Table 1 Suppl. Nine traits phenotyped in amphiploid *Festulolium* BC$_2$ mapping populations.

| Trait | Investigated year/date | | | | Notes |
|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | |
| Vigor index after planting | 5 Nov | | | | On a scale of 1 (poor growth) to 9 (high growth), where 5 indicates the median of each genotype |
| Winter hardiness index | | 24 Apr | 6 Apr | 14 Apr | On a scale of 1 (no sprouting) to 9 (very vigorous) according to the vigor of sprouting after snowmelt, where 5 indicates moderate sprouting across the stub but with some injured parts (Tamura *et al*. 2017) |
| Heading date | | after 1 May | after 1 May | | Date when the tips of ≥3 inflorescences appeared from the flag leaf sheath after 1 May |
| Dry matter at 5th cut [g] | | 15 Oct | 16 Oct | | In the last cutting before winter in each year, measured after drying at 70 °C for 72 h |
| Plant growth habit | | 23 May | 26 May | | On a scale of 1 to 9, where 3 is semi-erect, 5 is midway, and 7 is semi-prostrate, following the UPOV guideline for Festulolium, 2008 |
| Inflorescence length [cm] | | 17 Jun | 21 Jun | | Means of 3 inflorescences per clone (not investigated in FLBC2C in 2014) |
| Number of spikelets [inflorescence$^{-1}$] | | 17 Jun | 21 Jun | | Means of 3 inflorescences per clone (not investigated in FLBC2C in 2014) |
| Leaf width [mm] | | 3 Jun | | | Means of 4 leaves 1 lower than the flag leaves per clone |
| | | 6 Oct | 1 Oct | | Means of 4 randomly selected intact leaves per clone |
| Stubble width [cm] | | 17 Jul | 1 Oct | | Parallel to the 80-cm interval between clones |

Table 2 Suppl. *Lolium*/*Festuca* intron-flanking markers used in this study; CAPS - cleavage amplified polymorphic sequence, indel - insertion/deletion, A - FLBC2A, C - FLBC2C.

