An environment friendly engineered *Azotobacter* can replace substantial amount of urea fertilizer and yet sustain same wheat yield

Supplementary Figures and Tables

TAGACGAGGCACAGCATGACCCCGGCCAACCCGACCCTGAGCAACGAGCCGCAAGCGCCTCACGCCGAG

AGCGACGAGCTGCTTCCCGAGATCTTTCGCCAGACGGTGGAGCATGCGCCCATCGCCATTTCCATCACC

GACCTCAAGGCCAACATTCTTTACGCCAATCGCGCTTTCCGCACCATCACCGGCTACGGCAGCGAGGAA

GTGCTCGGCAAGAACGAATCGATCCTCTCCAACGGCACCACGCCGCGCCTGGTCTACCAGGCCCTGTGG

GGCTGGCTGGCGCAGAAGAAGCCCTGGTCCGGCGTGCTGGTCAACCGCCGCAAGGACAAGACCCTGTAC

CTGGCCGAACTGACCGTGGCGCCGGTGCTCAACGAGGCCGGCGAGACCATCTACTACCTGGGCATGCAC

CGCGACACCAGCGAATTGCACGAACTGGAACAACGCGTCAACAACCAGCGCCTGATGATCGAGGCGGTG

GTCAGCGCCGCCCCGGCGGCGATGGTGGTGCTCGACCGCCAGCACCGGGTGATGCTCTCCAACCCGAGC

TTCTGCCGCCTGGCCCGCGACCTGGTCGAGGATGGCAGCAGCGAGAGCCTGGTGGCGCTGCTGCGGGAA

AACCTCGCCGCCCCCTTCGAGACGCTGGAAAACCAGGGCAGCGCCTTCTCCGGCAAGGAGATCTCCTTC

GACCTGGGCGGCCGCTCGCCGCGCTGGCTGTCCTGCCACGGCCGGGCCATCCACATCGAGAACGAGCAG

GCCCACGTGTTCTTCGCGCCCACCGAGGAACGCTACCTGCTGCTGACCATCAACGACATCTCCGAGCTG

CGCCAGAAGCAGCAGGATTCGCGGCTCAACGCGCTGAAGGCGCTGATGGCCGAGGAAGAGCTGCTGGAA

GGCATGCGCGAGACCTTCAACGCCGCCATCCATCGCCTGCAGGGCCCGGCCAACCTGATCAGCGCGGCG

ATGCGCATGCTCGAACGGCGCCTCGGCGGCAAGGCCGGCAACGACCCGGTGCTGAGCGCCATGCGCGAA

GCCAGCACGGCCGGAATGGAGGCACTGGAGAACCTCAGTGGCTCCATTCCGGTGCGCATGGCCGAGTCC

AAGATGCCGGTCAACCTCAACCAGTTGATCCGCGAGGTGATCACCCTGTGCACCGACCAGTTGCTGGCC

CAGGGCATCGTCGTCGACTGGCAGCCGGCGCTGCGCCTGCCCTGGGTGATGGGCGGGGAAAGCAGCCTG

CGCAGCATGATCAAGCACCTGGTCGACAACGCCATCGAGTCCATGAGCCAGAACCAGGTCAGCCGCCGC

GAGCTGTTCATCAGCACCCGCGTGGAGAACCACCTGGTGCGCATGGAGATCACCGACAGCGGCCCGGGC

ATTCCGCCCGACCTGGTGCTGAAGGTGTTCGAGCCGTTCTTCTGCACCAAGCCGCCACACCGCGTCGGG

CGCGGCACGGGCCTGCCGGTGGTGCAGGAGATCGTCGCCAAGCACGCCGGCATGGTGCACGTAGACACC

GACTATCGCGAAGGCTGCCGGATCGTCGTCGAGCTGCCCTTCTCGGCCTCCACCTCTAGAGTCGA

Fig. S1. Base sequence of *A. chroococcum* CBD15 *nifL* gene. The red triplets ATG and TAG represent the initiator and the terminator codons.

