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BC39P – Plant Biochemistry

Plant Response to Stresses

Plants frequently encounter stresses. **Stresses** – external conditions that adversely affect growth, development, or productivity

Stresses trigger a wide range of plant responses, from altered gene expression and cellular metabolism, to changes in growth rates and crop yields.

Biotic and abiotic stresses can reduce average plant productivity by 65% to 87%, depending on the crop

Stresses can be:

biotic, imposed by other organisms or
abiotic, arising from an excess or deficit in the physical or chemical environment

Plant Response to Abiotic Stress

Environmental conditions that can cause stress are:

- water-logging
- drought
- high or low temperatures
- excessive soil salinity
- inadequate mineral in the soil
- too much or too little light

Phytotoxic compounds:

- ozone

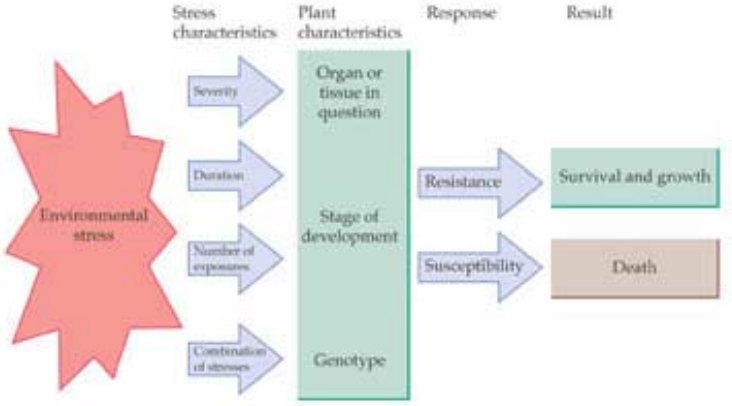
Resistance or sensitivity of plants to stress depends on:

- the species
- the genotype
- development age

Many factors determine how plants respond to environmental stress:

- the type of stress
- the number of times the plant is subjected to stress
- the duration
- the severity

- any additive or synergistic effects of multiple stresses
- the genotype
- developmental stage



Identifying which responses promote or maintain plant growth and development during stress is important for understanding the stress response process

Successful application of biotechnology and classical plant breeding techniques may lead to the development of stress-tolerant crop plants

Engineered mechanisms of stress tolerance might promote survival during periods of intense or prolonged stress or maintain high plant productivity under conditions of moderate environmental stress

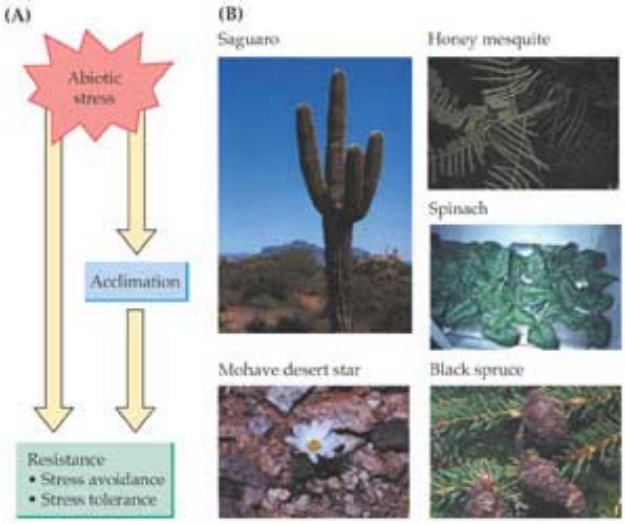
Stress resistance mechanisms

Stress resistance mechanisms can be grouped into two general categories:

1. Avoidance mechanisms
    - prevents exposure to stress
2. Tolerance mechanisms
    - permit the plant to withstand stress

Some resistance mechanisms are constitutive (**adaptations**, evolutional improvements) and are active before exposure to stress

In other cases, plants exposed to stress alter their physiology in response thereby **acclimating** themselves to an unfavourable condition



Constitutive adaptation of drought resistance

Saguaro cactus, a drought tolerant species – succulent photosynthetic stem
Honey mesquite, a drought avoiding species – deep roots
Mohave desert star, drought avoiding species - wet season life cycle

Acclimation to drought stress

During acclimation, the organism alters its homeostasis (steady state physiology) to accommodate shifts in its external environment.

Black spruce, freezing tolerance - osmotic adjustment

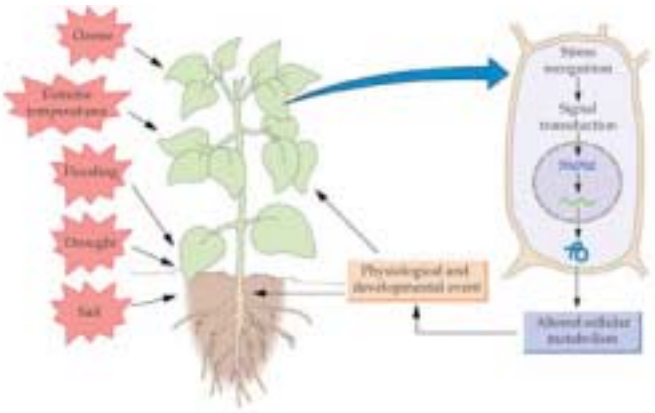
Changes in gene expression to stress

Stress-induced changes in metabolism and development can often be attributed to altered patterns of gene expression

A stress response is initiated when plants recognizes stress at the cellular level
Stress recognition activates signal transduction pathways that transmit information within the individual cell and throughout the plant

Ultimately, changes in gene expression, which occur at the cellular level, are integrated into a response by the whole plant that may modify growth and development and even influence reproductive capabilities.

Although much is not know how plants recognise stresses, considerable evidence indicates that the regulation of plant stress responses involves hormones – especially ***abscisic acid (ABA), jasmonic acid***, and ***ethylene*** – and secondary messengers, such as ***calcium***.



In response to stress some genes are expressed more strongly, whereas others are repressed.

The protein products of stress-induced genes often accumulate in response to unfavourable conditions.

Although most studies have focused on transcriptional activation of gene expression, growing evidence suggests that the accumulation of gene products is also influenced by post-transcriptional regulatory mechanisms that increase the amounts of specific mRNAs, enhance translation, stabilize proteins, alter protein activity, or some combination of these.

Stresses involving water deficit

Environmental conditions that can lead to water deficit in plants include:

- drought
- hypersaline conditions
- low temperatures
- transient loss of turgor at midday

Factors that can affect the response of a plant to water deficit stresses:

- duration of water deficiency
- the rate of onset
- if plant was acclimated to water stress

Tolerance to drought and salinity

Osmotic adjustment and its role in tolerance to drought and salinity

Osmotic adjustment is a biochemical mechanism that helps plants acclimate to dry and saline conditions

A plant cannot extract water from the soil unless the **water potential** in the roots is less than the water potential in the surrounding soil.

The plant root must therefore establish a water potential so that water flows towards the root surface from the soil.

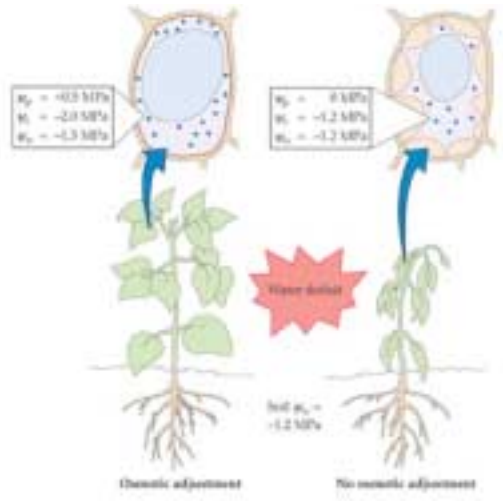
Some plants are highly sensitive to water stress and wilt when soil water potential becomes too low while other plants can endure dry or saline conditions without evident loss of turgor.

Many drought-tolerant plants can regulate their solute potentials to compensate for transient or extended periods of water stress by making osmotic adjustments, which results in a net increase in the number of solutes particles present in the plant cell.

The concentrations of solutes particles (osmolalities) achieved through osmotic adjustment exceed those that result when solutes are passively concentrated by dehydration.

Through decreasing the plant solute potential (Ψs), osmotic adjustment can drive root water potential (Ψw) to values lower than soil (Ψw), thus allowing water to move from soil to plant down a potential gradient.

Fig



Osmotic adjustment is believed to play a critical role in helping plants acclimate to drought or saline conditions

Osmotic adjustment occurs when the concentrations of solutes within a plant cell increases to maintain a positive turgor pressure within the cell. The cell actively accumulates solutes and as a result the solute potential (Ψs) drops, promoting the flow of water into the cell.

