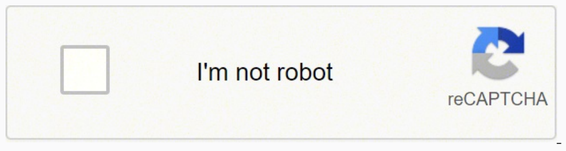

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Embryogenesis in plant tissue culture pdf

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ORIGINAL PAPER

Stress treatments and in vitro culture conditions influence microspore embryogenesis and growth of callus from anther walls of sweet pepper (*Capsicum annuum* L.)

Verónica Parra-Vega · Begoña Renau-Morata · Alicia Sifres · José M. Seguí-Simarro

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Abstract Production of doubled haploids (DHs) is a convenient tool to obtain pure lines for breeding purposes. Until now, the easiest and most useful approach to obtain pepper DHs is via anther culture. However, this method has an associated possibility of producing calli from anther wall tissues that would be coexisting in the anther locule with embryos derived from microspores. Using two established protocols for anther culture, Dumas de Vaulx et al. (Agronomie 2:983–988, 1981) and Supena et al. (Sci Hort 107:226–232, 2006a; Plant Cell Rep 25:1–10, 2006b) callus and embryo development was assessed in four sweet pepper cultivars. For all genotypes tested, the protocol of Dumas de Vaulx et al. (Agronomie 2:983–988, 1981) promoted both embryo development and callus growth, whereas the protocol of Supena et al. (Sci Hort 107:226–232, 2006a; Plant Cell Rep 25:1–10, 2006b) produced no callus but only embryos. However, differences in embryo production were observed among these genotypes. In parallel, anthers were exposed to a 35 °C inductive heat shock for 4, 8, 12 and 16 days, prior to culture at 25 °C. The duration of the heat shock had significant effects in embryo production, but also in callus generation. Callus generation increased with prolonged exposures to 35 °C. Embryo and callus origin was analyzed by flow cytometry, light microscopy and molecular markers. Tests conducted demonstrated a gametophytic origin for all of the embryos tested, and a sporophytic origin for all of the calli. Together, our results reveal that culture conditions have a significant influence on the presence of calli derived from anther walls, which could be minimized by reducing heat shock exposure and/or using a shed-microspore approach.

Keywords Androgenesis · *Capsicum annuum* · Heat stress · Microspore embryogenesis · In vitro culture

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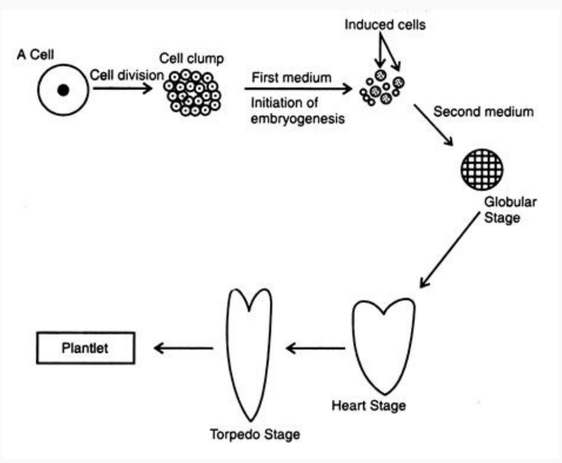
Introduction

Induction of androgenesis is one of the most convenient ways to obtain haploid and doubled haploid (DH) individuals. Pepper (*Capsicum annuum*) is, together with tomato and eggplant, one of the three solanaceous crops most recalcitrant to the induction of androgenic DHs (Seguí-Simarro et al. 2011). Apart from the spontaneous occurrence of some cases of in vivo androgenesis with no practical relevance (Campos and Morgan 1958), haploids in pepper were first obtained through parthenogenesis (reviewed in Regner 1996). Soon after their discovery by Guha and Maheshwari (1964), anther cultures were explored as a way to haploidy, and since then, they have been used as a tool to produce pepper DHs for breeding programs (Jiang and Li 1984; Dumas de Vaulx and Pochard 1986; Hwang and Paek 1998; Arnedo Andrés et al. 2004). Despite the recent progress made on isolated microspore culture (Lantos et al. 2009; Ferrie and Caswell 2011; Kim et al. 2012; Lantos et al. 2012), anther culture is still considered the method of choice for pepper DH production due to its simplicity (Germanà 2011). However, this technique carries a number of drawbacks. Among others, these include: a limited efficiency, producing only a few embryos per cultured anther; the uncontrollable secretory effect of the tapetum, which precludes a strict control of



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Analysis of genetic variability in various tissue culture-derived lemon plant populations using RAPD and flow cytometry

