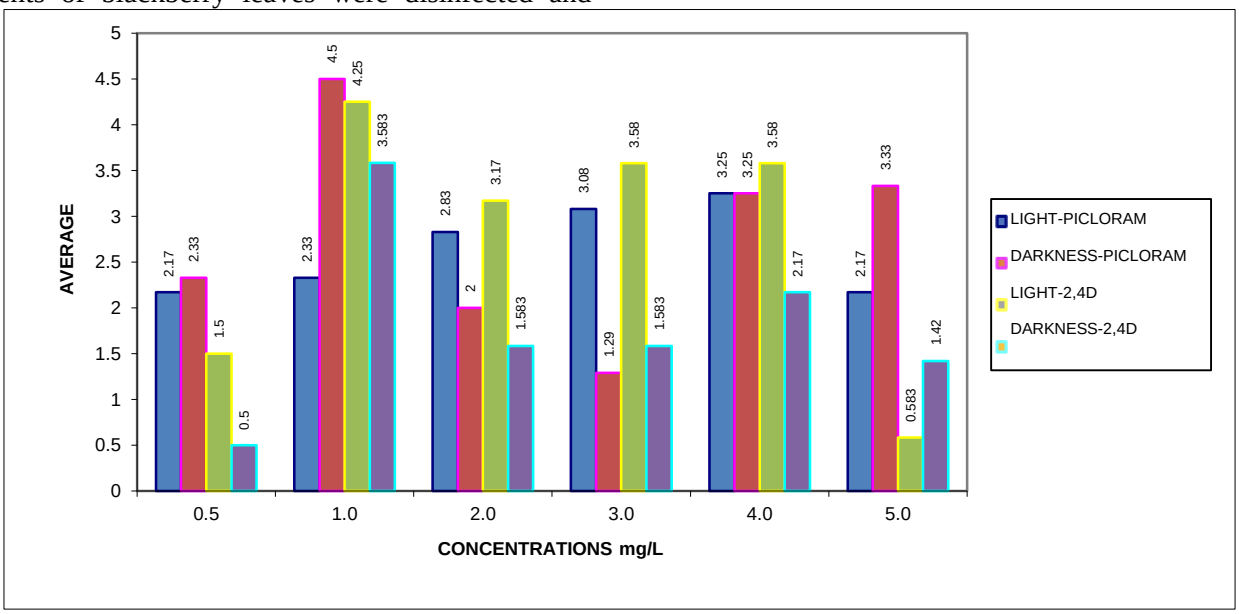

Fig. 5 Pro-embryogenic calluses obtained from thornless *R. glaucus* explants treated with Picloram and 2,4-D in the presence of light and darkness (Developed by the authors)

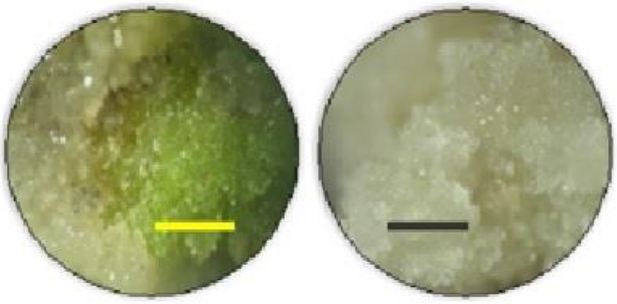

Fig. 6 Presence of pro-embryogenic callus from thornless *R. glaucus* leaves, with concentrations of 1.0 mg/l of the Picloram hormone, without light (Developed by the authors)

These results show similarity with those obtained by [14], which observed in *Musa* sp. *at 6* months of culture that in some zones of the compact callus there were loose cells that detached from it and formed the embryogenic culture, characterized by the presence of somatic embryos in different developmental states and, in many cases, these embryos remained attached to the calluses of origin with well-defined cell walls.

