

Table S1. QTLs for six deep-sowing tolerant traits in the F₂₃ population by single-environment mapping with composite interval mapping (CIM).

Trait	QTL	Bin	Sowing depth	QTL position		LOD	Additive (a)	Dominance (d)	Gene action		PVE (%)
				cM	Marker interval				d/a	Type	
RAT	qRAT-Ch.1-1	1.08	SW3	93.7	umc1085-umc2181	4.98	-0.16	0.01	0.06	A	4.35
			SW15	93.2	umc1085-umc2181	6.22	-0.21	0.03	0.14	A	5.37
			SW20	93.7	umc1085-umc2181	5.91	-0.18	-0.03	0.17	A	4.98
	qRAT-Ch.3-1	3.00-3.01	SW15	2.1	umc2108-umc2376	5.21	0.24	-0.27	1.13	D	4.74
	qRAT-Ch.5-1	5.04-5.05	SW3	101.9	umc2298-umc1482	6.97	0.11	0.02	0.18	A	6.50
			SW15	101.9	umc2298-umc1482	8.03	-0.28	-0.04	0.14	A	7.31
			SW20	101.9	umc2298-umc1482	7.45	0.25	0.03	0.12	A	6.83
	qRAT-Ch.7-1	7.06	SW3	3.4	umc1760-phi116	11.03	-0.29	0.05	0.17	A	10.12
			SW15	3.1	umc1760-phi116	8.84	-0.13	-0.01	0.08	A	8.18
			SW120	3.6	umc1760-phi116	12.81	-0.17	-0.02	0.12	A	11.96
MESL	qMESL-Ch.1-1	1.09	SW3	110.9	umc2047-bnlg1597	3.10	0.25	0.03	0.12	A	2.89
			SW15	110.8	umc2047-bnlg1597	8.84	-0.75	-0.11	0.10	A	8.34
			SW20	110.8	umc2047-bnlg1597	10.43	-0.91	-0.15	0.16	A	10.01
	qMESL-Ch.1-2	1.10	SW15	145.2	umc1534-umc2223	13.96	-1.17	0.10	0.09	A	13.12
	qMESL-Ch.3-1	3.04	SW15	55.3	umc1527-umc2261	7.10	-1.88	-0.21	0.11	A	11.60
			SW20	56.1	umc1527-umc2261	4.77	-1.03	-1.20	1.17	D	4.94
			SW3	88.1	umc1869-umc1775	4.08	-0.34	-0.02	0.06	A	3.81
	qMESL-Ch.4-1	4.06-4.07	SW15	88.0	umc1869-umc1775	10.02	-0.83	-0.09	0.11	A	9.46
			SW20	88.2	umc1869-umc1775	14.84	-1.25	-0.18	0.14	A	13.97
			SW15	119.1	umc1612-umc1313	4.44	-1.10	0.08	0.07	A	6.98
	qMESL-Ch.4-2	4.08	SW20	119.4	umc1612-umc1313	4.61	0.17	0.10	0.59	PD	8.42
			SW3	14.3	umc2311-umc2196	9.30	-1.01	-1.51	1.50	OD	8.11
			SW15	14.3	umc2311-umc2196	5.81	-0.61	0.97	1.59	OD	5.06
	qMESL-Ch.6-1	6.01	SW20	14.3	umc2311-umc2196	11.45	-1.14	-1.43	1.25	OD	10.03
			SW3	67.0	umc1585-umc2526	3.08	-0.80	-0.03	0.04	A	4.93
			SW15	66.9	umc1585-umc2526	2.29	-0.61	0.08	0.13	A	3.67
COLL	qCOLL-Ch.1-1	1.09	SW3	111.2	umc2047-bnlg1597	7.43	0.21	0.03	0.14	A	12.15
			SW15	111.0	umc2047-bnlg1597	6.36	0.19	-0.01	0.05	A	10.38
			SW20	111.4	umc2047-bnlg1597	8.61	0.28	-0.04	0.14	A	14.12
	qCOLL-Ch.3-1	3.08	SW3	114.1	phi088-umc2174	3.65	0.79	0.84	1.06	D	3.82
			SW20	114.1	phi088-umc2174	11.24	1.24	-1.58	1.27	OD	9.89
	qCOLL-Ch.4-1	4.08-4.09	SW20	130.5	umc1313-umc2287	5.51	-0.14	0.01	0.07	A	9.07
	qCOLL-Ch.9-1	9.05	SW15	102.3	umc2343-umc2341	3.23	0.11	-0.06	0.55	PD	5.83

