

## Article

# Potential and Constraints on In Vitro Micropropagation of *Juniperus drupacea* Labill.

Kostas Ioannidis <sup>1,\*</sup>, Ioanna Tomprou <sup>1</sup>, Danae Panayiotopoulou<sup>2</sup>, Stefanos Boutsios<sup>3</sup> and Evangelia N. Daskalakou<sup>3</sup>

<sup>1</sup> Laboratory of Sylviculture, Forest Genetics and Biotechnology, Institute of Mediterranean and Forest Ecosystems, Hellenic Agricultural Organization “DIMITRA”, Ilissia, 11528 Athens, Greece

<sup>2</sup> Library and Documentation Center, Institute of Mediterranean & Forest Ecosystems, Hellenic Agricultural Organization “DIMITRA”, Ilissia, 11528 Athens, Greece

<sup>3</sup> Laboratory Forest Management & Forest Economics, Institute of Mediterranean & Forest Ecosystems, Hellenic Agricultural Organization “DIMITRA”, Ilissia, 11528 Athens, Greece

\* Correspondence: ioko@fria.gr; Tel.: +30-210-7783-750 (K.I.)

**Table S1.** Effect of medium and plant growth regulator types and their concentrations on the average percentage of blastogenesis (%) of *Juniperus drupacea* explants in relation to their gender. (Means followed by the same letter do not differ statistically at  $p \leq 0.05$  according to the Duncan test.)

Female explants			Male explants		
Medium and PGR treatment	N	(%)	Medium and PGR treatment	N	(%)
DKW + 4 $\mu$ M mT	24	54.17 <sup>a</sup>	DKW + 4 $\mu$ M BA	24	62.50 <sup>a</sup>
DKW + 4 $\mu$ M TDZ	24	54.17 <sup>a</sup>	DKW + 4 $\mu$ M TDZ	24	58.33 <sup>ab</sup>
WPM + 4 $\mu$ M mT	24	54.17 <sup>a</sup>	DKW + 8 $\mu$ M mT	24	54.17 <sup>abc</sup>
WPM + 4 $\mu$ M BA	24	54.17 <sup>a</sup>	DKW + 4 $\mu$ M mT	24	54.17 <sup>abc</sup>
DKW + 8 $\mu$ M mT	24	50.00 <sup>ab</sup>	WPM + 4 $\mu$ M mT	24	54.17 <sup>abc</sup>
DKW + 4 $\mu$ M BA	24	50.00 <sup>ab</sup>	WPM + 8 $\mu$ M mT	24	50.00 <sup>abc</sup>
WPM + 8 $\mu$ M mT	24	50.00 <sup>ab</sup>	WPM + 4 $\mu$ M TDZ	24	50.00 <sup>abc</sup>
MS + 8 $\mu$ M BA	24	50.00 <sup>ab</sup>	MS + 4 $\mu$ M BA	24	50.00 <sup>abc</sup>
DKW + 8 $\mu$ M BA	24	45.83 <sup>abc</sup>	DKW + 8 $\mu$ M BA	24	45.83 <sup>abc</sup>
WPM + 4 $\mu$ M TDZ	24	45.83 <sup>abc</sup>	WPM + 8 $\mu$ M TDZ	24	45.83 <sup>abc</sup>
WPM + 8 $\mu$ M BA	24	45.83 <sup>abc</sup>	WPM + 4 $\mu$ M BA	24	45.83 <sup>abc</sup>
MS + 4 $\mu$ M mT	24	45.83 <sup>abc</sup>	MS + 4 $\mu$ M mT	24	45.83 <sup>abc</sup>
MS + 4 $\mu$ M TDZ	24	45.83 <sup>abc</sup>	MS + 8 $\mu$ M BA	24	45.83 <sup>abc</sup>
DKW + 8 $\mu$ M TDZ	24	41.67 <sup>abc</sup>	DKW + 8 $\mu$ M TDZ	24	41.67 <sup>abc</sup>
DKW + 1 $\mu$ M TDZ	24	41.67 <sup>abc</sup>	DKW + 2 $\mu$ M TDZ	24	41.67 <sup>abc</sup>
DKW + 2 $\mu$ M BA	24	41.67 <sup>abc</sup>	DKW + 2 $\mu$ M BA	24	41.67 <sup>abc</sup>
WPM + 2 $\mu$ M BA	24	41.67 <sup>abc</sup>	WPM + 8 $\mu$ M BA	24	41.67 <sup>abc</sup>
DKW + 2 $\mu$ M TDZ	24	37.50 <sup>abc</sup>	MS + 4 $\mu$ M TDZ	24	41.67 <sup>abc</sup>
WPM + 8 $\mu$ M TDZ	24	37.50 <sup>abc</sup>	DKW + 2 $\mu$ M mT	24	37.50 <sup>abc</sup>
WPM + 2 $\mu$ M TDZ	24	37.50 <sup>abc</sup>	DKW + 1 $\mu$ M TDZ	24	37.50 <sup>abc</sup>
MS + 8 $\mu$ M mT	24	37.50 <sup>abc</sup>	DKW + 1 $\mu$ M BA	24	37.50 <sup>abc</sup>
MS + 2 $\mu$ M TDZ	24	37.50 <sup>abc</sup>	WPM + 1 $\mu$ M mT	24	37.50 <sup>abc</sup>

