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(54) Title: PLANT PROPAGATION

Figure 1b



(57) Abstract: The present application relates generally to the field of plant propagation. In particular, the present invention relates to a method for the propagation of vegetatively reproducing plants and plants and plant parts produced by such methods. The invention also provides encapsulated propagules. The invention also provides various end uses for the encapsulated propagules and for plants grown from the same. The invention also provides a method for the modification of the architecture of rhizomes and rhizomes having modified architecture and a method for the modification of the architecture of stem cuttings and stem cuttings having modified architecture. The invention also provides a coating for a propagule and a propagule coated therewith.

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Plant Propagation

The present application relates generally to the field of plant propagation. In particular, the present invention relates to a method for the propagation of vegetatively reproducing plants and plants and plant parts produced by such methods. The invention also provides encapsulated propagules. The invention also provides various end uses for the encapsulated propagules and for plants grown from the same. The invention also provides a method for the modification of the architecture of rhizomes and rhizomes having modified architecture and a method for the modification of the architecture of stem cuttings and stem cuttings having modified architecture. The invention also provides a coating for a propagule and a propagule coated therewith.

Background

Recently, there has been much interest in alternative energy sources that can reduce our reliance on traditional energy sources such as coal, oil and nuclear power. One such alternative energy source is bio-fuels, which is fuel derived from crops (energy crops) which can be burned to produce heat and electricity or treated with enzymes to produce sugars that can be used to produce ethanol or hydrogen. *Miscanthus* is an example of a perennial grass that has been identified as an energy crop due to its high biomass yields.

There are a number of *Miscanthus* species, but currently the most productive clone is a sterile hybrid, *Miscanthus x giganteus*, resulting from a natural cross between *M. sacchariflorus* and *M. Sinensis. Miscanthus* is a seed-bearing grass but the hybrid M. x giganteus is a triploid which is therefore sterile and does not produce seed. It can therefore only be multiplied by vegetative means which has severely restricted its commercial introduction.

The main concern with seeded varieties of *Miscanthus* is their potential to become invasive, however the alternative to seeded varieties is to rely on vegetative propagation which is largely inefficient. Conventional means for the vegetative propagation of *Miscanthus* involves planting rhizome cuttings produced from dedicated multiplication crops, rhizomes being underground stems. As commercial *Miscanthus* crops develop over 20 or more years, their root complexes can become rather large and woody. Rhizomes that are suitable for establishing new crops are usually specifically produced in multiplication fields and are often planted at high density and harvested after 2-4 years. These conventional rhizomes are uneven in shape and can often have numerous root hairs and adventitious roots making them difficult to process using conventional farming equipment and difficult to store and handle due to the tangling of the root hairs and due to their large, uneven and bulky nature. Rhizome cuttings are obtained from these "root complexes", which are usually about 12-15cm long and weigh about 50g including the surrounding soil. Although these rhizomes look robust, they are rather sensitive to dehydration and therefore require careful storage and handling. The aforementioned factors make the multiplication of *Miscanthus* relatively expensive compared to other crops. For example, under typical growing conditions in Europe, the multiplication ratio of *Miscanthus* rhizomes is around 3-5 fold in one growing season, meaning than one rhizome will produce a maximum of five rhizomes after one year's growth. This is in contrast to cereals with a multiplication ratio of up to 80 fold and oilseed rape which has a multiplication rate of over 5,000 fold. Similarly, the costs of planting conventional *Miscanthus* rhizomes are also high compared with the costs of cleaning, processing and planting seeded crops.

Attempts have been made to solve the aforementioned problems by adapting the planting equipment to suit the rhizomes, including the introduction of precision planting, both of which have increased the reliability of crop establishment.

However, to date, no attempts have been made to adapt the rhizome itself to better suit conventional farming apparatus.

Many of the crops (perennial grasses) required for producing feedstock for fuel, feed and fibre do not posses rhizomes at all or not in sufficient quantities and therefore require stem propagation. However, similar problems exist with plant propagation based on stem cuttings. For example, sugarcane is an economically important crop for sugar production and for bio-fuel production, but production of sugarcane is labour-intensive and reliant on the use of specialist machinery. Cultivation of sugarcane involves a fairly lengthy process of about 2 years for the production of cane seed using micro-propagation techniques, followed by testing and breeding to develop new sugarcane cultivars. With high disease pressure, it can take up to five years for new cultivars to reach the market. The multiplication of sugarcane from seed cane then begins, a process which typically takes about 3 years before commercial production can begin. Typically, about 20% of the total growing area is reserved for multiplication, meaning that about 20% of the land is tied up for at least 3 years, if not continuously, for successive cycles of multiplication. The multiplication ratios over the 3 year period are also relatively inefficient showing around an 8-fold increase.

Some efficiency gains have been reported for rhizomatous crops such as turmeric and bamboo from the use of micro-propagation techniques. For example, a publication in the name of Nayak (Plant Growth Regulation 32: 41-47, 2000) describes the *in vitro* multiplication of turmeric using tissue culture techniques and further describes the induction of micro-rhizomes using sucrose and 6-benzyladenine and a specified photoperiod. Similarly, Lo-apirukkul *et al.*, (J Nat Med (2012) 66: 265-270) describe the micro-propagation of a Thai medicinal plant, *Curcuma comosa Roxb.*, and the induction of micro-rhizomes. The *in vitro* induction of rhizomes in bamboo has been reported by Kapoor and Rao (Plant Cell, Tissue and Organ Culture (2006) 85: 211-217). The efficiency

gains reported in the prior art methods involving the use of *in vitro* micro-propagation techniques show an improvement of about seven-fold compared to conventional methods. There therefore remains a need for ever more efficient methods for the propagation of vegetatively reproducing plants.

One of the major obstacles preventing wider cultivation of *Miscanthus* and other such crops is the inefficient propagation discussed above due, at least in-part, to its vegetative means for reproduction. There therefore remains a need for a more economically efficient means for the establishment of *Miscanthus* and other vegetatively reproducing crops.

