Improves Agricultural World
for Everyone

PSAP

Mediated Mitigation
In C_3 and C_4 Crop Plants

Mechanisms and Potential
For Stress Resilient Agriculture

PSAP

Potassium Salt of Active Phosphorus

Boost the Grower Farmers Income By 50% to 120%

PSAP Increases Plant Yield and Improves Produce Quality

Reduces Chemical Load in Crop and Improves Soil Health

Supports & Compliments Current Agriculture Technologies

PSAP Technology

Dedicated to Grower Farmers, Scientists,
Extension and Research Workers, Policy Makers

PSAP has a role in stress mitigation of crop plants even in changing environmental condition.

PSAP since its introduction in year 2009 - 10 never succumb to fail in any crop plant at any where in given situation.

PSAP is non toxic, eco-friendly having wide range of crop applicability and can be instrumental in our most needed, "Ever Green Revolution".

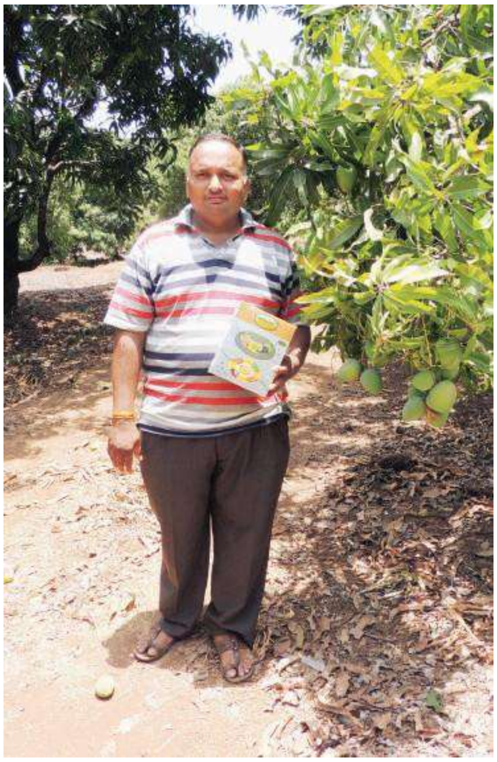
PSAP at Farmers Field

Excellent Quality Mangoes

May - 2018

Ulahas Vartak
Kelshi - Tal. Dapoli
Dist - Kokan - Maharashtra State

Alphonso Mangos

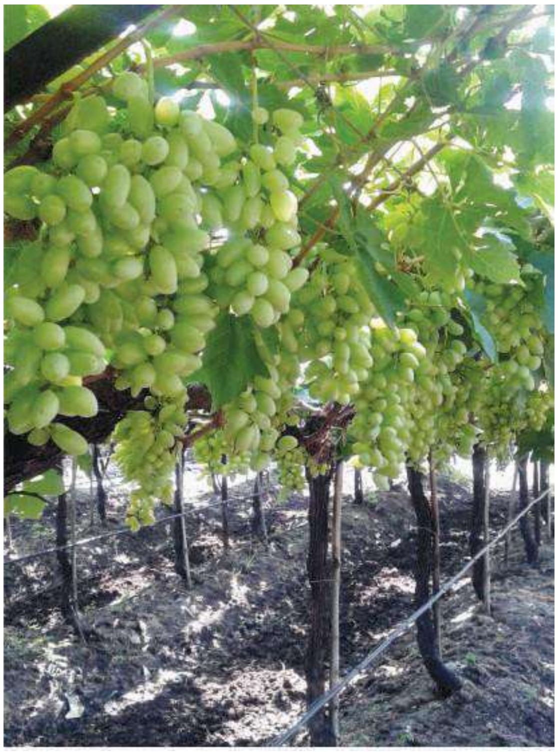


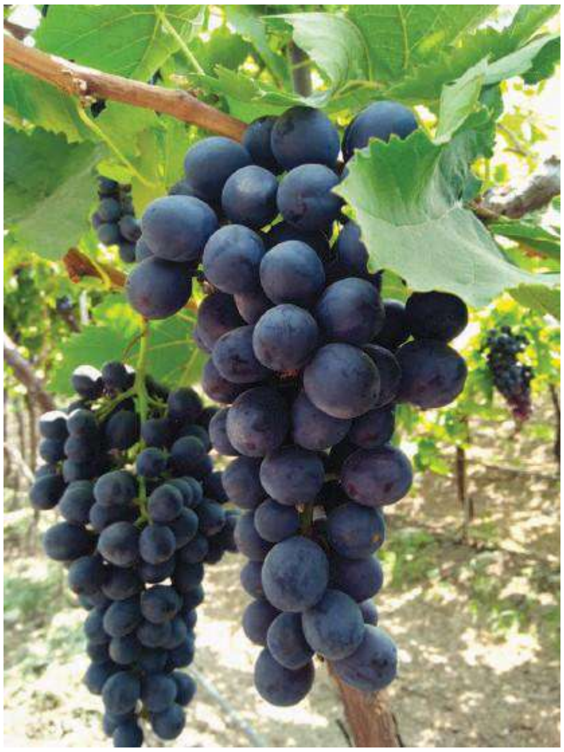
Vilas Oak
Palshet - Tal. Guhaghar
Dist - Kokan - Maharashtra State

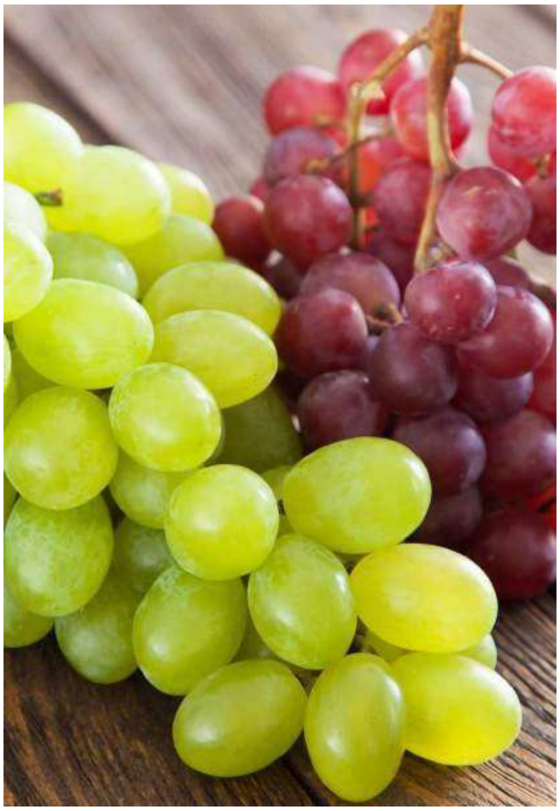
PSAP at Farmers Field

PSAP in Export Cultivation

More than hundred thousand Grape Growers are applying PSAP (ProPhite) regularly in their cultivation practices and earning more export income







PSAP at Farmers Field

PSAP Double Onion Farmer Income

May - 2018

PSAP Onion Harvest

1. Only 3 months period
2. Only 2 month irrigated
3. 70% A grade 250-300 gm each, 25 % Medium size 150-200 gm each, 5 % small 60-80gm size

87% more production in every year comparison

Saving

1. Urea 80%saved in Rs .1500
2. NPK 50%saved, Rrs.1165
3. Pesticide. 100% Saved Rs 2500

किसान का नाम : अशोक जाट, गांव : गन्गात खेडी,
प्रयोग का रकबा : २ बीगा,
फसल : प्याज/कांदा,
स्प्रे लिए. : ४, ६ पंप, १२ पंप, २० पंप और २४ पंप,
फसल में बडत, ८० कुंटल अनुमानित और १५० कुंटल प्राप्ति, ७० कुंटल अधिक
पी.एस.ए.पी. की लागत ३ किलो @१८००/-=५४००/-
और अधिक उपज के कारण
मुनाफा ४९०००/- (७००० किलो @७ प्रति किलो.)

Name : Ashok Jat

PSAP at Farmers Field

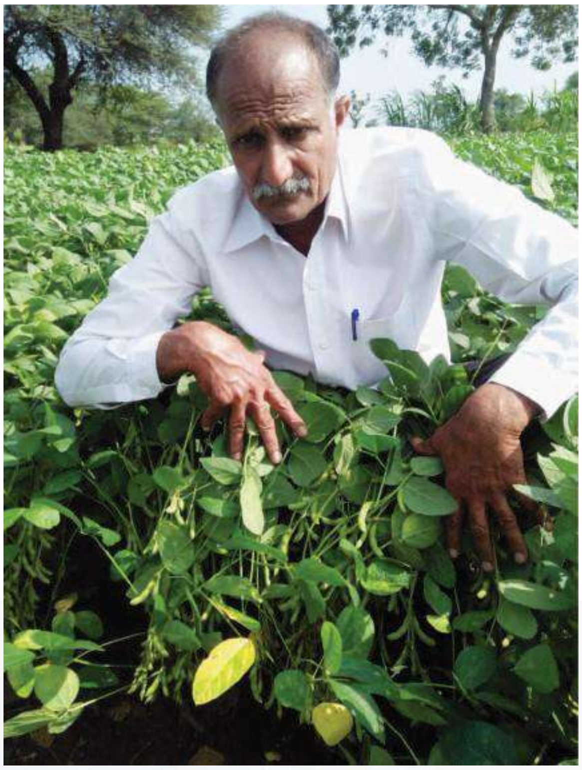
PSAP Double Soyabean Farmer Income

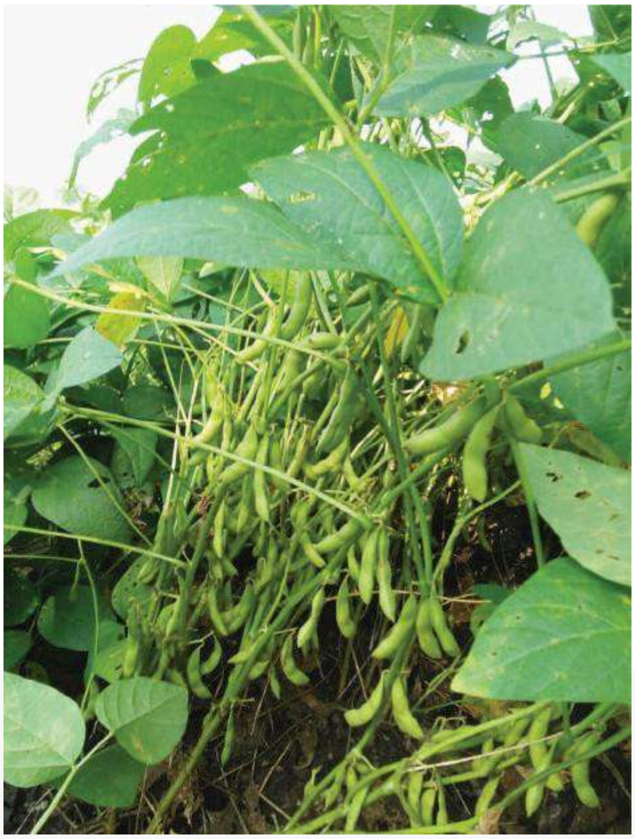
Production of Soyabean 50 qtl / Hectare
50% Increased in Yield 50% Reduction in Spray Chemicals

Feb - 2017

Islampur. Tal. Valva. Dist.Sangli.

Shrikrishn Hasabnis





PSAP at Farmers Field

PSAP in Sustainable Agriculture

More than 20,000 Growers are applying PSAP (ProPhite)
regularly in their Pomegranate Cultivation Practices.
They are satisfied with PSAP application.
Day by day number of PSAP
growers are increasing.

400 tonnes per hectare record
sugarcane harvested by applying PSAP

100 tonnes per hectare additional cane harvested by applying 30 kg PSAP

Farmer's name : Prashant Latpate

Address : Taluka- Ashta, District- Sangali, Maharashtra (INDIA)

PSAP used : 10kg per acre in 8 sprays

Total plot area : 77 R

Variety : Co 86032

Plantation : Adsali (16 Months)

Average millable canes : 35000 to 40000 per acre

Average no of internodes : 48 to 52

Average nodal length : 6 inchs

Average cane weight : 3.5 to 6 Kg

Previous season yield:120 tonnes per acre

For More Details- +919372618677

Foreword

It gives us immense pleasure while introducing PSAP technology. We hope, the data compiled in this book will benefit to the agricultural fraternity and to the nation at large. The content of book is not just a catalogue or a compilation of technical data. However it is about the invention of PSAP, purpose and potential, likely mode of action, and its role in pathways and metabolisms. PSAP methods and especially its application by the farmers to various crops will definitely help to grow the farmer's income in multifold. It is our proud privilege to announce that this is a first document of its kind ever written so exhaustively on any technology in agriculture as like PSAP. Here we have endeavored to explain the concept of PSAP to the readers in a lucid language; however, technical narration at certain places is inevitable, as the subject so demands. It is a matter of tremendous pride for all the scientists, who under the priceless guidance of **late Dr. N.R. Iyyangar, Director NCL-CSIR, Pune,** invented PSAP Molecule for the first time in India. Thus, it goes without saying that we are the pioneers of introducing PSAP technology in India. PSAP technology has been tested by various agricultural research Institutions and also on the farmer's fields at various places in our country. PSAP technology caters to the needs of the farmers in increasing crop productivity, reducing cost of cultivation and improving produce quality by retaining its inherent nutritional values. Technology also urges in reducing the chemical load on crop plants, there by reducing soil depletion rate and offering safety to the consumers with reduction in pesticide applications.

We are indebted to all our fellow-scientists, who not only extended their unstinted cooperation through-out the process of invention of the PSAP molecule, but who toiled hard from dawn to dust for achieving this goal by maintaining team spirit. Invention of PSAP Molecule is nothing but an outcome of untiring efforts, patience & perseverance of our scientists, who have proved that there is no limit to what one can achieve with sincere efforts, directed properly. **We are humble in our efforts, but proud of our achievement.**

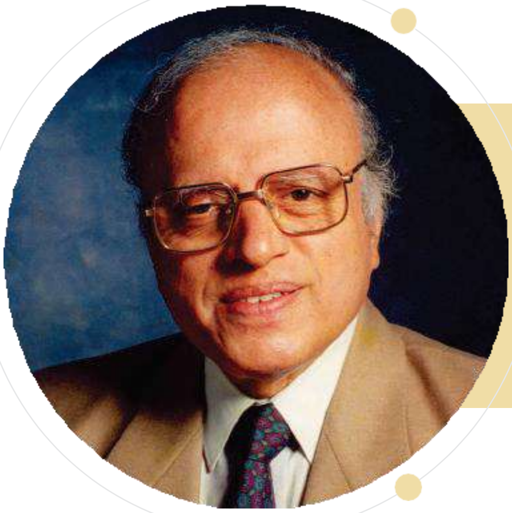
It is very often said that **"no human being is infallible"** and we also do not claim to be an exception to this Gospel Truth. Therefore, we shall be glad to receive any useful suggestions from our esteemed readers, which will help us carry out improvisation in our approach, if deemed appropriate.

PSAP Technology Felicitations

Hon’ble Radha Mohan Singh
Minister of Agriculture and Farmers’ welfare of India
New Delhi

Hon’ble Surya Pratap Shahi
Cabinet Minister of Agriculture in Uttar Pradesh Government

Hon’ble Dr. Singh
DDG-ICAR New Delhi, Sugarcon 2019
IIRS - Lucknow



M.S. Swaminathan
Founder Chairman
Ex-Member of Parliament (Rajya Sabha)

I am pleased to know Prashant P. Nandargikar and team of scientists under the guidance of late Dr. N. R. Iyyangar - Former Director NCL-CSIR Pune invented PSAP molecule.

PSAP alleviates abiotic and biotic stress in crop plants.

Sustainable agriculture becomes a mile stone in current environmental conditions. Developmental activities in crop varieties and production technology are gaining momentum than ever before.

To consolidate and confine these efforts PSAP will have major role to play.

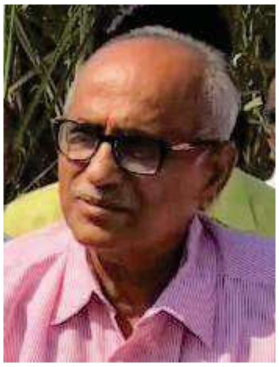
I congratulate PSAP promoters for their dedicated and farmer oriented approach while taking the technology to the fields.

I wish you all a great success in rediscovering the agriculture on residue free ground.

M S Swaminathan

M S Swaminathan

Sugar Industries and Cane Farmers



I have visited various on-farm trials and demonstrations conducted by the ISHA AGRO INDIA, Pune, in Maharashtra (Ahmadnagar, Beed, Satara, Sangli and Kolhapur districts); Karnataka (Belgavi, Bagalkot, Kalburg and Bellary districts) and Telangana (Mehbubnagar districts) and found 40% to 60% increase in sugarcane yield and quality improvement. We also tried to understand the response of farmers and observed that those who tried this product not only on sugarcane but also on other crops such as vegetables, cereals, flowers, fruits and oil seeds were happy and interested in using PSAP repeatedly.

The experiment conducted at CSRS Padegaon of Mahatma Phule Agricultural University, Rahuri Maharashtra (2014/15) has clearly brought out that foliar spray of PSAP (Potassium Salt of Active Phosphorus) has a significant effect on sugarcane yield with an average improvement of 18.26 t/ha in cane yield (At any given dose of P and K through soil). Besides, CCS percentage was improved by 0.26% (2.64 to 3.30 t/ha additional sugars.)

The reasons of the above spectacular yield and sugar improvement is that, a though in small quantity, the most active forms of P and K are readily available at the right place of metabolism (green leaves) where they are required. Activated Phosphorus in PSAP is manufactured by a catalytic process and Potash is attached to Phosphorus by split technology.

I STRONGLY RECOMMEND THE CONCERNED FARMERS AND SUGAR MILL MANAGEMENT THAT TAKE ADVANTAGE OF THIS NEW WONDERFUL TECHNOLOGY TO IMPROVE CANE YIELD AS WELL AS CANE QUALITY FOR COMING OUT OF THE CURRENT ADVERSE SITUATION.

Dr. D. G. Hapase
Director
VSI- Vasantdada Sugar Institute (1984 to 94)
Sugarcane Specialist
Central Sugarcane Research Station (1976 to 1984)
M : +91 98 230 37590

Preface

The world's population is increasing rapidly day by day and would demand more food from the limited natural resources such as land and water. Keeping in view the need of 7 billion people worldwide, agricultural productivity will have to be increased substantially by using available resource, which are being depleted rapidly. Thus to fulfil the basic needs of present and future populations is a more challenging task for farming communities and agricultural technologists.

Over the years agricultural scientists have developed various crop varieties and production technologies. Agricultural development organizations and funding agencies are doing their best to improve yield and quality of agricultural produce. All these efforts seem inadequate.

Nutrients play an important role in crop production. Phosphorus (P) plays a major role in metabolic processes, and potash (K) is important for inducing ability to tolerate various stress. Conventionally, these major crop nutrients are supplied through chemical fertilizers through soil. But 90% of phosphate gets fixed in soil and only 10% is absorbed by crop plants. Potash is given in ionic form, whereas its associated cation has a role that is not synergetic to the given potash.

To overcome these constraints on phosphorus and potash we invented PSAP-Potassium salt of active phosphorus-by using catalytic technology. The technical molecule of PSAP is 180% water soluble and quickly gets absorbed by green leaves.

Besides its vital role in plant metabolism, PSAP induces tolerance to abiotic and biotic stress. Hence the crop yield and quality improve to the best of their potential even under stress conditions. Application of PSAP increases plant productivity from 30% to 100% with remarkable improvement in produce quality along with reduction in the cost of cultivation. The inclusion of PSAP in farming will certainly boost the economy of farmers and earn substantial profits in farming. PSAP thus is a molecule of choice for doubling farmer's income by improving the yield and quality and reducing the input cost.

We attempt here to present PSAP technology to interest research workers in all areas of plant sciences. As a result of climate change it is expected that stress in many combinations such as abiotic + abiotic, abiotic + biotic, or biotic + biotic is going to affect crop performance adversely. We trust that information covered in this book will be useful in building strategies to counter abiotic and biotic stress in crop plants.

Prashant P. Nandargikar
+91 93 726 18677

Acknowledgements

We thank all the scientists, research workers, directors of research institutes, hon'ble vice chancellors of agriculture universities, technologists and administrators involved in research and development activities for sparing their valuable time to discuss and understand the impressive effects of PSAP (Potassium Salt of Active Phosphorus) on various crops. The discussions were very fruitful while exploring the various facets of application of PSAP to different crops including sugarcane.

The research trials of PSAP conducted at various institutions and on-farm demonstrations under the guidance of very eminent scientists helped us in studying the impact of PSAP on yield and quality of farm produce.

Even the guidance of cane staff from sugar mills helped us immensely while understanding the mode of action of PSAP and its performance under various soils and agro-climatic conditions under which sugarcane is grown. We are very grateful to all the staff for their guidance, support and technical help.

It is incredible that PSAP is absorbed quickly through foliage and does not have any residual effect on the plant or soil as compared to other fertilizer sources of elemental P and K. Besides, the quantum requirement of phosphorus and potash supplied through PSAP is very less compared to those applied through conventional fertilizer sources.

In the process of evaluation of PSAP, we received very valuable guidance from senior technologists as well as many innovative farmers who readily volunteered for conducting on-farm demonstrates cum trials.

Similarly, the management of some progressive sugar industries also readily supported our humble efforts and cooperated in conducting cane yield and quality evaluation in their area of operation. Without their support, we would not have come to the conclusion that PSAP definitely improves cane yield by 30%-60% and sugar recovery from 0.3 to 1.0% per unit.

Active Phosphorus and Potash of PSAP are one of their kinds in the world of chemistry. Those who have seen and experienced the performance of PSAP on the field are of unanimous opinion that, “PSAP molecule will help another green revolution in india and PSAP will turn around agriculture across the globe ”.

We very sincerely thank all those who helped us in evaluating this wonderful revolutionary molecule. During the discussions, they shared some insights on PSAP with different prospectives.

Ultimately, it is concluded that PSAP alleviates Abiotic and Biotic stress in crop plants. PSAP can transform agriculture from land to lab.

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4. PSAP

5. **Abiotic and Biotic Stress**

PSAP In C3 Crop Plants

6. PSAP

PSAP In C4 Crop Plants

7. **Challenges in Sugarcane**

10. **Abiotic Stress and Yield Losses**

14. PSAP

By 50% to 120%

Benefits Estimated from Farmer's Field Data and Test Reports from Various Institutions

Crops	Yield Increase 1	Reduction in Synthetic Chemicals 2	Quality Produce Fetches Higher Price to Farmer 3	Overall Increase in Farmers Income 4 = 1+2+3
Onion, Tomato, Potato, Chilly, Capsicum, Carrot, Turmeric, Ginger, Leafy vegetables, Guar, Citrus, Pumpkin, and Cucurbits	50% to 100%	50% to 70%	20% to 30%	**120%**
Oil Seeds, Cereals Grains, Fiber Crops, Paddy, Other Legume Crops, Soybeans, Pulses and Cotton	20% to 40%	25% to 50 %	5% to 10 %	**50%**
Avocado, Banana, Guava, Papaya, Fig, Orange, Tea, Watermelon, Clove, Mango, Pomegranate, Kiwi, Arecanut, Cacao, Walnut, Peach, Vanilla, Strawberry, Coffee, Pepper, Grapes, Cardamom, Cinnamon and other Herbs, Lychee, Apple, Stone Fruit, Olives, Tree nuts, Pepper and Ornamentals.	50% to 100%	50% to 100 %	20% to 30 %	**120%**

Challenges in Agriculture

Losses in Yield Potential

Increasing World Population, Depleting Natural Resources as well as Abiotic and Biotic Stress are the challenges in producing food

The world's population is increasing at an alarming rate and is expected to reach about ten billion by the end of 2050. The growing population will result in considerable additional demand for food and will also contribute towards climate, which is an alarming issue to the world's food safety.

- Increasing world population is putting pressure by way of extra demand for food grains.
- Natural resources are shrinking faster than before.
- Abiotic factors are leading to under utilization of the genetic potential of crops.
- Applications of too many toxic chemicals sprays to manage biotic stress is creating environment concerns.
- Soil health has been deteriorating because of over emphasis on synthetic fertilizers as well as due to low or zero application of organic matter to soil.
- Toxic residues are a serious health concern.

Because of the deleterious effects of various forms of stress, food productivity is decreasing, and to minimize these losses is a major concern of our nation to cope with the increasing food requirements.

Temperature stress are (high and low temperatures) is a major environmental factor affecting plant growth, development and also induces morphological, physiological and biochemical changes in plants.

According to a report of the Intergovernmental Panel on Climatic Change (IPCC Expert Meeting Report, 2007), the global mean temperature will rise 0.2^{0}C per decade in the coming years. This change in global temperature may alter the geographical distribution and the growing season of crops. Crop plants are often exposed to various environmental stress factors, such as drought, soil acidity, salinity and extreme temperatures, which severely affect soil productivity and crop production, worldwide. Bray (2000) and Paul H Moore (Bioen-Moore) estimated that global losses in crop production due to environmental stress factors are becoming increasingly serious..

Challenges in Agriculture

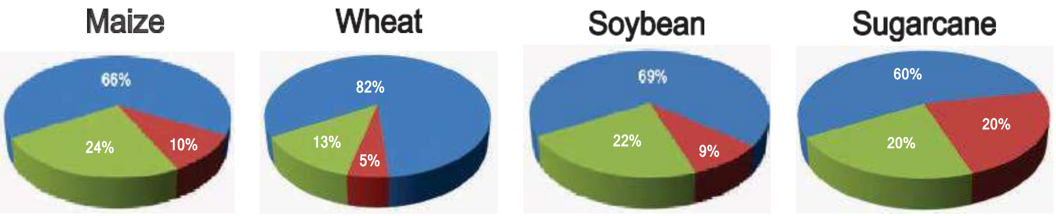
Losses in Yield Potential

Decreases in record yield capacity of crop plants by abiotic and biotic stress factors

🟦 Losses by abiotic stress

🟥 Losses by biotic stress

🟩 Present average yield



	Maize	Wheat	Soybean	Sugarcane
Record yield : (tonnes per hectare)	19.3	14.5	7.4	472
Average yield : (tonnes per hectare)	**4.6**	**1.9**	**1.7**	**90**

Record yields are yields under ideal conditions and decreases from the record yield capacities of maize, wheat, soybean and sugarcane caused by abiotic and biotic stress factors (Bray et al.,2000) and Sugarcane Biology, Yield and Potential for Improvement... Paul H Moore (Bioen-Moore).

Above figure shows that the relative decrease from the record yield i.e. potential yield (maximum yield under ideal growth conditions) caused by abiotic stress factors varies between 60% and 82% for maize, wheat, soybean and sugarcane. In case of wheat and soybean, record yields are 14.5 T/Ha and 7.4 T/Ha, respectively, but the current world averages are 1.9 T/Ha and 1.6 T/Ha, respectively.

When compared yield capacity losses in maize, wheat, soybean and sugarcane caused by abiotic stress factors are much higher than those caused by biotic ones. Most of the yield losses caused by abiotic stresses are attributed to drought, salinity, extreme temperatures, acidity, and impairments of the mineral nutritional status of plants, i.e., deficiencies and toxicities.

Cakmak (2002) reported that at least 60% of cultivated soils worldwide have growth-limiting problems arising from mineral nutrient deficiencies and toxicities. Combinations of such soil nutritional problems with other environmental stress factors such as drought, salinity and cold are responsible for severe losses in crop production worldwide.

Survival and productivity of crop plants exposed to environmental stresses are dependent on their ability to develop adaptive mechanisms to avoid or tolerate stress.

Challenges in Agriculture

Losses in Yield Potential

Accumulating evidence suggests that the nutritional status of plants greatly affects their ability to adapt to adverse environmental conditions.

In response to these temperature stresses, various approaches are being used, which can mitigate the effect of stress and lead to the adjustment of the cellular milieu and plant tolerance. In nature, stress does not generally come in isolation: a conglomerate of stresses acts in concerted manner. In response to these, there are stress signals that talk to each other, and plants have developed diverse mechanisms naturally for combating and tolerating stress.

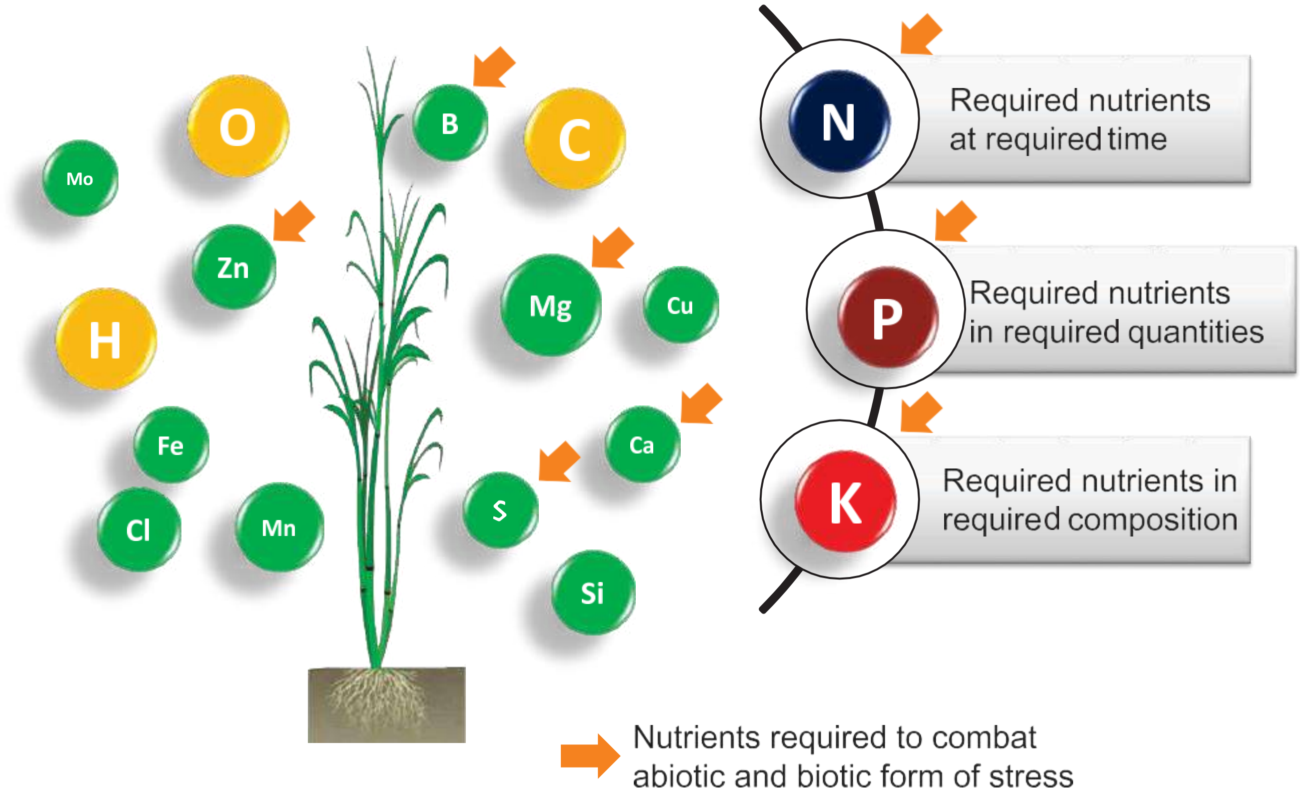
In this review, we have first emphasized high temperature stress followed by low temperature stress and their injurious effects on plants. Various mechanisms involved in cold and hot acclimation and their role towards membrane stabilization have also been discussed. The physiological and biochemical mechanisms pertaining to each stress, followed by the role of phosphorus, potassium, calcium and manganese are also important. Lastly, we propose how active Phosphorus and Potash play a role in alleviating abiotic and biotic stress in crop plants.