| Marker name (corresponding rice *TIGR* public locus) | (corresponding rice *RAP-DB* public locus) | Reference | Marker type | Restriction enzyme | Mapped population | Linkage group |
|---|---|---|---|---|---|---|
| Os01g01010 | Os01g0100100 | Tamura *et al*. 2009 | CAPS | *Xsp*I | | |
| Os01g01080 | Os01g0100900 | Tamura *et al*. 2009 | indel | | A | LG6 |
| Os01g02880 | Os01g0118000 | Tamura *et al*. 2009 | CAPS | *Afa*I | C | LG3 |
| Os01g08190 | Os01g0177100 | Tamura *et al*. 2012 | indel | | C | LG3 |
| Os01g25370 | Os01g0355900 | Tamura *et al*. 2009 | CAPS | *Afa*I | C | LG3 |
| Os01g34480 | Os01g0528800 | Tamura *et al*. 2009 | indel | | C | LG3 |
| Os01g35184 | Os01g0536000 | Tamura *et al*. 2012 | indel | | C | LG3 |
| Os01g36930 | Os01g0550100 | Tamura *et al*. 2009 | indel | | C | LG3 |
| Os01g43250 | Os01g0620100 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os01g49330 | Os01g0687500 | Tamura *et al*. 2009 | CAPS | *Taq*I | C | LG3 |
| Os01g53250 | Os01g0733600 | Tamura *et al*. 2009 | indel | | C | LG3 |
| Os01g55540 | Os01g0760600 | Tamura *et al*. 2009 | CAPS | *Dde*I | C | LG3 |
| Os01g57066 | Os01g0778700 | Tamura *et al*. 2012 | indel | | C | LG3 |
| Os01g60650 | Os01g0821700 | Tamura *et al*. 2009 | CAPS | *Mse*I | | |
| Os01g64720 | Os01g0867200 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os01g65660 | Os01g0878400 | Tamura *et al*. 2012 | indel | | | |
| Os01g65660 | Os01g0878400 | Tamura *et al*. 2012 | indel | | C | LG3 |
| Os01g66240 | Os01g0885600 | Tamura *et al*. 2009 | CAPS | *Sac*II | | |
| Os01g68710 | Os01g0915800 | Tamura *et al*. 2009 | indel | | | |
| Os01g73790 | Os01g0969100 | Tamura *et al*. 2012 | indel | | | |
| Os02g03260 | Os02g0125100 | Tamura *et al*. 2012 | indel | | A | LG6 |
| Os02g03890 | Os02g0131700 | Tamura *et al*. 2009 | CAPS | *Hae*III | | |
| Os02g14730 | Os02g0244300 | Tamura *et al*. 2012 | indel | | A | LG6 |
| Os02g17870 | Os02g0280000 | Tamura *et al*. 2009 | CAPS | *Taq*I | A | LG6 |
| Os02g29530 | Os02g0498700 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os02g30800 | Os02g0511900 | Tamura *et al*. 2009 | CAPS | *Afa*I | C | LG3 |
| Os02g36740 | Os02g0577100 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os02g43350 | Os02g0649700 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG6 |
| Os02g45650 | Os02g0680400 | Tamura *et al*. 2012 | indel | | A | LG6 |
| Os02g47310 | Os02g0701600 | Tamura *et al*. 2009 | CAPS | *Afa*I | A | LG5 |
| Os02g50360 | Os02g0736500 | Tamura *et al*. 2012 | indel | | | |
| Os02g57160 | Os02g0816600 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG6 |
| Os02g58650 | Os02g0833400 | Tamura *et al*. 2009 | CAPS | *Eco*RI | | |
| Os03g07300 | Os03g0169100 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os03g23950 | Os03g0355600 | Tamura *et al*. 2009 | indel | | AC | LG4 |
| Os03g27320 | Os03g0390700 | Tamura *et al*. 2012 | indel | | AC | LG4 |
| Os03g29950 | Os03g0412800 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os03g32490 | Os03g0439700 | Tamura *et al*. 2012 | indel | | AC | LG4 |
| Os03g36750 | Os03g0565200 | Tamura *et al*. 2009 | CAPS | *Afa*I | A | LG7 |
| Os03g38980 | Os03g0586800 | Tamura *et al*. 2009 | indel | | AC | LG4 |
| Os03g43760 | Os03g0638800 | Tamura *et al*. 2009 | CAPS | *Sau*3AI | A | LG4 |
| Os03g46410 | Os03g0666700 | Tamura *et al*. 2012 | indel | | AC | LG4 |
| Os03g50480 | Os03g0712700 | Tamura *et al*. 2009 | indel | | AC | LG4 |
| Os03g56300 | Os03g0774200 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG4 |
| Os03g60090 | Os03g0815200 | Tamura *et al*. 2009 | CAPS | *Afa*I | AC | LG4 |
| Os03g64210 | Os03g0859600 | Tamura *et al*. 2009 | indel | | | |
| Os04g02000 | Os04g0110600 | Tamura *et al*. 2009 | indel | | | |
| Os04g06790 | Os04g0151900 | Tamura *et al*. 2009 | indel | | AC | LG4 |
| Os04g11880 | Os04g0195000 | Tamura *et al*. 2009 | indel | | C | LG2 |
| Os04g16680 | Os04g0234600 | Tamura *et al*. 2009 | indel | | C | LG3 |
| Os04g23890 | Os04g0304200 | Tamura *et al*. 2012 | indel | | | |
| Os04g27860 | Os04g0346100 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG2 |
| Os04g30420 | Os04g0372700 | Tamura *et al*. 2009 | indel | | | |
| Os04g31210 | Os04g0381100 | Tamura *et al*. 2012 | indel | | | |
| Os04g32950 | Os04g0402100 | Tamura *et al*. 2009 | CAPS | *Alu*I | A | LG6 |
| Os04g37619 | Os04g0448900 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os04g43220 | Os04g0511600 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |

| Marker name (corresponding rice *TIGR* public locus) | (corresponding rice *RAP-DB* public locus) | Reference | Marker type | Restriction enzyme | Mapped population | Linkage group |
|---|---|---|---|---|---|---|
| Os04g45490 | Os04g0538100 | Tamura *et al*. 2012 | indel | | AC | LG2 |
| Os04g46620 | Os04g0551800 | Tamura *et al*. 2009 | indel | | | |
| Os04g48230 | Os04g0570800 | Tamura *et al*. 2009 | CAPS | *Eco*RI | AC | LG2 |
| Os04g54410 | Os04g0636600 | Tamura *et al*. 2009 | indel | | C | LG2 |
| Os05g01750 | Os05g0108000 | Tamura *et al*. 2012 | indel | | A | LG1 |
| Os05g01970 | Os05g0110300 | Tamura *et al*. 2012 | indel | | | |
| Os05g04190 | Os05g0132500 | Tamura *et al*. 2009 | CAPS | *Afa*I | C | LG1 |
| Os05g06330 | Os05g0155300 | Tamura *et al*. 2009 | CAPS | *Taq*I | | |
| Os05g11850 | Os05g0209100 | Tamura *et al*. 2009 | indel | | | |
| Os05g13780 | Os05g0224800 | Tamura *et al*. 2009 | CAPS | *Afa*I | A | LG4 |
| Os05g14170 | Os05g0230600 | Tamura *et al*. 2009 | CAPS | *Mse*I | | |
| Os05g19630 | Os05g0277300 | Tamura *et al*. 2009 | indel | | | |
| Os05g24550 | Os05g0310500 | Tamura *et al*. 2009 | indel | | | |
| Os05g28280 | Os05g0350500 | Tamura *et al*. 2009 | CAPS | *Hae*III | | |
| Os05g32140 | Os05g0387200 | Tamura *et al*. 2009 | indel | | AC | LG1 |
| Os05g38330 | Os05g0457700 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os05g43360 | Os05g0509200 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os05g48510 | Os05g0558900 | Tamura *et al*. 2009 | indel | | AC | LG5 |
| Os05g51700 | Os05g0595400 | Tamura *et al*. 2009 | indel | | A | LG1 |
| Os05g51754 | Os05g0596200 | Tamura *et al*. 2012 | indel | | AC | LG1 |
| Os06g04280 | Os06g0133900 | Tamura *et al*. 2009 | CAPS | *Dde*I | AC | LG7 |
| Os06g05180 | Os06g0143900 | Tamura *et al*. 2012 | indel | | | |
| Os06g11040 | Os06g0213400 | Tamura *et al*. 2009 | indel | | AC | LG7 |
| Os06g15420 | Os06g0265000 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os06g16350 | Os06g0274800 | Tamura *et al*. 2012 | indel | | AC | LG7 |
| Os06g21560 | Os06g0320100 | Tamura *et al*. 2009 | CAPS | *Alu*I | AC | LG7 |
| Os06g27760 | Os06g0472000 | Tamura *et al*. 2009 | indel | | AC | LG7 |
| Os06g41790 | Os06g0622900 | Tamura *et al*. 2009 | indel | | AC | LG7 |
| Os06g44890 | Os06g0659300 | Tamura *et al*. 2012 | indel | | | |
| Os06g49500 | Os06g0708700 | Tamura *et al*. 2012 | indel | | A | LG7 |
| Os06g50110 | Os06g0715000 | Tamura *et al*. 2009 | indel | | AC | LG5 |
| Os06g51029 | Os06g0725900 | Tamura *et al*. 2009 | indel | | | |
| Os07g01480 | Os07g0105600 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG2 |
| Os07g01760 | Os07g0108300 | Tamura *et al*. 2012 | indel | | AC | LG2 |
| Os07g01930 | Os07g0110300 | Tamura *et al*. 2009 | CAPS | *Ssp*I | C | LG7 |
| Os07g04840 | Os07g0141400 | Tamura *et al*. 2009 | indel | | | |
| Os07g19030 | Os07g0290800 | Tamura *et al*. 2009 | CAPS | *Mse*I | | |
| Os07g22350 | Os07g0406300 | Tamura *et al*. 2009 | indel | | C | LG2 |
| Os07g25430 | Os07g0435300 | Tamura *et al*. 2009 | indel | | AC | LG2 |
| Os07g30774 | Os07g0490200 | Tamura *et al*. 2009 | CAPS | *Mse*I | A | LG2 |
| Os07g30840 | Os07g0490800 | Tamura *et al*. 2009 | CAPS | *Taq*I | AC | LG2 |
| Os07g38620 | Os07g0573800 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os07g39630 | Os07g0585100 | Tamura *et al*. 2009 | CAPS | *Afa*I | A | LG2 |
| Os07g48920 | Os07g0688800 | Tamura *et al*. 2009 | CAPS | *Mse*I | | |
| Os07g49300 | Os07g0693700 | Tamura *et al*. 2012 | indel | | | |
| Os07g49320 | Os07g0693900 | Tamura *et al*. 2009 | indel | | C | LG2 |
| Os08g01350 | Os08g0104100 | Tamura *et al*. 2009 | CAPS | *Dde*I | C | LG7 |
| Os08g03390 | Os08g0127700 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os08g09940 | Os08g0199300 | Tamura *et al*. 2009 | CAPS | *Taq*I | AC | LG7 |
| Os08g15080 | Os08g0249400 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os08g23320 | Os08g0322600 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os08g27010 | Os08g0359000 | Tamura *et al*. 2009 | CAPS | *Taq*I | | |
| Os08g31810 | Os08g0412200 | Tamura *et al*. 2009 | CAPS | *Taq*I | | |
| Os08g33630 | Os08g0433100 | Tamura *et al*. 2009 | CAPS | *Taq*I | AC | LG7 |
| Os08g36774 | Os08g0471900 | Tamura *et al*. 2009 | indel | | C | LG5 |
| Os08g38720 | Os08g0496000 | Tamura *et al*. 2012 | indel | | AC | LG7 |
| Os08g41830 | Os08g0530400 | Tamura *et al*. 2009 | CAPS | *Taq*I | AC | LG7 |
| Os08g41910 | Os08g0531300 | Tamura *et al*. 2012 | indel | | | |
| Os08g44930 | Os08g0563300 | Tamura *et al*. 2009 | CAPS | *Alu*I | C | LG7 |
| Os09g03610 | Os09g0123200 | Tamura *et al*. 2009 | indel | | AC | LG5 |
| Os09g06499 | Os09g0240500 | Tamura *et al*. 2012 | indel | | | |