Summary of the Invention

The present invention aims to solve some of the aforementioned problems associated with the propagation of *Miscanthus* and other vegetatively reproducing plants, by providing a more efficient plant propagation process and by providing an encapsulated propagule which is in a form ready for planting.

The efficiency gains obtained by performing the methods of the invention give an improvement, depending on the plant in question, of between about 50-fold to about 200-fold compared to the prior art methods, which show an improvement of about seven-fold compared to conventional techniques.

According to the present invention, there is therefore provided a method for the propagation of a vegetatively reproducing plant, comprising the steps of:

(i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone and growing the plantlets and harvesting mini rhizomes or mini stem cuttings therefrom;

(iii) substantially encapsulating the mini rhizomes or mini stem cuttings produced from step (ii) in a plant growth medium.

The present invention also provides encapsulated mini rhizomes and encapsulated mini stem cuttings, which are collectively referred to herein as "encapsulated propagules". Also provided herein are various uses for the encapsulated propagules and various uses for the plants obtained therefrom.

The present invention also provides a biodegradable polymer for the coating of a propagule and propagules coated in the same.

Also provided is a method for altering the architecture of a rhizome or a stem cutting using plant hormones and/or by performing the methods of the invention.

Detailed Description of the Invention

The inefficiencies associated with conventional processes for the propagation of *Miscanthus* are overcome in the process of the present invention, which results in a greater productivity and in a more uniform end-product. Furthermore, the methods of the invention are more amenable to automation resulting in further efficiency gains compared to conventional processes. Furthermore, due to the more uniform size and shape of the mini rhizomes and mini stem cuttings produced by the methods, conventional agricultural equipment may readily be used in the planting process after minimal adaptation, where necessary, thereby removing any additional expense associated with having to produce customized equipment.

According to the present invention, there is therefore provided a method for the propagation of a vegetatively reproducing plant, comprising the steps of:

(i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone and growing the plantlets and harvesting mini-rhizomes or mini stem cuttings therefrom;

(iii) substantially encapsulating the mini-rhizomes or mini stem cuttings produced from step (ii) in a plant growth medium.

Micro-propagation is a standard, well known horticultural technique used for the rapid bulking up of large numbers of plantlets. The micro-propagation described in stage (i) of the method aims at producing large quantities of material suitable for entering stage (ii) of the method. The micro-propagation techniques used may be any conventional micro-propagation technique, such as tissue culture or rooted stem cuttings.

The micro-propagation results in plantlets which are ready to be multiplied, typically in a greenhouse. Conventional means for plant multiplication are used for this stage of the method. The greenhouse multiplication of the plantlets typically results in a 20-30 fold increase every 2-3 months. In the case of *Miscanthus* and *Arundo donax*, the micropropagation and multiplication results in every one plant generating about 10 plants in about an 8 week cycle; those 10 plants can then in turn generate about 100 plants in a further cycle of about 8 weeks.

Advantageously, micro-propagation followed by multiplication can result in every 1 (one) plant generating 10 million plantlets, plug plants or bare root cuttings (which refer to individual stems which can be separated from larger plants) in less than 6 months or in less than 8 months or in less than 10 months or in less than 12 months or in less than 14 months or in less than 16 months or in less than 18 months, depending on the plant in question.

Advantageously, micro-propagation followed by multiplication results in a decreased rate of mutation compared to conventional micropropagation, which again contributes to the overall efficiency gains brought about by performing the methods of the invention.

The resulting plantlets are then ready for the hormone treatment in stage (ii) of the process.

The terms “plantlet”, “plug plant” and “bare root cutting” are used interchangeably herein and are taken to mean the small plants produced following the micro-propagation and multiplication techniques of stage (i) of the method. The size of the plantlet will vary depending on the plant being propagated, and a skilled person will have at his or her disposal suitable means for generating plantlets from any given plant. In the case of *Miscanthus*, the plantlets are typically in the range of at least 2-5cm in length.

The term “rhizome” as used herein is used in its conventional sense to refer to an underground stem of a plant, which typically produces roots and shoots. The term “rhizomatous” plant as used herein refers to any plant capable of producing a rhizome. The term “mini-rhizome” is a term known in the art and refers to a whole rhizome from any given plant species which is about 10% of the size of a typical whole rhizome for that plant species, preferably about 5% of the size of a typical whole rhizome for that plant species. For example, a typical whole rhizome from *Miscanthus* is about 50g in weight and between about 12 to 15 cm in length, whereas a mini-rhizome from the same plant species is typically about 5g in weight, preferably about 4g or 3g or 2g in weight and between about 2 to 5 cm in length, preferably between about 1 to 2 cm in length.

The term “stem cutting” as used herein is used in its conventional sense where a piece of any given parent plant is removed and encouraged to grow as an

independent plant by placing the removed plant piece on a suitable growth medium, such as one or more of the following: soil, compost, potting mix, rock wool, perlite, vermiculite, coir, expanded clay pellets, hydrogel and water, which facilitates the growth of new roots and/or stems, which enable the stem cutting to become a plant independent of the source plant. The term "mini stem cutting" as used herein refers to a stem cutting from any given plant species which is about 10% of the size of a typical stem cutting for that plant species, preferably about 5% of the size of a typical stem cutting for that plant species. For example, a typical stem cutting from *Arundo donax* is about 100g in weight and between about 40 to 60cm in length, whereas a mini stem cutting from the same plant species is typically about 10g in weight, preferably about 4g or 3g or 2g in weight and between about 2 to 4cm in length. In the case of sugarcane, a conventional stem cutting is 30 to 40 cm in length, whereas a mini stem cutting from the same sugarcane species is typically between about 2 to 4cm in length.

