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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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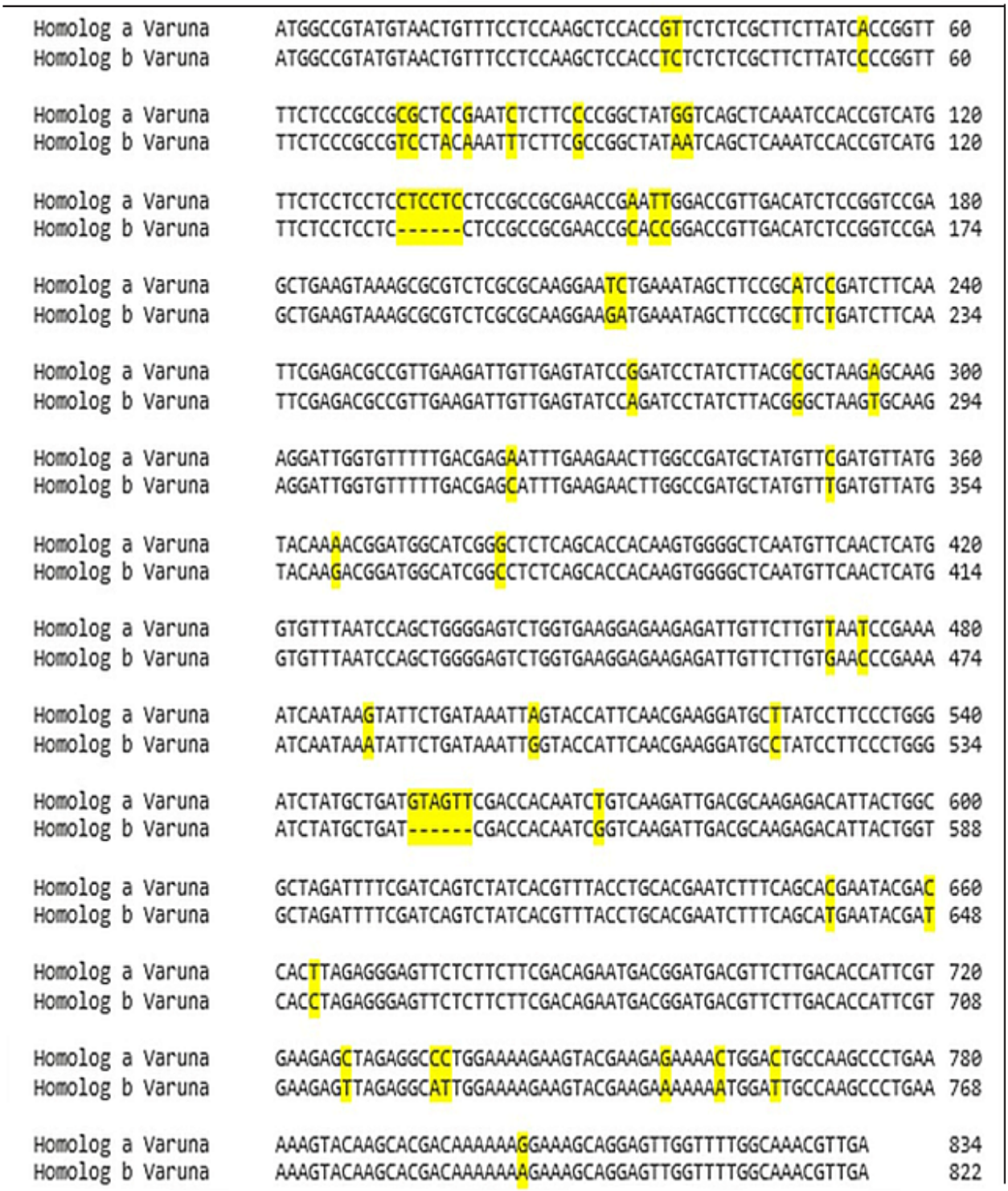
Supplementary data

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



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    MAVCNCFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSSAANRIGPLTSPVR  60
Homolog b Varuna    MAVCNCFLQAPPLSRFLSPVFSRRPTNFFAGYNQLKSTVMFSSS--SAANRTGPLTSPVR  58

Homolog a Varuna    AEVKRVSRKESEIASASDLQFETPLKIVEYPDPILRAKSKRIGVFDENLKNLADAMFDVM  120
Homolog b Varuna    AEVKRVSRKEDEIASASDLQFETPLKIVEYPDPILRAKCKRIGVFDEHLKNLADAMFDVM  118

Homolog a Varuna    YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG  180
Homolog b Varuna    YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG  178

Homolog a Varuna    IYADVVRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIR  240
Homolog b Varuna    IYAD--RPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIR  236

Homolog a Varuna    EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGKR    277
Homolog b Varuna    EELEALEKKYEEKNGLPSPEKVQARQKKKAGVGFGKR    273
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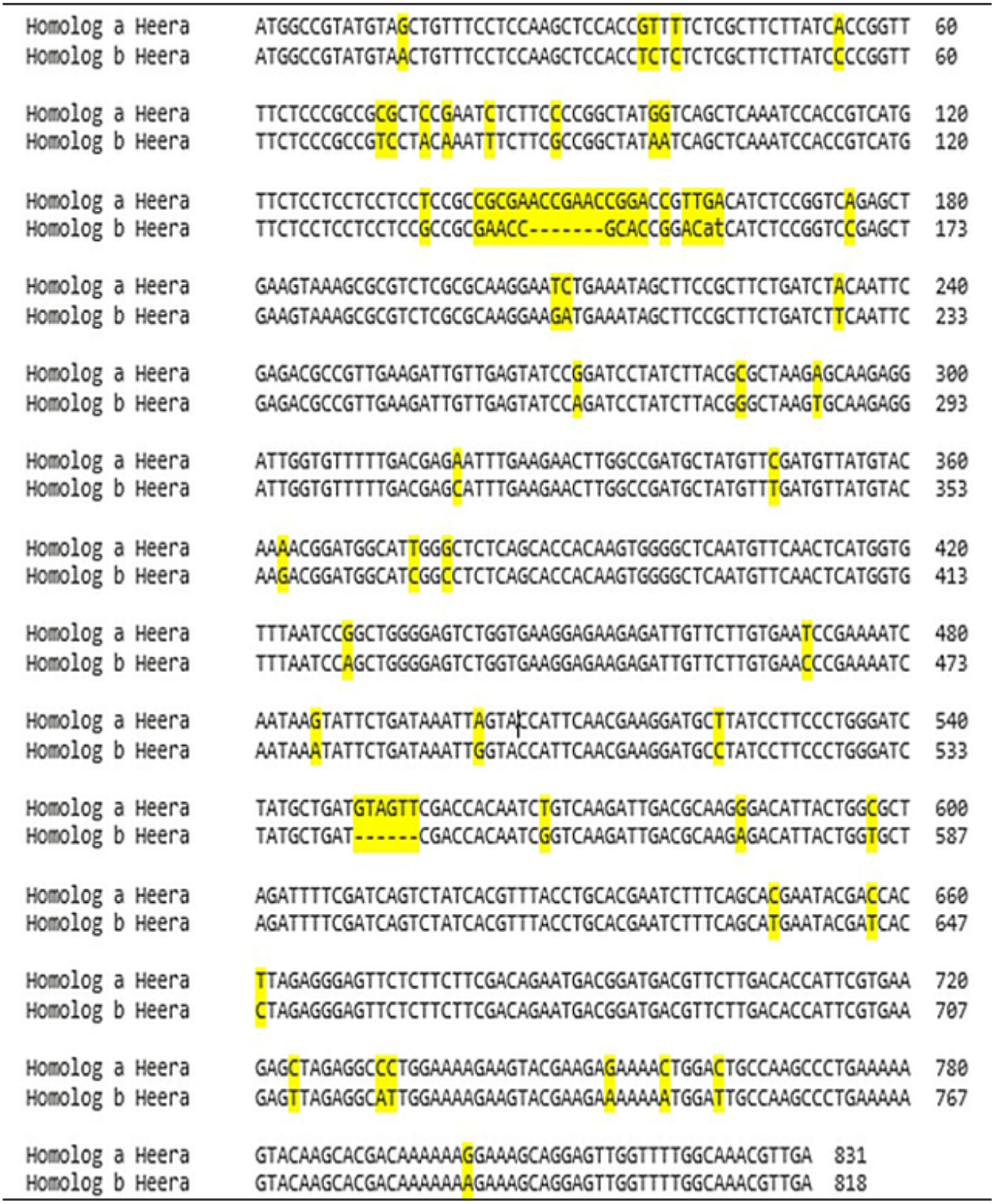
Supplementary Fig. 2. Amino acid sequence alignment of *DEF2* homologs ‘a’ and ‘b’ from *Brassica juncea* var. Varuna. Polymorphisms are highlighted in yellow boxes



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

```
