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Callus formation on *Gossypium barbadense* L. cultivar 'MSI' from leaf segments of *in vitro* germinated seeds

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ABSTRACT
Gossypium barbadense L.) has a high economic value and potential for industrial exploitation. The objective of this research was to determine the effect of different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and ascorbic acid in callus formation of cotton cultivar 'MSI'. The initial explant was leaf segments of plants obtained from seeds *in vitro* germinated. Six 2,4-D concentrations (2.26, 4.52, 6.76, 9.05, 11.31 and 13.57 µM) and three of ascorbic acid (30, 60 and 90 mg l^{-1}) were tested in culture medium in independent experiments. The percentages of total callus formation and callus with embryogenic appearance were evaluated, as well as the degree of oxidation of phenolic compounds using a scale designed for this purpose. The addition of 11.31 µM 2,4-D achieved 60% of total callus formation and 50% of callus with embryogenic appearance. The presence of oxidation of phenolic compounds was totally eliminated in the culture medium with 60 mg l^{-1} ascorbic acid. The use of 11.31 µM 2,4-D and 60 mg l^{-1} ascorbic acid in culture medium increased up to 90.33 and 88.05% the percentages of total callus formation and callus with embryogenic appearance, respectively.

Keywords: antioxidant, phenolic compounds, seeds, somatic embryogenesis

Formación de callos en *Gossypium barbadense* L. cultivar 'MSI' a partir de segmentos de hoja de semillas germinadas *in vitro*

RESUMEN
El algodón (*Gossypium barbadense* L.) tiene un alto valor económico y potencial para la explotación industrial. El objetivo de esta investigación fue determinar el efecto de diferentes concentraciones de ácido 2,4-diclorofenoxiacético (2,4-D) y ácido ascórbico en la formación de callos del cultivar de algodón 'MSI'. El explante inicial fueron segmentos de hoja de plantas obtenidas de semillas germinadas *in vitro*. Se probaron en medio de cultivo seis concentraciones de 2,4-D (2.26, 4.52, 6.76, 9.05, 11.31 y 13.57 µM) y tres de ácido ascórbico (30, 60 y 90 mg l^{-1}) en experimentos independientes. Se evaluaron los porcentajes de formación de callo total y de callo con apariencia embriogénica, así como el grado de oxidación de compuestos fenólicos mediante una escala diseñada para tal fin. La adición de 11.31 µM de 2,4-D logró un 60% de formación total de callo y un 50% de callo con apariencia embriogénica. La presencia de oxidación

de los compuestos fenólicos se eliminó totalmente en el medio de cultivo con 60 mg l^{-1} de ácido ascórbico. El uso de 11.31 µM de 2,4-D y 60 mg l^{-1} de ácido ascórbico en el medio de cultivo incrementó hasta 90.33 y 88.05% los porcentajes de formación de callo total y callo con apariencia embriogénica, respectivamente.

Palabras clave: antioxidante, compuestos fenólicos, embriogénesis somática, semillas

INTRODUCTION

Cotton (*Gossypium* spp.) is cultivated commercially in temperate and tropical regions of more than 50 countries, with a total coverage of 2.5% of the arable land in the world (WTO, 2018). It is an important crop that produces the principal source of renewable textile fiber. Moreover, oil extracted from the seeds and the shell of the seed has utility as raw forage and bedding for livestock, as fertilizer or fuel. The extracts are used in alternative medicine (Juturu *et al.*, 2015; Huang *et al.*, 2021).

G. hirsutum L. and *G. barbadense* L. are agronomically important cotton species. *G. barbadense* is natural from South America and spread out into Mesoamerica and the Caribbean. This species, also known as native cotton, country cotton, Creole cotton or colored cotton, belongs to the Malvaceae family (Roskov *et al*., 2016; Huang *et al.*, 2021). *G. barbadense* is resistant to drought conditions and soil salinity. However, the disadvantages are related to its long vegetative period, the fiber is thick and short in length. In addition, the crop presents high incidence of soil pathogens such as *Rhizoctonia solani* Kühn*, Pythium* spp*., Fusarium oxysporum* f. sp. *vasinfectum* and *Thielaviopsis basicola* (Berk. Y Broome) Ferraris, which causes seedling drop (Teruya, 2016). The worldwide production of this species is around 4.5% (Abdullaev *et al*., 2017).

In order to establish biotechnological improvement methods, an efficient and reproducible plant regeneration methodology is an essential requirement (Ghaemi *et al*., 2011; Martínez, 2018).