The plantlets produced in stage (i) of the process are then subjected to stage (ii) of the process, which comprises hormonal treatment of the plantlets with cytokinin(s) and/or auxin(s). Any one given plant hormone is applied at a rate of less than about 1000ppm, less than about 900ppm, less than about 800ppm, less than about 700ppm, less than about 600ppm, less than about 500ppm, less than about 400ppm, less than about 300ppm, less than about 200ppm, less than about 100ppm, less than about 90ppm, less than about 80ppm, less than about 70ppm, less than about 60ppm, less than about 50ppm, less than about 40ppm, less than about 30ppm, less than about 20ppm or less than about 10ppm.

The cytokinin may be selected from the group consisting of kinetin, zeatin, 6-benzylaminopurine, diphenyl urea and thidiazuron (TDZ). The auxin may be selected from the group consisting of indole-3-actetic acid (IAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), 2-phenylacetic acid (PAA) and indole-3-butyric acid (IBA). A combination of benzylaminopurine, TDZ and IAA is preferred in the

case of *Miscanthus* propagation. Preferably, the benzylaminopurine, TDA and IAA are each applied at a rate of less than about 1000ppm, less than about 900ppm, less than about 800ppm, less than about 700ppm, less than about 600ppm, less than about 500ppm, less than about 400ppm, less than about 300ppm, less than about 200ppm, less than about 100ppm, less than about 90ppm, less than about 80ppm, less than about 70ppm, less than about 60ppm, less than about 50ppm, less than about 40ppm, less than about 30ppm, less than about 20ppm or less than about 10ppm. The combinations and concentrations of plant hormones may readily be optimized, if necessary, by one skilled in the art depending on the plant to be propagated.

According to a preferred feature of the present invention, after stage (i) of the process and before, after or during the hormone treatment of stage (ii), but before the harvesting of mini rhizomes or mini stem cuttings, the plants may be subjected to a temporary abiotic or mechanical stress.

The abiotic stress may comprise subjecting the growing plant to any temporary environmental change compared to the normal growth conditions for the plant in question. For example, the stress may be any one or more of: (i) an osmotic stress (which may be caused by limited or excess salt or water compared to the normal levels of salt or water); (ii) a temperature stress (which may be caused by exposure of the plant to excessive heat or cold compared to normal growth conditions for the plant in question); (iii) a nutrient stress (which may be caused by a lack of nitrogen, phosphorous, sulphur etc.); or (iv) an oxidative stress. The exposure of the plants to the temporary abiotic and/or mechanical stress serves to encourage more bud formation.

The mechanical stress refers to any non-environmental stress resulting from a physical action to a plant, such as cutting parts of the plant. Preferably, the aboveground parts of the plant are cut back to a point just above a node.

Reference herein to a "temporary" stress is taken to mean exposure to a non-continuous stress, which may involve exposure of the plant to any one or more given stresses at intermittent periods. For example, the intermittent periods of stress may be 1, 2, 3, 4 or 5 or more separate occasions of exposure of the plant to a stress in between non-stress periods.

Stage (ii) of the process (contacting the plantlets with at least one plant hormone), which may or may not comprise exposure of the plants to an abiotic and/or mechanical stress, can last from between about 12 and 24 weeks depending on the plant in question and depending on whether sufficient buds have formed.

The contacting of the plantlets to at least one plant hormone may occur before, after or during exposure of the plants to a temporary abiotic or mechanical stress. Preferred plant hormones include benzylaminopurine, TDA and IAA, one or more of which may be applied at a rate of <1000ppm (less than one thousand parts per million).

Therefore, a preferred method for the propagation of a vegetatively reproducing plant comprises the steps of:

(i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone;

(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;

(iv) growing the plantlets and harvesting mini rhizomes or mini stem cuttings therefrom;

(v) substantially encapsulating the mini rhizomes or mini stem cuttings produced from step (iv) in a plant growth medium.

The hormone-treated plants are then grown in a field or greenhouse setting under optimal growth conditions. In the case of *Arundo donax* or other plants produced from stem cuttings, this growth phase may span a period of about 12 months. Shoots emerging from the new buds are removed and cut (by machine or hand) into short lengths resulting in mini stem cuttings.

In the case of *Miscanthus*, this growth phase may span a period of about 12 months or until the size of the plant is about 20cm in length and the rhizomes are between about 2 to 5g in weight and/or until the bud capacity of the rhizomes is around 20 buds per rhizome.

The rhizomatous plant material growing in the field or greenhouse may then be subjected to a light shredding or separation of the root complex in order to separate out the mini rhizomes therefrom. Advantageously, this shredding or separation process may be automated to directly take plant material from the field or greenhouse and to separate out the mini rhizomes from the root complex ready for the encapsulation stage.

The hormonal treatment followed by the growth cycle in the field or greenhouse leads to the generation of mini rhizomes from the plantlets of stage (i). Advantageously, in the case of *Miscanthus* propagation, the mini rhizomes are around 2 to 5g in weight compared to the about 30 to 50g weight of rhizomes produced by conventional production methods. A further advantage is that the *Miscanthus* mini rhizomes are also more uniform in shape and size than the rhizomes of conventionally-produced *Miscanthus* plants, meaning that they can be planted with minimal adaptation (where necessary) of available agricultural

machinery, thereby avoiding the expense associated with providing specially adapted or custom-made agricultural machinery.

The small, uniformly-shaped mini rhizomes or mini stem cuttings resulting from stage (ii) of the method are then in a form ready for encapsulation. The encapsulation process may advantageously be automated. The combined automation of the shredding (in the case of rhizomatous plants) and encapsulation processes will contribute towards further efficiency gains.

The mini rhizome or mini stem section or cutting may advantageously be encapsulated in any plant growth medium, such as compost, potting mix, peat, hydrogel, soil, rock wool, perlite, vermiculite, foam, syrofoam, pumice, coir, expanded clay pellets etc. The resulting encapsulated rhizome or encapsulated mini stem cutting propagule is a stable planting unit of less than about 10g, or less than about 15g for some crops, and is in a form ready for precision planting using minimally adapted conventional farming equipment or ready for storage until required.

