

Table S1. Phenotype, broad-sense heritability, and genotype \times environment interaction heritability of mesocotyl length in F_{2:3} families under 3 cm, 15 cm, and 20 cm sowing depth environments.

Sowing depth environment	Mean	Range	CV (%)	Skewness	Kurtosis	H_B^2	H_{GE}^2
3 cm	3.74 \pm 0.61	0.72~5.51	16.31	0.112	0.110	90.96	8.98
15 cm	10.95 \pm 2.57	3.30~15.75	23.47	0.450	0.301		
20 cm	13.09 \pm 5.88	4.00~19.34	44.92	0.982	0.337		

H_B^2 , broad-sense heritability; H_{GE}^2 , genotype \times environment interaction heritability; CV, coefficient of variation.

Table S2. Quality control of sequencing data for female parent W64A, male parent K12, and bulked LM-pool and SM-pool samples under 20 cm sowing environment, respectively.

Bulk	Clean reads	Data generated	Q30 (%)	GC (%)	Genome coverage (10×)	Averagedepth (×)	Alignment efficiency (%)
W64A	217,228,830	65,168,649,000	93.32	46.46	76.30	34.02	99.59
K12	198,744,069	59,623,220,700	93.10	45.81	76.78	30.85	99.48
LM-pool	282,373,223	84,711,966,900	93.66	45.98	86.08	41.10	99.58
SM-pool	342,998,856	102,899,656,800	93.20	46.02	87.39	50.10	99.13

Table S3. Annotation of variation SNPs information among W64A, K12, LM-pool, and SM-pool samples, respectively.

Variation SNP information	Total	W64A	K12	LM-pool	SM-pool
Downstream	2,224,362	1,035,464	1,069,307	1,528,150	1,549,083
Exon	15,825	6,950	8,116	11,189	10,970
Intergenic	16,271,598	7,629,206	7,621,337	10,505,125	10,668,917
Intron	637,413	314,325	314,055	448,811	451,889
Non_Synonymous_Coding	244,472	119,191	118,942	174,520	176,522
Splice_Site_Acceptor	2,495	1,200	1,250	1,720	1,725
Splice_Site_Donor	2,218	1,059	1,069	1,569	1,585
Start_Gained	9,740	4,667	4,784	6,854	6,866
Start_Lost	1,042	547	525	788	773
Stop_Gained	6,284	2,743	2,825	4,060	4,079
Stop_Lost	1,181	629	622	876	886
Synonymous_Coding	223,641	109,906	110,909	165,227	166,827
Synonymous_Stop	436	230	240	311	314
Unknown	17,008	7,884	7,938	11,897	11,806
Upstream	3,195,437	1,474,433	1,555,230	2,213,902	2,241,915
Utr_3_Prime	229,368	110,127	116,900	168,725	170,172
Utr_5_Prime	190,512	92,257	96,055	139,171	140,432

Table S4. Annotation of variation InDels information among W64A, K12, LM-pool, and SM-pool samples, respectively

Variation InDel information	Total	W64A	K12	LM-pool	SM-pool
Codon_Change_Plus_Codon_Deletion	4,764	4,555	4,256	4,687	4,726
Codon_Change_Plus_Codon_Insertion	2,580	2,494	2,340	2,539	2,562
Codon_Deletion	2,602	2,487	2,308	2,554	2,581
Codon_Insertion	4,378	4,204	3,977	4,331	4,358
Downstream	442,405	404,001	406,995	435,403	435,597
Exon	1,635	1,473	1,497	1,609	1,608
Exon_Deleted	13	13	11	13	13
Frame_Shift	18,223	17,178	16,857	18,007	18,060
Gene_Fusion	5	5	5	5	5
Gene_Fusion_Reverese	23	20	21	22	23
Intergenic	1,277,771	1,137,951	1,150,246	1,252,836	1,249,391
Intron	140,483	131,850	132,674	139,189	139,145
Splice_Site_Acceptor	959	894	906	953	946
Splice_Site_Donor	1,143	1,080	1,075	1,130	1,131
Start_Lost	110	105	104	109	110
Stop_Gained	229	210	207	225	227
Stop_Lost	81	76	79	80	81
Unknown	30,143	28,601	28,366	29,906	29,943
Upstream	689,777	628,463	632,705	678,423	678,950
Utr_3_Prime	50,622	47,914	48,119	50,181	50,199
Utr_5_Deleted	1	1	0	1	1
Utr_5_Prime	42,814	40,612	40,672	42,508	42,519

Table S5. Summary of quality preprocessing of RNA sequencing (RNA-Seq) data.