Tissue culture-based plant regeneration in cotton has been previously reported. Although, the majority of research are carried out in *G. hirsutum*. The petiole (Gawel and Robacker, 1990; Zhang *et al*., 2011), apical meristem (Gould *et al.,* 1991), cotyledon, hypocotyl, shoot apices and cotyledonary node (Agrawal *et al.,* 1997; Gupta *et al.,* 1997; Hemphill *et al*., 1998; Bajrovic *et al*., 2001; Aydin *et al*., 2004; Michel *et al*., 2008) are used as initial explants. However, the efficiencies of *in vitro* plant regeneration is limited when compared with other crops. Cotton has proven to be one of the most difficult crops to regenerate plants by tissue culture. As a consequence, the number of commercial cultivars and elite germplasm lines with better agronomic characteristics and fiber quality that can be regenerated are low. Besides, genotype dependent response restricts the cotton breeding and plants production by biotechnological methods (Ghaemi *et al*., 2011).

The auxin 2,4-dichlorophenoxyacetic acid (2,4-D) is the most widely used growth regulator in tissue culture for callus formation and it has been very useful for the induction of callus in cotton. For instance, in cultivars of *G. arboreum* and *G. hirsutums,* Khan *et al*. (2006) reported the callus formation with 9.04 µM 2,4-D combined with 0.464 µM kinetin. Also, they found that 2,4-D was ideal for the proliferation of callus from hypocotyl and cotyledonous leaf explants of all cultivars.

Specially, in *G. barbadense,* the plants regeneration by tissue culture techniques is limited to few cultivars (Gould *et al.*, 1991; Sakhanokho *et al.*, 2001; Efe, 2005; Xi-lian *et al*., 2016). According to Ahsan and Majidano (2014), the genotype, donor plant, growth regulators, type of sugar, culture medium, temperature and subculture timing are factors that affecting the tissue culture response in *Gossypium* spp. In this sense, the protocols for each cultivar must be optimized. The aim of this work was to form callus on the cotton cultivar 'MSI' from leaf segments of *in vitro* germinated seeds. This paper constitutes the first report of calli formation in cotton using this type of explant.

METERIALS AND METHODS

Plant material

Mature seeds of cotton (*Gossypium barbadense* L.) cv. 'MSI' were used as the initial plant material. The seeds with basic category were obtained from the gene bank of the Cotton Research Center of Antigua and Barbuda, Friars Hill Road.

Preparation of plant material

In order to obtain the leaf segments as initial explants, seeds were disinfected and germinated according to the methodology proposed by Teruya (2016) (Figure 1 A). Seedling with 5 cm height and at least three leaves were selected (Figure 1 B). Leaf segments of 1 cm^2 were used as the initial explant for callus formation (Figure 1 C). Four segments were placed over the surface of culture medium in a culture flask (Figure 1 D).

Culture media

The culture media used are specified in each experiment and its pH was adjusted to 5.7 with NaOH (1.0 N) or HCl (1.0 N) before sterilization by steam autoclave, at 121 ºC and 1.2 kg cm^{-2} pressure, for 20 minutes. Extra Hard Agar (BIOCEN, Cuba) was used as gelling agent for the culture media for callus formation. Culture flasks of 250 ml of total volume were used with 30 ml of culture medium each.

The metal plates for work at the laminar flow cabinet were sterilized in an oven at 180 °C for 2 h. The instruments (forceps and scalpels) were sterilized in a DENT-EQ model electric sterilizer (Germany) placed in a horizontal laminar flow chamber, where the plant material was handled under aseptic conditions.

In vitro culture conditions

All the experiments for callus formation were carried out in darkness at 27 ± 2 ºC.

Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on callus formation

The experiment was carried out with the objective of determining the effect of 2,4-D concentration (2.26, 4.52, 6.76, 9.05, 11.31 and 13.57 µM) on callus formation.

The composition of culture medium was MS salts and vitamins (100%) (Murashige and Skoog, 1962), 100 mg l^{-1} myo-inositol, 2,4-D concentration, 30 g l^{-1} sucrose and 8.0 g l^{-1} Extra Hard Agar. Ten culture flasks were used per treatment. Four explants per culture flask (40 explants per treatment) were placed horizontally by the abaxial side on the surface of culture medium (Figure 1 D). A control treatment without 2,4-D was used.