PSAP-Treated Healthy Pomegranate Plantation

Challenges in Agriculture

Mineral Elements, their Role and Application

8 Mineral elements combat different forms of stress



Mineral elements classified on the basis of their mobility within a plant and their tendency to re-translocate when deficient

Mobile	Immobile
Nitrogen (N)	Calcium (Ca)
Potassium (K)	Sulfur (S)
Magnesium (Mg)	Iron (Fe)
Phosphorus (P)	Boron (B)
Chlorine (Cl)	Copper (Cu)
Sodium (Na)	
Zinc (Zn)	
Molybdenum (Mo)	

Elements are listed in the order of their abundance in the plant.

Challenges in Agriculture

Mineral Elements, their Role and Application

1. Recommended balance nutrition. — Farmers tend to apply more nitrogen.
2. Uptake is poor. — Phosphate fertilizers.
3. Adequate K is very important in sugarcane. — Excess K is detrimental.
4. As per the recommendation 1 kg of chloride per acre is required to complete sugarcane cycle. — K is given as KCl-(Muriate of potash). Bag of 50 Kg. MoP has 25 kg of K and 25 kg. of Cl.
5. We tend to apply 25 to 50 times excess quantity of Cl. — Excess chloride in sugarcane has adverse impacts on soil as well as yield and quality of sugarcane.

Limitations of Phosphorus-based fertilizers

Solubility

Due to the composition, its solubility in water is very poor, varies from 5% to 22%

Fixation

Majority of applied Phosphorus get fixed in soil and 3 to 15% is only available for crops based on overall conditions

Uptake

Phosphorus is absorbed by diffusion and until it is dissolved in water, it cannot be absorbed through roots

Absorption

Absorption of these fertilizers through foliage is hardly 5 to 8 %, If its residue remain on plants, it supports the growth of phytopathogens

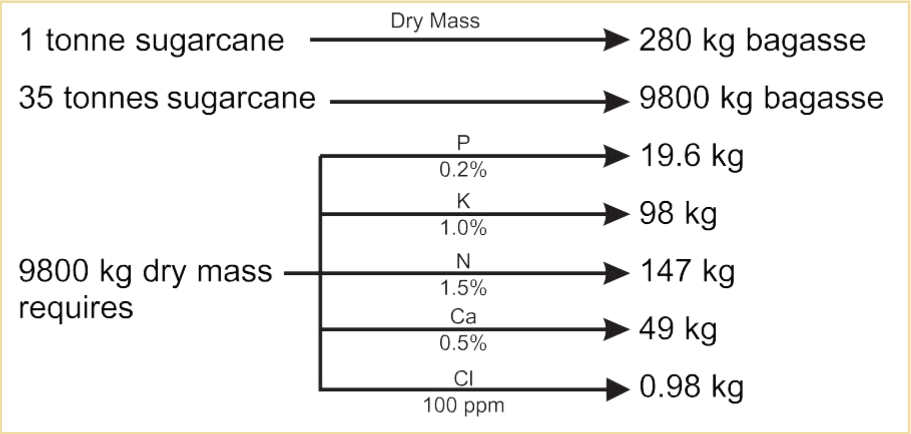
Availability

Availability of applied phosphorus fertilizer is uncertain, it may vary from 15 to 60 days based on soil and environmental conditions. Quantity of absorption is uncertain

Adequate element composition of plant tissue

Element	Chemical symbol	Concentration in dry matter (% or ppm)
Obtained from water and carbon dioxide (%)		
Hydrogen	H	6
Carbon	C	45
Oxygen	O	45
Obtained from the soil (%)		
Macronutrients		
Nitrogen	N	1.5
Potassium	K	1.0
Calcium	Ca	0.5
Magnesium	Mg	0.2
Phosphorus	P	0.2
Sulfur	S	0.1
Silicon	Si	0.1
Micronutrients (ppm)		
Chlorine	Cl	100
Iron	Fe	100
Boron	B	20
Manganese	Mn	50
Sodium	Na	10
Zinc	Zn	20
Copper	Cu	6
Nickel	Ni	0.1
Molybdenum	Mo	0.1

Source: Epstein 1972, 1999. The values for the non mineral elements (H, C, O) and macronutrients are percentages. The values for micronutrients are expressed in parts per million.



Nutrients according to their Biochemical Functions

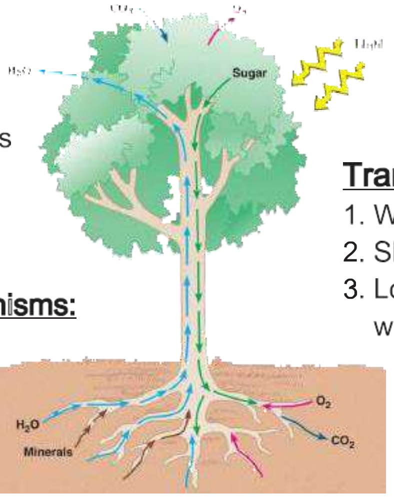
Mineral nutrient	Functions
Group 1	Nutrients that are part of carbon compounds - Precursors
N	Constituent of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexosamines, etc.
S	Component of cysteine, cystine, methionine, and proteins. Constituent of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, adenosine-5' -phosphosulfate, and 3-phosphoadenosine
Group 2	Nutrients that are important in energy storage or structural integrity
P	Component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid, etc. Has a key role in reactions that involve ATP.
Si	Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties, including rigidity and elasticity, of the cell wall.
B	Complexes with mannitol, mannan, polymannuronic acid, and other constituents of cell walls. Involved in cell elongation and nucleic acid metabolism.
Group 3	Nutrients that remain in ionic form - Recoup enzymatic activity
K	Required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electro neutrality.
Ca	Constituent of the middle lamella of cell walls and also forms part the cell wall in the form of calcium pectale. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a secondary messenger in metabolic regulation.
Mg	Required by many enzymes involved in phosphate transfer. Constituent of the chlorophyll molecule.

Mineral nutrient	Functions
Cl	Required for the photosynthetic reactions involved in O_2 evolution.
Mn	Required for activity of some dehydrogenases, decarboxylases, kinases, oxidases, and peroxidases. Involved with other cation-activated enzymes and photosynthetic O_2 evolution.
Na	Involved in the regeneration of phosphoenol pyruvate in C_4 and CAM plants. Substitutes for potassium in some functions.
Group 4	Nutrients that are involved in redox reactions
Fe	Constituent of cytochromes and non-heme iron proteins involved in photosynthesis, N_2 fixation, and respiration.
Zn	Constituent of alcohol dehydrogenase, glutamate dehydrogenase, carbonic anhydrase, etc.
Cu	Component of ascorbic acid oxidase, tyrosinase, monoamine oxidase, urecase, cytochrome oxidase, phenolase, laccase, and plastocyanin.
Ni	Constituent of urease. In N_2 - fixing bacteria, constituent of hydrogenases.
Mo	Constituent of nitrogenase, nitrate reductase, and xanthine dehydrogenase.

Source: After Evans and Sorger 1966 and Mengel and Kirkby 1987.

Transport in Plants

Physical forces drive the transport of materials in plants over a range of distances



Transport occurs on three scales

1. Within a cell - cellular level
2. Short distance cell to cell - tissue level
3. Long distance in xylem & phloem - whole plant level

Transport occurs by 3 mechanisms:

A. Osmosis & Diffusion
B. Activate Transport
C. Bulk Flow

Phosphorus

Application and Role

Three forms of Phosphorus are applied to crop plants

1. Nutrient based phosphorus : in phosphate form : PO_4^-

Phosphates are applied to crop plants as a source of nutrient. In the form of chemical fertilizers such as super phosphate, 00-52-34, 10-26-26, DAP (diammonium phosphate) (12-61), 19-19-19 as well as from organic matter. Solubility, fixation / leaching, availability and uptake are the problems associated with phosphorus and potash-based fertilizers. Even after spraying, these fertilizers are very poorly absorbed by foliage of majority crop plants and if remained on plant, the residue supports fungal growth. Hence managing PO_4^- in the field conditions is very difficult.

2. Fungicide-based phosphorus : in phosphite form : PO_3^-

Mono and di potassium salt of phosphorous acid and/or potassium salt of phosphonic acid or potassium phosphite, are some of the name of the molecule. They are normally applied by growers in combination with some fungicides such as captan, mancozeb. For pH correction of these fungicides as well as PO_3^- molecule has been reported in various references to have some specified role in management of diseases caused by oomycetes. However, it is phytotoxic if applied in excess. Carbon - Phosphite molecules are phosphonates also refer as PGR or fungicides like fosetly-Al and N-(phosphonomethyl) glycine herbicide.

3. Stress-alleviator-based phosphorus : in active Phosphate form : PSAP

PSAP is an autonomous form of phosphorus that plays a major role in biosynthesis of primary and secondary metabolites including SAP (shikimic acid pathway). It overcomes the limitation of phosphate and phosphites molecules in its application to crop plants. Application of PSAP along with fertilizers creates a synergetic effect. PSAP supports generation, storage and translocation of bio-energy in ATP/NADP bonds. The availability of ATP and a reductant in the form of NADPH helps the plants to scavange ROS and adapt to stress. Hence, recovery of various metabolic process from stress in PSAP-treated plants is very fast and effective. Active phosphorus alleviates abiotic and biotic stress in crop plants.

Active phosphorus is identified and authenticated as stress alleviator when applied with potash.

Phosphorus

In Stress

PSAP : Autonomous Combination of Phosphorus and Potash

Plants frequently cope with rapidly fluctuating and adverse environmental conditions because of their intrinsic metabolic capabilities. Variations in the outside environment could put the plant metabolism out of homeostasis and make it necessary for the plant to harbour some advanced genetic and metabolic mechanisms within its cellular system. Plants possess an array of protective mechanisms acquired during the course of evolution to combat adverse environmental situations. Such mechanisms cause metabolic re-programming in cells to facilitate routine physico-biochemical processes irrespective of the external situations. Without phosphorus in the environment no living organisms could exist. Phosphorus is present in all plant and animal tissue. It is necessary for such life processes as photosynthesis, the synthesis and breakdown of carbohydrates, and the transfer of energy within the plant. Phosphorus is taken up by the plant from the soil. Unless the soil contains adequate phosphorus or it is supplied to the soil from external sources, plant growth will be limited. Phosphorus does not occur as abundantly in soils as does the other major nutrients, nitrogen and potassium. Phosphorus occurs in both inorganic and organic forms in the soil. Small fraction of the total phosphorus is in a form available to plants.

	Nutrient-based phosphorus (PO_4^-)	**Fungicide-based phosphorus (PO_3^-)**	**Stress Alleviator-based active phosphorus**
Base	a. Phosphate : PO_4^- Synthetic fertilizer base b. Organic Phosphorus	a. Alkali metal salts fungicide base b. Carbon compound growth regulator base	Molecular combination of active phosphorus and potash catalytic base
Function	Phosphorus is a major plant nutrient that induces virtually all the biochemical processes and development phases of crop plants.	These products have fungicistic mode of action and / or regulate some metabolisms. However PO_4^- and PO_3^- phosphorus share antagonistic relationship. Hence do not replace each other.	Phosphorus in active form has important role in stress alleviation. Role of active phosphorus is complementary and supplementary to nutrient base phosphorus PO_4^- . Phosphorus and potash from PSAP get rapidly absorb and quickly translocate in crop plants.
Limition	**Synthetic fertilizers** • Solubility • Fixation/Leaching • Uptake • Absorption • Availability • Soil and water pollution **Organic phosphorus** • Very slowly available • Inadequately available • Soil bacterias are required • Poor source of phosphorus	**Alkali metal salts** • Crop wise specific application • Phytotoxic • No direct role in growth • PO_3^- unsuited in ATP generation **Carbon compounds** • Some compounds have MRL • May hinder growth metabolism • Debate is going on towards its environment-friendly utilization	Can be applied at any given stage as well as condition of crop plants

Convention and Comparison of all three forms of Phosphorus follows.

Phosphorus

Application and Role

Phosphate and PSAP

- Phosphates are produced by neutralizing phosphoric acid with base such as KOH and ammonia. Whereas potash is given as salts of chloride, sulphate, nitrate and phosphate as fertilizers to crop plants through soil.

  PSAP is produced by splitting phosphorus and attaching K with catalytic technology.

- Phosphate is a source of nutrients and plays a vital role in plant metabolism but has limitations in applications to crop plants. Due to uncertainty in its uptake by crop plants, phosphate-based fertilizers are not suppose to have role in stress. However, potash has a key role in stress management in crop plants.

  Active P and K from PSAP alleviate all forms of stress in crop plants.

- Due to the chemical composition of phosphatic fertilizers, they are poorly soluble in water e.g. mono potassium phosphate is 22% soluble i.e. 220 grams in one litre of water. DAP is approximately 20% water soluble.

  Technical molecule of PSAP is 180% water soluble i.e. 1800 grams in one liter.

- Phosphate fertilizers are not readily taken up by the foliage of many plants and require to be delivered through soil: 4% to 15% of applied P and K fertilizers get absorbed through roots depending on soil and environmental conditions.

  Majority of PSAP can easily be taken up by leaves, stems, and roots of crop plants.

- The mobility of phosphate fertilizers in the soil is limited leading to rapid localized depletion of phosphorus in the rhizosphere, resulting in phosphorus deficiency.

  PSAP does not get fixed in any kind of soil and is easily absorbed by roots.

- Potash fertilizers also have problems similar to phosphate fertilizers. In spite of potash being available in soil, potash deficiency gets noticed in crops.

  Combination of phosphorus and potash in PSAP is particularly very synergetic.

Phosphorus

Convention and Comparison

Phosphate and PSAP

- Fixation calls for frequent re-applications of phosphate fertilizers, which leads to leaching of phosphate into groundwater resulting in eutrophication of lakes, ponds and streams. It creates health issues for living beings. PSAP, in comparison with conventional fertilizers, is required in very small quantities and it does not get fixed in any kind of soil. Hence PSAP is very safe in soil.

- Phosphate fertilizers inhibit the beneficial symbiosis between roots and mycorrhizal fungi.

  PSAP supports the beneficial symbiosis between the roots and mycorrhizal fungi but may prevent the growth of pathogenic fungi and other soilborne pests by inhibiting oxidative phosphorylation in fungal pathogen.

- P and K fertilizers do not support the uptake of other micronutrients.

  PSAP supports the uptake of other micronutrients by increasing root biomass.

- Soil loses its quality and fertility as well as increases salinity of groundwater with excess application of P and K fertilizers.

  PSAP does not pollute groundwater and helps to improve the quality of soil. PSAP facilits the ionization of soil minerals and these ions can be easily taken up by roots.

Soyabean from PSAP-treated Plant **Soyabean from Control Plant**

Phosphorus

Convention and Comparison

Phosphate and Phosphite

- PO_3^- first came into limelight when it was thought to overcome the shortage of PO_4^- during Second World War.

- Since 1970, PO_3^- has been studied by many universities in USA, Europe, and elsewhere at molecular level for its role in fungicides, fertilizers and bio-stimulants.

- Potassium salt of phosphorous acid or potassium salt of phosphonic acid or potassium phosphite, is nothing but an ion compound of PO_3^- These are different names of the same molecule.

- Mono potassium salt $K^+(H_2PO_3)^-$ or di potassium salt $K_2^+(HPO_3)^-$ of PO_3^- is called potassium phosphite. Aluminum salt of organic phosphorous acid is a phosphonate called as a fosetyl - Al or aluminum tris (O-ethyl phosphonate), these phosphonates can be used as fungicide or pesticide.

- PO_3^- has direct and indirect mode of action on phytopathogens. It is being reported that fungal diseases caused by Oomycete are controlled by the PO_3^- to the extent of 60 to 70% in combination with appropriate fungicide.

- PO_3^- does not take part directly in photosynthesis, root growth, respiration and also does not take part in any ATP and NDPH synthesis like PO_4^-. Although the mobility of PO_3^- and PO_4^- are similar, there is no evidence to suggest that plant can take PO_3^- as a source of Po_4^-.

- PO_3^- after entering into the plant remains stable throughout the life cycle and no known metabolism in the plant can convert the PO_3^- into PO_4^- and use if as a nutrient. Higher concentration of PO_3^- hinder the uptake of PO_4^- and is toxic to almost all species. Hence PO_3^- is applied to crop plants very cautiously.

- The recommendation of PO_3^- is 1 to 3 kg per acre (if in liquid form i.e. 1 lit. to 3 lit. per acre) in 2 to 5 treatments in the season. It is reported that majority of crop plants can die with excess use of PO_3^- ion.

- PO_3^- was found to have a negative effect on the growth of PO_4^- deficient plants by suppressing the typical molecular and developmental responses to PO_4^- deficiency.

Phosphorus

Convention and Comparison

Phosphate and Phosphite

- The effect of PO_3^- on crop plants is not consistent, but depends strongly on the PO_4^- status of plants. In most cases, the deleterious effect of PO_3^- is evident in PO_4^--starved plants but not in PO_4^--sufficient plants.

- PO_3^- ion and nutrient PO_4^- are strangers and incompatible with each other. The transport path of both the ions in the plant is same. Hence it is reported, that PO_4^- sufficient plant does not respond to PO_3^- in disease incidence. PO_3^- has adverse effect on growth when plants are PO_4^- deficient. PO_3^- enters into the plant quite faster than PO_4^- does and hinders further uptake of PO_4^- as both share the same transport channel.

- The various defence pathways including SAP (Shikimic acid pathway) are assumed to be triggered to a limited level inspite of effective application and further utilization of both PO_3^- and Po_4^-

- Since the chemistry of PO_3^- and PO_4^- is mutually not supportive, that limits in the utilization of these ions even after proper applications of both.

- At though some products claim to be a combination of PO_3^- and PO_4^- in their formulation, due to their antagonism, combination fails to provide the synergy.

- PO_3^- and PO_4^- taken together consistently show that plants areincapable of directly using PO_3^- as phosphorus source and thus PO_3^- cannot substitute PO_4^- fertilizer.

- It appears that during enzymatic biochemical reactions in living organisms, PO_4^- binding sites recognize three of the four O atoms, and the remaining O that protrudes from the surface of PO_4^- molecule to become available for taking part in enzymatic reactions. Hence, PO_3^- can not take part in similar biochemical reaction as because its hydrogen atom protrudes from the surface of the enzyme instead of the O atom in PO_4^- Therefore, most of the enzymes involved in phosphoryl transfer reactions can readily differentiate between PO_4^- and PO_3^-.

- Role of PO_4^- in stress management is postulated but requires further study.

PSAP and Phosphite

- It is being reported that PO_3^- has direct and indirect modes of action against Oomycetous fungi. PO_3^- inhibits oxidative phosphorlylation, a process in fungal metabolism, directly. The chemical alteration produces elicitors which are recognized by the receptors that trigger the plants defence response, is the in-direct mode of PO_3^- action. For adequate results, PO_3^- is recommended in combination with fungicide. PO_3^- is not at all recommended and used for viral and bacterial infections and many other fungal diseases.

Active phosphorus from PSAP with a complex mode of action is believed to stimulate various metabolic processes in the plant to fight against invading fungal, viral and bacterial phytopathogens. Quick and effective recovery is noticed with standalone application of PSAP in post infection of various diseases. Triggering of elicitors response, synthesis of secondary metabolites, translocation of bio-energy, effective assimilation of nutrients, recovery from stress and bringing back the plant to normal conditions by increasing production of primary metabolites are the key roles supposed to be played by PSAP.

- PO_3^- is not recommended when crops are in the dormant stage or under stress. PO_3^- is believed to act as a preventive measure that is before any disease occurs.

PSAP is particularly effective when crops are in dormant the state or under stress. In such situations higher doses of PSAP (6 to 8 grams per litre of water) are applied through spray. If required, PSAP spray is repeated at 2 to 3 days intervals. PSAP has a definite role in preventive as well as curative measures.

- PO_3^- neither reduces fungicide application nor is recommended as a substitute for P and K fertilizers.

As found in different trials, PSAP lowers the chemical load on different crops from 50% to 100% without deteriorating quality or lowering yields. In fact, both yield and quality in PSAP-treated plants are higher in comparison with traditional fertilizer applications.

Phosphorus

Convention and Comparison

PSAP and Phosphite

- PSAP does not have any phytotoxicity or no know side effects even if used in excess or sprayed more frequently. This is not the case with PO_3^- . A higher dose of PO_3^- is reported to be phytotoxic to most plant species, and 8 grams of PO_3^- per litre of water is fatal to all the plant species.

  PSAP (6 to 8 grams per litre of water) is sprayed on sugarcane for luxuriant growth and quality improvement. Grape and pomegranate growers apply 4 to 12 sprays of PSAP in the season by using 6 to 16 kg PSAP per acre to achieve several advantages which can lead towards sustainable and profitable cultivation.

- PO_3^- has very a limited role under specific conditions in particular species of plant. It is being reported that application of PO_3^- in productive stage shrinks and hinders development further in plants.

  PSAP can be applied to any crop at a given stage to get the produce of uniform size, color, as well as higher brix level along with better keeping quality. PSAP induces defence responses of plants and also maintains better growth, achieving higher yields and quality.

- Once in the plant PO_3^- remain stable throughout the plant life cycle.

  PSAP suppose to breaks down inside the plant and may supplies both PO_3^- and PO_4^- in adequate quantities.

- Active phosphorus from PSAP can overcome the limitations of nutrient based PO_4^- in agriculture, whereas PO_3^- limits the advantages of PO_4^- .

- PO_3^- and PO_4^- compete with each other.

  PSAP does not have any antagonistic effects on PO_4^- . Hence phosphorus from PSAP is unique and one of its kind in agriculture.

For references and further studies
refer 11 to 16 reports from the list of references

Phosphorus

Convention and Comparison

PSAP and Phosphite

Comparison of PSAP with Potassium Phosphite

Spray Treatment : 8 gm / liter of water

Potassium Phosphite

Before spray

72 hours after spray

Spray Treatment : 8 gm / liter of water

PSAP

Before spray

72 hours after spray

Acid Test Experiment

Comparison of PSAP with Potassium Phosphite

Spray Treatment : 8 gm / liter of water

Potassium Phosphite

Before spray 72 hours after spray

Spray Treatment : 8 gm / liter of water

PSAP

Before spray 72 hours after spray

PSAP

An Innovation

Purpose and Potential

A group of farmers explained us, how difficult it is to manage Phosphorus and Potash, the important primary nutrients that are applied in agriculture. P and K elements play profound role in various metabolisms of the crop plants like, synthesis of bio-energy and its translocation, crop ability to stand in biotic and abiotic stresses and improvement in plant bearing capacity along with enhancement in overall yield and quality. After accessing chemical composition we found P & K fertilizers have five basic limitations .

» Solubility –	The solubility of these fertilizers in water is poor, hardly 20%.
» Fixation –	Majority of P and K fertilizers get fixed in soil and only 4% to 15% is available to crops depending on soil condition rest get leach out in water.
» Uptake –	Unless and until P and K do not dissolve in water droplets, their absorption is not possible through roots.
» Availability -	Availability of applied P and K to crops is uncertain because of fixation immobilization and leaching of these nutrients in soil and hence it's the uptake through roots is uncertain and varies from 15 to 60 days based on soil, water, and climatic conditions.
» Absorption -	Absorption of P and K fertilizers through foliage is hardly 5% to 8%. It is observed that susceptibility to pest and diseases increases in crops due to application of fertilizer sprays.

To overcome the above limitations, it was decided to activate phosphorus by using catalytic technology. Before changing to its isotopic form, Phosphorus is split and Potash is attached to Phosphorus. The molecule formed is called PSAP- Potassium Salt of Active Phosphorus. PSAP field trials conducted in many zones on various crops when the crops were under stress or showed low vigor and growth or suffered from poor yields and low quality.

When PSAP was applied, such crop recovered from the adverse situations and delivered yields higher by 30% to 100% compared to those from traditional practices. It was also noticed that there was substantial reduction in input cost on synthetic chemicals. This emerging technology will be useful in building strategies to counter abiotic and biotic stress in crop plants.

PSAP

Introduction

When Efforts Spurred by Intuition
a Molecule Evoked and Emerged as a PSAP

PSAP molecule is the experimental inclination of catalyst science.
Catalyst science is the science that starts where chemistry ends.

PSAP Technology

Proven in farmer's fields and tested by agricultural research institutations

☛ Increases crop productivity ☛ Reduces cost of cultivation

Improves produce quality and retains nutritional values

- PSAP has been tried and tested on farmers' fields. PSAP technology has proved that it increases crop yield spectacularly and improves produce quality. PSAP also induces tolerance to diseases, pest, and various types of stress in crop plants. Besides, PSAP can be applied to a wide range of crops and can be instrumental in bringing in the most needed all round agricultural revolution.

- PSAP applications are easy to handle and can be used without much change in existing agricultural practices.

- Application of PSAP complements existing agricultural production technology as well as emerging technologies such as precision agriculture.

- PSAP is very effective in almost all crops: it improves plant health, induces stress tolerance, reduces chemical load, increases yield (30%-100%) and improves quality of produce (sweetness, colour, size, aroma, luster and keeping quality) ultimately benefitting farmers and consumers in terms of food safety.

PSAP

Introduction

- PSAP technology is an innovative technology invented and introduced by Indian scientists in agriculture for the first time in India.

- Active phosphorus from PSAP is the highly soluble form of Phosphorus which along with Potash alleviates abiotic and biotic stress in crop plants, launched in the agricultural in year 2010.

- After working relentlessly for 6 years on the split technique, we developed the highly active Phosphorus and used it in PSAP.

- In PSAP, Potassium is given along with Phosphorus to support as well as rebuild various metabolic process in crop plants.

- Very few companies might have such a type of technology in their portfolio.

- In India, farmers from many states are applying PSAP on thousands of hectares, growing healthier crops and harvesting bumper yields with superior quality produce.

- PSAP can be used successfully on all kinds of crops like vegetables, fruits, cereals, flowers, herbs and spices as a supplementary fertilizer and has proved a very effective nontoxic formulation that triggers plant defences at higher level for longer period of time.

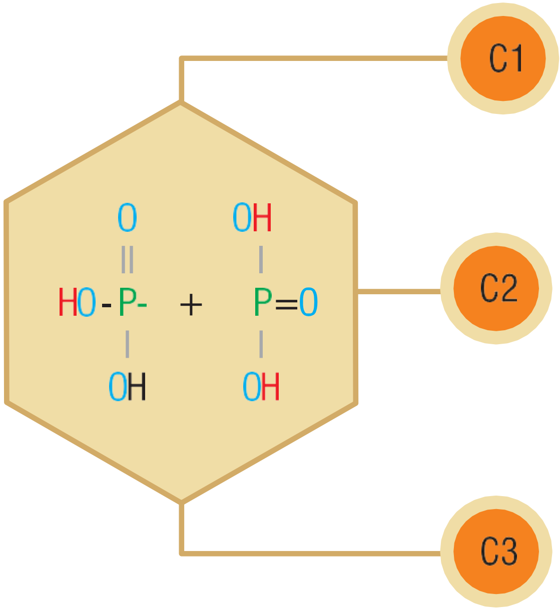
PSAP-treated plants react and respond very effectively against various diseases including the following,

(1) Downy mildews
(2) Damping off and seedling rots caused by Pythium sp, Rhizoctonia sp, Fusarium sp, etc.
(3) Phytophthora diseases such as late blight, collar rots, root rots, leather rots, red stele etc.
(4) White rust
(5) Scabs and diseases caused by Cercospora and Phomopsis.
(6) Bacterial blight caused by Xanthomonas campestris and others.
(7) Mosaic caused by viral infection.

PSAP

Introduction

In PSAP, **phosphorus is activated by catalytic technology**



C1 Dissociation of various O and H bonds in phosphorus base acids

C2 Before going to isotopic form phosphorus is split and potash is attached to this phosphorus

C3 Stabilization of phosphorus and potash

- PSAP a very pure salt, free of chloride, sodium and other detrimental elements.
- Elemental analysis of PSAP :

Phosphorous	40 % as P_2O_5
Potash	40 % as K_2O

- It is a sparkling white, free-flowing powder.
- PSAP is hygroscopic and absorbs moisture from air if kept open for short.
- Water solubility of PSAP is relatively high, 1800 grams of technical PSAP gets dissolved in a litre of water.
- pH of a PSAP solution in water is between 6 and 7.
- The shelf life of PSAP in original sealed packing is 2 years.

PSAP

Adaptability

- PSAP is the outcome of 6 years of untiring and in-depth rigorous research efforts. After launching the PSAP was tried and tested for many years in farmer's fields. Several agricultural institutes also validated the bio-efficacy of PSAP.

- PSAP is a non-toxic, people-friendly, plant-friendly and land-friendly formulation, which does not spoil the land and does not pollute ground water.

- PSAP applications are easy to handle and can be used without much change in current agricultural practices. Adaptability of PSAP under field conditions is excellent.

- PSAP is highly water soluble (180%: 1 Litre of water can dissolve 1.8 kg), very reactive, and does not get fixed in any kind of soil matrix.

- PSAP is compatible with some fungicides. PSAP can be sprayed at any given stage of crop plants. Higher doses of PSAP do not have any phytotoxicity.

- PSAP can be applied through foliar sprays, sprinkler, drip, as soil drench and root dip. Foliar application of PSAP is the most effective amongst all.

- PSAP passes easily through cuticles as well as stomata and is transported via xylem and phloem. PSAP is so effectively absorbed by the foliage and does not require any adjuvants such as stickers, spreaders, wetting agents. In comparison with conventional phosphate and potash-based fertilizers, PSAP is very rapidly taken up.

- PSAP solution can be prepared in any kind of water having pH range from 5 to 8. Pretreatment of water is not necessary while preparing PSAP solution.

- Application of PSAP complements the existing agricultural production technology. PSAP is having a major role in the economy and sustainability of agriculture.

- Phosphate-based fertilizers are absorbed through roots, and take a few days (depending on the soil and surrounding environmental conditions) whereas PSAP gets absorbed quickly in most of the soil under modarate environmental conditions.

