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Abstract

This study addresses the *in vitro* culture as an alternative to obtain compounds with cytotoxic activity from the medicinal plant *Jatropha curcas* (Euphorbiaceae). We determined the presence of cytotoxic compounds in both whole plants and dedifferentiated cells. We evaluated the effect of auxin, cytokinins and light on callus formation in cotyledon explants. We found that the most effective combination to induce callus was the auxin 2, 4-D (5 mM) with the cytokinin 6-BAP (2.5 mM), on Murashige-Skoog medium in darkness. We compared the callogenic potential among accessions from different geographic origins, finding that ARR-251107-MFG7 is most prone to form callus. The roots of *J. curcas* grown in field produced a compound chromatographically similar to the cytotoxic diterpene jatrophone. The profile of compounds extracted from the dedifferentiated cells was similar to that of the whole plant, including a relatively abundant stilbene-like compound. This study contributes to the future establishment of protocols to produce anti-cancer compounds from *J. curcas* cultivated in vitro.

Keywords: Cell culture, diterpenes, Euphorbiaceae, cytotoxic metabolites.

Introduction

The search for less toxic and more powerful anti-carcinogenic drugs is based on the fact that current drugs are scarcely selective and highly toxic to normal cells (Mohan et al. 2012). The most promising sources of such molecules are plants, especially those used for herbal medicine (Alonso-Castro et al. 2011). In this sense, *Jatropha curcas*, a plant native to Mesoamerica and recently rediscovered due to its potential as a source of raw material for biofuels (Salvador-Figueroa et al. 2014); it has been used both in traditional medicine, for the production of soaps, and as a source of energy (oil lamps) by various peoples of Asia, Africa and Mesoamerica (Kumar & Sharma 2008).

Furthermore, from the genus *Jatropha* different compounds with biological activity have been isolated (Devappa et al. 2010). Among them are: jatrophol, a molecule with rodenticide activity (Jing 2005); the curcusones *a*, *b* and *c*, and jatropholone *A*, with antineoplastic properties (Muangman et al. 2005; Naengchomnong et al. 1986, 1994; Theoduloz et al. 2009); hydroxyl-jatrophone and other diterpenes with antimetastatic activity (Devappa et al. 2011). The methanol extracts of the leaves have anti-metastatic and anti-proliferative activity (Balaji et al. 2009). That compounds have been isolated from different plant parts in several species of *Jatropha*, although the concentration is usually low (Goulart et al. 1993). This situation is not unique to this genus, as in other plants bioactive molecules are also found in low concentrations.

Given the above, the *in vitro* culture of dedifferentiated plant cells is an alternative for increasing the concentration of the compounds of interest (Roberts 2007). In this regard, Fett- Neto et al. (1994) obtained 100 times more taxoid in *Taxus cuspidata* callus than in the field plant. However, *in vitro* culture does not always improve the concentration of the metabolite of interest (Pletsch & Charlwood 1997), given the difficulties to obtain friable callus, the genetic variations throughout the culture and the formation of cell aggregates (Chattopadhyay et al. 2002).

Therefore, the objectives of this study were a) to establish a procedure for obtaining friable and fast growing calluses, and b) to evaluate the production of cytotoxic compounds in *J. curcas* dedifferentiated cells.

Materials and methods

Biological Materials

Five accessions of *J. curcas* (Table 3) representing the regions of Chiapas (Mexico) were used, from the Biosciences Center (CenBio, initials in Spanish) *Jatropha* Germplasm Bank of the Autonomous University of Chiapas (Mexico) located in the municipality of Tapachula, Chiapas (14.4976 N, 92.4774 W, 58 m a.s.l., annual average temperature 30.7 °C, annual average humidity 80 %, average rainfall of 2632.9 mm and andosol-type soil.) For *in vitro* culture 50 seeds of each of the accessions were collected. For the whole plant phytochemical analysis, samples of leaf, stem and root of the accession MAP-011107-G8 were used. Those samples were washed with tap water, dried at 60 °C 48 h and ground to particle size of 500 µm.

Production of dedifferentiated tissue

Cotyledons of different *J. curcas* accessions were used as explants for production of dedifferentiated tissue. In the first phase, the seeds of the accession MAP-011107-G8 were sown on MS medium (Murashige & Skoog 1962), after disinfection with sodium hypochlorite at 5%, following the procedure described by Soomro & Memon (2007), and kept in 2 d darkness and 2d in light. After that period, the seeds were cut transversely, the embryo was removed and cotyledons were sown on a MS medium supplemented with different hormone combinations, and under different lighting conditions. For this phase, we used a full-randomized design with 32

treatments including a control without hormones, with three replications. Explants were maintained for 20 d, at the end of which the dry weight of callus generated was quantified. Based on the treatment that produced the highest amount of callus, the optimization process was conducted based on the concentration of hormones, using a 6^2 factorial design, where the factors were the hormones (2, 4-D and BAP) at six levels each, with four replications. In these treatments the dry weight of callus was determined after 30 d of culture. Lastly, cotyledons of all accessions were placed under the best conditions to produce callus; this variable was quantified and compared between accessions.

