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Acta Societatis Botanicorum Poloniae
Journal homepage: pbsociety.org.pl/journals/index.php/asbp
ORIGINAL RESEARCH PAPER Received: 2010.06.10 Accepted: 2011.12.04 Published electronically: 2012.02.20 Acta Soc Bot Pol 81(1):53-60 DOI: 10.5586/asbp.2011.044

Propagation of goldenrod (*Solidago canadensis* L.) from leaf and nodal explants

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Abstract

Goldenrod (*Solidago canadensis* L.) is an invasive plant species in many countries except North America but a cut-flower species worldwide. There is a need to generate and propagate goldenrod clones efficiently for research and commercial purposes. A callus induction and plantlet regeneration system was developed by studying the influence of explant type and different concentrations of plant growth regulators. The highest callus production from leaf segments was obtained on Murashige and Skoog's medium (MS medium) supplemented with 1.0 mg/L naphthalene acetic acid (NAA) and 1.0 mg/L 6-benzylaminopurine (BA). Adventitious shoots could be regenerated directly from leaf explants without an intermediate callus phase with the highest shoot induction percentage of 87.2%. The largest number of adventitious shoots per leaf explant (3.2) was obtained on MS medium supplemented with 0.4 mg/L NAA and 2.0 mg/L BA. MS medium supplemented with 0.1 mg/L NAA and 1.0 mg/L BA was the best medium for axillary shoot regeneration from nodal segments. The highest root number and longest roots occurred on half-strength MS without the addition of any growth regulator. Rooted plantlets were then transferred to a soil-based growth medium, placed in a greenhouse, and acclimatized with 100% success. All surviving plants grew normally without showing any morphological variation when compared to those grow from seed. This regeneration protocol may be used to produce certain biotypes of goldenrod suitable for genetic transformation, rapid propagation of goldenrod for commercial purposes or for screening fungi and toxins as potential biocontrol agents against this weed.

Keywords: callus formation, micropropagation, adventitious shoots, tissue culture

Introduction

Goldenrod (*Solidago canadensis* L., Asteraceae), a landscape weedy plant species found commonly within its native range in North America, is also considered an ornamental plant [1]. Goldenrod is self-compatible and produces huge numbers of wind-dispersed seeds that germinate easily in a wide range of soil types [2], and can also reproduce asexually through rhizome propagation. Worldwide, it has naturalized in Europe, New Zealand, Australia, and parts of Asia to an invasive alien weed [3,4]. Due to its strong competitiveness, goldenrod is capable of displacing native plant communities, reducing plant diversity, and consequently resulting in serious ecological erosion in many countries [3,5-8].

Since introduced to Shanghai in 1935, goldenrod has escaped into greater areas in China and become a serious invasive species in eastern regions, especially around Shanghai, in Zhejiang, Jiangsu and Anhui provinces [8-10]. Chemical, ecological, mechanical, manual and biological control methods have been attempted to control goldenrod in China [11]. Although some short-term successes have been achieved, the goal of sustainable management has not been reached due to economic and environmental costs of those methods and continuous spread of the weed. It is necessary to study long-term strategies such as biological control for goldenrod. Previous reports have shown that in vitro culture of weed tissues has applications in screening and testing potential herbicide or biocontrol candidates [12-16]. The use of tissue culture allows for a rapid and efficient screening of microbial agents, and has been found to be representative of results obtained in whole-plant experiments in many studies [15,17,18].

Today the techniques of molecular biology pervade all types of biological research and have offered unparalleled opportunities to dissect the genetic basis and evolution of traits associated with the success of weedy and invasive plants. Fundamental knowledge of the genetic underpinnings of what makes a plant a weed will provide for new management strategies to mitigate the negative effects of weedy and invasive plants on food production and habitat destruction [19,20]. Therefore, establishment of a tissue culture system could allow further molecular ecology research and genetic transformation procedures that, in the future, may help us understand genetic basis of the enhanced performance of invasive plants species and the traits that have made weeds successful colonizers and troublesome pests. The tissue culture system could provide

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uniform and high quantity initial material within a limited time in an aseptic, temperature-controlled environment, and could also decrease potential harm to the environment because no seed is released. The aim of the present study was to develop a successful protocol for the propagation of *S. canadensis* clones in vitro, which will facilitate further study of this weed, including chemical/biological control and studies of functional characterization of the genes being responsible for weedy and invasive traits.