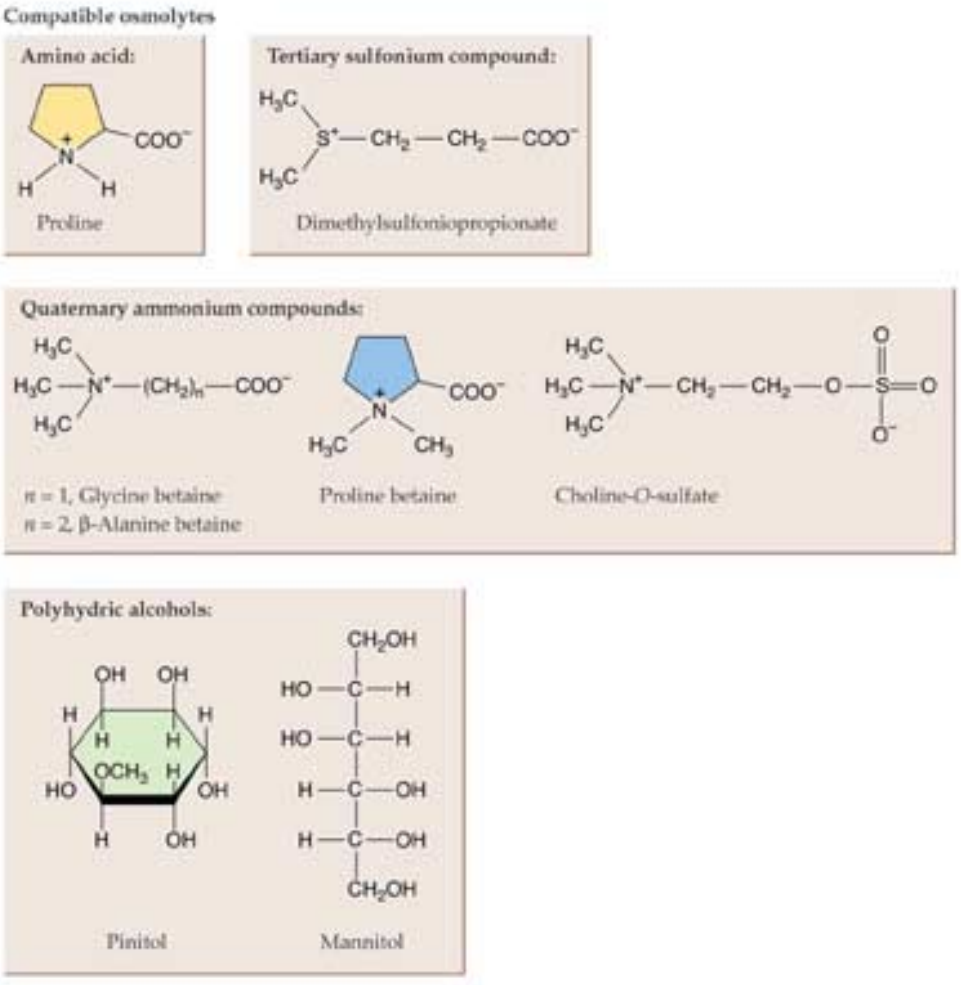
In cell that fails to adjust osmotically, solutes are concentrated passively but turgor is lost

Solutes that contribute to osmotic adjustments

Compatible solutes, are a small group of chemically diverse organic compounds that are highly soluble and do not interfere with cellular metabolism

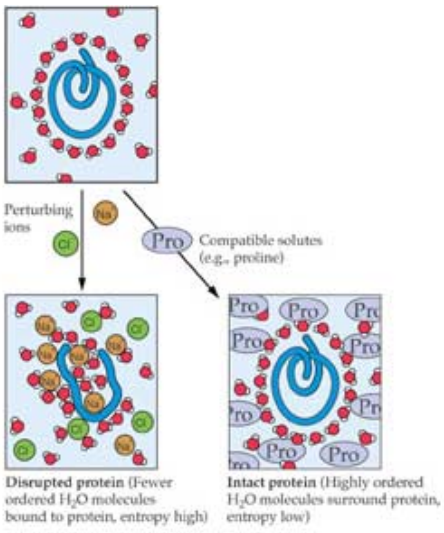
Synthesis and accumulation of organic osmolytes are widespread in plants

Examples: proline, dimethylsulfoniopropionate, proline betaine, choline-O-sulfate, pinitol and mannitol



Compatible solutes tend to be neutrally charged at physiological pH, either non-ionic or zwitterionic, and are excluded from hydration shells of macromolecules.

In contrast, many ions can enter the hydration shells of a protein and promote its denaturation.



The amino acid proline is accumulated by a taxonomically diverse set of plants and are maintained through a combination of synthesis and catabolism

Quaternary ammonium compound β-alanine betaine appears to be confined to representatives of a few genera in the Plumbaginaceae.

Compounds active in osmotic adjustment demonstrate distribution patterns that support water potential (osmotic) equilibria among various membrane-bound compartments of the cell

Membrane-associated carriers and transporters are also probably involved in differentially distributing osmolytes within the cell and throughout the plant

Some evidence indicates that the distribution of proline in osmotically stressed plants involves a transporter.

E.g. two *Arabidopsis* cDNA encoding proline transporters were cloned by functional complementation.

ProT2 transcripts, which encodes one of the proline transporters, were found in all tissue types examined and showed a marked increase in expression when plants were subjected to water deficit and salt stress

Radiotracers evidence indicates that glycine betaine accumulation in osmotically stressed plants resulted from increased rates of synthesis, whereas, with proline, synthesis and catabolism appears to be co-ordinately regulated in response to water stress.

Glycine betaine is synthesised and accumulated by many algae and higher plants and does not appear to be broken down by plants.

The amount of this osmolyte is thought to be dictated by rates of biosynthesis and by transport, through the phloem to growing tissues

Glycine betaine is synthesised from choline in a two-step pathway and occurs in the chloroplast.

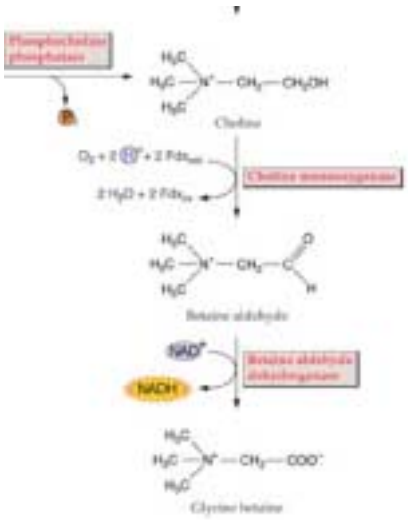
The first enzyme, **choline monooxygenase**, catalyses the oxidation of choline to betaine aldehyde

The second enzyme, **betaine aldehyde dehydrogenase**, catalyses the oxidation of betaine aldehyde to glycine betaine.

Both enzymes have been purified and cloned.

Genetic evidence indicates that accumulation of glycine betaine promotes salt tolerance

The activities of both enzymes increase several fold under conditions of osmotic stress The increased enzyme activity is accompanied by increases in the amounts of transcript which decline when the plants are irrigated with non-saline water.



Mannitol accumulation

In some plant species, salt stress inhibits sucrose synthesis and promotes accumulation of mannitol

Mannitol is the reduced form of the sugar mannose and is broadly distributed among plants and accounts for a substantial proportion of the sugars present in some plants such as celery.

In vitro radiolabelling shows that mannitol concentrations increase in response to osmotic stress

In sharp contrast to glycine betaine, mannitol accumulation appears to be regulated by competing pathways and decreasing rates of mannitol consumption and catabolism

In celery, salt stress inhibits sucrose synthesis but does not seem to affect the enzymes that synthesise mannitol

Also at the same time the rates of mannitol utilization decrease, particularly in sink tissues, such as young roots and leaves

Salt stress also down-regulate **NAD^+ -dependent mannitol dehydrogenase**, the enzyme that oxidises mannitol in celery

Enzyme activity, protein concentration, and mRNA abundance all decrease, bolstering mannitol accumulation.

Tobacco and *Arabidopsis*, both salt sensitive plants, have been genetically engineered to synthesise mannitol, a solute these plants do not generally contain mannitol

Transgenic plants expressing the *E. coli* gene for NAD^+-dependent mannitol-1-phosphate dehydrogenase, which converts fructose 6-phosphate to mannitol-1-phosphate, accumulated mannitol, although at low concentrations

The salt tolerance of the transgenic mannitol producing tobacco was improved relative to that of the controls.

Seeds of the mannitol-accumulating transgenic *Arabidopsis* were able to germinate in the presence of salt.

