Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v14i6.3183

Article

Regeneration of leaf explants of five raspberry genotypes

Monserrat Abigail Rosas-Rojas[1]
Neftalí Ochoa-Alejo[2]
Ma. del Carmen Rocha-Granados[1,§]

1 Facultad de Agrobiología 'Presidente Juárez'-Universidad Michoacana de San Nicolás de Hidalgo. Uruapan, Michoacán, México.

2 Departamento de Ingeniería Genética-Centro de Investigación y de Estudios Avanzados-Unidad Irapuato-Instituto Politécnico Nacional. Irapuato, Guanajuato, México.

Autora para correspondencia: carmen.rocha@umich.mx.

Abstract

The efficiency in raspberry regeneration, from leaf explants, is limited due to several factors, among which the age of the explant and the genotype stand out. The aim of this research was to determine the effect of growth regulators on oxidation and in vitro regeneration from leaf explants of five raspberry genotypes in 2021. Doses and combinations of auxins and cytokinins were tested to induce direct organogenesis in leaf explants of raspberry genotypes; 'C-6', 'Joan J.', 'A-1', 'UM-702' and 'Heritage'. The results showed that the regulator benzylaminopurine (BAP) decreased oxidation in genotypes 'C-6', 'Joan J.', 'A-1' and 'Heritage' by 36, 48, 60 and 68%, respectively, those that were supplemented with kinetin had a reduction in oxidation in the genotype 'C-6' (56%), when thidiazuron (TDZ) was added, oxidation decreased in the genotypes evaluated by 72, 64, 72, 84 and 68%, respectively. The greatest regeneration (number of shoots/explant) was with BAP (0.5 mg L-1) and TDZ (0.2 mg L-1) + indole butyric acid (IBA) (0.1 mg L-1) for the genotype 'C-6', and TDZ (0.2 mg L-1) + IBA (0.1 mg L-1) for 'Joan J.' and 'Heritage'. In 'A-1' and 'UMC-702', the use of TDZ (0.2 mg L-1) alone is suggested. It is concluded that the use of growth regulators, alone or combined, decreases oxidation in leaf explants, and increases the survival and regeneration of shoots in all genotypes evaluated.

Keywords:

Rubus idaeus L., organogenesis, plant hormones.

DOI: https://doi.org/10.29312/remexca.v14i6.3183

Introduction

Mexico is the fifth largest producer of raspberry (*Rubus idaeus* L.), in 2019, 128 848 t were produced, with a value of 5.154 billion dollars (FAOSTAT, 2022), of which Michoacán contributed 25 988 t (SIAP, 2020). Among the most successful varieties in this region are 'Heritage', 'Maling', 'Exploid', 'Adelita', 'Autum Bliss', 'Primavera' and 'Blazer' (Bascopé, 2013), which were generated through traditional techniques of crossing and selection; however, due to the perennial nature and their low genetic diversity, the programs of improvement and generation of new raspberry cultivars are limited (Hall *et al*., 2009).

Biotechnology provides tools to achieve genetic improvement in a rapid and targeted manner (Gutiérrez *et al*., 2003), from the clonal multiplication of plant species with desirable agronomic traits (Allccaco, 2016), in addition to the culture of plant organs and tissues that guarantees the quality and safety of plant material (Jadán *et al*., 2015). *In vitro* propagation methods for raspberry have been employed since the 80s; nevertheless, raspberry is highly recalcitrant so the explants usually present a large amount of phenolic compounds that affect the formation of adventitious shoots, coupled with the fact that each cultivar has its own requirements for *in vitro* multiplication (Wu *et al*., 2009).

The plants obtained by regeneration via organogenesis distinguished themselves by presenting outstanding traits such as greater number and length of canes, and fruits in raspberry plants (Debnath, 2014). Organogenesis is fundamental in the *in vitro* regeneration and multiplication of raspberries and includes the use of growth regulators. Several works have studied their effect, among which auxins and cytokinins stand out (González *et al*., 2009; Hunková *et al*., 2016), their concentration depends mainly on the species, tissue or organ, and on the main objective of the experiment (Adobkar *et al*., 2012).

Raspberry regeneration has been obtained from leaf segments and petioles (Kim and Dai, 2020), axillary buds and nodal meristems (Allccaco, 2016) and apical segments (Jadán *et al*., 2015) with the use of indole butyric acid (IBA), benzylaminopurine (BAP), gibberellins (GA) and thidiazuron (TDZ) (Jadán *et al*., 2015; Allccaco, 2016; Kim and Dai, 2020). During this process, oxidation occurs in the cells due to the stress caused by tissue cutting (Phineas and Kuman, 2013).

The oxidation of explants is due to the action of oxidase and tyrosinase enzymes that are released when tissues are injured (Jacinto, 2018). To counteract this, it is recommended to add antioxidants such as ascorbic acid, citric acid and adsorbents such as activated carbon to the culture medium, to make changes of culture medium when phenolization is observed or with a regular frequency, to keep the tissue in darkness in the growth chamber for about 15 days (Restrepo *et al*., 2018), as well as thermal shocks (Méndez-Álvarez and Abdelnour-Esquivel, 2014).

