

BnaC01.BIN2 and BnaC03.BIN2 modulate plant height and yield potential in *Brassica napus*

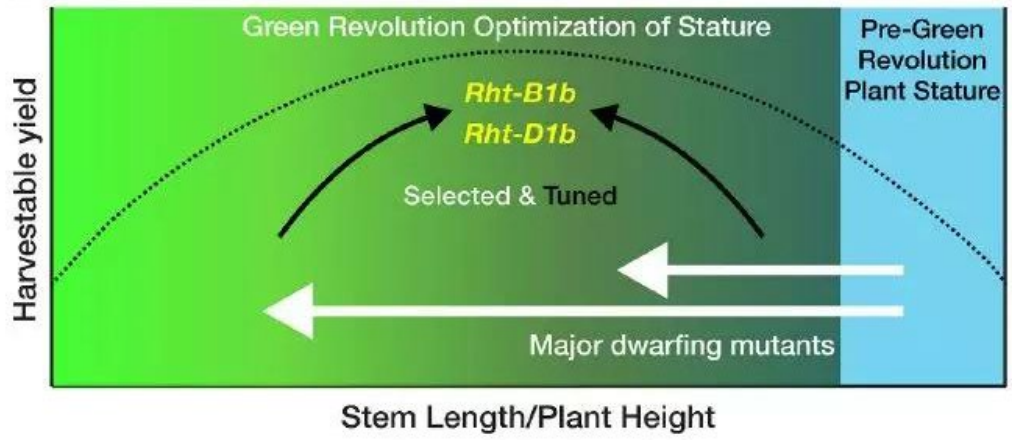
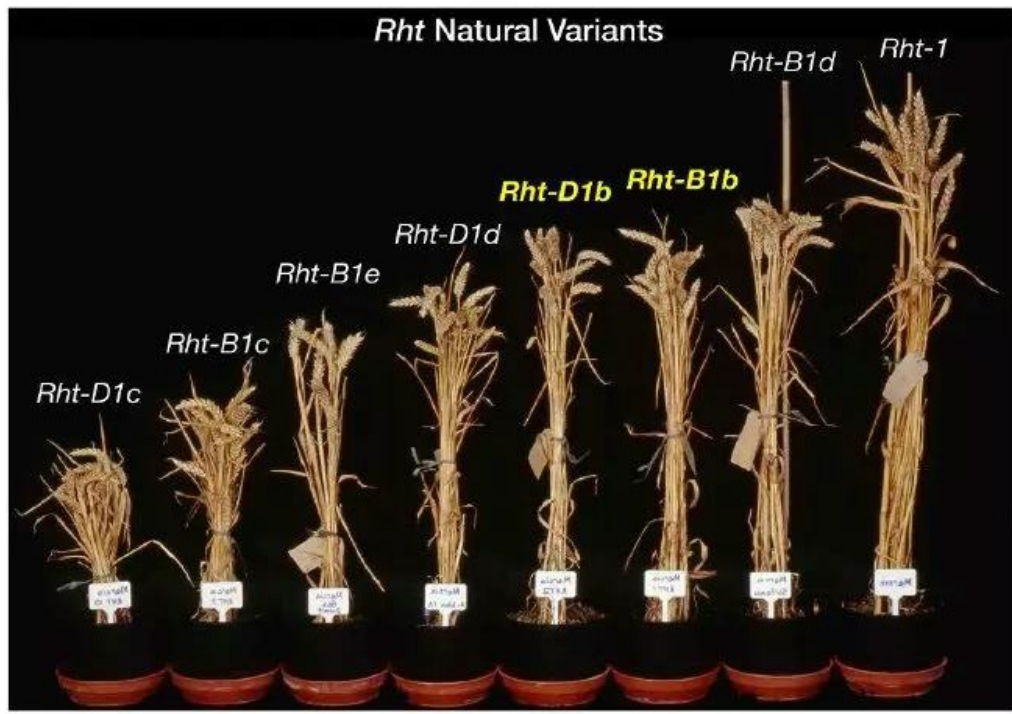
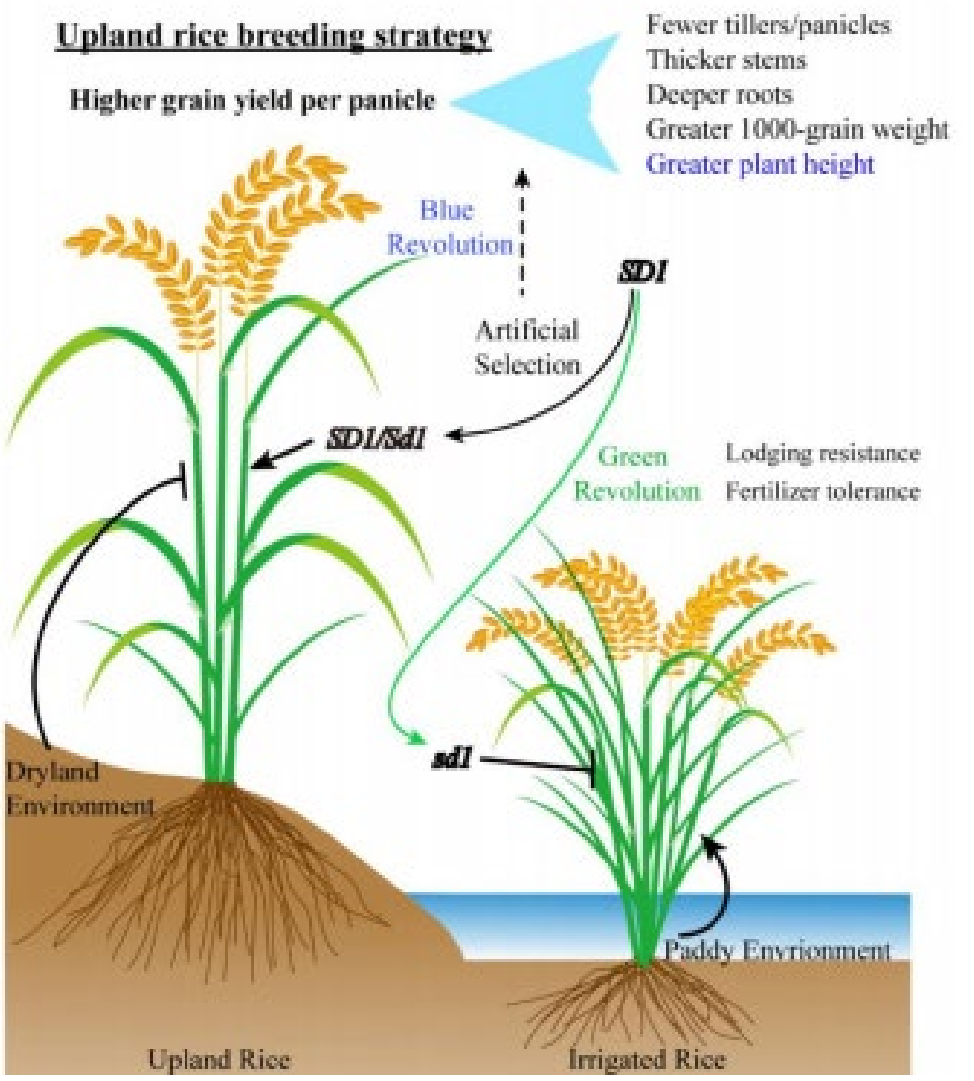
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Sydney, 2023

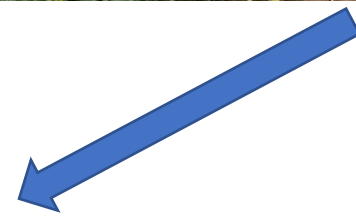
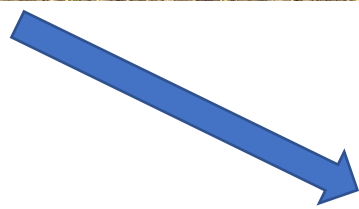


