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Supplementary Material

Genotypic variation in photosynthetic limitation responses to K deficiency of *Brassica napus* is associated with potassium utilisation efficiency

Zhifeng Lu[A,B], *Jianwei Lu*[A,B], *Yonghui Pan*[A,B], *Xiaokun Li*[A,B], *Rihuan Cong*[A,B] *and Tao Ren*[A,B,C]

[A]Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River) Ministry of Agriculture, Wuhan 43 0070, China.

[B]Microelement Research of Centre, Huazhong Agricultural University, Wuhan 43 0070, China.

[C]Corresponding author. Email: rentao@mail.hzau.edu.cn

Table S1. Potassium concentration threshold of each limitation that deserved center stage of photosynthesis of Huayouza No.9 and Zhongshuang No.11. S_L, MC_L and B_L denote stomatal, mesophyll conductance and biochemical limitations

Cultivar	K concentration (mmol g^{-1} DW)	Relationships among limitations	Dominant limitation
Huayouza No.9	0.0.21<K<0.32	$B_L<MC_L<S_L$	S_L
	0.17<K<0.21	$B_L<S_L<MC_L$	MC_L
	0.16<K<0.17	$S_L<B_L<MC_L$	MC_L
	K<0.16	$S_L<MC_L<B_L$	B_L
Zhongshuang No.11	0.27<K<0.36	$B_L<MC_L<S_L$	S_L
	0.21<K<0.27	$B_L<S_L<MC_L$	MC_L
	0.19<K<0.21	$S_L<B_L<MC_L$	MC_L
	K<0.19	$S_L<MC_L<B_L$	B_L

Table S2. Analysis of variance for cultivar and potassium effects on seed yield, shoot K content and K utilization efficiency (KUtE) at maturation stage, and for cultivar, potassium and leaf position effects on leaf area and dry matter, specific leaf weight, leaf thickness and density, leaf chlorophyll and K concentration, net CO_2 assimilation rate (A), stomatal conductance (g_s), mesophyll conductance (g_m) and electron transport rate (J)

*, ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively; ns indicates non-significance ($P > 0.05$)

Source of variation	Seed yield	shoot K content	KUtE	Leaf area	Leaf dry matter	Specific leaf weight	Leaf thickness	Leaf density	Chlorophyll concentration	K concentration	A	g_s	g_m	J
Cultivar (C)	**	**	**	**	**	**	*	**	**	**	**	ns	**	**
Potassium (K)	**	**	**	**	**	ns	**	**	**	**	**	**	**	**
C×K	ns	ns	ns	*	ns	ns	*	**	ns	**	ns	ns	ns	ns
Position (P)	-	-	-	**	**	**	**	**	**	**	**	**	**	**
C×P	-	-	-	**	ns	ns	ns	**	ns	**	ns	*	ns	ns
P×K	-	-	-	**	**	ns	**	ns	**	**	*	ns	**	ns
C×P×K	-	-	-	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns

Table S3. Sensitivity analysis of the estimation of mesophyll conductance (g_m) for variation in day respiration rate (R_d) values

Mesophyll conductance calculated by Harley *et al.* (1992) method using actual R_d calculated in this study and 50%, 25% elevated (or reduced) values. Values are means ± s.d. of three replicates per treatment. Different letters donate significant differences at $P < 0.05$ between g_m values obtained in this study (R_d) and each values estimations using different values of R_d

Cultivar	Treatment	Upper					Lower				
		(R_d) (mol CO_2 m^{-2} s^{-1})	(1.50 R_d) (mol CO_2 m^{-2} s^{-1})	(1.25 R_d) (mol CO_2 m^{-2} s^{-1})	(0.75 R_d) (mol CO_2 m^{-2} s^{-1})	(0.50 R_d) (mol CO_2 m^{-2} s^{-1})	(R_d) (mol CO_2 m^{-2} s^{-1})	(1.50 R_d) (mol CO_2 m^{-2} s^{-1})	(1.25 R_d) (mol CO_2 m^{-2} s^{-1})	(0.75 R_d) (mol CO_2 m^{-2} s^{-1})	(0.50 R_d) (mol CO_2 m^{-2} s^{-1})
H9	K_0	0.381±0.010c[1]	0.455±0.013a	0.416±0.011b	0.350±0.009d	0.322±0.008e	0.125±0.009bc	0.148±0.010a	0.136±0.009b	0.115±0.008cd	0.106±0.008d
	K_{30}	0.387±0.005c	0.465±0.007a	0.423±0.006b	0.356±0.005d	0.327±0.004e	0.144±0.014bc	0.169±0.015a	0.156±0.010ab	0.133±0.013cd	0.123±0.013d
	K_{60}	0.394±0.011c	0.477±0.015a	0.434±0.013b	0.364±0.010d	0.335±0.009e	0.161±0.013bc	0.188±0.014a	0.174±0.011ab	0.149±0.013cd	0.139±0.010d
	K_{120}	0.406±0.028bc	0.489±0.038a	0.445±0.032b	0.372±0.025cd	0.342±0.022d	0.185±0.012bc	0.213±0.012a	0.199±0.012ab	0.173±0.009cd	0.162±0.008d
Z11	K_0	0.346±0.022c	0.410±0.029a	0.376±0.025b	0.319±0.019d	0.295±0.017d	0.075±0.005c	0.091±0.006a	0.083±0.005b	0.068±0.005d	0.062±0.004d
	K_{30}	0.360±0.011c	0.428±0.015a	0.392±0.011b	0.331±0.010d	0.306±0.009e	0.130±0.011bc	0.156±0.012a	0.144±0.011ab	0.122±0.010cd	0.113±0.010d
	K_{60}	0.363±0.034bc	0.432±0.044a	0.395±0.023ab	0.334±0.021cd	0.308±0.026d	0.158±0.022abc	0.182±0.023a	0.169±0.023ab	0.145±0.018bc	0.135±0.018c
	K_{120}	0.371±0.024c	0.443±0.031a	0.405±0.027b	0.341±0.021d	0.315±0.018d	0.168±0.016bc	0.195±0.017a	0.181±0.017ab	0.156±0.016cd	0.145±0.015d

