PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :		(11) International Publication Number:
A01G 7/00, A01C 1/02	A1	WO 89/ 05575
		(43) International Publication Date: 29 June 1989 (29.06.89)

(21) International Application Number: PCT/US88/03934

(22) International Filing Date: 15 November 1988 (15.11.88)

(31) Priority Application Number: 135,034

(32) Priority Date: 18 December 1987 (18.12.87)

(33) Priority Country: US

(71) Applicant: UNIVERSITY OF FLORIDA [US/US]; 207 Tigert Hall, Gainesville, FL 32611 (US).

(72) Inventors: GRAY, Dennis, J. ; 5336 University Avenue, Leesburg, FL 32748 (US). CONGER, Bob, V. ; P.O. Box 1071, Knoxville, TN 37901-1071 (US).

(74) Agents: CLARKE, Dennis, P. et al.; Kerkam, Stowell, Kondracki & Clarke, 6404R Seven Corners Place, Falls Church, VA 22044-2010 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: QUIESCENT PLANT SOMATIC EMBRYOS AND METHOD FOR THEIR PRODUCTION

(57) Abstract

A method of producing synthetic seeds by inducing growth quiescence in plant somatic embryos by maintaining the embryos in an atmosphere having a relative humidity of 30-85 % or in an osmotically active environment having a moisture content of 30-85 % for a period of time sufficient to reduce the moisture content of the embryos to from 85-65 % to 4-15 % and for the embryos to cease growth.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**ML**	Mali
AU	Australia	**GA**	Gabon	**MR**	Mauritania
BB	Barbados	**GB**	United Kingdom	**MW**	Malawi
BE	Belgium	**HU**	Hungary	**NL**	Netherlands
BG	Bulgaria	**IT**	Italy	**NO**	Norway
BJ	Benin	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		

QUIESCENT PLANT SOMATIC EMBRYOS AND METHOD FOR THEIR PRODUCTION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to synthetic seed products and methods for their production. The present invention derived in part from work performed under Grant 82-CRCR-1-1086 from the U.S. Department of Agriculture.

Prior Art

Although seed is the primary means of planting most agronomic crops, asexual embryogenesis or the use of genetically uniform tissue culture-derived propagules, engineered to possess similar efficient handling qualities, would be advantageous for production of certain crops or cultivars. Potential applications of such "synthetic seed" include: (a) large-scale planting of outstanding genotypes for self-incompatible species that are difficult to propagate vegetatively, (b) production of genetically uniform seed for highly heterozygous crops that are currently propagated only from vegetative material, (c) propagation of novel genotypes produced by genetic engineering that are not meiotically stable, (d) commercialization and maintenance of proprietary germplasm containing intentionally introduced meiotic instability, and (e) maintenance of parental inbred lines.

Prior attempts to produce so-called "synthetic seed" products capable of producing genetically uniform tissue have included coating or encapsulating somatic plant embryos to preserve their

viability [Kitto et al, J. Amer. Soc. Hort. Sci., Vol. 110, pp. 277-282, 283-286 (1985), U.S. patents Nos. 4, 615,141; 4,583,320; 4,562,663].

The dehydration of plant embryogenic material has also been reported; however, it is not clear from these reports whether plant regeneration was achieved from the dried material [Nitzsche, Z. Pflanzenphysiol., Vol. 87, pp. 469-472 (1978); Nitzsche, Z. Pflanzenphysiol., Vol. 100, pp. 269-271 (1980)].

Somatic cell embryogenesis is the best prospect for synthetic seed production because somatic embryos are nearly identical to zygotic embryos, and the labor required for their production is low compared to other clonal propagation systems. However, an important difference between somatic and zygotic embryos is that the latter typically cease growth, becoming quiescent or dormant as water is lost, storage tissues mature, and the seed coat hardens. This arrested growth phase is the major factor accounting for the efficient storage and handling qualities of seed. A similar arrested growth phase induced during somatic embryo development would be essential to match the efficiency of seed propagation and would be a pivotal step in the development of synthetic seed technology.