MS + 4 $\mu$ M BA	24	37.50 <sup>abc</sup>	WPM + 2 $\mu$ M TDZ	24	37.50 <sup>abc</sup>
DKW + 2 $\mu$ M mT	24	33.33 <sup>abc</sup>	MS + 8 $\mu$ M mT	24	37.50 <sup>abc</sup>
DKW + 1 $\mu$ M BA	24	33.33 <sup>abc</sup>	MS + 2 $\mu$ M mT	24	37.50 <sup>abc</sup>
WPM + 2 $\mu$ M mT	24	33.33 <sup>abc</sup>	MS + 8 $\mu$ M TDZ	24	37.50 <sup>abc</sup>
WPM + 1 $\mu$ M TDZ	24	33.33 <sup>abc</sup>	MS + 2 $\mu$ M TDZ	24	37.50 <sup>abc</sup>
MS + 2 $\mu$ M mT	24	33.33 <sup>abc</sup>	MS + 2 $\mu$ M BA	24	37.50 <sup>abc</sup>
MS + 8 $\mu$ M TDZ	24	33.33 <sup>abc</sup>	DKW + 1 $\mu$ M mT	24	33.33 <sup>abc</sup>
MS + 1 $\mu$ M TDZ	24	33.33 <sup>abc</sup>	WPM + 2 $\mu$ M mT	24	33.33 <sup>abc</sup>
MS + 2 $\mu$ M BA	24	33.33 <sup>abc</sup>	WPM + 1 $\mu$ M TDZ	24	33.33 <sup>abc</sup>
DKW + 1 $\mu$ M mT	24	29.17 <sup>bc</sup>	WPM + 2 $\mu$ M BA	24	33.33 <sup>abc</sup>
WPM + 1 $\mu$ M mT	24	29.17 <sup>bc</sup>	WPM + 1 $\mu$ M BA	24	33.33 <sup>abc</sup>
WPM + 1 $\mu$ M BA	24	29.17 <sup>bc</sup>	MS + 1 $\mu$ M TDZ	24	33.33 <sup>abc</sup>
MS + 1 $\mu$ M mT	24	29.17 <sup>bc</sup>	DKW + No PGR	24	29.17 <sup>abc</sup>
MS + 1 $\mu$ M BA	24	29.17 <sup>bc</sup>	MS + 1 $\mu$ M mT	24	29.17 <sup>abc</sup>
DKW + No PGR	24	25.00 <sup>c</sup>	MS + 1 $\mu$ M BA	24	25.00 <sup>bc</sup>
WPM + No PGR	24	25.00 <sup>c</sup>	WPM + No PGR	24	20.83 <sup>c</sup>
MS + No PGR	24	20.83 <sup>c</sup>	MS + No PGR	24	20.83 <sup>c</sup>

MS: Murashige and Skoog medium, WPM: wood plant medium, DKW: Driver and Kuniyaki Walnut medium, No PGR: control, BA: 6-benzylaminopurine, TDZ: thidiazuron, mT: meta-topolin [6-(3-hydroxybenzylamino)purine].

**Table S2.** The effect of medium and plant growth regulator type and their concentrations on the average number of shoots per *Juniperus drupacea*'s explant in relation to their gender. (Means followed by the same letter do not differ statistically at  $p \leq 0.05$  according to Duncan test.)