Table S4. Sensitivity analysis of the estimation of mesophyll conductance (g_m) for the variation in CO_2 compensation point in the absence of mitochondrial respiration (Γ*)

Mesophyll conductance calculated by Harley *et al.* (1992) method using actual Γ* values calculated in this study and 10%, 5% elevated (or reduced) values. Values are means ± s.d. of three replicates per treatment. Different letters donate significant differences at $P < 0.05$ between g_m values obtained in this study (Γ*) and each values estimations using different values of Γ*

Cultivar	Treatment	Upper					Lower				
		(Γ*) (mol CO_2 m^{-2} s^{-1})	(1.10 Γ*) (mol CO_2 m^{-2} s^{-1})	(1.05 Γ*) (mol CO_2 m^{-2} s^{-1})	(0.95 Γ*) (mol CO_2 m^{-2} s^{-1})	(0.90 Γ*) (mol CO_2 m^{-2} s^{-1})	(Γ*) (mol CO_2 m^{-2} s^{-1})	(1.10 Γ*) (mol CO_2 m^{-2} s^{-1})	(1.05 Γ*) (mol CO_2 m^{-2} s^{-1})	(0.95 Γ*) (mol CO_2 m^{-2} s^{-1})	(0.90 Γ*) (mol CO_2 m^{-2} s^{-1})
H9	K_0	0.381±0.010c[1]	0.591±0.027a	0.463±0.015b	0.323±0.007d	0.281±0.005e	0.125±0.009c	0.157±0.013a	0.139±0.010b	0.113±0.008cd	0.104±0.007d
	K_{30}	0.387±0.005c	0.609±0.014a	0.473±0.008b	0.328±0.004d	0.285±0.003e	0.144±0.014bc	0.185±0.021a	0.161±0.016b	0.130±0.012cd	0.118±0.011d
	K_{60}	0.394±0.011c	0.636±0.032a	0.487±0.017b	0.335±0.008d	0.289±0.006e	0.161±0.013c	0.211±0.019a	0.181±0.015b	0.145±0.012cd	0.132±0.011d
	K_{120}	0.406±0.028c	0.665±0.083a	0.501±0.043b	0.341±0.020cd	0.294±0.014d	0.185±0.012c	0.246±0.017a	0.209±0.013b	0.167±0.011d	0.152±0.010d
Z11	K_0	0.346±0.022c	0.501±0.053a	0.413±0.031b	0.298±0.016d	0.262±0.012d	0.075±0.005bc	0.087±0.007a	0.081±0.006ab	0.071±0.004cd	0.067±0.004d
	K_{30}	0.360±0.011c	0.533±0.028a	0.432±0.016b	0.308±0.008d	0.269±0.006e	0.130±0.011c	0.168±0.016a	0.148±0.013b	0.120±0.009cd	0.109±0.008d
	K_{60}	0.363±0.034c	0.545±0.086a	0.437±0.049b	0.310±0.024cd	0.271±0.018d	0.158±0.022bc	0.201±0.030a	0.176±0.025ab	0.141±0.019c	0.128±0.017c
	K_{120}	0.371±0.024c	0.565±0.064a	0.449±0.035b	0.316±0.017d	0.276±0.012d	0.168±0.016bc	0.217±0.021a	0.190±0.018b	0.151±0.015cd	0.137±0.014d

Table S5. Sensitivity analysis of mesophyll conductance (g_m) response to p_1 and p_2 sets

RuBP regeneration is limited by either insufficient NADPH ($p_1 = 4$ and $p_2 = 8$) or insufficient ATP ($p_1 = 4.5$ and $p_2 = 10.5$ or $p_1 = 4$ and $p_2 = 9.33$). Values are means ± s.d. of four replicates per treatment. Different letters donate significant differences at $P < 0.05$ between g_m values obtained in this study ($p_1 = 4$, $p_2 = 8$) and each values estimations using different p_1 and p_2 inputs

