File S1

Supporting Material

Sequencing Late-NVL and Late-VL individuals

We designed primers using primer 3 (ROZEN and SKALETSKY 2000, Table S1 and Table S2) with the reference sequence of B73 and performed PCR on Control (F252), Late-VL and Late-NVL genotypes. PCR products were run on 1% agarose TAE 1X gel at 120 V and sequenced.

PCR Conditions

Reaction

PCR were performed in a final volume of 25 µl with :

DNA (5ng/µl)	10 ng
MgCl2	2mM
Buffer 5X [1]	1 X
dNTP (10 mM each)	0,2 mM
Primer F (10 µM)	0,4 µM
Primer R (10 µM)	0,4 µM
Taq polymerase [1]	1 unit

[1]: We used the Taq DNA polymerase and the Buffer 5X promega.

Thermocycling program:

Hot start

94°C for 2 min

Core

35 cycles of

94°C for 1 min

59°C for 1 min

72°C for 2 min 30

Final extension

72°C for 5 min

Table S1 Primer sequences

Primer name[a]	Sequence (5' - 3')	Annealing temperature used in PCR reaction (°C)
CL6-F1	TTGTGACCACCTCAACAGGA	60°C
CL6-F1b	ACCACCACCACTTCAAGCAT	60°C
CL6-F1c	AGACGCTGATGCCTCATGTT	60°C
CL6-F1d	AAATGCTCAGGAAGGGGTTT	60°C
CL6-F1e	GTCTTTGGACCGCTTGAGAG	60°C
CL6-F2	GATCGGTGTATCAGCGACAA	60°C
CL6-F3	CAGTACGCGCAGAGTCAGAG	60°C
CL6-F4	TCGAGGTAGAATATGCCATGC	58°C
CL6-F5	GTAGTCCGGTCCTACGCAGA	60°C
CL6-R1	GTATCGTGCCATGTCTGCAC	60°C
CL6-F1-R	TCCTGTTGAGGTGGTCACAA	60°C
CL6-R2	CTGCCATCCAAGATTCAGGT	60°C
CL6-R3	AAACCCCTTCCTGAGCATTT	60°C
CL6-R4	TTGTGTTGGGCATTTAACCA	58°C
CL6-R5	TGCTCCTTTGAATTTGATCC	60°C

[a] Each pair of primer is composed of a forward (F) and a reverse (R) primer. The same primers have been used for PCR amplification and sequencing.

Table S2 Primers combinations used for amplification and sequencing

	CL6-F1a	CL6-F1b	CL6-F1c	CL6-F1d	CL6-F1e	CL6-F2	CL6-F3	CL6-F4	CL6-F5
CL6-R1	X	X		X					
CL6-F1-R			X				X	X	X
CL6-R2						X			
CL6-R3							X		
CL6-R4			X						
CL6-R5			X		X			X	X

Evaluation of the association panel for developmental traits

The association panel was evaluated for 26 traits described in CAMUS-KULANDAIVELU *et al.* (2006) and for 13 new characters linked to development. Here we describe the phenotypic evaluation for these 13 additional traits (Table S3) The whole panel was tested at three different locations (Germany_Einbeck : 52°N, 10°E, France_Gif-sur-Yvette : 49°N, 2°E, Saint-Martin-de-Hinx : 43°N, 1.3°W). The two latest groups of lines were also evaluated at France_Mauguio (44°N, 4°E). French locations were evaluated three years (2002-2004) and Einbeck location one year (2005).

Lines were repeated twice at each location, following a complete block design. In order to limit competition effects, each block was organised into four sub-blocks corresponding to earliness groups based on a priori information. Each individual plot consisted of a row of 15 plants planted at a density of approximately six plants per square meter. Depending on the traits, it was measured as a whole for each plot or as an average of 5 plant measurements (details in Table S4).

Phenology traits were measured for the whole plant when 50% of plants reached the corresponding stage. Days to anthesis for male flowering (MFLW8) and days to silking for female flowering (FFLW8) were measured in thermal time (GDD : Growing Degree Days) following RITCHIE and NESMITH (1991) with parameter values (Tb=8° and To=30°C) that maximized correlations between sites.