Heera homolog a     MAVCSCFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSAANRTGPLTSPVRA  60
Heera homolog b     MAVCNCFLQAPPLSRFLSPVFSRRPTNFFAGYNQLKSTVMFSSS-SAANRTGPLTSPVRA  59

Heera homolog a     EVKRVSRKESEIASASDLQFETPLKIVEYPDPILRAKSKRIGVFDENLKNLADAMFDVMY  120
Heera homolog b     EVKRVSRKEDEIASASDLQFETPLKIVEYPDPILRAKCKRIGVFDEHLKNLADAMFDVMY  119

Heera homolog a     KTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPGI  180
Heera homolog b     KTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPGI  179

Heera homolog a     YADVVRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIRE  240
Heera homolog b     YAD--RPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIRE  237

Heera homolog a     ELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGKR        276
Heera homolog b     ELEALEKKYEEKNGLPSPEKVQARQKKKAGVGFGKR        273
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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    ATGGCCGTATGTAACTGTTTCCTCCAAGCTCCACCGTTCTCTCGCTTCTTATCACCGGTT 60
Homolog a Heera     ATGGCCGTATGTAGCTGTTTCCTCCAAGCTCCACCGTTTTCTCGCTTCTTATCACCGGTT 60

Homolog a Varuna    TTCTCCCGCCGCGCTCCGAATCTCTTCCCCGGCTATGGTCAGCTCAAATCCACCGTCATG 120
Homolog a Heera     TTCTCCCGCCGCGCTCCGAATCTCTTCCCCGGCTATGGTCAGCTCAAATCCACCGTCATG 120

Homolog a Varuna    TTctcctcctcctcctcctccGCCGCGAACCGAATTGGACCGTTGACATCTCCGGTCCGA 180
Homolog a Heera     TTCTCCTCCTC---CTCCTCCGCCGCGAACCGAACCGGACCGTTGACATCTCCGGTCAGA 177