Cultivar	Treatment	Upper			Lower		
		g_m (p_1=4, p_2=8) (mol CO_2 m^{-2} s^{-1})	g_m (p_1=4, p_2=9.33) (mol CO_2 m^{-2} s^{-1})	g_m (p_1=4.5, p_2=10.5) (mol CO_2 m^{-2} s^{-1})	g_m (p_1=4, p_2=8) (mol CO_2 m^{-2} s^{-1})	g_m (p_1=4, p_2=9.33) (mol CO_2 m^{-2} s^{-1})	g_m (p_1=4.5, p_2=10.5) (mol CO_2 m^{-2} s^{-1})
H9	K_0	0.381±0.010c[1]	0.509±0.011b	2.035±0.082a	0.125±0.009b	0.144±0.011b	0.180±0.026a
	K_{30}	0.387±0.005c	0.515±0.009b	2.072±0.067a	0.144±0.014b	0.169±0.019b	0.249±0.058a
	K_{60}	0.394±0.011b	0.531±0.019b	2.196±0.147a	0.161±0.013b	0.192±0.019b	0.329±0.074a
	K_{120}	0.406±0.028b	0.547±0.049b	2.325±0.378a	0.185±0.012b	0.228±0.018b	0.489±0.093a
Z11	K_0	0.346±0.022b	0.447±0.036b	1.636±0.256a	0.075±0.005b	0.089±0.005b	0.148±0.018a
	K_{30}	0.360±0.011c	0.469±0.019b	1.789±0.136a	0.130±0.011a	0.155±0.014a	0.139±0.037a
	K_{60}	0.363±0.034b	0.474±0.056b	1.842±0.411a	0.158±0.022b	0.188±0.031ab	0.267±0.133a
	K_{120}	0.371±0.024b	0.488±0.040b	1.941±0.304a	0.168±0.016b	0.205±0.024b	0.337±0.134a

Table S6. Sensitivity analysis of the estimation of photosynthetic limitations (S_L, stomatal limitation; MC_L, mesophyll conductance limitation; B_L, biochemical limitation; T_L (T_L= S_L+MC_L+B_L), total limitation) for the variation in mesophyll conductance (g_m) due to the biases of day respiration rate (R_d)

Quantitative photosynthetic limitations calculated by Grassi and Magnani (2005) method using g_m values listed in Table S3 as affected by R_d biases. Values are means ± s.d. of three replicates per treatment. Different letters donate significant differences at $P < 0.05$ between limitation values obtained in this study (using g_m values based on R_d) and each values estimations using different values of g_m based on R_d biases

Treatment	Limitation	H9					Z11				
		R_d (%)	1.50 R_d (%)	1.25 R_d (%)	0.75 R_d (%)	0.50 R_d (%)	R_d (%)	1.50 R_d (%)	1.25 R_d (%)	0.75 R_d (%)	0.50 R_d (%)
K_0	S_L	9.35±1.92a[1]	10.04±1.56a	9.79±1.54a	9.27±1.46a	9.00±1.41a	9.18±2.32a	10.21±2.58a	9.86±2.49a	9.42±2.33a	9.06±2.29a
	MC_L	10.32±2.45a	10.75±1.93a	10.41±1.89a	12.34±2.24a	13.38±2.43a	18.07±1.81a	16.43±1.65a	17.82±1.78a	20.28±2.03a	21.83±2.19a
	B_L	6.17±0.54a	6.47±0.54a	6.31±0.42a	5.96±0.40a	5.79±0.39a	12.02±2.01a	12.84±2.15a	12.43±2.08a	11.60±1.94a	11.16±1.87a
	T_L	25.84±1.84a	27.26±2.21a	26.51±1.87a	27.57±2.09a	28.17±1.99a	39.27±4.23a	39.48±4.25a	40.11±4.02a	41.30±4.45a	42.05±4.13a
K_{30}	S_L	8.47±1.85a	8.86±1.46a	8.67±1.45a	8.26±1.38a	8.05±1.34a	7.94±1.76a	8.05±1.79a	8.00±1.77a	7.98±1.61a	7.93±1.76a
	MC_L	5.93±1.54a	6.22±1.72a	6.83±1.36a	7.12±1.62a	7.73±1.72a	5.00±1.86a	6.55±1.91a	7.17±2.09a	7.43±1.68a	7.81±2.60a
	B_L	2.26±0.58a	2.32±0.33a	2.27±0.49a	2.17±0.41a	2.13±0.62a	4.01±1.07a	4.16±1.11a	4.09±1.10a	4.03±1.08a	3.97±1.06a
	T_L	16.66±2.63a	17.4±2.20a	17.77±2.17a	17.55±2.41a	17.91±2.16a	16.95±2.84a	18.76±3.14a	19.26±3.07a	19.44±3.39a	19.77±3.09a
K_{60}	SL	4.32±1.03a	4.53±0.82a	4.45±0.81a	4.54±0.78a	4.94±0.77a	4.50±0.94a	4.50±0.78a	4.41±0.92a	4.17±0.87a	4.07±0.85a
	MCL	3.04±0.95a	3.45±0.80a	3.80±1.02a	4.28±0.97a	4.30±0.85a	2.49±1.21a	2.62±0.90a	2.91±1.15a	3.47±1.02a	3.77±0.97a
	BL	0.51±0.20a	0.53±0.21a	0.52±0.22a	0.50±0.30a	0.49±0.27a	1.74±0.31a	1.79±0.32a	1.78±0.32a	1.74±0.29a	1.72±0.27a
	TL	7.87±1.81a	8.51±1.15a	8.77±1.33a	9.32±1.41a	9.73±1.13a	8.73±1.39a	8.91±1.39a	9.10±1.19a	9.38±1.56a	9.56±1.14a

Table S7. Sensitivity analysis of the estimation of photosynthetic limitations (S_L, stomatal limitation; MC_L, mesophyll conductance limitation; B_L, biochemical limitation; T_L (T_L= S_L+MC_L+B_L), total limitation) for the variation in mesophyll conductance (g_m) due to the biases of chloroplastic CO_2 compensation point (Γ*)

