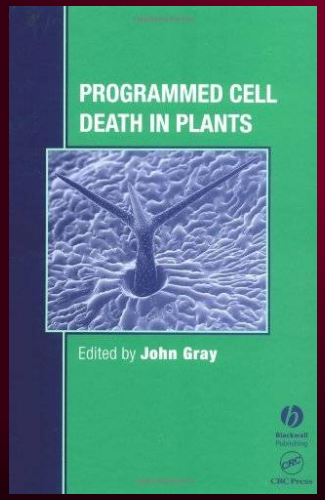
9) Senescence and programmed cell death (PCD)

a) Type of programmed cell death
b) PCD in plant life cycle
c) Senescence and plant hormones
d) Developmental PCD
e) PCD and plant responses to stress



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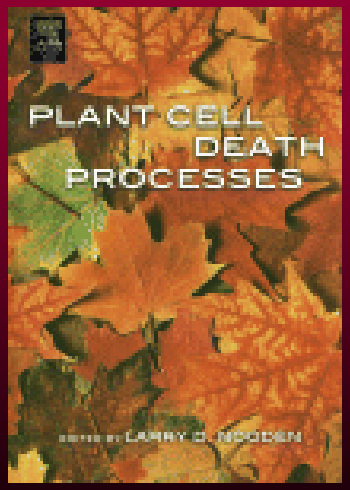


2004

2011

Shanker A (2011) Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives, InTech, Rijeka, Croatis

Programmed death - Programmed Cell Death (PCD) – is essential part of the growth and development of eukaryotic organisms and Their responses to stress

Organism itself controls initiation and process of death => „programmed death“

Examples of PCD in plants:

- **Cell death associated with hypersensitive response**
- **senescence**

a) Type of programmed cell death

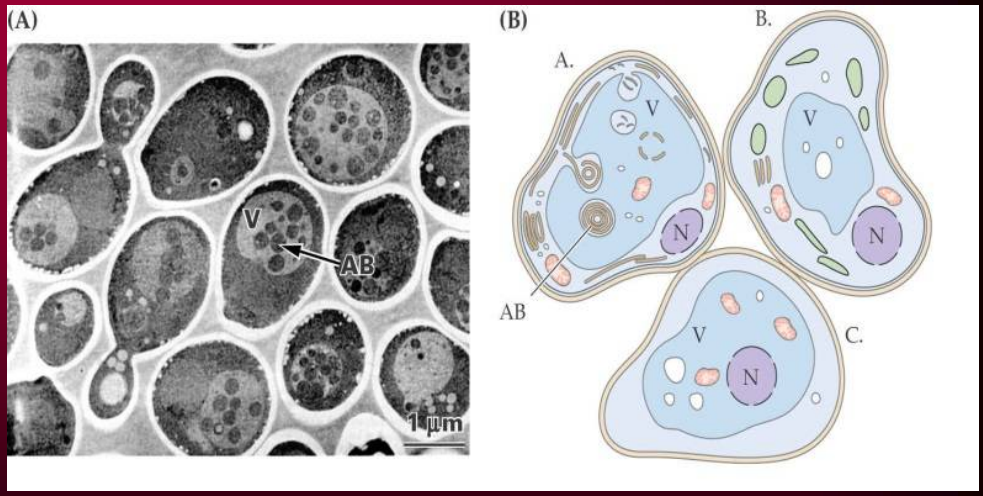
PCD in plants differs from PCD in animals.

Animals – apoptic cell is absorbed by phagocytosis
Plants – plant cell does not use phagocytosis (cell wall; absence of phagocytes)

Autophagy = process, by which plants lose their cytoplasm

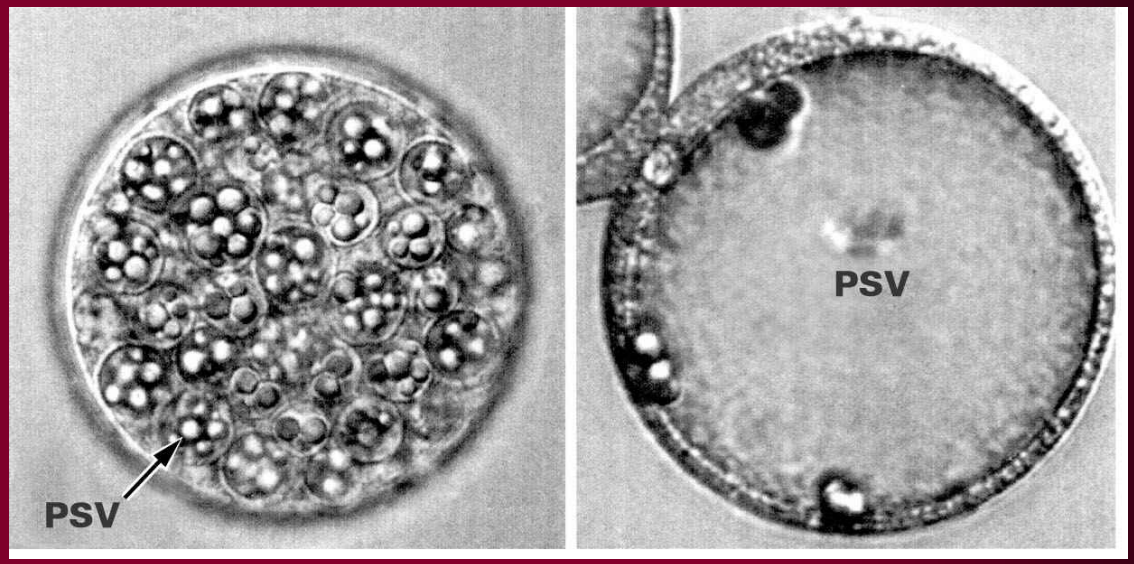
1. Autophagozomes (AB) = vesicles, which absorb a part of cytosol

Autophaagosomes are absorbed by central vacuole (V) and digested by hydrolytic enzymes



Saccharomyces Plants (morning glory - svlačec)

2. Autophagy in aleurone layer of cereal grain – small vacuoles accumulating proteins (PSV) fuse with central vacuole. The mechanisms how cell organelles are disposed in the vacuole is not known.



Vacuolar enzymes:

- **Enzymes with caspase 1 activity**

Caspase 1 = cysteine protease; plays a role in apoptosis and cleaves cytokins by proteolysis to their active forms

3. Autophagy at tracheid differentiation – cells at tracheid differentiation die. The vacuole ruptures and hydrolases (proteases, nucleases, phosphatase) spill out and degrade organelles and in the cell.

Autophagy → Changes induction, movement and recognition of signals leading to the PCD

Autophagy → Facilitates degradation of signals leading to PCD

Reactive oxygen
NO – nitric oxide (nitrogen oxide)
} Main signals mediating PCD

Plant specific PCD

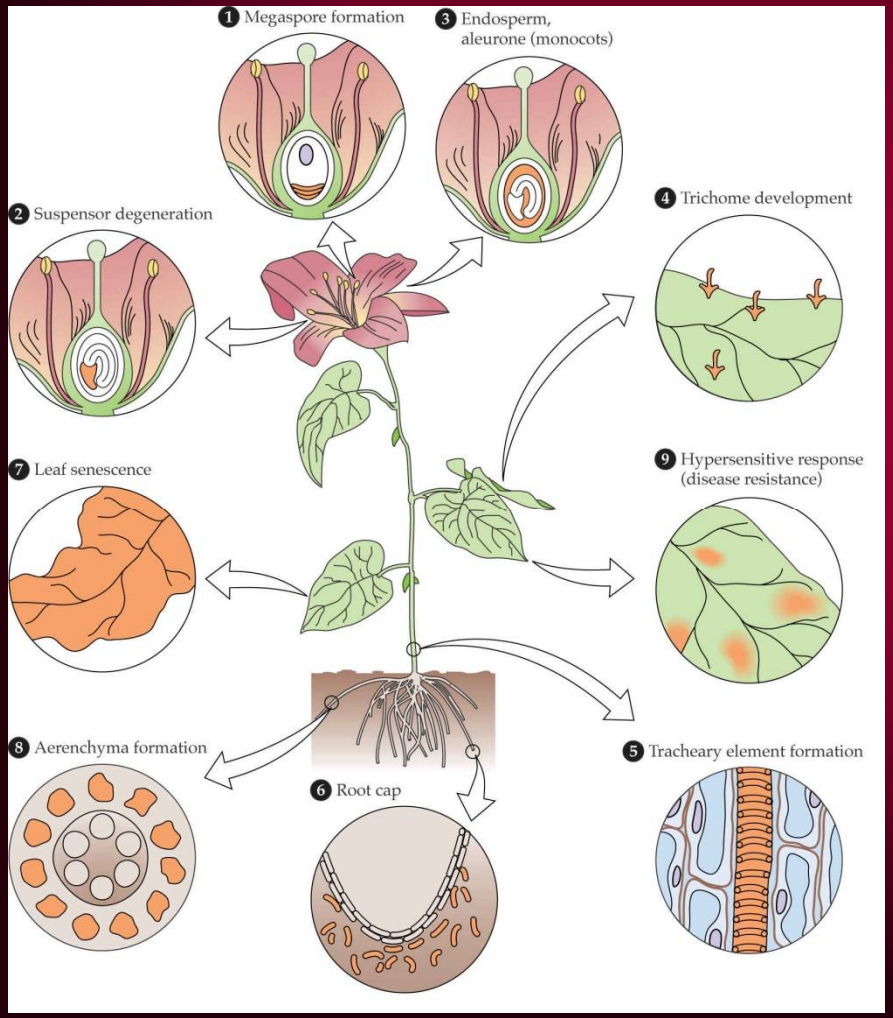
Endosperm formation in cereals – starch endosperm surrounded by aleurone layer

- endosperm accumulates storage material
- during maturation endosperm dies
- dead endosperm cells do not fall apart – they are mummified
- aleurone stays alive
- during germination mummified cells are digested by enzymes from aleurone

PCD = processes leading to PCD + processes of death itself

Plants: processes leading to PCD are **reversible**

b) PCD in plant life cycle



All phases of plant life cycle are influenced by PCD

+

PCD processes take place during responses to pathogen and stress

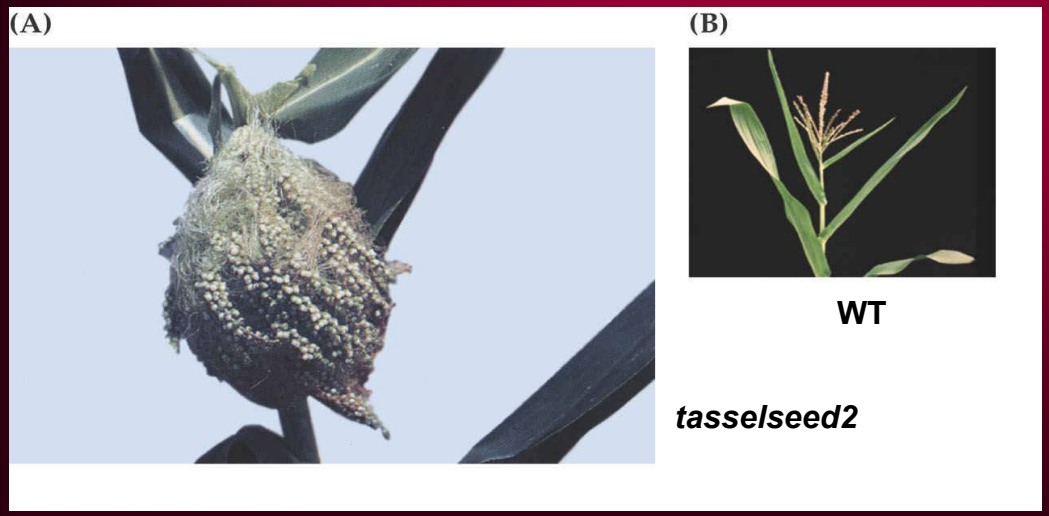
PCD results in developmental plasticity

PCD in reproduction development

Flower development is significantly influenced by PCD – plants with unisexual flowers (maize)

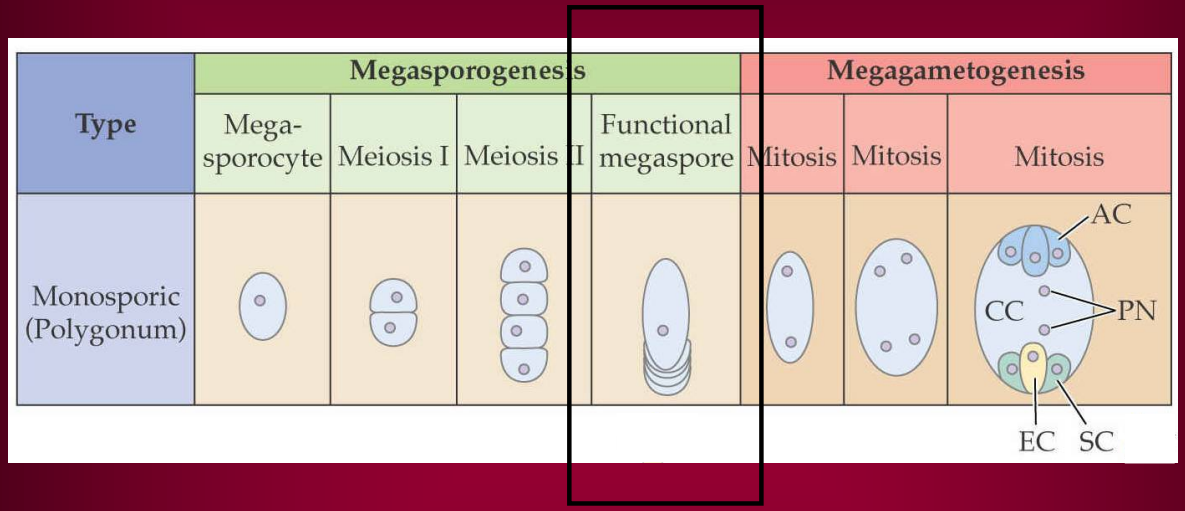
Early stages of flower development – primordia of female (gynoecium) and male (anthers) sexual organs exist in both types of flowers. Other development – primordium of one reproductive organ perishes = PCD

Mutant *tasselseed2* – in the tassel gynoecium develops



Gene *TASSELSEED2* controls PCD of gynoecium in the tassel

Development of female gametophyte (megaspore) – from immature egg embryonic sac develops. During development, 3 from 4 cells die = PCD



Microsporogenesis (pollen development) – tapetal cells die, content of cytoplasm (proteins, lipids) is deposited on the surface of pollen grain; death of tapetum = PCD