It is an object of the present invention to provide a synthetic seed product comprising dehydrated somatic embryos and a method for their production.

SUMMARY OF THE INVENTION

The above and other objects are achieved by the present invention which provides a synthetic seed product comprising dehydrated somatic plant embryos produced by the method described below which, upon rehydration and growth, yields plants which are essentially identical to the plant from which the somatic plant embryo is developed.

The method of the invention for producing the synthetic seed product comprises inducing growth quiescence in plant somatic embryos by maintaining the somatic embryos in an environment having a relative humidity or storage water content of from about 30% to about 85% for a period of time sufficient to reduce the moisture content of the embryos to from about 85-65% to about 4-15%, depending on plant species, and for the embryos to cease growth. The embryos are then capable of being stored for prolonged time periods (e.g., up to about 1 year) at this moisture range.

The invention further includes a method of developing plants by somatic embryogenesis employing the above-described dehydrated somatic plant embryos.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated on the discovery that quiescence may be induced in somatic plant embryos by dehydrating the embryos at a certain critical relative humidity or storage water content range. Attempts to achieve quiescence or dormancy by drying the somatic embryos in atmospheres or environments having a water content outside this range

result in products which cannot be stored and/or will not regenerate plants upon rehydration.

It will be understood that the terms "relative humidity" and "storage water content" refer to the water content of the atmosphere or environment to which the somatic embryos are exposed during dehydration. Thus, the embryo may be maintained in an atmosphere having a relative humidity of 30% to 85% or they may be placed in solutions or coated with compositions which are "osmotically active", i.e., materials which promote the osmotic transfer of moisture from the embryos to the surrounding medium.

The invention is applicable to both monocotyledonous and dicotyledonous plants.

In the drawing, FIG. 1 depicts the rate of water loss by somatic embryos. The solid line shows weight stabilization at about the 24 h mark. After 48 h the embryos were subjected to 60°C for 24 h to remove the remaining water (broken line for determination of fresh and stored water content).

The method of the invention reversibly arrests the characteristically rapid growth of plant somatic embryos. The quiescent embryos may then be stored at room temperature and growth leading to plant development is stimulated by rehydration when desired. This is a crucial stage in synthetic seed technology where fragile, metabolically active somatic embryos must be engineered to mimic durable, quiescent seed. The invention will find utility in all instances where clonal plant production with planting efficiencies of conventional seed is necessary.

According to the invention, plant somatic embryos are isolated from callus or suspension cultures and placed in a controlled, critical relative

humidity or storage water content, environment which causes dehydration. This results in a controlled final moisture content. Such embryos cease growth, decrease in size and cellular collapse occurs. The embryos are then stored at standard room temperatures. Upon rehydration, the embryos swell, resume growth and germinate into plants. Dehydrated embryos have been stored for up to 21 days at 23°C.

The important parameters of the invention include: 1) developmental stage of somatic embryos prior to dehydration, 2) storage water content, 3) storage time, 4) rehydration conditions. Only well developed somatic embryos possessing a morphology consistent with that of zygotic embryos survive the procedure. A relative humidity or storage water content of from about 30% to about 85%, preferably about 70%, is suitable for dehydration and storage. Studies have shown that orchardgrass embryos lose maximum water within 24 hours and contain 13% water when stored at 70% RH. This is identical to the moisture content of grass seeds stored at similar relative humidities which suggests that somatic embryos react similarly to zygotic embryos under these conditions. For rehydration, the embryos are placed on solidified medium containing mineral salts, vitamins, and sugars but no growth regulators.

Viability decreases with increasing storage time such that at 21 days of storage, 4% of orchardgrass somatic embryos produce green plants upon rehydration. Rehydrated embryos are incubated under 16 hours of fluorescent light per day for plant development. Green, rooted plants are established in potting mix in greenhouse pots following routine acclimatization treatments.

