

Figure S1. Callus induced from mature seeds after 10 days culture in different callus induction media. (A) Formula 1 [19] (auxin:cytokinin = 3:0.25) and (B) Formula 2 [31] (auxin:cytokinin = 2.5:0.15).

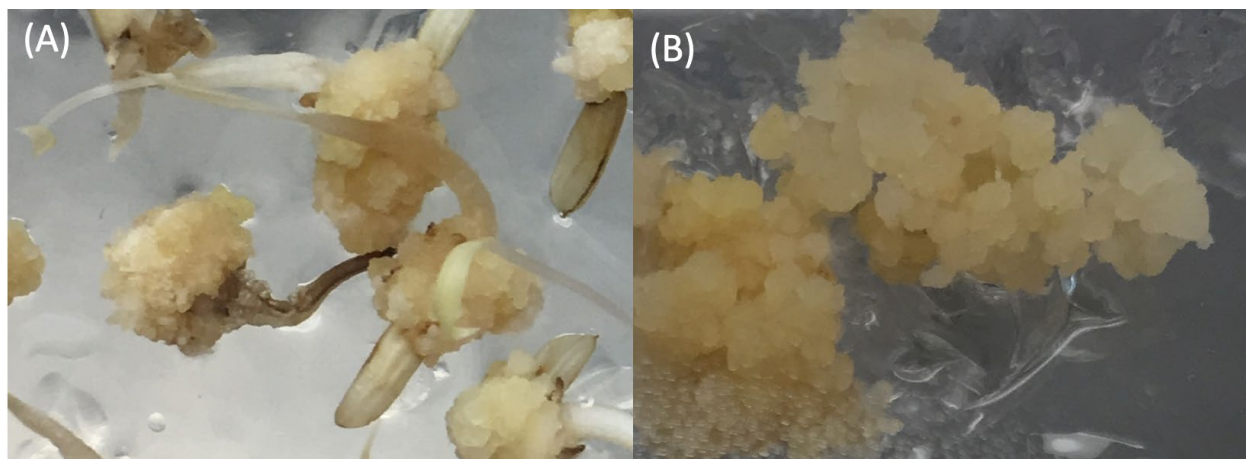


Figure S2. Calli from mature seeds after culture in callus induction medium (formula 5) for 14 d (A) and 16 days (B).

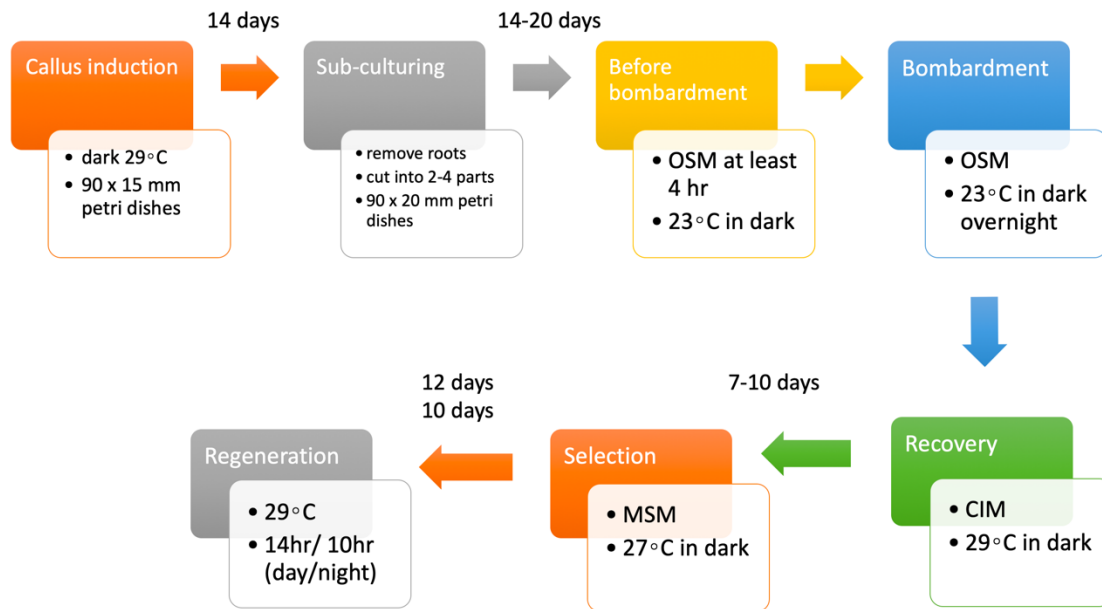


Figure S3. Biolistic bombardment workflow for Ciherang-Sub1 using mature seeds as explants.