Background

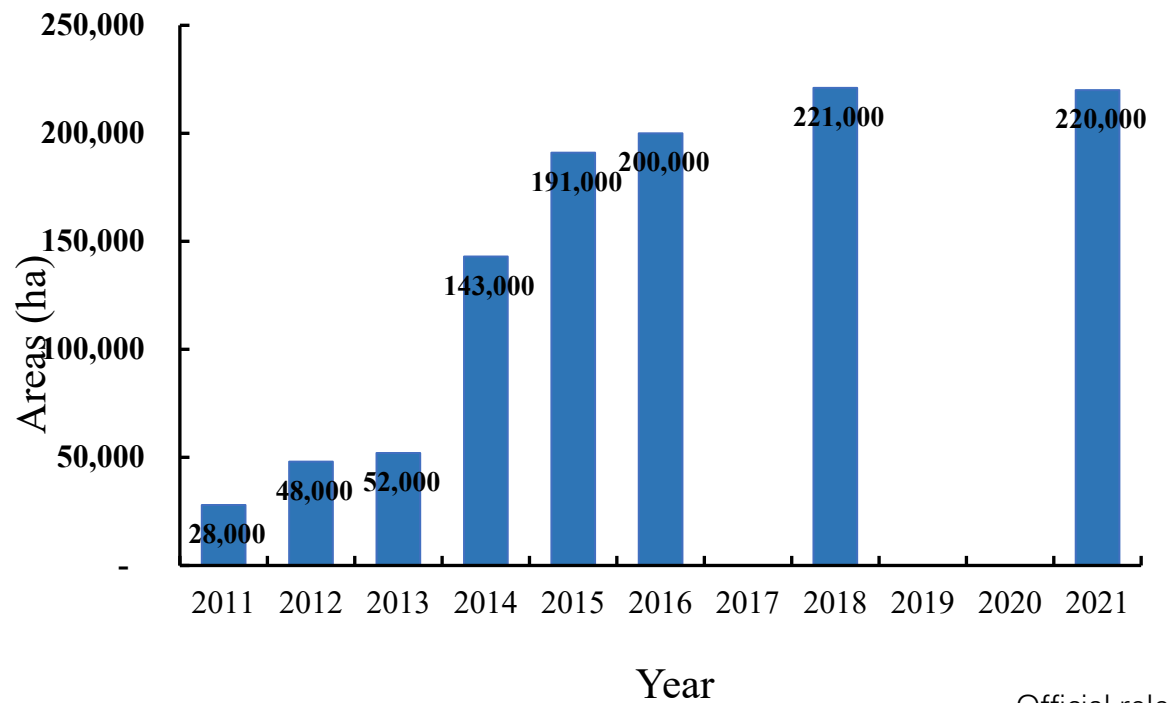
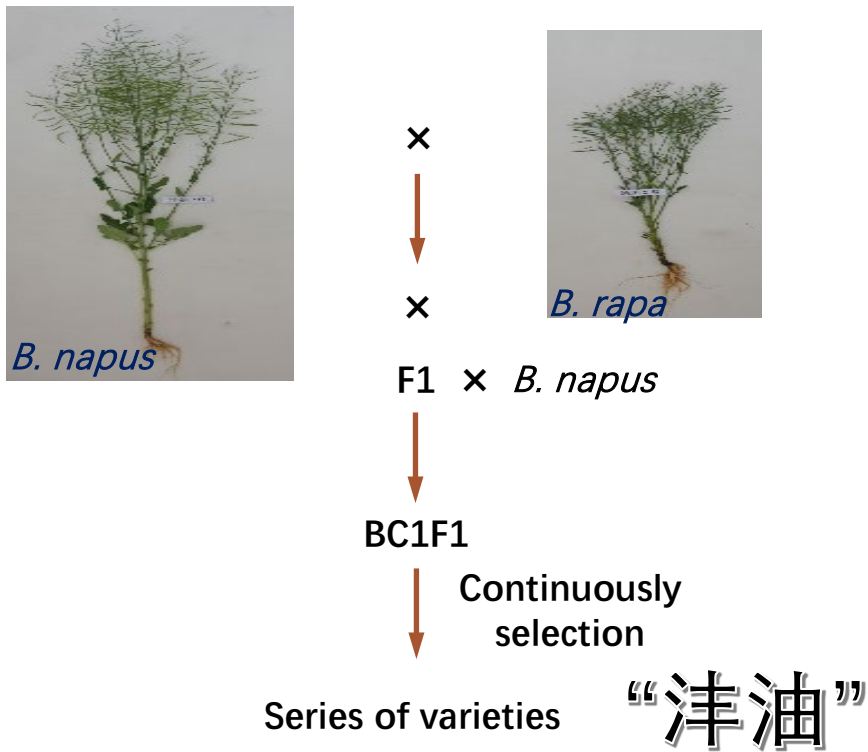


Introduction of semi-dwarf gene

Background



**Lower plant height
Adapt to mechanisation**



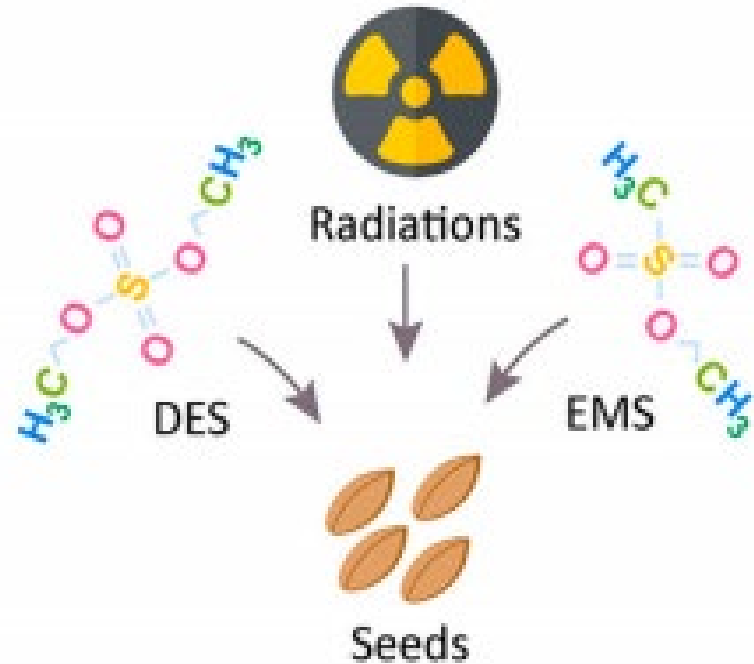
Official released data

Table 1 2008-2018 Plant height from National Regional trials

Traits\Varieties	QY7	QY10	ZYZ2	HYZ12	FY737
Plant height (cm)	168.3	170.3	168.7	182.8	154.2
Branch Initiation height (cm)	47.7	59.3	74.3	81.2	52.6
Main inflorescence length (cm)	61.3	66.4	58.9	59.9	53.1

New era of breeding program

Chemical and Physical Mutagenesis



Library construction of EMS-induced mutants

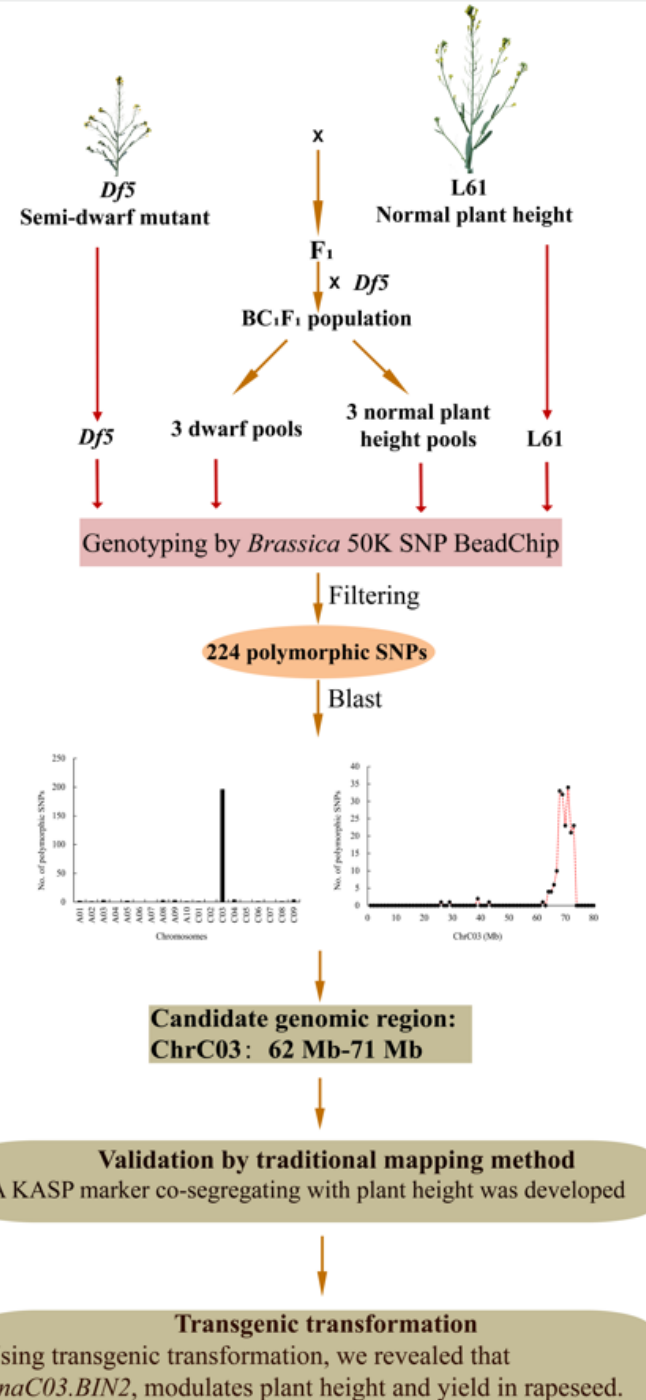
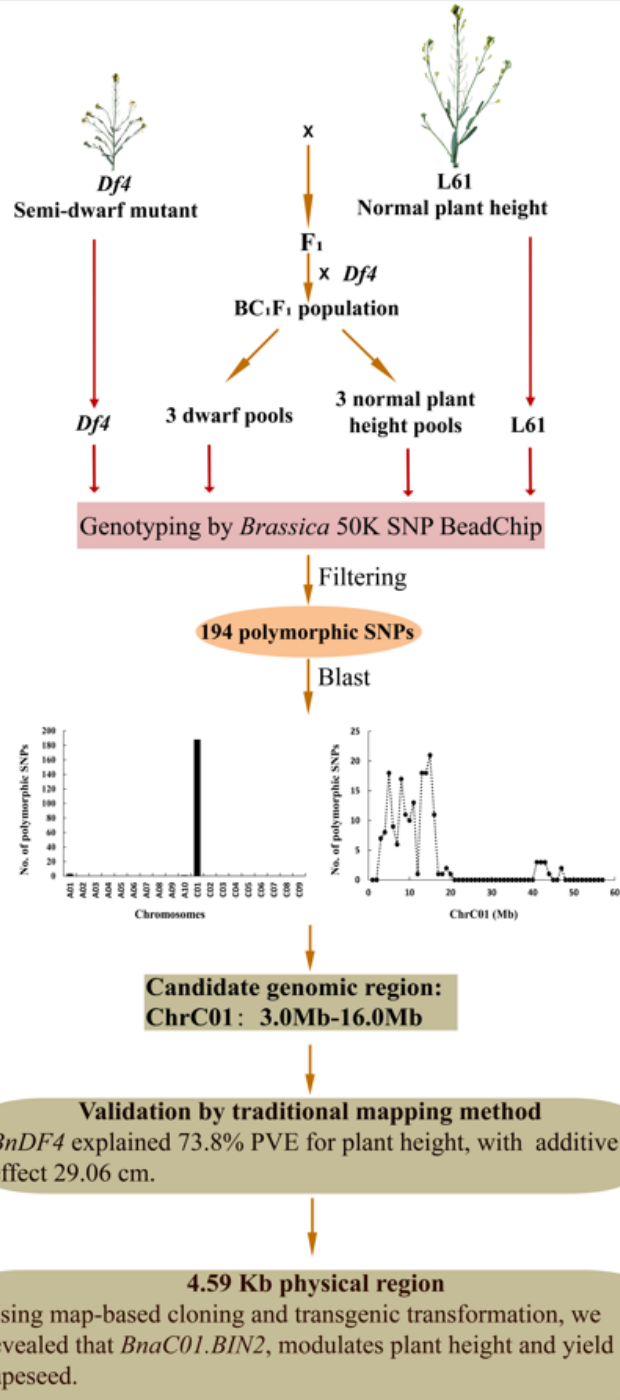
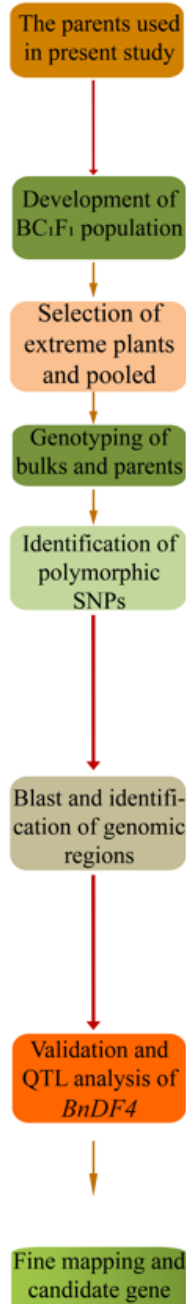


