TRANSFORMATION OF ARABIDOPSIS THALIANA: MODIFICATIONS OF VALVEKENS'S PROTOCOL

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* During the last eighteen months we have tried transforming Arabidopsis thaliana Landsberg erecta using numerous home-spun and published methods and have finally adopted the protocol of Valvekens et al (Dirk Valvekens, Marc Van Montagu, & Mieke Van Lijsebettens 1988. Proc. Natl. Acad Sci. 85, 5536-5540.). We have experienced a few problems with this method; they are:
(i) Depletion/degradation of auxin within shoot-induction medium (SIM) that results in the death of micro-calli. This phenomenon cannot be be prevented by regular transfer of explant material to fresh medium.
(ii) Inefficient regeneration of micro-calli in the presence of kanamycin.
(iii) Poor seed set from in vitro grown plants. Seed batches obtained from such plants usually germinate poorly.

* In attempting to overcome these problems we have found the following modifications to Valvekens's protocol useful.

1. Omit N6-(2-isopentenyl) adenine and indole-3-acetic acid from SIM and replace with 6- benzylaminopurine (0.4 ppm) and napthaleneacetic acid (0.2 ppm). Using this modified growth regulator regime we observe micro-calli by circa day 11, post co-cultivation.

2. Pick off micro-calli (2 to 3 days after they first become visible) from the background of yellowing root explants and transfer to fresh SIM (BAP 0.4 ppm, NAA 0.2 ppm) and continue incubation without selection. Shoot regeneration is very poor with selection, but in its absence circa 60% of micro-calli regenerate. We have not experienced any escapes by limiting selection (kanamycin 50ugml-1 or hygromycin 20ugml-1), to 11 days.

3. Transfer shoots and associated callus to GM medium (in 'Magenta' vessels, Sigma) and allow to bolt (circa 2-3 weeks). Cut bolted stems from their callus base and then root (circa 2-4 weeks) in GM medium containing indole-3-butyric acid (1 ppm). This usually ensures that roots connect with the shoot vascular system. We have found that IBA-induced rooting is approximately 25% efficient so it is prudent to set-up at least four 'like' explants to ensure success. Without roots, establishment of bolted stems in soil is poor.

4. Transfer rooted shoots to soil (80% sand + 20% Fisons' multipurpose compost) in a high humidity environment and wean to atmospheric humidity over a couple of days. Survival of successfully weaned plants is high (circa 90%). Silique ripening occurs circa 3-4 weeks from transfer of plants to soil. Seed set and subsequent germination are usually