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(54) Title: COMPOSITIONS, METHODS, AND SYSTEMS FOR MICROPROPAGATION OF PLANTS

(57) Abstract: The present invention provides compositions, methods, and systems for achieving very high multiplication rate of plants *in vitro* micropropagation.

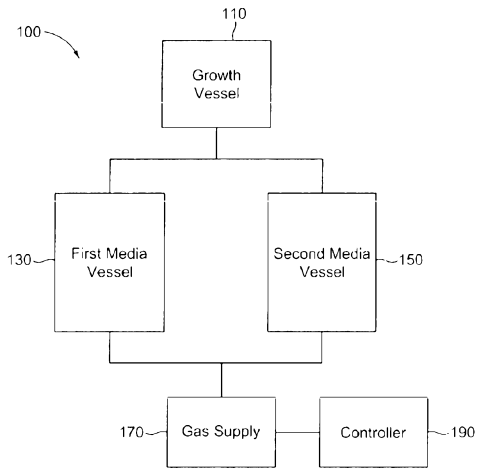

FIG.1

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COMPOSITIONS, METHODS AND SYSTEMS FOR MICROPROPAGATION OF PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to and the benefit of U.S. Provisional Patent Application Serial Nos. 61/510,955, filed July 22, 2011; 61/514,331, filed August 2, 2011; 61/515,735, filed August 05, 2011; 61/523,162, filed August 12, 2011; 61/523,205, filed August 12, 2011; 61/552,834, filed August 28, 2011; 61/607,838, filed March 7, 2012; and 61/618,344, filed March 30, 2012, each of which is hereby incorporated by reference in their entireties for all purposes.

TECHNICAL FIELD

The present invention relates generally to compositions, methods, and systems for plant propagation. In some embodiments, the present invention provides compositions, methods, and systems for the micropropagation of plants.

BACKGROUND

With increasing burdens on land to produce food and biomass for energy and materials additional attention is being placed on identifying and utilizing faster growing and more productive plants. Although many plants are suitable for such purposes, there is still a great need to develop compositions, methods, and systems for fast, economical plant propagation.

SUMMARY OF THE INVENTION

The present invention provides compositions, methods and systems for plant tissue culture, for example, compositions, methods and systems for plant micropropagation. In some embodiments, the compositions, methods and systems are used for plant *in vitro* micropropagation.

Media are provided for plant *in vitro* micropropagation. In some embodiments, bud induction media and shoot elongation/maintenance media are provided. In some embodiments, the bud induction media comprise one or more strong cytokinins, and the shoot elongation/maintenance media comprise one or more relatively weaker cytokinins. In some embodiments, the bud induction media comprise thidiazuron (TDZ) or analog thereof, and

the elongation and maintenance media comprise one or more cytokinins other than TDZ or an analog thereof. In some embodiments, the cytokinins other than TDZ are selected from the group consisting of N6-benzylaminopurine (BAP), meta-topolin (mT), zeatin, zeatin riboside, dihydrozeatin, kinetin, isopentenyladenine (ip, e.g., 2ip), adenine hemisulfate, dimethylallyladenine, N-(2-chloro-4-pyridyl)-N'- phenylurea) (4-CPPU), and analogs thereof. In some embodiments, the media can be used for plants *in vitro* micropropagation of monocots or dicots. In some embodiments, the media can be used for bamboo plants *in vitro* micropropagation.

In some embodiments, the bud induction medium comprises an effective amount of thidiazuron (TDZ) or analog thereof, and wherein the shoot elongation/maintenance medium comprises an effective amount of one or more cytokinins other than TDZ or an analog thereof. In some embodiments, the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is selected from the group consisting of N6-benzylaminopurine (BAP), meta-topolin (mT), zeatin, zeatin riboside, dihydrozeatin, kinetin, 2-isopentenyladenine (2ip), adenine hemisulfate, dimethylallyladenine, N-(2-chloro-4-pyridyl)-N'- phenylurea) (4-CPPU), and analogs thereof.

In some embodiments, the concentration of TDZ or analog thereof in the bud induction medium is effective to induce shoot buds. In some embodiments, the concentration of TDZ or analog in the bud induction media thereof is about 0.25 mg/L to about 100 mg/L, for example, about 0.5 mg/L to about 2 mg/L. Thus, the concentration of TDZ or analog thereof in the bud induction media can, for example, be about 0.25 mg/L, about 0.3 mg/L, about 0.4 mg/L, about 0.5 mg/L, about 0.6 mg/L, about 0.7 mg/L, about 0.8 mg/L, about 0.9 mg/L, about 1.0 mg/L, about out 1.5 mg/L, about 2.0 mg/L, about 2.5 mg/L, about 3 mg/L, about 4 mg/L, about 5 mg/L, about 6 mg/L, about 7 mg/L, about 8 mg/L, about 9 mg/L, about 10 mg/L, about 15 mg/L, about 20 mg/L, about 25 mg/L, about 30 mg/L, about 40 mg/L, about 50 mg/L, about 60 mg/L, about 70 mg/L, about 80 mg/L, about 90 mg/L or about 100 mg/L.

In some embodiments, the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is effective to elongate shoots. In some embodiments, the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium from about 0.01 mg/L to about 100 mg/L, for example, from about 0.25 mg/L to about 5 mg/L. Thus, the concentration of one or more cytokinins other than TDZ or analog thereof in the shoot

elongation/maintenance media can, for example, be about 0.01 mg/L, about 0.02 mg/L, about 0.03 mg/L, about 0.04 mg/L, about 0.05 mg/L, about 0.06 mg/L, about 0.07 mg/L, about 0.08 mg/L, about 0.09 mg/L, about 0.10 mg/L, about 0.15 mg/L, about 0.20 mg/L, about 0.25 mg/L, about 0.3 mg/L, about 0.35 mg/L, about 0.4 mg/L, about 0.45 mg/L, about 0.5 mg/L, about 0.6 mg/L, about 0.7 mg/L, about 0.8 mg/L, about 0.9 mg/L, about 1.0 mg/L, about 1.5 mg/L, about 2.0 mg/L, about 2.5 mg/L, about 3 mg/L, about 4 mg/L, about 5 mg/L, about 6 mg/L, about 7 mg/L, about 8 mg/L, about 9 mg/L, about 10 mg/L, about 15 mg/L, about 20 mg/L, about 25 mg/L, about 30 mg/L, about 40 mg/L, about 50 mg/L, about 60 mg/L, about 70 mg/L, about 80 mg/L, about 90 mg/L or about 100 mg/L.

In some embodiments, the bud induction medium and/or the shoot elongation/maintenance medium further comprise one or more auxins. In some embodiments, the auxins are selected from the group consisting of β-naphthoxyacetic acid (NAA), 2,4-Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, and analogs of each thereof. For example, the auxin is NAA or analogs thereof.

In some embodiments, the bud induction medium is a liquid medium. In some embodiments, the bud induction medium is a solid medium. In some embodiments, the shoot elongation/maintenance medium is a liquid medium. In some embodiments, the shoot elongation/maintenance media is a solid media.

Also provided are methods of *in vitro* micropropagation of plants. In some embodiments, the methods are used for micropropagating plants *in vitro*. In some embodiments, the methods comprise (a) incubating a plant tissue culture, explant or seed in a first medium, and (b) then incubating the plant tissue obtained from step (a) in a second medium. In some embodiments, the first medium is a bud induction medium, and the second medium is a shoot elongation and maintenance medium.

In some embodiments, when a bud induction medium and a shoot elongation and maintenance medium are used, the methods comprise: (a) incubating a plant tissue culture, explant or seed in a bud induction medium to induce shoot bud formation; and (b) incubating the shoot buds obtained in step (a) in a shoot elongation/maintenance medium.

In some embodiments, when a bud induction medium and a shoot elongation and maintenance medium are used, the methods further comprise: (c) incubating the shoots from step (b) in a bud induction medium to induce shoot bud formation; and (d) incubating the shoot buds obtained in step (c) in a shoot elongation/maintenance medium.

In some embodiments, when a bud induction medium and a shoot elongation and maintenance medium are used, the methods further comprise: (e) repeating the incubating step (c) and step (d) for at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or more additional cycles. There is no limit to how many times the cycling of step (c) and step (d) can be repeated. Buds and/or shoots obtained in step (a) or step (c) can be separated prior to the buds and/or shoots entering step (b) or step (d), respectively, wherein such separation can result in a single bud or shoot, 2 buds and/or shoots, 3 buds and/or shoots, or 4 or more buds and/or shoots per separation. Optimum separation for maximum, rapid production of bamboo copies of a single species, genotype or clone usually involves separating the buds and/or shoots obtained in step (a) or step (c) into 1-3 buds and/or shoots prior to entering into step (b) or step (d), respectively. Where there are 2 or more buds and/or shoots per separation this is known in the art as a clumping or "clump" of buds and/or shoots. Some variation in the methodologies of the present invention may be necessary so as to fine-tune the process for specific species, genotypes or clones of bamboo and such process variations are within the disclosure of this invention.

In some embodiments, the bud induction medium comprises an effective amount of thidiazuron (TDZ) or analog thereof, and wherein the shoot elongation/maintenance medium comprises an effective amount of one or more cytokinins other than TDZ or an analog thereof. In some embodiments, the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is selected from the group consisting of N6-benzylaminopurine (BAP), meta-topolin (mT), zeatin, zeatin riboside, dihydrozeatin, kinetin, 2-isopentenyladenine (2ip), adenine hemisulfate, dimethylallyladenine, N-(2-chloro-4-pyridyl)-N'- phenylurea) (4-CPPU), and analogs thereof.

In some embodiments, the concentration of TDZ or analog thereof in the bud induction medium is effective to induce shoot buds. In some embodiments, the concentration of TDZ or analog in the bud induction media thereof is about 0.25 mg/L to about 100 mg/L, for example, about 0.5 mg/L to about 2 mg/L. Thus, the concentration of TDZ or analog thereof in the bud induction media can, for example, be about 0.25 mg/L, about 0.3 mg/L, about 0.4 mg/L, about 0.5 mg/L, about 0.6 mg/L, about 0.7 mg/L, about 0.8 mg/L, about 0.9 mg/L, about 1.0 mg/L, about out 1.5 mg/L, about 2.0 mg/L, about 2.5 mg/L, about 3 mg/L, about 4 mg/L, about 5 mg/L, about 6 mg/L, about 7 mg/L, about 8 mg/L, about 9 mg/L, about 10 mg/L, about 15 mg/L, about 20 mg/L, about 25 mg/L, about 30 mg/L, about 40 mg/L,

about 50 mg/L, about 60 mg/L, about 70 mg/L, about 80 mg/L, about 90 mg/L or about 100 mg/L.

In some embodiments, the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is effective to elongate shoots. In some embodiments, the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium from about 0.01 mg/L to about 100 mg/L, for example, from about 0.25 mg/L to about 5 mg/L. Thus, the concentration of one or more cytokinins other than TDZ or analog thereof in the shoot elongation/maintenance media can, for example, be about 0.01 mg/L, about 0.02 mg/L, about 0.03 mg/L, about 0.04 mg/L, about 0.05 mg/L, about 0.06 mg/L, about 0.07 mg/L, about 0.08 mg/L, about 0.09 mg/L, about 0.10 mg/L, about 0.15 mg/L, about 0.20 mg/L, about 0.25 mg/L, about 0.3 mg/L, about 0.35 mg/L, about 0.4 mg/L, about 0.45 mg/L, about 0.5 mg/L, about 0.6 mg/L, about 0.7 mg/L, about 0.8 mg/L, about 0.9 mg/L, about 1.0 mg/L, about 1.5 mg/L, about 2.0 mg/L, about 2.5 mg/L, about 3 mg/L, about 4 mg/L, about 5 mg/L, about 6 mg/L, about 7 mg/L, about 8 mg/L, about 9 mg/L, about 10 mg/L, about 15 mg/L, about 20 mg/L, about 25 mg/L, about 30 mg/L, about 40 mg/L, about 50 mg/L, about 60 mg/L, about 70 mg/L, about 80 mg/L, about 90 mg/L or about 100 mg/L.

In some embodiments, the bud induction medium and/or the shoot elongation/maintenance medium further comprise one or more auxins. In some embodiments, the auxins are selected from the group consisting of β-naphthoxyacetic acid (NAA), 2, 4-Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, and analogs of each thereof. For example, the auxin is NAA or analogs thereof.

In some embodiments, the bud induction medium of step (a) and/or step (c) is a liquid medium. In some embodiments, the bud induction medium of step (a) and/or step (c) is a solid medium. In some embodiments, the shoot elongation/maintenance medium of step (b) and/or step (d) is a liquid medium. In some embodiments, the shoot elongation/maintenance media of step (b) and/or step (d) is a solid media.

In some embodiments the methods of the present invention may involve using a liquid media for one step and a solid media for the next step of a particular cycle. For example, the present invention encompasses methods whereby step (a) is accomplished using liquid media and step (b) is accomplished using solid media. Alternatively, if both steps (a) and (b) and/or steps (c) and (d) are both done using liquid media, then the present invention contemplates that the liquid media may be changed without moving the buds and/or shoots to another

container (e.g., test tube, bioreactor, jar, etc.). For example, if both steps (a) and (b) are accomplished using liquid media in a hydroponic setup, then the buds and/or shoots may remain in their fixed or unfixed position while the liquid media is replaced.

In some embodiments, the incubation of step (a) and/or step (c) lasts for a period that is sufficient to produce more than one shoot bud. For example, the period is set so as to produce at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 26, at least 27, at least 28, at least 30, or more shoot buds for each bamboo tissue culture, explant or seed placed in the bud induction medium of step (a) or (c). In some embodiments, the incubation of step (a) and/or step (c) lasts from about one hour to about three weeks, or more. For example, the incubation of step (a) and/or step (c) lasts from about 24 hours to about 60 hours. Thus, the incubation of step (a) and/or step (c) can last for about 24 hours, about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 35 hours, about 40 hours, about 45 hours, about 50 hours, about 55 hours, about 56 hours, about 57 hours, about 58 hours, about 59, hours, or about 60 hours. In some embodiments the incubation stage of step (a) or step (c) can last longer than 60 hours, e.g., about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, or longer.

In some embodiments, the incubation of step (b) and/or step (d) lasts for any desired period. In some embodiments, the incubation of step (b) and/or step (d) lasts from about 24 hours to about four weeks, or more. For example, the incubation of step (b) and/or step (d) lasts from about three days to about five days, or more. Thus, the incubation of step (b) and/or step (d) can last for about 24 hours, about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 35 hours, about 40 hours, about 45 hours, about 48 hours, about 50 hours, about 55 hours, about 56 hours, about 57 hours, about 58 hours, about 59, hours, about 60 hours, about 72 hours, about 96 hours or about 120 hours. In some embodiments the incubation of step (b) and/or (d) can last longer than 120 hours, e.g., about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, or longer.

In some embodiments, step (e) is repeated at least once, twice, three times, or more.

In some embodiments, steps (a) to (e) take approximately one week, two weeks, three weeks, four weeks, five weeks, six weeks, or more.

In some embodiments, the plant is a dicot species. In some embodiments, the plant is a monocot species. In some embodiments, the plant is a bamboo species. In some embodiments, the bamboo is *Phyllostachys edulisi* 'Moso', *Phyllostachys bissetti*, *Fargesia*

denudata, *Pleioblastus fortunei*, *Sasa Veitchii*, *Pleioblastus viridistriatus*, *Thamnocalamus crassinodus*, *Chusquea Culeo "Cana Prieta"*, *Bambusa Old Hamii*, *Phyllostachys Moso*, *Phyllostachys Atrovaginata*, *Dendrocalamus Asper*, *Guadua Angustifolia*, *Phylostachys Nigra*, *Fargesia rufa*, *Fargesia nitida*, *Borinda Boliana*, *Fargesia murielae*, *Pleioblastus fortune*, *Fargesia robusta*, or *Bambusa Oldhamii*.

In some embodiments, the plant is a non-bamboo species. In some embodiments, the non-bamboo plant species is Geranium spp. (e.g., Geranium rozanne), Hakonechloa macra (e.g., Hakonechloa macra 'Aureola', Hakonechloa macra 'All gold'), Helleborus (e,g., Helleborus 'Ivory Prince'), Phormium, Wasabi (e.g., Wasabi C2), Arundinaria (e.g., Arundinaria gigantean), or Solanum (e.g., Solanum tuberosum and Solanum tuberosum).

In some embodiments, the tissue culture is obtained from shoot clumps maintained on growth media as stock. In some embodiments, the explant is a segment of bamboo cane. In some embodiments, the segment of bamboo cane comprises an internode. In some embodiments, the segment of bamboo cane comprises a nodal section. In some embodiments, the nodal section comprises a single bud. In some embodiments, the bud is dormant or active. In some embodiments, the explant is taken from a plant of about 1 week old, about 2 weeks old, about 3 weeks old, about 1 month old, about 2 month old, about 3 months old, about half year old, about 1 year old, about 2 years old plant, about 3 years old, about 5 years old, or more. In some embodiments, a bamboo seed or a part thereof is used.

In some embodiments, the shoot buds obtained in step (a) and/or step (c) are separated prior to incubating the shoot buds in step (b) and/or step (d). In some embodiments, the separation produces groups of 1 to 3 shoot buds per separation prior to incubating the shoot buds in step (b) and/or step (d).

The present invention also provides alternative media and alternative methods for plant propagation. In some embodiments, the alternative media are selected from Stage 1 media, Stage 2 media, Stage 3 media, Stage 4 media, Stage 5 media, Stage 6 media, Stage 7 media, etc. as described herein.

In some embodiments, at least one Stage 1 medium and at least one Stage 2 medium are used.

In some embodiments, the Stage 1 and Stage 2 media are used sequentially during plant propagation. In some embodiments, the explants remain on the Stage 1 medium for about 1 to about 36 hours (e.g., when spiked media are used). In some embodiments, the explants remain on the Stage 1 medium for 10-120 days (e.g., when standard or reduced

media are used). In some embodiments, the explants stay on Stage 1 medium for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds (e.g., in each round, explants are transferred from an old Stage 1 medium to a fresh Stage 1 medium) before being transferred to the Stage 2 medium. In some embodiments, the explants remain on the Stage 2 medium for about 1 to about 36 hours (e.g., when spiked media are used). In some embodiments, the explants remain the Stage 2 medium for about 10-120 days (e.g., when standard or reduced media are used). In some embodiments, the explants stay on Stage 2 medium for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds (e.g., in each round, explants are transferred from an old Stage 2 medium to a fresh Stage 2 medium).

In some embodiments, the Stage 1 and Stage 2 media are used in rotation during plant propagation. In some embodiments, the rotation is continuous for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cycles. In some embodiments, the explants stay on Stage 1 medium for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds first before being transferred to the Stage 2 medium. In some embodiments, the explants stay on Stage 2 medium for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds first before being transferred back to the Stage 1 medium. In some embodiments, the explants are on the Stage 1 medium for about 1-36 hours (e.g., when spiked media are used) or more (e.g., when standard or reduced media are used) followed by being transferred to the Stage 2 medium. In some embodiments, the rotation is continuous until multiple shoots are observed. In some embodiments, the rotation takes about 1 week to about 24 months, depending on plant species and media.

Optionally, the multiplied shoots are then placed on a Stage 3 medium for further multiplication until desired number of shoots is obtained, depending on previous treatments. The explants on the Stage 3 medium can be further transferred to a Stage 4 medium. In some embodiments, the explants are on the Stage 3 medium for about 1-36 hours (e.g., when spiked media are used) followed by being transferred to the Stage 4 medium. In some embodiments, the explants are on the Stage 3 medium for about 10-120 days or more (e.g., when standard or reduced media are used) followed by being transferred to the Stage 4 medium. In some embodiments, the explants remain the Stage 4 medium for about 10-120 days.

Alternatively, the multiplied shoots obtained from a Stage 2 medium can be rotated between at least one Stage 3 medium and at least one Stage 4 medium. In some embodiments, the rotation is continuous for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cycles. In some embodiments, the rotation is continuous until desired number of shoots. In some

embodiments, the desired number of shoots is obtained by separation into new tubes and further expansion. In some embodiments, about one to ten shoots per tube are obtained per multiplication cycle.

In some embodiments, the explants are placed on a Stage 1 medium, a Stage 2 medium, and a Stage 3 medium in rotation, until desired number of shoots is obtained. In some embodiments, the multiplied shoots are then placed on a Stage 4 medium.

In some embodiments, the explants are placed on a Stage 1 medium and a Stage 2 medium in rotation, until desired number of shoots is obtained. In some embodiments, the multiplied shoots are then placed on a rotation of a Stage 3 medium and a Stage 4 medium.

In some embodiments, the explants are placed on a Stage 1 medium for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more rounds, and then transferred to a Stage 2 medium for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more rounds, until desired number of shoots is obtained. In some embodiments, the multiplied shoots are then placed on a rotation of a Stage 3 medium and a Stage 4 medium.

In some embodiments, the explants are placed on a Stage 1 medium first. In some embodiments, the explants are on the Stage 1 medium for about 1-36 hours (e.g., when spiked media are used). In some embodiments, the explants are on the Stage 1 medium for about 10-120 days or more (e.g., when standard or reduced media are used). In some embodiments, this step comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds of fresh Stage 1 medium. Then, the explants are kept on a rotation of a Stage 2 medium and a Stage 3 medium, until desired number of shoots is obtained. In some embodiments, the multiplied shoots are then placed on a Stage 4 medium. In some embodiments, the multiplied shoots remain on the Stage 4 medium for about 10-120 days.

Still optionally, the multiplied shoots obtained from a Stage 4 medium can be transferred onto a Stage 5 medium as described herein. In some embodiments, the shoots are placed on a Stage 5 medium for about 1-24 hours or more (e.g., when spiked media are used). In some embodiments, the shoots are placed on a Stage 5 medium for about 10 to 120 days (e.g., when standard or reduced media are used). In some embodiments, the shoots are transferred to small tissue culturing boxes, such as the magenta boxes.

Still optionally, the explants are kept on the Stage 5 medium first until the desired number of shoots is obtained, then transferred to a Stage 6 medium as described herein. In some embodiments, the shoots are placed on a Stage 6 medium for about 1-24 hours or more

(e.g., when spiked media are used). In some embodiments, the shoots are placed on a Stage 6 medium for about 10 to 120 days (e.g., when standard or reduced media are used).

In some embodiments, the explants obtained from the Stage 4 medium are placed on a rotation of a Stage 5 medium and a Stage 6 media, until the desired number of shoots is obtained.

Still optionally, the explants kept on the Stage 6 medium are transferred to a Stage 7 medium as described herein. In some embodiments, the shoots are placed on a Stage 7 medium for about 1-24 hours or more (e.g., when spiked media are used). In some embodiments, the shoots are placed on a Stage 7 medium for about 10 to 120 days (e.g., when standard or reduced media are used).

Still optionally, the multiplied shoots obtained from a Stage 4 medium can be transferred onto a rotation of a Stage 4 medium, a Stage 5 medium, and a Stage 6 medium as described herein.

Still optionally, the multiplied shoots obtained from a Stage 7 medium can be transferred one or more other additional media (e.g., a Stage 8, a Stage 9, etc.) for further propagation if needed.

In some embodiments, the alternative media comprise meta-topolin or an analogue thereof. In some embodiments, the alternative media comprise at least two other cytokinins. In some embodiments, the media supports multiplication cycles for a predetermined period of time. In some embodiments, the media support multiplication cycles for at least six months.

In some embodiments, said alternative media comprise at least three cytokinins. In some embodiments, the media supports multiplication cycles for a predetermined period of time. In some embodiments, the media support multiplication cycles for at least six months.

In some embodiments, said alternative media comprise at least one auxin and at least two cytokinins. In some embodiments, the media supports multiplication cycles for a predetermined period of time. In some embodiments, the media support multiplication cycles for at least six months.

In some embodiments, said alternative media comprise at least two auxins and at least two cytokinins. In some embodiments, the media supports multiplication cycles for a predetermined period of time. In some embodiments, the media support multiplication cycles for at least six months.

In some embodiments, said alternative media comprise at least two auxins and at least three cytokinins. In some embodiments, the media supports multiplication cycles for a

predetermined period of time. In some embodiments, the media support multiplication cycles for at least six months.

The present application also provides a system for plant micropropagation. In some embodiments, the system comprises a growth vessel, two or more media containers, and a power source for driving fluid into and/or out of the growth vessel. In some embodiments, the system further comprises a controller. In some embodiments, the system further comprises a light source and/or a gas source providing CO_2, O_2, N_2, or mixture thereof to the growth vessel.

In some embodiments, the system comprises:

a growth vessel for incubating plant tissue in a sterile or substantially sterile environment;

a first media container having a first fluid port and a second fluid port, the first fluid port fluidically coupleable to the growth vessel;

a second media container having a first fluid port and a second fluid port, the first fluid port fluidically coupleable to the growth vessel; and

a gas source fluidically coupleable to the second fluid port of the first media container and second fluid port of the second media container.

In some embodiments, the system further comprises a controller operable in a first operating mode in which pressurized gas is delivered from the gas source to the first media container to displace a first volume of liquid contained therein to the growth vessel, and a second operating mode in which pressurized gas is delivered from the gas source to the second media container to displace a second volume of liquid contained therein to the growth vessel.

In some embodiments, the controller is operable in a third operating mode in which liquid contained in the growth vessel is allowed to flow from the growth vessel into at least one of the first media container and the second media container.

In some embodiments, the controller is operable in a first incubation sequence in which the third operating mode is executed subsequent to the first operating mode.

In some embodiments, the controller is operable in a second incubation sequence in which the third operating mode is executed subsequent to the second operating mode.

In some embodiments, the controller is further operable in a plant propagation mode, in which the first incubation sequence and the second incubation sequence are executed.

In some embodiments, the growth vessel is elevated above the first and second media containers to allow liquid to flow from the growth vessel into at least one of the first media container and the second media container in the third operating mode.

In some embodiments, the system further comprises a manifold fluidically coupleable to the growth vessel, the first media container, and the second media container,

In some embodiments, the manifold is operable to control liquid flow between the growth vessel and the first media container and between the growth vessel and the second media container.

In some embodiments, the growth vessel includes a fluid conduit configured to siphon liquid from the growth vessel to at least one of the first media container and the second media container.

In some embodiments, the growth vessel is an ebb and flow bioreactor.

In some embodiments, the controller is operable to control fluid communication between the growth vessel and the first media container, between the growth vessel and the second media container, between the gas source and the first media container, and between the gas source and the second media container.

In some embodiments, the first media container comprises a bud induction medium as described herein, and the second media container comprises a shoot elongation/maintenance medium. In some embodiments, the bud induction medium comprises an effective amount of thidiazuron (TDZ) or analog thereof, and the shoot elongation/maintenance medium comprises an effective amount of one or more cytokinins other than TDZ or an analog thereof.

The present application also provides methods for exchanging liquid media in a bioreactor/plant growth system for the micropropagation of plant or plant tissue.

In some embodiments, the bioreactor comprising a growth vessel for incubating the plant tissue, a first media container fluidically coupleable to the growth vessel, a second media container fluidically coupleable to the growth vessel, and a gas source fluid fluidically coupleable to the first media container and the second media container

In some embodiments, the methods comprise:

establishing fluid communication between the first media container and the growth vessel;

fluidically isolating the second media container from the growth vessel;

establishing fluid communication between the gas source and the first media container;

delivering compressed gas to the first media container to displace a first volume of liquid from the first media container to the growth vessel;

allowing at least a portion of the first volume of liquid to flow from the growth vessel back into the first media container;

establishing fluid communication between the second media container and the growth vessel;

fluidically isolating the first media container from the growth vessel;

establishing fluid communication between the gas source and the second media container;

delivering compressed gas to the second media container to displace a second volume of liquid from the first media container to the growth vessel; and

allowing at least a portion of the second volume of liquid to flow from the growth vessel back into the second media container.

In some embodiments, the compressed gas is delivered to the first media container for approximately one minute.

In some embodiments, the compressed gas is delivered to the second media container for approximately one minute.

In some embodiments, the liquid is allowed to flow from the growth vessel back into the first media container for approximately 8 minutes.

In some embodiments, the liquid is allowed to flow from the growth vessel back into the second media container for approximately 8 minutes.

In some embodiments, said systems are used for plant propagation comprising a bud induction medium and a shoot elongation and maintenance medium. For example, the systems are used for plant propagations wherein a rotation of a bud induction medium and a shoot elongation and maintenance medium is involved.

In some embodiments, said systems are used for plant propagation comprising the alternative media as described herein. For example, the systems are used for plant propagations wherein (1) a rotation of a Stage 1 medium and a Stage 2 medium is involved; (2) a rotation of a Stage 2 medium and a Stage 3 medium is involved; (3) a rotation of a Stage 1 medium, a Stage 2 medium, and a Stage 3 medium is involved; (4) a rotation of a Stage 3 medium and a Stage 4 medium is involved; (5) a rotation of a Stage 4 medium and a Stage 5 medium is involved; (6) a rotation of a Stage 5 medium and a Stage 6 medium is involved; (7)

a rotation of a Stage 4 medium, a Stage 5 medium, and a Stage 6 medium is involved; and/or (7) a rotation of a Stage 6 medium and a Stage 7 medium is involved.

Also provided are devices and methods for intermittently exposing microenvironments of tissue culture plantlets to a liquid nutrient solution are described herein. In some embodiments, an apparatus includes a frame, a shelf assembly, and a drive assembly. In such embodiments, the drive assembly can be configured to engage the shelf assembly to facilitate an oscillating motion of the shelf assembly relative to the frame such that tissue culture plantlets are intermittently exposed to the liquid nutrient solution. In some embodiments, described herein relate generally to a rack and more particularly, to an oscillating rack for plant propagation vessels.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of a system of the invention.

FIG. 2 is a schematic illustration of a non-limiting embodiment of the system of FIG.1.

FIG. 3 is a schematic illustration of a media container of the system of FIG.2

FIG. 4 is a schematic illustration of a manifold of the system of FIG. 2.

FIG. 5A is a front view of a growth vessel of the system of FIG. 2.

FIG. 5B is a side view of the growth vessel of FIG. 5A.

FIG. 5C is a top view of the growth vessel of FIG. 5A.

FIG. 6 is a flowchart of a plant propagation sequence of the invention.

FIG. 7 is a table showing medium recipes of B-11, DIC 25/30 ST, DIC 25/30 DT, DKW, Wasabi, DIC 25/30, Lilly light, and Lilly dark. All components are represented in milligrams per/L except sugar which is represented in grams per/L.

FIG. 8 is a front perspective view of an oscillating rack, according to an embodiment.

FIG. 9 is a side perspective view of the oscillating rack of FIG. 8.

FIG. 10 is an enlarged exploded view of a portion of the oscillating rack labeled as Region Z in FIG. 9.

FIG.11 is a cross-sectional view of an upright included in the oscillating rack of FIG. 8, taken along line 4-4 in FIG. 8.

FIG.12 is a perspective view of a shelf assembly included in the oscillating rack of FIG. 8.

FIG. 13 is a perspective view of a portion of the shelf assembly of FIG. 12.

FIG. 14 is a cross-sectional view of a platform included in the portion of the shelf assembly of FIG. 6, taken along line 7-7 in FIG. 13.

FIG. 15 is a perspective view of bushings included in the shelf assembly of FIG. 11.

FIG. 16 is an exploded view of a drive assembly included in the oscillating rack of FIG. 8.

FIG. 17 is a side view of a portion of the oscillating rack of FIG. 8, in a first configuration.

FIG. 18 is a side view of the portion of the oscillating rack of FIG. 8, in a second configuration.

FIG. 19 is a side view of the portion of the oscillating rack of FIG. 8, in a third configuration.

DETAILED DESCRIPTION

More rapidly growing plants are available for cultivation and industrial uses, including plants in the subfamily Bambusoidea. The subfamily Bambusoideae (of the family Poaceae), comprises both woody and herbaceous bamboos. At present roughly 120 genera of temperate and tropical woody bamboos are recognized. Bamboos are versatile plants with many different applications. It has been estimated that approximately 2.2 billion people worldwide use bamboo to some extent, and in 1985 the global revenue attributable to bamboo was estimated around U.S. $4.5 billion. The market for bamboo is also expanding. Bamboo shoots are a staple of Asian cuisine, and bamboo is found in a number of products including toothpicks, brooms, poles for viticulture and arboriculture, landscaping materials, parquet flooring, laminate materials, furniture, handicrafts and other household items. In addition, bamboo is becoming an important source of textile material as a component of paper production and as a source of structural timber.

Plant species such as bamboo are considered environmentally friendly "green" products, which have extremely rapid growth rates. Despite rapid growth rates, other characteristics of these plants make it difficult to rapidly propagate these plants at an industrial scale. For example, many commercially important bamboos only flower at intervals of as long as 60-130 years. Compounding the difficulties of this long flowering cycle is the fact that many bamboos exhibit mass (or gregarious) flowering, with all plants in the population flowering simultaneously. For example, *Phyllostachys bambusoides* flowers at an

interval of 130 years, and in this species all plants of the same stock flower at the same time, regardless of differences in geographic locations or climatic conditions. After flowering, the bamboo dies. Bamboo's lengthy flowering interval and propensity for mass flowering makes it very difficult to obtain seeds for propagation. Compounding this problem is the fact that bamboo seeds, even when they are available, remain viable for no more than 3-6 months.

As a result of these difficulties with the propagation of bamboo and other fast growing plant species using traditional sexual reproduction, these plants are often propagated by asexual techniques such as clump division and cutting. These asexual propagation techniques, however, are insufficient to meet projected world demand because both their capacity to produce mass scale production, and their practical efficiency, are too low. In addition many asexual propagation methods have the downside of failing to eliminate pathogens present in the parent plants. Therefore, compositions, methods, and systems to achieve large scale production of plants are highly desirable. Micropropagation (also known as tissue culturing with the terms used interchangeably herein), is an excellent potential method that could be used to achieve this aim.

Micropropagation is not unlike growing plants from cuttings. However, unlike plants grown from cuttings, micropropagated plants are grown *in vitro* in sterile media. Typically, the growth media comprises a solid or semi-solid material such as agar, with the addition of various compounds such as nutrients, inorganic salts, growth regulators, sugars, vitamins and other compounds.

A benefit of tissue culturing plants is that the plants can be grown in a sterile environment so that they may more likely remain disease free. Other benefits include the ability to grow very large numbers of plants in a small space, the reduced water and nutrient needs of micropropagated plants, and the rapid multiplication of tissues that can in turn be used to yield more tissue culture material. Moreover micropropagation is very flexible and rapid upscaling is possible (within 1 year nearly one million plants can be produced from a single genotype). Such short time frames and large numbers cannot be rivaled by any conventional method. Tissue culturing also provides for the production of high quality plants which are easy to transport and deliver.

Some papers have published which address tissue culturing of plants. In practice, however (i.e., for large or mass scale propagation of bamboos), the compositions, methods, and systems described in these papers do not translate into commercially viable propagation systems.

The difficulties encountered in tissue culturing of plant species include high incidences of endogenous or surface contaminations and browning, factors related to dormancy or topophysis and hyperhydricity. The present disclosure provides compositions, methods, and systems that overcome these difficulties allowing the commercial-scale asexual production of plants.

Micropropagation in liquid culture media increases nutrient uptake and promotes growth. However, the advantages of *in vitro* culture in a liquid media are often counterbalanced by technical problems such as asphyxia, hyperhydricity, shear forces and the need for complex equipment.

International Patent Application Publication No. WO/2011/100762, which is incorporated herein by reference in its entirety, describes compositions and methods that are useful for bamboo *in vitro* propagation.

The present application discloses novel compositions, methods, and systems for the rapid *in vitro* propagation of plants. The present invention provides compositions and methods that can significantly increase plant tissue culture multiplication rate within a shorter time. In some embodiments, a strong cytokinin such as thidiazuron is utilized for a very brief period of time in either a solid or liquid induction medium to induce multiple shoot bud formation in explants of plant species. This bud induction treatment utilizing a media containing a strong cytokinin such as thidiazuron is followed by a shoot elongation and maintenance treatment whereby a relatively weaker cytokinin such as BAP, meta-topolin, 2ip, zeatin and or zeatin riboside is used to accomplish the shoot elongation and maintenance of the culture. This process, when alternated methodically resulted in culture multiplication rates between 2X and 28X within a 3-week culture cycle.

Definitions

The present invention provides compositions, methods, and systems for plant propagation. In some embodiments, the compositions comprise at least one plant hormone. As used herein, the verb "comprise" as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

In some embodiments, the compositions, methods, and systems are useful for plant in intro propagation. As used herein, the term "plant" refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom). This includes familiar

organisms such as but not limited to trees, herbs, bushes, grasses, vines, ferns, mosses and green algae. The term refers to both monocotyledonous plants, also called monocots, and dicotyledonous plants, also called dicots. Examples of particular plants include but are not limited to bamboo, corn, potatoes, roses, apple trees, sunflowers, wheat, rice, bananas, tomatoes, opo, pumpkins, squash, lettuce, cabbage, oak trees, guzmania, geraniums, hibiscus, clematis, poinsettias, sugarcane, taro, duck weed, pine trees, Kentucky blue grass, zoysia, coconut trees, brassica leafy vegetables (e.g. broccoli, broccoli raab, Brussels sprouts, cabbage, Chinese cabbage (Bok Choy and Napa), cauliflower, cavalo, collards, kale, kohlrabi, mustard greens, rape greens, and other brassica leafy vegetable crops), bulb vegetables (e.g. garlic, leek, onion (dry bulb, green, and Welch), shallot, and other bulb vegetable crops), citrus fruits (e.g. grapefruit, lemon, lime, orange, tangerine, citrus hybrids, pummelo, and other citrus fruit crops), cucurbit vegetables (e.g. cucumber, citron melon, edible gourds, gherkin, muskmelons (including hybrids and/or cultivars of cucumis melons), water-melon, cantaloupe, and other cucurbit vegetable crops), fruiting vegetables (including eggplant, ground cherry, pepino, pepper, tomato, tomatillo, and other fruiting vegetable crops), grape, leafy vegetables (e.g. romaine), root/tuber and corm vegetables (e.g. potato), and tree nuts (almond, pecan, pistachio, and walnut), berries (e.g., tomatoes, barberries, currants, elderberries, gooseberries, honeysuckles, mayapples, nannyberries, Oregon-grapes, see-buckthorns, hackberries, bearberries, lingonberries, strawberries, sea grapes, lackberries, cloudberries, loganberries, raspberries, salmonberries, thimbleberries, and wineberries), cereal crops (e.g., corn, rice, wheat, barley, sorghum, millets, oats, ryes, triticales, buckwheats, fonio, and quinoa), pome fruit (e.g., apples, pears), stone fruits (e.g., coffees, jujubes, mangos, olives, coconuts, oil palms, pistachios, almonds, apricots, cherries, damsons, nectarines, peaches and plums), vine (e.g., table grapes, wine grapes), fibber crops (e.g. hemp, cotton), ornamentals, and the like. For example, the plant is a species in the tribe of Camelineae, such as *C. alyssum*, *C. anomala*, *C. grandiflora*, *C. hispida*, *C. laxa*, *C. microcarpa*, *C. microphylla*, *C. persistens*, *C. rumelica*, *C. sativa*, *C. Stiefelhagenii*, or any hybrid thereof.

In some embodiments, the compositions, methods, and systems are useful for crop plant *in vitro* propagation. As used herein, the term "crop plant" refers to any plant grown for any commercial purpose, including, but not limited to the following purposes: seed production, hay production, ornamental use, fruit production, berry production, vegetable production, oil production, protein production, forage production, animal grazing, golf

courses, lawns, flower production, landscaping, erosion control, green manure, improving soil tilth/health, producing pharmaceutical products/drugs, producing food or food additives, smoking products, pulp production and wood production.

In some embodiments, the present invention provides plant parts derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "plant part" refers to any part of a plant including but not limited to the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, and the like. The two main parts of plants grown in some sort of media, such as soil, are often referred to as the "above-ground" part, also often referred to as the "shoots", and the "below-ground" part, also often referred to as the "roots". As used herein, the phrase "derived from" refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid or an amino acid derived from an origin or source may have all kinds of nucleotide changes or protein modification as defined elsewhere herein. As used herein, the term "a" or "an" refers to one or more of that entity; for example, "a gene" refers to one or more genes or at least one gene. As such, the terms "a" (or "an"), "one or more" and "at least one" are used interchangeably herein. In addition, reference to "an element" by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements.

In some embodiments, the present invention provides plant tissue derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "plant tissue" refers to any part of a plant. Examples of plant organs include, but are not limited to the leaf, stem, root, tuber, seed, branch, pubescence, nodule, leaf axil, flower, pollen, stamen, pistil, petal, peduncle, stalk, stigma, style, bract, fruit, trunk, carpel, sepal, anther, ovule, pedicel, needle, cone, rhizome, stolon, shoot, pericarp, endosperm, placenta, berry, stamen, and leaf sheath.

In some embodiments, the compositions, methods, and systems are useful for monocotyledon plants propagation. As used herein, the term "monocotyledon" or "monocot" refer to any of a subclass (Monocotyledoneae) of flowering plants having an embryo containing only one seed leaf and usually having parallel-veined leaves, flower parts in multiples of three, and no secondary growth in stems and roots. Examples include lilies;

orchids; rice; corn, grasses, such as tall fescue, goat grass, and Kentucky bluegrass; grains, such as wheat, oats and barley; irises; onions and palms.

In some embodiments, the compositions, methods, and systems are useful for bamboo plant *in vitro* propagation. As used herein, the term "bamboo" refers to plants in the subfamily of Bambusoideae. Representative genera of bamboo are described in International Patent Application Publication No. WO2011100762, which is incorporated herein by reference in its entirety.

In some embodiments, the present invention provides plants comprising the germplasm of the plants produced by the compositions, methods, and systems described herein. As used herein, the term "germplasm" refers to the genetic material with its specific molecular and chemical makeup that comprises the physical foundation of the hereditary qualities of an organism.

In some embodiments, the present invention provides offsprings derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "offspring" refers to any plant resulting as progeny from a vegetative or sexual reproduction from one or more parent plants or descendants thereof. For instance an offspring plant may be obtained by cloning or selfing of a parent plant or by crossing two parent plants and include selfings as well as the F1 or F2 or still further generations. An F1 is a first-generation offspring produced from parents at least one of which is used for the first time as donor of a trait, while offspring of second generation (F2) or subsequent generations (F3, F4, etc.) are specimens produced from selfings of F1's, F2's etc. An F1 may thus be (and usually is) a hybrid resulting from a cross between two true breeding parents (true-breeding is homozygous for a trait), while an F2 may be (and usually is) an offspring resulting from self-pollination of said F1 hybrids.

In some embodiments, the present invention provides crossings derived from the plants produced by the compositions, methods, and systems described herein, and methods of making and using such crossings. As used herein, the term "cross", "crossing", "cross pollination" or "cross-breeding" refer to the process by which the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of a flower on another plant.

In some embodiments, the present invention provides cultivars derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "cultivar" refers to a variety, strain or race of plant that has been produced by horticultural or agronomic techniques and is not normally found in wild populations.

In some embodiments, the present invention provides varieties derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "variety" refers to a subdivision of a species, consisting of a group of individuals within the species that are distinct in form or function from other similar arrays of individuals.

As used herein, the term "variety" or "cultivar" means a group of similar plants that by structural features and performance can be identified from other varieties within the same species. The term "variety" as used herein has identical meaning to the corresponding definition in the International Convention for the Protection of New Varieties of Plants (UPOV treaty), of Dec. 2, 1961, as Revised at Geneva on Nov. 10, 1972, on Oct. 23, 1978, and on Mar. 19, 1991. Thus, "variety" means a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether the conditions for the grant of a breeder's right are fully met, can be i) defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, ii) distinguished from any other plant grouping by the expression of at least one of the said characteristics and iii) considered as a unit with regard to its suitability for being propagated unchanged.

In some embodiments, the present invention provides genotypes derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "genotype" refers to the genetic makeup of an individual cell, cell culture, tissue, organism (e.g., a plant), or group of organisms.