| Marker name (corresponding rice *TIGR* public locus) | (corresponding rice *RAP-DB* public locus) | Reference | Marker type | Restriction enzyme | Mapped population | Linkage group |
|---|---|---|---|---|---|---|
| Os09g08660 | Os09g0261300 | Tamura *et al*. 2009 | indel | | | |
| Os09g15820 | Os09g0327400 | Tamura *et al*. 2009 | CAPS | *Ssp*I | | |
| Os09g20640 | Os09g0372800 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os09g20880 | Os09g0375600 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os09g23650 | Os09g0401300 | Tamura *et al*. 2012 | indel | | AC | LG5 |
| Os09g24650 | Os09g0413000 | Tamura *et al*. 2012 | indel | | AC | LG5 |
| Os09g26730 | Os09g0438700 | Tamura *et al*. 2009 | indel | | A | LG5 |
| Os09g30466 | Os09g0482680 | Tamura *et al*. 2009 | indel | | AC | LG5 |
| Os09g34970 | Os09g0521500 | Tamura *et al*. 2009 | indel | | AC | LG5 |
| Os10g02980 | Os10g0119300 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os10g10244 | Os10g0182000 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os10g11140 | Os10g0189100 | Tamura *et al*. 2009 | CAPS | *Taq*I | C | LG1 |
| Os10g17280 | Os10g0320400 | Tamura *et al*. 2009 | CAPS | *Sau*3AI | | |
| Os10g36470 | Os10g0508400 | Tamura *et al*. 2012 | indel | | AC | LG1 |
| Os10g39930 | Os10g0546600 | Tamura *et al*. 2009 | CAPS | *Taq*I | | |
| Os11g02580 | Os11g0118000 | Tamura *et al*. 2009 | indel | | A | LG4 |
| Os11g09280 | Os11g0199200 | Tamura *et al*. 2009 | indel | | AC | LG4 |
| Os11g20689 | Os11g0311300 | Tamura *et al*. 2009 | indel | | | |
| Os11g29380 | Os11g0484300 | Tamura *et al*. 2009 | CAPS | *Afa*I | A | LG4 |
| Os11g34130 | Os11g0544000 | Tamura *et al*. 2009 | CAPS | *Taq*I | | |
| Os11g36440 | Os11g0572700 | Tamura *et al*. 2012 | indel | | | |
| Os11g38020 | Os11g0592700 | Tamura *et al*. 2009 | CAPS | *Taq*I | AC | LG4 |
| Os11g43900 | Os11g0660500 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os11g47710 | Os11g0703400 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os11g48040 | Os11g0707800 | Tamura *et al*. 2009 | CAPS | *Taq*I | A | LG4 |
| Os12g02390 | Os12g0116000 | Tamura *et al*. 2009 | CAPS | *Mse*I | | |
| Os12g08810 | Os12g0190000 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os12g13320 | Os12g0235800 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os12g23180 | Os12g0420200 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |
| Os12g27830 | Os12g0464400 | Tamura *et al*. 2009 | CAPS | *Dde*I | A | LG4 |
| Os12g40550 | Os12g0597500 | Tamura *et al*. 2009 | CAPS | *Afa*I | | |
| Os12g42980 | Os12g0625000 | Tamura *et al*. 2009 | CAPS | *Dde*I | | |

Table 3 Suppl. *Festuca arundinacea* expressed sequence tag simple sequence repeat markers (Saha *et al*. 2004) used in this study; A - FLBC2A, C - FLBC2C.

