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OPEN Joint genome-wide association and transcriptome sequencing reveals a complex polygenic network underlying hypocotyl elongation in rapeseed (Brassica napus L.)

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Hypocotyl elongation is considered an important typical seedling trait contributing directly to an increase in and stabilization of the yield in Brassica napus, but its molecular genetic mechanism is poorly understood. In the present study, hypocotyl lengths of 210 lines were measured in an illuminated culture room. A genome-wide association study (GWAS) was performed with 23, 435 single nucleotide polymorphisms (SNPs) for hypocotyl length. Three lines with long hypocotyl length and three lines with short hypocotyl length from one doubled haploid line (DH) population were used for transcriptome sequencing. A GWAS followed by transcriptome analysis identified 29 differentially expressed genes associated with signif cant SNPs in *B. napus*. These genes regulate hypocotyl elongation by mediating f owering morphogenesis, dircadian dock, hormone biosynthesis, or important metabolic signaling pathways. Among these genes, BnaC07g46770D negatively regulates hypocotyl elongation directly, as well as fowering time. Our results indicate that a joint GWAS and transcriptome analysis has signif cant potential for identifying the genes responsible for hypocotyl elongation; The extension of hypocotyl is a complex biological process regulated by a polygenic network.

(Brassica napus L., $2 \mathbf{p}_{q} = 4\mathbf{x} = 38$; $\mathbf{p}_{q} \in \mathbf{AACC}$) i \mathbf{p}_{q} R rn irn il in. n i xni r, i r, l Bailr, Bail-l i 1 IBAH ,1 P. ni n nin nin lin, i.Wll-l n, li ir, lin, i 1r 1岡 n, i i, min ln rir ili i 🖪 🛱 il i n ir, r i $1 \ 10$ r, , lini 🛱 l lin, i ill n, in, n in ir, P. ra in B. napus. li 🛌 1 m 1 i ni r, 169 n mi , , , i - 1 i². M 🖬 ili(QTL) lin, i in B. napus ni i mm n i QTL¹.S l miin, iindha miindhai Sail - 1 i∎∎B. napus⁶. min i n 1 , n, il p, rn rain i n 14ng GER1, AILP1, PECT, ng FBP l m n li r n i lra i iraB. napus⁷. Hiji i jil ira i rai n 🖾 🛱 i l li r n i n Arabidopsis. Gl iin, lr. H , ka ll i r 🙀 Balr, ir, lir. in lin ու թվենայն l i 巖 🛱 in, 🛱 mi 🛱 File i e¹¹. n i CIRCADIAN CLOCK ASSOCIATED1 (CCA1), EARLY FLOWERING3 (ELF3), ELF4, i n l 1 ۴. lin, i in,l By talx n, ita -rqi pq l 🛱 pjra, li l pq l LUX ARRHYTHMO (LUX) ind nd Balr, ir, il l 🕅 Ì 📭 i 📭 i Patra B. napus.

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Ex re (EST) re , Per Hair, rali re reire, re, re rex-re ire reire reli ire Hair rali rali (GWAS) re re ira refaire reli i ire ralx iirelre ^{14 21}. Ire re Ba GWAS Ballre ire i i rel 210 B. napus ire re Ba 23,435 SNP. W l ra reira reire ire re Ballre ire re a contra i i ire re reire ire Ballre ire reilire i iBall-l i i, i reire Ballre ire reire GWAS re reira refaire raGre Orel Ba(GO) re KEGG Bar Ballre i reire B. napus.