Female explants			Male explants		
Medium and PGR treatment	N		Medium and PGR treatment	N	
DKW + 4 $\mu$ M mT	24	1.17 <sup>a</sup>	DKW + 4 $\mu$ M TDZ	24	1.17 <sup>a</sup>
DKW + 4 $\mu$ M TDZ	24	1.17 <sup>a</sup>	DKW + 4 $\mu$ M mT	24	1.08 <sup>ab</sup>
WPM + 4 $\mu$ M TDZ	24	1.08 <sup>a</sup>	DKW + 4 $\mu$ M BA	24	1.00 <sup>abc</sup>
MS + 4 $\mu$ M mT	24	1.08 <sup>a</sup>	WPM + 4 $\mu$ M mT	24	1.00 <sup>abc</sup>
WPM + 4 $\mu$ M mT	24	1.04 <sup>ab</sup>	MS + 4 $\mu$ M BA	24	1.00 <sup>abc</sup>
WPM + 4 $\mu$ M BA	24	1.04 <sup>ab</sup>	WPM + 4 $\mu$ M TDZ	24	0.96 <sup>abcd</sup>
MS + 4 $\mu$ M TDZ	24	1.00 <sup>abc</sup>	MS + 4 $\mu$ M mT	24	0.96 <sup>abcd</sup>
DKW + 4 $\mu$ M BA	24	0.92 <sup>abcd</sup>	MS + 4 $\mu$ M TDZ	24	0.88 <sup>abcd</sup>
WPM + 8 $\mu$ M mT	24	0.92 <sup>abcd</sup>	DKW + 8 $\mu$ M mT	24	0.83 <sup>abcdef</sup>
DKW + 8 $\mu$ M mT	24	0.88 <sup>abcd</sup>	DKW + 8 $\mu$ M BA	24	0.83 <sup>abcdef</sup>
DKW + 8 $\mu$ M TDZ	24	0.88 <sup>abcd</sup>	WPM + 4 $\mu$ M BA	24	0.83 <sup>abcdef</sup>
DKW + 8 $\mu$ M BA	24	0.88 <sup>abcd</sup>	MS + 8 $\mu$ M TDZ	24	0.79 <sup>abcdef</sup>
DKW + 2 $\mu$ M BA	24	0.79 <sup>abcd</sup>	DKW + 2 $\mu$ M mT	24	0.71 <sup>abcdef</sup>
MS + 4 $\mu$ M BA	24	0.79 <sup>abcd</sup>	DKW + 8 $\mu$ M TDZ	24	0.71 <sup>abcdef</sup>
DKW + 1 $\mu$ M TDZ	24	0.75 <sup>abcd</sup>	WPM + 8 $\mu$ M mT	24	0.71 <sup>abcdef</sup>
WPM + 8 $\mu$ M TDZ	24	0.75 <sup>abcd</sup>	MS + 8 $\mu$ M mT	24	0.71 <sup>abcdef</sup>
WPM + 2 $\mu$ M BA	24	0.75 <sup>abcd</sup>	MS + 2 $\mu$ M mT	24	0.71 <sup>abcdef</sup>
MS + 8 $\mu$ M BA	24	0.75 <sup>abcd</sup>	DKW + 2 $\mu$ M TDZ	24	0.67 <sup>abcdef</sup>
DKW + 2 $\mu$ M TDZ	24	0.71 <sup>abcd</sup>	DKW + 1 $\mu$ M TDZ	24	0.67 <sup>abcdef</sup>