The calli characteristics were described. After 30 days of culture, the total number of explants that formed callus and the number of callus with embryogenic appearance (nodular, bright yellow) were quantified and expressed as percentage (%) per culture flask. Besides, the percentage of callus with oxidation (presence of phenolic compounds in the medium) per culture flask was also

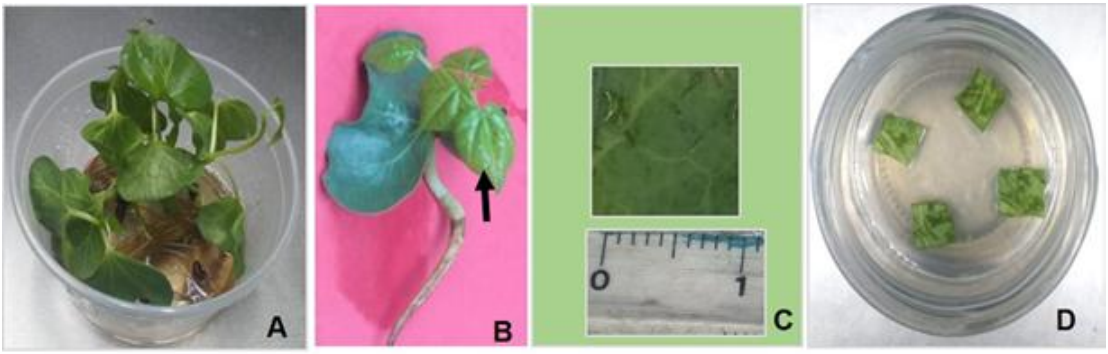


Figure 1. Explants used for callus formation in cotton (*Gossypium barbadense* L.) cultivar 'MSI'. (A, B) Plants obtained from *in vitro* germinated seeds, the arrow indicate the leaves used for callogenesis induction (C) Leaf segments of 1 cm^2 used as initial explant, (D) Leaf segments placed on the culture medium.

recorded. The degree of oxidation was classified according to qualitative scale designed for this purpose as: strong, medium, slight and no oxidation. The 2,4-D concentration with major number of callus formed was selected.

Effect of ascorbic acid on the oxidation of phenolic compounds

The experiment was carried out in order to evaluate the effect of ascorbic acid in controlling the oxidation of phenolic compounds of the explants. Three concentrations of ascorbic acid (30, 60 and 90 mg l^{-1}) were evaluated in the culture medium according to the results reported by Rodríguez *et al.* (2015). A control treatment without ascorbic acid was included. The culture medium was similar to that described previously and the 2,4-D concentration selected was used.

Ten culture flasks with four explants per flasks were employed by treatment. After 30 days of culture similar variables as describe above were evaluated.

Statistical analysis

During this study, a completely randomized experimental design was carried out to analyze the effect of 2,4-D on callus formation and the ascorbic acid effect on the oxidation. The experiments were repeated twice. To check the normality of the variables, the Shapiro-Wilk test was used. The Kruskal Wallis H test and the Mann-Whitney U test were used for comparison the groups. In all cases, the differences were established for $p \leq 0.05$. For the statistical analysis, the SPSS software package for Windows version 21.0 was used.

RESULTS AND DISCUSSION

The results indicated that it was possible to form callus from leaf segments of *in vitro* germinated seeds, in cotton 'MSI' cultivar in culture medium with 2,4-D. The positive response of callogenesis in this type of initial explant was not reported before. An important factor to consider for the establishment of an efficient plant regeneration protocol is the type of initial explant. In cotton *in vitro* culture, the use of different types of explants for the development of somatic embryogenesis has been described. Among the most used explants are those that come from sexual reproductive organs such as ovaries, ovules, zygotic embryos and anthers, roots, leaves and young seedling segments (cotyledons, hypocotyls) (Wu *et al*., 1998; Michel *et al*., 2018), cotyledonous axillary buds (González, 2015) and hypocotyl of mature zygotic embryos (Teruya, 2016). However, we did not find references in *G. barbadense* related to the use as initial explant of young leaf segments from seedlings obtaining from *in vitro* germinated seeds. This type of explant has been used successfully for the development of plant regeneration protocols in species such as: *Secale cereale* L. (Haliloglu and Murat, 2016), *Lavandula angustifolia* L. (Devasigamani *et al*., 2020) and *Styrax benzoin* Dryand (Nurwahyuni *et al*., 2020).

Effect of 2,4-dichlorophenoxyacetic acid on callus formation

All 2,4-D concentrations tested induced callus formation from the initial explant. After 10 days of culture, the beginning of callus formation was observed at the edges of the leaf segments used as explants. Two types of calli were formed; one friable, watery, and white coloration and the other, nodular, bright yellow color, granular, with embryogenic appearance (Figure 2). The highest percentage of total callus formation (60%) was obtained in culture medium with 11.31 µM 2,4-D, with significant differences with respect to the rest of the treatments ($p \leq 0.05$). Also, with this concentration the highest percentage of callus with embryogenic appearance was achieved (50%) (Figure 3). In previous reports, 2,4-D was an essential plant growth regulator for the induction of callogenesis in cotton (Michel *et al*., 2008). In the callus formation process, the growth regulator played a fundamental role (von Arnold *et al*., 2002).