Embryo development – zygote divides in 2 cells; one cell gives development of embryo, second cell gives development of suspensor; suspensor perishes after embryo formation = PCD

PCD in vegetative development

Growth of embryo – before germination, growth of embryo is mechanically limited by endosperm cells; once endosperm cells die, embryo can grow;
death of endosperm cells = PCD

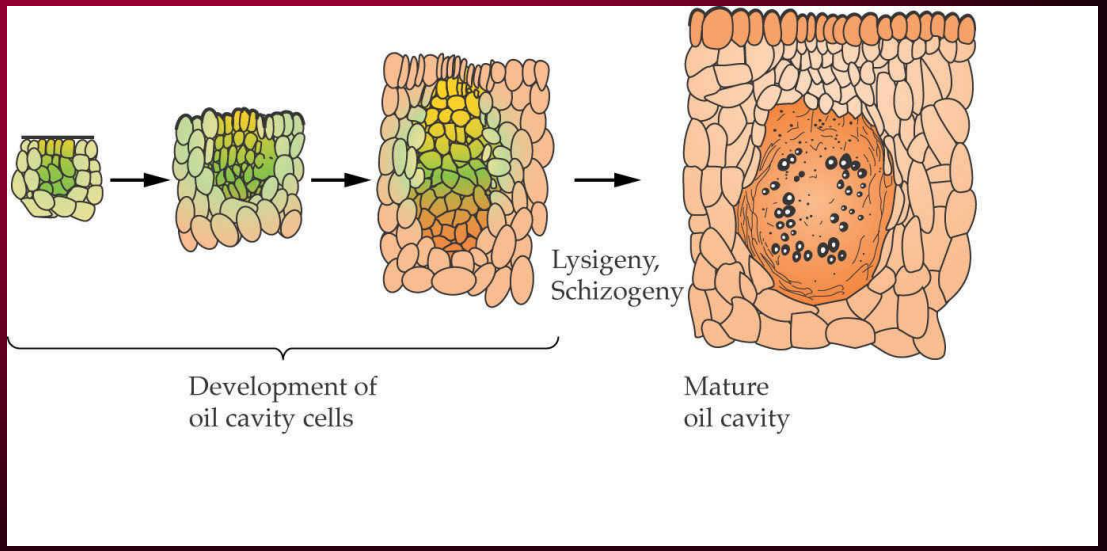
Differentiation of xylem tracheids – live tracheal cells have no conductive function; cytoplasm of tracheid elements dies and is removed; dead cells with only secondary cell wall function as tracheids;
death of treacheid elements = PCD

Organ formation – death of cells in some parts of leaves gives rise of typical shape of leaves in *Monstera* (Swiss cheese plant);
death of leaf cells = PCD

Monstera (*Monstera deliciosa*)

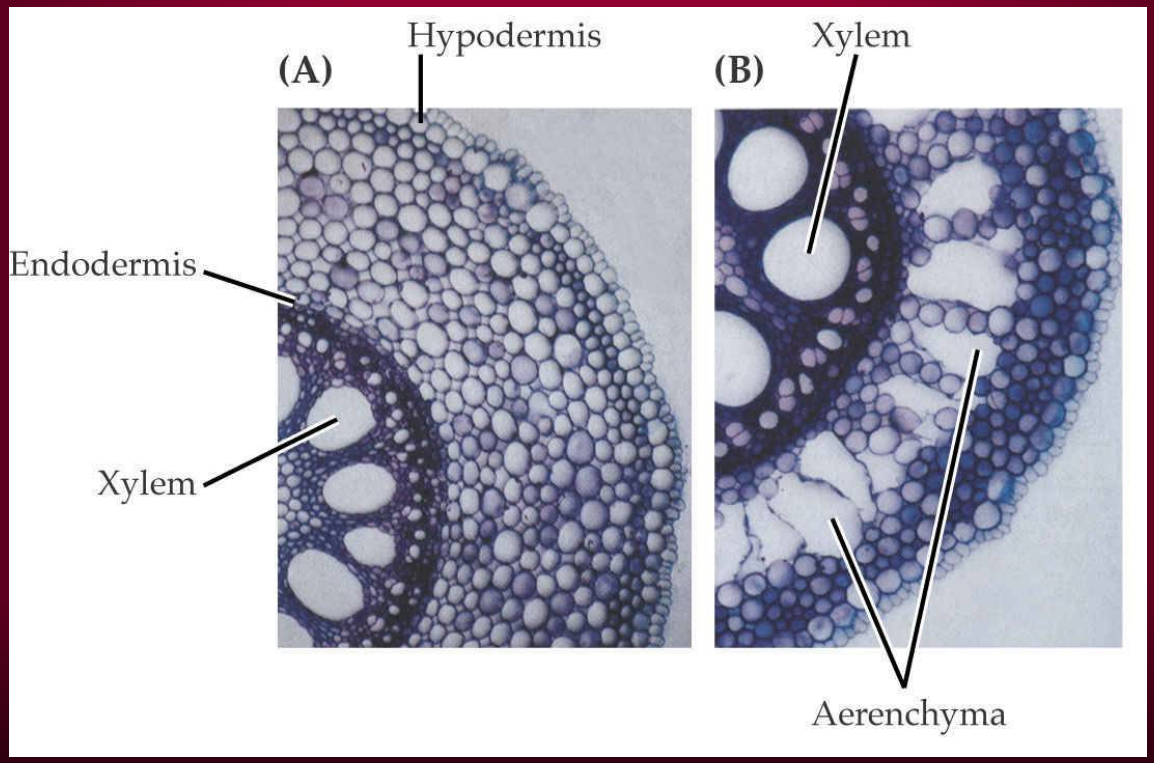
Formation of trichomes, thorns, etc. – green stems of cactus are replaced by leaves, which are reduced to thorns;
leaf reduction = PCD

Formation of glands on the surface of fruits - cells on the surface of fruits die = PCD; dead cells are full of essences and oil = lysigeny (schizogeny);

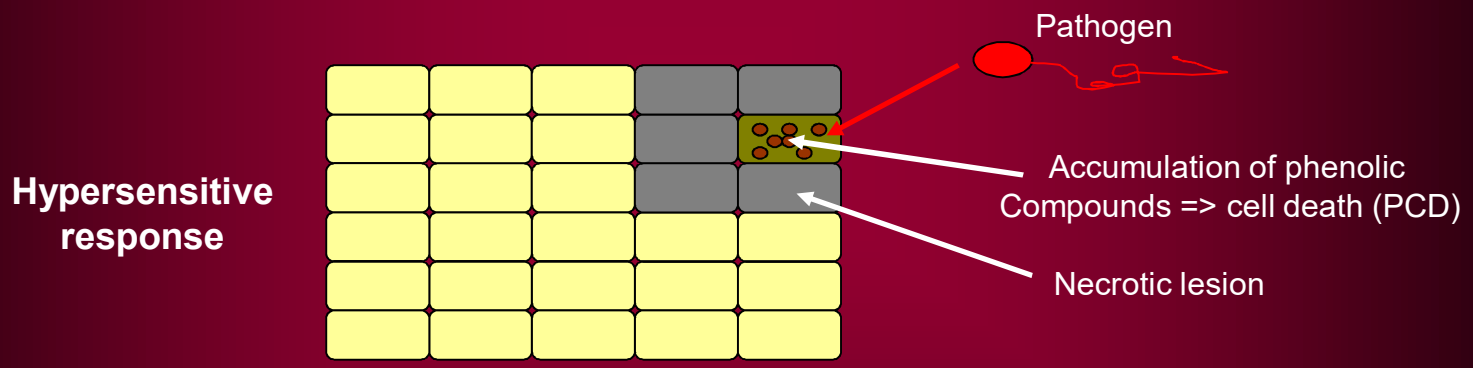


PCD as a part of plant responses to stress

Aerenchym formation – plants exposed to oxygen deficit (hypoxia) => cell wall and protoplast of root cells die = PCD; formation of channels for transport of air from stem.



Hypersensitive response – plant response to pathogen infection; host cell and surrounding cells die quickly - **PCD** => protection of other cells from infection

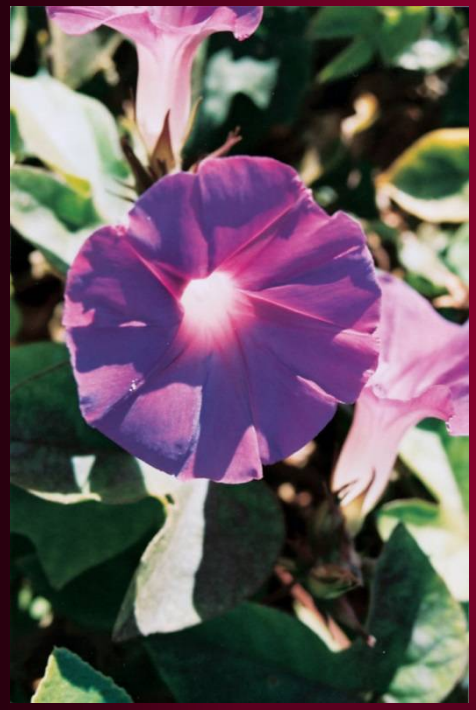


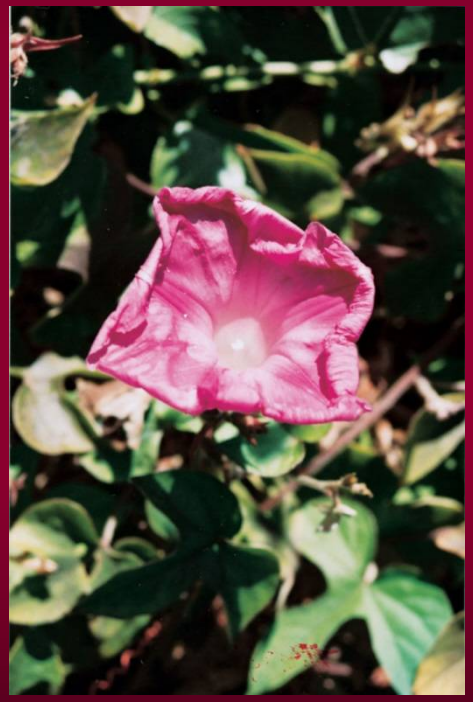
Senescence – example of PCD regulated by external factors

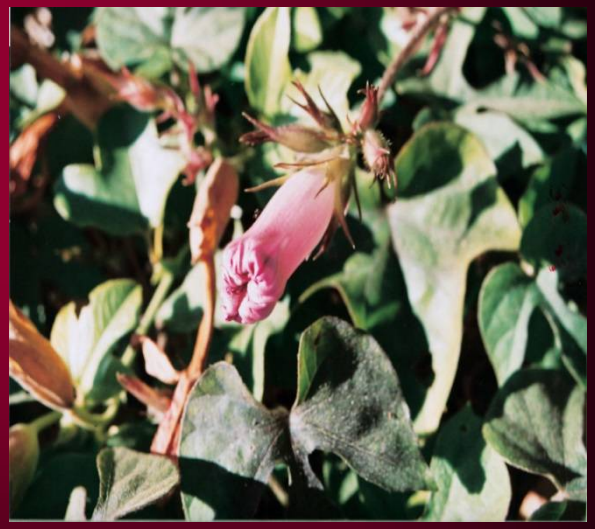
Senescence and death are final phases of development of all organs.

Senescence - natural, energy-dependent process controlled by own genetic program of the plant. However, senescence is dramatically regulated by external factors (day length, temperature)

Fast senescence - senescence of flower organs – during one day: flower opening 5.00 hrs, afternoon closure, change of color and shape, senescence and dying.







Povíjnice (*Ipomoea tricolor*)

Slow senescence - leaves (needle) of pine *Pinus longaeva* are replaced after 45 years

Mechanism of integration of senescence programs in development and life of organs or whole plants is not known.

Hypothesis „die now“ – signal „die now“ is continuously present – cells, tissues, organs respond to it in the moment when their individual program gives the command.

Signal „die now“ of particular cells can induce senescence in other cells.

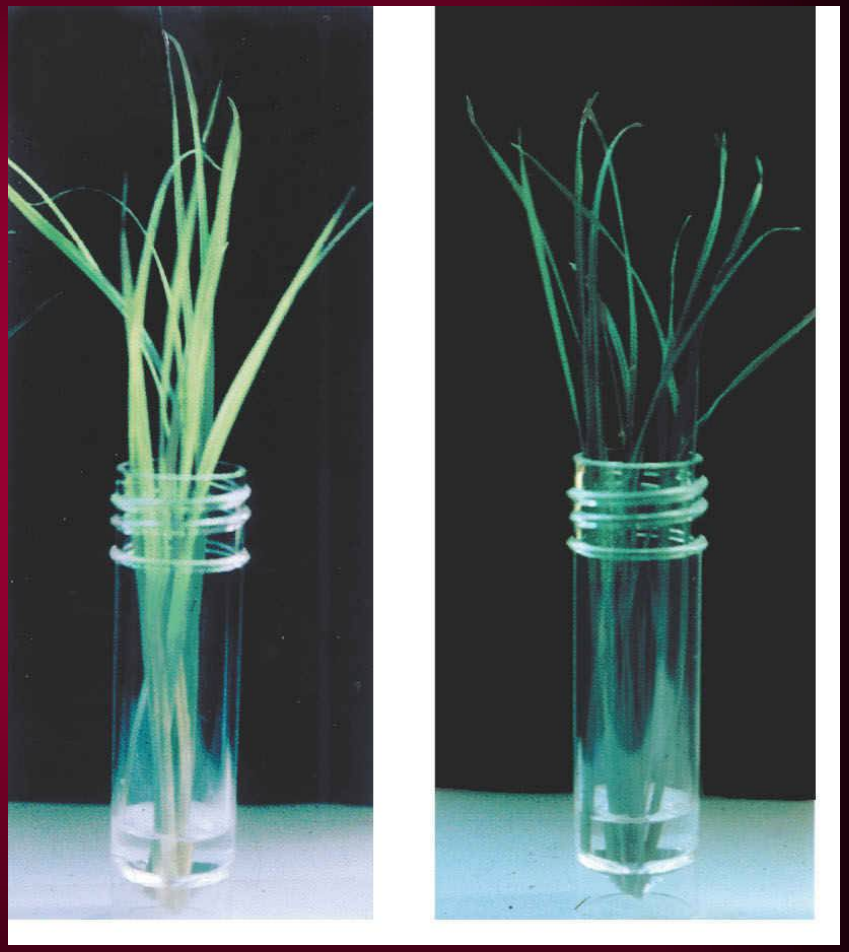
Phenotypes of genetic variants, hybrids or mutants:

