Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 21, Issue 4, 2021
PRINT ISSN 2284-7995, E-ISSN 2285-3952

GENETIC BIOENGINEERING IN AGRICULTURE - A MODEL SYSTEM FOR STUDY OF THE MECHANISM OF PROGRAMMED CELL DEATH

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Abstract

The behaviour of in vitro cell cultures is different from that of in vivo cells, when they are integrated into the organism. The selective death of cells, tissues and organs is a feature of plant development and survival. The process is called programmed cell death due to the organism's involvement in controlling of the initiation and execution of this process. The programmed cell death is an active, genetically controlled process that leads to the selective elimination of damaged cells. This complex process is present throughout the life of plants, from the seed germination to the maturation and senescence of plants. Cell death in plants has specific features due to the cell wall in particular but also of the presence of some specific structures of the plant cell, such as chloroplasts and vacuole. Exposure of plants to various stressors can induce oxidative stress and can be followed by cell death. However, cell death under abiotic stress conditions can also be a regulated process, meant to ensure the survival of plants. The programmed cell elimination plays an essential role in the desired modelling of plants, and this goal is the prerogative of genetic bioengineering, via cell cultures. The fascinating field of genetic bioengineering has a huge potential for the programmed modelling of the plants and obtaining new genotypes, with superior properties and high capacity to adapt to different environmental conditions, corresponding to the requirements of a sustainable management of modern agriculture.

Key words**:** *genetic bioengineering, cell death, programmed modelling*

INTRODUCTION

The concept of genetic bioengineering is a set of methods and laboratory techniques that allow the manipulation of genetic material, without the participation of sexual processes, with the aim of obtaining new organisms with new combinations of hereditary characters. Through modern genetic bioengineering techniques, it is possible to transfer genes from one organism to another or from one species to another and even the synthesis of organisms with modelled or programmed characteristics. The plant bioengineering support is in vitro culture, represented by techniques for plant organs cultivation, tissues and cells on artificial medium. The term in vitro completes the notion in vivo, which is represented by the cellular elements integrated in the universal connections of a plant or animal organism. Exposure of plants to various stressors such as hypoxia, water deficit, UV radiation, salinity, extreme temperatures, pollutants, toxins, heavy metals, etc., can induce oxidative stress and can be followed by cell death. The phenomenon of cell death in plants is essential in their life cycle. Programmed cell death (apoptosis) is an active, genetically controlled process that leads to the selective elimination of unwanted or damaged cells in eukaryotes [1, 9]. Cell death and cell proliferation, cell growth and cell differentiation, are well coordinated processes, thus ensuring the maintenance of homeostasis of plants tissues and organs [8].

Cell death in plants was classified into three types, depending on the morphological characteristics expressed by the affected cells [5, 9]:

a) Apoptosis-like cell death involving rapid nucleus degradation and loss of cell organization, a phenomenon encountered during plant development or under conditions of exposure to stressors. This form of degradation is signalled by mitochondria and is represented by nucleus shrinkage, chromatin condensation and DNA-laddering;

b) Cell death specific to plant senescence, a slow process associated with the recovery of cell content and the relocation of nutrients. After the complete degradation of the plastids, at the end of the cellular degradation process, occurs the nucleus and vacuole collapse;

c) Cell death induced by the collapse of the vacuole, which involves the action of proteases located in the vacuole, whose release into cytosol causes degradation of cell contents.

In vitro cell cultures are true systems for studying the mechanism of cell death in plants, due to the uniformity of the cultures, their accessibility and their low complexity. In this way, cell cultures reorient some directions of plant research, as well as certain concepts regarding the particularities of living matter.

MATERIALS AND METHODS

The death of specific cell sets is an essential part of plant and animal development. The phenomenon of programmed cell death in plants mean any process where the protoplast is removed as part of an event of cell development or adaptation in the life cycle of the plant. The process is called programmed cell death due to the fact that the organism controls the initiation and execution of this process. This review attempts to highlight some of the important issues on programmed cell death in plants and its presence throughout the entire life of plants, from the moment of seeds germination until their maturation and senescence. It is briefly presented how programmed cell death occurs during the vegetative growth of plants and their reproductive development, taking into account some factors of biotic and abiotic environment.