In spite of the harmful effects of invasiveness to many ecosystems, goldenrod is permitted for use as a herbal medicine ingredient in several countries [21-23]. In others, goldenrod and its related species are used as one of the popular cut-flower species, and an efficient in vitro propagation method for valuable clones could be of significant commercial value. Development of a tissue culture system could also be useful for genetic transformation and improvement of commercial production. However, there is limited information on the factors affecting callus induction and plantlet regeneration of this species. In this paper, an efficient system is described to provide a method for callus induction and plantlet regeneration of goldenrod.

Material and methods

Plant material and culture conditions

Nodal explants with axillary buds were collected from field-grown plants of goldenrod. The excised nodal segments (0.5-1 cm long) without leaves were surface sterilized in 70% (v/v) ethanol for 30 to 40 s, followed with 8-min in 1 g/L $HgCl_2$, and rinsed five times with autoclaved distilled water. The sterilized nodal explants were cultured on an induction medium supplemented with plant growth regulators (PGRs) and used as the starting plant material. After 3 months of culturing, nodal stems as well as leaves of the induced plantlets were used for microcuttings and callus induction. Murashige and Skoog's medium [24] supplemented with 30 g/L sucrose and 7 g/L agar was used for callus induction. 6-benzyladenine (BA) and a-naphthalene acetic acid (NAA) were added to the medium. The pH was adjusted to 5.8 ±0.2, and the medium was autoclaved at 121°C under 100 kPa for 20 min. The cultures were incubated at 25 ±2°C, 50 to 60% relative humidity under 12/ 12 h photoperiod with light intensity of 30 umol $m^{-2}s^{-1}$.

Initial cultures of goldenrod

In order to obtain abundant, uniform and clean starting materials quickly, six media supplemented with BA (0.1 and 0.2 mg/L) or NAA (0.05 and 0.1 mg/L) alone or varying combinations of NAA and BA (Tab. 1) were evaluated to determine the effect on promoting shoot growth and development. Plantlet height, leaf number, leaf area, root number and root length per explant were examined after 3-month culturing.

Proliferation of axillary shoots from nodal stems

The second or third node from the apex, cut in about 0.5 to 1 cm in length, were planted in culture media containing combinations of NAA (0.1 and 0.5 mg/L) and BA (0.5-4.0 mg/L) for axillary shoot induction (Tab. 2). The number of shoots induced, shoot length and the number of leaves were recorded after 30-d culturing.

Callus induction and adventitious shoots from leaf explants

The second or third leaf from the apex of 3-month-old plants were cut into 0.5 cm by 0.5 cm pieces, then leaf segments were placed on media supplemented with the growth regulators NAA (0.05-3.0 mg/L) and BA (0.1-3.0 mg/L), either separately or in combination (Tab. 3), to test their effects on induction of callus tissues and adventitious shoots from leaf explants. Resulting callus was then sub-cultured on shoot-inducing media containing different concentrations of BA and/ or NAA. Data on percentage of leaf explants forming callus, shoot, root, and adventitious shoots were recorded after 45-d culturing.

Rooting and acclimatization

Healthy shoots arising from callus, nodal stems, or leaf segments were transferred to MS medium supplemented with NAA (0.05, 0.1, 0.2, and 0.5 mg/L) or without growth regulators for root initiation and development (Tab. 3). To assess the concentration effect of mineral nutrients on rooting, these shoots were cultured on half strength MS medium. After 30 d, the number of roots generated, root length, and shoot height were recorded. Some of the rooted plantlets were removed from rooting media, washed, and then transferred to pots containing a mixture of sterile soil, peat and vermiculite in a ratio of 1:1:1. Newly potted plantlets were covered with polythene bags for 1 week before they were transfered to a research greenhouse. Survival percentage was recorded four weeks after transplanting.

Data analysis

All experiments used a randomized complete block design with 10 to 20 replicates per treatment. Each experiment was repeated at least once. Percentage data were subjected to arcsine transformation prior to analysis, and all data were analyzed using a One-Way Analysis of Variance (ANOVA). Means were separated according to the Duncan's test. Statistical tests were performed using SPSS17.0 package version and mean values that do not share the same letter are significantly different ($P = 0.05$).