- By drenching PSAP, soil born diseases such as collar rot, and root rot can be minimized. PSAP elicits the innate immunity response in developing roots, which destroys parasitic cyst nematodes without harming other bioagents.

- By giving PSAP through soil 10 to 15 days after application of phosphate fertilizers, postulates synergistic growth in crops.

- If PSAP is applied during germination, seeds are protected and percentage of germination increases significantly.

- PSAP helps the plant to take up other micronutrients by increasing the size of the plants vascular system and also enable micronutrients to overcome the osmotic forces while traveling across the cell wall.

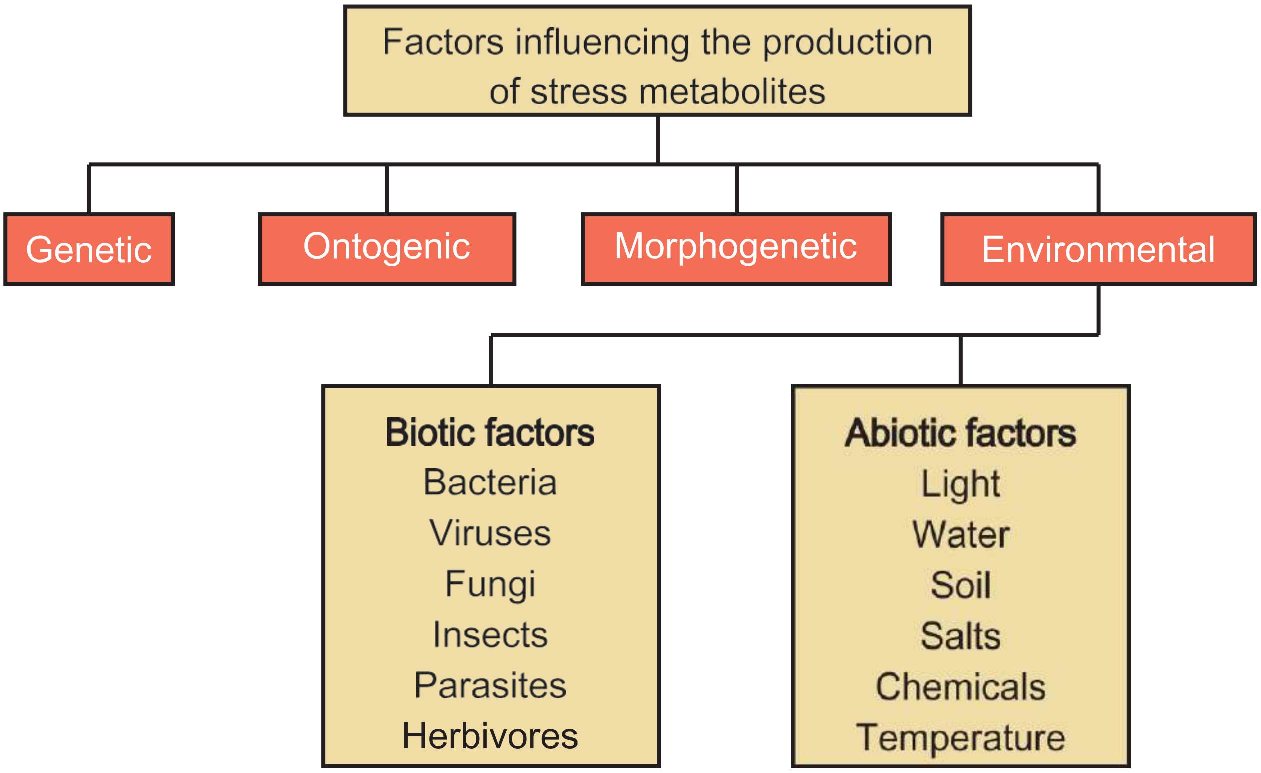
- PSAP enhances root development, which is evident from the profuse white roots along with increased biomass.

- PSAP given through soil does not inhibit the beneficial symbiosis between roots and mycorrhizal fungi.

PSAP

Crop Plants are Stronger and Healthier

- PSAP induces innate defence responses of crop plants against, pests and pathogens when applied to foliage or to soil.
- PSAP boosts the immune system of the plant by increased production of phenols, PR-proteins, amino acids, lignin, tannins, phytoalexins, flavonone and enzymes.
- PSAP increases phospholipids but decreases nucleoside triphosphates and lipid mass per mg mycelium, resulting in increase plasma membrane, distortion of hyphae, swelling of hyphal tip and prevention of fungal attack.
- PSAP increases cell turgidity and thickness providing resistance to stress due to mineral deficiencies and climatic extremes.
- Once within the plant, PSAP releases phosphorus and potash, which modulates the, accumulation of other nutrients through various growth stages of the plant.
- PSAP increases floral intensity, brix, TSS (total soluble solids), isoflavones, anthocyanin and fruit size.
- PSAP application to stressed and dormant crops induces uniform bud break. Early bud break enhances the chances of earlier flowering, and potentially greater harvest.
- The shelf life of the produce from PSAP-treated plants is longer.



Adverse climatic conditions creating abiotic stresses are among the principal causal factors of decline in agricultural productivity. As per the FAO report (2007), only 3.5% of the global land area is left unaffected by any environmental constraint. Dominant abiotic form of stress comprises drought, low or high temperatures, salinity and acidic conditions, light intensity, CO_2 contents of the atmosphere, submergence, anaerobiosis and nutrient starvation.

Water deficit or drought affects 64% of the global land area, flood (anoxia) 13% of the land area, salinity (6%), mineral deficiency (9%), acidic soils (15%) and, cold (57%). Out of the world's 5.2 billion ha of dryland agriculture, 3.6 billion ha is affected by the problems of erosion, soil degradation and salinity. Ruan et al. (2010) estimated that salt-affected soils account for 50% of the total irrigated land in the world.

Globally, the annual cost of land degradation by salinity in irrigated lands could be US$ 27.3 billion due to loss in crop production.The detrimental effect of salinity on plant growth is well established. The area under ever-increasing salinization was nearly 34 million irrigated hectares by 2011 (FAO, 2012). It is evident that such forms of stress affect large areas and significant cause qualitative and quantitative losses in crop production.

Abiotic and Biotic Stress

ABCD of Current Agriculture

A

Abiotic Stresss

- Global warming, increasing greenhouse gases
- Starving of nutrients and depleting soil health

B

Biotic Stresss

- Management of diseases and pests in crop plants
- Over emphasis on pesticides in stress management

C

Inherent Traits

- Metabolic disorders due to stress
- Understanding genomics and bio-mechanism of disorders

D

GM - Transgenic Crop Plants

- Breeding requires time and investments
- Yet the response of these plants to changing environment is not clearly understood

PSAP

Alleviates Abiotic Stress

- PSAP influences the production process of allelopathic compounds. Once in side the plant, PSAP triggers various metabolic at the molecular level and is also involved in nitrogen metabolism. Storage and transport of sugars in PSAP-treated plant are very efficient. PSAP plays a complementary role in Shikimic acid pathway (SAP). Path synthesizes chemicals that form part of the plant's defence response.

- PSAP improves defence responses of crop plants. Regular application of PSAP can reduce environmental stress due to drought, nutrient deficiencies, extreme temperatures and metal toxicity. PSAP strengthens seed vitality, improves stand quality, increases yield and reduces earlier decay of vegetables and fruit crops.

- PSAP promotes plant growth. PSAP increases photosynthesis, stimulates nutrient uptake, increases germination and boosts plant vigour.

- PSAP improves catalytic efficiency of various enzymes by upregulating their activities leading to cumulative synthesis of primary and secondary metabolites.

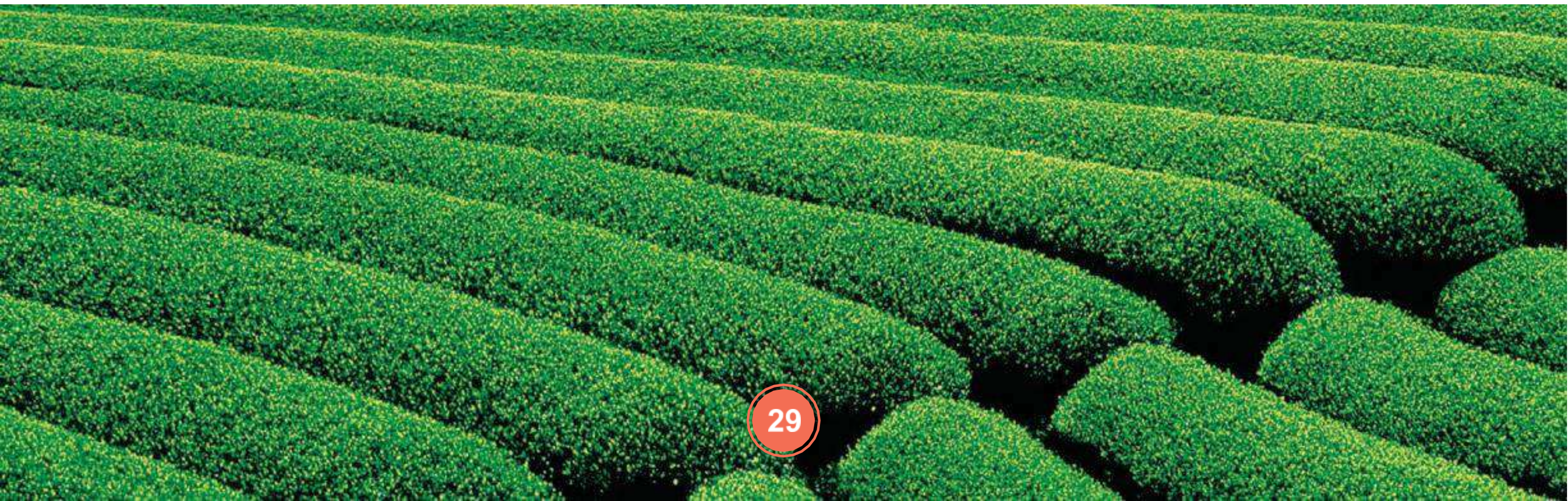
- PSAP-treated plants recover effectively and emphatically from stress occurring even due to changing climatic conditions.

- PSAP enhances beneficial symbioses between roots and mycorrhizal fungi.
  It does not support growth of phytopathogenic bacteria / fungi existing in the soil.

- PSAP promotes plant health in general and root health in particular.

- PSAP improves yield and quality over existing traditional soil or foliar fertilizers.

- PSAP increases pre and post harvest quality.



Impact of Abiotic Stress in Crop Plants and PSAP

Plants need light, water, carbon and mineral nutrients for their optimal development, growth and reproduction. Extreme conditions (below or above the optimal levels) limit plant growth and development. An unfavorable environment comprising extreme high or low temperature, salinity and drought poses a complex set of stress conditions. Plants can sense and react to stress in many ways that favour their sustenance. They remember past exposure to abiotic stress and even mechanisms to overcome them hence responses to repeated stress can be modified accordingly.

The most obvious effect of unfavourable conditions initially appears at the cellular level, followed by physiological symptoms. Water stress adversely affects plants physiology including photosynthesis. Prolonged water stress decreases leaf water potential and stomatal opening, reduces leaf size, suppresses root growth, reduces seed number, size, and viability, delays flowering and fruiting and limits plant growth and productivity.

Therefore, plants have evolved different mechanisms to optimize the consumption of water and manage their growth untill they are faced with adverse conditions. Exposure to low or high light intensities diminishes physiological processes and adversely influences growth and development of plants. Excess light induces photooxidation that increases the production of highly reactive oxygen intermediates to manipulate biomolecules and enzymes. Under severe conditions, loss in plant productivity is observed. Freezing (cold) injury and high temperatures are major causes of crop loss.

Various edaphic factors such as acidity, salinity, and alkalinity contamination with pollutants and anthropogenic perturbation severely affect plant development and adversely influence crop production .

Different levels of acidic conditions influence soil nutrients adversely and limit the easewith which they are availabile, due to which plants become nutrient deficient and lose their normal physiological pattern of growth and development. Early exposure to salinity leads to ion toxicity within the cell followed by disruption of osmotic balance if stress is continued for longer durations. The combined effect of these ionic as well osmotic shocks isaltered plant growth and development. Tolerance to salinity needs to be maintained or both osmotic and ionic homeostasis within the cells must be adjusted quickly. For combating salinity, plants usually try to avoid highly saline environments by keeping sensitive plant tissues away from the zone of high salinity or by exuding ions from roots or compartmentalizing ions away from the cytoplasm of physiologically active cells.

Plants under extreme cold conditions survive either through avoiding super cooling of tissue water or through freezing tolerance. Certain species of plants have developed an ability to tolerate super-cooling or freezing temperatures by increasing their anti-freezing response within a short photo period, a process normally known as cold acclimation .

After sensing stress, plants exhibit an immediate and effective response to initiate a complex stress-specific signalling cascade. Synthesis of phytohormones such as abcisic acid, jasmonic acid, salicylic acid and ethylene, accumulation of phenolic acids and flavonoids, elaboration of various antioxidants and osmolytes and activation of transcription factors (TFs), are initiated along with the expression of stress-specific genes to mount appropriate defence systems.

The most crucial aspect in mitigating stress in plants is to understand the fine-level molecular machinery and its networks operative under stress conditions. This includes elaborative elucidation of the abundance of metabolic pathways and their regulatory genes in plant varieties. Identification of multigenic traits involved in stress responses, exploration of linked markers for such genes, and investigation of the probabilities to pool important genes through breeding programmes are supposed to be the focus of stress mitigation strategies.

Another supporting strategy to alleviate stress from abiotic sources in plants includes the application of PSAP. Although many of the mechanisms related to stress tolerance in plants are known, a knowledge of "on-field response" of PSAP-treated crop plants to simultaneous exposure to multiple forms of stress is still require to explore.

Stress Mitigation Process of Crop Plants

Plants sense, manage, maintain or escape changing environmental conditions. Their perception of environmental stimuli and responses to abiotic forms of stress involves an interactive metabolic crosstalk within diverse biosynthetic networks and pathways. Root architecture is thought to be more sensitive to abiotic stimuli and reacts accordingly in soil. It is a complex phenomenon that involves dynamic and real-time changes at genetic, transcriptional, cellular, metabolic and physiological levels. The foremost and direct impact of drought, frost, salinity and heat is the creation of water-deficient conditions within cells, followed by a parallel development of biochemical, molecular and phenotypic responses to stress. In the environment, the sources of stress experienced by plants may be many and so is the complexity of their responses to multiple forms of stress in comparison to a sole source of stress. The complexity lies in activating specific genes expression followed by metabolic programming in cells in response to a given form of stress.

Tolerance or susceptibility to stress is a dynamic process involving multiple stages in a plant's development. Rather than imposing an additive effect on plants, abiotic stress responses may reduce or enhance susceptibility of plants to biotic stress caused by pests or pathogens. This becomes even more important when we take into account crops because, in many agricultural systems, most crops grow in suboptimal environmental conditions that limit the genetic potential of the plants for growth and development. Defence, repair, acclimation and adaptation are the major components of resistance to stress.

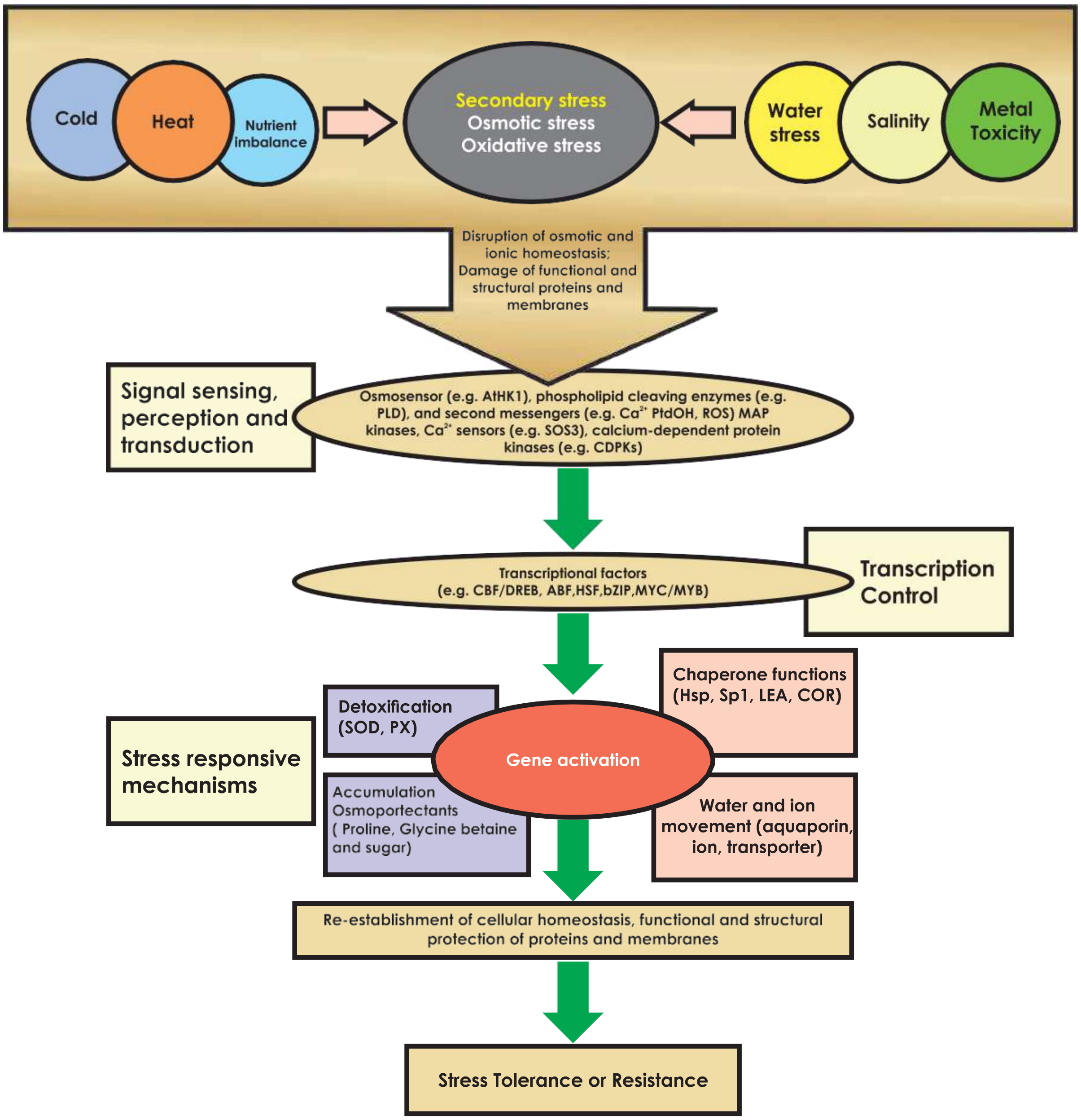
Plants are vulnerable to water stress. Environmental changes such as re-watering are more frequent under the globally changing climate. Under severe water deficit peroxidation may be induced leading to negative impact on the antioxidant metabolism. Re-watering decreases the level of peroxidation further and restores growth and development of new plant parts and stomatal opening. In roots, both drought and re-watering lead to high accumulation of hydrogen peroxide (H_2O_2). Drought responses vary from plant to plant in terms of the activity of superoxide dismutase (SOD), an enzyme that plays a central role in the antioxidant metabolism. In sugarcane, SOD activity remains unaffected by drought and the expression of FeSOD and Cu/ZnSOD is down-regulated.

In Alfalfa nodules, FeSOD and Cu/ZnSOD are up-regulated by moderate drought, indicating that responses differ among species and tissues. An elevated level of salts present in the soil is detrimental to plant cells, and different cells in a tissue respond differently to the stress caused by salinity. Stressed cells, irrespective of their location, whether at the root surface or within internal tissues, influence their neighbours and cause a change in their gene expression pattern over the duration of stress .

A drastic decrease in the osmotic potential of the soil occurs due to elevated salt levels, the ultimate result of which is ion toxicity coupled with water stress in plants. This situation can affect the vitality of plants by suppressing seed germination and the growth of the seedlings, hampering senescence, and finally causing death.

The role of salt overly sensitive (SOS) stress signaling pathway consisting of three majorly involved proteins, namely SOS1, SOS2, and SOS3, is well demonstrated. Salinity conditions cause decrease in the levels of such aromatic amino acids as cysteine, arginine and methionine. Proline accumulation within cells is a well-known strategy to alleviate salinity stress. Similarly, generation of nitric oxide (NO), activation of antioxidant enzymes and compounds, modulation of hormones, accumulation of glycine betaine and polyols are some other changes within plants due to salinity stress. This principally happens because of unavailability of water and mutilation in the nutrient availability caused by high salt concentrations that create much damage to plant tissues and ultimately affect productivity.

Gene Activation and Biochemical Responses



The complexity of the plant's response to abiotic stress.

Primary stresses, such as drought, salinity, cold, heat and chemical pollution are often interconnected, and cause cellular damage and secondary stress, such as osmotic and oxidative stress. The initial stress signals (e.g. osmotic and ionic effects, or temperature, membrane fluidity changes) trigger the downstream signaling process and transcription controls, which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes. Inadequate response at one or several steps in the signaling and gene activation may ultimately result in irreversible changes in cellular homeostasis and the destruction of functional and structural proteins and membranes, leading to cell death .

Some of the mechanisms that plants have evolved for adaptation to abiotic stresses

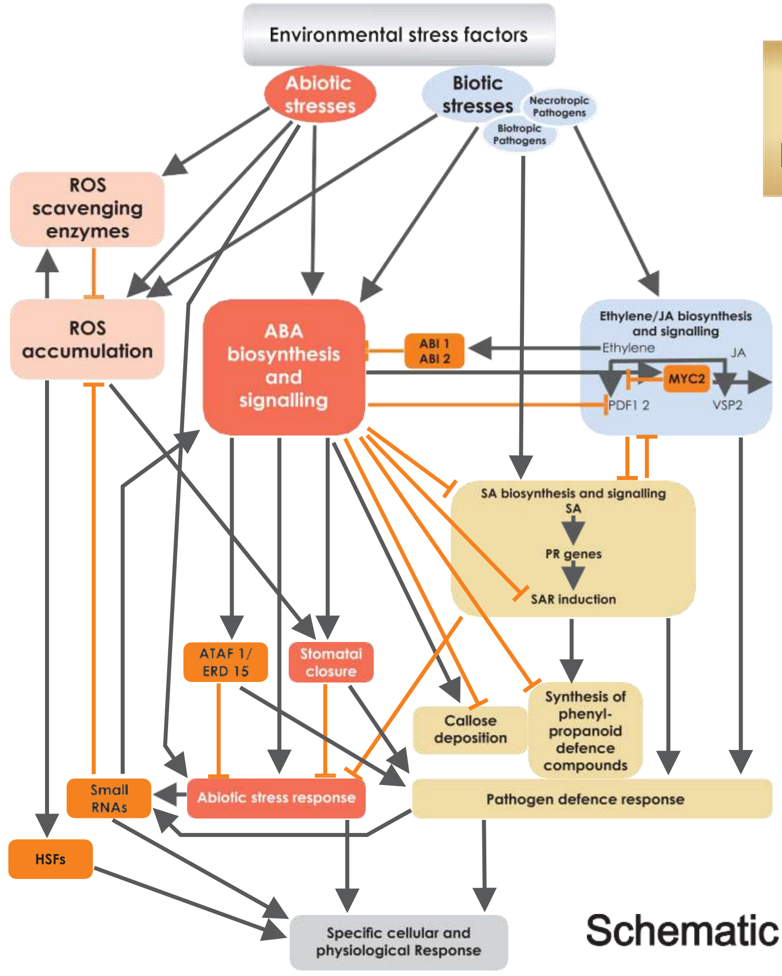
- Accumulation of osmo-protectants
- Superoxide radical scavenging mechanisms
- Exclusion or compartmentation of ions by efficient transporter and symporter systems
- Production of specific enzymes involved in the regulating of plant hormones.

Due to the continued rise in global temperature, heat stress is becoming an important agricultural problem as it badly affects crop production. Rising temperature has an adverse impact on morpho-anatomical, physiological, biochemical and genetic properties in plants. A thorough understanding of physiological responses of plants to heat and mechanisms of tolerance could lead to strategic development of better approaches to crop production management.

Heat affects plants at different developmental levels, and high temperature causes reduced seed germination, loss in photosynthesis and respiration and decrease in membrane permeability. Alterations in the level of phytohormones, primary and secondary metabolites, enhancement in the expression of heat shock and related proteins and production of reactive oxygen species (ROS) are some prominent responses of plants to heat stress. Strategies to mitigate heat stress involve activation of mechanisms that support maintenance of membrane stability and induction of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades. Besides, scavenging of ROS, accumulation of antioxidant metabolites and compatible solutes, chaperone signalling and transcriptional modulation are certain parallel activities that help cells to sustain heat stress. Multiple stress conditions impose more beneficial impacts on plants compared to those posed by any singal source of stress.

A combination different sources of stress ultimately reduces the detrimental effect of each other, thereby increasing the probability of better survival of plants. The cumulative impact of drought and accumulation of ozone (O_3) in plants results in greater tolerance. The combined effect was attributed to decreased values of stomatal conductance. Because of their elevated concentration, reduced glutathione and ascorbic acid effectively scavenge ROS, thereby causing a considerable drop in total ROS content. However, it is a difficult task to elucidate the response pattern of a plant against any single form of stress, particularly when it is growing in the field and subject to the cumulative impact of different sources of environmental stress. Multiple stresses occur simultaneously in field conditions and so, multifaceted mechanisms exist in the plants to cope with rapidly fluctuating adverse situations. Phytohormones are crucial to plant growth and development but they play critically role in tolerance to abiotic stress. Gene expression profiling has revealed that prioritization of signals done by protein switches like kinases, TFs and G-proteins are mostly regulated by hormones. Plants typically channel their physiological resources to adapt to abiotic stress, which makes them more susceptible to such biotic stress as herbivory and disease attack. ABA-dependent abiotic stress response pathways are predominant.

The role of plant hormones in regulating the interaction between biotic and abiotic stress.



ROS - Reactive Oxygen Species
ABA - Abscisic Acid
JA - Jasmonic Acid
SA - Salicylic Acid
PR - Pathogenesis-related
SAR - Systematic Acquired Resistance
HSF - Heat Shock Factor

Schematic Digram

Other defence pathways rooted through salicylic acid, jasmonic acid or ethylene also trigger responses to abiotic stress. For example, triggering ROS production to minimize loss during abiotic stress may defend plants against attacks by biotrophic pathogens, but it makes plants more susceptible to necrotrophic pathogens. The other hormone, jasmonic acid, is effective for in defence against necrotrophic pathogens.

The schematic diagram shows a cross-talk between hormones, transcription factors and other regulatory components when biotic and abiotic forms of stress occur concurrently. This complex network of interactions allows plants to respond in a highly specific fashion to the exact combination of environmental stresses encountered.

Gray arrows show induction or positive regulation, and orange lines show inhibition or repression. Events characteristic of the response to abiotic stress are shown in yellow, and those characteristic of biotic stress response are shows in blue. Transcription factors and other regulatory genes are represented by orange boxes.

The expression of phenylpropanoid biosynthetic pathway is precisely regulated in response to developmental signals, nutrient status and environmental stimuli such as light, heat and pathogen attack.

The accumulation of flavonoids and isoflavonoids under the attack of pathogens is used as a mechanism of detoxication of many species.

In most systems, the induction of isoprenoid and phenylpropanoid synthesis under stress is the result of increased transcription of genes encoding the corresponding biosynthetic enzymes. Metabolic adjustments in response to unfavourable conditions are dynamic and multifaceted and depend not only on the type and strength of stress but also on the cultivar and the plant species.

Metabolic rearrangements after drought are more profound than those after heat stress and the metabolic profile of plants exposed to a combination of drought and heat is more similar to that of drought-stressed than heat-stressed plants.

Generally, stress-tolerant plants have higher levels of stress-related metabolites under normal growth conditions and / or accumulate larger amounts of protective metabolites, such as proline and soluble sugars, under unfavorable conditions, indicating that their metabolism is prepared for adverse growth conditions.

Plant secondary metabolism and improved metabolite biosynthesis

Being sessile organisms, plants constantly interact with a multitude of variable and potentially damaging factors in their habitats that range from abiotic to biotic. The survival of floral diversity within an ecosystem thus requires elaborate mechanisms of defence. Among these, chemical defences represent the main trait of an innate immune system to cope with the hostile environment. Their metabolic plasticity evolves and exploits a range of inherent systems to create a rich repertoire of complex metabolites of adaptive significance for survival in diverse ecological niches.

These phytochemical derivatives of secondary metabolism confer a multitude of adaptive and evolutionary advantages to the producing plant. As a strategy for survival and generation of diversity at the organismic level, the ability to synthesize particular classes of secondary metabolites is often restricted to selected taxonomic groups. Apart from regulating the interaction between plants and their environment (biotic and abiotic), plant secondary metabolites also mediate certain physiological aspects of plant growth and development, symbiosis, and reproduction, and are important structural components of the wall (lignin). Secondary metabolism is the functional level of plant metabolism that is dispensable for growth and development but indispensable for the survival of the species.

The high degree of plasticity of secondary metabolism which, in contrast to primary metabolism, allows for structural and chemical modifications with almost unlimited restrictions is emphasized as a mechanical basis for the generation of chemical diversity. The diverse molecular changes that are associated with metabolism are understood to be preserved genetically, functionally and structurally to confer selective and adaptive advantages their hosts in diverse ecosystems.

A combination of gene duplication, neofunctionalization and positive selection is a mechanism for evolution of this diversity. The basis of genetic variation as being responsible for generating terrestrial organic diversity in response to plant-environment interactions has been established by research.

Despite this immense structural diversity, secondary metabolites derive their synthesis from limited products of primary metabolism. Ongoing research efforts have elucidated the basic biochemistry and molecular biology of some biosynthetic pathways of secondary metabolism, with most of the finding support that the diversification of secondary metabolism originates from elaboration of a few central intermediates.

Diverse abiotic stresses and the strategic defence mechanisms adopted by plants

Abiotic stress	Effects	Defence response
Salinity	Disturbed osmotic and ion homeostasis, membrane damage, nutrient imbalance	Osmolytes synthesis, stress responsive enzymes-detoxification, ion transporters
Heat	Higher transpiration, water deficiency, elevated evaporation	Induction of acclimation, synthesis of heat-shock proteins, induction of protein repair mechanisms
Drought	Decreased photosynthesis, water transport inhibition	Stomatal closure, rolling of leaves, stress-responsive enzymes, induction of osmolytes synthesis, responsible for lowering water potential
Chilling and cold	Decreased rate of biochemical reactions, decreased CO_2 fixation, ice-crystal mediated damage, free radical formation	Increased synthesis and accumulation of osmolytes, hydrophilic proteins, termination of growth
Intense light	Inhibited photosynthesis, increased photo oxidation, elevated generation of ROS	Increased production of scavengers of ROS, inactivation of photosynthesis, oxidation of proteins and lipids etc
Heavy metals	Bio-accumulation and protein damage	Generation of reactive oxygen radicals, deposition of excess metal in vacuoles
Submergence or flood	Anaerobiosis, respiration in mitochondria inhibited	Aerenchyma development

Although the consequences of heat, drought, salinity and chilling are different, the biochemical responses seem more or less similar. High light intensity and heavy metal toxicity also generate similar impacts but submergence or flooding leads to degenerative responses in plants where aerenchyma are developed to cope with anaerobiosis. It is therefore clear that adaptive strategies of plants against a variety of abiotic sources of stress are analogous. This observation may provide an important key for mounting a strategic tolerance to combined sources of abiotic stress in PSAP-treated crop plants.