Sample	Raw Date (bp)	Clean Date (bp)	Clean Date Rate (%)	Total Reads	Q20 (%)	Q30 (%)	GC (%)	Mapped Reads	Mapping Rate (%)
W64A(3 cm)	7,010,755,947	6,941,989,700	100.44	47,020,862	98.23	94.76	53.91	42,455,639	90.43
W64A(20 cm)	8,190,515,323	8,192,650,400	99.56	47,635,087	98.23	94.77	54.72	42,527,764	89.33
K12(3 cm)	9,280,368,041	7,852,391,550	84.53	55,602,589	97.7	93.45	53.63	48,326,857	86.81
K12(20 cm)	9,114,478,837	6,954,957,300	76.32	52,798,507	98.14	94.52	53.29	47,129,827	89.27
Mean	8,399,029,537	7,485,497,238	90.21	50,764,261	98.08	94.38	53.89	45,110,022	88.96

Table S6. Go annotation of 24 core conserved differentially expressed genes (DEGs) in all four comparisons by RNA-sequencing (RNA-Seq).

Gene ID	GO_annotation
Zm00001d039958	Molecular Function: N-acyltransferase activity (GO:0016410);;
MSTRG.44705	--
Zm00001d016500	--
MSTRG.38120	--
Zm00001d002005	Molecular Function: DNA binding (GO:0003677);; Cellular Component: nucleus (GO:0005634);; Biological Process: multicellular organism development (GO:0007275);; Molecular Function: metal ion binding (GO:0046872);;
Zm00001d016271	Molecular Function: nucleotide binding (GO:0000166);; Molecular Function: RNA binding (GO:0003723);; Cellular Component: spliceosomal complex (GO:0005681);; Biological Process: mRNA processing (GO:0006397);; Biological Process: RNA splicing (GO:0008380);; Cellular Component: nuclear speck (GO:0016607);;
Zm00001d020206	Biological Process: fatty acid biosynthetic process (GO:0006633);; Cellular Component: integral component of membrane (GO:0016021);; Molecular Function: 3-oxo-arachidoyl-CoA synthase activity (GO:0102336);; Molecular Function: 3-oxo-cerotoyl-CoA synthase activity (GO:0102337);; Molecular Function: 3-oxo-lignoceronyl-CoA synthase activity (GO:0102338);;
MSTRG.36969	--
Zm00001d048874	Molecular Function: magnesium ion binding (GO:0000287);; Biological Process: defense response (GO:0006952);; Biological Process: metabolic process (GO:0008152);; Molecular Function: ent-copalyl diphosphate synthase activity (GO:0009905);; Molecular Function: terpene synthase activity (GO:0010333);;
Zm00001d023604	--
Zm00001d048631	Molecular Function: protein serine/threonine kinase activity (GO:0004674);; Molecular Function: calcium ion binding (GO:0005509);; Molecular Function: ATP binding (GO:0005524);; Biological Process: cell surface receptor signaling pathway (GO:0007166);; Cellular Component: integral component of membrane (GO:0016021);; Molecular Function: polysaccharide binding (GO:0030247);;
MSTRG.21330	--
MSTRG.47158	Cellular Component: integral component of plasma membrane (GO:0005887);; Biological Process: carbohydrate transport (GO:0008643);; Cellular Component: integral component of membrane (GO:0016021);; Molecular Function: sugar transmembrane transporter activity (GO:0051119);;
Zm00001d031738	--
Zm00001d049020	Molecular Function: O-methyltransferase activity (GO:0008171);;
Zm00001d035172	Molecular Function: nucleotide binding (GO:0000166);; Molecular Function: ATP binding (GO:0005524);; Cellular Component: plasma membrane (GO:0005886);; Biological Process: signal transduction (GO:0007165);; Biological Process: plant-type hypersensitive response (GO:0009626);; Cellular Component: endomembrane system (GO:0012505);; Cellular Component: extrinsic component of plasma membrane (GO:0019897);; Molecular Function: ADP binding (GO:0043531);;
Zm00001d003648	Molecular Function: histone acetyltransferase activity (GO:0004402);; Cellular Component: nucleus (GO:0005634);; Biological Process: transcription, DNA-templated (GO:0006351);; Biological Process: regulation of transcription, DNA-templated (GO:0006355);; Molecular Function: zinc ion binding (GO:0008270);; Cellular Component: plasmodesma (GO:0009506);; Molecular Function: double-stranded methylated DNA binding (GO:0010385);; Molecular Function: histone binding (GO:0042393);; Biological Process: histone H3-K18 acetylation (GO:0043971);; Biological Process: histone H3-K23 acetylation (GO:0043972);; Biological Process: regulation of DNA methylation (GO:0044030);; Biological Process: histone H3-K14 acetylation (GO:0044154);; Biological Process: RNA-directed DNA methylation (GO:0080188);;
Zm00001d007745	Molecular Function: protein serine/threonine kinase activity (GO:0004674);; Molecular Function: calcium ion binding (GO:0005509);; Molecular Function: ATP binding (GO:0005524);; Cellular Component: cell (GO:0005623);; Biological Process: cell surface receptor signaling pathway (GO:0007166);; Biological Process: oligosaccharide metabolic process (GO:0009311);; Biological Process: response to salicylic acid (GO:0009751);; Biological Process: unidimensional cell growth (GO:0009826);; Biological Process: cellular water homeostasis (GO:0009992);; Cellular Component: integral component of membrane (GO:0016021);; Molecular Function: polysaccharide binding (GO:0030247);;
MSTRG.28112	--