Results

Initial cultures of goldenrod

PGR displayed different effects on the differentiation and growth of goldenrod shoot, leaf, and root (Tab. 1). Media containing NAA alone were generally superior to those with both NAA and BA in terms of shoot development. Plantlets produced the highest number of leaves (12.9) when cultured on MS medium with 0.1 mg/L BA + 0.05 mg/L NAA, while shoot height, leaf area, and root numbers were the lowest on this medium. In comparison to MS without PGR amendment, MS plus BA produced significantly smaller leaf area and shorter roots. When the concentration of BA was increased from 0.1 to 0.2 mg/L, shoot height was reduced from 8.6 cm to 5.4 cm. A low concentration of BA in the medium didn't promote shoot growth and development. A medium containing 0.05 mg/L NAA was superior to the PGR-free basal medium, and when at the 0.1 mg/L NAA level, the plantlets produced an average of 4.3 cm^2 leaf area and 6 roots per plantlet after 3 months of culturing (Tab. 1). The difference was significant ($P < 0.05$). Because plantlets grown on media containing 0.05 mg/L NAA were apparently more vigorous with taller shoots and large leaves, this medium was therefore used in all subsequent experiments (Fig. 1a).

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Tab. 1 Effect of plant growth regulators on initial culture of goldenrod.

PGR in medium						
BA (mg/L)	NAA (mg/L)	Shoot height (cm)	No. of leaves	Leaf area (cm^2)	Avg. root length (cm)	No. of roots
0	0	7.5 ±0.6 ab	10.7 ±0.9 bc	3.2 ±0.3 c	15.6 ±1.2 a	4.2 ±0.3 b
0	0.05	8.5 ±1.0 a	11.4 ±1.4 abc	4.3 ±0.3 a	14.1 ±4.2 a	6.0 ±0 a
0	0.1	7.7 ±1.8 a	10.6 ±0.5 bc	3.7 ±0.1 b	14.3 ±2.9 a	4.7 ±0.8 b
0.1	0	8.6 ±0.8 a	10.2 ±0.9 c	2.1 ±0.4 d	4.7 ±0.5 b	4.3 ±0.4 b
0.2	0	5.4 ±1.2 bc	10.8 ±0.8 bc	2.2 ±0.1 d	2.9 ±0.4 b	4.4 ±0.8 b
0.1	0.05	4.7 ±2.2 c	12.9 ±1.4 a	1.4 ±0.2 e	4.0 ±2.3 b	3.4 ±1.1 b
0.1	0.1	6.1 ±1.0 bc	12.4 ±1.1 ab	2.5 ±0.1 d	4.0 ±0.5 b	4.1 ±1.3 b

Data are means ± SE averaged over three experiments, each with 10 replicates, recorded three months after transfer to media with different concentrates of 1-naphthaleneacetic.acid and 6-benzylaminopurine. Means followed by the same letter within a column are not significantly different (Duncan's multiple range test, $P = 0.05$). BA – 6-benzylaminopurine; NAA – 1-naphthaleneacetic acid; PGR – plant growth regulator.

Tab. 2 Effect of plant growth regulators on axillary shoot formation from nodal segment of goldenrod.

PGR		No. of shoots	Avg. shoot	
BA(mg/L)	NAA (mg/L)	(/explant)	height (cm)	No. of leaves
0	0	1.0 ±0 e	3.5 ±0.1 a	8.3 ±0.3 ab
0.5	0	1.8 ±0.6 de	1.8 ±0.2 bc	8.7 ±0.3 a
1.0	0.1	7.5 ±1.4 ab	2.0 ±0.5 b	8.6 ±1.8 ab
1.0	0.5	4.8 ±1.4 bcd	2.0 ±0.8 bc	8.4 ±1.7 ab
2.0	0.1	4.3 ±1.9 cd	1.9 ±0.4 bc	9.2 ±1.7 a
2.0	0.5	5.5 ±1.7 abc	1.9 ±0.4 bc	9.8 ±1.4 a
3.0	0.1	8.2 ±4.2 a	1.1 ±0.2 d	7.7 ±1.7 abc
3.0	0.5	7.8 ±1.4 ab	1.2 ±0.2 d	7.8 ±1.8 abc
4.0	0.1	2.5 ±1.0 cde	1.3 ±0.4 cd	5.9 ±0.9 c
4.0	0.5	2.5 ±0.3 cde	1.3 ±0.4 cd	6.2 ±1.7 bc

Data are means ± SE averaged over three experiments, each with 10 replicates, recorded three months after transfer to media with different concentrates of 1-naphthaleneacetic acid and 6-benzylaminopurine. Means followed by the same letter within a column are not significantly different (Duncan's multiple range test, $P = 0.05$). BA – 6-benzylaminopurine; NAA – 1-naphthaleneacetic acid; PGR – plant growth regulator.