The results showed that 2,4-D is necessary for callus formation with this type of explant because in the culture medium without the growth regulator, no callus formation was observed and the explants were completely necrotic. There are different criteria about the 2,4-D concentration to be used in the calli formation. Several authors have reported low concentrations of this auxin (0.45 µM) as the best for embryogenic callus formation in

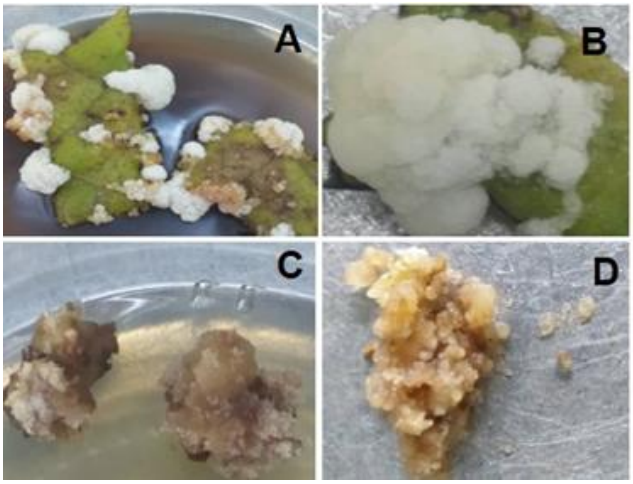
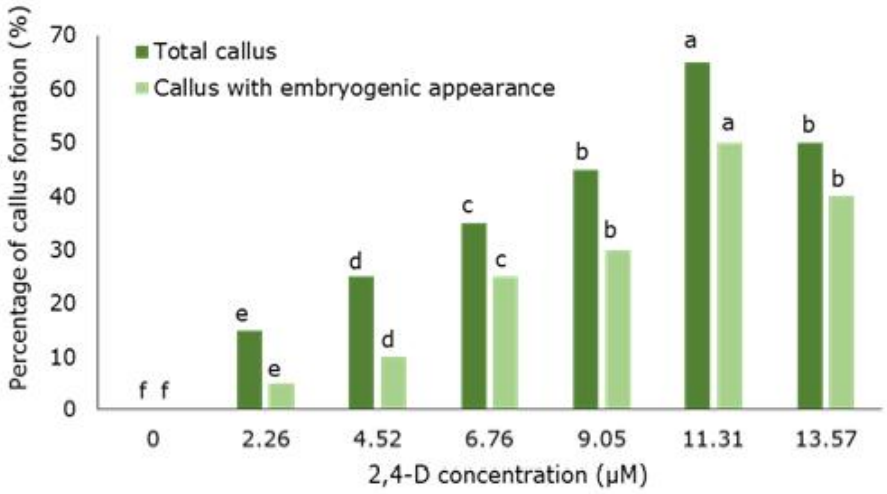


Figure 2. Callus formation in leaf segments of *in vitro* germinated seeds of *Gossypium barbadense* L. cultivar 'MSI' in culture medium with 2,4-D. A-B. Callus friable, watery, and white coloration (A-10 days of culture, B 30 days of culture). C-D Callus nodular, bright yellow color, granular (C-10 days of culture, D- 30 days of culture).



Different letters on the bars for each type of callus differ according to the Kruskal-Wallis H / Mann Whitney U tests for p≤0.05 (n = 40)

Figure 3. Callus formation in leaf segments of *in vitro* germinated seeds of *Gossypium barbadense* L. cultivar 'MSI' at 30 days of culture with 2,4-D.

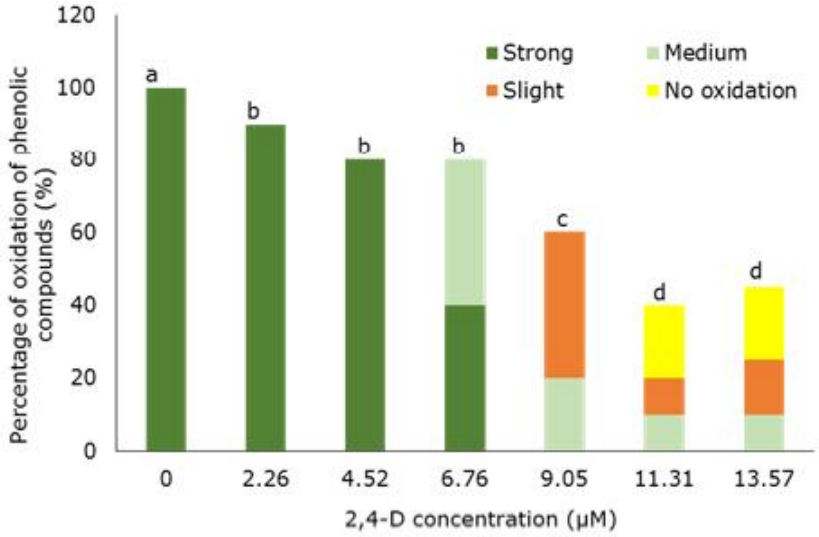
cotton (Zhang *et al*., 1999). According to Teruya (2016) in *G. barbadense* 'native cotton' in brown callus they found the highest induction of embryogenic appearance (82.5%) with 0.45 µM 2,4-D and 100 ml l^{-1} coconut water. The results in this work are not in agreement with those reported by mentioned author. The percentage of explants that formed callus was increased with higher concentrations (11.31 and 13.58 µM 2,4-D).