MESL+COLL	qCOLL-Ch.10-1	10.04-10.07	SW20	102.3	umc2343-umc2341	3.09	-0.09	0.03	0.33	PD	5.50
			SW15	126.4	umc1507-bnlg1185	3.00	-0.63	-0.71	1.13	D	3.02
			SW20	126.9	umc1507-bnlg1185	3.03	0.65	0.71	1.09	D	3.08
	qMESL+COLL-Ch.1-1	1.05-1.06	SW3	80.6	umc1076-umc1748	6.17	-0.18	-0.02	0.11	A	10.06
			SW15	81.0	umc1076-umc1748	7.39	-0.24	0.01	0.04	A	12.15
			SW20	81.0	umc1076-umc1748	4.91	-0.16	-0.12	0.75	PD	8.93
	qMESL+COLL-Ch.1-2	1.09	SW15	110.9	umc2047-bnlg1597	3.50	0.10	0.01	0.10	A	5.80
			SW20	111.0	umc2047-bnlg1597	5.68	0.18	-0.03	0.17	A	9.37
	qMESL+COLL-Ch.2-1	2.00-2.01	SW3	11.3	umc1419-phi96100	4.08	-0.89	1.00	1.12	D	4.13
			SW15	10.9	umc1419-phi96100	8.92	-1.91	-1.98	1.04	D	9.05
			SW20	11.0	umc1419-phi96100	10.75	-2.30	2.21	0.96	D	11.01
	qMESL+COLL-Ch.4-1	4.08	SW3	120.1	umc1612-umc1313	3.03	-0.64	-0.23	0.36	PD	5.54
			SW15	120.3	umc1612-umc1313	9.21	-1.97	2.00	1.02	D	9.40
			SW20	120.3	umc1612-umc1313	3.97	-0.88	-0.79	0.90	D	4.08
	qMESL+COLL-Ch.6-1	6.01	SW15	14.9	umc2311-umc2196	6.54	0.73	1.01	1.38	OD	5.81
			SW20	14.9	umc2311-umc2196	9.06	1.00	-1.34	1.34	OD	8.15
	qMESL+COLL-Ch.9-1	9.00-9.01	SW20	54.7	umc2393-phi028	5.00	0.59	0.88	1.49	OD	4.49
	qMESL+COLL-Ch.10-1	10.02-10.03	SW15	85.9	umc2034-umc2349	3.08	-0.63	0.60	0.95	D	3.16
			SW20	85.9	umc2034-umc2349	6.16	1.27	1.11	0.87	D	6.30
	qMESL+COLL-Ch.10-2	10.04	SW15	122.2	umc1077-umc1678	3.88	0.79	0.82	1.04	D	3.97
			SW20	122.2	umc1077-umc1678	9.89	-1.18	1.60	1.36	OD	8.92
MESL/COLL	qMESL/COLL-Ch.4-1	4.08	SW3	120.0	umc1612-umc1313	8.98	-0.98	1.11	1.13	D	8.43
			SW15	120.0	umc1612-umc1313	10.27	-1.12	-1.09	0.97	D	9.56
			SW20	120.0	umc1612-umc1313	11.27	-1.07	-1.19	1.11	D	10.69
			SW15	95.3	bnlg1460-umc1289	6.24	-0.84	0.55	0.65	PD	5.88
	qMESL/COLL-Ch.8-1	8.03	SW20	95.6	bnlg1460-umc1289	7.93	-0.94	-0.49	0.52	PD	7.35
			SW3	85.2	umc2121-umc1387	5.94	-1.03	-1.16	1.13	D	5.59
SDL	qMESL/COLL-Ch.9-1	9.04	SW20	85.5	umc2121-umc1387	8.99	-1.16	-1.01	0.87	D	8.50
			SW15	44.6	umc1770-bnlg2204	3.87	-1.03	-0.63	0.61	PD	6.58
	qSDL-Ch.1-1	1.03-1.04	SW3	88.0	umc1748-umc1085	7.87	-1.62	-0.79	0.49	PD	12.21
			SW15	88.0	umc1748-umc1085	3.14	-0.66	0.42	0.64	PD	4.87
	qSDL-Ch.1-2	1.06-1.08	SW15	106.4	umc2240-umc2047	6.93	-1.44	-0.5	0.49	PD	10.75
			SW20	106.6	umc2240-umc2047	5.35	1.10	-1.21	1.10	D	5.48
	qSDL-Ch.1-3	1.08-1.09	SW3	77.9	umc1048-umc1860	4.48	-1.24	-0.16	0.13	A	7.33
			SW20	77.7	umc1048-umc1860	3.60	1.89	0.94	0.50	PD	6.04
	qSDL-Ch.5-1	5.03-5.04	SW3	70.0	umc1585-umc2526	3.85	1.09	0.10	0.09	A	6.26
			SW15	70.0	umc1585-umc2526	6.71	1.86	0.11	0.06	A	10.91