RESULTS AND DISCUSSIONS

Biotic stressors cause a multitude of processes in plants, which begins with the perception of stress by specialized receptors and ends with the expression of a battery of target genes [25]. Biotic stress produces effects both on primary metabolism and on secondary metabolism, reflected in the change in the activity of some enzymes, given that all chemical processes are catalysed by enzymes.

Reactive oxygen species are a normal product in the cellular metabolism of plants. A variety of environmental stressors lead to the overproduction of oxygen-reactive species, which cause oxidative damage and ultimately can cause cell death (Figure 1). In addition to their destructive activity, they are also described as secondary messengers in various cellular processes, including increasing plant tolerance to biotic and abiotic stress [25].

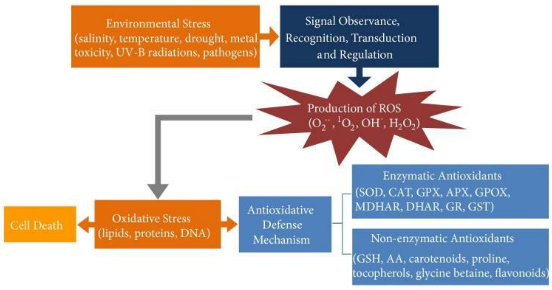

Fig. 1. The relationship between stressors, oxidative damage and cell death in plants
Source: [25].

Plants have a complex system of defence against oxidative stress, composed of enzymatic and non-enzymatic components for detoxifying reactive oxygen species. In plant cells, the systems for producing and detoxifying reactive oxygen species are found in various cellular organs, such as chloroplast, mitochondria and peroxisome. In fact, mitochondria play an essential role in promoting and achieving programmed cell death in plants.

The response of plants to abiotic stress is determined by several factors, such as their genotype and stage of development, as well as the duration, intensity, periodicity and synergistic effects of multiple stresses or pollutants [2, 3, 22]. In plants, stress triggers a wide range of responses, from altered gene expression and cellular metabolism to changes in growth rate and yield per unit area. From this point of view, sustainable crop management strategies reduce the negative effects of various stressors, increase the chances of food security and at the same time protect the environment [2, 7, 16-21].

One example of programmed cell death like response of the plant to stress is the aerenchyma formation, a specialized tissue in cortical root cells located between the endoderm and hypodermis. The formation of the aerenchyma is a response to limiting the access of oxygen to the roots (hypoxic stress). This response leads to the removal of cortical cells from the root and forming a space that facilitates the movement of oxygen in the roots [4, 5, 9].

Another example of programmed plant cell death is the host plant's resistance to pathogens, translated by the rapid death of the host cell. This genetically programmed response is a consequence of new transcription and translation processes and is given by some incompatible interactions between disease-resistant plants and non-virulent pathogens. The whole process is controlled by resistance genes (R), which respond to pathogens that carry specific avirulence genes (Avr). In the absence of the R allele in the host and Avr in the pathogen, the disease sets in because the plant does not recognize the pathogen and takes place a compatibility reaction [9].

Host response is an active process that requires transcription and translation. Peptides and oligosaccharides produced by the pathogen or as a result of the pathogen-host interaction applied to a plant with have resistance genes induce some biochemical and cellular responses. However, cell death in a resistant host is not necessarily the result of direct toxic effects.

Many scientific results show that in plants, the response of cells to the pathogen is apoptosis [4, 9, 10, 15]. Although programmed cell death in plants has some similarities to apoptosis in animals, it still has important differences (Figure 2), as plants do not have an immune system and do not contain phagocytes, and the plant-specific cell wall prevents the formation of apoptotic bodies [4]. It is also considered that in plants apoptotic corpuscles would be functionally irrelevant, as their phagocytosis by adjacent cells would not be possible in the presence of cell walls [9].

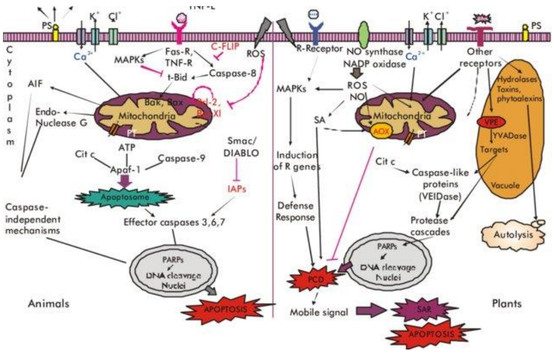

Fig. 2. Main mechanisms for programmed cell death in plants and animals
Source: [4].