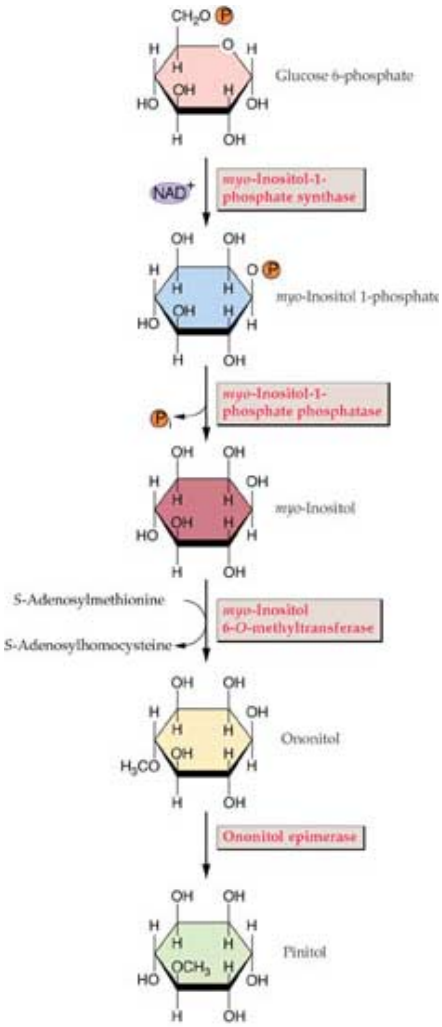
Accumulation of Pinitol in Response to Salt Stress

D-pinitol, a cyclic sugar alcohol, is a major solute in members of the Pine Family and Bean Family

In general, its concentrations are higher among halophytic species and those adapted to drought.

Pinitol accumulation occurs in the salt-tolerant legume *Sesbania* spp and in the facultative halophyte, *Mesembryanthemum crystallinum*, when irrigated with solutions containing sodium chloride.

In leaves, pinitol is localised to the chloroplast and cystol but not in the vacuoles



Osmotin

Osmotin, an abundant alkaline protein, was discovered in cultured tobacco cells that had been acclimated to 428 mM NaCl; in these cells osmotin accounted for as much as 12% of the total protein.

This mature protein has a molecular size of 26-kDa and is localised in the vacuole

Osmotin is classified as a **pathogenesis related** (PR) protein because in early studies it was found to accumulate after pathogen infection

The protein has been shown to have *in vitro* anti-fungal activity, inhibiting the growth of fungal hyphae and causing hyphae and spores to lyse

Transcription of an osmotin gene is induced by at least 10 signals: ABA, ethylene, auxin, infection by TMV, salinity, lack of water, cold, UV light, wounding, and fungal infection.

Many of the gene products induced in response to water deficit are regulated at the transcriptional level.

Many of the stress-induced genes are regulated by ABA, a plant hormone that is increased in response to water deficit and low temperature

ABA plays a role in several responses to water stress, most notably stomata closure and induction of gene expression

ABA biosynthesis mutants have been used to demonstrate that *Arabidopsis*, maize, and tomato require increased concentrations of ABA to express several water-deficit genes

Flooding and oxygen deficit

Plants can be damaged not only by the absence of water but also by too much water, which blocks the entry of oxygen into the soil so that roots and other organs cannot carry out respiration.

Like most eukaryotic organisms plants are obligate aerobes: oxygen is the terminal electron acceptor in the mitochondrial electron transfer chain.

Under normal aerobic conditions, plants can oxidize 1 mol of hexose sugar through glycolysis, the citric acid cycle, and oxidative phosphorylation to yield 30 to 32 mol of ATP.

In the absence of oxygen, ATP production of oxygen is greatly diminished because glycolysis can yield only 2 mol of ATP per mol of hexose

Intracellular ratios of ATP and ADP decline as mitochondrial ATP production is inhibited.

The supply of oxygen to plant roots is influenced by several factors, including:

- soil porosity
- water content
- temperature
- root density
- presence of competing algae and aerobic microorganisms

Oxygen concentration in root tissues also vary according to:

- root depth
- root thickness
- the volume of interchangeable gaseous spaces
- cellular metabolic activity

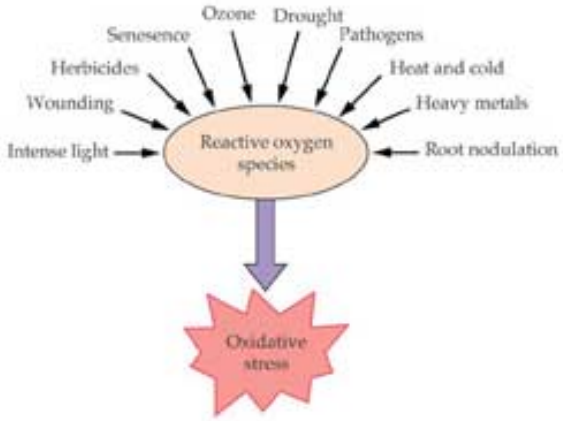
Oxidative Stress

Oxidative stress results from conditions promoting the formation of active oxygen species that damage or kill cells

Environmental factors that cause oxidative stress include:

- air pollution (increased amounts of ozone or sulfur dioxide)
- oxidant forming herbicides e.g. paraquat dichloride
- heavy metals
- drought
- heat and cold stress
- wounding
- UV light
- intense light that stimulate **photoinhibition**

Oxidative stress also occurs in response to pathogen infection and during senescence



Reactive oxygen species (ROS) are formed during certain redox reactions and during incomplete reduction of oxygen or oxidation of water by the mitochondrial or chloroplast electron transfer chains

Formation of singlet oxygen subsequently stimulates the production of other ROS such as hydrogen peroxide, superoxide anion, hydroxyl and perhydroxyl radicals.

Superoxide anions are produced in the chloroplast when electrons are directly from Photosystem I (PSI) to oxygen. These reactive molecules, especially hydroxyl, are highly destructive to lipids, nucleic acids, and proteins.

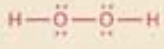
ROS such as superoxide anion and hydrogen peroxide are required for lignification and function as signals in the defence response to pathogen infection.

Tropospheric ozone and oxidative stress in plants

One of the best characterised causes of oxidative stress is exposure to high concentrations of ozone.

Anthropogenic hydrocarbons and oxides of nitrogen (NO, NO_2) and sulfur (SO_x) react with solar UV radiation to generate ozone (O_3).

Stratospheric ozone is beneficial because it shields the earth from UV irradiation, but tropospheric ozone is harmful to life because it is a highly reactive oxidant.

Compound	Shorthand notation(s)	Structural representation(s)	Sources
Molecular oxygen (triplet ground state)	O_2 ; $^3\Sigma$		Most common form of dioxygen gas
Singlet oxygen (first excited singlet state)	$^1O_2$; $^1\Delta$		UV irradiation, photoinhibition, photosystem II e^- transfer reactions (chloroplasts)
Superoxide anion	$O_2^{\cdot-}$		Mitochondrial e^- transfer reactions, Mehler reaction in chloroplasts (reduction of O_2 by iron–sulfur center F_X of Photosystem I), glyoxysomal photorespiration, peroxisome activity, plasma membrane, oxidation of paraquat, nitrogen fixation, defense against pathogens, reaction of O_3 and OH^- in apoplastic space
Hydrogen peroxide	H_2O_2		Photorespiration, β-oxidation, proton-induced decomposition of $O_2^{\cdot-}$, defense against pathogens
Hydroxyl radical	$OH^{\cdot}$		Decomposition of O_3 in presence of protons in apoplastic space, defense against pathogens
Perhydroxyl radical	$O_2H^{\cdot}$		Reaction of O_3 and OH^- in apoplastic space
Ozone	O_3		Electrical discharge or UV radiation in stratosphere, reactions involving combustion products of fossil fuels and UV radiation in troposphere

The negative effects of ozone on plants include:

- decreased rates of photosynthesis
- leaf injury
- reduced growth of shoots and roots
- accelerated senescence
- reduced crop yield

Plants vary in their ability to survive in high ozone environments

Resistance to ozone utilizes either avoidance or tolerance

Avoidance involves physically excluding the pollutant by closing the stomata, the principal site at which ozone enters the plant

Tolerance can result from biochemical responses that induce or activate the antioxidant defence system and possibly also various repair mechanisms

Although ozone-induced injury can increase the susceptibility of the plant to pathogens, ozone exposure paradoxically can induce pathogen resistance by up-regulating the antioxidant enzymes that are induced by the **hypersensitive response** (HR) and **systemic acquired resistance** (SAR)

Ozone Damage

Ozone causes oxidative damage to biomolecules

Most likely, damage occurs after ozone is taken up through the stomata, which results in oxidative destruction of lipids and proteins of the plasma membrane and production free radicals or other reactive intermediates.

