

Plant growth regulators influence *in vitro* growth and mesembrine-type alkaloid production of *Mesembryanthemum tortuosum*

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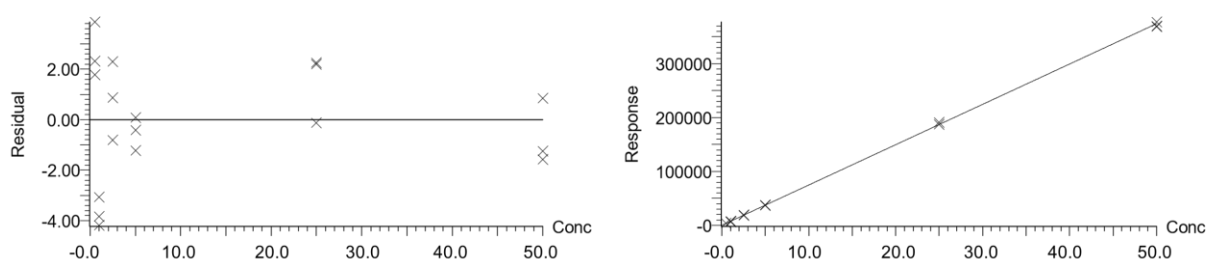


Figure S1: Calibration curve and residual plot for mesembrenone

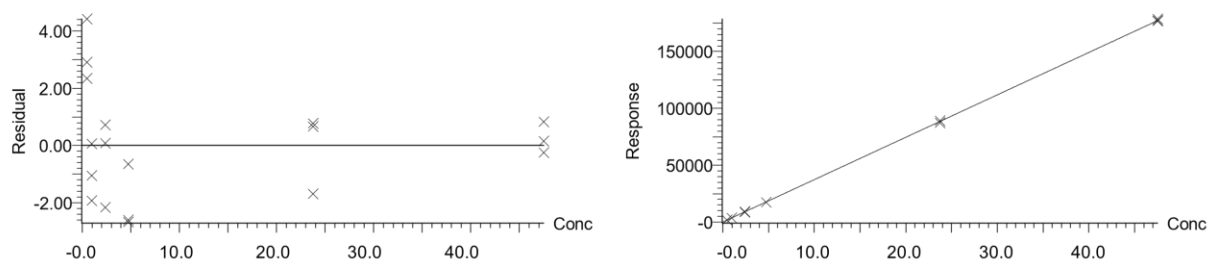


Figure S2: Calibration curve and residual plot for mesembranol

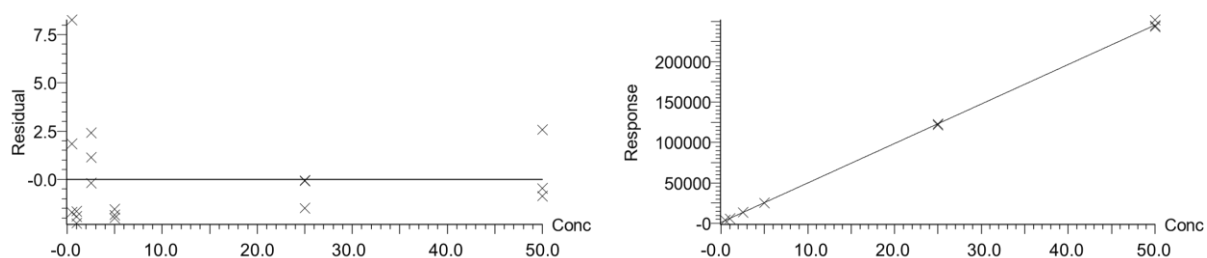


Figure S3: Calibration curve and residual plot for mesembrenol

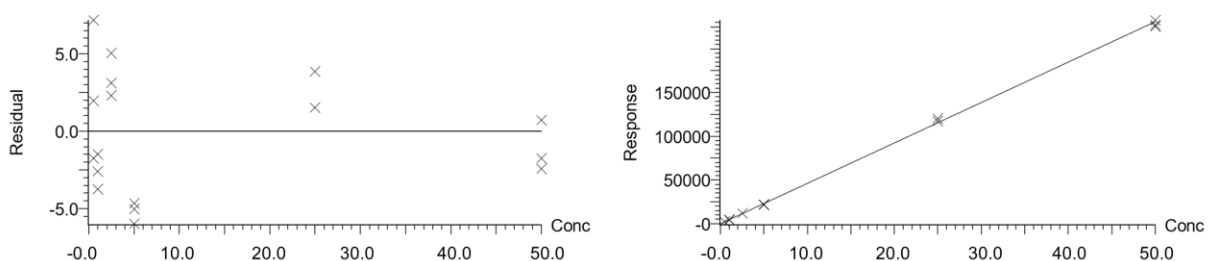


Figure S4: Calibration curve and residual plot for mesembrine

Table S1: Linearity, limit of detection (LOD), and limit of quantification (LOQ) of UPLC-PDA method