AATTCTCATGTTTGACAGCTTATCATCGATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTGAC

GCAGTCAGGCACCGTGTATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGC

TGTAGGCATAGGCTTGGTTATGCCGGTACTGCCGGGCCTCTTGCGGGATATCGTCCATTCCGACAGCATC

GCCAGTCACTATGGCGTGCTGCTAGCGCTATATGCGTTGATGCGATTTCTATGCGCACCCGTTCTCGGAG

CACTGTCCGACCGCTTTGGCCGCTGCCCAGTCCTGCTCGCTTCGCTACTTGGAGCCACTATCGACTACGC

GATCATGGCGACCACACCCGTCCTGTGGATC

Fig. S2. Base sequence of the 381 bp *Eco*RI-*Bam*HI fragment from pBR322, carrying the *Tet* promoter.

TAGACGAGGCACAGCATGACCCCGGCCAACCCGACCCTGAGCAACGAGCCGCAAGCGCCTCACGCCGAGA

GCGACGAGCTGCTTCCCGAGATCTTTCGCCAGACGGTGGAGCATGCGCCCATCGCCATTTCCATCACCGA

CCTCAAGGCCAACATTCTTTACGCCAATCGCGCTTTCCGCACCATCACCGGCTACGGCAGCGAGGAAGTG

CTCGGCAAGAACGAATCGATCCTCTCCAACGGCACCACGCCGCGCCTGGTCTACCAGGCCCTGTGGGGCT

GGCTGGCGCAGAAGAAGCCCTGGTCC**AATTCTCATGTTTGACAGCTTATCATCGATAAGCTTTAATGCGG**

TAGTTTATCACAGTTAAATTGCTGACGCAGTCAGGCACCGTGTATGAAATCTAACAATGCGCTCATGTCA

TCCTCGGCACCGTCACCCTGGATGCTGTAGGCATAGGCTTGGTTATGCCGGTACTGCCGGGCCTCTTGCG

GGATATCGTCCATTCCGACAGCATCGCCAGTCACTATGGCGTGCTGCTAGCGCTATATGCGTTGATGCGA

TTTCTATGCGCACCCGTTCTCGGAGCACTGTCCGACCGCTTTGGCCGCTGCCCAGTCCTGCTCGCTTCGC

TACTTGGAGCCACTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCTTCTGCACCAAGCC

GCCACACCGCGTCGGGCGCGGCACGGGCCTGCCGGTGGTGCAGGAGATCGTCGCCAAGCACGCCGGCATG

GTGCACGTAGACACCGACTATCGCGAAGGCTGCCGGATCGTCGTCGAGCTGCCCTTCTCGGCCTCCACCT

CTAGAGTCGA

Fig. S3. Sequence of bases of the *nifL* region of *Azotobacter chroococcum* HKD15. The sequence shown in normal straight letters represents the bases of the *nifL* gene, while the sequence shown in bold represents the bases of the DNA fragment from pBR322 containing the *Tet* promoter. The red triplets ATG and TAG represent the initiator and the terminator codons.

Table S1. Production of indole acetic acid (IAA) by *Azotobacter chroococcum* CBD15 and by *Azotobacter chroococcum* HKD15

	IAA production * (ppm/mg protein)	
	In the absence of tryptophan	In the presence of tryptophan (50 µg/ml)
Azotobacter chroococcum CBD15	4.7 (0.9)	9.4 (0.5)
Azotobacter chroococcum HKD15	4.3 (0.4)	10.9 (0.9)

*Average of 3 experiments; standard deviation in parenthesis.

Table S2 A. Population of *Bacteria* in general in the rhizosphere soil of wheat plants. Nutrient agar was used as the selective medium.