Quantitative photosynthetic limitations calculated by Grassi and Magnani (2005) method using g_m values listed in Table S4 as affected by Γ^* biases. Values are means ± s.d. of three replicates per treatment. Different letters donate significant differences at $P < 0.05$ between limitation values obtained in this study (using g_m values based on Γ*) and each values estimations using different values of g_m based on Γ^* biases

Treatment	Limitation	H9					Z11				
		Γ* (%)	1.10 Γ* (%)	1.05 Γ* (%)	0.95 Γ* (%)	0.90 Γ* (%)	Γ* (%)	1.10 Γ* (%)	1.05 Γ* (%)	0.95 Γ* (%)	0.90 Γ* (%)
K_0	S_L	9.35±1.92a[1]	10.21±2.10a	9.86±2.07a	9.23±1.90a	8.95±1.03a	9.18±2.32a	10.01±2.00a	9.75±1.76a	9.29±1.86a	9.08±1.03a
	MC_L	10.32±2.45a	10.70±2.54a	10.80±2.75a	11.88±2.10a	12.39±2.48a	18.07±1.81a	19.15±2.83a	19.22±2.13a	19.31±1.93a	19.33±2.00a
	B_L	6.17±0.54a	6.57±0.94a	6.35±0.37a	5.94±0.52a	5.76±1.15a	12.02±2.01a	12.64±2.53a	12.31±2.01a	11.71±2.34a	11.44±2.30a
	T_L	25.84±1.84a	27.48±1.74a	27.01±3.24a	27.05±2.93a	27.10±4.22a	39.27±4.23a	41.80±5.63a	41.28±4.45a	40.31±5.08a	39.85±4.77a
K_{30}	S_L	8.47±1.85a	9.08±1.98a	8.75±1.73a	8.20±1.27a	7.94±1.59a	7.94±1.76a	8.09±1.62a	8.02±1.44a	7.89±1.58a	7.82±1.60a
	MC_L	5.93±1.54a	6.81±1.02a	6.94±1.23a	7.21±2.08a	7.82±1.70a	5.00±1.86a	6.84±1.41a	7.12±1.38a	7.58±1.64a	7.62±1.51a
	B_L	2.26±0.58a	2.38±0.49a	2.30±0.41a	2.15±0.55a	2.09±0.42a	4.01±1.07a	4.21±0.84a	4.12±1.10a	3.94±0.79a	3.86±0.82a
	T_L	16.66±2.63a	18.27±2.92a	17.99±3.96a	17.56±2.90a	17.85±3.01a	16.95±2.84a	19.14±3.12a	19.26±3.28a	19.41±4.00a	19.30±3.67a
K_{60}	SL	4.32±1.03a	4.61±1.10a	4.48±0.49a	4.84±1.02a	5.13±0.83a	4.50±0.94a	4.77±0.95a	4.63±0.83a	4.39±0.88a	4.27±0.93a
	MCL	3.04±0.95a	3.74±1.17a	4.29±0.98a	4.76±1.59a	4.92±1.10a	2.49±1.21a	1.98±0.40a	2.44±0.77a	2.86±0.77a	3.07±0.89a
	BL	0.51±0.20a	0.54±0.22a	0.52±0.12a	0.49±0.20a	0.48±0.10a	1.74±0.31a	1.80±0.36a	1.79±0.32a	1.76±0.35a	1.74±0.37a
	TL	7.87±1.81a	8.89±2.04a	9.29±2.25a	10.09±2.26a	10.53±2.02a	8.73±1.39a	8.55±1.71a	8.86±1.44a	9.01±1.80a	9.08±1.67a

Table S8. Sensitivity analysis of the estimation of photosynthetic limitations (S_L, stomatal limitation; MC_L, mesophyll conductance limitation; B_L, biochemical limitation; T_L (T_L= S_L+MC_L+B_L), total limitation) for the variation in mesophyll conductance (g_m) due to different p_1 and p_2 inputs

Quantitative photosynthetic limitations calculated by Grassi and Magnani (2005) method using g_m values listed in Table S5 as affected by p_1 and p_2 sets. Values are means ± s.d. of four replicates per treatment. Different letters donate significant differences at $P < 0.05$ between limitation values obtained in this study (using g_m values based on $p_1 = 4$, $p_2 = 8$) and each values estimations using different values of g_m based on different p_1 and p_2 inputs.

