

### Improving the drought tolerance of a Brazilian soybean variety using *Agrobacterium*-mediated transformation

Brazil is the second-largest soybean producing country in the world. However, severe droughts have affected production in Brazil, thus the development of drought-tolerant soybean is required. For soybean, it has been difficult to produce transformants because the transformation efficiency is very low. Although transformation of Brazilian soybean varieties BR-16 using bio-ballistic had been reported, there is no report of *Agrobacterium*-mediated transformation of the Brazilian varieties. As the plants transformed by *Agrobacterium* generally have low copy numbers of transgenes, the establishment of an *Agrobacterium*-mediated transformation method will make it easier to obtain transformants stably expressing a gene of interest and allow the rapid fixation of inserted gene in transgenic plants.

We succeeded in improving the transformation efficiency of Brazilian soybean cultivars and were able to establish the transformation method using *Agrobacterium* (Fig. 1a-b). The transformation efficiency was 1.74% when we used the reporter *GUS* ( $\beta$ -glucuronidase) gene for the transformation. This efficiency can enable us to produce the transgenic soybean varieties at a practical level. We confirmed that the copy numbers of the transgene are low. Stress-inducible AREB (ABRE (ABA- responsive element)-binding factor) transcription factors play important roles in regulating stress responses and tolerances. We obtained transgenic events having *AREB1* stress-tolerance gene using *Agrobacterium* methods. The transgenic events showed drought tolerance in the greenhouse (Fig. 1c-e).

We are conducting an evaluation of transgenic soybean lines in the field. We expect to produce transgenic soybean varieties with high yield under drought conditions in the future. In addition, we expect to produce transgenic soybean varieties with various kinds of useful genes using the *Agrobacterium*-mediated transformation methods.

1) Kanamori N, et al. (2011) JIRCAS Working Report **71**:75-79.

2) Marinho JP\*, Kanamori N\*, et al. (2015) Plant Mol. Biol. Rep. (Marinho JP and Kanamori N contributed equally to this work)

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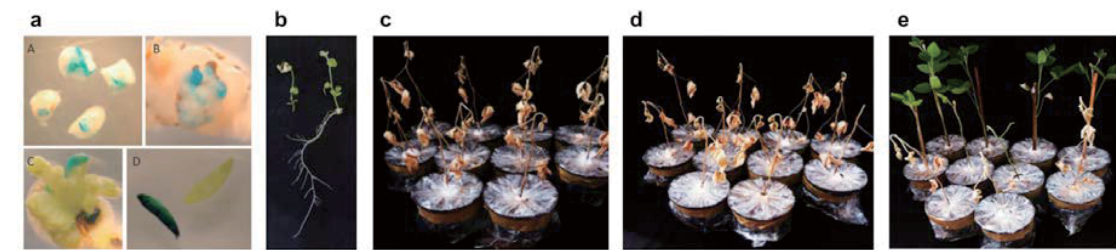


Fig. 1. Establishment of the transformation method applied to Brazilian soybean variety BR16 using *Agrobacterium*, and drought tolerance of a transgenic soybean expressing *AREB1* gene in the greenhouse.

**a)** GUS activity in the tissues of soybean after being infected by *Agrobacterium* carrying a binary vector with BAR and GUS genes. **A:** transient *gus* gene expression around the embryonic tip region after 5 days of co-cultivation; **B:** stable expression in meristems of embryonic tip cultured on shoot elongation medium for 1 week; **C:** stable expression in regenerated adventitious shoot cultured on shoot elongation medium for 2 weeks; **D:** leaflets with (left) GUS activity from a transgenic plant or without (right) from a non-transgenic plant. The figures were from Kanamori et al. (2011).

**b)** Root elongation of transgenic soybean plant. A differentiated shoot (left) developed roots (right) on a rooting medium. The figure was from Kanamori et al. (2011).

**c-e)** Survival rates of soybean plants of the genotypes 1Ea15 and 1Ea2939 transformed with 35S:AtAREB1 and non-transgenic BR 16 after 17 days of withholding irrigation followed by 7 days of rewatering. **c:** BR 16; **d:** 1Ea15; **e:** 1Ea2939. Figures **c**, **d**, and **e** were adapted from Marinho JP et al. (2015).

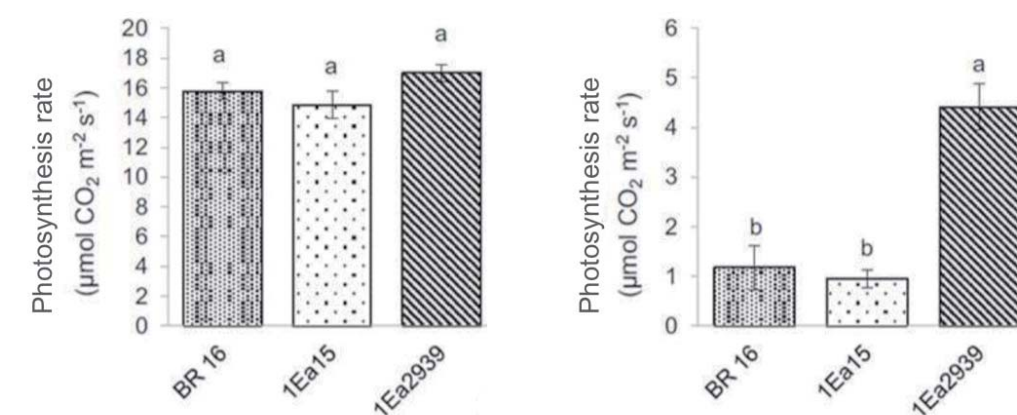


Fig. 2. Photosynthetic rate of soybean transgenic lines 1Ea15 and 1Ea2939 transformed with 35S:AtAREB1 and non-transgenic BR 16, grown under well-watered conditions (left) and under water deficit (right). Values represent mean  $\pm$  standard error; n=9 replicates. In each water condition, means followed by the same lowercase letters do not differ by the Tukey test ( $p \leq 0.05$ ). The figures were adapted from Marinho JP et al. (2015).