V. Orbović · M. Ćalović · Z. Viloria · B. Nielsen · F. G. Gmitter Jr. · W. S. Castle · J. W. Grosser

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Abstract Five populations of lemon plants [*Citrus limon* (L.) Burm] obtained from undeveloped ovules through different tissue culture procedures were examined for the presence of somaclonal and irradiation-induced genetic variation. Tested groups were: (1) nucellar seedlings; (2) organogenic, regenerated via adventitious buds from nucellar seedling internodes; (3) embryogenic population, regenerated from non-irradiated nucellar callus via somatic embryogenesis; (4) embryogenic population, regenerated from irradiated nucellar callus via somatic embryogenesis; and (5) protoplast-derived, regenerated via somatic embryogenesis. Genomic DNA samples from 360 plants (72 from each group) were screened for polymorphism among RAPD fingerprints amplified by 10 decamer primers. Among all tested plants, genetic variation was detected only within the group of plants recovered from irradiated embryogenic calli. Out of 72 plants from that group, three had RAPD fingerprints different from the rest of the population, and fourth plant was found to be cytochimeric, consisting of diploid and tetraploid cells as revealed by flow cytometry. In all other populations of regenerated plants, we did not come across any plants with changed ploidy level.

Keywords *Citrus limon* · Organogenesis · Protoplasts · Somaclonal variation · Somatic embryogenesis

Abbreviations

BA	6-Benzyladenine
2,4-D	2,4-Dichlorophenoxyacetic acid
MES	4-Morpholinoethanesulfonic acid

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Introduction

The term 'somaclonal variation' is used to refer to the phenotypic and genotypic variations of both qualitative and quantitative traits that occur in plants regenerated from cell and tissue cultures (Jain 2001). These variations are frequently epigenetic and unstable, and as such not inherited but when they are genetic and stably inherited, they have a great potential in plant improvement programs compared to traditional breeding which is difficult and time consuming. In vitro culture alone or combined with mutagenic induction that includes treatments with physico-chemical and biological agents has been utilized with the aim of recovering plants with increased genetic variability and selecting mutants as a potential source of new commercial cultivars. With this approach, induction of somaclonal variation has been successful at identifying potential new varieties in different crops such as wheat (Gao et al. 1991), potato (Arihara et al. 1995), and citrus (Grosser et al. 1996).

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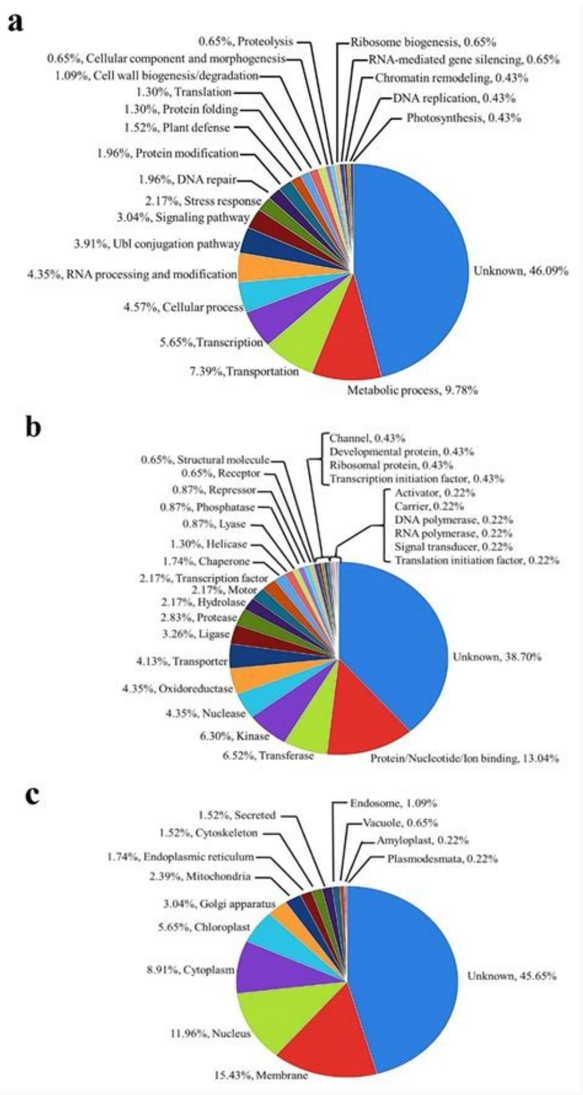

Importance of somatic embryogenesis in plant tissue culture. Organogenesis and embryogenesis in plant tissue culture. Somatic embryogenesis in plant tissue culture pdf. Somatic embryogenesis in plant tissue culture ppt. Zygotic embryogenesis in plant tissue culture. Somatic embryogenesis in plant tissue culture. Embryogenesis in plant tissue culture slideshare. Embryogenesis in plant tissue culture biology discussion.

