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PHYTOMORPHOLOGY

Isolation, genetic mapping and expression studies of a peptide deformylase gene, *DEF2*, from bold-seeded and small-seeded varieties of the oilseed crop, *Brassica juncea* (Indian mustard) and analysis of its over-expression and down-regulation on seed size of transgenic plants

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Supplementary data

Supplementary Table 1. Primers for amplification of full-length sequences of *DEF2*

| Primer Name | Primer sequence (5'-3') |
|-------------|------------------------------|
| DEF2- FP | GTCCATGGTCTAGCCATGGCCGTATGTA |
| DEF2- RP | CGTCTAGATCAACGTTTGCCAAAACCAA |

Supplementary Table 2. SOE-ing primers utilized for development of transgene cassettes

| Construct | Primer name | Primer sequence (5' – 3') |
|----------------------------------|--------------------|--|
| pDEF2 (over-expression cassette) | DEF2 SO-1 | TCGACTAGTTCTCATCCCCTTTTAAACCAA |
| | DEF2 SO-2 | TACATACGGCCATGGCTAGACCATGTGTATGTT TTTAATCTTGTTTGTATTG |
| | DEF2 SO-3 | CAATACAAACAAGATTAAAAACATACACATGG TCTAGCCATGGCCGTATGTA |
| | DEF2 SO-4 | CGGGTACCGAGCTCGAATTCTCAACGTTTGCCA AAACCAA |
| | DEF2 SO-5 | TTGGTTTTGGCAAACGTTGAGAATTCGAGCTCG GTACCCG |
| | DEF2 SO-6 | ACGTCTAGAAAGCTTGGTACAATCAGTAAA TTG |
| pASDEF2 (antisense cassette) | ASDEF2 SO-1 | TCGACTAGTTCTCATCCCCTTTTAAACCAA |
| | ASDEF2 SO-2 | TTGGTTTTGGCAAACGTTGAGTGTATGTTTTTA ATCTTGTTTGTATTG |
| | ASDEF2 SO-3 | CAATACAAACAAGATTAAAAACATACACTCAAC GTTTGCCAAAACCAA |
| | ASDEF2 SO-4 | CGGGTACCGAGCTCGAATTCATGGTCTAGCCA TGGCCGTATGTA |
| | ASDEF2 SO-5 | TACATACGGCCATGGCTAGACCATGAATTCGAG CTCGGTACCCG |
| | ASDEF2 SO-6 | ACGTCTAGAAAGCTTGGTACAATCAGTAAATTG |