- „stay green“
- necrotic
- diseased

Mutation in genes, which regulate timing or localization of normal senescence or PCD

Analysis of mutants: revealing of processes controlling senescence or PCD

cad1 **– *constitutively activated cell death 1*;**
phenotype similar to injury typical for hypersensitive response; 32x increased level of salicylic acid;
codes protein involved in immune responses of animals



WT ***Stay-green***

Senescence is highly regulated process – three basic phases:

Initiation phase

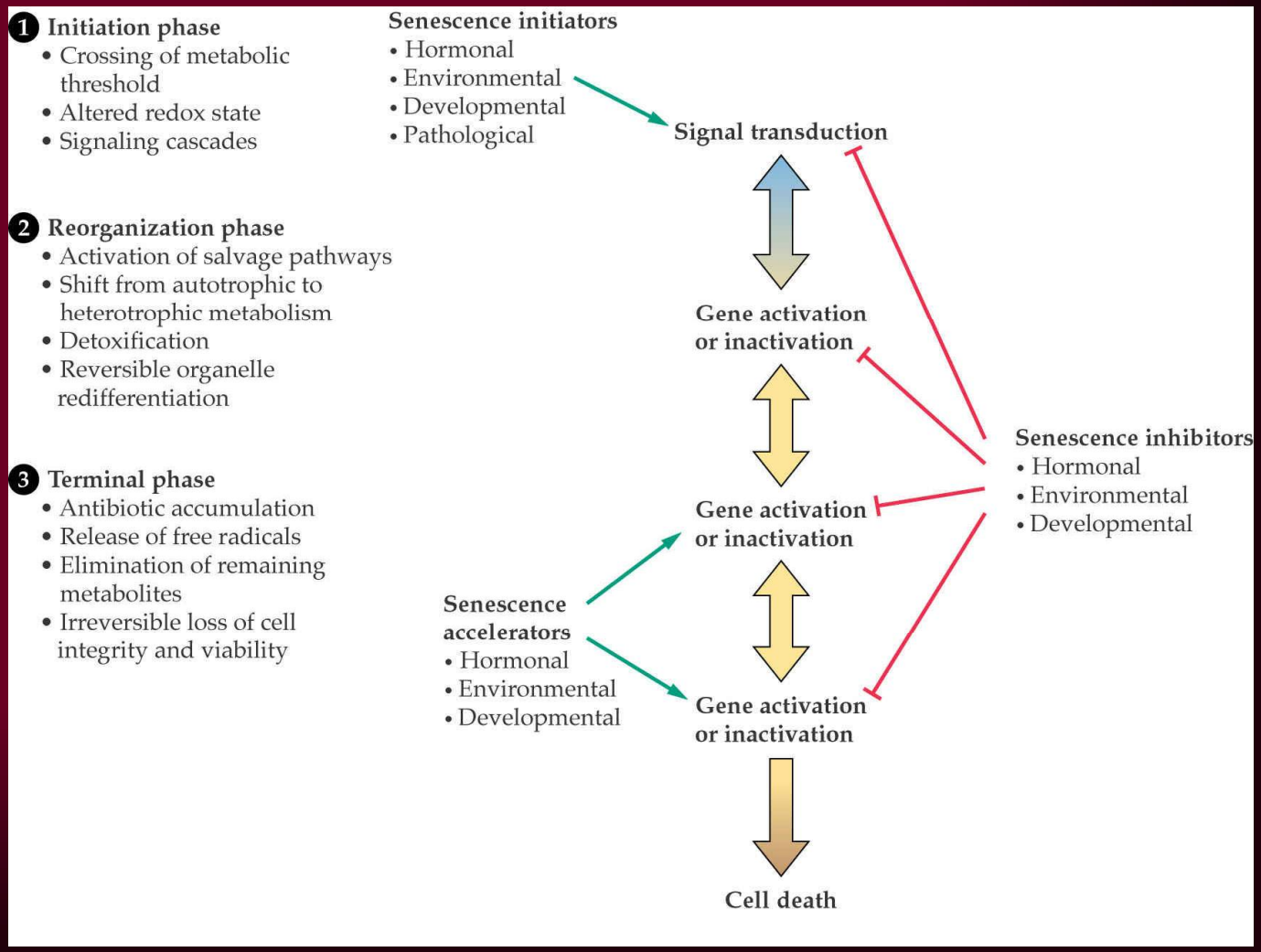
Activation and inactivation of genes

Reorganization phase

Re-differentiation of cellular structures and remobilization of material

Terminal phase

Initiation of irreversible processes

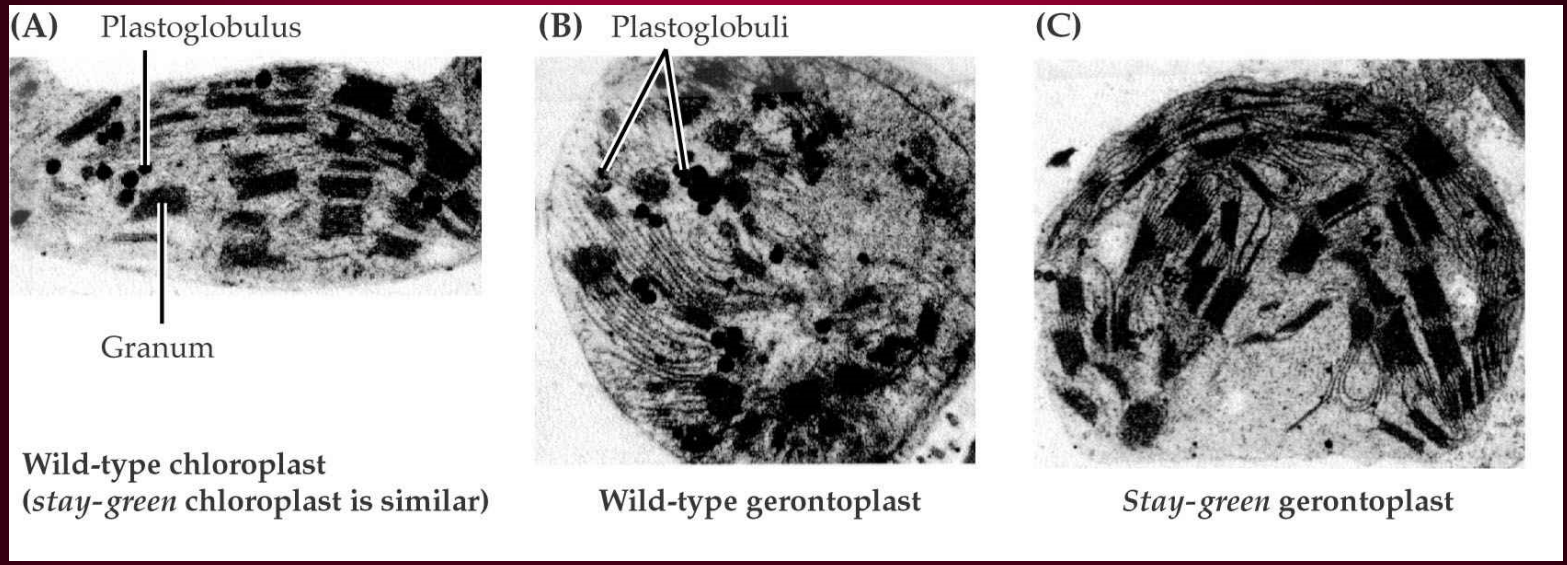


Senescence of leaves and fruits is characterized by dramatic changes of main organelles, specifically of plastids in mesophyll cells and parenchyma of fruit pericarp.

Chloroplasts → Gerontoplasts

Grana decay, increasing number of plastoglobuli

Grana are preserved, plastoglobuli are not present

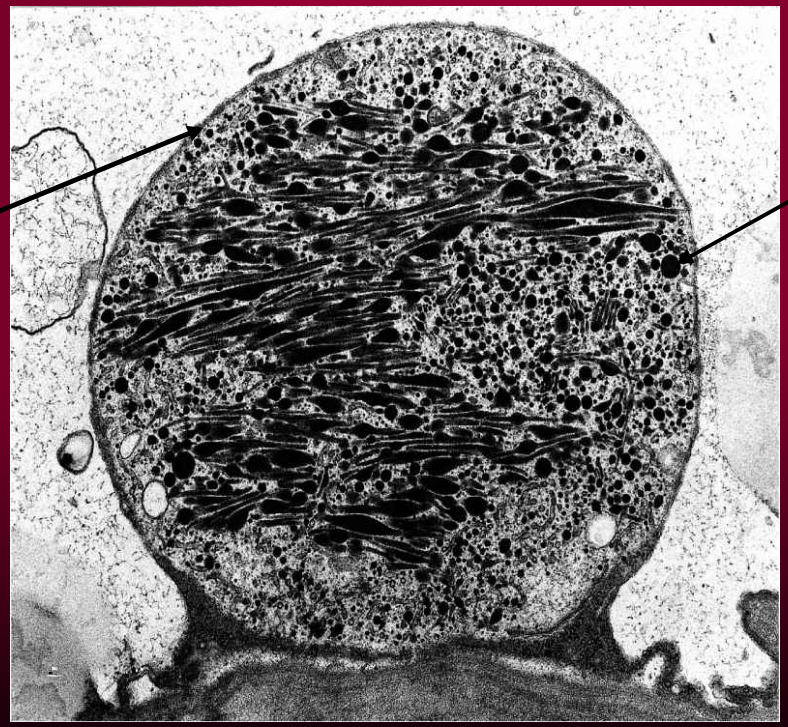


WT **Stay green**

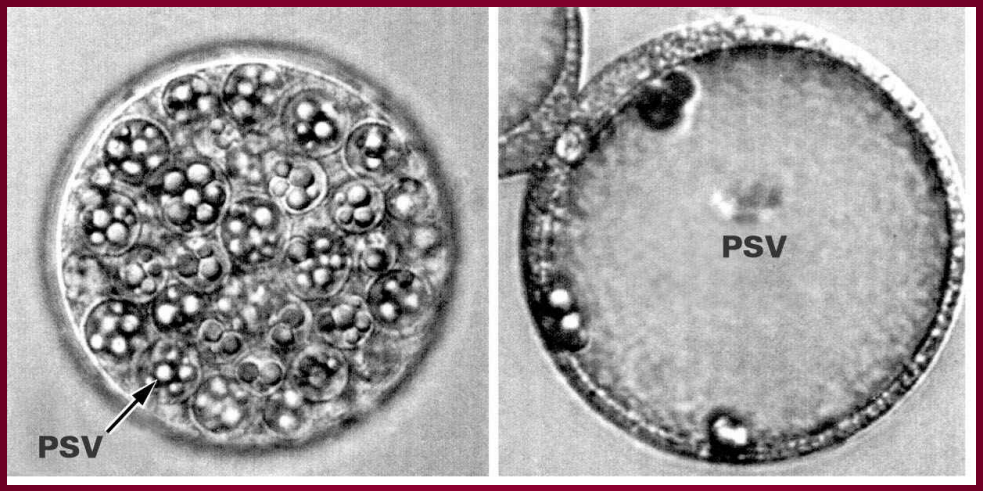
Gooseberry
(mochyně)

Chloroplasts → Chromoplasts

Chromoplasts of gooseberry – cell has very thin cytoplasm; whole cell is full of plastids containing carotenoids



Cotyledons and endosperm are reservoir of proteins – during senescence small vacuoles are changed from reservoir organelles To big central vacuole



Cotyledons and endosperm function as reservoir of lipids as well. Lipids are collected in organelles- oleosomes

During senescence glyoxysomes are formed – play a role In gluconeogenesis = formation of sugars from lipids

The changes in cellular compartmentalization provide evidence for high organization of senescence process

Activation of specific genes controlling predetermined cell events

Decay of organelles: **First:** chloroplasts (thylakoid proteins, stromatal enzymes)

The last: nuclei

Senescence-down-regulated genes (*SDGs*) – genes, which are repressed during senescence (proteins involved in photosynthesis)

Senescence-associated genes (*SAGs*) – genes, which are activated during senescence (hydrolytic enzymes – proteases, ribonucleases, lipases, chloroplast degrading enzymes…)

Classification of *SAG* based on functional activity of proteins, which code for:

1) Genes coding proteolytic enzymes – three types of cysteine proteases:

   a) enzymes inducing cereal germination
   b) enzymes similar to papain = enzyme from papaya
   c) enzymes modifying proteins

2) Genes coding components of proteolytic system (aspartic proteases, ubiquitin)

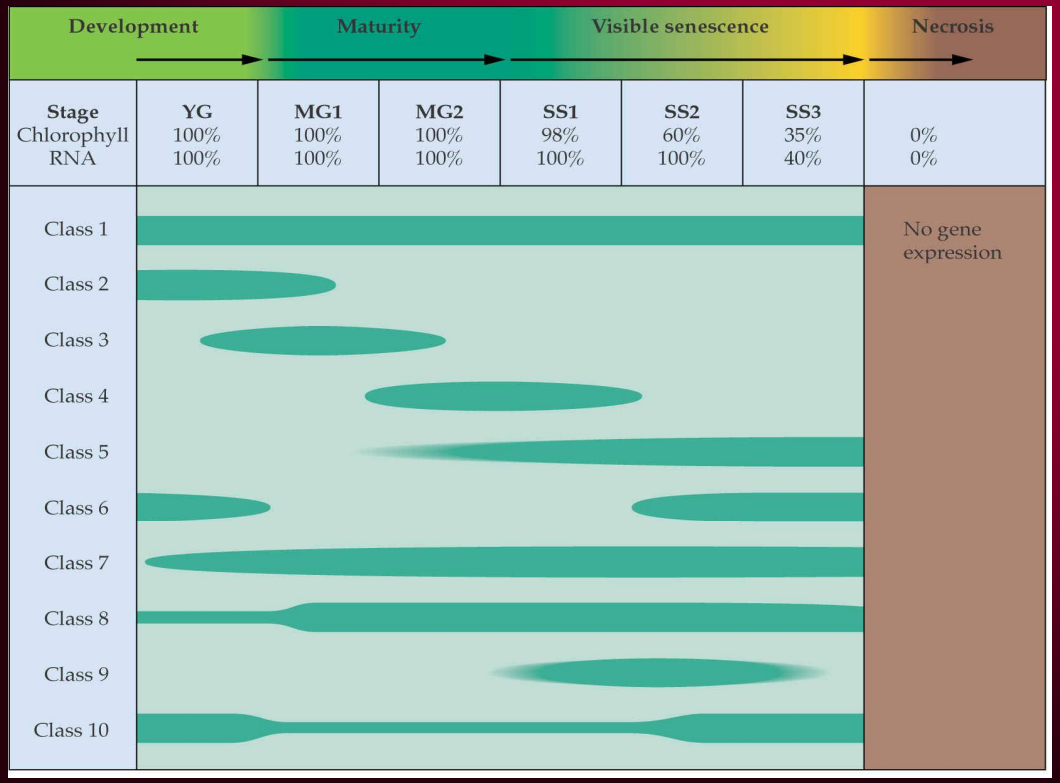
3) Genes coding proteins involved in plant defense against pathogens – antifungal proteins, chitinases, pathogenesis-related proteins

4) Genes coding proteins, which protect cell against oxidative damage induced by metal ions

Variability in *SAG* expression – genes expressed:

- in different stages of senescence
- only in aging or in opposite only not aging organs
- only in specific organs
- by influence of stress, hormones (ABA, ethylene), by deficit of carbohydrates

Stages of leaf senescence in *Brassica napus*



YG – fully developed leaves

MG1 – leaves from flowering plants

MG2 – leaves from plants forming siliques

SS1 – leaves with 98% of chlorophyll

SS2 – leaves with 60% of chlorophyll

SS3 – leaves with 35% of chlorophyll

Mutants in genes involved in senescence

- genes regulating initiation of whole senescence program = genes functioning at the beginning of senescence signaling pathways

- genes coding individual enzymes of metabolic pathways = genes functioning later in signaling pathway

Gregor Mendel – study of pea senescence – gene *I* (previously *B*) – regulates degree of cotyledon greening

Mutant in gene *I* has defect in enzyme (PaO), which digest chlorophyll. It shows delayed senescence.