Plant to PSAP Empirical Interactions and Metabolic Alterations

After elucidating secondary metabolic pathways, gene regulations, enzymes involved, and factors affecting various important metabolites, accumulated evidence has mode if possible to model these systems and engineer plant metabolic pathways for enhanced metabolite production. A multitude of factors, the complex integrated regulatory mechanisms and coordinated networks of metabolic routes leading to the synthesis of specific metabolites, as well as the general plasticity and adaptability of the various biosynthetic pathways, shape the profiles and fluxes of plant secondary metabolites.

Exploitation of the plant's capacity to synthesize metabolites presents numerous exciting opportunities but also equally complex challenges. Much of this rich chemical diversity arises from a limited pool of chemical scaffolds which are subsequently modified though specific chemical substitutions as catalyzed by substrate and/or regio-specific enzymes. The enzyme-driven reactivity and regio- and stereo-chemistry during the multi-step conversion of substrates into precise products in the bio-catalytic landscape of secondary metabolism is one of the lucrative key points of exploitation. The bio-mimetic exploitation of enzymes, particularly those that exhibit strict stereo specificity, is an interesting aspect in the production. Equally intriguing is the synthesis of novel metabolites by protein engineering aimed at altering the substrate specificity of biosynthetic enzymes. Application of recombinant DNA methods to restructure metabolic networks can improve the production of metabolites and proteins by altering pathway distributions and rates. Recruitment of heterologous proteins enables extension of existing pathways to obtain new chemical products, alter post transnational protein processing, and degrade recalcitrant wastes. Transgenic plants with altered enzyme activities have also become a powerful tool to study the control architecture of secondary metabolites.

The fact that the synthesis and accumulation of secondary metabolites remains under the influence of the environment adds to the multiple dimensions of the metabolic manipulation level points for enhanced production, which seems to be very effective in PSAP-treated crop plants. Following this direct logic, varied levels of metabolic perturbation through manipulation of environmental factors, either singly or in combination, have been reported to trigger positive abrupt activation of qualitative and quantitative changes in the accumulation of secondary metabolite in plants.

Understanding the physiology of the pathway is as essential, as understanding transport, pH and cellular and subcellular compartmentation. Genomic sequencing of the target plant species using proteomics and metabolomics as tools for linking the genes with the secondary metabolite pathways would be a useful and promising approach to obtain deeper insights into the stress-mitigating mechanisms in PSAP-treated crop plants and to deploy them to increase productivity. Studying the alleviation of abiotic stress in PSAP-treated field-grown plants will open new gateways for scientists to unearth novel strategies to mitigate such stress. Study of omics may help in understanding these complex PSAP-plant interactions and metabolic alterzations.

Genomic and biochemical approaches and an appreciation of molecular evolution and environmental influences, as well as structural enzymology, hold great promise for altering the complex plant secondary metabolic pathways towards synthesis and accumulation of desired bioactive compounds. These are required to be established in PSAP-treated crop plants. However, the regulatory architectures of these pathways, and the ways in which these are integrated into broader metabolic networks, are less well understood, often making it hard to predict the results of over expressing a single gene or multiple genes within a particular pathway. Several attempts to dissect secondary metabolism for the purpose of improving bioactive metabolites using classical genetics have yielded, to some extent and in some species, positive results. It must also be tried in PSAP-treated crop plants. Understanding the basic network of metabolic intermediates and enzyme forms the fundamental basis of unraveling these attributes. Knowledge of the spatial and temporal regulatory architectures of secondary metabolic pathways, and the ways in which they are integrated into broader metabolic networks is the key to establishing the mode of action of PSAP at molecular level.

Transcription factors, a diverse group of proteins that recognize specific DNA sequences in the promoter of genes, negotiate the regulation of gene expression at the level of transcription. TFs mediate the assembly of the basal transcription machinery resulting in the activation of RNA polymerase II and mRNA synthesis. The control of specific sets of genes within the metabolic network is accomplished by the interaction among TFs, between TFs and non-DNA-binding proteins and between TFs and cis-regulatory elements in organized hierarchical gene regulatory networks. Empirical observations on PSAP-treated crop plants and data show that there is a great potential for a broad range of applications, ranging from improving the production of certain secondary metabolites to revealing new pathways in plants.

For further developing the full potential of metabolic engineering, it is thus necessary to increase our knowledge about plant secondary metabolism at the level of the intermediates, enzymes and genes in PSAP-treated plants.

PSAP

Mediated Mitigation of Biotic Stress

- PSAP inhibits the growth of fungi and induces the defence response quickly in crops.
- PSAP causes a number of changes in the phytopathogen metabolism, some of which may lead to stimulation of the host's defence mechanism.
- PSAP causes the phytopathogens to release elicitors, which are active metabolites that trigger the host's defence response.
- PSAP increases the plant`s resistance to pests and diseases.
- PSAP is involved in many enzymatic activities that affect ethylene biosynthesis, phenylalanine ammonia lyase (PAL) activation, lignin biosynthesis and phytoalexin accumulation.
- The enzyme PAL involved in phenyl propanoid biosynthesis, is activated earlier in PSAP-treated plants. Lignin, one of the syntheses from this pathway also gets accumulated earlier. Lignin is believed to play an important role in defence response.
- Phytoalexins, or plant antibiotics, are also produced more rapidly around the infection site in PSAP-treated Plant.
- PSAP induces activity much earlier in the 6 - phosphogluconate & pentose phosphate pathway with quick accumulation of sugars. It seems that glucose metabolism remains under normal enzymatic control in PSAP-treated plants but is completely disrupted within 12 hours in untreated plant, as a result of disease development.
- Ethylene is an early indicator of plant stress response and has been proposed to have to signalling function. PSAP-treated plants produce ethylene earlier than untreated.
- PSAP-treated plants regenerate bio-energy, remove the blockades and reform the cell-to-cell communication systems very effectively in reformation.

PSAP

Mediated Mitigation of Biotic Stress

PSAP induces Plant Defenses against Pathogens and Herbivores

Plants represent a rich source of nutrients for many organisms including bacteria, fungi, protists, insects, and vertebrates. Although lacking an immune system comparable to animals, plants have developed a stunning array of structural, chemical, and protein-based defenses designed to detect invading organisms and stop them before they are able to cause extensive damage.

Humans depend almost exclusively on plants for food, and plants provide many important non-food products including wood, dyes, textiles, medicines, fossil oil, cosmetics, soaps, rubber, plastics, inks, and industrial chemicals. Plants that can defend themselves from pathogens and herbivores are essential in order to protect our food supply.

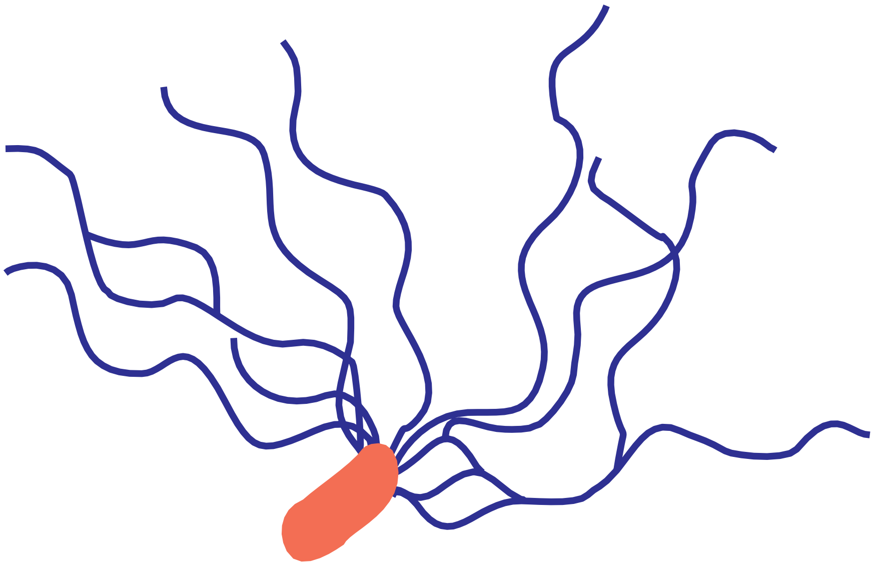
Disease resistance in plants involves two distinct forms of chemical communication, pathogen recognition and defence. Both are essential components of a highly complex, multifaceted defence response, which begins with non-self recognition through the perception of pathogen-derived signal molecules and results in the production, inter alia, of antibiotically active compounds (phytoalexins) and cell wall-reinforcing material around the infection site.

Plant Disease and Resistance

Broadly defined, disease is a physiological abnormality or a significant disruption in the “normal” health of a plant. Disease can be caused by living (biotic) agents, including fungi and bacteria, or by environmental (abiotic) factors such as nutrient deficiency, drought, lack of oxygen, excessive temperature, ultraviolet radiation, or pollution. In order to protect themselves from damage, plants have developed a wide variety of constitutive and inducible defenses. Constitutive (continuous) defences include many preformed barriers such as cell walls, waxy epidermal cuticles, and bark.

These substances not only protect the plant from invasion but also give the plant strength and rigidity. In addition to preformed barriers, virtually all living plant, cells have the ability to detect invading pathogens and respond with inducible defences including the production of toxic chemicals, pathogen-degrading enzymes, and deliberate cell suicide. Plants often wait until pathogens are detected before producing toxic chemicals or defence-related proteins because of the high energy costs and nutrient requirements associated with their production and maintenance.

Detection and Response to Microbial Pathogens

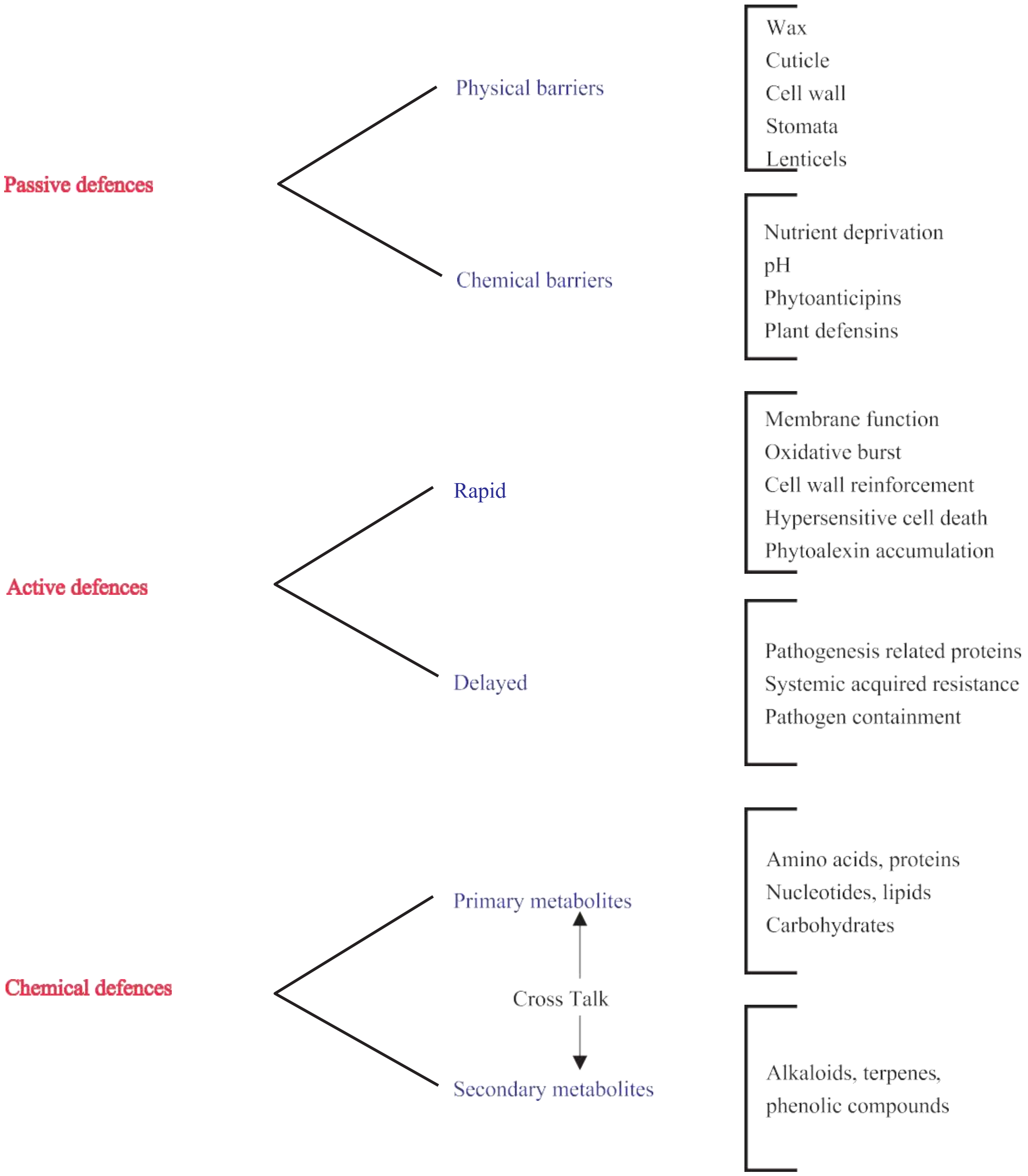


Bacterial flagella are often recognized by plants during basal resistance.

Plants have developed multiple layers of sophisticated surveillance mechanisms that recognize potentially dangerous pathogens and rapidly respond before those organisms have a chance to cause serious damage. These surveillance systems are linked to specific pre-programmed defence responses. Basal resistance, also called innate immunity, is the first line of pre-formed and inducible defences that protect plants against entire groups of pathogens. Basal resistance can be triggered when plant cells recognize microbe-associated molecular patterns (MAMPs) including specific proteins, lipopolysaccharides, and cell wall components commonly found in microbes. The result is that living plant cells become fortified against attack. Non-pathogens as well as pathogens are capable of triggering basal resistance in plants due to the widespread presence of these molecular components in their cells.

Plant dynamics and coordination of defence responses

Disease resistance mechanisms may be conveniently classified as either passive or active. Passive mechanisms include the barriers presented by the cuticle, cell wall and phytoanticipins. Active mechanisms are those activated only upon pathogen challenge and restrict the invading pathogen. Wound repair mechanisms, such as cork layers, papillae, lignitubers and the expression of systemic acquired resistance retard the colonization and spread of pathogens that survive or escape the initial defence responses.



Active defence responses are most likely to be effective if they are expressed in combination. The rapid release of reactive oxygen species and the deposition of papillae, lignin and cross-linked hydroxyproline-rich glycoproteins at the point of penetration of the cell wall are followed by rapid hypersensitive cell death and phytoalexin accumulation. Lytic enzymes accumulate in intercellular spaces and vacuoles; systemic acquired resistance is activated; and wounds and tissue damage repaired. Plants use these weapons in coordination to form a potent arsenal against invading pathogens.

The failure or delay of these responses, invariably leads to susceptibility. Disease resistance depends on the speed, localization and magnitude of these responses.

PSAP effectively induces plant defence responses at the molecular level and hence regular application of PSAP is of immense importance.

Time	Events involved in the coordination of defence response on challenge by pathogens
Minutes	Membrane depolarization and electrolyte leakage Reactive oxygen generation Expression of genes involved in phytoalexin biosynthesis
Hours	Oxidative burst Membrane lipid peroxidation Rise in salicylic acid levels Cytoplasmic aggregation, cell collapse and hypersensitive cell death Phytoalexin accumulation Cell wall reinforcements
Days	Accumulation of pathogenesis-related proteins Systemic acquired resistance

Passive defences

To gain access to nutrients or to the replication machinery available within the host cell, pathogens must first breach the natural barriers presented by healthy plants. These barriers may be physical (the cuticle, cell wall, stomatal aperture or lenticel) or chemical including inhibitory compounds or the absence of stimulatory compounds needed for pathogen development.

Physical barriers

Structural defences

The plant cell

All plant tissues contain preformed structural barriers that help limit pathogen attachment invasion and infection. The cell wall is a major line of defence against fungal and bacterial pathogens. In PSAP-treated plants, there is an excellent structural barrier that also incorporates a wide variety of chemical defences that can be rapidly activated when the cell detects the presence of potential pathogens. All plant cells have a primary cell wall, which provides structural support and is essential for turgor pressure, and many also form a secondary cell wall that develops inside of the primary cell wall after the cell stops growing.

The primary cell wall consists mostly of cellulose, a complex polysaccharide consisting of thousands of glucose monomers linked together to form long polymer chains. These chains are bundled into fibers, also known as microfibrils, which give strength and flexibility to the wall. The cell wall may also contain two groups of branched polysaccharides: cross-linking glycans and pectins. Cross-linking glycans include hemicellulose fibers that give the wall strength via cross-linkages with cellulose. Pectins form hydrated gels that help “cement” neighboring cells together and regulate the water content of the wall. Soft-rot pathogens often target pectins for digestion using specialized enzymes that cause cells to break apart: these organisms are extremely common and anyone who has seen fruits or vegetables become brown and “mushy” has seen these pathogens in action.

Synthesis of pectins occurs more efficiently in PSAP-treated plants, and at the fruit maturity stage, pectins are translocated to seeds. Hence the occurrence of spongy tissue, a physiological disorder and a quality issue in alphonso mangoes, is reduced in PSAP-treated plants. Many cell walls also contain lignin, a heterogeneous polymer composed of phenolic compounds that give the cell rigidity. Lignin is the primary component of wood, and cell walls that become “lignified” are highly impermeable to pathogens and difficult for small insects to chew. Cutin, suberin, and waxes are fatty substances that may be deposited in either primary or secondary cell walls (or both) and outer protective tissues of the plant body, including bark.

Cell walls contain proteins and enzymes that actively work to reshape the wall during cell growth yet thicken and strengthen the wall during induced defence. When a plant cell detects the presence of a potential pathogen, enzymes catalyse an oxidative burst that produces highly reactive oxygen molecules capable of damaging the cells of invading organisms. Reactive oxygen molecules also help strengthen the cell wall by catalysing cross-linkages between cell wall polymers, and they serve as a signal to neighboring cells that an attack is under way. Plants cell also respond to microbial attack by rapidly synthesizing and depositing callose between the cell wall and the cell membrane adjacent to the invading pathogen. Callose deposits, called papillae, are polysaccharide polymers that impede cellular penetration at the site of infection, and these are often produced as part of the induced basal defence response very rapidly in PSAP-treated plants.

Some plant cells are highly specialized for plant defence. Idioblasts (“crazy cells”) help protect plants against herbivory because they contain toxic chemicals or sharp crystals that tear the mouth parts of insects and mammals as they feed. There are many classes of idioblasts including pigmented cells, sclereids, crystalliferous cells and silica cells. Pigmented cells often contain bitter-tasting tannins that make plant parts undesirable as a food source.

Young red wines often contain high levels of tannins that give wine a sharp, biting taste. Sclereids are irregularly-shaped cells with thick secondary walls that are difficult to chew. The rough texture of PSAP-treated plants is caused by thousands of sclereid stone cells that can abrasively wear down the teeth of feeding animals. Stinging nettles produce stinging cells shaped like hypodermic needles that break off when disturbed and inject highly irritating toxins into herbivore tissues. Some stinging cells contain prostaglandins, hormones that amplify pain receptors in vertebrate animals and increase the sensation of pain.

Crystalliferous cells contain crystals of calcium oxalate, which are abundant in PSAP-treated plants and may tear mouth parts of herbivores when chewed and can be toxic if ingested.

Plant Tissues and Specialized Appendages

The epidermis constitutes the outermost protective tissue system of leaves, floral parts, fruits, seeds, stems, and roots of plants until they undergo considerable secondary growth. It is the first line of defence against invading pathogens and consists of both specialized and unspecialized cells. The epidermal cells of aerial plant parts are often covered in a waxy cuticle that not only prevents water loss from the plant, but also prevents microbial pathogens from coming into direct contact with epidermal cells and thereby limits infection. The hydrophobic nature of the cuticle also prevents water from collecting on the leaf surface, an important defence against many fungal pathogens that require standing water on the leaf surface for spore germination.

However, some fungal pathogens including Fusarium solani produce cutinases that degrade the cuticle and allow the fungi to penetrate the epidermis.

It is observed in PSAP-treated plants that the epidermis layer is structured to combat invading pathogens.

Chemical barriers

Proteins and Enzymes in Plant defence

Many plants and seeds contain proteins that specifically inhibit pathogen and pest enzymes by forming complexes that block active sites or alter enzyme conformations, ultimately reducing enzyme function. These proteins are generally small and rich in the amino acid cysteine. They include defensins, amylase inhibitors, lectins, and proteinase inhibitors. Unlike simple chemicals such as terpenoids, phenolics and alkaloids, proteins require a great deal of plant resources and energy to produce; consequently, many defensive proteins are only made in significant quantities after a pathogen or pest has attacked the plant provided if they have sufficient nutrients in reserve. Once activated, however, defensive proteins and enzymes effectively inhibit fungi, bacteria, nematodes, and insect herbivores.

One group of phytoanticipins, the saponins, are plant glycosides with surfactant (wetting agent) properties. Saponins bind sterols in pathogen cell membranes, destroying membrane integrity and function. In this way saponins are toxic to organisms containing sterols in their membranes. Inactive saponin precursor molecules appear to be stored in vacuoles of intact plant cells, but hydrolase enzymes released following wounding or infection convert these precursors to active, antimicrobial forms.

Several lines of evidence suggest that saponins are involved in disease resistance and host range determination.

Defensins are small cysteine-rich proteins that display broad antimicrobial activity. They are widely distributed and may be present in most plants. Defensins can be found in virtually all types of plant tissues including leaves, pods, tubers, fruit, roots, bark, and floral tissues. They exhibit a wide range of biological activities that serve to inhibit the growth of many fungi and bacteria. Some defensins also inhibit digestive proteins in herbivores.

The precise mechanisms employed by defencins in PSAP-treated plants to inhibit fungi and bacteria are required to be characterized further.

Digestive enzyme inhibitors are proteins that block the normal digestion and absorption of nutrients by vertebrate and invertebrate herbivores. Alpha-amylase inhibitors are proteins commonly found in legumes that bind to amylase enzymes and inhibit starch digestion. Lectins are non-enzymatic proteins and glycoproteins that bind to carbohydrates and exhibit a wide range of functions including disruption of digestion in insects and agglutination of blood cells in vertebrates. Ricin is a powerful toxin produced in castor beans. It combines a lectin molecule with an N-glycoside hydrolase that enters animal cells and inhibits protein synthesis. Ricin is a highly potent toxin.

Protease inhibitors are typically produced in response to herbivore attack and inhibit digestive enzymes including trypsin and chymotrypsin. They occur widely in nature. Herbivore feeding often triggers a series of molecular signalling events that induce systemic production of these compounds in distal tissues that contribute to the protection of undamaged plant parts from subsequent attacks by a wide range of herbivore pests.

Hydrolytic enzymes are produced by some plants in response to pathogens and often accumulate in extracellular spaces where they degrade the cell walls of pathogenic fungi. Chitinases are enzymes that catalyse the degradation of chitin, a polymer with a backbone similar to cellulose, that is present in the cell walls of true fungi.

Glucanases are enzymes that catalyse the degradation of glycosidic linkages in glucans, a class of polymers similar to cellulose that is present in the cell walls of many oomycetes (water molds). In vitro analysis has verified the anti-fungal properties of these compounds, and transgenic plants expressing high levels of these enzymes exhibit increased resistance to a wide range of both foliar and root pathogens. Lysozymes are hydrolytic enzymes that are capable of degrading bacterial cell walls.

Active defences

Rapid

Plant responses to infection are complex and there is no universal model or sequence of events that accurately describes the dynamics of resistance. Almost every host-parasite interaction is unique in the details of the activation, localization, timing and magnitude of each component of the defence response. Resistance is rarely absolute, and whether a plant is resistant or susceptible depends on the sum of many individual responses.

A pattern is emerging that indicates that the outcome of many, if not all, host-parasite interactions depends on complex interactions between host and pathogen cells. These interactions are conditioned by host and pathogen gene expression, are mediated by chemical signal transduction pathways and involve dynamic interactions between elicitors, enhancers, suppressors, receptors and secondary signals. The dynamics of the interaction is sensitive to environmental fluctuations and is regulated by feedback from both the host and the pathogen. It is the complexity of plant-pathogen interactions that defines the multitude of possible outcomes.

Changes in membrane function

Most studies on the earliest stages of the host-parasite interaction conclude that the host membrane is involved in pathogen recognition and signal transduction. Membrane permeability changes rapidly following the exposure of plant cell suspension cultures to fungal and bacterial elicitors, usually leading to a loss of cellular electrolytes such as K^+ and an uptake of H^+. At the same time, there is often an influx of Ca^{2+}, a key intracellular signal in plants that is involved in the activation of enzymes and gene expression. The experimental blocking of Ca^{2+} transport across membranes in inoculated bean cells also inhibits gene activation and subsequent defence responses.

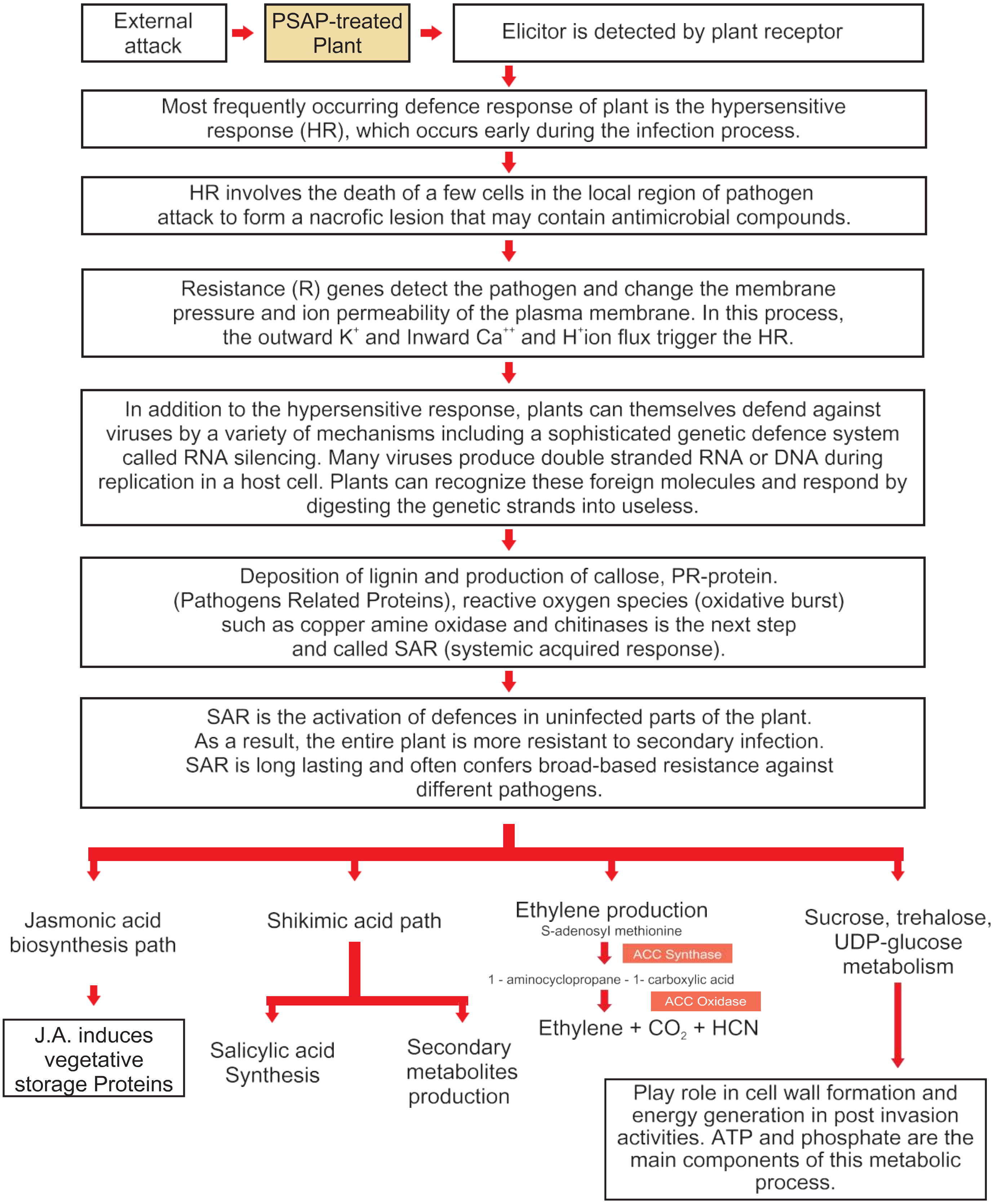
The oxidative burst

Membranes are also the sites where the oxidative burst occurs. The term 'oxidative burst' was first used to describe a rapid increase in respiration observed in neutrophils involved in the immune response of mammals. This increased level of respiration is now known to be due to the generation of reactive oxygen species, especially hydrogen peroxide and the superoxide anion (O_2^-), through the addition of electrons to O_2 catalysed by the membrane-bound enzyme NADPH oxidoreductase. Reactive oxygen species are also produced by errors in electron transport during respiratory and photosynthetic reactions in plant cells. Cells are normally protected from the damaging effects of reactive oxygen by superoxide dismutase, various peroxidases and catalyases and by natural antioxidants such as carotenes. The rapid oxidative burst generates levels of reactive oxygen species that initiate membrane lipid peroxidation and cell death. The oxidative burst in plants is associated with the release of local and systemic signals that trigger gene expression and the oxidative cross-linking of host cell wall components. Levels of ROS accumulate at the infection court that are sufficient to kill microorganisms in vitro. Experimental suppression of the oxidative burst shows that it is involved in initiating later defence responses.

PSAP

Mediated Mitigation of Biotic Stress

Active and Rapid Responses of Crop Plants



External attack → PSAP-treated Plant → Elicitor is detected by plant receptor

Most frequently occurring defence response of plant is the hypersensitive response (HR), which occurs early during the infection process.

HR involves the death of a few cells in the local region of pathogen attack to form a nacrofic lesion that may contain antimicrobial compounds.

Resistance (R) genes detect the pathogen and change the membrane pressure and ion permeability of the plasma membrane. In this process, the outward K^+ and Inward Ca^{++} and H^+ion flux trigger the HR.