It is preferred to conduct the dehydration for a period of time in the range of from about 24 hr to about 336 hr although actual dehydration time will vary from species to species and may lie outside this broad range.

It sill be understood that the dehydrated embryos may be encapsulated in a coating which will preserve the critical moisture content therein.

The present invention which provides synthetic seeds in an efficient and inexpensive manner will have a significant impact on crop production in the future. Commercial applications exist for two broad crop categories. Firstly, synthetic seeds could be used to produce self-incompatible crops that normally must be vegetatively propagated, for example, potato as well as certain fruit and nut trees. In such crops, this would allow for direct planting of non-grafted varieties and would provide a significant alternative for germplasm preservation that presently depends on perpetual maintenance of living plants. Secondly, utilization of elite germplasm for crops that are economically grown only from seeds would be facilitated. Examples include forest trees, some forage grasses and vegetables, most major agronomic crops and important sources of oil such as coconut and oil palm. In this regard, synthetic seeds would be essential for utilization of future genetically engineered cultivars containing meiotically unstable foreign genes. Intentionally introduced meiotic instability would then become an available tool for commercialization of proprietary germplasm. Maintenance of parenteral inbred lines as an adjunct to conventional breeding would be facilitated by synthetic seeds. In breeding programs, the ability to

commercially propagate a new hybrid without "trueing up" the seed and/or producing parental inbreds would become possible.

Dehydration by controlling the relative humidity of the atmosphere to which the embryos are exposed may be effected according to a variety of systems.

In a so-called static system, embryos positioned in unsealed petri dishes are placed in a closed system containing an atmosphere having the desired relative humidity.

In a flowing system, air or controlled oxygen and/or CO_2 mixtures having the requisite relative humidity are passed into and out of a chamber containing the embryos in petri dishes.

Humidities are generated utilizing either aqueous inorganic salt mistures, aqueous glycerol mixtures or by mixing high humidity air or gas (bubbled through water) with low humidity air or gas (passed through desiccant) to achieve a specific final relative humidity.

In the static system, the salt or glycerol mixture is placed in the bottom of the container. In the flowing system, air or gas is bubbled through the salt or glycerol mixture before passing through the chamber containing the embryos.

Suitable salt solutions for generating atmospheres having requisite relative humidities include:

1) Calcium chloride ($CaCl_2$) saturated in water at 20-29°C produces a 31% RH.
2) Saturated sodium nitrate ($NaNO_3$) solutions at 20-29°C gives 46-47% RH.
3) Saturated sodium chloride (NaCl) at 20-24°C gives 74-75% RH.
4) Saturated zinc sulfate ($ZnSO_4$) at 20-29°C gives 86-97% RH.

Table 1 sets forth glycerol/water (v/v concentrations) mixtures and the relative humidities generated by each.

TABLE 1

% Glycerol	Relative Humidity
100%	0% RH
85%	42% RH
70%	64% RH
60%	73% RH
50%	82% RH

Dehydration may also be achieved by exposing the embryos to "osmotically active" materials which promote the transfer of the requisite amount of water from the embryos to the material. The embryos may be either treated with an osmotically active solution prior to encapsulation in a synthetic seed coat or directly encapsulated in an osmotically active seed coat matrial that causes dehydration to a desired level.

Osmotically active materials include:

1) inorganic salts (buffers)
2) organic buffers
3) glycerol
4) polyethylene glycol
5) sugars
6) oils (lipids)
7) synthetic polymers, e.g., polyethylene glycols, etc.
8) organic polymers, e.g., alginates, etc.
9) mixtures of non-osmotically active polymers with any of the above osmotically active substances.

The invention is illustrated by the following non-limiting examples.