Tomato mutant in gene *GREENFLESH* – expressed in leaves and fruits (presence of chlorophyll in maturating fruits)

Mutants in „*stay-green*“ – blockade In activity of enzymes, which degrade chlorophyll

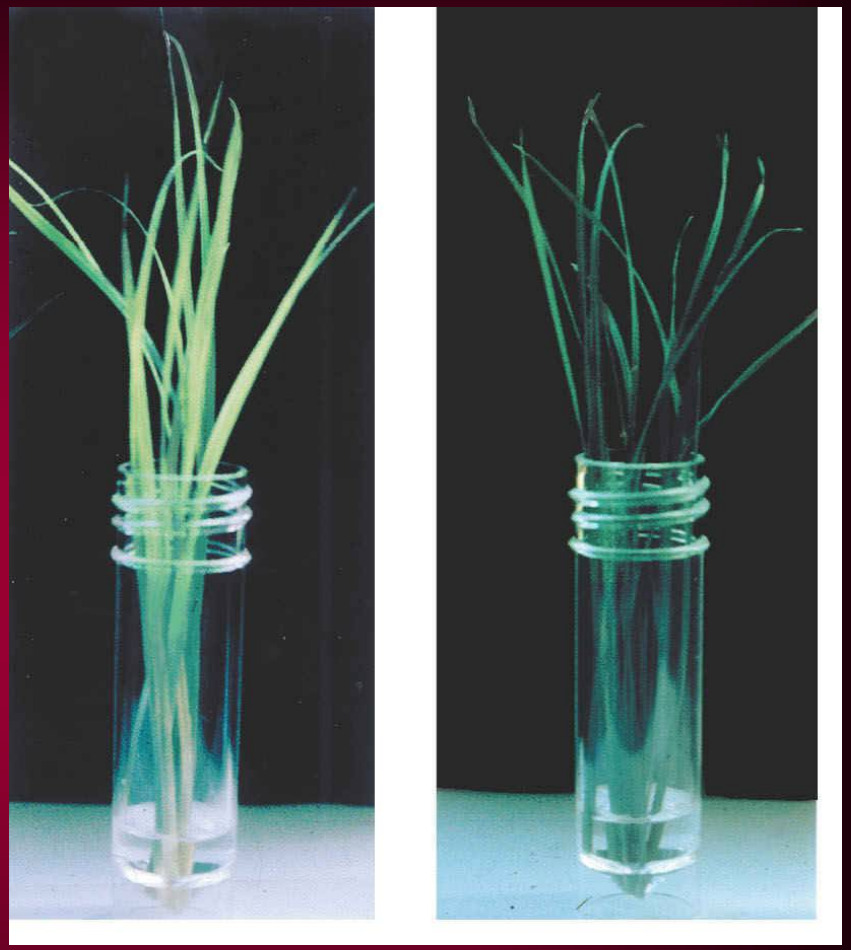
⇩

Plants are green for a longer time

„Stay-green“ cereals – economic importance

⇩

1985 record yield of maize in Illinois (24 thousands kg/ha) – *stay-green* line



WT *Stay-green*

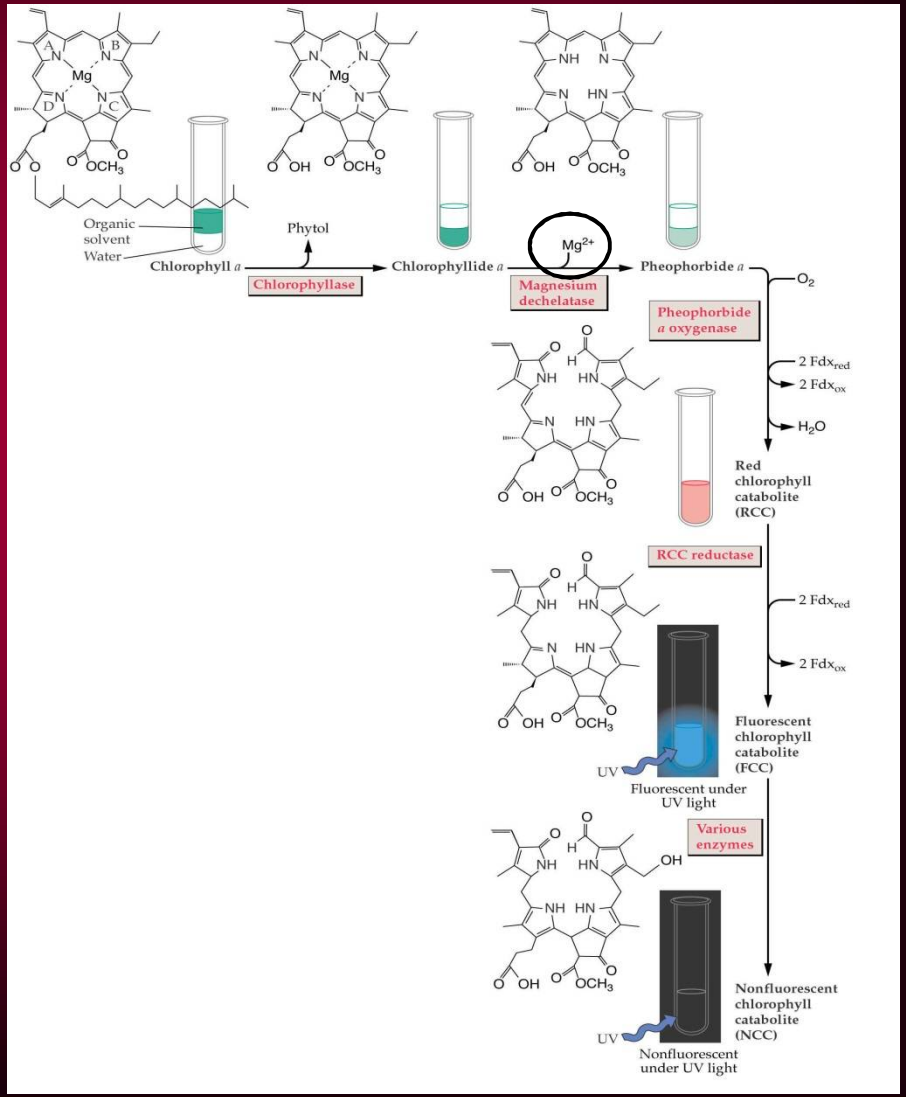
Stay-green lines are important in developing countries

Analysis of stay-green mutant *sid* (in grasses) resulted in identification of biochemical pathway controlling chlorophyll degradation.

Chlorophyll degradation is complex process involving complex enzymatic pathway and taking place in several subcellular parts.

Critical points:

- enzymatic removing of Mg^{2+}
- opening of circle and rise of colorless tatrapyrrole



Loss of chlorophyll is associated with decreasing or increasing of content of carotenoids, in dependence on plant species.

Chlorophyll degradation

↓

Exposing carotenoid layer (yellow-orange pigment)

↓

Color combination in fall leaves

Ougham H et al. (2008) New Phytologist 179: 9-13

Question: Why leaves color not only in winter but also in summer.

Conference: „Origin and evolution of autumn colors“, Oxford, March 2008. Topic – significance of leaf coloring for a plant

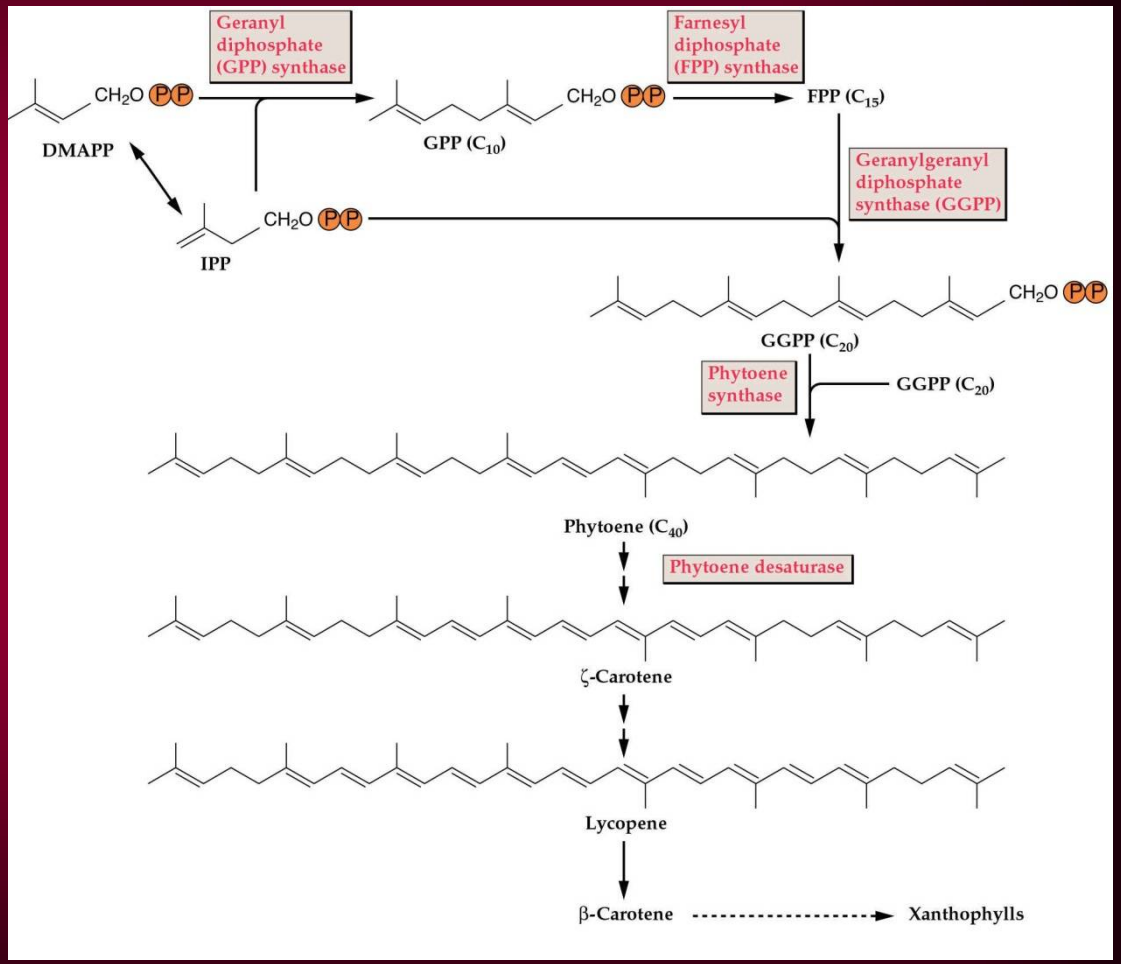
Anthocyanin functions: - physiological (photoprotective, antioxidative, supply)
- signaling – yellow color, but not red one, attracts aphids

Analysis of mutants with abnormal colored fruits or leaves

Important genes cloned playing role in carotenoid biosynthesis

Xanthophylls: zeaxanthin

Receptor of blue light mediating stomata opening



c) Senescence and plant hormones

Factors

External Internal → Senescence

Plant hormones:

Ethylene (CH_2-CH_2)

Cytokinins

ABA, GA

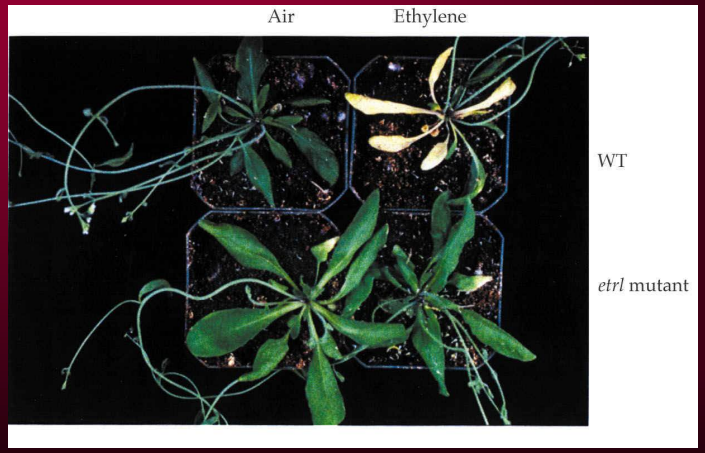
1) Hormone interaction

2) Different plant responses to the same hormone

3) Interaction of hormones with external and other internal factors (plant age)

Ethylene (gaseous hormone) – stimulates senescence

- reduction of leaf growth and induction of yellowing
- reduction of expression of genes associated with photosynthesis
- expression of *SAG*