Ozone may react with ethylene and other alkenes in the apoplastic fluid to form hydroxyl radicals, superoxide ion, and hydrogen peroxide

Ozone and ROS not decomposed in the apoplast will react with membrane lipids to form reactive lipid peroxidases, which will perpetuate ROS formation

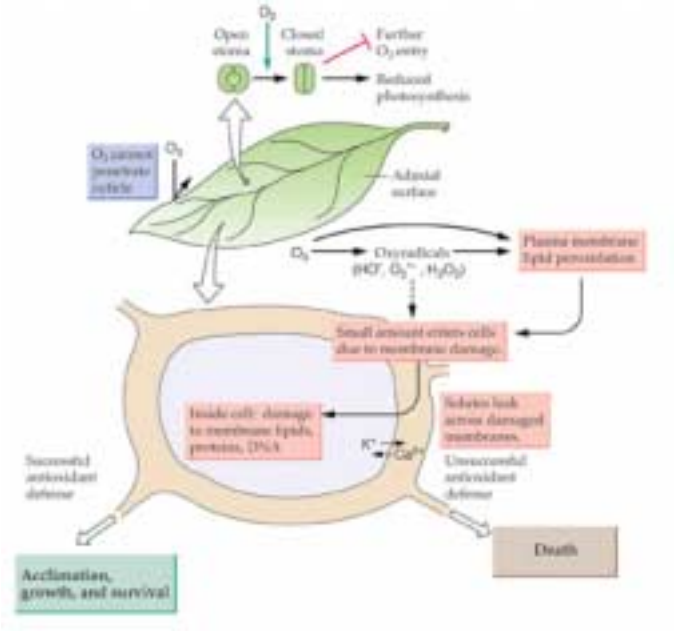
Some ozone and ROS may enter the cytoplasm after membrane damage and promote further production of radicals inside the cell

Ozone-stimulated damage to the plasma membrane

- alters ion transport
- increases membrane permeability
- inhibits H^+-pump activity
- collapses membrane potential
- increases Ca_2^+ uptake from the apoplasm

Within the cell, the presence of ROS and damaged biomolecules may trigger the antioxidant defence system.

Ozone stimulate the wound induced production of ethylene as well as the accumulation of salicylic acid



Tolerance to oxidative stress

Increased synthesis of antioxidants and antioxidant enzymes can improve tolerance to oxidative stress

Stress conditions, antioxidants and antioxidant enzymes

Antioxidant or antioxidant enzyme	Stress condition
Anionic peroxidases	Chilling, high CO_2
Ascorbate peroxidase	Drought, high CO_2, high light intensity, ozone, paraquat
Catalase	Chilling
Glutathione	Chilling, drought, γ-irradiation, heat stress, high CO_2, ozone, SO_2
Glutatione reductase	Chilling, drought, high CO_2, ozone, paraquat
Polyamines	Deficiency of K, P, Ca, Mg, Mn, S, or B; drought, heat, ozone
Superoxide dismutase	Chilling, high CO2, high light, increased O_2, ozone, paraquat, SO_2

Tolerant or acclimated plants may contain higher concentrations of antioxidant factors to minimize damage from ROS

Production of salicylic acid and ethylene

The initial signal in response to ROS is probably the molecules themselves
An increase in cystolic concentrations of Ca_2+ can serve as a second messenger, with plant hormone modulating the response

Ozone exposure results in increased amounts of H_2O_2, which stimulate the production of SA
This results in a transient increase in the number of transcripts that encode defence-related secondary metabolites e.g. phytoalexins, cellular barrier molecules e.g. lignins, callose, and extensins

PR proteins e.g. (1→3) β-glucanase, chitinase, gluthatione *S*-transferase and phenylalanine ammonia lyase.

Ozone can increase the resistance to pathogens by overlap with HR and SAR

Ozone fumigation can also result in ethylene production by inducing increases in ACC synthase and ACC oxidase gene transcription.

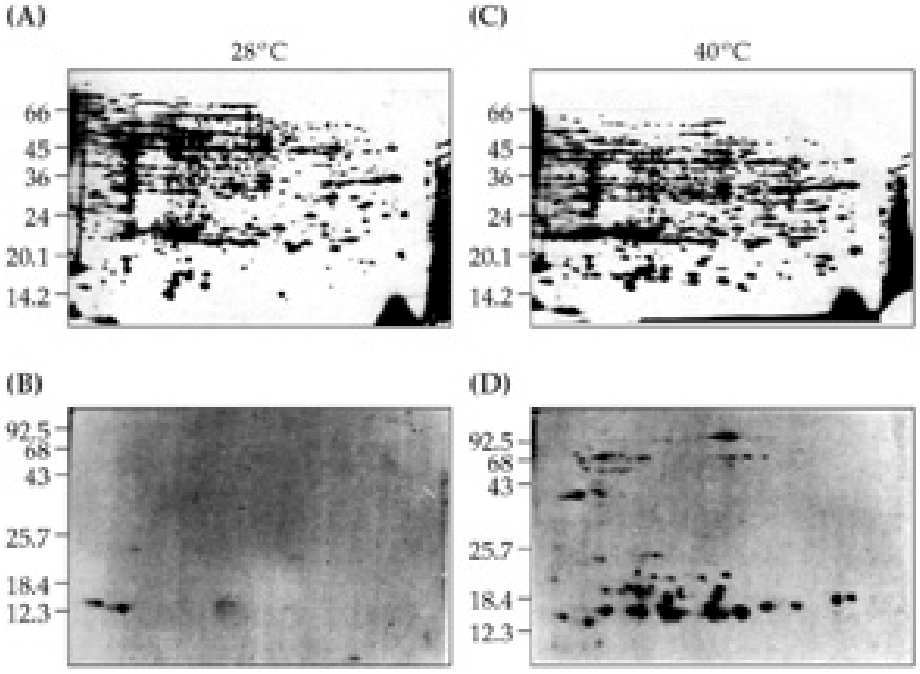
Overproduction of ethylene may be responsible for localized cell death in ozone treated plants

Heat stress

Plants exposed to heat exhibit a characteristic set of cellular and metabolic responses, many of which are conserved in all organisms

The signature response to heat stress is a decrease in the synthesis of normal proteins, accompanied by an accelerated transcription and translation of new proteins known as **heat shock proteins** (HSPs).

This response is observed when plants are exposed to temperatures at least 5°C above their optimal growing conditions
HSPs can be visualized easily on two-dimensional SDS-Gels



HSPs, low mol wt accumulate in soybean seedlings in response to high temperatures
A + B at 28C, C + D at 40C. A + C silver stained, B + D by fluorography

In addition to altering patterns of gene expression, heat also damages cellular structures, including organelles and the cytoskeleton, and impairs membrane function

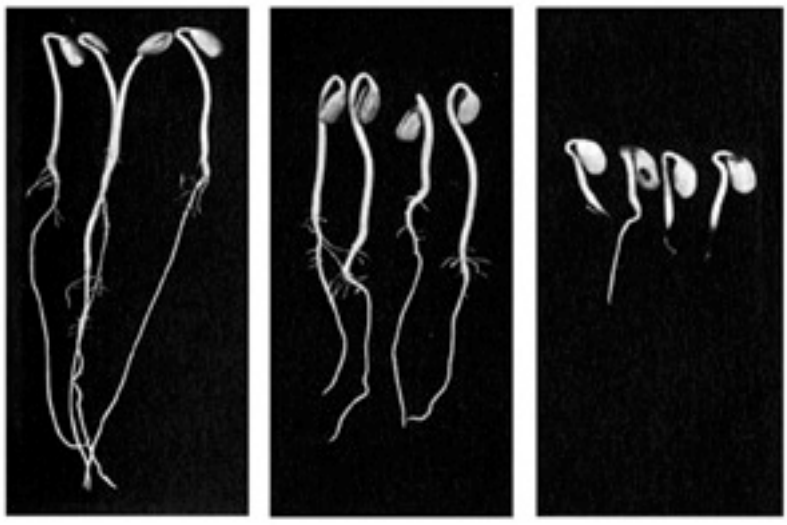
Heat shock may arise under numerous temporal and developmental circumstances, with results ranging from retarded growth to damaged organs to plant death

In the field, heat shock may arise in leaves, when transpiration is insufficient or when stomata are partially or fully closed and irradiance is high; in germinating seedlings, when the soil is warmed by the sun; in organs with reduced capacity for transpiration (e.g. fruits); and overall from high ambient temperatures.

Duration and severity of the stress, susceptibility of different cell types, and stage of development a; influence the ability of a particular genotype to survive heat stress

An acclimated plant can survive exposure to high temperature that other wise be lethal.

E.g. thermotolerance can be induced in seedlings. Soybean seedling grown at 28C. On the left, the seedlings were not subjected to additional heat; in the middle, pre-treatment for 2-hr at 40C then transferred to 45C. On the right, seedlings transferred to high temperature without pre-treatment



Despite the capacity for acclimation, there is, of course, a limit to how much heat a plant can withstand.

Some major HSPs are conserved among all living organisms, both prokaryotic and eukaryotic.

Many of these proteins function as chaperones and are involved in refolding proteins denatured by heat.

Classes of HSPs

Five classes based on their approx. molecular masses

Protein class	Size (kDa)	Location
HSP100	100-114	cytoplasm
HSP90	80-94	cytoplasm, ER
HSP70	69-71	ER, cytoplasm, mitochondria
HSP60	10-60	chloroplasts, mitochondria
smHSP	15-30	cytoplasm, chloroplast, ER, mitochondria

Members of HSP100 family includes proteins found in bacteria, yeast, trypanasomes, mammals, and plants

HSP90 family are found in bacteria and in the cystolic, nuclear, and ER of eukaryotic cells, where they function as molecular chaperones and may have long-lived interactions with particular target proteins.