The lowest percentages of explants with oxidation of phenolic compounds (40 and 45%) were obtained in treatments with the higher 2,4-D concentrations (11.31 and 13.58 µM) with significant differences with the rest of the treatments (Figure 4). Considering the results, 11.31 µM was selected for the rest of the experiments.

Effect of ascorbic acid on the oxidation of phenolic compounds

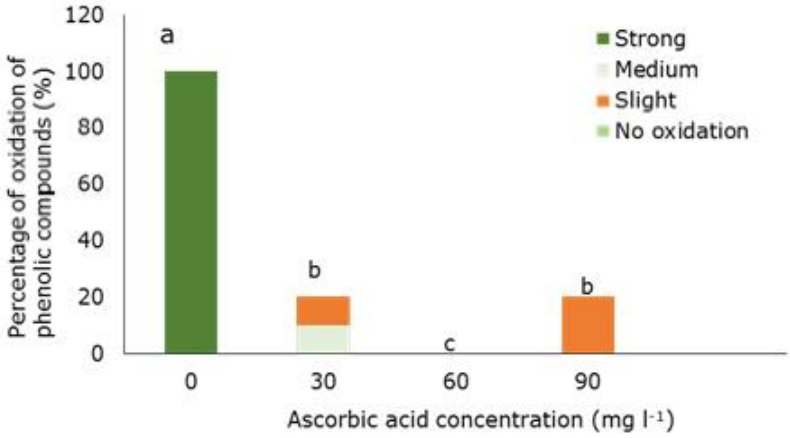
As described previously, different degree of oxidation of phenolic compounds was observed in treatments with calli formed, but

it was reduced with the addition of ascorbic acid. With 60 mg l^{-1} ascorbic acid in culture medium, the lower oxidation of phenolic compounds by the explant, with significant differences compared to the control, as well as with treatments 30 and 90 mg l^{-1}. With 30 mg l^{-1} ascorbic acid, the oxidation of phenolic compounds by the explant was not totally eliminated. In this treatment, 10% of the calli presented a slight oxidation in the culture medium according to the scale established to classify the intensity of this variable. With the addition of 90 mg l^{-1} to the culture medium, it was not possible to increase the control of the oxidation of phenolic compounds. In this treatment, despite presenting 15% of the calluses with oxidation of phenolic compounds, it was classified as medium (Figure 5).



Different letters on the bars significant differences according to the Kruskal-Wallis H test / Mann-Whitney U test for p≤0.05 (n = 40)

Figure 4. Percentage of callus with oxidation of phenolic compounds in the culture medium with different concentrations of 2,4-D for the formation of callus in *Gossypium barbadense* L. cv. 'MSI' after 30 days of culture.



Different letters on the bars differ according to the Kruskal-Wallis H test / Mann-Whitney U test for p≤0.05 (n = 40)

Figure 5. Effect of ascorbic acid on the oxidation of phenolic compounds in *Gossypium barbadense* L. cv. 'MSI' callus after 30 days of culture.

In the control (without antioxidant) a strong oxidation of phenolic compounds in the explants was observed which was exuded into the culture medium. The addition of ascorbic acid (60 mg l^{-1}) eliminated the oxidation of these compounds and the calli were nodular, bright yellow and with embryogenic appearance (Figure 6). No dark brown pigments were observed in any of the callus formed.

The percentage of callus formation and those with embryogenic appearance increased with the addition of ascorbic acid to the culture medium regardless of the added concentration. With 60 mg l^{-1}, the total percentage of callus formation (90.33%) and callus with embryogenic appearance (88.05%) was significantly increased with significant differences with respect to the rest of the treatments (Table 1). This result showed that the addition of this concentration of ascorbic acid could increase the explant absorption of the nutrients present in the culture medium and callus formation.