| | | |
|------------------|---|-----|
| Homolog a Varuna | ATGGCCGATGTAAGTGTTCCTCCAAGCTCCACCGTTCTCTCGCTTCTTATCACC GGTT | 60 |
| Homolog b Varuna | ATGGCCGATGTAAGTGTTCCTCCAAGCTCCACCTCTCTCTCGCTTCTTATCCCC GGTT | 60 |
| Homolog a Varuna | TTCTCCCGCCGCGCTCCGAATCTCTTCCCGGCTATGGTCAGCTCAAATCCACC GTCATG | 120 |
| Homolog b Varuna | TTCTCCCGCCGCTCTACAAATTTCTTCCCGGCTATAATCAGCTCAAATCCACC GTCATG | 120 |
| Homolog a Varuna | TTCTCCTCCTCCTCCTCCTCCGCCGGAACCGAATTGGACC GTTGACATCTCCGGTCCGA | 180 |
| Homolog b Varuna | TTCTCCTCCTC-----CTCCGCCGGAACCGCACCGGACC GTTGACATCTCCGGTCCGA | 174 |
| Homolog a Varuna | GCTGAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGCATCCGATCTTCAA | 240 |
| Homolog b Varuna | GCTGAAGTAAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTTCAA | 234 |
| Homolog a Varuna | TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGCGCTAAGAGCAAG | 300 |
| Homolog b Varuna | TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGATCCTATCTTACGGGCTAAGTGAAG | 294 |
| Homolog a Varuna | AGGATTGGTGTTTTTGACGAGAAATTTGAAGAAGCTGGCCGATGCTATGTTGATGTTATG | 360 |
| Homolog b Varuna | AGGATTGGTGTTTTTGACGAGCATTTTGAAGAAGCTGGCCGATGCTATGTTGATGTTATG | 354 |
| Homolog a Varuna | TACAAACCGGATGGCATCGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG | 420 |
| Homolog b Varuna | TACAAGACCGGATGGCATCGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG | 414 |
| Homolog a Varuna | GTGTTAATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTAAATCCGAAA | 480 |
| Homolog b Varuna | GTGTTAATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAACCCGAAA | 474 |
| Homolog a Varuna | ATCAATAAGTATTCTGATAAATTAGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG | 540 |
| Homolog b Varuna | ATCAATAAATATTCTGATAAATTGGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG | 534 |
| Homolog a Varuna | ATCTATGCTGATGTAGTTCGACCACAATCTGTCAAGATTGACGCAAGAGACATTACTGGC | 600 |
| Homolog b Varuna | ATCTATGCTGAT-----CGACCACAATCGGTCAAGATTGACGCAAGAGACATTACTGGT | 588 |
| Homolog a Varuna | GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCATGAATACGAC | 660 |
| Homolog b Varuna | GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCATGAATACGAT | 648 |
| Homolog a Varuna | CACCTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGT | 720 |
| Homolog b Varuna | CACCTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGT | 708 |
| Homolog a Varuna | GAAGAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAAC TGGACTGCCAAGCCCTGAA | 780 |
| Homolog b Varuna | GAAGAGTTAGAGGCATTGGAAAAGAAGTACGAAGAAAAAATGGATTGCCAAGCCCTGAA | 768 |
| Homolog a Varuna | AAAGTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | 834 |
| Homolog b Varuna | AAAGTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | 822 |

Supplementary Fig. 1. cDNA sequence alignment of *DEF2* homologs 'a' and 'b' from *Brassica juncea* var. Varuna. Polymorphisms are highlighted in yellow boxes

| | | |
|------------------|--|-----|
| Homolog a Varuna | MAVCNCF LQAPPF SRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSSAANRIGPLTSPVR | 60 |
| Homolog b Varuna | MAVCNCF LQAPPLSRFLSPVFSRRPTNFFAGYNQLKSTVMFSS--SAANRTIGPLTSPVR | 58 |
| Homolog a Varuna | AEVKRVS RKESEIASASDLQFETPLKIVEYPPDILRAKSKRIGVFDENLKNLADAMFDVM | 120 |
| Homolog b Varuna | AEVKRVS RKEDEIASASDLQFETPLKIVEYPPDILRAKCKRIGVFDENLKNLADAMFDVM | 118 |
| Homolog a Varuna | YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG | 180 |
| Homolog b Varuna | YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG | 178 |
| Homolog a Varuna | IYADVWRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLLEGVLFDFRMTDDVLDITIR | 240 |
| Homolog b Varuna | IYAD--RPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLLEGVLFDFRMTDDVLDITIR | 236 |
| Homolog a Varuna | EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGR | 277 |
| Homolog b Varuna | EELEALEKKYEEKNGLPSPEKVQARQKKKAGVGFGR | 273 |

Supplementary Fig. 2. Amino acid sequence alignment of *DEF2* homologs ‘a’ and ‘b’ from *Brassica juncea* var. Varuna. Polymorphisms are highlighted in yellow boxes