The encapsulation material may also comprise compounds, such as plant hormones (such as cytokinins or auxins), plant growth regulators, mycorrhiza, endophytic organisms, symbiotic organisms or other beneficial organisms, surfactants, gels, fungicides, nematicides, insecticides, organic and inorganic nutrients, water, polymer and organic-based super absorbents and stabilization compounds etc. to aid the storage of the mini rhizomes and mini stem cuttings so as to prevent any loss of material due to deterioration and to enhance the survival and performance of the propagules once they are planted in the field.

According to a preferred feature of the present invention, the encapsulated material is then substantially coated in a biodegradable polymer, which preferably has a melting point of between 30 to 65ºC.

Preferably the polymer is selected from one or more of wax, polyester, petroleum-based paraffin or plastic, polysaccharide or any plant-based plastic.

Preferably, the coating also comprises a fibre component comprising at least up to 20%, at least up to 30%, at least up to 40%, at least up to 50%, at least up to 60%, at least up to 70%, at least up to 80% or at least up to 90% of the coating.

The fibre may be one or more of the following: (i) fibre from agricultural biomass residue (for example, cereal straw, cotton, peanut hulls, soy straw, corn fodder); (ii) dedicated fibres (for example, *Miscanthus*, *Arundo*, sugarcane, bagasse, hemp, Kenaf); (iii) processed fibres (for example, paper, recycled cardboard, wood flour, wood saw dust); and (iv) artificial or processed fibres (for example, nylon, polyester, cotton).

The coating may also comprise fungicides, endophytic organisms, plant nutrients, hormones, dyes or other means for identification, such as barcodes or transponders or the like, to aid sorting.

The coating is applied to the encapsulated propagule by dipping (at least once) or by co-extrusion or by thermally forming the coating around the propagule. The coating covering the propagule is less than 1mm (millimetre) thick, preferably less than 0.5mm thick.

According to a preferred embodiment of the present invention, there is therefore provided a method for the propagation of a vegetatively reproducing plant, comprising the steps of:

(i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone and growing the plantlets and harvesting mini-rhizomes or mini stem cuttings therefrom;

(iii) substantially encapsulating the mini-rhizomes or mini stem cuttings produced from step (ii) in a plant growth medium; and

(iv) substantially coating the encapsulated mini rhizome or mini stem cutting of step (iii) in a biodegradable polymer.

According to a further preferred embodiment of the present invention, there is provided a method for the propagation of a vegetatively reproducing plant, comprising the steps of:

(i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone;

(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;

(iv) growing the plantlets and harvesting mini rhizomes or mini stem cuttings therefrom;

(v) substantially encapsulating the mini rhizomes or mini stem cuttings produced from step (iv) in a plant growth medium; and

(vi) substantially coating the encapsulated mini rhizome or mini stem cutting of step (v) in a biodegradable polymer.

A preferred method for the propagation of a rhizomatous plant comprises the steps of:

(i) micro-propagation of plant material from a rhizomatous plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone and growing the plantlets;
(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;
(iv) growing the plantlets and harvesting mini rhizomes therefrom;
(v) shredding or separation of the root complex to separate out the mini rhizomes therefrom;
(vi) substantially encapsulating the mini rhizomes produced in step (iv) or (v) in a plant growth medium; and optionally
(vii) substantially coating the encapsulated mini rhizome of step (vi) in a biodegradable polymer.

The preferred aspects of steps (i) to (vii) above are as described hereinabove.

A preferred method for the propagation of a plant from a stem cutting comprises the steps of:

(i) micro-propagation of plant material from a plant stem followed by multiplication to produce plantlets;
(ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone and growing the plantlets;
(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;
(iv) growing the plantlets and harvesting mini stems from the shoots produced;
(v) substantially encapsulating the mini stems produced in step (iv) in a plant growth medium; and optionally
(vi) substantially coating the encapsulated mini stem of step (v) in a biodegradable polymer.

The preferred aspects of steps (i) to (vi) above are as described hereinabove.

Advantageously, the methods of the invention may be applied to any plant capable of vegetative reproduction. In particular, the methods of the invention are particularly suited to rhizomatous and stem propagated plants. For example, the methods of the invention are particularly suited to energy grasses, such as *Miscanthus* (elephant grass), *Pennisetum purpureum* (napier grass), *Panicum virgatum* (switch grass), energy cane (the *Saccharum* complex), *Arundo donax* (Giant Reed), Sugarcane, *Bambusa* (bamboo), *Curcuma*, *Humulus* (hop), asparagus, *Zingiber* (ginger), iris, genus *Erianthus* , *Fallopia sachalinensis* (Igniscum), *Ipomoea batatas* (sweet potato) and wasabi. In addition there are applications for other food and medicinal crops propagated using rhizomes or stems, such as strawberry (genus *Fragaria),* the medicinal herb nettle (genus *Urtica*) and turmeric.

Arundo donax (giant reed), *Pennisetum purpureum* (napier grass), energy cane, *Saccharum officinarum* (sugar cane), *Hevea* (rubber) and *Manihot* (cassava), for example, are more suited to multiplication using stem cuttings. When multiplying using stem cuttings, the plantlets, derived from micro-propagation are planted and grown to a suitable height and any aboveground growth is cut back to a point just above a node. This procedure encourages more bud formation which may be further stimulated by the application of cytokinins and auxins. The shoots emerging from the new buds are removed and cut up by machine into short lengths to form mini stem cuttings which are then ready to be encapsulated. Although not all of the stem cuttings that are produced will contain buds, the multiplication rate that is achieved is many times greater than would be achieved by conventional procedures.