Although some regulators of programmed cell death observed in animal systems have been identified in plants, the corresponding genes in plants have not yet been isolated. This may suggest that plants have developed other ways to control host resistance to various stressors and have also developed their own ways of regulating cell death processes that differ from those in animals.

Plant cells subjected to cell death synthesize self-destructing substances, which are placed in the vacuole, and rupture of the vacuole causes cell death. The condition of the cells can be monitored with fluorescein diacetate (FDA). The presence of fluorescence in cells shows that they are alive, and the absence of the phenomenon indicate cell death. If fluorescence is missing and condensation of the cytoplasm is present, it means that the cell has been subjected to cell death. In some in vitro experiences with animal tumour cells were identified near-infrared fluorescent carboxylate cyanine (NIRF) [24].

The cells at the root tips, which come from the initial cells of the meristem moved to the periphery by the newly formed cells, protect the apical meristem during seed germination and seedling growth. These peripheral cells die after a few days and this death is part of the normal development of the plant. Cells that die from the root tips shrink and take on different profiles [13].

Programmed cell death occurs in most plant tissue types and is involved in numerous processes, from seeds germination to plant senescence. Thus, when the seed germinates, hydrolytic enzymes are secreted from the aleuronic layer and the starch endosperm is degraded while the cells of the aleuronic layer remain alive. The programmed death of the cells of the aleuronic layer takes place gradually during germination, by progressive vacuolation, followed by the death and collapse of the protoplast. The cells of the aleuronic layer die via autolysis only after the starch reserves have been mobilized.

During the vegetative growth of plants and other structures or processes are accompanied or come by programmed cell death. Some of the most important plant development processes in which programmed cell death is involved are: embryo formation, degeneration of aleuronic layer, formation of root aerenchyma, anthers degeneration, pollen self-incompatibility, remodelling the leaves shape, leaf senescence, etc.

Sclerenchyma cells are dead and cell walls are thickened to perform mechanical function. The bark is made up of specific cells with a suberified cell wall to protect the internal tissues from dehydration (the protoplast of these cells is removed). The growth of the stem is also accompanied by cell death, the division of the cells in the cambium causing the death of the cells in the bark layer [15].

By the death of some sub-epidermal cells from the surface of the citrus fruits, are formed some cavities in which the essential oils are stored. The mechanism by which differentiation of secretory canals and cavities in which volatile oils are collected, found in many plant species, is called lysogeny [6]. Programmed cell death has also been observed in suspended embryogenic cultures in some plant species [12]. Totipotent cells divide asymmetrically and form pairs of cell one of which develop into a somatic embryo, while in the other it occurs the stop of DNA synthesis and her death.

Cell death also accompanies a number of processes specific to the reproductive development of plants, such as sex determination, gamete formation, fertilization and embryogenesis. Cell death occurs in various tissues and reproductive organs and sometimes even these organs are subject to extinction [8].

By programmed elimination of excessed cells in the processes of morphogenesis, cell death is involved in remodelling the shape of the plant leaves (Figure 3).

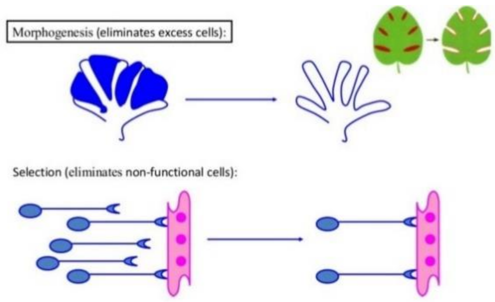

Fig. 3. Programmed leaf remodelling in morphogenesis processes and programmed elimination of non-functional cells in embryogenesis
Source: [14].

In the early stages of flower development in unisexual plants, the flower contains primordia for both male and female sexual organs. Subsequently, either the male or female organs are removed by programmed cell death. Abortion of stamens or carpels primordia is a type of programmed cell death that ensures the formation of unisexual flowers. In maize, for example, a monoecious plant, the male inflorescence is spatially separated from the female inflorescence. The young flowers in the panicle contain both the primordia of the stamens and the gynoecium, the latter stopping their development, due to the phenomenon of increased vacuolation of cells and loss of organettes.