Treatment	Limitation	H9			Z11		
		p_1=4, p_2=8 (%)	p_1=4, p_2=9.33 (%)	p_1=4.5, p_2=10.5 (%)	p_1=4, p_2=8 (%)	p_1=4, p_2=9.33 (%)	p_1=4.5, p_2=10.5 (%)
K_0	S_L	9.35±1.92a[1]	9.96±2.52a	10.00±2.49a	9.18±2.32a	10.32±2.87a	11.97±3.33a
	MC_L	10.32±2.45a	11.61±3.70a	13.71±2.68a	18.07±1.81a	17.51±2.91a	16.31±1.04a
	B_L	6.17±0.54a	6.41±0.57a	6.57±0.62a	12.02±2.01a	12.73±2.01a	14.57±2.22a
	T_L	25.84±1.84a	27.97±2.34a	30.27±2.95a	39.27±4.23a	40.56±2.12a	42.85±4.69a
K_{30}	S_L	8.47±1.85a	8.86±1.95a	9.39±2.10a	7.94±1.76a	8.13±1.79a	7.88±1.76a
	MC_L	5.93±1.54a	7.75±2.80a	10.27±3.27a	5.00±1.86a	7.51±1.48a	9.43±3.70a
	B_L	2.26±0.58a	2.33±0.71a	2.45±0.93a	4.01±1.07a	4.23±1.18a	3.97±1.11a
	T_L	16.66±2.63a	18.94±2.60a	22.11±4.40a	16.95±2.84a	19.87±3.15a	21.58±2.42a
K_{60}	SL	4.32±1.03a	4.52±1.43a	4.72±1.12a	4.50±0.94a	4.47±1.03a	5.11±1.06a
	MCL	3.04±0.95a	4.39±1.27a	6.95±1.37a	2.49±1.21a	3.39±0.59a	4.52±1.88a
	BL	0.51±0.20a	0.53±0.32a	0.58±0.48a	1.74±0.31a	1.77±0.33a	1.81±0.31a
	TL	7.87±1.81a	9.44±2.41a	10.25±4.01a	8.73±1.39a	9.62±1.27a	10.44±2.01a

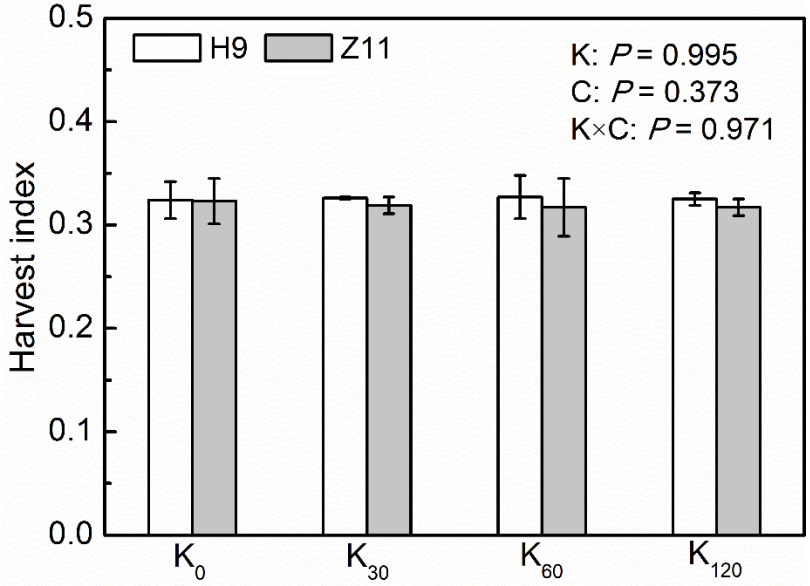

Fig. S1. Harvest index of Huayouza No. 9 (H9) and Zhongshuang No. 11 (Z11) as affected by contrasting K rates. Data are mean ± s.d. of four replications. K and C indicate K treatments and cultivars, and K × C indicates the interaction.

Methods S1. Detailed materials and methods

Study site and growth conditions

A field experiment was conducted at Wuxue county, Hubei province, central China (30° 06′46″N, 115° 36′9″E) during the 2014–2015 oilseed rape growing season. With a subtropical monsoon climate, the mean temperature of the whole season and wintertime (from December 2014 to February 2015) was 12.2 and 6.5°C, respectively. The total precipitation during the oilseed rape cropping season was 670.0 mm, with wintertime accounting for 33.2% of the total. The soil was a sandy loam with pH value (1 : 2.5 soil: DI water) 5.7, organic matter 37.1 g kg^{-1}, total N 2.0 g kg^{-1}, NH_4OAc-K 45.3 mg kg^{-1}, Olsen-P 14.6 mg kg^{-1} and hot-water soluble B 0.82 mg kg^{-1} in the topsoil layer (0–20 cm). As stated by Zou *et al*. (2011), the soil is a K-deficient type, which would cause yield reduction without K fertilizer addition.

Experimental design

The field experiment was designed as a split-plot experiment with K fertilizer level as the main plot and cultivar as the subplot, with four replicates. The K treatments included 0 kg K_2O ha^{-1} (K_0), 30 kg K_2O ha^{-1} (K_{30}), 60 kg K_2O ha^{-1} (K_{60}) and 120 kg K_2O ha^{-1} (K_{120}). The cultivars were Huayouza No. 9 (H9) and Zhongshuang No. 11 (Z11) with higher and lower KUtE, respectively.

To ensure that nutrients other than K can satisfy the need for plant growth, 180 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 1.6 kg B ha^{-1} were applied for all treatments (Wang *et al.* 2014). The N, P, K, and B fertilizers used in the experiment consisted of urea (46% N), superphosphate (12% P_2O_5), potassium chloride (60% K_2O), and borax (10.8% B), respectively. The N fertilizer was applied in three splits: 60% prior to transplanting (i.e., BBCH 15-16) (Lancashire *et al.* 1991), 20% at the over-wintering stage (i.e., BBCH 29), and 20% at the initiation of stem elongation (i.e., BBCH 30). Moreover, all the P, K, and B fertilizers were applied as basal fertilizer. The experimental field was ploughed and levelled with a rotary tiller,

and the basal fertilizers were incorporated during the process. Each plot measured 20 m^2 with a length of 10 m and a width of 2 m, and they were halved for sample collection and yield measurement.