The most important antecedent reported to date on somatic embryogenesis in *R. glaucus* was reported by [14], which claimed that the formation and development of somatic embryos is influenced mainly by the type of explant and the auxin stimulus, presenting the best responses when immature zygotic embryos are cultured, preferably in the presence of 1.0 mg/l de AIB, conditions under which the embryogenic process is predominantly induced by the indirect route.

[9] reported that for thorny *R. glaucus*, adding auxin 2,4-D (1.0 mg/l) in the culture medium induces granular and brownish callus formation; this condition may be compared with the appearance of embryogenic callus; therefore, probably for thorny blackberry 1.0 mg/l of 2,4-D induces pro-embryo formation (Fig. 7).

Fig. 7 Brown callus formation in darkness (left) and green with light (right) (Developed by the authors)

In addition to the foregoing, with the Picloram hormone in higher concentrations, the most predominant callus was of friable type under light and darkness (Figs. 8 and 9); however, the appearance of embryogenic callus only occurred in darkness; therefore, the texture of the friable callus is only suitable for embryogenesis with this growth regulator during photoperiods without light.

Fig. 8 Friable callus texture (Developed by the authors)

Studies on rubber by [16] showed that in the medium for callogenesis initiation, adding substances, such as calcium, saccharose, and hormones permits stimulating the formation of friable lobes in calluses. In addition, four to six subcultures should be performed on the same initiation medium to stimulate friable callus formation. The quality of friable tissue is not a unique and sufficient characteristic to define complete embryogenesis; it is also necessary for the callus to acquire a strong proliferation capacity.

In the study conducted on thornless *R. glaucus*, during callus establishment, constant subcultures were conducted in the same medium; this coincides with that described by [17] regarding the stimulus for friable callus formation.

Fig. 9 Compact callus texture (greenish appearance) (Developed by the authors)

Similar behavior was reported by [4], which found compact and hard calluses in MS medium with 0.1 mg/l NAA and 0.5 mg/l BAP; however, the medium including 1.0 mg/l 2,4-D, 0.9 mg/l NAA, and 0.5 mg/l BAP resulted in friable and soft calluses in callus induction and proliferation in cultivars from Troyer and Feutrell's citrange leaf explants.

3.2. Proembryogenic Callus Multiplication

Once embryogenic calluses were obtained, they were fragmented (initial weight 1.2 g) and subjected to various multiplication treatments to stimulate the appearance of mature embryos.

The random effects ANOVA, applied as support for embryogenic callus multiplication, indicated that the highest weight averages corresponded to the 3.0 mg/l 2,4-D treatment combined with 3.0 mg/l BAP, for a mean of 3.5 under light and 2.79 under darkness (Fig. 10). The appearance of calluses was greenish under light (photosynthetic) and brown under darkness

(oxidized) (Fig. 11). Calluses exposed to media without growth regulators (WGR) showed a cream-colored appearance under light conditions and brown (oxidized) under darkness (Fig. 12).

According to [17], the types of growth regulators and their concentrations are essential parameters for callus development. [18] and [17] demonstrated that although adding kinetin and BAP to the culture medium can darken callus color, in the callogenesis induction medium, both regulators promote the induction of somatic embryogenesis and subsequent conversion of embryos to seedlings.

Studies conducted on thorny *R. glaucus* demonstrated that adding auxins and cytokines to the culture medium stimulated embryogenic and organogenic responses. Overall, auxin presence in the medium was more effective in inducing embryogenic processes [15].