According to another aspect of the present invention, there is provided a substantially encapsulated mini rhizome or substantially encapsulated mini stem

cutting produced by the method according to the invention. The encapsulated mini rhizome or encapsulated mini stem cutting may be substantially coated in a biodegradable polymer.

The present invention also provides a planting unit weighing less than about 25g, or less than about 20g, or less than about 15g or less than about 10g, or less than about 5g, or less than about 2g, comprising a substantially uniformly-shaped mini rhizome or mini stem cutting substantially contained within a plant growth medium and optionally coated in a biodegradable polymer. The weight of the planting unit described above does not include the growth medium. In one embodiment of the present invention, the planting unit comprises a rhizome from a *Miscanthus* plant, energy cane, *Arundo donax* or sugarcane plant.

The invention also provides a method for the production of plants, comprising the steps of growing an encapsulated mini rhizome or mini stem cutting produced by the methods according to the invention. Also provided are plants obtainable from such an encapsulated mini rhizome or mini stem cutting and progeny and ancestors thereof.

Advantageously, plants produced from the mini stem cuttings or mini rhizomes produced by the methods according to the present invention have increased vigour. Taking *Miscanthus* as an example, this is manifested by an increase in the number of tillers (200 to 400% increase); increased height (up to a 30% increase); increased root volume (up to 300% increase); increased belowground buds (up to a 400% increase); increased canopy closure (up to a 200% increase); and increased leaf area (up to an 800% increase).

In the case of *Miscanthus*, 60 to 90 days after germination, the plants produced by the methods of the invention resemble conventionally produced plants that are one year or older.

According to the present invention, there is provided use of an encapsulated mini rhizome or mini stem cutting, or plants produced thereform, in increasing plant vigour relative to conventionally grown plants of the same species.

Advantageously, the mini stem cuttings and mini rhizomes produced by the methods of the present invention offer starting material for plant propagation having the same growth capacity but of greatly reduced weight compared to conventional starting material, thereby saving transportation costs, storage and handling costs etc. For example, the weight of the starting material (mini stem cuttings and mini rhizomes) will be about 0.25 tonnes per hectare compared to 1.5 tonnes per hectare in the case of conventionally grown *Miscanthus*, 6 tonnes per hectare for conventionally grown Napier grass and 10 tonnes per hectare for conventionally grown sugarcane.

Advantageously, the mini stem cuttings or mini rhizomes produced by the methods of the present invention allow the harvest cycle of the crop plants grown therefrom to be shortened compared to the harvest cycle of the same plant grown by conventional methods. The mini stem cuttings or mini rhizomes produced by the methods of the present invention offer multiple planting windows compared to the planting window for the same plant grown by conventional methods. According to the present invention, there is provided use of an encapsulated mini rhizome or mini stem cutting or plants produced therefrom in altering the harvest cycle of a plant.

The plants produced by growing an encapsulated mini rhizome or mini stem cutting may also find use in the production of bio-fuels or bio-ethanol. According to the present invention, there is provided use of an encapsulated mini rhizome or mini stem cutting or plant produced therefrom in biofuel or bioethanol production.

Advantageously, the mini stem cuttings and mini rhizomes produced by the methods of the present invention may find use in bioremediation applications for cleaning up areas having contaminated water. According to the present invention, there is provided use of an encapsulated mini rhizome or mini stem cutting or plant produced therefrom in bioremediation.

According to another aspect of the present invention, there is provided a method for altering rhizome architecture, comprising the steps of:

(i) micro-propagation of plant material from a rhizomatous plant to produce plantlets; and

(ii) contacting the plantlets from step (i) with plant hormones selected from the group consisting of benzylaminopurine, TDA and IAA;

(iii) obtaining substantially uniformly-shaped mini rhizomes weighing less than about 25g.

The aforementioned method for altering rhizome architecture may also comprise the additional steps of shredding and/or encapsulation of the rhizomes as described hereinabove.

A preferred method for altering rhizome architecture comprises the steps of:

(i) micro-propagation of plant material from a rhizomatous plant followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with a plant hormone selected from the group consisting of benzylaminopurine, TDA and IAA and growing the plantlets;

(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;

(iv) growing the plantlets and harvesting mini-rhizomes therefrom;

(v) shredding or separation of the root complex to separate out the mini rhizomes therefrom;

(vi) obtaining substantially uniformly-shaped mini rhizomes weighing less than about 25g.

According to another aspect of the present invention, there is provided a method for altering stem architecture, comprising the steps of:

(i) micro-propagation of plant material from a plant stem to produce plantlets; and

(ii) contacting the plantlets from step (i) with a plant hormone selected from the group consisting of benzylaminopurine, TDA and IAA;

(iii) obtaining substantially uniformly-shaped mini stems weighing less than about 25g.

A preferred method for altering stem architecture comprises the steps of:

(i) micro-propagation of plant material from a plant stem followed by multiplication to produce plantlets;

(ii) contacting the plantlets or a part thereof from step (i) with a plant hormone selected from the group consisting of benzylaminopurine, TDA and IAA and growing the plantlets;

(iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;

(iv) growing the plantlets and harvesting mini stems from the shoots produced;

(v) obtaining substantially uniformly-shaped mini stems weighing less than about 25g.

The present invention provides mini rhizomes and mini stem cuttings obtainable by the methods described herein. The method for altering rhizome or mini stem

architecture results in mini rhizomes or mini stem cuttings weighing less than about 25g or weighing less than about 20g or weighing less than about 15g or weighing less than about 10g or weighing less than about 5g or weighing less than about 2g.

Preferably, the benzylaminopurine, TDA and IAA are each (individually, not in combination) applied at a rate of less than about 1000ppm, less than about 900ppm, less than about 800ppm, less than about 700ppm, less than about 600ppm, less than about 500ppm, less than about 400ppm, less than about 300ppm, less than about 200ppm, less than about 100ppm, less than about 90ppm, less than about 80ppm, less than about 70ppm, less than about 60ppm, less than about 50ppm, less than about 40ppm, less than about 30ppm, less than about 20ppm or less than about 10ppm.