WPM + 8 µM BA	24	0.71 <sup>abcd</sup>	DKW + 2 µM BA	24	0.67 <sup>abcdef</sup>
MS + 8 µM mT	24	0.71 <sup>abcd</sup>	WPM + 8 µM TDZ	24	0.67 <sup>abcdef</sup>
WPM + 2 µM TDZ	24	0.67 <sup>abcd</sup>	MS + 8 µM BA	24	0.67 <sup>abcdef</sup>
MS + 8 µM TDZ	24	0.67 <sup>abcd</sup>	MS + 2 µM BA	24	0.67 <sup>abcdef</sup>
MS + 2 µM mT	24	0.63 <sup>abcd</sup>	DKW + 1 µM mT	24	0.63 <sup>abcdef</sup>
MS + 1 µM TDZ	24	0.63 <sup>abcd</sup>	WPM + 2 µM TDZ	24	0.63 <sup>abcdef</sup>
DKW + 2 µM mT	24	0.58 <sup>abcd</sup>	WPM + 1 µM mT	24	0.58 <sup>abcdef</sup>
WPM + 1 µM TDZ	24	0.58 <sup>abcd</sup>	WPM + 8 µM BA	24	0.58 <sup>abcdef</sup>
MS + 1 µM mT	24	0.58 <sup>abcd</sup>	WPM + 2 µM BA	24	0.58 <sup>abcdef</sup>
DKW + 1 µM BA	24	0.54 <sup>abcd</sup>	MS + 2 µM TDZ	24	0.58 <sup>abcdef</sup>
WPM + 2 µM mT	24	0.54 <sup>abcd</sup>	MS + 1 µM TDZ	24	0.58 <sup>abcdef</sup>
MS + 2 µM TDZ	24	0.54 <sup>abcd</sup>	DKW + 1 µM BA	24	0.54 <sup>abcdef</sup>
MS + 2 µM BA	24	0.54 <sup>abcd</sup>	WPM + 2 µM mT	24	0.54 <sup>abcdef</sup>
DKW + 1 µM mT	24	0.50 <sup>abcd</sup>	WPM + 1 µM TDZ	24	0.54 <sup>abcdef</sup>
WPM + 1 µM mT	24	0.50 <sup>abcd</sup>	WPM + 1 µM BA	24	0.50 <sup>bcdef</sup>
WPM + 1 µM BA	24	0.46 <sup>abcd</sup>	MS + 1 µM mT	24	0.42 <sup>cdef</sup>
MS + 1 µM BA	24	0.38 <sup>bcd</sup>	DKW + No PGR	24	0.33 <sup>def</sup>
DKW + No PGR	24	0.33 <sup>cd</sup>	MS + 1 µM BA	24	0.33 <sup>def</sup>
WPM + No PGR	24	0.33 <sup>cd</sup>	MS + No PGR	24	0.25 <sup>ef</sup>
MS + No PGR	24	0.29 <sup>d</sup>	WPM + No PGR	24	0.21 <sup>f</sup>

MS: Murashige and Skoog medium, WPM: wood plant medium, DKW: Driver and Kuniyaki Walnut medium, No PGR: control, BA: 6-benzylaminopurine, TDZ: thidiazuron, mT: meta-topolin [6-(3-hydroxybenzylamino)purine]. Means followed by the same letter do not differ statistically at  $p \leq 0.05$  according to the Duncan test.

**Table S3.** Effect of medium and plant growth regulator types and their concentrations on the average shoot length per *Juniperus drupacea*'s explant in relation to their gender. (Means followed by the same letter do not differ statistically at  $p \leq 0.05$  according to Duncan test.)

Female explants			Male explants		
Medium and PGR treatment	N	(mm)	Medium and PGR treatment	N	(mm)
DKW + 4 µM BA	11	2.94 <sup>a</sup>	DKW + 1 µM mT	8	2.69 <sup>a</sup>
DKW + 4 µM mT	13	2.90 <sup>ab</sup>	DKW + 1 µM TDZ	9	2.67 <sup>a</sup>
DKW + 4 µM TDZ	13	2.83 <sup>abc</sup>	WPM + 1 µM TDZ	8	2.57 <sup>a</sup>
DKW + 2 µM TDZ	9	2.78 <sup>abcd</sup>	DKW + 8 µM TDZ	10	2.53 <sup>ab</sup>
DKW + 2 µM mT	8	2.75 <sup>abcd</sup>	WPM + 2 µM BA	8	2.52 <sup>ab</sup>
WPM + 2 µM mT	8	2.75 <sup>abcd</sup>	DKW + 8 µM mT	13	2.50 <sup>ab</sup>
DKW + 8 µM TDZ	10	2.73 <sup>abcd</sup>	MS + 4 µM TDZ	10	2.50 <sup>ab</sup>
WPM + 4 µM BA	13	2.62 <sup>abcd</sup>	MS + 1 µM mT	7	2.47 <sup>ab</sup>
DKW + 1 µM mT	7	2.57 <sup>abcde</sup>	WPM + 8 µM BA	10	2.47 <sup>ab</sup>
DKW + 2 µM BA	10	2.55 <sup>abcde</sup>	MS + 8 µM TDZ	9	2.47 <sup>ab</sup>
WPM + 4 µM mT	13	2.54 <sup>abcde</sup>	WPM + 4 µM mT	13	2.45 <sup>ab</sup>
WPM + 4 µM TDZ	11	2.51 <sup>abcde</sup>	WPM + 2 µM TDZ	9	2.44 <sup>ab</sup>
DKW + 8 µM BA	11	2.50 <sup>abcde</sup>	MS + 2 µM TDZ	9	2.44 <sup>ab</sup>
DKW + 1 µM BA	8	2.50 <sup>abcde</sup>	WPM + 4 µM BA	11	2.44 <sup>ab</sup>
DKW + 8 µM mT	12	2.48 <sup>abcde</sup>	DKW + 4 µM TDZ	14	2.43 <sup>abc</sup>
WPM + 1 µM mT	7	2.46 <sup>abcde</sup>	WPM + 1 µM mT	9	2.40 <sup>abcd</sup>
MS + 8 µM TDZ	8	2.44 <sup>abcde</sup>	MS + 8 µM mT	9	2.40 <sup>abcd</sup>