EXAMPLE 1

Basal portions of young orchardgrass leaves were cultured on Schenk and Hildebrandt (SH) medium containing 7 g/l agar and 30 µM 3,6-dichloro-O-anisic acid (dicamba, Velsicol Chemical Corp., Chicago, IL) as described by Hanning et al, L. Theor. Appl. Genet., Vol. 63, pp. 155-159 (1982). Friable, embrogenic calli were isolated from leaf cultures after 4 wk and were maintained by monthly subcultures on identical medium.

Embryos were manually selected from callus cultures 4 wk after subculture and approximately 30 were placed in each empty 100 x 15-mm sterile plastic petri dish. Only morphologically normal embryos were chosen. Abnormal embryos, i.e., those fused with others or with misshapen scutellar tissues, were excluded. Specific details of selection criteria are provided in Table 1. The dishes were kept at 23°C in

the dark at a controlled, relative humidity of 70% ± 5%. Dehydration commenced immediately under these conditions. For germination, the dehydrated embryos were rehydrated in the dark for 48 h on SH medium lacking dicamba, then placed in a 16-8 h diffuse cool white fluorescent light (30 µmol photons $s^{-1}m^{-2}$)-dark cycle at 25°-10°C. Non-dehydrated embryos were included as controls.

TABLE 2

EFFECT OF DEVELOPMENTAL STAGE ON THE ABILITY OF SOMATIC EMBRYOS TO GERMINATE AFTER DEHYDRATION [a]

Response	Developmental Stage			
	A	B	C	D
No germination	150/100[b]	120/80	108/72	150/100
Germination, no further growth	0	22/14	33/22	0
Germination, viable plants	0	8/5	9/6	0
Total	150/100	150/100	150/100	150/100

[a] A, translucent proembryos, i.e, externally undifferentiated embryos as defined by Merry [Bull. Torrey Bot. Club, Vol. 68, pp. 585-598 (1941), up to 300 µm in diameter; B, well-developed white, opaque embryos up to 1000 µm long with scutella and coleoptiles; C, larger, well-developed white, opaque embryos up to 1500 µm long; D, enlarged well-developed translucent embryos over 1500 µm long. Embryos of each stage were dehydrated for 7 d and then allowed to imbibe water. Embryos that produced root hairs, roots, coleptiles, and/or shoots but exhibited no further growth were considered to have survived desiccation and to have germinated. Those that germinated to produce green leaves and roots resulted in viable plants.

[b] Number/percentage of total.

During dehydration, somatic embryos decreased in size, became yellowish and brittle, and their outer cell walls were collapsed. When placed on fresh medium, they enlarged and became white again within 15 min. All embryos enlarged whether viable or not. After enlargement, the embryos were morphologically indistinguishable from those never subjected to dehydration. Root hair growth was the first morphologic evidence of germination and was apparent as early as 6 d after placement of dehydrated embryos on fresh medium. Development of root hairs was previously documented for germinating orchardgrass somatic embryos with time-lapse photomicrography [Gray et al, Trans. Am. Microsc. Soc. Vol. 104, pp. 395-399 (1985)] as well as for zygotic embryos of barley (Hordeum vulgare L.) germinated in vitro [Norstog, Ohio J. Sci., Vol. 55, pp. 340-342 (1955)]. Zygotic embryos of many gramineous species typically produce root hairs upon germination [Brown, Phytomorphology, Vol. 10, pp. 215-223 (1960); Brown, Phytomorphology, Vol. 15, pp. 274-284 (1965).

Somatic embryos were initated and developed continuously in callus cultures so that several ontogenetic stages were present at a given time [Gray et al, Protoplasma, Vol. 122, pp. 196-202 (1984)]. Therefore, the effect of developmental stage on the ability of embryos to survive dehydration and subsequently germinate was investigated to provide guidelines for manual selection. Results on embryo survival and germination for four stages that could be repeatedly isolated from cultures were evaluated (Table 2). Only embryos that were white and opaque with well-developed scutellar and coleoptilar regions before dehydration were capable of germination after

imbibition; however, many of these produced only roothairs or roots.

