In Vitro Shoot Regeneration from Leaves of *Hypericum* sp.

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Significance to Industry: Species in the genus *Hypericum* L. have valuable ornamental merit and considerable potential for breeding and improvement. Breeding efforts at the N.C. State Mountain Horticultural Crops Research and Extension Center have produced the hybrid *Hypericum* H2003-004-016 from crosses including *H. frondosum* 'Sunburst'*, H. galioides* 'Brodie'*,* and *H. kalmianum*. To further enhance the ornamental qualities of this hybrid, an *in vitro* shoot regeneration protocol was developed as a foundation for future ploidy manipulations and mutation treatments. The greatest regeneration of shoots per leaf segment was achieved at concentrations of 5 μM benzylamino purine (BA) and 2.5 μM indoleacetic acid (IAA).

Nature of Work: The genus *Hypericum* L. contains approximately 370 species worldwide. *H. frondosum, H. galioides,* and *H. kalmianum* have desirable ornamental characteristics and environmental tolerances that make them promising species for breeding and improvement. All three species demonstrate broad adaptability and have showy, golden flowers. In addition to attractive flowers, *H. frondosum* also has bluish-green foliage and a compact growth form. Although generally more open in habit than the other two species, *H. kalmianum* has the desirable characteristic of being cold-hardy to USDA zone 4. *Hypericum galioides* is particularly tolerant to the hot, humid conditions in the southeast United States. At the N.C. State Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, NC, these three species have been crossed, through multiple generations, to develop hybrid H2003-004-016. The hybrid exhibits a dense, compact growth form, narrow, bluish foliage, and an abundance of showy, golden flowers.

Tissue culture techniques can be a useful tool in furthering the improvement of ornamental features. The development of *in vitro* regeneration systems provides an ideal platform for further improvements through ploidy manipulations, mutation treatments, and transgenic applications. Previous *in vitro* regeneration studies of *Hypericum* species have included *H. perforatum* (3,4,5,6), *H. heterophyllum* (1)*,* and *H. frondosum* (8). Benzylamino purine (BA) is a commonly used cytokinin for the *in vitro* regeneration of *Hypericum.* However, a newer cytokinin, meta-topolin (mT), has shown promise in the regeneration of *Spathiphyllum floribundum* (9), *Musa* AAB (7), *Aloe polyphylla* (2)*,* and *Pelargonium* × *hederaefolium* 'Bonete' (10). In this study, mT and BA were investigated in order to develop a reliable and efficient shoot regeneration protocol from leaves of *Hypericum* H2003-004-016.

Young leaves of *Hypericum* H2003-004-016 were collected from glass house grown plants at the MHCREC and surface sterilized under a laminar flow hood for 17 min. in 20% commercial bleach, followed by three rinses of 5 min. each in sterile deionized water. The leaves were then sectioned with a scalpel into 5 mm (0.2 in.) long pieces and placed abaxial side down on petri dishes containing medium composed of Murashige and Skoog basal salts and vitamins, 3% sucrose, and solidified with 0.8% agar. Media was supplemented with either BA or mT at concentrations of 5, 10, and 15 μM in combination with indoleacetic acid (IAA) at concentrations of 0, 1.25, 2.5 or 5 μM. Plates were incubated in the dark at 23°C (73.4°F).

Each cytokinin (BA or mT) was treated as a separate experiment with a completely randomized factorial design. There were at least seven replicates (plates) per treatment and five subsamples (leaf segments) per replicate. After approximately five weeks, data were collected on the percentage of regenerative callus and number of shoots produced per callus by each treatment. Data were subjected to multiple regression analyses (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC).

Results and Discussion: Production of regenerative callus and shoots was successfully achieved *in vitro* for *Hypericum* H2003-004-016. Shoots were induced in all treatments combining BA with IAA. In the mT treatments, all treatments produced shoots except for 5 μM mT + 0 μM IAA, 10 μM mT + 0 μM IAA, 15 μM mT + 0 μM IAA, and 15 μM mT + 1.25 μM IAA.

Regression analysis of treatments utilizing BA in combination with IAA demonstrated that BA concentration, IAA concentration, and the interaction between BA and IAA had a significant effect on the production of regenerative callus and number of shoots ($P<0.05$). From the regression models, a surface response plot was generated to show the response and interaction between PGRs and their affect on regenerative callus production and number of shoots (Figures 1 and 2). The optimal treatment in the BA experiment was 5 μM BA + 2.5 μM IAA and produced approximately 18 shoots per callus.

Regression analysis of treatments utilizing mT in combination with IAA indicated that mT and IAA concentrations had a significant effect on the production of regenerative callus ($P<0.05$) and number of shoots per callus ($P<0.10$). From the regression models, a surface response plot was generated to show the response and interaction between PGRs and their affect on production of regenerative callus and number of shoots (Figures 3 and 4). The optimal treatment in the mT experiment was 5 μM mT + 5 μM IAA and produced approximately 10 shoots per callus.

Protocols developed by this study will be used in the future experiments focused on the development of allopolyploids and induced mutants with more diverse and improved ornamental characteristics.