MS + 4 $\mu$ M mT	11	2.44 <sup>abcde</sup>	MS + 8 $\mu$ M BA	11	2.38 <sup>abcde</sup>
MS + 2 $\mu$ M mT	8	2.43 <sup>abcde</sup>	WPM + 2 $\mu$ M mT	8	2.38 <sup>abcde</sup>
WPM + 8 $\mu$ M BA	11	2.41 <sup>abcde</sup>	DKW + 8 $\mu$ M BA	11	2.37 <sup>abcde</sup>
MS + 4 $\mu$ M BA	9	2.39 <sup>abcde</sup>	DKW + 2 $\mu$ M BA	10	2.37 <sup>abcdef</sup>
MS + 1 $\mu$ M mT	7	2.39 <sup>abcde</sup>	DKW + 4 $\mu$ M mT	13	2.37 <sup>abcdef</sup>
WPM + 8 $\mu$ M TDZ	9	2.37 <sup>abcde</sup>	WPM + 4 $\mu$ M TDZ	12	2.36 <sup>abcdef</sup>
DKW + 1 $\mu$ M TDZ	10	2.35 <sup>abcde</sup>	WPM + 1 $\mu$ M BA	8	2.34 <sup>abcde</sup>
WPM + 2 $\mu$ M BA	10	2.35 <sup>abcde</sup>	DKW + 2 $\mu$ M mT	9	2.33 <sup>abcde</sup>
MS + 4 $\mu$ M TDZ	11	2.35 <sup>abcde</sup>	MS + 2 $\mu$ M BA	9	2.33 <sup>abcde</sup>
WPM + 8 $\mu$ M mT	12	2.33 <sup>abcde</sup>	MS + 2 $\mu$ M mT	9	2.31 <sup>abcde</sup>
MS + 8 $\mu$ M mT	9	2.32 <sup>abcde</sup>	DKW + 4 $\mu$ M BA	15	2.30 <sup>abcdef</sup>
WPM + 2 $\mu$ M TDZ	9	2.27 <sup>abcdef</sup>	DKW + 2 $\mu$ M TDZ	10	2.23 <sup>abcdef</sup>
MS + 2 $\mu$ M TDZ	9	2.27 <sup>abcdef</sup>	MS + 4 $\mu$ M BA	12	2.22 <sup>abcdef</sup>
WPM + 1 $\mu$ M TDZ	8	2.24 <sup>abcdef</sup>	MS + 1 $\mu$ M TDZ	8	2.19 <sup>abcdef</sup>
MS + 1 $\mu$ M TDZ	8	2.20 <sup>abcdef</sup>	MS + 1 $\mu$ M BA	6	2.18 <sup>abcdef</sup>
MS + 2 $\mu$ M BA	6	2.18 <sup>abcdef</sup>	WPM + 8 $\mu$ M TDZ	11	2.18 <sup>abcdef</sup>
WPM + 1 $\mu$ M BA	7	2.14 <sup>bcdef</sup>	MS + 4 $\mu$ M mT	11	2.11 <sup>bcdef</sup>
MS + 8 $\mu$ M BA	12	2.13 <sup>cdef</sup>	WPM + 8 $\mu$ M mT	12	2.10 <sup>bcdef</sup>
MS + 1 $\mu$ M BA	5	2.04 <sup>def</sup>	WPM + No PGR	5	1.90 <sup>cdef</sup>
DKW + No PGR	6	1.83 <sup>efg</sup>	DKW + 1 $\mu$ M BA	9	1.89 <sup>def</sup>
WPM + No PGR	6	1.58 <sup>fg</sup>	DKW + No PGR	7	1.86 <sup>bcdef</sup>
MS + No PGR	6	1.33 <sup>g</sup>	MS + No PGR	5	1.80 <sup>bcdef</sup>

MS: Murashige and Skoog medium, WPM: wood plant medium, DKW: Driver and Kuniyaki Walnut medium, No PGR: control, BA: 6-benzylaminopurine, TDZ: thidiazuron, mT: meta-topolin [6-(3-hydroxybenzylamino)purine]. Means followed by the same letter do not differ statistically at  $p \leq 0.05$  according to the Duncan test.