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Establishing an abundant and efficient explant system for the *Agrobacterium*-mediated transformation of zoysiagrass

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Introduction Zoysiagrass (*Zoysia japonica* Steud .) is one of the most important warm season turf grass species native to the Far East . This turf grass is distinguished by a low nutrient requirement , and tolerance to environmental stresses such as drought and salinity . However , thedifficult seed germination and callus production , has seriously blocked attempts to perform biotechnology on the species .Our aim has been to develop a system for regeneration from embryo drived callus of zoyiagrass that can be used for *A grobacterium* -mediated transformation with genes to improve cold tolerance . . To ensure a abundant and constant supply of explantsmature seeds and embryos isolated from mature seeds were used for callus induction . .

Materials and methods Mature seeds and embryos isolated from mature seeds of zoysiagrass (Figure 1) were incubated on M5 induction medium (MS basal medium supplemented 30 g/L sucrose and 5 mg/L 2 ,4-D) , darkness and 26±1°C , to induce calli . Then calli were transferred to the regeneration medium (MS basal medium supplemented 30 g/L maltose and 1 mg/L kinetin) .

Result The was a difference in the efficiency of callus induced from mature seeds and embryos (Table 1 & Figure 2) . After incubated on M5 induction medium for 3 months , 90 .7% calli developed from embryos . Under the same conditions , i only 50 . 8% of mature seeds produced calli . After transferring onto the regeneration medium , shoots generated efficiently (Figure 3) .

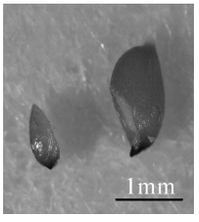

Figure 1 *Embryos isolated from mature seeds .*

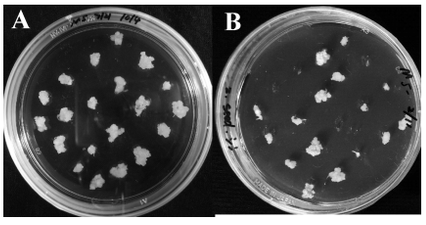

Figure 2 *Calli derived from embryos (A) or mature seeds (B) .*

Figure 3 *Regenerated shoots .*

Conclusions and prospects Zoysiagrass is well known for its slow growth and seed p dormancy , due to a hard , waxy outer glume around the caryopsis (seed) and a high content of ABA in seeds . In the current experiment , dissecting the embryo from the mature seed proved to be an effective way of inducing callus formation of zoyiagrass . This methodology will provide an abundant and constant supply of explants for zoysiagrass transformation . A simmialr method isused to get callus for transformation in some cereal species , such as barley (Hensel , 2007) . However , there has been no previous report that this approach is useful for callus induction inforage or turf grasses . Three kinds of fructosyltransferase genes involved fructan biosynthesis are being transformed into calli of zoysiagrass by the *A grobacterium*-mediated method . In most transformation procedures for forage and turf grasses calli were employed as the explant and embryogenic calli is considered to bea key factor affecting transformation frequency (Wang & Ge , 2006) . An alternativemethod where embryos are infected directly with *A grobacterium* and then cultured for callus formation will be attemptedcalli .

Table 1 *Callus induction rates from mature seeds or embryos .*

Inoculated material	Callus induction rate %
Mature seeds	50 .8
Embryos	90 .7

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