

**Additional file 4– Primer sequences used for RT-qPCR analyses, amplicon length and PCR efficiency.**

The corresponding primer PCR efficiency (E) of one cycle in the exponential phase has been calculated according to the equation  $E=10^{(-1/\text{slope})}$

| Brachypodium<br>Dof genes | Primer sense           | Primer antisense        | Amplicon<br>size (bp) | T <sub>m</sub> (°C) | Slope | E <sup>*</sup> |
|---------------------------|------------------------|-------------------------|-----------------------|---------------------|-------|----------------|
| <i>BdDof1</i>             | CATGCGGTTTGTGCTATGTC   | CGTGGTTCTTCTTTGGTTC     | 128                   | 78.4                | -3.14 | 2.08           |
| <i>BdDof2</i>             | ATGTGGAGACGTGGAAGGTC   | CAGCCTTCCGCTTGTAGTTG    | 177                   | 81.1                | -3.06 | 2.13           |
| <i>BdDof3</i>             | TCATCATCAGTTCGGAGCAG   | TGTCCATGAACCAAGACGTG    | 89                    | 78.6                | -3.48 | 1.94           |
| <i>BdDof4</i>             | TCGATCTCCATGACTCCATC   | GATCCATTGTTCCGCAGTTC    | 146                   | 73.5                | -3.30 | 2.01           |
| <i>BdDof5</i>             | GCACCGGTGTTACTGAATTAGG | CGAACGACACAAGACCATACC   | 100                   | 70.8                | -3.57 | 1.90           |
| <i>BdDof6</i>             | GGAGGGAATTGGAGAAGTTG   | AATCCCTACCTTTGCCATC     | 94                    | 71.5                | -3.11 | 2.09           |
| <i>BdDof7</i>             | ATCGATGGAGGGGTGTTATG   | CATCCACACCATGAGCAAAC    | 79                    | 68.5                | -3.54 | 1.91           |
| <i>BdDof8</i>             | GGGAAGAGATCTTCAGAGCAG  | CTCCATACATGCTGCCATTG    | 82                    | 74.2                | -3.59 | 1.90           |
| <i>BdDof9</i>             | GCGGAAATGATAGGGGATAG   | CAAACCCAGCCAAAAGAGAG    | 103                   | 72.1                | -3.53 | 1.92           |
| <i>BdDof10</i>            | GGGCCAACTTTAGGAGACTTG  | CACAAAGCTGAAACCACTGC    | 99                    | 73.4                | -3.02 | 2.14           |
| <i>BdDof11</i>            | AAGTACGAGCCGTTTCGATTC  | TTGAGAGCCCAACACTTTGC    | 108                   | 79.87               | -3.18 | 2.06           |
| <i>BdDof12</i>            | AAAGAGGAGAGGAGAGATGAGG | CACTAGGATCACCGTATGACACC | 109                   | 68                  | -3.01 | 2.14           |
| <i>BdDof13</i>            | AAAGCAGGCTTTGAGACGAG   | CCGTGTATGCAAGAACAAGC    | 110                   | 72.4                | -3.64 | 1.88           |
| <i>BdDof14</i>            | TGCGGATGTAGGTTTCTGTG   | TGTAGATTTCAAGGCCAAGC    | 129                   | 74.7                | -3.59 | 1.90           |
| <i>BdDof15</i>            | GCTCATGCTGTGCTGTTTTG   | CGACACGATGGTTAGCTTTG    | 109                   | 74.4                | -3.57 | 1.90           |
| <i>BdDof16</i>            | CAATGATCTGGCAACGTAGG   | CTGGCAATCTGACCATGAAC    | 126                   | 74.1                | -3.58 | 1.90           |
| <i>BdDof17</i>            | GCTCACTCCTGTTTGCTTGTC  | TCTTTGCAAGCTCCACTGC     | 145                   | 71.6                | -3.56 | 1.91           |
| <i>BdDof18</i>            | TATTTCTACCGCTCCATC     | AACTCAAGATGACGCCCAAC    | 116                   | 72.1                | -3.43 | 1.95           |
| <i>BdDof19</i>            | TTCATGCTGCCTTCTGCTTC   | GTACACTCCATTTCCTTACC    | 101                   | 68.2                | -3.56 | 1.91           |
| <i>BdDof20</i>            | CCTGGTCCTGTGTAGATCAATG | GCTGATGCACAAAACACCTC    | 75                    | 68.4                | -3.53 | 1.92           |
| <i>BdDof21</i>            | GATGCCCTTTTCTGTTCTG    | GCGATGCAACCTTACAAACC    | 127                   | 70.4                | -3.55 | 1.91           |
| <i>BdDof22</i>            | GCTCGATCGCTTTTGATTACC  | TCATGGATCCAAACCCTAGC    | 92                    | 72.5                | -3.53 | 1.92           |
| <i>BdDof23</i>            | TCCGGATTCAAGCTCTTCG    | TCTTCCGTGGAGGTGGAG      | 95                    | 80                  | -3.33 | 1.99           |
| <i>BdDof24</i>            | ATATGAGAGCAGCAGCATGG   | GCAAATGCAAGGTAGAGCAAC   | 110                   | 71.4                | -3.50 | 1.93           |
| <i>BdDof25</i>            | ACCACCAGCAGATCAAGAGC   | ACGACGTCGAGGAAGATGTC    | 117                   | 80.1                | -3.02 | 2.14           |
| <i>BdDof26</i>            | CGGATGGTGTCAACTGTGG    | GGCATGTCATCTTGATCTG     | 73                    | 75.9                | -3.16 | 2.07           |
| <i>BdDof27</i>            | GCTCATCCGATGCATTTCTG   | CATAGTATCGGACGGGGAAG    | 114                   | 78.4                | -3.15 | 2.08           |
| <i>BdGAPDH</i>            | CTCCCGCTATTTCGTTTGTC   | TGAAGATGTTGGAGCTGACG    | 76                    | 71.5                | -3.07 | 2.11           |