S1 Preparation of Plant Materials with a Dual Root System:

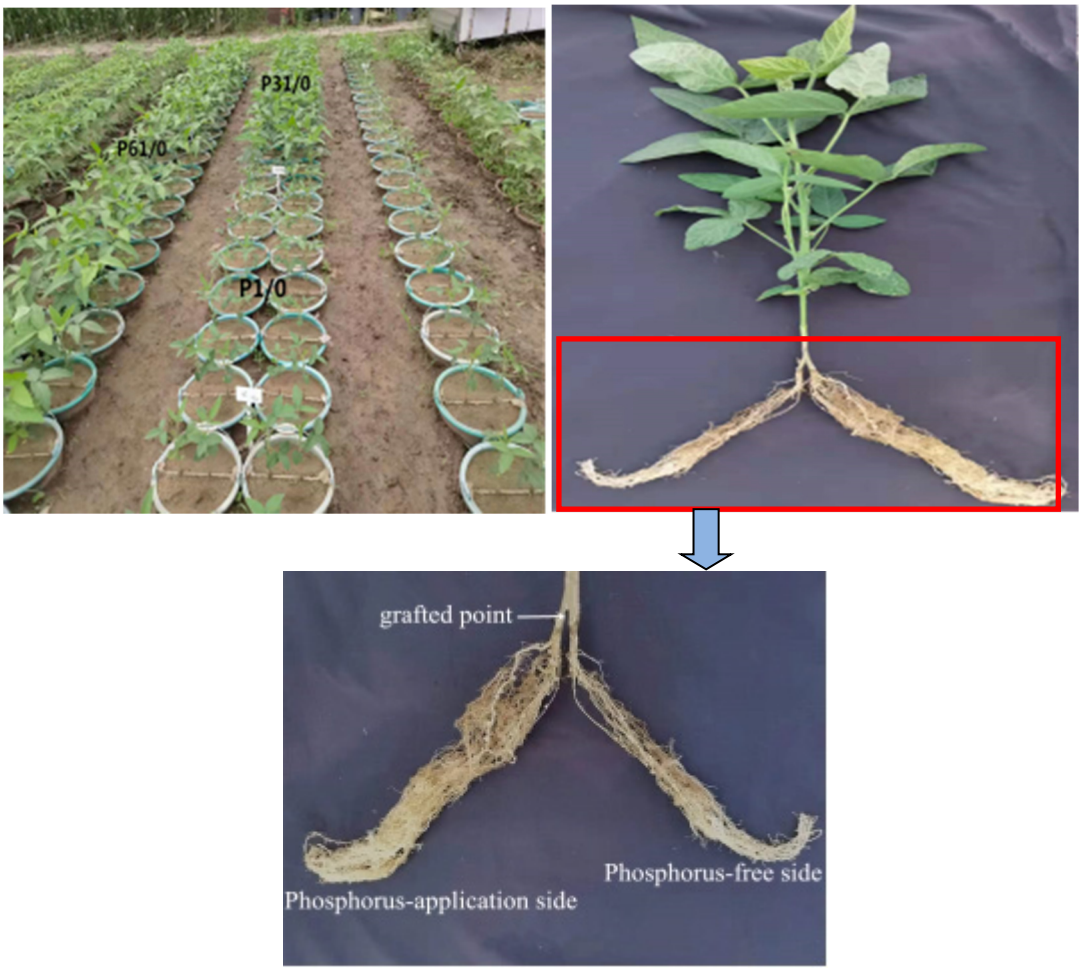
Potted cultivation with river sand was used in this study. Each plastic pots was 0.30 m in diameter and 0.28 m in height. Each was divided into two equal parts in the middle with a custom-made polycarbonate plastic plate that fit the inside of the pot well. The top of the plastic plate was 2 cm lower than the edge of the pot, and the gaps between the pot and the partition plate were all sealed with glue. Two drainage holes 1 cm in diameter were drilled at the bottom of the pot, with one on each side of the partition plate. Each pot was filled with 20 kg of river sand. Before use, the sand was thoroughly washed with tap water and then rinsed twice with distilled water.

The soybean seeds were sown in the fine-sand medium at a depth of 2 cm and cultured in an incubator at 30°C for approximately 3 days. When the distance between the growing point of the cotyledon and the tip of root reached approximately 7-10 cm, the root system of soybean seedlings was exposed by rinsing with distilled water. First, a 0.5-1.0-cm-long incision (the seedling was not severed) was made upward with a sterilized blade from the root to the cotyledon at the site above the middle point of the hypocotyls in one soybean seedling. In the other seedling, a similar slash was made downward from the cotyledon to the root.

After the two seedlings were cross-inserted through their cuts, the grafting site was fixed with a grafting clip. The roots of the two seedlings were separately planted into the fine-sand medium divided by the partition plate in the plastic pot, with the grafting site exactly above the partition plate.