| | | | | |
|-----------------|---|--------------------------|-------------------------------|-----|
| Homolog a Heera | ATGGCCGATGTAGCTGTTTCTCCAAGCTCCACC | GTTTTCTCGTTCTTATCACCGGTT | 60 | |
| Homolog b Heera | ATGGCCGATGTAACTGTTTCTCCAAGCTCCACC | TCTCTCTCGTTCTTATCCCCGGTT | 60 | |
| Homolog a Heera | TTCTCCCGCCGCGCTCCGAATCTCTTCCCGGCTATGGT | CAGCTCAAATCCACCGTCATG | 120 | |
| Homolog b Heera | TTCTCCCGCCGTCCTACAATTTCTTCCCGGCTATAAT | CAGCTCAAATCCACCGTCATG | 120 | |
| Homolog a Heera | TTCTCCTCCTCCTCCTCCGCCGCGAACC | CGAACC | CGGACCGTTGACATCTCCGGTCCAGAGCT | 180 |
| Homolog b Heera | TTCTCCTCCTCCTCCTCCGCCGAACC | -----GCACCGGACat | CATCTCCGGTCCAGAGCT | 173 |
| Homolog a Heera | GAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGCTTCTGATCTACAATTC | | 240 | |
| Homolog b Heera | GAAGTAAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTTACAATTC | | 233 | |
| Homolog a Heera | GAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGGCTAAGAGCAAGAGG | | 300 | |
| Homolog b Heera | GAGACGCCGTTGAAGATTGTTGAGTATCCAGATCCTATCTTACGGGCTAAGTGCAAGAGG | | 293 | |
| Homolog a Heera | ATTGGTGTTTTTGACGAGAATTTGAAGAACTTGGCCGATGCTATGTTGATGTTATGTAC | | 360 | |
| Homolog b Heera | ATTGGTGTTTTTGACGAGCATTTTGAAGAACTTGGCCGATGCTATGTTGATGTTATGTAC | | 353 | |
| Homolog a Heera | AAAACGGATGGCATTGGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTG | | 420 | |
| Homolog b Heera | AAGACGGATGGCATTGGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTG | | 413 | |
| Homolog a Heera | TTTAATCCGGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAATCCGAAAATC | | 480 | |
| Homolog b Heera | TTTAATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAACCCGAAAATC | | 473 | |
| Homolog a Heera | AATAAGTATTCTGATAAATTAGTAFCATTCAACGAAGGATGCTTATCCTTCCCTGGGATC | | 540 | |
| Homolog b Heera | AATAAATATTCTGATAAATTGGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGGATC | | 533 | |
| Homolog a Heera | TATGCTGATGTAGTTTGACCACAATCTGTCAAGATTGACGCAAGGGACATTACTGGCGCT | | 600 | |
| Homolog b Heera | TATGCTGAT-----CGACCACAATCGTCAAGATTGACGCAAGAGACATTACTGGTGCT | | 587 | |
| Homolog a Heera | AGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCACGAATACGATCAC | | 660 | |
| Homolog b Heera | AGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCATGAATACGATCAC | | 647 | |
| Homolog a Heera | TTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGTGAA | | 720 | |
| Homolog b Heera | CTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGTGAA | | 707 | |
| Homolog a Heera | GAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAACTGGACTGCCAAGCCCTGAAAAA | | 780 | |
| Homolog b Heera | GAGTTAGAGGCATTGGAAAAGAAGTACGAAGAAAAATGGATTGCCAAGCCCTGAAAAA | | 767 | |
| Homolog a Heera | GTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | | 831 | |
| Homolog b Heera | GTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | | 818 | |

Supplementary Fig. 3. cDNA sequence alignment of *DEF2* homologs 'a' and 'b' from *Brassica juncea* var. Heera. Polymorphisms are highlighted in yellow boxes.

| | | 7 | |
|-----------------|--------|-----|----|
| Heera homolog a | MAVCS | CF | 60 |
| Heera homolog b | MAVCN | CF | 59 |
| Heera homolog a | EVKR | 120 | |
| Heera homolog b | EVKR | 119 | |
| Heera homolog a | KTDG | 180 | |
| Heera homolog b | KTDG | 179 | |
| Heera homolog a | YADV | 240 | |
| Heera homolog b | YAD-- | 237 | |
| Heera homolog a | ELEALE | 276 | |
| Heera homolog b | ELEALE | 273 | |