Proliferation of plantlets from nodal stems

The nodal cutting technique provided a rapid single-step, whole-plant proliferation system that could be used for large-scaled micro propagation. Axillary shoots were initiated from NS within 15 d of culture. On the MS medium without PGR, an average of 3.5 cm shoot length was recorded whereas multiple shoots were not obtained with this medium. Shoot multiplication and growth occurred in all media with BA + NAA (Fig. 1b). The results showed a variable shoot-forming capacity depending on the combination of growth regulators used in the culture medium (Tab. 2). The number of shoots per explant increased (1.8-8.2) with an increase in BA concentration from 0.5 to 3.0 mg/L. A high BA concentration, however, affected axillary shoot formation negatively; the number of shoots generated on the explant basis was reduced significantly with an increase of BA concentration to 4.0 mg/L when compared to that with 1.0 to 3.0 mg/L (Tab. 2). The concentration of BA also influenced the growth of axillary shoots; at 3.0 mg/L, formed shoots appeared stunted with the average height decreased to 1.1-1.2 cm as opposed to >2.0 cm at lower concentrations, and the difference was significant ($P < 0.05$). Increasing BA concentration to 4.0 mg/L also decreased the leaf number on axillary shoots (Tab. 2). Furthermore, at 3.0 mg/L BA concentration, formed leaves showed symptoms of vitrification, and as the BA concentration increased to 4.0 mg/L vitrification became more serious. Although the combination of 3.0 mg/L BA and 0.1 mg/L NAA showed the highest incidence of axillary shoot formation (8.2 shoots per nodal stem), shoot quality was poorer than that in media with 1.0 mg/L BA and 0.1 mg/L NAA in terms of shoot length and leaves number (Tab. 2). Based on these criteria, we conclude that the optimal medium for plantlet multiplication from nodal stem cuttings is MS medium supplemented with 1.0 mg/L BA and 0.1 mg/L NAA. This medium induced an average of 7.5 shoots, with a height of 2.0 cm and 8.6 leaves per shoot after 1 month of culture. No significant difference was observed in leaf or shoot number, or in shoot length between the NAA concentration 0.1 mg/L and 0.5 mg/L in the presence of BA.

Callus induction from leaf explants and shoot induction from leaf explants callus

After 5-7 d culture, cutting edge of leaves slightly turned upward, calli formed on the cut surfaces about 15 d culturing. Explants on the PGR-free MS basal medium did not produce any callus and explants died after a few days. Callus was not formed and the leaf tissue only increased their size when MS was supplemented with BA alone. Calli were formed on MS medium supplemented with NAA alone. However, calli were formed in high percentages when MS was amended with >1.0 mg/L NAA plus >0.4 mg/L BA, and callus induction was markedly enhanced with combinations of NAA and BA at higher concentrations (Tab. 3). These results showed that both NAA and BA played an important role in callus formation from goldenrod leaf explants, and the interaction of cytokinin and auxin can be concentration dependent. The highest frequency of callus induction (100%) occurred with 1.0 mg/L NAA + 1.0 or 2.0 mg/L BA and 2.0 mg/L NAA + 0.6 mg/L BA (94.4%). All original explants were completely covered with calli in medium

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Tab. 3 Effect of plant growth regulators on callus induction, shoot formation, multiple adventitious shoots appearance and root induction from the leaf segments of goldenrod.