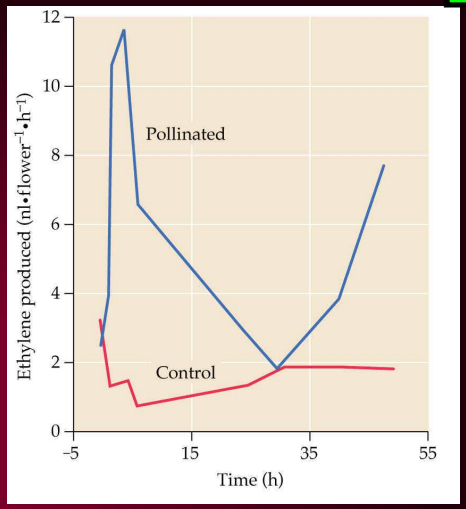
[Ethylene] ↑ → leaf senescence

petal senescence after pollination →

fruit senescence - maturation

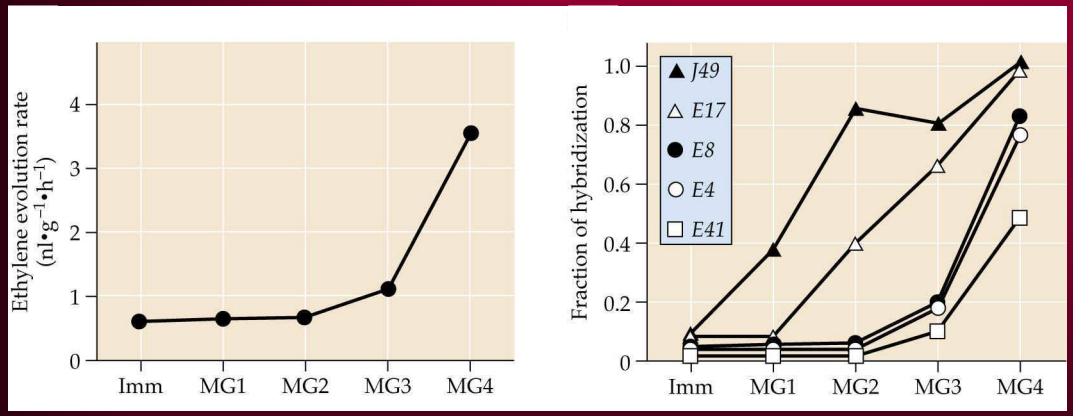
↓

Model plant - tomato



Petunia

Ethylene production → Expression of genes associated with fruit maturation



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Change of color, texture and fruit taste

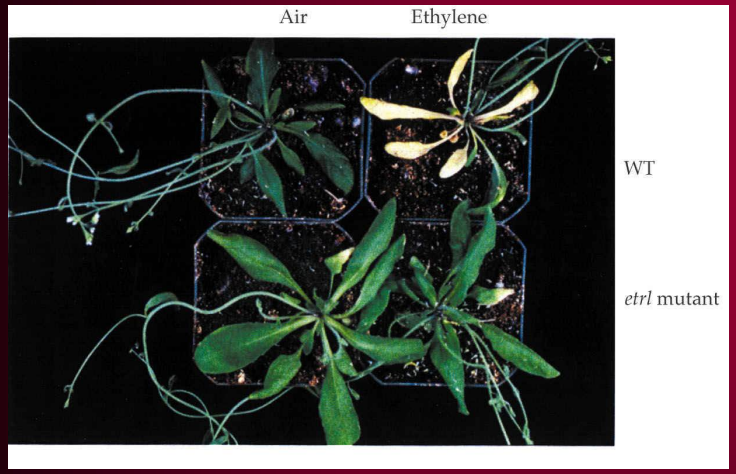
Stages of fruit maturation: Imm – immature
MG – mature green

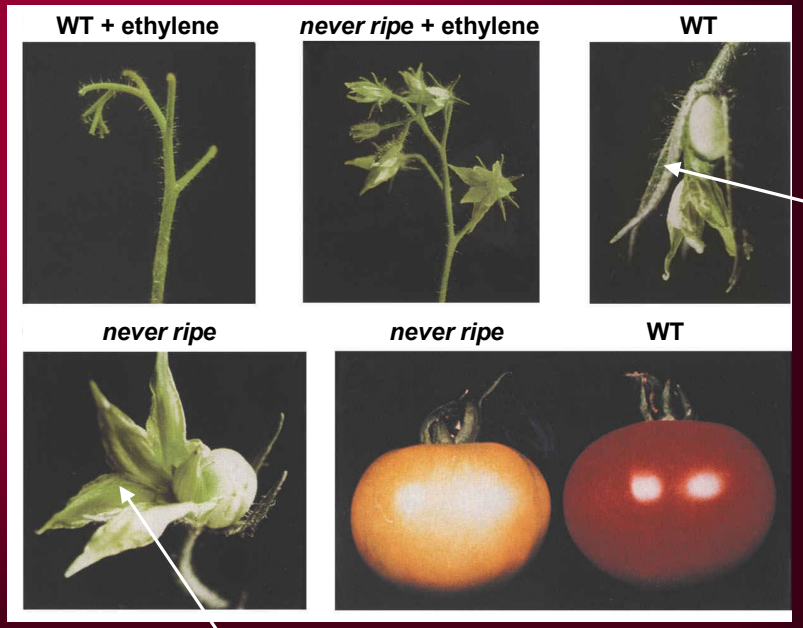
Plants with changes in ethylene receptor show changes in senescence

Arabidopsis mutant – *etr1* – insensitive to ethylene; *ETR1* code for ethylene receptor

Tomato mutant – *never ripe* – insensitive to ethylene

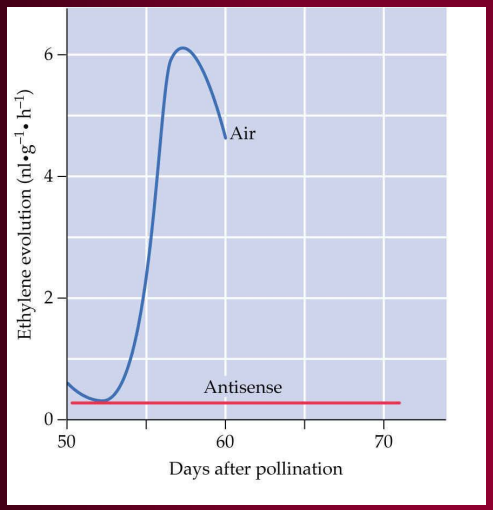
Delayed senescence of fruits and flowers

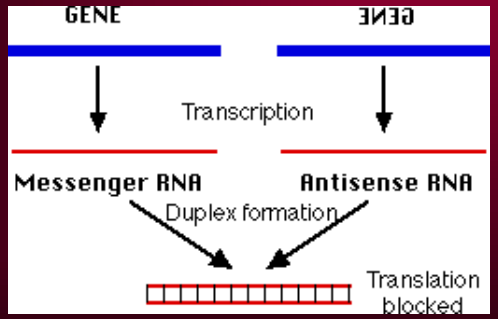


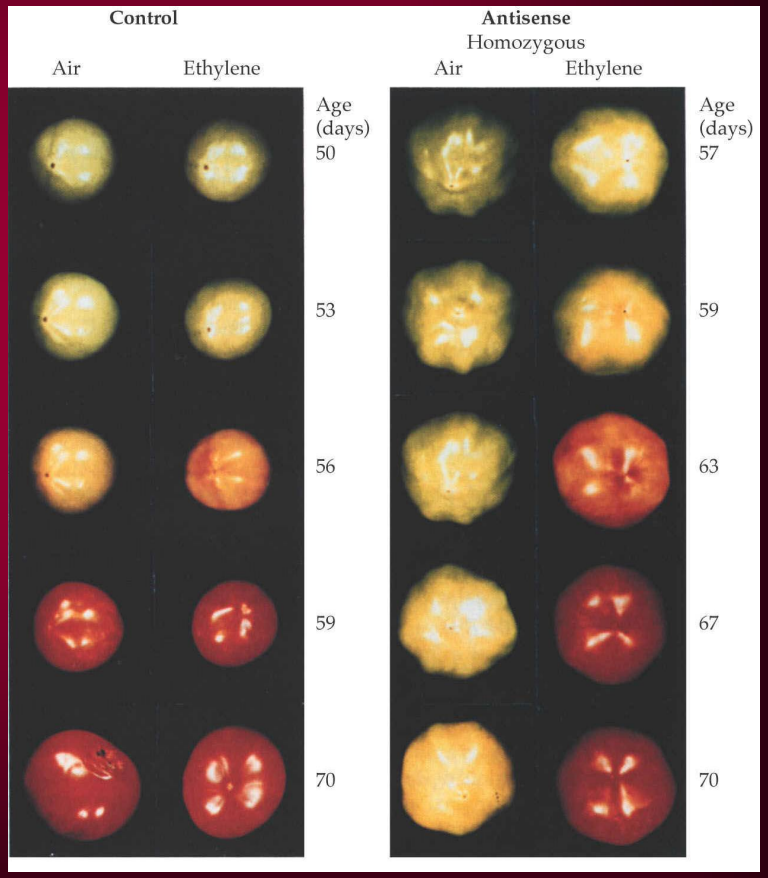


Plants with changes in ethylene biosynthesis

Transgenic plants – tomato expressing antisense genes involved in ethylene biosynthesis => production of enzymes blocked => production of ethylene blocked => poor fruit maturation







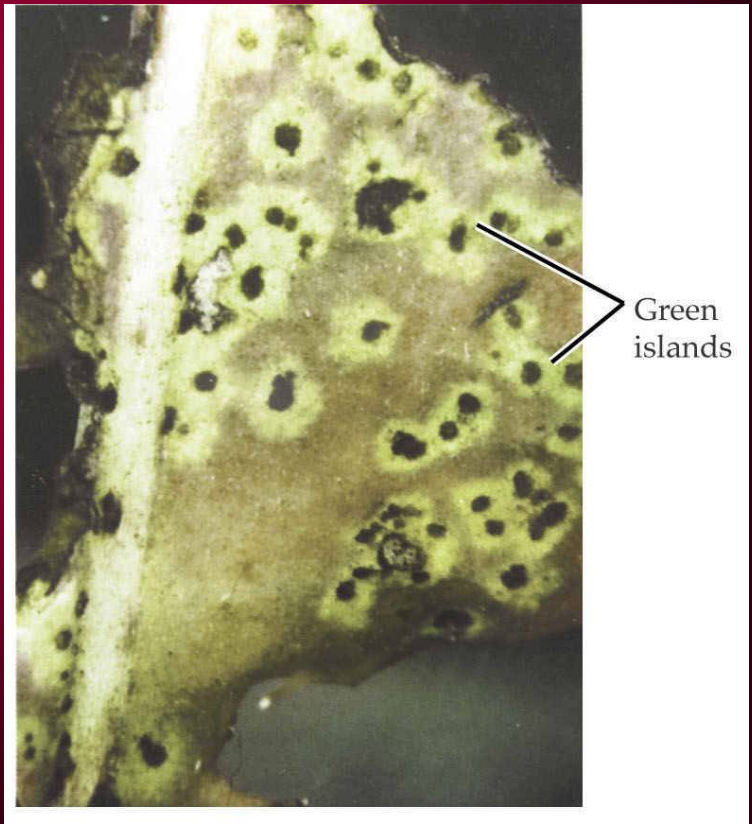
Normal plants = ethylene production

Antisense plants = no ethylene production

Cytokinins – suppresses senescence

- concentration of cytokinins decreases in aging tissues
- Application of cytokinins causes senescence delay

Cytokinins exist in the zone surrounding
The place of pathogen infection => delay
In senescence in the place around pathogen
(ageless zone) => green islands



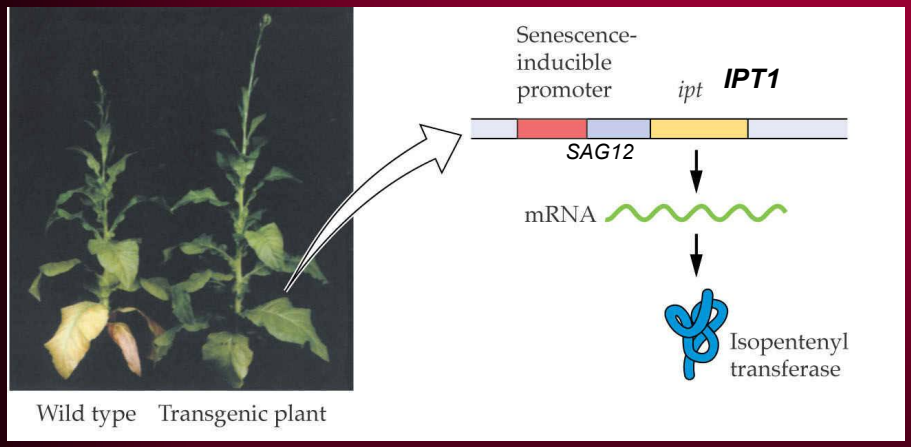
Identification of role of cytokinins in senescence – molecular methods

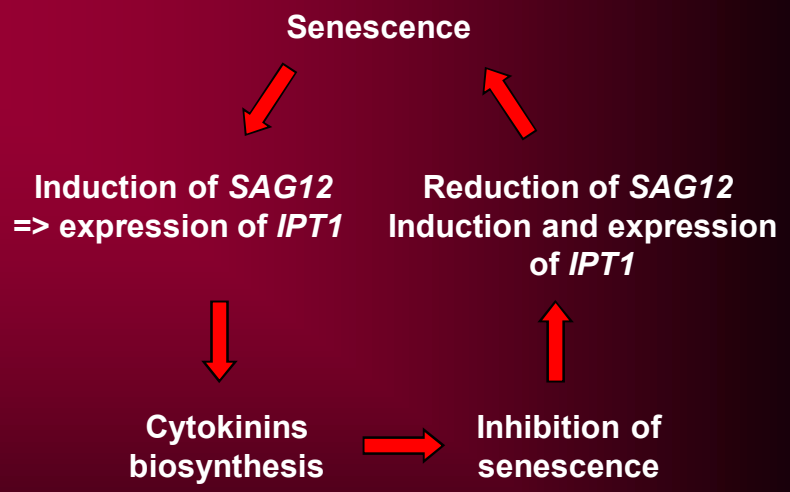
Gene ***IPT*** from *A. tumefaciens* – codes isopentenyl transferase – catalyzes biosynthesis of cytokinins

Transgenic plants containing *IPT* – delayed senescence + additional phenotypes

Difficult to determine, whether delayed senescence is direct consequence of increased level of cytokinins, or consequence of secondary effects

Smart experiment





Production of cytokinins causes delay of senescence → Practical impact

Possible explanation of antisenescence activity of cytokinins

1) Tissue with higher level of cytokinins play a role in metabolite economy of plants => accumulation of nutrition => senescence does not occur

