Assessment of genetic stability in somatic embryogenesis regenerated plants of *Magnolia dealbata*.

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INTRODUCTION

- *Magnolia dealbata* belongs to the family Magnoliaceae, it is an endemic and endangered species from Mexico with medicinal and ornamental importance (Alonso-Castro *et al.*, 2014).

- In *vitro* propagation is an alternative that could help in decreasing pressure on natural populations (Chandrika *et al*., 2008).

INTRODUCTION

- Plant tissue culture is recognized as one of the valuable components of biotechnology methods because of its potential to rapid multiplication of true-to-type genotypes and to conserve valuable germoplasm (Dhir and Shekhawat, 2013).

INTRODUCTION

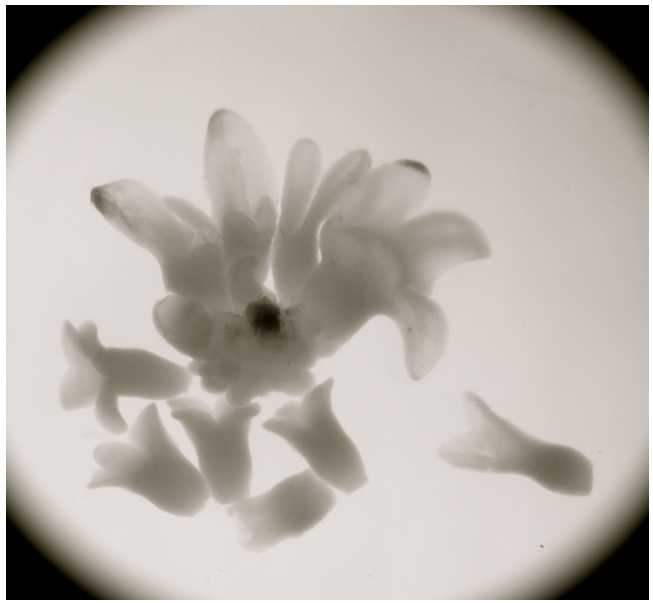
- In general, clonal propagation through tissue culture should generate individuals identical to the mother plant from which they were sub-cultured (Carloni *et al*., 2014).

- Although *M. vovidesii* (considered before as *M. dealbata*) has been propagated by somatic embryogenesis, there are no reports on in *vitro* propagation of *M. dealbata* including the assessment of the genetic identity.

INTRODUCTION

The purpose of this study is to determine the effects of genotype, concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) on secondary somatic embryos in repetitive cycles and the genetic stability of regenerated plants of *M. dealbata*.

MATERIALS AND METHODS



3 individuals of *M. dealbata*

3 mature fruis selected

Seeds

surface-sterilize

120 zygotic embryos

primary somatic embryogenic cultures (WP + 4.5 µM) (WP + 9 µM) 60 days

callus

(WP + 4.5 µM) (WP + 9 µM) 60 days

secondary somatic embryogenic cultures (x2)

medium WP + 0.05 % activated charcoal 30 days

18 explants with somatic embryos

360 torpedo or cotyledonary stage primary embryos were isolated

germination

(WP)

DNA extraction (154 regenerants)

amplification SSR

Genetic stability

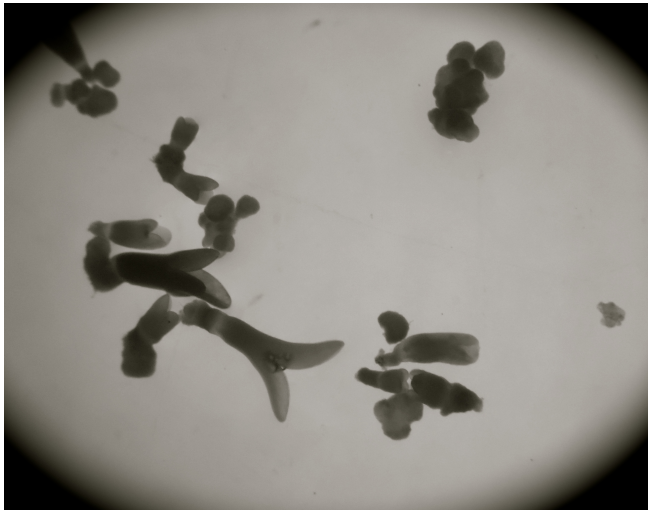
counts

somatic embryos at different developmental stages

RESULTS AND DISCUSSION

Table 1. Effect of PGR, cycles and genotypes on the development of somatic embryos from explants of *Magnolia dealbata*.