PGR					
NAA (mg/L)	BA (mg/L)	Callus formation (%)[a]	Shoot formation (%)[a]	Multiple adventitious shoots (/explant[b])	Root induction (%)[a]
0	0	-	-	-[c]	-
1	0	-	-	-	100 a
2	0	25.0 ±8.3 fg	-	-	100 a
3	0	38.9 ±9.6 def	-	-	100 a
0.05	0.1	-	-	-	100 a
0.1	0.1	-	-	-	100 a
0.5	0.1	-	-	-	100 a
0	1	-	-		-
0.1	1	16.7 ±5.6 g	-	-	-
0.2	1	53.7 ±11.6 d	-	-	-
1	1	100 a	-	-	-
2	1	80.6 ±1.0 c	15.8 ±1.2 d	0.4 ±0.1 cd	50.8 ±9.6 d
0	2	0	0	0	0
0.2	2	33.4 ±5.3 ef	77.4 ±6.1 a	2.0 ±0.9 b	-
0.4	2	31.6 ±5.9 h	80.6 ±4.8 a	3.2 ±0.2 a	-
1	2	100 a	35.6 ±3.9 c	1.2 ±0.1 c	-
2	2	84.8 ±5.3 c	45.8 ±4.2 b	0.9 ±0.1 cd	25.5 ±3.5 e
2	0.6	94.4 ±9.6 ab	8.3 ±2.9 e	0.2 ±0.1 d	66.7 ±9.6 c
2	0.4	86.1 ±12.7 bc	-	-	85.7 ±0 b
2	0.2	44.4 ±9.6 de	-	-	100 a
3	3	100 a	-	-	20.8 ±5.9 f

[a] Data are means ± SE of three experiments consisting of 20 replicates each recorded 45 d after transfer to media with different concentrates of 1-naphthaleneacetic acid and 6-benzylaminopurine. [b] Data are means ± SE of three experiments each with 10 replicates, recorded 45 d after transfer to media with different concentrates of 1-naphthaleneacetic acid and 6-benzylaminopurine. [c] Not responding. BA – 6-benzylaminopurine; NAA – 1-naphthaleneacetic acid; PGR – plant growth regulator.

supplemented with 1.0 mg/L NAA + 1.0 mg/L BA after 18 days, or with 1.0 mg/L NAA + 2.0 mg/L BA after 45 days. The amount of calli produced in the former case was significantly higher than that in other PGR combinations. The morphology of callus varied with different BA:NAA ratios in the cultural medium; calli from higher ratios were more compact and green in color (Fig. 1c), while those obtained from a lower ratio (0.6:2 for example) were friable, yellowish to brown in color (Fig. 1d). Calli formed on media with a moderate amount and equal portions of PGRs (1:1 mg/L) appeared friable, transparent and light green (Fig. 1e). We conclude that the amount of PGRs for optimal callus induction is 1.0 mg/L NAA + 1.0 mg/L BA in MS medium. Calli obtained with a high concentration of NAA (2.0 mg/L) plus BA (0.6 mg/L) could form full plantlets without an induction process for differentiation (Fig. 1d), which suggests that they developed through embryogenesis. The combination of very low NAA (0.1 mg/L) and BA (0.1 mg/L) concentrations in MS medium failed to produce any callus. Instead, rhizogenesis occurred on the surface of leaf explants.

Calli produced on NAA (1.0 mg/L) plus BA (1.0 mg/L) MS medium were transferred to the basal medium amended with different concentrations of BA in combination with a low concentration of NAA for shoot regeneration, but the response was generally poor for the majority of treatments selected (data not shown). MS medium supplemented with 3.0 mg/L BA and 0.2 mg/L NAA produced about 1 shoot/callus for 25.0% of calli after three months (Fig. 1g).

Adventitious shoot formation from leaf explants

Several morphogenic events were assessed to determine the effect of NAA and BA combinations (Tab. 3). Adventitious shoots were induced directly from the leaf segments without going through the callus phase on MS containing 0.6-2.0 mg/L BA and 2.0-0.2 mg/L NAA (Fig. 1f), and roots were formed on a medium containing NAA . These shoots formed near the wound areas well covered by callus. The percentage of responding explants was a function of the auxin/cytokinin ratio. As the BA concentration increased (0.6 to 2.0 mg/L) in the presence of descending NAA (2.0-0.2 mg/L), the shoot-forming percentage increased substantially (from 8.3% to 77.4%) but the root-forming percentage was reduced significantly (from 100% to 25.5%) due to the changing ratio of the auxin to the cytokinin (Tab. 3). The average number of shoots regenerated per leaf explant also increased with the reduction of NAA concentration from 2.0 to 0.2 mg/L (Tab. 3). The highest adventitious shoots induction percentage (87.2%) was obtained with 0.4 mg/L NAA and 2.0 mg/L BA, followed by 0.2 mg/L NAA and 2.0 mg/L BA (77.4%). On the average 3.2 shoots could be produced from a leaves segment on the medium containing 0.4 mg/L NAA and 2.0 mg/L BA within 30 d of culture. Low concentrations of auxins and cytokinins (0.1 mg/L BA), or further reduction of NAA concentration (0.05 mg/L) failed to induce goldenrod shoot formation.