In addition to the hypersensitive response, plants can themselves defend against viruses by a variety of mechanisms including a sophisticated genetic defence system called RNA silencing. Many viruses produce double stranded RNA or DNA during replication in a host cell. Plants can recognize these foreign molecules and respond by digesting the genetic strands into useless.

Deposition of lignin and production of callose, PR-protein. (Pathogens Related Proteins), reactive oxygen species (oxidative burst) such as copper amine oxidase and chitinases is the next step and called SAR (systemic acquired response).

SAR is the activation of defences in uninfected parts of the plant. As a result, the entire plant is more resistant to secondary infection. SAR is long lasting and often confers broad-based resistance against different pathogens.

Jasmonic acid biosynthesis path

J.A. induces vegetative storage Proteins

Shikimic acid path

Salicylic acid Synthesis

Secondary metabolites production

Ethylene production

S-adenosyl methionine

ACC Synthase

1 - aminocyclopropane - 1- carboxylic acid

ACC Oxidase

Ethylene + CO_2 + HCN

Sucrose, trehalose, UDP-glucose metabolism

Play role in cell wall formation and energy generation in post invasion activities. ATP and phosphate are the main components of this metabolic process.

Cell wall reinforcement

The first visible response to attempted penetration of plant cell walls by pathogens is often the intensification of cytoplasmic streaming followed by the accumulation of host cytoplasm under the site of attempted penetration. These cytoplasmic aggregates are thought to contain the cellular apparatus for the synthesis of cell wall fortifications. Most pathogens must penetrate host cell walls at some stage, either as germ tubes, hyphae or haustoria. If the cell can respond quickly enough to repair or reinforce the cell wall, penetration efficiency may be reduced and pathogen development retarded.

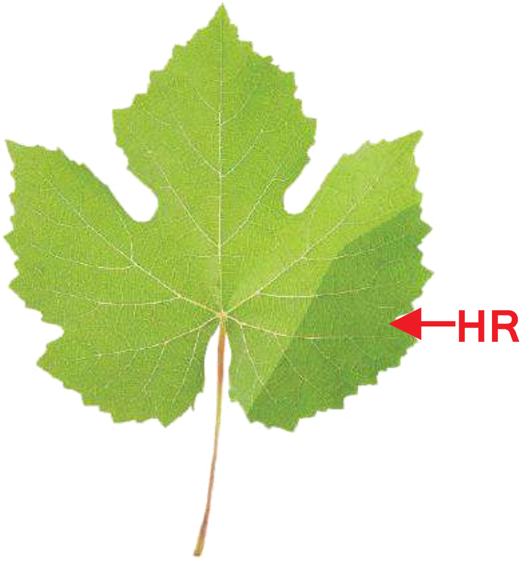
A number of different types of cell wall fortifications are produced in response to the attempted penetration of plant cell walls. Some pathogens induce the deposition of a papilla, a reinforcement composed of a branched Beta-1,3 glucan callus along with silicon, lignin and proteins, between the host cell wall and the plasma membrane, directly under the penetration peg. The rapid deposition of papillae is a common response of cereals to attempted penetration of epidermal cells by the powdery mildew fungus. Papillae in resistant cultivars form more rapidly and are more difficult to penetrate than those formed by susceptible cultivars. As a result, haustorial development is prevented. Lignitubers are lignified callose deposits that ensheath invading hyphal tips.

In PSAP-treated plants, a rapid response with timely activation of cell wall reinforcement is the more likely succeed interaction. Hydroxyproline-rich glycoproteins are structural proteins in plant cell walls involved in the organization of secondary cell wall thickening. Genes encoding hydroxryproline-rich glycoprotein biosynthesis are transcribed in advance of the invading hyphae, making cell walls tougher.

Hydrogen peroxide, released during the oxidative burst following pathogen challenge, causes extensive cross-linking between hydroxyproline-rich glycoproteins and other cell wall components, making the walls even more resistant to microbial digestion. Cross-linked hydroxyproline-rich glycoproteins also provide a focus for lignin deposition on the plant cell wall. The rapid deposition of lignin and suberin following infection is associated with resistance to non-pathogens and to avirulent pathogens in many plants, including cereals, member of solanaceae, brassicas, melons and carrots.

Lignin deposited on plant cell walls ahead of invading hyphae increase their resistance to fungal penetration. Lignin also binds to hyphal tips and bacterial cells, preventing further growth and movement and restricting the diffusion of pathogen enzymes and toxins and the uptake of water and nutrients by the pathogen. Furthermore, precursor molecules and free radicals produced during lignin biosynthesis are toxic to pathogens and inactivate pathogen enzymes, toxins, elicitors or suppressors. The effect of lignin can be further enhanced by the release of reactive oxygen species and the activation of phenol oxidase enzymes that convert phenolic compounds to more toxic complex polymerized phenolics and quinones during the defence response.

Hypersensitive Response



HR lesion on leaf

Pathogens have developed countermeasures able to suppress basal resistance in certain plant species. If a pathogen is capable of suppressing the basal defence, plants may respond with another line of defence namely the hypersensitive response (HR), which is characterized by deliberate suicide of plant cells at the site of infection. Although drastic compared to basal resistance, the response may limit pathogen access to water and nutrients by sacrificing a few cells in order to save the rest of the plant. The response is typically more pathogen-specific than basal resistance and is often triggered when gene products in the plant cell recognize the presence of specific disease-causing effector molecules introduced into the host by the pathogen. Bacteria, fungi, viruses, and microscopic worms called nematodes are capable of inducing the HR in plants.

Once the HR has been triggered, plant tissues may become highly resistant to a broad range of pathogens for an extended period of time. This phenomenon is called systemic acquired resistance (SAR) and represents a heightened state of readiness in which plant resources are mobilized in case of further attack.

Recovery form Virus Infection

In addition to the hypersensitive response, plants can defend themselves against viruses by a variety of mechanisms including a sophisticated genetic defence system called RNA silencing. Many viruses produce double-stranded RNA or DNA during replication in a host cell. Plants can recognize these foreign molecules and respond by digesting the genetic strands into useless fragments and halting the infection. Plants that are infected with viruses will often exhibit chlorosis and mottling, but disease symptoms may eventually disappear if RNA silencing is successful, a process called recovery. In addition, the plant will retain a template of the digested genetic strand that can be used to quickly respond to future attack by similar viruses, a process analogous to the memory of vertebrate immune systems. In PSAP-treated plants, it is observed that recovery from viral infections was quick and lasted longer.

Active defences

Delayed

Pathogen containment and wound repair

While earlier responses retard the development of pathogens, later responses restrict their spread and contain the damage to host tissues. The ability of a plant to repair tissue damage may contribute to its ability to fight off secondary infections by opportunistic pathogens. Infected areas of fleshy tissues, roots, fruits and bark are sealed by layers of cork cells with thick, suberized walls. Wound cork is produced by a secondary meristem, the cork cambium, formed from mature parenchyma tissue in response to the damage caused by infection. In some cases, such as in the response of potato tuber tissue to the powdery scab pathogen, cork barriers appear to seal the infected area and prevent further colonization by the pathogen. Wounded tree trunks often secrete gums that effectively seal the wound from opportunistic pathogens. If pathogen growth is retarded by environmental conditions or other disease resistance mechanisms, induced barriers may also prevent further colonization by the pathogen or by secondary invaders. Tyloses are ingrowths of the protoplasts of xylem parenchyma through xylem vessel pits into the lumen of xylem vessels. They are thought to impede the progress of fungal and bacterial vascular wilt pathogens. If tyloses form rapidly enough ahead of the advancing pathogen they may restrict colonization or the spread of propagules in the xylem.

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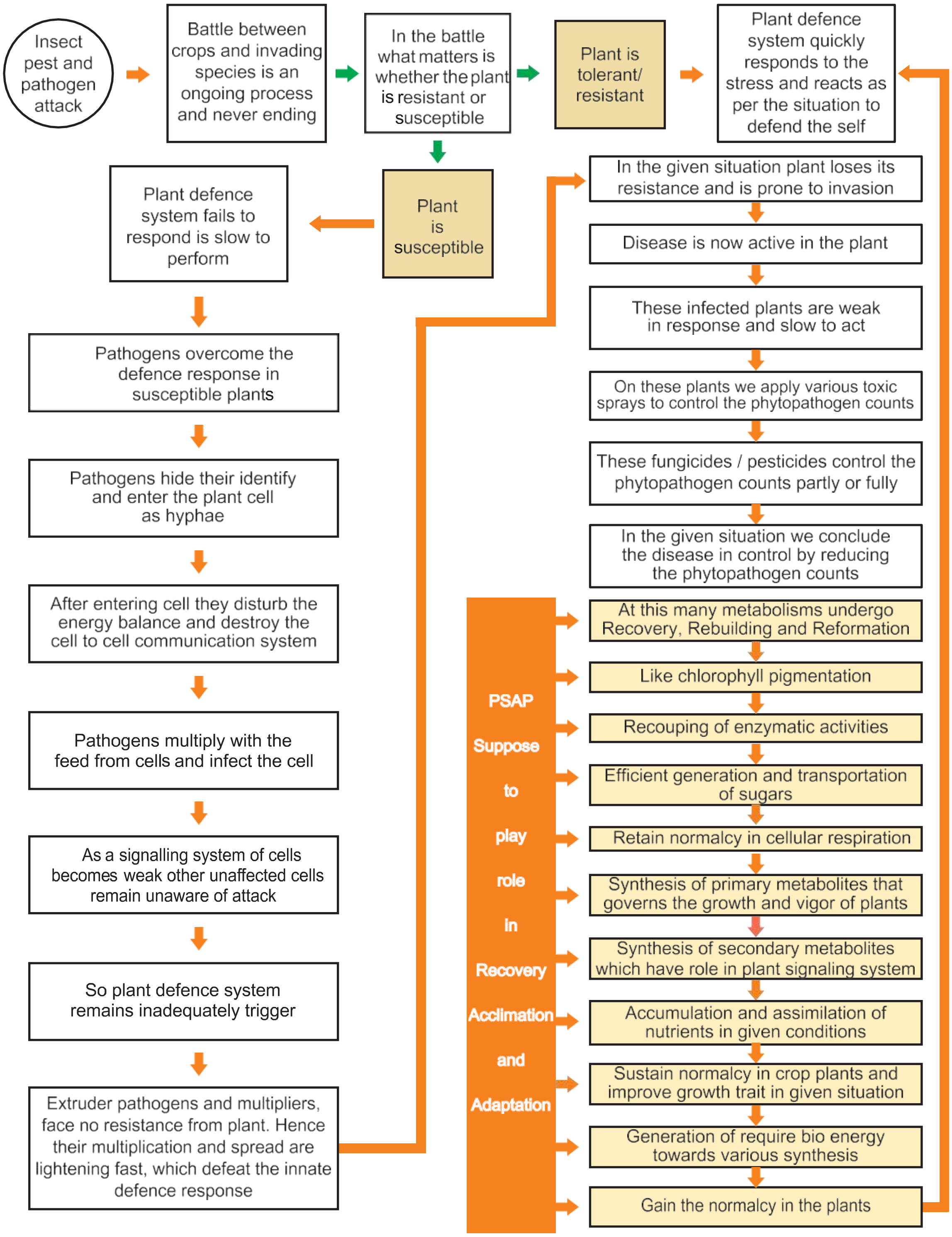
The formation of tyloses involves a cost to the plant, as they not only block the spread of the pathogen but also reduce the translocation of water, possibly causing wilt symptoms. In PSAP-treated plants, recovery from such a situation is unique requiring further study.

PSAP-treated crop plants: recovery, acclimation and adaption



Insect pest and pathogen attack

Battle between crops and invading species is an ongoing process and never ending

In the battle what matters is whether the plant is resistant or susceptible

Plant is tolerant/ resistant

Plant defence system quickly responds to the stress and reacts as per the situation to defend the self

Plant is susceptible

Plant defence system fails to respond is slow to perform

Pathogens overcome the defence response in susceptible plants

Pathogens hide their identify and enter the plant cell as hyphae

After entering cell they disturb the energy balance and destroy the cell to cell communication system

Pathogens multiply with the feed from cells and infect the cell

As a signalling system of cells becomes weak other unaffected cells remain unaware of attack

So plant defence system remains inadequately trigger

Extruder pathogens and multipliers, face no resistance from plant. Hence their multiplication and spread are lightening fast, which defeat the innate defence response

In the given situation plant loses its resistance and is prone to invasion

Disease is now active in the plant

These infected plants are weak in response and slow to act

On these plants we apply various toxic sprays to control the phytopathogen counts

These fungicides / pesticides control the phytopathogen counts partly or fully

In the given situation we conclude the disease in control by reducing the phytopathogen counts

PSAP Suppose to play role in Recovery Acclimation and Adaptation

At this many metabolisms undergo Recovery, Rebuilding and Reformation

Like chlorophyll pigmentation

Recouping of enzymatic activities

Efficient generation and transportation of sugars

Retain normalcy in cellular respiration

Synthesis of primary metabolites that governs the growth and vigor of plants

Synthesis of secondary metabolites which have role in plant signaling system

Accumulation and assimilation of nutrients in given conditions

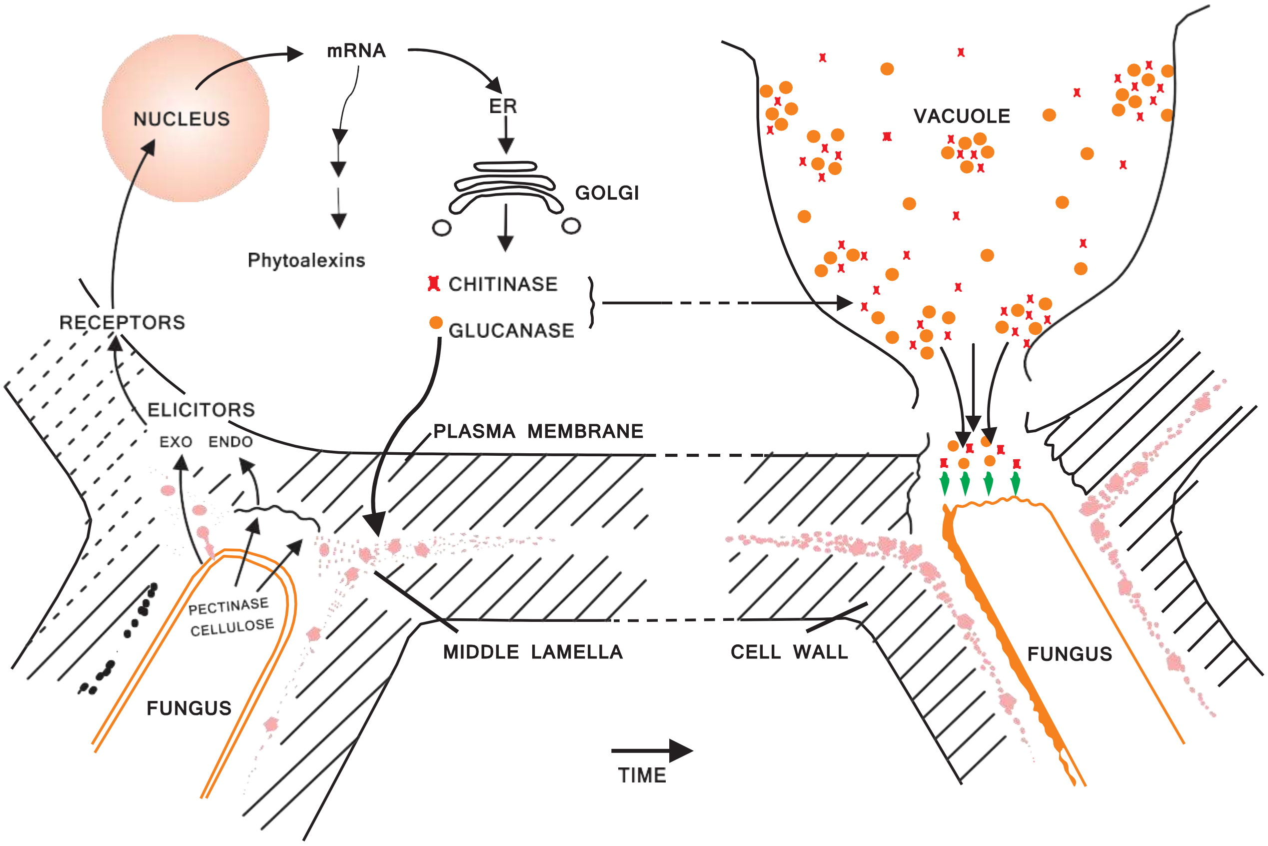
Sustain normalcy in crop plants and improve growth trait in given situation

Generation of require bio energy towards various synthesis

Gain the normalcy in the plants

Pathogenesis-Related Proteins (PR Proteins)

During the massive shift in cellular metabolism and gene expression, plants synthesize many novel proteins following infection. Some of these novel proteins may be enzymes involved in synthesizing phytoalexins and some may have no role in disease resistance at all. However, pathogenesis-related proteins have B-glucanase, chitinase or lysozyme activity. Some are related to plant defensins whereas others are proteinase inhibitors that disrupt pathogen nutrition. Pathogenesis-related proteins are sometimes present at low levels before infection and are induced following stress, wounding or flowering, indicating that they may have a wider function in plant growth and development than just disease rsistance.



Chitinase and Beta-1 ,3-glucanase in plant defence against pathogen attacks.

Chitinase and glucanase accumulate in vacuoles, although some glucanase is secreted within intercellular space. These enzymes dissolve fungal cell walls and the fragments released lead to the death of hypersensitive cells and the synthesis phytoalexins. Cellular de-compartmentalization during hypersensitive cell death ambushes the pathogen by a flood of hydrolytic enzymes released from the vacuole. Hydrolytic enzymes have antiviral, antibacterial and antifungal activity. Plants genetically transformed to overproduce glucanases, chitinases and ribozyme-inactivating proteins show about 50% reduction in disease severity. Paradoxically, some pathogens exploit the lytic activity of PR proteins to increase their virulence. Glucanases elicited by some viruses increase the porosity of plant cell walls, thus facilitating the movement of viral particles between cells. Pathogenesis-related proteins accumulate over several days, reaching a maximum about seven to ten days after the initial infection. In contrast, gene-for-gene resistance is determined within hours of the initial attack. These results show that hydrolytic enzymes reduce susceptibility to disease if they are present at the time of challenge, as in plants with systemic acquired resistance, a response that protects plants against re-infection. Accumulation of PR-proteins seems to be rapid in PSAP-treated plants.

Systemic Acquired Resistance (SAR)

It has been known since the early twentieth century that plants surviving an attack by a pathogen become systemically protected against subsequent infections. Systemic acquired (also called induced) resistance protects plants against a wide range of pathogens and not just the pathogen that induced the response. The expression of SAR does not make plants immune but reduces disease severity.

The development of systemic acquired resistance involves three steps:

1) The development of a slowly expanding necrotic lesion. Induction of systemic resistance may be associated with other localized responses such as hypersensitive cell death, phytoalexins accumulation, papilla deposition and lignification.

2) Systematic translocation to the phloem of a signal released two or three days after the appearance of the inducing lesion. This signal is graft- transmissible and is not specific to a cultivar, species or genus but ceases to be active once plant beings to flower. The entire signal originates from the induction site.

3) Primins of the rest of the plant against further pathogen challenge.
Defence responses such as the rapid release of ROS, hypersensitive cell death, phytoalexins accumulation, and enhanced levels of PR proteins are expressed more rapidly and intensely in PSAP-treated plants than in untreated plants.

The identity of the signal that triggers SAR has been the subject of intense study in PSAP-treated plants. Several molecules can induce the features characteristic of SAR, including salicylic acid, B-ionone and jasmonic acid. The entire response is, however, apparently mediated by a complex signal transduction pathway regulated by a number of stress signals.Salicylic acid, a precursor of aspirin widely distributed in the plant kingdom, plays a key role in SAR. Salicylic acid binds to at least two proteins found in plant cell membranes.

One of the above two proteins shows catalase activity, which is inhibited upon binding, causing a localized build-up of hydrogen peroxide. This form of reactive oxygen causes a number of changes in plant cells that increase their resistance to pathogens. The second, high-affinity, salicylic acid-binding protein appears to activate gene expression directly. Levels of salicylic acid rise rapidly around necrotic lesions in plants and remain high in plants that have acquired resistance. Although it must be present for SAR to be expressed, salicylic acid is not translocated over long distances in plants and presumably interacts with another systemic signal.

We have learned to trigger SAR artificially by spraying plants with PSAP to alleviate stress. PSAP is gaining favour in the agricultural community because it is much less toxic to humans and wildlife than fungicides or antibiotics, and its protective effects last much longer.

Detection and Response to Insect Herbivores

Mechanical damage caused by insects is not generally considered a “true” plant disease although plants have developed surveillance systems designed to recognize insect pests and respond with specific defence mechanisms. Plants can distinguish between general wounding and insect feeding by the presence of elicitors contained in the saliva of chewing insects. In response, plants may release volatile organic compounds (VOCs), including monoterpenoids, sesquiterpenoids and homoterpenoids. These chemicals may repel harmful insects or attract beneficial predators that prey on the destructive pests. Feeding on one part of a plant can induce systemic production of these chemicals in undamaged plant tissues and, once released, these chemicals can act as signals to neighboring plants

Feeding on one part of a plant can induce systemic production of these chemicals in undamaged plant tissues and, once released, these chemicals can act as signals to neighboring plants to begin producing similar compounds. Production of these chemicals exacts a high metabolic cost from the host plant, which is why many of these compounds are not produced in large quantities until after insects have begun to feed.

Chemical Defences

Primary and Secondary Plant Metabolites

Plant chemicals, also called plant metabolites, can be divided into two major categories: primary metabolites and secondary metabolites. Primary metabolites are substances produced by all plant cells that are directly involved in growth, development, or reproduction. Examples include sugars, proteins, amino acids, and nucleic acids. Secondary metabolites are not directly involved in growth or reproduction but they are often involved in plant defence. These compounds usually belong to one of three large chemical classes: terpenoids, phenolics, and alkaloids.

Secondary Metabolism and Metabolites

Secondary metabolism comprises a coordinate series of coupled enzymatic conversions that uses limited amounts of the products of primary or central metabolism as substrates or intermediates. Secondary metabolism uses a highly organized systematic mechanism that integrates into the developmental, morphological and biochemical regulatory patterns of the entire plant metabolic network. The inevitable link between metabolic fluxes of central metabolism and the synthesis of secondary metabolites further substantiates the existence of coordinated gene expression networks the interface of the two types of metabolism.

Accumulating evidence suggest that many transcriptional factors (TFs) coordinate the transcriptional activation of secondary metabolism genes concurrently with the expression of genes in upstream pathways of primary metabolism. For example, transketolase activity has been identified as an important determinant of photosynthetic and phenylpropanoid metabolism, and the provision of precursors by primary metabolism co-limit has been shown to the flux into the shikimate pathway and phenylpropanoid metabolism. A slight modification in transketolase activity significantly alters phenylpropanoid metabolism. There is no fixed & commonly agreed upon system for classifying secondary metabolism.

However, based on their biosynthetic origins, they can be broadly divided into three main groups: terpenes, phenolics, nitrogen and sulfur containing compounds.

A number of these phytochemicals have well defined eco-physiological and defence adaptive roles in plants. More than 2,00,000 secondary metabolites are known and many more continue to be discovered: 1700 of these are known to be VOC's with high vapour pressure under normal conditions to be vapourized into the atmosphere. These volatiles are involved in a range of ecological functions, including indirect plant defence against insects, pollinator attraction, plant to plant communication and plant-pathogen interactions.

The low-molecular-weight compounds, namely nitric oxide, ethylene, jasmonic acid, methyl jasmonate, isoprene usually act as stress signals. Isoprene, nitric oxide and other compounds also directly act as antioxidants and are involved in scavenging of ROS, thermo-tolerance and adoption to environmental stress.

Alkaloids, such phenolic compounds as flavonoids, tannins, anthocyanin, coumarin, lignin, phytoalexins and terpenes and terpenoids, are phytochemical derivatives of secondary metabolism. These secondary metabolites are involved in the acetate-mevalonate, acetate-malonate and shikimic acid path.

Alkaloids : Nitrogen Compounds

Alkaloids encompass about 12,000 low-molecular-weight natural products. The principal requirement for classification as an alkaloid is the presence of a basic nitrogen atom at any position in the molecule, which does not include nitrogen in an amide or peptide bond. As implied by this exceptionally broad definition, alkaloids form a group of structurally diverse and genetically unrelated molecules. As opposed to most types of secondary metabolites the similar chemical structures of which are derived from related synthetic pathways, the many classes of alkaloids have unique origins.

Alkaloids synthesis and accumulation are associated with a variety of cell types in different plants, including epidermis, endodermis, pericycle, phloem parenchyma, phloem sieve elements and companion cells, specialized mesophyll and lacticifers. Alkaloids are commonly synthesized with amino acids as the starting precursor molecules. Alkaloids are derived from various sources and categories as tropane and nicotine, Amarryllidaceae, piperidine, indole alkaloids and benzophenanthridine although some purine-derived alkaloids

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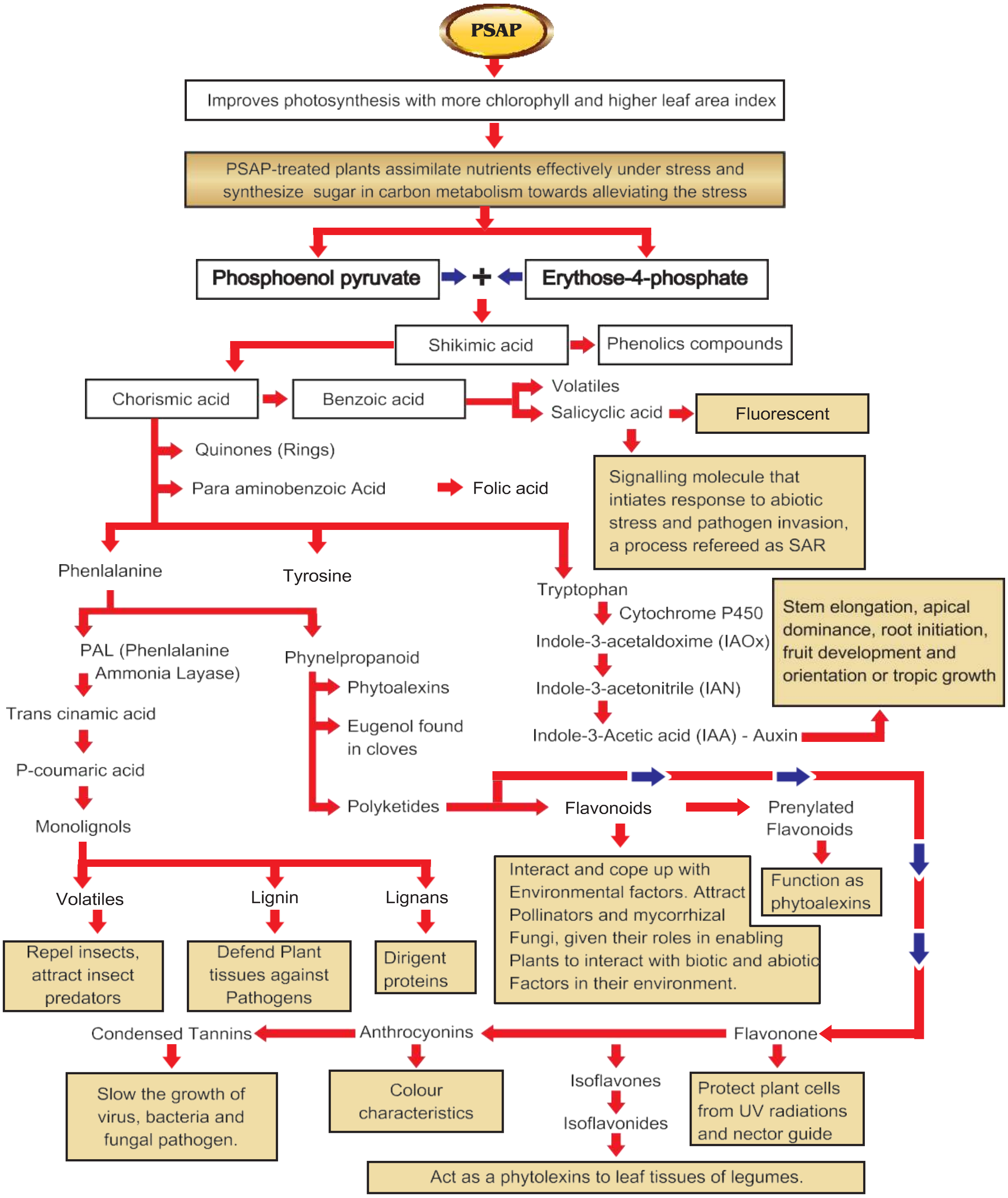
PSAP in Shikimic Acid Path – Secondary Metabolism



PSAP

Improves photosynthesis with more chlorophyll and higher leaf area index

PSAP-treated plants assimilate nutrients effectively under stress and synthesize sugar in carbon metabolism towards alleviating the stress

Phosphoenol pyruvate + **Erythose-4-phosphate**

Shikimic acid

Phenolics compounds

Chorismic acid

Benzoic acid

Volatiles

Salicyclic acid

Fluorescent

Signalling molecule that intiates response to abiotic stress and pathogen invasion, a process refereed as SAR

Quinones (Rings)

Para aminobenzoic Acid

Folic acid

Phenlalanine

Tyrosine

Tryptophan

Cytochrome P450

Indole-3-acetaldoxime (IAOx)

Indole-3-acetonitrile (IAN)

Indole-3-Acetic acid (IAA) - Auxin

Stem elongation, apical dominance, root initiation, fruit development and orientation or tropic growth

PAL (Phenlalanine Ammonia Layase)

Trans cinamic acid

P-coumaric acid

Monolignols

Phynelpropanoid

Phytoalexins

Eugenol found in cloves

Polyketides

Flavonoids

Prenylated Flavonoids

Function as phytoalexins

Interact and cope up with Environmental factors. Attract Pollinators and mycorrhizal Fungi, given their roles in enabling Plants to interact with biotic and abiotic Factors in their environment.

Volatiles

Lignin

Lignans

Repel insects, attract insect predators

Defend Plant tissues against Pathogens

Dirigent proteins

Condensed Tannins

Anthrocyonins

Flavonone

Slow the growth of virus, bacteria and fungal pathogen.

Colour characteristics

Isoflavones

Isoflavonides

Protect plant cells from UV radiations and nector guide

Act as a phytolexins to leaf tissues of legumes.

are also known. Due to the toxic nature of alkaloids, their synthesis provides a general defensive mechanism to the producing organism. For example, caffeine, cocaine, morphine and nicotine derived from amino acids aspirate, lysine, tyrosine, and tryptophan have powerful effect on animal physiology. It is toxic to both insects and fungi.