In some embodiments, the present invention provides clones derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "clone" refers to a cell, group of cells, a part, tissue, organism (e.g., a plant), or group of organisms that is descended or derived from and genetically identical or substantially identical to a single precursor. In some embodiments, the clone is produced in a process comprising at least one asexual step.

In some embodiments, the present invention provides hybrids derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "hybrid" refers to any individual cell, tissue or plant resulting from a cross between parents that differ in one or more genes.

In some embodiments, the present invention provides inbreds derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "inbred" or "inbred line" refers to a relatively true-breeding strain.

In some embodiments, the present invention provides populations derived from the plants produced by the compositions, methods, and systems described herein. As used herein, the term "population" means a genetically homogeneous or heterogeneous collection of plants sharing a common genetic derivation.

In some embodiments, the present invention provides bioreactors for bioculture of plant species. As used herein, the term "bioreactor" refers to any vessel, device or system capable of holding, supporting and/or growing viable tissue. In other words, the term "bioreactor" as used herein may refer to a growth vessel that holds viable plant tissue, various other components internal or external to the growth vessel that are required for or aid the holding, supporting and/or growing of viable plant tissue, and any subsystem thereof.

In some embodiments, the present invention provides ebb and flow bioreactors for bioculture of plant species. As used herein, the term "ebb and flow bioreactor" refers to any bioreactor designed to nourish the viable tissue by cyclical inflow and outflow of nutrient media to and from the bioreactor, respectively. Ebb and flow bioreactors are frequently employed in hydroponics. As used herein, a "plant propagation system" is a bioreactor for growing viable plant tissue.

Compositions

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction.

Micropropagation can first begin with the selection of plant material to be propagated. Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants. Once the plant material is chosen for culture, the collection of explant(s) begins and is dependent on the type of tissue to be used, including stem tips, anthers, petals, pollen and others plant tissues. The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones). Usually the medium is thickened with agar to create a gel which supports

the explant during growth. Some plants are easily grown on simple media but others require more complicated media for successful growth; the plant tissue grows and differentiates into new tissues depending on the medium. For example, media containing cytokinins are used to create branched shoots from plant buds.

Multiplication is the taking of tissue samples produced during the first stage and increasing their number. Following the successful introduction and growth of plant tissue, the establishment stage is followed by multiplication. Through repeated cycles of this process, a single explant sample may be increased from one to hundreds or thousands of plants. Depending on the type of tissue grown, multiplication can involve different methods and media. If the plant material grown is callus tissue, it can be placed in a blender and cut into smaller pieces and recultured on the same type of culture medium to grow more callus tissue. If the tissue is grown as small plants called plantlets, hormones are often added that cause the plantlets to produce many small offshoots that can be removed and recultured.

The next stage ("pretransplant" stage) involves treating the plantlets/shoots produced to encourage root growth and "hardening." It is performed *in vitro* , or in a sterile or substantially sterile environment. "Hardening" refers to the preparation of the plants for a natural growth environment. Until this stage, the plantlets have been grown in "ideal" conditions, designed to encourage rapid growth. Due to lack of necessity, the plants are likely to be highly susceptible to disease and often do not have fully functional dermal coverings and will be inefficient in their use of water and energy. *In vitro* conditions are high in humidity and plants grown under these conditions do not form a working cuticle and stomata that keep the plant from drying out, when taken out of culture the plantlets need time to adjust to more natural environmental conditions. Hardening typically involves slowly weaning the plantlets from a high-humidity, low light, warm environment to what would be considered a normal growth environment for the species in question.

In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or (more commonly) potting compost for continued growth by conventional methods. This stage is often combined with the "pretransplant" stage.

Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol or bleach) is required. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly

into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganized growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces to culture and which to discard. As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.

The tissue obtained from the plant to culture is called an explant. Based on work with certain model systems, particularly tobacco, it has often been claimed that a totipotent explant can be grown from any part of the plant. However, this concept has been vitiated in practice. In many species explants of various organs vary in their rates of growth and regeneration, while some do not grow at all. The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also the risk of microbial contamination is increased with inappropriate explants. Thus it is very important that an appropriate choice of explant be made prior to tissue culture.

The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth regulating substances including auxins and cytokinins. Some explants, like the root tip, are hard to isolate and are contaminated with soil microflora that become problematic during the tissue culture process. Certain soil microflora can form tight associations with the root systems, or

even grow within the root. Soil particles bound to roots are difficult to remove without injury to the roots that then allows microbial attack. These associated microflora will generally overgrow the tissue culture medium before there is significant growth of plant tissue. Aerial (above soil) explants are also rich in undesirable microflora. However, they are more easily removed from the explant by gentle rinsing, and the remainder usually can be killed by surface sterilization. Most of the surface microflora do not form tight associations with the plant tissue. Such associations can usually be found by visual inspection as a mosaic, de-colorization or localized necrosis on the surface of the explant.

An alternative for obtaining uncontaminated explants is to take explants from seedlings which are aseptically grown from surface-sterilized seeds. The hard surface of the seed is less permeable to penetration of harsh surface sterilizing agents, such as hypochlorite, so the acceptable conditions of sterilization used for seeds can be much more stringent than for vegetative tissues.

Tissue cultured plants are clones, if the original mother plant used to produce the first explants is susceptible to a pathogen or environmental condition, the entire crop would be susceptible to the same problem, and conversely any positive traits would remain within the line also. Plant tissue culture is used widely in plant science; it also has a number of commercial applications. Applications include:

1. Micropropagation is widely used in forestry and in floriculture. Micropropagation can also be used to conserve rare or endangered plant species.
2. A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. pathogen resistance/tolerance.
3. Large-scale growth of plant cells in liquid culture inside bioreactors as a source of secondary products, like recombinant proteins used as biopharmaceuticals.
4. To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
5. To cross-pollinate distantly related species and then tissue culture the resulting embryo, which would otherwise normally die (Embryo Rescue).
6. For production of doubled monoploid (dihaploid) plants from haploid cultures to achieve homozygous lines more rapidly in breeding programs, usually by treatment with colchicine which causes doubling of the chromosome number.
7. As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.

8. Certain techniques such as meristem tip culture can be used to produce clean plant material from infected stock, such as potatoes and many species of soft fruit.

9. Micropropagation using meristem and shoot culture to produce large numbers of identical individuals.

Medium and methods used for plant micropropagation have been described at least in M. R. Ahuja, *Micropropagation of woody plants*, Springer, 1993, ISBN 0792318072, 9780792318071; Narayanaswamy, *Plant cell and tissue culture*, Tata McGraw-Hill Education, 1994, ISBN 0074602772, 9780074602775; Singh and Kumar, *Plant Tissue Culture*, APH Publishing, 2009, ISBN 8131304396, 9788131304396; Y.P.S. Bajaj *High-tech and micropropagation V*, Springer, 1997, ISBN 3540616063, 9783540616061; Trigiano and Gray, *Plant Tissue Culture, Development and Biotechnology*, CRC Press, 2010, ISBN 1420083260, 9781420083262; Gupta and Ibaraki, *Plant tissue culture engineering Volume 6 of Focus on biotechnology*, Springer, 2006, ISBN 1402035942, 9781402035944; Jain and Ishii, *Micropropagation of woody trees and fruits Volume 75 of Forestry sciences,* Springer, 2003, ISBN 1402011350, 9781402011351; and Aitken-Christie et al., *Automation and environmental control in plant tissue culture*, Springer, 1995, ISBN 0792328418, 9780792328414, each of which is incorporated herein by reference in its entirety.

Medium and methods for bamboo micropropagation have been described in International Patent Application Publication No. WO2011100762, which is incorporated herein by reference in its entirety.

The present invention provides media that can be used for *in vitro* micropropagation of plants, such as bamboo plants. The media can be liquid, semi-solid, or solid, and the physical state of the media can be varied by the incorporation of one or more gelling agents. Any gelling agent known in the art that is suitable for use in plant tissue culture media can be used. Agar is most commonly used for this purpose. Examples of such agars include Agar Type A, E or M and Bacto™Agar. Other exemplary gelling agents include carrageenan, gellan gum (commercially available as PhytaGel™, Gelrite® and Gelzan™), alginic acid and its salts, and agarose. Blends of these agents, such as two or more of agar, carrageenan, gellan gum, agarose and alginic acid or a salt thereof also can be used. Typically, the media comprises agar, with the addition of various compounds such as nutrients, inorganic salts, growth regulators, carbon source, vitamins and other compounds.

In some embodiments, the media comprise one or more minimum nutrition necessary for plant growth, such as amino acids, macroelements, microelements, aluminum, boron,

chlorine (chloride), chromium, cobalt, copper, iodine, iron, lead, magnesium, manganese, molybdenum, nitrogen (nitrates), potassium, phosphorous (phosphates), silicon, sodium, sulphur (sulphates), titanium, vanadium, zinc, inositol and undefined media components such as casein hydrolysates or yeast extracts. For example, the media can include any combination of NH_4NO_3; KNO_3; $Ca(NO_3)_2$; K_2SO_4; $MgSO_4$; $MnSO_4$; $ZnSO_4$; K_2SO_5; $CuSO_4$; $CaCl_2$; KI; $CoCl_2$; H_3BO_3; Na_2MoO_4; KH_2PO_4; $FeSO_4$; Na_2EDTA; Na_2H_2PO4; inositol (e.g., myo-inositol); thiamine; pyridoxine; nicotinic acid; glycine; riboflavin; ascorbic acid; and silicon standard solution. The media may further comprise other nutrition components which have been described, for example, in International Patent Application Publication No. WO2011100762. It is known to those in the art that one or more components mentioned above can be omitted without affecting the function of the media.

The media can comprise one or more carbon source, such as a sugar. Non-limiting exemplary sugars include sucrose, glucose, maltose, galactose and sorbitol or combinations thereof.

Exemplary concentrations of the components described above are shown in Table 1. The concentrations of these components can be adjusted based on plant species, tissue type, and purposes, etc, without substantially affecting the media function.

Table 1. Exemplary Concentrations

Component	**Concentrations (mg/L in all unless otherwise noted)**
NH_4NO_3	about 800-about 2500
KNO_3	about 900-about 3000
$Ca(NO_3)_2$	0-about 800
K_2SO_4	0-about 800
$MgSO_4$	about 150-about 550
$MnSO_4$	about 8.0-about 26.0
$ZnSO_4$	about 4.0-about 12.0
$CuSO_4$	about 0.010-about 0.4
$CaCl_2$	about 200-about 660
KI	about 0.4-about 1.5
$CoCl_2$	about 0.010-about 0.4
H_3BO_3	about 3.0-about 9.0
Na_2MoO_4	about 0.10-about 0.4
KH_2PO_4	about 80-about 250
K_2SO_5	about 0 - about 1500
$FeSO_4$	about 25-about 90
Na_2EDTA	about 35-about 120
$Na_2H_2PO_4$	about 80-250
myo-Inositol	about 50-about 150
Thiamine	about 0.2-about 20

Component	Concentrations (mg/L in all unless otherwise noted)
Pyridoxine	about 0 - about 5
Nicotinic acid	about 0 - about 5
Glycine	about 0 - about 5
Riboflavin	about 0 - about 5
Ascorbic Acid	about 0 - about 5
Sugar	about 15 g/L -about 45 g/L
Gelling agent*	about 2.5 g/L -about 8.0 g/L
*The amount of gelling agent may vary depending on the type of the agent, and the type of the media (e.g., semi-solid or solid media)	

The media further comprise one or more effective amount of plant growth regulators. Examples of plant growth regulators include plant hormones, such as auxins and compounds with auxin-like activity, cytokinins and compounds with cytokinin-like activity. The term "cytokinin" refers to a class of plant growth regulators that are characterized by their ability to stimulate cell division and shoot organogenesis in tissue culture. Non-limiting examples of cytokinins include, N^6- benzylaminopurine (BAP) (a.k.a. N^6-benzyladenine (BA)), meta-topolin, zeatin, kinetin, thiadiazuron (TDZ), meta-topolin, 2-isopentenyladenine (a.k.a., 6-γ-γ-(dimethylallylamino)-purine or 2ip), adenine hemisulfate, dimethylallyladenine, 4-CPPU (N-(2-chloro-4-pyridyl)-N'- phenylurea)), and analogs thereof. The term "auxin" refers to a class of plant growth regulators that are characterized principally by their capacity to stimulate cell division in excised plant tissues. In addition to their role in cell division and cell elongation, auxins influence other developmental processes, including root initiation. Non-limiting examples of β-naphthoxyacetic acid (NAA), 2,4- Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, and analogs thereof. More cytokinins and auxins are described in WO2011100762, US5211738, US20100240537, US20060084577, US20030158043, and Aremu et al. (*Plant Cell Tiss. Organ. Cult.*, DOI 10.1007/s11240-011-0007-7, 2011), which are incorporated by reference in their entireties.

In some embodiments, other plant hormones such as abscisic acid, gibberellic acid, analogs thereof, or combination thereof also can be included in the media. In some embodiments, active charcoal can be added in the media to improve cell growth and development.

If present in a media, each cytokinin can be present in an amount from about 0.001 mg/L-about 100 mg/L and all amounts in between. For example, the concentration of a cytokinin is about 0.001, 0.01, 0.1, 1, 2, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2,

4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, mg/L or more.

In some embodiments, the media comprise meta-topolin and/or its analogues. In some embodiments, meta-topolin is present in an amount equal to or greater than 1.5 mg/L, equal to or greater than 2.0 mg/L, equal to or greater than 2.5 mg/L, equal to or greater than 3.0 mg/L, equal to or greater than 3.5 mg/L, equal to or greater than 4.5 mg/L or equal to or greater than 5.0 mg/L. In other embodiments, meta-topolin is present in an amount of 3.2 mg/L or 5.36 mg/L. In another embodiment, the amount of meta-topolin cannot be less than 1.5 mg/L, cannot be less than 2.0 mg/L, cannot be less than 2.5 mg/L, cannot be less than 3.0 mg/L, cannot be less than 3.5 mg/L, cannot be less than 4.5 mg/L or cannot be less than 5.0 mg/L. In some embodiments, meta-topolin and/or its analogues can be included in any amount up to 200 mg/L. In some embodiments, the media is used for bamboo micropropagation. In some embodiments, the bamboo plant is selected from the species consisting of Arundinaria, Bambusa, Borinda, chusquea, Dendrocalamus, fargesia, Guadua, Phyllostachys, Pleioblastus and Thamnocalamus.

In some embodiments, the media comprise thidiazuron and/or its analogues. In some embodiments, thidiazuron and/or its analogues can be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, thidiazuron and/or its analogues can also be included in any amount up to 200 mg/L.

If present in a media, each auxin can be present in an amount from about 0.001 mg/L-about 100 mg/L and all amounts in between. For example, the concentration of an auxin is about 0.001, 0.01, 0.1, 1, 2, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,

20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or about 100 mg/L.

In some embodiments, the media comprise NAA and/or its analogues. In some embodiments, NAA and/or its analogues can be present at 0.001 mg/L, 0.01, 0.0125, 0.015, 0.0175, 0.02, 0.0225, 0.025, 0.0275, 0.03, 0.0325, 0.035, 0.0375, 0.04, 0.0425, 0.045, 0.0475, 0.05, 0.0525, 0.055, 0.0575, 0.06, 0.0625, 0.065, 0.0675, 0.07, 0.0725, 0.075, 0.0775, 0.08, 0.0825, 0.085, 0.0875, 0.09, 0.0925, 0.095, 0.0957, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, NAA and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, the media comprise IBA and/or its analogues. In some embodiments, IBA and/or its analogues an be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.075, 0.08, 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, IBA and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, the media comprise BAP and/or its analogues. In some embodiments, BAP and/or its analogues an be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.06, 0.07, 0.0725, 0.075, 0.0775, 0.08, 0.0825, 0.085, 0.0875, 0.09, 0.0925, 0.095, 0.0975, 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73,

74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, BAP and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, the media comprise 2ip and/or its analogues. In some embodiments, 2ip and/or its analogues an be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.075, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4,0, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, 2ip and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, the media comprise DPU and/or its analogues. In some embodiments, DPU and/or its analogues an be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, DPU and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, the media comprise CPPU and/or its analogues. In some embodiments, CPPU and/or its analogues an be present at 0.001 mg/L, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.5, 2.75, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/L. In some embodiments, CPPU and/or its analogues can be included any amount up to 200 mg/L.

In some embodiments, one or more cytokinins in combination with one or more other cytokinins or auxins, and auxins in combination with other auxins or cytokinins can also be utilized in ratios. For example, in some embodiments, any two cytokinins and/or auxins in

pairs disclosed herein can be included in the following exemplary ratios: 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1; 9:1, 8:1, 7:1, 6.9:1, 6.8:1, 6.7:1, 6.6:1, 6.5:1, 6.4: 1, 6.3:1, 6.2:1, 6.1:1, 6:1, 5.9:1, 5.8:1, 5.7:1, 5.6:1, 5.5:1, 5.4:1, 5.3:1, 5.2:1, 5.1:1, 5:1; 4:1, 3:1, 2:1, 1:1, 0.75:1, 0.5:1, 0.25:1, 0.1:1, 0.075:1, 0.05:1, 0.025:1 or 0.001:1. These ratios can also be utilized between meta-topolin (and analogues) with thidiazuron (and analogues), with NAA (and analogues), with BAP (and analogues), with IBA (and analogues), with 2ip (and analogues), with DPU (and analogues) and/or with CPPU (and analogues). Similarly, the ratios can be utilized between thidiazuron (and analogues) with NAA (and analogues), with BAP (and analogues), with IBA (and analogues), with 2ip (and analogues), with DPU (and analogues) and/or with CPPU (and analogues). The ratios can also be utilized between NAA (and analogues) with BAP (and analogues), with IBA (and analogues), with 2ip (and analogues), with DPU (and analogues) and/or with CPPU (and analogues). The ratios can also be utilized between BAP (and analogues) with IBA (and analogues), with 2ip (and analogues), with DPU (and analogues) and/or with CPPU (and analogues). In short, each of the cytokinins and/or auxins or its analogues can be included with a second cytokinin and/or auxin disclosed herein according to any of the disclosed ratios.

In some embodiments, the present invention provides at least two types of media that are used in *in vitro* micropropagation. The first media, referred herein as the "bud induction media", comprises at least one strong cytokinin, such as a thidiazuron, or analogs thereof. The second media, refereed herein as the "shoot elongation/maintenance media", comprises one or more cytokinins other than the cytokinin in the bud induction media. For example, the cytokinins are selected from meta-topolin, kinetin, isopentenyl adenine (iP), zeatin, trans-zeatin, zeatin riboside, dihydrozeatin, benzyleadenin (BAP), benzyladenosine ([9R]BAP), analogs thereof, or combination thereof. Other cytokinins available for use in tissue culture can also be substituted for the above cytokinins to achieve similar effects.

Both the bud induction media and the shoot elongation/maintenance media comprise components of a minimum media for plant tissue culture, such as carbon source and salts. In some embodiments, both media can comprise one or more components selected from NH_4NO_3, KNO_3, $Ca(NO_3)_2$, K_2SO_4, $MgSO_4$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, K_2SO_5, $CaCl_2$, Kl, $CoCl_2$, H_3BO_3, Na_2MoO_4, KH_2PO_4, $FeSO_4$, Na_2EDTA, $Na_2H_2PO_4$, Glycine, myo-Inositol, Thiamine, Pyridoxine, Nicotinic acid, and Riboflavin.

In some embodiments, the bud induction media comprises only one strong cytokinin, for example, thidiazuron (TDZ), or an analog thereof. In some embodiments, the bud induction media comprises one additional cytokinin. In some embodiments, the bud induction media further comprises one or more auxin, such as NAA, 2,4-D, IBA, IAA, picloram, or analogs thereof.

In some embodiments, the concentration of the strong cytokinin (e.g., TDZ) in the bud induction media is about 0.25 mg/L (± 10%) to about 100 mg/L (± 10%), for example, is about 0.2 mg/L (± 10%), about 0.5 mg/L (± 10%), about 1.0 mg/L (± 10%), about 5 mg/L (± 10%), about 10 mg/L (± 10%), about 20 mg/L (± 10%), about 30 mg/L (± 10%), about 40 mg/L (± 10%), about 50 mg/L (± 10%), about 60 mg/L (± 10%), about 70 mg/L (± 10%), about 80 mg/L (± 10%), about 90 mg/L (± 10%), or about 100 mg/L (± 10%). For example, the concentration of TDZ is about 0.2 (± 10%) to about 20 (± 10%) mg/L, about 0.4 (± 10%) to about 10 (± 10%) mg/L, or about 0.5 (± 10%) to about 2 (± 10%) mg/L.

In some embodiments, the concentration of the auxin in the bud induction media is about 0.01 mg/L (± 10%) to about 10 mg/L (± 10%), for example, is about 0.01 mg/L (± 10%), about 0.05 mg/L (± 10%), about 0.1 mg/L (± 10%), about 0.5 mg/L (± 10%), about 1 mg/L (± 10%), about 5 mg/L (± 10%), or about 10 mg/L (± 10%).

In some embodiments, the shoot elongation/maintenance media comprises one or more cytokinins other than TDZ, such as meta-topolin, kinetin, isopentenyladenine (ip, e.g., 2ip), zeatin, trans-zeatin, zeatin riboside, dihydrozeatin, benzyleadenin (BAP), benzyladenosine ([9R]BAP), analogs thereof. In some embodiments, the shoot elongation/maintenance media further comprise one or more auxin, such as NAA, 2,4-D, IBA, IAA, picloram, or analogs thereof.

In some embodiments, the concentration of cytokinin in the shoot elongation/maintenance media is about 0.01 mg/L (± 10%) to about 100 mg/L (± 10%), for example, is about 0.01 mg/L (± 10%), about 0.05 mg/L (± 10%), about 0.1 mg/L (± 10%), about 0.5 mg/L (± 10%), about 1 mg/L (± 10%), about 5 mg/L (± 10%), about 10 mg/L(± 10%), about 20 mg/L(± 10%), about 30 mg/L(± 10%), about 40 mg/L(± 10%), about 50 mg/L(± 10%), about 60 mg/L(± 10%), about 70 mg/L(± 10%), about 80 mg/L(± 10%), about 90 mg/L(± 10%), or about 100 mg/L(± 10%). In some embodiments, the concentration of the cytokinin in the shoot elongation/maintenance media is about 0.01 (± 10%) to about 20 (± 10%) mg/L, about 0.1 (± 10%) to about 10 (± 10%) mg/L, or about 0.25 (± 10%) to about 5 (± 10%) mg/L.

In some embodiments, the concentration of the auxin in the bud induction media is about 0.01 mg/L (± 10%) to about 50 mg/L (± 10%), for example, is about 0.01 mg/L (± 10%), about 0.05 mg/L (± 10%), about 0.1 mg/L (± 10%), about 0.5 mg/L (± 10%), about 1 mg/L (± 10%), about 5 mg/L (± 10%), about 10 mg/L(± 10%), about 20 mg/L(± 10%), about 30 mg/L(± 10%), about 40 mg/L(± 10%), or about 50 mg/L(± 10%). In some embodiments, the concentration of the auxin in the shoot elongation/maintenance media is about 0.01 (± 10%) to about 20 (± 10%) mg/L, about 0.02 (± 10%) to about 10 (± 10%) mg/L, or about 0.05 (± 10%) to about 5 (± 10%) mg/L.

Non-limiting concentrations of the components in the bud induction media and shoot elongation/maintenance media are shown in Table 2. One or more components in table 2 can be omitted or replaced without affecting the function of the media. The concentration of each component can be adjusted without affecting the function of the media.

Table 2. Exemplary concentrations of bud induction media and shoot elongation & maintenance media

Media	**Bud Induction media (mg/L)**	**Shoot elongation & maintenance media (mg/L)**
NH_4NO_3	about 800-about 2500, e.g., 1650	about 800-about 2500, e.g., 1650
KNO_3	about 900-about 3000, e.g., 1900	about 900-about 3000, e.g., 1900
$Ca(NO_3)_2$	0-about 800, e.g., 0	0-about 800, e.g., 0
K_2SO_4	0-about 800, e.g., 0	0-about 800, e.g., 0
$MgSO_4$	about 150-about 550, e.g., 370	about 150-about 550, e.g., 370
$MnSO_4$	about 8.0-about 26.0, e.g., 16.9	about 8.0-about 26.0, e.g., 16.9
$ZnSO_4$	about 4.0-about 12.0, e.g., 8.6	about 4.0-about 12.0, e.g., 8.6
$CuSO_4$	about 0.010-about 0.4, e.g., 0.025	about 0.010-about 0.4, e.g., 0.025
$CaCl_2$	about 200-about 660, e.g., 440	about 200-about 660, e.g., 440
KI	about 0.4-about 1.5, e.g., 0.83	about 0.4-about 1.5, e.g., 0.83
$CoCl_2$	about 0.010-about 0.4, e.g., 0.025	about 0.010-about 0.4, e.g., 0.025
H_3BO_3	about 3.0-about 9.0, e.g., 6.2	about 3.0-about 9.0, e.g., 6.2
Na_2MoO_4	about 0.10-about 0.4, e.g., 0.25	about 0.10-about 0.4, e.g., 0.25
KH_2PO_4	about 80-about 250, e.g., 170	about 80-about 250, e.g., 170
K_2SO_5	as necessary	as necessary
$FeSO_4$	about 25-about 90, e.g., 55.7	about 25-about 90, e.g., 55.7
Na_2EDTA	about 35-about 120, e.g., 74.6	about 35-about 120, e.g., 74.6
Na_2H_2PO4	about 80-250, e.g., 170	about 80-250, e.g., 170
myo-Inositol	about 50-about 150, e.g., 100	about 50-about 150, e.g., 100

Media	Bud Induction media (mg/L)	Shoot elongation & maintenance media (mg/L)
Thiamine	about 0.2-about 0.6, e..g, 0.4	about 0.2-about 0.6, e..g, 0.4
Sugar	about 15 gr-about 45 gr, e.g. 30 gr.	about 15 gr-about 45 gr, e.g. 30 gr.
agar (solid)	about 2.5 gr-about 8.0 gr, e.g., 4.5 gr	about 2.5 gr-about 8.0 gr, e.g., 4.5 gr
PH	about 5.5-6.5, e.g., 5.7	about 5.5-6.5, e.g., 5.7
HORMONES		
NAA	about 0.05	about 0.01 – 50
BAP	about 1	about 0.01 – 50
TDZ	about 0.25 – about 100	0
ST-10	about 0.01 – about 50	about 0.01 – about 50
2ip	0	about 6

In some embodiments, the media can be selected from the ones listed in the table shown in FIG. 7, or any medium equivalent thereof.

In addition to the "bud induction media" and "shoot elongation/maintenance media" combination described above, the present invention provides other alternative media combinations for plant propagation. For example, provided are media combinations comprising at least one Stage 1 medium and at least one Stage 2 medium. In some embodiments, the number assigned to a media within a given process is maintained when a certain media is used more than one time. For example, certain embodiments disclosed herein include cycling explants or shoots in a rotation of media. For example, an explant may be placed in a Stage 1 media followed by a Stage 2 media and then returned back to its Stage 1 media. In this context, when exposure to a media is repeated, it retains its lowest Stage number within the particular process.

In some embodiments, to begin the process, a Stage 1 media can be obtained or prepared. Stage 1 media include a pH that is generally hospitable to plants (typically from 4.0-7.0 or 4.5-6.5). Stage 1 media disclosed herein can include (i) meta-topolin; (2) at least three cytokinins; (3) the cytokinin meta-topolin or an analogue thereof in combination with at least two other cytokinins; (4) at least one auxin and at least two cytokinins; (5) at least two auxins and at least two cytokinins or (6) at least two auxins and at least three cytokinins. In certain embodiments, Stage 1 media must include more than 1 auxin. In other embodiments, Stage 1 media must include more than 1 cytokinin. In further embodiments, Stage 1 media must include more than 1 auxin and more than 1 cytokinin.

In some embodiments, the cytokinins and auxins are chosen from one or more of meta-topolin, thidiazuron, NAA, IBA, BAP, 2ip, CCPU and DPU. In additional embodiments, the cytokinins and auxins are chosen from one or more of meta-topolin or analogues thereof, thidiazuron or analogues thereof, NAA or analogues thereof, IBA or analogues thereof, BAP or analogues thereof, 2ip or analogues thereof, CCPU or analogues thereof and DPU or analogues thereof.

In some embodiments, the media and at least two other cytokinins. In some embodiments, the media supports multiplication cycles for at least six months.

In some embodiments, the media comprise at least three cytokinins. In some embodiments, the media can support multiplication cycles for at least six months. In some embodiments, provided are media comprising the cytokinin meta-topolin or an analogue thereof in combination with at least two other cytokinins.

In some embodiments, the media comprise at least one auxin and at least two cytokinins. In some embodiments, the media can support multiplication cycles for at least six months. In some embodiments, at least one cytokinin is meta-topolin or an analogue thereof.

In some embodiments, the media comprise at least two auxins and at least two cytokinins. In some embodiments, the media can support multiplication cycles for at least six months.

In some embodiments, the media comprise at least two auxins and at least three cytokinins. In some embodiments, the media can support multiplication cycles for at least six months.

In some embodiments, the micropropagated plants are grown *in vitro* in sterile media.

In some embodiments, the media can be liquid, semi-solid, or solid, and the physical state of the media can be varied by the incorporation or removal of one or more gelling agents. Any gelling agent known in the art that is suitable for use in plant tissue culture media can be used. Agar is most commonly used for this purpose. Examples of such agars include Agar Type A, E or M and Bacto® Agar (Becton Dickinson & Co.). Other exemplary gelling agents include carrageenan, gellan gum (commercially available as PhytaGel™ (Sigma-Aldrich), Gelrite® (Sigma-Aldrich) and Gelzan™ (Caisson Labs)), alginic acid and its salts, and agarose. Blends of these agents, such as two or more of agar, carrageenan, gellan gum, agarose and alginic acid or a salt thereof also can be used.

Examples of plant growth regulators include abscisic acid (ABA), triacontanol, phloroglucinol, auxins and compounds with auxin-like activity, cytokinins and compounds with cytokinin-like activity. Exemplary auxins include 4-fluorophenoxyacetic acid (FA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 3-bromooxindole-3-aceitc acid, 4-bromophenoxyacetic acid, dicamba, p-chlorophenoxyacetic acid (CPA) indole-3-propinoic acid (IPA), 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram and combinations thereof. Exemplary cytokinins include meta-topolin, thidiazuron, N-(2-chloro-4-pyridyl)-N-phenylurea (CCPU), 1,3-diphenylurea (DPU), adenine hemisulfate, benzyladenine, dimethylallyladenine, kinetin, zeatin, riboside, adenosine, meta-topolin riboside, meta-topolin-9-glucoside, ortho-topolin, ortho-topolin riboside, ortho-topolin-9-glucoside, para-topolin, para-topolin riboside, para-topolin-9-glucoside, ortho-methoxytopolin, ortho-methoxytopolin riboside, meta-methoxytopolin, meta-methoxytopolin riboside, meta-methoxytopolin-9-glucoside and combinations thereof as well as plant extracts having cytokinin-like activity, such as coconut water, banana powder, malt extract, pineapple powder or tomato powder.

Gibberellic acid also can be included in the media. A sugar or combination of sugars can be included in the media and can serve as a carbon source. Such sugars are known to those of ordinary skill in the art. Exemplary sugars include fructose, sucrose, glucose, maltose, galactose mannitol and sorbitol or combinations thereof. Other exemplary additives (with suggested but non-limiting functions) include polyamines (regeneration enhancer); citric acid, polyvinylpyrodine (PVP) and sodium thiosulfate (anti-browning agents); $CaNO_3$ or calcium gluconate (hyperhydricity reducer); paclobutrazol or ancymidol (multiplication enhancer); acetyl salicylic acid (ethylene inhibitor) and p-chlorophenoxyisobutyric acid (PCIB) and triiodobenzoic acid (TIBA) (anti-auxins).

In some embodiments, basal media can be Murashige and Skoog (MS). Suitable nutrient salts also include, without limitation, Anderson's Rhododendron, Chu's N-6, DKW, Gamborg's B-5, Hoaglands No. 2, Kao & Michayluk, Nitsch & Nitsch, Schenk and Hildebrant, Vacin and Went, Whites and WPM, available from commercial sources such as Caisson Laboratories, Inc or Phytotechnology Laboratories.

One example of a Stage 1 media includes meta-topolin. Another non-limiting example includes meta-topolin, thidiazuron, NAA and BAP. Another non-limiting example includes meta-topolin, NAA and BAP. Another non-limiting example includes meta-topolin, NAA, BAP and IBA. Another non-limiting example includes meta-topolin, thidiazuron,

NAA, BAP and IBA. Another non-limiting example includes thidiazuron, NAA, BAP and 2ip. Another non-limiting example includes thidiazuron, NAA and 2ip. Another non-limiting example includes meta-topolin, thidiazuron, NAA, BAP, IBA and 2ip. Another non-limiting example includes meta-topolin, IBA, 2ip and BAP. Another non-limiting example includes meta-topolin, thidiazuron, CPPU, NAA and BAP. Another non-limiting example includes meta-topolin, thidiazuron, DPU, NAA and BAP. Another non-limiting example includes thidiazuron, CPPU, BAP, IBA and 2ip. Another non-limiting example includes CPPU, DPU, NAA and BAP. Another non-limiting example includes meta-topolin, thidiazuron, CPPU, DPU, NAA, BAP, IBA and 2ip. Each of these non-limiting examples can also include analogues of meta-topolin, thidiazuron, NAA, BAP, DPU, CPPU, IBA and/or 2ip.

The Stage 1 media is then placed into test tubes or other appropriate containers (including jars, boxes, jugs, cups, sterile bag technology, bioreactors, temporary immersion vessels, etc. wherein when not specified are collectively referred to as "tubes"). These tubes can be capped or covered and autoclaved to sterilize the tubes and media. In another embodiment, sterilization is achieved by autoclaving at 5-25 pounds pressure psi at a temperature of 200° F - for 200° F 10-25 minutes. In another embodiment, sterilization is achieved by autoclaving at 15 pounds pressure psi at a temperature of 250° F for 15-18 minutes. Liquid media can be subjected to filter sterilization.

After the explants are allowed to establish themselves on Stage 1 media, the cell cultures grown from the explants are transferred into a Stage 2 media. Stage 2 media disclosed herein can include (i) meta-topolin; (2) at least three cytokinins; (3) the cytokinin meta-topolin or an analogue thereof in combination with at least two other cytokinins; (4) at least one auxin and at least two cytokinins; (5) at least two auxins and at least two cytokinins or (6) at least two auxins and at least three cytokinins. In certain embodiments, Stage 2 media must include more than 1 auxin. In other embodiments, Stage 2 media must include more than 1 cytokinin. In further embodiments, Stage 2 media must include more than 1 auxin and more than 1 cytokinin.

In some embodiments, the cytokinins and auxins are chosen from one or more of meta-topolin, thidiazuron, NAA, IBA, BAP, 2ip, CCPU and DPU. In additional embodiments, the cytokinins and auxins are chosen from one or more of meta-topolin or analogues thereof, thidiazuron or analogues thereof, NAA or analogues thereof, IBA or analogues thereof, BAP or analogues thereof, 2ip or analogues thereof, CCPU or analogues thereof and DPU or analogues thereof.

In some embodiments, one example of a Stage 2 media includes meta-topolin. Another non-limiting example includes meta-topolin, thidiazuron, NAA and BAP. Another non-limiting example includes meta-topolin, NAA and BAP. Another non-limiting example includes meta-topolin, NAA, BAP and IBA. Another non-limiting example includes meta-topolin, thidiazuron, NAA, BAP and IBA. Another non-limiting example includes thidiazuron, NAA, BAP and 2ip. Another non-limiting example includes thidiazuron, NAA and 2ip. Another non-limiting example includes meta-topolin, thidiazuron, NAA, BAP, IBA and 2ip. Another non-limiting example includes meta-topolin, IBA, 2ip and BAP. Another non-limiting example includes meta-topolin, thidiazuron, CPPU, NAA and BAP. Another non-limiting example includes meta-topolin, thidiazuron, DPU, NAA and BAP. Another non-limiting example includes thidiazuron, CPPU, BAP, IBA and 2ip. Another non-limiting example includes CPPU, DPU, NAA and BAP. Another non-limiting example includes meta-topolin, thidiazuron, CPPU, DPU, NAA, BAP, IBA and 2ip. Each of these non-limiting examples can also include analogues of meta-topolin, thidiazuron, NAA, BAP, DPU, CPPU, IBA and/or 2ip.

In particular embodiments disclosed herein, both Stage 1 and Stage 2 media include meta-topolin. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, NAA and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, NAA and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, NAA, BAP and IBA. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, NAA, BAP and IBA. In another non-limiting embodiment, both Stage 1 and Stage 2 media include thidiazuron, NAA, BAP and 2ip. In another non-limiting embodiment, both Stage 1 and Stage 2 media include thidiazuron, NAA and 2ip. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, NAA, BAP, IBA and 2ip. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, IBA, 2ip and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, CPPU, NAA and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, DPU, NAA and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include thidiazuron, CPPU, BAP, IBA and 2ip. In another non-limiting embodiment, both Stage 1 and Stage 2 media include CPPU, DPU, NAA and BAP. In another non-limiting embodiment, both Stage 1 and Stage 2 media include meta-topolin, thidiazuron, CPPU, DPU,

NAA, BAP, IBA and 2ip. Each of these non-limiting examples can also include analogues of meta-topolin, thidiazuron, NAA, BAP, DPU, CPPU, IBA and/or 2ip.

In some embodiments, examples of Stage 1 and Stage 2 media are described in WO/2011/100762, which is incorporated herein by references in its entirety. For example, Stage 1 and Stage 2 media can be selected from the group consisting of Media b-12c(i-v); Media CW2(i-v); Media CW3(i-v); Media b-9(i-v); Media CW4(i-v); Media CW1(i-v); Media CW5(i-v); Media CW6(i-v); Media b-10(i-v); Media b-11(i-v); Media b-1 (i-v); Media b-4(i-v); Media b-6(i-v).

In some embodiments, examples of Stage 1 and Stage 2 media are selected from the ones described below:

Media b-12c(i-v):

Component	**Standard b-12c-i**	**Standard b-12c-ii**	**Standard b-12c-iii**	**Standard b-12c-iv**	**Standard b-12c-v**
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$Ca(NO_3)_2$	225-775	410-690	495-605	550	550±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.020-0.030	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media CW2(i-v):

Component	**Standard CW2-i**	**Standard CW2-ii**	**Standard CW2-iii**	**Standard CW2-iv**	**Standard CW2-v**
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2

Component	Standard CW2-i	Standard CW2-ii	Standard CW2-iii	Standard CW2-iv	Standard CW2-v
$Ca(NO_3)_2$	225-775	410-690	495-605	550	550±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8.0-26.0	12.0-22.0	15.0-19.0	16.9	16.9±0.2
$ZnSO_4$	4.0-12.0	6.0-10.0	8.0-9.0	8.6	8.6±0.2
$CuSO_4$	0.012-0.37	0.02-0.03	0.02-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.02-0.028	0.025	0.025±.002
H_3BO_3	3.0-9.0	4.0-8.0	5.0-7.0	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112			74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media CW3(i-v):

Component	Standard CW3-i	Standard CW3-ii	Standard CW3-iii	Standard CW3-iv	Standard CW3-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$Ca(NO_3)_2$	225-775	410-690	495-605	550	550±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.02-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.05-0.15	0.07-0.12	0.09-0.11	0.1	0.1±0.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
IBA	0.1-0.3	0.15-0.25	0.17-0.22	0.2	0.2±0.1

Component	Standard CW3-i	Standard CW3-ii	Standard CW3-iii	Standard CW3-iv	Standard CW3-v
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media b-9(i-v):

Component	Standard b-9-i	Standard b-9-ii	Standard b-9-iii	Standard b-9-iv	Standard b-9-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8.0-26.0	12.0-22.0	15.0-19.0	16.9	16.9±0.2
$ZnSO_4$	4.0-12.0	6.0-10.0	8.0-9.0	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.4-1.25	0.6-1.05	0.7-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media CW4(i-v):

Component	Standard CW4-i	Standard CW4-ii	Standard CW4-iii	Standard CW4-iv	Standard CW4-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15 -19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02

Component	Standard CW4-i	Standard CW4-ii	Standard CW4-iii	Standard CW4-iv	Standard CW4-v
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.05-0.15	0.07-0.12	0.09-0.11	0.1	0.1±0.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
IBA	0.1-0.3	0.15-0.25	0.17-0.22	0.2	0.2±0.1
Thidiazuron	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media CW1(i-v):

Component	Standard CW1-i	Standard CW1-ii	Standard CW1-iii	Standard CW1-iv	Standard CW1-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2
Silicon Solution mL	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2

Media CW5(i-v):

Component	Standard CW5-i	Standard CW5-ii	Standard CW5-iii	Standard CW5-iv	Standard CW5-v

Component	Standard CW5-i	Standard CW5-ii	Standard CW5-iii	Standard CW5-iv	Standard CW5-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
IBA	0.1-0.3	0.15-0.25	0.17-0.22	0.2	0.2±0.1
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2
Silicon Solution mL	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2

Media CW6(i-v):

Component	Standard CW6-i	Standard CW6-ii	Standard CW6-iii	Standard CW6-iv	Standard CW6-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.4-1.2	0.6-1.0	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
K_2SO_5	181.8-545.6	272.8-454.6	327.4-400.0	363.75	363.75±.02
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2

Component	Standard CW6-i	Standard CW6-ii	Standard CW6-iii	Standard CW6-iv	Standard CW6-v
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.05-0.15	0.07-0.12	0.09-0.11	0.1	0.1±0.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
IBA	0.1-0.3	0.15-0.25	0.17-0.22	0.2	0.2±0.1
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Thidiazuron	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media b-10(i-v):

Component	Standard b-10-i	Standard b-10-ii	Standard b-10-iii	Standard b-10-iv	Standard b-10-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4.0-12.0	6.0-10.0	8.0-9.0	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37.-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2

Media b-11(i-v):

Component	Standard b-11-i	Standard b-11-ii	Standard b-11-iii	Standard b-11-iv	Standard b-11-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2

Component	Standard b-11-i	Standard b-11-ii	Standard b-11-iii	Standard b-11-iv	Standard b-11-v
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Meta-topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media b-1(i-v):

Component	Standard b-1-i	Standard b-1-ii	Standard b-1-iii	Standard b-1-iv	Standard b-1-v
NH_4NO_3	600-1800	900-1500	1080-1320	1200	1200±2
$Ca(NO_3)_2$	838-2515	1257-2096	1510-1844	1677	1677±2
K_2SO_4	121-363	181-302	218-266	242	242±2
$MgSO_4$	270-830	410-690	500-610	555	555±2
$MnSO_4$	12.6-38.0	19.0-31.7	22.8-27.8	25.35	25.35±.02
$ZnSO_4$	6.4-19.5	9.6-16.2	11.5-14.0	12.9	12.9±0.2
$CuSO_4$	0.01-0.05	0.02-0.04	0.033-0.041	0.037	0.037±0.002
$CaCl_2$	48-144	72-120	85-105	96	96±2
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27.0-84.0	40.0-70.0	50.0-60.0	55.7	55.7±0.2
Na_2EDTA	37.0-112.0	55.0-94.0	67.0-82.0	74.6	74.6±0.2
$Na_2H_2PO_4$	42-128	63-106	75-95	85	85±2
myo-Inositol	100-300	150-250	180-220	200	200±2
Thiamine	0.4-1.4	0.6-1.1	0.8-1.0	0.9	0.9±0.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Glycine	1-3	1.5-2.5	1.75-2.25	2	2±1
Riboflavin	10-30	15-25	18-22	20	20±2
BAP	0.1-0.3	0.15-0.25	0.17-0.22	0.2	0.2±0.1
NAA	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Thidiazuron	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02

Component	Standard b-1-i	Standard b-1-ii	Standard b-1-iii	Standard b-1-iv	Standard b-1-v
2ip	7-23	11-19	13-17	15	15±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Carrageenan g/L	3-11	4-10	5-8	7	7±2

Media b-4(i-v):

Component	Standard b-4-i	Standard b-4-ii	Standard b-4-iii	Standard b-4-iv	Standard b-4-v
NH_4NO_3	600-1800	900-1500	1080-1320	1200	1200±2
$Ca(NO_3)_2$	838-2515	1257-2096	1510-1844	1677	1677±2
K_2SO_4	121-363	181-302	218-266	242	242±2
$MgSO_4$	270-830	410-690	500-610	555	555±2
$MnSO_4$	12.6-38.0	19.0-31.7	22.8-27.8	25.35	25.35±.02
$ZnSO_4$	6.4-19.5	9.6-16.2	11.5-14.0	12.9	12.9±0.2
$CuSO_4$	0.01-0.05	0.02-0.04	0.03-0.04	0.037	0.037±.002
$CaCl_2$	48-144	72-120	85-105	96	96±2
H_3BO_3	3.0-9.0	4.0-8.0	5.0-7.0	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	42-128	63-106	75-95	85	85±2
myo-Inositol	100-300	150-250	180-220	200	200±2
Thiamine	0.4-1.4	0.6-1.1	0.8-1.0	0.9	0.9±0.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Glycine	1-3	1.5-2.5	1.75-2.25	2	2±1
Riboflavin	10-30	15-25	18-22	20	20±2
BAP	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
NAA	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
2ip	10-30	15-25	18-22	20	20±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Carrageenan g/L	3-11	4-10	5-8	7	7±2

Media b-6(i-v):

Component	Standard b-6-i	Standard b-6-ii	Standard b-6-iii	Standard b-6-iv	Standard b-6-v
NH_4NO_3	600-1800	900-1500	1080-1320	1200	1200±2
$Ca(NO_3)_2$	838-2515	1257-2096	1510-1844	1677	1677±2
K_2SO_4	121-363	181-302	218-266	242	242±2
$MgSO_4$	270-830	410-690	500-610	555	555±2
$MnSO_4$	12.6-38.0	19.0-31.7	22.8-27.8	25.35	25.35±.02
$ZnSO_4$	6.4-19.5	9.6-16.2	11.5-14.0	12.9	12.9±0.2
$CuSO_4$	0.01-0.05	0.02-0.04	0.033-0.041	0.037	0.037±.002

Component	Standard b-6-i	Standard b-6-ii	Standard b-6-iii	Standard b-6-iv	Standard b-6-v
$CaCl_2$	48-144	72-120	85-105	96	96±2
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50 -60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	42-128	63-106	75-95	85	85±2
myo-Inositol	100-300	150-250	180-220	200	200±2
Thiamine	0.4-1.4	0.6-1.1	0.8-1.0	0.9	0.9±0.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Glycine	1-3	1.5-2.5	1.75-2.25	2	2±1
Riboflavin	10-30	15-25	18-22	20	20±2
NAA	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
2ip	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	12-37	15-35	20-30	25	25±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2
Carrageenan g/L	1-3	1.5-2.5	1.75-2.25	2	2±1

Media B-9N2

Component	Standard B-9N2-i	Standard B-9N2-ii	Standard B-9N2-iii	Standard B-9N2-iv	Standard B-9N2-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
K_2SO_4	242-726	363-605	436-532	484	484±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.02-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
KI	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3.0-9.0	4.0-8.0	5.0-7.0	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	100-300	150-250	180-220	200	200±2
Thiamine	0.45-1.35	0.67-1.13	0.8-1.0	0.9	0.9±0.2
NAA	0.15-0.45	0.22-0.38	0.27-0.33	0.3	0.3±0.2
BAP	1-3	1.25-2.75	1.5-2.5	2	2±0.5

Component	Standard B-9N2-i	Standard B-9N2-ii	Standard B-9N2-iii	Standard B-9N2-iv	Standard B-9N2-v
Thidiazuron	0.25-0.75	0.37-0.63	0.45-0.55	0.5	0.5±.2
Meta-Topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media B-12C CPPU

Component	Standard B-12C CPPU-i	Standard B-12C CPPU-ii	Standard B-12C CPPU-iii	Standard B-12C CPPU-iv	Standard B-12C CPPU-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$Ca(NO_3)_2$	225-775	410-690	495-605	550	550±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
KI	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
Casein Hydroxylate g/L	1-3	1.5-2.5	1.75-2.25	2	2±1
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
Meta-Topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
CPPU	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media B-12C DPU

Component	Standard B-12C CPU-i	Standard B-12C CPU-ii	Standard B-12C CPU-iii	Standard B-12C CPU-iv	Standard B-12C CPU-v
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2

Component	Standard B-12C CPU-i	Standard B-12C CPU-ii	Standard B-12C CPU-iii	Standard B-12C CPU-iv	Standard B-12C CPU-v
$Ca(NO_3)_2$	225-775	410-690	495-605	550	550±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
KI	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	27-84	40-70	50-60	55.7	55.7±0.2
Na_2EDTA	37-112	55-94	67-82	74.6	74.6±0.2
$Na_2H_2PO_4$	85-255	120-210	150-190	170	170±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±0.2
Casein Hydroxylate g/L	1-3	1.5-2.5	1.75-2.25	2	2±1
NAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
BAP	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.2
Thidiazuron	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
Meta-Topolin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
DPU	0.36 – 1.12	0.56-0.94	0.67-.083	0.75	0.75±.02
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

In some embodiments, the non-cytokinin components are those found in Anderson's Rhododendron, Chu's N-6, DKW, Gamborg's B-5, Hoaglands No. 2, Kao & Michayluk, Nitsch & Nitsch, Schenk and Hildebrant, Vacin and Went, Whites and WPM, available from commercial sources. Particular media can have higher or lower levels of macronutrients than those provided in the preceding tables and others will lack nitrates. In some embodiments, the media have higher or lower levels of macronutrients and lack nitrates. In some embodiments, the media have higher levels of macronutrients and lack nitrates.

