## **Supplementary material**

Table S1. Effect of water regime and genotype on different growth parameters: spike dry matter (Spike DM), culm dry matter (Culm DM), leaf dry matter (Leaf DM), root nitrogen content (Root N), flag leaf nitrogen content (Flag leaf N) and spike nitrogen content (Spike N)

For each genotype and treatment data shown are the means of the four replications. Means followed by different letters were significantly different (P < 0.05) by Tukey's b test. WW, well watered plants; WS water stressed plants; G: Genotype; T: Treatment; G × T: genotype by treatment interaction. The associated sum of squares type III and probabilities (ns, not significant; \*P < 0.05; \*\*P < 0.01; \*\*P < 0.001) are shown

		Spike	Culm	Leaf	Root	Flag leaf	Spike
Т	G	DM	DM	DM	Ν	Ν	Ν
		$(g plant^{-1})$	$(g plant^{-1})$	$(g plant^{-1})$	(%)	(%)	(%)
ΜM	KS194	0.78 a	2.20 ab	0.61 a	1.48 b	3.58 a	3.13 b
	KS230	1.07 a	2.10 a	0.72 a	1.22 ab	3.28 a	3.55 c
	RIL2108	1.05 a	2.78 ab	0.70 a	1.34 ab	4.36 b	2.57 a
	RIL2510	0.95 a	2.88 b	0.67 a	1.09 a	4.55 b	2.53 a
	Mean	0.96	2.49	0.67	1.28	3.94	2.95
SW	KS194	0.30 a	1.27 a	0.23 a	0.81 a	3.17 bc	2.58 b
	KS230	0.39 b	1.62 b	0.40 c	0.80 a	2.44 a	3.15 c
	RIL2108	0.68 d	1.51 ab	0.24 ab	0.67 a	2.99 b	2.18 a
	RIL2510	0.58 c	1.69 b	0.32 b	0.66 a	3.58 c	2.01 a
	Mean	0.49	1.52	0.30	0.73	3.05	2.48
IOVA	G	0.45***	1.54***	0.08**	0.28*	6.21***	5.90***
	Т	1.81***	7.44***	1.14***	2.40***	6.44***	1.74***
AN	G x T	0.13 ns	0.75*	0.02 ns	0.12 ns	0.94*	0.04 ns

## Table S2. Effect of water regime and genotype on the root dry matter weight (Root DM), and the root length (Root L) in each of the three different soil sections, as well as the total values for both traits through all the soil sections

For each trait the number following the acronym refers to the soil section where the trait was estimated: 1 refers to soil upper section (0.00–0.26 m); 2 refers to soil middle section (0.26–0.52 m); and 3 refers to soil bottom section (0.52–0.78 m), while "T" before the acronym refers to the total trait value through the three soil sections. Data shown is the mean of the four replications of each genotype in each treatment. Means followed by different letters were significantly different (P < 0.05) by the Tukey's b test. WW, well watered plants; WS, water stressed plants; G, genotype; T, treatment; G × T, genotype by treatment interaction. The associated sum of squares type III and probabilities ( $^*P < 0.05$ ;  $^{**}P < 0.01$ ;  $^{***}P < 0.001$ ) are shown