Homolog a Varuna    GCTGAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGCATCCGATCTTCAA 240
Homolog a Heera     GCTGAAGTAAAGCGCGTCTCGCGCAAGGAATCTGAAATAGCTTCCGCTTCTGATCTACAA 237

Homolog a Varuna    TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGCGCTAAGAGCAAG 300
Homolog a Heera     TTCGAGACGCCGTTGAAGATTGTTGAGTATCCGGATCCTATCTTACGCGCTAAGAGCAAG 297

Homolog a Varuna    AGGATTGGTGTTTTTGACGAGAATTTGAAGAACTTGGCCGATGCTATGTTCGATGTTATG 360
Homolog a Heera     AGGATTGGTGTTTTTGACGAGAATTTGAAGAACTTGGCCGATGCTATGTTCGATGTTATG 357

Homolog a Varuna    TACAAAACGGATGGCATCGGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG 420
Homolog a Heera     TACAAAACGGATGGCATTGGGCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATG 417

Homolog a Varuna    GTGTTTAATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTTAATCCGAAA 480
Homolog a Heera     GTGTTTAATCCGGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAATCCGAAA 477

Homolog a Varuna    ATCAATAAGTATTCTGATAAATTAGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG 540
Homolog a Heera     ATCAATAAGTATTCTGATAAATTAGTACCATTCAACGAAGGATGCTTATCCTTCCCTGGG 537

Homolog a Varuna    ATCTATGCTGATGTAGTTCGACCACAATCTGTCAAGATTGACGCAAGAGACATTACTGGC 600
Homolog a Heera     ATCTATGCTGATGTAGTTCGACCACAATCTGTCAAGATTGACGCAAGGGACATTACTGGC 597

Homolog a Varuna    GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCACGAATACGAC 660
Homolog a Heera     GCTAGATTTTCGATCAGTCTATCACGTTTACCTGCACGAATCTTTCAGCACGAATACGAC 657