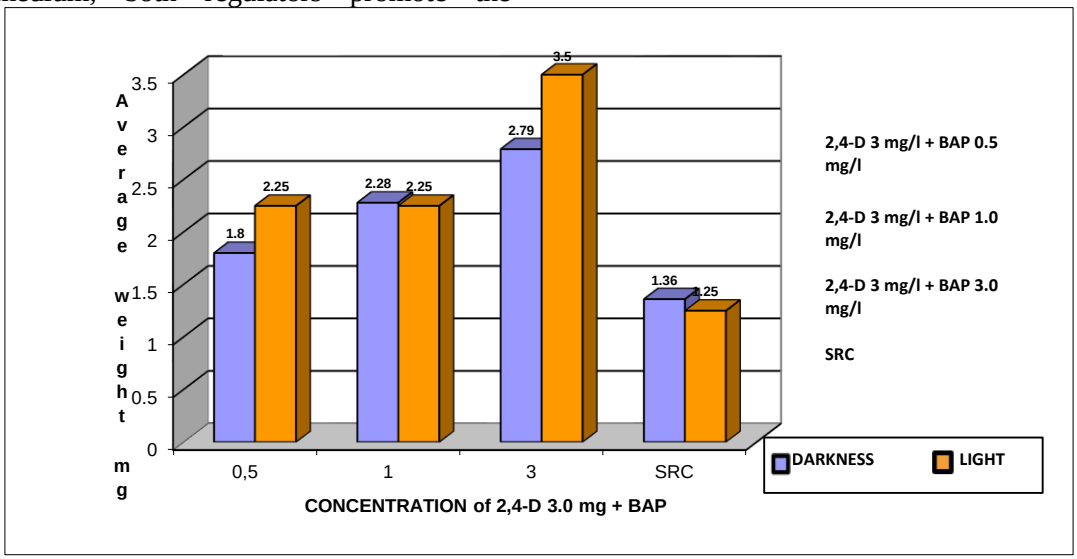

Fig. 10 Average callus weight in thornless R. glaucus explants in 2,4-D culture medium (3.0 mg/l) combined with BAP (0.5, 1.0, 3.0 mg/l and WGR) (Developed by the authors)

Fig. 11 Brown callus formation under darkness (left) and green under light (right) and in the combination of 2,4-D 3.0 mg/l + BAP 3.0 mg/l. 2 millimeters (Developed by the authors)

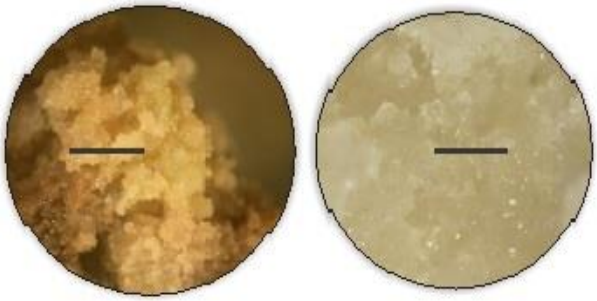

Fig. 12 Callus formation under a 12-h light photoperiod (right) and darkness (left) in WGR culture medium (Developed by the authors)

A similar situation to that reported by [1] was noted here during the multiplication phase of embryogenic callus of thornless *R. glaucus*, obtained in only one of the ten treatment calluses, with appearance of sprouts of likely organogenic origin in the combination of 2,4-D 3.0 mg/l + BAP 3.0 mg/l, in presence of light. Sprouts originated after 15 days of formation of the aerial part of seedlings (Fig. 13).

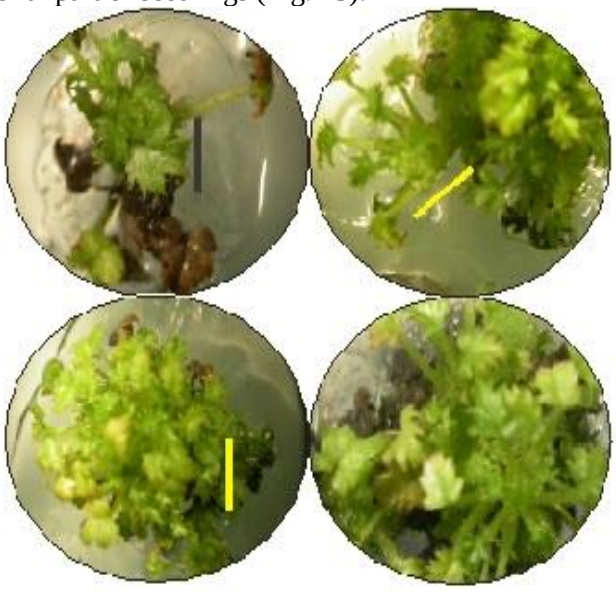

Fig. 13 In vitro formation of seedlings from sprouts of organogenic origin 2,4-D 3.0 mg/l^{-1}L + BAP 3.0 mg/l^{-1}, in the presence of light 3 millimeters (Developed by the authors)

