

Table 1 Suppl. Evaluation of three methods to induce polyploidization using antimitotic agents in *Lolium multiflorum* (*Lm*), *Lolium perenne* (*Lp*), *Festuca pratensis* (*Fp*), and *Festulolium* (*Lm* × *Fp*). In the first method (method 1 - rooted tiller dipping in greenhouse), *Lm* tillers obtained from plants grown on soil in the greenhouse were treated with three antimitotic agents at different concentrations. During the second method (method 2 - antimitotic agent supplemented to *in vitro* culture medium), different concentrations of the antimitotic agent supplemented to the culture medium were tested on smaller explants of *Lp*, *Lm*, *Fp*, and *Lm* × *Fp*. In the third method (method 3 - shock pretreatment with antimitotic agent), small explants of *Lp*, *Lm*, *Fp*, and *Lm* × *Fp* were treated for a short period of time with a high dosage of colchicine followed by multiplication on culture medium with or without colchicine. ‘Explants’ indicate the number of explants subjected to the polyploidization method, ‘survival rate’ indicates the number of explants that survived the treatment after four weeks and were sufficiently grown to be measured by flow cytometry (FCM) (also referred to as plantlets); ‘mixo’ and ‘tetra’ indicate the number of plantlets confirmed as mixoploid and tetraploid, respectively, by the initial measurement by FCM, with, indicated between brackets, the percentage of mixoploids and tetraploids calculated on the number of surviving plantlets (conc. - concentration).

Method	Explant type	Antimitotic agent pretreatment			growth		Species (genotypes)	Explants	Survival rate (%)	Polyploidization efficiency	
		type	conc.	duration	type	conc.				mixo (%)	tetra (%)
1) rooted tiller dipping in greenhouse	tillers (1-2 cm above and below meristem)	trifluralin	746 μM (0.02%)	4 h	potting soil	<i>Lm</i> (3)	13	0 (0%)			
			(0.25 g L ⁻¹)								
			1 491 μM (0.04%) (0.5 g L ⁻¹)				35	0 (0%)			
				2 983 μM (0.07%) (1 g L ⁻¹)				39	0 (0%)		
		oryzalin	722 μM (0.02%) (0.25 g L ⁻¹)	4 h	potting soil	<i>Lm</i> (3)	18	0 (0%)			
			1444 μM (0.04%) (0.5 g L ⁻¹)				35	0 (0%)			
				2 887 μM (0.08%) (1 g L ⁻¹)				34	0 (0%)		
		colchicine	1 878 μM (0.06%) (0.75 g L ⁻¹)	4 h	potting soil	<i>Lm</i> (3)	11	0 (0%)			
3 755 μM (0.12%) (1.5 g L ⁻¹)	36		0 (0%)								
		5 007 μM (0.15%) (2 g L ⁻¹)				17	0 (0%)				

2) antimitotic agent supplemented to <i>in vitro</i> culture medium	meristems of 0.5 cm	no	2 h	potting soil	<i>Lm</i> (6)	7 511 µM (0.23%) (3 g L ⁻¹)	16	0 (0%)		
						188 µM (0.01%) (0.075 g L ⁻¹)	23	0 (0%)		
						376 µM (0.01%) (0.15 g L ⁻¹)	47	11 (23%)	0 (0%)	0 (0%)
						939 µM (0.03%) (0.375 g L ⁻¹)	26	0 (0%)		
						1 878 µM (0.06%) (0.75 g L ⁻¹)	63	2 (3%)	1 (50%)	0 (0%)
						3 755 µM (0.12%) (1.5 g L ⁻¹)	61	3 (5%)	2 (67%)	0 (0%)
	no	no		trifluralin	<i>Lp</i> (2)	1 µM (2.47E-05 %)	60	38 (63%)	7 (18%)	0 (0%)
						3 µM (7.4E-05%)	60	40 (67%)	10 (25%)	0 (0%)
						10 µM (0.0002%)	60	34 (57%)	8 (24%)	0 (0%)
				oryzalin	<i>Lp</i> (2)	1 µM (2.89E-05 %)	60	18 (30%)	3 (17%)	0 (0%)
						3 µM (8.66E-05%)	60	10 (17%)	2 (20%)	0 (0%)
						10 µM (0.0003%)	60	4 (7%)	0 (0%)	0 (0%)
	no	no		colchicine	<i>Lp</i> (3)	0 µM	45	18 (40%)	0 (0%)	0 (0%)
						10 µM (0.0003%)	60	41 (68%)	7 (17%)	0 (0%)
					<i>Lp</i> (4)	30 µM (0.001%)	157	107 (68%)	10 (6%)	0 (0%)
						100 µM (0.003%)	150	75 (50%)	20 (13%)	2 (1%)
						200 µM	36	0 (0%)		

