Use of Basta (Phosphinothricin) Selection for Genetic Transformation of Regen SY Alfalfa

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Genetic transformation is a powerful tool to understand developmental, biochemical, and physiological processes in plants, as well as to create plants with novel traits that might be difficult or impossible to achieve through conventional breeding. Genotypes from Regen SY alfalfa germplasm are amenable to *Agrobacterium*-mediated transformation and regeneration using a well-established protocol [1,2]. In this protocol, a marker gene, often kanamycin or hygromycin resistance, allows selection of transformed cells using the corresponding antibiotic. One approach to understanding multigene phenomena is to supertransform previously transformed plants using multiple selectable markers. We sought to use basta (phosphinothricin, PPT) resistance (conferred by the *bar* gene) as a selectable marker to supertransform plants that were already resistant to kanamycin and hygromycin. Using the original protocol of Austin et al. [1], initial experiments required relatively large amounts of PPT (8 mg/L) for effective selection, yet we were unable to recover transgenic plants. Because PPT mode of action is inhibition of glutamine synthase, we hypothesized high levels of PPT were required for selection due to a large amount of supplemental glutamine in the media (B5h, B5h0) used for initial culture and selection steps, and that high levels of PPT might inhibit embryo and shoot development. To test this, we first generated PPT "kill curves" by incubating alfalfa leaf explants on B5h medium with and without glutamine. In the absence of glutamine, only 2 mg/L PPT was required for effective killing of non-transformed cells. We subsequently used *Argrobacterium* strain LBA4404 harboring a pCambia3301-based binary vector that uses bar as the selectable marker in transformation experiments using 2 mg/L PPT for selection. Explants co-cultivated with the *Agrobacterium* developed callus tissue, proembryos, and embryos. Control explants not co-cultivated with *Agrobacterium* failed to form callus tissue. Inclusion of 2 mg/L PPT appeared to inhibit shoot development, so we have subsequently reduced PPT to 1 mg/L for embryo development and eliminated it for shoot development. Our results indicate that glutamine supplementation of B5h and B5h0 media is not required for somatic embryogenesis, and prevents effective selection by PPT. Our modifications of the established transformation protocol allow transformation of Regen SY alfalfa using the *bar* gene as the selectable marker, which will offer additional flexibility and options for supertransformation.

1. Austin, S., Bingham, E.T., Mathews, D.E. et al. 1995. Production and field performance of transgenic alfalfa (*Medicago sativa* L.) expressing alpha-amylase and manganese-dependent lignin peroxidase. Euphytica 85, 381–393. https://doi.org/10.1007/BF00023971
2. Samac, D.A., Austin-Phillips, S. 2006. Alfalfa (*Medicago sativa* L.). In: Wang, K. (ed) *Agrobacterium* Protocols. Methods in Molecular Biology, vol 343. Humana Press. https://doi.org/10.1385/1-59745-130-4:301