Determination of jatrophone content in field plants

Three grams (± 0.1 g) of particles of different plant parts were subjected to extraction by triplicate using refluxing (60 °C, 20 cycles) with 80 mL hexane in Soxhlet equipment. The hexane was evaporated in a rotary evaporator to 50 °C and the yield (extract weight • sample weight^{-1}) was calculated. The separation and identification of jatrophone was performed by thin layer chromatography using silica gel 60 plates of 5 x 20 cm (Sigma-Aldrich®, Fluka, Germany) washed with MetOH (Hycel®, Mexico, purity 99.8%) activated at 50 °C for 5 min. For this, the residue obtained as previously described was dissolved in hexane to achieve concentrations of 0.1 g • mL^{-1}. An aliquot (15 µL) of each of the extracts and of a mixture of jatrophone (10 mM) with jatropholone *a* and *b* (4 mM based on jatropholone *a* dissolved in Hexane: Ethyl Acetate 7:3, kindly provided by Dr. Guillermo Schmeda-Hirschmann of the University of Talca, Chile), were placed individually in the chromoplate lanes. The chromatogram was developed at 28 °C as a mobile phase a mixture of Hexane: Ethyl Acetate 7:3. The compounds were revealed using the procedure reported by Pertino et al. (2007). Under the above conditions the Rf values for

jatrophone and jatropholone *a* and *b* mixture were 0.772 and 0.817 (given that isomers are not separated with this eluent mixture), respectively.

The positive hexanic extracts for jatrophone were subjected to column chromatography packed with silica gel 60 (Merck®, Mexico). The column preparation and elution was performed using the method proposed by Goulart et al. (1993) with flow of 0.6 mL • min^{-1}. The eluate was received in 2 mL fractions and the presence of jatrophone was verified by thin layer chromatography. Fractions where the compound of interest was present were blended, then the solvent was evaporated in an oven at 50 °C and the residue was dissolved in 1 mL methanol (J.T. Baker®, Mexico, purity 99.9%).

The solids dissolved in MetOH from the previous phase were analyzed on a gas chromatograph (Thermo®, Focus-GC-Milan, Italy), coupled to a mass spectrum (Thermo®, MS DSQ-II). The chromatography was performed at an intermediate polarity column (Thermo®-5ms SQC, Milan, Italy) of 30 m x 0.25 mm, D.I. 0.25 x 0.25 µm using helium as gas carrier. The temperatures in the column of the injector and the ionization chamber used were based on those reported by Wang et al. (2009). The standards were analyzed similarly, separately. The chromatographic and spectrometric data were processed by Xcalibur© data system (Version 2.0.7, 2007). The fragmentograms obtained from each of the compounds present in the extracts were compared with those stored in the NIST 02© (2005) database.

Determining metabolites in dedifferentiated cells

Of each of the accessions, 2 g of callus (dry weight) were taken and subjected to an extraction and hemi-purification process, following the procedure described for evaluating jatrophone content in field plants. We performed two sequential extractions, the first one with hexane and

131 then the residue with ethanol 96 °GL (Goulart *et al*., 1993). Extracts were analyzed by GC-MS,
132 as described previously.

133 *Data analysis*

134 The concentrations of jatrophone and jatropholone *a* and *b* were obtained based on the areas
135 under the curve of the samples peaks in relation to those of the standards. The concentration of
136 an abundant stilbene-like compound (**1**) in callus was calculated using the standard of jatrophone
137 as a reference. Data from all assays were subjected to analysis of variance (ANOVA) and
138 comparison of means (Tukey $\alpha \leq 0.05$).

139

140 **Results**

141 *Effect of auxins and cytokinins on the production of callus*

142 All hormone treatments induced callus formation in cotyledons of *J. curcas*, accession MAP-011
143 107-G8. In all cases, the callogenesis started on the edge where the cutting was made and then
144 covered the rest of the explant. Table 1 shows the callus induction by the different phytohormone
145 treatments and lighting conditions. It was observed that exogenous phytohormones under any
146 lighting condition did not influence the formation of callus, as they were statistically similar to
147 the control ($p \leq 0.05$). The auxin 2,4- dichlorophenoxyacetic acid (2,4-D) presented lower values
148 of callus formation, but its callogenic effect was potentiated by combining it with the cytokinin
149 6-benzylaminopurine (BAP), either under light or darkness. Besides this, it was observed that
150 calluses from treatments with light showed signs of differentiation, producing chlorophyll and
151 compacting themselves, while those cultivated in darkness remained undifferentiated and were
152 friable (Figure 1).