Homolog a Varuna    CACTTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCGT 720
Homolog a Heera     CACTTAGAGGGAGTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCGT 717

Homolog a Varuna    GAAGAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAACTGGACTGCCAAGCCCTGAA 780
Homolog a Heera     GAAGAGCTAGAGGCCCTGGAAAAGAAGTACGAAGAGAAAACTGGACTGCCAAGCCCTGAA 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    MAVCNCFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSSSSSAANRIGPLTSPVR 60
Homolog a Heera     MAVCSCFLQAPPFSRFLSPVFSRRAPNLFPGYGQLKSTVMFSSS-SSAANRTGPLTSPVR 59

Homolog a Varuna    AEVKRVSRKESEIASASDLQFETPLKIVEYPDPILRAKSKRIGVFDENLKNLADAMFDVM 120
Homolog a Heera     AEVKRVSRKESEIASASDLQFETPLKIVEYPDPILRAKSKRIGVFDENLKNLADAMFDVM 119

Homolog a Varuna    YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG 180
Homolog a Heera     YKTDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPG 179

Homolog a Varuna    IYADVVRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIR 240
Homolog a Heera     IYADVVRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIR 239

Homolog a Varuna    EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGKR 277
Homolog a Heera     EELEALEKKYEEKTGLPSPEKVQARQKRKAGVGFGKR 276
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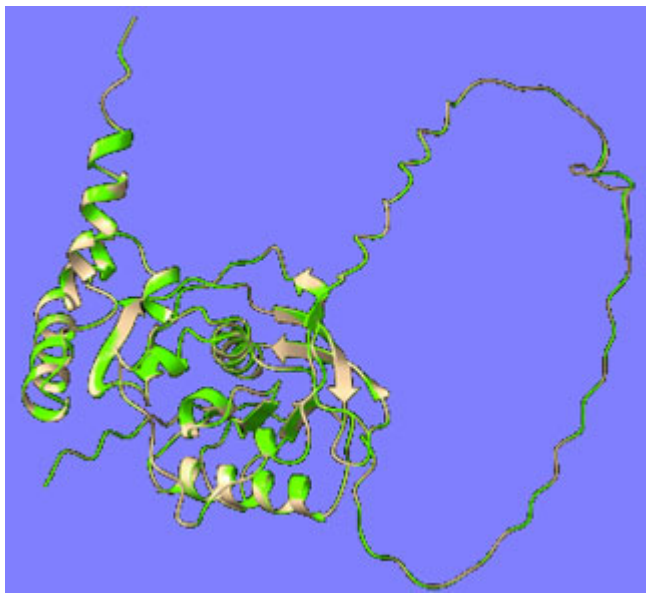
Supplementary Fig. 6. Comparative alignment of amino acid sequences for *DEF2* homolog 'a' in *Brassica juncea* vars. Varuna and Heera. Polymorphisms are highlighted in yellow boxes.