Compound	Linear range ($\mu\text{g/mL}$)	Regression equation	r^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Mesembranol	0.5-100	$Y = 3730.05X + 43.62$	0.9999	0.256	0.777
Mesembrenol	0.5-100	$Y = 4883.63X + 1184.20$	0.9998	0.222	0.672
Mesembrenone	0.5-100	$Y = 7486.93X + 40.84$	0.9997	0.218	0.659
Mesembrine	0.5-100	$Y = 4623.09X - 104.33$	0.9992	0.332	1.007

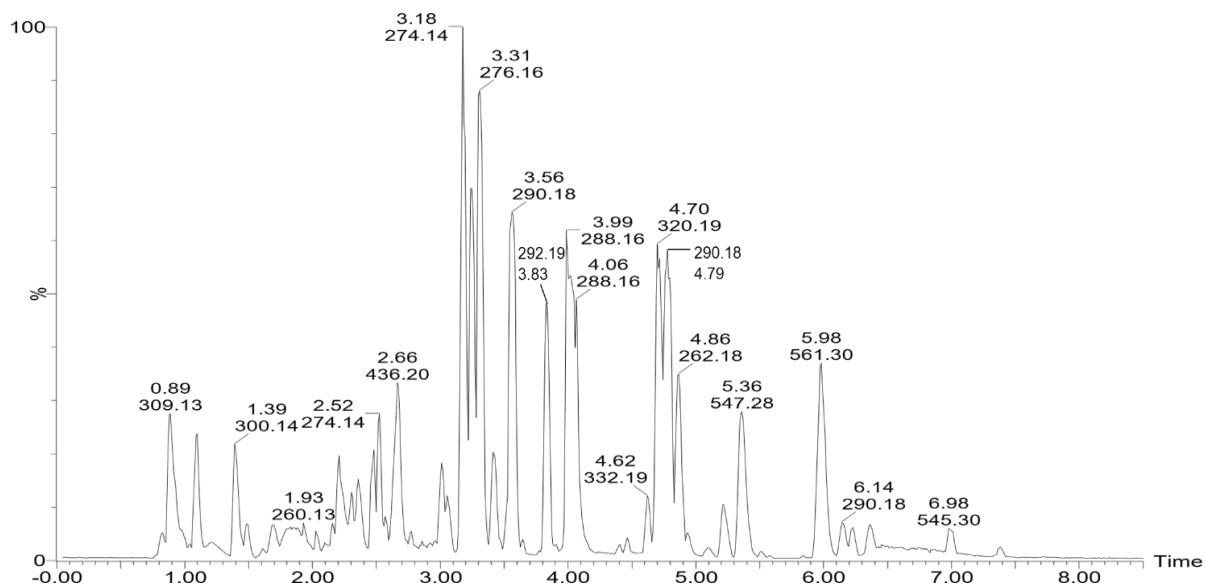


Figure S5: Representative chromatogram obtained from UPLC-MS analysis of a *Mesembryanthemum tortuosum* sample from the 20 μ M indole-3-butyric acid treatment. Mass (m/z) and retention times of mesembrine-type alkaloids are as follows: mesembrenone (m/z = 288.16, 4.06 min), mesembranol (m/z = 292.19, 3.83 min), mesembrenol (m/z = 290.18, 3.56 min), mesembrine (m/z = 290.18, 4.79 min)