153 To obtain the appropriate callus production, different concentrations of phytohormones 2,4-D
154 and BAP were evaluated under darkness. In the overall analysis of the treatments, the
155 combinations of 2,4-D and BAP at 7.5 + 2 µM and 5 + 2.5 µM produced the largest amount of
156 callus, were statistically different from the other treatments, but equal among them (Table 2).
157 Statistically significant differences were found among the amounts of cell callus formed in the
158 five accessions evaluated (Table 3). The ARR-251107-MFG7 accession was superior in its
159 capacity of callogenesis.

160 *Cytotoxic compounds in mother plants.*

161 The yield of the hexane extracts depended of the type of tissue of the *J. curcas* studied. The
162 highest extract concentration was found in the roots, which had 1.6 - 1.8 times more extract than
163 leaves (30 ± 2 mg extract • g dry weight^{-1}) and bark (27.1 ± 3 mg extract • g dry weight^{-1}).
164 Separation by thin layer chromatography of the components in all the hexane extracts showed a
165 compound with Rf similar to jatropholone (0.814), while for jatrophone (Rf = 0.772) only one
166 spot with similar Rf (0.768) was found in the root extract (Figure 2). Separation and analysis by
167 gas chromatography-mass spectrometry of jatrophone positive fractions revealed that the root
168 hexane extract contains, in addition to other compounds, jatrophone and jatropholones *a* and *b*
169 (Table 4). Figure 3 shows the chromatogram and mass spectrum of the fraction in which
170 jatrophone was identified.

171 *Production of compounds in dedifferentiated cells*

172 Although 19 compounds (alkanes, fatty acids, among others) were found under the assayed
173 conditions of gas chromatography- mass spectrometry, no jatrophone or jatropholones were
174 detected in dedifferentiated cells. A major stilbene-like compound (**1**) was found, which was

present in all extracts with retention time of 27.21 min (Figure S1) and whose fragmentogram showed the following ions and relative abundances: [**280** (**8**), 279 (12), 273 (14), 167 (53), 166 (14), 165 (19), 163 (62), 150 (52), 149 (32), 148 (40), 147 (32), **145** (**100**), 112 (13), 110 (13), 101 (7), 83(11), 70 (40), 68 (24), 57 (19), 53 (19)].

There were no differences ($p = 0.635$) in the amount of (**1**) produced by type of callus. It was found that this metabolite is dependent on genotype ($p = 0.029$) as the accession ARR-1251107-MFG7 produced almost 30 mg•g callus^{-1}, while the remainder produced less than one milligram. Compared to the accumulation in roots of the accession MAP-011107-G8 cultivated in the field (1.152 mg• root^{-1}), calluses produced 26 times more stilbene-like compound (**1**).

Discussion

Although a variety of compounds with cytotoxic activity isolated from *J. curcas* has been reported (Misra & Misra 2010; Ravindranath et al. 2004; Van den Berg et al. 1995), our study focused on three compounds: jatrophone, jatropholones *a* and *b*, which were present, especially in the roots. In particular, jatrophone is a diterpene present in the genus *Jatropha,* which had not been previously reported in *J. curcas.* Unlike the findings by Goulart et al. (1993), who detected jatrophone in *J. elliptica* using hexane as eluent in column chromatography, in this study it was detected in fractions where the elution solvent polarity was increased (Hexane: Ethyl Acetate 6:1).

Further studies of the extracts are required to corroborate the presence of jatrophone and discard the possibility that we are dealing with other terpenoid compounds with similar chemical structure. However, comparison of the mass spectrum of the compound detected [**312** (**14**), 297

197 (4), **284** (**29**), 216 (36), 202 (33), **187** (**34**), 175 (45), 173 (83), **159** (**100**), 147 (30), 145 (70), 133
198 (30), 130 (45), 119 (30), 107 (35), 105 (56), 91 (32), **79** (**40**), 77 (50), 69 (35)] with that of the
199 jatrophol, a terpenoid with the same molecular weight (Naengchomnong et al. 1994), showed
200 that they have different ionization patterns [312 (100) 297 (11), 281 (43), 253 (34), 240 (37), 225
201 (35)]. Conversely, we emphasize that the compound identified as jatrophone has a ionization
202 pattern very similar to that of the standard [**312** (**15**), **297** (**6**), 241 (67), 189 (66), 187 (34), 175
203 (31), 173 (43), 160 (75), **159 (100)**, 147 (41), 145 (38), 115 (39), 91 (43), 81 (51), 79 (43), 53
204 (67)] and to that reported by Pertino et al. (2007) for jatrophone.

205 In this work a maximum of 2038 µg of jatrophone • g dry sample^{-1} was found, which represents
206 22.6 more times the maximum reported for *J. gossypiifolia* (90 µg•g sample^{-1}; Kupchan et al.
207 1976) and 1.4 more times than in *J. isabelli* (1450 µg•g sample^{-1}; dos Santos and Goulart 1999),
208 but 4.9 times less than in *J. elliptica* (10 000 µg•g sample^{-1}; Goulart et al. 1993). The *J. curcas*
209 accession MAP-011107-G8 has a high amount of jatrophone, although it is possible that the
210 yields are affected by the extraction method; Kupchan et al. (1976) performed a purification of
211 jatrophone using liquid-liquid phases, while this extraction was carried out in solid phase.
212 Although these results are promising with respect to other species, it must be remembered that in
213 all cases the extraction is performed at the root, which involves waiting for the development of
214 the latter and the complete sacrifice of the plant.