| Marker name | Mapped population | Linkage group |
| --- | --- | --- |
| NFA001 | | |
| NFA002 | | |
| NFA005 | | |
| NFA008 | | |
| NFA011 | | |
| NFA013 | | |
| NFA014 | | |
| NFA015 | | |
| NFA016 | | |
| NFA017 | | |
| NFA018 | | |
| NFA020 | | |
| NFA021 | | |
| NFA026 | | |
| NFA029 | | |
| NFA030 | C | LG2 |
| NFA031 | | |
| NFA033 | | |
| NFA034 | | |
| NFA035 | | |
| NFA037 | | |
| NFA039 | | |
| NFA040 | | |
| NFA043 | | |
| NFA045 | | |
| NFA046 | | |
| NFA047 | | |
| NFA048 | A | LG6 |
| NFA051 | | |
| NFA057 | | |
| NFA059 | AC | LG5 |
| NFA060 | | |
| NFA062 | | |
| NFA065 | | |
| NFA067 | | |
| NFA068 | | |
| NFA070 | | |
| NFA071 | AC | LG4 |
| NFA072 | | |
| NFA073 | AC | LG1 |
| NFA074 | | |
| NFA075 | AC | LG1 |
| NFA077 | | |
| NFA079 | | |
| NFA082 | | |
| NFA084 | | |
| NFA086 | | |
| NFA087 | | |
| NFA088 | A | LG1 |
| NFA089 | | |
| NFA090 | | |
| NFA092 | AC | LG2 |
| NFA094 | | |
| NFA096 | | |
| NFA097 | AC | LG2 |
| NFA098 | | |
| NFA101 | | |
| NFA103 | | |
| NFA104 | C | LG4 |

| Marker name | Mapped population | Linkage group |
| --- | --- | --- |
| NFA105 | | |
| NFA107 | | |
| NFA108 | | |
| NFA109 | | |
| NFA110 | | |
| NFA111 | | |
| NFA112 | A | LG1 |
| NFA113 | | |
| NFA115 | | |
| NFA116 | | |
| NFA117 | | |
| NFA119 | A | LG6 |
| NFA120 | A | LG1 |
| NFA123 | | |
| NFA126 | | |
| NFA129 | | |
| NFA130 | | |
| NFA131 | | |
| NFA134 | AC | LG1 |
| NFA135 | | |
| NFA138 | A | LG1 |
| NFA140 | AC | LG1 |
| NFA142 | | |
| NFA145 | | |
| NFA146 | | |
| NFA153 | | |
| NFA154 | | |
| NFA155 | A | LG1 |
| NFA157 | | |

Table 4 Suppl. *Lolium perenne* expressed sequence tag simple sequence repeat markers (Studer *et al*. 2010) used in this study; A - FLBC2A, C - FLBC2C.

| Marker name | Mapped population | Linkage group |
|---|---|---|
| G01_001 | | |
| G01_002 | | |
| G01_007 | | |
| G01_010 | | |
| G01_013 | | |
| G01_022 | A | LG2 |
| G01_024 | | |
| G01_025 | | |
| G01_027 | | |
| G01_031 | | |
| G01_035 | | |
| G01_037 | | |
| G01_038 | | |
| G01_039 | | |
| G01_040 | | |
| G01_043 | | |
| G01_044 | | |
| G01_045 | | |
| G01_046 | | |
| G01_047 | | |
| G01_048 | | |
| G01_054 | | |
| G01_063 | | |
| G01_073 | AC | LG7 |
| G01_080 | | |
| G01_081 | | |
| G01_086 | | |
| G01_090 | | |
| G01_094 | | |
| G01_095 | C | LG5 |
| G01_097 | | |
| G01_098 | | |
| G02_004 | | |
| G02_016 | | |
| G02_017 | | |
| G02_018 | | |
| G02_021 | | |
| G02_022 | | |
| G02_025 | | |
| G02_029 | | |
| G02_032 | | |
| G02_035 | | |
| G02_037 | | |
| G02_041 | | |
| G02_043 | | |
| G02_047 | | |
| G02_048 | AC | LG7 |
| G02_049 | | |
| G02_053 | | |
| G02_057 | | |
| G02_058 | | |
| G02_069 | | |
| G02_079 | | |
| G02_080 | | |
| G02_081 | | |
| G02_092 | | |
| G02_098 | | |
| G03_002 | | |
| G03_003 | | |
| G03_010 | | |
| G03_013 | AC | LG4 |