Df 4
Df 5

Are these mutants useful for breeding in rapeseed?

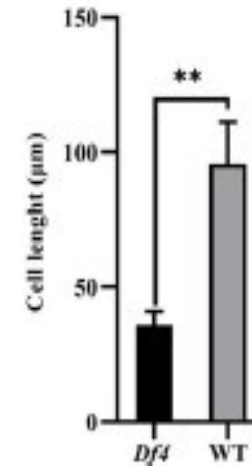
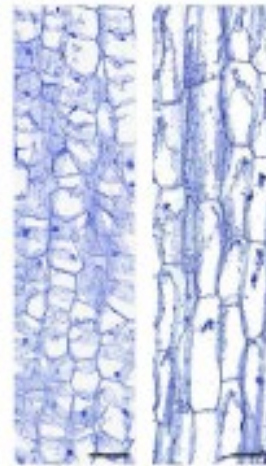
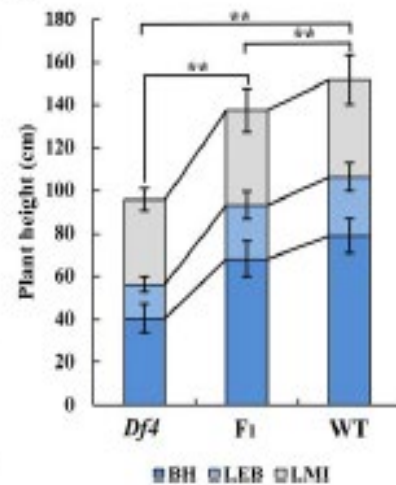
Methods

Gene Location Strategy



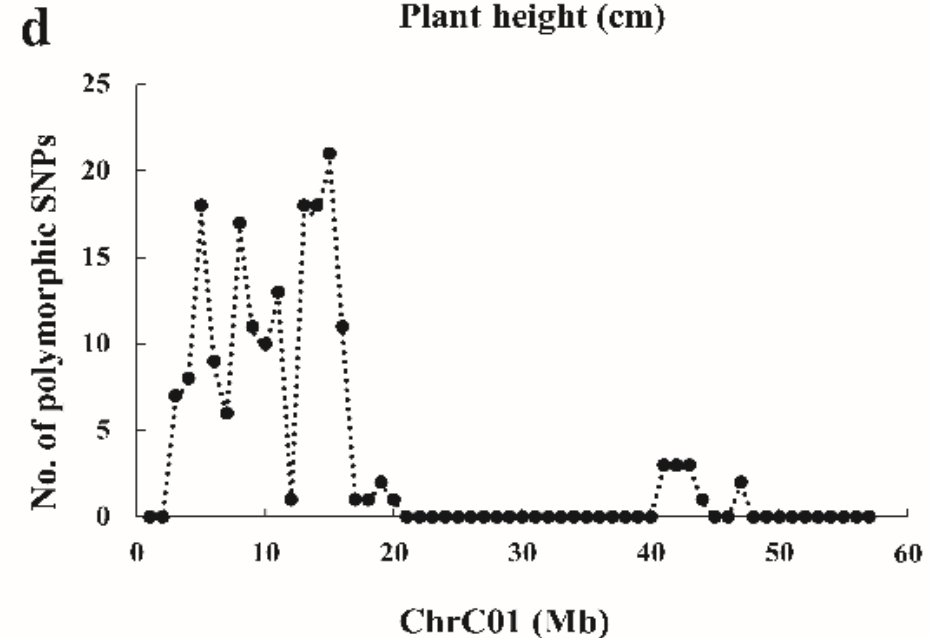
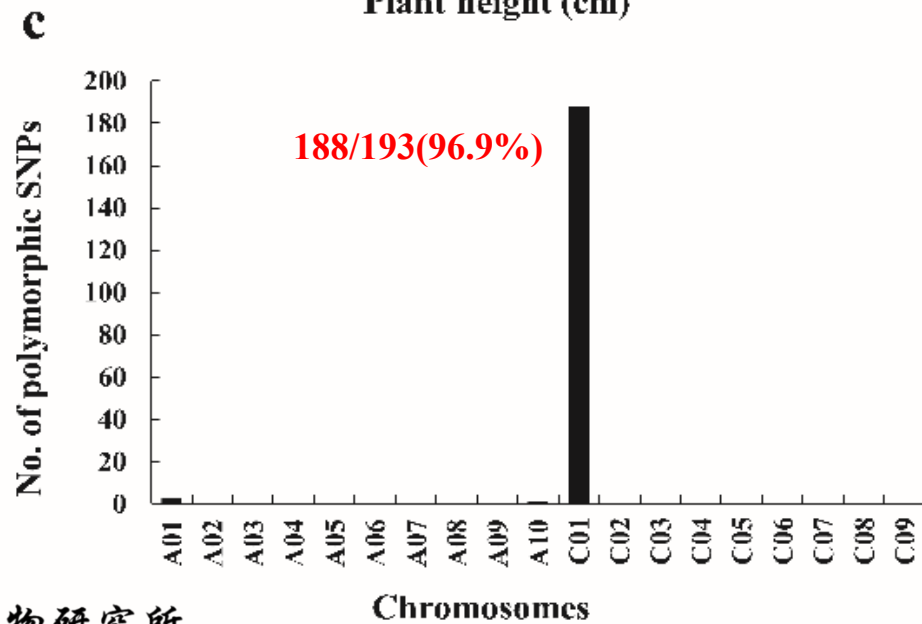
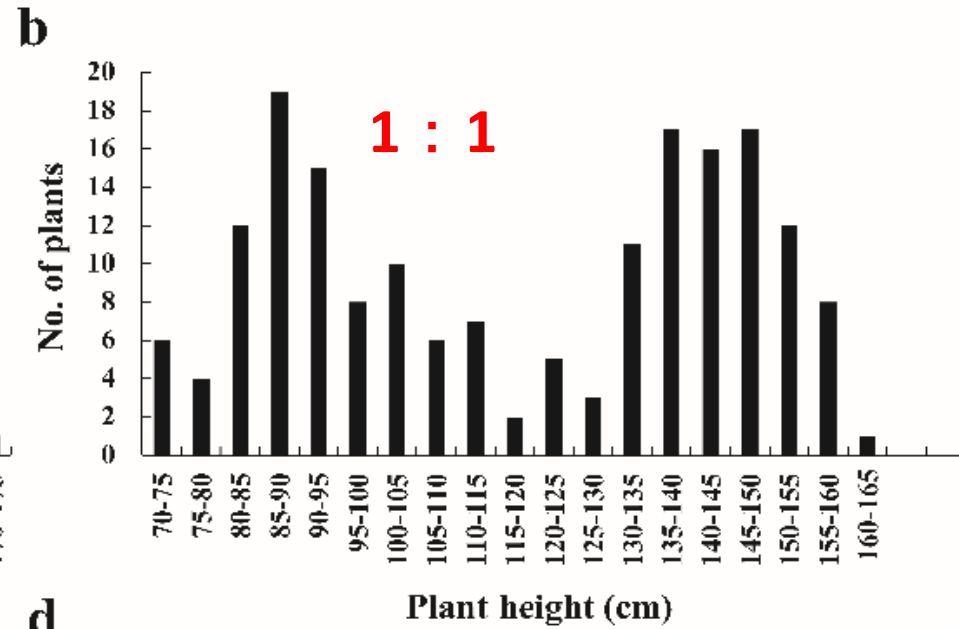
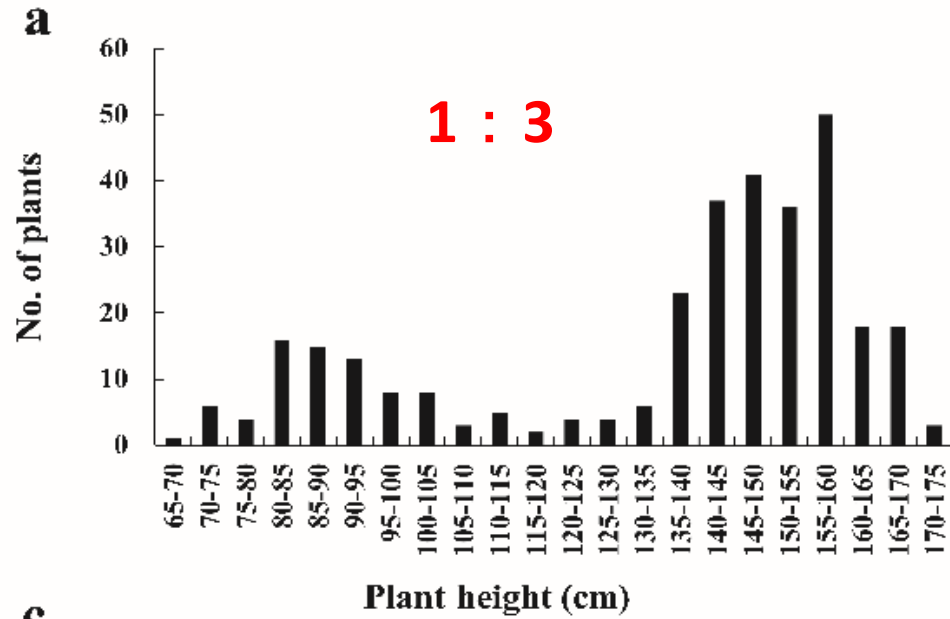
Results

Characterization of *Df4*



- Shorter stature and curled leaves.
- Stem cell elongation was suppressed.