Media disclosed herein also include spiked media. Spiked media are those in which the concentration of at least one cytokinin and/or auxin in the media described above is increased by, for example and without limitation, 1%, 3%, 5%, 7%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 100%, 105%,

110% or 200%. In other embodiments, the concentration of at least one cytokinin and/or auxin in the media described above is increased by, for example and without limitation, 1-10%, 5-15%, 10-20%, 15-25%, 20-30%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, 90-100%, 95-105%, 100-110%, 105-115%, 110-120%, 115-125%, 120-130%, 125-135%, 130-140%, 135-145%, 140-150%, 145-155%, 150-160%, 155-165%, 160-170%, 165-175%, 170-180%, 175-185%, 180-190%, 185-195%, 190-200%, 195-205%, 3-6%, 7-17%, 12-22%, 17-27%, 22-32%, 27-37%, 32-42%, 37-47%, 42-52%, 47-57%, 52-62%, 57-67%, 62-72%, 67-77%, 72-82%, 77-87%, 82-92%, 87-97%, 92-102%, 97-107%, 102-112%, 107-117%, 112-122%, 117-127%, 122-132%, 127-137%, 132-142%, 137-147%, 142-152%, 147-157%, 152-162%, 157-167%, 162-172%, 167-177%, 172-182%, 177-187%, 162-172%, 167-177%, 182-192%, 187-197%, 192-202%, 197-207%, or 200-210%. When more than one cytokinin and/or auxin is spiked, the concentrations of each raised cytokinin and/or auxin can be raised by the same amount or a different amount than other cytokinins and/or auxins in the media.

The following tables provide non-limiting examples of spiked media disclosed herein. Each media includes the components described in its respective table above or adjusted as described in the next paragraph with the following adjustments to cytokinin levels.

Media b-12c(i-v):

Component	**Spiked b-12c-i**	**Spiked b-12c-ii**	**Spiked b-12c-iii**	**Spiked b-12c-iv**	**Spiked b-12c-v**
NAA	0.1	0.1	0.05	0.05	0.5
BAP	2	1	1.25	1	1.5
Thidiazuron	0.75	0.7	1	0.75	1.25
Meta-topolin	10	5	5	6	7.5

Media CW2(i-v):

Component	**Spiked CW2-i**	**Spiked CW2-ii**	**Spiked CW2-iii**	**Spiked CW2-iv**	**Spiked CW2-v**
NAA	0.05	0.05	0.2	0.1	0.05
BAP	1	2	1	2	5
Meta-topolin	20	10	7.5	10	5

Media CW3(i-v):

Component	**Spiked CW3-i**	**Spiked CW3-ii**	**Spiked CW3-iii**	**Spiked CW3-iv**	**Spiked CW3-v**
NAA	0.1	0.1	0.4	0.1	0.5
BAP	1	1	3	1	2
IBA	0.2	0.75	0.2	0.5	1
Meta-topolin	8	16	9	30	5

Media b-9(i-v):

Component	Spiked b-9-i	Spiked b-9-ii	Spiked b-9-iii	Spiked b-9-iv	Spiked b-9-v
NAA	1	2	0.5	0.25	0.05
BAP	11	5	50	10	1
Thidiazuron	0.5	1	1.5	1.75	0.25
Meta-topolin	9	8	7	6	500

Media CW4(i-v):

Component	Spiked CW4-i	Spiked CW4-ii	Spiked CW4-iii	Spiked CW4-iv	Spiked CW4-v
NAA	0.1	0.1	0.1	0.1	0.1
BAP	1	1	2	3	4
IBA	0.4	0.2	1	0.5	0.2
Thidiazuron	0.25	0.5	1	5	0.3
Meta-topolin	50	40	5	15	10

Media CW1(i-v):

Component	Spiked CW1-i	Spiked CW1-ii	Spiked CW1-iii	Spiked CW1-iv	Spiked CW1-v
NAA	2	0.05	0.05	10	0.15
BAP	1	100	150	80	5
Meta-topolin	85	300	100	5	25

Media CW5(i-v):

Component	Spiked CW5-i	Spiked CW5-ii	Spiked CW5-iii	Spiked CW5-iv	Spiked CW5-v
NAA	0.05	0.15	0.05	0.1	3
BAP	1	1	5	1	4
IBA	0.2	0.4	0.2	1	5
Meta-topolin	1000	890	75	30	5

Media CW6(i-v):

Component	Spiked CW6-i	Spiked CW6-ii	Spiked CW6-iii	Spiked CW6-iv	Spiked CW6-v
NAA	0.1	0.1	2	0.4	0.5
BAP	3	1	4	1.5	2
IBA	0.5	0.2	0.4	0.3	1
Thidiazuron	0.25	0.25	0.5	10	3
Meta-topolin	450	20	10	5	7.5

Media b-10(i-v):

Component	Spiked b-10-i	Spiked b-10-ii	Spiked b-10-iii	Spiked b-10-iv	Spiked b-10-v
NAA	0.05	0.05	1	0.25	0.05
BAP	1.3	3	1.6	2	1
Meta-topolin	65	70	75	80	20

Media b-11(i-v):

Component	Spiked b-11-i	Spiked b-11-ii	Spiked b-11-iii	Spiked b-11-iv	Spiked b-11-v
NAA	0.05	0.5	1	1.75	0.05
BAP	4	3	2	1	1
Thidiazuron	5	10	15	20	0.5
Meta-topolin	750	43	12	300	125

Media b-1(i-v):

Component	Spiked b-1-i	Spiked b-1-ii	Spiked b-1-iii	Spiked b-1-iv	Spiked b-1-v
BAP	0.2	0.4	0.6	0.8	0.3
NAA	3	0.75	0.5	1	0.5
Thidiazuron	7.5	75	9	150	3.5
2ip	80	60	45	30	20

Media b-4(i-v):

Component	Spiked b-4-i	Spiked b-4-ii	Spiked b-4-iii	Spiked b-4-iv	Spiked b-4-v
BAP	30	10	7	5	5
NAA	4	3	2	1	1
Thidiazuron	0.75	75	7.5	5	0.75
2ip	60	20	80	40	25

Media b-6(i-v):

Component	Spiked b-6-i	Spiked b-6-ii	Spiked b-6-iii	Spiked b-6-iv	Spiked b-6-v
NAA	1	1	4	10	1
Thidiazuron	1	2	5	10	0.25
2ip	15	7.5	5	5	5

Media B-9N2

Component	Spiked B-9N2-i	Spiked B-9N2-ii	Spiked B-9N2-iii	Spiked B-9N2-iv	Spiked B-9N2-v
NAA	1	0.6	0.3	0.4	0.30
BAP	4	3	1.5	2	1
Thidiazuron	0.75	0.25	0.5	0.5	0.25
Meta-Topolin	10	5	5	6	5

Media B-12C CPPU

Component	Spiked B-12C CPPU-i	Spiked B-12C CPPU-ii	Spiked B-12C CPPU-iii	Spiked B-12C CPPU-iv	Spiked B-12C CPPU-v
NAA	0.75	1	0.05	1	0.05
BAP	1.25	2	1	1	2
Thidiazuron	1	1.5	1	0.75	1.5
Meta-Topolin	6	10	8	5	5
CPPU	0.8	1.5	1.5	0.75	0.75

Media B-12C DPU

Component	Spiked B-12C CPU-i	Spiked B-12C CPU-ii	Spiked B-12C CPU-iii	Spiked B-12C CPU-iv	Spiked B-12C CPU-v
NAA	0.1	0.1	1.8	3	0.3
BAP	1.5	2	1.8	2	1.5
Thidiazuron	1.5	1.5	1.8	17	3
Meta-Topolin	10	9	5	6	5
DPU	4	3	1.8	0.75	0.75

Additional spiked media can include any standard media described above with the addition or adjustment to the following cytokinin and/or auxin concentrations:

Media AA

Component	AA-i	AA-ii	AA-iii
BAP	1	1	1
Thidiazuron	2.5	5	10

Media AB

Component	AB-i	AB-ii	AB-iii	AB-iv
BAP	5	10	20	40

Media AC

Component	AC-i	AC-ii	AC-iii	AC-iv	AC-v
BAP	1	1	1	1	1
CPPU	2.5	7.5	10	25	50

As explained more fully below, when a spiked media is utilized, explants or shoots generally (but not necessarily) remain on the spiked media for a shorter period of time than those kept on non-spiked media and following culture on a spiked media, the explants or shoots are transferred to a media containing standard, reduced or no levels of cytokinins and/or auxins (those containing reduced or no cytokinins and/or auxins are both referred to as "reduced" media herein).

One or more compositions of the media disclosed herein can also be adjusted based on the plant species.

In some embodiments, the media disclosed herein can be used for bamboo tissue culture. Representative genera of bamboo are described in WO/2011/100762, which is incorporated herein by reference in its entirety.

In some embodiments, Stage 1 media are selected from the group consisting of b-12c-v media, B-12C-CPPU-v media, B-12C DPU-v, B-9N2-v media or b-10-v media or spiked versions thereof.

In some embodiments, Stage 2 media are selected from the group consisting of CW1-v media; CW2-v media; CW3-v media; CW4-v media; CW5-v media; CW6-v media, B-12C-CPPU-v media, B-12C DPU-v, B-9N2-v media or spiked and reduced/standard versions thereof for 10-120 day cycles (as modified for spiked media as described more fully below).

In some embodiments, one or more Stage 3 media can be included. In some embodiments, Stage 3 media are selected from the group consisting of Br-2-v media; Ech-v media or Amel-v media or spiked and reduced/standard versions thereof (as modified for spiked media as described more fully below).

In some embodiments, non-limiting examples of Stage 2, Stage 3, Stage 4, Stage 5, Stage 6, Stage 7 media include:

Media Ech(i-v):

Component	**Standard Ech-i**	**Standard Ech-ii**	**Standard Ech-iii**	**Standard Ech-iv**	**Standard Ech-v**
NH_4NO_3	825-2475	1237-2063	1485-1815	1650	1650±2
KNO_3	950-2850	1425-2375	1710-2090	1900	1900±2
$MgSO_4$	185-555	275-465	330-410	370	370±2
$MnSO_4$	8-26	12-22	15-19	16.9	16.9±0.2
$ZnSO_4$	4-12	6-10	8-9	8.6	8.6±0.2
$CuSO_4$	0.01-0.37	0.02-0.03	0.022-0.028	0.025	0.025±.002
$CaCl_2$	220-660	330-350	400-480	440	440±2
Kl	0.40-1.25	0.60-1.05	0.75-0.90	0.83	0.83±.02
$CoCl_2$	0.012-0.378	0.020-0.030	0.022-0.028	0.025	0.025±.002
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	85-255	120-210	150-190	170	170±2
$FeSO_4$	13-42	20-34	25-30	27.8	27.8±0.2
Na_2EDTA	18-56	28-46	33-41	37.3	37.3±0.2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.35-0.45	0.4	0.4±0.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Glycine	1-3	1.5-2.5	1.75-2.25	2	2±1
NAA	0.05-0.15	0.07-0.12	0.09-0.11	0.1	0.1±0.02
IAA	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	2.7-8.2	4.1-6.8	4.9-6.1	5.5	5.5±0.2

Media BR-2(i-v):

Component	**Standard BR-2-i**	**Standard BR-2-ii**	**Standard BR-2-iii**	**Standard BR-2-iv**	**Standard BR-2-v**
NH_4NO_3	700-2100	1050-1750	1260-1540	1400	1400±2
$Ca(NO_3)_2$	973-2919	1459-2433	1752-2140	1946	1946±2

Component	Standard BR-2-i	Standard BR-2-ii	Standard BR-2-iii	Standard BR-2-iv	Standard BR-2-v
K_2SO_4	606-1818	909-1515	1091-1333	1212.5	1212.5±0.2
$MgSO_4$	370-1110	555-925	665-815	740	740±2
$MnSO_4$	16.9-50.7	25.4-42.3	30.5-37.1	33.8	33.8±0.2
$ZnSO_4$	8.6-25.8	12.9-21.5	15.5-18.0	17.2	17.2±0.2
$CuSO_4$	0.02 – 0.08	0.03-0.07	0.04-0.06	0.05	0.05±.02
$CaCl_2$	72-216	108-180	130-158	144	144±2
H_3BO_3	3-9	4-8	5-7	6.2	6.2±0.2
Na_2MoO_4	0.12-0.36	0.18-0.31	.22-.28	0.25	0.25±.02
KH_2PO_4	72-342	202-338	243-297	270	270±2
$FeSO_4$	16.68-50.04	25.02-41.70	30.06-36.66	33.36	33.36±.02
Na_2EDTA	22.38-67.14	33.57-55.95	40.36-49.16	44.76	44.76±.02
myo-Inositol	100-300	150-250	180-220	200	200±2
Thiamine	0.4-1.4	0.6-1.2	0.8-1.0	0.9	0.9±0.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Glycine	1-3	1.5-2.5	1.75-2.25	2	2±1
Riboflavin	10-30	15-25	18-22	20	20±2
Ascorbic Acid	50-150	75-125	90-110	100	100±2
NAA	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Sugar g/L	15-45	22-37	27-33	30	30±2
Carrageenan g/L	4-12	6-10	7-9	8	8±2
Charcoal g/L	150-450	220-370	270-330	300	300±2

Media Amel(i-v):

Component	Standard Amel-i	Standard Amel-ii	Standard Amel-iii	Standard Amel-iv	Standard Amel-v
NH_4NO_3	410-1240	620-1030	740-910	825	825±2
$Ca(NO_3)_2$	475-1425	710-1190	855-1045	950	950±2
$MgSO_4$	90-280	140-230	160-200	185	185±2
$MnSO_4$	4.20-12.70	6.30-10.60	7.65-9.25	8.45	8.45±.02
$ZnSO_4$	2.0-6.5	3.0-5.5	3.5-5.0	4.3	4.3±0.2
$CuSO_4$	.0062-0188	.0094-.0156	.0115-.0135	0.0125	0.0125±.0002
$CaCl_2$	110-330	165-285	195-240	220	220±2
Kl	.20-.62	.31-.52	.37-.45	0.415	0.415±.002
H_3BO_3	1.5-4.6	2.3-4.0	2.8-3.4	3.1	3.1±0.2
$CaCl_2$	.006-.018	.009-.015	.011-.013	0.0125	0.0125±.0002
Na_2MoO_4	.06-.18	.09-.15	.11-.13	0.125	0.125±.002
KH_2PO_4	40-130	60-110	75-95	85	85±2
$FeSO_4$	6.8-20.9	10.4-17.5	12.5-15.5	13.9	13.9±0.2
Na_2EDTA	9.3-27.9	13.9-23.3	16.8-20.4	18.65	18.65±.02
$Na_2H_2PO_4$	40-130	60-110	75-95	85	85±2
myo-Inositol	50-150	75-125	90-110	100	100±2
Thiamine	0.2-0.6	0.3-0.5	0.36-0.44	0.4	0.4±.2
Pyridoxine	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
Nicotinic acid	1-3	1.5-2.5	1.75-2.25	2	2±1

Component	Standard Amel-i	Standard Amel-ii	Standard Amel-iii	Standard Amel-iv	Standard Amel-v
Riboflavin	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
NAA	0.2-0.8	0.3-0.7	0.4-0.6	0.5	0.5±0.2
IBA	0.5 – 1.5	0.7 – 1.3	0.9-1.1	1	1±0.5
Sugar g/L	15-45	22-37	27-33	30	30±2
Agar g/L	1.5-4.5	2.0-4.0	2.5-3.5	3	3±2
Carrageenan g/L	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2
Charcoal g/L	2.5-7.5	3.7-6.2	4.5-5.5	5	5±2

In some embodiments, non-limiting examples of spiked versions of these media include:

Media Ech(i-v):

Component	Spiked Ech-i	Spiked Ech-ii	Spiked Ech-iii	Spiked Ech-iv	Spiked Ech-v
NAA	0.2	0.1	0.1	0.3	2
IAA	0.5	0.5	1	0.05	0.75

Media BR-2(i-v):

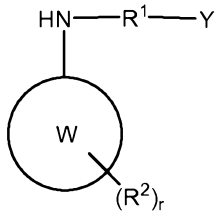
Component	Spiked BR-2-i	Spiked BR-2-ii	Spiked BR-2-iii	Spiked BR-2-iv	Spiked BR-2-v
NAA	1	1.5	2	50	5

Media Amel(i-v):

Component	Spiked Amel-i	Spiked Amel-ii	Spiked Amel-iii	Spiked Amel-iv	Spiked Amel-v
NAA	1	1.5	0.5	0.5	3
IBA	12	10	5	2	1

Cytokinins and Analogs

Compounds useful according to the present disclosure include meta-topolin analogues having a general formula



wherein W is an aryl or heteroaryl;

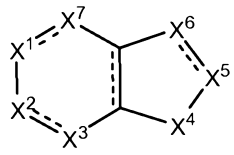
R1 is substituted or unsubstituted alkyl wherein any C in the alkyl can be substituted with O, N or S;

each R2 is independently H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, cyano, C1-C6 alkyloxy, aryl or heteroaryl each optionally substituted with a C1-C6 alkyl, SH, NHR3, CO2R3 or halogen;

R3 is H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, carboxylic group, ester group, aldehyde or cyano;

r is 0 to 8.

In some embodiments, W is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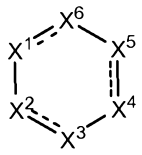
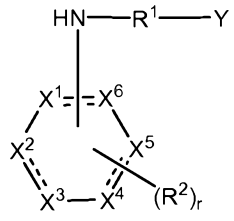


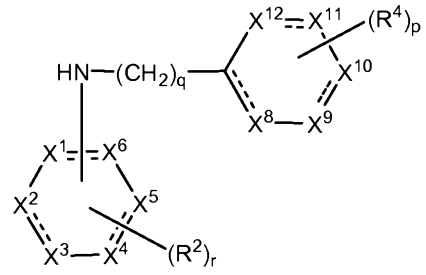
wherein a dashed line represents the presence or absence of a bond;

X1-X^7 is each independently selected from C, N, O, S with the proviso that the X linking the ring to N is C.

In some embodiments, the compounds have a structure,


. wherein a dashed line represents the presence or absence of a bond.

In some embodiments, the compounds have a structure,



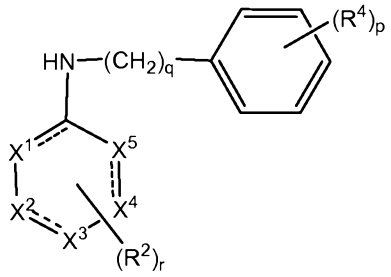
wherein a dashed line represents the presence or absence of a bond;

X8-X12 is each independently selected from C, N, O, S;

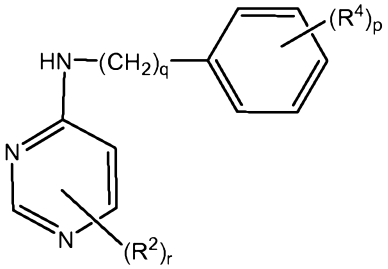
each R4 is independently H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, cyano, C1-C6 alkyloxy, aryl or heteroaryl each optionally substituted with a C1-C6 alkyl, SH, NHR3, CO2R3 or halogen;

R3 is H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, carboxylic group, ester group, aldehyde or cyano; p is 0 to 5; and q is 0 to 6.

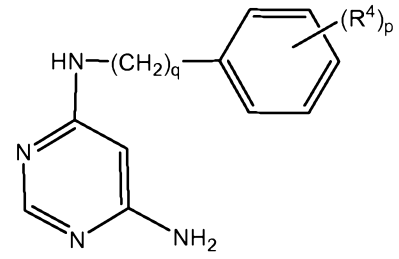
In some embodiments, the compounds have a structure,



In some embodiments, the compounds have a structure,



Further still, compounds can have structures selected from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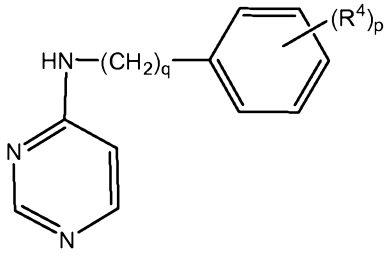
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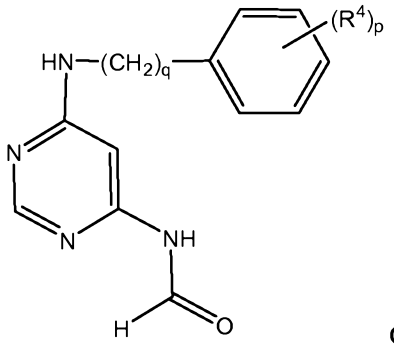
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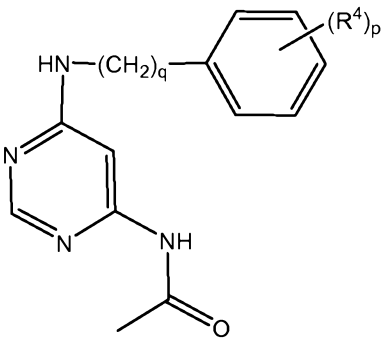
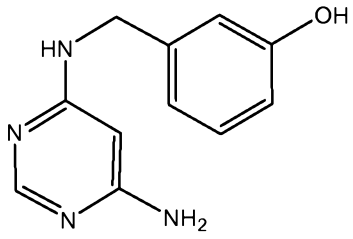

.

In one embodiments, R4 is OH.

In some embodiments, compounds have a structure selected from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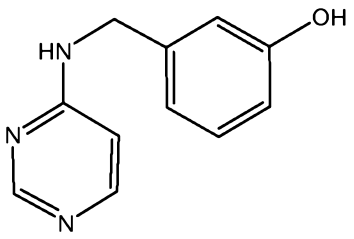
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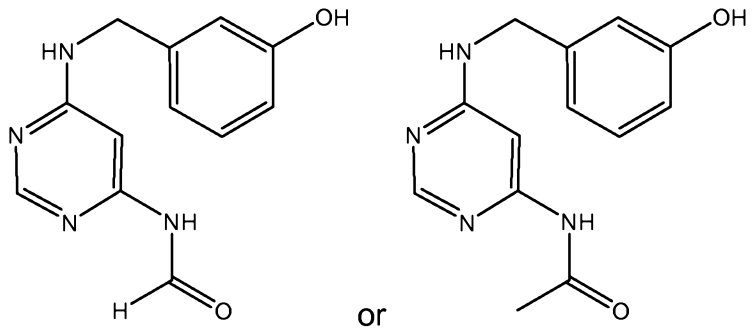
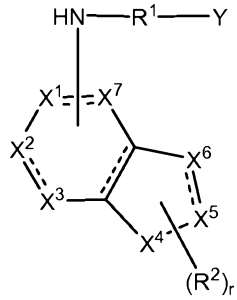
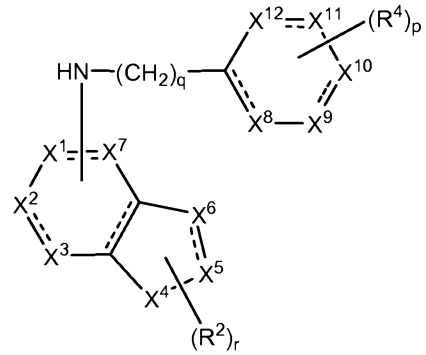


In some embodiments, the compounds have a structure,



wherein a dashed line represents the presence or absence of a bond.

In another embodiment, the compounds have a structure



wherein a dashed line represents the presence or absence of a bond;

X8-X12 is each independently selected from C, N, O, S;

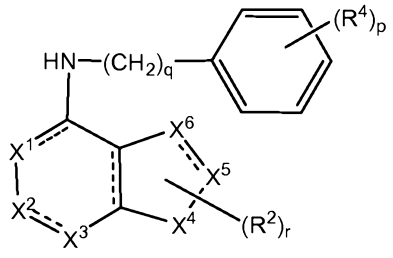
each R4 is independently H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, cyano, C1-C6 alkyloxy, aryl or heteroaryl each optionally substituted with a C1-C6 alkyl, SH, NHR3, CO2R3 or halogen;

R3 is H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, carboxylic group, ester group, aldehyde or cyano;

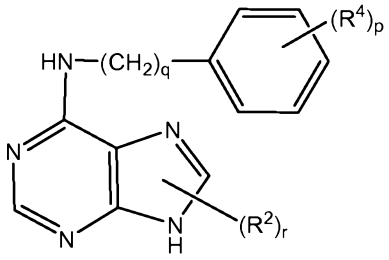
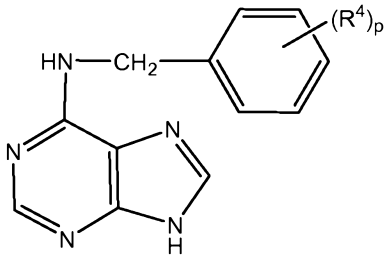
p is 0 to 5; and

q is 0 to 6.

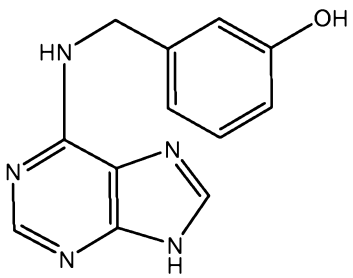
In some embodiments, the compounds have a structure



In still some embodiments, the compounds have a structure


or

.

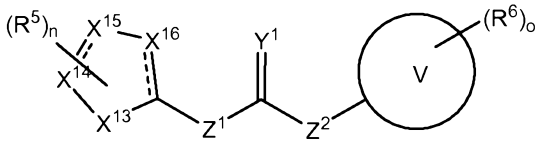
In some embodiments, the compound is meta-topolin, also known as 6-(3-hydroxybenzylamino)-purine, and by the abbreviation mT, having a empirical formula of C12H10N5OH, a molecular weight of 241.25, and the following structural formula:



wherein said meta-topolin is a derivative of a willow tree or a poplar tree.

Meta-topolin analogues particularly include, without limitation, meta-topolin riboside, meta-topolin-9-glucoside, ortho-topolin, ortho-topolin riboside, ortho-topolin-9-glucoside, para-topolin, para-topolin riboside, para-topolin-9-glucoside, ortho-methoxytopolin, ortho-methoxytopolin riboside, meta-methoxytopolin, meta-methoxytopolin riboside and meta-methoxytopolin-9-glucoside. In particular embodiments, referred to herein as "mT limited embodiments", 6-(3-fluorobenzylamino)purine (FmT), 6-(3-flurobenzylamino)purine-9-riboside (FmTR) and/or 6-(3-methoxybenzylamino)purine-9-riboside (memTR) can be excluded from the class of meta-topolin analogs.

Compounds useful according to the present disclosure include thidiazuron analogues having a general formula



wherein V is an aryl or heteroaryl;

each R5 and R6 is each independently H, OH, C1-C6 alkyl, C1-C6 alkylene, C1-C6 alkynyl, halogen, cyano, C1-C6 alkyloxy, aryl or heteroaryl each optionally substituted with a C1-C6 alkyl or halogen;

n is 0 to 4;

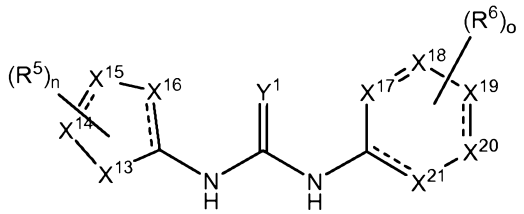
o is 0 to 5

X13-X16 is each independently selected from C, N, O, S;

Z1 and Z2 are each independently NH, O, SH or CH or Z1 and Z2 can be combined to form a substituted or unsubstituted aryl or heteroaryl; and

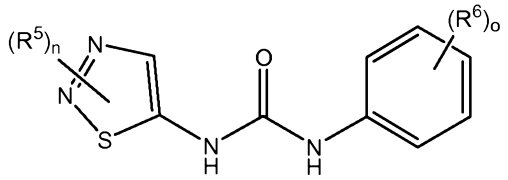
Y1 is O or S.

In another embodiment, compounds have a structure



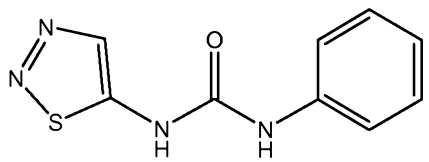
wherein X17-X21 is each independently selected from C, N, O, S.

In other embodiments, compounds include



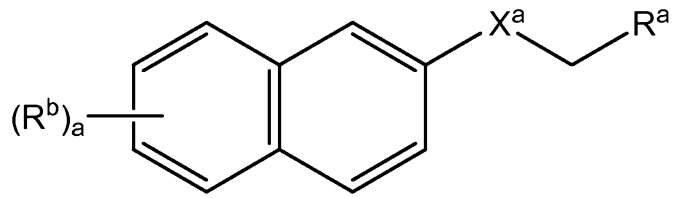
.

In one embodiment, the compound is thidiazuron, also known as 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea and 5-phenylcarbamoylamino-1,2,3-thiadiazole, has the empirical formula of C9H8N4OS, a molecular weight of 220.25 and the following structural formula



.

Compounds useful according to the present disclosure include B-naphthoxyacetic analogues having a general formula:



or a salt thereof;

wherein Ra is COR3, CO2R3, CONR3R4, or CN;

each Rb is independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

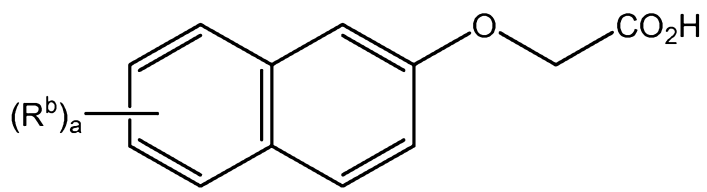
a is 1, 2, 3, 4, 5, 6, or 7;

Xa is NH, S or O;

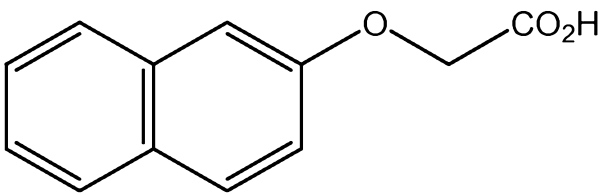
each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

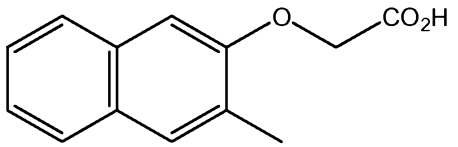
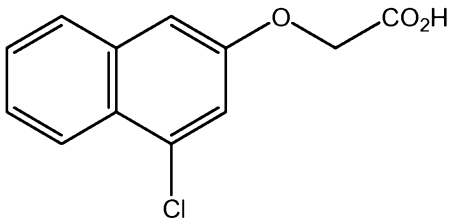
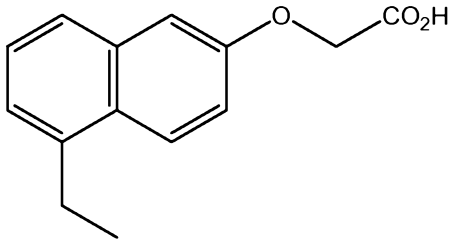
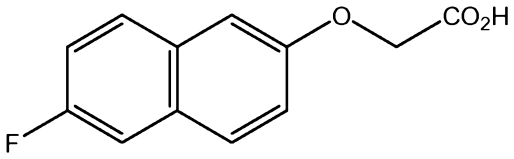
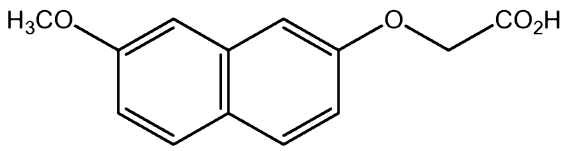
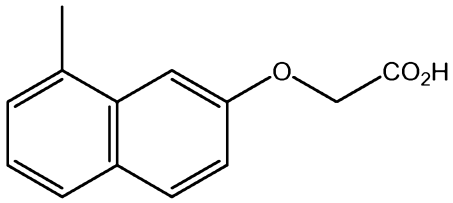
In another embodiment, compounds have a structure:



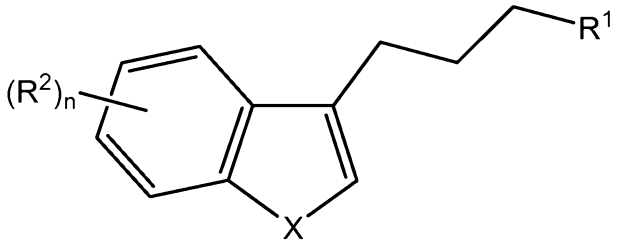
In one embodiment, the compound is B-naphthoxyacetic acid (NAA), also known as acetic acid, (2-naphthalenoxy)-(9CI) and has a CAS Number of 120-23-0, has the empirical formula of C12H10O3, a molecular weight of 202.21 and the following structural formula:



Other examples of NAA analogues may include, but are not limited to:

Compounds useful according to the present disclosure include indole butyric acid (IBA) analogues having a general formula:



or a salt thereof;

wherein R1 is COR3, CO2R3, CONR3R4, or CN;

each R2 is independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

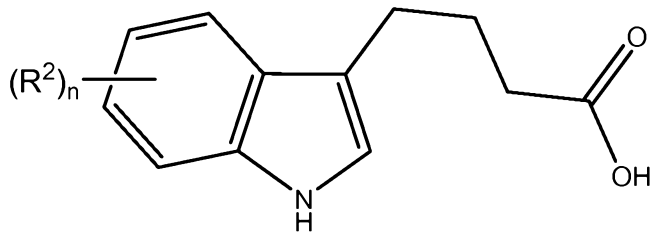
n is 1, 2, 3, or 4;

X is NH, S or O;

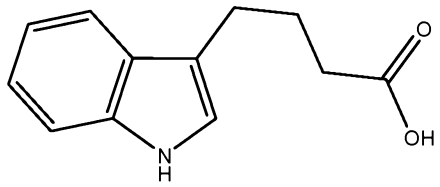
each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

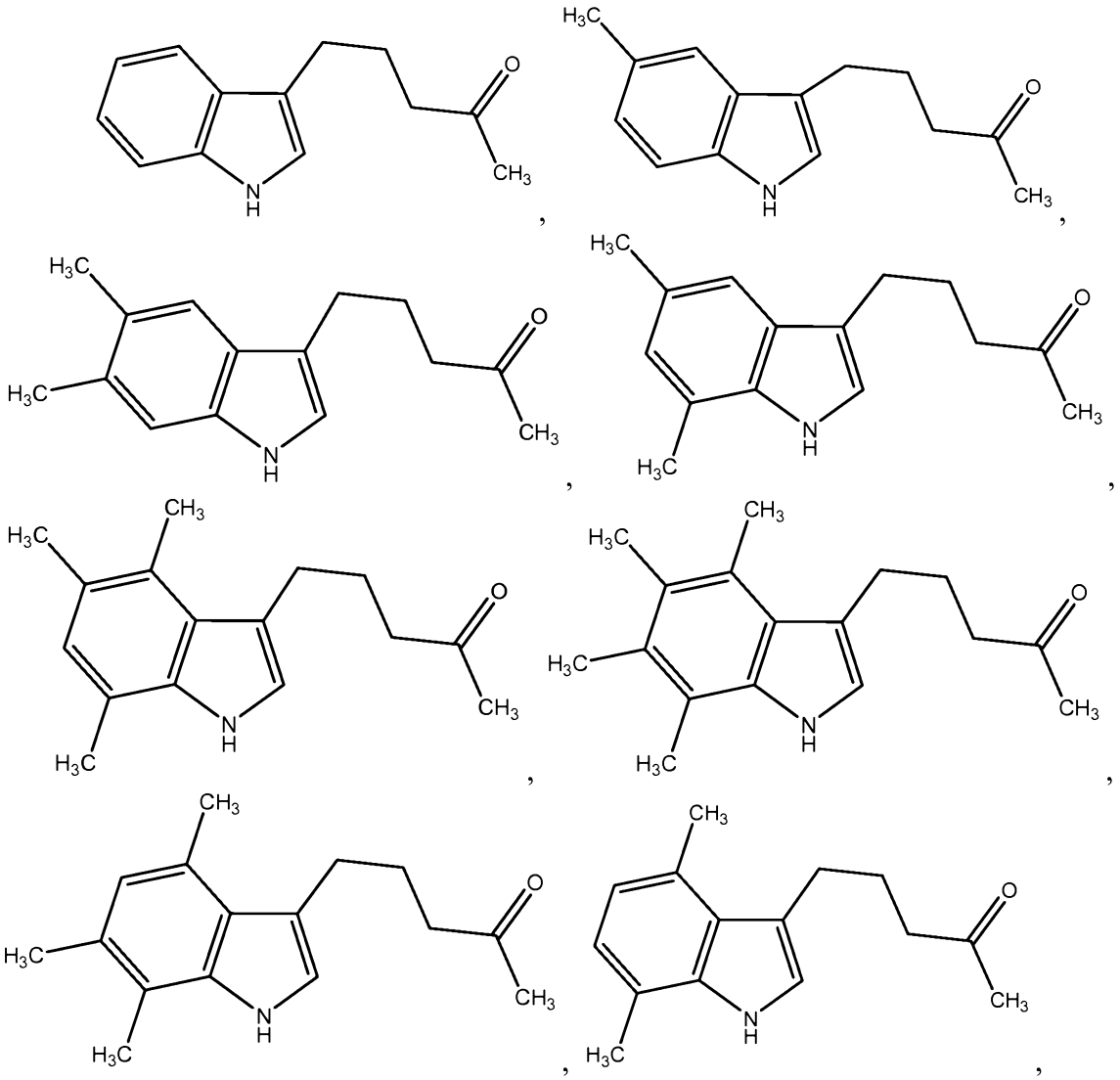
In another embodiment, compounds have a structure:

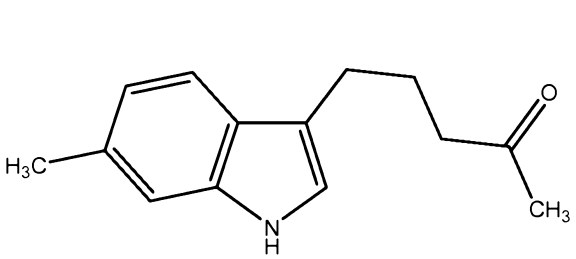


In one embodiment, the compound is indole butyric acid (IBA), also known as 1-Indole-3-butanoic acid, and has a CAS Number of 133-32-4, has the empirical formula of C12H13NO2, a molecular weight of 203.24, and the following structural formula:



Other examples of IBA analogues may include, but are not limited to:

