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Research Note

Plant regeneration *in vitro* from immature embryos of lesser burnet (*Sanguisorba minor* Scop.)

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Efficient and reproducible shoot regeneration has been established from immature zygotic embryos of lesser burnet on MS medium containing 4μM BAP and 10μM NAA. Regenerated shoots were best rooted in half-strength MS medium supplemented with 10μM NAA and later established in the greenhouse.

Lesser burnet (*Poterium sanguisorba* L. = *Sanguisorba minor* Scop.) is an evergreen herbaceous perennial forage plant that grows widely on poor soils in arid and cold areas. Its nutritional quality is similar to alfalfa and sainfoin but does not induce bloat (Rodriguez and Bermejo 1986). This plant has pinkish white flowers and is used as ornamental plant and culinary herb for medicinal purposes.

Because of the potential usefulness of lesser burnet for cattle production in Central Anatolia, we are interested in developing an efficient regeneration system to assist in breeding. Shoot regeneration in lesser burnet has been achieved previously from different types of explants (Babaoğlu and Yorgancılar 2000). However, shoot regeneration from immature embryos of lesser burnet has not yet been reported. The present study describes a rapid, efficient and simple regeneration system from immature zygotic embryos of three lesser burnet cultivars, namely, Gözlü, Altınova and Bünyan.

Approximately 12–14 days after pollination, immature fruits were harvested from field-grown plants of three lesser burnet cultivars and surface-sterilised in 30% commercial bleach (Axion) for 20min, followed by three rinses in sterile distilled water. Immature zygotic embryos were removed from healthy fruits and cultured in 100mm diameter petri dishes containing regeneration medium. The number of explants producing shoots and the number of shoots per explant were scored after eight weeks of culture.

Regeneration medium consisted of MS mineral salts and vitamins (Murashige and Skoog 1962), 3% sucrose, 0.7% agar and 1μM, 2μM, 4μM and 8μM 6-benzylaminopurine (BAP) and 1.5μM, 3.6μM and 10μM α-naphthaleneacetic acid (NAA), pH was adjusted to 5.6 with 1M NaOH or 1M HCl before autoclaving at 121°C, 1.4kg cm^{-2} for 20min. All cultures were incubated at 24°C under cool white fluorescent lamps with a 16h photoperiod.

Regenerated shoots from cultivar Altınova were excised and rooted in half-strength MS medium supplemented with 2.5μM, 5μM and 10μM NAA or 2.5μM, 5μM and 10μM indolebutyric acid (IBA) in Magenta GA-7™ culture vessels. The rooted plantlets were then acclimatised in growth cabinets at 90% relative humidity during the first seven days, by placing in a sterile soil mix (70% sand : 30% clay) in pots. Plant establishment was achieved, taking care to avoid desiccation by gradually reducing the humidity to 40%.

Each treatment had four replicates, each containing five explants in both regeneration and rooting experiments. Significance was determined by analysis of variance (ANOVA) and the differences between the means were compared by Duncan's multiple range test using an MSTAT-C computer program (Michigan State University).

After two weeks in culture, green shoot initials formed on developing callus and produced shoots at later stages. Effects of cultivars and growth regulators on shoot regeneration were significant ($P < 0.05$). The highest frequency of explants producing shoots (75%) and the greatest number of shoots per explant (7.25) were achieved from immature embryos of the cultivar Altınova cultured on MS medium containing 4μM BAP and 10μM NAA ($P < 0.05$). Cultivar Altınova had a higher regeneration capacity than Gözlü and Bünyan. Shoot regeneration via organogenesis and embryogenesis has been reported from immature zygotic embryos of many crop species, e.g. apple (Daigny *et al*. 1996). The results obtained in previous studies and in the current work confirm the regeneration potential of immature embryo explants. Babaoğlu and Yorgancılar (2000) also achieved shoot regeneration from hypocotyl and leaf explants of less-

er burnet on media containing NAA and TDZ (Babaoğlu and Yorgancılar 2000), but failed to induce regeneration on media containing BAP, kinetin and zeatin in the absence or presence of NAA. However, these authors found that TDZ plus NAA played a major role in shoot regeneration by organogenesis from hypocotyl and petiole explants.

Regenerated shoots of cv. Altınova were separated and cultured in half-strength MS medium supplemented with various levels of NAA or IBA. Rooting started one week after placement on rooting media. The highest frequency (80%) of healthy, vigorous and profile rooting occurred in a medium containing 10µM NAA (average 4.4 roots explant^{-1}; $P < 0.05$). The rooted shoots were transferred to a growth cabinet and established within 10–15 days. Later, they were transplanted to the greenhouse where they continued growth without showing signs of stress, abnormality or stunting; the plants flowered and set fertile seeds.

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