The oxidation of phenolic compounds was observed initially in the explants during the callus formation process. This problem is common during the isolation of explants by the accumulation and oxidation of phenolic compounds (Jones and Saxena, 2013). Those are released by damaged cells during the process of dissecting the leaves into explants. The cut parts of the leaves darken rapidly, the oxidized products are toxic to the rest of the explant and diffuse into the culture medium, darkening it. This problem is particularly serious in the isolation of explants of woody species. The tissues of these species are richer in phenolic compounds, precursors of lignin synthesis. Some techniques to reduce this problem include the use of different carbon sources (Kumar *et al*., 2015) and antioxidant substances such as polyvinylpyrolidone (PvP), ascorbic acid and citric acid to counteract this effect (Aguirre *et al.,* 2010).

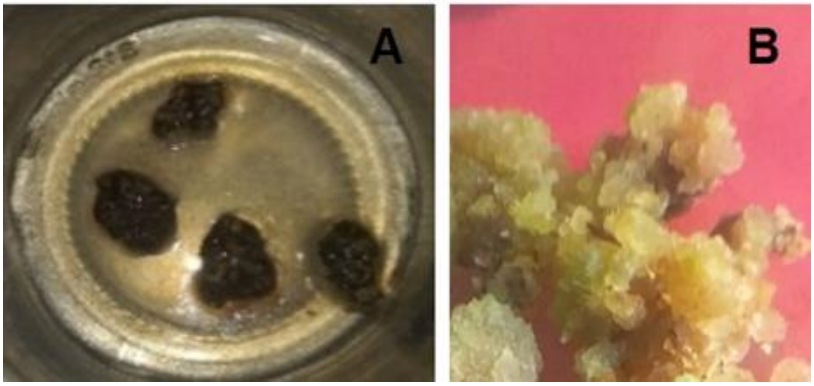


Figure 6. Calli formed from 1 cm^2 leaf segments of *Gossypium barbadense* L. cv. 'MSI' *in vitro* germinated seeds using culture medium with 2,4-D. (A) Control, callus with oxidation of phenolic compounds in culture medium without ascorbic acid, (B) nodular, bright yellow color callus formed with 11.31 µM 2,4-D and 60 mg l^{-1} ascorbic acid.

Table 1. Effect of ascorbic acid on the oxidation of phenolic compounds in callus formation in *Gossypium barbadense* L. cv. 'MSI' after 30 days of culture.

Ascorbic acid (mg l^{-1})	Percentage of callus formation (%)			
	Total		Callus with embryogenic appearance	
	Media	Range Mean	Mean	Range Mean
0	34.17	29.87 d	22.00	27.53 d
30	44.33	48.60 c	37.00	33.98 c
60	90.33	105.00 a	88.05	104.83 a
90	85.50	103.53 b	83.50	101.65 b

Range means with uncommon letters within the same column differ according to the Kruskal-Wallis H / Mann-Whitney U test for p≤0.05 (n = 40)

The data evidenced that the use of ascorbic acid in the medium achieve the control of the oxidation of phenolic compounds in callus formation in *G. barbadense* cultivar MSI. There are few references related to the addition of antioxidants in cotton callus culture. In this regard, Davis *et al.* (1974) reported the inclusion of 5 mg l^{-1} ascorbic acid in callus culture of *G. hirsutum*. Besides, Katterman *et al.* (1977) formed callus in *G. barbadense* from cotyledons of germinating cotton seedlings by use of a strong reducing agent (dithiothreitol) that was superior to ascorbic acid. Azofeifa (2009) referred that the oxidation of phenolic compounds is influenced by different factors such as the genotype, type, size and age of the explant, as well as the time of year in which the explants are collected. This author also indicated that ascorbic acid is a very common tricarboxylic acid in plants, and key compounds of the Krebs cycle. In other species, Ancasi *et al*. (2019) reported the effectiveness of adding ascorbic acid and citric acid to the culture medium (100 mg l^{-1} of each) to overcome specific oxidation problems associated with tissue culture during *in vitro* propagation of banana (*Musa paradisiaca* L.).

The results of this work indicated that the addition of ascorbic acid to the culture medium was necessary to control of the oxidation of phenolic compounds from the explants during callus formation in cotton cv. MSI. The concentration of 60 mg l^{-1} ascorbic acid was the most effective in reducing phenolic oxidation, which contributed to a greater number of total callus and callus with embryogenic appearance. So, this concentration was selected for the culture medium in callus formation with a view to carrying out further studies.

CONCLUSIONS

The use of segments from the young leaves of plants obtained from *in vitro* germinated seeds is an important factor for callus formation in *G. barbadense* cv. 'MSI'. The callus formation with embryogenic appearance is influenced by the concentration of 2,4-D and the antioxidant effect of ascorbic acid. A high percentage of total calli and callus with embryogenic appearance is achieved in the presence of 11.31 µM 2,4-D and 60 mg l^{-1} ascorbic acid.