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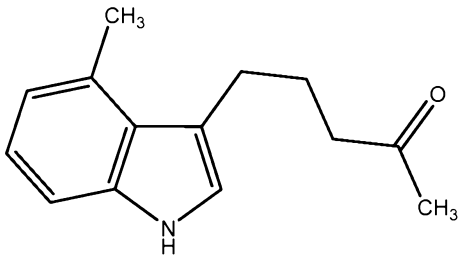
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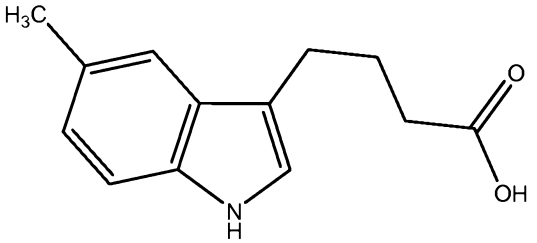
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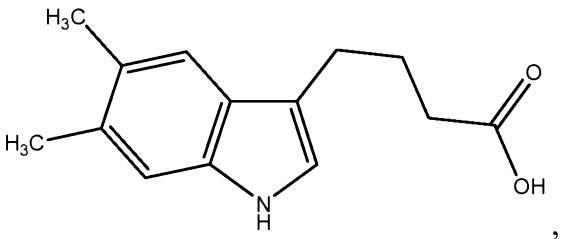
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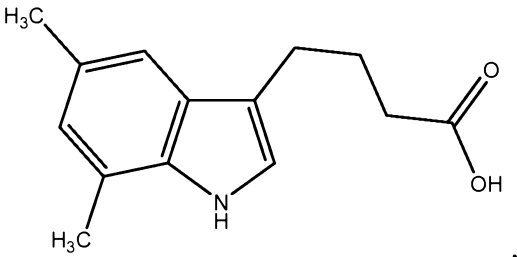
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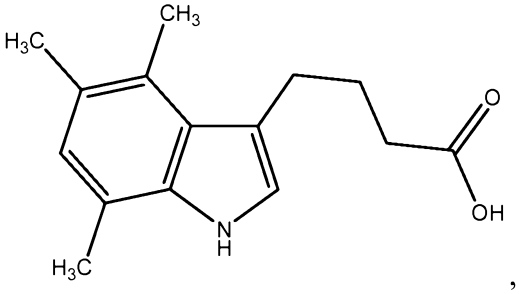
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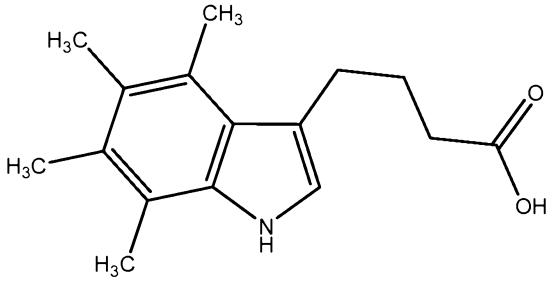
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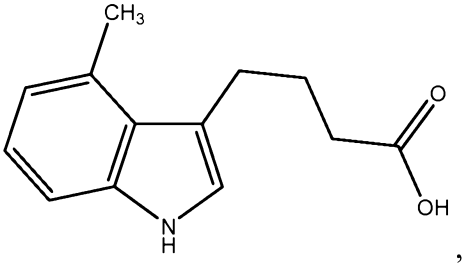
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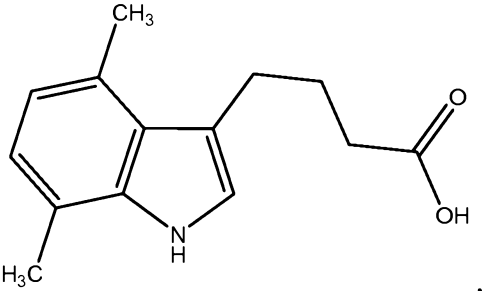
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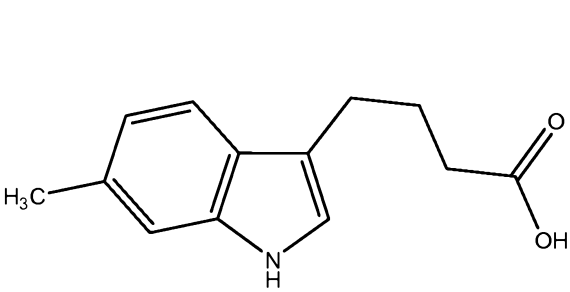
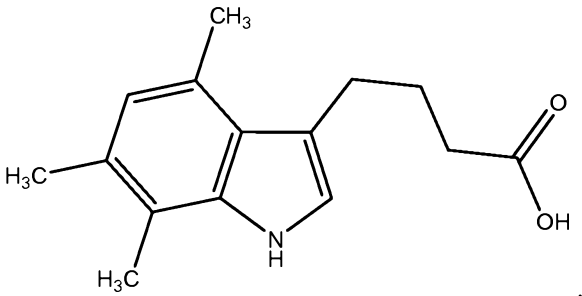

, and

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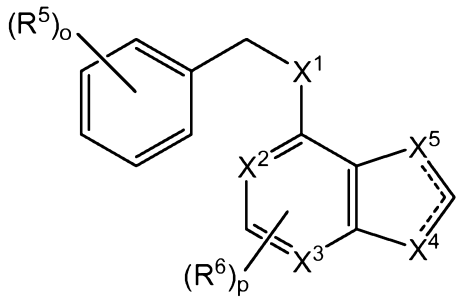
Compounds useful according to the present disclosure include benzylaminopurine (BAP) analogues having a general formula:



or a salt thereof;

wherein a dashed line represents the presence or absence of a bond;

each R5 and each R6 is independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

o is 0, 1, 2, 3, 4, or 5;

p is 0, 1, or 2;

X1 is NH, S or O;

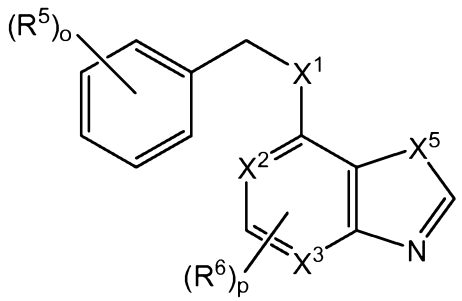
X4 is -N= and X5 is –NH-, -S-, or –O-; or X5 is -N= and X4 is –NH-, -S-, or –O-;

X2 and X3 and are independently N or CR6;

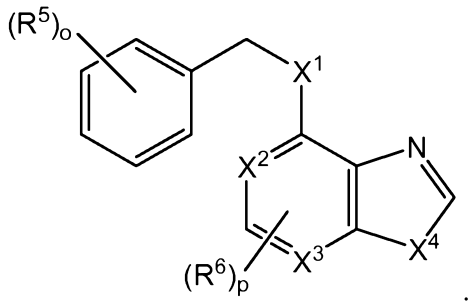
each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

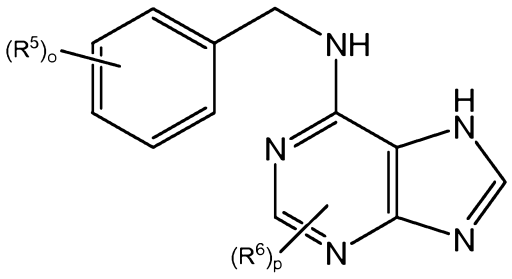
In some embodiments, X4 is -N= and X5 is –NH-, -S-, or –O-, and the dashed line represents the presence or absence of a bond. Thus, compounds of according to the formula below are contemplated.



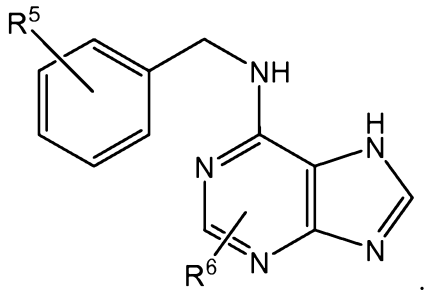
In other embodiments, X5 is -N= and X4 is –NH-, -S-, or –O-. Thus, compounds of the formula below are contemplated.



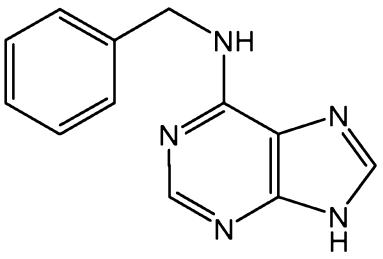
In another embodiment, the compounds have a structure:



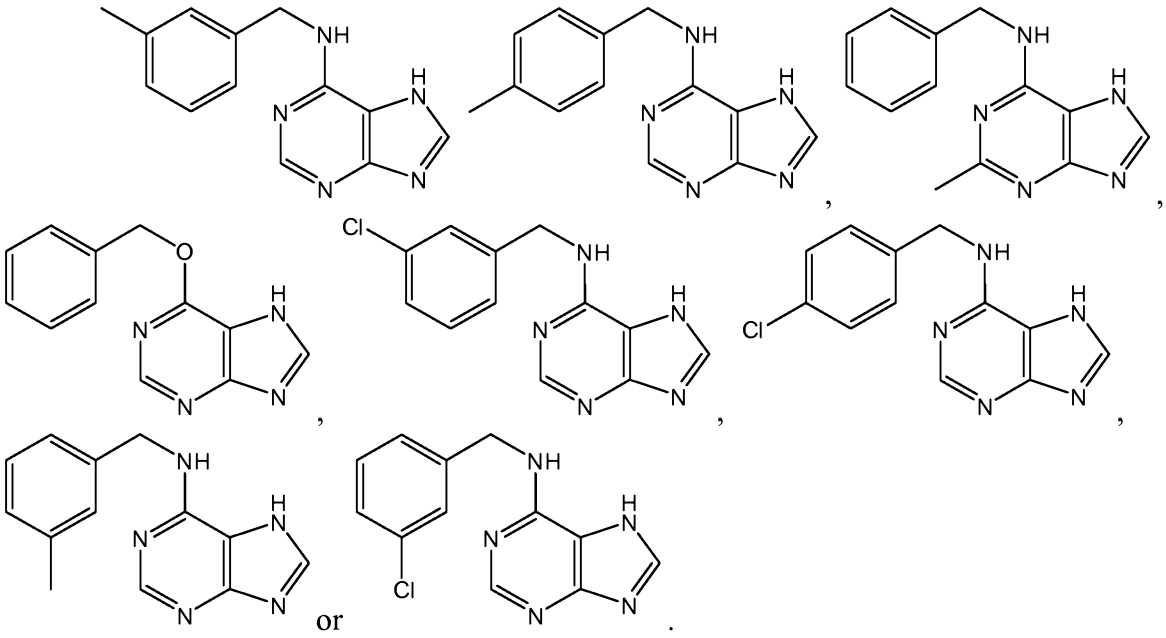
In another embodiment, the compounds have a structure:



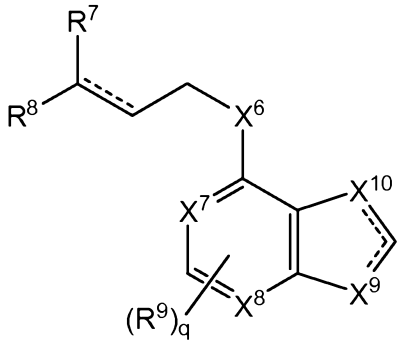
In one embodiment, the compound is benzylaminopurine (BAP), also known as 9H-Purin-6-amine, N-(phenylmethyl)-, which has a CAS Number of 1214-39-7, an empirical formula of C12H11N5, a molecular weight of 225.25, and the following structural formula:



Other examples of BAP analogues may include, but are not limited to:



Compounds useful according to the present disclosure include 6-y-y-(dimethylallylamino)-purine (2ip) analogues having a general formula:



or a salt thereof;

wherein a dashed line represents the presence or absence of a bond;

wherein R7, R8, and each R9 are independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

q is 0, 1, or 2;

X6 is NH, S or O;

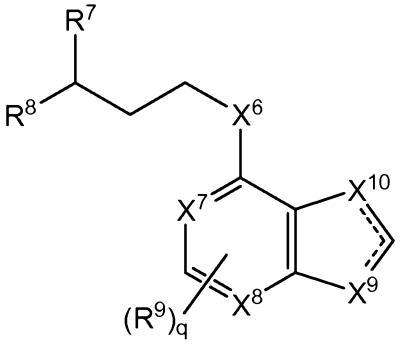
X9 is -N= and X10 is –NH-, -S-, or –O-; or X10 is -N= and X9 is –NH-, -S-, or –O-;

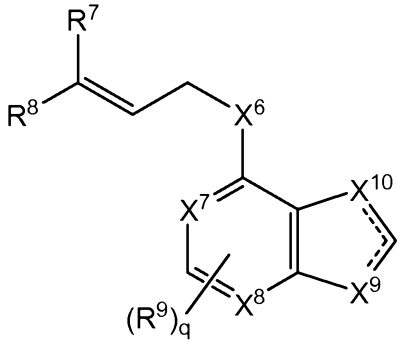
X7 and X8 and are independently N or CR9; and

each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

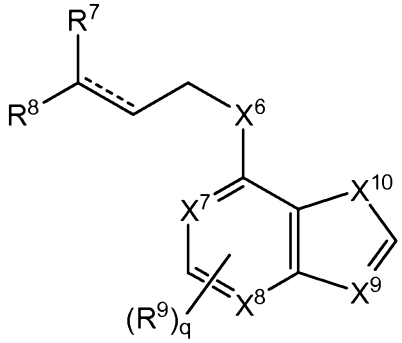
each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

In some embodiments, the dashed line represents the presence or absence of a bond. Thus, compounds of according to the formulas below are contemplated.

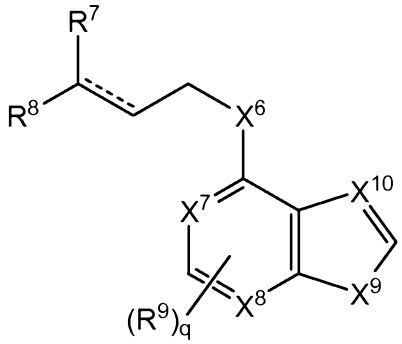




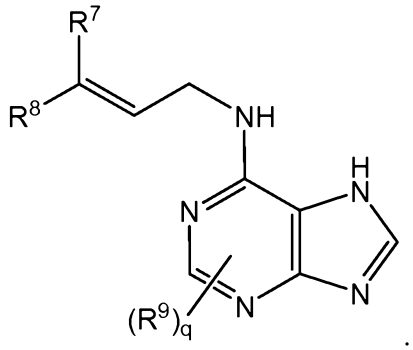
In some embodiments X9 is -N= and X10 is –NH-, -S-, or –O-. Thus, compounds according the formula below are contemplated.



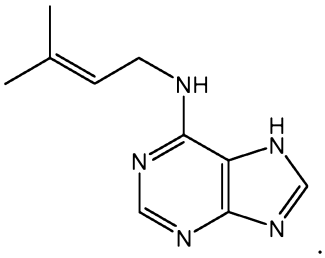
In other embodiments, X10 is -N= and X9 is –NH-, -S-, or –O-. Thus, compounds having the structure shown below are contemplated.



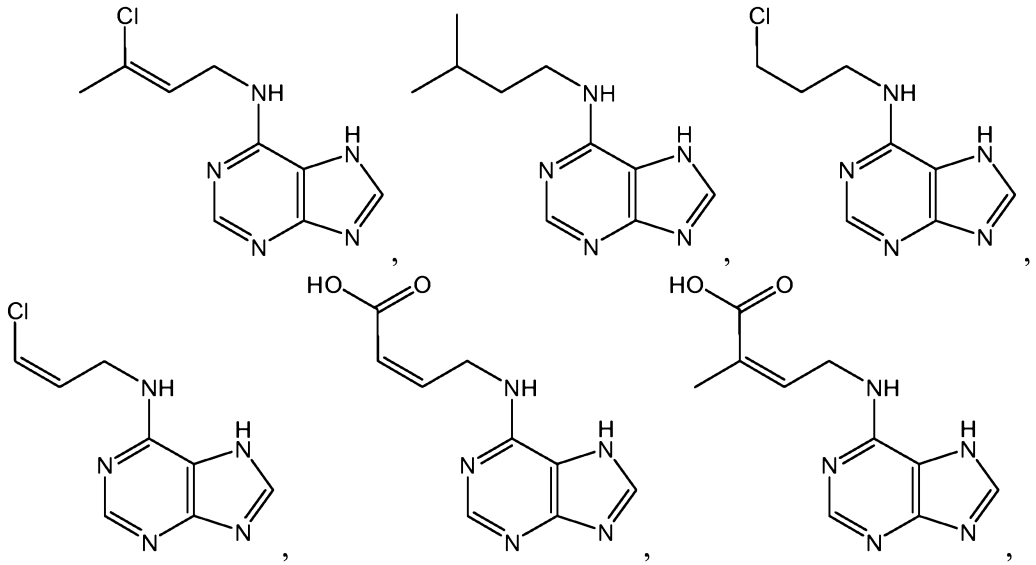
In another embodiment, compounds have a structure:

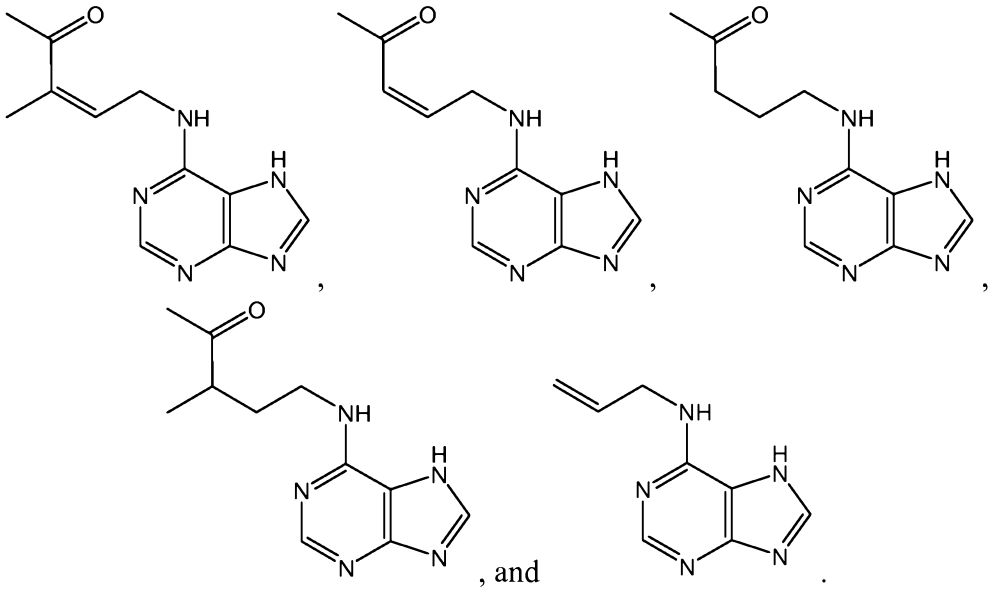


In one embodiment, the compound is 6-y,y,-(dimethylallylamino)-purine (2ip) or DAP, also known as 9H-purin-6-amine, N-(3-methyl-2-butene-1-yl)-, having a CAS No. 2365-40-4, an empirical formula of C10H13N5, a molecular weight of 203.24, and the following structural formula:

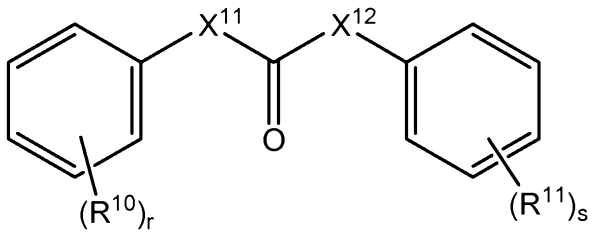


Other examples of 2ip analogues may include, but are not limited to:





Compounds useful according to the present disclosure include N,N-diphenylurea (DPU) analogues having a general formula:



or a salt thereof;

wherein each R10 and each R11 is independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

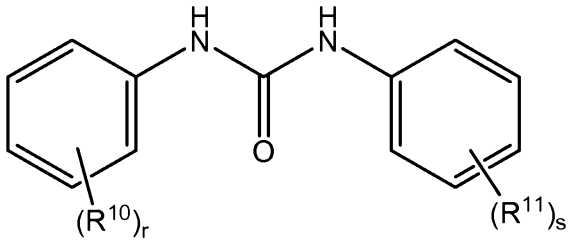
r and s are independently 0, 1, 2, 3, 4, or 5;

X11 and X12 are independently NR10, S, or O;

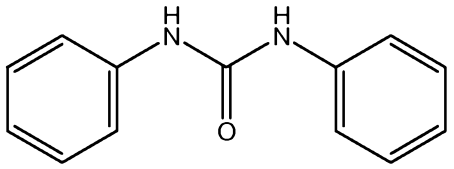
each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

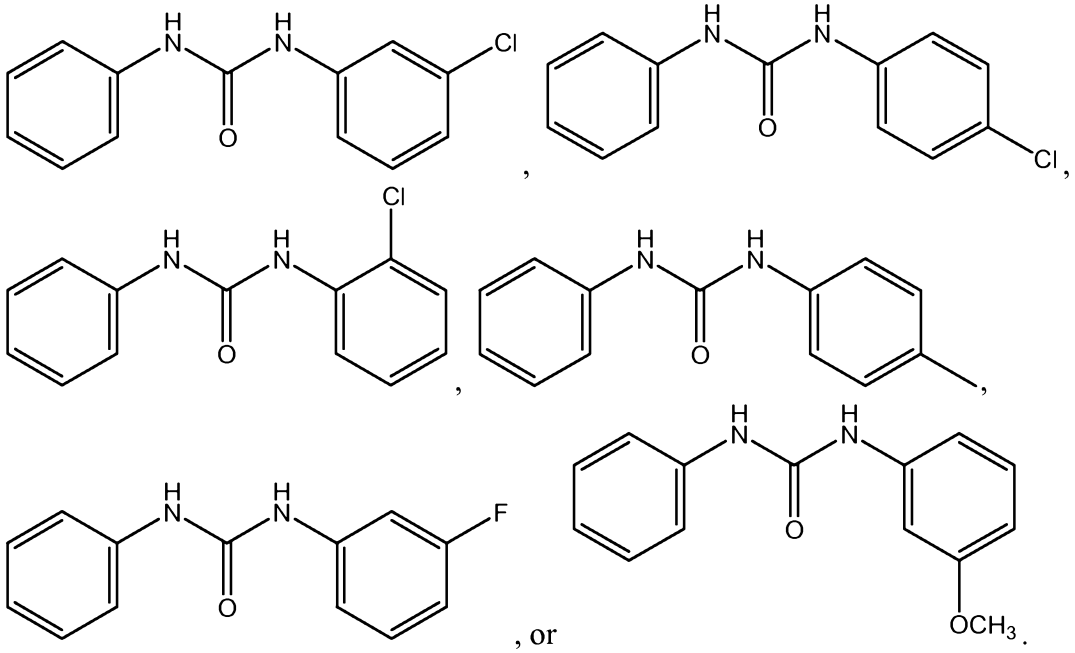
In another embodiment, compounds have a structure:



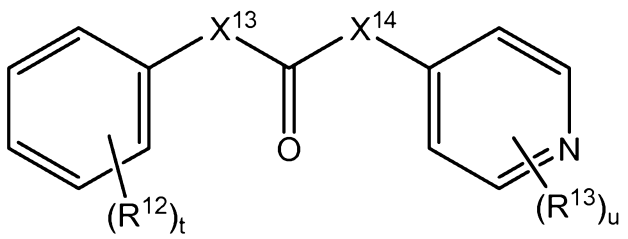
In one embodiment, the compound is N,N-diphenylurea (DPU), which is represented by a formula:



Other examples of DPU analogues may include, but are not limited to:



Compounds useful according to the present disclosure include N-pyridinyl-N'-phenylurea (PPU used interchangeably with CPPU herein) analogues having a general formula:



or a salt thereof;

wherein each R12 and each R13 is independently R3; OR3; F; Cl; Br; I; CN; NO2; OCF3; CF3; NR2R3; SR3, SOR3, SO2R3, CO2R3, COR3, CONR3R4, CSNR4R5; or optionally substituted aryl or optionally substituted heteroaryl, wherein each substituent of aryl or heteroaryl is independently C1-C6 alkyl, F, Cl, Br, or I;

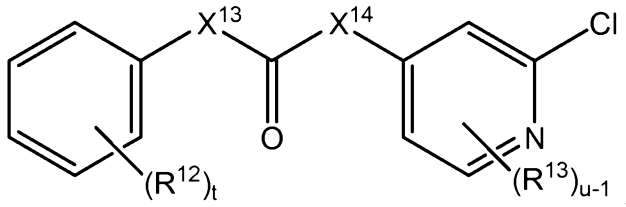
t and u are independently 0, 1, 2, 3, 4, or 5;

X13 and X14 are independently NR12, S, or O;

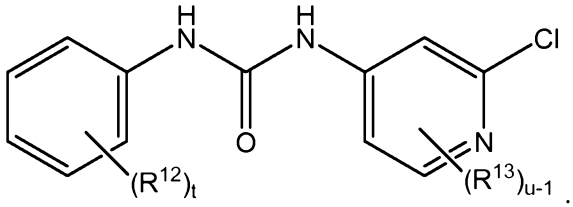
each R3 is independently H, C1-C6 alkyl, C2-C6 alkenyl, or C2-C6 alkynyl; and

each R4 is independently R3 or optionally substituted phenyl, wherein each substituent of phenyl is independently C1-C6 alkyl, F, Cl, Br, or I.

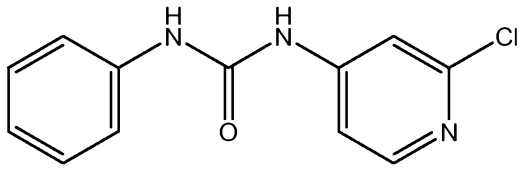
In one embodiment, compounds have a structure:



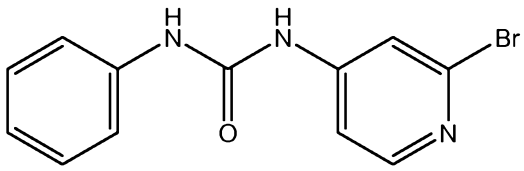
In another embodiment, compounds have a structure:



In one embodiment, the compound is N-(2-chloropyridin-4-yl)-N'-phenylurea, which is represented by a formula:



Other examples of PPU analogues may include, but are not limited to: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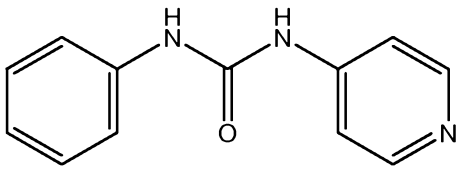
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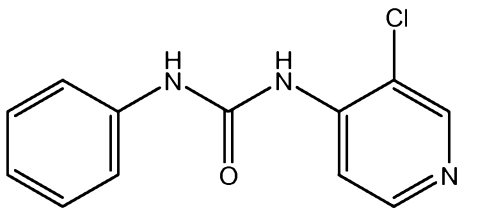
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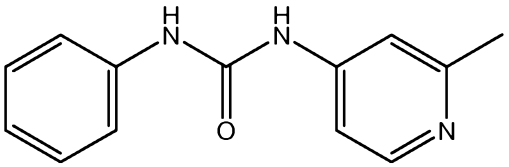
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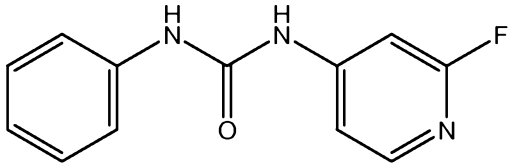
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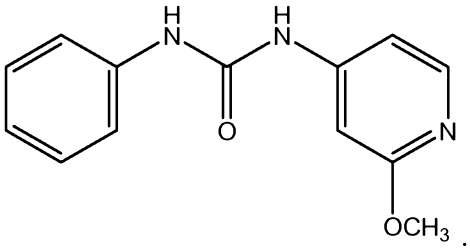
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Plant Propagation System

Bioreactors offer a promising way of scaling-up micropropagation processes, making it possible to work in large containers with a high degree of control over culture parameters (e.g., pH, aeration, oxygen, carbon dioxide, hormones, nutrients, etc.). Bioreactors are also compatible with the automation of micropropagation procedures, utilizing artificial intelligence, which reduces production costs.

Previously bioreactors have been mostly applied to microbial technology, cell culture, and somatic embryogenesis and prior to the present invention, bioreactors for plant micropropagation were rare, complicated, and expensive.

To solve these problems, the present invention provides novel compositions, methods, and systems for the micropropagation of plants using a bioreactor. Without wishing to be bound by any theory, the present invention achieves greatly improved plant micropropagation through controlling/reducing exposure of the shoots/plantlets to toxic components that build-up in the growth compositions/environment (e.g., certain plant hormones, such as TDZ) and/or are produced as by-products of plant growth (e.g., phenolics).

A plant propagation system 100 according to an embodiment is schematically illustrated in FIG. 1. The system 100 is configured for large scale multiplication of plants. In some embodiments, the system 100 is used for large scale multiplication of monocot plants. In some embodiments, the system 100 is used for large scale multiplication of bamboo. The

system 100 includes a growth vessel 110, a first media container 130, a second media container 150, a gas source 170, and a controller 190.

The growth vessel 110 is configured to incubate plant tissue in a sterile or substantially sterile environment. The growth vessel 110 may be any suitable container capable of providing a sterile or substantially sterile environment for the plant tissue and nutrient media. The growth vessel 110 may further be of any suitable material and any desirable shape. For example, the growth vessel 110 may be transparent to permit visual observation and light stimulation of the plant tissue, and may be constructed to reduce shear forces on the incubated tissue.

In some embodiments, the growth vessel 110 comprises one or more type of light source suitable for plant growth. Alternatively, the growth vessel is transparent to permit light stimulation provided outside of the growth vessel 110.

In some embodiments, the growth vessel 110 is connected to a gas source. In some embodiments, the gas source provides carbon dioxide, oxygen, nitrogen, or combinations thereof. In some embodiments, the provided gas or mixture of gas is sterile or substantially sterile. The ratio of the gas mixture provided to the growth vessel 110 can be predetermined readily controlled depending on any of a variety of factors including, for example, the type of plants being grown in the plant propagation system 100.

The first media container 130 and second media container 150 are configured to contain a liquid and a gas and are each fluidically coupleable to both the growth vessel 110 and the gas source 170. Additional media containers can be includes depending on any of a variety of factors including, for example, the type of plants being grown in the plant propagation system 100. The media containers 130, 150 may contain identical liquid media, or media that differs in content and/or composition. The media containers 130, 150 can be fluidically coupled to the growth vessel 110 in any suitable manner. For example, in some embodiments, the growth vessel 110 can have multiple fluid exchange ports (not shown), and each media container 130, 150 can be coupled to a separate media exchange port. Each connection may be direct and continuous, or include a controllable valve (e.g., manual or under electronic control of the controller 190). In some embodiments, the growth vessel 110 can have a single fluid exchange port (not shown) for connecting all media containers 130, 150, and a manifold (not shown) to control exchange of liquid media between the media containers 130, 150 and the growth vessel 110. The manifold can include any number of fluid communicators and valves (manual, electronically actuated, hydraulically actuated, etc.)

to control liquid exchange between the media containers 130, 150 and the growth vessel 110. In some embodiments, the growth vessel 110 can have multiple fluid exchange ports (not shown) for connecting each media container.

The gas source 170 can be any device or system suitable for delivering pressurized gas to the media containers 130, 150. The gas source 170 can include one or more of, but is not limited to, compressed tanks of gas and gas pumps. Any number of gas sources 170 may be employed. For example, in some embodiments, each media container 130, 150 may be connected to a different gas source 170. In some embodiments, a manifold can be used to connect the gas source 170 to the media containers 130, 150. The manifold can include any number of fluid communicators and valves (manual, electronically actuated, hydraulically actuated, etc.) to control the pressurized gas supply to the media containers 130, 150. The manifold and valves can be coupled to the controller 190 to allow automated and/or electronic control of the gas supply to the medial containers 130, 150. The gas employed by the gas source 170 may be any gas that does not compromise the liquid media in the media containers 130, 150. Examples of such gases include any inert gas, oxygen, nitrogen, carbon dioxide, gas of an atmospheric composition, and combinations thereof.

The gas source 170 is operable to change the gas pressure in the media containers 130, 150 by delivering a mass of pressurized gas to one or both of the medial containers 130, 150. In some embodiments, the gas source 170 is configured to deliver pressurized gas to the first media container 130 or the second media container 150 to raise the gas pressure in the first media container 130 or the second media container 150 up to about 1 pound per square inch (psi), e.g., about 0.7 psi, about 0.8 psi, about 0.9 psi, about 1 psi, about 1.1 psi, or about 1.2 psi. The increased gas pressure in the first media container 130 or the second media 150 causes at least a portion of the liquid media contained in the first media container 130 to be displaced from the first media container 130 to the growth vessel 110, or at least a portion of the liquid media contained in the second media container 150 to be displaced from the second media container 150 to the growth vessel 110. The gas source 170 can be deactivated or isolated to allow the gas pressure in the first media container 130 or the second media container 150 to return to its original (e.g., atmospheric) value. As a result, the displaced portion of the liquid media in the growth vessel 110 returns back to the first or the second media container. The combination of pressurization and deactivation of the gas source 170 results in "pulsing" of the first media and/or the second media contacting the plant tissue in the growth vessel 110.

In some embodiments, during the deactivation stage, media container equalizes pressure, and automatic siphoning drainage begins, emptying media back to original media container. In some embodiments, the siphoning drainage rate is about 500 to 1000 mls/minute, such as 600-720 mls/minute.

In some embodiments, during the drainage, the plants are partially submerged in media for about 2 to about 4 minutes, such as about 2.5 to about 3 minutes.

In some embodiments, after the media is drained, and before the next media comes into the vessel, the plants inside the vessels are dried for a predetermined time. In some embodiments, the plants are dried for about 3-6 minutes, for example, about 5 minutes.

The pulsing process can be repeated for the first media container 130 or the second media container 150 for any number of cycles depending on, for example, the type of plants being grown in the plant propagation system 100. For example, the repeated cycles in total take about half hour to about three weeks, e.g., about 24 hours to about 60 hours, or about 24 hours to about four weeks., e.g., about three days to about five days. In some embodiments, the first media container holds a bud induction medium that includes the plant hormone TDZ, and the second media container hold a shoot elongation/maintainence medium that does not include TDZ.

In some embodiments, the pulsing process can be alternated between the first media container 130 and the second media container 150. In some embodiments, the pulsing process can be repeated for the first media container 130 for a predetermined number of cycles and then switched to the second media container for a predetermined number of cycles. In some embodiments, the pulsing process can be repeated and alternated between the first media container 130 and the second media container 150 according to any of a variety of predetermined patterns depending on, for example, the type of plants being grown in the plant propagation system 100. For example, the pulsing process can repeated for the first media for about half hour to about three weeks, e.g., about 24 hours to about 60 hours, and then repeated for the second media for about 24 hours to about four weeks., e.g., about three days to about five days.

Operation of the plant propagation system 100 is controlled either manually, or by the controller 190 which may be a processor, a computing device, or any programmable/configurable device or system as is known in the art. The controller 190 is configured for electronically control of the gas source 170, and controls at least activation and deactivation of the gas source 170. In some embodiments, the controller 190 is

configured for electronic control of a manifold that connects the gas source 170 to each of the media containers 130, 150 to enable selection of one of the media containers. In some embodiments, the controller 190 is configured for electronic control of a manifold that connects the media containers 130, 150 with the growth vessel 110 to enable control of liquid flow between the media containers 130, 150 with the growth vessel 110. Furthermore, and not inconsistent with various embodiments and combinations thereof, the controller 190 may be connected to, and configured for control of, multiple gas sources, multiple manifolds, and/or multiple valves. Control of other aspects of the system 100 not illustrated herein (e.g., control of a gas exchange system, a temperature control system, etc.) are within the scope of this invention.

The controller 190 is operable in a first operating mode in which it causes the gas source 170 to deliver pressurized gas to the first media container 130 to displace a first volume of liquid contained therein to the growth vessel 110. Additionally, the controller 190 is operable in a second operating mode in which it causes the gas source 170 to deliver pressurized gas to the second media container 150 to displace a second volume of liquid contained therein to the growth vessel 110. In some embodiments, the first and second operating modes are run for a predetermined time. In some embodiments, the first and second operating modes are run for about one minute ± half minute, e.g., about 30 seconds, about 40 seconds, about 50 seconds, about 60 seconds, about 70 seconds, about 80 seconds, or about 90 seconds. As described above, the first and second operating modes can be repeated and/or alternated according to a predetermined pattern.

Additionally, after the first operating mode or second operating mode has been executed (i.e., the growth vessel 110 contains liquid from one of the media containers 130, 150), the controller 190 is operable to be run in a third operating mode. In the third operating mode, the liquid in the growth vessel 110 is allowed to return to its respective media container, for example, by deactivating the gas source 170. In some embodiments, the third operating mode is run for a predetermined time. In some embodiments, the third operating mode is run for about eight minutes, e.g., about 7 minutes, 7.5 minutes, 8 minutes, 8.5 minutes, etc.

In some embodiments, the controller 190 is operable to run a first incubation sequence comprising one or more cycles of the first operating mode-third operating mode sequence. In some embodiments, the first incubation sequence is run from about 1 hour to about 3 weeks, e.g., from about 24 hours to about 60 hours. The controller 190 is also operable to run a

second incubation sequence comprising one or more cycles of the second operating mode-third operating mode sequence. In some embodiments, the second incubation sequence is run from about 24 hours to about 4 weeks, and e.g., 3 days to about 5 days.

In some embodiments, the controller 190 is operable to run a plant propagation sequence comprising one or more cycles of the first incubation sequence each followed by the second incubation sequence. The number of cycles of the plant propagation sequence may range from one to eight, or more.

In some embodiments, one, or more, or all of the parts of the growth system can be sterilized by any known methods, such as autoclaving.

In some embodiments, the media is driven into or out of the growth vessel by gas pressure. In some embodiments, the media is driven into or out of the growth vessel by other forces, such as gravity, electricity, etc.

Referring now to FIGS. 2-5C, an exemplary embodiment of a plant propagation system 200 is shown. The system 200 is similar in operation to the system 100 described above, thus unless stated otherwise, various components of the system 200 may be of similar design and function to that of other embodiments. For example, the growth vessel 210 may be similar to the growth vessel 110.

The illustrated embodiment of FIG. 2 includes a growth vessel 210, a manifold 240, a first media container 230, a second media container 250, a gas pump 270 as the gas source, and a timer-controlled circuit 290 as the controller. The single gas source 270 is attached to the first media container 230, and may be removed and reattached to the second media container 250. Advantageously, the use of a filter 252 (see FIG. 3 also) for each media container 230, 250 prevents any potential contamination of the liquid media in the media containers by the air pump 270, even upon switching. Alternatively, two gas sources can be used, with each source attached to media container 230 or 250, under the control of the timer-controlled circuit 290, so no reattachment is needed. As illustrated in FIG. 3, each media container 230, 250 has a first fluid port 232 in fluid communication or otherwise coupleable with port 222 of the growth vessel 210 to enable exchange of fluid between the media containers and the growth vessel. Each media container 230, 250 also has a second fluid port 236 in fluid communication or otherwise coupleable with the gas source 270 to change the gas pressure inside the media container. The ports 232 and 236 are formed on an adapter 238 that seals the media container to prevent contamination, a bulkhead adapter for example. As illustrated, the second fluid port 236 is additionally fitted with a filter 252 (e.g. a vent filter

with stepped hose barbs) to prevent contamination of the fluid in the media container during gas exchange with the gas pump 270.

FIGS. 2 and 4 illustrate a non-limiting design of a manifold 240 that connects to tubing 242 and 244 from the media containers 230, 250, respectively. FIG. 4 illustrates valves 246 and 248 formed on manifold 240 for controlling flow from each tubing 242, 244, respectively. Any suitable 2-way valve may be employed such as, for example, ball valves, gate valves, butterfly valves, etc. Valves 246, 248 may be under electronic control of the timer 290, and/or under manual control. In the setup illustrated in FIG. 2, valve 246 is open to fluidly couple the growth vessel 210 and the first media container 230. Valve 248 is closed to fluidly isolate the second media container 250 from the growth vessel 210 as well as the first media container 230. In this manner, intermixing of fluids between the first media container 230 and the second media container 250 is prevented.

Control of the gas pump 270 may be achieved by switching on/off the power supply of the gas pump by the timer-controlled circuit 290. In an embodiment, the gas pump is a 1 psi pump which when powered by the circuit 290 (during the first or second operating mode, for example), pumps gas into the connected first media container 230 to increase the pressure to 1 psi. The gas pump 270 can be deactivated or otherwise turned off when circuit 290 shuts off the power to the gas pump 270 (during the third operating mode, for example), thereby allowing the pressure in the first media container to equalize by allowing the pumped gas to flow back into the gas pump.

As illustrated in FIGS. 5A-C, the growth vessel 210 includes a closure 212 for accessing the interior of the growth vessel, and a handle 216 for ease of transportation. Though illustrated as formed on a front portion, the closure 212 and the handle 216 may be formed on any other part of the growth vessel 210. In an embodiment, the growth vessel 210 is an ebb and flow bioreactor.

The growth vessel 210 can also have a gas exchange port 220 and a fluid exchange port 222 formed on the growth vessel, although any number of gas exchange ports and fluid exchange ports are within the scope of the invention. The ports 220 and 222 are fitted with adapters for enabling fluid communication with the interior of the growth vessel 210, while maintaining sterility. In an embodiment, the ports 220 and 222 are fitted with bulkhead adapters. The growth vessel 210 also includes a fluid conduit 226 attached to the port 222 for exchanging fluid with the interior of the growth vessel. The conduit 226 is of sufficient length and has a lumen of appropriate cross-section to enable siphoning of fluid from the

floor of the growth vessel 210 and into the selected media container 230 as is described in more detail below for the system 100 of FIG. 1.

In an embodiment, growth vessel 210 is used for large scale multiplication of plants. In some embodiments, growth vessel 210 is used for large scale multiplication of bamboo. In some embodiments, growth vessel further is used for pre-rooting and rooting the cultures. Referring again to the system 100 in FIG. 1, in use, the plant tissue to be incubated (e.g. bamboo tissue) is placed in the growth vessel 110, which is then placed at a height relative to the media containers 130, 150 to achieve the siphoning effect. First and second liquid media are placed in media containers 130, 150, respectively. In an embodiment, the first and second liquid media are different. The gas source 170 is connected to media containers 130, 150, and the controller 190 is connected to the gas source, and any other components requiring electronic control, as discussed.

Operation of an exemplary plant propagation sequence 300 of the controller 190 is described herein as illustrated in FIG. 6. While illustrated in a stepwise manner, it is noted that the order of execution of these steps need not necessarily follow so. At 302, controller 190 starts the plant propagation sequence. At 304, controller 190 starts the first incubation sequence. At 306, the controller 190 establishes fluid communication between the first media container 130 and the growth vessel 110. The controller 190 also establishes fluid communication between the gas source 170 and the first media container 130. The controller 190 also fluidically isolates the second media container 150 from the growth vessel 110. At 308, the controller enters the first operating mode and drives the gas source 170 to deliver pressurized gas to the first media container 130 to displace a first volume of liquid contained therein to the growth vessel 110. At 310, the controller then enters the third communication mode and allows at least a portion of the first volume of liquid in the growth vessel 110 to return to the first media container 130 by deactivating the gas source 170.

At 312, if the first incubation sequence is not complete, the controller 190 returns to 308, and enters the first operating mode again. If the first incubation sequence is complete, the controller, at 314, starts the second incubation sequence. At 316, the controller establishes fluid communication between the second media container 150 and the growth vessel 110. The controller also establishes fluid communication between the gas source 170 and the second media container 150. The controller also fluidically isolates the first media container 130 from the growth vessel 110. At 318, the controller enters the second operating mode and drives the gas source 170 to deliver pressurized gas to the second media container

150 to displace a second volume of liquid contained therein to the growth vessel 110. At 320, the controller then enters the third communication mode and allows at least a portion of the second volume of liquid in the growth vessel 110 to return to the second media container 150 by deactivating the gas source 170.