HSP70 proteins are essential for normal cell function. Some members are expressed constitutively; others are induced by heat or cold.

HSP60 protein family are thought to function as molecular chaperones and are present in bacteria cystol (GroEL), the mitochondria matrix, and the chloroplast stroma.
HSP60 proteins are abundant even at normal temperatures; their major role is thought o involve protein assembly.
In plants the most studied HSP60 protein is chaperonin 60, a nuclear-encoded chloropast protein that is involved in Rubisco assembly but does not increase in response to heat stress.
In-vitro, HSP60 protein prevents other proteins from aggregating at physiologically relevant temperatures and is important in protein refolding as temperature increases.

Plants contain 5 or 6 classes of low molecular mass (smHSPs), whereas other eukaryotes have only a single class of smHSP.
One explanation for this difference is that these proteins are distributed in different compartments in plants: 2 in the cystol, 1 in the chloroplast' 1 in the ER, 1 in the mitochondrion, and possibly another in a membrane compartment that has not been defined.

HSP18.1 from pea has been demonstrated to prevent protein aggregation in response to high temperature in vitro.

Expression of HSP

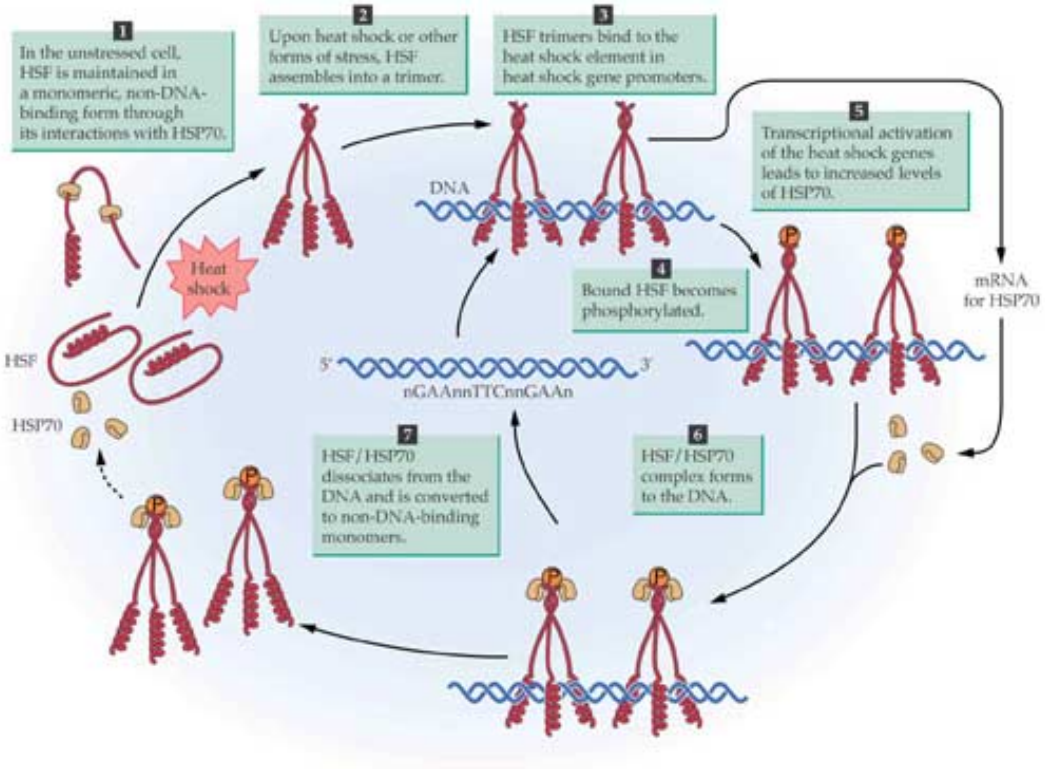
Expression of many HSPs is controlled by a transcription factor that recognises a conserved promoter sequence.

The heat shock transcription factor is expressed constitutively but must be activated during heat stress to recognise its DNA target, the heat shock element (HSE).

The HSE is made up of 5-bp repeats in alternating orientation with the consensus sequence nGAAn

An HSF-regulated promoter may contain 5 to 7 of these repeats close to the TATA box.

Like other HSFs, the HSF of Arabidopsis (ATHSF1) can only bind DNA as trimers; heat stress is required for trimerization.



In the unstressed cell, HSF is maintained as a monomer and cannot bind DNA.
On heat shock, the HSF is assembled into a trimer capable of binding a specific DNA sequence that increases the synthesis of HSP70.

Plant defence systems

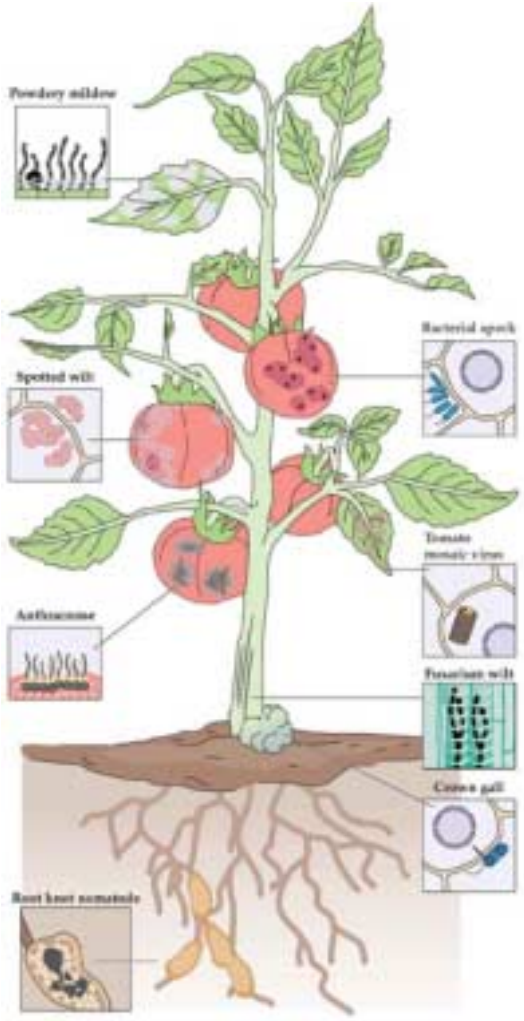
This section will highlight the essential prerequisites for pathogen recognition and the induction of localised defence responses

Complex responses frequently save plants from destruction by pathogens

Only a very small proportion of pathogen infections are likely to result in a diseased plant

Ways in which plant pathogens cause disease

Roots and shoots of plants are always in contact with plant pathogens. Each pathogen has evolved a specific way to invade plants



Some species directly penetrate surface layers by using mechanical pressure or enzymatic attack
Others pass through natural openings (stomata or lenticels). A third group invades only tissue that has been previously wounded.

Once inside the plant, one of three main attack strategies is deployed to utilise the host plant as substrate: **necrotrophy**, in which the plant cells are killed; **biotrophy**, in which the plant cells remain alive; and **hemibiotrophy**, in which the pathogen initially keeps cells alive but kills them at later stages of infection

Four main reasons account for most failures of pathogen to infect plants successfully

- The plant species attacked is unable to support the life-strategy requirements of the particular pathogen, and thus is considered to be a non-host

- The plant possesses preformed structural barriers or toxic compounds that confine successful infection to specialised pathogen species (nonhost resistance)

- On recognition of the attacking pathogen, defence mechanisms are activated such that the invasion remains localised

- Environmental conditions change and the pathogen perish before the infection process has reached the point at which it is no longer influenced by adverse external stresses

The first three interactions are said to represent genetic incompatibility, but only the third mechanism of resistance depends exclusively on induced defence responses to limit pathogen attack

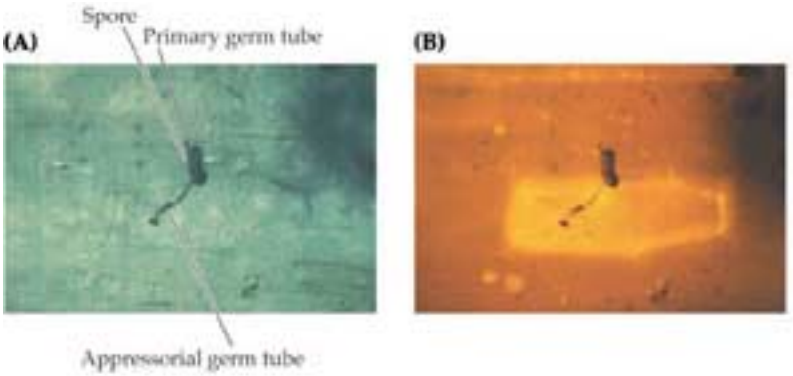
Hypersensitive response

Activated defences can result in the hypersensitive response, a localised cell death program that prevents pathogen spread.