The method for altering rhizome architecture is particularly suited to the rhizomes of *Miscanthus* plants, but may also be used for other rhizomatous plants.

The combinations and concentrations of plant hormones may readily be optimized, if necessary, by one skilled in the art depending on the plant to be propagated. The resulting mini rhizomes or mini stem cutting having modified architecture may then be used in a method for the production of a plant, comprising the steps of growing a rhizome or an encapsulated rhizome or a mini stem cutting or encapsulated mini stem cutting obtainable by the aforementioned methods for altering rhizome or mini stem architecture.

According to another aspect of the present invention, there is provided use of benzylaminopurine, TDA and IAA to modify the architecture of rhizomes, particularly *Miscanthus* rhizomes, or to modify the architecture of mini stems.

According to another aspect of the present invention, there is provided use of benzylaminopurine, TDA and IAA to increase the yield of rhizomatous plants or the yield of plants propagated using stem cuttings compared to the yields obtained using conventional production methods. The use of benzylaminopurine, TDA and IAA is as described above in the method for plant propagation.

In such use, the benzylaminopurine, TDA and IAA are each (individually, not in combination) applied at a rate of less than about 1000ppm, less than about 900ppm, less than about 800ppm, less than about 700ppm, less than about 600ppm, less than about 500ppm, less than about 400ppm, less than about 300ppm, less than about 200ppm, less than about 100ppm, less than about 90ppm, less than about 80ppm, less than about 70ppm, less than about 60ppm, less than about 50ppm, less than about 40ppm, less than about 30ppm, less than about 20ppm or less than about 10ppm.

The architecture of the rhizomes and mini stem cuttings is modified such that the resulting mini rhizomes or mini stem cuttings after application of the plant hormones are substantially uniform in shape and weight less than about 25g or weigh less than about 20g or weigh less than about 15g or weigh less than about 10g or weight less than about 5g or weigh less than about 2g.

According to a further aspect of the present invention, there is provided a coating for a propagule comprising a biodegradable polymer, which preferably has a melting point of between 30 to 65ºC. The invention also provides propagules coated with such a biodegradable polymer.

Preferably the propagule is encapsulated prior to being coated in the biodegradable polymer. The propagule may advantageously be encapsulated in any plant growth medium, such as compost, potting mix, peat, hydrogel, soil, rock wool, perlite, vermiculite, foam, syrofoam, pumice, coir, expanded clay

pellets etc. The encapsulation material may also comprise compounds, such as plant hormones (such as cytokinins or auxins), plant growth regulators, mycorrhiza, endophytic organisms, symbiotic organisms or other beneficial organisms, surfactants, gels, fungicides, nematicides, insecticides, organic and inorganic nutrients, water, polymer and organic based super absorbents and stabilization compounds etc. to aid the storage of the propagule so as to prevent any loss of material due to deterioration and to enhance the survival and performance of the propagules once they are planted in the field.

Preferably the polymer is one or more of wax, polyester, petroleum-based paraffin or plastic, polysaccharide or any plant-based plastic.

The coating may comprise Preferably, the coating also comprises a fibre, which may comprise at least up to 20%, at least up to 30%, at least up to 40%, at least up to 50%, at least up to 60%, at least up to 70%, at least up to 80% or at least up to 90% of the coating.

The fibre may be one or more of the following (i) fibre from agricultural biomass residue (for example, cereal straw, cotton, peanut hulls, soy straw, corn fodder); (ii) dedicated fibres (for example, *Miscanthus*, *Arundo*, sugarcane, bagasse, hemp, Kenaf); (iii) processed fibres (for example, paper, recycled cardboard, wood flour, wood saw dust); and (iv) artificial or processed fibres (for example, nylon, polyester, cotton).

The coating may also comprise fungicides, endophytic organisms, plant nutrients, hormones, dyes or other means for identification, such as barcodes or transponders or the like, to aid sorting.

The coating is applied to the (encapsulated) propagule by, for example, dipping (at least once) or by co-extrusion or by thermally forming the coating around the

propagule. The coating covering the (encapsulated) propagule is less than 1mm (millimetre) thick, preferably less than 0.5mm thick.

According to a further aspect of the present invention, there is provided use of a biodegradable polymer, which preferably has a melting point of between 30 ºC to 65ºC, for the coating of a propagule.

A "propagule" as defined herein is any plant part which is capable of being grown or regenerated into a whole plant. A propagule may therefore comprise, rhizomes, mini rhizomes, stem cuttings, mini stem cuttings, tubers, seeds etc.

In the case where the propagule is a seed, any commercial or other seed variety may optionally first be encapsulated as described above and then coated in a biodegradable polymer as described. The seed may be a transgenic or non-transgenic seed. Preferred seeds include melon and tomato seeds.

Figures

The present application will now be described with reference to the following Figures which are by way of illustration alone, in which:

Figure 1a shows a rhizome from *Miscanthus* produced by conventional methods (weighing about 30g) and Figure 1b shows a mini rhizome from *Miscanthus* produced by the methods of the present invention (weighing around 2g).

Figure 2 shows *Miscanthus* plantlets undergoing micro-propagation according to stage (i) of the method of the invention.

Figure 3 shows the crop establishment in the field of *Miscanthus* plants produced from the encapsulated mini rhizomes (right hand half of the picture) produced by

the methods of the present invention compared to plant produced from conventional rhizomes (left hand half of the picture).

Examples

The present application will now be described with reference to the following examples.

Example 1: Micropropagation and multiplication of Miscanthus

Explant material was selected from disease-free *Miscanthus* plant material. The explant material was surface sterilized, rinsed in sterilized water and seeded onto an agar-based growth medium containing nutrients (sucrose and other standard nutrients) and plant hormones to encourage cell division. The resulting callus was then re-plated onto a growth medium infused with nutrients and plant hormones (cytokinins and auxins) to stimulate cell differentiation into roots and shoots and the production of plantlets. Repeated cycles of this process allow a single explant sample to be increased from one to thousands of plantlets.