Rapeseed was sown in prepared seedbeds on 22 September 2014. Approximately 30 d after sowing, oilseed rape seedlings with five leaves (i.e., BBCH 15-16, 3–4 g dry weight $plant^{-1}$) were randomly selected and transplanted by hand in double rows spaced approximately 0.3 m apart with 0.2–0.3 m between plants, and 112 500 plants ha^{-1}. The oilseed rape was grown under rain-fed conditions. Weeds, pests and disease stress were controlled by spraying with herbicides, insecticide and fungicide. No obvious weeds, insect pests, or disease infestation occurred during the cropping season.

Plant and leaf tagging

Both rapeseed cultivars contained a total of 9 to 10 leaves (i.e., BBCH 19) 75 d (at the over-wintering stage) after transplanting, with no difference between K treatments. The rapeseed grew in rosulates, and all leaves were staggered with each other to avoid obvious absorption differences of light energy due to leaf overlap. Therefore, 18 upper (the 3rd fully expanded leaf from apex downwards) and lower leaves (the 7th fully expanded leaf from apex downwards) were tagged in each of the four replicate plots for the measurements. Additionally, 16 uniform plants were tagged for determining the plant dry matter of each plot.

Leaf gas exchange and fluorescence measurements

Tagged leaves were used for simultaneous leaf gas exchange and chlorophyll fluorescence measurements using a portable open circuit infrared gas analysis system (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) equipped with an integrated leaf chamber fluorometer (LI-6400-40). At least four measurements, on either the upper or lower leaves of each K treatment, were carried out each day in the late morning (11:00–13:00) under a

light-saturating photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} (with 90% red light and 10% blue light). The CO_2 concentration in the leaf chamber (C_a) was set at 400 μmol mol^{-1} air, leaf temperature was controlled at 20 ± 0.2°C, relative humidity was maintained at 55 ± 4%, and the flow rate was 500 μmol s^{-1}. After reaching a steady state, typically after 10 min, gas exchange parameters, steady-state fluorescence yield (F_s) and maximum fluorescence (F_m') with a light-saturating pulse (0.8s) of approx. 8000 μmol m^{-2} s^{-1} were recorded.

Twenty minutes after acclimation to saturating light conditions, measurements were taken on three leaves for each treatment to construct A/C_i curves. The C_a was decreased stepwise from 400 to 300, 250, 200, 150, 100, 50 μmol CO_2 mol^{-1}, and then increased from 50 to 400, 600, 800, 1000, 1200, 1500, 1800 μmol CO_2 mol^{-1} at a constant PPDF of 1200 μmol m^{-2} s^{-1} at 20 ± 0.2°C and 50 ± 4% relative humidity. In all cases, parameters were recorded after the gas exchange rate had stabilized at the given C_a. Additionally, three dead leaves (obtained by submerging the leaves in boiling water until no variable chlorophyll fluorescence was detected) per treatment were used to analyse the leakage in the leaf chamber (Flexas *et al.* 2007). The mean value for each treatment did not differ significantly. Therefore, the average leakage was used to correct the measured gas exchange parameters.

The effective quantum efficiency of the PSII photosystem (Φ_{PSII}, Genty *et al.* 1989) was then calculated as follows:

$$\Phi_{PSII} = \frac{F_m' - F_s}{F_m'} \quad (1)$$

The linear electron transport rate on the basis of chlorophyll fluorescence (J) can be determined as:

$$J = \Phi_{PSII} \mathrm{PPFD} \alpha\beta \quad (2)$$

where α is the leaf absorptance, and β is the fraction of light absorbed by PSII. The product αβ was determined as the slope of the relationship

between Φ_{PSII} and Φ_{CO_2} obtained by varying the light intensity under nonphotorespiratory conditions in a low O_2 atmosphere (<1.0%) (Valentini *et al.* 1995).

The variable *J* method proposed by Harley *et al.* (1992) was used to calculate g_m and C_c.

$$g_m = \frac{A}{C_i - \frac{\Gamma^*\left(J + 8(A + R_d)\right)}{J - 4(A + R_d)}} \quad (3)$$

$$C_c = C_i - \frac{A}{g_m} \quad (4)$$

where A, C_i and J were determined as previously described, and the day respiration rate (R_d) and the apparent CO_2 photocompensation point (C_i^*) were measured by the Laisk method as reported by Brooks & Farquhar (1985). Briefly, the response of A to C_i was generated at three PPFD values of 75, 150, 500 μmol m^{-2} s^{-1}, respectively, with each having five different C_a values in chamber (i.e., 50, 80, 100, 120 and 150 μmol CO_2 mol^{-1}). A linear regression was then fitted to each A/C_i curve. The *x*-axis and *y*-axis of intersection point of three A/C_i curves were defined as C_i^* and R_d (von Caemmerer *et al.* 1994). Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration calculated from C_i^* and R_d as:

$$\Gamma^* = C_i^* + \frac{R_d}{g_m} \quad (5)$$

The A/C_c curves were therefore constructed by converting A/C_i curves according to Eqn 4. The variable *J* method, which estimates g_m by combining chlorophyll fluorescence and leaf gas exchange measurements, is sensitive to many sources of bias. Recently, Gu and Sun (2014) identified three sources of uncertainty: (1) errors in the estimates of R_d and Γ^*; (2) biases in the measurement of C_i, A and J; and (3) different assumptions with respect to processes that limit RuBP regeneration, that is, errors in p_1 and p_2. We accounted for leakage during measurement, and there seemed to be no unreliable errors of C_i, A or J. To identify the effects of R_d and Γ^* on g_m estimates, sensitivity analyses were conducted

using actual Γ* values calculated in this study and 10%, 5% elevated (or reduced) values, actual R_d and values elevated (or reduced) by 10% or 5% (Tables S3–S4, available as Supplementary Material to this paper) (Harley *et al.* 1992).