Genetic analysis and cloning of *Df4*



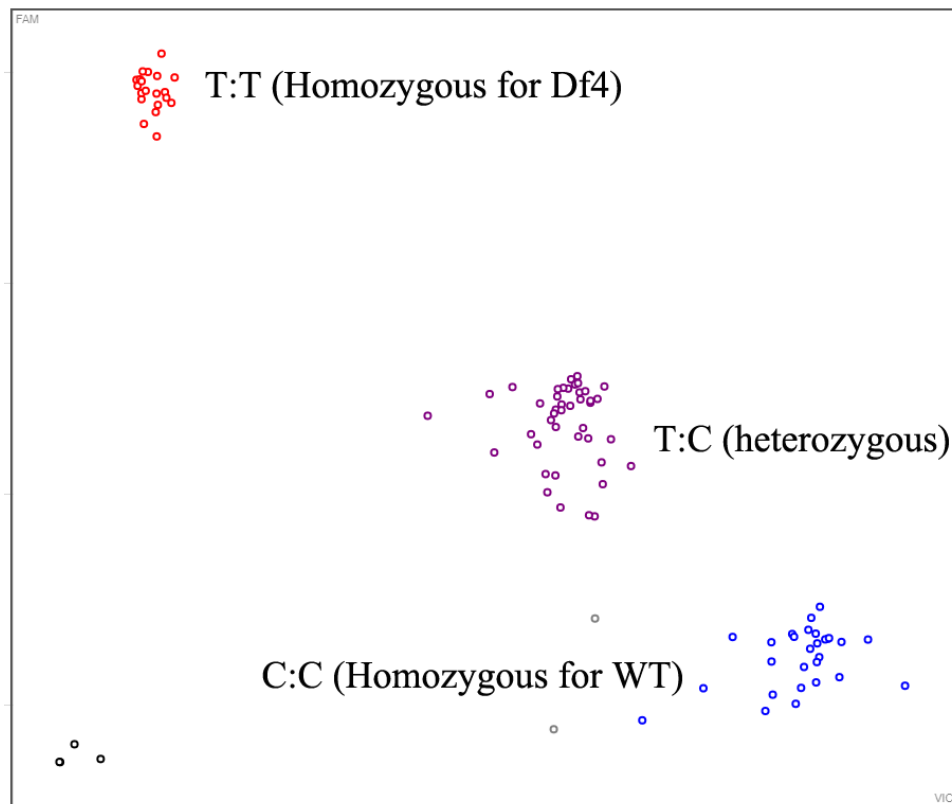
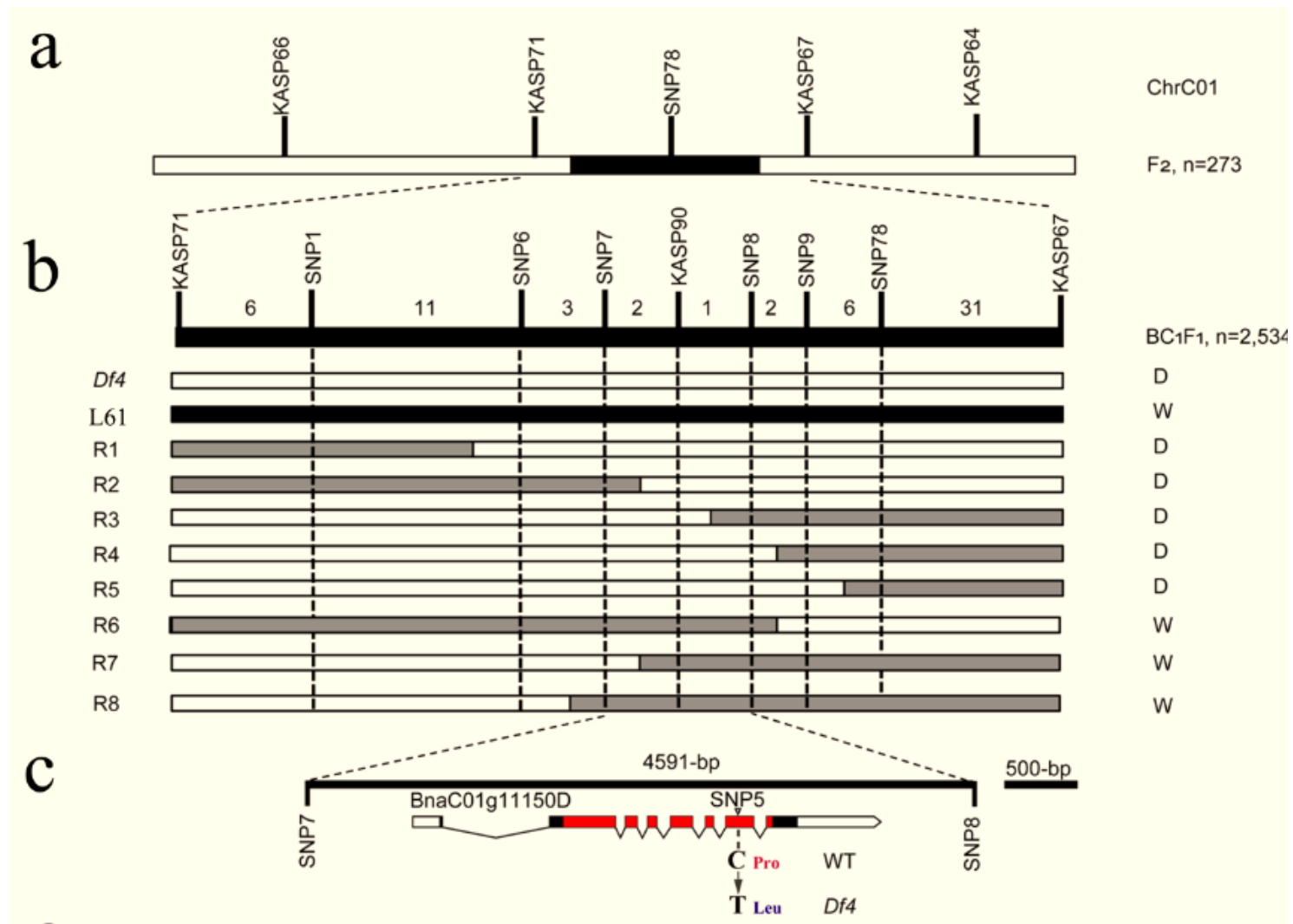


Table S1 Chromosomal locations and primer sequences for the KASP markers used in this study

Marker	SNP position			Genotype			Primers		
	Chr	Darmor	ZS11	DW2	SC4	Westar	Primer Allele X	Primer Allele Y	Primer Common
KASP66	chrC01	3328364	3687335	AA	GG	GG	GAAGGTGACCAAGTTCA TGCTGAGCTGAGCGTAA TAGGTAAGCATAGC	GAAGGTCGGAGTCAACGG ATTGGAGCTGAGCGTAAT AGGTAAGCATAGT	TTTGCCTTAGTTGATCCGT GAGTGTT
KASP71	chrC01	6375324	7593571	AA	GG	GG	GAAGGTGACCAAGTTCA TGCTGTTACAGGGCAG ACGACAATG	GAAGGTCGGAGTCAACGG ATTGTTTACAGGGCAGA CGACAATA	TCACCACTATGTCACATT TATTACTTTGG
KASP90	chrC01	6945960	8086086	TT	CC	CC	GAAGGTGACCAAGTTCA TGCTCCGAGATAAAGGCCA CATCC	GAAGGTCGGAGTCAACGG ATTCCGAGATAAAGGCACA TCT	GCAGCCTTGATGCAAAAT CAATG
KASP78	chrC01	7032414	8177018	GG	AA	AA	GAAGGTGACCAAGTTCA TGCTTGAAATGTGCTTCC TTGTGCGAA	GAAGGTCGGAGTCAACGG ATTTGAAATGTGCTTCCCTT TGTCGAG	ATATCAAACCGTTGTCC AGAAAGAG
KASP67	chrC01	7322793	8509890	CC	AA	AA	GAAGGTGACCAAGTTCA TGCTGCAGAAAAGTTCCG TACTCCCAGTTAAGT	GAAGGTCGGAGTCAACGG ATTGCAGAAAAGTTTCGTA CTCCCAGTTAAGG	GTTGTAICTGAAATCTGT CGGCTCTG
KASP64	chrC01	16248674	19850779	GG	AA	GG	GAAGGTGACCAAGTTCA TGCTAATCACTCAAAGG TTGAAAAGAATCCA	GAAGGTCGGAGTCAACGG ATTTCACTCAAAGTTGA AAAGAATCCG	TAATTCAAGATTCATCC GTTTCTCG

- Development of kompetitive allele-specific PCR marker (KASP) and SNP for high-throughput genotyping



- Targeted gene was located within 4,591bp region by linkage analysis using 2,534 individual plants from BC₁F₁ population.
- Only one gene was found with one mutation site, from Cytosine (C) to Thymine (T).