When well-developed embryos were separated by size, more large than small germinated (22 vs. 14%) but neither exhibited further growth. However, both produced nearly equivalent percentages of viable green plants (5 or 6%). These results correlate with zygotic embryogenesis in wheat (Triticum aestivum L.), where embryo size was not predictive of later growth [Symons et al, J. Exp. Bot., Vol. 34, pp. 1541-1550 (1983)]. Dehydrated proembryos did not germinate. This was probably due to insufficient development before dehydration inasmuch as proembryos never subjected to dehydration similarly did not germinate. These observations are in agreement with those of Norstog [Am. J. Bot., Vol. 52, pp. 538-546 (1965)] who demonstrated that isolated zygotic barley proembryos could not germinate unless further embryonic development occurred. Enlarged translucent embryos did not germinate, despite having a morphology typical of well-developed embryos. Such embryos were in early stages of disorganization leading to embryogenic callus formation [Conger et al, Science, Vol. 221, pp. 850-851 (1983); Gray, Protoplasma, Vol. 122, pp. 196-202 (1984)]. Only white somatic embryos possessed an abundance of starch (determined in fresh specimens with I_2-KI staining) which was localized primarily in scutellar and coleoptilar tissues as demonstrated histologically [Gray et al, Plenum Press (1985), pp. 49-57; Gray et al, Protoplasma, Vol. 122, pp. 196-202 (1984)]. Translucent embryos possessed little or no starch. This suggests that orchardgrass embryos must contain starch to survive dehydration.

Freshly isolated somatic embryos were periodically (every 4 h) weighed during dehydration at 23°C to estimate water-loss rate. Embryo weight decreased rapidly and became constant after 24 h (FIG. 1). Freshly isolated embryos contained 75% water and dehydrated embryos contained 13%. The initial and final water contents of the orchardgrass somatic embryos were similar to those found in grass and cereal seeds during ripening and storage at 70% relative humidity [Osborne, Can. J. Bot., Vol. 61, pp. 3568-3577 (1983); Owen, Commonwealth Agricultural Bureaux, Vol. 11, pp. 52-53 (1956); Symons et al, supra]. However, the 24-h period for maximum water loss was much less than the 10 d reported for grains of barley, oats and wheat [Meredith et al, N.Z.J. Sci., Vol. 18, pp. 501-509 (1975)].

The effect of storage period on germination was evaluated by rehydrating white opaque embryos (450 for each period) after 0 (control), 7, or 21 d of dehydration (Table 3). After 7 d, 18% of the embryos germinated but exhibited no further growth, whereas 8% germinated to become normal-appearing plants with green leaves and roots, However, at 21 d, only 8% germinated with abbreviated growth and 4% produced plants. In the control group, the figures were 40 and 32%, respectively.

TABLE 3

EFFECT OF STORAGE PERIOD ON THE GERMINATION RATE OF QUIESCENT SOMATIC EMBRYOS[a]

Response	Days of Dehydrated Storage		
	0	7	21
No germination	126/28[b]	333/74	396/88
Germination, no further growth	180/40	81/18	36/8
Germination, viable plants	144/32	36/8	18/4
TOTAL	450/100	450/100	450/100

[a] Well-developed white, opaque embryos (stages B B and C in Table 2) were maintained in a dehydrated state at 23°C for 0, 7, or 21 d and then allowed to imbibe water. Embryos that produced root hairs, roots, coleoptiles, and/or shoots but no further growth were scored as germinated. Those that germinated to produce green leaves and roots resulted in viable plants.

[b] Number/percentage of total.

EXAMPLE 2

Embryogenic cultures of grape were prepared from anthers, ovules or young leaves using methods as described by Gray and Mortensen, Plant Cell Tissue and Organ Culture, Vol. 9, pp. 73-89 (1987). Perennial cultures of Vitis longii 'Microsperma', Vitis rupestris 'St. George', Vitis vinifera 'Thompson Seedless', experimental hybrids B-1, B-2, B-3 and B-4 were established and successfully dehydrated, stored and germinated as described below.