In vitro regeneration, via direct organogenesis, is a required phase in the development protocols of Mexican raspberry varieties through biotechnological tools, such as diploidization by chemical agents, or for genetic transformation. The objective of this work was to obtain basic information on the effect of growth regulators, auxins and cytokinins, on oxidation and *in vitro* regeneration of segments of raspberry (*Rubus idaeus* L.) leaves in the genotypes 'C-6', 'Joan J.', 'A-1', 'UMC-702' and 'Heritage'.

Materials and methods

Plant material

In this experimental work, the following five genotypes of red raspberry (*Rubus idaeus* L.) were used: 'Joan J.', 'Heritage', 'A-1', 'UM-702' and 'C-6', the first two are commercial materials and the last three were generated in the berry genetic improvement program of the 'Presidente Juárez' Faculty of Agrobiology of the Michoacan University of San Nicolás de Hidalgo.

DOI: https://doi.org/10.29312/remexca.v14i6.3183

In vitro establishment of vegetative material

Axillary and apical shoots from the raspberry germplasm bank of the berry greenhouse were disinfected using the methodology described by Granados-Rubio (2017) and were established *in vitro* in MS culture medium (Murashige and Skoog, 1962) with mineral salts in 100% concentration, vitamins and sucrose 30 g L^{-1}. For the proliferation of the shoots, the medium was added with 2 mg L^{-1} of BAP (Minas and Neocleous, 2007), the pH was adjusted to 5.7 ±1, the culture medium was gelled with 8 g L^{-1} of agar and 20 ml of medium were poured into bottles of 100 ml capacity. They were sterilized in autoclave at 15 psi pressure for 15 min.

The explants were placed in a growth room at 16/8 h light/darkness and a temperature of 24 ±1 °C. After three weeks, the shoots that did not show contamination were placed in the same proliferation medium, in order to have enough explants to establish the experiments on the effect of growth regulators on the oxidation and regeneration of raspberry from leaf segments.

Oxidation and regeneration of leaf segments

To determine the effect of growth regulators on raspberry oxidation and regeneration, leaf segments of each genotype (established *in vitro*) were placed in a basic MS culture medium added with cytokinins [kinetin (Kin), BAP and TDZ] and auxin (IBA) at concentrations of 1, 1.5, 2, 2.5 and 3 mg L^{-1} for Kin and BAP and 0.2, 0.4, 0.6, 0.8 and 1 mg L^{-1} for TDZ, alone or combined with 0.1 mg L^{-1} of IBA. From the seedlings propagated *in vitro*, the seeding of the explants was carried out under the following procedure: inside the laminar flow hood with the light off, sterile distilled water with ascorbic acid (50 mg L^{-1}) was placed in Petri dishes to prevent oxidation of the explants; next, leaves were dissected in sections of approximately 1 cm^2 and placed inside each bottle with culture medium, which were kept in the growth room under conditions of darkness for eight days; after this period of time, they were subjected to a photoperiod of 16/8 h of light/ darkness and 24 ±1 °C. After three weeks, they were sub-cultured in a fresh culture medium with the same conditions as the previous medium.

Experimental design

A completely randomized experimental design was used, with 34 treatments and a control with 5 repetitions, each experimental unit consisted of 5 explants per bottle. With the data obtained, a univariate analysis of variance was made and the variables that showed significant differences were subjected to Duncan's test (*p*# 0.5) (Duncan, 1995) to compare means between treatments with the statistical program of SAS version 9.0 (SAS, 2002).

The variables evaluated were: 1) oxidation of explants; it was determined by the following formula: oxidation (%)= number of oxidized explants x 100/ total number of explants established; 2) regenerated explants: the percentage of regeneration was determined by the following formula: regeneration (%)= number of explants with shoots x 100/ total number of explants established; and 3) the coefficient of multiplication: it was determined by the following formula: coefficient of multiplication = number of final seedlings/number of explants established.

Results

Effect of auxins and cytokinins on the oxidation of explants

Table 1 includes the results of the effect of regulators on the oxidation of the explants of the five genotypes analyzed. The genotype 'C-6' treated with TDZ (0.2 to 1 mg L^{-1}) showed percentages of oxidation from 8 to 16%, when 0.2 mg L^{-1} + 0.1 mg L^{-1} of IBA was used there was no oxidation (0%), while in the control (72%) was observed. The explants of 'Joan J.' that were established with TDZ presented oxidation from 0 to 28%, this decreased when TDZ was combined with IBA, where the oxidation was from 0 to 12% and in the control treatment it was (64%). Oxidation increased radically with 3 mg L^{-1} of kinetin (84%).