According to [19], a condition exhibited by embryogenic calluses, with prolonged darkness exposure, generally leads to organogenesis, which is described as possible when the media is supplemented

with organic sources. For thornless *R. glaucus*, embryogenic calluses remained exposed for prolonged periods up to 12 h of illumination, resulting in the low-frequency appearance of organogenic sprouts. For thorny *R. glaucus*, [15] reported the appearance of multiple axillary stems from cytokine stimulus and, to a lesser degree, auxin stimulus.

[10] found that the 2.0 mg/l 2,4-D + 2.0 mg/l NAA combination is necessary to produce calluses with embryogenic structures during a short time in *V. odorata* L., whereas the use of thidiazuron (TDZ) also produced embryogenic calluses, but took more time.

[12] reported that *in vitro* callogenesis induction in *E. uniflora* for embryogenesis, after 30 days of culture under a 16-h photoperiod, resulted in calluses originating from nodal segments of treatment T4 (NAA 10.0 mM + TDZ 5.0 mM), which revealed the development of putative embryogenic structures with isodiametric and elongated cells, with morphological structures similar to the globular and torpedo stages observed in somatic embryos of the species Myrtaceae Acca sellowiana and Myrcianthes communis. Calluses from nodal segments of treatment T3 (5.0 mM NAA + 5.0 mM TDZ) and leaves of treatment T6 (10.0 mM 2,4-D + 5.0 mM BAP) did not develop somatic embryos but became compact green structures.

4. Conclusion

The vegetative reproduction of blackberry *(Rubus glaucus*) is not the most appropriate because of the application of laborious methodologies to carry a transplant to the field, at the same time genetically heterogeneous plants are generated, which is complex for the establishment of commercial crops. In accordance with the above, other reproduction techniques are required through *in vitro* sowing of different explants that induce the formation of shoots, roots, or flowers, either from organogenesis or embryogenesis.

Proliferation of thornless *R. glaucus* embryogenic callus was obtained in added complete MS medium in concentrations of 1.0 mg/l 2,4-D under light conditions; the best response in embryogenic callus proliferation corresponded to a concentration of 1.0 mg/l with Picloram under darkness, and appearance of thornless *R. glaucus* organogenic sprouts from embryogenic callus was achieved with treatment 2,4-D 3.0 mg/l + BAP 3.0 mg/l, in the presence of light.

The ANOVA yielded that statistically significant differences exist among the different treatments ($p < 0.05$), where callus formation with the Picloram hormone at different concentrations, with presence of light and darkness, indicated that the greatest callus formation was verified with the 1.0 mg/l concentration and under darkness (4.92 calluses on average), followed by a 4.0 mg/l concentration (3.75 calluses on average).

Pro-embryogenic callus formation with Picloram hormone at 1% and darkness had the highest callus formation with 4.5, followed by auxin 2,4-D at 1%, with presence of light, with 4.25 pro-embryogenic calluses and the Picloram hormone at 1% concentration with light, developing, on average, 3.58 calluses.

With the Picloram hormone, the most predominant callus was the friable type under light and dark conditions; the appearance of calluses was greenish in light (photosynthetic) and brown in darkness (oxidized).

It is advisable to conduct tests that allow embryo germination, either in solid or liquid media (cell suspensions), making modifications to the medium with different combinations of auxins than those used in this study, and even with a single auxin.

It is recommended to evaluate the field behavior of *R. glaucus* plants without stingers from callogenesisto establish possible morphological differences and determine their production quality.

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