	Globular	Heart shape	Torpedo	Cotiledonary
9 µM 2,4-D	980[a]	599[a]	735[a]	1172[a]
F1020	371[a]	245[a]	400[a]	516[a]
C1	70	76	172	180
C2	232	155	171	228
C3	69	14	57	108
F1015	568[a]	320[a]	250[a]	546[a]
C1	121	17	42	180
C2	386	256	170	299
C3	61	47	38	67
F1012	41[a]	34[a]	85[a]	110[a]
C1	18	23	5	31
C2	23	11	67	53
C3	0	0	13	26
4.5 µM 2,4-D	764[a]	498[a]	475[a]	1324[a]
F1020	105[a]	100[a]	131[a]	433[a]
C1	45	32	32	240
C2	41	63	54	126
C3	19	5	45	67
F1015	331[a]	177[a]	162[a]	486[a]
C1	157	70	27	213
C2	44	44	53	125
C3	130	63	82	148
F1012	328[a]	221[a]	182[a]	405[a]
C1	105	29	9	119
C2	139	150	105	182
C3	84	42	68	104



HS-cycle
9 µM 2,4-D, 1012: r^2=0.99, p=0.01

T-cycles
4.5 µM 2,4-D, 1015: r^2=0.99, p=0.02

A strong genotype response is known across all types of explants and species. The differences are usually associated with the variations between genotypes in susceptibility to genetic programming and reprogramming of embryogenically competent cells by external factors (Wilhelm *et al.*2005).

RESULTS AND DISCUSSION

Table 2. Effect of PGR, cycles and genotypes on the induction of secondary somatic embryogenesis from explants of *Magnolia dealbata*.

Genotype	Response	4.5 µM 2,4-D			9 µM 2,4-D		
		C1	C2	C3	C1	C2	C3
1020	N	20	30	30	20	30	30
	% ER	75	43	26.7	55	36.7	13.3
	TN	262	576	298	360	1111	213
	MN	13.1	48	18.6	12.9	46.3	17.8
	SE	52.4	192	74.5	51.4	185.2	71
1015	N	20	30	30	20	30	30
	% ER	25	43	33.3	45	60	36.7
	TN	349	284	136	498	786	248
	MN	17.5	11.8	8.5	17.8	32.8	15.5
	SE	69.8	47.3	34	71.7	131	62
1012	N	15	30	30	20	16	20
	% ER	40	23	36.7	30	25	15
	TN	467	266	423	77	154	39
	MN	23.4	9.5	36.4	9.6	12.8	4.9
	SE	93.4	38	105.8	38.5	51.3	19.5
Total	N		235			216	
	% ER		38.4^a			35.2^a	
	TN		3061^a			3486^a	
	MN		19.6^a			18.9^a	
	SE		78.6^a			75.7^a	

NE: explants number; % ER: embryogenic response; TN: total number of somatic embryos; MN: mean number of somatic embryos; SE: somatic embryos per explant.

Response	2,4-D µM	Genotye	Cycle	Response	2,4-D µM	Genotye	Cycle
% ER	4.5	1020^a	1^a	TN	4.5	1020^a	1^a
		1015^a	2^a			1015^a	2^a
		1012^a	3^a			1012^a	3^a
	9	1020^a	1^a		9	1020^a	1^a
		1015^a	2^a			1015^a	2^a
		1012^a	3^a			1012^a	3^a
MN	4.5	1020^a	1^a	SE	4.5	1020^a	1^a
		1015^a	2^a			1015^a	2^a
		1012^a	3^a			1012^a	3^a
	9	1020^a	1^a		9	1020^a	1^a
		1015^a	2^a			1015^a	2^a
		1012^a	3^a			1012^a	3^a

It is well known that genotype has an effect on expression of regeneration competence. It was found that some genotypes respond well whereas others are recalcitran, which suggests that regeneration is genetically controlled (Shoemaker *et al*. 1991), but our data do not support it.