Example 2: Hormone Manipulation

The plantlets were then planted in compost and grown in a greenhouse until 5 to 10 shoots were established. The plantlets were then subjected to a temporary drought by withholding watering of the plants for a period of 1 day. The shoots, when about 10cm tall, were subjected to a separation procedure which involved separating, by hand, each shoot having a dedicated root system and potting this shoot into compost. The potted shoots were then treated with a combination of benzylaminopurine, TDA and IAA each being at a rate of <1000ppm (less than one thousand parts per million) to enhance shoot and root initiation.

After about 30 to 45 days, the aboveground plant tissue was removed and the rooting systems removed from the pots and lightly separated. These mini

rhizomes were then ready for the encapsulation process described in Example 4 below.

Example 3: Using Mini Stem Cuttings

Arundo donax (giant reed), *Pennisetum purpureum* (napier grass) and energy cane are more suited to multiplication using stem cuttings. The *Arundo donax* , *Pennisetum purpureum* , sugarcane and energy cane plantlets were subjected to micro-propagation and multiplication as described in Example 1. Plantlets derived from micro-propagation and multiplication were planted onto compost and grown to a height of about 10cm. The aboveground growth was cut back to a point just above a node to encourage more bud formation, which was further stimulated by the application of benzylaminopurine, TDA and IAA, each being at a rate of <1000ppm (less than one thousand parts per million). Shoots emerging from the new buds were removed and cut by machine into short lengths to form mini stem cuttings. These mini stem cuttings were then encapsulated as described in Example 4.

Example 4: Encapsulation

Encapsulation was carried out using machinery available in the horticultural industry for encapsulating seeds or cuttings but adapted so as to fully encase the mini-rhizome or mini stem cutting. The mini-rhizome or stem cutting was introduced into a flow of compost containing cytokinins, auxins, mycorrhiza, surfactants, gels, fungicides and insecticides. The compost surrounding the mini-rhizome or stem cutting was then compressed. The resulting unit of mini-rhizome or mini stem cutting in compressed compost was then wrapped in a paper binding to increase the stability of the encased propagule. The encased propagule was then stored until required for planting.

Claims:

1. A method for the propagation of a vegetatively reproducing plant comprising the steps of:
   (i) micro-propagation of plant material from a vegetatively reproducing plant followed by multiplication to produce plantlets;
   (ii) contacting the plantlets or a part thereof from step (i) with at least one plant hormone;
   (iii) exposing the plantlets before, after or during step (ii) above to an abiotic or mechanical stress;
   (iv) growing the plantlets and harvesting mini rhizomes or mini stem cuttings therefrom;
   (v) substantially encapsulating the mini rhizomes or mini stem cuttings produced from step (iv) in a plant growth medium.

2. Method according to claim 1, wherein said encapsulated mini rhizome or mini stem cutting is substantially coated in a biodegradable polymer.

3. Method according to claim 2, wherein said biodegradable polymer has a melting point of between 30 to 65ºC.

4. Method according to claim 2 or 3, wherein said biodegradable polymer comprises one or more of wax, polyester, petroleum-based paraffin or plastic, polysaccharide or any plant-based plastic.

5. Method according to any of claims 2 to 4, wherein said coating comprises a fibre component of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the coating.

6. Method according to claim 5, wherein said fibre comprises one or more of the following: (i) fibre from agricultural biomass residue; (ii) dedicated fibres; (iii) artificial or processed fibres.

7. Method according to any of claims 2 to 6, wherein said coating comprises one or more of the following: fungicides, endophytic organisms, plant nutrients, hormones, dyes or other means for identification, such as barcodes or transponders.

8. Method according to any preceding claim, wherein said vegetatively reproducing plant is a rhizomatous plant or wherein said plant is propagated using a stem cutting.

9. Method according to claim 8, wherein said plant is selected from *Miscanthus* (elephant grass), *Pennisetum purpureum* (napier grass), *Panicum virgatum* (switch grass), energy cane (the *Saccharum* complex), *Arundo donax* (giant reed), sugarcane, *Bambusa* (bamboo), *Curcuma*, *Humulus* (hop), asparagus, *Zingiber* (ginger), iris, genus *Erianthus* , *Fallopia sachalinensis* (Igniscum), strawberry (Genus *Fragaria)*, herb Nettle (Genus *Urtica*), *Hevea* (rubber) and *Manihot* (cassava), turmeric, wasabi and *Ipomoea batatas* (sweet potato).

10. Method according to any preceding claim, wherein said mechanical stress comprises cutting back of aboveground parts.

11. Method according to any preceding claim, wherein said abiotic stress is an osmotic stress.

12. Method according to any preceding claim, wherein said micro-propagation comprises tissue culture.

13. Method according to any preceding claim, wherein said plant hormone comprises cytokinins and auxins.

14. Method according to claim 13, wherein said plant hormones comprise benzylaminopurine, TDZ and IAA.

15. A substantially encapsulated mini rhizome or mini stem cutting obtainable by the method according to any of claims 1 to 14.

16. A substantially encapsulated mini rhizome or mini stem cutting according to claim 15 substantially coated in a biodegradable polymer.

17. A planting unit weighing less than about 25g, comprising a substantially uniformly-shaped rhizome or uniformly-shaped stem cutting substantially contained within a plant-growth medium and optionally coated in a biodegradable polymer.

18. A propagule substantially coated in a biodegradable polymer.

19. A propagule according to claim 18 which is substantially encapsulated in a plant growth medium before being substantially coated in a biodegradable polymer.

20. A propagule according to claim 18 or 19, wherein said biodegradable polymer has a melting point of between about 30 to about 65ºC.

