Organogenesis from *Hypericum frondosum* leaves

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Significance to Industry: The genus *Hypericum* contains approximately 370 species found throughout the world. Commonly known as St. John's wort, the genus has received considerable attention for its medicinal qualities. In addition, several species, including *Hypericum frondosum*, have significant commercial value to the ornamental horticulture industry. *H. frondosum* 'Sunburst' grows 2-4 ft, has blue-green foliage and golden flowers. It is reasonably drought tolerant and cold hardy to USDA zone 5. The development of improved lines of *H. frondosum* would be beneficial to the ornamental horticulture industry. However, the improvement of ornamental qualities is often limited by the lack of effective plant regeneration systems. In vitro regeneration systems are useful tools in genetic improvement programs to facilitate the recovery of somaclonal variants and to manipulate ploidy levels, which increases ornamental characteristics and expands breeding opportunities.

Nature of Work: Despite the diversity of *Hypericum*, the regeneration of plants via organogenesis and somatic embryogenesis procedures have only been reported for limited species with pharmaceutical value. For example, a few studies have described the successful organogenesis and somatic embryogenesis from leaf segments of *H. perforatum* (5). Other studies have described organogenesis systems from seed for *H. perforatum* and *H. heterophyllum* (1,3), from hypocotyl segments for *H. perforatum* (4) and from nodal buds for *H. brasiliense* (2). In the present study, we report on the development of an organogenesis system from leaves for *H. frondosum*.

In all experiments, not fully expanded leaves from *Hypericum frondosum* 'Sunburst' were obtained from glass house grown plants maintained at the Mountain Horticultural Crops Research and Extension Center, Mills River, NC. Leaves were surface sterilized for 17 min in 20% commercial bleach and rinsed 3 times in sterile deionised water (5 mins each wash).

Leaves were then scored on the underside with a scalpel blade and cut into 5 mm (0.2 in) segments and plated on to different media. The callus induction medium contained Murashige and Skooge (MS) basal salts and vitamins supplemented with 3% sucrose. The pH was adjusted to 5.75 ± 0.03 and the media was solidified with 0.8% agar. The effect of growth regulators was tested using benzylamino purine (BAP) (0, 1.25, 2.5, 5.0 and 10 µM) in combination with 2,4-dichlorophenoxyacetic acid (2,4-D) or Indoleacetic acid (IAA) (0, 0.5, 2.5, 5.0 µM). Cultures were maintained in the dark at 23 C (73.4 F).

Ten leaf segments were cultured per plate, replicated 5 times representing a total of 30 observations per treatment. The frequency of callus and shoot production was determined after 8 weeks and data were analyzed using (PROC GLM; SAS version 9.1, SAS Institute., Cary, N.C., 1988).

For rooting induction, medium salt type and strength were tested. Elongated shoots (2.0 cm long) (0.79 in) were isolated and transferred to either full- or half-strength MS, Shenck and Hilderbrandt (SH) or Gamborgs B5 salts supplemented with 5 µM indolebutyric acid (IBA), 3% sucrose and solidified with 0.8 g/l agar. Seven shoots were placed in 180 ml (6.1 oz) baby food jars and cultured at 23 ^{o}C (73.4 F) under cool white fluorescent lights (130 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with a 16 h photoperiod. The experiment was replicated 10 times representing 70 observations per treatment. The frequency of rooting and average number of roots were determined after 4 weeks and rooted plants were transferred to the glass house.

Results and Discussion: In vitro shoot regeneration protocols were successfully developed from leaf segments of *Hypericum frondosum* (Table 1). While callus was observed using BAP in combination with 2,4-D and IAA, shoot formation was only observed using a combination of BAP and IAA. In contrast, studies on *H. perforatum* reported that 2,4-D was essential for shoot formation (5). Species specific in vitro regeneration protocols are often observed in woody plants, even for closely related species (4).

The interaction between BAP and IAA significantly affected both callus formation and shoot regeneration ($P<0.05$). In general, callus formation increased with increasing concentrations of BAP and IAA (Table 1). Highest shoot formation was observed with a combination of 10 µM BAP and 2.5 µM IAA. Decreased concentrations of BAP and lower or higher concentrations of IAA resulted in attenuated shoot formation (Table 1). In contrast, 4.5 µM BAP was optimal for shoot regeneration in *H. perforatum* and *H. heterophyllum* (1,5), again highlighting species specific differences.

Elongated shoots obtained from callus produced roots on all media evaluated. However, the basal salt concentration significantly influenced the number of shoots producing roots and the total number of roots. Higher frequencies of root formation were observed on half-strength MS media supplemented with 5 µM IBA (Table 2). Similar results have been observed for *H. perforatum* (5) and *H. canariense* (5). It is likely that the higher ionic strength of the other media combinations may have an inhibitory effect on woody plant species.

In this study we described an efficient protocol for the regeneration of plantlets via callus induced from leaf segments. The results highlight the species specific nature of regeneration protocols. Protocols developed in this study will be used to assist in mutation and ploidy manipulation studies.

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Table 1. Effect of cytokinin and auxin concentration on callus initiation and shoot formation from leaf segments of *H. frondosum*.

		Regeneration Response	
BAP (µM)	IAA (µM)	Callus[1]	Shoot
0	0	0a	0a
1.25	0	8.4 ± 2.3b	0a
2.5	0	38.7 ± 6.2d	0a
5	0	36.1 ± 6.1d	0a
10	0	22.3 ± 4.8c	0a
0	0.5	0a	0a
1.25	0.5	92.5 ± 3.6f	0a
2.5	0.5	88.4 ± 3.3f	11.5 ± 3.3b
5	0.5	100g	7.3 ± 2.2ab
10	0.5	100g	33.8 ± 5.6c
0	2.5	63.7 ± 6.3e	0a
1.25	2.5	97.5 ± 1.7g	10.0 ± 3.3b
2.5	2.5	96.3± 1.8g	11.0 ± 3.0b
5	2.5	100g	26.3 ± 5.8c
10	2.5	100g	51.25 ± 5.6d
0	5	14.7 ± 63.6b	0a
1.25	5	92.2 ± 2.6fg	0a
2.5	5	93.2± 2.9fg	0a
5	5	92.2 ± 3.1fg	16.8 ± 4.2b
10	5	98.3 ± 1.6fg	0a

[1]Means in each column followed by the same letter are not significantly different at $P<0.05$.

Table 2. Effect of basal salt composition on root formation and the number of roots of *H. frondosum*.

	Rooting Media			
	SH[1,2]	B5	MS	½ MS
Rooting (%)	79.8 ± 5.0ab	69.1 ± 7.5b	68.3 ± 9.6b	88.0 ± 6.6a
Average roots per shoot	1.8 ± 0.15a	1.8 ± 0.15a	3.1 ± 0.34b	4.2 ± 0.38c

[1]Means in each row followed by the same letter are not significantly different at $P<0.05$.
[2]SH – Shenck and Hilderbrandt basal salts ; B5 – Gamborgs B5 basal salts and vitamins; MS – Murashige and Skooge basal salts and vitamins; 1/2MS – half strength Murishige and Skooge basal salts.