Recognition of a genetically incompatible pathogen results in the activation of a complex series of defence responses. The process is coordinated both temporally and spatially to ensure that the only necessary numbers of plant cells are recruited from primary metabolism into a defensive roll.

This rapid and highly localised induction of plant defence responses results in the creation of unfavourable conditions for pathogen growth and reproduction; at the same time, the responding cell detoxify and impair the spread of harmful enzymes and toxins produced by the pathogen.

Full activation of this intense response to the pathogen occurs within 24-hr and invariably leads either directly or indirectly to localised cell and tissue death



HR of barley epidermal cells to attack by germinating spores of the barley powdery mildew fungus (A) the stained extracellular fungal structure – primary germ tube and appressorial germ tube and spore. (B) the corresponding whole cell autofluorescence of the same barley cell at 310 mn; only the cell undergoing HR exhibits autofluorescence.

The rapid activation of defence of reactions in association with host cell death is frequently called **hypersensitive response**

Because the dead cells contain high concentrations of molecules with antimicrobial properties, opportunist necrotrophic organisms do not subsequently attack them.

Preformed defence – secondary metabolites

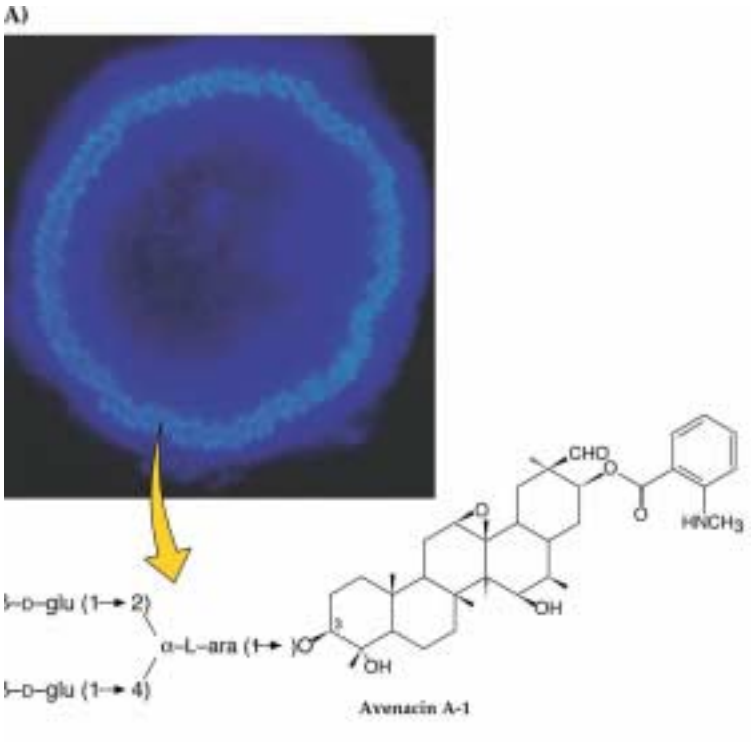
Most healthy plants possess many different secondary metabolites with antimicrobial properties. These compounds may be present in their biological active form or may be store as inactive precursors that are converted to their active forms by host enzymes in response to pathogen attack or tissue damage.

Two well-characterized classes of pre-formed inhibitors are the **saponins** and the **glucosinolates**

Saponins are glycosylated compounds, classified as either triterpinoids, steroids, or steroidal glycoalkaloids.

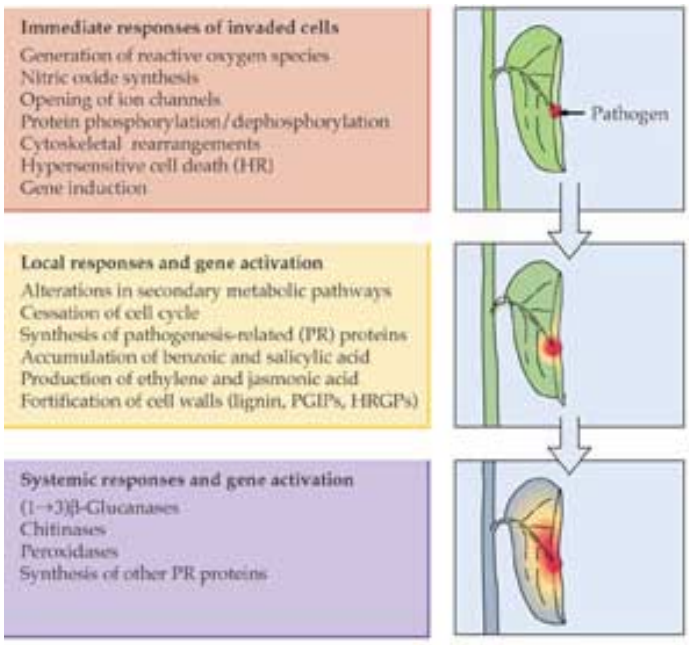
A biologically active triterpinoid saponin found in the roots of oat plants, **avenacin A-1**, is highly effective against the root infecting take-all fungus, a major pathogen of cereal roots.
This pathogen affects wheat and barley, but not oat plants.

The peripheral location of avenacin in healthy oat roots is revealed during when root sections are viewed under UV fluorescence



Biochemistry of plant defence reactions

Plant resistance is correlated with the activation of a diverse set of defence mechanisms. The response involves transcriptional activation of numerous defence-related genes, opening of ion channels, modification of protein phosphorylation status, and activation of preformed enzymes to undertake specific modifications to primary and secondary metabolism



Temporal order of plant defence responses, both locally and systemically

Hypersensitive response

HR results in rapid, localized cell death. Necrotic flecks resulting from dead plant cells often form at sites of attempted pathogen attack

Reactive oxygen species (ROS)

In many incompatible interactions, the production of ROS is often the first response detected, occurring within 5 min

The typical ROS species detected are superoxide (O_2) and hydrogen peroxide (H_2O_2). The mechanism plants have for producing superoxide from molecular oxygen probably involves a plasma membrane-associated NADPH oxidase, similar but not identical to that used in mammalian neutrophils for defence.

The superoxide anions produced outside the plant cells usually are rapidly converted to H_2O_2, a molecule that can cross the plasma membrane and enter the plant cell

H_2O_2 is eventually removed from cells by conversion to H_2O through the action of the enzyme catalase, ascorbate peroxidase, or glutathione peroxidase.

Several roles in plant defence have been proposed for ROS:

Hydrogen peroxide maybe directly toxic to pathogens. In the presence of Fe, H_2O_2 gives rise to the extremely reactive hydroxyl radical (OH).

It may contribute to the structural reinforcement of plant cell walls, either by cross-linking various hydroxyproline and proline rich glycoprotein to the polysaccharide matrix or by increasing the rate of lignin polymer formation by way of peroxidase enzyme activity, both of which would make the plant cell wall more resistant to microbial perpetration and enzymatic degradation.

A signalling role for some ROS is also likely:

H_2O_2 induces benzoic acid 2 hydrolase (BA 2-H) enzyme activity, which is required for biosynthesis of SA

H_2O_2 is known to induce genes for proteins involved in certain cell protection mechanisms, e.g. glutathione S-transferase.

Production of ROS may also substantially alter the redox balance in the responding cells

Production of nitric acid

Nitric oxide, a signalling molecule in mammals, and is induced during incompatibility interactions in plants

NO is used by mammals to regulate various biological processes of the immune, nervous, and vascular systems.

In plants, rapid *de novo* synthesis of NO accompany the recognition of avirulent pathogenic bacteria

Although a localised HR is a consistent feature of genetically incompatible interactions, the rapid burst of ROS production is insufficient to induce plant cell death but may able to inhibit pathogen growth

NO has the capacity to potentiate induction of plant cell death by ROS

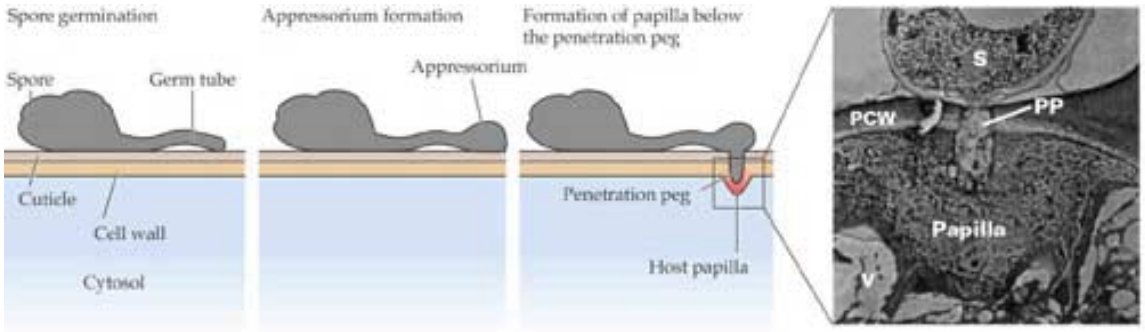
NO is known to bind heme and thereby could inhibit catalase and ascorbate peroxidase, which detoxifies H_2O_2
Adding NO-generating compounds to plant cell suspension cultures and leaves lead to the accumulation of mRNAs from several genes involved in defence and cell protection.