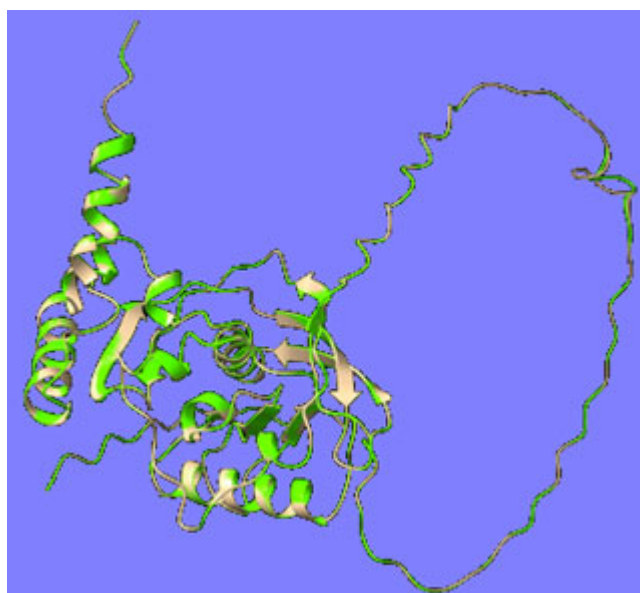
Supplementary Fig. 4. Amino acid sequence alignment of *DEF2* homologs ‘a’ and ‘b’ from *Brassica juncea* var. Heera. Polymorphisms are highlighted in yellow boxes

| | | |
|------------------|---|-----|
| Homolog a Varuna | ATGGCCGTATGTA ^A CTGTTTCTCCAAGCTCCACCGTT ^T CTCTCGTTCTTATCACCGGTT | 60 |
| Homolog a Heera | ATGGCCGTATGTAG ^A CTGTTTCTCCAAGCTCCACCGTT ^T CTCTCGTTCTTATCACCGGTT | 60 |
| Homolog a Varuna | TTCTCCCGCCGCGCTCCGAATCTTCCCCGGCTATGGTCAGCTCAAATCCACCGTCATG | 120 |
| Homolog a Heera | TTCTCCCGCCGCGCTCCGAATCTTCCCCGGCTATGGTCAGCTCAAATCCACCGTCATG | 120 |
| Homolog a Varuna | TTctcctcctc ^{ctc} ctcctccGCCGGAACCGAAT ^{TT} GGACCGTTGACATCTCCGGT ^{CC} GA | 180 |
| Homolog a Heera | TTCTCCTCTC ⁻⁻⁻ CTCCTCCGCCGGAACCGA ^{ACC} GGACCGTTGACATCTCCGGT ^C AGA | 177 |
| Homolog a Varuna | GCTGAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGC ^{ATC} GATCT ^T CAA | 240 |
| Homolog a Heera | GCTGAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGC ^{TTC} GATCT ^A CAA | 237 |
| Homolog a Varuna | TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGCGTAAGAGCAAG | 300 |
| Homolog a Heera | TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGCGTAAGAGCAAG | 297 |
| Homolog a Varuna | AGGATTGGTGTTTTGACGAGAATTTGAAGAACTTGGCCGATGCTATGTTGATGTTATG | 360 |
| Homolog a Heera | AGGATTGGTGTTTTGACGAGAATTTGAAGAACTTGGCCGATGCTATGTTGATGTTATG | 357 |
| Homolog a Varuna | TACAAAACGGATGGCAT ^C GGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG | 420 |
| Homolog a Heera | TACAAAACGGATGGCAT ^T GGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG | 417 |
| Homolog a Varuna | GTGTTTAATCC ^A GCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGT ^T AATCCGAAA | 480 |
| Homolog a Heera | GTGTTTAATCC ^{GG} GCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGT ^G AATCCGAAA | 477 |
| Homolog a Varuna | ATCAATAAGTATTCTGATAAATTAGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG | 540 |
| Homolog a Heera | ATCAATAAGTATTCTGATAAATTAGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG | 537 |
| Homolog a Varuna | ATCTATGCTGATGTAGTTCGACCACAATCTGTCAAGATTGACGCAAG ^A GACATTACTGGC | 600 |
| Homolog a Heera | ATCTATGCTGATGTAGTTCGACCACAATCTGTCAAGATTGACGCAAG ^{GG} GACATTACTGGC | 597 |
| Homolog a Varuna | GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCACGAATACGAC | 660 |
| Homolog a Heera | GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCACGAATACGAC | 657 |
| Homolog a Varuna | CACTTAGAGGGAGTTCTTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCTG | 720 |
| Homolog a Heera | CACTTAGAGGGAGTTCTTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCTG | 717 |
| Homolog a Varuna | GAAGAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAACCTGGACTGCCAAGCCCTGAA | 780 |
| Homolog a Heera | GAAGAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAACCTGGACTGCCAAGCCCTGAA | 777 |
| Homolog a Varuna | AAAGTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | 834 |
| Homolog a Heera | AAAGTACAAGCACGACAAAAAAGGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA | 831 |