215 In regard to the study of induction for dedifferentiation, it was found that when the exogenous
216 phytohormones auxin and cytokinin were at low concentrations did not influence the formation
217 of cell callus. The formation of callus in the control treatment was remarkable (Table 1). The
218 results of this study show that explants of *J. curcas* tend spontaneously to *in vitro* callogenesis,
219 denoting a high endogenous hormone load or high responsiveness of the plant tissues to healing,

since callogenesis invariably started at the cutting sites of the explant. In this sense, Sujatha et al. (2005) mention that *J. curcas* leaf explants develop callus from the cutting margins. In other species, *in vitro* spontaneous callogenesis has been observed, which has been linked either to high hormone concentrations or to its *de novo* synthesis. In order to determine the cause of spontaneous callogenesis in *J. curcas,* studies are needed regarding the dynamics of hormone concentration in intact tissues and *in vitro* cultured.

Although auxin 2,4- D presented the lowest values of callus formation, its effect was potentiated when combined with cytokinin BAP. For this reason wider ranges of concentration of both hormones were explored and found that at higher concentrations (greater than 2.5 μM in the case of auxins and above 2μM in the case of cytokinins) there is an increased production of callus. Similar results were obtained by Kaewpoo & Te- Chato (2009), who evaluated different growth regulators in epicotyl and hypocotyl explants of *J. curcas* finding friable, soft and slightly yellow callus in all treatments with 2,4- D (6.78×10^{-6} M), while compact callus or shoots were obtained with the other auxins evaluated (IBA or NAA). In the present study friable callus induction was observed when using NAA although in lesser amounts, which is consistent with results obtained by Shrivastava & Banerjee (2008) who at assessing concentrations from 2×10^{-5} M to 5.37×10^{-6} M of NAA obtained the highest amount of callus at the highest concentration.

In the study for optimization of concentrations, it was observed that the largest amount of callus is formed at concentrations of 5 μM and 7.5 μM of 2, 4-D, which are statistically equal (Table 2). However, we suggest the use of the lowest effective concentration, since high concentrations of auxin are almost always detrimental to the production of secondary metabolites (Kim et al. 2007).

In the case of the selection of cytokinins it is advisable to use BAP at 2.5 µM, since when combined with auxin callogenesis is potentiated, which did not occur with other cytokinins. This coincides with the study of Kalidass et al. (2010). They studied the effect of BAP in the production of *Catharanthus roseus* callus and found that increasing the levels of BAP resulted in higher dry weight of callus. In other studies with explants of *J. curcas* effective concentrations of BAP for callogenesis were found, similar to the findings in the present study (Datta et al. 2007; Rajore & Batra 2007; Wei et al. 2004).

In regard to lighting conditions, it was found that it does not influence the amount of callus formed, however, it was significant in the differentiation of callus tissue. Since the long-term objective is obtaining dedifferentiated cell cultures that can be maintained in bioreactors, the condition of darkness is preferable. Paz et al. (2006) mention something similar. They found that under lighting, friable calluses could be conditioned to a vitrification process, which they did not observe in the conditions of darkness. Also, Pletsch & Charlwood (1997) found that the production of jatrophone in cultures *in vitro* of *J. elliptica* decreases up to 15 times under continuous lighting. There are other studies which conclude that in the condition of darkness calluses can accumulate more secondary metabolites compared to those grown under light (Banthorpe et al. 1986; Mukundan & Hjortso 1991; Yazaki et al. 2001).

The purpose of this research was the evaluation of cytotoxic compounds in dedifferentiated cells; however, none of the three compounds in which the study focused was detected in calluses. It is worth noting that jatrophone was found in the mother plant but not in the calluses, probably because of two main reasons: 1) Calluses do not produce jatrophone. Charlwood et al. (1990) point out that it is common that dedifferentiated cells lack the capacity for synthesizing isoprenoids present in the mother plant and this is related to its disorganized nature; similar results are

reported in the biosynthesis of heavier terpenes such as steroids (Lui & Staba 1979). Other possibility is that *in vitro* cultures may have the ability to synthesize the compounds produced by the field plant but lack the capacity to accumulate them (Banthorpe et al. 1986). 2) *In vitro* cultures produce smaller quantities than those detectable by the equipment used (lower than 3 µg • ml^{-1}). In this regard Pletsch & Charlwood (1997) detected minimum amounts of jatrophone (3 µg •g of dry $weight^{-1}$), which were at the detection limit. Nevertheless, cell calluses accumulated a large amount of the compound **(1)** in relation to that found in the field plant (26 times more), which deserves further studies for identification and biological activity.

In this study, a compound similar to jatrophone in root hexane extracts of *J. curcas* grown in the field was identified, while the best treatment for callus induction was the addition of 2, 4-D 5 µM, together with BAP 2.5 µM in dark conditions. However, the evaluation of the synergy between 2,4- D and NAA is recommended, as well as the evaluation of BAP in concentrations higher than the ones evaluated in the current optimization. Lastly, when evaluating the possible differences between accessions, ARR-251107-MFG7 turned out to be the one, which produced the greatest amount of callus. In the identification of metabolites in dedifferentiated cells, we could not identify jatrophone, although a stilbene-like compound was found in concentrations 26 times higher than in the plant field.

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Figure 1(on next page)

Influence of illumination on the appearance of Jatropha curcas calli

Callus induced in conditions of light and darkness from cotyledon explants of *Jatropha curcas*. A) Developing photosynthetic callus. B) Developing callus of “sugary” and friable appearance.

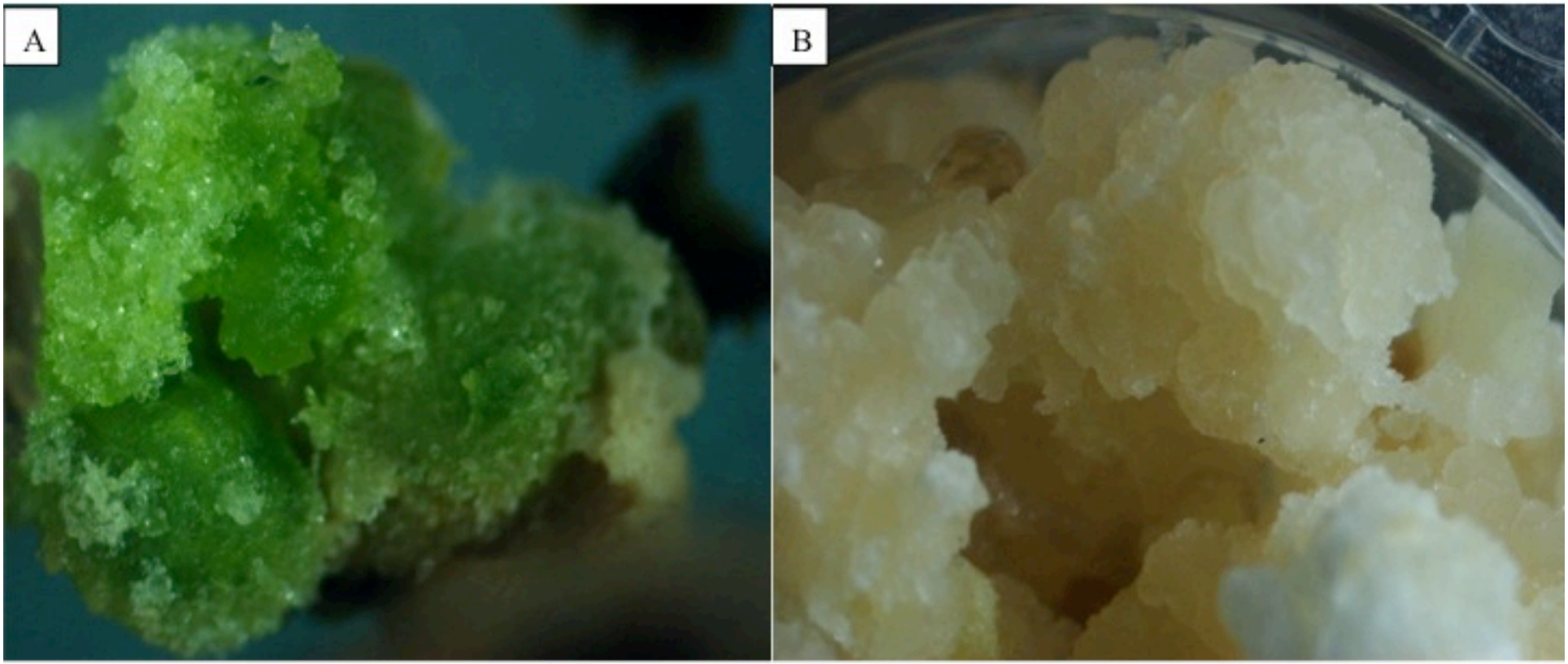
A
B

Figure 2 (on next page)

Thin layer chromatogram of crude hexane extracts from different tissues of Jatropha curcas MAP- 011107-G8.

Extracts were diluted to 0.1 $g \cdot mL^{-1}$ and revealed with sulphuric anisaldehyde. Lanes: 1) mixture of standards Jatrophone 10 mM + Jatropholone at 4 mM + *b*; 2) leaf extract; 3) bark extract; 4) root extract. In lane 1 band **“a”** represents the jatropholones (Rf = 0.817); and band **“b,”** the jatrophone (Rf = 0.772).

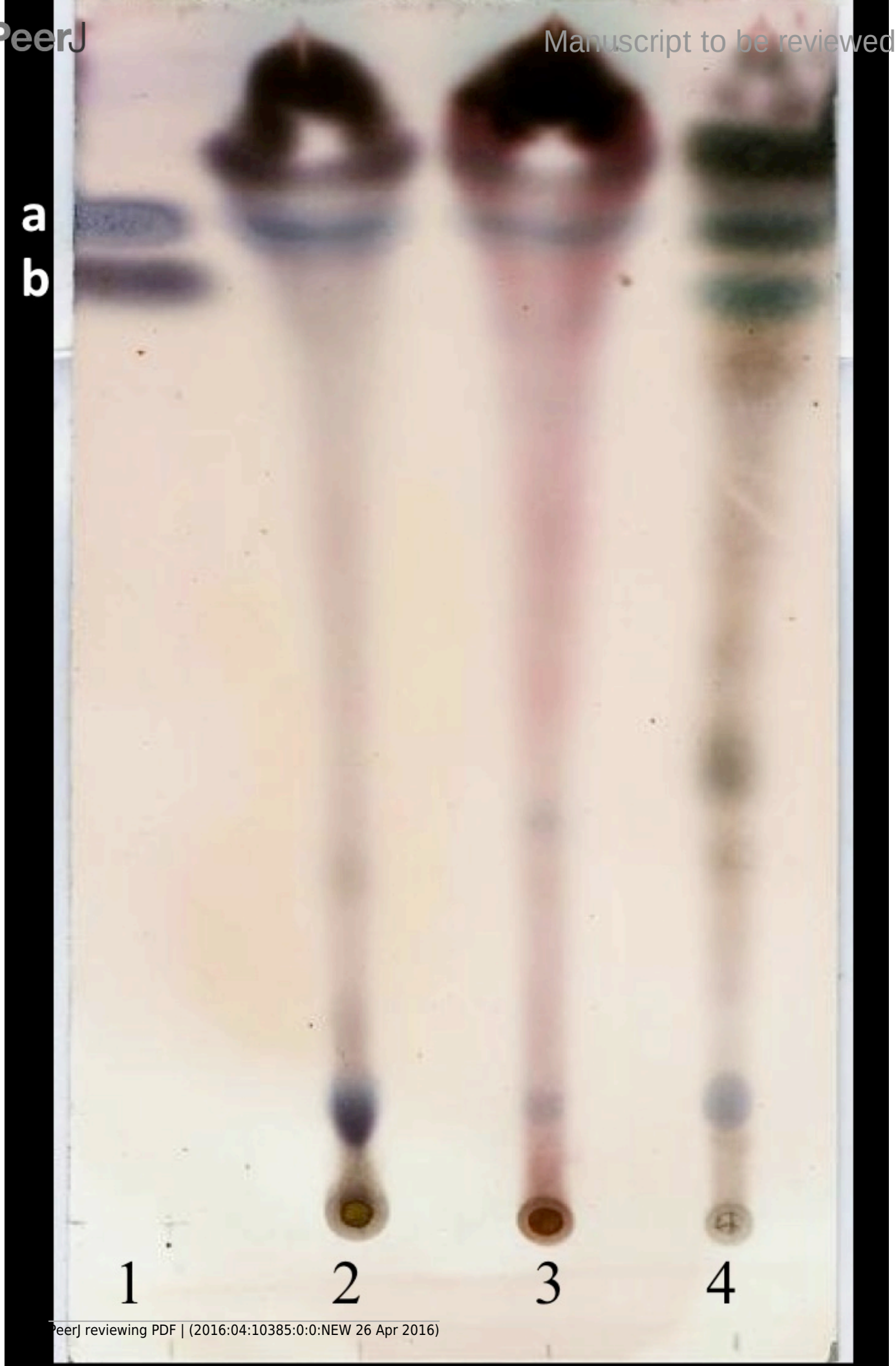
a
b
1
2
3
4

Figure 3(on next page)

Analysis of hexane extracts of Jatropha curcas by GC-MS

A) Chromatogram of a fraction of the root hexane extract of *Jatropha curcas* MAP-011107-G8, where the peak corresponding to jatrophone is circled. B) Fragmentogram of jatrophone–retention time 20.57 min, molecular weight is circled 312g • $gmol^{-1}$.

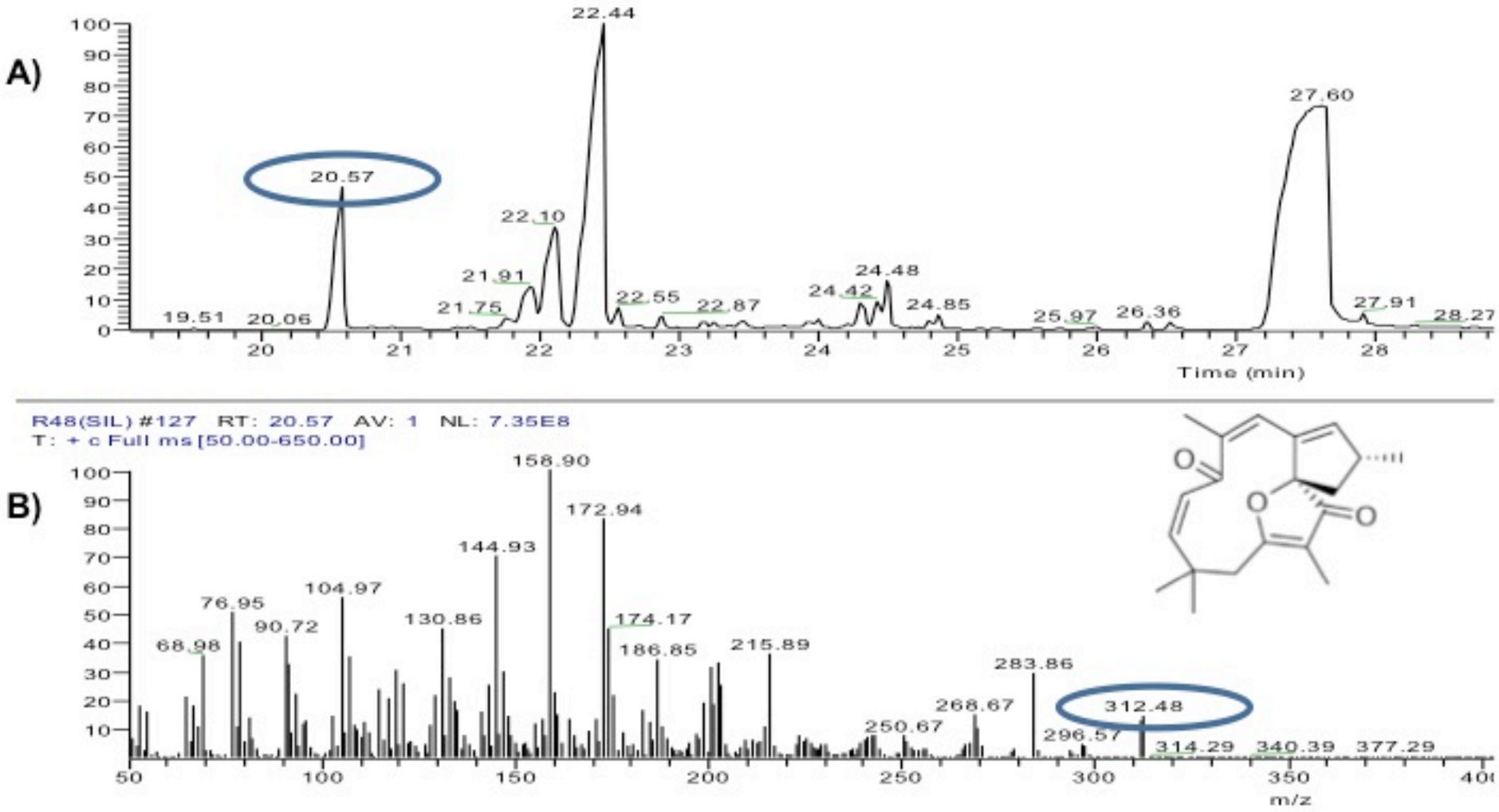
A)
22.44
27.60
20.57
22.10
21.91
24.48
24.42
22.55
22.87
24.85
21.75
19.51
20.06
25.97
26.36
27.91
28.27
Time (min)
B)
R48(SIL) #127 RT: 20.57 AV: 1 NL: 7.35E8
T: + c Full ms [50.00-650.00]
158.90
172.94
144.93
104.97
76.95
90.72
68.98
130.86
174.17
186.85
215.89
283.86
268.67
312.48
250.67
296.57
314.29
340.39
377.29
m/z

Table 1(on next page)

Effect of phytohormones and lighting conditions on callus dry weight (mg) obtained from cotyledon explants of *Jatropha curcas*.

Each treatment was repeated three times and each replicate consisted of 20-30 cuttings of a seed cotyledon from accession MAP-011107-G8. Means with different letters are significantly different ($p \leq 0.05$). Formulation of Medium: Basal Medium MS + 3% sucrose (p/v) + 500 mg• L^{-1} PVP. Auxins: 2,4-D (2,4-dichlorophenoxyacetic acid), NAA (naphthalene acetic acid), IAA (indole-3-acetic acid). Cytokinins: KIN (kinetin), BAP (6-benzylaminopurine), SAD (adenine sulfate).