| Marker name | Mapped population | Linkage group |
| --- | --- | --- |
| G03_015 | | |
| G03_016 | | |
| G03_020 | | |
| G03_023 | | |
| G03_030 | | |
| G03_035 | | |
| G03_039 | | |
| G03_045 | | |
| G03_049 | | |
| G03_052 | | |
| G03_054 | | |
| G03_055 | | |
| G03_065 | AC | LG4 |
| G03_069 | | |
| G03_071 | | |
| G03_072 | | |
| G03_073 | | |
| G03_074 | | |
| G03_089 | | |
| G03_092 | | |
| G03_095 | | |
| G03_096 | | |
| G03_099 | | |
| G04_002 | A | LG7 |
| G04_020 | | |
| G04_024 | | |
| G04_027 | | |
| G04_028 | AC | LG7 |
| G04_034 | AC | LG4 |
| G04_040 | | |
| G04_045 | | |
| G04_048 | | |
| G04_052 | | |
| G04_054 | | |
| G04_056 | AC | LG4 |
| G04_059 | | |
| G04_061 | | |
| G04_064 | | |
| G04_065 | | |
| G04_067 | | |
| G04_072 | | |
| G04_074 | | |
| G04_081 | | |
| G04_085 | | |
| G04_098 | | |
| G04_099 | C | LG4 |
| G05_014 | | |
| G05_022 | | |
| G05_023 | C | LG3 |
| G05_024 | | |
| G05_028 | | |
| G05_030 | | |
| G05_033 | | |
| G05_035 | | |
| G05_041 | | |
| G05_044 | | |
| G05_046 | | |
| G05_050 | | |
| G05_052 | | |
| G05_056 | | |
| G05_065 | | |
| G05_068 | | |

| Marker name | Mapped population | Linkage group |
|---|---|---|
| G05_070 | | |
| G05_071 | | |
| G05_073 | | |
| G05_076 | AC | LG2 |
| G05_081 | | |
| G05_088 | | |
| G05_089 | | |
| G05_092 | | |
| G05_094 | | |
| G05_099 | | |
| G05_100 | | |
| G05_107 | | |
| G05_108 | | |
| G05_112 | | |
| G05_121 | | |
| G05_122 | | |
| G05_124 | | |
| G05_127 | AC | LG5 |
| G05_129 | | |
| G05_130 | | |
| G05_134 | | |
| G05_139 | | |
| G06_006 | | |
| G06_021 | | |
| G06_022 | | |
| G06_029 | | |
| G06_041 | | |
| G06_043 | A | LG4 |
| G06_049 | A | LG1 |
| G06_072 | A | LG7 |
| G06_078 | | |
| G06_079 | | |
| G06_088 | | |
| G06_089 | AC | LG4 |
| G06_096 | C | LG5 |
| G07_024 | | |
| G07_025 | | |
| G07_037 | | |
| G07_038 | | |
| G07_045 | A | LG5 |
| G07_056 | | |
| G07_057 | | |
| G07_058 | | |
| G07_065 | AC | LG5 |
| G07_066 | | |
| G07_071 | | |
| G07_074 | | |
| G07_075 | | |
| G07_083 | | |
| G07_088 | | |
| G07_098 | | |

Table 5 Suppl. Variation in investigated agronomic traits between two amphiploid *Festulolium* BC$_2$ mapping populations FLBC2A and FLBC2C. Lp - *Lolium perenne*, $h^2$ - broad-sense heritability; [a] - scores (1 - 9) were assigned as described in Table 1 Suppl.