Zm00001d015759	Molecular Function: DNA-binding transcription factor activity (GO:0003700);; Cellular Component: nucleus (GO:0005634);; Biological Process: transcription, DNA-templated (GO:0006351);; Biological Process: response to water deprivation (GO:0009414);; Biological Process: ethylene-activated signaling pathway (GO:0009873);; Biological Process: cutin biosynthetic process (GO:0010143);; Biological Process: wax metabolic process (GO:0010166);; Molecular Function: sequence-specific DNA binding (GO:0043565);;
MSTRG.26409	Molecular Function: DNA binding (GO:0003677);; Molecular Function: glutathione transferase activity (GO:0004364);; Cellular Component: nucleus (GO:0005634);; Cellular Component: cytoplasm (GO:0005737);; Biological Process: toxin catabolic process (GO:0009407);; Biological Process: response to toxic substance (GO:0009636);;
Zm00001d049000	Molecular Function: DNA binding (GO:0003677);; Cellular Component: nucleus (GO:0005634);; Biological Process: transcription, DNA-templated (GO:0006351);; Biological Process: regulation of transcription, DNA-templated (GO:0006355);; Molecular Function: metal ion binding (GO:0046872);;
Zm00001d045931	--
Zm00001d004209	--

Table S7. Go annotation of 15 candidate genes in cQMES4.