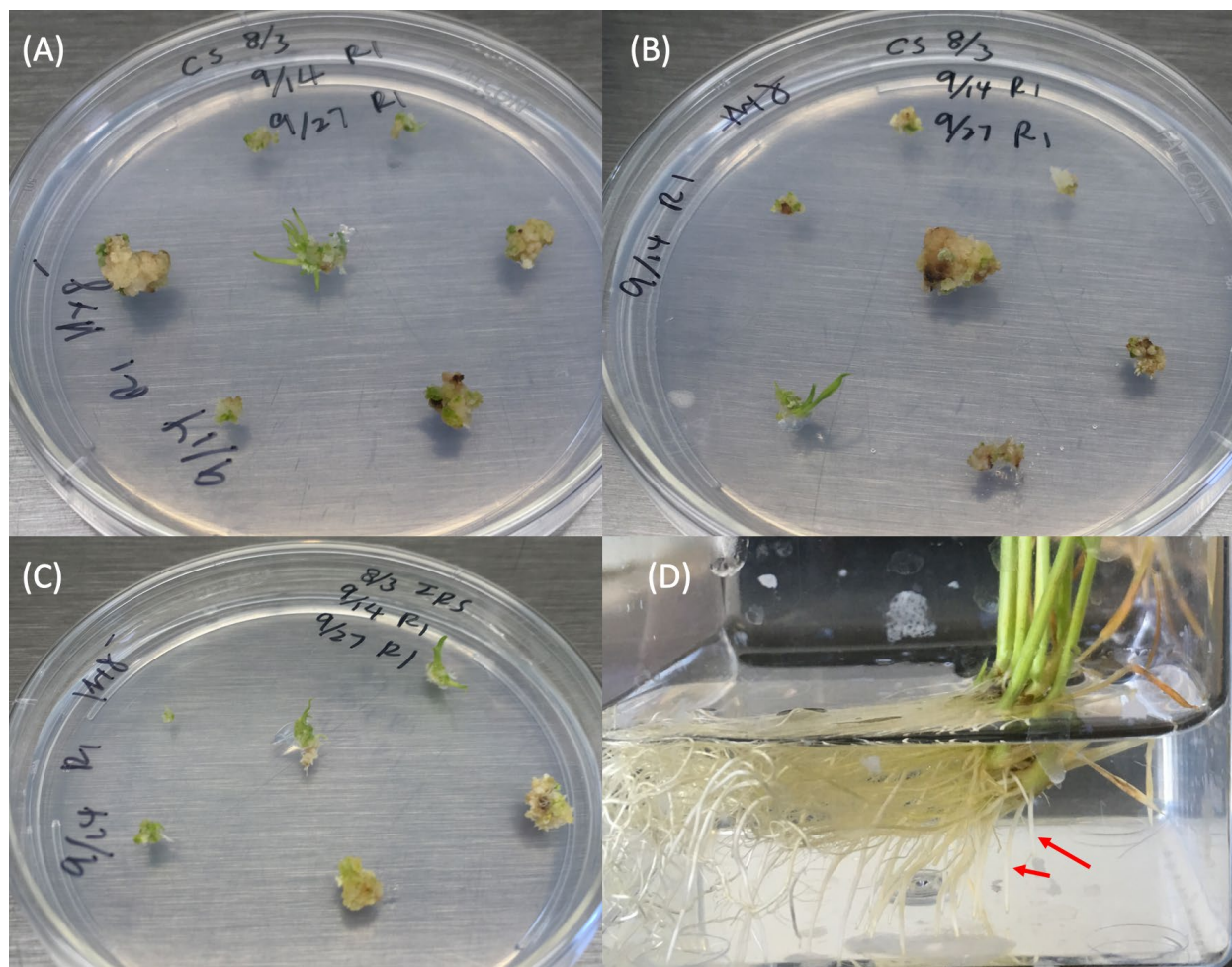


Figure S4. Untransformed calli derived from mature seeds, after 13 days of culture on optimal regeneration medium (formula 3) without antibiotic added. (A) and (B) Ciherang-Sub1. (C) IR64-Sub1. (D) Plant acclimation in fresh tap water. Red arrows indicate the newly formed roots.