At 322, if the second incubation sequence is not complete, the controller 190 returns to 318 and enters the second operating mode again. If the second incubation sequence is complete but (as determined at 324) the plant propagation sequence is not complete, the controller 190 returns to step 304 and starts the first incubation sequence again. If the plant propagation sequence is complete, the controller 190 exits the plant propagation process at 326. In an embodiment, the controller 190 includes a visual and/or audio indicator for signaling the end of the plant propagation sequence.

Aspects of the invention are hence beneficial for providing a semi-fully or fully automated, enclosed plant propagation system that is fully programmable for independently controlling, for multiple media, the pulsing time (i.e. the activation/deactivation time) and the incubation time, as well as for controlling the total number of incubation cycles for the entire set of available liquid media. Desirably, all components of the system are autoclavable, and hence reusable. Significant cost savings are realized by reduction in labor, oversight, and contamination loss.

Non-limiting examples of plant micropropagation systems include those described in U.S. Patent. Nos. 3,578,431; 4,320,594; 4,669,217; 4,908,315; 4,934,096; 5,049,505; 5,088,231; 5,104,527; 5,119,588; 5,139,956; 5,171,683; 5,184,420; 5,186,895; 5,212,906; 5,225,342; 5,558,984; 5,597,731; and US 6,753,178. More examples of plant micropropagation systems can be found in Etienne et al. (*Bioreactors in coffee micropropagation*, Braz. J. Plant Physiol., 18(1):45-54, 2006); Ziv (*Bioreactor Technology for Plant Micropropagation Horticultural Reviews*, Volume 24, Edited by Jules Janick ISBN 0-471-33374-3); and Paek et al. (*Application of bioreactors for large-scale micropropagation systems of plants*, *In vitro* Cell. Dev. Biol.-Plant 37:149-157, March-April 2001).

It is understood that plant propagation systems of the invention also include the systems derived from the exemplary systems described herein, by adding one or more parts/features of the systems known to one skilled in the art.

Racks for Plant Micropropagation

For many plant species there are well known processes for micropropagation. In some instances, for example, it is desirable to intermittently expose cultivated plant tissue within a growth medium to a liquid nutrient solution. Some known systems designed to perform this function are inadequate and prone to breakage and/or mechanical failure. Furthermore, known systems are not sufficiently robust for a large-scale application. Thus, a need exists for improved apparatus for intermittently exposing microenvironments of tissue culture plantlets to a liquid nutrient solution.

In some embodiments, an apparatus includes a frame, a shelf assembly supported on the frame, and a drive assembly coupled to the shelf assembly. In such embodiments, the drive assembly can be configured to impart an oscillating motion to the shelf assembly relative to the frame such that tissue culture plantlets in propagation vessels and supported on the shelf assembly are intermittently exposed to a liquid nutrient solution.

FIGS. 8 and 9 are perspective views of an oscillating rack 100, according to an embodiment. An oscillating rack 100 includes a frame 110, a shelf assembly 140, and a drive assembly 170. The frame 100 includes uprights 111, an upper cross member 120, and a base 125. The components of the frame 100 can be formed from any suitable material. For example, in some embodiments, the frame 100 can be formed from aluminum. In other embodiments, the frame 100 can be formed from an aluminum alloy, steel, and/or steel alloy and can be of any suitable gauge or thickness.

The uprights 111 can be any suitable configuration and extend upwardly from the base 125, as described in further detail herein. The upper cross member 120 can be any suitable size, shape, or configuration. For example, in some embodiments, the upper cross member 120 can be a formed (e.g., mechanically bent) or extruded C-channel. In other embodiments, the upper cross member 120 can be a substantially closed or solid structure, such as, for example, box tubing or bar stock. The upper cross member 120 is configured to be coupled to an upper portion of the uprights 111. In this manner, the upper cross member 120 can increase the rigidity and/or strength of an upper portion of the oscillating rack 100.

The base 125 can be any suitable platform or structure. For example, while shown in FIGS. 8-10 as being substantially I-shaped (e.g., including a cross member coupled to two support members perpendicularly aligned to the cross member), in other embodiments, the base 125 can be any shape or configuration. In some embodiments, for example, the base 125 can be a substantially rectangular structure. In other embodiments, the base 125 can include stiffening members and/or the like. For example, in some embodiments, the base 125

can include a sheet metal portion coupled (e.g., screwed, welded, riveted, or otherwise fastened) to a top surface of the base 125 configured to increase the rigidity and/or strength of the base 125. Furthermore, in the embodiments shown in FIGS. 8-10, the base 125 includes a set of caster wheels such that the oscillating rack 100 can be moved or repositioned.

As shown in FIG. 10, the base 125 includes a support member 126 that extends from a top surface of the base 125. More specifically, the support members 126 are fixedly coupled (e.g., welded) to the top surface of the base 125 and receive at least a portion of the one of the uprights 111. Furthermore, one of the support members 126 includes a drive shaft opening 127 and a rocker shaft opening 128 configured to receive a drive shaft 172 and a rocker shaft 182 of the drive assembly 170, respectively.

The uprights 111 include a set of walls that define a substantially C-Shaped cross-section (FIG. 11) and a cover 112 (FIG. 10). As shown in FIGS. 8-10, the uprights 111 are configured to extend from the top surface of the base 125. More specifically, the uprights 111 are disposed around the support members 126 such that the uprights 111 extend away from the top surface of the base 125. Similarly stated, the support members 126 are disposed within a volume 115 defined by the set of walls that define the C-shaped cross-section. In this manner, the uprights 111 can be coupled (e.g., welded and/or fastened) to the base 125 and/or support members 126. The uprights 111 can further define any number of holes configured to receive portions of the oscillating rack 100. For example, at least one upright 111 can include a drive shaft opening 113 and a rocker shaft opening 114 configured to receive a drive shaft 172 and a rocker shaft 182, respectively, included in the drive assembly 170, respectively, and positioned to align with the drive shaft opening 127 and the rocker shaft opening 128, respectively, in the support member 126.

In some embodiments, the uprights 111 can define any number of holes and/or protrusions configured to engage a portion of a lighting system (not shown) and/or control system (not shown). In some embodiments, each cover 112 can be coupled to a respective upright 111 such that the cover 112 and the upright 111 house a set of electrical components (not shown) within the volume 115. For example, in some embodiments, the volume 115 can contain wires, switches, relays, electronic devices (e.g., a programmable logic controller (PLC) including, for example, at least a processor, a memory, and a network interface), and/or the like. In some embodiments, at least one upright 111 can include a sensor bracket 121. In such embodiments, a sensor can be disposed on the sensor bracket 121 and can

indicate and/or monitor the position of the shelf assembly 140 relative to the frame 110, as further described herein.

The shelf assembly 140 is rotatably coupled to the uprights 111 (see e.g., FIG. 8) and includes a set of shelves 141, a set of outer bushings 150, a set of inner bushings 155, and a set of linkages 145. For example, as shown in FIG. 12, the shelf assembly 140 can include any suitable number of shelves 141 configured to be vertically stacked. Furthermore, the shelves 141 are operatively coupled together via the linkages 145 (e.g., the linkages 145 transfer at least a portion of a force to cause each shelf 141 of the shelf assembly 140 to pivot simultaneously, as further described herein). While shown in FIG. 12 as including two linkages 145, in some embodiments, a shelf assembly can include any suitable number of linkages 141. For example, in some embodiments, a shelf assembly can include a set of four linkages 141 such that a first set of two linkages 145 are disposed on a first side of the shelves 141 and a second set of two linkages 145 are disposed on a second side of the shelves 141.

As shown in FIG. 13, each shelf 141 includes a set of platforms 142 coupled together by support tubes 144 (e.g., a first support tube 144 is disposed on a first side of a shelf 141 and a second support tube 144 is disposed on a second side of a shelf 141). As shown in FIG. 14, the platforms 142 define a cross-sectional shape defining a double return, thereby increasing the strength and rigidity of the platform 142. Furthermore, at least one of the support tubes 144 of a shelf 141 is configured to be coupled to the linkages 145.

The outer bushing 150 and the inner bushing 155 (FIG. 15) are configured to rotatably couple the shelf assembly 140 to the uprights 111. More specifically, the inner bushings 155 are rigidly coupled to the support tubes 144 of the shelves 141 and the outer bushings 150 are rigidly coupled to the uprights 111. In this manner, the inner bushings 155 can be rotatably disposed within an opening 151 defined by the outer bushings 150. Thus, the shelves 141 can pivot about the inner bushings 155, disposed within the openings 151 of the outer bushings 150, in response to at least a portion of a force exerted by the drive assembly 170.

Referring now to FIG. 16, the drive assembly includes a motor 171, a drive gear 173, and a rocker assembly 180. The motor 171 can be any suitable motor defining any suitable torque and/or output speed. For example, in some embodiments, the motor 171 is a Bison 650AC. As shown in FIG. 10, the motor 171 is configured to be coupled to at least one of the uprights 111 such that a drive shaft 172 extends from the motor 171 through the drive shaft opening 113 of the upright 111 and the drive shaft opening 127 of the support member 126.

The drive gear 173 is configured to be disposed about the drive shaft 172 and is housed within the volume 115 defined by the upright 111.

The rocker assembly 180 includes a rocker gear 181, a rocker shaft 182, a bearing 183, a mounting bracket 184, and a rocker bushing 186. The rocker gear 181 can be any suitable size and/or define any suitable number of teeth. Furthermore, the rocker gear 181 is disposed within the volume 115 and is operably coupled to the drive gear 173, for example via a chain (not shown). The arrangement of the drive gear 173 and the rocker gear 181 can be such that a desired gear ratio is defined between the drive gear 173 and the rocker gear 181.

The rocker shaft 182 is configured to be inserted into the rocker gear 181 and the bearing 183 and extends through the rocker shaft opening 128 of the support member 126 and the rocker shaft opening 114 of the upright 111. The bearing 183 can be used to facilitate the rotation of the rocker shaft 182 and/or to reduce wear on the rocker assembly 180. The rocker shaft 182 is further configured to be inserted through the rocker bushing 186 and is fixedly coupled (e.g., welded) to the mounting bracket 184. With the rocker shaft 182 coupled to the mounting bracket 184, the mounting bracket 184 can be coupled to the support tube 144 of a first shelf 141. Thus, with the mounting bracket 184 coupled to the first shelf 141 and the rocker shaft 182, the shelf assembly 140 is operably coupled to the motor 171.

For example, FIGS. 17-19 illustrate a portion of the oscillating rack 100 in a first configuration, a second configuration, and a third configuration, respectively. As seen in FIG. 17, the oscillating rack 100 can be in the first configuration such that platforms 142 of the shelves 141 are substantially parallel to a horizontal axis (e.g., the shelves 141 are parallel to the ground). In some embodiments, the shelves 141 can be substantially perpendicular to the linkages 145, while the oscillating rack 100 is in the first configuration.

[1031] As shown in FIG. 18, the oscillating rack 100 can be moved towards the second configuration by rotating the rocker gear 181 in the direction of the arrow AA. More specifically, the motor 171 (not shown in FIG. 18) can be electrically engaged (e.g., placed in the "on" position via, for example, a control panel) such that the motor 171 rotates the drive shaft 172 and the drive gear 173. The motor 171 can be configured to rotate the drive shaft 172 at any given output speed. For example, in some embodiments, the motor 171 can be configured to rotate the drive shaft 172 at a rate between 0.5 RPM and 1 RPM.

As described above, the rocker gear 181 is operably coupled to the drive gear 173 via a chain. Thus, the chain transfers a portion of the rotational force produced by the motor 171 to the rocker gear 181 such that the rocker gear 181 rotates in the direction of the arrow AA.

With the mounting bracket 184 coupled to the first shelf 141 (as described above), a portion of the rotational force, produced by the motor 171, is applied to the first shelf 141. In this manner, a first end of the first shelf 141 is urged to move in the direction of the arrow BB and a second end of the first shelf 141 is urged to move in the direction of the arrow CC. Moreover, with the linkages 145 coupled to the each of the shelves 141, the linkages 145 transfer a portion of the rotational force produced by the motor 171 to each of the shelves 141. Therefore, each shelf 141 is configured to move concurrently with the first shelf 141 in response to at least a portion of the rotational force produced by the motor 171. In addition, when in use with, for example, cultivated plant tissue, the pivoting motion of the shelves 141 can be such that a set of portions of the plant tissue, such as the roots, disposed on a surface of the platforms 142 are intermittently tilted so that the portions (e.g., roots) are alternately immersed in, and free of, a liquid nutrient contained in the vessels. Expanding further, the pivoting motion of the shelves 141 is such that the shelves 141 are placed at an angle relative to the horizontal axis, thus, the liquid nutrients flow in the direction of the arrow DD.

As shown in FIG. 19, the oscillating rack 100 can be moved from the second configuration towards the third configuration by rotating the rocker gear 181 in the direction of the arrow EE (substantially opposite the direction AA). With the rocker gear 181 being moved in the direction of the arrow EE, the first end of the first shelf 141 is urged to move in the direction of the arrow FF (substantially opposite the direction BB) and the second end portion of the first shelf 141 is urged to move in the direction of the arrow GG (substantially opposite the direction CC). Furthermore, the linkages 145 urge each of the shelves 141 of the shelf assembly 140 to move concurrently with the first shelf 141. Thus, when in use with, for example, cultivated plant tissue, the pivoting motion of the shelves 141 in the direction EE can be such that the liquid nutrient can be urged to flow in the direction of the arrow HH such that the cultured plant tissues (e.g., the roots) are alternately immersed in, and free of, the liquid nutrient contained in the vessels.

When in use, the oscillating rack 100 can be configured to oscillate between the second configuration and the third configuration. In some embodiments, the oscillating rack 100 can oscillate between the second configuration and the third configuration with a given cycle time. For example, in some embodiments, the cycle time can be 25 seconds (e.g., an oscillating time of 15 seconds and a hold time in the second configuration or the third configuration for 10 seconds before moving in the opposite direction). In other embodiments, the cycle time can be any other suitable length of time. In some embodiments, the oscillating

rack 100 can include a sensor (described above). In such embodiments, the sensor, such as a magnetic sensor, can be configured to sense the position of the shelf assembly 140 relative to the frame 100. The sensor can be configured to be in electrical communication with, for example, a programmable logic controller. The programmable logic controller and the sensor can detect a system malfunction. For example, in some embodiments, the programmable logic controller can be configured to send an electrical signal to an output device to generate a suitable output if the sensor does not sense the position of the shelf assembly 140 for predetermined time period (e.g., 35 seconds). The output can be an audible alarm, a flashing light, a telephone call, an email, and/or any other suitable notification.

The components described herein can be made using any suitable manufacturing technique. For example, in some embodiments, some components can be extruded. In some embodiments, the components can be formed (e.g., bent). In such embodiments, the components can include any suitable feature such that the component defines a specific material characteristic. For example, the platforms 142 are described above as including a double return configured to increase the strength and/or rigidity of the platforms 142. In some embodiments, other components can include similar features. For example, in some embodiments, the uprights 111 can include a double return. In other embodiments, the linkages 145 can include a double return.

The components described herein can be assembled in any suitable manner. For example, in some embodiments, components can be welded. In other embodiments, at least a portion of the components can be mechanically fastened. For example, in some embodiments, portions of the components described herein can be assembled (e.g., coupled) via bolts and nuts, screws, pins, and/or the like. In some embodiments, a portion of the components can be assembled using self-clinching nuts (e.g., PEM nuts) in conjunction with bolts or screws.

[1037] While various embodiments have been described above, it should be understood that they have been presented by way of example only, and not limitation. Where schematics and/or embodiments described above indicate certain components arranged in certain orientations /or positions, the arrangement of components may be modified. Similarly, where methods and/or events described above indicate certain events and/or procedures occurring in certain order, the ordering of certain events and/or procedures may be modified. While the embodiments have been particularly shown and described, it will be understood that various changes in form and details may be made.

Although various embodiments have been described as having particular features and/or combinations of components, other embodiments are possible having a combination of any features and/or components from any of embodiments as discussed above.

Methods for Plant Micropropagation

Micropropagated plants can begin from a selected piece of plant tissue, called an "explant" or "mother plant." This explant is the source of cells to be developed during the tissue culturing process. For example, the explant can be any segment or collection of cells from apical meristems, terminal buds, axillary buds, adventitious buds, accessory buds, pseudo-terminal buds, cambium, lateral meristem, lateral bud, vegetative buds, reproductive buds, mixed buds, shoot segments, shoot apices, stem segments, immature nodal sections from stems, lateral shoots, seedlings, seeds, shoots starting to rise from the ground, immature flower buds, inflorescences, crown segments, leaf segments, or any part thereof. In one embodiment, the explant is taken from a plant of about 1 week old, about 2 weeks old, about 3 weeks old, about 1 month old, about 2 month old, about 3 months old, about half year old, about 1 year old, about 2 years old plant, about 3 years old, about 5 years old, or more. The plant from which the explant is obtained can be grown in any suitable conditions, including but not limited to growing in a growth chamber, growing in a greenhouse, growing in a field, or growing in a tissue culture container (petri dish, margenta box, etc.). In some embodiments, the explant is tissue culture obtained from shoot clumps maintained as stock on growth media. In some embodiments, the explant is a nodal section having one or more axillary bud, which can be dormant or active. In some other embodiments, the explant is a seed or a part thereof.

The present invention provides methods for *in vitro* micropropagation of plant, for example, gymnosperm plants, angiosperm plants, monocot plants, dicot plants, crops, agriculturally/economically/environmentally important plants, etc.

In some embodiments, the methods disclosed herein can be used for *in vitro* micropropagation of a gymnosperm plant. For example, the methods can be used for *in vitro* micropropagation of the plants in the family/order of *Cycadaceae, Zamiaceae, Ginkgoaceae, Welwitschiaceae, Gnetaceae, Ephedraceae, Pinaceae, Araucariaceae, Podocarpaceae, Sciadopityaceae, Cupressaceae,* or *Taxaceae*.

In some embodiments, the methods disclosed herein can be used for *in vitro* micropropagation of an angiosperm plant. For example, the methods can be used for *in vitro*

micropropagation of the plants in the family/order of Ceratophyllum, Chloranthaceae, eudicots, magnoliids, or monocots.

In some embodiments, the methods disclosed herein can be used for *in vitro* micropropagation of a dicot plant. For example, the methods can be used for *in vitro* micropropagation of the plants in the family/order of *Buxaceae, Didymelaceae, Sabiaceae, Trochodendraceae, Tetracentraceae, Ranunculales, Proteales, Aextoxicaceae, Berberidopsidaceae, Dilleniaceae, Gunnerales, Caryophyllales, Saxifragales, Santalales, rosids, Aphloiaceae, Geissolomataceae, Ixerbaceae, Picramniaceae, Strassburgeriaceae, Vitaceae, Crossosomatales, Geraniales, Myrtales, Zygophyllaceae, Krameriaceae, Huaceae, Celastrales, Malpighiales, Oxalidales, Fabales, Rosales, Cucurbitales, Fagales, Tapisciaceae, Brassicales, Malvales, Sapindales, asterids, Cornales, Ericales, Boraginaceae, Icacinaceae, Oncothecaceae, Vahliaceae, Garryales, Solanales, Gentianales, Lamiales, Bruniaceae, Columelliaceae, Desfontainiaceae, Eremosynaceae, Escalloniaceae, Paracryphiaceae, Polyosmaceae, Sphenostemonacae, Tribelaceae, Aquifoliales, Apiales, Dipsacales,* or *Asterales*.

In some embodiments, the methods disclosed herein can be used for *in vitro* micropropagation of a monocot plant. For example, the methods can be used for *in vitro* micropropagation of the plants in the family/order of *Acorales, Alismatales, Asparagales, Dioscoreales, Liliales, Pandanales, Petrosaviales, Dasypogonaceae, Arecales, Commelinales, Poales,* or *Zingiberales*.

In some embodiments, the methods disclosed herein can be used for *in vitro* micropropagation of a bamboo species, such as *Phyllostachys edulis* (e.g., *Phyllostachys edulisi* 'Moso'*)*, *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia.* In some embodiments, the bamboo species is *Phyllostachys edulis*, Moso.

In some embodiments, the methods are used for rapid bamboo *in vitro* micropropagation. High shoot multiplication rate can be achieved in the methods disclosed herein. As used herein, the phrase "multiplication rate" refers to the multiplication fold of plant shoots obtained in a micropropagation process by starting from a single explant. For example, in the situation where the explant is a nodal section comprising a single bud, and 3 shoots are obtained after a micropropagation cycle, the multiplication rate is 3X. In some

embodiments, by using the bud induction media and the shoot elongation/maintenance media, a multiplication rate of at least about 2X to about 30X can be achieved after micropropagation. For example, about 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X, 11X, 12X, 13X, 14X, 15X, 16X, 17X, 18X, 19X, 20X, 21X, 22X, 23X, 24X, 25X, 26X, 27X, 28X, 29X, about 30X, or more can be achieved within about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, or about 28 days, or more.

In some embodiments, the present invention is based on the unexpected discovery that a pulsed treatment of an explant on a first medium comprising a strong cytokinin, such as TDZ, followed by a treatment of the explant on a second medium comprising one or more cytokinins other than TDZ, e.g., cytokinins that are relatively weaker than TDZ, such as meta-topolin, kinetin, isopentenyl adenine (iP, e.g., 2ip), zeatin, trans-zeatin, zeatin riboside, dihydrozeatin, benzyleadenin (BAP), or benzyladenosine ([9R]BAP), can provide rapid *in vitro* micropropagation with unexpected high multiplication rate.

In some embodiments, the methods comprise using a bud induction medium and a shoot elongation/maintenance media, wherein the bud induction medium comprises a strong cytokinin, such as TDZ, and the shoot elongation/maintenance medium comprises a relatively weaker cytokinin, such as meta-topolin, kinetin, isopentenyl adenine (iP, e.g., 2ip), zeatin, trans-zeatin, zeatin riboside, dihydrozeatin, benzyleadenin (BAP), or benzyladenosine ([9R]BAP).

Examples of a bud induction medium are described herein. In some embodiments, a bud induction medium comprises one or more strong cytokinin or analog thereof. In some embodiments, the bud induction medium comprises only one strong cytokinin, wherein the cytokinin is TDZ or analog thereof. In some embodiments, the concentration of the strong cytokinin (e.g., TDZ) in the bud induction media is about 0.25 mg/L to about 100 mg/L, for example, about 0.5 mg/L to about 2 mg/L.

Examples of a shoot elongation/maintenance media are described herein. In some embodiments, a shoot elongation/maintenance medium comprises one or more cytokinin that is relatively weaker cytokinin, such as a cytokinin other than TDZ. In some embodiments, the shoot elongation/maintenance medium comprises only one relatively weaker cytokinin, such as BAP, meta-topolin, ip (e.g., 2ip), zeatin, zeatin riboside, or combination thereof. In some embodiments, the shoot elongation/maintenance medium comprises more than one

cytokinins. In some embodiments, the concentration of a cytokinin in a shoot elongation/maintenance medium is about 0.01 mg/L to about 100 mg/L, for example, 0.25 mg/L to about 5 mg/L.

In some embodiments, the bud induction medium and/or the shoot elongation/maintenance medium comprises one or more auxin, such as β-naphthoxyacetic acid (NAA), 2,4- Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, or analogs thereof. In some embodiments, the bud induction medium and/or the shoot elongation/maintenance medium comprises NAA. In some embodiments, the concentration of an auxin in the media is 0.01 mg/L to about 50 mg/L, for example, about 0.25 mg/L to about 0.5 mg/L.

In some embodiments, the methods comprise (a) incubating a tissue culture, explant or seed/seed part in a bud induction medium to induce shoot bud formation; (b) incubating the shoot buds obtained in step (a) in a shoot elongation/maintenance medium.

The methods can further comprise (c) incubating the shoots from step (b) in a bud induction medium to induce shoot bud formation; and (d) incubating the shoot buds obtained in step (c) in a shoot elongation/maintenance medium.

In some embodiments, the methods further comprises (e) repeating the incubating steps (c) and step (d) for at least once.

In some embodiments, both the bud induction medium and the shoot elongation/maintenance medium are liquid media. The advantage of liquid media is that the one can replace old media with fresh media, or replace one type of media with another type of media quickly and easily, without transferring the seedlings of plant from one container to another. Therefore, in some embodiments, the whole micropropagation process is achieved in a single container, for example, in a bioreactor.

In some embodiments, the bud induction media and/or the shoot elongation/maintenance media are semi-solid or solid media. In some embodiments, liquid media and semi-solid or solid media can be used subsequently with any desired order. For example, the bud induction medium in step (a) and/or step (c) is liquid, semi-solid, or solid; the shoot elongation/maintenance medium in step (b) and/or step (d) is liquid, semi-solid, or solid.

In some embodiments, the incubation period of step (a) or step (c) lasts for about one hour to about three weeks. For example, the incubation period of step (a) or step (c) lasts for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 6 hours, about 7 hours, about

8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 30 hours, about 36 hours, about 42 hours, about 48 hours, about 54 hours, about 60 hours, about 1 week, about 1.5 weeks, about 2 weeks, about 2.5 weeks, about 3 weeks, about 3.5 weeks, about 4 weeks, or more.

In some embodiments, the incubation period of step (b) or step (d) lasts for about 0.5 week, about 1 week, about 1.5 weeks, about 2 weeks, about 2.5 weeks, about 3 weeks, about 3.5 weeks, about 4 weeks, about 4.5 weeks, about 5 weeks, about 5.5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, or more.

The incubation periods in the steps can be adjusted depending on the species of the plant, type of the explant, a desired multiplication rate. Without wishing to be bound by any theory, in some embodiments, for a bamboo species, the incubation period in step (a) or step (c) can be about 1 hour to about 3 weeks, for example, about 24 hours to about 60 hours; and the incubation period in step (b) or step (d) can be about 24 hours to about 4 weeks, for example, about 3 days to about 5 days.

The shoot multiplication rate can be further improved by repeating step (c) and step (d). For example, the multiple shoots developed after treatment of step (a) and treatment of step (b) can be subjected to one or more round of treatment of step (c) and treatment of step (d). In some embodiments, treatment in step (c) and treatment of step (d) are conducted at least once, at least twice, at least three times, at least four times, at least five times, at least six times, at least seven times, at least eight time, or more. Since the treatments in all steps are in short periods, a very short total time is needed to reach a very high shoot multiplication rate.

In some embodiments, each step of (a) to (e) can also be repeated before conducting the next step, by replacing old media with fresh media, for once, twice, three times, or more.

In some embodiments, starting from a single explant, the present methods can provide about 10X to about 30X shoot multiplication rate in approximately three weeks. In addition, at least about 500, at least about 1,000, at least about 2,000, at least about 3,000, at least about 4,000, at least about 5,000, at least about 6,000, at least about 7,000, at least about 8,000, at least about 9,000, at least about 10,000, at least about 20,000, at least about 30,000, at least about 40,000, at least about 50,000, at least about 60,000, at least about 70,000, at least about 80,000, at least about 90,000, at least about 100,000, or more plant shoots can be obtained within about 6 weeks, about 10 weeks, about 2 months, about 2.5 months, about 3 months, about 4 months, about 5 months, about 6 months.

To further improve the shoot multiplication rate, a separation step can be added during or immediately after one or more steps selected from steps (a), (b), (c), and (d). For example, multiple shoot buds produced in step (a) and/or step (c), or multiple shoots produced in step (c) and/or step (c) can be separated into individual pieces, and each of the separated pieces can be placed in an individual container comprising fresh media. For example, multiple shoot buds developed in a bud induction medium can be divided into individual pieces, and placed either on a fresh bud induction medium, or on a fresh shoot elongation/maintenance medium; multiple shoots developed in a shoot elongation/maintenance medium can be separated into individual pieces, and placed either on a fresh shoot elongation/maintenance medium, or a fresh bud induction medium. Each separated piece may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more shoot buds or shoots.

The present invention also provides methods for plant micropropagation using a plant growth system described herein.

In some embodiments, a plant growth system of the present invention is used for plant micropropagation. In some embodiments, it is used for bamboo micropropagation. In some embodiments, it is used for micropropagation of *Phyllostachys edulisi* 'Moso', *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia,* Nigra Henon, Rufa, or Nigra.

Referring again to the system 100 in FIG. 1, in use for plant micropropagation, the plant propagation sequence starts with placing an explant into the growth vessel 110. In some embodiments, the first media container 130 comprises a bud induction medium as described herein, and the second media container 150 comprises a shoot elongation/maintenance medium.

In some embodiments, the bud induction medium comprises an effective amount of thidiazuron (TDZ) or analog thereof, and wherein the shoot elongation/maintenance medium comprises an effective amount of one or more cytokinins other than TDZ or an analog thereof. In some embodiments, the concentration of TDZ or analog thereof in the bud induction medium is about 0.25 mg/L to about 100 mg/L, e.g., from 0.5 mg/L to about 2 mg/L. In some embodiments, one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is selected from the group consisting of N^6-benzylaminopurine (BAP), meta-topolin (mT), zeatin, kinetin, 2-isopentenyladenine (2ip),

adenine hemisulfate, dimethylallyladenine, N-(2-chloro-4-pyridyl)-N'- phenylurea) (4-CPPU), and analogs of each thereof. In some embodiments, the concentration of the one or more cytokinins other than TDZ or an analog thereof is from about 0.01 mg/L to about 100 mg/L, e.g., from about 0.25 mg/L to about 5 mg/L. In some embodiments, the bud induction medium and/or the shoot elongation/maintenance medium further comprises one or more auxins, such as β-naphthoxyacetic acid (NAA), 2,4- Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, and analogs of each thereof.

In some embodiments, the first incubation sequence of 304 lasts for about half hour to about three weeks, e.g., for about 24 hours to about 60 hours, and the second incubation sequence of 314 lasts for about 24 hours to about four weeks, e.g., for about three days to about five days.

In some embodiments, the length the plant propagation sequence is determined by the multiplication rate reached. In some embodiments, the multiplication rate is from at least about 1,000 to at least about 100,000 within about 3 weeks to about 6 months.

As far as the inventors know, this is the first time that such a system has been used for plant micropropagation, especially for bamboo micropropagation. The methods using a bioreactor are unique at least for the following reasons:

1. The methods are suitable for both small scale (e.g., laboratory) and large scale (e.g., industrial) plant micropropagation.

2. The methods allow the pulsing micropropagation technology described herein to be used in a more efficient way (e.g., recycled medium; less labor; more accurate control; less contamination; etc.).

3. The methods enable greatly improved shoot/plant multiplication over prior methods (e.g., micropropagation using solid medium, and micropropagation using liquid medium without a bioreactor).

4. The methods enable greater plant survival rate over prior methods, particularly for certain plant species, such as Moso bamboo.

5. Bamboo releases phenolics which are harmful to the shoots/plants when they buildup in the media/environment, which has been a problem with using solid medium. Moving from solid growth environment (e.g., plant micropropagation in tissue culture tubes/boxes) to the liquid environment and combining pulsing methods and a bioreactor system, the present invention achieves a major improvement in number of shoots/plants that

are obtained, as well as improving the resultant plants' health and ability to produce full size plants. Without wishing to be bound by any theory, the inventors believe these achievements are the result of controlling/reducing the exposure of the shoots/plantlets to toxic components in the growth compositions (e.g., certain plant hormones, such as TDZ) and/or plant produced by-products (e.g., phenolics), by utilizing the bioreactor systems of the present invention.

In addition to the methods described above which are based on using "bud induction media" and "shoot elongation/maintenance media" combination, the present invention also provides alternative plant micropropagation methods based on using "Stage 1 media", "Stage 2 media", "Stage 3 media", and/or more media.

In embodiments, the methods comprising using at least one "Stage 1 media" and at least one "Stage 2 media", and an explants. A variety of appropriate explants can be used in accordance with the present disclosure. In certain embodiments according to the present disclosure, immature nodal sections from stems can be used as the explant material. In one embodiment, the explants can be new growth canes with the lateral shoots just breaking the sheath at nodal section(s). New growth canes include those obtained from the plant within a current season or year, wherein such new growth canes can be obtained from any node on the plant. In one particular embodiment, explant material includes or is limited to the third node from the base of a cane.

In some embodiments, the plant is a bamboo. Detailed methods for collecting and initially disinfecting bamboo explants are described in WO/2011/100762, which is incorporated herein by reference in its entirety. In some embodiments, the disinfectant such as dichloroisocyanuric acid, dichloroisanuric acid, trichlorotriazinetriona, mercuric chloride, hydrogen peroxide, FungiGoneTM (bioWorld, Inc., Dublin, OH), plant preservatives can be used. In some embodiments, following the initial disinfection, the outer sheaths of a bamboo can be peeled off and discarded and the remaining piece can be put into an approximately 1% to about 50% solution of a commercial bleach or a similar disinfecting solution. In some embodiments, the bleach can be heated to about 20-60°C, such as 23-50°C. In some embodiments, sonication and vacuum infiltration of the tissue can also be used with the described disinfection procedures.

In some embodiments, the multiplication process can continue substantially indefinitely by continuing to separate and multiply shoots. In some embodiments, the multiplication cycles can be repeated without initiating new explants for at least 1 month, at least 3 months, at least 6 months, at least 12 months, at least 24 months, at least 36 months, or

more. In some embodiments, the multiplication cycles include 1-10 days per cycle, 2-9 days per cycle, 3-6 days per cycle, 0.5-3 days per cycle, 4-5 days per cycle, 0.5-1 day per cycle, 10-120 days per cycle, etc.

The present invention has many advantages. Without wishing to be bound by any theory, the methods disclosed herein do not require the use of seeds or inflorescence to start plants, or selection of diseased starting plants, or the use of antibiotics, somatic embryogenesis, pseudospiklets, or induction and/or reversion of flowering. For successful growth following tissue culture, the produced plants do not require watering directly on the pot but remain robust with overhead watering and do not require multiple adjustments to light intensity or humidity conditions prior to transfer to a greenhouse or other growing conditions. Moreover, media can be free from polyaspartic acid(s), seaweed concentrates and/or surfactants. These improvements over prior methods provide even additional advantages related to the health of produced plants and efficiency of growth and processing.

In some embodiments, the present invention can be used for grass propagation. In some embodiments, the micropropagated plants have not been genetically modified. Other particular embodiments exclude the use of timentin and/or kanamycin in the micropropagation procedure.

In some embodiments, when the methods are used for bamboo propagation, explants from a bamboo plant between the age of 3 months and 3 years are used. In some embodiments, a node from the cane with the lateral shoot just breaking the sheath can be used as the explants. In some embodiments, each nodal section can be cut into 3-5 millimeter sections with the shoot intact. In some embodiments, the outer sheaths can be peeled off and discarded and the remaining nodal section piece put into a 10% bleach solution with a final concentration of 0.6% sodium hydrochloride. In some embodiments, the explant in bleach solution can be placed onto a Lab Rotators, Adjustable speed, Barnstead/Lab line orbital Shaker (model number KS 260) shaker table for 1 hour at 6-9 revolutions per minute. The explants can then be put into a 1% bleach solution with a final concentration of 0.06% sodium hydrochloride, and be placed back onto the shaker table for 30 minutes. This 1% bleach solution step can then be repeated.

In some embodiments, individual explants can then be placed on a Stage 1 media (15-25 mL) within a tube and the tubes can be placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-54 $\mu mole/m^2/s^2$. The initial Stage 1 media can be b-12c-iv at a pH of 5.7. The explants can then be transferred to

fresh b-12c-iv media every 10-120 days (usually every 21 days), with contaminated tubes being discarded.

In some embodiments, if a spiked version of the b-12c-iv media is utilized, the explants can be placed in the spiked media for 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 hours before transition to a "standard" media disclosed herein or to a media containing substantially reduced or no cytokinins ("reduced" media as used herein) for the remainder of the 10-120 day cycle. Additional time periods for placement in a spiked media include anywhere between 0.1 and 240 hours and can include, without limitation, 0.1-0.5 hours, 0.3-2.5 hours, 2.5-6 hours, 1-10 hours, 5-15 hours, 10-20 hours, 15-25 hours, 20-30 hours, 25-35 hours, 30-40 hours, 35-45 hours, 40-50 hours, 45-55 hours, 50-60 hours, 55-65 hours, 60-70 hours, 65-75 hours, 70-80 hours, 75-85 hours, 80-90 hours, 85-95 hours, 90-100 hours, 95-105 hours, 100-110 hours, 105-115 hours, 110-120 hours, 115-125 hours, 120-130 hours, 125-135 hours, 130-140 hours, 135-145 hours, 140-150 hours, 145-155 hours, 150-160 hours, 155-165 hours, 160-170 hours, 165-175 hours, 170-180 hours, 175-185 hours, 180-190 hours, 185-195 hours, 190-200 hours, 195-205 hours, 200-210 hours, 205-215 hours, 210-220 hours, 215-225 hours, 220-230 hours, 225-235 hours, 230-240 hours, 235-245 hours, 240-250 hours, 3-6 hours, 7-17 hours, 12-22 hours, 17-27 hours, 22-32 hours, 27-37 hours, 32-42 hours, 37-47 hours, 42-52 hours, 47-57 hours, 52-62 hours, 57-67 hours, 62-72 hours, 67-77 hours, 72-82 hours, 77-87 hours, 82-92 hours, 87-97 hours, 92-102 hours, 97-107 hours, 102-112 hours, 107-117 hours, 112-122 hours, 117-127 hours, 122-132 hours, 127-137 hours, 132-142 hours, 137-147 hours, 142-152 hours, 147-157 hours, 152-162 hours, 157-167 hours, 162-172 hours, 167-177 hours, 172-182 hours, 177-187 hours, 162-172 hours, 167-177 hours, 182-192 hours, 187-197 hours, 192-202 hours, 197-207 hours, 202-212 hours, 207-217 hours, 212-222 hours, 217-227 hours, 222-232 hours, 227-237 hours, 232-242 hours, 237-247 hours or 242-252 hours. Placement in spiked media can also be 0.5 hours less than a cycle in standard or reduced media, 1 hour less than a cycle in standard or reduced media and all time periods in between 1 and 240 hours less than a cycle in standard or reduced media. Alternatively, in place of spending the remainder of the cycle in the standard or reduced media, explants can be placed on a spiked media for a period of time followed by culture on a standard or reduced media for the full cycle time (i.e. 10-120 days not reduced by time spent in the spiked media).

Media containing no cytokinins or substantially reduced cytokinins can be a reduced b-9 media, reduced CW2 media, reduced b-10 media, reduced b-11 media, reduced b-12c media, reduced b-1 media, reduced b-4 media, reduced b-6 media, reduced CW1 media, reduced CW3 media, reduced CW4 media, reduced CW5 media, reduced CW6 media, reduced B-9N2 media, reduced B-12C CPPU media, reduced B-12C DPU media with all cytokinins and/or auxins removed or can have at least one cytokinin and/or auxin's amount reduced by 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, 100%, 1-10%, 5-15%, 10-20%, 15-25%, 20-30%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, 90-100%, 3-6%, 7-17%, 12-22%, 17-27%, 22-32%, 27-37%, 32-42%, 37-47%, 42-52%, 47-57%, 52-62%, 57-67%, 62-72%, 67-77%, 72-82%, 77-87%, 82-92% or 87-97%. Non-limiting examples of reduced media include (embodiments with no cytokinins or auxins not shown in table format):

Media B-9N2

Component	**Reduced B-9N2-i**	**Reduced B-9N2-ii**	**Reduced B-9N2-iii**	**Reduced B-9N2-iv**	**Reduced B-9N2-v**
NAA	0.15	0.01	0.1	0.15	0.1
BAP	1	0.75	0.9	1	0.5
Thidiazuron	0.2	0.25	0.2	0.25	0.25
Meta-Topolin	4	5	4.5	5	5

Media B-12C CPPU

Component	**Reduced B-12C CPPU-i**	**Reduced B-12C CPPU-ii**	**Reduced B-12C CPPU-iii**	**Reduced B-12C CPPU-iv**	**Reduced B-12C CPPU-v**
NAA	0.05	0.04	0.025	0.04	0.05
BAP	1	1	0.5	0.9	1
Thidiazuron	0.2	0.3	0.75	0.7	0.5
Meta-Topolin	3	3	5	4.5	2.5
CPPU	0.75	0.5	0.75	0.7	0.5

Media B-12C DPU

Component	**Reduced B-12C CPU-i**	**Reduced B-12C CPU-ii**	**Reduced B-12C CPU-iii**	**Reduced B-12C CPU-iv**	**Reduced B-12C CPU-v**
NAA	0.01	0.05	0.025	0.05	0.05
BAP	0.25	1	0.75	1	0.5
Thidiazuron	0.2	0.6	0.75	0.75	0.3
Meta-Topolin	2	3	4	5	5
DPU	0.75	0.75	0.6	0.75	0.4

Alternatively, the cytokinins noted above can be replaced with weaker cytokinins at similar or higher levels. Exemplary weaker cytokinins include zeatin and kinetin.

Contaminated tubes can be identified by bacterial discoloration of the agar or by visible surface contamination. These explants can stay on the chosen b-12c-iv media for 3-4 10-120 day cycles (usually 21 day cycles) or as modified in the spiked procedure (spiked media for a period of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 hours before transition to a standard or reduced media for the remainder of the 10-120 day cycle or for a full 10-120 day cycle). Additional time periods for placement in a spiked media include anywhere between 0.1 and 240 hours and can include, without limitation, 0.1-0.5 hours, 0.3-2.5 hours, 2.5-6 hours, 1-10 hours, 5-15 hours, 10-20 hours, 15-25 hours, 20-30 hours, 25-35 hours, 30-40 hours, 35-45 hours, 40-50 hours, 45-55 hours, 50-60 hours, 55-65 hours, 60-70 hours, 65-75 hours, 70-80 hours, 75-85 hours, 80-90 hours, 85-95 hours, 90-100 hours, 95-105 hours, 100-110 hours, 105-115 hours, 110-120 hours, 115-125 hours, 120-130 hours, 125-135 hours, 130-140 hours, 135-145 hours, 140-150 hours, 145-155 hours, 150-160 hours, 155-165 hours, 160-170 hours, 165-175 hours, 170-180 hours, 175-185 hours, 180-190 hours, 185-195 hours, 190-200 hours, 195-205 hours, 200-210 hours, 205-215 hours, 210-220 hours, 215-225 hours, 220-230 hours, 225-235 hours, 230-240 hours, 235-245 hours, 240-250 hours, 3-6 hours, 7-17 hours, 12-22 hours, 17-27 hours, 22-32 hours, 27-37 hours, 32-42 hours, 37-47 hours, 42-52 hours, 47-57 hours, 52-62 hours, 57-67 hours, 62-72 hours, 67-77 hours, 72-82 hours, 77-87 hours, 82-92 hours, 87-97 hours, 92-102 hours, 97-107 hours, 102-112 hours, 107-117 hours, 112-122 hours, 117-127 hours, 122-132 hours, 127-137 hours, 132-142 hours, 137-147 hours, 142-152 hours, 147-157 hours, 152-162 hours, 157-167 hours, 162-172 hours, 167-177 hours, 172-182 hours, 177-187 hours, 162-172 hours, 167-177 hours, 182-192 hours, 187-197 hours, 192-202 hours, 197-207 hours, 202-212 hours, 207-217 hours, 212-222 hours, 217-227 hours, 222-232 hours, 227-237 hours, 232-242 hours, 237-247 hours or 242-252 hours. Placement in spiked media can also be 0.5 hours less than a cycle in standard or reduced media, 1 hour less than a cycle in standard or reduced media and all time periods in between 1 and 240 hours less than a cycle in standard or reduced media. If an explant is cultured on a particular spiked media type (e.g. b-12c), when transferred to a standard or reduced media, the standard or reduced media can be of the same type (e.g. standard or reduced b-12c) or of a different type (e.g. standard or reduced CW1, CW2, CW6, b6, b9 etc.).

In particular embodiments disclosed herein, culture periods are less than 12 weeks, less than 9 weeks, or less than 6 weeks.