21. A propagule according to any of claims 18 to 20, wherein said biodegradable polymer is selected from one or more of wax, polyester,

petroleum-based paraffin or plastic, polysaccharide or any plant-based plastic.

22. A propagule according to any of claims 18 to 21, wherein said coating comprises a fibre component of up to 20%, up to 30%, up to 40%, up to 50%, up to 60%, up to 70%, up to 80% or up to 90% of the coating.

23. A propagule according to claim 22, wherein said fibre comprises one or more of the following: (i) fibre from agricultural biomass residue; (ii) dedicated fibres (for example, *Miscanthus*, *Arundo*, sugarcane, bagasse, hemp, Kenaf); (iii) artificial or processed fibres.

24. A propagule according to any of claims 18 to 23, wherein said coating comprises one or more of the following: fungicides, endophytic organisms, plant nutrients, hormones, dyes, barcode or transponder.

25. A propagule according to any of claims 18 to 24, wherein said coating is applied to the encapsulated propagule by dipping (at least once), co-extrusion or by thermally forming the coating around the propagule.

26. A propagule according to any of claims 18 to 25, wherein said propagule is selected from a rhizome, mini-rhizome, stem cutting, mini stem cutting, tuber and seed.

27. A method for altering rhizome architecture, comprising the steps of:
(i) micro-propagation of plant material from a rhizomatous plant to produce plantlets; and
(ii) contacting the plantlets from step (i) with plant hormones selected from the group consisting of benzylaminopurine, TDA and IAA;

(iii) obtaining substantially uniformly-shaped rhizomes weighing less than about 25g.

28. A mini rhizome obtainable by a method according to claim 27.

29. A method for altering stem architecture, comprising the steps of:
(i) micro-propagation of plant material from a plant stem to produce plantlets; and
(ii) contacting the plantlets from step (i) with a plant hormone selected from the group consisting of benzylaminopurine, TDA and IAA;
(iii) obtaining substantially uniformly-shaped mini-stems weighing less than about 25g.

30. A mini stem cutting obtainable by a method according to claim 29.

31. A method for the production of a plant, comprising the steps of growing an encapsulated mini rhizome or mini stem cutting obtainable by a method according to any of claims 1 to 14 and 27 and 29.

32. Plants obtainable by a method according to claim 31 and progeny and ancestors thereof.

33. Plant according to claim 32 having increased vigour relative to corresponding conventionally propagated plants.

34. Harvestable parts of a plant according to claim 32 or 33, wherein said harvestable plant parts comprise aboveground biomass and/or stem cuttings and/or rhizomes.

35. Use of a plant according to claim 32 or 33 and/or use of harvestable parts of a plant according to claim 34 in the production of bio-fuels or bioethanol.

36. Use of a plant according to claim 32 or 33 in bioremediation.

37. Use of a plant according to claim 32 or 33 in altering the harvest cycle of a plant.

Figure 1a

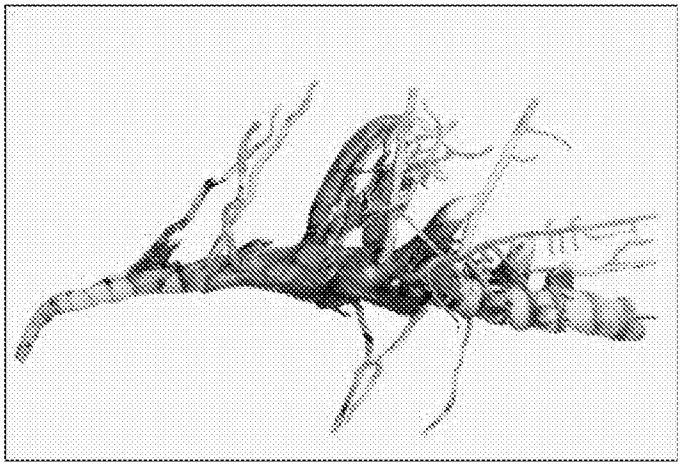


Figure 1b



Figure 2

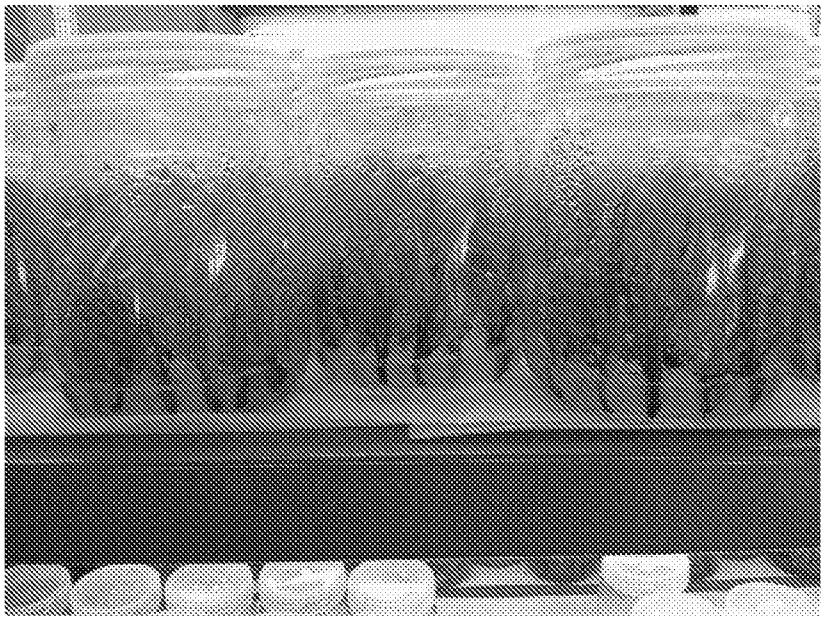


Figure 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/051543

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H4/00 A01H5/04 A01H5/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBL, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	page 300, column 2, line 15 - page 302, column 2, line 5	3,5,9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2013	02/10/2013

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/051543

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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