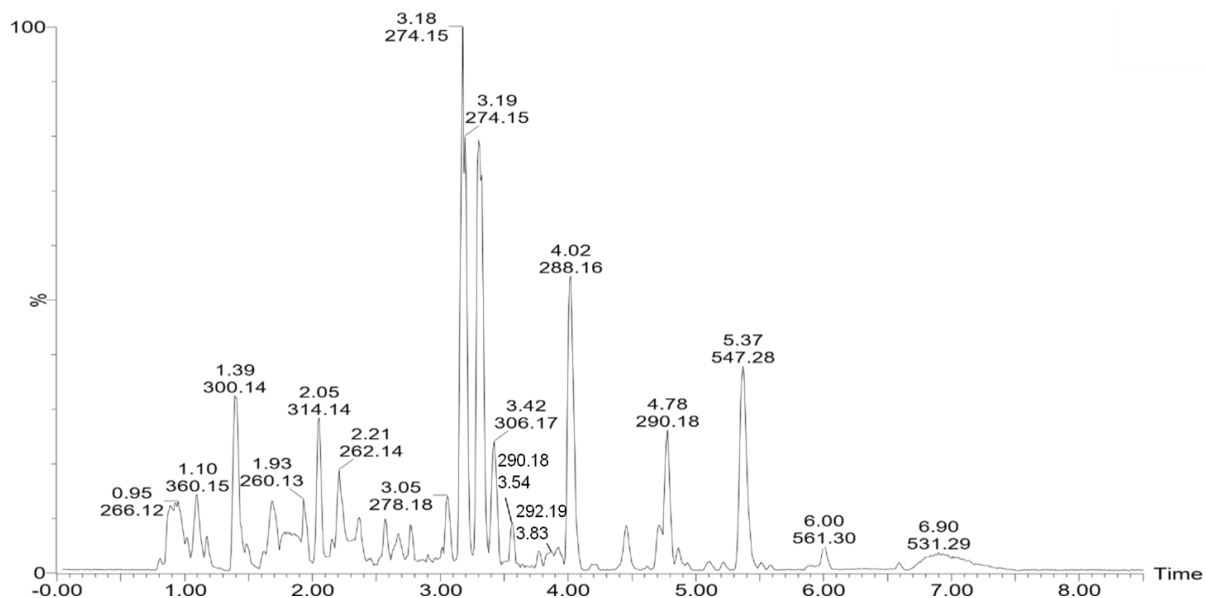


Figure S6: Representative chromatogram obtained from UPLC-MS analysis of a *Mesembryanthemum tortuosum* sample from the 20 μ M naphthaleneacetic acid treatment. Mass (m/z) and retention times of mesembrine-type alkaloids are as follows: mesembrenone (m/z = 288.16, 4.02 min), mesembranol (m/z = 292.19, 3.83 min), mesembrenol (m/z = 290.18, 3.54 min), mesembrine (m/z = 290.18, 4.78 min)