Root induction and acclimatization of plantlets

NAA has been used for rooting wild as well as cultivated

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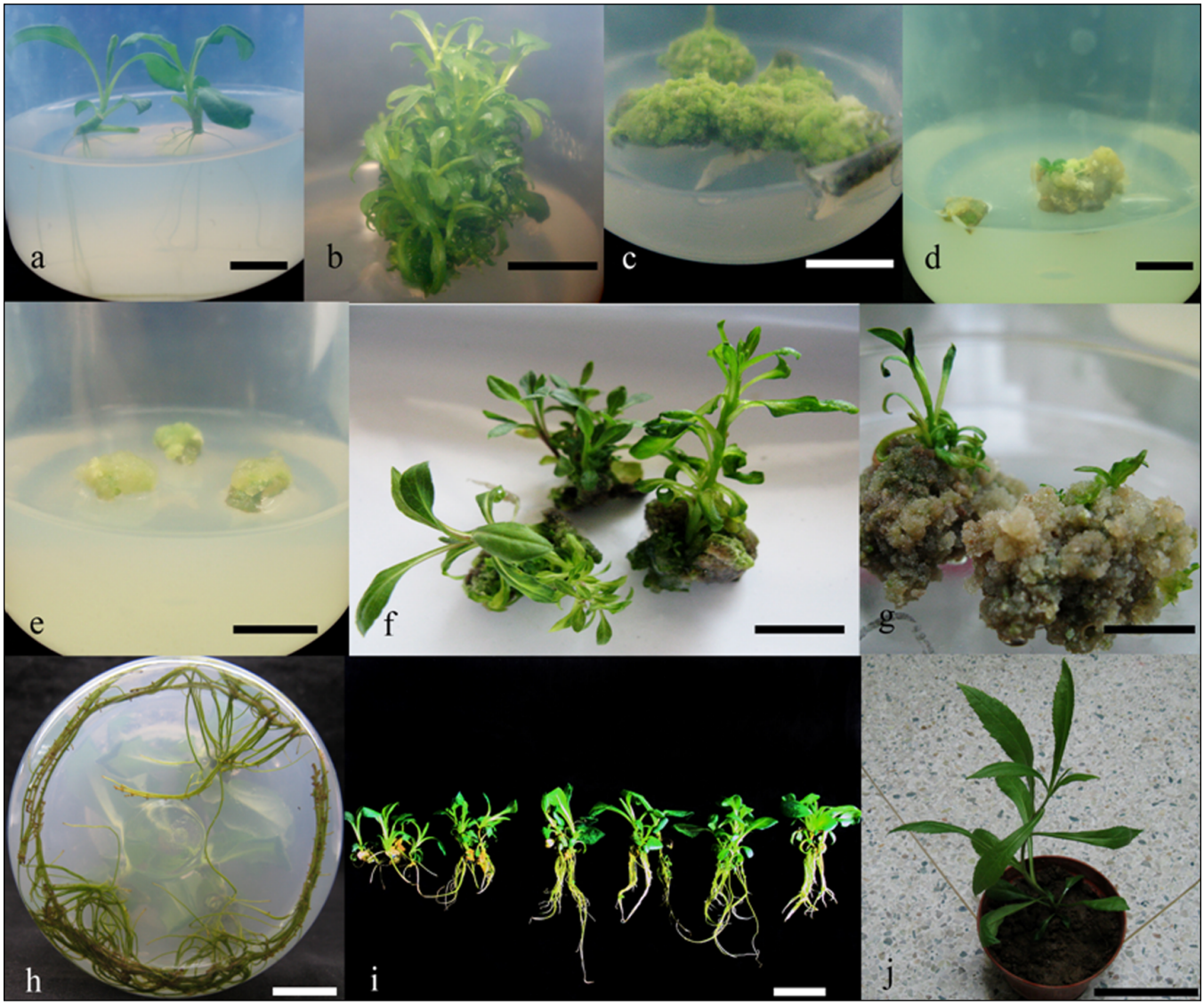

Fig. 1 The regeneration of plantlets from leaf segments and axillary shoot formation from stem explants of goldenrod. **a** Initial cultures. **b** Axillary shoot from nodal segment. **c-e** Different types of callus induced from leaf segment. **f** Adventitious shoots formed from leaf segments. **g** Adventitious shoots formed from leaf callus. **h** Well-rooted plantlet in half-strength Murashige and Skoog's medium. **i** Plantlet derived from various rooting media. **j** Plants regenerated from callus without any visual (somaclonal) morphological variation, one month after transplanting. **a-i** Scale bars: 1 cm. **j** Scale bar: 10 cm.

