

Dissecting Heterosis During the Ear Inflorescence Development Stage in Maize *via* a Metabolomics-based Analysis

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SUPPLEMENTAL INFORMATION

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Supplementary Figure S1. Sequential fine mapping of *hIEW2b*.

Supplementary Figure S2. Hierarchical clustering analysis of metabolomics data.

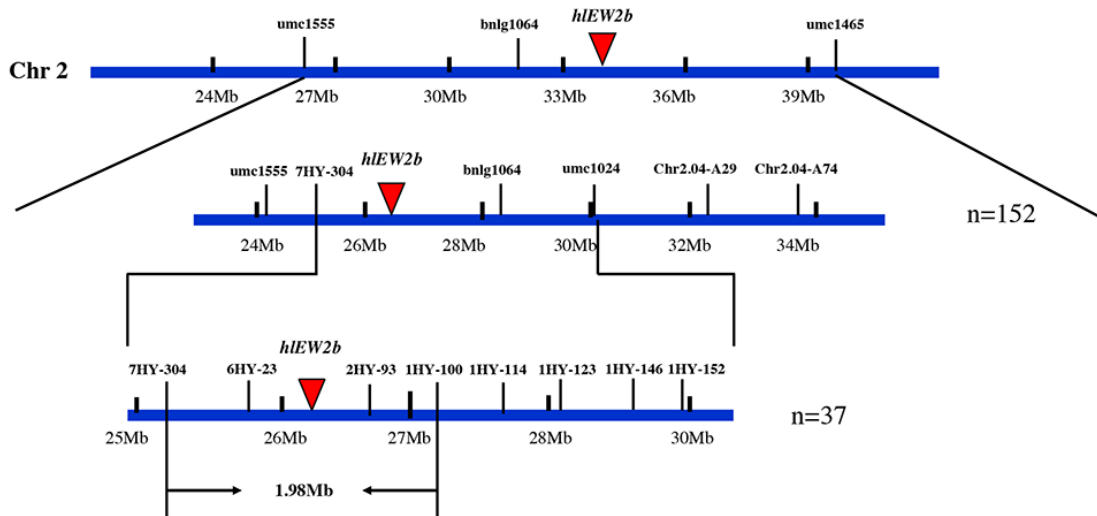
Supplementary Table S1. Functional annotation of candidate genes in the mapping region of *hIEW2b*.

Supplementary Table S2. Maize ear traits of Ix9801 and sub-CSSL₁₆ in two environments.

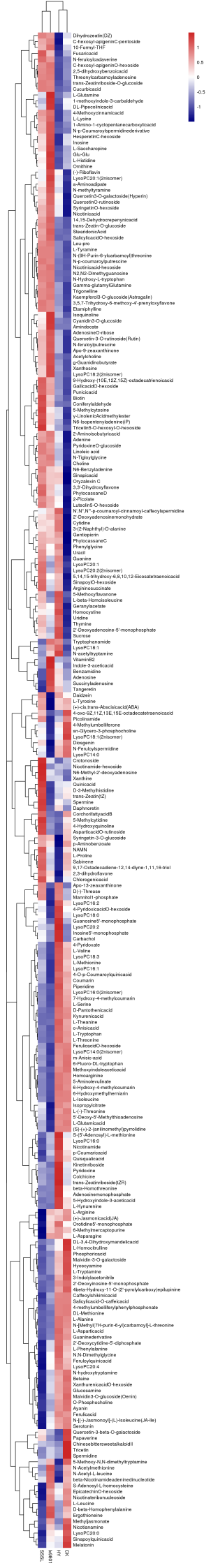
Supplementary Table S3. The heterotic effects analysis for *hIEW2b* on ear width trait in the testcross of Zheng58 × sub-CSSL₁₆.

Supplementary Table S4. Molecular markers developed for fine mapping of *hIEW2b* on chromosome 2.

Supplementary Table S5. Primers used for detecting relative gene expression level.



Supplementary Figure S1. Sequential fine mapping of *hIEW2b*. Locations of the fine-mapped regions on chromosome 2 are presented. The *hIEW2b* locus was roughly mapped between the simple sequence repeat markers 7HY-304 and *umc1024* on chromosome 2, and subsequently fine-mapped between markers 7HY-304 and 1HY-100, with a physical distance of 1.98 Mb.



Heatmap showing relative metabolite abundance across three samples: H2O, H2, and H4. The color scale ranges from -1 (blue) to 1 (red). The y-axis lists 1000 metabolites, including various amino acids, sugars, and organic acids.