qSDL-Ch.8-1	8.06-8.07	SW20	70.0	umc1585-umc2526	8.70	2.45	0.18	0.07	A	14.17
		SW3	142.7	umc2711-bnlg1828	3.25	1.69	-0.57	0.34	PD	5.49
		SW15	141.9	umc2711-bnlg1828	4.01	2.06	-1.44	0.70	PD	6.75
		SW20	142.3	umc2711-bnlg1828	4.66	2.39	-1.62	0.68	PD	7.84

RAT, emergence rate; MESL, mesocotyl length; COLL, coleoptile length; MESL+COLL, total length between mesocotyl and coleoptile, MESL/COLL, length ratio of mesocotyl to coleoptile; SDL, seedling length; SW3, SW15, and SW20 indicate the sowing depths of 3cm, 15cm, and 20cm, respectively; LOD, logarithm of odds; additive (a) effect positive values indicate that K12 carries the allele for an increase in corresponding deep-sowing trait, whereas negative values indicate that W64A carries the allele and contributes to an increase in corresponding trait; the QTL action [$|$ dominance (d)/additive (a) $|$; additive (A), $|d/a|=0.00\sim0.20$; partial-dominance (PD), $|d/a|=0.21\sim0.80$; dominance (D), $|d/a|=0.81\sim1.20$; over-dominance (OD), $|d/a|>1.20$]; PVE, phenotypic variance explained by the QTL.

Table S2. The functional annotations of corresponding candidate genes in 13 major QTLs and meta-QTLs (MQTLs) regions.