Soil sampling date in relation to seed sowing date	Inoculation of wheat seeds (*A. chroococcum*)	Urea applied to soil (kg N / hectare)	Bacteria / gm of rhizosphere soil* (x 10^7)
14 Days before	None	None	1.2 (0.2)
33 Days after	None	None	3.8 (0.5)
33 Days after	CBD15	None	4.9 (1.1)
33 Days after	HKD15	None	5.2 (1.0)
33 Days after	None	120	3.7 (2.0)
90 Days after	None	None	1.6 (0.4)
90 Days after	CBD15	None	1.8 (0.1)
90 Days after	HKD15	None	2.0 (0.3)
90 Days after	None	120	1.7 (0.1)
125 Days after	None	None	4.4 (0.1)
125 Days after	CBD15	None	5.2 (0.2)
125 Days after	HKD15	None	5.2 (0.3)
125 Days after	None	120	3.9 (1.6)
140 Days after**	None	None	2.8 (1.1)
140 Days after	CBD15	None	2.9 (1.2)
140 Days after	HKD15	None	3.4 (1.4)
140 Days after	None	120	3.1 (2.1)

* Standard deviation in parenthesis.

** Harvesting was done between 129 to 136 days after sowing.

78 **Table S2 B.** Population of *Fungi* in general in the rhizosphere soil of wheat plants.

79 Martin's Rose Bengal agar was used as the selective medium.

80

Soil sampling date in relation to seed sowing date	Inoculation of wheat seeds (*A. chroococcum*)	Urea applied to soil (kg N / hectare)	*Fungi* / gm of rhizosphere soil* (x 10^3)
14 Days before	None	None	1.2 (0.2)
33 Days after	None	None	1.8 (0.3)
33 Days after	CBD15	None	2.1 (0.4)
33 Days after	HKD15	None	1.7 (0.3)
33 Days after	None	120	1.6 (0.2)
90 Days after	None	None	0.5 (0.2)
90 Days after	CBD15	None	1.6 (0.7)
90 Days after	HKD15	None	1.0 (0.1)
90 Days after	None	120	0.6 (0.2)
125 Days after	None	None	4.0 (0.4)
125 Days after	CBD15	None	3.8 (0.3)
125 Days after	HKD15	None	4.0 (0.7)
125 Days after	None	120	3.6 (0.2)
140 Days after**	None	None	2.0 (1.3)
140 Days after	CBD15	None	2.5 (1.2)
140 Days after	HKD15	None	2.2 (0.6)
140 Days after	None	120	1.9 (1.5)

81

82 * Standard deviation in parenthesis.

83 ** Harvesting was done between 129 to 136 days after sowing.

84

Table S2 C. Population of *Actinomycetes* in general in the rhizosphere soil of wheat plants. Ken Knight's agar was used as the selective medium.

Soil sampling date in relation to seed sowing date	Inoculation of wheat seeds (*A. chroococcum*)	Urea applied to soil (kg N / hectare)	*Actinomycetes* / gm of rhizosphere soil* (x 10^4)
14 Days before	None	None	3.3 (0.6)
33 Days after	None	None	4.0 (0.9)
33 Days after	CBD15	None	4.0 (0.7)
33 Days after	HKD15	None	5.3 (2.1)
33 Days after	None	120	5.0 (1.0)
90 Days after	None	None	2.9 (0.6)
90 Days after	CBD15	None	2.7 (0.3)
90 Days after	HKD15	None	3.1 (0.9)
90 Days after	None	120	5.1 (1.7)
125 Days after	None	None	9.6 (2.7)
125 Days after	CBD15	None	9.0 (0.6)
125 Days after	HKD15	None	10.6 (3.4)
125 Days after	None	120	8.6 (1.6)
140 Days after**	None	None	5.7 (1.8)
140 Days after	CBD15	None	4.9 (1.7)
140 Days after	HKD15	None	6.5 (1.9)
140 Days after	None	120	7.6 (1.5)

* Standard deviation in parenthesis.

** Harvesting was done between 129 to 136 days after sowing.