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v14i6.3183

Table 1. Percentage of oxidation of raspberry leaf explants of genotypes 'C-6', 'Joan J.', 'A-1', 'UMC-702' and 'Heritage' cultured *in vitro* and treated with growth regulators (BAP, kinetin and TDZ) alone or in interaction with indole butyric acid (IBA).

Growth regulator	(mg L^{-1})	'C-6'	'JOAN J.'	'A-1'	'UMC-702'	'HER'
Coefficient of variation		63.15	61.64	37.63	27.56	28.71
Benzylaminopurine	0.5	44 bcdefgh	40 bcdefghi	48 defg	72 ab	36 ef
	1	84 a	44 bcdefgh	92 abc	84 ab	88 ab
	1.5	52 abcdef	56 abcde	16 ghi	64 bc	76 abc
	2	48 abcdefg	56 abcde	96 ab	72 ab	20 fgh
	2.5	64 abcd	68 abc	88 abc	64 bc	88 ab
	3	80 ab	72 abc	100 a	84 ab	100 a
Benzylaminopurine + indole butyric acid	0.5+0.1	60 abcde	16 fghij	100 a	40 c	44 de
	1+0.1	60 abcde	44 bcdefgh	84 abc	64 bc	0 h
	1.5+0.1	60 abcde	20 efghij	60 bcdef	88 ab	76 abc
	2+0.1	44 bcdefgh	28 defghij	68abcde	68 bc	44 de
	2.5+0.1	52 abcdef	40 bcdefghi	56 cdef	92 ab	64 cd
	3+0.1	36 cdefghi	44 bcdefg	76 abcd	88 ab	28 efg
Kinetin	0.5	48 abcdefg	68 abc	88 abc	100 a	96 a
	1	36 cdefghi	72 abc	80 abcd	100 a	100 a
	1.5	40 cdefgh	64 abcd	100 a	100 a	96 a
	2	72 abc	40 bcdefghi	92 abc	100 a	100 a
	2.5	16 fghi	76 ab	84 abc	100 a	96 a
	3	28 defghi	84 a	76 abcd	100 a	100 a
Kinetin + indole butyric acid	0.5+0.1	60 abcde	48 abcdefg	40 efgh	100 a	96 a
	1+0.1	32 defghi	52 abcdef	68 abcde	100 a	100 a
	1.5+0.1	44 bcdefgh	36 cdefghij	100 a	80 ab	100 a
	2+0.1	40 cdefgh	64 abcd	76 abcd	88 ab	100 a
	2.5+0.1	56 abcde	48 abcdefg	100 a	100 a	100 a
	3+0.1	44 bcdefgh	72 abc	100 a	88 ab	100 a
Thidiazuron	0.2	12 ghi	4 ij	4 i	84 ab	16 fgh
	0.4	8 hi	24 efghij	16 ghi	72 ab	16 fgh
	0.6	12 ghi	0 j	32 fghi	76 ab	12 fgh
	0.8	16 fghi	28 defghij	20 ghi	64 bc	8 gh
	1	16 fghi	12 ghij	60 bcdef	68 bc	24 efgh
Thidiazuron + indole butyric acid	0.2+0.1	0 i	4 ij	8 hi	4 d	12 fgh
	0.4+0.1	16 fghi	12 ghij	36 efghi	0 d	8 gh
	0.6+0.1	8 hi	0 j	28 fghi	0 d	12 fgh
	0.8+0.1	8 hi	8 hij	36 efghi	0 d	16 fgh
	1+0.1	24 efghi	4 ij	4 i	0 d	0 h
Control	0	72 abc	64 abcd	76 abcd	84 ab	68 bc

Different letters in the same column indicate differences significant at 0.05.

The genotype 'A-1' showed lower oxidation with TDZ at concentrations of 0.2 mg L^{-1} and 1 mg L^{-1} plus 0.1 mg L^{-1} of IBA (4%). Whereas doses of 3 mg L^{-1} of BAP, 0.5 mg L^{-1} of BAP plus 0.1 mg L^{-1} of IBA or 1.5, 2.5 and 3 mg L^{-1} of kinetin combined with 0.1 mg L^{-1} of IBA showed 100% oxidation (Table 1). The genotype 'UMC-702' showed 0% oxidation in explants exposed to 0.4 - 1 mg L^{-1} of TDZ + 0.1 mg L^{-1} of IBA, explants exposed to kinetin, alone or combined with IBA, presented 100% oxidation (Table 1). TDZ decreased oxidation in the 'Heritage' genotype. Where concentrations from 0.2 to 1 mg L^{-1} of TDZ + 0.1 mg L^{-1} of IBA showed percentages of oxidation

DOI: https://doi.org/10.29312/remexca.v14i6.3183

from 0 to 16%, while kinetin (0.5 to 3 mg L^{-1}) + 0.1 mg L^{-1} of IBA induced oxidation from 96 to 100%, values higher than the control (68%).