Candidate genes	Encoded protein	Gene ontology (GO) annotation
GRMZM2G065635	Brassinosteroid synthesis 1	response to brassinosteroid (GO:0009741); brassinosteroid biosynthetic process (GO:0016132); brassinosteroid homeostasis (GO:0010268); jasmonic acid mediated signaling pathway (GO:0009867); leaf development (GO:0048366)
GRMZM2G124532	PhytochromeB1 (PHYB1)	photoreceptor activity (GO:0009881); detection of visible light (GO:0009584); red, far-red light phototransduction (GO:0009585)
GRMZM2G326335	Phenylalanine ammonia lyase homolog 10 (PAL10)	L-phenylalanine catabolic process (GO:0006559); cinnamic acid biosynthetic process (GO:0009800); phenylalanine ammonia-lyase activity (GO:0045548)
GRMZM2G040638	Peroxidase (POD)	peroxidase activity (GO:0004601); regulation of circadian rhythm (GO:0042752);plant-type cell wall (GO:0009505)
GRMZM2G107499	Protein LNK1	circadian rhythm (GO:0007623); regulation of transcription, DNA-templated (GO:0006355)
GRMZM2G403620	Protein rough sheath 2 (RS2)	response to auxin (GO:0009733); response to gibberellin (GO:0009739); response to salicylic acid (GO:0009751); response to jasmonic acid (GO:0009753); leaf morphogenesis (GO:0009965); leaf formation (GO:0010338)
GRMZM2G422419	Auxin-responsive protein SAUR71	response to auxin (GO:0009733)
GRMZM2G419267	Beta-2-xylosyltransferase (xt1)	UDP-xylosyltransferase activity (GO:0035252); xylosyltransferase activity (GO:0042285); glycoprotein 2-beta-D-xylosyltransferase activity (GO:0050513); shoot system development (GO:0048367)
GRMZM2G404443	Cytokinin dehydrogenase 6 precursor	cytokinin metabolic process (GO:0009690); cytokinin dehydrogenase activity (GO:0019139)
GRMZM2G167986	Cytochrome P450 CYP78A1	monooxygenase activity (GO:0004497); oxidoreductase activity (GO:0016491); regulation of timing of plant organ formation (GO:0090709)
GRMZM2G103773	Brassinosteroid-deficient dwarf 1	microtubule bundle formation (GO:0001578); monooxygenase activity (GO:0004497); brassinosteroid homeostasis (GO:0010268); brassinosteroid biosynthetic process(GO:0016132)
GRMZM2G033871	Auxin responsive protein (SAUR)	response to auxin (GO:0009733)
GRMZM2G146108	Auxin responsive protein (SAUR)	response to auxin (GO:0009733)
GRMZM2G157727	PhytochromeA1 (PHYA1)	photoreceptor activity (GO:0009881); detection of visible light (GO:0009584); red, far-red light phototransduction (GO:0009585)
GRMZM2G057935	PhytochromeC1 (PHYC1)	photoreceptor activity (GO:0009881); detection of visible light (GO:0009584); red, far-red light phototransduction (GO:0009585)

GRMZM2G040736	Cytokinin response regulator 1	phosphorelay signal transduction system (GO:0000160)
GRMZM2G107228	Peroxidase (POD)	peroxidase activity (GO:0004601); response to oxidative stress (GO:0006979); plant-type cell wall (GO:0009505);
GRMZM2G138268	Auxin-responsive protein (IAA10)	auxin-activated signaling pathway (GO:0009734)
GRMZM2G167794	Auxin-responsive protein (IAA11)	auxin-activated signaling pathway (GO:0009734)
GRMZM2G174249	Invertase cell wall 8 (incw8)	hydrolase activity, hydrolyzing O-glycosyl compounds (GO:0004553);
GRMZM2G143328	Transcription factor MYB53	sequence-specific DNA binding (GO:0043565); DNA binding (GO:0003677)
GRMZM2G410499	Auxin responsive protein (SAUR)	response to auxin (GO:0009733)
GRMZM2G130043	Starch synthase V	glycogen (starch) synthase activity (GO:0004373); starch metabolic process (GO:0005982); starch synthase activity (GO:0009011); starch biosynthetic process (GO:0019252)
GRMZM2G003501	3-ketoacyl-CoA synthase	response to cold (GO:0009409); response to light stimulus (GO:0009416)
GRMZM2G015049	Auxin responsive protein SAUR50 (SAUR)	response to auxin (GO:0009733)
GRMZM2G017852	U-box domain-containing protein 44	ubiquitin-protein transferase activity (GO:0004842); abscisic acid biosynthesis (GO:0010115)
GRMZM2G045171	Sucrose synthase 6	sucrose synthase activity (GO:0016157); transferase activity (GO:0016740); sucrose metabolic process (GO:0005985)
GRMZM2G455658	Nana2-like 1	lignin metabolic process (GO:0009808); unidimensional cell growth (GO:0009826); plant-type secondary cell wall biogenesis (GO:0009834); brassinosteroid biosynthetic process (GO:0016132)
GRMZM2G102163	Receptor-like serine/threonine-protein kinase ALE2	protein kinase activity (GO:0004672); cell surface receptor signaling pathways (GO:0007166); protoderm histogenesis (GO:0010068); cell differentiation (GO:0030154); cuticle development (GO:0042335), plant organ formation (GO:1905393),
GRMZM2G102216	Glutathione S-transferase family protein	glutathione transferase activity (GO:0004364); auxin-mediated signaling pathways (GO:0010930)
GRMZM2G475683	SAUR12-auxin-responsive SAUR family member (SAUR)	response to auxin (GO:0009733)
GRMZM2G174834	AP2-EREBP transcription factor	DNA-binding transcription factor activity (GO:0003700)
GRMZM2G460406	L-ascorbate peroxidase 3 peroxisomal	response to reactive oxygen species (GO:0000302); cytochrome-c peroxidase activity (GO:0004130); L-ascorbate peroxidase activity (GO:0016688); hydrogen peroxide catabolic process (GO:0042744)
GRMZM2G361993	Auxin-responsive protein SAUR32 (SAUR)	response to auxin(GO:0009733)
GRMZM2G088212	Catalase 1 (CAT1)	catalase activity (GO:0004096); peroxidase activity (GO:0004601); circadian rhythm (GO:0007623); response to abscisic acid (GO:0009737); response to salicylic acid (GO:0009751)
GRMZM2G059212	Callose synthase 3	1,3-beta-D-glucan synthase activity (GO:0003843); (1->3)-beta-D-glucan biosynthetic process (GO:0006075); regulation of cell shape (GO:0008360); cell wall organization (GO:0071555)
GRMZM2G050705	Glycosyltransferase family protein 28	dolichol-linked oligosaccharide biosynthetic process (GO:0006488); transferase activity (GO:0016740)
GRMZM2G305856	Transcription factor MYB59-like	DNA binding (GO:0003677)