Results

 Phenotypic variations and correlation analysis. Ex
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 LD analysis. All 23,435 SNP ir
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SNP		С	e	Aee	Р	MAF	P-a e	R ² (%)
Bre	16110 1- 587456	C07		A/G	44303399	0.49	1.94E-06	4.82
Bre	16110 1- 670992	C07		G/A	44218661	0.50	7.25E-07	
Br	16110 1- 671315	C07		A/G	44218337	0.49	7.66E-07	
Br	16110 1- 685258	C07		A/G	44204592	0.50	6.87E-07	

C/A

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16110 1- 685428

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 BnaC07g46660D, BnaC07g46770D, r. BnaC07g47470D)
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 Six DEG (BnaC07g45590D, BnaC07g45710D, BnaC07g45560D, BnaC07g45560D, BnaC07g45710D, BnaC07g46560D, RaaC07g45660D, r. BnaC07g47470D)
 i.r. 30
 gT r. i.r.

 1aC07g46060D, BnaC07g46560D, BnaC07g46660D, F, BnaC07g46560D l li rir F, ir, F, Bra. F x fn l, BnaC07g46060D F, BnaC07g46560D l li rir F, i i Br, i ir, F, F, ri l, BnaC07g46060D F, BnaC07g47470D i i ir, BF BF i / l i BF fn , BnaC07g46060D F, BnaC07g47470D i i ir, BF BF i / l li nin r 🕅 P, r, r, l xi , n i n P. nyra lirany nyxinyiny piny i njra piny iny i piny Allxrainy iny ny xiny rany ny ny P. 1 1 ni. P. , ir, P,



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AT4G37750	l n	AP2/EREBP	P,	rnil	P.	i	169	1	阙	1	r (CCA1)	i

Materials and Methods

Population structure, relative kinship, and linkage disequilibrium. T link STRUCTURE 2.3.4⁵⁶ **P** 5,334 SNP i AA BB in, in, N rnixi i i i ∭25⁷. A , ₽, i i n ili mara i >0.7 i mara i maira la mara la cara i a cara i mara la cara i mara la cara i mara la cara i mara la cara i mara i mar i i<0.7 in raix lin i n ስበ ስበ 0^{59} li r

GWAS and statistical analysis. l i r, (Q, PC) r, ir, i (K) r, i

 l
 GWAS ir, rn l (GLM, Q, PCA, PCA+K, r, Q+K). Si ri r, l i i r, i

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 Nuclear RNA extraction and RNA sequencing.
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DEG identification and gene annotations. $l - li = r_{q}$, r_{q} , r_{q mir. i ni rlir. 🛱 🖪 l-**P** 64

References

1. B r, r, P. et al. C the i the ir, r, r, i i ilin, l ir, i lir, r, Fill ir, i ir, il (B i r, L.). TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik **120**, 271 281, i: 10.1007/00122-009-1133- (2010).