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      *      20      *      40      *      60      *      80      *      100      *
Df4   : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
WT    : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
ZS11  : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
Westar : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
Tapidor : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
Darmor : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
no21217 : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
B.oleracea : MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV : 115
MADDREMPAAVVDGHDQVTGHIISTTIGGKNGEPKQTI SYMAERVVGTGSFGIVFQAKCLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLV

      120      *      140      *      160      *      180      *      200      *      220      *
Df4   : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
WT    : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
ZS11  : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
Westar : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
Tapidor : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
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no21217 : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
B.oleracea : MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC : 230
MEYVPESLYRVLKHYSTANQRMPLVYVKLYMYQIFRGLAYIHNVAGVCHRDLKPQNLLVDPLTHQVKICDFGSAKQLVKGEANISYICSRFYRAPELIFGATEYTTSDIWSAGC

      240      *      260      *      280      *      300      *      320      *      340
Df4   : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
WT    : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
ZS11  : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
Westar : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
Tapidor : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
Darmor : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
no21217 : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
B.oleracea : VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF : 345
VLAELLGQPLFPGENAVGQLVEI IKVLGTPTRREEIRCMNPHYTDFRFPQIKAMPVHKKI FHKRMPPEAIDFASRLLOYSPSLRACTALEACAHFFDELREP NARLPNGRPFPPPLF

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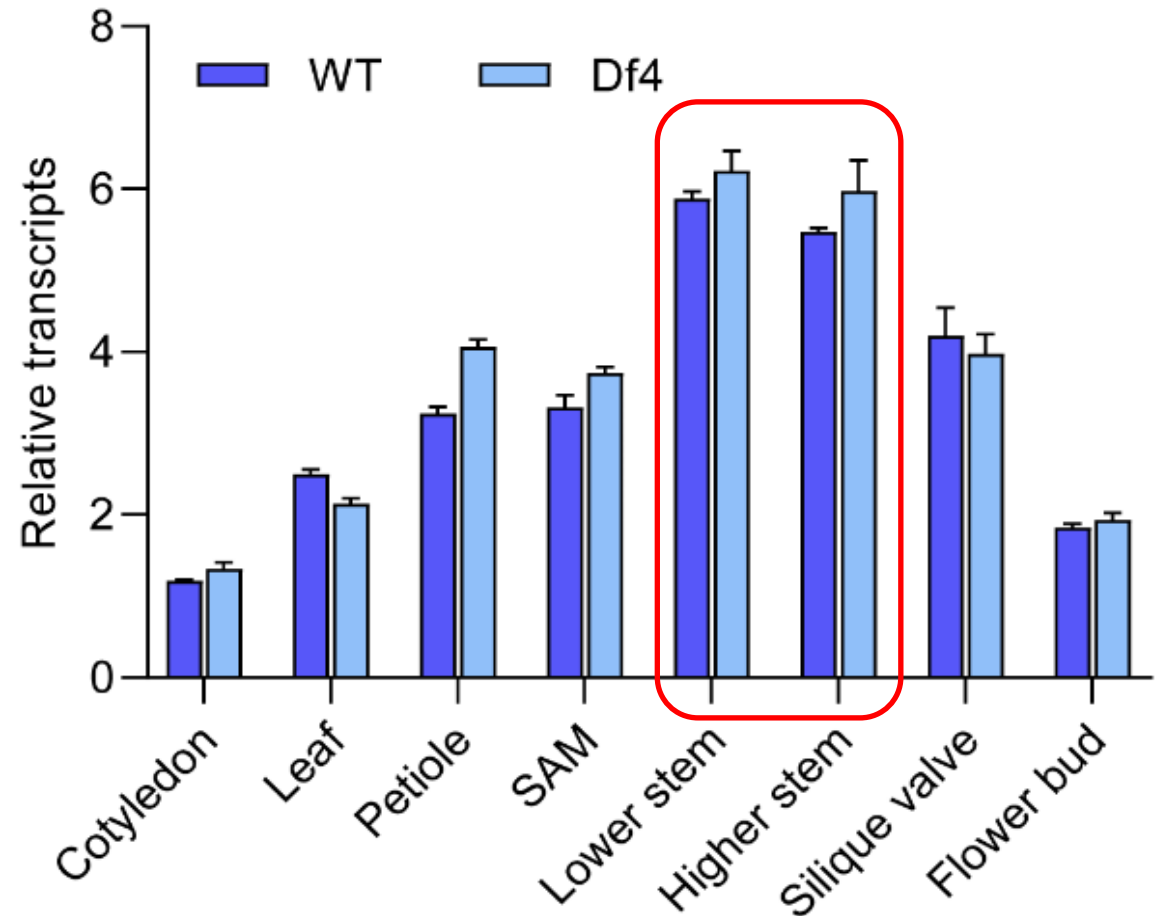
- BnaC01g11150D (Darmor-*bzh* reference), encodes a glycogen synthase kinase (GSK3) protein, similar to *Arabidopsis* BIN2;
- Pro to Leu at the 285 point site.