Asteraceae species [25-27]. In the current study, all media containing NAA promoted rhizogenesis and the rooting incidence generally reached 100% (Tab. 4). Root formation was noticed from basal cut portion of the shoot after 7-d incubation (Fig. 1h) when the regenerated shoots were transferred to MS medium containing NAA. Roots were also induced in half-strength MS medium without the addition of any PGR, with the highest number of roots produced (11.4 roots/shoot). The half-strength MS medium alone was sufficient for satisfactory rhizogenesis, while the full-strength MS with NAA showed varied rooting responses (Tab. 4, Fig. 1i). A low amount of callus growth was observed with high NAA concentrations (>0.5 mg/L), hindering the growth and development of goldenrod seedlings, including reduced number of roots, root length and shoot height. The effect was the worst when 0.5 mg/L NAA was used (Tab. 4). The low concentration of auxin didn't help the rooting when compared to the PGR-free medium, but there was no significant difference in plantlet height between culturing in half-strength MS and full-strength MS media. However the number of roots produced on half-strength MS exceeded that on the full-strength MS.

Well-developed plantlets were transferred to pots containing a mixture of sterilized soil, peat and vermiculite. Regenerated plantlets established successfully with a 100% survival rate and all surviving plants did not exhibit any visible variation in morphology or growth characteristics in comparison to the donor plant (Fig. 1j).

Discussion

It has been reported that direct plant regeneration from nodal stems explants could be successfully obtained in some Asteraceae plants [25,28], Shen et al. [28] reported a number of 4.6 shoots per stem in 0.5 mg/L NAA + 1.0 mg/L BA from the wild growing croftonweed, Borthakur et al. [25] reported similar result on *Eupatorium adenophorum*. In the study, we achieved a significantly better proliferation frequency (7.5 shoots/nodal stem explant) for stem explants, high frequency shoot proliferation was obtained using leaf explants (3.2 shoots

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Tab. 4 Effect of Murashige and Skoog's medium strength and concentration of auxin on root formation of goldenrod generated from shoot tissues.

Nutrient composition (mg/L)	Height of shoot (cm)	No. of roots	Average root length (cm)
MS + NAA 0.05	1.8 ±0.1 bc	7.6. ±1.7 bc	4.0 ±0.3 b
MS + NAA 0.1	1.9 ±0.2 abc	8.6 ±0.9 b	3.9 ±0.4 b
MS + NAA 0.2	1.9 ±0.1 abc	5.6 ±1.2 cd	2.2 ±0.1 c
MS + NAA 0.5	1.7 ±0 c	5.0 ±1.0 d	1.3 ±0.1 c
½ MS	2.2 ±0.2 ab	11.4 ±1.1 a	4.3 ±0.3 b
MS	2.3 ±0.4 a	7.3 ±0.8 bcd	6.6 ±1.2 a

Data are means ±SE of three experiments each with 10 replicates, recorded 30 d after transfer to media with/without different concentrates of 1-naphthaleneacetic acid. Means followed by the same small letter within a column are not significantly different at P = 0.05 according to Duncan's multiple range test. MS – Murashige and Skoog's medium; NAA – 1-naphthaleneacetic acid.

per leaf explant) on MS medium supplemented with 0.4 mg/L NAA and 2.0 mg/L BA, and it is the first report on tissue culture for the production of multiple shoots directly from leaf segments of mature *S. canadensis* plants. Adventitious shoots obtained from callus are often genetically unstable, and may show somaclonal variations [29-32]. The callus induction stage can be optimized by direct induction of shoots, and this strategy has been proved efficient and reliable. Proliferation of adventitious shoots from leaf explants of goldenrod can be a useful method for multiplication of this plant, allowing for rapid propagation of selected clone. It can be also very useful for the genetic transformation.

Previous reports showed that PGR requirements for callus induction vary depending on the explants types in Asteraceae species [33-35], and such variations can be attributed to the physiological condition of the explant, which is determined by genetic factors [36]. In the present study, leaf is a better explant source for the species with its 100% of callus induced in MS medium supplemented with BA and NAA. This result was fairly consistent with other species tested [28,33,37]. The intercalary meristems distributed in leaves might be responsible for the higher regeneration potential.