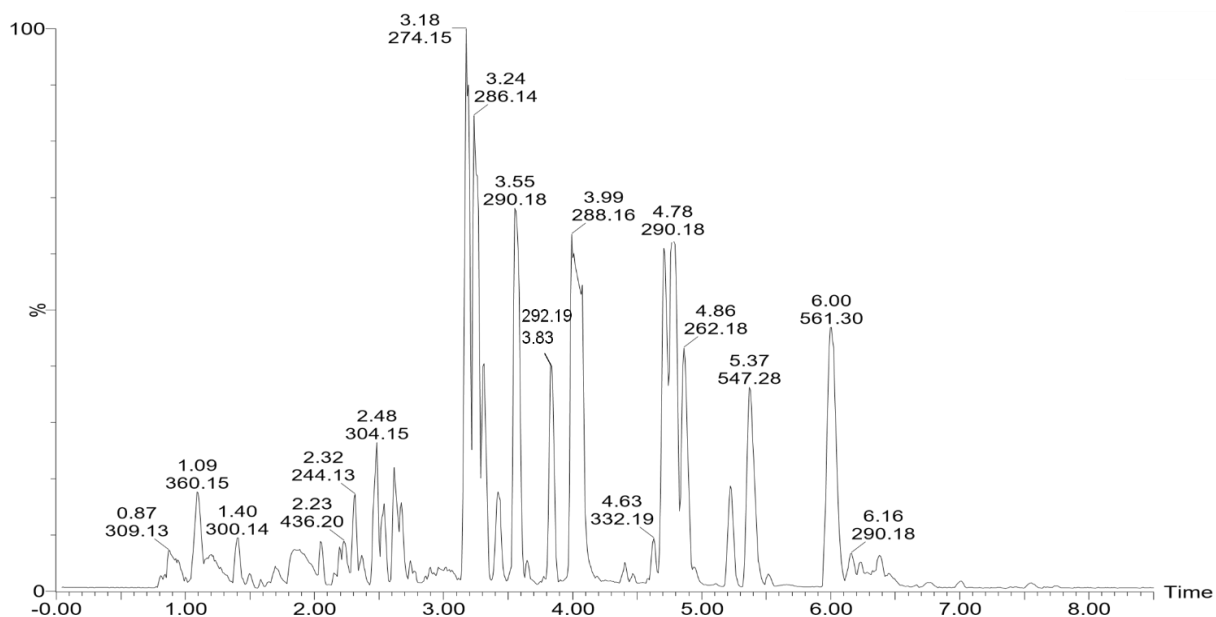


Figure S7: Representative chromatogram obtained from UPLC-MS analysis of a *Mesembryanthemum tortuosum* sample from the 20 μ M 6-benzylaminopurine treatment. Mass (m/z) and retention times of mesembrine-type alkaloids are as follows: mesembrenone (m/z = 288.16, 3.99 min), mesembranol (m/z = 292.19, 3.83 min), mesembrenol (m/z = 290.18, 3.55 min), mesembrine (m/z = 290.18, 4.78 min)