In the presence of inhibitors of NO production, the HR diminishes, disease symptoms become more severe, and bacterial growth is increased.

These findings indicate that NO and ROS play an important synergistic role in the rapid activation of a wide repertoire of defence responses after pathogen attack.

Cell wall fortification

Cell wall fortifications and extracellular activities contribute to plant disease resistance responses
Minute papillae often form directly beneath the sites at which biotrophic fungi attempt to penetrate the plant cell wall



These papillae, which a re primarily composed of callose [(1 → 3) β-glucan polymer] and lignin (a highly complex phenolic polymer) are thought to act as physical barrier, blocking fungal penetration into plant cells

Induced callose deposition within plasmodesmata is also likely to block virus cell-to-cell movement.

Extracellular basic **hydroxyproline-rich glycoproteins** (HRGPs) contribute to fortifying the cell wall in two ways

Preformed HRGPs cross-link rapidly to the wall matrix by way of tyrosine reacting with induced H_2O_2
Later, de novo HRGP synthesis initiates addition lignin polymerisation to further reinforce cell walls

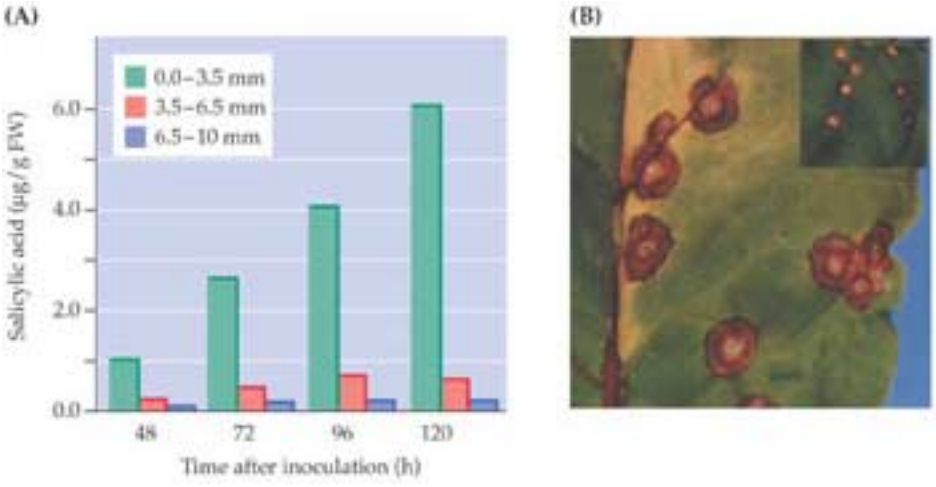
Another class of defence-related extracellular proteins are polygalacturonase-inhibiting proteins (PGIPs), which inhibit a specific subclass of necrotrophic pathogen cell wall-degrading enzymes called polygalacturonidases (PGs).

PGIPs possible retard PG function, resulting in an increased abundance of oligogalacturonides with chains more than 8-units long; this in turn, may trigger additional defence responses

Benzoic acid and salicylic acid

Free acids and glucoside conjugates of the phenolic compounds benzoic acid (BA) and SA accumulate to high concentrations in the vicinity of incompatible infection sites.

Both SA and BA are derived from the phenylpropanoid pathway and have many roles in plant defence responses



(A) Detailed analysis of TMV necrotic lesions forming on *N* gene-expressing resistant tobacco leaves reveal that the total SA contents are greatest in the centre of the lesion and rapidly diminish with distance from the centre

(B) Constitutive expression in tobacco plants of the *Psuedomonas putida nah*G gene (encodes the enzyme salicylate hydrolase and hydrolyses SA to catechol) leads to continual removal of induced SA. This causes substantial increase in TMV multiplication in the tobacco leaves through a weakening of several *N* gene-mediated resistance to TMV

Jasmonic acid and ethylene

Jasmonic acid (JA) is an oxylipin-like hormone derived from oxygenated linolenic acid.

Increases in JA in response to pathogen/insect attack occur both locally and systematically.

Spraying methyl-JA onto plants increases their resistance to some (but not all) necrotrophic fungi, but not to biotrophic fungi or bacteria

The gaseous hormone ethylene is frequently synthesised during both incompatible and compatible interactions

By blocking ethylene biosynthesis, it was found that ethylene is apparently not required for several *R-avr* gene-mediated resistance responses

When ethylene signal is eliminated during compatible interactions, the severity of chlorotic and necrotic symptoms frequently is greatly reduced and the plants appear to be more disease tolerant.

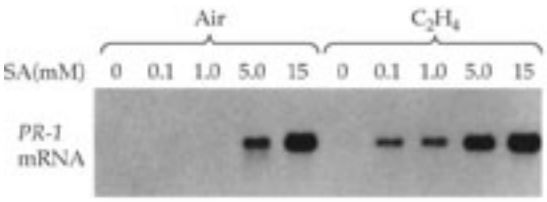
Ethylene is required to mediate both resistance against necrotrophic fungal pathogens and non -ost resistance against soil borne fungal species that are not ordinarily plant pathogens.

Ethylene and JA are required for activation of proteinase inhibitor (PI) genes and certain PR and chitinase genes.

PR proteins

Pathogenesis (PR) related proteins and other defence-related proteins include fungal cell wall-degrading enzymes, chitinases, glucanases, anti-microbial polypeptides, and components of signal transduction cascades. SA-mediated signal transduction cascades regulate the transcriptional activation of many PR genes

Ethylene and SA have been shown to act synergistically, further enhancing the expression of PR genes.



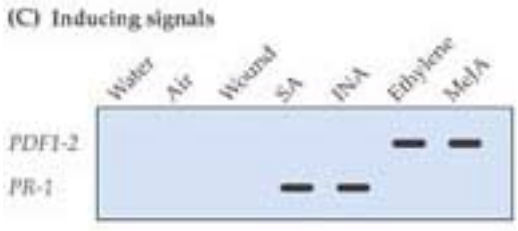
The cooperative effect of two defence signalling molecules, gaseous ethylene and salicylic acid on the accumulation of pathogenesis-related mRNA

Other PR defence proteins, such as lipoxygenase, may contribute to defence by generating secondary signal molecules such as JA and lipid peroxidases

Plant defensins are a third type of defence-related genes with demonstrated antimicrobial activity.
Plant defensins are small (<7 kDa) cysteine-rich peptides that accumulate in the periphery of the plant plasma membrane and are frequently found in dry plant seeds.

In *Arabidopsis*, induction of the defensin *PDF1-2* gene transcript is regulated by defence signalling cascades that require ethylene or Me-jasmonate.

Application of these 2 signaling molecules does not induce the pathogenesis-related *PR-1* gene is activated by dichloroisonicotinic acid (INA) and SA.