Supplementary Fig. 5. Comparative alignment of cDNA sequences for *DEF2* homolog 'a' in *Brassica juncea* vars. Varuna and Heera. Polymorphisms are highlighted in yellow boxes

| | | |
|------------------|--|-----|
| Homolog a Varuna | MAVCHCFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSAANRIGPLTSPVR | 60 |
| Homolog a Heera | MAVCS CFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSAANRTGPLTSPVR | 59 |
| Homolog a Varuna | AEVKRVS RKESEIASASDLQFETPLKIVEY PDPILRAKSKRIGVFDENLKNLADAMFDVM | 120 |
| Homolog a Heera | AEVKRVS RKESEIASASDLQFETPLKIVEY PDPILRAKSKRIGVFDENLKNLADAMFDVM | 119 |
| Homolog a Varuna | YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVFPNEGCLSFPG | 180 |
| Homolog a Heera | YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVFPNEGCLSFPG | 179 |
| Homolog a Varuna | IYADVWRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGV LFFDRMTDDVLD TIR | 240 |
| Homolog a Heera | IYADVWRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGV LFFDRMTDDVLD TIR | 239 |
| Homolog a Varuna | EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGR | 277 |
| Homolog a Heera | EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGR | 276 |

Supplementary Fig. 6. Comparative alignment of amino acid sequences for *DEF2* homolog 'a' in *Brassica juncea* vars. Varuna and Heera. Polymorphisms are highlighted in yellow boxes.



Supplementary Fig. 7. Superimposed structure of *DEF2* homolog 'a' protein from Varuna (beige) vs Heera (green). The models were generated using SWISS-MODEL and superimposed using ChimeraX