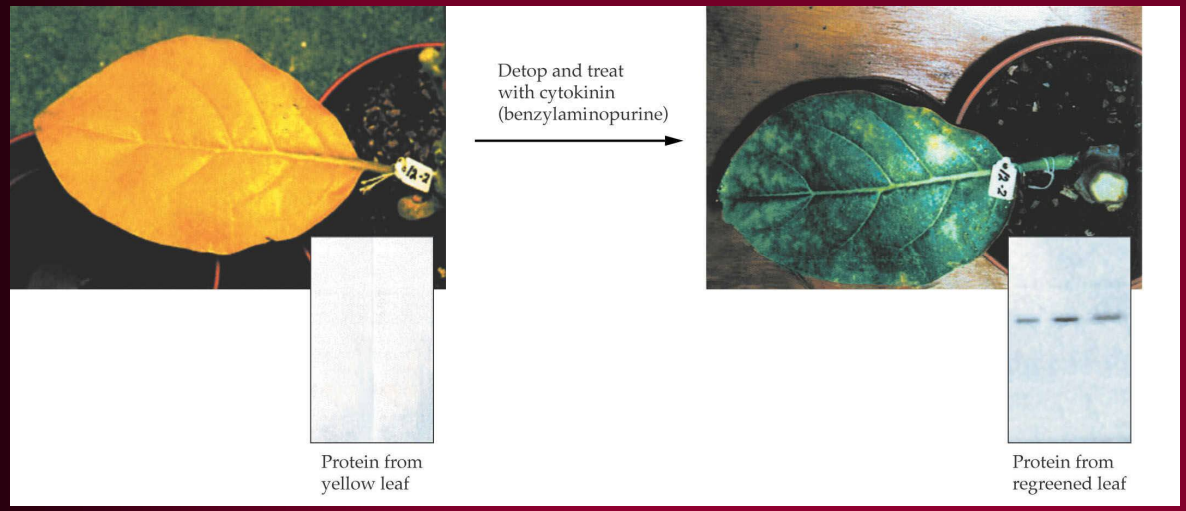
2) Cytokinins can suppress expression of key genes: *SAG*

3) Cytokinins activate transcription of chloroplast genes. Activity of cytokinins depend on light and age of cells and leaves

Duál aktivity of cytokinins:

- action on long distances (stimulation of differentiation and metabolite economy)
- local effect (in aging cells cytokinins suppress senescence)

Regreening = an example of reversibility of senescence in plants



- suppression of *SAG* expression
- activation of genes for plastid formation
- conversion of gerontoplasts to chloroplasts

Role of other hormones in senescence – is not known very well

ABA – mostly stimulates senescence

GA – generally delays senescence

Auxins – stimulates as well as delay senescence

d) Developmental PCD

Xylogenesis (xylem formation) – **1st model example** of developmentally regulated cell death

Xylogenesis:

- initiated during embryogenesis
- continues during life of plant – **differentiation of tracheids** (xylem cells – transport of water to plant)

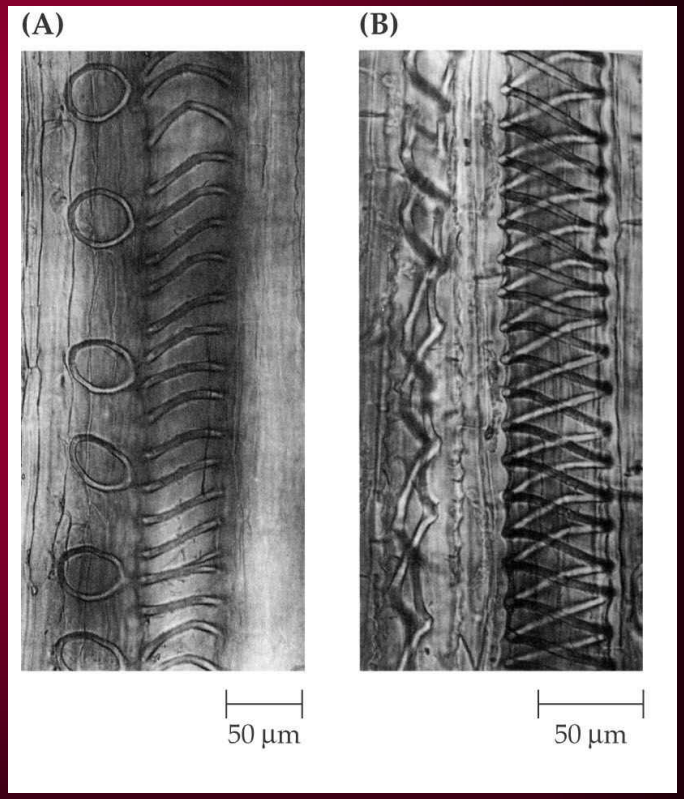
Formation of secondary cell wall (lignification)

↓

Cell death and autolysis of cell content

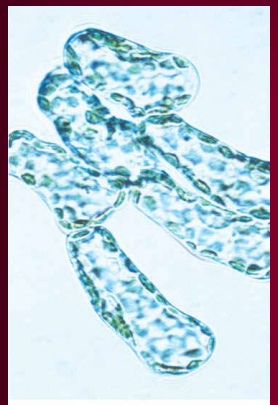
↓

Cell cover = sec. cell wall = **tracheids (TE)**



Tracheids (tracheary elements)

Development of TE from mesophyll cells of *Zinnia*



Isolated mesophyll cells

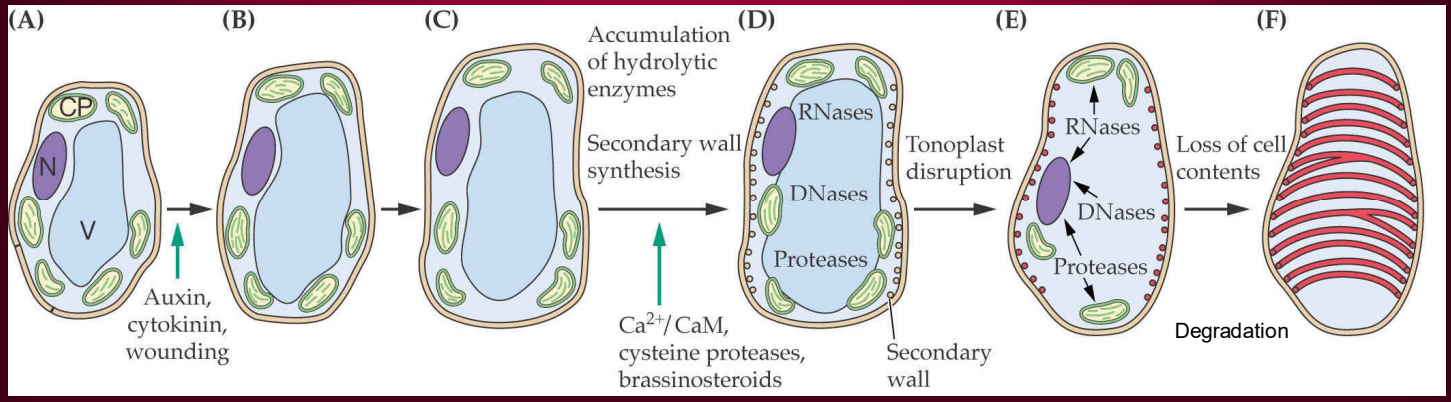
Culture *in vitro*

+ auxin

+ cytokinins

Differentiated tracheary elements

***Zinnia* (Ostálka)**



Induction by hormones **Dedifferentiation** **Precursor TE cell** **Immature TE with sec. cell wall (lignification)** **Tonoplast rupture** **TE = cell tracheids**

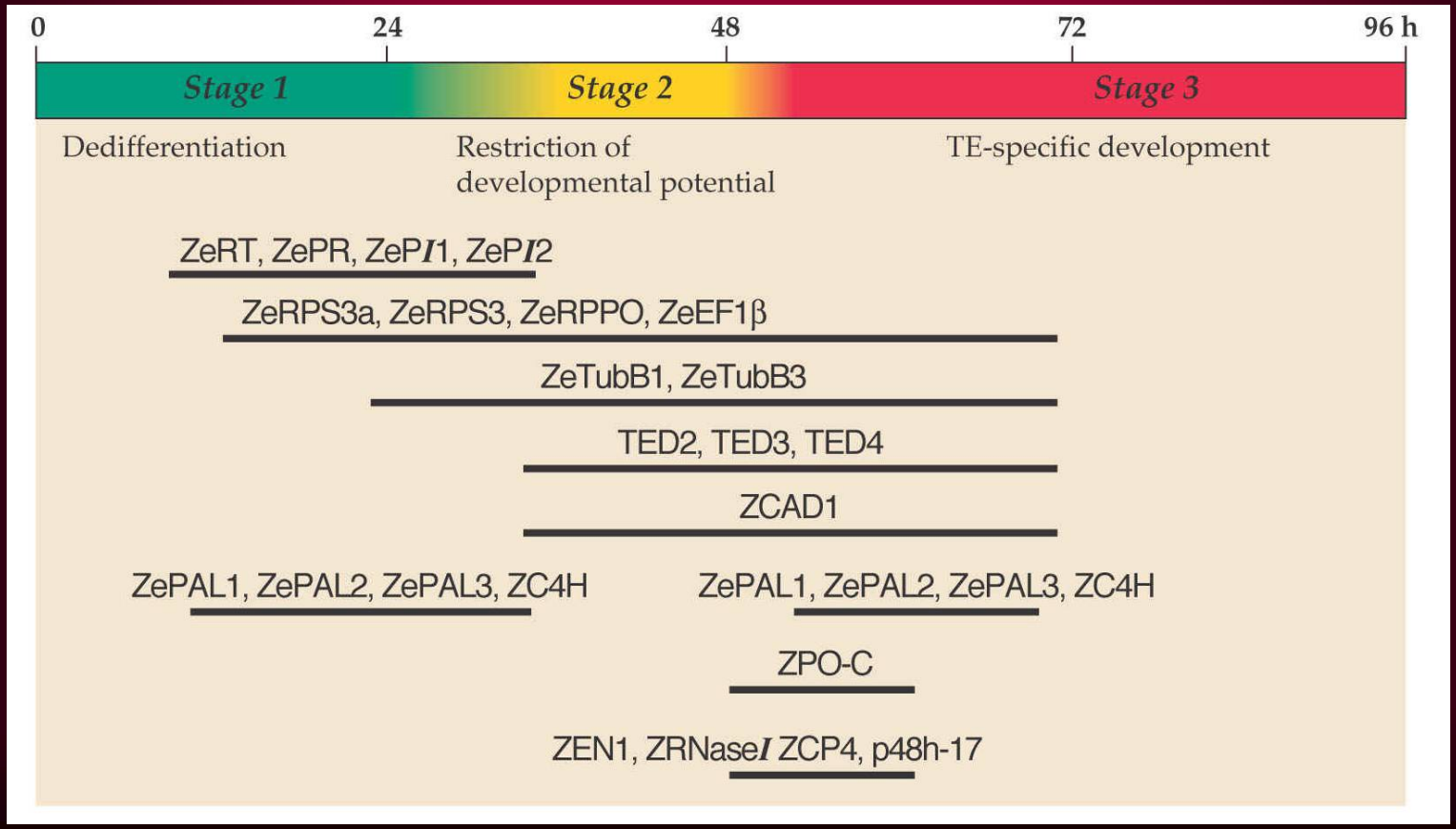
In vitro process of differentiation of TE in cell population happens synchronously

3 phases of tracheid differentiation tracheid ~ 4 days ← Possibility of biochem. and mol. analyses

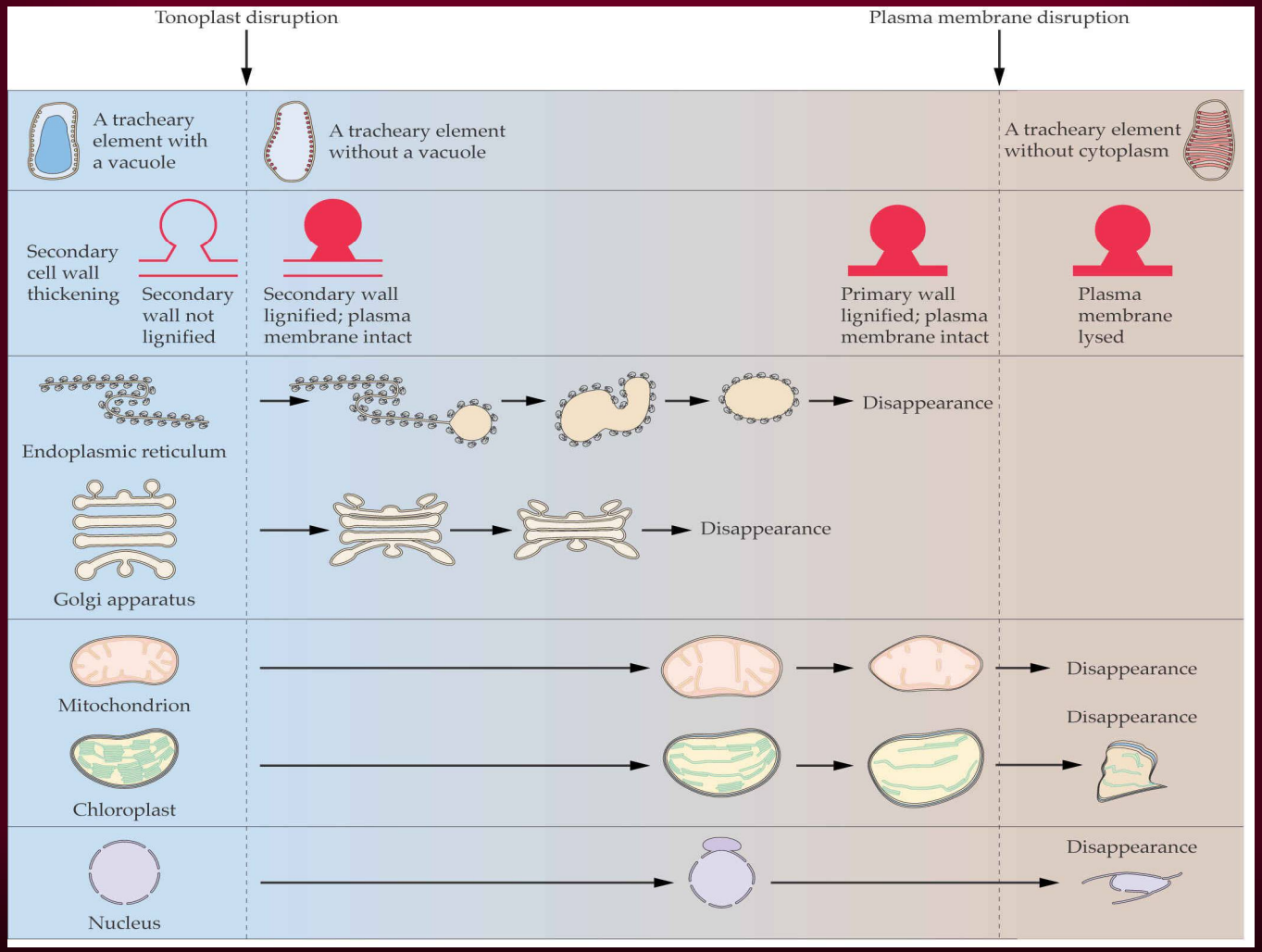
Expression of genes involved in responses to damage (PI - Protease inhibitors) (RP – Ribosomal proteins) (EF – Elongation factors)

Expression of TE differentiation-related genes (TED, unknown function)

Expression of genes participating in synthesis of cytoskeleton components (TUB - tubulin). Activation of genes coding proteins of cell wall (arabinogalactan-like, extensin-like)



Once secondary cell wall is deposited, protoplast degenerates – autolysis: tonoplast ruptures and Lytic vacuolar enzymes (cysteine proteases, nucleases, serine proteases) are released into cytosol. After tonoplast rupture, changes of organelle organization and cell wall are evident.