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    ATGGCCGTATGTAACTGTTTCCTCCAAGCTCCACCTCTCTCTCGCTTCTTATCCCCGGTT  60
Homolog b Heera     ATGGCCGTATGTAACTGTTTCCTCCAAGCTCCACCTCTCTCTCGCTTCTTATCCCCGGTT  60

Homolog b Varuna    TTCTCCCGCCGTCCTACAAATTTCTTCGCCGGCTATAATCAGCTCAAATCCACCGTCATG  120
Homolog b Heera     TTCTCCCGCCGTCCTACAAATTTCTTCGCCGGCTATAATCAGCTCAAATCCACCGTCATG  120

Homolog b Varuna    TTCTCCTCCTCCTCCGCCGCGAACCGCACCGGACCGTTGACATCTCCGGTCCGAGCTGAA  180
Homolog b Heera     TTCTCCTCCTCCTCCGCCGCGAACCGCACCGGACCGTTGACATCTCCGGTCCGAGCTGAA  180

Homolog b Varuna    GTAAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTTCAATTCGAG  240
Homolog b Heera     GTAAAGCGCGTCTCGCGCAAGGAAGATGAAATAGCTTCCGCTTCTGATCTTCAATTCGAG  240

Homolog b Varuna    ACGCCGTTGAAGATTGTTGAGTATCCAGATCCTATCTTACGGGCTAAGTGCAAGAGGATT  300
Homolog b Heera     ACGCCGTTGAAGATTGTTGAGTATCCAGATCCTATCTTACGGGCTAAGTGCAAGAGGATT  300

Homolog b Varuna    GGTGTTTTTGACGAGCATTTGAAGAACTTGGCCGATGCTATGTTTGATGTTATGTACAAG  360
Homolog b Heera     GGTGTTTTTGACGAGCATTTGAAGAACTTGGCCGATGCTATGTTTGATGTTATGTACAAG  360

Homolog b Varuna    ACGGATGGCATCGGCCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTGTTT  420
Homolog b Heera     ACGGATGGCATCGGCCTCTCAGCACCACAAGTGGGGCTCAATGTTCAACTCATGGTGTTT  420

Homolog b Varuna    AATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAACCCGAAAATCAAT  480
Homolog b Heera     AATCCAGCTGGGGAGTCTGGTGAAGGAGAAGAGATTGTTCTTGTGAACCCGAAAATCAAT  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    GTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCGTGAAGAGTTAGAG  720
Homolog b Heera     GTTCTCTTCTTCGACAGAATGACGGATGACGTTCTTGACACCATTCGTGAAGAGTTAGAG  720

Homolog b Varuna    GCATTGGAAAAGAAGTACGAAGAAAAAAATGGATTGCCAAGCCCTGAAAAAGTACAAGCA  780
Homolog b Heera     GCATTGGAAAAGAAGTACGAAGAAAAAAATGGATTGCCAAGCCCTGAAAAAGTACAAGCA  780

Homolog b Varuna    CGACAAAAAAAGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA 822
Homolog b Heera     CGACAAAAAAAGAAAGCAGGAGTTGGTTTTGGCAAACGTTGA 822
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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    VKRVSRKEDEIASASDLQFETPLKIVEYPDPILRAKCKRIGVFDEHLKNLADAMFDVMYK  120
Homolog b Heera     VKRVSRKEDEIASASDLQFETPLKIVEYPDPILRAKCKRIGVFDEHLKNLADAMFDVMYK  120

Homolog b Varuna    TDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPGIY  180
Homolog b Heera     TDGIGLSAPQVGLNVQLMVFNPAGESGEGEEIVLVNPKINKYSDKLVPFNEGCLSFPGIY  180

Homolog b Varuna    ADRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIREELE  240
Homolog b Heera     ADRPQSVKIDARDITGARFSISLSRLPARIFQHEYDHLEGVLFFDRMTDDVLDTIREELE  240

Homolog b Varuna    ALEKKYEEKNGLPSPEKVQARQKKKAGVGFGKR        273
Homolog b Heera     ALEKKYEEKNGLPSPEKVQARQKKKAGVGFGKR        273
```

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