3) shock pretreatment with antimitotic agent	meristems of 0.5 cm	no			colchicine	0 µM	<i>Lm</i> (4)	88	59 (67%)	10 (17%)	0 (0%)
						30 µM (0.001%)		103	96 (93%)	5 (5%)	0 (0%)
						100 µM (0.003%)		77	29 (38%)	14 (48%)	1 (3%)
						200 µM (0.006%)	<i>Lm</i> (1)	30	0 (0%)		
	meristems of 0.5 cm	no			colchicine	0 µM	<i>Fp</i> (2)	7	1 (14%)	0 (0%)	0 (0%)
						30 µM (0.001%)	<i>Fp</i> (4)	44	27 (61%)	1 (4%)	0 (0%)
						100 µM (0.003%)		35	23 (66%)	6 (26%)	0 (0%)
		no			colchicine	100 µM (0.003%)	<i>Lm</i> × <i>Fp</i> (8)	155	30 (19%)	18 (60%)	2 (7%)
	meristems of 0.5 cm	colchicine	500 µM (0.02%)	24 h	/	/	<i>Lp</i> (2)	30	10 (33%)	3 (30%)	0 (0%)
			1 000 µM (0.03%)	24 h				30	19 (63%)	15 (79%)	0 (0%)
			2 000 µM (0.06%)	24 h	/	/	<i>Lp</i> (4)	90	26 (29%)	5 (19%)	1 (4%)
							<i>Lm</i> (4)	81	58 (72%)	23 (40%)	1 (2%)
							<i>Fp</i> (4)	41	19 (46%)	11 (58%)	0 (0%)
							<i>Lm</i> × <i>Fp</i> (8)	81	64 (79%)	28 (44%)	3 (5%)
		colchicine	2 000 µM (0.06%)	24 h	colchicine	30 µM (0.001%)	<i>Lp</i> (4)	79	29 (37%)	4 (14%)	3 (10%)
						100 µM (0.003%)		60	5 (8%)	0 (0%)	1 (20%)
	meristems of 0.5 cm	colchicine	2 000 µM (0.06%)	24 h	colchicine	30 µM (0.001%)	<i>Lm</i> (4)	125	79 (63%)	41 (52%)	4 (5%)
						100 µM (0.003%)		89	11 (12%)	7 (64%)	2 (18%)
		colchicine	2 000 µM (0.06%)	24 h	colchicine	30 µM (0.001%)	<i>Fp</i> (4)	68	28 (41%)	8 (29%)	1 (4%)
						100 µM (0.003%)		34	4 (12%)	1 (25%)	0 (0%)
	meristems of 0.5 cm	colchicine	2 000 µM (0.06%)	24 h	colchicine	30 µM (0.001%)	<i>Lm</i> × <i>Fp</i> (8)	121	91 (75%)	59 (65%)	0 (0%)

100 µM (0.003%)	202	19 (9%)	16 (84%)	0 (0%)
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Table 2 Suppl. Overview of the concentrations of antimitotic agents used in different notations.

Antimitotic	conc. [μM]	conc. [g L^{-1}]	volume%
trifluralin	1	0.0003	0.0000
	3	0.0010	0.0001
	10	0.0034	0.0002
	746	0.2500	0.0184
	1491	0.5000	0.0368
	2983	1.0000	0.0735
oryzalin	1	0.0003	0.0000
	3	0.0010	0.0001
	10	0.0035	0.0003
	722	0.2500	0.0208
	1444	0.5000	0.0417
	2887	1.0000	0.0833
colchicine	10	0.0040	0.0003
	30	0.0120	0.0009
	100	0.0399	0.0031
	188	0.0750	0.0058
	200	0.0800	0.0061
	376	0.1500	0.0116
	500	0.1997	0.0154
	939	0.3750	0.0289
	1000	0.3994	0.0307
	1878	0.7500	0.0577
	2000	0.7989	0.0615
	3755	1.5000	0.1154
	5007	2.0000	0.1538
	7511	3.0000	0.2308