Effect of auxins and cytokinins on adventitious shoot regeneration in leaf explants

Growth regulators had an effect on the number of explants that formed shoots in the genotypes studied. Explants of the genotype 'C-6' had their highest rate of shoot formation with TDZ (0.2 mg L^{-1}) with 1.4 shoots per explant; increasing the dose and combining with IBA decreased shoot induction. It was also observed that kinetin, alone or combined with IBA, did not induce regeneration (Table 2, Figure 1A). The explants of the 'Joan J.' genotype showed a greater formation of shoots with 0.6 mg L^{-1} of TDZ and 0.1 mg L^{-1} of IBA, where 1.6 shoots per explant were obtained (Table 2, Figure 1B).

In the genotype 'A-1', TDZ (0.2 mg L^{-1}) induced regeneration of 0.92 shoots per explant, this represented 36% more than the control treatment (Table 2, Figure 1C). In the case of 'UM-702', it was observed that low doses of TDZ (0.2 mg L^{-1}) + IBA (0.1 mg L^{-1}) induced the regeneration of shoots (1.12 shoots per explant) (Table 2, Figure 1D). In the 'Heritage' genotype, regeneration was obtained with TDZ at the dose of 0.2 mg L^{-1}, alone or in combination with 0.1 mg L^{-1} of IBA (40%), while in the control treatment no regeneration was obtained (Table 2, Figure 1C).

Table 2. Comparison of the coefficient of multiplication and results of Duncan's test for shoots obtained from raspberry leaf explants of genotypes 'C-6', 'Joan J.', 'A-1', 'UMC-702' and 'Her' cultured *in vitro* and treated with growth regulators (BAP, KIN and TDZ) alone or in interaction with IBA.

Growth regulator	*(mg L^{-1})*	*'C-6'*	*'JOAN J.'*	*'A-1'*	*'UMC-702'*	*'HER'*
Coefficient of variation		239.1	117.48	175.69	139.1	176.81
Benzylaminopurine	0.5	0.12 bc	0.12 d	0 d	0.04 fg	0.04 e
1	0 c	0.24 cd	0 d	0.18 defg	0 e	
1.5	0.16 bc	0.08 d	0.16 bcd	0.04 fg	0.44 bcde	
2	0.16 bc	0.08 d	0.04 d	0.2 defg	0.08 e	
2.5	0.08 c	0.04 d	0.08 cd	0.6 b	0.04 e	
3	0 c	0.04 d	0 d	0.04 fg	0 e	
Benzylaminopurine + indole butyric acid	0.5+0.1	0.08 c	0.36 cd	0 d	0.24 cdefg	0 e
1+0.1	0.16 bc	0 d	0 d	0.2 defg	0.16 e	
1.5+0.1	0.12 bc	0.08 d	0.08 cd	0.04 fg	0.08 e	
2+0.1	0.2 bc	0 d	0.08 cd	0.04 fg	0 e	
2.5+0.1	0.04 c	0 d	0 d	0 g	0.12 e	
3+0.1	0.24 bc	0 d	0.36 b	0.04 fg	0.2 e	
Kinetin	0.5	0.08 c	0 d	0 d	0 g	0 e
1	0 c	0 d	0 d	0 g	0 e	
1.5	0 c	0 d	0 d	0 g	0 e	
2	0 c	0 d	0 d	0 g	0 e	
2.5	0 c	0 d	0 d	0 g	0 e	
3	0.04 c	0 d	0.08 cd	0 g	0 e	
Kinetin + indole butyric acid	0.5+0.1	0 c	0 d	0 d	0 g	0 e
1+0.1	0 c	0 d	0 d	0 g	0 e	
1.5+0.1	0.04 c	0 d	0 d	0.04 fg	0 e	

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v14i6.3183

Growth regulator	*(mg L $^{-1}$)*	*'C-6'*	*'JOAN J.'*	*'A-1'*	*'UMC-702'*	*'HER'*
2+0.1	0 c	0.04 d	0 d	0 g	0 e	
2.5+0.1	0 c	0 d	0 d	0 g	0 e	
3+0.1	0 c	0 d	0 d	0.04 fg	0 e	
Thidiazuron	0.2	1.4 a	1.12 a	0.92 a	0.37 bcde	0.84 ab
	0.4	1 a	1 ab	0.24 bcd	0.04 fg	0.32 de
	0.6	1 a	0.88 ab	0.36 b	0.36 cdef	0.8 abc
	0.8	0.2 bc	0.08 d	0 d	0.08 efg	0.68 abcd
	1	0.2 bc	0.4 cd	0.16 bcd	0.2 defg	0.28 de
Thidiazuron	0.2 +0.1	0.8 ab	1.24 a	0.32 bc	1.12 a	0.96 a
+ indole	0.4+0.1	0.2 bc	1.2 a	0.12 bcd	0.4 bcd	0.68 abcd
butyric acid	0.6+0.1	0.2 bc	1.6 a	0.12 bcd	0.16 defg	0.36 cde
	0.8+0.1	0 c	0.32 cd	0.2 bcd	0.44 bcd	0 e
	1+0.1	0.2 bc	0.6 bc	0.08 cd	0.52 bc	0.24 de
Control	0	0 c	0.04 d	0.08 cd	0 g	0 e

Different letters in the same column indicate differences significant at 0.5.