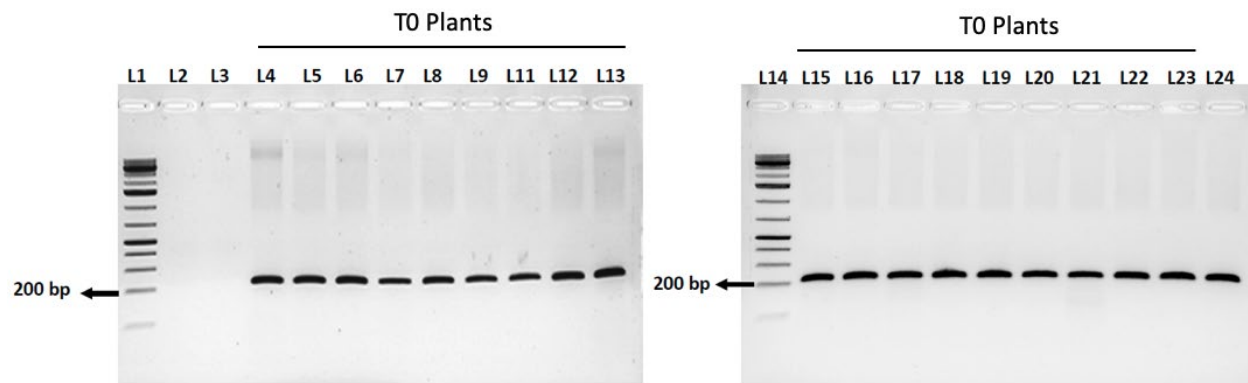


Figure S5. Validation of Cas9 gene presence in transgenic plants (T₀) derived from immature embryos with Cas9-specific primers: L1 and L14: 1kb+ ladder; L2: water control; L3: wild type Ciherang-Sub1; L4-L23: T₀ plants (Plant no.: Plant 1, Plant 2, Plant 3, Plant 4, Plant 5, Plant 6, Plant 7, Plant 8, Plant 9, Plant 10, Plant 11, Plant 12, Plant 13, Plant 14, Plant 15, Plant 16, Plant 17, and Plant 18) and L24: positive control

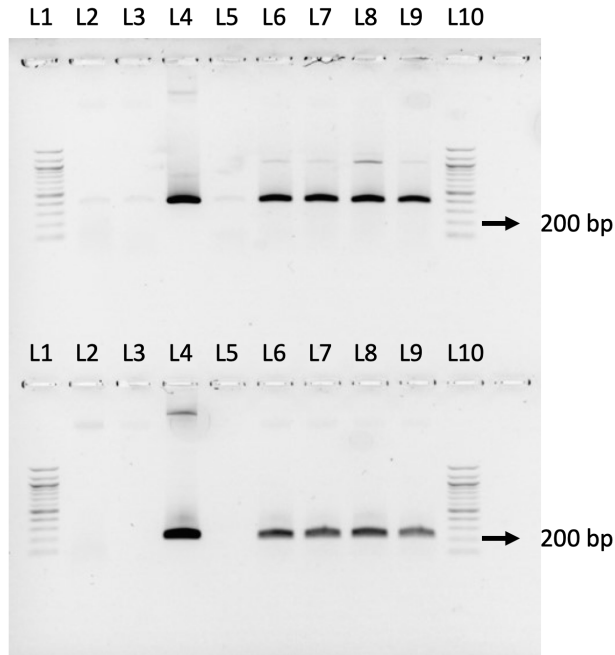


Figure S6. Validation of transgenic plants (T_0) derived from mature seeds with HPT primers and Cas9 specific primers. Upper panel HPT primers; lower panel Cas9 specific primers. L1 and L10: 1kb+ ladder; L2: Ciherang; L3: wild type Ciherang-Sub1; L4: positive control; L5: water control; and L6-L9: transformed plants (Plant no.: Bombardment 1 leaf 1, Bombardment 1 leaf 2, Bombardment 2, and Bombardment 3).