Cyanogenic glycosides are also a particularly toxic class of nitrogenous compounds that break down to produce hydrogen cyanide (HCN), a lethal chemical that halts cellular respiration in aerobic organisms. Plants that produce cyanogenic glycosides also produce enzymes that convert these compounds into hydrogen cyanide, including glycosidases and hydroxynitrile lyases, but they are stored in separate tissues or compartments within the plant; when herbivores feed on these tissues, the enzymes and substrates mix and produce the lethal hydrogen cyanide. Glucosinolates, also known as mustard oil glycosides, are sulfur-containing compounds synthesized by members of the mustard family and produce cyanide gas when broken down by enzymes called thioglucosidases. The enzyme tryptophan decarboxylase has been identified with differential expression during stress and development. It is suggested that the enzyme plays a dual role in primary and secondary defence. PSAP applications to stressed plants recoup the enzymes and restore the disrupted enzymatic activities. Alkaloids in response to biotic stress are supposed to accumulate effectively in PSAP-treated plants. The role of PSAP role in cellular respiration requires to be characterized.

Phenolics / Phenolic Componds

Phenolics are another large class of secondary metabolites produced by plants to defend themselves against pathogens. They are produced primarily via the shikimic acid and malonic acid pathways in plants, and include a wide variety of defence-related compounds including flavonoids, anthocyanins, phytoalexins, tannins, lignin, coumarin and furanocoumarins.

Flavonoids

Flavonoids are one of the largest classes of phenolics. Built upon a flavones skeleton, flavonoids are the most widespread and diverse class of low-molecular-weight phenolic compounds and are derived from a combination of the shikimic acid and the acetate pathways. Flavonoids can occur as monomers, dimers, and higher oligomers and are constituents of a variety of plant parts, including, leaves, fruits, seeds, flowers, and roots, with over 4000 different variants identified so far. Plants that produce them, flavonoids provide protection against ultraviolet radiation, invading pathogens, and herbivores. One of the ascertained functions of flavonoids in plants is their protective role against microbial invasion. Numerous flavonoids have been characterized as antifungal, antibacterial, antiviral and antioxidant are several consistent lines of evidence support the role of flavonoids in radical scavenging, chelating, and oxidant activities against various ROS.

Anthocyanins

Anthocyanins are colourful water-soluble flavonoid pigments produced by plants to protect foliage from the damaging effects of ultraviolet radiation. Anthocyanins are responsible for the showy colors of many plants and are present in high concentrations in flowers, fruits, and the leaves of deciduous plants in fall. Phytoalexins are isoflavonoids with antibiotic and antifungal properties that are produced in response to pathogen attack. These toxic molecules disrupt pathogen metabolism or cellular structure but are often pathogen specific in their toxicity. Examples include medicarpin produced by alfalfa (medicago sativa) are rishitin produced by both tomatoes and potatoes (solanacea family).

Phytoalexins

Phytoalexins are low-molecular-weight antibiotics produced by plants in response to infection. Their toxicity is non-selective and the chemical affinity of most phytoalexins for lipids suggests that they accumulate in cell membranes. For phytoalexins to play a role in disease resistance, they must accumulate to inhibitory levels at the infection court and restrict further development of the pathogen.

Since 1909, when phytoalexins were discovered, over 350 phytoalexins have been found in over 100 plant species from 30 families of dicotyledons and monocotyledons. Phytoalexins have been isolated from all parts of plants but different organs may accumulate different phytoalexins. The chemical structure of phytoalexins is diverse but, with one exception, they are small organic compounds synthesized through one of the three secondary metabolic pathways: the acetate-malonate, actetate-mevalonate, and shikimic acid pathways.

Most plant species produce several, chemically related phytoalexins, presenting a toxic cocktail to any invading pathogen. For example, many legumes synthesize phenylpropanoid phytoalexins via the shikimic acid and acetate-malonate pathways, whereas most solanaceous plants produce terpenoid phytoalexins via the acetate-mevalonate pathway. French beans produce at least five phenylpropanoid phytoalexins, and potato synthesizes at least four terpenoids. Phytoalexins are believed to be synthesized in cells adjacent to the infection site in response to a signal produced either by the invading pathogen or by infected host cells and are packaged in lipid vesicles and exported to the infected cell.

Consequently, the infected cell becomes a toxic micro-environment for the invading pathogen. Phytoalexins accumulation is often associated with hypersensitive cell death. However, phytoalexins synthesis requires gene expression and the activation of complex biochemical pathways involving perhaps 20 enzymes, which must occur in living cells. Many steps in their synthesis are sensitive to regulation by the host and the pathogen.

Some plants, such as soybean and chickpea, synthesize phytoalexins upon infection, but convert a proportion into inactive sugar conjugates held in reserve in vacuoles. If the initial defence response fails to check pathogen growth, enzymes that cleave the sugar molecule are activated and the phytoalexins reserves are rapidly released. Phytoalexins synthesis is localized in cells immediately surrounding the infection court. There is no evidence that they are dispersed within the plant. In a number of interactions, resistance is lost if phytoalexins synthesis is blocked by inhibitors of enzymes involved in the synthesis and is reduced in mutants that are slow to accumulate phytoalexins.

Resistance is increased in plants transformed to express novel phytoalexins or if exogenous phytoalexins are applied. For example, although the biochemical precursor of resveratrol is widely distributed in the plant kingdom, only grapevine and peanut have the enzyme required to complete its synthesis. When the genes encoding this enzyme are transformed into tobacco, resveratrol is synthesized in response to infection.

Like other active defence responses, the success of phytoalexins accumulation depends on the speed, location and magnitude of the response. There is a good experimental correlation between resistance and rapid, localized phytoalexins accumulation in many host-parasite interactions. There is evidence that phytoalexins accumulate faster and to higher concentrations in resistant cultivars. In resistant plants, gene transcription begins within one hour of recognition, phytoalexins appear within four hours and concentrations peak to fungi toxic levels about 18-24 hours after the challenge. These events are delayed and more diffuse in susceptible plants. Phytoalexin synthesis is not universal among crop plants.

Wheat and cucumber apparently do not produce phytoalexins, yet effectively resist most pathogenic fungi and bacteria. However, in many interactions the rapid accumulation of toxic concentrations of phytoalexins at the infection court plays a decisive role in the expression of resistance.

Tannins

Tannins are phenolic compounds that exhibit complex and highly variable chemical structures. Tannins are water-soluble flavonoid polymers produced by plants and stored in vacuoles. They are broadly categorized into hydrolysable and condensed tannins, based on whether acids or enzymes can hydrolyze the components or whether they condense the components to polymers. Tannins are the most widespread polyphenols in plants after lignin. In plants, condensed tannins may act as deterrents to feeding in reproductive tissue and developing fruit and also impart astringency to fresh fruit juices and wine. Tannins are characteristic chemical defence of plants and act as quantitative and dosage-dependent barriers to predators that may feed on plants. Due to their antibiotic, antifeedant, or biostatic effects on a variety of organisms that consume them, tannins act principally by binding to the virus and /or protein of the host cell membrane and thus arresting adsorption of the virus. Similarly, bacterial and fungal enzymes and toxic proteins may be bound by tannins and inactivated in a similar manner. This propensity to bind to proteins also presumably accounts for the fact that polyphenols inhibit virtually every enzyme that has been tested in vitro.

Polyphenols and protein complexes are essentially a surface phenomenon, maximized at or near the isoelectric point of the protein. Interactions are dynamic and the time independent; conformational flexibility in both the polyphenol and the protein is an important complementary factor that leads to strong interactions. Through their aromatic nuclei and phenolic groups, polyphenols act as multidenatate ligands on the protein surface and the efficacy of binding increases as the number of polyphenol galloyl groups increases. Tannins are toxic to insects because they bind to salivary proteins and digestive enzymes including trypsin and chymotrypsin resulting in protein inactivation. Insect herbivores that ingest high amounts of tannins fail to gain weight and may eventually die.

Lignin

Lignin is a highly branched heterogeneous polymer found principally in the secondary cell walls of plants, although primary walls can also become lignified. Lignin consists of hundreds or thousands of phenolic monomers and is a primary component of wood. Because it is insoluble, rigid, and virtually indigestible, lignin provides an excellent physical barrier to pathogen attack.

Coumarins

Coumarins are simple phenolic compounds widespread in vascular plants and appear to function in different capacities in various plant defence mechanisms against insect herbivores and fungi. Coumarins are derived through the shikimic acid pathway common to bacteria, fungi and plants but absent in animals. Coumarins are a highly active group of molecules with wide ranging of antimicrobial activity against both fungi and bacteria. These cyclic compounds behave as natural pesticidal defence compounds for plants and also are starting point for the synthesis of new derivatives with improved antifungal activity. The haloginated coumarin derivatives work very effectively in vitro to inhibit fungal growth. Some coumarin derivatives have higher antifungal activity against a range of soilborne plant pathogenic fungi and are more stable.

Furanocoumarins

Furanocoumarins are a type of coumarins that are specially phytotoxic are produced by a wide variety of plants in response to pathogen or herbivore attack. Normally these compounds are not toxic, until they are activated by light (UV-A) and go into a highenergy electronic state, which enables them to insert themselves into the double helix of DNA and to bind to the pyramidine bases, thus blocking transcription and repair, eventually leading to cell death. They may be activated very early by ultraviolet light in presence of PSAP and then can be highly toxic to certain vertebrates and invertebrates. In fact, grapefruit juice contains small quantities of furanocoumarins, which greatly increase the absorption of certain drugs into the bloodstream from the intestines. Some medicines carry warning labels cautioning people to avoid drinking grapefruit juice while taking the drugs in order to avoid an accidental overdose.

Terpenes and terpenoids

Terpenes, one of the largest and perhaps most structurally diverse groups of the secondary metabolites, are all synthesized from two precursors, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). Plants invariably use the mevalonate pathway in the cytosolic compartment and the non-mevalonate pathway in plastids, an aspect that signifies sub cellular compartmentalization of the pathway. The cytosolic mevalonate pathway provides the precursor for sesquiterpenes and sterols, whereas the plastidial MEP pathway furnishes the monoterpene, diterpene and carotenoids. The chemical diversity of plant terpenoids is a reflection of their multiple biological activities in nature. In plants that produce them, isoprenoids serve numerous biochemical functions including electron transport chains, as components of membrane (sterols), sub-cellular targeting and regulation (prenylation of protein), as photosynthetic pigments (carotenoids, side chains of chlorophyll) as hormones (gibberellins, brassinosteroids, abscisic acid, cytokinins) and, as plant defence compounds and attractants for pollinators.

Monoterpenoids and sesquiterpenoids are primary components of essential oils, which are highly volatile compounds that contribute to the fragrance of plants that produce them. Essential oils often function as insect toxins and many protect against fungal and bacterial attack.

Many spices, seasonings, condiments, and perfumes are made using essential oils that function as insect toxins in plants but are relatively harmless to humans. Examples include peppermint and spearmint, basil, oregano, rosemary, sage, savory, thyme, black pepper, cinnamon and bay leaf.

Diterpenoids include gossypol, a terpenoid produced by cotton that has strong antifungal and antibacterial properties. Triterpenoids are similar in molecular structure to plant and animal sterols and steroid hormones. Phytoectysones are mimics of insect molting hormones. When produced by plants such as spinach, they disrupt larval development and increase insect mortality. The fresh scent of lemon and orange peels is the result of a class of triterpenoids known as limonoids. Azadirachtin is a very powerful limonoid isolated from neem trees. Some insects are repelled by concentrations as low as a few parts per million. Citronella is an essential oil isolated from lemon grass and contains high limonoid levels and has become a popular insect repellent. Saponins are glycosylated triterpenoids (triterpenoids with attached sugars) that are present in the cell membranes of plant species.

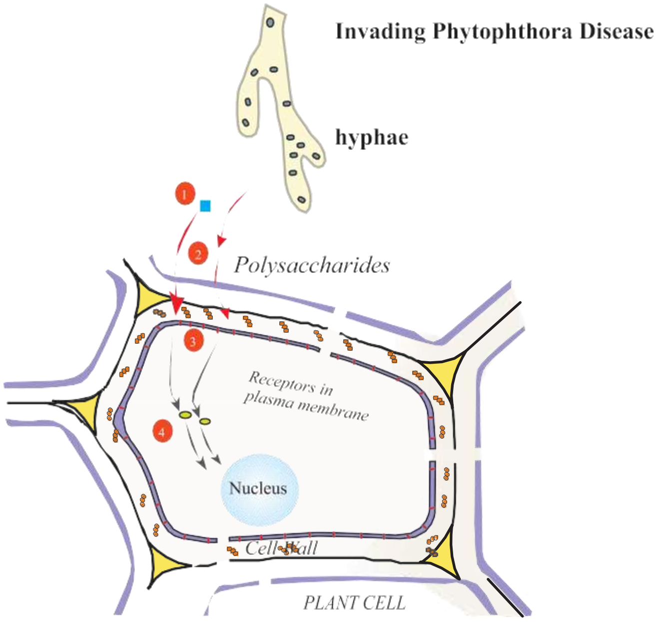
These substances have detergent (soap-like) properties and disrupt the cell membranes of invading fungal pathogens.However, some fungal pathogens have developed counter-measures to these plant defences: Botrytis cinerea, Fusarium oxysporum, and Septoria lycopersici are all capable of degrading saponins and causing disease in susceptible saponin-producing plants.

PSAP

Mediated Mitigation of Biotic Stress

PSAP is a systemic molecule that is mobile in both xylem and phloem. It moves from old leaves to new leaves and vice versa as well as from leaves to roots and vice versa. The action of PSAP on a pathogen is direct as well as indirect and complex.

Shows how the plant reacts to invasion by a pathogen without the presence of PSAP.



① Some molecules from the pathogen are recognized directly but

② Phytophthora masks this recognition with suppressors; thereafter

③ Recognition fails at the host cell interface, and

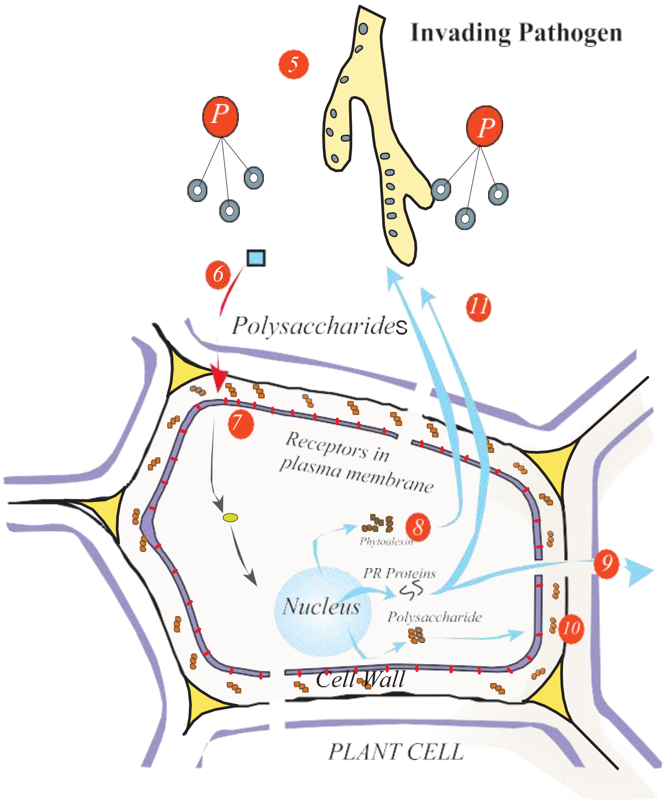
④ Only a weak signal goes to cell nucleus thereby delaying the plant response to pathogen without PSAP.

PSAP is directly fungistatic, i.e. it stops the growth of the plant pathogen and inhibits the formation of spores. This causes the release of stress metabolites (chemicals) by the pathogen which are recognized by the plant as signals or as elicitors, causing the plant to enhance its defence response. One of the results of the defence response by the plant is the accumulation of phytoalexins (immune bodies); these cause an immune response similar to that in humans. In addition, hypersensitive cell death occurs (death of infected cells) as well as lignification and cell wall fortification (cell walls are thickened) take place. Lytic enzymes (which dissolve the walls of diseased cells) are also produced by the plant which, in combination with rest of the hyper-resistant response, can kill the pathogen.

PSAP

Mediated Mitigation of Biotic Stress

Plant response to pathogen in the presence of PSAP



5 Pathogen is recognized by the defence system in presence of PSAP.

6 Suppressors either under produced or not produced.

7 Recognition of the phytophathogen by plant cell.

8 PSAP encourages defensive molecules such as phytoalexins and PR proteins, and attacks the pathogen directly.

9 Defensive molecules send alarm signals to cells that have not been attacked yet.

10 Polysaccharides strengthen the cell wall adding additional protection.

11 Pathogen is limited or killed by PSAP plant response in the process.

In short, Application of PSAP enhances the activity of the plant's dynamic defence system. This induces the formation of necrotic blocking zones (dead cells limiting the spread of resultant lesion), rapid changes within the cell, production of ethylene and the death of hypersensitive cells(death of infected cells). Production of lytic enzymes, thickening of cell walls and phytoalexin accumulation in infected plants take place quickly.

PSAP

Mediated Mitigation of Biotic Stress

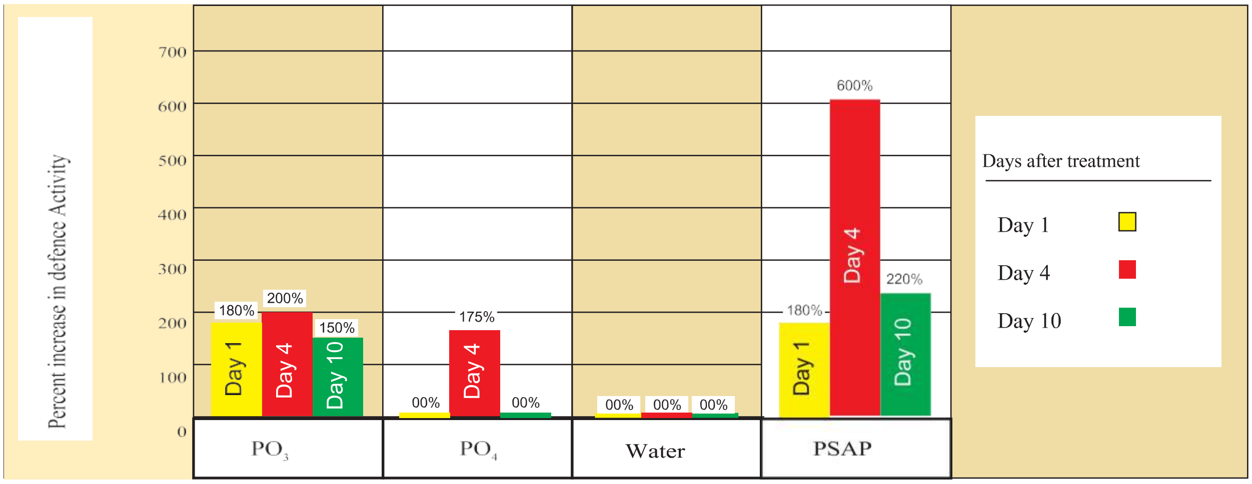
Over all

PSAP is not only profoundly effective in the Shikimic acid pathway the mere presence of PSAP in a plant increases the production of allelopathic compounds in various pathways. These key metabolic pathways produce hundreds of compounds that are involved in fighting against pests and pathogens. Many of these compounds have a role in the management of abiotic forms of stress. Some of these compounds include,

- Lignins and tannins
- Phytoalexins
- Essential amino acid
- PR - protein
- ATP and NADP
- Cholorophyll
- Phenolic compounds
- Ethylene
- Jasmonates
- Salicyclic acid
- Auxinx / cytokinins
- Abscissic acid

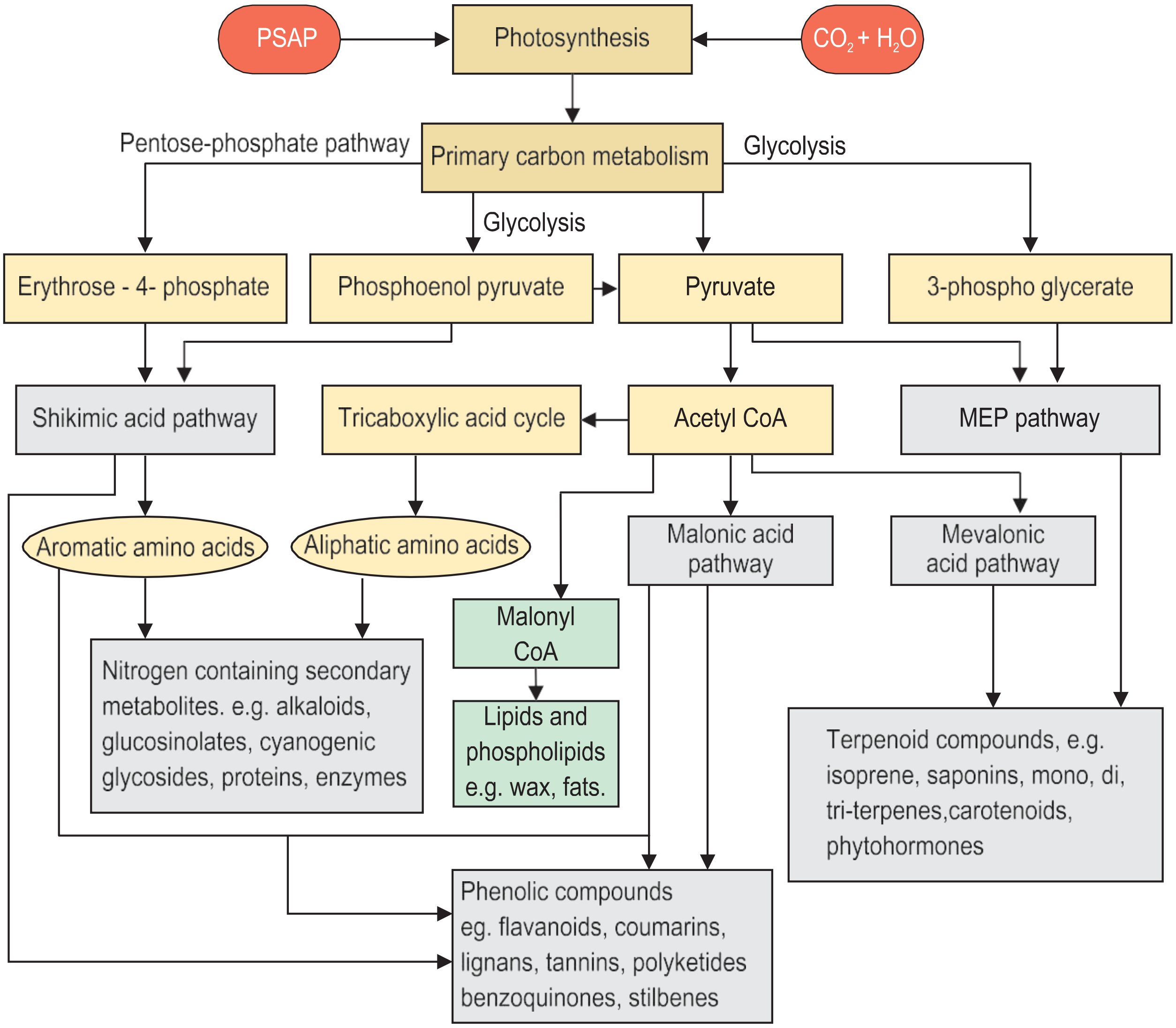
A hypothical comparison of three sources of phosphorus is presented in the graph with reference to the role of phosphorus in inducing a response to stress, based on our studies.

As can be seen, PSAP induces a response that is not only greater but also last longer.



induced in defence response to stress (DRS) plotted against time in percentage.

PO_3^- : Phosphite / Phosphonate : Nominal increment in DRS
PO_4^- : Phosphate : Hardly any increment in DRS
Water : No increment in DRS
PSAP : Fully Charged DRS



Impact of PSAP on a plant in terms of metabolites produced
(light yellow : primary metabolites and gray : secondary metabolites)

Primary metabolites such as amino acids, proteins, nucleotides, carbohydrates and lipids have a direct role in the plant metabolic processes of respiration, photosynthesis and use of nutrients through assimilation, transport and translocation. These primary metabolites are involved in plant growth, vigour and maturity.

A plant also produces secondary metabolites, which include of toxic chemicals and pathogen-degrading enzymes and can kill plant cell. Production of secondary metabolites is very expensive as it makes great demands on energy and nutrients. Many times, under in stressful conditions, growth is compromised to ensure survival.

Influence of autonomous combination of Phosphorus and Potash

Active phosphorus greatly influences photosynthesis and carbon metabolism. Under phosphate deficiency, the accumulation of carbohydrates in roots increases significantly, registering positive correlations among the concentration of phosphate in the environment, the concentrate of active phosphorus in the plant and the concentration of hexose phosphate in leaves, starch precursor molecule in chloroplasts and of sugar in cytosol.

With the addition of 5.0% of total P in the form of active phosphorus, a significant increment in the concentration of total sugars during the blooming stage was observed. It is a positive effect, because a high concentration of total sugars in the plant leads to early production and increases yield. On the other hand, active phosphorus is a nutrient that has influence on the stability of the chlorophyll molecule. Leaves treated with high concentrations of active P and K turn dark green, indicating a possible change in the concentration of chlorophyll.

The role of active phosphorus and potash from PSAP in various metabolic processes of plants is complex and not yet fully understood. The responses of PSAP-treated plants are co related to various strategical mechanisms to authenticate its role further. Although there is no consensus so far on its physiological function as a P source for plant nutrition, experimental evidence has shown that active phosphorus can alleviate abiotic and biotic form of stress.

Active phosphorus and potash from PSAP play an important role in increasing plant resistance to abiotic stress. Potash from PSAP has a major role in the survival of crop plants under abiotic stress conditions. Active potash from PSAP has many essential roles in such physiological processes, as photosynthesis, translocation of photosynthates into sink organs, maintenance of turgidity and activation of enzymes under stress conditions.

PSAP-treated plants can modulate a wide range of adaptive or resistance mechanisms to maintain productivity and ensure survival under a variety of environmental forms of stress such as drought, chilling, frost, high temperatures, soil salinity or sodicity and nutrients imbalance.

Low-temperature stress affects the fluidity of membrane lipids, which may alter membrane structure. Low temperature also affects photosynthetic electron transport, stomatal conductance, rubisco activity, and CO_2 fixation in plants due to conversion of O_2 to ROS.

PSAP

Mediated Mitigation of Biotic Stress

Potassium in required amounts can protect plants from oxidative damage caused by chilling or frost. Decrease in yield and increase in leaf damage induced by frost under field conditions can be alleviated by the application of PSAP. It is observed that adequate supply of PSAP potash enhances total plant yield many fold depending upon the plant geometry. PSAP is directly or indirectly involved in several physiological and biochemical processes during plant growth such as cell elongation, cell division, cell wall biosynthesis, membrane function, nitrogen(N) metabolism, photosynthesis and uracil synthesis.

PSAP increases the activities of antioxidants in plants and thereby alleviates ROS damage induced by temperature stress. PSAP improves sugar transport in the plant, which helps to improve both germination and grain formation. This in turn improves yield by increasing the tolerance to high allow temperature. PSAP application also improves carbohydrate metabolism and decreases phenolic compounds in leaves under abiotic stress. This in turn reduces the production of ROS and enhances the rate of photosynthesis and reduces damage to cells.

PSAP also seems to involve in the activation of many enzymes in plant systems mostly in oxidation-reduction, decarboxylation and hydrolytic reactions and hence might be playing a role in detoxification of ROS. PSAP can reduce the adverse effects of temperature stress indirectly by enhancing the rate of photosynthesis and nitrogen metabolism in the plant body. PSAP reduces interveinal chlorosis, brown necrotic spots on leaves and premature leaf drop. PSAP has some role in lowering, the production of free radicals of oxygen and increases the activity of anti-oxidative compounds and enzymes under temperature stress.

Potassium is required by plants in amounts similar to or greater than nitrogen. Uptake of K by the plant is highly selective and closely coupled to metabolic activity at all levels in plants, within individual cells and tissues and in long-distance transport via xylem and phloem. Potassium takes part in many essential processes: enzyme activation, protein synthesis, photosynthesis, phloem transport, osmoregulation, cation-anion balance, stomatal movement and light-driven nastic movements. Potassium has been described as the "quality element" for crop production. Potassium increases the protein content of plants, starch content of grains and tubers, vitamin C and soluble solids in fruits. Potassium improves fruit colour and flavour and increases the size of fruits and tubers, it reduces the incidence of pests and diseases, enhances storage and shipping quality and extends shelf life. The crucial importance of K in quality formation stems from its role in promoting the production of photosynthates, their transport to storage organs such as fruits, grains and tubers and their conversion into starch, proteins, vitamins, oils, etc.

PSAP

Mediated Mitigation of Biotic Stress

With shortage of K, many metabolic processes are affected, including photosynthesis, translocation and enzyme production, at the same time, the rate of dark respiration is increased. The result is reduction in plant growth and quality. Effectiveness of K depends on its nature, i.e. whether it exists as a free ion in solution or as an electrostatically bound cation.

Potash is bound with active phosphorus in PSAP, a synergetic combination. The profound effects of PSAP are evident in various metabolic process.

1. Chlorophyll development and photosynthesis.

2. Starch formation : starch synthesis is triggered by K from PSAP more effectively than conventional application of potash. Starch builds cellulose and reduces lodging.

3. Sugar transport system uses energy in the form of ATP. If K is inadequate, less ATP is available and the transport system breaks down, causing photosynthates to build up in leaves and the rate of photosynthesis is reduced. Translocation of sugars and starch is ensured with regular application of PSAP synergetic potash combination in plants.

4. The activation of enzymes by K and its involvement in adenosine triphosphate (ATP) production is important in regulating the rate of photosynthesis. Solar Energy + CO_2 + H_2O = Sugar + O_2 + ATP -The electrical charge balance at the site of ATP production is maintained with synergetic potash ion (initial high energy product).

5. Rate of a reaction is controlled by the rate at which PSAP-potash enters a given reaction.

6. Application of such potash effectively controls stomata opening and closure reducing water loss and wilting.

7. PSAP-potash prevents premature cell death more effectively than conventional potash.

8. Uptake of water and nutrients by osmosis by accumulating of K in plant, roots produces a gradient of osmatic pressure that draws water into the roots. When K supply is reduced, translocation of nitrates, phosphates, calcium, magnesium and amino acids is depressed. Accumulation of PSAP-potash regulates metabolism.