RESULTS AND DISCUSSION

Table 3. Variation frequency (%) of mutated plants of *Magnolia dealbata* at Mmc277 (1), Mmc493 (2), Mmc076 (3), Mmc182 (4), Mmc059 (5), Mmc001 (6), Mmc187 (7) and Mmc051 (8) loci.

		Loci							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
9 µM 2,4-D									
N. analysed plants	71								
N. mutated plants		26	0	31	6	39	5	22	15
Variation frequency (%)		36.6	0	43.6	8.4	54.9	7.0	30.9	21.1
4.5 µM 2,4-D									
N. analysed plants	83								
N. mutated plants		18	1	4	22	50	1	39	24
Variation frequency (%)		21.6	1.2	4.8	26.5	60.2	1.2	46.9	28.9

The eight loci showed different degrees of variability, indicating that a specific locus is particularly susceptible to DNA rearrangements.

RESULTS AND DISCUSSION

Table 4. The frequency (%) within each concentration, families and induction cycles are presented as the frequency of perfect genotypes (FPG). The mean of the mutation frequencies of the alleles observed in the eight microsatellite in each variable is presented as MMF (mean mutation frequency). The mutation frequency was obtained by dividing the number of mutated alleles by the total number of alleles analysed.

	No. of genotypes	No. of perfect genotypes	% FPG	Mutation frequency
	9 µM 2,4-D		48.29[a]	37.29[a]
F1020	24	8	33.3[a]	56.52[a]
C1	22	8	36.3	56.52
C2	11	7	63.6	23.08
C3	10	8	80	16.67
F1015	16	8	50[a]	23.53[a]
C1	14	8	57.1	23.53
C2	12	8	66.6	23.53
C3	11	8	72.7	7.14
F1012	13	5	61.5[a]	31.82[a]
C1	11	8	72.7	21.05
C2	11	8	72.7	21.05
C3	9	8	88.8	6.25
	4.5 µM 2,4-D		44.8[a]	42.67[a]
F1020	22	8	36.4[a]	50.00[a]
C1	16	8	50	37.5
C2	13	8	61.5	37.5
C3	12	8	66.6	23.08
F1015	23	9	39.1[a]	39.13[a]
C1	15	9	60	22.22
C2	14	9	64.2	22.22
C3	20	9	45	39.13
F1012	17	10	58.5[a]	38.89[a]
C1	11	9	81.8	15.38
C2	12	6	75	21.43
C3	14	9	64.2	26.67

%FPG-%ER

4.5 µM 2,4-D, 1020: r=0.99, p=0.021

%FPG-cycles

9 µM 2,4-D: r^2=0.53, p=0.02

MMF-cycles

4.5 µM 2,4-D, 1012: r^2=0.99, p= 0.02

There is a risk of mutations accumulating in embryogenic tissues during the somatic embryogenic process. The high mutation rates found might reflect the plasticity to adapt stress, which also includes adaptation to in vitro conditions according with Burg *et al*. (2007).

Plant tissues in vitro are exposed to high levels of oxidative stress and reactive oxygen species (ROS) are known to cause DNA damage, incluiding microsatellite instability (Wilhelm *et al*.2005), it´s possible that *M. dealbata* is ROS such as *M. grandiflora*.

CONCLUSION

- The variables tested (genotype, PGR concentration and cycles) had no effect on secondary somatic embryogenesis of *Magnolia dealbata*.

- Genetic fidelity was assessed using SSR marker which has revealed the polimorphic nature (≈51%) of the regenerants indicating a high level of genetic instability among the regenerants.

CONCLUSION

- Our results provide preliminary evidence for protocol of somatic embryogenesis of *Magnolia dealbata* in inducing genetic instability. All these facts should be taken into consideration for clonal propagation, because it could represent a very serious problem for preserving the genetic integrity of the regenerated plants. On other hand, that induce genetic variability, could contribute to plant genetic improvement programs.