Process that occurs after the fertilization of an ovule to produce a fully developed vegetable embryonic embryonic development, also the vegetable embryenesis is a process that occurs after fertilization of a ovum to produce a vegetable embryon completely developed. This is a pertinent stage in the plant life cycle that is followed by the gorgence and germination. [1] The zygote produced after fertilization must be subjected to several cell divisions and differentiations to become a mature embryon. [1] A final phase embryon has five main components, including the Apical Meristem, Hypocotyl, Meristem RaÃz, RaÃz Cap and Cotched. [1] Unlike embryonic development in animals, and specifically in humans, the embryonic development development results in an immature form of the plant, lacking most structures such as leaves, stems and reproductive structures. [2] However, both plants and animals, including humans, go through a phytothel stage that evolved independently [3] and that causes a development limitation that limits morphological diversification. [4] [6] [6] Embrusogenesis is naturally produced as a result of individual or double fertilization of ovum, resulting in two different structures: the embryon of the plant and endosperm that develop in a seed. [8] The Zygote passes through several cellular differences and divisions to produce a mature embryon. These morphogenic events form the basic cell pattern for the development of the body of the s seesion and the primary tissue layers; We also program the meristematist tissue formation regions. The following morphogenic events are only particular for eudicos, and non-monocotas. Six moments in Embrigenesis Two cell stadiums Globuular stage cardiac stage torpedo Stagematuration Endospermsingle Celled apical meristem (SAM)root apical meristem (RAM) Two cell stages After fertilization, zygote and endosperm are present within the ovule, as seen in stage I ofIllustration on this page. Then, the cigot is subjected to an asymmetric cross-section that gives rise to two cells: a small apical cell that rests on a large basal cell. [9] [10] These two cells are very different, and give rise to different structures, establishing polarity in the embryo. Apical cell The small apical cell is at the top and contains most of the cytoplasm, the aqueous substance that is found inside the cells, from the original cygote. [11] It gives rise to the hypocothyl, shoots the apical meristem and the cots. [11] Basal cell The large basal cell is at the bottom and consists of a large vacuola [11] and gives rise to the hypophysis [9] and the suspense. [9] Eight Cell Stage After two rounds of longitudinal division and a round of cross-section, an eight-cell embryo is the result. [12] Stage II, in the above illustration, indicates how the embryo looks during the eight-cell stage. According to Laux et al., there are four distinct domains during the eight cell stage. [13] The first two domains contribute to the embryo itself. The apical embryo domain gives rise to the meristem apical brothing and cotiledones. The second domain, the domain of the central embryo, gives rise to the hypocothyl, the meristema apical root and parts of the cotiledones. The third domain, the domain of the basal embryo, contains the hypophysis. Hypophysis will later lead to the root and lid. The last domain, the suspense, is the region at the bottom, which connects the embryo to the endosperm for nutritional purposes. Sixteen cell stages produce additional cell divisions, leading to the stage of sixteen cells. The four domains are still present, but are more defined with the presence of more cells. The important aspect of this stage is the introduction of protoderm, which is a meristematic tissue that will lead to the[12] The prototel is the external layer of cells in the embryo itself. [12] GLOBULAR STAGE The name of this stage is indicative of the appearance at this point in embryogenesis; It is spherical or globular. Stage III, in the photograph above, represents how the embryo looks during the globular stage. 1 is indicating the location of the endosperm. The important component of the globular phase is the introduction of the rest of the primary merestematic tissue. The protoderm was already introduced during the stage of sixteen cells. According to Evert and Eichhorn, the Meristem Ground and the procambium begin during the globular stage. [12] The land Meristem will continue to form the ground tissue, which includes the marrow and cortex. The procambium will eventually form the vascular tissue, which includes xylema and floem. Heart Stage According to Evert and Eichhorn, the stage of the heart is a transition period where the cots finally begin to form and lengthen. [12] It is given to this name in Eudicots because most of the plants of this group have two cotiledones, which gives the embryo a heart-shaped appearance. The meristem apex is among the coiledons. Stage IV, in the above illustration, indicates how the embryo looks at this point of development. 