PSAP

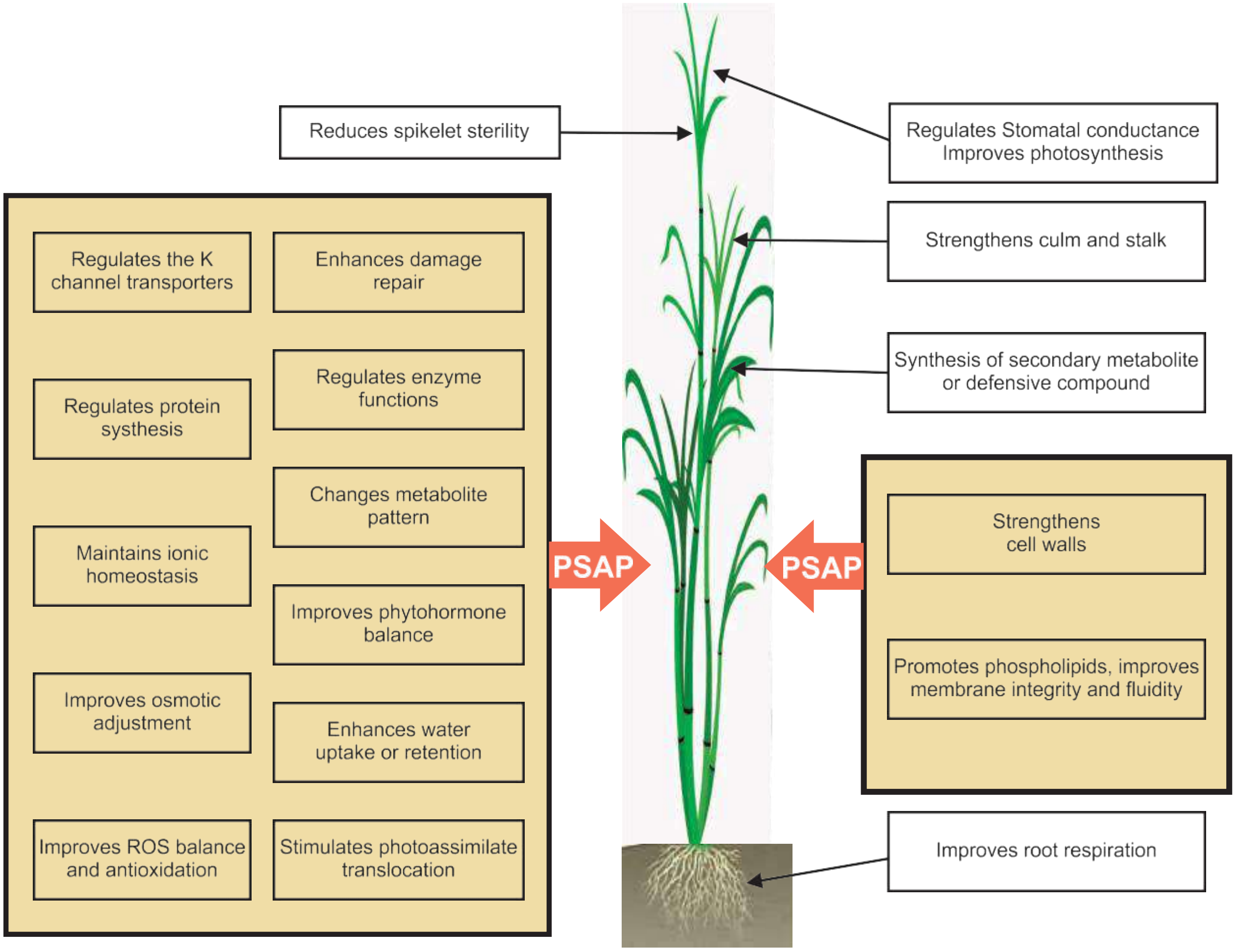
Mediated Mitigation of Biotic Stress

9. `K' activates at least sixty enzymes involved in plant growth. Enzymes serve as catalysts for biochemical reactions, being used but not consumed in the process. They bring together other molecules to ensure that the required chemical reaction can take place. Potash from PSAP promotes enzyme activity more efficiently than conventional K$^+$ion.

10. PSAP potash changes the physical shape of the enzyme molecule, exposing the appropriate chemically active sites for reaction in stress. Synergetic potash from PSAP seems to have a major role in alleviating stress.

11. PSAP potash helps to stabilize the pH between 6 and 7 which is optimum for most enzyme reactions e.g. optimum the pH of plasma membrane 1'1$^+$ - AT Pase is 6.0

12. Potash is required at every major step in protein synthesis. The reading of genetic code in plant cells to produce proteins and enzymes that regulate all growth processes is possible with adequate ` K '. PSAP potash adds value to `K' assimilation in crops.



The role of PSAP-Potash in plants

PSAP

Mediated Mitigation of Biotic Stress

Stressed vine yard

Stress free vine yard after sprays of PSAP

PSAP

Mediated Mitigation of Biotic Stress

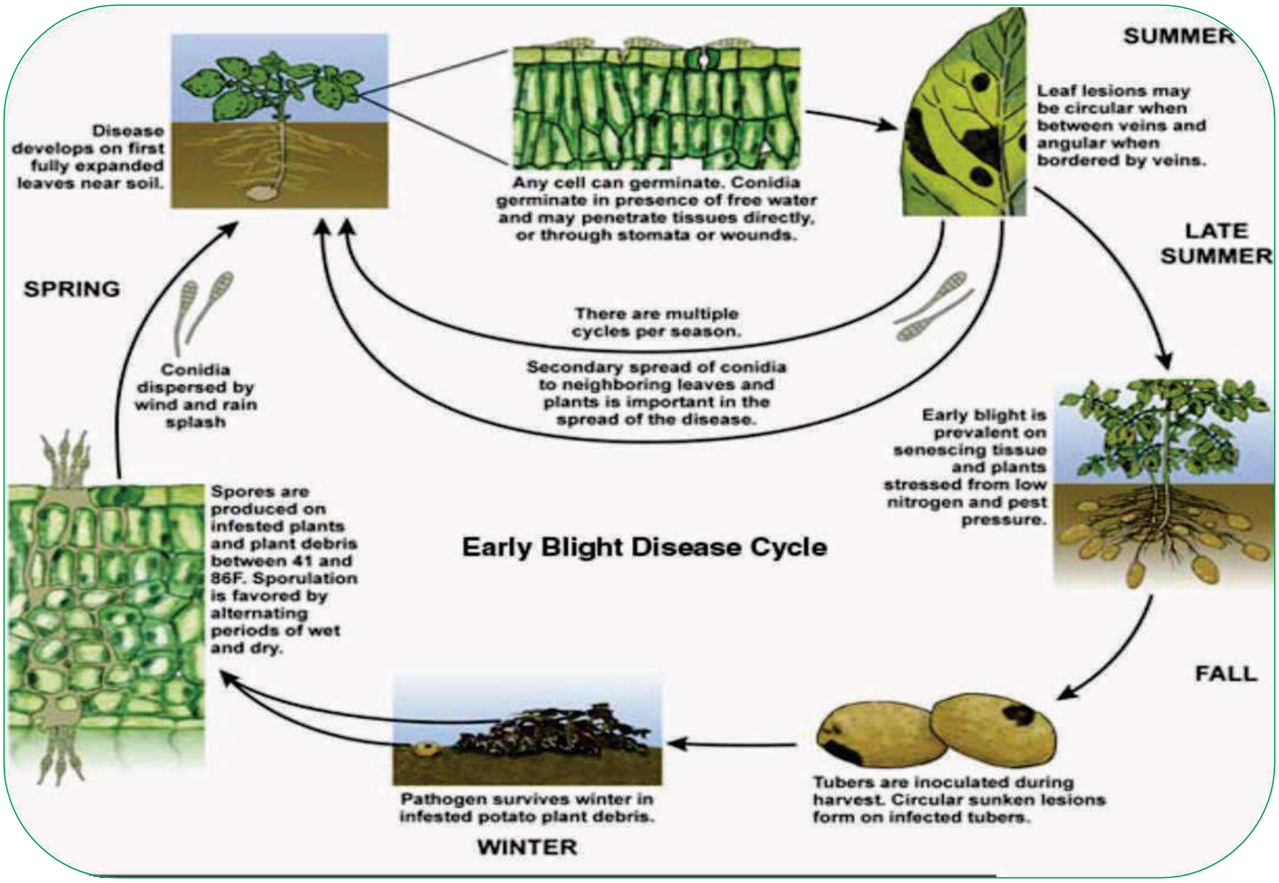
PSAP-treated plants must be evoking many mechanisms to overcome the probable stress and to defend themselves. Recognition of non-self, transcriptional reprogramming and accumulation of secondary metabolites in PSAP-treated plant and pathogen interactions require further basic research. A multiomics approach to identify regulatory nodes in the transcriptional network of SAR in PSAP-treated plants can be the elaborated for further studies.

Identification, characterization and detection of the response to stress in PSAP-treated crop plants with respect to the perspectives will suggest a set of strategies.

- Genes, proteins and metabolites in response to stress and PSAP interactions
- Adapted functions of genes, proteins and metabolites
- Temporal and spatial monitoring of cell physiology, deposits and ultra structural changes
- New metabolic pathways
- Reverse genetics (from metabolites to proteins togenes)
- Gene network mapping or interconnecti ons, signature or signal molecules, phenotype

Cellular level	Multi-omics	Identification and characterization
Genome	Genomics	Sequencing of genes and whole genomes
Transcriptome	Transcriptomics	Gene expression profiling/transcript abundance
Proteome	Proteomics	Protein identification and functional characterization
Glycome	Glycomics	Understanding protein glycosylation
Interactome	Interactomics	Understanding protein-protein interactions
Transcriptome	Transcriptomics	Unbiased or targeted analysis of cellular metabolites
Proteome	Proteomics	Addressing phenotypic changes under stress

- These diseases are caused by the water mold pathogens. These pathogens are not true fungi and as a result, not all fungicides are effective against such pathogens. Late blight have been controlled by metalaxyl or mefenoxam. However resistance to these fungicides has created great difficulties. For pink rot there is hardly any fungicide to control the counts of pink rot .
- In such situation, application of PSAP is very effective in controling pink rot and late blight in potato.
- Foliar application of PSAP should begin at tuber initiation.
- A dose of 4 grams per litre of water and 200 - 400 litres of water per acre of 2-weeks intervals is necessary.
- Three to four applications are sufficient to control the diseases.
- Post-harvest applications are useful to keep tubers healthy.



Source : http://www.potatodiseases.org

PSAP

Mediated Mitigation of Biotic Stress

PSAP

- Improves resistance to downy mildew.
- Ensures superior root formation.
- Boosts resistance to stress, disorders and diseases.
- Offers better growth, earlier bud formation, blossom and fruit set.
- Improves both yield and quality of grapes.

Recommendation of PSAP for grape vines

Add PSAP to your spraying schedule. We do not recommend any other change in your existing spraying schedule. We only suggest need base pesticides spray in PSAP treated grape wine.

PSAP spraying schedule in the foundation pruning

- Start applying PSAP from the first pruning, i.e. when shoots are 10 to 15 cm in length or after the 3 to 4 true leaves stage.
- Repeat the PSAP spray at 12-15 days interval. Apply 2 to 3 sprays. The spray intervals may need to be reduced to 7 days for two to three sprays when weather is particularly favourable for disease development.
- As the weather becomes normal, revert to normal intervals and with the usual dose.
- Spray should be applied as normal high volume sprays to provide good coverage of leaves as well as of the developing grape bunches. Over wetting of vines should be avoided as this results in run-off and loss of the active ingredient.

PSAP spraying schedule in forward or fruit pruning

- Spray volume should as per the stages and canopy as mentioned below.
- Although berries are resistant to infection once at "pea size", but the stems or peduncles remain sensitive to infection. Peduncle infection occurring later in the season will cause individual berries or sections of bunch get damage. However earlier PASP protection against midsummer leaf infection will improve the quality of final produce.

Growth staged volume of spray solution required per acre at 4 gm PSAP/litre

Stage	No. of sprays	Spray volume	% Canopy
Shoot length 2.5 to 25 cm.	One	200-300 liters	70%
25 cm shoot length to pre flowering.	One	300-400 liters	80%
Flowering to pea-size berries	Two	300-400 liters	90%
After Fruit Set 60 days after pruning	One	400 liters	100%

PSAP

Mediated Mitigation of Biotic Stress

PSAP is applied by various methods including foliar sprays, trunk injection, soil incorporation, drip, drench and bare-root dip. For foliar sprays, apply PSAP with sufficient water for adequate coverage of foliage, according to crop and growth stage.

1. Fill the spray tank with ½ of the required volume of water.
2. Add PASP slowly to the tank and agitate by hydraulic or mechanical means.
3. Continue to fill the tank with water to the desired volume while agitating.
4. Continue agitation if possible when applying.
5. Do not use with any biological control agent or pH controller.
6. Do not mix with oxidizing agent, spreaders, stickers, or wetting agents.

Soil application

- Soil application : use 4 grams PSAP per liter. Use of total quantity will depend on the age and canopy of the plant. For example in mango, small trees need 15 litres medium sized plants need 20-30 litres and a large tree 30-50 liters for soil drench.

Spraying method

- For spray, dissolve 4 grams of PASP in one litre of water.
- Spray the solution early in the morning or in the evening, after 4 p.m.
- Spraying should be carried preferably when atmospheric temperature is less than 30^0C for effective absorption through foliage.
- While spraying apply zero or foggy spray so that droplets will be absolutely tiny.
- Spray the leaves thoroughly from top and bottom.
- Make sure there is no rain or sprinkle application within two hours of spraying.
- Wash your hands properly after spraying.
- PSAP is a non-toxic, harmless and does not have any side effects on any crop.
- For any inquiry please talk to our local dealers or our field officers from your area.

Compatibility

- PSAP is compatible with most commonly used fungicides.
- To determine the compatibility of PSAP with other products use a jar compatibility test. Add the correct proportions of each product and the appropriate quantity of water, thoroughly mix, then let stand for 3-5 minutes. if the mixture remains in solution or can be re-mixed readily, the products are considered compatible. Try spray on small area.
- Make applications prior to disease development in conjunction with good agricultural management practices. Use the higher rate when disease pressure is severe.
- Allow foliage to completely dry after application. Do not apply when conditions favor wet tissue for prolonged periods (more than2 hours).

PSAP

Crop wise Application and Doses

- Use 4 to 6 grams of PSAP per litre of water.
- Apply 75-400 litres of PSAP solution per acre per spray, depending on the stages and canopy.
- Spray PSAP solution throughly inside out of leaves.
- Prepare the PSAP solution only in water. Normally it is recommended not to mix any other chemical while preparing the PSAP solution.
- PSAP spray can be applied at any stage.

Crop-wise Spray Schedule

DAP : Days After Planting

Crops	Spray	Number Of Sprays Recommended	Interval between two sprays	Qty Require / Acre / Season
Onion, Potato, Capsicum, turmeric, ginger, carrot, guar, pumpkin, cucurbit and citrus crops	20-30 DAP	3 to 5	15 to 25 days	3 to 5 kg
Brassica crops, bulb and leafy vegetables crops	20-30 DAP	2 to 4	15 to 20 days	3 to 4 kg
Oil seeds, cereal grains, fiber crops, paddy, soybeans, other legume crops and cotton	20-25 DAP	3 to 5	15 to 20 days	3 to 5 kg
Avocado, banana, papaya, guava, fig, orange, mango, watermelon, pomegranate, arecanut, cacao, walnut, peach, vanilla, strawberry, coffee, clove, tea, pepper, cardamom, cinnamon and other herbs, lychee, apple, strawberry, stone fruit crops, kiwi, olives, grape, tree nut crops and ornamentals.	30 to 60 days after planting or just before flowering	6 to 12	10 to 15 days	12 to 18 kg

PSAP

Profitable Sustainable Agricultural Practices

In C4 Crop Plants

Sugarcane

Potential to Double Net Income of Millions of Cane Farmer
Increases Mill Receipt By 30% and Profits Nearly By 100%
Extra Cane Meets Additional Ethanol Requirement in EBP

Challenges in Sugarcane

Yield Potential Losses

Sugarcane is the most important plant source of sugar and ethanol and is cultivated in more than 80 countries in the tropics and the subtropics. However, environmental factors can lower its yield and jeopardize the prospect to meet the increasing demands for bio-ethanol and sugarcane-derived bye products. The development of stress-tolerant plants is fundamental to maintaining and increasing of crop yields. Biotechnology to enhance sugarcane productivity and stress tolerance provides a comprehensive account of both theoretical and practical aspects of sugarcane production. It contains extensive coverage of genome mapping and molecular breeding in sugarcane and presents the status of the elucidation and improvement of plant genomes of economic interest.

Average sugarcane yield in India is 25 to 30 tonnes per Acre per Year (65 to 75 t/ha.)

Losses in sugarcane yield are estimated at 70% to 80% because of marginal conditions.

Yield	**Annual yield (t/ha)**	**Physiological limit**	**Yield losses % wise**
Theoretical maximum	472	**Crop characteristics** - Phenology - Physiology - Architecture - Cell characteristics	0.00
Experimental maximum	220	**Environmental constraints** - Water - CO_2 - Radiation - Temperature - Nitrogen, phosphorous and potash imbalance	50 to 55 ↓
Commercial maximum	150	**Agronomic constraints** - Weeds - Pests - Diseases - Mineral toxicity - Salinity / Sodicity	20 to 25 ↓
Average	80	Based on farmer practices	70 to 80 Overall losses

Reference from- Sugarcane biology, yield and potential for improvement by Paul H. Moore, USDA. Bray et. al. 2000

C4 Type Crop Plant

Sugarcane

Sugarcane is a C4 type crop plant

C4 plants are highly productive

Low photorespiration

Stomata partly closed during the day

Most suited for hot, dry environments

Minimum loss in respiration

Sugarcane potentially produces 1 Lakh biochemical

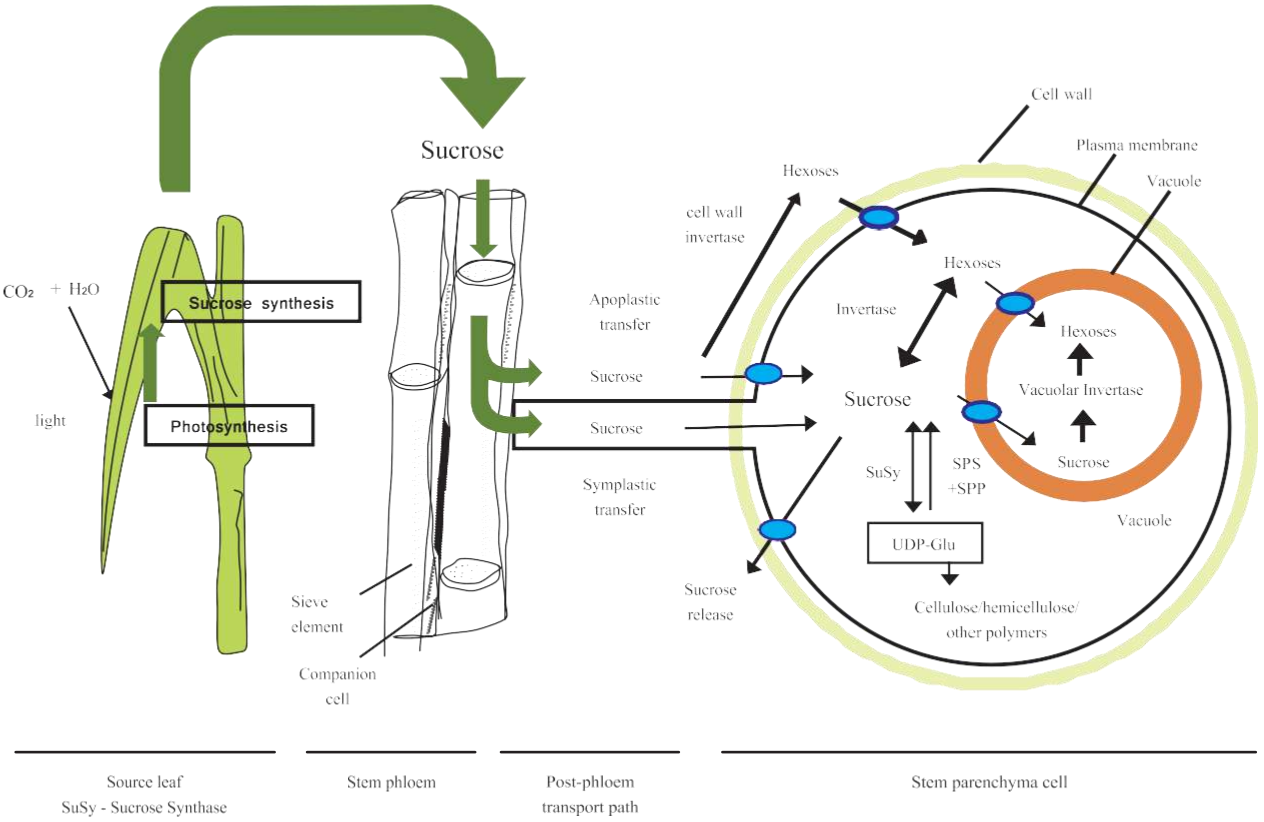
Greater photosynthetic efficiency

Lose less water than C3 crop plants, but produce the same amount of Carbohydrate (water efficient, growth efficient)

Part of carbon fixation is done in a "different" cell (CO_2 is concentrated in bundle sheath cells to increase the likelihood of creating sugars, instead of photorespiration)

Some of C4 plants : sorghum, maize, sugarcane, crabgrass and bermuda grass.

Synthesis, Transport and Storage of Sugars

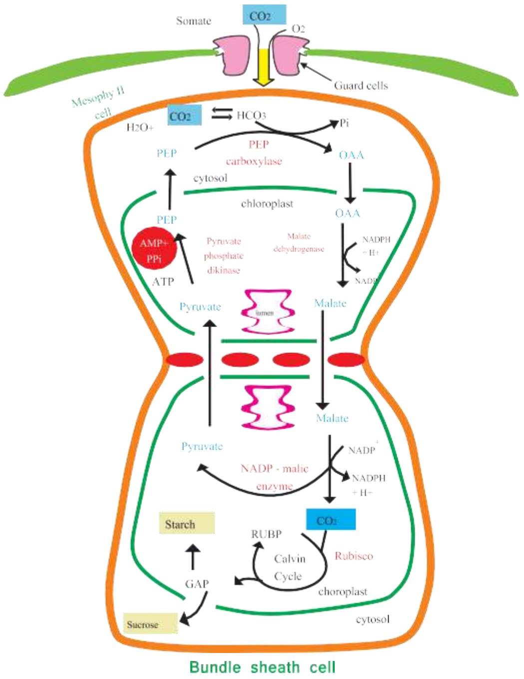


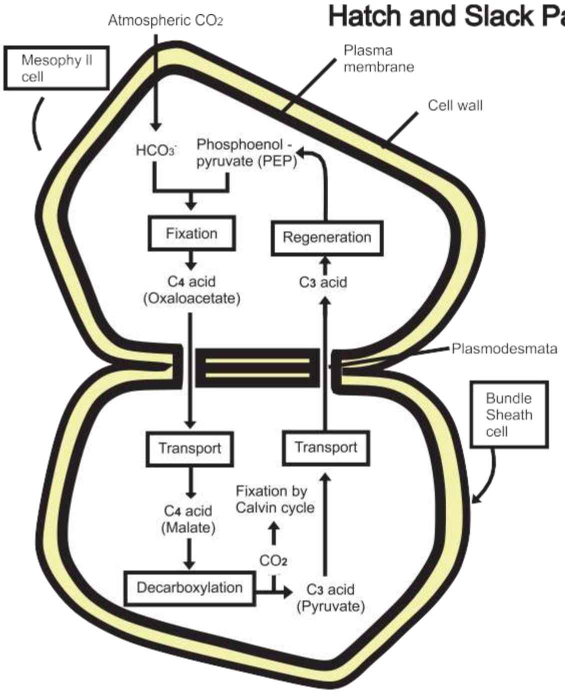
- Sucrose is the principal form of carbohydrate translocated throughout the plant by the phloem.
- Sucrose is synthesized in the cytosol in 10 steps.
- Starch is an insoluble stable carbohydrate. Starch is synthesized in the chloroplast in 7 steps.

C4 Type Crop Plant

Sugarcane

Under marginal environmental conditions, chlorophyll is decreased, leaf area index is smaller as well as hatch and slack pathways are disrupted.
The application of PSAP restores leaf normalcy for an extended period because of which more photosynthetic activity and higher sugar synthesis towards accumulation in the marginal conditions.





Hatch and Slack Pathway

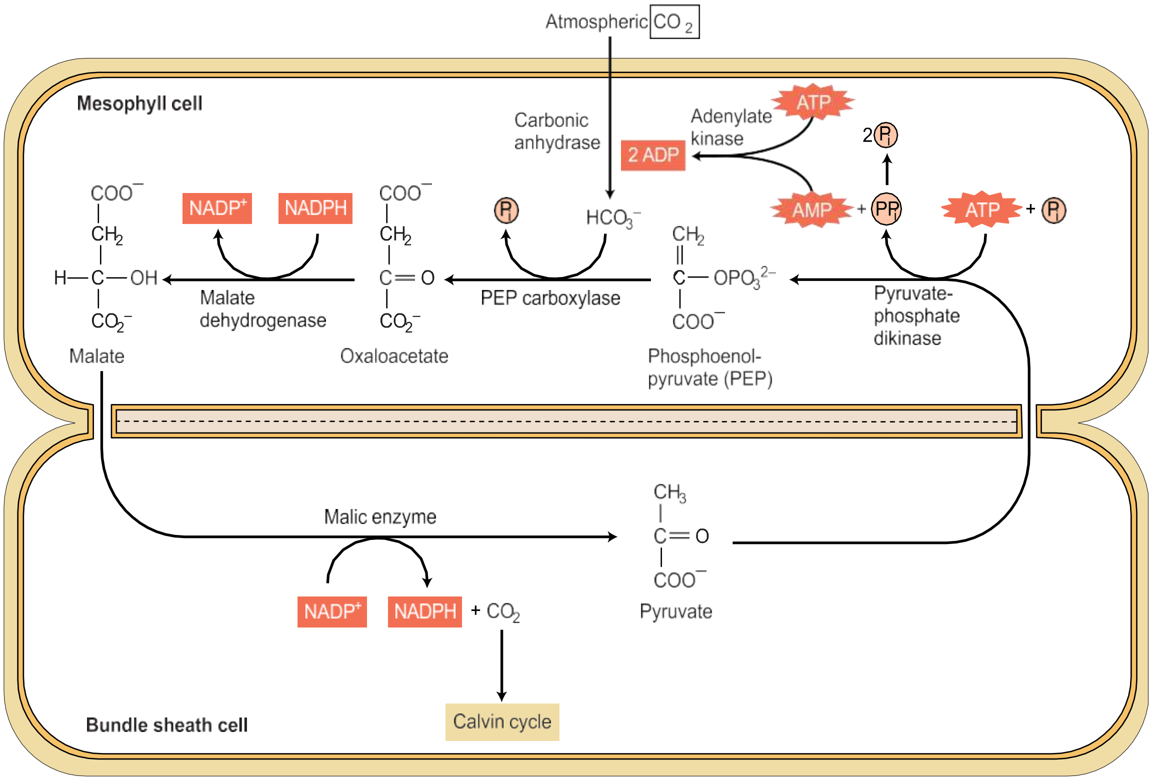
In a Sugarcane Plant

- The initially observed compound in this case is oxalo acetate, so this is referred to as the C4 pathway. Similarly, normal photosynthesis is called the C3 pathway.
- CO_2 fixation takes place in mesophyll cells, even when CO_2 levels are low, producing a C4 product used for the Calvin Cycle. Mesophyll cells are responsible for CO_2 capture.
- Losses less water than C3 plants, but produce the same amount of CHO.
- The Calvin Cycle takes place in the bundle sheath cells (around the veins of the leaf), where sugars are made. Interior bundle sheath cells (further away from atmospheric O_2) use CO_2 released from the Calvin cycle. C4 product acts as a carbon shuttle.
- This "separation in space" between the two cell types essentially eliminates the oxygenase reaction in rubisco and thereby blocks photorespriration.

This pathway uses a CO_2 concentrating mechanism to permit photosynthesis to surpass photorespiration. C4 crop plants lose only about half as much water as C3 crop plants when producing the same amount of carbohydrates.

In Hot and Dry Climates

Sugarcane Reduces Photorespiration and Water Loss



The C4 photosynthetic pathway.

The hydrolysis of two ATP molecules drives the cycle in the direction of the arrows, thus pumping CO_2 from the atmosphere to the Calvin Cycle in the chloroplasts from bundle sheath cells.

Two features of the C4 cycle in sugarcane overcome the deleterious effects of higher temperature on photosynthesis. First, the affinity of PEP carboxylase for its substrate, HCO_3^- , is sufficiently high that the enzyme is saturated by HCO_3^- in equlibrium with air levels of CO_2. Furthermore, because the substrate is HCO_3^- , O_2 is not a competitor in the reaction.

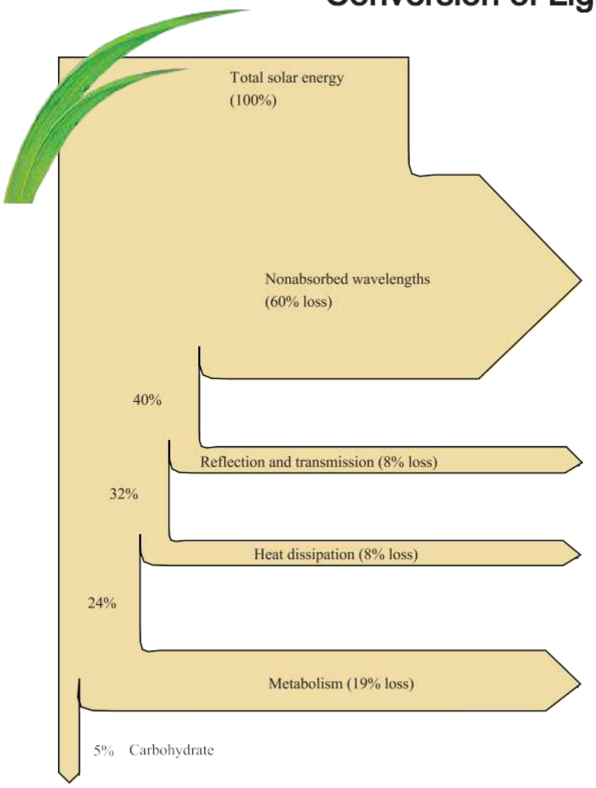
This high activity of PEP carboxylase enables C4 plants to reduce the stomatal aperture and thereby conserve water while fixing CO_2 at rates equal to or greater than those of C3 plants. The second beneficial feature is the suppression of photorespiration resulting from the concentration of CO_2 in bundle sheath cells. These features enable sugarcane to photosynthesize more efficiently at high temperatures than C3 plants, and are probably the reason for the relative abundance of sugarcane plants in drier, hotter climates.