Most of the earlier researchers found BA to be the most effective cytokinin for inducing shoot development in other species of the Asteraceae as well as in plants of other families [28,37-40]. Our observation showed that BA was also significantly effective for proliferation of goldenrod. Our study clearly indicates the requirement of low auxin: high cytokinin ratio for shoot induction from stem explants and leaf explants. It is confirmed that the balance between NAA and BA is a determining factor for morphogenic variations [41-43]. This pattern of BA impact was also observed on other species of Asteraceae [28,37,40]. Previous reports show that somatic embryos or adventitious shoots could be proliferated directly from explants using only 2,4-D, BA, or NAA + BA [40,44-46]. This different response to PGR may have some relation to the endogenous growth regulator levels in the explants. Our data also indicate the importance of PGR amounts for in vitro proliferation of goldenrod from leaf explants. Low concentrations of auxins and cytokinins (0.1 mg/L BA), or further reduction of NAA concentration (0.05 mg/L) failed to induce goldenrod shoot formation, and this result may be due to higher levels of endogenous auxins produced in goldenrod leaves. In the natural environment, goldenrod grows rapidly, exhibiting highly invasive behavior. This growth pattern may be related to relatively higher levels of endogenous growth regulators.

In previous reports, NAA alone or combined with BA, was used to induce callus in other species of the Asteraceae family [27,33,47,48]. In our study, callus from goldenrod leaf explants under high auxin: low cytokinin ratio medium also grew well. Furthermore, we found that the callus induction was affected by auxin and cytokinin ratio as well as their actual concentrations. The interaction of cytokinin and auxin can be concentration dependent. The combination of very low NAA (0.1 mg/L) and BA (0.1 mg/L) concentrations in MS medium failed to produce any callus. Instead, rhizogenesis occurred on the surface of leaf explants. Low amounts of NAA and BA induced callus formation from leaf explants of croftonweed [37]. Such variations may be attributed to physiological conditions of explants, which can be determined by genetic factors regulating the production of endogenous growth regulators in the leaves of goldenrod. Our study showed a low yield of shoots from leaf explants callus of goldenrod in MS medium with NAA and BA. However, in some cases, other types of cytokinin appeared more effective than BA for shoot generation [41,49]. This different response to PGR could be a reflection of differences of endogenous growth regulator levels in this weed or different tissue sensitivities to the PGR [50]. But unfortunately no additional types of cytokinin were included in the current study, and further study is warranted for investigation of possible effect of other types of cytokinin on shoot induction from the leaf calli.

During the initial cultures of goldenrod, NAA is very effective in shoot elongation but at a very low concentration. One possible explanation for this result is that rooting was promoted by low auxin, and better rooting promoted plant growth and development. The presence of exogenous BA appeared to inhibit root growth and development, and this observation is consistent with an earlier report [38]. However, Feito et al. [51] found that higher levels of cytokinin but lower levels of IAA and ABA were favorable to spontaneous rooting. The influence of endogenous hormones and their concentration on rooting may vary with different plant tissues/species and require further investigation. However, during root induction, the low concentration of auxin didn't help the rooting when compared to the PGR-free medium. This result is consistent with what was observed on other Asteraceae species [25-28]. It is proposed that the half-strength MS may be sufficient for satisfactory rooting of goldenrod plants. This recipe may also have practical applications to commercial propagation of goldenrod for cut flowers. The phenomenon of easy root formation also provided additional evidence for high propagation rate of this weed.

In conclusion, this study successfully developed an in vitro propagation system for *S. canadensis*. High frequency of callus induction can be achieved for goldenrod using leaves as explants. Combinations of BA and NAA can be more effective for callus formation than either PGR used alone. High proliferation and survival rate was achieved, which facilitated uniform and vigorous growth of regenerated plantlets in field conditions. It was showed that goldenrod plantlets could be readily generated through micropropagation as well as through adventitious shoot regeneration from leaf explants. It

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was also demonstrated that adventitious shoots of goldenrod could be regenerated directly without going through the callus formation stage, and this method can produce true types of the plant rapidly and efficiently for further research and commercial uses.

Acknowledgements

The study was funded by National Basic Research Program of China-973 Program (2009CB119202), National Science Foundation of China (31070482), the Special Scientific Research Program for Non-profit Professions (No. 200709017) and the 111 project (B07030) respectively.

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