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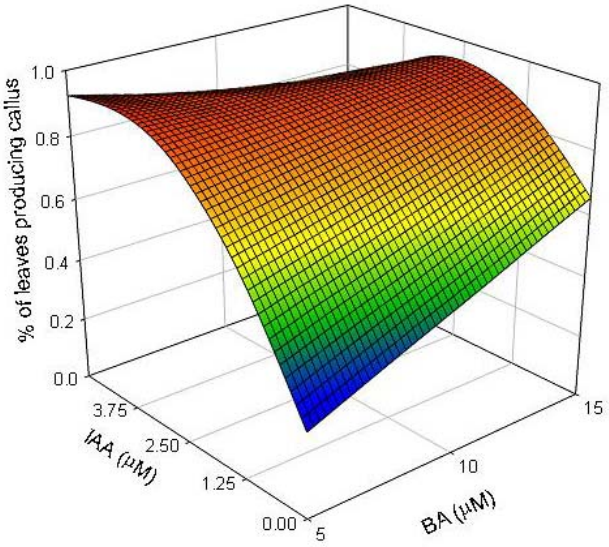

Figure 1. Effect of benzylamino purine (BA) and indoleacetic acid (IAA) on percentage of leaf segments producing regenerative callus.
($y=0.082 + 0.037*BA + 0.398*IAA – 0.0415*IAA^2 – 0.0123*BA*IAA$, $P<0.001$, $r^2=0.83$)

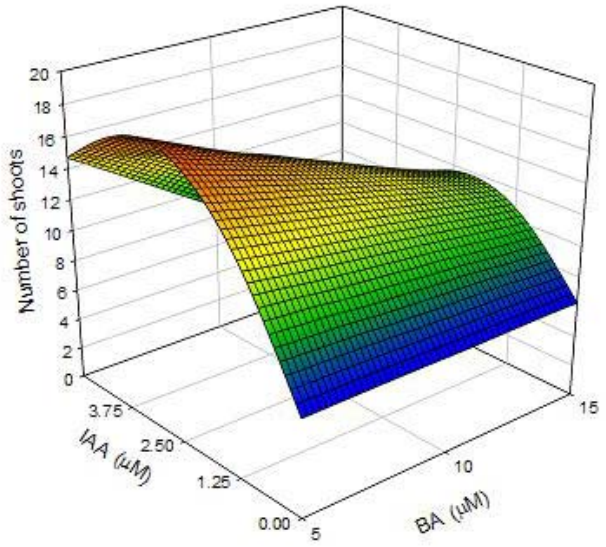

Figure 2. Effect of benzylamino purine (BA) and indoleacetic acid (IAA) on number of shoots per leaf segment.
($y=6.25 – 0.0014*BA + 8.58*IAA – 1.14*IAA^2 – 0.24*BA*IAA$, $P<0.05$, $r^2=0.61$)

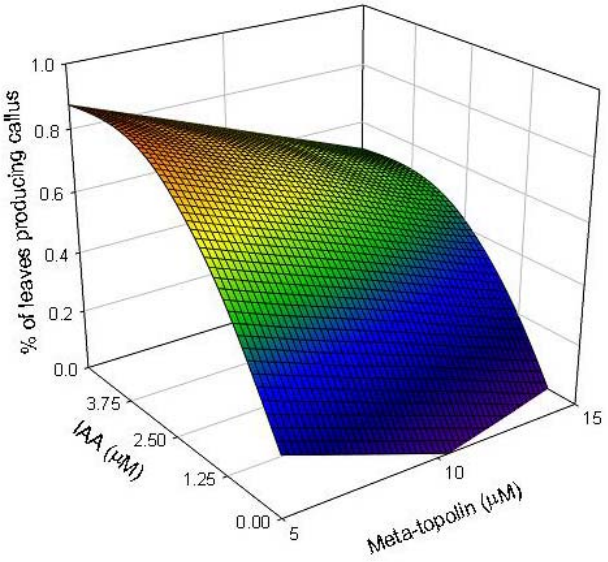

Figure 3. Effect of meta-topolin (mT) and indoleacetic acid (IAA) on percentage of leaf segments producing regenerative callus.
($y=0.38 – 0.037*mT + 0.34*IAA – 0.041*IAA^2$, $P<0.05$, $r^2=0.81$)

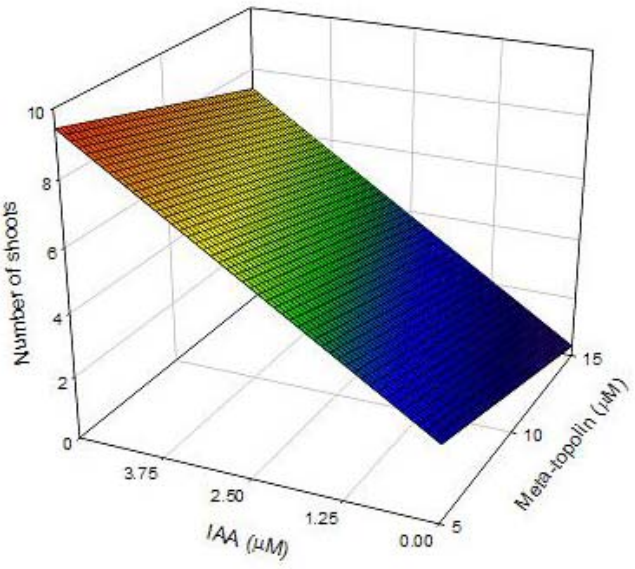

Figure 4. Effect of meta-topolin (mT) and indoleacetic acid (IAA) on number of shoots per leaf segment.
($y=3.49 – 0.21*mT + 1.49*IAA$, $P<0.05$, $r^2=0.49$)