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Functional Plant Biology

Supplementary Material

***BrRD20* improves abiotic stress resistance in chrysanthemum**

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1 TTAACACTTACAGAGGAGAAGCCAAAGGATTAGAGAGGTTATGGGAGAAGAGTCAGAGGCTTTTGCCACGACGGCGCGTGTAGCTCCAGTG 17
M G E E S E A F A T T A P L A P V

92 ACCGGTGAGCGAAAAGTAAGGAACGACTTGGAGGAAACATTACCTAAACCATATTTGGCAAGAGCACTAGTAGCTCCAGATACAGAGCAT 47
T G E R K V R N D L E E T L P K P Y L A R A L V A P D T E H

182 CCGAATGGATCAGAAGGTCATGACAGCAAAGGCATGAGTGTATGCAACAACATGTTGCTTTTTTCGACCAAAAACGGCGATGGAATCGTC 77
P N G S E G H D S K G M S V M Q Q H V A F F D Q N G D G I V

272 TATCCTTGGGAGAGTATGCAGGATTCGGTGACCTCGGTTTCAACCCAATCTCTTCTGTTTTTTGGGCCATATTCATAAACTTTGCGTTT 107
Y P W E T Y A G F R D L G F N P I S S V F W A I F I N F A F
Ca²⁺-binding EF-hand domain (62-89aa) amphipathic α -helix (93-106aa)

362 AGCTACGTTACACTCCGAGTTGGTTGCCATACCACTATTGCCTGTTATATCGACAACATTCACAAAAGCCAAGCATGGGAGTGATTCA 137
S Y V T L P S W L P S P L L P V Y I D N I H K A K H G S D S
Proline-knot (114-123aa)

452 AGCACATATGATACCGAAGGAAGGTATGTCCCGTTAATCTCGAGAACATCTTTAGCAAATATGCGTTAACGGCTCCAAAATAAAATAACA 167
S T Y D T E G R Y V P V N L E N I F S K Y A L T A P N K I I

542 TTAAAAGAGCTTTGGAAGTAAACCGAGGAAAACCGAATGGCAATCGATCCTTTTGGATGGCTTGGCAATAAAGTTGAATGGCTACTAGTC 197
L K E L W N L T E G N R M A I D P F G W L A N K V E W L L V

632 TATCTTCTGCAAAGGATGAGGACGGGTTCTGTCTAAAGAAGCTGTGAGAGGTGCTTTGATGCAAGTTTCTTTGAATACTGTGCTAAA 227
Y L L A K D E D G F V S K E A V R G V F D A S F F E Y C A K

722 AAGAATAAAGAGAAGGCCGATTCTCGAAGCAAGACTAAAGCATTAATTTGCTTATCTTTAAATTTGATGTTGTATTATTATGCTC
K N K E K A D S R K Q D *
C-terminal hydrophilic domain (126-235aa)

812 TCAATCTTAATTAGAAAAGGACAGAAAAACAAAACTCTATGTGGAATAAAACGTTTGTTCTTGGATATATTGTTGTGTAATATTTA

902 TTGAATAAATATAAAATGAGATTTTCATCAAAAAAAAAAAAAAAAAA

Fig. S1, Supplementary data

DNA sequences of *BrRD20*. Start and stop codons are boxed. Caleosin superfamily domain is underlined. The gray shade is Ca²⁺-binding EF-hand domain, the yellow shade is amphipathic α -helix, the red shade is Proline-knot, and the green shade is C-terminal hydrophilic domain (Hu et al., 2013).

Table S1a. Supplementary data

Primers used for sequencing and cDNA cloning

Primer name	Nucleotide sequence 5' to 3'
BrRD20-F	5'-ATGGGAGAAGAGTCAGAG-3'
BrRD20-R	5'-GATAAGCAAATTAATGC-3'
Oligo(dT) ₁₇ adapter primer	5'-GACTCGAGTGCACATCG(T) ₁₇ -3'
adapter primer	5'-GACTCGAGTGCACATCG-3'

Oligo(dT) ₁₆ anchor primer	5'-GACCACGCGTATCGATGTCGAC(T) ₁₆ <u>M</u> -3'
anchor primer	5'-GACCACGCGTATCGATGTCGAC-3'
5'RACE-BrRD20-F	5'-TCGATATAAACAGGCAATA-3'
3'RACE-BrRD20-F	5'-AAAACGGCGATCCAATCGT-3'

Notes: Underlines for the degenerate bases used in the primers represent M = A or C. Other abbreviations: F = forward primer; R = reverse primer.

Table S1b. Supplementary data

Primers used for quantitative real-time PCR

Primer name	Nucleotide sequence 5' to 3'
BrRD20-F	5'-CGATCCTTG GAGACACCATT -3'
BrRD20-R	5'-ACCTCCACCTCTTCCCAAGT -3'
BrRD20-F(transgenic lines)	5'-GCATGGATAACGCTATCA-3'
BrRD20-R(transgenic lines)	5'-AAGCCAAGAATATTA AAA-3'
BrACTIN-F	5'-GCTCAGTCCAAGAGAGGTATTC-3'
BrACTIN-R	5'-GCTCGTTGTAGAAAGTGTGATC-3'
CmACTIN-F	5'-GATGACGCAGATCATGTTCG-3'
CmACTIN-R	5'-AGCATGTGGAAGTGCATACC-3'