| Trait | year | date | FLBC2A mean | SD | min. | max. | BC$_1$ parent | Lp parent | $h^2$ | FLBC2C mean | SD | min. | max. | BC$_1$ parent | Lp parent | $h^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vigor index after planting [a] | 2013 | 5 Nov | 5.7 | 0.8 | 2.7 | 7.7 | 5.0 | 3.0 | 47 | 5.9 | 1.1 | 2.3 | 8.0 | 3.0 | 2.3 | 63 |
| Winter hardiness index [a] | 2014 | 24 Apr | 5.3 | 1.1 | 2.0 | 7.7 | 5.0 | 2.7 | 68 | 6.3 | 0.9 | 4.0 | 8.0 | 6.0 | 2.7 | 62 |
|  | 2015 | 6 Apr | 4.2 | 0.9 | 1.3 | 6.0 | 5.0 | 3.0 | 41 | 5.2 | 1.3 | 1.0 | 7.3 | 5.0 | 3.3 | 56 |
|  | 2016 | 14 Apr | 3.6 | 1.5 | 1.0 | 7.0 | 6.7 | 2.3 | 54 | 4.1 | 1.7 | 1.0 | 8.0 | 5.0 | 3.3 | 57 |
| Heading date (days after 1 May) | 2014 | – | 34.0 | 1.6 | 30.3 | 41.0 | 33.7 | 34.3 | 83 | 34.9 | 1.8 | 32.3 | 41.7 | 37.3 | 36.7 | 73 |
|  | 2015 | – | 31.9 | 2.1 | 28.0 | 47.3 | 33.0 | 31.0 | 82 | 34.0 | 3.1 | 30.0 | 46.0 | 34.0 | 34.0 | 81 |
| Dry matter before winter [g] | 2014 | 15 Oct | 22.2 | 6.1 | 2.8 | 35.6 | 21.0 | 16.7 | 51 | 24.2 | 7.4 | 2.2 | 39.5 | 15.2 | 3.1 | 63 |
|  | 2015 | 16 Oct | 20.9 | 8.4 | 0.0 | 43.4 | 16.5 | 14.5 | 59 | 18.9 | 8.6 | 0.0 | 44.2 | 10.1 | 5.1 | 63 |
| Plant growth habitat [a] | 2014 | 23 May | 5.7 | 1.1 | 2.7 | 8.3 | 5.7 | 5.3 | 71 | 5.4 | 1.0 | 2.7 | 7.7 | 5.0 | 4.7 | 70 |
|  | 2015 | 26 May | 4.8 | 0.9 | 2.7 | 7.7 | 3.7 | 6.0 | 62 | 4.3 | 0.8 | 3.0 | 7.0 | 5.0 | 4.0 | 61 |
| Inflorescence length [cm] | 2014 | 17 Jun | 23.3 | 3.5 | 14.3 | 33.3 | 29.4 | 18.0 | 75 |  |  |  |  |  |  |  |
|  | 2015 | 21 Jun | 19.7 | 2.8 | 11.6 | 26.6 | 22.2 | 15.5 | 70 | 23.2 | 2.6 | 14.9 | 27.9 | 24.9 | 20.4 | 62 |
| Number of spikelets [inflorescence$^{-1}$] | 2014 | 17 Jun | 17.8 | 1.9 | 11.6 | 23.0 | 18.7 | 15.6 | 76 |  |  |  |  |  |  |  |
|  | 2015 | 21 Jun | 16.2 | 1.5 | 11.4 | 19.6 | 16.4 | 15.0 | 71 | 18.9 | 1.7 | 14.6 | 23.0 | 20.0 | 19.3 | 65 |
| Leaf width [mm] | 2014 | 3 Jun | 6.7 | 0.7 | 5.0 | 9.1 | 7.4 | 5.2 | 68 | 7.4 | 0.8 | 5.7 | 9.6 | 7.5 | 5.9 | 71 |
|  | 2014 | 6 Oct | 4.9 | 0.5 | 3.6 | 7.0 | 5.9 | 4.2 | 60 | 5.1 | 0.6 | 3.8 | 6.7 | 6.8 | 3.8 | 61 |
|  | 2015 | 1 Oct | 4.3 | 0.5 | 3.3 | 7.1 | 4.6 | 4.0 | 48 | 4.0 | 0.5 | 2.9 | 6.0 | 5.8 | 3.8 | 62 |
| Stubble width [cm] | 2014 | 17 Jul | 22.5 | 3.2 | 13.0 | 29.7 | 24.7 | 15.0 | 56 | 23.5 | 3.6 | 9.3 | 31.0 | 17.7 | 10.7 | 71 |
|  | 2015 | 1 Oct | 22.7 | 5.6 | 0.0 | 31.3 | 24.3 | 18.7 | 59 | 23.1 | 5.6 | 0.0 | 30.7 | 17.7 | 10.3 | 63 |

Table 6 Suppl. Parameter values of linkage maps based on the presence/absence of *Festuca pratensis*-derived alleles of two amphiploid *Festulolium* BC$_2$ mapping populations FLBC2A and FLBC2C. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ - Chi-squared goodness-of-fit test for 1:1 segregation; NS - not significant; ND - no data.

| Linkage group | FLBC2A number of markers | distance [cM] | mean interval [cM] | mean *F. pratensis*-derived allele frequency [%] | segregation distortion | FLBC2C number of markers | distance [cM] | mean interval [cM] | mean *F. pratensis*-derived allele frequency [%] | segregation distortion |
|---|---|---|---|---|---|---|---|---|---|---|
| LG1 | 19 | 74.7 | 4.2 | 58 | * | 13 | 29.4 | 2.5 | 75 | *** |
| LG2 | 19 | 31.8 | 1.8 | 48 | NS | 21 | 59.1 | 3.0 | 52 | NS |
| LG3 | ND |  |  |  |  | 24 | 30.6 | 1.3 | 48 | NS |
| LG4 | 33 | 33.0 | 1.0 | 38 | ** | 24 | 56.5 | 2.5 | 46 | NS |
| LG5 | 25 | 67.2 | 2.8 | 47 | NS | 21 | 40.3 | 1.9 | 49 | NS |
| LG6 | 17 | 52.4 | 3.3 | 51 | NS | ND |  |  |  |  |
| LG7 | 30 | 42.1 | 1.5 | 53 | NS | 23 | 40.9 | 1.9 | 48 | NS |
| Total | 143 | 301.2 | 2.2 | 48 | NS | 126 | 256.2 | 2.1 | 52 | NS |

