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Vegetative propagation of elite *Eucalyptus* clones as food source for honeybees *(Apis mellifera)*; adventitious roots versus callus formation

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ABSTRACT

Summer and autumn in Israel are highly arid with not enough plants in bloom offering nectar and pollen to support the local apiary. This leads to decline in colony health and honey production. To increase food sources for honeybees, we initiated a project to clone elite *Eucalyptus* trees exhibiting constant and rich blooming from late summer to early winter. We induced adventitious roots from cuttings of two mature *Eucalyptus* trees of which nectar production and honeybees' attraction was measured: *Eucalyptus brachyphylla* and *Eucalyptus* x *trabutii*. During the rooting process, a high frequency of cylindrical callus formation instead of roots was obtained. To shed light on the inner anatomy of the callus chunks, we compared their cell organization and cell-wall composition to those of roots. Whereas in the root, cells were organized in circumferential symmetry, no symmetry was found in the callus. Instead, a more chaotic accumulation of meristematic-like cells with sporadic clusters of tracheary elements laid in different directions were observed. The outer cell layer of the callus often included swollen cells with thin cell walls. Most callus cells stained more strongly for cellulose and lignin than

cells in the root meristem. In addition, specific antibodies to methylesterified and de-methylesterified pectin showed differential staining of callus vs. root cells indicating cell wall differences. Strikingly, roots were seen to differentiate from the chaotic cell organization of the callus, albeit at low rates. Further investigation of the cellular and molecular mechanisms underlying callus formation, are required.

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KEYWORDS

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Supplementary material

Movie S1. HREM imaging of a root forming callus initiated from a E. brachyphylla cutting. Beginning with a 3D reconstruction of the callus, and continues with a sequence of the transverse sections (2.6 µL each) performed in the sample. The numbers on the sections point on the circular structures surrounding the tracheary elements along the callus which diverge, converge, and disappear alternately, until a root is formed.

Table S1. Nectar volume and sugar concentration measurements.

E × trabutii – Nectar secretion observation

	#	08/10/2018					09/10/2018						10/10/2018						11/10/2018				
		$06^{\underline{43}}$	$09^{\underline{15}}$	$11^{\underline{57}}$	$14^{\underline{20}}$	$16^{\underline{20}}$	$06^{\underline{50}}$	$08^{\underline{55}}$	$11^{\underline{00}}$	$13^{\underline{21}}$	$15^{\underline{20}}$	$17^{\underline{20}}$	$06^{\underline{40}}$	$08^{\underline{50}}$	$11^{\underline{04}}$	$13^{\underline{00}}$	$15^{\underline{00}}$	$17^{\underline{00}}$	$06^{\underline{50}}$	$09^{\underline{30}}$	$11^{\underline{30}}$	$13^{\underline{30}}$	
Nectar Volume (NV;µL)	1	19	10	1	1	0	21	5	0	1	1	3	43	5	0	0	0	1	41	0	0	0	
	2	7	10	3	1	2.5	52	2	8	2	0	2	70	0	0	0	0	0	0	0	0	0	
	3	16	0	9	2	0.5	30	0	2	0	0	1	63	0	0	0	0	0	19	0	0	0	
	4	27	4	0	2.5	2.5	50	0	0	0	0	1	48	0	0	0	0	0	27	0	0	0	
	5	25	1	0	2	0	20	0	0	2	0	1	22	2	0	0	0	0	0	0	0	0	
	6	40	7	3	2	0.5	55	4	0	0	1	0	47	7	0	0	0	0	54	0	0	0	
	7	14	0	0	2.5	1	45	0	0	0	1	0	68	10	0	0	0	0	0	0	0	0	
	8	26	0	0	1	0	12	0	0	0	1	0	36	4	0	0	0	0	0	0	0	0	
	9	17	0	0	2	0	23	0	0	0	1	0	25	0	0	0	0	0	20	0	0	0	
	10	20	0	0	1	0	25	1	0	0	1	0	26	0	0	0	0	0	70	0	0	0	
Nectar concentration (NC; %)	1	14	30	N/D	30	-	17	22	-	16	N/D	22	0.25	21	-	-	-	22	20	-	-	-	
	2	14.5	23	28	30	30	16	28	N/D	12	-	N/D	0.2	N/D	-	-	-	-	-	-	-	-	
	3	13	-	25	20	30	17	-	N/D	-	-	N/D	0.2	N/D	-	-	-	-	20	-	-	-	
	4	14	26	-	12.5	26	16	-	-	-	-	N/D	0.26	N/D	-	-	-	-	27	-	-	-	
	5	10	22	-	21	-	19	-	-	14	-	N/D	0.2	20.25	-	-	-	-	-	-	-	-	
	6	15	19	26	30	30	18	32	-	-	N/D	-	0.28	20	-	-	-	-	20	-	-	-	
	7	16	-	-	30	30	14	-	-	-	N/D	-	0.19	20	-	-	-	-	-	-	-	-	
	8	16	-	-	32	-	20	-	-	-	N/D	-	0.26	20	-	-	-	-	-	-	-	-	
	9	11	-	-	30	-	16.5	-	-	-	N/D	-	0.19	-	-	-	-	-	30	-	-	-	
	10	12	-	-	32	-	16	32	-	-	N/D	-	0.19	-	-	-	-	-	20	-	-	-	**Sum**
Sugar production (mg; NC×NV)	1	2.66	3	-	0.3	0	3.57	1.1	0	0.16	0	0.66	10.7	1.05	0	0	0	0.22	8.2	0	0	0	**31.67**
	2	1.01	2.3	0.84	0.3	0.75	8.32	0.56	0	0.24	0	0	14	0	0	0	0	0	0	0	0	0	**28.32**
	3	2.08	0	2.25	0.4	0.15	5.1	0	0	0	0	0	12.6	0	0	0	0	0	3.8	0	0	0	**26.38**
	4	3.78	1.04	0	0.31	0.65	8	0	0	0	0	0	12.4	0	0	0	0	0	7.29	0	0	0	**33.55**
	5	2.5	0.22	0	0.42	0	3.8	0	0	0.28	0	0	4.4	0.40	0	0	0	0	0	0	0	0	**12.02**
	6	6	1.33	0.78	0.6	0.15	9.9	1.28	0	0	0	0	13.1	1.4	0	0	0	0	10.8	0	0	0	**45.4**
	7	2.24	0	0	0.75	0.3	6.3	0	0	0	0	0	12.9	2	0	0	0	0	0	0	0	0	**24.51**
	8	4.16	0	0	0.32	0	2.4	0	0	0	0	0	9.36	0.8	0	0	0	0	0	0	0	0	**17.04**
	9	1.87	0	0	0.6	0	3.79	0	0	0	0	0	4.75	0	0	0	0	0	6	0	0	0	**17.01**
	10	2.4	0	0	0.32	0	4	0.32	0	0	0	0	4.94	0	0	0	0	0	14	0	0	0	**25.98**
																						Average (4 Days)	**26.18**