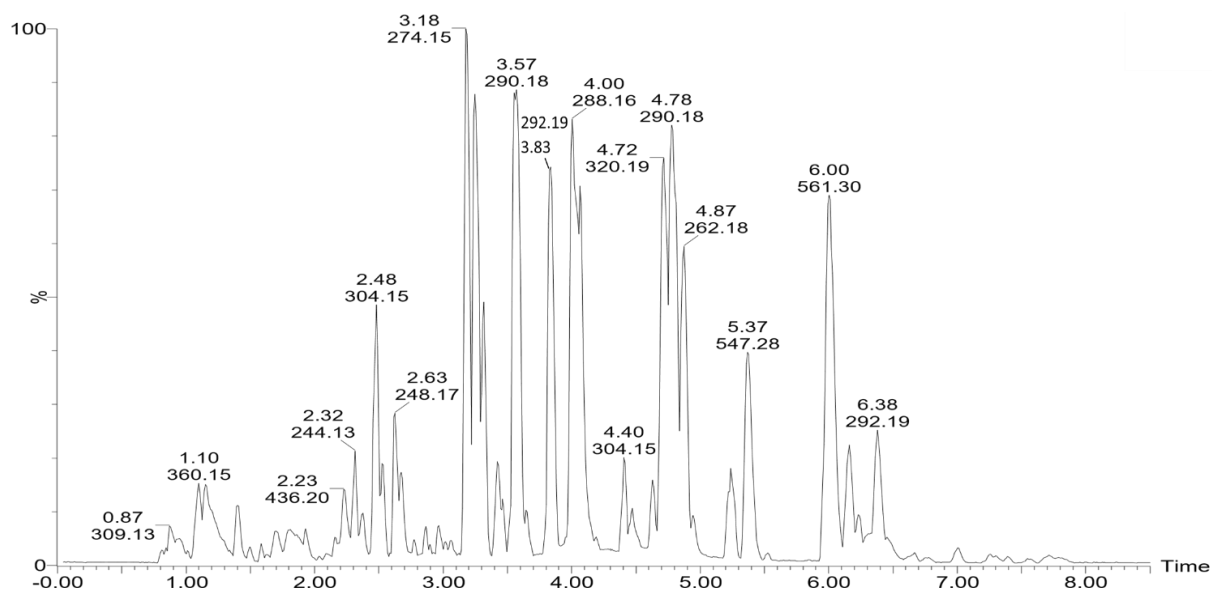


Figure S8: Representative chromatogram obtained from UPLC-MS analysis of a *Mesembryanthemum tortuosum* sample from the 20 μ M kinetin treatment. Mass (m/z) and retention times of mesembrine-type alkaloids are as follows: mesembrenone (m/z = 288.16, 4.00 min), mesembranol (m/z = 292.19, 3.83 min), mesembrenol (m/z = 290.18, 3.57 min), mesembrine (m/z = 290.18, 4.78 min)

Table S2: Effects of plant growth regulator treatments on mean number of new leaf pairs of *Mesembryanthemum tortuosum* plantlets

Treatment	n	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	6	1.942 $\pm$ 0.617	1.000	a
5 IBA	6	2.233 $\pm$ 0.678	.970	a
10 IBA	6	1.933 $\pm$ 1.003	1.000	a
15 IBA	6	2.292 $\pm$ 1.134	.983	a
20 IBA	6	1.722 $\pm$ 0.722	.992	a
Pooled control	24	1.944 $\pm$ 1.265	—	a
2.5 NAA	6	0.389 $\pm$ 0.143	<.001	b
5 NAA	6	0.048 $\pm$ 0.074	<.001	c
10 NAA	6	0.111 $\pm$ 0.272	<.001	bc
15 NAA	6	0.000 $\pm$ 0.000	<.001	c
20 NAA	6	0.028 $\pm$ 0.068	<.001	c
Pooled control	24	1.944 $\pm$ 1.265	—	a
2.5 BAP	6	7.725 $\pm$ 5.000	.198	a
5 BAP	6	9.167 $\pm$ 4.367	.059	a
10 BAP	6	9.542 $\pm$ 5.847	.139	a
15 BAP	6	8.972 $\pm$ 4.269	.061	a
20 BAP	6	9.008 $\pm$ 4.719	.086	a
Pooled control	24	1.944 $\pm$ 1.265	—	a
2.5 KIN	6	3.639 $\pm$ 0.992	.049	a
5 KIN	6	5.000 $\pm$ 2.876	.254	ab
10 KIN	6	4.528 $\pm$ 1.488	.044	a
15 KIN	6	6.542 $\pm$ 4.807	.325	ab
20 KIN	6	5.083 $\pm$ 2.419	.140	ab
Pooled control	24	1.944 $\pm$ 1.265	—	b

Table S3: Effects of plant growth regulator treatments on mean number of roots of *Mesembryanthemum tortuosum* plantlets

Treatment	n	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	6	3.467 $\pm$ 3.996	.817	c
5 IBA	6	5.150 $\pm$ 2.504	.090	bc
10 IBA	6	8.292 $\pm$ 5.704	.179	abc
15 IBA	6	11.550 $\pm$ 3.679	.007	ab
20 IBA	6	14.181 $\pm$ 4.659	.007	a
Pooled control	24	1.462 $\pm$ 1.327	—	c
2.5 NAA	6	9.579 $\pm$ 4.206	.032	ab
5 NAA	6	14.960 $\pm$ 2.531	<.001	a
10 NAA	6	9.679 $\pm$ 2.272	.001	b
15 NAA	6	11.083 $\pm$ 5.345	.043	ab
20 NAA	6	13.044 $\pm$ 2.959	.001	ab
Pooled control	24	1.462 $\pm$ 1.327	—	c
2.5 BAP	6	0.000 $\pm$ 0.000	<.001	b
5 BAP	6	0.042 $\pm$ 0.102	<.001	bc
10 BAP	6	0.000 $\pm$ 0.000	<.001	c
15 BAP	6	0.000 $\pm$ 0.000	<.001	b
20 BAP	6	0.000 $\pm$ 0.000	<.001	b
Pooled control	24	1.462 $\pm$ 1.327	—	a
2.5 KIN	6	0.083 $\pm$ 0.204	.001	b
5 KIN	6	0.056 $\pm$ 0.136	<.001	b
10 KIN	6	0.083 $\pm$ 0.204	.001	b
15 KIN	6	0.000 $\pm$ 0.000	<.001	b
20 KIN	6	0.000 $\pm$ 0.000	<.001	b
Pooled control	24	1.462 $\pm$ 1.327	—	a