For dehydration experiments, grape somatic embryos were carefully separated from proliferating cultures. Only embryos with well-developed hypocotyl-radical axes and discrete, separated cotyledons were selected. Pluricotyly, which was characteristic of many embryos, was tolerated in experimental samples. Grape embryos were subjected to the same dehydration procedures as orchardgrass in Example 1 and the morphological changes were generally similar (i.e., decreased size with yellowing, brittleness and cellular collapse). Imbibition was rapid and restored the white, opaque nature of the embryos. However, criteria to assess germination in grape differed from those set for orchardgrass. Embryos that produced only a root and/or became yellow to green were considered to have survived dehydration. Embryos that, in addition to the above responses, produced shoots with green leaves were scored as viable.

In order to assess the effect of dehydration on survival, samples of 200 well-developed embryos were dehydrated and stored for 21 days. Embryos were subsequently placed on Murashige and

Skoog's medium [Physiologia Plantarum, Vol. 15, pp. 473-497 (1962)] containing 30g sucrose per liter, pH 5.4 for imbibition and growth. Responses were scored after 4 weeks of incubation under fluorescent light at 23°C. Germination was greater for grape compared to carrot or orchardgrass. Data for line B-1 are presented in Table 4. At 21 days, only 23% of imbibed embryos remained white and did not respond (Table 4). Forty- three percent germinated with no further growth and 34% produced rooted green plants. This response was greater than that obtained for a control group of embryos, never subjected to dehydration, where only 5% produced green plants.

The relatively poor response of controls is explained by considering the nature of grape seed. Seeds of grape exhibit typical dormancy which is most commonly broken by cold stratification (3 months at 4°C). Grape somatic embryos were also dormant with regard to germination but remained metabolically active and underwent secondary embryogenesis. This accounted for the low germination rate of controls. In grape, somatic embryo dormancy is broken by either cold temperatures or treatments with gibberellic acid or benzyladenine (BA). For example, approximately 50% of freshly isolated V. longii somatic embryos germinated into green plants when cultured on medium containing 1 µM BA. Dehydration appeared to be another dormancy-breaking treatment. Because germination occurred readily after imbibition and was not dependent on other treatments, dehydrated embryos must be considered to be non-dormant or quiescent. Thus, dehydrated grape somatic embryos germinate better than controls.

TABLE 4

Germination rate of dehydrated grape somatic embryos from experimental line B-1 as compared to control. White opaque embryos with well-developed embryo axes and discrete unfused cotyledons were maintained in a dehydrated state at 23°C for 0 to 21 days and then allowed to imbibe water. Those that produced roots and/or became yellow to green with no further growth were scored as germinated. Embryos with roots and shoots with green leaves were viable.

	Days of Dehydrated Storage	
Response	0	21
No germination	85/85[z]	46/23
Germination - no further growth	10/10	86/43
Germination - viable plants	5/5	68/34
Total	100/100	200/100

[z] Number/percentage of total

EXAMPLE 3

Osmotic Transfer of H_2O Prior to Encapsulation

To obtain a 13% embryo water content, embryos are mixed with a solution of glycerol (87%) and water (13%). They are stored in this solution and are rehydrated by rinsing in a solution of 100% water or liquid culture medium.

EXAMPLE 4

Osmotic Transfer of H_2O to Encapsulating Coating

Embryos are mixed with synthetic resin formulated so as to remove embryo water osmotically down to desired level. The resin is formed into drops around individual embryos and then polymerized by either heat, UV light or chemical hardener depending on the resin. This results in dehydrated, quiescent synthetic seeds. To germinate, water is added, solubilizing the resin, rehydrating the embryo and causing germination. Instead of drops, the resin could be formed into a continuous polymerized strand containing evenly-spaced embryos. Such a "synthetic seed string" would make planting crops less labor- and machinery-intensive.