Table 7 Suppl. Quantitative trait loci for winter hardiness and other agronomic traits in the amphiploid *Festulolium* BC[2] mapping populations FLBC2A and FLBC2C; [a] - defined as the additive effect of *Festuca pratensis* alleles, [b] - scores (1 - 9) were assigned as described in Table 1 Suppl, [c] - values of the second logarithm of odds (LOD) peak in parentheses, [d] - values of the second and third LOD peaks in parentheses.

| Traits | year | FLBC2A LG | max. LOD pos. [cM] | 1.5-LOD supp. interval [cM] | max. LOD value | LOD threshold | $R^2$ [%] | weight [a] | FLBC2C LG | max. LOD pos. [cM] | 1.5-LOD supp. interval [cM] | max. LOD value | LOD threshold | $R^2$ [%] | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vigor index after planting [b] | 2013 | 4 | 11.8 | 8.4-12.8 | 3.9 | 2.5 | 10.4 | 0.6 | 2 | 28.7 | 0.0-34.1 | 2.6 | 2.3 | 9.9 | -0.7 |
| Winter hardiness index [b] | 2014 | 4 | 11.8 | 9.7-13.8 | 2.8 | 2.3 | 7.7 | 0.7 | | | | | | | |
| | 2015 | 1 | 33.2 | 22.3-44.4 | 2.6 | 2.3 | 7.0 | 0.5 | 1 | 6.4 | 0.0-21.6 | 3.0 | 2.3 | 11.6 | 1.1 |
| | | 5 | 18.9 (36.8)[c] | 7.0-22.4 (24.9-58.3)[c] | 3.4 | | 9.0 (8.5)[c] | 0.6 (0.5)[c] | | | | | | | |
| | | 2 | 21.8 | 0.0-31.1 | 2.5 | | 6.6 | -0.5 | | | | | | | |
| | 2016 | | | | | | | | 1 | 6.4 | 0.0-12.8 | 4.2 | 2.4 | 15.8 | 1.6 |
| Heading date (days after 1 May) | 2014 | | | | | | | | 7 | 21.6 | 11.6-35.1 | 3.1 | 2.3 | 11.7 | 1.2 |
| | 2015 | 7 | 7.9 | 2.0-20.6 | 2.7 | 2.1 | 7.4 | 1.2 | | | | | | | |
| Dry matter before winter [g] | 2014 | | | | | | | | 2 | 29.1 | 3.0-33.1 | 2.8 | 2.4 | 10.8 | -4.9 |
| Plant growth habitat [b] | 2014 | | | | | | | | 4 | 35.1 | 26.6-56.5 | 2.4 | 2.3 | 9.2 | -0.6 |
| Inflorescence length [cm] | 2014 | 7 | 11.4 | 8.9-16.9 | 4.9 | 2.4 | 12.9 | 2.5 | | | | | | | |
| | 2015 | 7 | 4.6 | 0.0-18.1 | 3.5 | 2.4 | 9.7 | 1.7 | | | | | | | |
| | | 2 | 31.8 | 12.0-31.8 | 3.5 | | 9.6 | 1.7 | | | | | | | |
| No. spikelets [inflorescence$^{-1}$] | 2015 | 7 | 11.4 | 0.0-19.1 | 3.7 | 2.3 | 10.2 | 0.9 | | | | | | | |
| Leaf width [mm] | 2014 (spring) | 7 | 15.9 (25.6, 21.6)[d] | 9.9-25.6 (24.7-25.6, 20.6-22.7)[d] | 5.4 (4.6, 3.9)[d] | 2.5 | 14.0 (12.1, 10.4)[d] | 0.5 (0.5, 0.5)[d] | | | | | | | |
| | 2014 (autumn) | 7 | 37.1 (14.5)[c] | 30.7-42.1 (0.0-19.1)[c] | 2.7 (2.6)[c] | 2.4 | 7.2 (6.9)[c] | 0.3 (0.3)[c] | | | | | | | |
| Stubble width [cm] | 2014 | 4 | 11.8 | 7.4-13.8 | 2.8 | 2.4 | 7.5 | 1.8 | | | | | | | |
| | 2015 | | | | | | | | 1 | 16.1 | 0.0-29.4 | 2.3 | 2.2 | 8.9 | 3.8 |