

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).

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

| | | | | | | | | | | | | | | |
|--|------------------------|------------|--------------------------|------|---|---|------------|----------------------|----------------------------------|-----|----------|----------|---------|---------|
| 3) shock pretreatment with antimitotic agent | meristems of 0.5 cm | colchicine | 500 μ M (0.02%) | 24 h | / | / | colchicine | 0 μ M | <i>Lm</i> (4) | 88 | 59 (67%) | 10 (17%) | 0 (0%) | |
| | | | | | | | | 30 μ M (0.001%) | | 103 | 96 (93%) | 5 (5%) | 0 (0%) | |
| | | | | | | | | 100 μ M (0.003%) | | 77 | 29 (38%) | 14 (48%) | 1 (3%) | |
| | | | | | | | | 200 μ M (0.006%) | <i>Lm</i> (1) | 30 | 0 (0%) | | | |
| | meristems of 0.5 cm | colchicine | 1 000 μ M (0.03%) | 24 h | / | / | colchicine | 0 μ M | <i>Fp</i> (2) | 7 | 1 (14%) | 0 (0%) | 0 (0%) | |
| | | | | | | | | 30 μ M (0.001%) | <i>Fp</i> (4) | 44 | 27 (61%) | 1 (4%) | 0 (0%) | |
| | | | | | | | | 100 μ M (0.003%) | | 35 | 23 (66%) | 6 (26%) | 0 (0%) | |
| | meristems of 0.5 cm | colchicine | 2 000 μ M (0.06%) | 24 h | / | / | colchicine | 100 μ M (0.003%) | <i>Lm</i> \times <i>Fp</i> (8) | 155 | 30 (19%) | 18 (60%) | 2 (7%) | |
| | | | | | | | | | <i>Lp</i> (2) | 30 | 10 (33%) | 3 (30%) | 0 (0%) | |
| | | | | | | | | | | 30 | 19 (63%) | 15 (79%) | 0 (0%) | |
| | | | | | | | | | <i>Lp</i> (4) | 90 | 26 (29%) | 5 (19%) | 1 (4%) | |
| | | | | | | | | | <i>Lm</i> (4) | 81 | 58 (72%) | 23 (40%) | 1 (2%) | |
| | meristems of 0.5 cm | colchicine | 2 000 μ M (0.06%) | 24 h | / | / | colchicine | | <i>Fp</i> (4) | 41 | 19 (46%) | 11 (58%) | 0 (0%) | |
| | | | | | | | | | <i>Lm</i> \times <i>Fp</i> (8) | 81 | 64 (79%) | 28 (44%) | 3 (5%) | |
| | | | | | | | | | <i>Lp</i> (4) | 79 | 29 (37%) | 4 (14%) | 3 (10%) | |
| | | | | | | | | | | 60 | 5 (8%) | 0 (0%) | 1 (20%) | |
| | | | | | | | | | | | | | | |
| | colchicine | colchicine | 2 000 μ M (0.06%) | 24 h | / | / | colchicine | 30 μ M (0.001%) | <i>Lm</i> (4) | 125 | 79 (63%) | 41 (52%) | 4 (5%) | |
| | | | | | | | | | 100 μ M (0.003%) | | 89 | 11 (12%) | 7 (64%) | 2 (18%) |
| | | | | | | | | | | | | | | |
| | colchicine | colchicine | 2 000 μ M (0.06%) | 24 h | / | / | colchicine | 30 μ M (0.001%) | <i>Fp</i> (4) | 68 | 28 (41%) | 8 (29%) | 1 (4%) | |
| | | | | | | | | | 100 μ M (0.003%) | | 34 | 4 (12%) | 1 (25%) | 0 (0%) |
| | colchicine | colchicine | 2 000 μ M (0.06%) | 24 h | / | / | colchicine | 30 μ M (0.001%) | <i>Lm</i> \times <i>Fp</i> (8) | 121 | 91 (75%) | 59 (65%) | 0 (0%) | |

100 μ M (0.003%)

202

19 (9%)

16 (84%)

0 (0%)

Table 2 Suppl. Overview of the concentrations of antimitotic agents used in different notations.

| Antimitotic | conc. [μ M] | 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 |