285
▼

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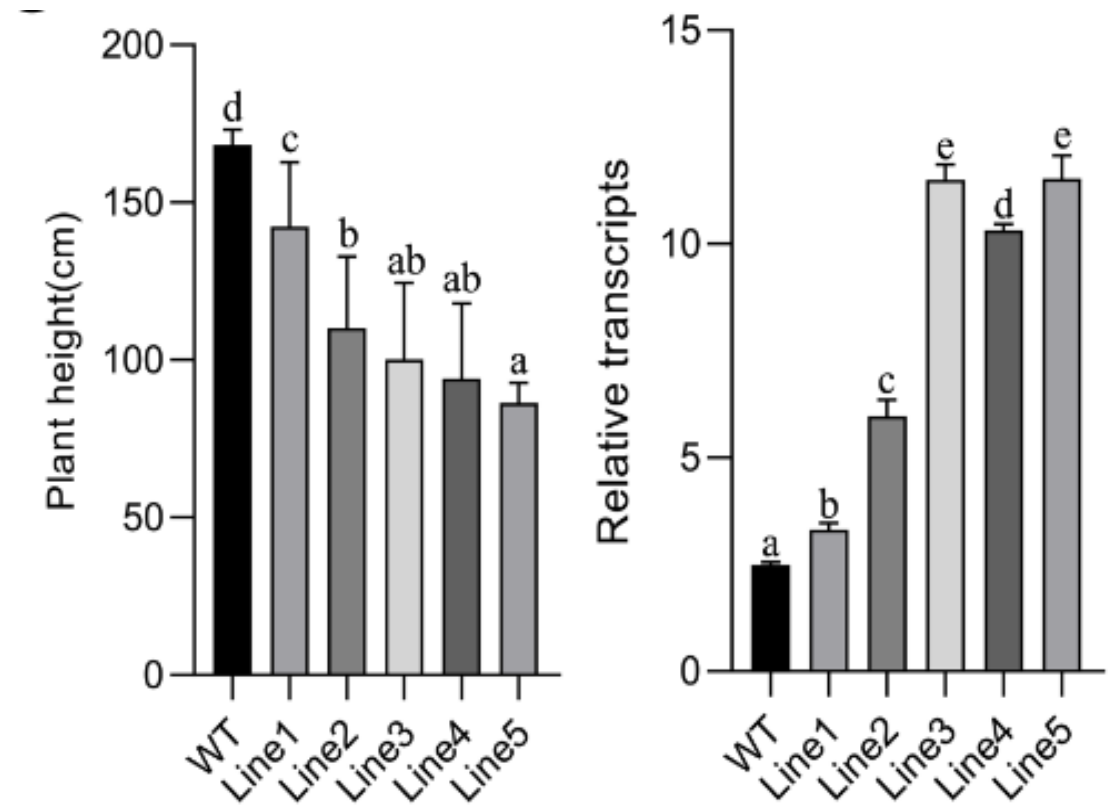
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BnaC01.BIN2-WT: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Bn_BnaA01.BIN2: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Bl_BloC01.BIN2: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Br_BraA01.BIN2: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
At_BIN2:       CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Rs_XP018483190: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Al_XP002870026: CMNPHYTD FRFPQ IKAHPWHKIFHKRMPPEAIDF
Os_KAB8080:     CMNPNYTEF KFPQ IKAHPWHKIFHKRMPPEAIDL
Cn_EHA8591:     CMNPNYTEF RFPQ IKAHPWHKIFHKRMPPEAIDL
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Av_KFP84225:   EMNPNYTEF KFPQ IKAHPWTKVFRPRTPEAIAL
Ce_AAD45354:   SMNPNYKEF KFPQ IKAHPWNKVF RVHTPAEAIDL
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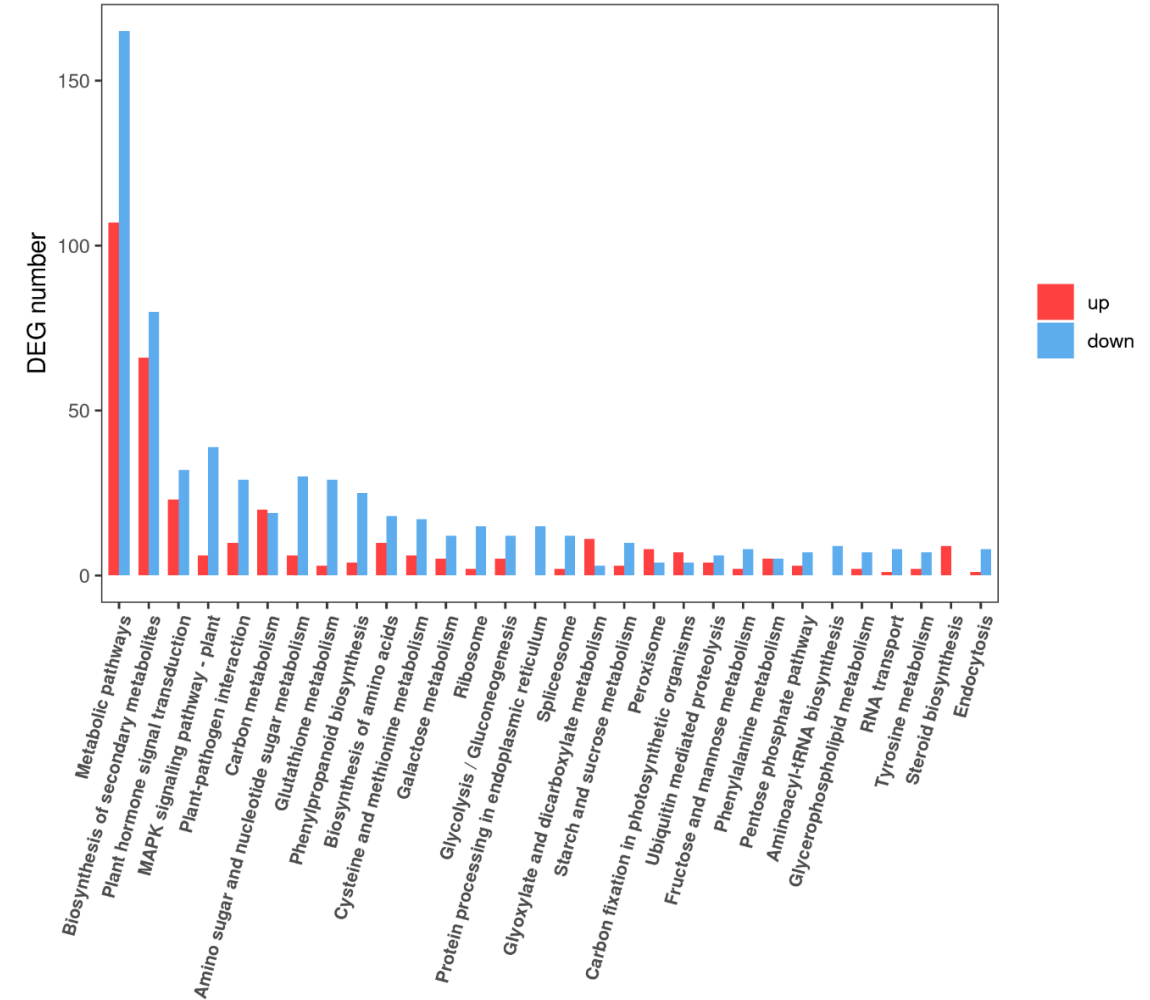
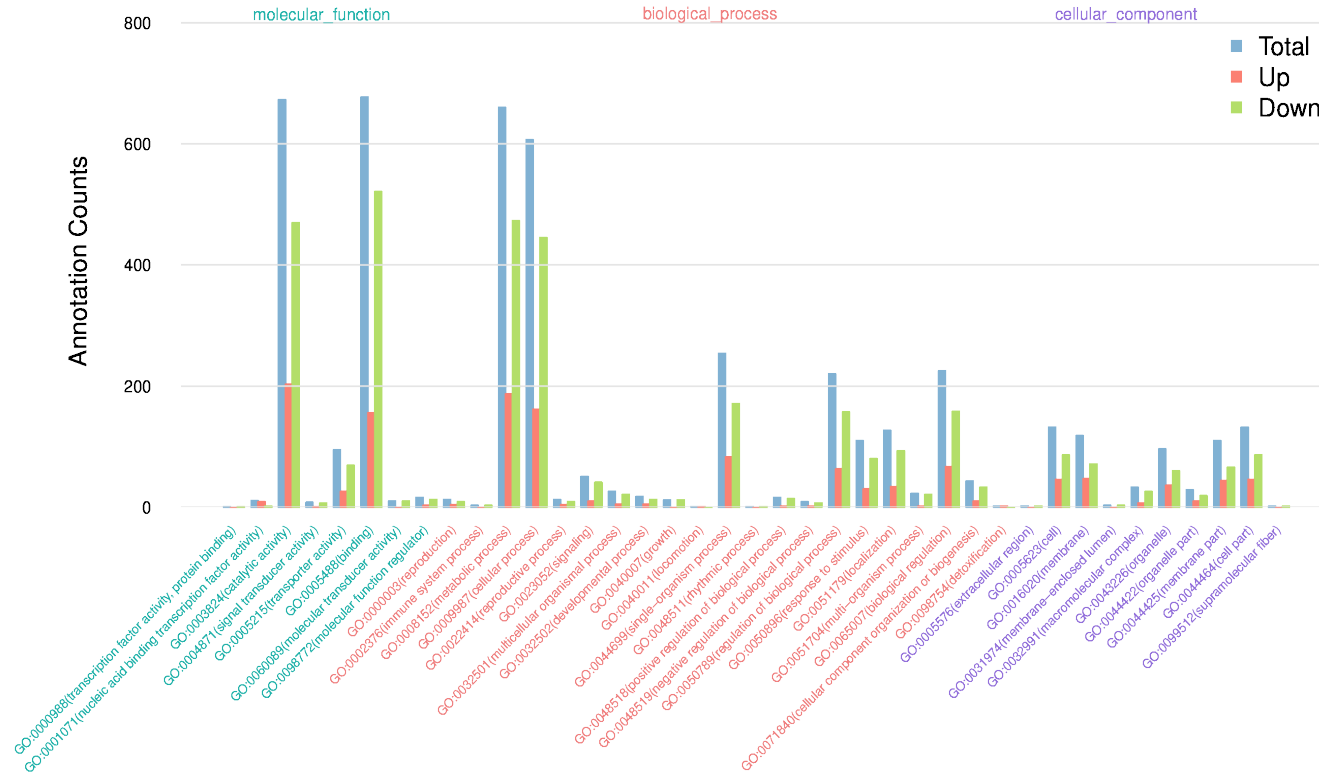
- Highly conserved;
- High in expression in stem, especially the lower stem;
- Named *BnaC01.BIN2*.

Transgenic validation of *BnaC01.BIN2*



- Transgenic results validated gene function of *BnaC01.bin2*, with 27/34 showed dwarfism in T1, ranking 20-80%;
- Plant heights were negatively correlated with relative transcripts of *BnaC01.bin2* in different transgenic lines.

Transcriptome analysis of *Df4*



- 2,298 DEGs obtained between *Df4* and L74;
- 719 (31%) were up-regulated, 1579 (69%) were down-regulated;
- Catalytic activity, transporter activity, metabolic pathways, etc were most significantly enriched.

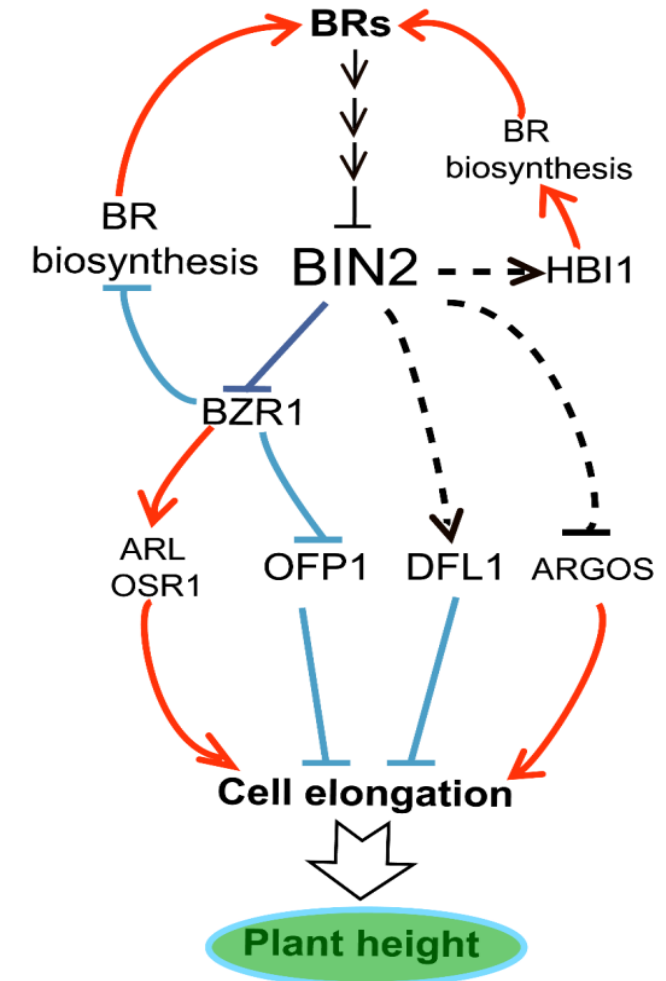
Table 11 Expression changes for cell elongation related genes

Name	AT Locus ^a	BN Locus ^b	log ₂ FoldChange ^c	P-value	Short description
ARL	AT2G44080	BnaC03T0254100ZS	-2.8894	2.03E-07	Positively regulates
		BnaC04T0598300ZS	-1.1149	0.000159	shoot cell elongation
		BnaA03T0215500ZS	-3.1049	5.43E-07	(Hu et al. 2006)
		BnaC04T0046200ZS	-3.3715	8.1E-09	
		BnaA05T0042400ZS	-3.5692	3.52E-11	
ARGOS	AT3G59900	BnaA07T0210000ZS	-2.3772	4.27E-10	Positively regulates
		BnaC06T0221400ZS	-1.3762	7.83E-05	shoot cell elongation (Hu et al. 2003)
DFL1	AT5G54510	BnaC03T0145500ZS	1.2135	2.01E-05	Negatively regulates
		BnaA02T0132700ZS	1.5205	1.27E-22	shoot cell elongation
		BnaC02T0166500ZS	1.6982	1.06E-15	(Nakazawa et al. 2001)
ORS1	AT2G41230	BnaC03T0238200ZS	-1.9082	0.001754	Positively regulates
		BnaC04T0580700ZS	-3.3046	3.18E-05	shoot cell elongation
		BnaA04T0263600ZS	-3.1056	1.16E-05	(Feng et al. 2011)

- GO and KEGG analysis revealed Brassinosteroid (BR) regulation related pathways;
- Included genes relate to cell elongation, such as Auxin-regulated Gene involved in Organ Size(ARGOS)-like(ARL), organ Size Related 1 (OSR1) and Dwarf in Light1 (DFL1) .