According to the Farquhar model, A and J can be linked as follows:

$$A = \frac{J(C_c - \Gamma^*)}{p_1 C_c + p_2 \Gamma^*} - R_d \quad (6)$$

RuBP regeneration can be limited by insufficient of either NADPH or ATP. If it is limited by NADPH, $p_1 = 4$ and $p_2 = 8$; if it is limited by ATP, $p_1 = 4$ and $p_2 = 9.33$ or $p_1 = 4.5$ and $p_2 = 10.5$. Sensitivity analyses for wrong assumptions regarding p_1 and p_2 in g_m estimates were conducted for all three sets (Table S5, available as Supplementary Material to this paper). Finally, sensitivity analysis for photosynthetic limitations was conducted based on these calculated g_m values (Tables S6–S8, available as Supplementary Material to this paper), and the conclusion was drawn that g_m is significantly influenced by varying Γ^*, R_d (Tables S3–S4, available as Supplementary Material to this paper), and p_1 and p_2 inputs (Table S5, available as Supplementary Material to this paper). However, all g_m estimates repeated the same pattern with the variation of actual g_m for each treatment and cultivar; that is, the impact of K on g_m did not significantly change.

Quantitative limitation analysis

There was no obvious influence imposed by K deficiency on photosynthesis by upper leaves in either cultivar, thus only limitations (stomatal limitations, S_L; mesophyll conductance limitations, MC_L; biochemical limitations, B_L) of lower leaves were investigated by using the quantitative limitation analysis method proposed by Grassi and Magnai (2005). Because the fluorescence derived linear electron transport rate (J) is tightly coupled with the maximum rate of Rubisco-catalysed carboxylation ($V_{c,max}$) (Galmés *et al.* 2007; Gallé *et al.* 2009; Varone *et al.* 2012), a minor

modification was adopted when calculating B_L by using J instead of $V_{c,max}$ (Gallé *et al.* 2009; Varone *et al.* 2012). This substitution can avoid possible errors in the determination of $V_{c,max}$ (Bernacchi *et al.* 2002; Gallé *et al.* 2009). Accordingly, g_s, g_m and J were used to calculate the proportions of the three limitations. Relative changes in light-saturated assimilation are expressed in terms of relative changes in stomatal, mesophyll conductance, and biochemical capacity as in Eqn 7.

$$\frac{\mathrm{d}A}{A} = S_L + MC_L + B_L = l_s \cdot \frac{\mathrm{d}_{g_{sc}}}{g_{sc}} + l_{mc} \cdot \frac{\mathrm{d}_{g_m}}{g_m} + l_b \cdot \frac{\mathrm{d}_J}{J} \quad (7)$$

where l_s, l_{mc}, and l_b are the corresponding relative limitations calculated as Eqns 8 to 10, g_{sc} is stomatal conductance of CO_2 (g_s/1.6).

$$l_s = \frac{g_{tot}/g_{sc} \cdot \partial A/\partial C_c}{g_{tot} + \partial A/\partial C_c} \quad (8)$$

$$l_m = \frac{g_{tot}/g_m \cdot \partial A/\partial C_c}{g_{tot} + \partial A/\partial C_c} \quad (9)$$

$$l_b = \frac{g_{tot}}{g_{tot} + \partial A/\partial C_c} \quad (10)$$

where g_{tot} is the total conductance of CO_2 from leaf surface to carboxylation sites determined as Eqn 11. $\partial A/\partial C_c$ was calculated as the slope of A/C_c response curves over a C_c range of 50–100 μmol mol^{-1} (Tomás *et al.* 2013).

$$g_{tot} = \frac{1}{1/g_{sc} + 1/g_m} \quad (11)$$

Then, the relative change of A, g_{sc}, g_m and J in Eqn 7 can be approximated by (Chen *et al.* 2015)

$$\frac{\mathrm{d}A}{A} \approx \frac{A_{\max}^{\mathrm{ref}} - A}{A_{\max}^{\mathrm{ref}}} \quad (12)$$

$$\frac{\mathrm{d}g_{\mathrm{sc}}}{g_{\mathrm{sc}}} \approx \frac{g_{\mathrm{sc}}^{\mathrm{ref}} - g_{\mathrm{sc}}}{g_{\mathrm{sc}}^{\mathrm{ref}}} \quad (13)$$

$$\frac{\mathrm{d}g_{\mathrm{m}}}{g_{\mathrm{m}}} \approx \frac{g_{\mathrm{m}}^{\mathrm{ref}} - g_{m}}{g_{\mathrm{m}}^{\mathrm{ref}}} \quad (14)$$

$$\frac{\mathrm{d}J}{J} \approx \frac{J_{\mathrm{max}}^{\mathrm{ref}} - J}{J_{\mathrm{max}}^{\mathrm{ref}}} \quad (15)$$