																	STDEV	**9.59**	

E. brachyphylla – Nectar secretion observation

	#	19/11/2018					20/11/2018						21/11/2018					22/11/2018		
		06^{30}	08^{30}	11^{15}	14^{36}	16^{15}	06^{30}	08^{30}	10^{30}	12^{30}	14^{30}	16^{00}	06^{30}	08^{45}	10^{45}	12^{45}	15^{40}	07^{30}	09^{30}	
Nectar Volume (NV;µL)	1	9	0	0	0	2	38	5	3	1	1.5	5	35	6	2	0	1	23.00	0	
	2	10	0	0	0	0	12	0	0	0	0	0.5	16	2	0	0	1	7.50	0	
	3	10	0	0	0	0	25	8	0	1	0.5	0.5	15	2.5	0	0	1	4.00	0	
	4	22	20	0	0	2	11	7	0	0	2.5	2	24	5	1.5	0	1	34.00	0	
	5	10	13	0	0	6	30	12	1	0.5	0	0		1	0	0	0	11.00	0	
	6	26	0	2	0	0	69	9	1	5	5	4	40	17	2.5	1.5	7	36.00	2	
	7	20	0	0	0	6	20	2.5	0.5	0	2	0	44	15	1.5	0	3	45.00	4	
	8	20	0	0	0	0	12	0.5	0	0	0	0	10	19	0	0	0	0.00	0	
	9	15	0	0	0	5	36	2.5	0	0	0	0.5	13	13	0	0	0	3.50	0	
	10	15	0	0	0	2	45	0	0	0	0	0	20	13	0	0	0	8.50	0	
Nectar concentration (NC; %)	1	32	-	-	-	28	13	10	12	29	32	31	21	16	29	-	42	15	-	
	2	23	-	-	-	-	11	-	-	-	-	27	11	17	-	-	39	15	-	
	3	21	-	-	-	-	11	14	-	29	21	23	11	16	-	-	35	13	-	
	4	23	28	-	-	24	10	14	-	-	30	32	12	13	33	-	35	13	-	
	5	21	30	-	-	10	13	15	31	14	-	-			-	-	-	15	-	
	6	26	-	30	-	-	17	29	16	10	31	22	30	17	35	51	22	16	44	
	7	31	-	-	-	23	20	25	16	-	18	-	12	15	35	-	35	17	28	
	8	17	-	-	-	-	18	15	-	-	-	-	12	19	-	-	-	-	-	
	9	21	-	-	-	30	12	15	-	-	-	-	11	13	-	-	-	15	-	
	10	19	-	-	-	31	13	15	-	-	-	-	12	13	-	-	-	15	-	**Sum**
Sugar production (mg; NC×NV)	1	2.88	0	0	0	0.56	4.94	0.5	0.36	0.29	0.48	1.55	7.35	0.96	0.58	0	0.42	3.45	0	**24.32**
	2	2.3	0	0	0	0	1.32	0	0	0	0	0.13	1.76	0.34	0	0	0.39	1.12	0	**7.37**
	3	2.1	0	0	0	0	2.75	1.12	0	0.29	0.10	0.11	1.65	0.4	0	0	0.35	0.52	0	**9.4**
	4	5.06	5.6	0	0	0.48	1.1	0.98	0	0	0.75	0.64	2.88	0.65	0.49	0	0.35	4.42	0	**23.4**
	5	2.1	3.9	0	0	0.6	3.9	1.8	0.31	0.07	0	0	0	0	0	0	0	1.65	0	**14.33**
	6	6.76	0	0.6	0	0	11.7	2.61	0.16	0.5	1.55	0.88	12	2.89	0.87	0.76	1.54	5.76	0.88	**49.5**
	7	6.2	0	0	0	1.38	4	0.625	0.08	0	0.36	0	5.28	2.25	0.52	0	1.05	7.65	1.12	**30.52**
	8	3.4	0	0	0	0	2.16	0.07	0	0	0	0	1.2	3.61	0	0	0	0	0	**10.44**

	9	3.15	0	0	0	1.5	4.32	0.38	0	0	0	0	1.43	1.69	0	0	0	0.52	0	**12.99**
	10	2.85	0	0	0	0.62	5.85	0	0	0	0	0	2.4	1.69	0	0	0	1.275	0	**14.68**
																		Average (4 Days)		**19.69**
																		STDEV		**12.83**