Regulation of PCD during tracheid formation

Auxins

Cytokinins – role in induction of PCD in plants and animals – 2002; elements of signaling pathway are known very poorly

Recent results – involvement of NO (nitric oxide)

NO – reactive water and lipid soluble gas; involved in many biological processes:

- **stomata closure**
- **seed germination**
- **root development**
- **expression of defense genes**

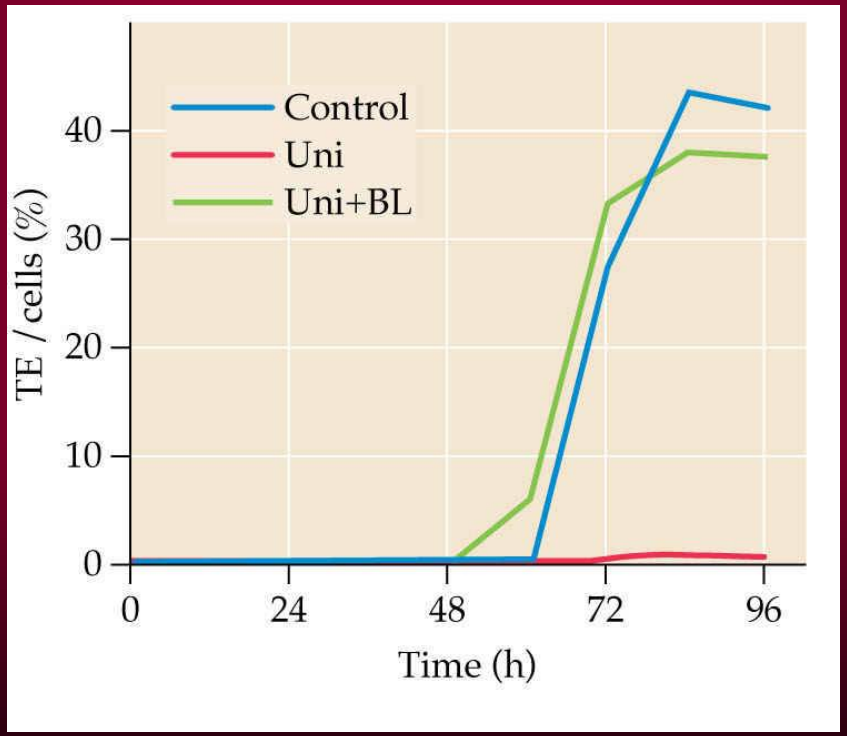
2002 – cytokinins induce formation of NO in *Arabidopsis*, tobacco, parsley, etc.

Cytokinins induce synthesis of NO in xylem cells => inhibition of respiration => => PCD (tracheid formation)

Brassinosteroids

– application of uniconazol (inhibitor of BR synthesis) results in blockage of tracheid differentiation and reduction of expression of late genes

– at simultaneous application of uniconazol and brassinosteroids, effect of uniconazol is suppressed



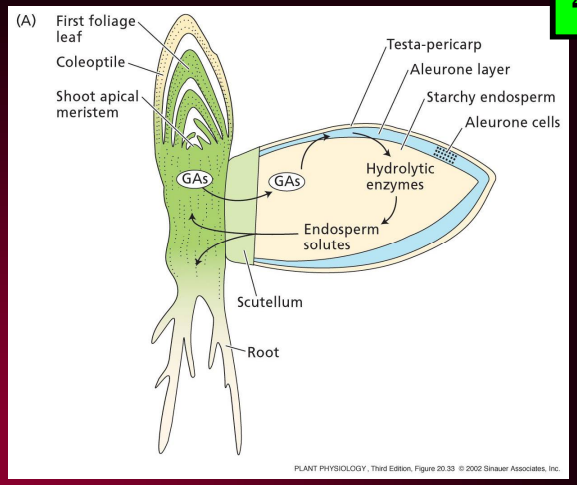
PCD of endosperm and aleurone cells -
- 2nd model example of developmentally regulated cell death

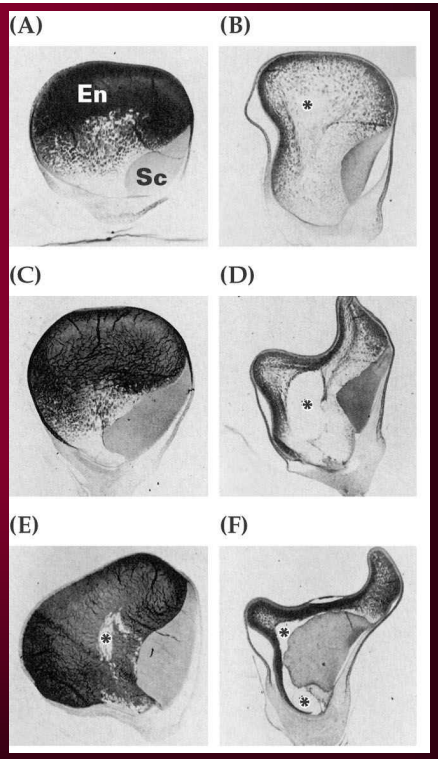
2 types of cells – 2 different ways of PCD

- **starch endosperm**
- **aleurone cells**

Starch endosperm – dead cells, but their content is not degraded – cell is mummified; at germination, endosperm is degraded by enzymes released from aleurone

shrunken2 **– maize mutant, precocious death of starch endosperm and cell degradation; during PCD nuclear DNA is cut to big fragments; differentially from WT, *shrunken2* cells autolyse and endosperm shrunkens – rise of cavities (*)**





Days after pollination

28

32

40

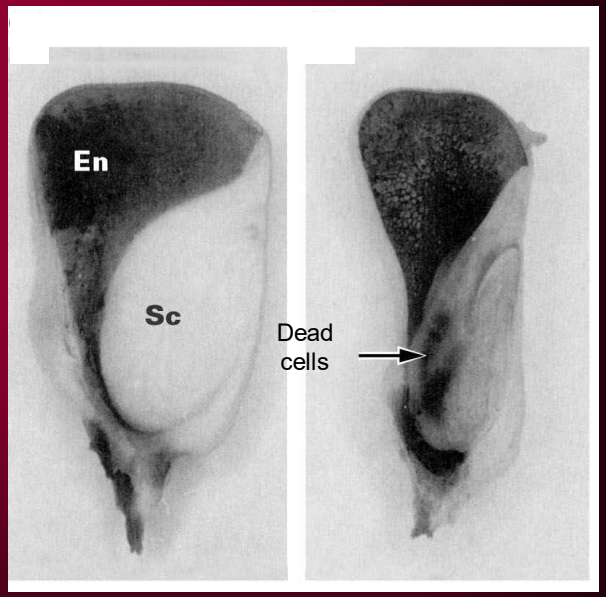
WT *shrunken2*

shrunken2 – overproduction of ethylene

WT – application of ethylene => increased amount of cell death and deformations

AVG (inhibitor of ethylene biosynthesis) – reduces fragmentation of DNA in *shrunken2* and decreases size of cavity in deformed grains.

Ethylene plays important role in PCD of starch endosperm



WT WT + ethylene

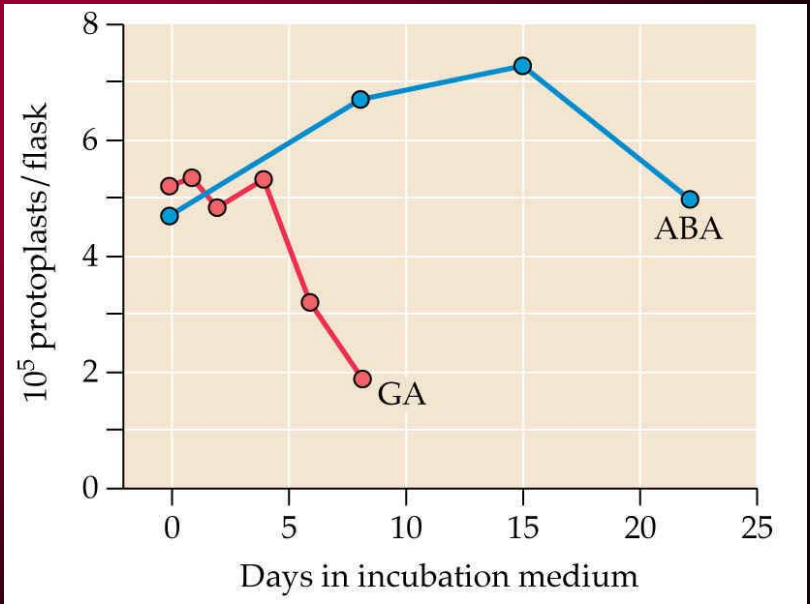
Aleurone cells – stay alive until germination and till all reserves of endosperm are mobilized

End of germination → changes in aleurone:
- vacuolization
- death
- protoplast disintegration

Plant hormones **ABA** and **GA** regulate PCD of aleurone:

GA – stimulates beginning of PCD – causes cell death 8 days after application

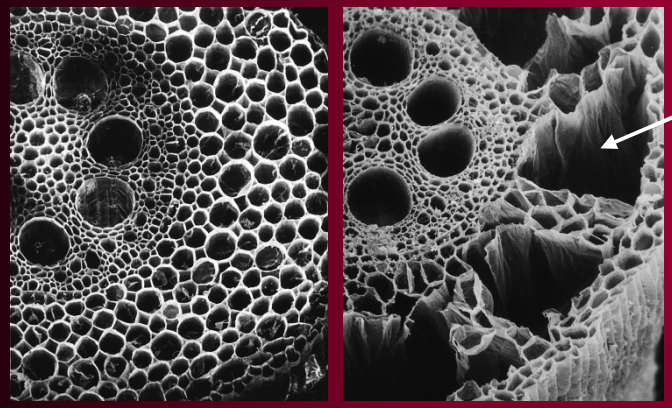
ABA – delays PCD – causes delay of cell death about 6 months



e) PCD and plant responses to stress

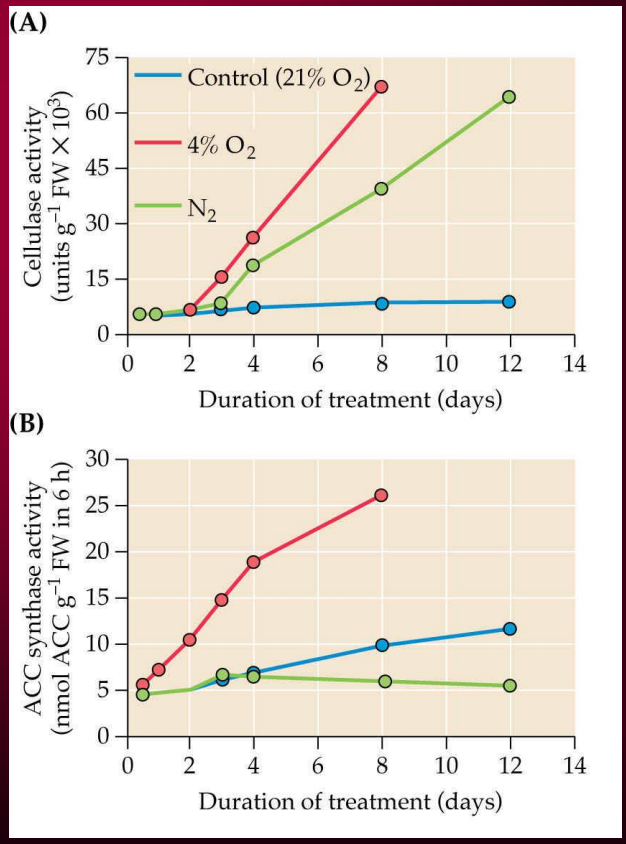
Hypoxia – starts at soil submersion

- aerenchym formation – fast process, consisting in removing of cortical cells including cell wall and formation of spaces (channels) for oxygen transport



Hypoxia → High level of cellulases

Activity of ACC synthase



Cells undergoing hypoxia show higher level of Ca^{2+} in cytosol.