Т	G	Root DM 1	Root DM 2	Root DM 3	<b>Total Root DM</b>	Root L 1	Root L 2	Root L 3	<b>Total Root L</b>
		(g)	(g)	(g)	(g)	m	m	m	m
WM	KS194	0.18 a	0.07 a	0.07 a	0.31 a	4.04 a	2.16 a	1.75 a	7.95 a
	KS230	0.14 a	0.07 a	0.10 b	0.32 a	4.18 a	3.06 a	4.23 c	11.47 b
	RIL2108	0.30 b	0.08 a	0.09 b	0.48 b	7.36 b	3.16 a	2.92 b	13.44 b
	RIL2510	0.26 b	0.11 b	0.13 c	0.49 b	6.67 b	5.15 b	4.94 c	16.75 c
	Mean	0.22	0.08	0.10	0.40	5.56	3.38	3.46	12.40
SM	KS194	0.18 a	0.09 a	0.05 a	0.32 a	3.01 a	2.02 a	1.20 a	6.22 a
	KS230	0.22 ab	0.11 b	0.09 b	0.42 ab	5.11 b	3.09 b	2.35 b	10.55 bc
	RIL2108	0.25 ab	0.10 ab	0.09 b	0.44 ab	4.28 ab	2.22 a	1.86 ab	8.36 ab
	RIL2510	0.32 b	0.13 c	0.10 b	0.55 b	6.57 c	3.25 b	2.54 b	12.36 c
	Mean	0.24	0.11	0.08	0.43	4.74	2.65	1.99	9.38
IOVA	G	0.08***	0.01***	0.014***	0.20***	44.00***	18.99***	24.17***	223.46***
	Т	0.00 ns	0.00***	0.00**	0.01 ns	5.37*	4.31***	17.39***	73.33***
AN	G×T	0.020*	0.001 ns	0.001 ns	0.02 ns	17.44**	4.64***	4.07*	24.33*



**Fig. S1.** Averaged values through all the soil sections for (*a*) root weight density (RWDa), (*b*) root length density (RLDa) and (*c*) specific root length (SRLa) for genotypes KS194, KS230, RIL2108 and RIL2510. Data include both well-watered (WW, white bars) and waterstressed (WS, black bars) plants. Errors bar represent the standard error of the mean (SEM). Means followed by different letters were significantly different (P < 0.05) by Tukey's b test. Genotype, treatment, and genotype by treatment interaction were significant for all traits except for RWDa the treatment was not significant.



**Fig. S2.** Relationships between plant time-integrated water use efficiency ( $WUE_{Aerial DM}$ ) versus (*a*)  $\delta^{13}$ C of the spike and (*b*)  $\delta^{18}$ O of the spike. Data include both well-watered (WW, open circles) and water-stressed (WS, filled circles) plants. The fitting line is only included for the significant relationships. r<sup>2</sup> and probability is shown: \*\*\*\* P < 0.0001.



**Fig. S3.** Simple linear regression between flag leaf  $\delta^{18}$ O and  $\delta^{13}$ C. Correlation data include both well-watered (WW, open circles) and water-stressed (WS, filled circles) plants. The fitting line is only included in the significant relationships. r<sup>2</sup> and probabilities are shown :  ${}^{**}P < 0.01$ ;  ${}^{***}P < 0.001$ ; and  ${}^{****}P < 0.001$ ).



**Supplementary Figure** 

**Fig. S4.** Relationships between: root weight density (RWD, upper: *a*–*c*), root length density (RLD, middle: *d*–*f*) and specific root length (SRL, lower: *g*–*i*) versus aerial dry matter (Aerial DM). The number behind the trait refers to the soil section where the trait was estimated: 1, upper section (0.00–0.26 m); 2, middle section (0.26–0.52 m); and 3, soil bottom section (0.52–0.78 m). Data include both well-watered (WW, open circles) and water-stressed (WS, filled circles) plants. The fitting line is only included in the significant relationships. r<sup>2</sup> and probabilities are shown: \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; and \*\*\*\*P < 0.0001.



**Fig. S5.** Relationship between the plant cumulative transpiration per unit of Root DM ( $T_{cum}$ /TRoot DM) versus (a)  $\delta^{13}$ C and (b)  $\delta^{18}$ O of the spike. Data include both well-watered (WW, open circles) and water-stressed (WS, filled circles) plants. The fitting line is only included in the significant relationships. r<sup>2</sup> and probabilities are shown:  ${}^{*}P < 0.05$ ;  ${}^{***}P < 0.001$ ; and  ${}^{****}P < 0.0001$ .