Programmed cell death also occurs in the dehiscence of anthers, which ensures the release of pollen from pollen sacs. The dehiscence of the anther occurs by the degeneration of the tissue between the stoma and the connective tissue of the pollen sacs. Several cell types are involved in this process, being associated with increased activity of a cysteine protease [9]. In the tomato’s anthers (Figure 4), along with the death of the group of cells under the stoma, there is also the degeneration of the protoplast of the epidermal cells adjacent to the stoma, endothecia and connective tissue [23].

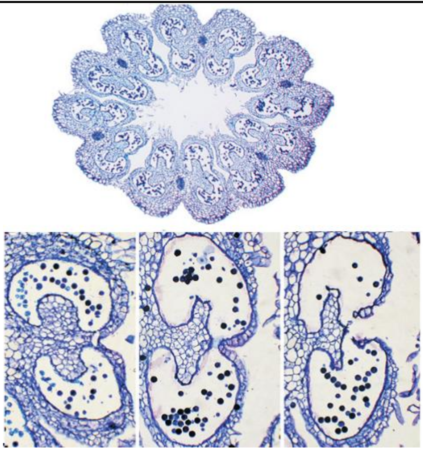

Fig. 4. Tomato anthers and a progressive loss of cell layers of the connective tissue
Source: [23].

Exposure of plants to various stressors such as hypoxia, water deficit, UV radiation, salinity, extreme temperatures, pollutants, toxins, heavy metals, etc., can induce oxidative stress and can be followed by cell death. Plants have developed mechanisms to counteract or tolerate adverse environmental conditions to a certain extent which, if exceeded, can lead to the death of a part of the plant or its death in its entirety. However, cell death in plants under conditions of abiotic stress can also be a regulated process, meant to ensure their survival. For example, the adaptation of plants to drought or intense lighting is often accompanied by the covering of their surface with a protective layer of dead single-celled hairs, which come from programmed cell death [11]. Any organism includes a series of polarizations and correlations. In plants, the genetic information responsible for form and function during ontogenesis creates a network of stresses in tissues, organs, cells, cytoplasm, chromosomes, etc. Tensions at various levels are interdependent, and an intervention in a certain point in organism can have a quantitative or qualitative consequence at another point, through the cell polarization phenomenon. Although there are obvious differences between animal and plant cells, some phenomena that accompany cell death in the cells of the two regna are similar, evidence of their descent from a common ancestor [9]. In plants, programmed cell death has specific features due to the cell wall, in particular. The uniqueness of some features of cell death in plants also results from the presence of other structures specific to the plant cell, such as chloroplasts and vacuole. In most situations of cell death in plants, the cell wall is preserved after degradation of the protoplast and reuse of its components. In the case of the hypersensitive response, cell death is accompanied by organ destruction, membrane collapse plasma and its separation from the cell wall [8]. The phenomenon of programmed cell death in plants is as important as cell division. This aspect of plant life is directly involved in the response to pathogens or stressors. The laws of in vitro cell variability are not fully known and therefore, many results are variable and even unpredictable. But future research in the field of biology and genetic engineering will certainly elucidate these unknowns.

CONCLUSIONS

Cell death is a complex phenomenon that accompanies plant growth and development from the time of seed germination to the maturation and senescence of plants. Exposure of plants to various stressors induces oxidative stress and may be followed by cell death. Genetic bioengineering and agricultural biotechnologies, through cell cultures, are a model system for studying programmed cell death in plants. In embryogenic phytovitrocultures, totipotent cells are divided asymmetrically and form pairs of cells, one of which develops into a somatic embryo, while the other involves the interruption of DNA synthesis and the occurrence of cell death. Programmed cell death plays an essential role in morphological modelling of the plant. The elucidation by researchers of all the phenomena specific to cell death in plants may bring in the future new arguments regarding the existence of unique mechanisms in the living world for the realization of certain processes and functions. Also, the deeper knowledge of the structure of matter and genetic processes in plants is the basis on which one can consciously act to direct these processes in order to modern plant improvement and obtain new genotypes with improved characteristics.

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