

Each plastic pot was 0.30 m in diameter and 0.28 m in height and was divided into two equal parts in the middle with a custom-made polycarbonate plastic partition plate. The gap between the plate and the pot was sealed with glue. Two drainage holes 1 cm in diameter were drilled at the bottom of the pot. Each pot was filled with 20 kg of sand.

Dual root system of soybean

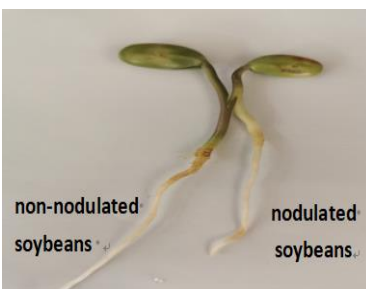
Soybeans sand culture experiments



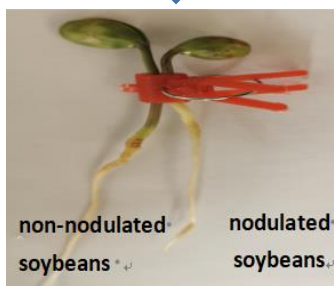
Dual Root System



Seeds were seeded into fine-sand medium and were cultured in a growth chamber at 30°C for approximately 3 days. Cut long slightly, the nodulated soybean had an upward incision, the non-nodulated soybean plants had a downward incision



Two seedlings were cross-inserted into the cuts of each other

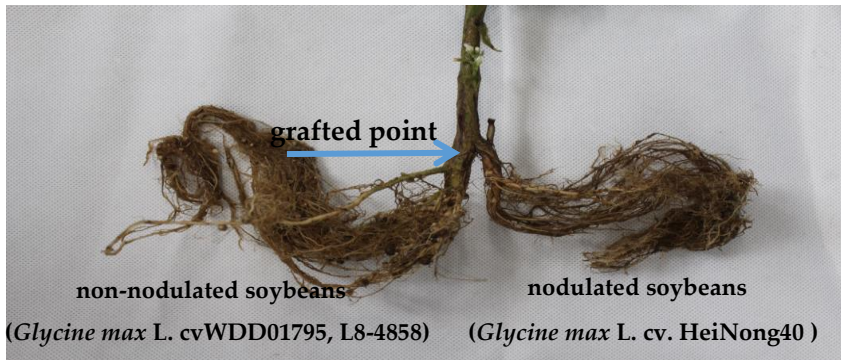


Fixed using a grafting clip



Two seedlings were separately planted into fine-sand medium of each half of the pot divided by the partition plate

Sampled soybeans



The soybean plant with dual root systems, the roots of a soybean plant with a dual root system at the time of sampling, with non-nodulated roots on the right and nodulated roots on the left side.

Figure S1. Preparation of plant materials with a dual root system

Table S1. Ingredients of universal nutrient solution for sand culture

Inorganic Salts	Concentration (mg/L)	Inorganic Salts	Concentration (mg/L)
KH ₂ PO ₄	136.00	ZnSO ₄ ·7H ₂ O	0.22
MgSO ₄	240.00	MnCl ₂ ·4H ₂ O	4.90
CaCl ₂	220.00	H ₃ BO ₃	2.86
Na ₂ MoO ₄ ·H ₂ O	0.03	FeSO ₄ ·7H ₂ O	5.57
CuSO ₄ ·5H ₂ O	0.08	Na ₂ EDTA	7.45

The corresponding ¹⁵NH₄¹⁵NO₃ concentrations of these treatments were 0 mg/L (N0), 71.4 mg/L (N25), 142.8 mg/L (N50), 214.2 mg/L (N75), and 285.6 mg/L (N100), respectively.

Table S2. Change in nodules of two soybean root systems

	Treatments	Nodules on Both Sides		Nodules on Single Side
		N+	N-	N-
Nodules number (per plant)	N0	336.0 ± 34.54a	336.0 ± 34.54a	492.8 ± 45.42a
	N100	139.7 ± 12.00b	348.5 ± 21.29a	189.5 ± 20.75b
Nodules weight (g/plant)	N0	1.36 ± 0.232a	1.36 ± 0.232a	1.42 ± 0.244a
	N100	0.49 ± 0.127b	1.39 ± 0.222a	0.91 ± 0.109b

Changes in number and dry weight of root nodules under N0 and N100 treatments in two soybean double-root systems. In the dual root system, the values are the means ± standard error (*n* = 4). Different lowercase letters indicate significant differences between means at the 5% level.