Adventitious shoot regeneration in cultures of *Humulus lupulus* L. (hop) cvs. Brewers Gold and Nugget

**Abstract** A very efficient protocol for plant regeneration from two commercial *Humulus lupulus* L. (hop) cultivars, Brewers Gold and Nugget has been established, and the morphogenetic potential of explants cultured on Adams modified medium supplemented with several concentrations of cytokinins and auxins studied. Zeatin at 4.56 μM produced direct caulogenesis and caulogenic calli in both cultivars. Subculture of these calli on Adams modified medium supplemented with benzylaminopurine (4.4 μM) and indolebutyric acid (0.49 μM) promoted shoot regeneration which gradually increased up to the third subculture. Regeneration rates of 60 and 29% were achieved for Nugget and Brewers Gold, respectively. By selection of callus lines, it has been possible to maintain caulogenic potential for 14 months. Regenerated plants were successfully transferred to field conditions.

**Key words** Hop · *Humulus lupulus* · Plant regeneration

**Abbreviations** BAP Benzylaminopurine · IAA Indoleacetic acid · IBA Indolebutyric acid · TDZ Thidiazuron

**Introduction**

Hop (*Humulus lupulus* L., Cannabaceae) is essential for the brewing industry. The resins (alpha and beta acids) and essential oils produced by the lupulin glands of female flowers (cones) are responsible for the characteristic aroma and flavour of beer.

In Spain, two cultivars are traditionally grown: H-7 and H-3. Gas chromatographic analyses have confirmed that they are Southern Brewer and Brewers Gold cvs., respectively, but they must be considered as botanical ecotypes, as their agronomic and chemical behaviour are clearly different (J.L. Benitez, personal communication). In recent years, other cultivars, in high demand by the market, have been introduced. With respect to vegetative behaviour and resin production and quality, cv. Nugget appears to be one of the most promising (Benitez et al. 1994).

Few papers dealing with the in vitro culture of hop have been published, and of these, most have concerned virus eradication by meristem culture and cloning (Vine and Jones 1969; Adams 1975; Samyn and Welvaert 1983; Probasco and Winslow 1986). Plant regeneration from hop callus has only been reported by Motegi (1979) for cvs. Shinshawase and Italy-2, and by Batista et al. (1996), who recently reported successful plant regeneration from stem- and petiole-derived callus of the spontaneous clone Bragança and from petiole-derived callus of cv. Brewers Gold. Connell and Heale (1986) and Heale et al. (1989) noted plant regeneration from callus of cv. Challenger, and cvs. Eastwell Golding and Earlybird Golding, respectively. Direct organogenesis in hop has only been reported by Rakouský and Matousek (1994) for two Bohemian hops.

We have studied the direct and indirect regeneration ability of two commercial hop cultivars, Brewers Gold and Nugget, and established an efficient protocol for plant regeneration from callus.

**Material and methods**

Hop rhizomes of cvs. Brewers Gold and Nugget were provided by SAE Fomento del Lúpulo from their fields in León (Spain). They were washed with 4% sodium hypochlorite for 30 min, followed by 30 min under tap water. Rhizomes cultured on wet perlite under controlled conditions (25°C, 16 h photoperiod) sprouted after 10 days. Developed shoots were cut into nodal segments.
Table 1: Callus, root and shoot formation on internodal segments of two hop cultivars after 30 days of culture on Adams modified medium with several hormone combinations (values within columns with no common letter differ significantly at \( P=0.05 \))