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- ra , V. et al. G 🖪 ra i i i na in 35 lini 🖬 l n in i (O 🖬 i). DNA Research 22, 133–145, i: 10.1093/ P, / 046 (2015).
- in in in (B i r, L.). DNA
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- 38. L, Y. et al. J in line line i ili i rara ir, i 1 ir, ni i il i 🖡 li 🛛 🖬 1 r. in the i Proceedings of the National Academy of Sciences of the United States of America 107, 19585 19590, i: 10.1073/ r .1006105107 (2010).
- i i ma in in, in ins n P. 39. C i, E. et al. C m in lin (H li 🖪 P.P. L.). TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik 126, 1337 1356, i: 10.1007/00122-013-2056-2 (2013).
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- , P. X. et al. A M li P. A P. G P. i P. IP. -C genetics 5, 1000551, i: 10.1371/ P. l. P. 1000551 (2009). n i i i in A i i li 🖡 . PLoS Pa -010
- 43. Wi, Letal. Gran i iran 🍘 ira i railx ir, r, 🎯 i i S 1 ir B i P. ini ¢η. **r**, *Plant Biotechnol J* **14**, 1368 1380, i: 10.1111/ i.12501 (2016).
- r, Y. et al. A r, IA i i MYB-li r, i i r, , MYBH, I F rn l i r, Journal of experimental botany 64, 3911 3922, i: 10.1093/x / 223 (2013). Sallen in Saran in xin
- 45. Z, Y. et al. T n, n, xin, i 🍘 n, i in A i i:in, l n n, 🖉 n P450 CYP79B2 n, CYP79B3. Genes & development 16, 3100 3112 (2002).
- 46. M, P.D. I. I. TOCIIE, C.F. I. Ci. I.F. P. F. r, ir, A i i. The Plant Cell Online 15, ъi. 223 236, i:10.1105/.006734 (2002). 47. Vr. r. , F. V Ir. J.-P. & Vr. D S r. D. Oli r. Ir. : lir.
- 6 in Aii. BioEssays 27, 275 284, i: 10.1002/ i .20199 (2005).
- 48. M i, N. i : Pl **F**, G H fn **F**, C **F**, l C S Yil. American Journal of Plant Sciences 05, 2178 2187, i: 10.4236/ .2014.514231 (2014).
- 49. B , ix, C., M ll, C. M. & H 🕫 , F. G. Ci in Cl Gr, Uni Illan Cr, I Ba A i I IT i Molecular plant 8, 1135 1152, i: 10.1016/ rn l .2015.03.003 (2015). 50. Li, F. et al. C i i **r**
- ll n i i i 🏊 i Sail - 1 iin Bin L. Pq. BMC plant biology **11**, 168 (2011).
- 51. L, X. et al. Un llin, rulx i (B i 🖪 L.) i ir, xir i i nora in BMC Genomics 16, 379, i: 10.1186/ 12864-015-1607-0 (2015).
- 52. S i, T. et al. B i 🖪 ra reirereii x re i 🖪 🕅 Plant and Soil 358, 61 74, 🖗 inin i: 10.1007/ 11104-012-1219-2 (2012).
- 53. Cl, B. et al. E 🖾 II 📓 I i i nin -NliiBi 🖡 il r, ra . Science 345, 950 953 (2014). 54. N i, M. & T i, N. E in i n 🗈 DNA 🔥 🕼 i . Proc 5th World Cong Genet Appl niin n 🕅 ni *Livestock Prod* **21**, 405 412 (1983).
- 55. Li, . & M, S. V. P M n Bininan n 🕅 i . Bioinformatics 21, 2128 2129 : min n i m (2005).
- lin 56. P i , J. ., S r, , M. & D r, , IIB, P. Ir, r, in ta lil P. 🗃 . Genetics 155, 945 959 (2000).
- 57. E 📭 , G., r , S. & G 1 , J. D $p_{i} \in \Omega$ in ii lin ST UCTU E: in lin ir. Molecular ecology 14, 2611 2620 (2005).
- il 🗥 📓 O. J. & V 👘 🖡 , X. SPAG Di: r, 16 il ni 58. H řΩ. inii l İ i **r**, l l. Molecular ecology notes **2**, 618 620 (2002).
- 59. Y, J. et al. A ni raix -ra lra n lill l n. Nature genetics iina in R, **38,** 203 208 (2006).
- 60. Pi, A. L. et al. Pir, il ra r, r, By i i inin na-i i i r. i . Nature genetics 38, 904 909, i: 10.1038/r, 1847 (2006).
- 61. B **B**, P. J. *et al.* TASSEL: iinga in mlxiini ra 1 . Bioinformatics 23, 2633 2635 (2007).
- 62. B r. mini, Y. & H 1 i il 🖡 1 ,Y.C. n, llin, talil in Journal of the Royal Statistical Society. Series B (Methodological), 289 300 (1995).
- 63. Tryll, C. et al. Di ryil ry ry ry i x iry ry 🕎 i . T n II, C. et al. 27 Protocols 7, 562 578 (2012). . i n l i l B n fai NAx in r i T H r C ir Nature
- 64. irnen, O. et al. en en n i ra ra li 8 P. ilil . Plant Journal for Cell & Molecular Biology 37, 914 939 (2004).

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Additional Information

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