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Conflict of interest

The authors have no conflict of interest to declare. The work is genuinely under taken and all the data in the paper is never published or in consideration for publication in any other journal.

Authors contribution

Conceptualization SJMM, LLTNMEGV, Formal Analysis SJMM, LLTN, Investigation LLTN, SJMM, Methodology SJMM, LLTN, Supervision SJMM, Writing- first draft SJMM, LLTN, Writing-Revision and Edition SJMM, LLTNMEGV.

REFERENCES

Abdullaev AA, Salakhutdinov IB, Egamberdiev SS, Khurshut EE, Rizaeva SM, Ulloa M, Abdurakhmonov Y (2017) Genetic diversity, linkage desequilibrium, and association mapping analyses of *Gossypium barbadense* L. germplasm. PLOS ONE 14(1): 30

Agrawal DC, Banerjee AK, Kolalal RR, Dhage AB, Kulkarni WV, Nalawade SM, Hazra S, Krishtamurthy KV (1997) *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep 16: 647-652

Aguirre G, Jean B, Ligue L (2010) Aplicaciones del cultivo de tejidos en la multiplicación y conservación de los recursos fitogenéticos. Universidad Mayor de San Simón, Cochabamba

Ahsan MZ, Majidano MS (2014) Regeneration of cotton (*Gossypium hirsutum* L.) through asexual methods, a review. Amer Eura J Agric & Environ Sci 14(12): 1478-1486

Ancasi RG, Montero JR, Ferreira NJ, Muñoz I (2019) Determinación un mejor medio de cultivo en la fase de establecimiento para la propagación *in vitro* de plátano (*Musa paradisiaca* L). Journal of the Selva Andina Research Society 7(2): 104-11

Aydin Y, Ipekci Z, Talas-Oðraþ T, Zehir A, Bajrovic K, Gozukirmizi N (2004) High Frequency Somatic Embryogenesis in Cotton. Biologia Plantarum 48: 491-495; doi:10.1023/B:BIOP.0000047142.07987.e1

Azofeifa A (2009) Problemas de oxidación y oscurecimiento de explantes cultivados *in vitro*. Agronomía Mesoamericana 1: 153-175

Bajrovic K, Aydin Y, Talas-Ogras T, Ipekci Z, Altinkut A, Gencer O, Gozukirmizi N(2001) Direct plant regeneration from node and apex explants of cotton in Turkey. Biotechnol Biotech Eq 15 (2): 73-76

Davis DG, Dusbabek KE, Hoerauf RA (1974) *In vitro* Culture of Callus Tissues and Cell Suspensions from Okra (*Hibiscus esculentus* L.) and Cotton (*Gossypium hirsutum* L.). *In Vitro* 9(6): 395–398

Devasigamani L, Devarajan R, Ramuoganathan, Rafath H, Padman M, Govinda Raju MV, Giridhar L, Chetan HC, Kappa N (2020) *Lavandula angustifolia* L. plants regeneration from *in vitro* leaf explants-derived callus as conservation strategy. Biotecnología Vegetal 20(2): 75 – 82

Efe L (2005) Callus formation and plant regeneration from two cotton species (*Gossypium hirsutum* L., and *G. barbadense* L.). Pak J Bot 37(2): 227-236

Ghaemi M, Majd A, Fallahian F, Bezdi G (2011) Comparison of callus induction and somatic embryogenesis of some Iranian cottons (*Gossypium* spp.) with Coker 312 and histology of somatic embryogenesis. African Journal of biotechnology 10(15): 2915-2922

Gawel NJ, Robacker CD (1990) Somatic embryogenesis in two *Gossypium hirsutum* genotypes on semi-solid versus liquid proliferation media. Plant Cell Tiss Org Cult 23: 201-20

González JA (2015) Evaluación *in vitro* de materiales de algodón *Gossypium hirsutum* L. en relación a la capacidad de regeneración y respuesta a estrés abiótico. Tesis para optar al título de Magister en Genética Vegetal. Universidad Nacional de Rosario, Rosario, Argentina

Gould J, Banister S, Hasegawa O, Fahima M, Smith RH (1991) Regeneration of *Gossypium hirsutum* and *G. barbadense* from shoot apex tissues for transformation. Plant Cell Reports 10: 12-16

Gupta SK, Srivastava AK, Singh PK, Tuli R (1997) *In vitro* proliferation of shoots and regeneration of cotton. Plant Cell Tiss Org Cult 51: 149-152

Haliloglu K, Murat A (2016) Efficient regeneration system from rye leaf base segments. SpringerPlus 5: 2-8