GRMZM2G450233	Peroxidase 5 (POD5)	peroxidase activity (GO:0004601); response to oxidative stress (GO:0006979)
GRMZM2G044358	GA 3-oxidase 2 isoform X1	gibberellin 3-beta-dioxygenase activity (GO:0016707)
GRMZM2G027723	Cellulose synthase 2	plant-type primary cell wall biogenesis (GO:0009833); cellulose synthase activity (GO:0016759); cellulose synthase (UDP-forming) activity (GO:0016760); cellulose biosynthetic process (GO:0030244); cell wall organization (GO:0071555)
GRMZM2G022904	Cytokinin hydroxylase	monooxygenase activity (GO:0004497); oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (GO:0016705); trans-zeatin biosynthetic process (GO:0033466)
GRMZM2G011463	Auxin-responsive SAUR family member (SAUR37)	response to auxin (GO:0009733)
GRMZM2G065928	Absciscic acid 8-hydroxylase 4	multicellular organism development (GO:0007275); brassinosteroid homeostasis (GO:0010268); (+)-abscisic acid 8'-hydroxylase activity (GO:0010295); brassinosteroid biosynthetic process (GO:0016132); abscisic acid catabolic process (GO:0046345)
GRMZM2G112984	Pectinesterase	pectinesterase activity (GO:0030599); cell wall modification (GO:0042545); aspartyl esterase activity (GO:0045330); pectin catabolic process (GO:0045490); pectinesterase inhibitor activity (GO:0046910)
GRMZM2G447271	Laccase 21 (LAC21)	oxidoreductase activity (GO:0016491); lignin catabolic process (GO:0046274)
GRMZM2G336337	Laccase 22 (LAC22)	oxidoreductase activity (GO:0016491); lignin catabolic process (GO:0046274)
GRMZM2G092174	PhytochromeB2 (PHYB2)	detection of visible light (GO:0009584); red, far-red light phototransduction (GO:0009585); photoreceptor activity (GO:0009881)
GRMZM2G126834	ARR-B-transcription factor 1	phosphorelay signal transduction system (GO:0000160); cytokinin-activated signaling pathway (GO:0009736)
GRMZM2G122340	Cytokinin oxidase 11	cytokinin metabolic process (GO:0009690); cytokinin dehydrogenase activity (GO:0019139)

Table S3. Summary of quality preprocessing of RNA-Sequencing (RNA-Seq) data in both parents (W64A and K12).