1

Treatments	Lighting	
	Light	Darkness
Control	189 ± 8^{abc}	183 ± 37^{abc}
2,4-D	135 ± 36^{c}	136 ± 14^{c}
NAA	168 ± 18^{abc}	190 ± 42^{abc}
AIA	178 ± 12^{abc}	201 ± 30^{abc}
KIN	166 ± 36^{abc}	152 ± 40^{bc}
BAP	168 ± 12^{abc}	207 ± 33^{abc}
SAD	154 ± 37^{bc}	128 ± 8^{c}
2,4-D + KIN	199 ± 33^{abc}	235 ± 52^{abc}
2,4-D + BAP	289 ± 107^{a}	280 ± 13^{ab}
2,4-D + SAD	154 ± 30^{bc}	234 ± 39^{abc}
NAA + KIN	163 ± 18^{abc}	245 ± 34^{abc}
NAA + BAP	170 ± 42^{abc}	239 ± 95^{abc}
NAA + SAD	183 ± 23^{abc}	208 ± 41^{abc}
AIA + KIN	197 ± 46^{abc}	203 ± 52^{abc}
AIA + BAP	156 ± 37^{bc}	205 ± 34^{abc}
AIA + SAD	227 ± 18^{abc}	201 ± 46^{abc}

2

Table 2 (on next page)

Combined effect of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and cytokinin 6-benzylaminopurine (BAP) on the formation of cell callus in cotyledons of *Jatropha curcas* accession MAP-011107-G8

The data were taken 30 days after culture in darkness. Each treatment had three replicates and each repetition consisted of 20 to 30 cuttings in a cotyledon. Means having different letters are significantly different ($p \leq 0.05$). Formulation of Medium: Basal Medium MS + 3% sucrose (p/v) + 500 mg • L^{-1} PVP.

1

BAP (µM)	2,4 D (µM)					
	0	**1**	**2.5**	**5**	**7.5**	**10**
0	196 ± 10^{efghij}	138 ± 10^{j}	145 ± 7^{ij}	186 ± 20^{ghij}	142 ± 10^{j}	191 ± 20^{efghij}
0.5	194 ± 10^{efghij}	271 ± 110^{bcdefgh}	153 ± 21^{hij}	156 ± 30^{hij}	194 ± 20^{efghij}	208 ± 40^{defghij}
1	152 ± 20^{hij}	199 ± 20^{defghij}	246 ± 31^{cdefghij}	285 ± 30^{abcdefg}	303 ± 10^{abcdefg}	313 ± 20^{abcde}
1.5	140 ± 20^{j}	0.190 ± 20^{fghij}	376 ± 24^{ab}	223 ± 20^{cdefghij}	245 ± 30^{cdefghij}	267 ± 30^{bcdefghi}
2	203 ± 60^{defghij}	0.221 ± 40^{abc}	342 ± 46^{cdefghij}	209 ± 60^{defghij}	**405 ± 11^{a}**	319 ± 20^{abcd}
2.5	211 ± 10^{defgihj}	0.219 ± 30^{defghij}	375 ± 33^{ab}	**402 ± 20^{a}**	311 ± 10^{abcdef}	307 ± 20^{abcdefg}

2

Table 3 (on next page)

Jatropha curcas accessions representative of regions in Chiapas State (Mexico) used in this study and their callogenic ability when induced with 2,4-D (5 µM) and BAP (2.5 µM).

* Populations located in Chiapas, Mexico. Source: Ovando-Medina et al. (2011). **Coefficient of variation was 64.9 %. The data were taken 30 days after culture. Each accession had three replicates and each repetition consisted of 20 to 30 cuttings of a cotyledon. Means having different letters are significantly different from the others ($p \leq 0.05$). Composition of medium: Medium MS (1962), 3% sucrose p/v and 500 mg•Lof Polyvinylpyrrolidone + 2, 4-D (5 µM) + BAP (2.5 µM).

1

Accession*	Latitude	Longitude	Population	Callus dry weight (mg)**
ARR-251107-MFG7	16°11.231’	93°54.516’	Isthmus	1014 ± 578[a]
MAP-011107-G8	15°25.505’	92°53.554’	Soconusco	402 ± 23[ab]
JIQ-090208-AG1	16°40.012’	93°39.242’	Center	175 ± 31[b]
PUJ-030 508-S4	16°16.430’	92°17.550’	Frailesca	204 ± 19[b]
CDCU-030208-F4	15°40.473’	92°00.129’	Border	207 ± 50[b]

2

Table 4(on next page)

Compounds identified in the root hexane extract of *Jatropha curcas* accession MAP-011 107-G8, by gas chromatography-mass spectrometry.

Yields were calculated based on the areas under the peak curve of purified standards.

1

Structure	Molecular Weight ($g \bullet gmol^{-1}$)	Retention time (min)	Yield (mg compound•g sample^{-1})
Jatrophone	312	20.57	2.038
Jatropholone *a*	295	22.1	6.331
Jatrotropholone *b*	296	22.3	1.668

2