<table>
<thead>
<tr>
<th>Hormone combination (µM)</th>
<th>Brewers Gold</th>
<th>Nugget</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calli (%)</td>
<td>Roots (%)</td>
<td>Shoots (%)</td>
</tr>
<tr>
<td>BAP 2.2</td>
<td>87.3 a, b, c</td>
<td>16.2 b</td>
</tr>
<tr>
<td>BAP 4.4</td>
<td>76 c, d</td>
<td>11.1 b</td>
</tr>
<tr>
<td>BAP 8.8</td>
<td>79.4 b, c, d</td>
<td>0 d</td>
</tr>
<tr>
<td>BAP 4.4</td>
<td>IAA 5.71</td>
<td>88.89 a, b</td>
</tr>
<tr>
<td>BAP 4.4</td>
<td>IBA 0.49</td>
<td>96 a</td>
</tr>
<tr>
<td>BAP 4.4</td>
<td>IBA 4.99</td>
<td>95.56 a</td>
</tr>
<tr>
<td>Zeatin 2.28</td>
<td>76.97 c, d</td>
<td>0 d</td>
</tr>
<tr>
<td>Zeatin 4.56</td>
<td>71.05 d</td>
<td>1.05 d</td>
</tr>
<tr>
<td>Zeatin 9.12</td>
<td>73 d</td>
<td>0 d</td>
</tr>
<tr>
<td>Zeatin 4.56</td>
<td>IAA 5.71</td>
<td>96 a</td>
</tr>
<tr>
<td>Zeatin 4.56</td>
<td>IBA 0.49</td>
<td>85 b, c</td>
</tr>
<tr>
<td>Zeatin 4.56</td>
<td>IBA 4.99</td>
<td>96 a</td>
</tr>
<tr>
<td>TDZ 0.45</td>
<td>79 b, c, d</td>
<td>0 d</td>
</tr>
<tr>
<td>TDZ 2.27</td>
<td>73 d</td>
<td>0 d</td>
</tr>
<tr>
<td>TDZ 4.45</td>
<td>87.87 a, b, c</td>
<td>0 d</td>
</tr>
</tbody>
</table>

(1 cm), sterilized with 7.5% Domestos for 20 min followed by three rinses with sterile distilled water and cultured in vitro on Adams modified medium (Adams 1975) – multiplication medium – as previously described (Gurriarán et al. 1991). Multiplication medium contained MS salts (Murashige and Skoog 1962), WS vitamins (Wetmore and Soroking 1955) and 3% glucose, supplemented with 4.4 µm benzylaminopurine (BAP) and 0.5 µm indolebutyric acid (IBA), solidified with 0.7% agar (Roko), pH 5.2. The micropropagation chain was maintained by subculturing three rinses with sterile distilled water and cultured in vitro on BAP- or zeatin-containing medium significantly increased the number of explants forming calli, except when Nugget explants were cultured on BAP-containing medium. The callusogenic response of Brewers Gold explants was increased when IAA was added to the medium; however, IAA decreased the number of Nugget explants forming calli when added to medium supplemented with BAP and there were no significant differences when added to medium with zeatin. The use of TDZ led to the lowest percentages of callus formation for Nugget, whereas Brewers Gold showed no differences between these treatments and the use of other cytokinins alone.

Calli ranged in colour from pale yellow to dark brown, and only callus grown on medium with 4.56 µm zeatin showed green compact areas on the surface after 30 days.

Explants from both cultivars did not develop roots in media with TDZ. BAP rarely promoted root formation on Nugget explants, while 11.1% of Brewers Gold explants developed roots. Zeatin rarely induced roots in Brewers Gold. The highest percentages of rooted explants were observed when 4.99 µm IBA was added to medium with cytokinin, where almost half of the explants showed roots (Table 1). Roots emerged either directly from explants or from the formed callus.

Direct shoot regeneration was only observed in internodal segments cultured on media with BAP or zeatin alone, although at very low rates (Table 1). Brewers Gold explants regenerated shoots only with 4.4 µm BAP or 4.56 µm zeatin. Nugget internodal segments showed direct regeneration at all zeatin concentrations, while only the highest BAP concentration promoted shoot formation in this cultivar.

To investigate whether other explants show a similar morphogenic pattern as internodal segments, Nugget leaves were cultured with BAP or zeatin alone, at the same concentrations as indicated in Table 1. Two percent shoot regeneration was obtained either with

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**Results and discussion**

All the hormonal combinations assayed promoted callus formation after 30 days in both Nugget and Brewers Gold internodal segments (mainly at the basal end of the explant), but there were some differences between cultivars (Table 1). The addition of IBA to BAP- or zeatin-containing medium significantly