where $A_{\mathrm{max}}^{\mathrm{ref}}$, $g_{\mathrm{sc}}^{\mathrm{ref}}$, $g_{\mathrm{m}}^{\mathrm{ref}}$ and $J_{\mathrm{m}}^{\mathrm{ref}}$ are the reference values of net CO_2 assimilation rate, stomatal conductance, mesophyll conductance, and the rate of electron transport, respectively, defined as the maximum value measured under light saturation. Grassi and Magnai (2005) used the maximum value of seasonal *A* as a reference to evaluate the photosynthetic limitations for each determination. In the current study, the maximum *A* was generally reached concomitantly with maximum g_s, g_m and *J* in the K_{120} treatment. Therefore, treatment K_{120} was used as a reference, which means, there was no limitation present in the lower leaves under 120 kg K_2O ha^{-1}. Whenever one of the three parameters was larger in any one of the three K treatments (K_0, K_{30} or K_{60}) than that in the K_{120} treatment, its corresponding limitation was set to zero. In this way, the limitations in the lower leaves of different K treatments could be quantified. Finally, total limitations were defined as the sum of S_L, MC_L and B_L.

It is noteworthy that quantitative limitations were calculated on the basis of g_m. A sensitivity analysis with different estimated values was conducted to elucidate the effects of methodological artefacts on photosynthetic limitations and their proportions (Tables S6–S8, available as Supplementary Material to this paper). It seems that those biases did not cause strong effects on the final results as mentioned in Fig. 3.

Total dry matter, leaf area index, specific leaf weight and leaf density

After the determination of photosynthesis parameters, six tagged leaves and eight tagged plants (with shoot cut off at the cotyledonary node) in each plot were collected to determine their individual leaf area, dry matter of specific plant organs, and total dry matter. Each leaf, together with a reference green card (25 cm^2), was digitally scanned using an Epson ES-1200C scanner (Epson, Long Beach, CA, USA), and the leaf area was determined using ImageJ software (National Institutes of Health, Bethesda, Maryland) (Sack *et al.* 2003; Battie-Laclau *et al.* 2014). Leaf dry matter and total dry matter were determined by weighing after oven drying at 65°C for 48 h. Specific leaf weight was calculated by dividing leaf dry matter by leaf area. Total green leaf area was measured using a leaf-area meter (Li-2200, Li-Cor Inc., Lincoln, NE, USA) and expressed as the total one sided leaf area that is green per unit ground area of leaf area index (LAI). According to Witkowski and Lamont (1991), dividing leaf specific weight by leaf thickness is defined as leaf density. Following the method of Tomás *et al.* (2013), at least thirty measurements were performed at a magnification of 200× in microscope fields of semi-thin (0.8 μm) cross-sections to determine leaf thickness.

K and chlorophyll concentration

Dried leaves and plants were milled, and approximately 0.15 g of powder was digested with H_2SO_4-H_2O_2 as described by Thomas *et al.* (1967). The K concentration in the digestion solution was measured by a flame photometer (M-410, Cole-Parmer, Chicago, IL, USA). The shoot K uptake was determined as the product of plant tissue K concentration multiplied by total dry matter.

Fresh leaf discs of approximately 0.5 g were prepared using a 1 cm^2 circular punch for chlorophyll concentration determination according to the method of Arnon (1949). Leaf discs were extracted with 50 mL of 80% acetone in the dark for 48 h at 25°C until they were blanched. The concentrations of chlorophyll a and b were determined by measuring the absorbance of the extract with a spectrophotometer (UV2102 PCS, Unico,

China) at 663 and 645 nm. Total chlorophyll was expressed as the sum of chlorophyll a and b. There were four replications each for the chlorophyll and K concentration determination.

Yield and K utilization efficiency

The rest of the eight tagged plants from each plot were cut at ground level 5 d prior to harvest, and separated into seeds, stems and pod walls. Aboveground biomass was the sum of the dry weight of each part after oven drying at 65°C for 48 h. After that, the K concentration of each part was determined. At maturity (8 May 2015; i.e., BBCH 99), plants from a 10 m^2 area in each plot were harvested and threshed, and the seeds were dried to determine seed yield.

K content (K_{shoot}) and K utilization efficiency (KUtE) were calculated as follows.

$$\mathrm{K_{shoot}} = \mathrm{K_{seed}} \cdot \mathrm{B_{seed}} + \mathrm{K_{stem}} \cdot \mathrm{B_{stem}} + \mathrm{K_{pood\ wall}} \cdot \mathrm{B_{pood\ wall}} \quad (16)$$

$$\mathrm{KUtE} = \frac{\mathrm{B_{seed}}}{\mathrm{K_{shoot}}} \quad (17)$$

where K_{seed}, K_{stem}, and $K_{pod\ wall}$ are the K concentrations of seeds, stems and pod walls; B_{seed}, B_{stem}, and $B_{pod\ wall}$ are the dry biomass of seeds, stems and pod walls.

Statistical analysis

Data were analyzed using descriptive statistical analyses to obtain means and the range of variability and standard deviation (SD). All data were subjected to two-way analysis of variance (*ANOVA*) with SPSS 18.0 software (SPSS, Chicago, IL, USA). The difference between mean values was compared using Duncan's multiple range test at the $P < 0.05$ level of significance. Graphics and regression analysis were performed using

OriginPro 8.5 software (OriginLab Corporation, Northampton, MA, USA).

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