Gene ID	GO_annotation
Zm00001d051554	Molecular Function: iron ion binding (GO:0005506); Cellular Component: endoplasmic reticulum membrane (GO:0005789); Biological Process: multicellular organism development (GO:0007275); Biological Process: abscisic acid metabolic process (GO:0009687); Biological Process: brassinosteroid homeostasis (GO:0010268); Molecular Function: (+)-abscisic acid 8'-hydroxylase activity (GO:0010295); Cellular Component: integral component of membrane (GO:0016021); Biological Process: sterol metabolic process (GO:0016125); Biological Process: brassinosteroid biosynthetic process (GO:0016132); Molecular Function: oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen (GO:0016709); Molecular Function: heme binding (GO:0020037); Biological Process: abscisic acid catabolic process (GO:0046345);
Zm00001d051577	Molecular Function: DNA binding (GO:0003677); Molecular Function: DNA-binding transcription factor activity (GO:0003700); Cellular Component: nucleus (GO:0005634); Biological Process: transcription, DNA-templated (GO:0006351); Biological Process: regulation of transcription, DNA-templated (GO:0006355); Biological Process: procambium histogenesis (GO:0010067); Biological Process: phloem or xylem histogenesis (GO:0010087); Biological Process: positive regulation of transcription, DNA-templated (GO:0045893); Molecular Function: metal ion binding (GO:0046872);
Zm00001d051569	Molecular Function: DNA binding (GO:0003677); Molecular Function: DNA-binding transcription factor activity (GO:0003700); Cellular Component: nucleus (GO:0005634); Biological Process: transcription, DNA-templated (GO:0006351); Biological Process: response to blue light (GO:0009637); Biological Process: flower development (GO:0009908); Biological Process: positive regulation of flower development (GO:0009911);
Zm00001d051687	Molecular Function: Rho GDP-dissociation inhibitor activity (GO:0005094); Molecular Function: GTPase activator activity (GO:0005096); Cellular Component: cytoplasm (GO:0005737); Biological Process: cell tip growth (GO:0009932); Biological Process: root epidermal cell differentiation (GO:0010053);
Zm00001d051792	Cellular Component: chromatin (GO:0000785); Molecular Function: chromatin binding (GO:0003682); Molecular Function: guanyl-nucleotide exchange factor activity (GO:0005085); Cellular Component: nucleus (GO:0005634); Cellular Component: cytosol (GO:0005829); Biological Process: response to UV (GO:0009411); Biological Process: entrainment of circadian clock (GO:0009649); Molecular Function: photoreceptor activity (GO:0009881); Biological Process: response to UV-B (GO:0010224); Biological Process: protein-chromophore linkage (GO:0018298); Molecular Function: protein homodimerization activity (GO:0042803);
Zm00001d051787	Cellular Component: endoplasmic reticulum (GO:0005783); Biological Process: fatty acid biosynthetic process (GO:0006633); Biological Process: response to cold (GO:0009409); Biological Process: response to light stimulus (GO:0009416); Molecular Function: fatty acid elongase activity (GO:0009922); Cellular Component: membrane (GO:0016020); Cellular Component: integral component of membrane (GO:0016021); Biological Process: pollen tube development (GO:0048868); Molecular Function: 3-oxo-arachidoyl-CoA synthase activity (GO:0102336); Molecular Function: 3-oxo-cerotoyl-CoA synthase activity (GO:0102337); Molecular Function: 3-oxo-lignoceronyl-CoA synthase activity (GO:0102338);
Zm00001d051800	Cellular Component: endoplasmic reticulum (GO:0005783); Cellular Component: lipid droplet (GO:0005811); Cellular Component: organelle membrane (GO:0031090); Molecular Function: metal ion binding (GO:0046872); Molecular Function: plant seed peroxidase activity (GO:1990137);
Zm00001d051802	Biological Process: auxin-activated signaling pathway (GO:0009734); Biological Process: regulation of growth (GO:0040008);
Zm00001d051829	Molecular Function: ubiquitin-protein transferase activity (GO:0004842); Cellular Component: nucleus (GO:0005634); Cellular Component: cytoplasm (GO:0005737); Cellular Component: plasma membrane (GO:0005886); Biological Process: regulation of abscisic acid biosynthetic process (GO:0010115); Biological Process: leaf senescence (GO:0010150); Biological Process: regulation of chlorophyll catabolic process (GO:0010271); Biological Process: regulation of chlorophyll biosynthetic process (GO:0010380); Molecular Function: transmembrane receptor protein serine/threonine kinase binding (GO:0070696); Biological Process: negative regulation of abscisic acid biosynthetic process (GO:0090359);
Zm00001d051837	Biological Process: sucrose metabolic process (GO:0005985); Molecular Function: sucrose synthase activity (GO:0016157);
Zm00001d051847	Molecular Function: calmodulin binding (GO:0005516); Cellular Component: vacuole (GO:0005773); Cellular Component: vacuolar membrane (GO:0005774); Cellular Component: endoplasmic reticulum (GO:0005783); Cellular Component: plasma membrane (GO:0005886); Biological Process: lignin metabolic process (GO:0009808); Biological Process: unidimensional cell growth (GO:0009826); Biological Process: plant-type secondary cell wall biogenesis (GO:0009834); Cellular Component: membrane (GO:0016020); Cellular Component: integral component of membrane (GO:0016021); Biological Process: brassinosteroid biosynthetic process (GO:0016132); Molecular Function: oxidoreductase activity, acting on CH-OH group of donors (GO:0016614); Molecular Function: delta24-sterol reductase activity (GO:0050614); Molecular Function: flavin adenine dinucleotide binding (GO:0050660);
Zm00001d051520	Molecular Function: DNA binding (GO:0003677); Cellular Component: nucleus (GO:0005634); Biological Process: transcription,

DNA-templated (GO:0006351);; Biological Process: regulation of transcription, DNA-templated (GO:0006355);;