Explants can be taken off the media after the third cycle if multiplication is occurring. If multiplication is not occurring or not occurring to a significant degree, explants can be left on the media for a fourth cycle.

Live shoots can next be transferred to a Stage 2 media (if standard b-12c used in the previous step or a Stage 3 media if a basic spiked procedure was used), such as b-9, CW1, CW2, CW3, CW4, CW5, CW6, b-6, B-9N2, B-12C CPPU or B-12C DPU at a pH of 5.7. The cultures can stay on this Stage 2 media until the desired number of shoots is obtained by separation into new tubes and further expansion. Generally, the range of time includes 10-120 day cycles (usually 14-21 day cycles) between which the cultures are assigned to go through another multiplication round or transitioned to a Stage 3 or Stage 4 media, for example, b-10-iv or b-11-iv at a pH of 5.7 for further multiplication.

In particular embodiments disclosed herein, culture periods are less than 12 weeks, less than 9 weeks, or less than 6 weeks.

Alternatively, live shoots can also be placed on a spiked b-9, CW1, CW2, CW3, CW4, CW5, CW6, b-6, B-9N2, B-12C CPPU, B-12C DPU media for a period of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 hours before transition to a same or different type of standard or reduced media for the remainder of the 10-120 day cycle or for a full 10-120 day cycle. Additional time periods for placement in a spiked media include anywhere between 0.1 and 240 hours and can include, without limitation, 0.1-0.5 hours, 0.3-2.5 hours, 2.5-6 hours, 1-10 hours, 5-15 hours, 10-20 hours, 15-25 hours, 20-30 hours, 25-35 hours, 30-40 hours, 35-45 hours, 40-50 hours, 45-55 hours, 50-60 hours, 55-65 hours, 60-70 hours, 65-75 hours, 70-80 hours, 75-85 hours, 80-90 hours, 85-95 hours, 90-100 hours, 95-105 hours, 100-110 hours, 105-115 hours, 110-120 hours, 115-125 hours, 120-130 hours, 125-135 hours, 130-140 hours, 135-145 hours, 140-150 hours, 145-155 hours, 150-160 hours, 155-165 hours, 160-170 hours, 165-175 hours, 170-180 hours, 175-185 hours, 180-190 hours, 185-195 hours, 190-200 hours, 195-205 hours, 200-210 hours, 205-215 hours, 210-220 hours, 215-225 hours, 220-230 hours, 225-235 hours, 230-240 hours, 235-245 hours, 240-250 hours, 3-6 hours, 7-17 hours, 12-22 hours, 17-27 hours, 22-32 hours, 27-37 hours, 32-42 hours, 37-47 hours, 42-52 hours, 47-57 hours, 52-62 hours, 57-67 hours, 62-72 hours, 67-77 hours, 72-82 hours, 77-87 hours, 82-92 hours, 87-97 hours, 92-102 hours,

97-107 hours, 102-112 hours, 107-117 hours, 112-122 hours, 117-127 hours, 122-132 hours, 127-137 hours, 132-142 hours, 137-147 hours, 142-152 hours, 147-157 hours, 152-162 hours, 157-167 hours, 162-172 hours, 167-177 hours, 172-182 hours, 177-187 hours, 162-172 hours, 167-177 hours, 182-192 hours, 187-197 hours, 192-202 hours, 197-207 hours, 202-212 hours, 207-217 hours, 212-222 hours, 217-227 hours, 222-232 hours, 227-237 hours, 232-242 hours, 237-247 hours or 242-252 hours. Placement in spiked media can also be 0.5 hours less than a cycle in standard or reduced media, 1 hour less than a cycle in standard or reduced media and all time periods in between 1 and 240 hours less than a cycle in standard or reduced media.

Generally, one-ten shoots per tube can be obtained per multiplication cycle.

Following removal from the multiplication process, the shoots can be transferred to small tissue culturing boxes (known as "magenta boxes") for 10-120 days (usually 14-21 days) containing a Stage 3, Stage 4 or Stage 5 media, in this Example, BR-2 at a pH of 5.7 for 10-120 days (usually 14-21 days) or Amel at a pH of 5.7 for 10-120 days (usually 14-21 days). As above, shoots can be placed in spiked media for shorter time periods followed by placement into a standard or reduced media for the remainder of or for a full 10-120 day cycle.

In particular embodiments disclosed herein, culture periods are less than 12 weeks, less than 9 weeks, or less than 6 weeks.

As will be understood by one of ordinary skill in the art, when spiked media are used, the use of the spiked media increases the number of media stages within a particular process due the following use of a standard or reduced media. If spiked media are used at only one stage, the process generally expands by 1 media stage. If spiked media are used at two stages, the process generally expands by 2 media stages. If spiked media are used at three stages, the process generally expands by 3 media stages, etc.

Alternatively, the following procedures may also be used (Stage 1, Stage 2, Stage 3, etc, media are defined elsewhere herein):

Individual explants can then be placed on a Stage 1 media (15-25 mL) within a tube and the tubes placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-90 $\mu mole/m^2/s^2$. The Stage 1 media can be standard b-12c-iv at a pH of 5.7 or spiked b-12c-iv media. If placed on standard b-12c-iv, the explants can be transferred to fresh b-12c-iv media every 10-120 days (usually every 21 days), with contaminated tubes being discarded. If on spiked b-12c-iv media, the explants can remain on the spiked media for 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,

19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 hours and then be transferred to a media without spiked components (standard or reduced) for the remainder of the 10-120 day cycle or for a full 10-120 day cycle. Additional time periods for placement in a spiked media include anywhere between 0.1 and 240 hours and can include, without limitation, 0.1-0.5 hours, 0.3-2.5 hours, 2.5-6 hours, 1-10 hours, 5-15 hours, 10-20 hours, 15-25 hours, 20-30 hours, 25-35 hours, 30-40 hours, 35-45 hours, 40-50 hours, 45-55 hours, 50-60 hours, 55-65 hours, 60-70 hours, 65-75 hours, 70-80 hours, 75-85 hours, 80-90 hours, 85-95 hours, 90-100 hours, 95-105 hours, 100-110 hours, 105-115 hours, 110-120 hours, 115-125 hours, 120-130 hours, 125-135 hours, 130-140 hours, 135-145 hours, 140-150 hours, 145-155 hours, 150-160 hours, 155-165 hours, 160-170 hours, 165-175 hours, 170-180 hours, 175-185 hours, 180-190 hours, 185-195 hours, 190-200 hours, 195-205 hours, 200-210 hours, 205-215 hours, 210-220 hours, 215-225 hours, 220-230 hours, 225-235 hours, 230-240 hours, 235-245 hours, 240-250 hours, 3-6 hours, 7-17 hours, 12-22 hours, 17-27 hours, 22-32 hours, 27-37 hours, 32-42 hours, 37-47 hours, 42-52 hours, 47-57 hours, 52-62 hours, 57-67 hours, 62-72 hours, 67-77 hours, 72-82 hours, 77-87 hours, 82-92 hours, 87-97 hours, 92-102 hours, 97-107 hours, 102-112 hours, 107-117 hours, 112-122 hours, 117-127 hours, 122-132 hours, 127-137 hours, 132-142 hours, 137-147 hours, 142-152 hours, 147-157 hours, 152-162 hours, 157-167 hours, 162-172 hours, 167-177 hours, 172-182 hours, 177-187 hours, 162-172 hours, 167-177 hours, 182-192 hours, 187-197 hours, 192-202 hours, 197-207 hours, 202-212 hours, 207-217 hours, 212-222 hours, 217-227 hours, 222-232 hours, 227-237 hours, 232-242 hours, 237-247 hours or 242-252 hours. Placement in spiked media can also be 0.5 hours less than a cycle in standard or reduced media, 1 hour less than a cycle in standard or reduced media and all time periods in between 1 and 240 hours less than a cycle in standard or reduced media. These explants can stay on b-12c-iv media or spiked b-12c-iv media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths can be removed. At the time of transfer to the third cycle, explants can be transitioned to a Stage 2 media or Stage 3 media (depending on whether spiked procedures are used), in this Example, standard b-12c-iv supplemented with 7 g/L carageenan rather than the 5.5 g/L provided above or a spiked b-12c-iv supplemented with 7 g/L carageenan rather than the 5.5 g/L provided above for 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 hours followed by transition to standard or reduced b-12c-iv. Additional time periods for placement in a spiked media include anywhere between 0.1 and

240 hours and can include, without limitation, 0.1-0.5 hours, 0.3-2.5 hours, 2.5-6 hours, 1-10 hours, 5-15 hours, 10-20 hours, 15-25 hours, 20-30 hours, 25-35 hours, 30-40 hours, 35-45 hours, 40-50 hours, 45-55 hours, 50-60 hours, 55-65 hours, 60-70 hours, 65-75 hours, 70-80 hours, 75-85 hours, 80-90 hours, 85-95 hours, 90-100 hours, 95-105 hours, 100-110 hours, 105-115 hours, 110-120 hours, 115-125 hours, 120-130 hours, 125-135 hours, 130-140 hours, 135-145 hours, 140-150 hours, 145-155 hours, 150-160 hours, 155-165 hours, 160-170 hours, 165-175 hours, 170-180 hours, 175-185 hours, 180-190 hours, 185-195 hours, 190-200 hours, 195-205 hours, 200-210 hours, 205-215 hours, 210-220 hours, 215-225 hours, 220-230 hours, 225-235 hours, 230-240 hours, 235-245 hours, 240-250 hours, 3-6 hours, 7-17 hours, 12-22 hours, 17-27 hours, 22-32 hours, 27-37 hours, 32-42 hours, 37-47 hours, 42-52 hours, 47-57 hours, 52-62 hours, 57-67 hours, 62-72 hours, 67-77 hours, 72-82 hours, 77-87 hours, 82-92 hours, 87-97 hours, 92-102 hours, 97-107 hours, 102-112 hours, 107-117 hours, 112-122 hours, 117-127 hours, 122-132 hours, 127-137 hours, 132-142 hours, 137-147 hours, 142-152 hours, 147-157 hours, 152-162 hours, 157-167 hours, 162-172 hours, 167-177 hours, 172-182 hours, 177-187 hours, 162-172 hours, 167-177 hours, 182-192 hours, 187-197 hours, 192-202 hours, 197-207 hours, 202-212 hours, 207-217 hours, 212-222 hours, 217-227 hours, 222-232 hours, 227-237 hours, 232-242 hours, 237-247 hours or 242-252 hours. Placement in spiked media can also be 0.5 hours less than a cycle in standard or reduced media, 1 hour less than a cycle in standard or reduced media and all time periods in between 1 and 240 hours less than a cycle in standard or reduced media. Following the third cycle, explants can be cleaned. The explants can be kept on b-12c-iv supplemented with 7 g/L carageenan rather than the 5.5 g/L provided above for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months. When multiple cycles are used, explants can be cultured in standard media for all cycles, on spiked media followed by standard media for all cycles or on spiked media followed by reduced media for all cycles. Alternatively, explants can be exposed to one or more of these treatments across cycles in any combination and order.

In particular embodiments disclosed herein, culture periods are less than 12 weeks, less than 9 weeks, or less than 6 weeks.

Once an explant exhibits multiple shoots, it can be either maintained on its current media when shooting occurred (with transfer to fresh media every 10-120 days) or transferred to a subsequent media. Non-limiting subsequent media include, without limitation a b-9 media, a CW1 media, a CW2 media a CW3 media, a CW4 media, a CW5 media, a CW6

media or a b-6 media at a pH of 5.7 or spiked versions of the same followed by transition to a standard or reduced media. The cultures can stay on the current or subsequent media until the desired number of shoots is obtained by separation into new tubes and further expansion. Generally, the range of time includes 10-120 day cycles (usually 21 day cycles) between which the cultures can be assigned to go through another multiplication round or transitioned to a next stage media, such as a BR-2 media at a pH of 5.7 for 10-120 days (usually 21 days) in “magenta boxes" or a Amel media at a pH of 5.7 for 10-120 days (usually 14-21 days).

In particular embodiments disclosed herein, culture periods are less than 12 weeks, less than 9 weeks, or less than 6 weeks.

In even more particular non-limiting embodiments, the following species can be micropropagated in the following media (at a pH of 5.5-5.7) according to procedures described in the proceeding paragraphs [000198]-[0002-1]:

Arundinaria gigantea: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Bambusa balcoa: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Bambusa vulgaris: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v spiked and reduced versions thereof;

Bambusa vulgaris ‘Vitatta’: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Bambusa Oldhamii: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Bambusa tulda: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus brandesii:b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus asper: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus hamiltoni: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus giganteus: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus membranaceus: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Dendrocalamus strictus: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Gigantochloa aspera: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Gigantochloa scortechini: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua culeata: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua aculeata 'Nicaragua': b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua amplexifolia: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua angustifolia: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua angustofolia bi-color: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Guadua paniculata: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Melocanna bambusoides: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Neohouzeaua dullooa (Teinostachyum): b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Ochlandra travancorica: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Phyllostachys edulis 'Moso': b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Phyllostachys nigra: b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Phyllostachys nigra 'Henon': b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof;

Schizostachyum lumampao:b-9-v, CW1-v, CW3-v, CW4-v, CW5-v, CW6-v, B-9N2-v, B-12C CPPU-v, B-12C DPU-v or spiked and reduced versions thereof.

In some embodiments, the methods comprise using the bioreactors of the present invention. In some embodiments, the methods comprising using the racks of the present invention. In some embodiments, the methods comprise using the bioreactors and the racks of the present invention. In some embodiments, when two or more media described above are used in rotation during micropropagation, a bioreactor described herein can be used, for example, when a Stage 1 and a Stage 2 media are used in rotation, when a Stage 3 and a Stage 4 media are used in rotation, and/or when a Stage 1, a Stage 2, and a Stage 3 media are used in rotation, etc.

Kits

The present invention also provides kits for plant propagation. In some embodiments, the kits include one or more media of the present invention. In some embodiments, the kits include one or more explants of a plant species.

For example, the kits can include one or more bud induction medium, shoot elongation/maintenance media, b-9-i media, b-9-ii media, b-9-iii media, b-9-iv media, b-9-v media, spiked b-9-i media, spiked b-9-ii media, spiked b-9-iii media, spiked b-9-iv media, spiked b-9-v media, reduced b-9-i media (reduced media are described below), reduced b-9-ii media, reduced b-9-iii media, reduced b-9-iv media, reduced b-9-v media, CW2-i media, CW2-ii media, CW2-iii media, CW2-iv media, CW2-v media, spiked CW2-i media, spiked CW2-ii media, spiked CW2-iii media, spiked CW2-iv media, spiked CW2-v media, reduced CW2-i media, reduced CW2-ii media, reduced CW2-iii media, reduced CW2-iv media, reduced CW2-v media, b-10-i media, b-10-ii media, b-10-iii media, b-10-iv media, b-10-v media, spiked b-10-i media, spiked b-10-ii media, spiked b-10-iii media, spiked b-10-iv media, spiked b-10-v media, reduced b-10-i media, reduced b-10-ii media, reduced b-10-iii media, reduced b-10-iv media, reduced b-10-v media, b-11-i media, b-11-ii media, b-11-iii media, b-11-iv media, b-11-v media, spiked b-11-i media, spiked b-11-ii media, spiked b-11-iii media, spiked b-11-iv media, spiked b-11-v media, reduced b-11-i media, reduced b-11-ii media, reduced b-11-iii media, reduced b-11-iv media, reduced b-11-v media, b-12c-i media, b-12c-ii media, b-12c-iii media, b-12c-iv media, b-12c-v media, spiked b-12c-i media, spiked b-12c-ii media, spiked b-12c-iii media, spiked b-12c-iv media, spiked b-12c-v media, reduced b-12c-i media, reduced b-12c-ii media, reduced b-12c-iii media, reduced b-12c-iv

media, reduced b-12c-v media, b-1-i media, b-1-ii media, b-1-iii media, b-1-iv media, b-1-v media, spiked b-1-i media, spiked b-1-ii media, spiked b-1-iii media, spiked b-1-iv media, spiked b-1-v media, reduced b-1-i media, reduced b-1-ii media, reduced b-1-iii media, reduced b-1-iv media, reduced b-1-v media, b-4-i media, b-4-ii media, b-4-iii media, b-4-iv media, b-4-v media, spiked b-4-i media, spiked b-4-ii media, spiked b-4-iii media, spiked b-4-iv media, spiked b-4-v media, reduced b-4-i media, reduced b-4-ii media, reduced b-4-iii media, reduced b-4-iv media, reduced b-4-v media, b-6-i media, b-6-ii media, b-6-iii media, b-6-iv media, b-6-v media, spiked b-6-i media, spiked b-6-ii media, spiked b-6-iii media, spiked b-6-iv media, spiked b-6-v media, reduced b-6-i media, reduced b-6-ii media, reduced b-6-iii media, reduced b-6-iv media, reduced b-6-v media, CW1-i media, CW1-ii media, CW1-iii media, CW1-iv media, CW1-v media, spiked CW1-i media, spiked CW1-ii media, spiked CW1-iii media, spiked CW1-iv media, spiked CW1-v media, reduced CW1-i media, reduced CW1-ii media, reduced CW1-iii media, reduced CW1-iv media, reduced CW1-v media, CW3-i media, CW3-ii media, CW3-iii media, CW3-iv media, CW3-v media, spiked CW3-i media, spiked CW3-ii media, spiked CW3-iii media, spiked CW3-iv media, spiked CW3-v media, reduced CW3-i media, reduced CW3-ii media, reduced CW3-iii media, reduced CW3-iv media, reduced CW3-v media, CW4-i media, CW4-ii media, CW4-iii media, CW4-iv media, CW4-v media, spiked CW4-i media, spiked CW4-ii media, spiked CW4-iii media, spiked CW4-iv media, spiked CW4-v media, reduced CW4-i media, reduced CW4-ii media, reduced CW4-iii media, reduced CW4-iv media, reduced CW4-v media, CW5-i media, CW5-ii media, CW5-iii media, CW5-iv media, CW5-v media, spiked CW5-i media, spiked CW5-ii media, spiked CW5-iii media, spiked CW5-iv media, spiked CW5-v media, reduced CW5-i media, reduced CW5-ii media, reduced CW5-iii media, reduced CW5-iv media, reduced CW5-v media, CW6-i media, CW6-ii media, CW6-iii media, CW6-iv media, CW6-v media, spiked CW6-i media, spiked CW6-ii media, spiked CW6-iii media, spiked CW6-iv media, spiked CW6-v media, reduced CW6-i media, reduced CW6-ii media, reduced CW6-iii media, reduced CW6-iv media, reduced CW6-v media, B-9N2-i media, B-9N2-ii media, B-9N2-iii media, B-9N2-iv media, B-9N2-v media, spiked B-9N2-i media, spiked B-9N2-ii media, spiked B-9N2-iii media, spiked B-9N2-iv media, spiked B-9N2-v media, reduced B-9N2-i media, reduced B-9N2-ii media, reduced B-9N2-iii media, reduced B-9N2-iv media, reduced B-9N2-v media, B-12C CPPU-i media, B-12C CPPU-ii media, B-12C CPPU-iii media, B-12C CPPU-iv media, B-12C CPPU-v media, spiked B-12C CPPU-i media, spiked B-12C CPPU-ii media, spiked B-12C CPPU-iii media, spiked B-12C CPPU-iv media,

spiked B-12C CPPU-v media, reduced B-12C CPPU-i media, reduced B-12C CPPU-ii media, reduced B-12C CPPU-iii media, reduced B-12C CPPU-iv media, reduced B-12C CPPU-v media, B-12C DPU-i media, B-12C DPU-ii media, B-12C DPU-iii media, B-12C DPU-iv media, B-12C DPU-v media, spiked B-12C DPU-i media, spiked B-12C DPU-ii media, spiked B-12C DPU-iii media, spiked B-12C DPU-iv media, spiked B-12C DPU-v media, reduced B-12C DPU-i media, reduced B-12C DPU-ii media, reduced B-12C DPU-iii media, reduced B-12C DPU-iv media, reduced B-12C DPU-v media, Br-2-i media, Br-2-ii media, Br-2-iii media, Br-2-iv media, Br-2-v media, spiked Br-2-i media, spiked Br-2-ii media, spiked Br-2-iii media, spiked Br-2-iv media, spiked Br-2-v media, reduced Br-2-i media, reduced Br-2-ii media, reduced Br-2-iii media, reduced Br-2-iv media, reduced Br-2-v media, Ech-i media, Ech-ii media, Ech-iii media, Ech-iv media, Ech-v media, spiked Ech-i media, spiked Ech-ii media, spiked Ech-iii media, spiked Ech-iv media, spiked Ech-v media, reduced Ech-i media, reduced Ech-ii media, reduced Ech-iii media, reduced Ech-iv media, reduced Ech-v media, Amel-i media, Amel-ii media, Amel-iii media, Amel-iv media, Amel-v media, spiked Amel-i media, spiked Amel-ii media, spiked Amel-iii media, spiked Amel-iv media, spiked Amel-v media, reduced Amel-i media, reduced Amel-ii media, reduced Amel-iii media, reduced Amel-iv media, and/or reduced Amel-v media. In another embodiment, the kits can comprise one or more containers for the tissue culturing process including without limitation, tubes, jars, boxes, jugs, cups, sterile bag technology, bioreactors, temporary immersion vessels, etc. In another embodiment the kits can comprise instructions for the tissue culturing of bamboo. In another embodiment, the kits comprise combinations of the foregoing. Components of various kits can be found in the same or different containers. Additionally, when a kit is supplied, the different components of the media can be packaged in separate containers and admixed immediately before use. Such packaging of the components separately may permit long-term storage without losing the active components' functions. Alternatively, media can be provided pre-mixed.

The present invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the Figures, are incorporated herein by reference in their entirety for all purposes.

EXAMPLE

Example 1.

Micropropagation of Phyllostachys Edulis "Moso" by Stage 1 to Stage 5 media - I

Starting with a bamboo plant between the ages of approximately 3 months and approximately 3 years, a node from the cane with the lateral shoot just breaking the sheath is used as the explant. Each nodal section is cut into 3-5 millimeter sections with the shoot intact. Some explants, including explants taken from canes 1 year or older are pre-rinsed by shaking them in a jar of 70% isopropyl alcohol for 3 seconds followed by rinsing them under running tap water for 1 minute. Other explants are not pre-rinsed.

The outer sheaths are peeled off and discarded and the remaining nodal section piece put into a 10% bleach solution. The explant in bleach solution is placed onto a Lab Rotators, Adjustable speed, Barnstead/Lab line orbital Shaker (model number KS 260) shaker table for 1 hour at 6-9 revolutions per minute. For some implants, including those taken from canes 1 year or older, this step is modified by adding a few drops of Tween 20 to the 10% bleach solution and soaking the explants for 45 minutes rather than 1 hour. The explants are then put into a 1% bleach solution, and placed back onto the shaker table for 30 minutes. This 1% bleach solution step is then repeated and can then be rinsed with sterile distilled water.

Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-90 μmole/m2/s2. In this Example the Stage 1 media is spiked b-12c-iv otherwise as described in Example 10. These explants stay on spiked b-12c-iv media for 2 8-118 day cycles (usually 12 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, spiked b-12c-iv supplemented with 0.5 – 3 g/L (optimum is 2 g/L) casein hydroxylate. Following the third cycle, explants are cleaned. The explants are kept on spiked b-12c-iv supplemented with 0.5 – 3 g/L casein hydroxylate for 8-118 day cycles (usually 12 day cycles) until multiple shoots were observed. Observation of multiple shoots generally occurs within 3-15 months.

[0291] Once the explant exhibits multiple shoots, it is transferred to a Stage 3 media, in this Example reduced B-9N2-iv at a pH of 5.7 for 10-120 day cycles (usually 21 day cycles). Shoots are rotated between this Stage 3 media and a Stage 4 media, in the Example, reduced B-9N2-iv liquid (agar removed) for continued rotating 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new tubes and

further expansion. One-ten shoots per tube are generally obtained per multiplication cycle. The shoots are then placed in a Stage 5 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 2

Micropropagation of Phyllostachys Edulis "Moso" by Stage 1 to Stage 5 media - II

Explants are chosen and disinfected as in Example 1. Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-90 μmole/m2/s2. In this Example the Stage 1 media is spiked b-12c-iii otherwise as described in Example 10. These explants stay on spiked b-12c-iii media for 2 10-120 day cycles (usually 14 day cycles) (note in this example even though the media is spiked, the time period is not shortened because b-12c-iii can be considered a weakly spiked media). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, reduced B-9N2-iii. Following 1 or 2 10-120 day cycles (usually 14 day cycles), explants are cleaned and placed back in Stage 1 media for additional 1 or 2 10-120 day cycles (usually 14 day cycles). The explants are kept on this rotation of Stage 1 and State 2 media until multiple shoots are observed. Observation of multiple shoots generally occurs within 3-15 months.

Once the explant exhibits multiple shoots, it is transferred to a Stage 3 media, in this Example reduced B-9N2-iii at a pH of 5.7 for 10-120 day cycles (usually 21 day cycles). Shoots are rotated between this Stage 3 media and a Stage 4 media, in the Example, reduced B-9N2-iii liquid (agar removed) for continued rotating 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube are generally obtained per multiplication cycle. The shoots are then placed in a Stage 5 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 3.

Micropropagation of Bambusa Old Hamii by Stage 1, Stage 2, and Stage 3 media

Explants are chosen and disinfected as in Example 1. Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of

36-90 μmole/m2/s2. In this Example the Stage 1 media is spiked b-12c-ii otherwise as described in Example 10. These explants stay on spiked b-12c-ii media for 2 8-118 day cycles (usually 12 day cycles). Between cycles, excess sheaths are removed. Explants remain on cycles of spiked b-12c-ii media until multiple shoots were observed. Observation of multiple shoots generally occurs within 3-15 months.

Once the explant exhibits multiple shoots, it is transferred to a Stage 2 media, in this Example reduced b-10-iv at a pH of 5.7 for 10-120 day cycles (usually 21 day cycles). Shoots remain on reduced b-10-iv media for continued rotating 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube are generally obtained per multiplication cycle. The shoots are then placed in a Stage 3 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 4.

Micropropagation of Phyllostachys Edulis "Moso" by Stage 1 to Stage 4 media

Explants are chosen and disinfected as in Example 1. Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 200-500 μmole/m2/s2. In this Example the Stage 1 media is spiked b-12c-iv otherwise as described in Example 10. These explants stay on spiked b-12c-iv media for 2 8-118 day cycles (usually 12 day cycles). Between cycles, excess sheaths are removed. Explants remain on cycles of spiked b-12c-ii media until multiple shoots were observed. Observation of multiple shoots generally occurs within 3-15 months.

Once the explant exhibits multiple shoots, it is transferred to a Stage 2 media, in this Example reduced B-9N2-iv at a pH of 5.7 for 10-120 day cycles (usually 21 day cycles). Shoots are rotated between this Stage 2 media and a Stage 3 media, in the Example, spiked b-11-iv for continued rotating 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube are generally obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 5.

Micropropagation of Bamboo Plants by Stage 1, Stage 2, Stage 3, Stage 4, Stage 5, and/or Stage 6 media

Starting with a bamboo plant between the ages of 3 months and 3 years, a node from the cane with the lateral shoot just breaking the sheath is used as the explant. Each nodal section is cut into 3-5 millimeter sections with the shoot intact. The outer sheaths are peeled off and discarded and the remaining nodal section piece put into a 10% bleach solution with a final concentration of 0.6% sodium hydrochloride. The explant in bleach solution is placed onto a Lab Rotators, Adjustable speed, Barnstead/Lab line orbital Shaker (model number KS 260) shaker table for 1 hour at 6-9 revolutions per minute. The explants are then put into a 1% bleach solution with a final concentration of 0.06% sodium hydrochloride, and placed back onto the shaker table for 30 minutes. This 1% bleach solution step is then repeated.

Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-90 μmole/m2/s2. The initial Stage 1 media in this Example is spiked b-12c-iv at a pH of 5.7. The explants remain on spiked b-12c-iv media for 1-36 hours after which they are transferred to a Stage 2 media, in this Example, reduced b-12c-iv media for the remainder of a 10-120 day (usually 21 day) cycle.

Contaminated tubes are discarded. Contaminated tubes are identified by bacterial discoloration of the agar or by visible surface contamination. These explants undergo 3-4 Stage 1/2 media rotations, each including 10-120 day cycles (usually 21 day cycles). A subset of the explants are on Stage 1 spiked b-12c-iv media for 1-36 hours followed by transfer to a Stage 2 reduced b-12c-iv media for the remainder of the cycle for each cycle (rotation between Stage 1 and Stage 2 media). Other explants alternate between culture on the spiked/reduced protocol and culture on standard b-12c-iv media (rotation between Stage 1, Stage 2 and Stage 3 media). Alternatively, explants could begin the process in standard b-12c-iv media for the first 10-120 day cycle and then transition to the spiked/reduced protocol for one or more of the following cycles.

Explants are taken off the media after the third cycle if multiplication is occurring. If multiplication is not occurring or not occurring to a significant degree, explants are left on media for a fourth cycle. Spiked/reduced or standard media is chosen based on the particular explant's previous treatment and treatment parameters (all cycles on spiked/reduced or alternating between spiked/reduced and standard).

Live shoots are next transferred to a Stage 3 or Stage 4 media (depending on previous treatments), in this Example, standard b-9-iv at a pH of 5.7. The cultures stay on b-9-iv media until the desired number of shoots is obtained by separation into new tubes and further expansion. Generally, the range of time includes 10-120 day cycles (usually 14-21 day cycles) between which the cultures are assigned to go through another multiplication round in Stage 3 or Stage 4 media or transitioned to a Stage 4 or Stage 5 media, in this Example, standard b-10-iv at a pH of 5.7 for further multiplication. One-ten shoots per tube can be obtained per multiplication cycle.

[0303] Following removal from the multiplication process, the shoots are transferred to small tissue culturing boxes (known as "magenta boxes") for 10-120 days (usually 14-21 days) containing a Stage 5 or Stage 6 media, in this Example, standard BR-2-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 6.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into jars containing a Stage 1 media, in this Example, standard b-12c-iv with a pH of 5.7 (liquid; 30-40 mL) in a regulated clean growth chamber at a temperature of from 65°F-70°F and a full spectrum light level of 36-90 μmole/m2/s2. The explants are transferred to fresh standard b-12c-iv media every 10-120 days (usually every 21 days), with contaminated tubes being discarded. Contaminated tubes are identified by bacterial discoloration of the agar or by visible surface contamination. These explants stay on standard b-12c-iv media for 3-4 10-120 day cycles (usually 21 day cycles).

The cultures are then transferred onto a Stage 2 media, in this Example, spiked b-11-iv (liquid) in jars on a rotating shelf that provides 6-9 revolutions per minute. The cultures remained on spiked b-11-iv media at a pH of 5.7 for 0.5-12 hours and are then transferred to Stage 3 reduced b-12c-iv media for the remainder of the 10-120 day cycle (usually 14 day cycles) until the desired number of shoots can be obtained by separation into new jars and further expansion. One-fifteen shoots per jar can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, standard Ech-iv at a pH of 6 for 10-120 days (usually 14-21 days).

Example 7.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, spiked b-12c-iv for a period of 24-48 hours followed by transfer of the cultures to a Stage 2 reduced b-12c-iv media for 10-21 days (generally 14).

Shoots are then transferred to a Stage 3 media, in this Example, spiked b-9-iv in magenta boxes (40-50 mL). They remain on spiked b-9-iv media for 12-36 hours followed by transfer to Stage 4 reduced b-9-iv media or reduced CW-2-iv media for the remainder of the 10-120 day cycles (usually 14 day cycles) until the desired number of shoots is obtained by separation into new boxes and further expansion. One-twenty shoots per box can be obtained per multiplication cycle. The shoots are then placed on a Stage 5 media, in this Example, spiked BR-2-iv for 5-10 hours followed by transfer to Stage 6 reduced BR-2-iv for the remainder of the 10-120 day (usually 14-21 days) cycle.

Example 8.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, spiked b-12c-ii at a pH of 5.7. Explants remain on spiked b-12c-ii media for 3-18 hours followed by transfer to standard b-12c-iv media for 10-21 days (usually 14 days). Three-eighteen hours in spiked b-12c-ii followed by 10-21 days in standard b-12c-iv cycles are repeated until explants begin to multiply. Shoots are then transferred into a Stage 3 media, in this Example, standard b-1-iv at a pH of 5.5 for 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, spiked Br-2-iv at a pH of 5.7 for 1-10 hours followed by standard Br-2-iv for 14-21 days.

Example 9.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, spiked b-12c-iv for 1-24 hours. Shoots are then transferred into a Stage 2 media, in this Example, standard b-10-iv media for 10-120 days (generally 14). Rotation through Stage 1 and Stage 2 media continues until explants begin to multiply. Once multiplication has begun, shoots are transferred to standard b-4-iv media at a pH of 5.5 for 10-120 day cycles (usually 21 day cycles) until the

desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, spiked Br-2-iv at a pH of 5.7 for 1-10 hours followed by the Stage 5 media, standard Amel-iv for 10-120 days (usually 14-21 days).

Example 10.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, standard b-12c-iv media as described in Example 1. Shoots are then transferred into a Stage 2 media, in this Example, spiked b-9-iv at a pH of 5.5 for 4-24 hours. Following culture in the spiked b-9-iv media for 4-24 hours, shoots are placed in Stage 3 standard b-9-iv media for 10-120 days (usually 21 days). Rotation between spiked and standard b-9-iv media is repeated until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 11.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into boxes containing a Stage 1 media, in this Example, standard b-10-iv (40-50 mL). Shoots are maintained on a rotation of standard b-10-iv media (10-120 day cycles (usually 21 day cycles)), Stage 2 spiked b-10-iv media (1-10 hours) and Stage 3 reduced b-10-iv media (10-120 day cycles (usually 10 day cycles) until the desired number of shoots is obtained by separation into new boxes and further expansion. One-twenty shoots per box can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 media, in this Example, Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 12.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, spiked b-12c-i for 1-24 hours followed by Stage 2 reduced b-10-i for 10-21 days (generally 14) for 3 or 4 cycles. Shoots are then transferred into a Stage 3 media, in this Example, spiked b-9-iv at a pH of 5.5 for 1-5 hours followed by transfer to a stage 4, standard b-9-iv media for 10-120 day cycles (usually 21 day cycles) until the desired number of shoots is obtained by separation into new

tubes and further expansion. A spiked and standard or reduced B-6 media rotation at a pH of 5.5 can also be used. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 5 media, in this Example, Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 13.

Explants are chosen and disinfected as in Example 1. The explants are then transferred into tubes containing a Stage 1 media, in this Example, standard b-12c-iv as described in Example 1. Shoots are then transferred into a Stage 2 media, in this Example, spiked b-10-iv at a pH of 5.5 for 0.5-3 hours followed by standard b-10-iv media as a Stage 3 media for 10-120 day cycles (usually 21 day cycles). The spiked to standard b-10-iv cycles are repeated until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 3 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 14.

Starting with a bamboo plant between the ages of 3 months and 3 years, a node from the cane with the lateral shoot just breaking the sheath is used as the explant. Each nodal section is cut into 3-5 millimeter sections with the shoot intact. Some explants, including explants taken from canes 1 year or older are pre-rinsed by shaking them in a jar of 70% isopropyl alcohol for 3 seconds followed by rinsing them under running tap water for 1 minute. Other explants are not pre-rinsed.

The outer sheaths are peeled off and discarded and the remaining nodal section piece put into a 10% bleach solution. The explant in bleach solution is placed onto a Lab Rotators, Adjustable speed, Barnstead/Lab line orbital Shaker (model number KS 260) shaker table for 1 hour at 6-9 revolutions per minute. For some implants, including those taken from canes 1 year or older, this step is modified by adding a few drops of Tween 20 to the 10% bleach solution and soaking the explants for 45 minutes rather than 1 hour. The explants are then put into a 1% bleach solution, and placed back onto the shaker table for 30 minutes. This 1% bleach solution step is then repeated.

Individual explants are then placed on a Stage 1 media (15-25 mL) within a tube and the tubes are placed into a regulated clean growth chamber at a temperature of from 65°F-

70°F and a full spectrum light level of 36-90 μmole/m2/s2. In this Example, the Stage 1 media is standard b-12c-iv at a pH of 5.7. The explants are transferred to fresh b-12c-iv media every 10-120 days (usually every 21 days), with contaminated tubes being discarded. These explants stay on b-12c-iv media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, spiked b-12c-i supplemented with 7 g/L carageenan (rather than the 5.5 g/L provided above) for a period of 0.5-10 hours followed by placement in standard b-12c-iv for 10-120 days (usually 21). Following the third cycle, explants are cleaned. The explants are kept on a rotation of these Stage 2 and Stage 3 media for the stated time periods until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 2 media/Stage 3 media rotation or transferred to a Stage 4 media, in this Example, when used standard b-9-iv at a pH of 5.7. Alternatively to using one of the b-9 media, a CW1 media at a pH of 5.7 can also be used. The cultures stay on Stage 2/3 media or Stage 4 media until the desired number of shoots is obtained by separation into new tubes and further expansion. Generally, the range of time includes 10-120 day cycles (usually 21 day cycles) between which the cultures are assigned to go through another multiplication round or are transitioned to a Stage 4 or Stage 5 media, in this Example, standard BR-2-iv at a pH of 5.7 for 10-120 days (usually 21 days) in “magenta boxes".

Example 15.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into jars containing a Stage 1 media, in this Example, spiked b-12c-iv (liquid; 30-40 mL). These explants stay on spiked b-12c-iv media for 0.5-24 hours followed by placement in Stage 2 reduced b-12c-i for 10-120 days (usually 21). This process is repeated two times. Between cycles, excess sheaths are removed.

At the time of transfer to the third cycle, explants are transitioned to a Stage 3 media, in this Example, standard b-12c-iv supplemented with 7 g/L carageenan (rather than the 5.5 g/L) for 10-120 day cycles (usually 21 day cycles). Following the third cycle, explants are cleaned. The explants are kept on the Stage 3 standard b-12c-iv supplemented with 7 g/L carageenan for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 3 media or transferred to a Stage 4 media, in this Example, spiked b-11-iv (liquid) at a pH of 5.7 in jars on a rotating shelf that provides 6-9 revolutions per minute. Shoots remain on spiked b-11-iv for 0.5-24 hours followed by transition to a Stage 5 standard b-11-iv. The cultures remain on Stage 3 or Stage 4/5 media for 10-120 day cycles (usually 14 day cycles) until the desired number of shoots is obtained by separation into new jars and further expansion. One-fifteen shoots per jar can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 or Stage 6 media, in this Example, spiked Ech-iv at a pH of 6 for 1-24 hours. Shoots are then transitioned to a Stage 5 or 7 media that is standard Ech-iv for 10-120 days (usually 21 days).

Example 16.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into tubes containing a Stage 1 media in this Example, spiked b-12c-iv. These explants stay on spiked b-12c-iv media for 0.5-24 hours followed by transfer to a Stage 2 standard b-12c-iv media for 10-120 day cycles (usually 21 day cycles). This rotation is repeated 3 times. Between cycles, excess sheaths are removed. At the time of transfer to the fourth cycle, explants are transitioned to a Stage 3 media, in this Example, spiked b-12c-iv supplemented with 7 g/L carageenan (rather than the 5.5 g/L) for 1-24 hours followed by transfer to Stage 4 reduced b-12c-ii media. Following the fourth cycle, explants are cleaned. The explants are kept on Stage 3/4 media rotation for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 3/4 media or transferred to a Stage 5 media, in this Example standard b-9-iv in magenta boxes (40-50 mL) (CW1 media or spiked and reduced and/or standard versions thereof can also be used). They remain on b-9-iv media for 10-120 day cycles (usually 14 day cycles) until the desired number of shoots is obtained by separation into new boxes and further expansion. One-twenty shoots per box can be obtained per multiplication cycle. The shoots are then placed in a Stage 5 or Stage 6 media, in this Example, BR-2-iv for 10-120 days (usually 14-21 days).

Example 17.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into tubes containing a Stage 1 media, in this Example, standard b-12c-iv as described in Example 28. These explants stay on standard b-12c-iv media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, standard b-12c-iv supplemented with 7 g/L carageenan (rather than the 5.5 g/L). Following the third cycle, explants are cleaned. The explants are kept on standard b-12c-iv supplemented with 7 g/L carageenan for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 2 media or transferred to a Stage 3 media, in this Example spiked b-1-iv at a pH of 5.5 for 5-10 hours followed by transfer to Stage 4 no cytokinin b-1 media for 10-120 day cycles (usually 21 day cycles). The stage 3/4 media rotation is repeated until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 3 or Stage 5 media, in this Example, spiked Br-2-iv at a pH of 5.7 for 1-10 hours followed by reduced Br-2-i for 10-120 days (usually 14-21 days).

Example 18.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into tubes containing a Stage 1 media, in this Example, spiked b-12c-iv. These explants stay on spiked b-12c-iv media for 0.5-10 hours followed by transfer to a Stage 2 no cytokinin b-12c media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 3 media, in this Example, spiked b-12c-iii supplemented with 7 g/L carageenan (rather than the 5.5 g/L) for 0.5-10 hours followed by transfer back to the Stage 2 no cytokinin b-12c media. Following the third cycle, explants are cleaned. The explants are kept on the Stage 3/2 media rotation for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 3/2 media rotation or transferred to a Stage 4 media, in this Example spiked b-4-iv at a pH of 5.5 for 12-36 hours followed by Stage 5 no cytokinin b-4-iv for 10-120 days (usually 21 day

cycles) until the desired number of shoots is obtained by separation into new tubes and further expansion. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 4 or Stage 6 media, in this Example, Br-2-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 19.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into tubes containing a Stage 1 media, in this Example, standard b-12c-iv also as described in Example 28. These explants stay on b-12c-iv media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, standard b-12c-iv supplemented with 7 g/L carageenan rather than the 5.5 g/L provided above. Following the third cycle, explants are cleaned. The explants are kept on standard b-12c-iv supplemented with 7 g/L carageenan for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 2 media or transferred to a Stage 3 media, in this Example standard b-9-iv at a pH of 5.5 for 10-120 day cycles (usually 21 days) until the desired number of shoots is obtained by separation into new tubes and further expansion. A b-6 media at a pH of 5.5 can also be used. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 3 or Stage 4 media, in this Example, spiked Amel-iv media at a pH of 5.7 for 0.5-24 hours followed by transfer to a Stage 4 or 5 no cytokinin Amel media for 10-120 days (usually 14-21 days).

Example 20.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into boxes containing a Stage 1 media, in this Example, spiked b-10-iv (40-50 mL). Explants remain on spiked b-10-iv media for 1-5 hours and are then transferred to a Stage 2 no-cytokinin b-10 media for 1-5 days followed by transfer to Stage 3 reduced b-10-ii media for 5-115 days (usually 18 days). Between cycles, excess sheaths are removed, and the rotation is repeated two times. At the time of transfer to the third rotation, explants are transitioned to a Stage 4 media, in this Example, spiked b-10c-i supplemented with 7 g/L carageenan (rather than the 5.5 g/L) for 1-5 hours, followed by transfer to a Stage 5 no

cytokinin b-10c for 1-5 days and Stage 6 reduced b-12c for 5-115 days (usually 18 days). Following the third cycle, explants are cleaned. The explants are kept on the Stage 4/5/6 media rotation until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months. Cultures are maintained on the Stage 4/5/6 media rotation until the desired number of shoots is obtained. One-twenty shoots per box can be obtained per multiplication cycle. The shoots are then placed in a Stage 7 media, in this Example, standard Amel-iv at a pH of 5.7 for 10-120 days (usually 14-21 days).

Example 21.

Explants are chosen and disinfected as in Example 10. The explants are then transferred into tubes containing a Stage 1 media, in this Example, standard b-12c-iv as described in Example 28. These explants stay on standard b-12c-iv media for 2 10-120 day cycles (usually 21 day cycles). Between cycles, excess sheaths are removed. At the time of transfer to the third cycle, explants are transitioned to a Stage 2 media, in this Example, standard b-12c-iv supplemented with 7 g/L carageenan rather than the 5.5 g/L provided above. Following the third cycle, explants are cleaned. The explants are kept on standard b-12c-iv supplemented with 7 g/L carageenan for 10-120 day cycles (usually 21 day cycles) until multiple shoots are observed. Observation of multiple shoots can occur within 3-15 months.