Figure 1. Different numbers of explants showing the degree of oxidation and *in vitro* regeneration of raspberry leaf sections of genotypes 'C-6'(A), 'Joan J.' (B), 'A-1' (C), 'UM-702' (D) and 'Heritage' (E), all growing in a basic MS medium added with 0.2 mg L $^{-1}$ of TDZ and 0.1 mg L $^{-1}$ of IBA.

The most relevant data of this study, encompassed in the previous paragraph, show that there is a correlation between oxidation and regeneration of the shoots obtained from sections of leaves in all raspberry genotypes used in this research, the lower oxidation the greater the regeneration of explants.

Discussion

Oxidation of explants

The results show that growth regulators can influence oxidation and survival of explants. The *in vitro* culture of woody plants is limited by the occurrence of lethal brownings, these are related

DOI: https://doi.org/10.29312/remexca.v14i6.3183

to oxidative stress (Turrens, 2003) that originates from the cuts of the explant, the composition of the medium, volume and capacity of the bottle of culture, among others (Abdelwahd *et al*., 2008). In most protocols, stress is caused in the explants, this induces the production of phenolic compounds and several reactive oxygen species (Phineas and Kuman, 2013). Oxidative stress can be attributed to the use of growth regulators; the cytokinin BAP is one of the regulators with the most reports of this effect (Azofeifa *et al*., 2009).

In our research we observed that the oxidation of raspberry explants is influenced by the genotype and by the type of growth regulator used. This coincides with other studies that indicate that regeneration in plants is genotype-dependent, as regeneration has been obtained for some genotypes, since the recalcitrance of *Rubus* tissues is a limitation (Palomo-Ríos *et al*., 2018). Zawadzka and Orlikowska (2006) observed raspberry genotypes *in vitro* that showed chlorotic and recalcitrant leaves at regeneration.

Chlorosis in raspberry plants and oxidation of explants increase substantially when tissues are exposed to long periods of fluorescent light in culture media added with cytokinins of type 6-bencyl adenine (BA) or isopentenyl adenine (2iP), since these interfere with the proper functioning of intracellular calcium and increase the concentration of some proteins involved in the proper functioning of photosystem II (Murvanidze *et al*., 2022).

Regeneration and multiplication of adventitious shoots

Regeneration protocols in berries should contain the correct doses and combinations of growth regulators (auxins and cytokinins) in the culture medium (Cappelletti *et al*., 2016). *In vitro* morphogenesis is affected by factors such as: genotype, age, position and orientation of the explant in the culture medium (Kumar and Reddy, 2011). In this research, it was observed that growth regulators influenced regeneration; however, each genotype had a different responsiveness; the use of BAP (0.5 mg L^{-1}), alone or combined with IBA (0.1 mg L^{-1}), induced the regeneration of adventitious shoots in the genotype 'C-6', the rest of the genotypes showed greater regeneration with TDZ (0.2 mg L^{-1}).

These results agree with Meng *et al*. (2004), where the use of BAP (1 mg L^{-1}) and IBA (0.1 mg L^{-1}) in raspberry cv. 'Marion' induced regeneration by 70%, while 46% was observed in the cultivar 'Sunberry'. Kim and Dai (2020) obtained in the 'Joan J.' genotype a regeneration of 70% with 2.5 µM (0.56 mg L^{-1}) of BAP + 1 µM (0.216 mg L^{-1}) of TDZ. The combination of BAP with TDZ promotes cell proliferation as the multiplication of new shoots accelerates (Bairú *et al*., 2007). The effect of cytokinins on regeneration can be attributed to the fact that they act as a positive activator of cell division, BAP belongs to this group, which are the key hormones for the induction of shoots in various tissues and organs (Bustillo-Avendaño *et al*., 2018; Howell *et al*., 2003).

Some studies have shown that morphogenetic processes are regulated in the first instance by cytokinins, which act on the central zone of the explants and subsequently auxins intervene in the process on the peripheral cells of the explant (Schaller *et al*., 2015). BAP is used for *in vitro* culture of woody species to induce multiplication because these plants have a higher endogenous hormonal load compared to herbaceous plants (Bairú *et al*., 2007) and when used in young tissues, the morphogenic potential for differentiation increases (Mazumdar *et al*., 2020).

In this research, it was observed that kinetin did not induce regeneration in any of the genotypes evaluated. Nevertheless, Zawdzka and Orlikowska (2006) reported the effect of the combination of BAP + kinetin on the regeneration of five raspberry cultivars, as cytokinins stimulate cell division and vegetative propagation (Taiz and Zeiger, 2010).