Zm00001d051529 Molecular Function: ATP binding (GO:0005524);; Biological Process: phenylpropanoid metabolic process (GO:0009698);; Biological Process: pollen exine formation (GO:0010584);; Molecular Function: 4-coumarate-CoA ligase activity (GO:0016207);;

Zm00001d051925 Molecular Function: protein serine/threonine kinase activity (GO:0004674);; Molecular Function: transmembrane receptor protein serine/threonine kinase activity (GO:0004675);; Molecular Function: ATP binding (GO:0005524);; Cellular Component: plasma membrane (GO:0005886);; Biological Process: cell surface receptor signaling pathway (GO:0007166);; Biological Process: protoderm histogenesis (GO:0010068);; Cellular Component: integral component of membrane (GO:0016021);; Molecular Function: kinase activity (GO:0016301);; Biological Process: cell differentiation (GO:0030154);; Biological Process: cuticle development (GO:0042335);; Biological Process: protein autophosphorylation (GO:0046777);; Biological Process: plant organ formation (GO:1905393);;

Zm00001d051928 Molecular Function: structural constituent of nuclear pore (GO:0005487);; Molecular Function: phospholipid binding (GO:0005543);; Cellular Component: nuclear envelope (GO:0005635);; Cellular Component: cytosol (GO:0005829);; Biological Process: RNA export from nucleus (GO:0006405);; Biological Process: protein import into nucleus (GO:0006606);; Biological Process: negative regulation of auxin mediated signaling pathway (GO:0010930);; Molecular Function: structural constituent of nuclear pore (GO:0017056);; Cellular Component: nuclear periphery (GO:0034399);; Cellular Component: nuclear pore central transport channel (GO:0044613);; Biological Process: mRNA transport (GO:0051028);; Biological Process: ribonucleoprotein complex export from nucleus (GO:0071426);;

Table S8. Accession numbers to NCBI database.

Accession	PRJNA822510
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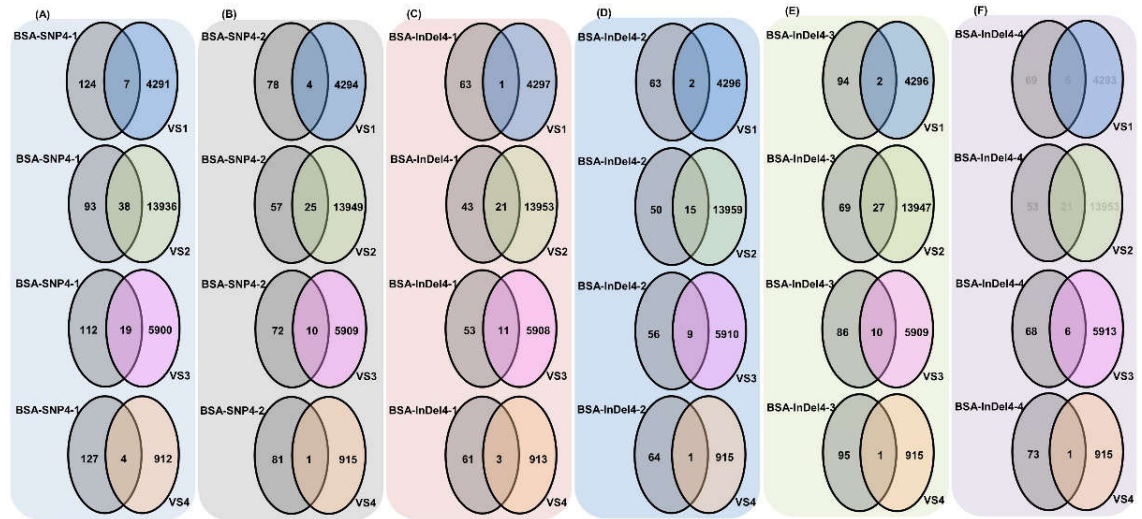


Figure S1. Venn diagram of genes between BSA-SNP4-1 (A), BSA-SNP4-2 (B), BSA-InDel4-1 (C), BSA-InDel4-2 (D), BSA-InDel4-3 (E), and BSA-InDel4-4 (F) region by BSA strategy and four comparisons (VS1 [W64A(20 cm)_VS_K12(20 cm)], VS2 [W64A(3 cm)_VS_K12(3 cm)], VS3 [W64A(20 cm)_VS_W64A(3 cm)], and VS4 [K12(20 cm)_VS_K12(3 cm)]) via RNA-sequencing, respectively.