Table S4: Effects of plant growth regulator treatments on mean root lengths of *Mesembryanthemum tortuosum* plantlets

Treatment	n	Mean $\pm$ SD	p (vs. pooled control)	Group
2.5 IBA	6	5.171 $\pm$ 4.459	.925	a
5 IBA	6	5.126 $\pm$ 4.311	.924	a
10 IBA	6	2.686 $\pm$ 1.868	.995	a
15 IBA	6	6.266 $\pm$ 4.372	.672	a
20 IBA	6	6.282 $\pm$ 2.588	.303	a
Pooled control	24	3.283 $\pm$ 4.240	—	a
2.5 NAA	6	3.465 $\pm$ 0.529	1.000	a
5 NAA	6	3.381 $\pm$ 0.605	1.000	a
10 NAA	6	2.489 $\pm$ 0.950	.958	a
15 NAA	6	2.439 $\pm$ 0.991	.948	a
20 NAA	6	2.970 $\pm$ 0.394	.999	a
Pooled control	24	3.283 $\pm$ 4.240	—	a
2.5 BAP	6	0.000 $\pm$ 0.000	.011	b
5 BAP	6	0.033 $\pm$ 0.082	.012	b
10 BAP	6	0.000 $\pm$ 0.000	.011	b
15 BAP	6	0.000 $\pm$ 0.000	.011	b
20 BAP	6	0.000 $\pm$ 0.000	.011	b
Pooled control	24	3.283 $\pm$ 4.240	—	a
2.5 KIN	6	0.141 $\pm$ 0.345	.017	b
5 KIN	6	0.041 $\pm$ 0.099	.012	b
10 KIN	6	0.032 $\pm$ 0.078	.012	b
15 KIN	6	0.000 $\pm$ 0.000	.011	b
20 KIN	6	0.000 $\pm$ 0.000	.011	b
Pooled control	24	3.283 $\pm$ 4.240	—	a

Table S5: Effects of plant growth regulator treatments on total mesembrine-type alkaloid contents of *Mesembryanthemum tortuosum* samples

Treatment	n	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	3	3.536 $\pm$ 0.108	.012	b
5 IBA	3	2.613 $\pm$ 0.105	.002	c
10 IBA	3	4.995 $\pm$ 0.180	.635	a
15 IBA	3	3.578 $\pm$ 0.043	.014	b
20 IBA	3	3.626 $\pm$ 0.143	.014	b
Pooled control	12	5.564 $\pm$ 0.842	—	a
2.5 NAA	3	1.330 $\pm$ 0.110	<.001	b
5 NAA	3	0.590 $\pm$ 0.156	<.001	c
10 NAA	3	0.685 $\pm$ 0.060	<.001	c
15 NAA	3	0.730 $\pm$ 0.030	<.001	c
20 NAA	3	0.670 $\pm$ 0.098	<.001	c
Pooled control	12	5.564 $\pm$ 0.842	—	a
2.5 BAP	3	4.545 $\pm$ 0.056	.173	a
5 BAP	3	4.225 $\pm$ 0.049	.070	b
10 BAP	3	3.430 $\pm$ 0.010	.011	c
15 BAP	3	3.440 $\pm$ 0.036	.011	c
20 BAP	3	3.440 $\pm$ 0.082	.010	c
Pooled control	12	5.564 $\pm$ 0.842	—	ab
2.5 KIN	3	6.810 $\pm$ 0.691	.315	abc
5 KIN	3	7.765 $\pm$ 0.181	.007	a
10 KIN	3	5.510 $\pm$ 0.346	1.000	c
15 KIN	3	5.935 $\pm$ 0.244	.903	bc
20 KIN	3	7.360 $\pm$ 0.408	.027	ab
Pooled control	12	5.564 $\pm$ 0.842	—	c