| | | |
|------------------|---|-----|
| Homolog b Varuna | ATGGCCGTATGTAAGTGTTCCTCCAAGCTCCACCTCTCTCTCGTTCTTATCCCCGGTT | 60 |
| Homolog b Heera | ATGGCCGTATGTAAGTGTTCCTCCAAGCTCCACCTCTCTCTCGTTCTTATCCCCGGTT | 60 |
| Homolog b Varuna | TTCTCCCGCCGTCTACAAATTTCTTCGCCGGCTATAATCAGCTCAAATCCACCGTCATG | 120 |
| Homolog b Heera | TTCTCCCGCCGTCTACAAATTTCTTCGCCGGCTATAATCAGCTCAAATCCACCGTCATG | 120 |
| Homolog b Varuna | TTCTCCTCCTCCTCCGCCGGAACCGCACCGGACCGTTGACATCTCCGGTCCGAGCTGAA | 180 |
| Homolog b Heera | TTCTCCTCCTCCTCCGCCGGAACCGCACCGGACCGTTGACATCTCCGGTCCGAGCTGAA | 180 |
| Homolog b Varuna | GTAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTCAATTCGAG | 240 |
| Homolog b Heera | GTAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTCAATTCGAG | 240 |
| Homolog b Varuna | ACGCCGTTGAAGATTGTTGAGTATCCAGATCCTATCTTACGGGCTAAGTGCAAGAGGATT | 300 |
| Homolog b Heera | ACGCCGTTGAAGATTGTTGAGTATCCAGATCCTATCTTACGGGCTAAGTGCAAGAGGATT | 300 |
| Homolog b Varuna | GGTGTTTTGACGAGCATTTGAAGAACTTGGCCGATGCTATGTTTGATGTTATGTACAAG | 360 |
| Homolog b Heera | GGTGTTTTGACGAGCATTTGAAGAACTTGGCCGATGCTATGTTTGATGTTATGTACAAG | 360 |
| Homolog b Varuna | ACGGATGGCATCGGCCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTGTTT | 420 |
| Homolog b Heera | ACGGATGGCATCGGCCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTGTTT | 420 |
| Homolog b Varuna | AATCCAGCTGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTAACCCGAAAATCAAT | 480 |
| Homolog b Heera | AATCCAGCTGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTAACCCGAAAATCAAT | 480 |
| Homolog b Varuna | AAATATTCTGATAAATTGGTACCATTCAACGAAGGATGCCTATCCTTCCCTGGGATCTAT | 540 |
| Homolog b Heera | AAATATTCTGATAAATTGGTACCATTCAACGAAGGATGCCTATCCTTCCCTGGGATCTAT | 540 |
| Homolog b Varuna | GCTGATCGACCACAATCGGTCAAGATTGACGCAAGAGACATTACTGGTGCTAGATTTTCG | 600 |
| Homolog b Heera | GCTGATCGACCACAATCGGTCAAGATTGACGCAAGAGACATTACTGGTGCTAGATTTTCG | 600 |
| Homolog b Varuna | ATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCATGAATACGATCACCTAGAGGGA | 660 |
| Homolog b Heera | ATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCATGAATACGATCACCTAGAGGGA | 660 |
| Homolog b Varuna | GTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGTGAAGAGTTAGAG | 720 |
| Homolog b Heera | GTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTTCGTGAAGAGTTAGAG | 720 |
| Homolog b Varuna | GCATTGAAAAGAAGTACGAAGAAAAAATGGATTGCCAAGCCCTGAAAAAGTACAAGCA | 780 |
| Homolog b Heera | GCATTGAAAAGAAGTACGAAGAAAAAATGGATTGCCAAGCCCTGAAAAAGTACAAGCA | 780 |
| Homolog b Varuna | CGACAAAAAAGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA 822 | |
| Homolog b Heera | CGACAAAAAAGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA 822 | |

Supplementary Fig. 8. Comparative alignment of cDNA sequences for *DEF2* homolog 'b' in *Brassica juncea* vars. Varuna and Heera.

| | | |
|------------------|--|-----|
| Homolog b Varuna | MAVCNCFLQAPPLSRFLSPVFSRRPTNFFAGYNQLKSTVMFSSSSAANRTGPLTSPVRAE | 60 |
| Homolog b Heera | MAVCNCFLQAPPLSRFLSPVFSRRPTNFFAGYNQLKSTVMFSSSSAANRTGPLTSPVRAE | 60 |
| Homolog b Varuna | VKRVSrKEDEIASASDLQFETPLKIVEYDPDILRAKCKRIGVFDEHLKNLADAMFDVMYK | 120 |
| Homolog b Heera | VKRVSrKEDEIASASDLQFETPLKIVEYDPDILRAKCKRIGVFDEHLKNLADAMFDVMYK | 120 |
| Homolog b Varuna | TDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVFPNEGCLSFPGIY | 180 |
| Homolog b Heera | TDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVFPNEGCLSFPGIY | 180 |
| Homolog b Varuna | ADRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFDRMTDDVLDTIREELE | 240 |
| Homolog b Heera | ADRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFDRMTDDVLDTIREELE | 240 |
| Homolog b Varuna | ALEKKYEEKNGLPSPEKVQARQKKKAGVGFGR | 273 |
| Homolog b Heera | ALEKKYEEKNGLPSPEKVQARQKKKAGVGFGR | 273 |

Supplementary Fig. 9. Comparative alignment of amino acid sequences for *DEF2* homolog 'b' in *Brassica juncea* vars. Varuna and Heera.