Changes in Ca^{2+} concentration is fast

Role of cytosolic Ca^{2+} in hypoxia

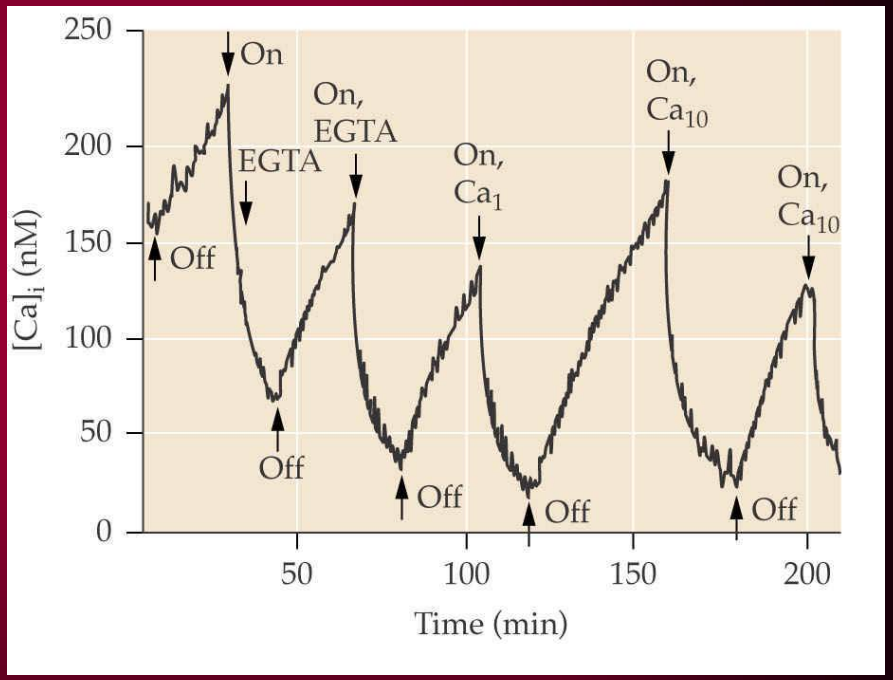
$[Ca]_i$ – cytosolic Ca^{2+}

On – oxidization of medium switched on

Off – oxidization of medium switched off – hypoxia starts

Ca_1 – 1 mM external Ca^{2+}

Ca_{10} – 10 mM external Ca^{2+}

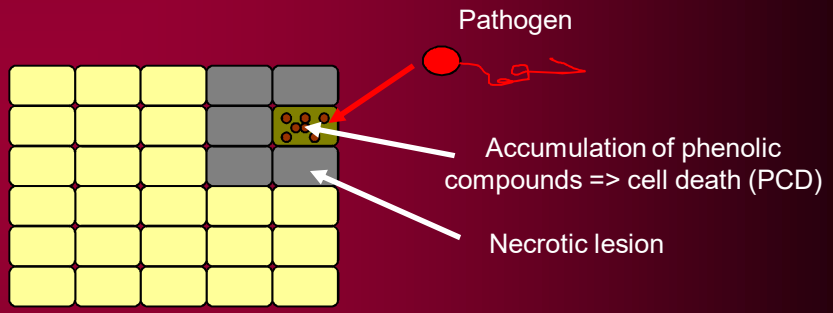


Changes in levels of cytosolic Ca^{2+} in cultured cells of maize

Responses to pathogen

Death of host cell is one of the basic character of plant resistance to pathogen.

Form of resistance to pathogens – hypersensitive response (HR) = localized cell deaths



HR is character of incompatible interaction between plant and avirulent pathogen.

Incompatible interaction is controlled by gene of resistance *R* in plant. It allows plant to recognize pathogen and respond to pathogen, which carries gene of avirulence *Avr*.

In the absence of one of gene *R* or *Avr* compatible interaction occurs – plant is not able to recognize pathogen and disease break out.

Cell death may be symptoms of disease during compatible interaction. This form of cell death is not programmed and it is a consequence of killing the host by pathogen (toxins secreted by pathogen).

Basic question:

Is the cell death during HR suicide (geneticall programmed death) or murder (death as a consequence of toxicity of products produced by pathogen)?

Recent research leans to hypothesis of suicide.

Genetic evidences that cell death at HR is programmed

Lesion-mimic mutants (paranoid mutants) – show HR in the absence of pathogen

↓

HR comes out from endogenous genetic program of cell death

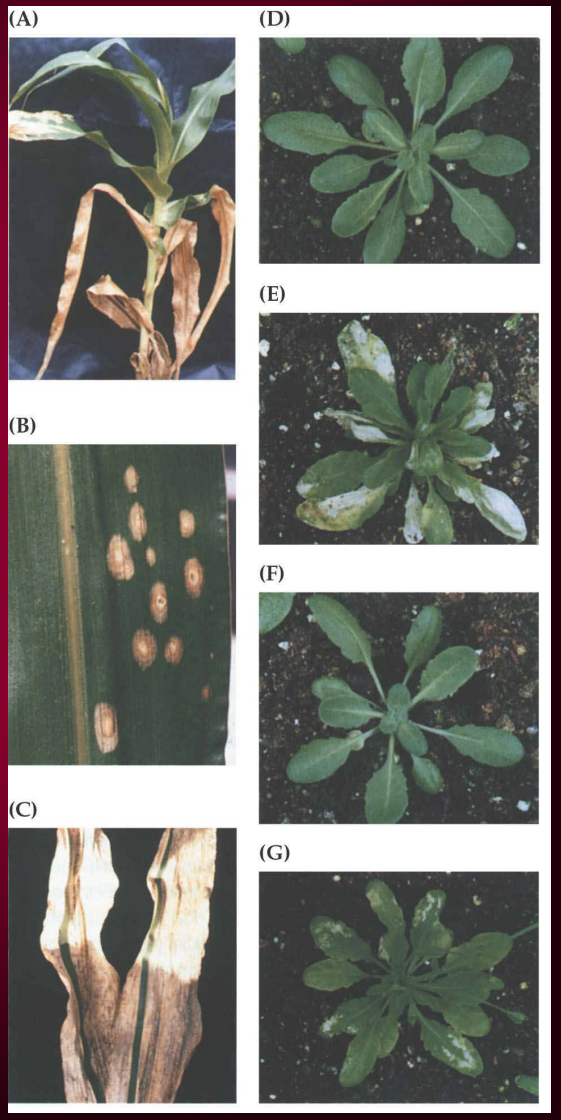
↙ ↘

Mutation in gene for resistance to pathogen

Mutation in gene controlling metabolic pathways

↘ ↙

HR



Triggering PCD

Oxidative burst – process, when production of reactive oxygen species begins after inoculation by a pathogen

Superoxide O_2^- (unstable; poorly passing membrane)

↓

Hydrogen peroxide H_2O_2 (highly toxic; easily passing membrane)

↓

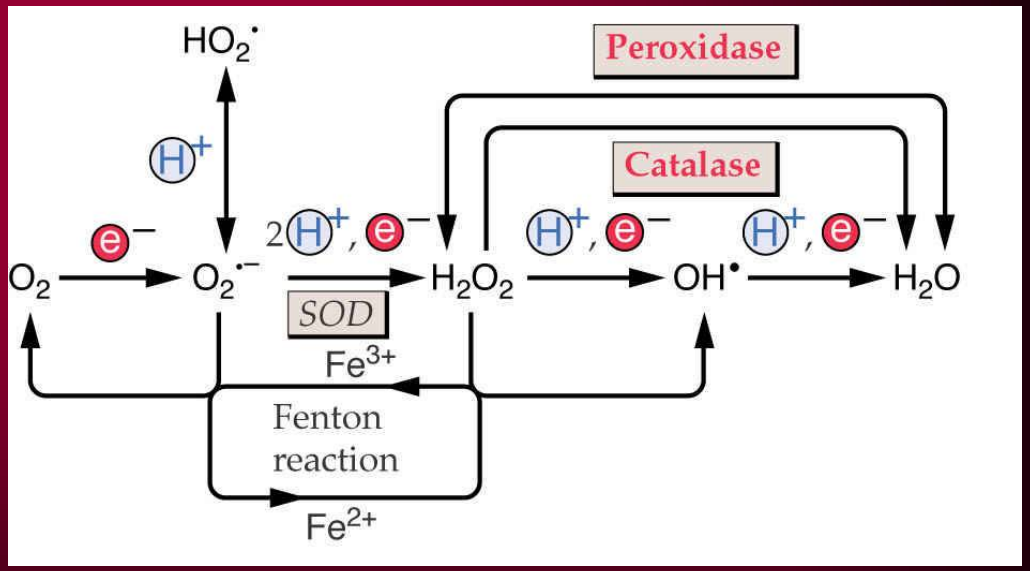
Intermediates (destructive)

↓

Cell death

Inhibition of formation of superoxide results in the reduction of cell death extent.

O^{2-} - superoxide
H_2O_2 – hydrogen peroxide
$^1O_2$ – singlet oxide
OH* – hydroxyl radical

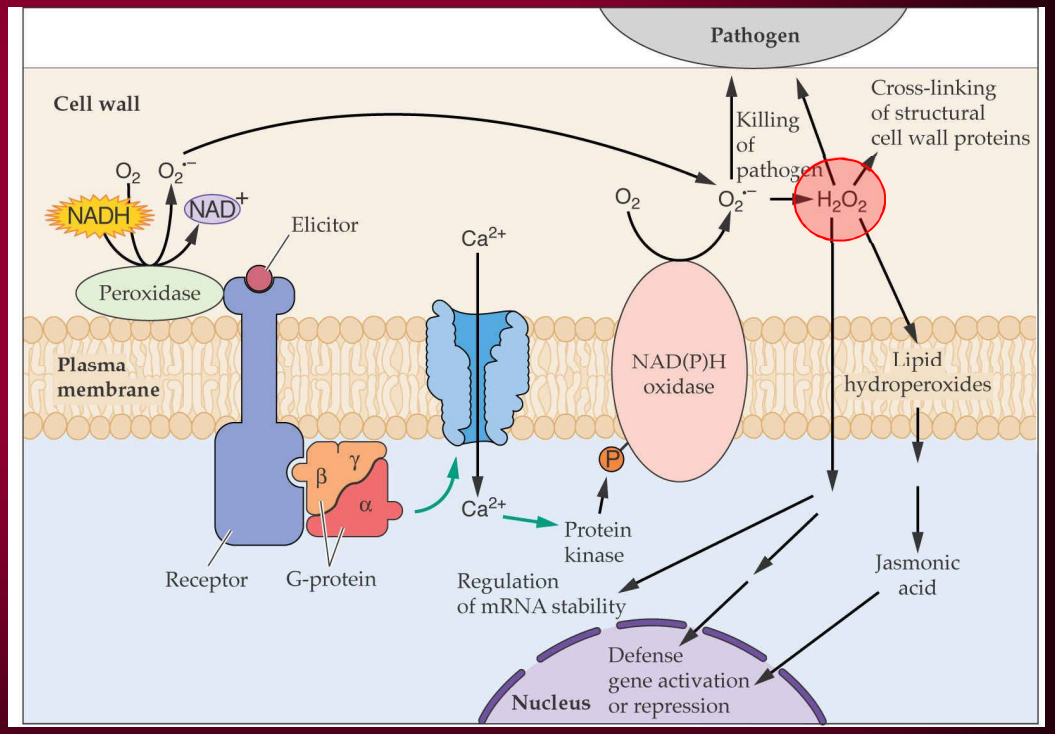


SOD = superoxide dismutase

Intermediates of reactive oxygen species in low concentrations can function as signal molecules, which trigger other pathways leading to PCD.

Hydrogen peroxide – induces transcription of genes, coding antioxidant proteins

Lower risk of cell death



Genes involved in reactive oxygen species signaling

RCD – Radical-induced Cell Death

EXE1 – Executer1

Search for other elements involved in the network of gene regulation of PCD mediated by reactive oxygen species

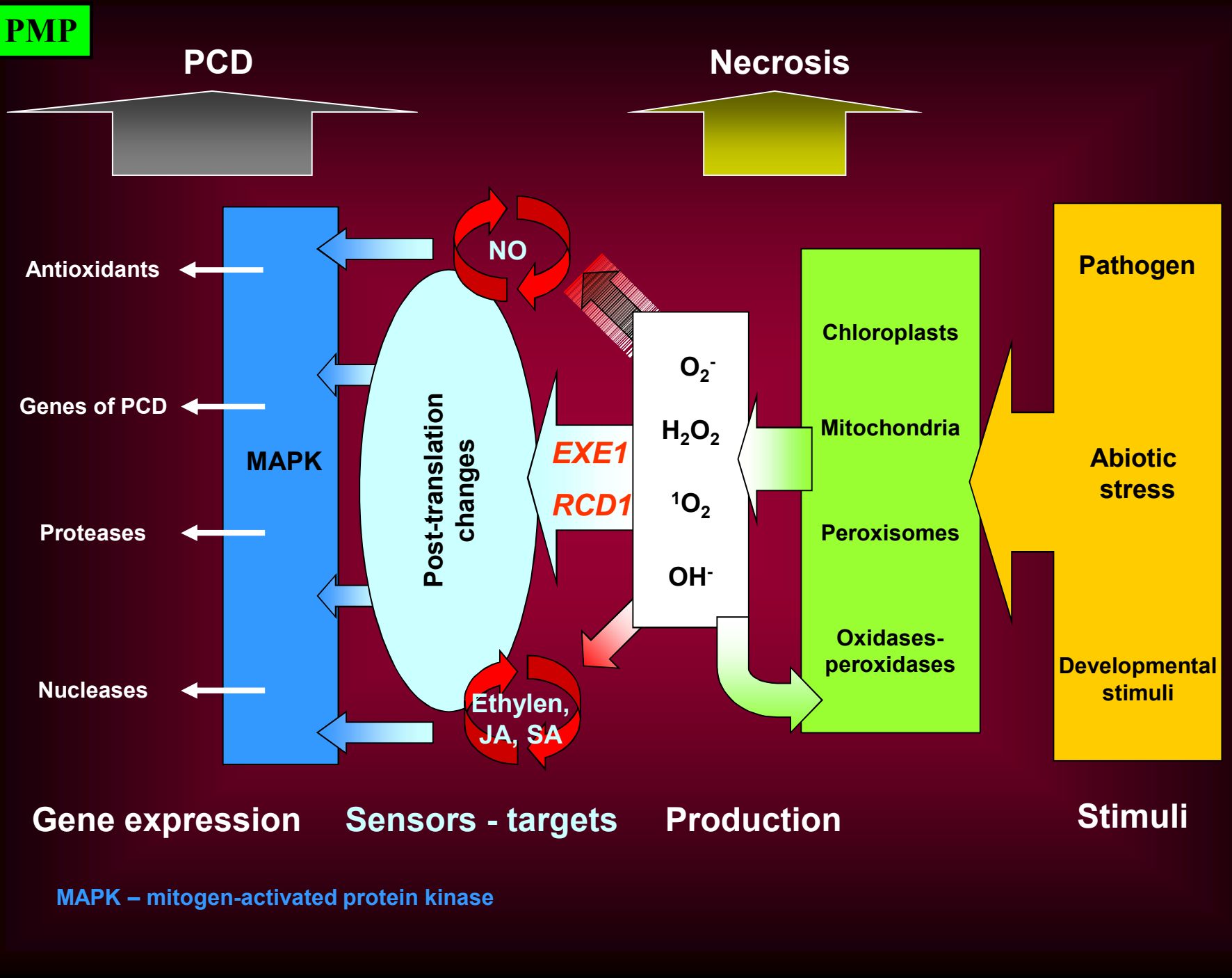
UPDATE 2007

Queval G et al. (2007) Plant J 52: 640 - 657

PCD induced by light and mediated by $^1O_2$ depends on functional receptor of blue light CRY1. Mechanisms is different from the mechanism of PCD induced by light and mediated by O_2^- and H_2O_2 during photosynthesis.

Photoperiod affects signaling pathways leading to PCD and mediated by H_2O_2. Photoperiod determines whether plant exposed to stress will acclimatize or choose the pathway leading to PCD.

PMP



MAPK – mitogen-activated protein kinase