Table S6: Effects of plant growth regulator treatments on mesembrenone concentrations of *Mesembryanthemum tortuosum* samples

Treatment	N	Mean $\pm$ SD	<i>p</i> (vs. paired control)	Group
2.5 IBA	3	3.377 $\pm$ 0.091	<.001	c
5 IBA	3	2.455 $\pm$ 0.113	<.001	d
10 IBA	3	4.665 $\pm$ 0.267	.039	b
15 IBA	3	3.295 $\pm$ 0.061	<.001	c
20 IBA	3	3.355 $\pm$ 0.090	<.001	c
Paired IBA control	3	5.885 $\pm$ 0.090	—	a
2.5 NAA	3	0.820 $\pm$ 0.144	.003	b
5 NAA	3	0.305 $\pm$ 0.018	.006	b
10 NAA	3	0.290 $\pm$ 0.031	.006	b
15 NAA	3	0.280 $\pm$ 0.043	.006	b
20 NAA	3	0.235 $\pm$ 0.032	.006	b
Paired NAA control	3	5.133 $\pm$ 0.356	—	a
2.5 BAP	3	4.215 $\pm$ 0.250	.176	ab
5 BAP	3	3.980 $\pm$ 0.044	.145	a
10 BAP	3	3.255 $\pm$ 0.145	.055	c
15 BAP	3	3.225 $\pm$ 0.015	.066	bc
20 BAP	3	3.215 $\pm$ 0.044	.064	bc
Paired BAP control	3	5.390 $\pm$ 0.524	—	abc
2.5 KIN	3	6.640 $\pm$ 0.171	.001	b
5 KIN	3	7.560 $\pm$ 0.235	.001	a
10 KIN	3	5.356 $\pm$ 0.211	.141	de
15 KIN	3	5.780 $\pm$ 0.122	.006	cd
20 KIN	3	7.160 $\pm$ 0.393	.020	abc
Paired KIN control	3	4.835 $\pm$ 0.143	—	e

Table S7: Effects of plant growth regulator treatments on mesembranol concentrations of *Mesembryanthemum tortuosum* samples

Treatment	n	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	3	0.060 $\pm$ 0.005	.138	b
5 IBA	3	0.075 $\pm$ 0.011	.774	b
10 IBA	3	0.180 $\pm$ 0.049	.262	ab
15 IBA	3	0.150 $\pm$ 0.006	.004	a
20 IBA	3	0.140 $\pm$ 0.008	.012	a
Pooled control	12	0.089 $\pm$ 0.022	—	b
2.5 NAA	3	0.165 $\pm$ 0.015	.008	a
5 NAA	3	0.060 $\pm$ 0.015	.326	bc
10 NAA	3	0.045 $\pm$ 0.006	.025	c
15 NAA	3	0.045 $\pm$ 0.020	.174	bc
20 NAA	3	0.120 $\pm$ 0.027	.568	abc
Pooled control	12	0.089 $\pm$ 0.022	—	b
2.5 BAP	3	0.110 $\pm$ 0.017	.632	a
5 BAP	3	0.090 $\pm$ 0.024	1.000	a
10 BAP	3	0.065 $\pm$ 0.014	.448	a
15 BAP	3	0.060 $\pm$ 0.010	.189	a
20 BAP	3	0.070 $\pm$ 0.015	.666	a
Pooled control	12	0.089 $\pm$ 0.022	—	a
2.5 KIN	3	0.095 $\pm$ 0.010	.988	a
5 KIN	3	0.110 $\pm$ 0.030	.862	a
10 KIN	3	0.070 $\pm$ 0.026	.877	a
15 KIN	3	0.070 $\pm$ 0.027	.881	a
20 KIN	3	0.080 $\pm$ 0.004	.920	a
Pooled control	12	0.089 $\pm$ 0.022	—	a