Table 5 Expression changes for ethylene and WRKY related genes

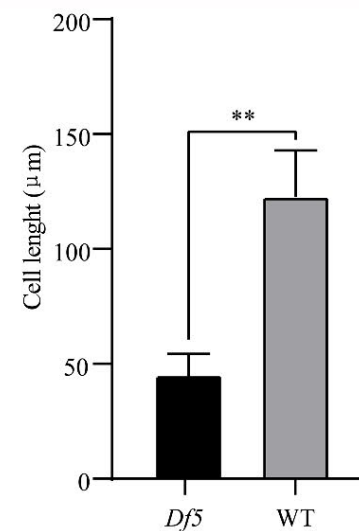
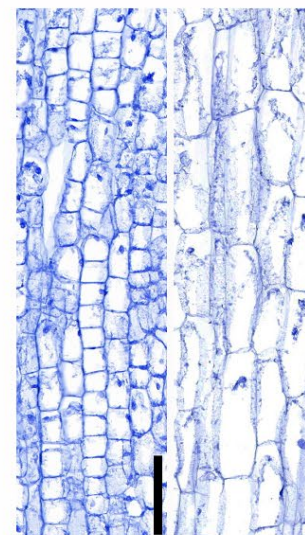
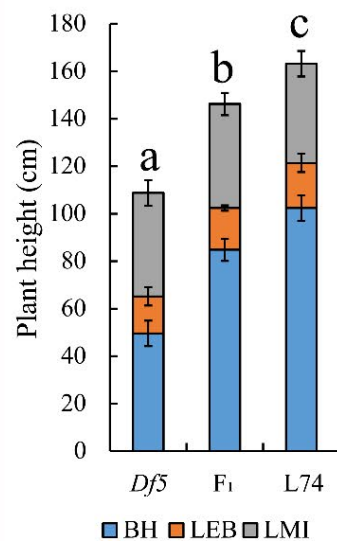
Name	AT-Locus ^a	BN-Locus ^b	log ₂ FoldChange ^c	P-value ^d	Short description ^e
<i>BR6OX1</i>	AT5G38970	BnaC04T0421500ZS	2.82	1.60E-07	BR biosynthesis
		BnaA04T0132600ZS	1.28	7.80E-05	(Shimada et al. 2003)
<i>BR6OX2</i>	AT3G30180	BnaC07T0322400ZS	1.30	1.30E-10	BR biosynthesis
		BnaA06G0369900ZS	1.13	0.00354	(Shimada et al. 2003)
<i>DWF4</i>	AT3G50660	BnaA09T0472400ZS	1.33	6.00E-05	BR biosynthesis
		BnaC08G0308400ZS	1.46	0.00018	(Choe et al. 1998)
<i>CPD</i>	AT5G05690	BnaA02T0020500ZS	1.15	6.80E-06	BR biosynthesis
<i>CYP90D1</i>	AT3G13730	BnaA05T0414100ZS	2.55	1.10E-26	BR biosynthesis (Kim
		BnaA01T0357900ZS	1.90	7.30E-13	et al. 2005a)
		BnaC01T0445000ZS	1.91	3.70E-12	
		BnaC05T0466200ZS	2.06	3.10E-14	
<i>HBI</i>	AT2G18300	BnaA06T0312700ZS	1.82	3.20E-08	Positively Regulates
		BnaA07T0020400ZS	1.39	1.80E-06	BR biosynthesis(Cai
		BnaC09T0120000ZS	1.80	1.20E-07	et al. 2021)
		BnaC07T0041100ZS	1.69	1.70E-12	
		BnaC03T0510600ZS	1.65	2.10E-06	

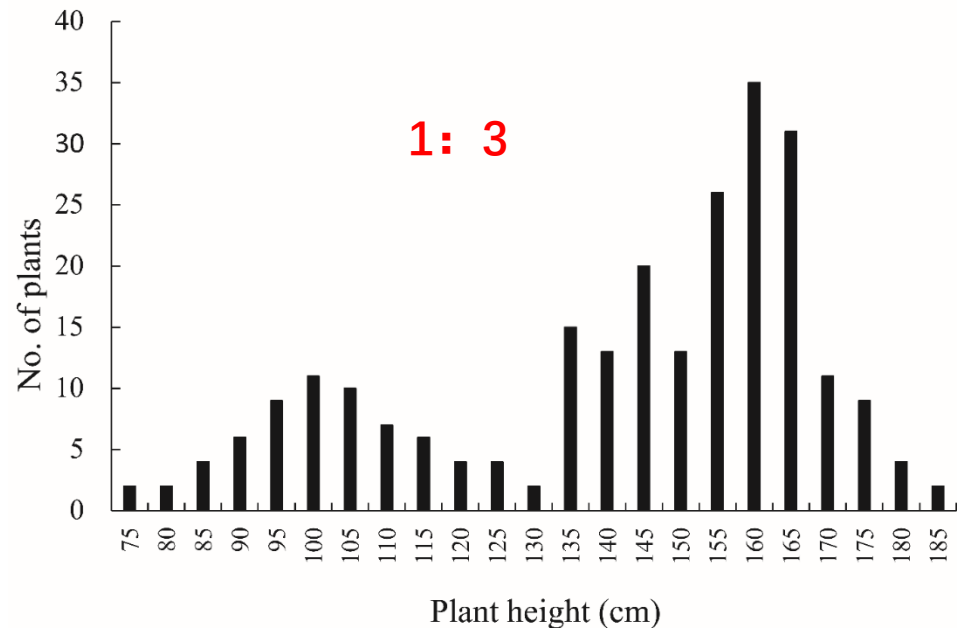


Working model of the BIN2 function in cell elongation and BR biosynthesis.

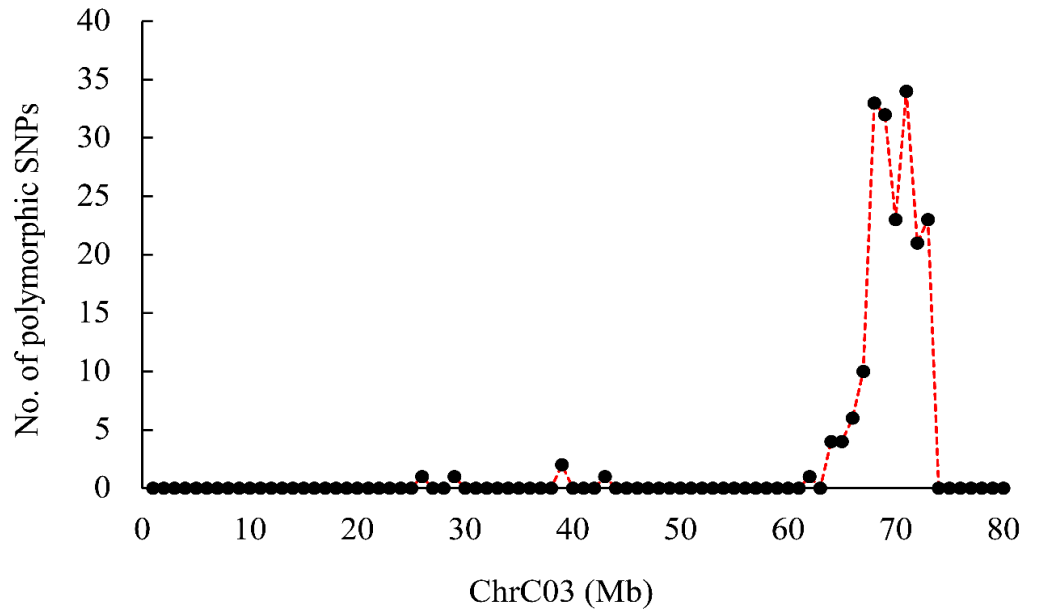
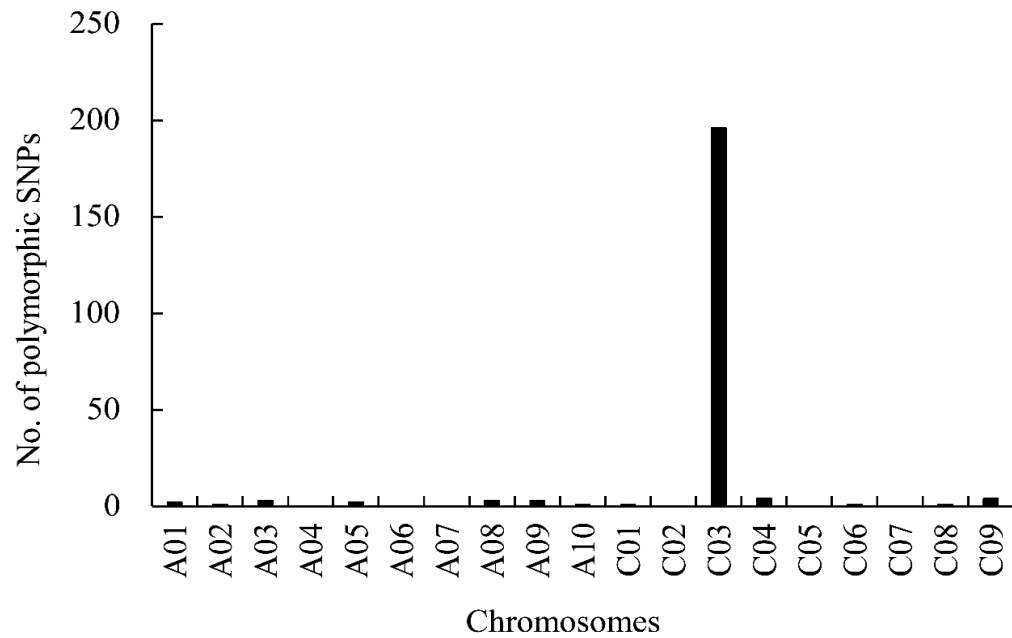
- BnDF4 encodes a GSK-like kinase similar to BIN2 in Arabidopsis, and is a key repressor of BR signaling

Characterization of *Df5*





- Controlled by an incomplete dominant gene;
- 196 out of 234 polymorphic SNP (87.5%) were located in C03 62-71Mb