Architecture traits were measured as an average of 5 plants (plant height : PTHT, ear insertion height : EARHT, leaf number : LFNB, leaf number above top ear : LFNBa, leaf number below top ear : LFNb) at least 5 days after flowering. The 5 first leaves were marked early during cycle before senescence.

For ear and yield components, top and secondary ears of the whole plot were separately harvested in order to weight 100 grains of top ears to estimate thousand kernel weight (TKW) after 48 hours at 120°C. Yield was estimated as the total kernel weight (KW) divided by the number of harvested plants. The kernel row number (ROWNB) were averaged on 5 top ears.

Panicle architecture was described as an average on 5 plants for panicle length (PnL1), panicle length above branches (PnL2), panicle length below branches (PnL3), number of branches (BRNB) as 4 classes (1 : 0-3, 2 : 4-10, 3 : 11-15, 4 : >15).

A global ANOVA of the data was performed to test the significance of genotype, location and genotype-by-location interaction. Considering that genotype-by-location interaction was generally low compared to genotype effect, we used the adjusted mean (estimated using the LSMEANS statement in the GLM procedure of SAS) of each genotype for further analyses.

Table S3 Description and abbreviation of the 13 supplementary phenotypic traits measured on the association panel

category	abbreviation	name	description
phenology	MFLW8	anthesis (GDD[a])	50% of plants exhibit anthers (male flowering)
phenology	FFLW8	silking (GDD[a])	50% of plants exhibit silks (female flowering)
plant architecture	PTHT	plant height (cm)	tassel included
plant architecture	EARHT	ear insertion height (cm)	from soil to insertion node
plant architecture	LFNB	leaf number	at least 5 days after flowering, taking into account early dead leaves
plant architecture	LFNBa	leaf number above top ear	
plant architecture	LFNBb	leaf number below top ear	
ear and yield components	ROWNB	kernel row number	number of top ear kernel row
ear and yield components	TKW	thousant kernel weight (g)	based on 100 grains of top ear
panicle architecture	PnL1	panicle length (cm)	
panicle architecture	PnL2	panicle length above top secondary branch (cm)	
panicle architecture	PnL3	length from the first branch to the top branch of the panicle (cm)	L1-L2
panicle architecture	BRNB	panicle branch number (qualitative)	1 (0-3), 2 (4-10), 3 (11-15), 4 (>15)

[a] : Growing Degree Days, thermal time following RITCHIE and NESMITH (1991), Tb=8°C, To=30°C

Table S4 Description of evaluation trial sites for the 13 supplementary phenotypic traits measurements

category	abbreviation	plant measurement	plot measurement	average trial number	average plot number	trial number	site number	KWS_einbeck: 2005	mauguio: 2002	mauguio: 2003	moulon: 2002	moulon: 2003	moulon: 2004	SMH: 2002	SMH: 2003	SMH: 2004
phenology	MFLW8	0	1	7.29	14.84	9	4	1	1	1	1	1	1	1	1	1
phenology	FFLW8	0	1	7.18	14.55	9	4	1	1	1	1	1	1	1	1	1
plant architecture	PTHT	1	0	7.18	14.57	9	4	1	1	1	1	1	1	1	1	1
plant architecture	EARHT	1	0	7.13	14.45	9	4	1	1	1	1	1	1	1	1	1
plant architecture	LFNB	1	0	3.96	6.95	5	4	1	NA	1	NA	1	NA	NA	1	1
plant architecture	LFNBa	1	0	5.81	11.71	7	4	1	1	NA	1	1	1	1	NA	1
plant architecture	LFNBb	1	0	2.62	4.15	3	3	1	NA	NA	NA	1	NA	NA	NA	1
ear and yield components	ROWNB	1	0	5.62	11.05	8	4	1	1	1	1	1	1	1	1	NA
ear and yield components	TKW	0	1	5.53	10.86	8	4	1	1	1	1	1	1	1	1	NA
panicle architecture	PnL1	1	0	5.36	10.89	7	3	NA	1	1	1	NA	1	1	1	1
panicle architecture	PnL2	1	0	5.36	10.89	7	3	NA	1	1	1	NA	1	1	1	1
panicle architecture	PnL3	1	0	5.36	10.89	7	3	NA	1	1	1	NA	1	1	1	1
panicle architecture	BRNB	1	0	5.60	11.44	7	3	NA	1	1	1	NA	1	1	1	1

References

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