5 Indicates the position of the cotiledons. Stage Pro Embryo This stage is defined by the continuous growth of the cots and the lengthening of the axis. [12] In addition, programmed cell death should occur during this stage. This is done throughout the growth process, like any other development. [14] However, in the development stage of the torpedo, the parts of the suspense complex must be completed. [14] The suspense complex is shortened because at this point of development most of the endosperm nutrition has been used, and there must be room for the mature embryo. [11] After the suspense complex has gone, the embryo is completely developed. [13] Stage V, in the above illustration, indicates how the embryo looks at this point of development. Madura Laphase, or postambryonic development, involves maturation of cells, cells, It involves cell growth and macromolé storage (such as oils, starches and proteins) required as a "food and energy supply" during germination and growth of seed. The appearance of a mature embryon is seen in stage VI, in the previous illustration. Dormancy The End of Embrigenesis is defined by an arrested development phase, or stopping in growth. This phase usually coincides with a necessary component of growth called Dormcurity. Dorenance is a period in which a seed can not germinate, even in optimal environmental conditions, until a specific requirement is met. [15] The rupture of the Dorenance, or the determination of the specific requirement of the seed, can be pretty difficult. For example, a seed coat can be extremely thick. According to Evert and Eichhorn, very thick seed layers must undergo a process called scarceness, to deteriorate the coating. [12] In other cases, the seeds must experience stratification. This process exposes the seed to certain environmental conditions, such as cold or smoke, to break the gorgence and start germination. The auxin role is a hormone related to the elongation and regulation of plants. [16] He also performs an important role in the polarity of the establishment with the embryon of the plant. Research has shown that gymnastry hypocotyl and angiosperms show transport auxin to the extreme raisin of the embryon. [17] Hypothesis that the embryonic pattern is regulated by the Auxin transport mechanism and the polar positioning of the cells within the ovum. The importance of auxin was, in its investigation, when carrot embryos, in different stages, were subjected to auxin transport inhibitors. The inhibitors who were subjected to these carrots could not progress at subsequent stages of embrigionnesis. the globular stage of embryosis, embryos continued the spherical expansion. In addition, oblong embryos continued axial growth, without the introduction of cotilledons. During the development stage of the heart embryo, there wasGrowth axes in hypochothetics. Additional research on the inhibition of auxin transport, carried out in Brassica Jounce, show that after germination, cotyledons merged and not two separate structures. [18] Alternative forms of embryographic embryo Somatics Somatical embryos are formed from vegetable cells that normally do not participate in the development of embryos, ie, ordinary vegetable tissue. An endosperm or a layer of seed is not formed around a somatical embryon. The applications of this process include: Clonal propagation of genetically uniform vegetable material; virus elimination; supply of fabrics of origin for genetic transformation; generation of entire plants from solo cells called protoplasts; Development of Sinthetic Seed Technology. The cells derived from competent origin tissue are cultivated to form an undifferentiated mass of cells called callus. The growth regulators in the tissue culture medium can be manipulated to induce the formation of callus and subsequently changed to induce embryos to form the callus. The proportion of different plant growth regulators needed to induce calling or embryo formation varies according to the type of plant. The substantial cell division also seems to be important in the development of somatical embryos, and although the lack of training of the suspension cell is lethal for cigotic embryos, it is not lethal for somatical embryos. Androgencies The androgen process allows a mature vegetable embryon to be formed from a grain of reduced or immature pollen. [19] The Androgen is usually presented under stressful conditions. [19] The embryos resulting from this mechanism can germinate in fully functional plants. As mentioned, the embryon is the result of a single grain of pollen. Pollen grains They make up three cells â € "a vegetative cell containing two generative cells. According to Maraschin et al., Androgencies should be triggered during the substantial division of microspores. [19] However, once the vegetative cell begins to produce starch and proteins, the Androgense can no longer occur. Maraschin and others, others, that this embryogenesis mode consists of three phases. The first phase is the acquisition of embryonic potential, which is the repression of gametofites formation, so that the differentiation of cells occurs. Then, during the start of cell divisions, multicellular structures begin to form, which are contained in the exine wall. The last step of androgenesis is the formation of patterns, where embryonic structures are released from the wall of exile, so that the formation of patterns will continue. After these three phases occur, the rest of the process adjusts to standard embryogenic events. Growth of plants and shoots The embryonic tissue is composed of active growth cells and the term is normally used to describe the early formation of tissue in the early stages of growth. It may refer to different stages of the sporophytic and gametofite plant; including the growth of embryos in seedlings, and merestematic tissues,[20] that are in a persistent embryonic state,[21] to the growth of new shoots in stems.[22] Both in gymnospermas and angiospermas, the young plant contained in the seed, begins as a developing egg formed after fertilization (sometimes without fertilization in a process called apomixis) and becomes a plant embryo. This embryonic condition also occurs in the shoots that form in stems. Brotes have tissue that has been differentiated but has not become complete structures. They may be in a state of rest, sleeping during the winter or when the conditions are dry, and then begin to grow when the conditions become adequate. Before they begin to grow in stem, leaves or flowers, it is said that the shoots are in an embryonic state. 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