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Query 1 GGTGTTGAGGTCACCAGGTGAAAATGATGCAGGCCGGGGCGCCGCCGCCACCATGTCCAT 60
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 675 GGTGTTGAGGTCACCAGGTGAAAATGATGCAGGCCGGGGCGCCGCCGCCACCATGTCCAT 616
Query 61 GCCGCTGGACCCCGTGACCGAGGAGGCCGAGCCGGCGGTGGCTGAGAAGCCTCGCCGGCG 120
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 615 GCCGCTGGACCCCGTGACCGAGGAGGCCGAGCCGGCGGTGGCTGAGAAGCCTCGCCGGCG 556
Query 121 CCGGCCGAGGCGGAGCTACGAGTACCACGGCATCCGGCAGCGGCCGTGGGGGCGGTGGTC 180
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Sbjct 555 CCGGCCGAGGCGGAGCTACGAGTA CCACGGCATCCGGCAGCGGCCGT GGGGGCGGTGGTC 496
Query 181 GTCGGAGATCCGCGACCCCGTCAAGGGCGTCCGCCTCTGGCTCGGCACCTTCGACACCGC 240
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Sbjct 495 GTCGGAGATCCGCGACCCCGTCAAGGGCGTCCGCCTCTGGCTCGGCACCTTCGACACCGC 436
Query 241 CGTCGAAGCCCGCTCGCCTACGACGCCGAGGCCGCCGCATCCACGGCTGGAAAGCCCG 300
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Sbjct 435 CGTCGAAGCCCGCTCGCCTACGACGCCGAGGCCGCCGCATCCACGGCTGGAAAGCCCG 376
Query 301 GACAAACTTCCCACCCGCGATCTTTCTTCGCCCGCGCCGCGTGCAGCCGCTCTGCTT 360
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Query 361 CTTGCTCAACGACAACGGCCTCATCACAATCGGAGAAgcgccgaccgacgacgcccgcgc 420
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Query 421 gacgtcgacgtcgacgacggaggcgctccggcgacgcgcgATACAACTGGAGTGCTGCTC 480
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Sbjct 255 GACGTCGACGTCGACGACGGAGGCGTCCGGCGACGCGCGCATAAACTGGAGTGCTGCTC 196
Query 481 GGACGACGTGATGGACAGCCTCCTCGCCGGCTACGACGTGGCCAGCGGCGACGACATATG 540
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Sbjct 195 GGACGAC GTGATGGACAGCCTCCTCGCCGG CTACGACGTGGCCAGCGGCGACGACATATG 136
Query 541 GACATGGACATCTGGAGCCTCCTCCACCTCTGTTAACCAAGAGATCAAGACCCCATCGAT 600
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Sbjct 135 GACATGGACATCTGGAGCCTCCTCCACCTCTGTTAACCAAGAGATCAAGACCCCATCGAT 76
Query 601 C 601
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Sbjct 75 C 75

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Figure S7. *SUB1A-1* sequence alignment between Ciherang-Sub1 and the second exon of *SUB1A-1* (NCBI Accession DQ011598.1) with the two gRNAs marked in yellow.

Table S1. Multiple comparisons for the shoot elongation rate among T₁ plants, Ciherang, and Ciherang-Sub1.

	Ciherang	Ciherang-Sub1	Plant 1	Plant 12	Plant 16	Plant 17	Plant 18
Ciherang-Sub1	0.00097	-	-	-	-	-	-
Plant 1	0.94808	0.00058	-	-	-	-	-
Plant 12	0.59060	0.00226	0.93535	-	-	-	-
Plant 16	0.94808	0.00057	1.00000	0.64593	-	-	-
Plant 17	0.93535	0.00057	1.00000	0.85778	0.94808	-	-
Plant 18	0.25915	0.09419 ^a	0.25915	0.49611	0.23746	0.23746	-
Plant 3	0.59060	0.00015	0.61329	0.30516	0.59060	0.59060	0.04790 ^b

^aNo significant difference between Ciherang-Sub1 plants and T₁ plants derived from plant # 18.

^bThere was significant difference between T₁ plants derived from plant #18 and T₁ plants derived from plant #3.

Table S2. Multiple comparisons for the leaf chlorophyll content after 3 days of recovery among T₁ plants, Ciherang, and Ciherang-Sub1.

	Ciherang	Ciherang-Sub1	Plant 1	Plant 12	Plant 16	Plant 17	Plant 18
Ciherang-Sub1	0.00097	-	-	-	-	-	-
Plant 1	0.89779	0.00018	-	-	-	-	-
Plant 12	0.69488	0.00018	0.77070	-	-	-	-
Plant 16	0.73488	0.00026	0.69488	0.88511	-	-	-
Plant 17	0.19869	0.00121	0.15207	0.19702	0.11085	-	-
Plant1 8	0.67814	0.00013	0.68105	0.67814	0.89779	0.22085	-
Plant 3	0.19869	0.00121	0.16266	0.22085	0.25724	0.93660	0.22085

Table S3. Multiple comparisons for the leaf chlorophyll content after 7 days of recovery among T₁ plants, Ciherang, and Ciherang-Sub1.

	Ciherang	Ciherang-Sub1	Plant 1	Plant 12	Plant 16	Plant 17	Plant 18
Ciherang-Sub1	0.0168	-	-	-	-	-	-
Plant 1	0.9484	0.0168	-	-	-	-	-
Plant 12	0.9335	0.0085	0.8904	-	-	-	-
Plant 16	0.8904	0.0214	0.6904	0.6740	-	-	-
Plant 17	0.8628	0.0214	0.8904	0.4874	0.9900	-	-
Plant 18	0.9803	0.0085	0.9900	0.8628	0.8904	0.8628	-
Plant 3	0.9803	0.0085	0.9484	0.9335	0.6159	0.6159	0.9335