In this research, the addition of TDZ to the culture medium stimulated the regeneration of shoots in the genotypes 'Joan J.' and 'A-1', which agrees with the results obtained by Fiola *et al*. (1990), where TDZ had a greater effect than BAP on the induction of organogenesis in cotyledons and leaves of *Rubus fruticosus*, the optimal dose in leaf explants was 5-20 µM (1.13-4.5 mg), this similarly occurred in the formation of shoots from axillary buds and apical shoots in blackberry,

DOI: https://doi.org/10.29312/remexca.v14i6.3183

where concentrations of 0.25, 0.5, 0.75 and 1 mg L^{-1} induced regeneration percentages of 60, 70, 100, 80 and 75%, respectively (Jadán *et al*., 2015).

In the raspberry cultivars 'Autumn Bliss', 'Canby', 'Summit' and 'Sentry', it was observed that TDZ was significantly more effective than BAP, the medium added with 1 µM (0.23 mg) of TDZ induced leaf regeneration (Turk, 1994). Debnath *et al*. (2014) reported 70% regeneration with 4.5 µM (1.01 mg) of TDZ with 4.2 shoots per explant and a coefficient of multiplication of 1.7 in a bioreactor system and by increasing the dose to 5 µM, a regeneration percentage of 96% was obtained in the cv. 'MD-ETC E-1'.

Ruíz-Anchondo *et al*. (2018) observe that the *in vitro* micropropagation of raspberry cv. Heritage, from meristems and internodes, is favored when BAP (4.44 µM) and GA (1.44 µM) are used in the culture medium, while Georgieva *et al*. (2020) find that the proliferation capacity is higher in the cv. Magdalena (3.9 shoots/explant) relative to the cv. Willamette (2.6 shoots/explant) in a medium added with 0.5 mg L^{-1} of BAP and 0.01 mg L^{-1} of IBA, concentrations of regulators lower than those used in our study.

TDZ has been shown to be effective in the regeneration of many recalcitrant species (Liu *et al*., 2003). Unlike other cytokinins, TDZ is resistant to cytokinin oxidase, so it is quite stable in plant tissues (Dewir *et al*., 2018). The need for cytokinins is extremely variable and depends on the endogenous content of the species and the genotype, as this has a marked effect on the ability to regenerate under *in vitro* conditions (Hunková *et al*., 2016).

The doses used influence the processes to which it gives rise, for example, when low doses of TDZ are used, it induces organogenesis; using high doses leads to embryogenesis; but high concentrations can be toxic to the development of *in vitro* cultures (Ling *et al*., 2013).

Conclusions

The degree of oxidation of the explants and the regeneration of raspberry from leaf sections depend largely on the growth regulators used in the culture medium and on the genotype or variety used for this purpose. Cytokinins (BAP), alone or combined with auxins (IBA), decrease oxidation in the explants of genotypes 'C-6' 'Joan J.' and 'Heritage', while TDZ, alone or combined with IBA, has a broader effect because it decreases oxidation and also promotes the regeneration of explants in the five genotypes evaluated.

Acknowledgements

The authors express their gratitude to the National Council of Science and Technology (CONACYT, for its acronym in Spanish) for the master's degree scholarship granted to carry out this research project and to the Scientific Research Council (CIC, for its acronym in Spanish) of the UMSNH for the financing of the project.

Bibliography

1 Abdelwahd, R.; Hakam, N.; Labhilili, M. and Udupa, S. M. 2008. Use of an adsorbent and antioxidasnts to reduce the effects of leached phenolics in *in vitro* plantlet regeneration of faba bean. Afr. J. Biotechnol. 7(8):997-1002.

2 Adobkar, I.; Ahmed, M. S. and Elshabed, M. 2012. Plant tissue culture media. Annarita L. and MR Laura. Ed. *In*: recent advances in plant *in vitro* culture. IntechOpen. Doi: 10.5772/50569.

3 Allccaco, J. C. 2016. Estandarización para la propagación clonal *in vitro* de *Rubus idaeus* var. Heritage "frambuesa roja" de importancia comercial. Tesis Licenciada en Biología. Lima, Perú. Universidad Ricardo Palma, Facultad de Ciencias Biológicas. 123 p.

4 Azofeifa-Delgado, A. 2009. Problemas de oxidación y oscurecimiento de explantes cultivados *in vitro*. Agron. Mesoam. 20(1):153-175.

DOI: https://doi.org/10.29312/remexca.v14i6.3183

5 Bairú, M. W.; Stirk, W. A.; Dolezal, K. and Staden, J-Van. 2007. Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can metapolin and its derivates serve as replacement for benzyladenine and zeatin. Plant Cell Tissue and Organ Culture. 90(1):15-23.

6 Bascopé, J. A. 2013. Realidad productiva de la frambuesa EE. UU. y México. Informe de Experto. Santiago, Chile. Oficina de Estudios y Políticas Agrarias. 42 p.