Hemphil JK, Maier CGA, Chapman KD (1998) Rapid *in vitro* regeneration of cotton (*Gossypium hirsutum* L.). Plant Cell Rep 17(4): 273-278

Huang G, Huang JQ, Chen XY, Zhu YX (2021) Recent advances and future perspectives in cotton research. Annual review of plant biology 72: 437-462

Jones AM, Saxena PK (2013) Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.: a novel approach to reduce oxidative browning in plant tissue culture. PLoS One 8(10):e76802; doi: 10.1371/journal.pone.0076802

Juturu VN, Mekala GK, Kirti PB (2015) Current status of tissue culture and genetic transformation research in cotton (*Gossypium* spp.). Plant Cell, Tissue and Organ Culture (PCTOC) 120: 813-839

Katterman FR, Williams MD, Clay WF (1977) The influence of a strong reducing agent upon the initiation of callus from the germinating seedlings of *Gossypium barbadense*. Physiologia Plantarum 40(2): 98-100

Khan T, Singh AK, Pant RC (2006) Regeneration via somatic embryogenesis and organogenesis in different cultivars of cotton (*Gossypium* spp.). *In Vitro* Cell Dev Biol-Plant 42: 498–50

Kumar GP, Subiramani S, Govindarajan S, Sadasivam V, Manickam V, Mogilicherla K, Narayanasamy J (2015) Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium*

hirsutum L.) cv. SVPR-2. Biotechnology Reports 7: 72-80

Martínez SJ (2018) Regeneración de plantas de sorgo granífero [*Sorghum bicolor* (L.) Moench] cultivar 'CIAP 132R-05' vía embriogénesis somática. Tesis presentada en opción al grado científico de Doctor en Ciencias Agrícolas. Universidad Central Marta Abreu de Las Villas, Santa Clara, Cuba

Michel Z, Hilaire KT, Mongomaké K, Georges AN, Justin KY (2008) Effect of genotype, explants, growth regulators and sugars on callus induction in cotton (*Gossypium hirsutum* L.). Australian Journal of Crop Science 2(1): 1-9

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tabacco tissue culture. Physiologia Plantarum 15: 473-497

Nurwahyuni I, Situmorang M, Sinaga R (2020) Plant Regeneration through Callus Cultures from Leaf Explant of Sumatra Benzoin (*Styrax benzoin*). International Journal of Forestry Research 2020: 8860178; doi: 10.1155/2020/8860178

Rodríguez M, Gómez Kosky R, Martínez SJ, Pons M, Pérez M, La O, Romero C (2015) Formación de embriones somáticos a partir de semillas inmaduras en *Sorghum bicolor* variedad CIAP 132-R. Acta Biol Colomb 20(2): 237-245

Roskov Y, Abucay L, Orrell T, Nicolson D, Flann C, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A (2016) Annual Checklist. Available in: www.catalogueoflife.org/annual-checklist/2016 Accessed 09/01/2021

Sakhanokho HF, Zipf A, Rajasekaran K, Saha S, Sharma GC (2001) Induction of highly embryogenic calli and plant regeneration in upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) cottons. Crop Science 41(4): 1235-1240

Teruya MS (2016) Evaluación de fitorreguladores del crecimiento en la inducción de callo embriogénico en *Gossypium barbadense* L. 1753 'algodón nativo' color pardo. Tesis para optar el Título Profesional de Licenciada en Biología, Facultad de Ciencias Biológicas Escuela Profesional De Biología, Universidad Ricardo PALMA, Lima, Perú

von Arnold S, Sabala I, Bozhkov P, Dyachok J, Filonova L (2002) Developmental pathways of somatic embryogenesis. Plant Cell Tiss Organ Cult 69: 233-249

WTO (2018) Cotton in the World Economy. World Trade Organization Switzerland. Available in: www.wto.org Accessed 29/1/2021

Wu JY, She JM, Cai XN, Bajaj YPS (1998) Establishment of callus culture, somatic embryogenesis, and the regeneration of cotton plants. In: Bajaj YPS (ed). Cotton, Biotechnology in agriculture and forestry Vol 42, pp. 37-47. Springer, Berlin

Xi-lian D, Yan-ying Q, Qiong L, Jia-yan G, Quan-jia C (2016) The Study of Regeneration System from Different Sea Island Cotton (*Gossypium barbadense* L.) Cultivars. Biotechnology Bulletin 32(1): 97-102

Zhang BH, Feng R, Li XL, Li FL (1999) Direct induction of cotton somatic embryogenesis. Chinese Sci Bull 44:766 – 767

Zhang C, Yu S, Fan S (2011) Inheritance of somatic embryogenesis using leaf petioles as explants in upland cotton. Euphytica 181: 55-63; doi: 10.1007/s10681-011-0380-7

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