H2O
H2
H4

Supplementary Figure S2. Hierarchical clustering analysis of metabolomics data.

The heat map was plotted using the \log_2 -transformed mean values of metabolomics data.

Supplementary Table S1. Functional annotation of candidate genes in the mapping region of *hlEW2b*.

Chr.	Candidate genes	Annotation ^a
2	Zm00001d002872	Unknown
2	Zm00001d002873	UPF0426 protein chloroplastic
2	Zm00001d002874	OSJNBa0038O10.24 protein
2	Zm00001d002875	Encodes alpha-helical IF (intermediate filament)-like protein.
2	Zm00001d002876	Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain
2	Zm00001d002878	Uncharacterized ACR YggU family COG1872 containing protein expressed
2	Zm00001d002879	Unknown
2	Zm00001d002880	Isopropylmalate dehydrogenase 2
2	Zm00001d002881	Putative pentatricopeptide repeat-containing protein
2	Zm00001d002882	SKP1-interacting partner 15
2	Zm00001d002884	Integral membrane protein
2	Zm00001d002885	Putative CCR4-associated factor 1 homolog 3
2	Zm00001d002886	Unknown
2	Zm00001d002887	Unknown
2	Zm00001d002888	MtN21 nodulin protein-like

2	Zm00001d002889	Soluble inorganic pyrophosphatase
2	Zm00001d002890	Unknown
2	Zm00001d002891	Chaperone DnaJ-domain superfamily protein
2	Zm00001d002893	WAT1-related protein
2	Zm00001d002896	TUB-transcription factor 4
2	Zm00001d002897	Peroxidase 14
2	Zm00001d002898	Peroxidase 12
2	Zm00001d002899	Peroxidase 12
2	Zm00001d002900	Unknown
2	Zm00001d002901	Peroxidase 12
2	Zm00001d002902	Peroxidase 12
2	Zm00001d002904	PLC-like phosphodiesterases superfamily protein
2	Zm00001d002905	Protein kinase superfamily protein
2	Zm00001d002906	Unknown
2	Zm00001d002907	RNA polymerase II Rpb4 core protein
2	Zm00001d002912	Glyoxal oxidase-related protein
2	Zm00001d002915	RNA polymerase II Rpb4 core protein
2	Zm00001d002916	Glyoxal oxidase-related protein
2	Zm00001d002917	RNA-binding region RNP-1

2	Zm00001d002919	Tetratricopeptide repeat (TPR)-like superfamily protein
2	Zm00001d002922	Inositol polyphosphate multi kinase beta
2	Zm00001d002925	AP-4 complex subunit mu
2	Zm00001d002928	DUF740 family protein
2	Zm00001d002929	Auxin response factor 17
2	Zm00001d002930	Unknown
2	Zm00001d002933	DDB1- and CUL4-associated factor homolog 1
2	Zm00001d002934	NAC domain-containing protein 30

a) Annotations were obtained from MaizeGDB databases ([http:// www.maizegdb.org](http://www.maizegdb.org)).

Supplementary Table S2. Maize ear traits of Ix9801 and sub-CSSL₁₆ in two environments.

Traits	Environments	Parents		
		Ix9801	sub-CSSL ₁₆	P-value
Ear width(mm)	E1	48.38±1.44	48.44±1.04	9.42E-01
	E2	46.84±3.05	47.01±2.01	9.22E-01
Ear length(cm)	E1	11.86±2.34	11.95±1.66	8.06E-01
	E2	12.03±2.79	12.18±1.14	5.79E-01
Kernel row number	E1	12.40±0.89	13.20±1.09	2.42E-01
	E2	13.20±0.89	12.80±1.10	5.80E-01
Kernel number per row	E1	24.00±2.65	22.80±1.30	3.90E-01
	E2	23.40±2.07	23.00±1.08	7.40E-01
Ear weight(g)	E1	87.45±2.46	83.63±5.94	2.79E-01
	E2	90.42±1.91	91.04±4.85	7.97E-01

E1 and E2 are Xinxiang 2015, Changge 2015, China, respectively.

*, ** indicate significant differences at the 0.05 and 0.01 probability level.

Supplementary Table S3. The heterotic effects analysis for *hIEW2b* on ear width trait in the testcross of Zheng58 × sub-CSSL16.