Once the explant exhibits multiple shoots, it is either maintained on its Stage 2 media or transferred to a Stage 3 media, in this Example spiked b-9-iv at a pH of 5.5 for 0.5-24 hours followed by transfer to a Stage 4 no cytokinin b-4 media for 10-120 days. The Stage 2 or Stage 3/4 media rotation cycles (usually 21 day cycles) continue until the desired number of shoots is obtained by separation into new tubes and further expansion. A spiked or standard b-6 media at a pH of 5.5 can also be used. One-ten shoots per tube can be obtained per multiplication cycle. The shoots are then placed in a Stage 3 or Stage 5 media, in this Example, spiked Amel-iv at a pH of 5.7 for 24 hours followed by transfer to standard Amel-iv for 10-120 days (usually 14-21 days).

As will be understood by one of ordinary skill from the provided examples, the tissue culturing method for individual species includes slight variations in media, timing and growth conditions. These variations for individual species require optimization based on factors including location, desired outcome, starting material, etc.

For each of the species provided in the examples listed above, in particular embodiments, each can be initiated and/or multiplied in b-9-i media, b-9-ii media, b-9-iii

media, b-9-iv media, b-9-v media, spiked b-9-i media, spiked b-9-ii media, spiked b-9-iii media, spiked b-9-iv media, spiked b-9-v media, reduced b-9-i media (reduced media are described below), reduced b-9-ii media, reduced b-9-iii media, reduced b-9-iv media, reduced b-9-v media, CW2-i media, CW2-ii media, CW2-iii media, CW2-iv media, CW2-v media, spiked CW2-i media, spiked CW2-ii media, spiked CW2-iii media, spiked CW2-iv media, spiked CW2-v media, reduced CW2-i media, reduced CW2-ii media, reduced CW2-iii media, reduced CW2-iv media, reduced CW2-v media, b-10-i media, b-10-ii media, b-10-iii media, b-10-iv media, b-10-v media, spiked b-10-i media, spiked b-10-ii media, spiked b-10-iii media, spiked b-10-iv media, spiked b-10-v media, reduced b-10-i media, reduced b-10-ii media, reduced b-10-iii media, reduced b-10-iv media, reduced b-10-v media, b-11-i media, b-11-ii media, b-11-iii media, b-11-iv media, b-11-v media, spiked b-11-i media, spiked b-11-ii media, spiked b-11-iii media, spiked b-11-iv media, spiked b-11-v media, reduced b-11-i media, reduced b-11-ii media, reduced b-11-iii media, reduced b-11-iv media, reduced b-11-v media, b-12c-i media, b-12c-ii media, b-12c-iii media, b-12c-iv media, b-12c-v media, spiked b-12c-i media, spiked b-12c-ii media, spiked b-12c-iii media, spiked b-12c-iv media, spiked b-12c-v media, reduced b-12c-i media, reduced b-12c-ii media, reduced b-12c-iii media, reduced b-12c-iv media, reduced b-12c-v media, b-1-i media, b-1-ii media, b-1-iii media, b-1-iv media, b-1-v media, spiked b-1-i media, spiked b-1-ii media, spiked b-1-iii media, spiked b-1-iv media, spiked b-1-v media, reduced b-1-i media, reduced b-1-ii media, reduced b-1-iii media, reduced b-1-iv media, reduced b-1-v media, b-4-i media, b-4-ii media, b-4-iii media, b-4-iv media, b-4-v media, spiked b-4-i media, spiked b-4-ii media, spiked b-4-iii media, spiked b-4-iv media, spiked b-4-v media, reduced b-4-i media, reduced b-4-ii media, reduced b-4-iii media, reduced b-4-iv media, reduced b-4-v media, b-6-i media, b-6-ii media, b-6-iii media, b-6-iv media, b-6-v media, spiked b-6-i media, spiked b-6-ii media, spiked b-6-iii media, spiked b-6-iv media, spiked b-6-v media, reduced b-6-i media, reduced b-6-ii media, reduced b-6-iii media, reduced b-6-iv media, reduced b-6-v media, CW1-i media, CW1-ii media, CW1-iii media, CW1-iv media, CW1-v media, spiked CW1-i media, spiked CW1-ii media, spiked CW1-iii media, spiked CW1-iv media, spiked CW1-v media, reduced CW1-i media, reduced CW1-ii media, reduced CW1-iii media, reduced CW1-iv media, reduced CW1-v media, CW3-i media, CW3-ii media, CW3-iii media, CW3-iv media, CW3-v media, spiked CW3-i media, spiked CW3-ii media, spiked CW3-iii media, spiked CW3-iv media, spiked CW3-v media, reduced CW3-i media, reduced CW3-ii media, reduced CW3-iii media, reduced CW3-iv media, reduced CW3-v media, CW4-i media, CW4-ii media,

CW4-iii media, CW4-iv media, CW4-v media, spiked CW4-i media, spiked CW4-ii media, spiked CW4-iii media, spiked CW4-iv media, spiked CW4-v media, reduced CW4-i media, reduced CW4-ii media, reduced CW4-iii media, reduced CW4-iv media, reduced CW4-v media, CW5-i media, CW5-ii media, CW5-iii media, CW5-iv media, CW5-v media, spiked CW5-i media, spiked CW5-ii media, spiked CW5-iii media, spiked CW5-iv media, spiked CW5-v media, reduced CW5-i media, reduced CW5-ii media, reduced CW5-iii media, reduced CW5-iv media, reduced CW5-v media, CW6-i media, CW6-ii media, CW6-iii media, CW6-iv media, CW6-v media, spiked CW6-i media, spiked CW6-ii media, spiked CW6-iii media, spiked CW6-iv media, spiked CW6-v media, reduced CW6-i media, reduced CW6-ii media, reduced CW6-iii media, reduced CW6-iv media, reduced CW6-v media, B-9N2-i media, B-9N2-ii media, B-9N2-iii media, B-9N2-iv media, B-9N2-v media, spiked B-9N2-i media, spiked B-9N2-ii media, spiked B-9N2-iii media, spiked B-9N2-iv media, spiked B-9N2-v media, reduced B-9N2-i media, reduced B-9N2-ii media, reduced B-9N2-iii media, reduced B-9N2-iv media, reduced B-9N2-v media, B-12C CPPU-i media, B-12C CPPU-ii media, B-12C CPPU-iii media, B-12C CPPU-iv media, B-12C CPPU-v media, spiked B-12C CPPU-i media, spiked B-12C CPPU-ii media, spiked B-12C CPPU-iii media, spiked B-12C CPPU-iv media, spiked B-12C CPPU-v media, reduced B-12C CPPU-i media, reduced B-12C CPPU-ii media, reduced B-12C CPPU-iii media, reduced B-12C CPPU-iv media, reduced B-12C CPPU-v media, B-12C DPU-i media, B-12C DPU-ii media, B-12C DPU-iii media, B-12C DPU-iv media, B-12C DPU-v media, spiked B-12C DPU-i media, spiked B-12C DPU-ii media, spiked B-12C DPU-iii media, spiked B-12C DPU-iv media, spiked B-12C DPU-v media, reduced B-12C DPU-i media, reduced B-12C DPU-ii media, reduced B-12C DPU-iii media, reduced B-12C DPU-iv media, reduced B-12C DPU-v media, or combinations thereof according to the various procedures described herein.

Example 22.

Methods and Materials for Pulsing Method

General procedures for bamboo tissue culture are described in the International Patent Application Publication No. WO2011100762, which is incorporated herein by reference in its entirety.

The bud induction media and shoot elongation media used in Examples 1-3 are described in the table below.

Description of media components for the bud induction and shoot elongation/maintenance media

Media	Bud Induction media (mg/L)	Shoot elongation & maintenance media (mg/L)
NH_4NO_3	1650	1650
KNO_3	1900	1900
$Ca(NO_3)_2$	0	0
K_2SO_4	0	0
$MgSO_4$	370	370
$MnSO_4$	16.9	16.9
$ZnSO_4$	8.6	8.6
$CuSO_4$	0.025	0.025
$CaCl_2$	440	440
KI	0.83	0.83
$CoCl_2$	0.025	0.025
H_3BO_3	6.2	6.2
Na_2MoO_4	0.25	0.25
KH2PO4	170	170
K_2SO_5	as necessary	as necessary
$FeSO_4$	55.7	55.7
Na_2EDTA	74.6	74.6
$Na_2H_2PO_4$	170	170
myo-Inositol	100	100
Thiamine	0.4	0.4
Sugar	30gr.	30gr.
agar	4.5gr	5gr
PH	5.7	5.7
HORMONES		
NAA	0.05	0.01 - 50
BAP	1	0.01 - 50
TDZ	0.25 - 100	0
ST-10	0.01 - 50	0.01 - 50
2ip	0	6

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Example 23.

Micropropagation of Bamboo by Pulsing Method - I

In this example, *Phyllostachys edulis*, MOSO tissue cultures were inoculated in a bioreactor. Bud induction medium was inoculated into the bioreactor and the cultures were

incubated in the growth room for 48 hours. After 48 hours, the bud induction medium was replaced with the shoot elongation and maintenance medium, and the bamboo plants were cultured for an additional period of 5 days. The above cycle was repeated two more times for a total of three bud induction treatments and three shoot elongation and maintenance treatments.

At the end of the treatment period, data were collected to determine culture multiplication rates. A multiplication rate of 28X was achieved compared to the control treatment in the gelled media where the multiplication rate was 2X.

Example 24.

Micropropagation of Bamboo by Pulsing Method - II

In this example, *Phyllostachys edulis*, MOSO cane segments were collected from the green house and surface sterilized before placing the nodal segments in the bud induction media. The nodal segments had one single bud, which could be dormant or active. The cultures were incubated in the growth room for a period of 1 hour to 3 weeks, with an optimal culture incubation time of 2 weeks.

The dormant or active axillary bud developed shoots during the bud induction phase. The shoot buds were isolated at the end of the bud induction phase and cultured on the bud elongation and maintenance media for a period of 1 to 3 weeks.

During this time, the single axillary bud developed into multiple shoots. The process of bud induction and shoot elongation/maintenance was repeated several times to get shoot multiplication rates enough to produce 100,000 plants from a single axillary bud.

Example 25.

Micropropagation of Bamboo by Pulsing Method - III

In this example, *Phyllostachys edulis*, MOSO seeds were surface sterilized and placed on the bud induction media. The cultures were incubated in the growth room for a period of 1 hour to 3 weeks.

The seeds germinated in the bud induction media and produced multiple shoots. The seed derived multiple shoots were then placed on the shoot elongation/maintenance media for a period of 1 to 3 weeks.

The process of the bud induction and bud elongation/maintenance was repeated several times to achieve shoot multiplication rates enough to produce 100,000 plants from a single seed explant.

Example 26.

Micropropagation of Bamboo by Pulsing Method - VI

In this example, tissue cultures of *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia* are inoculated in a bioreactor. Bud induction medium is inoculated into the bioreactor and the cultures are incubated in the growth room for 48 hours. After 48 hours, the bud induction medium is replaced with the shoot elongation and maintenance medium, and the bamboo plants are cultured for an additional period of 5 days. The above cycle is repeated two more times for a total of three bud induction treatments and three shoot elongation and maintenance treatments.

At the end of the treatment period, data are collected to determine culture multiplication rates. A multiplication rate of about 10X to about 28X is achieved for each of the bamboo species compared to the control treatment in the gelled media where the multiplication rate is 2X.

Example 27.

Micropropagation of Bamboo by Pulsing Method - V

Cane segments of *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia* are collected from the green house and surface sterilized before placing the nodal segments in the bud induction media. The nodal segments have one single bud, which can be dormant or active. The cultures are incubated in the growth room for a period of 1 hour to 3 weeks, with an optimal culture incubation time of 2 weeks.

The dormant or active axillary bud develops shoots during the bud induction phase. The shoot buds are isolated at the end of the bud induction phase and cultured on the bud elongation and maintenance media for a period of 1 to 3 weeks.

During this time, the single axillary bud develops into multiple shoots. The process of bud induction and shoot elongation/maintenance is repeated several times to get shoot multiplication rates enough to produce 100,000 plants from a single axillary bud.

Example 28.

Micropropagation of Bamboo by Pulsing Method - VI

Seeds of *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia* are surface sterilized and placed on the bud induction media. The cultures are incubated in the growth room for a period of 1 hour to 3 weeks.

The seeds germinate in the bud induction media and produce multiple shoots. The seed derived multiple shoots are then placed on the shoot elongation/maintenance media for a period of 1 to 3 weeks.

The process of the bud induction and bud elongation/maintenance is repeated several times to achieve shoot multiplication rates enough to produce 100,000 plants from a single seed explant.

Example 29.

Micropropagation of Bamboo by Using a Bioreactor

- Materials and Methods

Shoot cultures of Moso, Nigra Henon, Rufa and Nigra plants were grown in the Ebb and flow bioreactor as described in the figures. Liquid multiplication media B11 and Dic 25/30 ST were used.

Procedure

Approximately 100 to 300 micro shoots of the different Bamboo species were inoculated into the Ebb and flow bioreactors using sterile techniques. The bioreactors were sealed and incubated in the growth chamber (25°C and a photo period of 16/8). The cultures were exposed to Dic 25/30 ST liquid media for a total of 5 days and B11 media for a total of 2 days. The media cycling (pulsing) was repeated for a period of 3 to 6 weeks. The Media was completely changed to fresh media once every 3 weeks.

- Results

The micro shoots responded to the media pulsing treatment and rapidly multiplied. An estimated number of 1000 to 10000 plants were produced from each bioreactor with in a culture period of 3 to 6 weeks.

Example 30.

Micropropagation of Bamboo by Using a Bioreactor - II

- Materials and Methods

Shoot cultures of Moso, Phylostachys Nigra 'Henon', Fargesia rufa, Fargesia nitida, Chusquea culeou, Borinda Boliana, Fargesia murielae, Fargesia nitida jiuzhaigou, Pleioblastus fortune, Chusquea culeou 'Cana Prieta', Fargesia denudate, Fargesia robusta 'Pingwu', Fargesia robusta 'Wolong', Phylostachys Nigra, Dendrocalamus, and Variagated Old Hamii plants are grown in the Ebb and flow bioreactor as described in the Figures. Liquid multiplication media for each bamboo species is listed in the table below.

Description of media components for the bud induction and shoot elongation/maintenance media

Species	**Media 1**	**Media 2**	**Pulsing duration**	**Culture stage**
Moso	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Phylostachys Nigra 'Henon'	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Fargesia rufa	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Fargesia nitida	B11		Media 1 continuous	Multiplication
Chusquea culeou	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Borinda Boliana	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Fargesia murielae	B11		Media 1 continuous	Multiplication
Fargesia nitida jiuzhaigou	B11		Media 1 continuous	Multiplication
Pleioblastus fortunei	B11		Media 1 continuous	Multiplication
Chusquea culeou 'Cana Prieta'	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Fargesia denudata	B11	DIC 25/30 ST	168 hr media 1 and 168 hr media 2	Multiplication
Fargesia robusta 'Pingwu'	B11	DIC 25/30	168 hr media 1 and	Multiplication

		ST	168 hr media 2	
Fargesia robusta 'Wolong'	B11		Media 1 continuous	Multiplication
Phylostachys Nigra	DIC 25/30 DT	DIC 25/30 ST	168 hr media 1 and 168 hr media 2	Multiplication
Dendrocalamus	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Variagated Old Hamii	DIC 25/30 ST		Media 1 continuous	Multiplication

Recipes of B11, DIC 25/ 30 ST, and DIC 25/ 30 DT are shown in FIG. 7.

- Procedure

Micro shoots of the different Bamboo species are inoculated into the Ebb and flow bioreactors using sterile techniques. The bioreactors are sealed and incubated in the growth chamber (25°C and a photo period of 16/8). The cultures are exposed to Dic 25/30 ST liquid media and B11 media according to the conditions specified in Table 4. The media cycling (pulsing) is repeated for a period of 3 to 6 weeks. The Media is completely changed to fresh media once every 3 weeks.

- Results

The micro shoots responded to the media pulsing treatment and rapidly multiplied. An estimated number of 1000 to 10000 plants are produced from each bioreactor with in a culture period of 3 to 6 weeks.

Example 31.

Micropropagation of Other Plants

- Materials and Methods

Shoot cultures of *Geranium rozanne*, *Hakonechloa macra* 'Aureola', *Hakonechloa macra* 'All gold', Helleborus Ivory Prince, Phormium, Wasabi C2, *Arundinaria gigantean*, *Solanum tuberosum*, and *Solanum tuberosum* plants are grown in the Ebb and flow bioreactor as described in the figures. Liquid multiplication media for each bamboo species is listed in the table below.

Description of media components for the bud induction and shoot elongation/maintenance media

Species	Media 1	Media 2	Pulsing duration	Culture stage
Geranium rozanne	DKW		Media 1 continuous	Multiplication
Hakonechloa macra 'Aureola'	DIC 25/30		Media 1 continuous	Multiplication
Hakonechloa macra 'All gold'	DIC 25/30		Media 1 continuous	Multiplication
Helleborus 'Ivory Prince'	DIC 25/30		Media 1 continuous	Multiplication
Phormium	DIC 25/30		Media 1 continuous	Multiplication
Wasabi C2	Wasabi		Media 1 continuous	Multiplication
Arundinaria gigantea	B11	DIC 25/30 ST	48 hr media 1 and 120 hr media 2	Multiplication
Solanum tuberosum	Lilly light		Media 1 continuous	Multiplication
Solanum tuberosum	Lilly dark		Media 1 continuous	Tuberization

Recipes of B11, DKW, DIC 25/30 ST, Wasabi, DIC 25/30, Lilly light and Lilly dark are shown in FIG. 7.

- Procedure

Micro shoots of the different plant species listed in table 5 are inoculated into the Ebb and flow bioreactors using sterile techniques. The bioreactors are sealed and incubated in the growth chamber (25°C and a photo period of 16/8). The cultures are exposed to suitable medium under the conditions according to in Table 5. The media cycling (pulsing) is repeated for a period of 3 to 6 weeks. The Media is completely changed to fresh media once every 3 weeks.

- Results

The micro shoots responded to the media pulsing treatment and rapidly multiplied. An estimated number of 1000 to 10000 plants are produced from each bioreactor with in a culture period of 3 to 6 weeks.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention.

Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein,

can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes. Also incorporated by reference herein are nucleic acid sequences and polypeptide sequences deposited into the GenBank, which are cited in this specification.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

CLAIMS

What is claimed is:

1. A method of *in vitro* micropropagating bamboo comprising:

(a) incubating a bamboo tissue culture, explant or seed in a bud induction medium to induce shoot bud formation,

(b) incubating the shoot buds obtained in step (a) in a shoot elongation/maintenance medium;

(c) incubating the shoots from step (b) in a bud induction medium to induce shoot bud formation;

(d) incubating the shoot buds obtained in step (c) in a shoot elongation/maintenance medium; and

(e) repeating the incubating steps (c) and step (d) at least one additional cycle; wherein the bud induction medium comprises an effective amount of thidiazuron (TDZ) or analog thereof, and wherein the shoot elongation/maintenance medium comprises an effective amount of one or more cytokinins other than TDZ or an analog thereof.

2. The method of claim 1, wherein the bud induction medium of step (a) and/or step (c) is a liquid medium.

3. The method of claim 1, wherein the bud induction medium of step (a) and/or step (c) is a solid medium.

4. The method of claim 1, wherein the shoot elongation/maintenance medium of step (b) and/or step (d) is a liquid medium.

5. The method of claim 1, wherein the shoot elongation/maintenance medium of step (b) and/or step (d) is a solid medium.

6. The method of claim 1, wherein the incubation of step (a) and/or step (c) lasts from about one hour to about three weeks.

7. The method of claim 1, wherein the incubation of step (b) and/or step (d) lasts from about 24 hours to about four weeks.

8. The method of claim 1, wherein the incubation of step (a) and/or step (c) lasts from about 24 hours to about 60 hours.

9. The method of claim 1, wherein the incubation of step (b) and/or step (d) lasts from about three days to about five days.

10. The method of claim 1, wherein step (e) is repeated at least once.

11. The method of claim 1, wherein steps (a) to (e) take approximately three weeks.

12. The method of claim 1, wherein the bamboo is *Phyllostachys edulisi* 'Moso', *Phyllostachys bissetti, Fargesia denudata, Pleioblastus fortunei, Sasa Veitchii, Pleioblastus viridistriatus, Thamnocalamus crassinodus, Chusquea Culeo "Cana Prieta", Bambusa Old Hamii, Phyllostachys Moso, Phyllostachys Atrovaginata, Dendrocalamus Asper,* or *Guadua Angustifolia, Phylostachys Nigra, Fargesia rufa*, *Fargesia nitida*, *Borinda Boliana*, *Fargesia murielae*, *Pleioblastus fortune*, *Fargesia robusta*, and *Bambusa Oldhamii*.

13. The method of claim 1, wherein the bamboo explant is a segment of bamboo cane.

14. The method of claim 13, wherein the segment of bamboo cane comprises an internode.

15. The method of claim 1, wherein the concentration of TDZ or analog thereof in the bud induction medium is about 0.25 mg/L to about 100 mg/L.

16. The method of claim 1, wherein the concentration of TDZ or analog thereof in the bud induction medium is about 0.5 mg/L to about 2 mg/L.

17. The method of claim 1, wherein the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is selected from the group consisting of N^6-benzylaminopurine (BAP), meta-topolin (mT), zeatin, kinetin, 2-isopentenyladenine (2ip), adenine hemisulfate, dimethylallyladenine, N-(2-chloro-4-pyridyl)-N'- phenylurea) (4-CPPU), and analogs of each thereof.

18. The method of claim 17, wherein the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is from about 0.01 mg/L to about 100 mg/L.

19. The method of claim 18, wherein the concentration of the one or more cytokinins other than TDZ or an analog thereof in the shoot elongation/maintenance medium is from about 0.25 mg/L to about 5 mg/L.

20. The method of claim 1, wherein the bud induction medium and/or the shoot elongation/maintenance medium further comprises one or more auxins.

21. The method of claim 20, wherein the one or more auxins are selected from the group consisting of β-naphthoxyacetic acid (NAA), 2,4- Dichlorophenoxy acetic acid (2,4-D), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), picloram, and analogs of each thereof.

22. The method of claim 20, wherein the one or more auxins is NAA or analogs thereof.

23. The method of claim 1, wherein the shoot buds obtained in step (a) and/or step (c) are separated prior to incubating the shoot buds in step (b) and/or step (d).

24. The method of claim 23, wherein said separation produces groups of 1 to 3 shoot buds per separation prior to incubating the shoot buds in step (b) and/or step (d).

25. A system, comprising:

a growth vessel for incubating plant tissue in a sterile or substantially sterile environment;

a first media container having a first fluid port and a second fluid port, the first fluid port fluidically coupleable to the growth vessel;

a second media container having a first fluid port and a second fluid port, the first fluid port fluidically coupleable to the growth vessel;

a gas source fluidically coupleable to the second fluid port of the first media container and second fluid port of the second media container; and

a controller operable in a first operating mode in which pressurized gas is delivered from the gas source to the first media container to displace a first volume of liquid contained therein to the growth vessel, and a second operating mode in which pressurized gas is delivered from the gas source to the second media container to displace a second volume of liquid contained therein to the growth vessel.

26. The system of claim 25, wherein the controller is operable in a third operating mode in which liquid contained in the growth vessel is allowed to flow from the growth vessel into at least one of the first media container and the second media container.

27. The system of claim 26, wherein the controller is operable in a first incubation sequence in which the third operating mode is executed subsequent to the first operating mode.

28. The system of claim 27, wherein the controller is operable in a second incubation sequence in which the third operating mode is executed subsequent to the second operating mode.

29. The system of claim 28, wherein the controller is further operable in a plant propagation mode in which the first incubation sequence and the second incubation sequence are executed.

30. The system of claim 26, wherein the growth vessel is elevated above the first and second media containers to allow liquid to flow from the growth vessel into at least one of the first media container and the second media container in the third operating mode.

31. The system of claim 25, further comprising a manifold fluidically coupleable to the growth vessel, the first media container, and the second media container,

wherein the manifold is operable to control liquid flow between the growth vessel and the first media container and between the growth vessel and the second media container.

32. The system of claim 25, wherein the growth vessel includes a fluid conduit configured to siphon liquid from the growth vessel to at least one of the first media container and the second media container.

33. The system of claim 25, wherein the growth vessel is an ebb and flow bioreactor.

34. The system of claim 25, wherein the controller is operable to control fluid communication between the growth vessel and the first media container, between the growth vessel and the second media container, between the gas source and the first media container, and between the gas source and the second media container.

35. A method of exchanging liquid media in a bio-reactor for the micropropagation of plant tissue, the bio-reactor comprising a growth vessel for incubating the plant tissue, a first media container fluidically coupleable to the growth vessel, a second media container fluidically coupleable to the growth vessel, and a gas source fluid fluidically coupleable to the first media container and the second media container, the method comprising:

establishing fluid communication between the first media container and the growth vessel;

fluidically isolating the second media container from the growth vessel;

establishing fluid communication between the gas source and the first media container;

delivering compressed gas to the first media container to displace a first volume of liquid from the first media container to the growth vessel;

allowing at least a portion of the first volume of liquid to flow from the growth vessel back into the first media container;

establishing fluid communication between the second media container and the growth vessel;

fluidically isolating the first media container from the growth vessel;

establishing fluid communication between the gas source and the second media container;

delivering compressed gas to the second media container to displace a second volume of liquid from the first media container to the growth vessel; and

allowing at least a portion of the second volume of liquid to flow from the growth vessel back into the second media container.

36. The method of claim 35, wherein compressed gas is delivered to the first media container for approximately one minute.

37. The method of claim 35, wherein compressed gas is delivered to the second media container for approximately one minute.

38. The method of claim 35, wherein the liquid is allowed to flow from the growth vessel back into the first media container for approximately 8 minutes.

39. The method of claim 35, wherein the liquid is allowed to flow from the growth vessel back into the second media container for approximately 8 minutes.

40. A media for micropropagating bamboo wherein said media comprises meta-topolin or an analogue thereof and at least two other cytokinins wherein the media supports multiplication cycles for at least six months.

41. A media for micropropagating bamboo wherein said media comprises at least three cytokinins and supports multiplication cycles for at least six months.

42. A media for micropropagating bamboo wherein said media comprises at least one auxin and at least two cytokinins and supports multiplication cycles for at least six months.

43. A media for micropropagating bamboo wherein said media comprises at least two auxins and at least two cytokinins and supports multiplication cycles for at least six months.

44. A media for micropropagating bamboo wherein said media comprises at least two auxins and at least three cytokinins and supports multiplication cycles for at least six months.

45. A media for micropropagating bamboo wherein said media comprises meta-topolin or an analogue thereof and supports multiplication cycles for at least six months.

46. A media according to any of claims 40-45 wherein said media supports multiplication cycles for at least one year.

47. A media according to claim 1 wherein said meta-topolin or analogue thereof is present in an amount from 0.0125 mg/L – 10 mg/L.

48. A media according to any of claims 40-47 wherein said media comprises thidiazuron or an analogue thereof.

49. A media according to any of claims 40-48 wherein the media comprises NAA and BAP.

50. A media according to any of claims 40-48 wherein the media comprises NAA and thidiazuron.

51. A media according to any of claims 40-48 wherein the media comprises NAA and IBA.

52. A media according to any of claims 40-48 wherein the media comprises NAA and 2ip.

53. A media according to any of claims 40-48 wherein the media comprises BAP and thidiazuron.

54. A media according to any of claims 40-48 wherein the media comprises BAP and IBA.

55. A media according to any of claims 40-48 wherein the media comprises BAP and 2ip.

56. A media according to any of claims 40-48 wherein the media comprises IBA and thidiazuron.

57. A media according to any of claims 40-48 wherein the media comprises IBA and 2ip.

58. A media according to any of claims 40-48 wherein the media comprises thidiazuron and 2ip.

59. A media for micropropagating bamboo wherein said media comprises NAA, BAP, meta-topolin and thidiazuron or analogues thereof wherein the media supports multiplication cycles for at least six months.

60. A media for micropropagating bamboo wherein said media comprises NAA, BAP, meta-topolin and thidiazuron and CCPU and/or DPU or analogues thereof wherein the media supports multiplication cycles for at least six months.

61. A media according to claim 59 wherein said media is a standard or spiked b-12c media, a standard or spiked b-9 media, a standard or spiked b-11 media, or a standard or spiked B-9N2 media.

62. A media according to claim 60 wherein said media is a standard or spiked B-12C CPPU media or a standard or spiked B-12C CPU media.

63. A media for micropropagating bamboo wherein said media comprises NAA, BAP and meta-topolin or analogues thereof wherein the media supports multiplication cycles for at least six months.

64. A media according to claim 63 wherein said media is a standard or spiked CW2 media, a standard or spiked CW1 media or a standard or spiked b-10 media.

65. A media for micropropagating bamboo wherein said media comprises NAA, BAP, meta-topolin and IBA or analogues thereof wherein the media supports multiplication cycles for at least six months.

66. A media according to claim 65 wherein said media is a standard or spiked CW3 or a standard or spiked CW5 media.

67. A media for micropropagating bamboo wherein said media comprises NAA, BAP, meta-topolin, thidiazuron and IBA or analogues thereof wherein the media supports multiplication cycles for at least six months.

68. A media according to claim 67 wherein said media is a standard or spiked CW4 or a standard or spiked CW6 media.

69. A media for micropropagating bamboo wherein said media comprises NAA, BAP, thidiazuron and 2ip or analogues thereof wherein the media supports multiplication cycles for at least six months.

70. A media according to claim 69 wherein said media is a standard or spiked b-1 or a standard or spiked b-4 media.

71. A media for micropropagating bamboo wherein said media comprises NAA, thidiazuron and 2ip or analogues thereof wherein the media supports multiplication cycles for at least six months.

72. A media according to claim 71 wherein said media is a standard or spiked b-6 media.

73. A method of micropropagating bamboo comprising culturing bamboo explants and/or shoots in a media of any one of claims 40-72.

74. A method of micropropagating bamboo according to claim 73 wherein said bamboo is Phyllostachys bissetti; Fargesia denudata; Pleioblastus fortunei; Sasa Veitchii; Pleioblastus viridistriatus; Thamnocalamus crassinodus; Chusquea Culeo “Cana Prieta”; Bambusa Old Hamii; Phyllostachys Moso; Phyllostachys Atrovaginata; Dendrocalamus Asper; or Guadua Angustifolia.

75. A method of micropropagating bamboo according to claim 74 wherein said bamboo is Arundinaria gigantea; Bambusa balcoa; Bambusa vulgaris; Bambusa vulgaris ‘Vitatta’; Bambusa Oldhamii; Bambusa tulda; endrocalamus brandesii; Dendrocalamus asper; Dendrocalamus hamiltoni; Dendrocalamus giganteus; Dendrocalamus membranaceus; Dendrocalamus strictus; Gigantochloa aspera; Gigantochloa scortechini; Guadua culeata; uadua aculeata ‘Nicaragua’; Guadua amplexifolia; Guadua angustifolia; Guadua angustofolia bi-color; Guadua paniculata; Melocanna bambusoides; eohouzeaua dullooa (Teinostachyum);

Ochlandra travancorica; Phyllostachys edulis 'Moso'; Phyllostachys nigra; Phyllostachys nigra 'Henon'; or Schizostachyum lumampao.

76. A media comprising, consisting essentially of or consisting of spiked b-9-i media, spiked b-9-ii media, spiked b-9-iii media, spiked b-9-iv media, spiked b-9-v media, reduced b-9-i media, reduced b-9-ii media, reduced b-9-iii media, reduced b-9-iv media, reduced b-9-v media, spiked CW2-ii media, spiked CW2-iii media, spiked CW2-iv media, spiked CW2-v media, reduced CW2-i media, reduced CW2-ii media, reduced CW2-iii media, reduced CW2-iv media, reduced CW2-v media, spiked b-10-i media, spiked b-10-ii media, spiked b-10-iii media, spiked b-10-iv media, spiked b-10-v media, reduced b-10-i media, reduced b-10-ii media, reduced b-10-iii media, reduced b-10-iv media, reduced b-10-v media, spiked b-11-i media, spiked b-11-ii media, spiked b-11-iii media, spiked b-11-iv media, spiked b-11-v media, reduced b-11-i media, reduced b-11-ii media, reduced b-11-iii media, reduced b-11-iv media, reduced b-11-v media, spiked b-12c-i media, spiked b-12c-ii media, spiked b-12c-iii media, spiked b-12c-iv media, spiked b-12c-v media, reduced b-12c-i media, reduced b-12c-ii media, reduced b-12c-iii media, reduced b-12c-iv media, reduced b-12c-v media, spiked b-1-i media, spiked b-1-ii media, spiked b-1-iii media, spiked b-1-iv media, spiked b-1-v media, reduced b-1-i media, reduced b-1-ii media, reduced b-1-iii media, reduced b-1-iv media, reduced b-1-v media, spiked b-4-i media, spiked b-4-ii media, spiked b-4-iii media, spiked b-4-iv media, spiked b-4-v media, reduced b-4-i media, reduced b-4-ii media, reduced b-4-iii media, reduced b-4-iv media, reduced b-4-v media, spiked b-6-i media, spiked b-6-ii media, spiked b-6-iii media, spiked b-6-iv media, spiked b-6-v media, reduced b-6-i media, reduced b-6-ii media, reduced b-6-iii media, reduced b-6-iv media, reduced b-6-v media, spiked CW1-i media, spiked CW1-ii media, spiked CW1-iii media, spiked CW1-iv media, spiked CW1-v media, reduced CW1-i media, reduced CW1-ii media, reduced CW1-iii media, reduced CW1-iv media, reduced CW1-v media, spiked CW3-i media, spiked CW3-ii media, spiked CW3-iii media, spiked CW3-iv media, spiked CW3-v media, reduced CW3-i media, reduced CW3-ii media, reduced CW3-iii media, reduced CW3-iv media, reduced CW3-v media, spiked CW4-i media, spiked CW4-ii media, spiked CW4-iii media, spiked CW4-iv media, spiked CW4-v media, reduced CW4-i media, reduced CW4-ii media, reduced CW4-iii media, reduced CW4-iv media, reduced CW4-v media, spiked CW5-i media, spiked CW5-ii media, spiked CW5-iii media, spiked CW5-iv media, spiked CW5-v media, reduced CW5-i media, reduced CW5-ii media, reduced CW5-iii media, reduced CW5-iv media, reduced

CW5-v media, spiked CW6-i media, spiked CW6-ii media, spiked CW6-iii media, spiked CW6-iv media, spiked CW6-v media, reduced CW6-i media, reduced CW6-ii media, reduced CW6-iii media, reduced CW6-iv media, reduced CW6-v media, B-9N2-i media, B-9N2-ii media, B-9N2-iii media, B-9N2-iv media, B-9N2-v media, spiked B-9N2-i media, spiked B-9N2-ii media, spiked B-9N2-iii media, spiked B-9N2-iv media, spiked B-9N2-v media, reduced B-9N2-i media, reduced B-9N2-ii media, reduced B-9N2-iii media, reduced B-9N2-iv media, reduced B-9N2-v media, B-12C CPPU-i media, B-12C CPPU-ii media, B-12C CPPU-iii media, B-12C CPPU-iv media, B-12C CPPU-v media, spiked B-12C CPPU-i media, spiked B-12C CPPU-ii media, spiked B-12C CPPU-iii media, spiked B-12C CPPU-iv media, spiked B-12C CPPU-v media, reduced B-12C CPPU-i media, reduced B-12C CPPU-ii media, reduced B-12C CPPU-iii media, reduced B-12C CPPU-iv media, reduced B-12C CPPU-v media, B-12C DPU-i media, B-12C DPU-ii media, B-12C DPU-iii media, B-12C DPU-iv media, B-12C DPU-v media, spiked B-12C DPU-i media, spiked B-12C DPU-ii media, spiked B-12C DPU-iii media, spiked B-12C DPU-iv media, spiked B-12C DPU-v media, reduced B-12C DPU-i media, reduced B-12C DPU-ii media, reduced B-12C DPU-iii media, reduced B-12C DPU-iv media or reduced B-12C DPU-v media.

77. A method of micropropagating bamboo comprising culturing bamboo explants and/or shoots in a spiked and reduced media of claim 76.

78. A method of micropropagating bamboo according to claim 77 wherein said bamboo is Phyllostachys bissetti; Fargesia denudata; Pleioblastus fortunei; Sasa Veitchii; Pleioblastus viridistriatus; Thamnocalamus crassinodus; Chusquea Culeo “Cana Prieta”; Bambusa Old Hamii; Phyllostachys Moso; Phyllostachys Atrovaginata; Dendrocalamus Asper; or Guadua Angustifolia.

79. A method of micropropagating bamboo according to claim 78 wherein said bamboo is Arundinaria gigantea; Bambusa balcoa; Bambusa vulgaris; Bambusa vulgaris ‘Vitatta’; Bambusa Oldhamii; Bambusa tulda; endrocalamus brandesii; Dendrocalamus asper; Dendrocalamus hamiltoni; Dendrocalamus giganteus; Dendrocalamus membranaceus; Dendrocalamus strictus; Gigantochloa aspera; Gigantochloa scortechini; Guadua culeata; uadua aculeata ‘Nicaragua’; Guadua amplexifolia; Guadua angustifolia; Guadua angustofolia bi-color; Guadua paniculata; Melocanna bambusoides; eohouzeaua dullooa (Teinostachyum);

Ochlandra travancorica; Phyllostachys edulis ‘Moso’; Phyllostachys nigra; Phyllostachys nigra ‘Henon’; or Schizostachyum lumampao.