Sample code	Raw Date Size (bp)	Clean Date Size (bp)	Clean Date Rate (%)	Total Reads	Q20 percentage (%)	Q30 percentage (%)	GC (%)	Mapped Reads	Mapping Rate (%)
K12-MES(SW3)	9280368041	7852391550	84.53	55602589	97.7	93.45	53.63	48326857	86.81
K12-MES(SW20)	9114478837	6954957300	76.32	52798507	98.14	94.52	53.29	47129827	89.27
W64A-MES(SW3)	7010755947	6941989700	100.00	47020862	98.23	94.76	53.91	42455639	90.43
W64A-MES(SW20)	8190515323	8192650400	99.56	47635087	98.23	94.77	54.72	42527764	89.33
K12-COL(SW3)	7962591997	6660647300	84.73	46565669	98.1	94.46	54.61	42068030	90.28
K12-COL(SW20)	8351880567	8315574300	99.25	53620385	98.17	94.63	54.9	47996023	89.58
W64A-COL(SW3)	7735085100	7252831295	89.37	51232339	98.09	94.4	53.6	46106931	90.24
W64A-COL(SW20)	7222181400	6834207251	90.46	45931682	98.16	94.58	54.67	41423237	90.24
Average	8108482152	7375656137	90.58	50050890	98.1	94.45	54.17	44754288	89.52

MES, mesocotyl; COL, coleoptile; SW3 and SW20 indicate the sowing depth environment of 3cm and 20cm, respectively.

Table S4. Sequences of primers used in quantitative real-time PCR (qRT-PCR) analysis.

Gene ID	Forward primer (5' to 3')	Reverse primer (5' to 3')
GRMZM2G126010 (Actin-1)	CGATTGAGCATGGCATTGTCA	CCCACTAGCGTACAACGAA
GRMZM2G157727	ATGAACTCGATCGCATCCCT	CACATATCTGGGGCTCTCGT
GRMZM2G045171	ATGGACCACAGGTATCATTCTCA	TGGCATTGTGAACGCATAGTG
GRMZM2G017852	TCCTCAAGTGGTTCAGGGAGT	CAGGTCAGCTATGGTGGGTATG
GRMZM2G450233	TGACCAACGCAACCATGAAG	TCCTGATGAACCTGGCTGAC
GRMZM2G088212	CCGAATCCAAAGACCAAT	ATGCCAACATCGTCAAAGAG

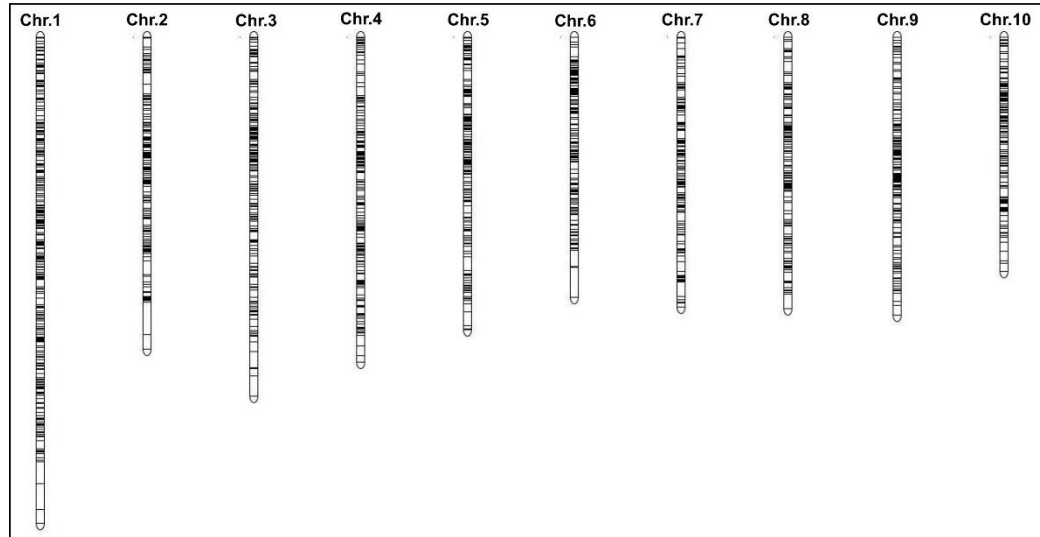


Figure S1. Consensus map constructed across three original mapping datasets.