Traitsa	Mean±SD			d/ab	Mode of effectc
	sub-CSSL16	Zheng58	Zheng58 × sub-CSSL16		
Ear width (mm)	48.44±1.04	48.58±0.9	52.7±1.30	59.86	OD

a) Phenotype of ear width was collected in Xinxiang 2015.

b) The ratio of the estimated dominance to the absolute value of additive effect (d/a , degree of dominance) >1 , $=1$, <1 were regarded as exhibiting overdominance (OD), dominance (D) and partial-dominance (PD), respectively.

c) Mode of genetic effect manifestation.

Supplementary Table S4. Molecular markers developed for fine mapping of *hIEW2b* on chromosome 2.

Marker	AGP v4 position	Forward Primer	Reverse Primer
umc1555	25121821	ATAAAACGAACGACTCTCTCACCG	ATATGTCTGACGAGCTTCGACACC
7HY-304	25177782	ACGTGGTCAATTGCACAGAC	TGTCGCGCTTGTAACCAAAA
6HY-23	25651399	GGGAAGGAGTGGAGGTGGT	TGCATACCCGACTAGCAGTTC
2HY-93	26045713	CTACCTCTTCGCCTCCGC	CCATTTCGCTTTTGCCCT
1HY-100	27934745	GTAGTTTGCTGCTTTTTTGTGTT	CTTGATTCGCTCTCTTAGTTCCT
1HY-114	28088080	CCCGCTTAGGTATCTATCTGC	ATCTTCTGGTTCTGCCGTGA
1HY-123	28153007	CAAATCCAGAAGGAAGAAAAACT	TAGAACGGAGAGGAGCACAAAG
bnlg1064	28258664	CTGGTCCGAGATGATGGC	TCCATTTCTGCATCTGCAAC
1HY-146	29463038	CACGGGATTGTGGTAGTAAAAG	TTCTGGAGGTTGTTATTTGCC
1HY-152	29467416	GAGAACC AAAACGGCAAGAA	AGCGGACTACTGCTGACCAT
umc1024	30533873	CCTTTTTTCGCCTCGCTTTTTTAT	TCGTCGTCTCCAATCATACTG
Chr2.04-A29	32857673	CCTTTGCTGCTTTGCGACT	GCGTTGGGACCCTACTCG
Chr2.04-A74	33353765	GACCCCAACAAACCAGTG	CACTTCCCCTCCGTCCC
umc1465	39701635	GTACTTCAAAGCACATGAATGCGA	AGTCTCGACTGCCAATTTACAAA

Supplementary Table S5. Primers used for detecting relative gene expression level.

Gene ID	Forward Primers	Reverse Primers
Zm00001d011699	GCCTGTGGCCATTCAATTAC	GGTACCAAAGCAGGAGACGA
Zm00001d013140	AGTACATGCGAATGCGACAG	CACATCCTTGACGGTGATGA
Zm00001d034023	CACAGGCCCAGAATTTGATT	TGAAATTCCACCATCAGCAA
Zm00001d034320	GTTGGAAGAGGACAGGCAAC	AGCTAAAAAGCTGCCCATCA
Zm00001d026685	CCCCGAGGTTACACATTCAG	ACCACCTGACAATGCACAGA
Zm00001d030895	CTTTGAGGAAGCCGAAGAAG	TGTTCCACCGGTAGCAATAAG
Zm00001d041352	GGGATTGCCTCCTCCATC	GCAACTACGGTGATCCCTTG
Zm00001d007094	GCTTTTGGATGGTGTCAAAA	ATTGCCAGAAAGGTCAATGC
Zm00001d003017	GCTTCAAATTCCTGGTGCAT	GCCGCAGACAAGTTAGCAAT
Zm00001d017271	ATTGAGGAGATTGCTTTGAAGA	CTCCTTGGGCAATAGGATCA
Zm00001d007180	CGCTGTTCCCACACTACGAG	GTAGGAGCTCTTGCCGAGGT
Zm00001d029031	GGTGCTGCCCTACTACGAGA	TAGGAGCTCTTGCCGAGGT
Zm00001d022254	ACTGCTTCCAAGGGATCGTC	AGCGCCTCCTTGATGTTGT
Zm00001d008376	CTGGGCCTCACCCTACCT	ACAAAGCTTGCGATCCTCAG
Zm00001d051927	CTTGCTCGAGTCATTCACCA	TTGGCACAATCTCATCAACC
β -Actin	CAATGGCACTGGAATGGT	ATCTTCAGGCGAAACACG