Genetic Transformation of Rhodesgrass (Chloris Gayana Kunth.) by Particle Bombardment

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Genetic transformation of rhodesgrass (*Chloris gayana* Kunth.) by particle bombardment

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Introduction Rhodesgrass (*Chloris gayana* Kunth) has been cultivated as one of the most important warm-season grasses in the world. One of the major limitations for cattle production on forage grasses, especially warm-season grasses is poor digestibility if compared to temperate grasses (Gondo *et al*., 2003). It is believed that the low digestibility of warm-season grasses is due to high lignin contents (Akashi *et al*., 2003). Recently, modification of the lignin content of plants appears to be feasible using genetic engineering strategies. We have established a methodology for high-frequency somatic embryogenesis and multiple shoot formation from seed-derived shoot apical meristems in rhodesgrass. Also, we have studied several factors involved in particle bombardment transformation.

Materials and methods Shoot apices as initial explants were isolated from aseptically germinated seedlings and were cultured in vitro. Embryogenic calli and the multiple shoot forming calli could be induced and maintained on MS basal medium with various combinations of 2,4-D and BAP. The induced embryogenic calli and multiple shoot forming calli were bombarded in a particle inflow gun with pAHC25, containing a modified bialaphos resistance gene (*bar*) and the GUS reporter gene. Following bombardment, cells were incubated at 27℃ for 1 day and assayed for GUS activity. Transgene expression of regenerated plants was confirmed by PCR amplification analysis.

Results The best response of embryogenic calli and the multiple shoot forming calli was observed with 2.0 mg/L 2,4-D and 0.1 mg/L 2,4-D+2.0 mg/L BAP, respectively. Also, many bialaphos resistance cells were obtained from the bombarded calli. These regenerated plants displayed GUS activity and integration of the transgenes were confirmed by PCR amplification.

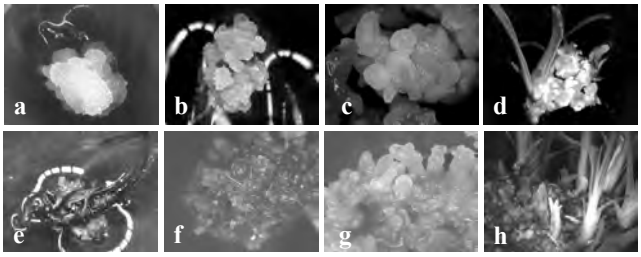

Figure 1 Differentiation of regeneration from somatic embryogenic calli and multiple shoot forming calli in rhodesgrass. a) Primary callus. b) Embryogenic callus. c) Somatic embryogenic callus. d) Germination of somatic embryo and plant regeneration. e) Primary callus. f) Multiple shoot forming callus. g) Multiple shoot formation under dark conditions. h) Multiple shoots under light conditions.

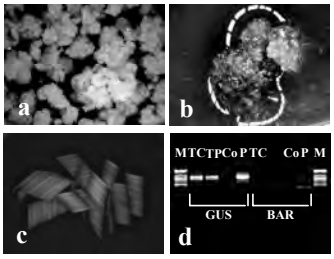

Figure 2 Transformation of rhodesgrass using particle bombardment. a) Transient GUS expression 24 hours after bombardment. b) Multiple shoot formation under bialaphos selection conditions. c) GUS expression in leaf tissue after transformation. d) Detection of *bar* and the GUS gene on transformed callus and plants by PCR. M: 100bp DNA ladder, TC: Transformed callus, TP: Transformed plant, Co: Non-transformed plant, P: Plasmid pAHC25 DNA.

Conclusions In this report, we induced embryogenic calli and multiple shoot forming calli. These multiple shoot forming calli provided target tissue for genetic transformation using particle bombardment of rhodesgrass. The recovered transgenic plants were established in soil and acclimatised in the green house.

References

Gondo, T., Y. Ishii, R. Akashi and O. Kawamura (2003). Efficient Embryogenic Callus Induction Derived from Mature Seeds and the Examination of the Genetic Transformation Condition by Particle Bombardment in Bahiagrass (P*aspalum notatum* Flugge). *Grassland Science* 49,33-37

Akashi, R., T. Gondo and O. Kawamura (2003). Fundamental Studies on the Improvement of Some Warm-season Grasses by Means of Plant Biotechnology. *Grassland Science* 49 (1): 79-87