CLAIMS:

1. A method of producing synthetic seeds by inducing growth quiescence in plant somatic embryos comprising maintaining said somatic embryos in an atmosphere having a relative humidity of from about 30% to about 85% or in an osmotically active environment having a moisture content of from about 30% to about 85% for a period of time sufficient to reduce the moisture content of said embryos to from about 85-65% to about 4-15% and for said embryos to cease growth.

2. The method of claim 1 wherein the temperature of said atmosphere or environment is maintained at from about 15°C to about 35°C.

3. The method of claim 1 wherein said embryos are maintained in said atmosphere or environment for from about 24 hr to about 336 hr.

4. The method of claim 1 wherein said plant embryos are monocotyledonous.

5. The method of claim 1 wherein said plant embryos are dicotyledonous.

6. A synthetic seed product comprising the dehydrated somatic plant embryos produced by the method of claim 1 which, upon rehydration and growth, yields plants essentially identical to the plant from which the somatic plant embryos is developed.

7. The synthetic seed product of claim 6 wherein said plant embryos are monocotyledonous.

8. The synthetic seed product of claim 6 wherein said plant embryos are dicotyledonous.

9. In a method of developing plants by somatic embryogenesis the improvement comprising employing the dehydrated somatic plant embryos produced by the method of claim 1.

10. The method of claim 9 wherein said somatic plant embryos are monocotyledonous.

11. The method of claim 9 wherein said somatic plant embryos are dicotyledonous.

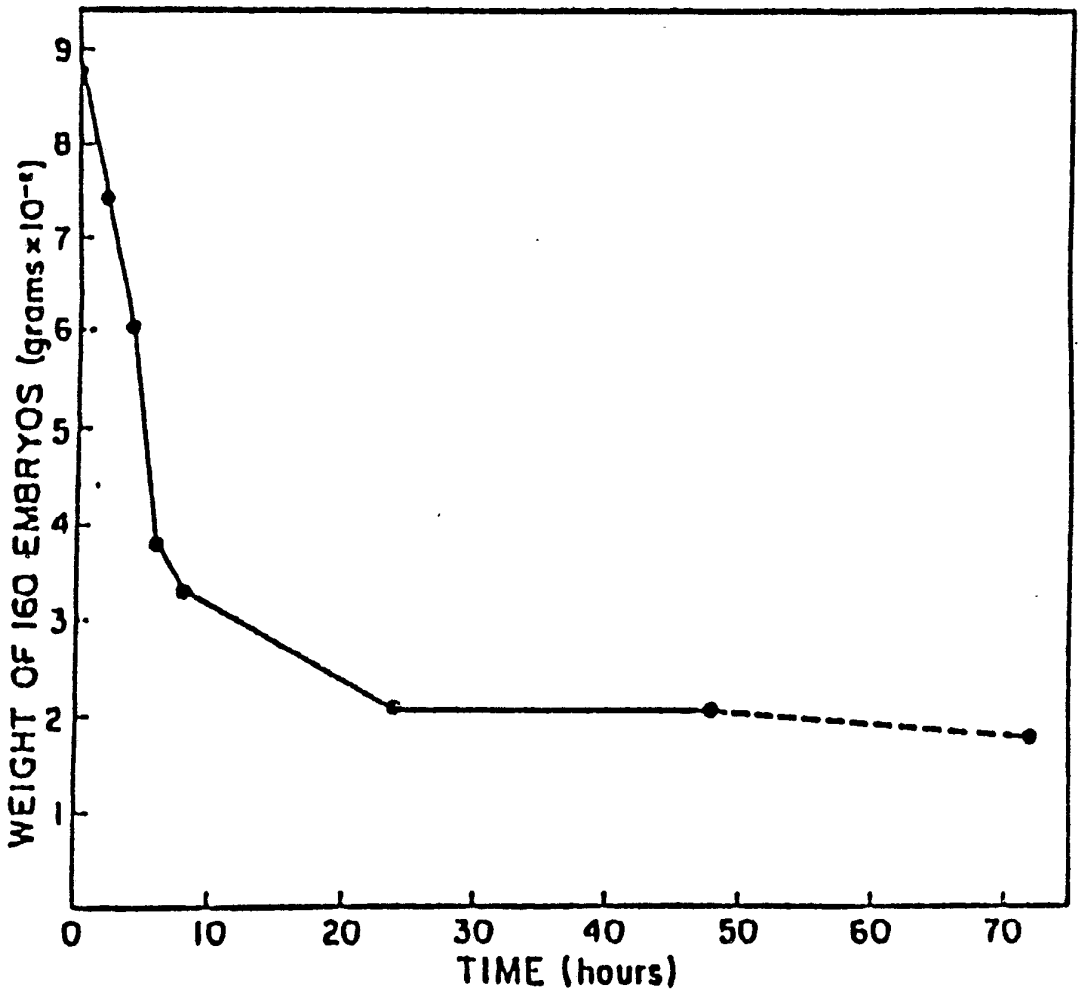

WEIGHT OF 160 EMBRYOS (grams x 10⁻⁴)
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
TIME (hours)

FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03934

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 01 G 7/00; A 01 C 1/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 01 G; A 01 H; A 01 C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	WO, A, 87/04044 (PLANT GENETICS INC.) 16 July 1987 see page 6, line 1 - page 8, line 18; page 33, lines 12-19	1-3,5,6,8, 9,11
A	--	4,7,10
X	US, A, 4615141 (JANICK et al.) 7 October 1986 see column 2, line 45 - column 5, line 35; figure 1 cited in the application	1-11
	--	
X	EP, A, 0141373 (PLANT GENETICS INC.) 15 May 1985 see page 4, line 28 - page 8, line 35; page 20, line 1 - page 21, line 29 cited in the application	1-3,5,6, 8,9,11
A	--	4,7,10
	./.	

* Special categories of cited documents: [10]

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

"E" earlier document 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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th March 1989	20. 04. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

International Application No. PCT/US 88/03934

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A, 0107141 (PLANT GENETICS INC.) 2 May 1984 see page 6, line 6 - page 7, line 6; page 14, line 25 - page 15, line 27 cited in the application	1-3,5,6, 8,9,11
A	--	4,7,10
A	Journ. Am. Soc. Horticultural Science, volume 110, no. 2, 1985, (Alexandria, US), S.L. Kitto et al.: "Production of synthetic seeds by encapsulating asexual embryos of carrot" pages 277-282 see the whole document cited in the application	1-11
A	-- Zeitschrift Pflanzenphysiol., volume 100, 1980, W. Nitzsche: One year storage of dried carrot callus", pages 269-271 see the whole document cited in the application	1-11
A	-- Zeitschrift Pflanzenphysiol., volume 87, 1978, W. Nitzsche: "Erhaltung der Lebensfähigkeit in getrocknetem Kallus", pages 469-472 see the whole document cited in the application	1-11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8803934 SA 25498

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 12/04/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8704044	16-07-87	AU-A- 6897887	28-07-87
		EP-A- 0250584	07-01-88
		JP-T- 63502241	01-09-88
		US-A- 4777762	18-10-88
US-A- 4615141	07-10-86	None	
EP-A- 0141373	15-05-85	AU-A- 3464284	02-05-85
		JP-A- 60214811	28-10-85
		US-A- 4583320	22-04-86
		US-A- 4780987	01-11-88
		US-A- 4779376	25-10-88
EP-A- 0107141	02-05-84	AU-A- 1981083	19-04-84
		JP-A- 59102308	13-06-84
		US-A- 4562663	07-01-86
		US-A- 4583320	22-04-86
		AU-B- 558835	12-02-87
		CA-A- 1223443	30-06-87
		DE-A- 3375684	24-03-88