80. A kit comprising a media according to any one of claims 40-72 or 76.

5

81. A rack for plant propagation as substantially described and shown herein.

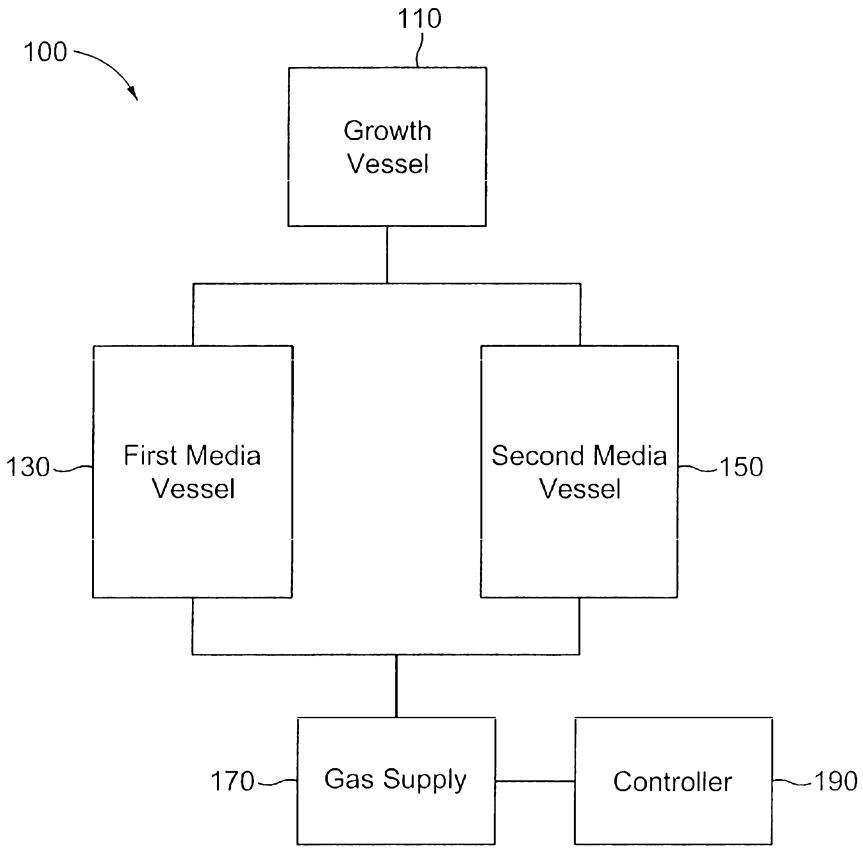
100
110
Growth Vessel
First Media Vessel
130
Second Media Vessel
150
170
Gas Supply
Controller
190

FIG.1

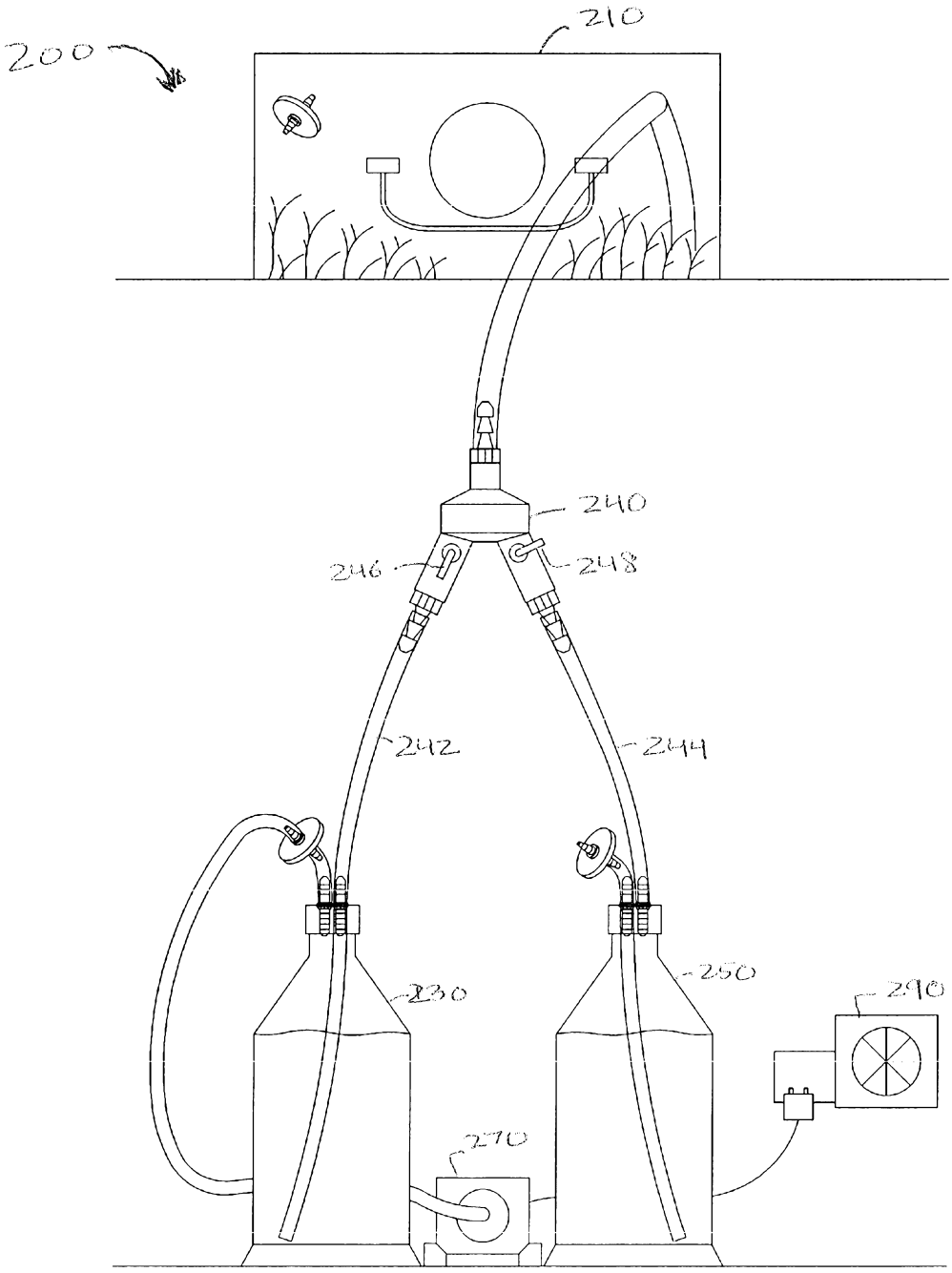

FIG.2

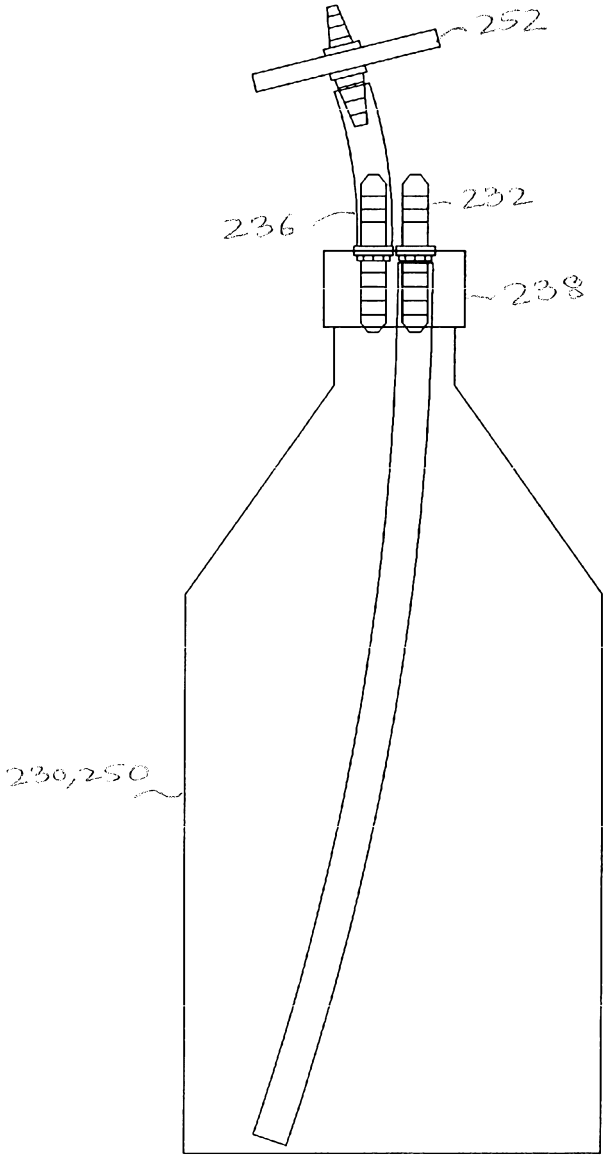

FIG.3

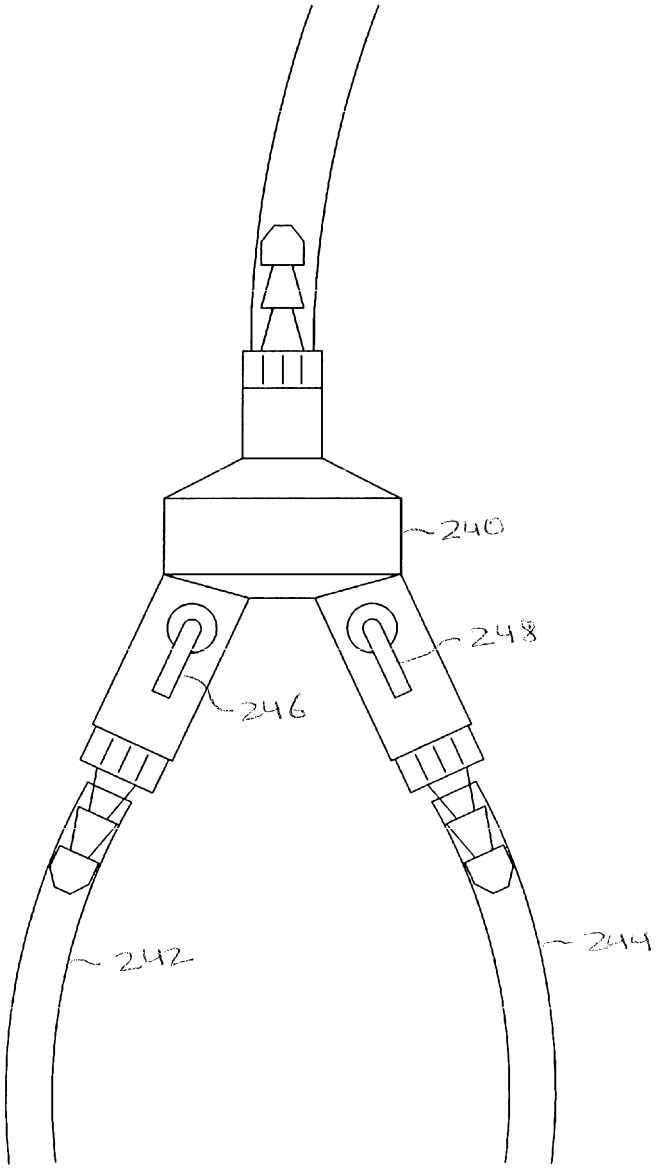

FIG.4

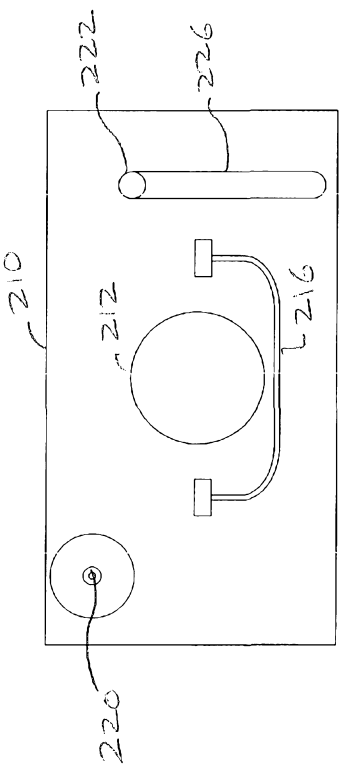

FIG.5A

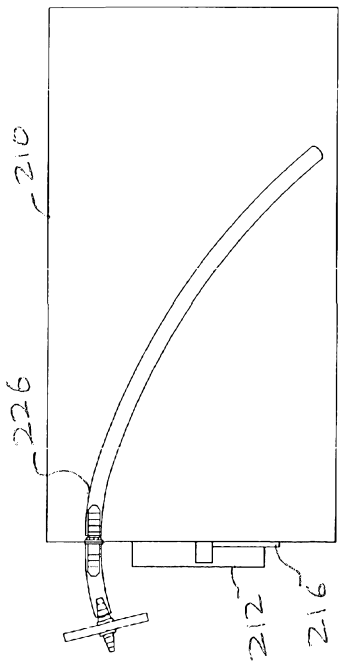

FIG.5B

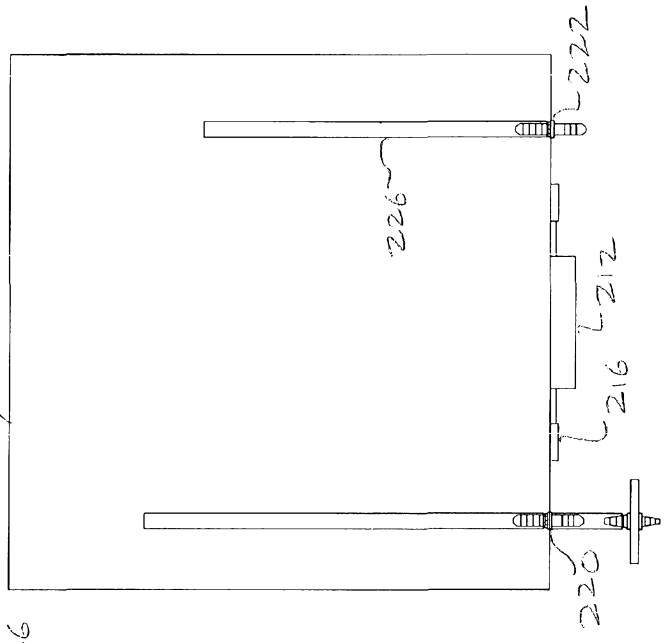

FIG.5C

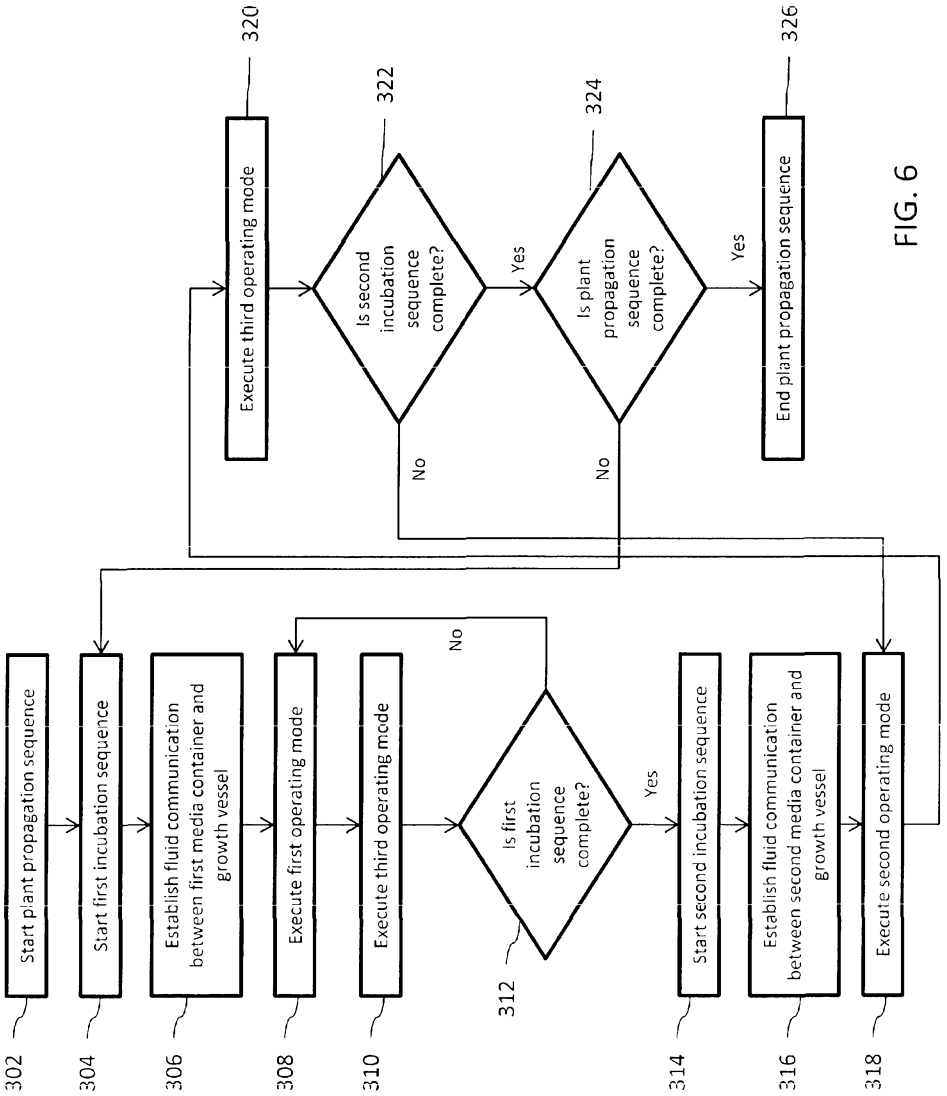
Start plant propagation sequence
302
Start first incubation sequence
304
Establish fluid communication between first media container and growth vessel
306
Execute first operating mode
308
Execute third operating mode
310
Is first incubation sequence complete?
312
No
Yes
Start second incubation sequence
314
Establish fluid communication between second media container and growth vessel
316
Execute second operating mode
318
Execute third operating mode
320
Is second incubation sequence complete?
322
No
Yes
Is plant propagation sequence complete?
324
No
Yes
End plant propagation sequence
326

FIG. 6

Componet								
media	B-11	Dic 25/30 ST	Dic 25/30 DT	DKW	Wasabi	Dic 25/30	Lilly light	Lilly dark
NH4NO3	1650	1650	1650	1416	1650	1650	1650	1650
KNO3	1900	1900	1900	0	1900	1900	1900	1900
Ca(NO3)2	0	0	0	1367	0	0	0	0
K2SO4	0	0	0		0	0	0	0
MgSO4	370	370	370	740	370	370	370	370
MnSO4	16.9	16.9	16.9	27.8	16.9	16.9	16.9	16.9
ZnSO4	8.6	8.6	8.6	17.2	8.6	8.6	8.6	8.6
cuSO4	0.025	0.025	0.025	0.05	0.025	0.025	0.025	0.025
CaCl2	440	440	440	96	440	440	440	440
KI	0.83	0.83	0.83	0	0.83	0.83	0.83	0.83
CoCl2	0.025	0.025	0.025	0	0.025	0.025	0.025	0.025
H3BO3	6.2	6.2	6.2	0.62	6.2	6.2	6.2	6.2
Na2MoO4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
KH2PO4	170	170	170	270	170	170	170	170
K2SO5			0	1215	0	0	0	0
FeSO4	55.7	55.7	27.8	27.8	27.8	27.8	55.7	55.7
Na2EDTA	74.6	74.6	37.3	37.3	37.3	37.3	74.6	74.6
Na2H2PO4	170	170	170	0	0	170	170	170
myo-inositol	100	100	100	1000	100	100	100	100
Thiamine	0.4	0.4	0.4	16	0.4	0.4	0.4	0.4
Pyridoxine	0	0	0	0	0	0	0	0
Nicotinic acid	0	0	0	2	0	0	0	0
Glycine	0	0	0	2	0	0	0	0
Riboflavin	0	0	0	0	0	0	0	0
AscorbicAcid	0	0	0	0	0	0	0	0
HORMONES								
Adenine Sulfate	0						0	0
NAA	0.05	0.05	0.05	0	0	0.05	0	0.02
IBA	0	0	0	0	0	0	0	0
IAA	0	0	0	0	0.5	0	1	0
BAP	1	2.5	2.5	0.35	0.5	2.5	0	0
ST-200	0.5	0	0.1	0	0	0	0	0
ST-10	5	5	5	0	0	0	0	0
2ip	0	6	6	0	0	6	2-4	0
Sugar	30gr.	30gr.	30gr.	30gr.	30gr.	30gr.	30gr.	60gr.
agar	0	0	0	0	0	0	0	0
Carrageenan	0	0	0	0	0	0	0	0
Charcoal	0	0	0	0	0	0	0	0
PH	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7

FIG. 7

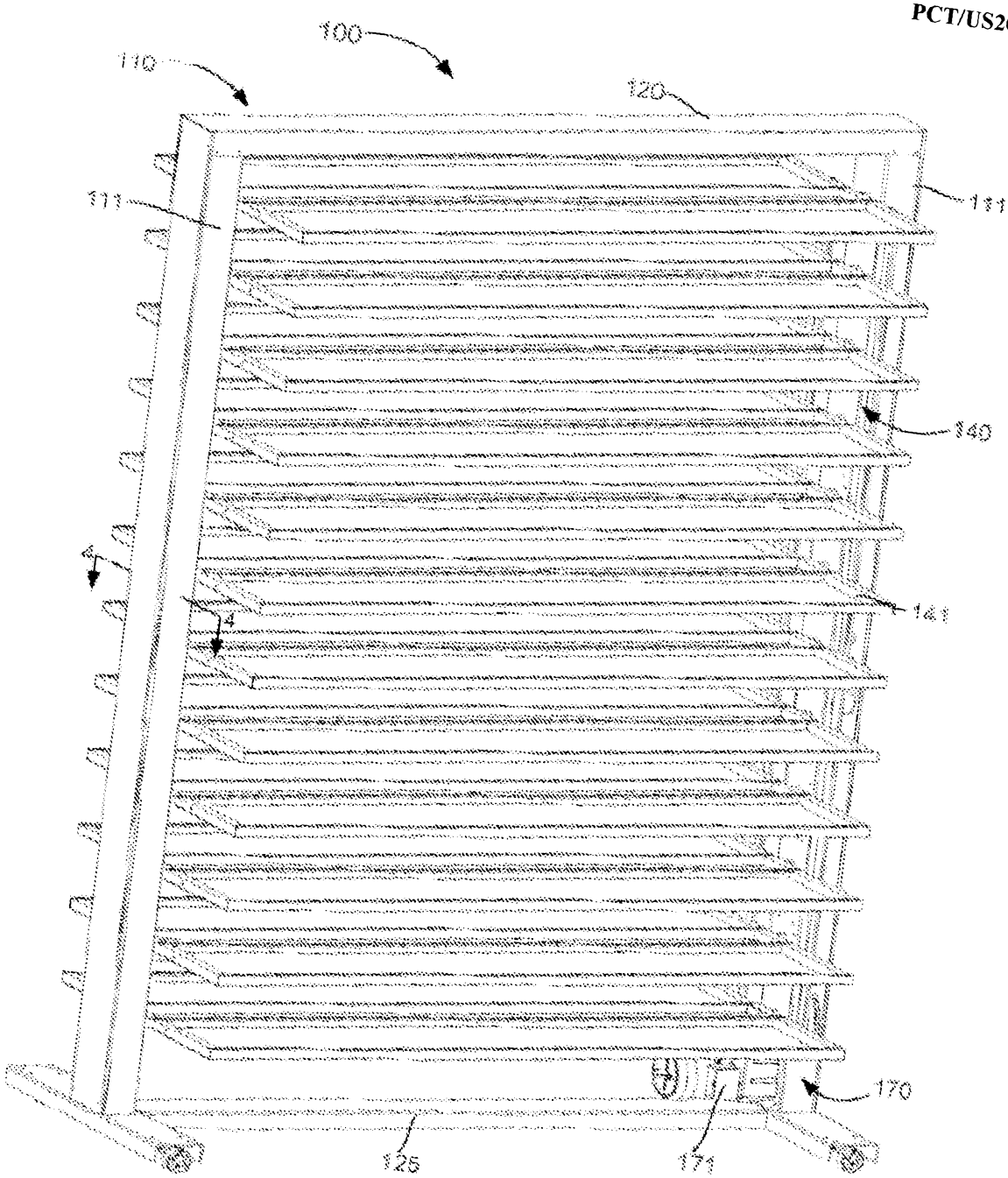

FIG. 8

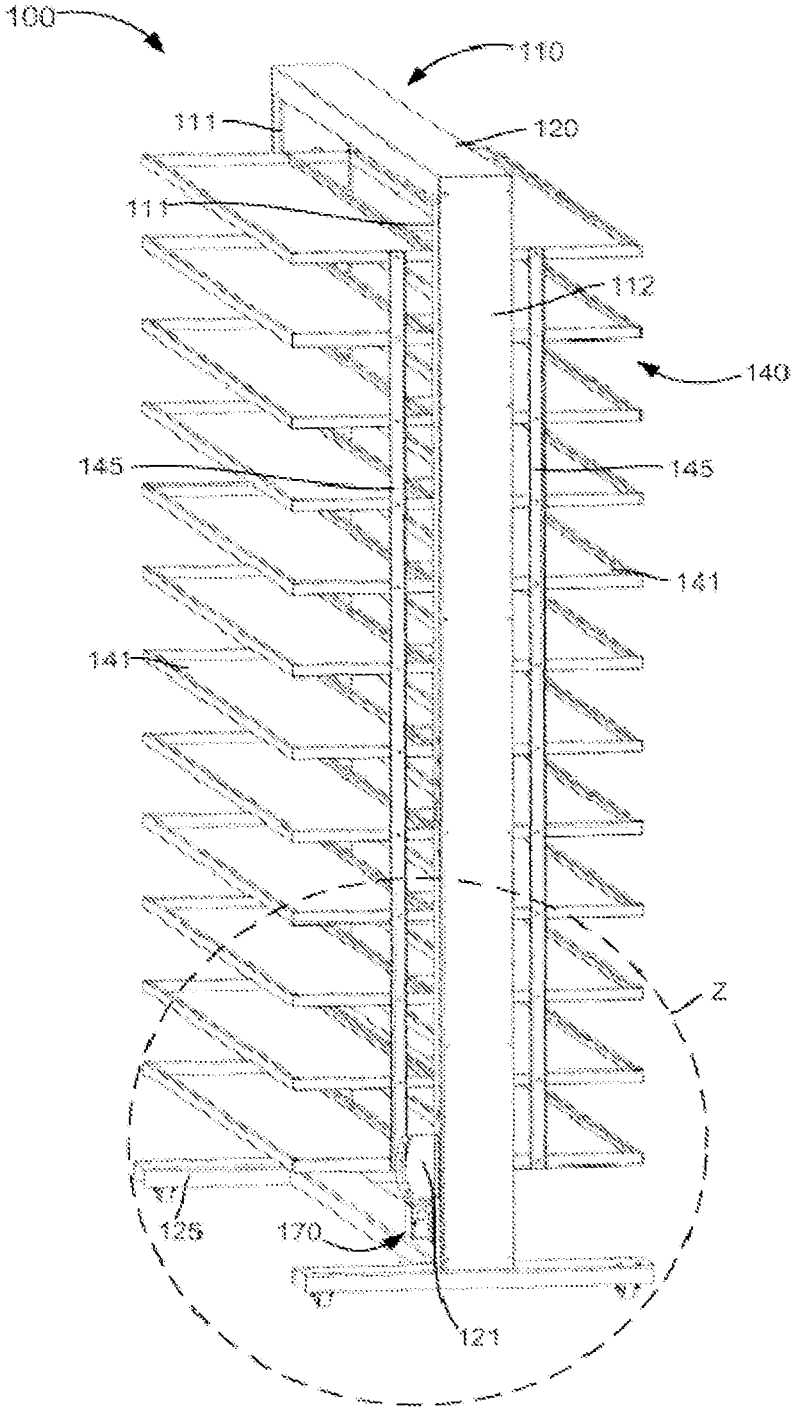

FIG. 9

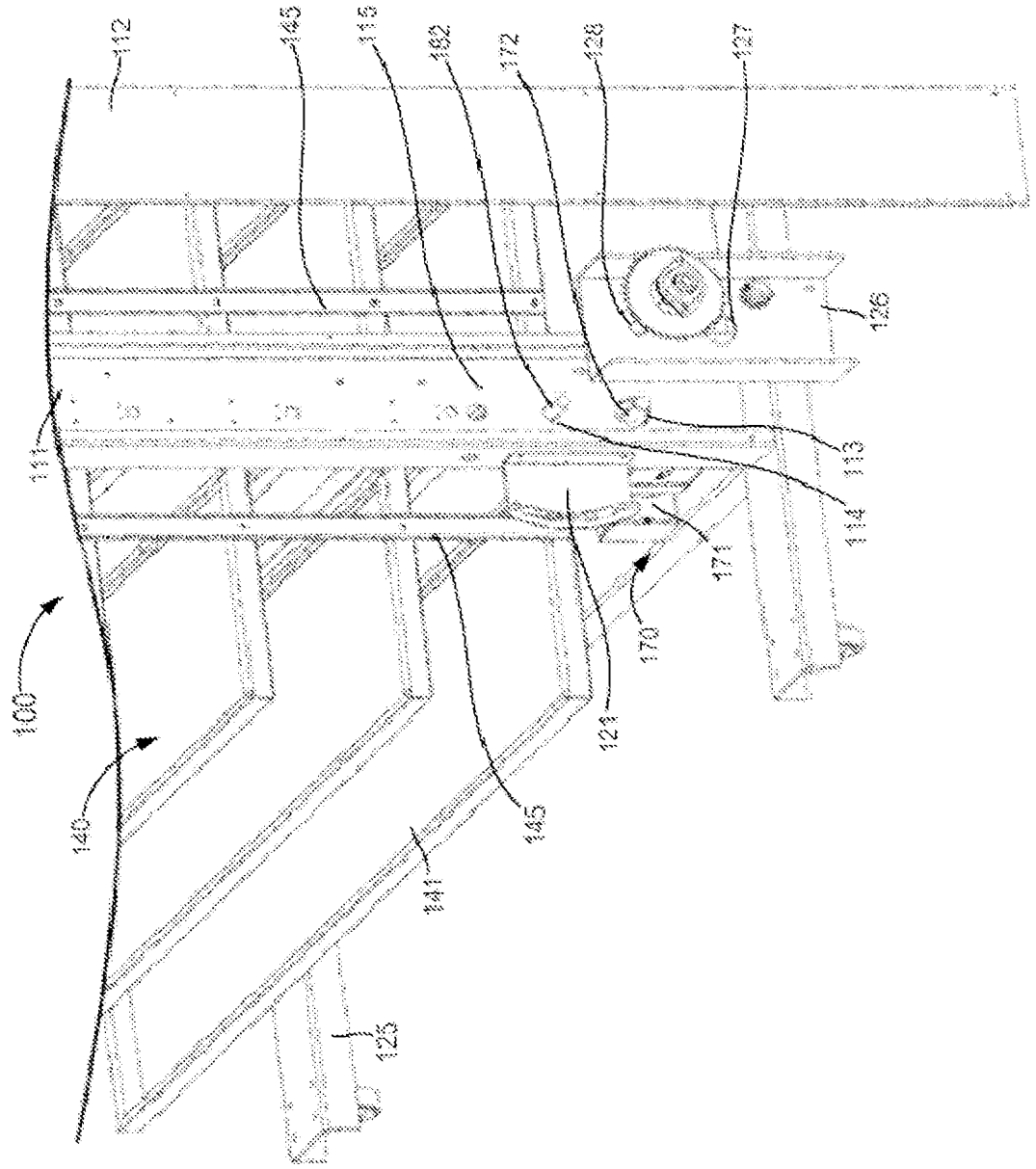
112
145
116
192
172
128
127
126
111
113
114
171
170
100
121
140
145
141
125

FIG. 10

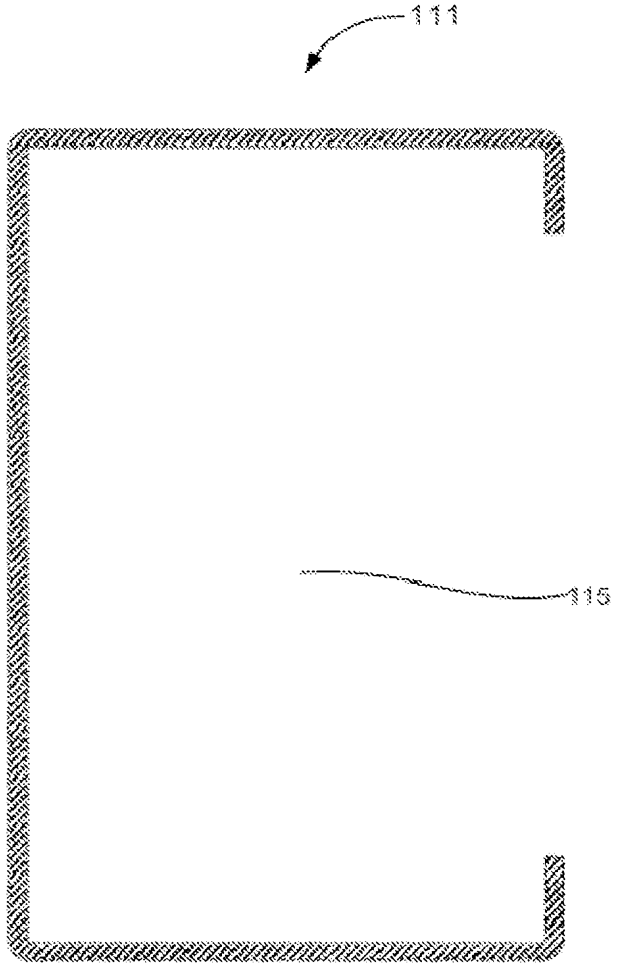

FIG. 11

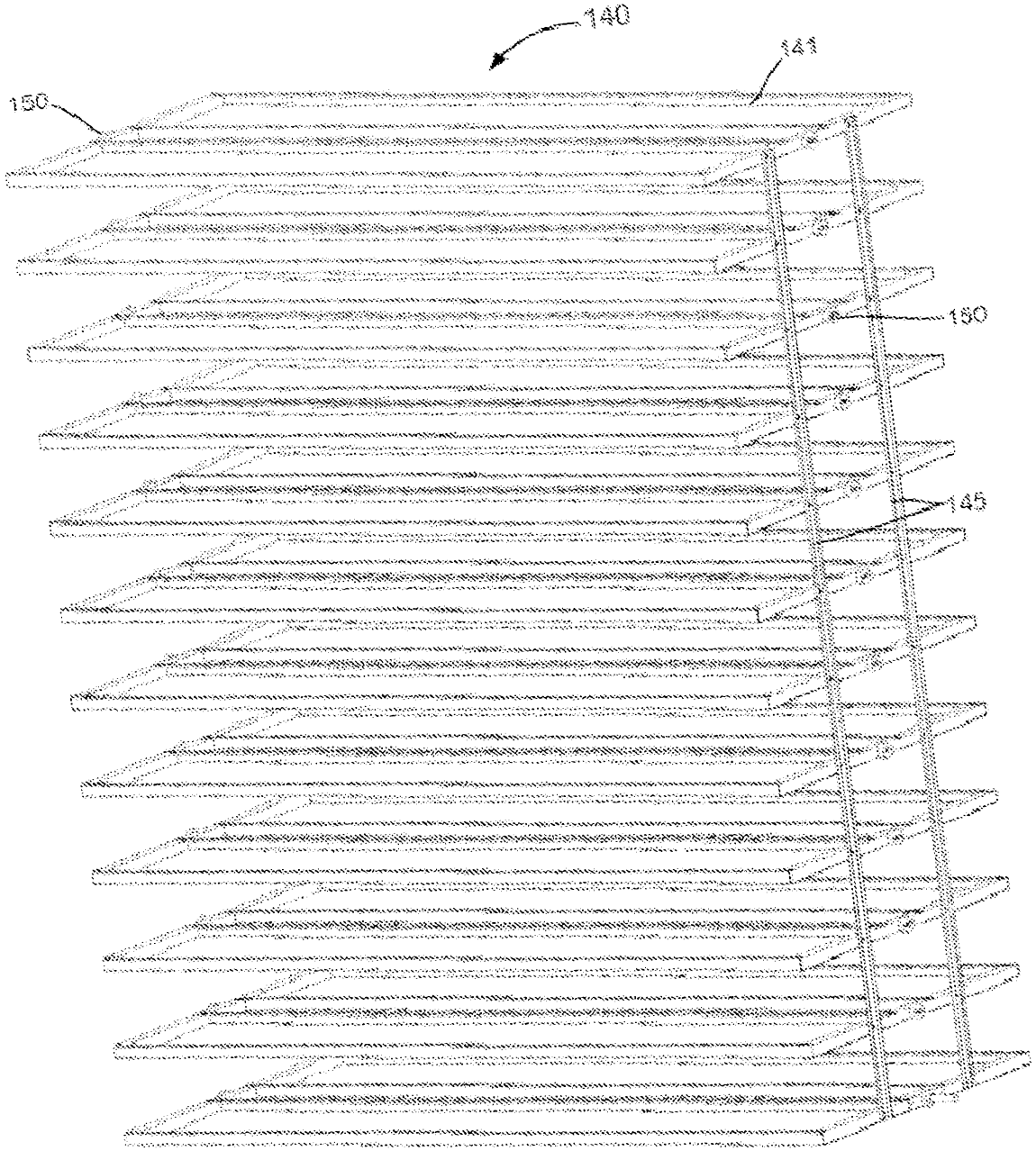

FIG. 12

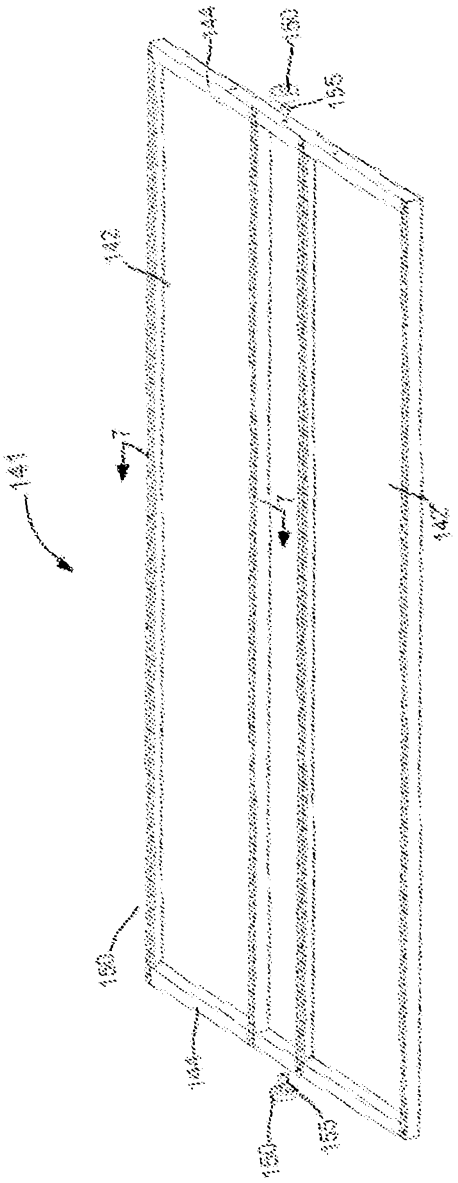

FIG. 13

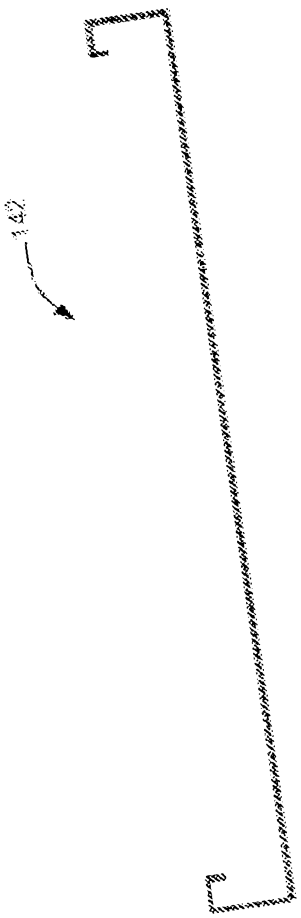

FIG. 14

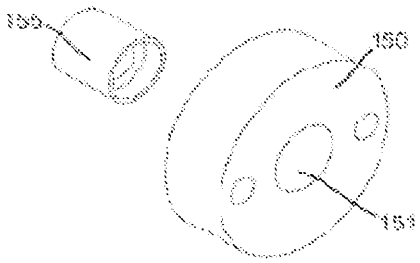

FIG. 15

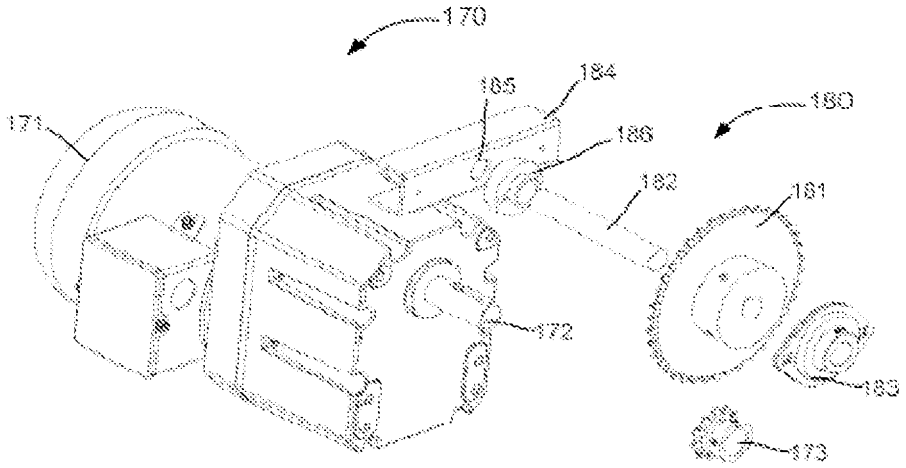

FIG 16

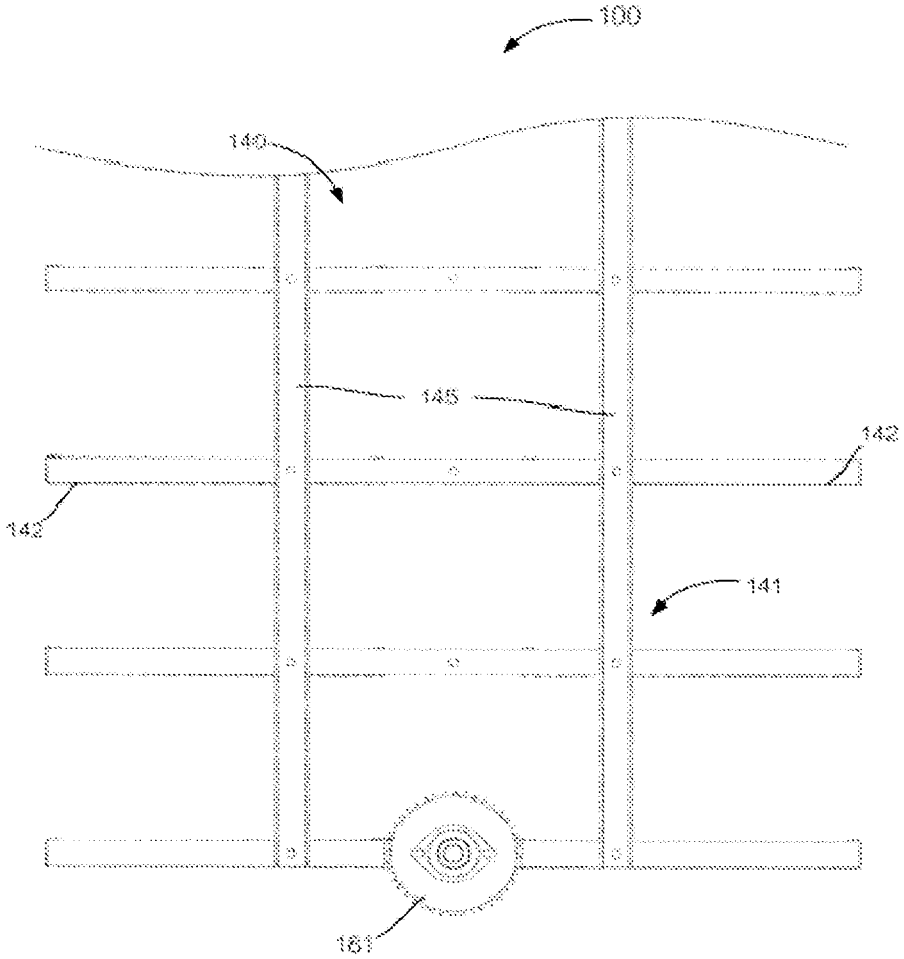

FIG. 17

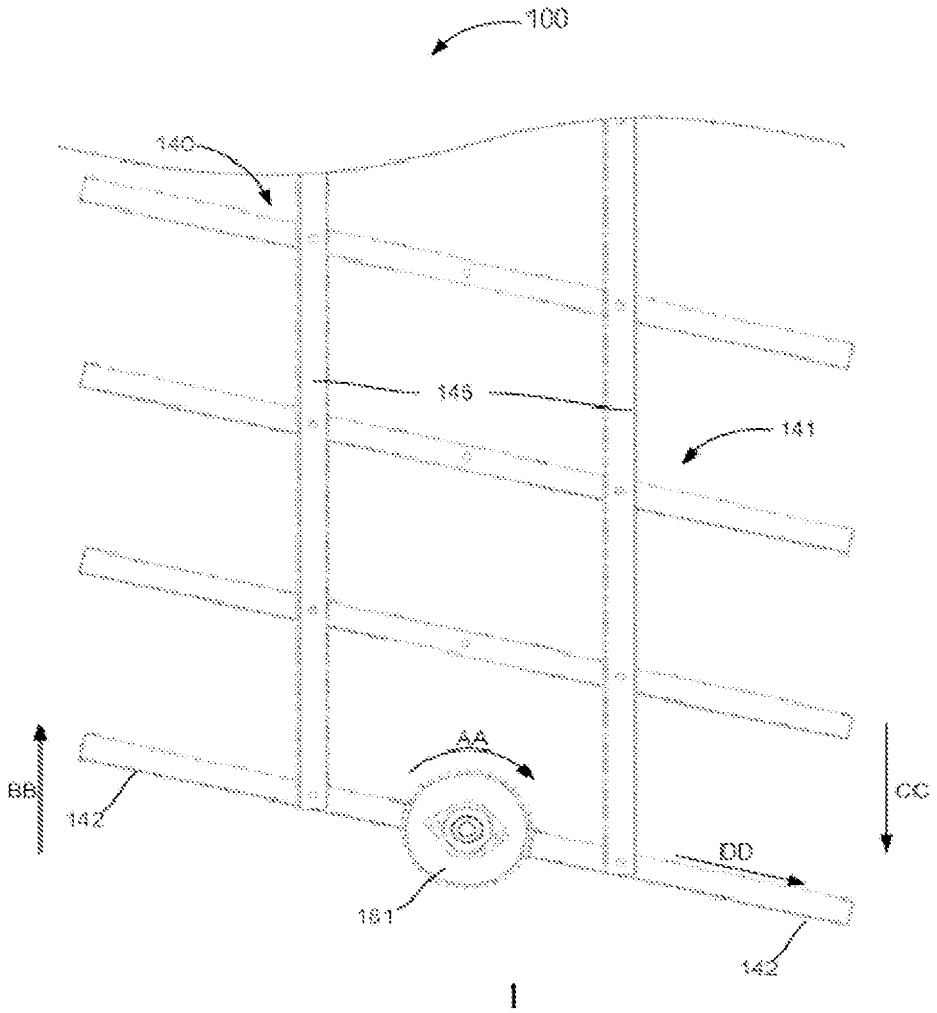

FIG. 18

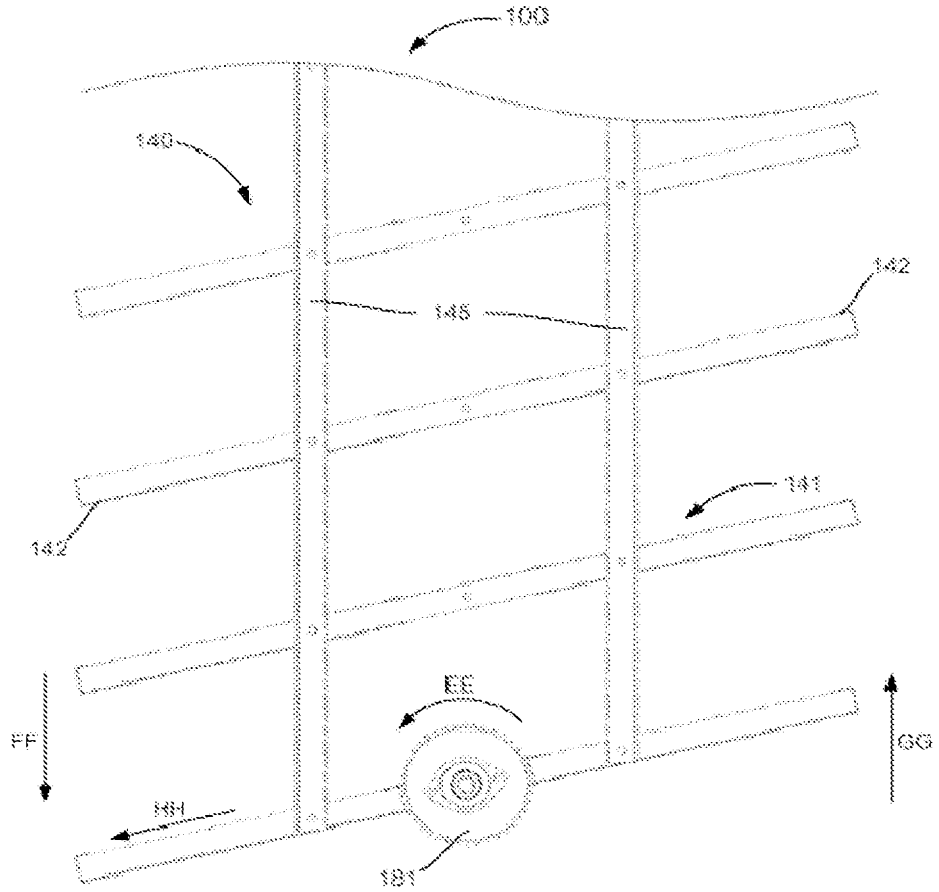

FIG. 19