Dual Root System

After a week, the grafting clip was removed. In one seedling, the top part above the grafting site was cut, leaving only the grafting site and the lower part. As a result, a seedling containing two root systems and one aboveground part was obtained. Two successfully grafted soybean seedlings were left in each pot to generate a one-plant/two-root-system soybean.



the root morphology of a dual-root soybean plant under P31/0 treatment at initial flowering stage, with the Phosphorus-application side on the left and the Phosphorus-free side on the right.

Table S1. The elements of P or K in different P level treatments

treatment	inorganic salts concentration(mg/L)			
	KH_2PO_4	K_2SO_4	KCl	$NaH_2PO_4·2H_2O$
P1	4.39	42	36	—
P31	136	—	—	—
P61	136	—	—	150.96
P0		43.5	37.25	

Table S2. Concentrations of elements in nutrient medium of the sand culture

inorganic salts	concentration (mg/L)	inorganic salts	concentration (mg/L)
$(NH4)_2SO_4$	235.80	$ZnSO_4·7H_2O$	0.22
$MgSO_4$	240.00	$MnCl_2·4H_2O$	4.90
$CaCl_2$	220.00	H_3BO_3	2.86
$Na_2MoO_4·H_2O$	0.03	Fe–EDTA*	—
$CuSO_4·5H_2O$	0.08	—	—

Table S3. Primer sequences of key genes for nodulation and nitrogen fixation in soybean

Genes	Primer sequences
GmEXP2- F	GAGTGGGTGGTAGGGGGTTA
GmEXP2- R	AACGGGTTGCTAATGCTGGG
GmSPX5-F	TGGATGCCAACGAACTCAAC
GmSPX5-R	TGAGCGAAGTAGAGCACCAGTTT
GmALN1-F	TATCCTACAACCGTGTCAAAGGAAAAACTA
GmALN1-R	AAGAACACCAGCACTTAAGAGCCCC
GmACP1-F	CTGTATCAACAGGCTGTAACTGCTACCAAC
GmACP1-R	TTGTATGTAACTGCTGTCTTACCATTGTATTTATC
GmUR9-F	TGTGTCTTGTAATTCTGGTTTTTGTTATCTTTATC
GmUR9-R	AAGGACACTAGGAAATTATTGGAATAGTAGAAAC
Gm-PUR5-F	GATTGGAGGCTTCGGTGGTTTATTC
Gm-PUR5-R	TCATTCACACTCATCGCAACCAGG
GmHIUH-F	TTGGAGAGGATCTGTTTTCAAATGAAGAG
GmHIUH-R	GTGACGAATTACTTGCTGTCCGTTGAC
Gm18S-F	CCATAAACGATGCCGACCAG
Gm18S-R	AGCCTTGCGACCATACTCCC
nifD- F	5'-ATATGGAGGCAAGACCATCG-3'
nifD - R	5'-CCCGTAGTCCCATCTCTTCA-3'
nifK - F	5'-AAGATGATTGCGGTTTCCAC-3'
nifK - R	5'-GTCCCAAAAATGCTCCA GAA-3'
nifH - F	5'-GGACTCGACTCGCCTTATTC- 3'
16S-F	5'-GCGGGAAGATAATGACGGTA-3'
16S-R	5'-ACTCGCAGTTCCACTCACCT-3'

Table S4.Effect of phosphorus supply on the number of nodules in double-root soybeans

Treatments		The number of nodules	
		P+	P-
V4	P1/0	32.33±2.33 b	28.00±3.21 b
	P31/0	86.33±2.40 a**	51.67±1.45 a
	P61/0	92.33±1.45 a**	57.33±2.19 a
R1	P1/0	46.67±1.20 c**	35.67±1.33 c
	P31/0	93.33±0.88 b**	54.00±1.15 b
	P61/0	108.33±3.48 a**	64.67±0.89 a

Note: All data in the table are expressed as the mean ± standard error (n=4). Different lowercase letters indicate the differences between treatments at a significance level of 5%. Longitudinal comparison. ** denote a significant difference between the P+ side and the P− side at the 1% level, respectively.

Table S5.Effect of P supply on the number of IC、UC and Bt in nodules of double-root soybeans

Treatments	P1/0		P31/0		P61/0	
	P+	P-	P+	P-	P+	P-
IC	1.7±0.2 b	1.6±0.1 a	2.5±0.2 a**	1.8±0.1 a	2.1±0.2 a	1.7±0.1 a
UC	3.4±0.2 a	3.9±0.2 a	0.8±0.1 b**	1.9±0.1 b	1.3±0.2 b**	2.7±0.3 ab
Bt	26.3±1.7 b**	15.7±1.1 b	70.5±3.2 a**	30.6±1.5 a	68.8±3.9 a**	28.1±2.2 a

Note: All data in the table are expressed as the mean ± standard error (n=8). Different lowercase letters indicate the differences between treatments at a significance level of 5%. Longitudinal comparison. ** denote a significant difference between the P+ side and the P− side at the 1% level, respectively. IC: infected cell; UC: uninfected cell;Bt: bacteroid, IC and UC are the quantities in an area of 17.40μm×13.88μm under a transmission electron microscope (4000×)，Bt are the quantities in an area of 7.78μm×6.21μm under a transmission electron microscope (20,000×).