7 Bustillo-Avendaño, E.; Ibañez, S.; Sanz, O.; Sousa, B. J. A.; Gude, I.; Perianez-Rodríguez, J.; Micol, J. L.; Del Pozo, J. C.; Moreno-Risueno, M. A. and Pérez-Pérez, J. M. 2018. Regulation of hormonal control, cell reprogramming and patterning during *de novo* root organogenesis. Plant Physiology. 176(2):1709-1727.

8 Cappelletti, R.; Sabbadini, S. and Mezzetti, B. 2016. The use of TDZ for the efficient *in vitro* regeneration and organogénesis of strawberry and blueberry cultivars. Scientia Horticulturae. 207:117-124.

9 Debnath, S. C. 2014. Bioreactor-induced adventitious shoot regeneration affects genotype-dependent morphology but maintains clonal fidelity in red raspberry. *In vitro* Cellular and Developmental Biology-Plant. 50(6):777-788.

10 Dewir, Y. H.; Nurmansyah, H.; Naidoo, Y. and Teixeira da Silva, J. A. 2018. Thidiazuron-induced abnormalities in plant tissue cultures. Plant Cell Reports. 37(11):1451-1470.

11 Duncan, D. B. 1995. Multiple range and multiple F test. Biometrics. 11(1):1-42.

12 FAOSTAT. 2022. Database. Avalable online: https://www.fao.org/faostat/en/#home.

13 Fiola, J. A.; Hassan, M. A.; Swartz, H. J.; Bors, R. H. and McNicols, R. 1990. Effect of thidiazuron, light fluence rates and kanamycin on *in vitro* shoot organogenesis from excised Rubus cotiledons and leaves. Plant Cell Tissue and Organ Culture . 20(3):223-222.

14 Georgieva, M.; Kondakova, V. and Yancheva, S. 2020. A comparative study on raspberry cultivars in micropropagation. Bulgarian J. Agric. Sci. 26(3):527-532.

15 González, M. V.; López, M.; Valdes, A. E. and Ordas, R. J. 2009. Micropropagation of three berry fruit species using nodal segments from field-Grown plants. Annals Appl. Biol. 137(1):73-78.

16 Granados-Rubio, K. 2017. Variación somaclonal *in vitro* de frambuesa (*Rubus ideaus* L.) var. Josephine. Tesis de licenciatura. Facultad de Agrobiología "Presidente Juárez". Universidad Michoacana de San Nicolás de Hidalgo. Uruapan, Michoacán, México. 46 p.

17 Gutiérrez, M. A.; Santacruz, R. F.; Cabrera, P. J. L. y Rodríguez, G. B. 2003. Mejoramiento genético vegetal *in vitro*. Revista Digital Científica y Tecnológica e- Gnosis. 1(4):0-19.

18 Hall, H. K.; Hummer, K. E.; Jamieson, A. R.; Jennings, S. N. and Weber, C. A. 2009. Raspberry breeding and genetics. Plant Breeding Review. 32:44-62.

19 Howell, S. H.; Lali, S. and Che, P. 2003. Cytokinins and shoot development. Trends in Plant Science. 8(9):453-459.

20 Hunková, J.; Libiakova, G. and Gajdosova, A. 2016. Shoot proliferation ability of selected cultivars of *Rubus* spp. as influenced by genotype and cytokinin concentration. J. Central Eur. Agric. 17(2):379-390.

21 Jacinto, A. M. E. 2018. Evaluación de tres niveles de auxinas y citoquininas para la obtención de plantas madre de rosa (*Rosa* sp.) variedad Freedom en condiciones *in vitro*. Rev. de la carrera de ingeniería Agronómica-UMSA. 4(2):1073-1081.

22 Jadán, M.; Ruíz, J.; Soria, N. and Mihai, R. A. 2015. Synthetic seed production and the induction of organogenesis in blackberry (*Rubus glaucus* Benth). Romanian Biotechnological Letters. 20(1):10134-10142.

DOI: https://doi.org/10.29312/remexca.v14i6.3183

23 Kim, C. and Dai, W. 2020. Plant regeneration of redraspberry (*Rubus idaeus* L.) cultivars 'Joan J' y 'Polana'. *In Vitro* Cellular & Developmental Biology-Plant. 56(3):390-397.

24 Kumar, N. and Reddy, M. P. 2011. *In vitro* plant propagation: a review. J. For Environ Science. 27(3):61-72.

25 Ling, A. P. K.; Tan, K. P. and Hussein, S. 2013. Comparative effects of plant growth regulators on leaf and stem explants of *Labisla pumila* var. Alata. J. Zhejrang University Science B. 14(7):621-631.

26 Liu, C. Z.; Murch, S. J.; Demerdash, M. E. L. and Saxeria, P. K. 2003. Regeneration of the egyptian medicinal plant *Artemesia juddaica* L. Plant Cell Reports. 21:525-530.