Table S8: Effects of plant growth regulator treatments on mesembrenol concentrations of *Mesembryanthemum tortuosum* samples

Treatment	n	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	3	0.065 $\pm$ 0.010	.634	ab
5 IBA	3	0.040 $\pm$ 0.005	.967	b
10 IBA	3	0.110 $\pm$ 0.033	.245	ab
15 IBA	3	0.096 $\pm$ 0.009	.023	a
20 IBA	3	0.070 $\pm$ 0.009	.396	ab
Pooled control	12	0.048 $\pm$ 0.024	—	b
2.5 NAA	3	0.332 $\pm$ 0.025	.001	a
5 NAA	3	0.201 $\pm$ 0.020	.001	b
10 NAA	3	0.320 $\pm$ 0.028	.001	a
15 NAA	3	0.375 $\pm$ 0.020	<.001	a
20 NAA	3	0.295 $\pm$ 0.036	.009	ab
Pooled control	12	0.048 $\pm$ 0.024	—	c
2.5 BAP	3	0.145 $\pm$ 0.018	.005	a
5 BAP	3	0.080 $\pm$ 0.008	.124	ab
10 BAP	3	0.065 $\pm$ 0.004	.546	b
15 BAP	3	0.085 $\pm$ 0.007	.069	ab
20 BAP	3	0.100 $\pm$ 0.006	.015	a
Pooled control	12	0.048 $\pm$ 0.024	—	b
2.5 KIN	3	0.030 $\pm$ 0.005	.570	a
5 KIN	3	0.050 $\pm$ 0.009	1.000	a
10 KIN	3	0.035 $\pm$ 0.009	.847	a
15 KIN	3	0.035 $\pm$ 0.002	.780	a
20 KIN	3	0.050 $\pm$ 0.006	1.000	a
Pooled control	12	0.048 $\pm$ 0.024	—	a

Table S9: Effects of plant growth regulator treatments on mesembrine concentrations of *Mesembryanthemum tortuosum* samples

Treatment	N	Mean $\pm$ SD	<i>p</i> (vs. pooled control)	Group
2.5 IBA	3	0.045 $\pm$ 0.007	.040	b
5 IBA	3	0.040 $\pm$ 0.010	.086	ab
10 IBA	3	0.050 $\pm$ 0.010	.292	ab
15 IBA	3	0.035 $\pm$ 0.010	.054	ab
20 IBA	3	0.055 $\pm$ 0.013	.665	ab
Pooled control	12	0.068 $\pm$ 0.007	—	a
2.5 NAA	3	0.015 $\pm$ 0.006	.001	b
5 NAA	3	0.027 $\pm$ 0.005	<.001	b
10 NAA	3	0.030 $\pm$ 0.006	.002	b
15 NAA	3	0.030 $\pm$ 0.006	.002	b
20 NAA	3	0.020 $\pm$ 0.016	.095	ab
Pooled control	12	0.068 $\pm$ 0.007	—	a
2.5 BAP	3	0.075 $\pm$ 0.009	.829	ab
5 BAP	3	0.075 $\pm$ 0.006	.599	a
10 BAP	3	0.045 $\pm$ 0.003	.003	b
15 BAP	3	0.070 $\pm$ 0.005	.989	a
20 BAP	3	0.055 $\pm$ 0.011	.565	ab
Pooled control	12	0.068 $\pm$ 0.007	—	a
2.5 KIN	3	0.045 $\pm$ 0.010	.149	ab
5 KIN	3	0.045 $\pm$ 0.005	.015	b
10 KIN	3	0.050 $\pm$ 0.004	.026	b
15 KIN	3	0.050 $\pm$ 0.007	.118	ab
20 KIN	3	0.070 $\pm$ 0.014	.999	ab
Pooled control	12	0.068 $\pm$ 0.007	—	a