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In vitro rooting of *Rehmannia glutinosa* L.

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Objectives

R. glutinosa can be propagated from seeds or by division of tuberous roots. However, this method can result in delayed root harvesting and low propagation rate. *In vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines. Since *Rehmannia* roots contain higher medical value compared to other plant parts, efficient *in vitro* techniques to boost production of root portion are looked for. Therefore, this study was conducted to determine the most suitable medium for rapid *in vitro* root proliferation from young stem explants of *R. glutinosa.*

Materials and Methods

The most suitable medium was selected from the preliminary experiment. Then, root regeneration was determined by using 4 different concentrations of the medium. About 10 - 20 mm of 7 shoot segments were cultured on the respective medium. The same procedure was followed for sterilizing the medium and culture conditions, as those mentioned above. Different auxins at different concentrations were used in combination with SH medium for efficient root regeneration in *R. glutinosa.* Five segments of 10 - 20 mm long shoots were cultured in a magenta box containing 50 mL of the respective medium.

Results

Plant tissue culture plays a vital role for plant improvement. Therefore, efficient protocols are crucial for saving time and costs associated with molecular work. Establishment of reliable protocols for root regeneration is also an important factor for all plants whose roots have an economic value. In this study, we found that a 4-fold dilution of SH medium was the most efficient for *in vitro* rooting of *R. glutinosa*. This information provides useful indications for future applications on commercial root production via gene transformation. Nonetheless, further studies are needed to investigate the effect of other plant hormones on *R. glutinosa* root regeneration.

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Table 1. Effect of different media on root regeneration and growth from excised stems of *Rehmannia glutinosa* L. after 4 weeks of *in vitro* culture.

Medium	Regeneration frequency* (%)	No. of roots per explant**	Root length[a](mm)
SH	100	4.5 ± 0.5	32.2 ± 1.4
B5	100	3.9 ± 0.4	28.5 ± 1.9
MS	100	2.5 ± 0.5	22.3 ± 1.3

* Regeneration frequency (%) = No. of explants with root differentiation/All explants × 100

** From a total of 100 stem explants.

[a] Values represent the mean ± standard deviation of 50 roots.

Table 2. Effect of SH media at different concentrations on root regeneration and growth from excised stems of *Rehmannia glutinosa* L. after 4 weeks of *in vitro* culture.

Medium	Regeneration frequency* (%)	No. of roots per explant**	Root length[a](mm)
¼ SH	93	5.3 ± 0.4	42.3 ± 1.5
½ SH	90	4.8 ± 0.3	39.5 ± 1.9
SH	100	4.4 ± 0.5	35.5 ± 1.4
2 SH	82	2.6 ± 0.5	28.2 ±1.3

* Regeneration frequency (%) = No. of explants with root differentiation/All explants × 100

** From a total of 100 stem explants.

[a] Values represent the mean ± standard deviation of 50 roots.

Table 3. Response of different concentrations of auxins on root regeneration and growth from excised stem of *Rehmannia glutinosa* L. after 4 weeks of *in vitro* culture.

Medium	Concentration (μM)	Regeneration frequency* (%)	No. of roots per explant**	Root length[a] (mm)
Control	0.0	100	4.3 ± 0.5	31.1 ± 1.5
SH + NAA	1	90	3.6 ± 0.5	26.3 ± 1.4
	3	40	2.8 ± 0.5	20.0 ±1.1
	5	-	-	-
SH + IBA	1	70	3.4 ± 0.5	26.0 ± 1.4
	3	60	2.3 ± 0.3	18.2 ±1.3
	5	20	1.8 ± 0.5	16.6 ± 1.2
SH + IAA	1	90	2.3 ± 0.4	24.3 ± 1.4
	3	-	-	-
	5	-	-	-

* Regeneration frequency (%) = No. of explants with root differentiation/All explants × 100

** From a total of 100 stem explants.

- No response

[a] Values represent the mean ± standard deviation of 50 roots.