

## Histological approach to understand *in vitro* regeneration ability on sweet basil

M. Montone <sup>(1,2)</sup>, A. Cassetti <sup>(1)</sup>, B. Ruffoni <sup>(1)</sup>, L. Pistelli <sup>(2,3)</sup> & M. Savona <sup>(1)\*</sup>

<sup>(1)</sup> CREA Research Centre for Vegetable and Ornamental Crops (CREA-OF), Corso Degli Inglese 508, 18038 Sanremo, Italy

<sup>(2)</sup> Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

<sup>(3)</sup> Interdepartmental Research Centre “Nutraceuticals and Food for Health”, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

\* Corresponding author: [marco.savona@crea.gov.it](mailto:marco.savona@crea.gov.it) ; Tel.: +39-0184-694829

### Abstract

*Ocimum basilicum* L. (Lamiaceae), commonly known as sweet basil, is an aromatic species economically important worldwide for its food, ornamental and pharmaceutical purposes. Micropropagation represents a powerful technique for the species. New efficient *in vitro* regeneration protocols are strictly required for genome editing applications, for example, to improve resistance to pathogens. Five commercial cultivars (‘Prospera’, ‘Paoletto’, ‘Italiko FT’, ‘Dark Opal’, and ‘Bolloso Napoletano’) were evaluated for *in vitro* germination and regeneration aptitude. Histological detection was conducted to verify the presence of early meristematic activity during *in vitro* regeneration process.

Seeds were surface sterilized with ethanol (70% v/v) and hypochlorite solution (1,5% v/v), cultured at  $23 \pm 1$  °C, for *in vitro* germination, on Petri dishes containing standard basal medium (MS) + 0,3% sucrose + 0,6% agar (pH 5.7). Among the cultivars, ‘Prospera’ ensured the highest germination percentage (96%) combined with the lowest average germination time (0.65 days).

For each cultivar, *de novo* shoot regeneration trial was set up, under different hormonal induction conditions, using roots (MS + 1.0 mg L<sup>-1</sup> TDZ), hypocotyls (MS + 2.0 mg L<sup>-1</sup> TDZ + 0.1 mg L<sup>-1</sup> IBA), cotyledons (MS + 2.25 mg L<sup>-1</sup> BAP) and cotyledonary nodes, i.e. CNs (MS + 0.5 mg L<sup>-1</sup> BAP + 0.25 mg L<sup>-1</sup> IAA) as starting plant materials. Significant differences occurred among cultivars and explant types; for example, the best regeneration potential was recorded on roots, through nodule-like meristems (NLMs), precursors of shoots, especially in ‘Prospera’ (100%). CNs also showed a good morphogenic aptitude, reaching 72.5% of direct regeneration events in ‘Paoletto’. Cotyledons ensured the highest cv-related response: ‘Prospera’ (36%) and ‘Paoletto’ (25.50%) were more efficient than ‘FT Italiko’ (3.8%) and ‘Dark opal’ (10%). Hypocotyls were the explants with the lowest regeneration competence in all cultivars tested.

Based on economic and commercial interest, 20-day-old *in vitro* roots and cotyledons of three selected cultivars (‘Prospera’, ‘Paoletto’, and ‘Italiko FT’) were embedded in paraffin and stained with toluidine blue 0.25 M for histological analysis. Microscopic detection was suitable for identifying early meristematic organization in both roots and cotyledons grown in induction substrates, compared to the control without hormonal induction. Among the *O. basilicum* cultivars analyzed, different intensities of meristematic activity were detected. In roots, presence of meristematic centres was observed with the ability to generate shoot primordia; in cotyledons, both direct, with shoot primordia formation, and indirect, through callus, organogenesis pathways were observed.

A strong cultivar-dependent morphogenic aptitude was recorded in *O. basilicum* L., especially in these cultivars with high commercial interest. Up to now, this is the first report on the histological observations of *in vitro* regeneration in sweet basil, as a tool that can improve the understanding of *de novo* morphogenesis and *in vitro* regeneration mechanism. These results represent a valuable starting point for future New Genomic Techniques experiments in *Ocimum basilicum* L.

**Keywords:** morphogenesis, *Ocimum basilicum*, cultivar response, germination, explant types

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