27 Mazumdar, P.; Basu, A.; Paul A.; Mahanta, C. and Sahoo, L. 2020. Age and orientation of cotyledonary leaf explants determine the efficiency of *de novo* plant regeneration and *Agrobacterium tumefaciens*-mediated transformation in *Jatropha curcas* L. South African J. Bot. 76(2):337-344.

28 Méndez-Álvarez, D. y Abdelnour-Esquivel, A. 2014. Establecimiento *in vitro* de *Terminalia amazonia* Gmel. Excell. Rev. Forestal Mesoamericana Kurú. 11(27):07-21.

29 Meng, R.; Chen, T. H. H.; Fino, C. E. and Li, Y. 2004. Improving *in vitro* plant regeneration from leaf and petiole explant of 'Marion' blackberry. HortScience. 39(2):316-320.

30 Minas, G. J. and Neocleous, D. 2007. A protocol for rapid clonal micropropagation *in vitro* of primocane-fruiting red raspberry cultivars. Sistema Internacional de Ciencia y Tecnología Agrícolas AGRIS. 7 p.

31 Murashige, T. y Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15(3):473-497.

32 Murvanidze, N.; Ameye, M.; Geelen, D. and Werbrouck, S. P. O. 2022. A calmodulin antagonist protects in vitro raspberries agaimst disturbed photosynthesis caused by constant light and cytokinin. Plant Cell, Tissue and Organ Culture. 148(1):73-80.

33 Palomo-Ríos, E.; Quesada, M. A.; Matas, A. J.; Pliego-Alfaro, F. and Mercado, J. A. 2018. The history and status of genetic transformation in berry crops. *In*: The Genomes of Rosaceous-Berries and Their Wild Relatives. Springer Nature. Spain. 139-160 pp.

34 Phineas, J. A. A. and Kumar, S. P. 2013. Inhibition of phenylpropanoid biosynthesis in Artemisa annua L.: A novel approach to reduce oxidative browning in plant tissue culture. PlosOne. 8(10):/e76802:1-13.

35 Restrepo-Osorio, C.; Gómez-Velasquez, F. A.; Gil-Correal, A.; Torres-Bonilla, J. M. and Urrea-Trujillo, A. I. 2018. *In vitro* propagation of avocado *Persea americana* Mill. cv. Hass through morfogénesis. Acta Agron. 67(1):160-167.

36 Ruíz-Anchondo, T.; Martínez, J. A.; Carrillo-Castillo, T.; Parra-Quezada, R. A.; Ojeda-Barrios, D. L. y Hernández-Rodríguez, A. 2018. Establecimiento *in vitro* de dos cultivares liberados de frutillas: fresa y frambuesa. Revista Mexicana de Ciencias Agrícolas. 9(4):799-812.

37 Schaller, G. E.; Bishopp, A. and Kieber, J. J. 2015. The Ying-Yang of hormones: cytokinin and auxin interactions in plant development. The Plant Cell. 27(1):44-63.

38 SIAP. 2021. Sistema de información agroalimentaria y pesquera. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA). Atlas agroalimentario. https://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2020/Atlas-Agroalimentario-2020.

39 SAS. 2002. Statistical Analysis System. Institute Inc. SAS/STAT. User's Guide, version 9.0. Carey, N.C.

40 Taiz, L. and Zeiger, E. 2010. Plant Phisiology. 5th Ed. Sinaeur Associates Inc., Massachusetts. 778 p.

DOI: https://doi.org/10.29312/remexca.v14i6.3183

41 Turrens, J. 2003. Mithochondrial formation of reactive oxygen species. J. Physiol. 552(2):335-344.

42 Turk, B. A.; Swartz, H. J. and Zimmerman, R. H. 1994. Adventitious shoot regeneration from *in vitro*- cultured leaves of Rubus genotipes. Plant Cell. Tissue and Organ Culture. 38:11-17.

43 Wu, J. H.; Miller, S. A.; Hall, H. K. and Mooney, P. A. 2009. Factors affecting the efficiency of micropropagation fron llateral buds and shoots tips of Rubus. Plant Cell Tisssue and Organ Culture. 99:17-25.

44 Zawadzka, M. and Orlikowska, T. 2006. Factors modifying regeneration *in vitro* of adventitious shoot in five red raspberry cultivars. Journal of fruit and Ornamental. Plant Research. 14:105-115.

Regeneration of leaf explants of five raspberry genotypes

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 June 2023
Date accepted: 01 August 2023
Publication date: 24 August 2023
Publication date: August 2023
Volume: 14
Issue: 6
Electronic Location Identifier: e3183
DOI: 10.29312/remexca.v14i6.3183

Categories

Subject: Articles

Keywords:

Keywords:

Rubus idaeus L.
organogenesis
plant hormones.

Counts

Figures: 1
Tables: 2
Equations: 0
References: 44
Pages: 0