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*      20      *      40      *      60      *      80      *      100     *      120     *
Df5      : --MADDKEVP-AAVVNGHDQVTGHIISTTIGGKNGEPKQTSYMAERVVGTGSFGIVFQAKLETGETVAIKKVLQDRRYKNRELQLMRVMDHPNVVCLKHCFFSTTSKDELFLNLVMEYVPESLYRVLKHY : 129
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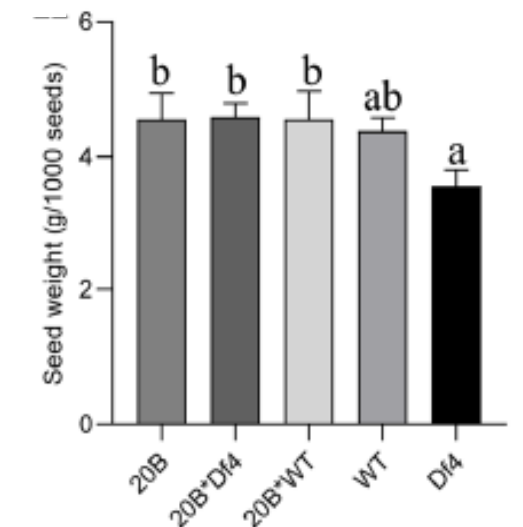
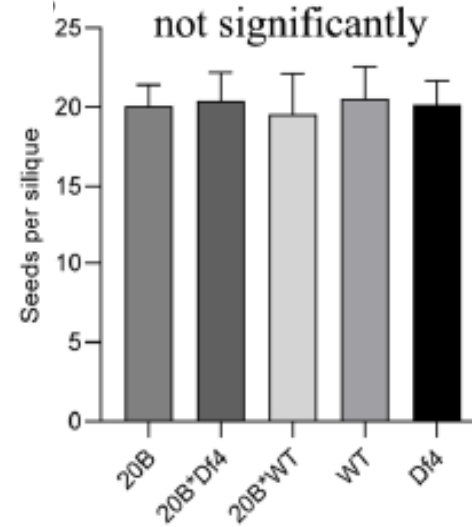
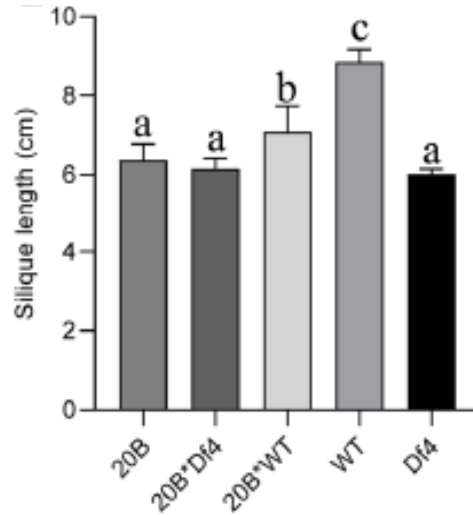
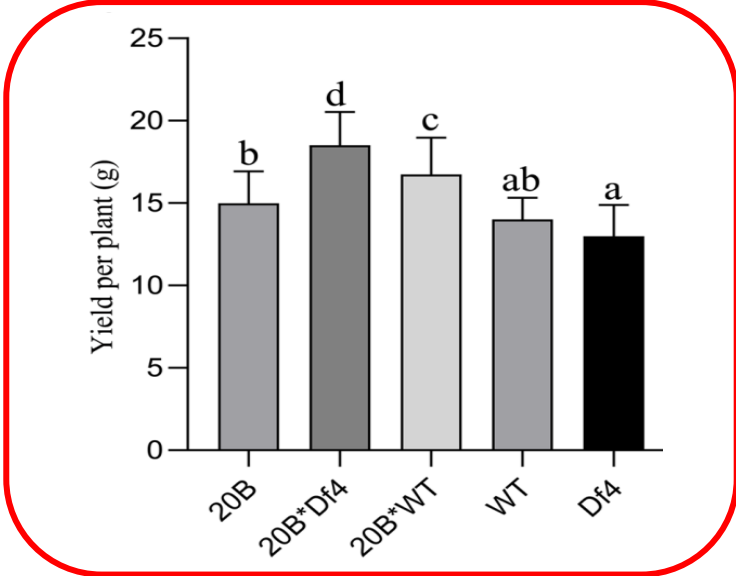
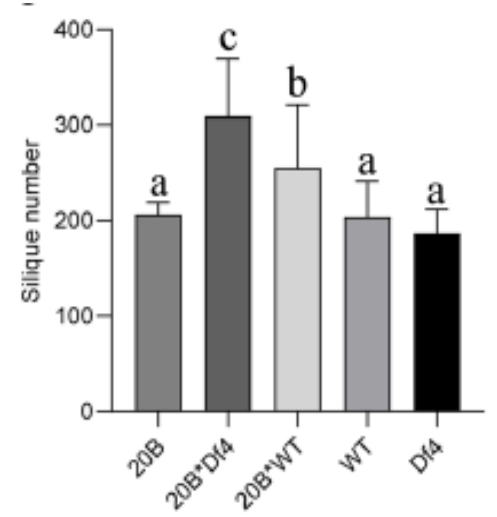
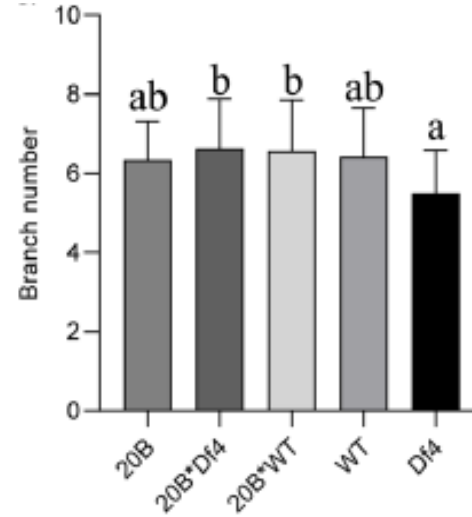
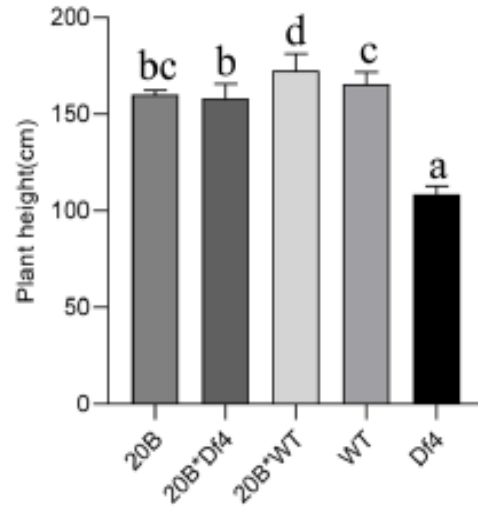
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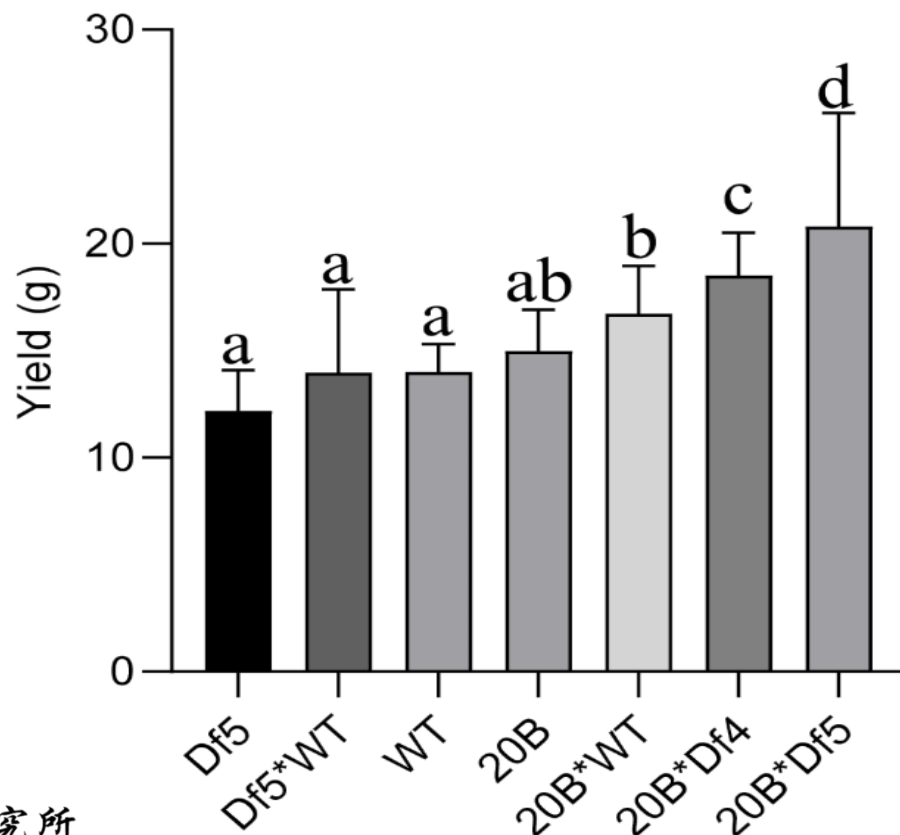
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- There are 6 copies of BIN2 in *B. napus*;
- *BnaC03.BIN2* and *BnaC01.BIN2* were the most homologous pair;
- Similar mutation point was observed in *BnaC03.BIN2* of *Df5*.

Can these apply to breeding program?





- Reduced in plant height, not affected in branch number, total plant silique number and seed number per pod;
- Significant higher yield in terms of hybrid crossing compared to *df4*.

Summary

- *Df4* and *Df5* are both gain-of-function mutants, homologous mutant genes;
- *BnaC01.BIN2* and *BnaC03.BIN2* have involved in BR signaling related regulation;
- Over parental heterosis in both *Df4* and *Df5* ;
- KASP markers were developed for both *Df4* and *Df5* for high-throughput identification for large-scale selection for breeding;
- More environments are needed for yield potential testing.

Acknowledgements





Thank you!