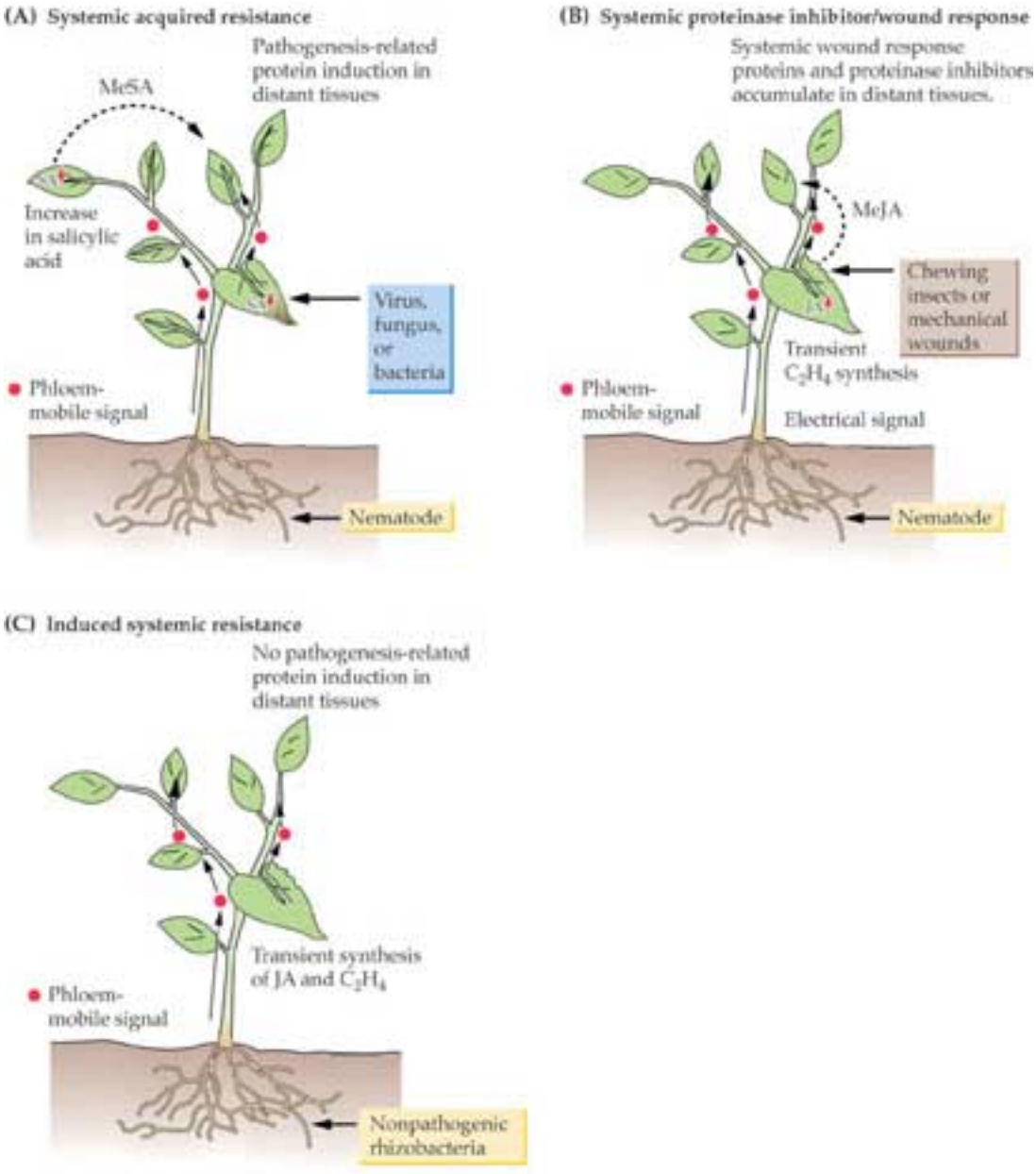
Phytoalexins

Phytoalexins are low-molecular-mass, lipophilic antimicrobial compounds that accumulate rapidly at sites of incompatible pathogen infection.
phytoalexins includes both organic and inorganic secondary metabolites
Biosynthesis of phytoalexins occurs only after primary metabolic precursors are diverted into a novel secondary metabolic pathway.
E.g. phenylalanine is diverted into the synthesis of various flavonoid phytoalexins by the de novo synthesis of phenylalanine ammonia lyase (PAL).

Systemic plant defence responses

Within minutes of pathogen, insect, or nematode attack, plant defence responses are activated locally.
Within hours, defence responses are also sometimes elaborated in tissues far from the invasion site and even in neighbouring plants. The type of systemic responses induced, however is determined by the identity of the attacking organism.

Induced systemic responses to fungi, bacteria, and viruses are distinct from the response to insect.
Nematodes appear to induce a mixture of the two
Root colonising non-pathogenic bacteria induce another type of response



Systemic acquired resistance (SAR)

Fungi, bacteria, and viruses activate systemically a specific subset of PR-type gene by a mechanism known as **systemic acquired resistance** (SAR) in which local necrosis formation at the initial site of pathogen invasion triggers both a local increase in SA accumulation and the formation of a phloem-mobile signal.

For SAR to occur, the initial infection must result in formation of necrotic lesions, either as part of the HR or as symptom of disease.

Subsequently, in distal plant tissue, SA concentrations increase and volatile methyl-SA (MeSA) is released.

Together, these signals induce the synthesis of various pathogen-related proteins in the non-invaded parts of the plants

SAR activation leads to a marked reduction in disease symptoms after subsequent infection of many pathogenic species.

In tobacco, for example, *N* gene-mediated resistance against TMV protects the plant against later infection by the identical TMV strain and by most other tobacco pathogens tested.

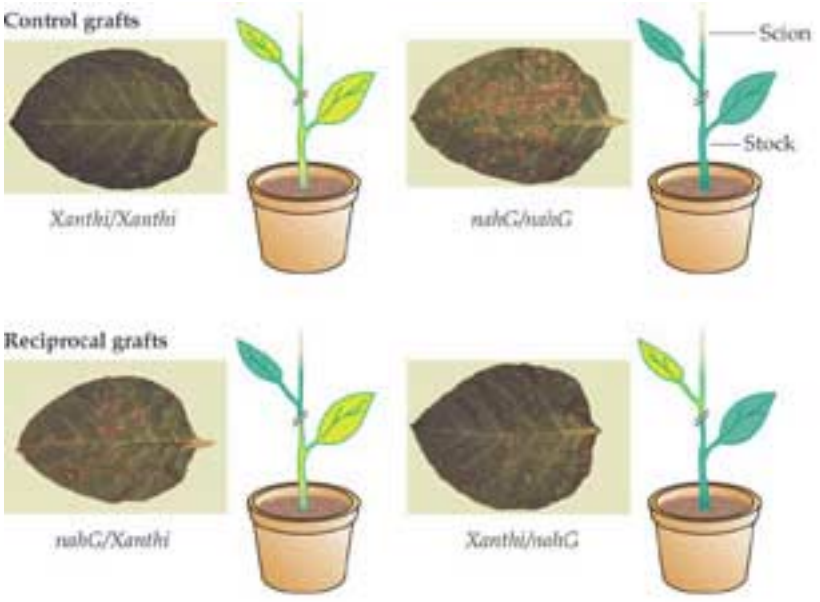
Various synthetic chemicals induce SAR. Two of the most potent are 2,6-dichloroisonicotinic acid (INA) and benzo-(1,2,3)-thiodiazole-7-carbothionic acid S-m ethyl ester (BTH)

Role of SA in activating SAR

The requirement for SA in SAR activation was demonstrated by using transgenic tobacco and *Arabidopsis* plants expressing the *nah*G gene (salicylic hydrolase). These plants do not accumulate free SA and were incapable of activating SAR

Studies of in vivo 14C-labeled SA in tobacco have shown that as much as 70% of the increase in SA concentrations observed in uninfected tissue after TMV infection is the result of SA translocation from the infected leaves.

A series of grafting experiments between wild type and transgenic *nah*G expressing plants suggests that for induction of SAR, SA need only to be present in the distal plant organs.



Reciprocal grafts and control grafts were generated by using two types of tobacco plants which expressed either the *N* resistance gene alone (*Xanthi*) or the *N* gene in combination with the *nah*G transgene

The 4 types of grafted plants were inoculated with TMV on the lower rootstock leaves; 7 days later, the same TMV isolate was inoculated onto the scion leaves.

The above photos show the infection types on the scion leaves 5 days after the second TMV inoculation.

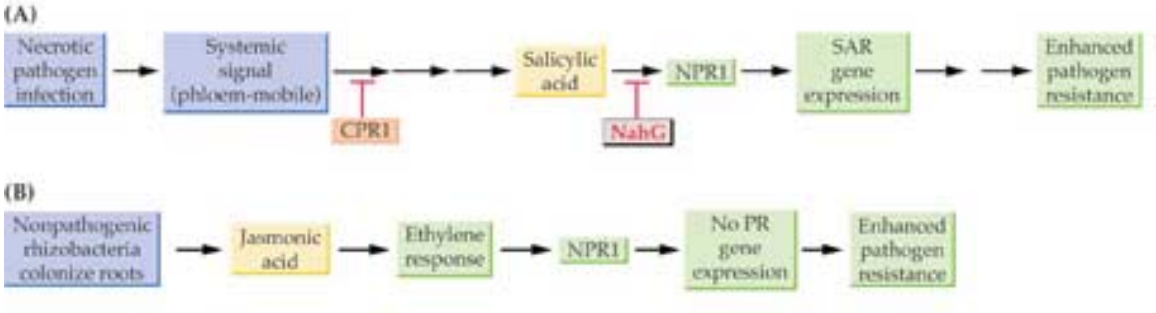
*nah*G scion grafted onto *Xanthi* rootstock (*nah*G/*Xanthi*) were unable to mount a SAR response.
Xanthi/*nah*G grafts demonstrated SAR response similar to those of the control Xanthi/Xanthi grafts
*nah*G/*nah*G lacking SA were unable to mount SA response

Although SA is mobile, it is probably not the mobile signal that activates SAR

Induces systemic resistance (ISR)

Non-pathogenic root-colonizing rhizobacteria cause induced system resistance.
Rhizobacteria that promote specific plant growth, for example *P. fluorescens*, induce a systemic resistance response that does not depend on SA or PR protein accumulation.

Instead, ISR requires both JA and ethylene signalling and also the SAR regulatory protein NPRI



The requirement for NPRI protein indicates that pathogen-induced SAR and rhizobacteria induced ISR converge during the latter parts of the signalling pathway.

Reference

Buchanan, Gruissem and Jones (2000) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists