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Original Article

A Brief Introduction to an Altering Plant Characteristics Without Genetic Engineering

Bill Carrington[1]* and Marc George[2]

[1]*Department of Cell/Tissue Culture, Bill Berry Farms Global Inc, California, USA*

[2]*Department of Molecular/Cellular Endo-Biochemist, Concordia University Chicago, Illinois, USA*

Abstract

Background: New strains of plants can have improved properties and can provide useful resources. However, when new genes are introduced into plants by recombinant technology, the chromosomes of the resulting genetically modified plant cells can be altered in unpredictable and sometimes unfortunate ways. For example, introduced DNA can be inserted into essential genes and interrupt the function of such essential genes. New methods for generating new varieties of plants, for culturing new types of plant cells, and for extracting useful materials from such new plants and plant cells are needed.

Although new strains of plants have been made by protoplast fusion, the methods currently employed do not efficiently provide a high proportion of hybrid cells with desired characteristics. When such low efficiency methods are employed laborious cultivation of many plantlets may be needed, followed by extensive screening of the plantlets for selected trait (s) as well as for desirable functional agronomic characteristics before a suitable plant line can be identified.

Summary: This invention involves improved methods of generating hybrid plant cells and hybrid plants by somatic cell fusion without electric shock. The methods do not require recombinant alteration of cellular chromosomes by currently available genetic engineering procedures. For example, the inventive methods do not involve transformation of cells by insertion into plant chromosomes or transient expression of coding regions from expression cassettes, expression vectors, viral vectors, plasmids or other vectors commonly used for genetic engineering. Instead the nuclei of fused somatic cells can naturally exchange genetic information by homologous recombination using processes like those that occur naturally during sexual reproduction of plants. New types of hybrid cells are therefore formed that have desirable traits and improved characteristics.

***Corresponding author:** Bill Carrington, Department of Cell/Tissue Culture, Bill Berry Farms Global Inc, California, USA, Tel: +1 6162158223; E-mail: bill@billberryfarmstissueculture.com; bill@bbforganicsolutions.com

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Detailed Description

The invention relates to methods of generating hybrid plants that do not involve genetic engineering. Gentle methods are employed that optimize the efficiency of hybrid cell formation and improve the survival and outgrowth of hybrid cells. The methods can be used for the fusion and breeding of sexuallycompatible or incompatible plant species. The methods generally involve at least removal of plant cell walls to make protoplasts, protoplast fusion and cell wall regeneration. Improved efficiency and cell survival is obtained when the methods include at least two or three of the following steps prior to removal of cell walls: pre-treatment of plants, plant preparation and/or treatment of shoots, roots or somatic embryos. The methods can also include improved processes for cell wall regeneration, hybrid cell recovery, and outgrowth. Hardy plants with a variety of new propertied can be generated by the methods described herein.

Although other researchers have performed protoplast fusions in the past, the procedures described herein not only improve the percentage of successful fusions but also yield more robust fusions that provide hybrid plant strains with useful new properties that are hardy and resistant to environmental stress.

Plant Material

As described in the section entitled “Types of Fusions” a large variety of plant types can be fused to generate new and useful hybrid plant lines. However, when selecting plant materials and cells for fusion, younger plants are a good source of cells for fusion because they tend to have fewer mutant cells and they have been subjected to less environmental stress that might weaken them.

Types of fusions

The methods described herein can be employed for protoplast fusion between all herbaceous and Cannabaceae family. The following are examples of fusions that can be made using the procedures described herein, where the X signifies a protoplast fusion via the methods described herein.

- Rutaceae RUE or CITRUS FAMILY X Cannabis (Examples: lemon, lime, grape fruit, orange, mandarine, kumquat (Citrus), rue (Ruta), prickly-ash (Zanthoxylum))
- Rubiaceae COFFEE FAMILY X Cannabis (Examples: bedstraw, madder (Galium, Rubia), quinine tree (Cinchona), coffee (Coffea), yohimbine (Pausinystalia), buttonbush (Cephalanthus), West Indian jasmine (Ixora), morinda, noni (Morinda), pentas (Pentas), Ipecacuanha (Psychotria).

- Solanaceae TOMATO, PEPPER, POTATO &Lamiaceae FAMILY X Cannabis (Examples: potato/tomato (Solanum, incl. Lycopersicon), chili pepper/sweet pepper (Capsicum), basil, thai basil, sweet basil, angel trumpet (Brugmansia), tobacco (Nicotiana), petunia (Petunia), tomatillo (Physalis), jimsonweed (Datura), saint joseph's wort)
- Cannabaceae family all types and species can undergo protoplast fusion with any other Cannabaceae family members, including any other type or species of Cannabaeae (Examples: Sativa hybrid, Indica hybrid, Sativa, Indica, hemp hybrid)

Protoplasts of any species or type of Cannabaceae (such as Sativa hybrid, Indica hybrid, Sativa, Indica, hemp hybrid) can be fused with protoplasts.

Results

Figure 1 is a photomicrograph showing fusion of two female Cannabis cells of the same strain that are fused to improve the properties of the strain.

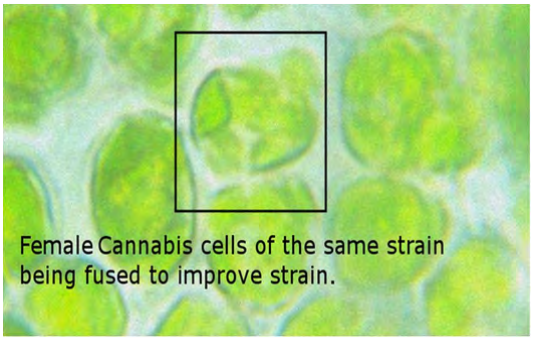

Figure 1: Photomicrograph illustrating fusion of two female Cannabis cells of the same strain that are fused to improve the properties of the strain.

Figure 2 graphically illustrates the THC content of Sativa and Indica control plant seeds that have not been generated from hybrid cells as described in this Example (left-most bars), compared to Sativa and Indica hybrid plant somatic synthetic seeds enerated from hybrid cells as described in this Example (right-most bars). The middle bars in figure 1 show the THC content of Sativa and Indica tissue culture plantlets.

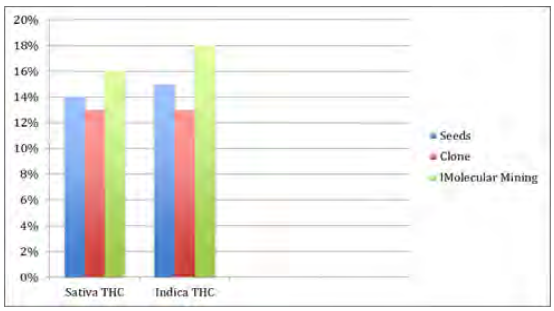

Figure 2: Shows graphically illustrates the THC content of Sativa and Indica control plant seeds that have not been generated from hybrid cells as described in this Example (left-most bars), compared to Sativa and Indica hybrid plant somatic synthetic seeds generated from hybrid cells as described in this Example (right-most bars). The middle bars in figure 1 show the THC content of Sativa and Indica tissue culture plantlets.

Example 5: Fusion of Basil and Cannabis Protoplasts

This Example illustrates that the methods described herein are useful for fusing cells or protoplasts from different species.

The materials described in Example 1 and the methods described in Examples 2 and 3 were utilized to generate an interspecies hybrid between basil cells and cannabis cells.Protoplast fusions are readily spotted by their appearance of an overly large polykaryon (multi-nuclear protoplast or cell). Use of cell with two different sizednuclei or two differently sized cells facilitates identification of fusions. In addition, infiltration or injection of cellular dyes into one cell type also makes it easier to see fusion of the cannabis and basil protoplasts.

Figure 3 shows a blue-dyed basil cell fused with a cannabis cell. As illustrated, the hybrid cell is enclosed by a cellular membrane.

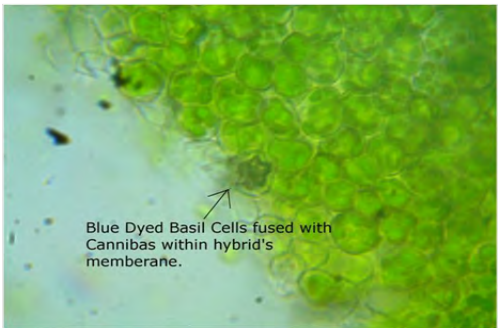

Figure 3: Blue-dyed basil cell fused with a cannabis cell. As illustrated, the hybrid cell is enclosed by a cellular membrane.

Figure 4 shows another fusion between a blue-dyed basil cell and a cannabis cell.

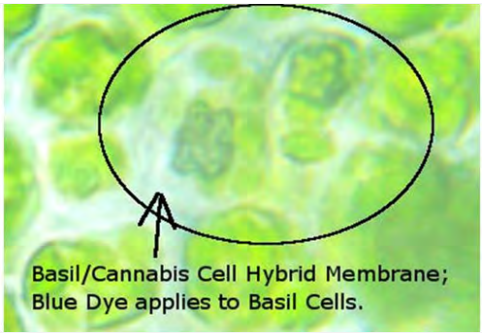

Figure 4: Fusion between another blue-dyed basil cell and a cannabis cell.

All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby specifically incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

Conclusion

"Through new method Type of Fusions" a large variety of plant types (cannabis fusions with cannabis and cannabis fusions with basil.

Enhancing strength, yield, characteristics, and in all creating a new super cell hybrid) to be fused so to generate a new and useful hybrid plant lines. However, when selecting plant materials and cells for fusion, Young mature plants of 2 years of age are a good source of cells for fusion because they tend to have fewer mutant cells and they have been subjected to less environmental stress that might weaken them. When designing experiments meant to interact with specific pathways. Introduction of molecules of interest ideally is carefully timed in order to match the process of interest or the process to that, which is altered. At this point the dyes (e.g., those described in Example 1) can be added to one of the protoplast (female cannabis cell to female cannabis cell and or female cannabis cell to basil cell) preparations so that one population of protoplasts or nucleic can be identified during manipulations such as protoplast fusion. In addition, DNA and other plant components can be added to the protoplasts to modulate the genetic make-up and/or the enzymatic/hormonal composition of the plant cells ("Cell Mutation or Modulation"). And from there cell to plant.

The following statements of the invention are intended to describe and summarize various embodiments of the invention according to the foregoing description in the specification.

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3. Altered plants material from Molecular Mining LLC.
4. Lab used Somatic Synthetic Seeds Organization.

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