C4 Type Crop Plant

Sugarcane

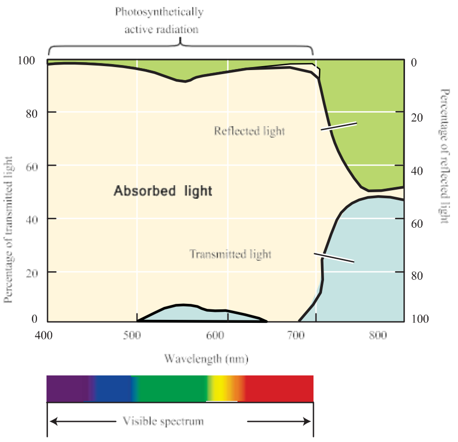
Photosynthesis Activities

Conversion of Light Energy into Sugars



Sugarcane is very efficient in conversion of solar energy into carbohydrates. Of the total incident energy, 5% is converted into biomass.

The chemical reaction of photosynthesis is driven by light
$6CO_2 + 6H_2O$ (Light / plant) -> $C_6H_{12}O_6 + 6O_2$



Leaf Anatomy Maximizes Light Absorption

Roughly 1.3 kW/m^2 of radiant energy from the sun reaches the Earth, but only about 5% of this energy can be converted into carbohydrates by leaves through photosynthesis. The reason this percentage is so low is that a major fraction of the incident light is of a wavelength either too short or too long to be absorbed by the photosynthetic pigments. Of the absorbed light energy, a significant fraction is lost as heat, and a smaller amount is lost as fluorescence. Radiant energy from the Sun consists of many different wavelengths of light. Only photons of wavelengths from 400 to 700 nm are utilized in photosynthesis, and about 85 to 90% of this PAR (photosyntheticaly active readiation) is absorbed by the leaf; the remainder is either reflected from the leaf surface or transmitted through the leaf. Because chlorophyll absorbs very strongly in the blue and the red regions of the spectrum, the transmitted and reflected light are vastly rich in green hence the green colour of vegetation.

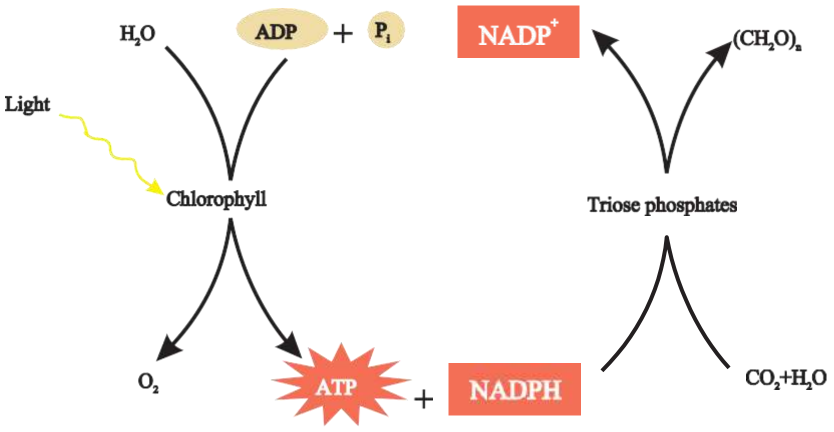
Photosynthesis Activities

Sugars, NADPH and ATP Synthesis in Chloroplast

The process of photosynthesis in plants involves a series of steps and biochemical reactions that use solar energy, water and carbon dioxide to produce organic compounds and oxygen. There are two main sets of reactions: energy-transduction reactions (commonly called light reactions) and carbon-fixation reactions (commonly called dark reactions).

In the energy transduction reactions, solar energy is converted into chemical energy in the form of two energy-transporting molecules, ATP and NADPH. When solar energy reaches plant cells and excites special chlorophyll molecules, they release a high-energy electron. The process of photosynthesis in plants involves a series of steps and reactions that use solar energy, water, and carbon dioxide to produce organic compounds. One of the first steps in this complex process depends on chlorophyll and other pigment molecules.

Chlorophyll is the green pigment molecule that makes plants appears green. In photosynthesizing plant cells, chlorophyll molecules are embedded in stacked membranes (thylakoids) contained in special membrane-bound organelles called chloroplasts. The chlorophyll molecules are arranged in discrete units known as photosystems, each of which contains hundreds of pigment molecules (chlorophyll plus others) arranged into an "antenna complex" surrounding a reaction centre.



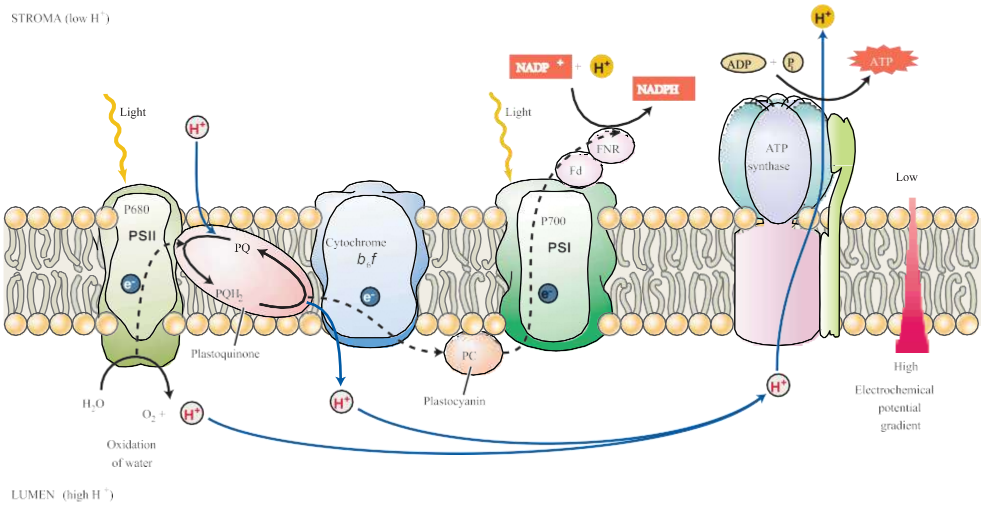
ADP - Adenosine Di-Phosphate
ATP - Adenosine Tri-Phosphate

Photosynthesis Activities

Sugars, NADPH and ATP Synthesis in Chloroplast

NADP and ATP are Energy Bonds
NADP - Nicotinamide Adenine Dinucleotide Phosphate

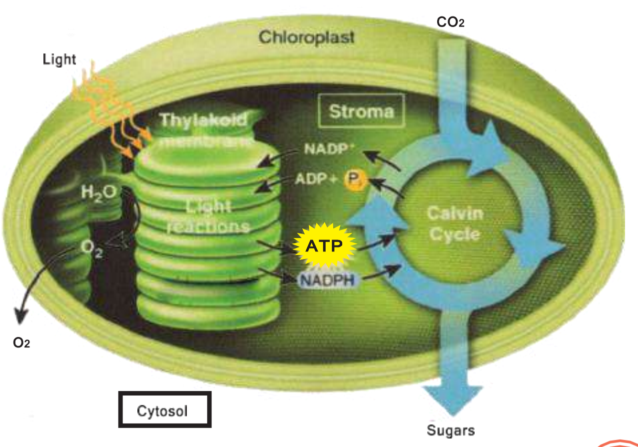
Active phosphorus from PSAP has role in Photosynthesis



The transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes. Water is oxidized and protons are released in the lumen by PSII (Photosystem 2). PS I reduces NADP+ to NADPH in the stroma, via the action of feroxin (Fd) and the flavoprotein feroxin–NADP reductase (FNR). Protons are also transported into the lumen by the action of the cytochrome b6 f complex and contribute to the electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical potential gradient is used to synthesize ATP in the stroma. Reduced plastoquinone (PQH2) and plastocyanin transfer electrons to cytochrome b6 f and to PSI, respectively. Broken lines represent electron transfer; solid lines represent proton movement.

The light reactions in the thylakoid membrane produce O2, ATP, and NADPH.

The Calvin Cycle in the stroma uses CO2, ATP, and NADPH to make carbohydrates, such as sugars.



In chloroplasts, ATP synthesis is driven by light-dependent photophosphorylation. Photosynthesis take place in the specialized internal membranes of the chloroplasts called thylakoids. Reactions are also called as thylakoid reactions. The end products of these thylakoid reactions are high-energy compounds ATP and NADPH, which are used for the synthesis of sugars in carbon fixation reactions.

Photosynthesis Activities

Sugars, NADPH and ATP Synthesis in Chloroplast

When light hits a pigment molecule in the antenna complex, the light energy “excites” the molecules, causing its electrons to jump to a higher level of energy. This excited state is temporary, and when the electrons fall back to a lower energy level, energy is released. This released energy can be transferred to a neighboring pigment molecule and so on, creating a chain of excited pigment molecules that ultimately deliver the energy to the photosystem's reaction centre. The reaction centre contains special chlorophyll molecules that have a specific response to absorbing energy rather than only transferring it. The resulting high-energy electrons in chlorophyll transfer only themselves to an electron-acceptor molecule, which begins the flow of electrons, that plays a key role in the rest of photosynthesis.

The release of this electron sets off a chain of electron-trading and energy-transferring events between several intermediary molecules, and the last molecule to form and hold the electrons in this chain is $NADP^+$.

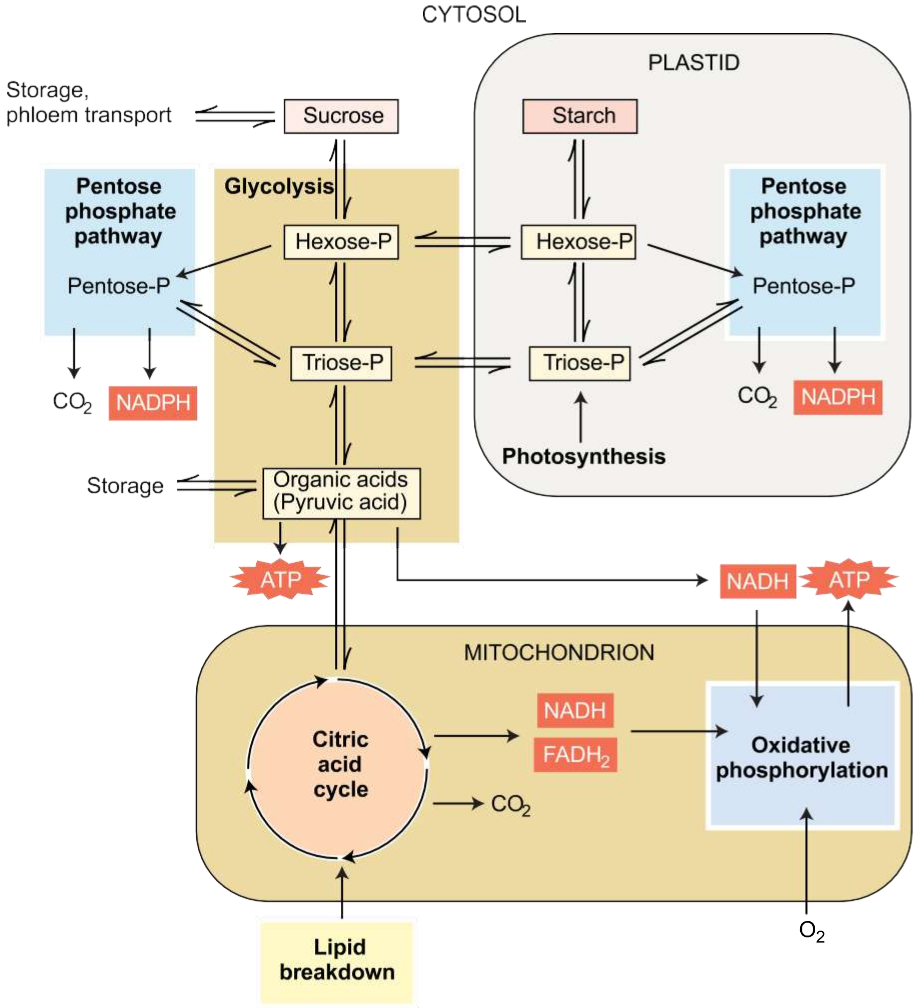
The electrons from the excited chlorophyll molecules need to be replaced, and these electrons come from water. With the help of enzymes and solar energy, water is split (photolysis) into electrons, protons (H^+), and oxygen. The electrons go to the chlorophyll, while the protons contribute to a proton gradient that is used to power the synthesis of a second energy-carrying molecule, ATP. Oxygen is a by-product of the whole process.

The chemical energy in NADPH and ATP is then used to power steps in the subsequent carbon-fixation reactions.

In a cycle of reactions called the Calvin Cycle or C3 pathway, the carbon-containing molecule resulting from this first fixation reaction is converted into various compounds using the energy from ATP and NADPH. The products of the Calvin cycle include a simple sugar that is subsequently converted into such carbohydrates as glucose, sucrose, and starch, which serve as important energy sources for the plant. The cycle also regenerates molecules of the initial reactant that more CO_2 will bond with in another turn of the cycle. Interest in learning from and applying how plants activate and convert CO_2 into useful products is particularly high, as CO_2 is abundant in the atmosphere but is chemically stable and requires a large amount of energy to convert into compounds that are useful in industrial processes.

ATP Synthesis in Mitochondria

Cellular Respiration



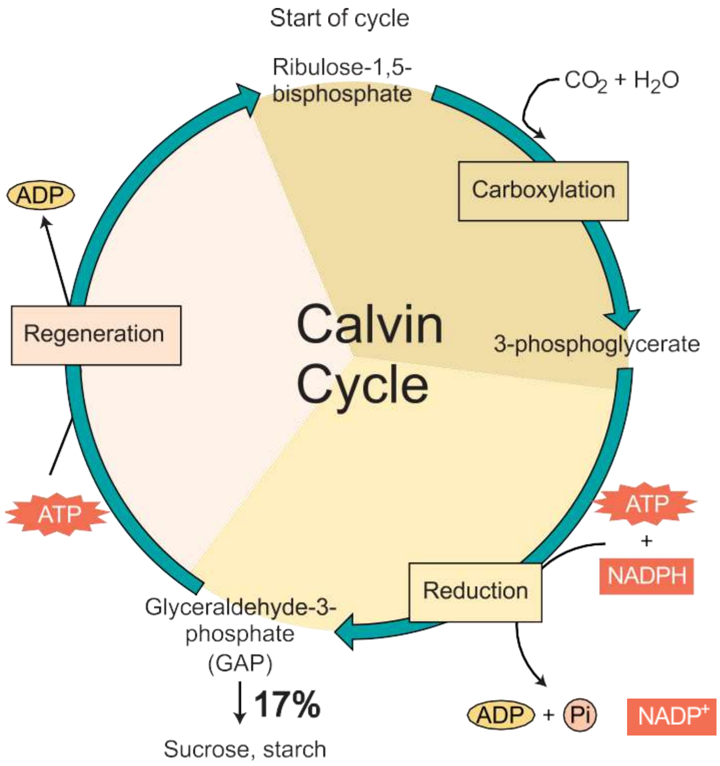
In mitochondria, the energy for ATP synthesis derives from the oxidation of NADH by oxidative phosphorylation.

Overview of respiration.

Substrates for respiration are generated by other cellular processes and enter the respiratory pathways. Glycolysis and the pentose phosphate pathways in the cytosol and plastid convert sugars to organic acids, via hexose phosphates and triose phosphates, generating NADH or NADPH and ATP. The organic acids are oxidized in the mitochondrial citric acid cycle, and the NADH and $FADH_2$ produced provide the energy for ATP synthesis by the electron transport chain and ATP synthase in oxidative phosphorylation. In gluconeogenesis, carbon from lipid breakdown is broken down in the glyoxysomes, metabolized in the citric acid cycle, and then used to synthesize sugars in the cytosol by reverse glycolysis, i.e. gluconeogeonesis.

GAP Precursor for Sucrose and Starch Synthesis

Triose phosphate (GAP) that is generated in the Calvin Cycle 17% is exported for starch and sucrose synthesis



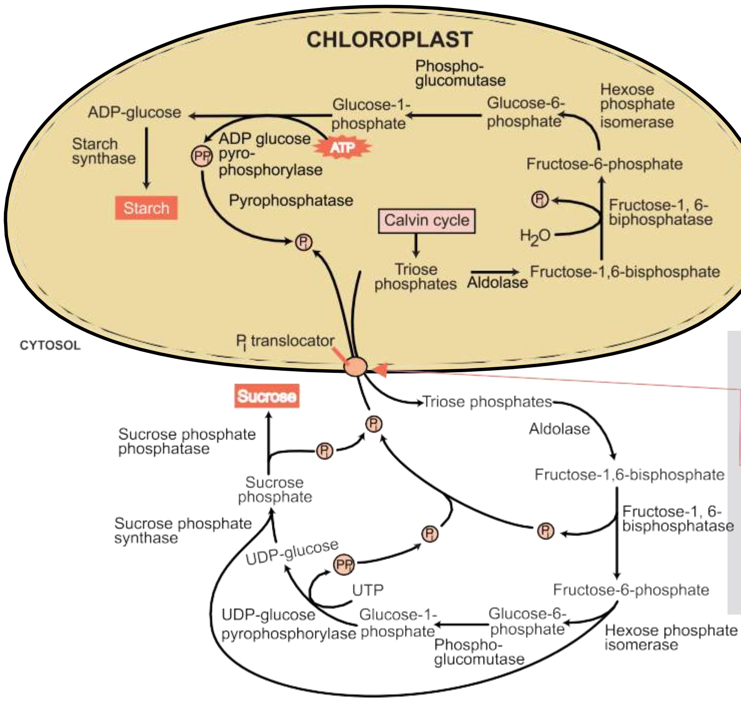
Thermodynamic efficiency of the Calvin Cycle is close to 90%. In 12 steps, CO_2 and H_2O are converted into fructose – 6 –phosphate. The conversion is achieved through three basic mechanisms:

A) Carboxylation of the CO_2 acceptor; ribulose – 1, 5-bisphosphate, forming two molecules of 3-phosphoglycerate, the first stable intermediate of the calvin cycle.

B) Reduction of 3-phosphoglycerate, forming glyceraldehyde – 3 – phosphate, a carbohydrate. When photosynthesis reaches a steady state, 83% of triose phosphate (glyceraldehyde – 3 – phosphate) contributes to regeneration of the ribulose –1,5 bisphosphate and 17% is exported to cytosol for synthesis of sucrose and other metabolites that are converted, into starch in the chloroplast.

C) Regeneration of the CO_2 acceptor ribulose – 1, 5-bisphosphate from glyceraldehyde –3 phosphate.

Phosphate Concentration at Translocator



Application of PSAP drives the synthesis of sucrose which gets accumulated in stress. Hence greater sugar recovery likely in PSAP-treated sugarcane.

Pi - Phosphate concentration is high; therefore, sucrose is formed

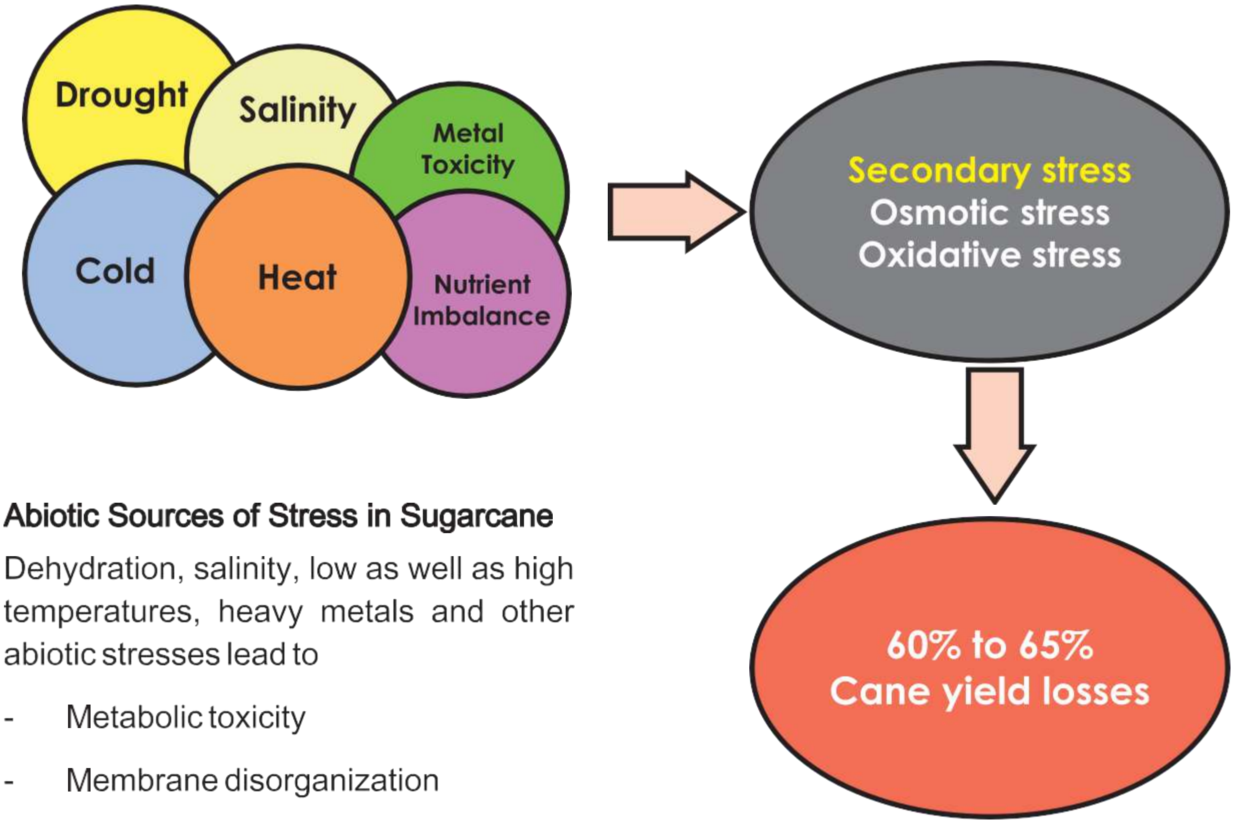
Pi - Phosphate concentration is low; therefore, starch is formed

oroplast and the cytosol, respectively. When the cytosolic Pi concentration is high, chloroplast triose phosphate is exported to the cytosol via the Pi in exchange for Pi, and sucrose is synthesized. When the cytosolic Pi concentration is low, triose phosphate is retained within the chloroplast, and starch is synthesized.

The relative concentrations of orthophosphate and triose phosphate are major factors that control whether photosynthetically fixed carbon is partitioned as starch in the chloroplast or as sucrose in the cytosol. The two compartments communicate with one another via the phosphate triose phosphate translocator, also called the phosphate translocator, a strict stoichiometric antiporter. The phosphate translocator catalyses the movement of orthophosphate and triose phosphate in opposite directions between chloroplasts and cytosol. A low concentration of orthophosphate in cytosol limits the export of triose phosphate from the chloroplast through the translocator, thereby promoting the synthesis of starch. Conversely, an abundance of orthophosphate in the cytosol inhibits starch synthesis within the chloroplast and promotes the export of triose phosphate into cytosol, where it is converted to sucrose. Orthophosphate and triose phosphate control the activity of several regulatory enzymes in the sucrose and starch biosynthetic pathways. The chloroplast enzyme ADP-glucose pyrophosphorylase is the key enzyme that regulates the synthesis of starch from glucose- 1-phosphate. This enzyme is stimulated by 3-phosphoglycerate and inhibited by orthophosphate. A high concentration ratio of 3-phosphoglycerate to orthophosphate is typically found in illuminated chloroplasts that are actively synthesizing starch. Reciprocal conditions prevail in the dark.

GAP Precursor for Sucrose and Starch Synthesis

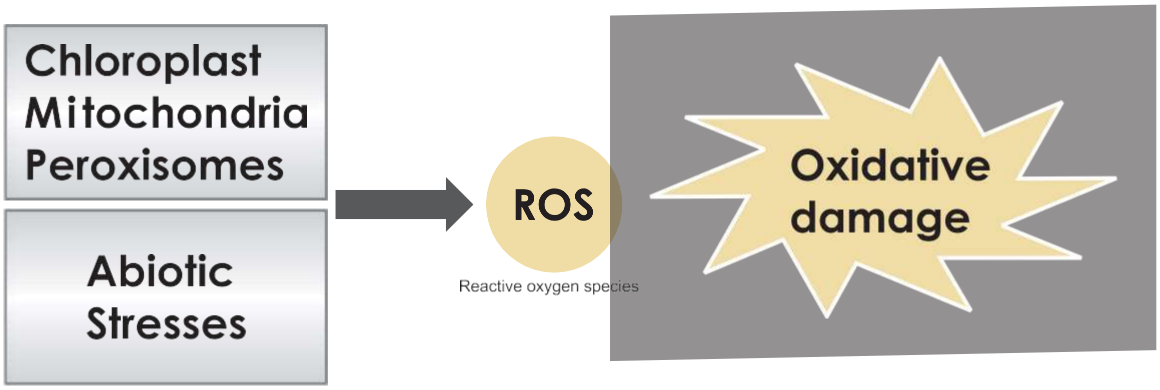
Major abiotic stress factors that restrict
Sugarcane yield and quality



Abiotic Sources of Stress in Sugarcane

Dehydration, salinity, low as well as high temperatures, heavy metals and other abiotic stresses lead to

- Metabolic toxicity
- Membrane disorganization
- Generation of ros
- Inhibition of photosynthesis
- Reduced nutrient acquisition
- Altered hormonal levels



10% to 15% Reduction in Sugarcane Yield

- Observed at temperatures 38 °C higher.
- Sucrose synthesis is reduced.
- Photosynthetic activity decreases.
- Attack of stem borer increases.

Sugarcane survives at a maximum temperature approaching 45 °C.
However, there is little growth at temperatures above 40 °C.

Effects of High temperature stress on sugarcane plants

High temperature stress induces morphological and anatomical as well as physiological and biochemical changes in plants. It induces changes in water relations, accumulation of compatible osmolytes, decrease in photosynthesis, hormonal changes and cell membrane thermostability.

High temperatures stress (> 40°C) can cause scorching of leaves and twigs, sunburn of leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition and damage and reduced yield in plants.

High temperature stress induces the rapid production and accumulation of ROS.

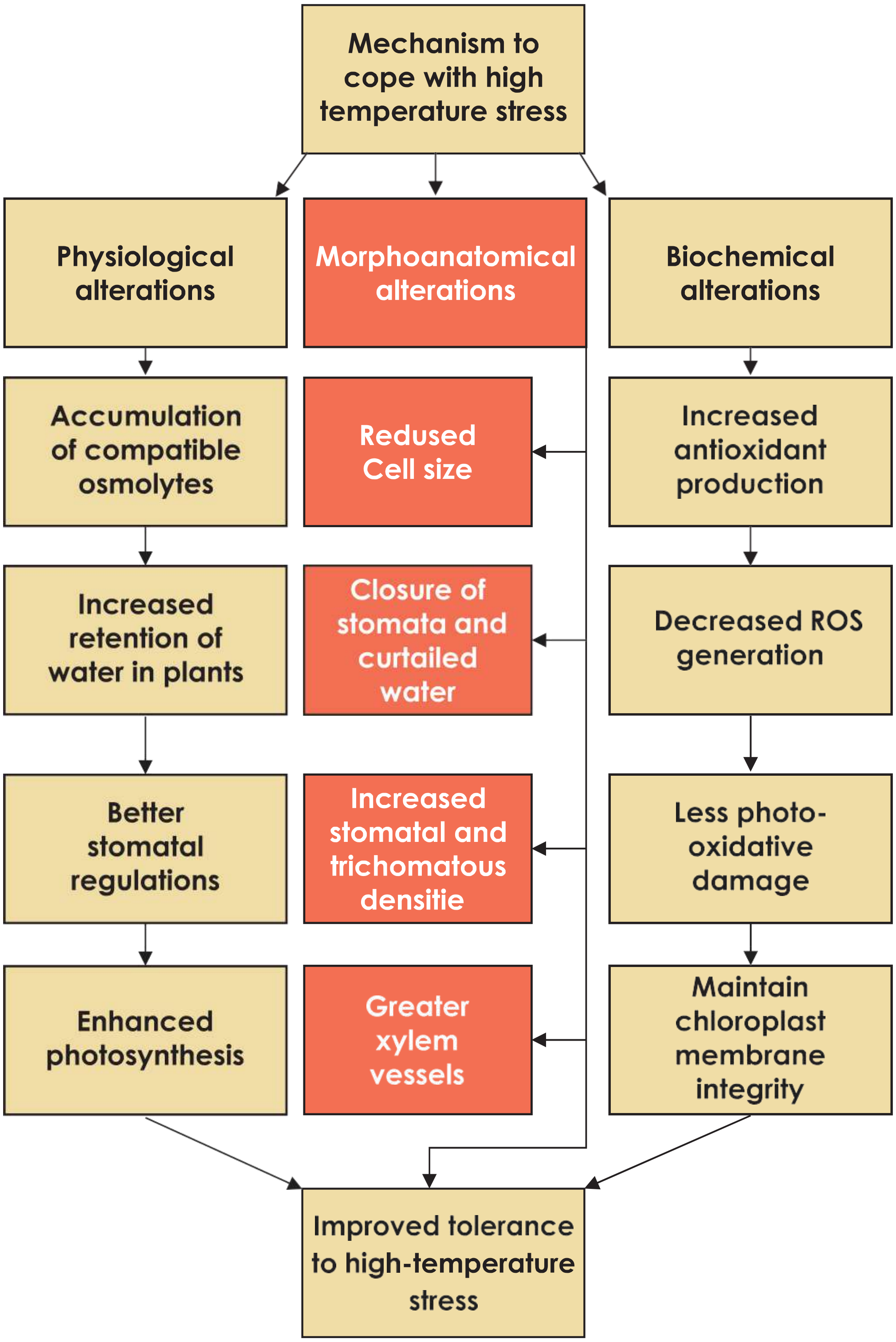
These high levels of ROS are harmful to all cellular compounds and negatively influence cellular metabolic processes. The detoxification of these ROS is very important, and plants have evolved complex strategies to deal with them. Plant cells typically respond to increase in ROS levels by increasing the expression and activity of ROS-scavenging enzymes and increasing their production of antioxidants in order to maintain redox homeostasis.

Environmental stress in plants has been associated with production of activated forms of oxygen, including hydrogen peroxide (H_2O_2), singlet oxygen, superoxide, and the hydroxyl radical. Reactive oxygen species are produced continuously as by products of different metabolic pathways, which are located in different cellular compartments such as chloroplasts, mitochondria and peroxisomes.

Through a variety of reactions, O_2^- leads to the formation of H_2O_2, OH and other ROS.

The ROS comprising O_2^-, H_2O_2, $_1O^2$, HO_2^-, OH, ROOH, ROO^+ and RO^+ are highly reactive and toxic and causes damage to proteins, lipids, carbohydrates and DNA which ultimately results in cell death. Accumulation of ROS as a result of high temperature stress is a major cause of loss of crop productivity worldwide.

Sugarcane plants cope with high temperature stress
Morphological, physiological and biochemical alterations



Approaches to induce tolerance to high temperature stress

The mechanisms through which plants can cope with high-temperature stress are distributed in plants that can cope with by physiological, morpho-anatomical and biochemical alterations. Under high-temperature stress, plants accumulate compatible osmolytes, which help to increase the retention of water in plants for better stomatal regulation and increase rate of photosynthesis.

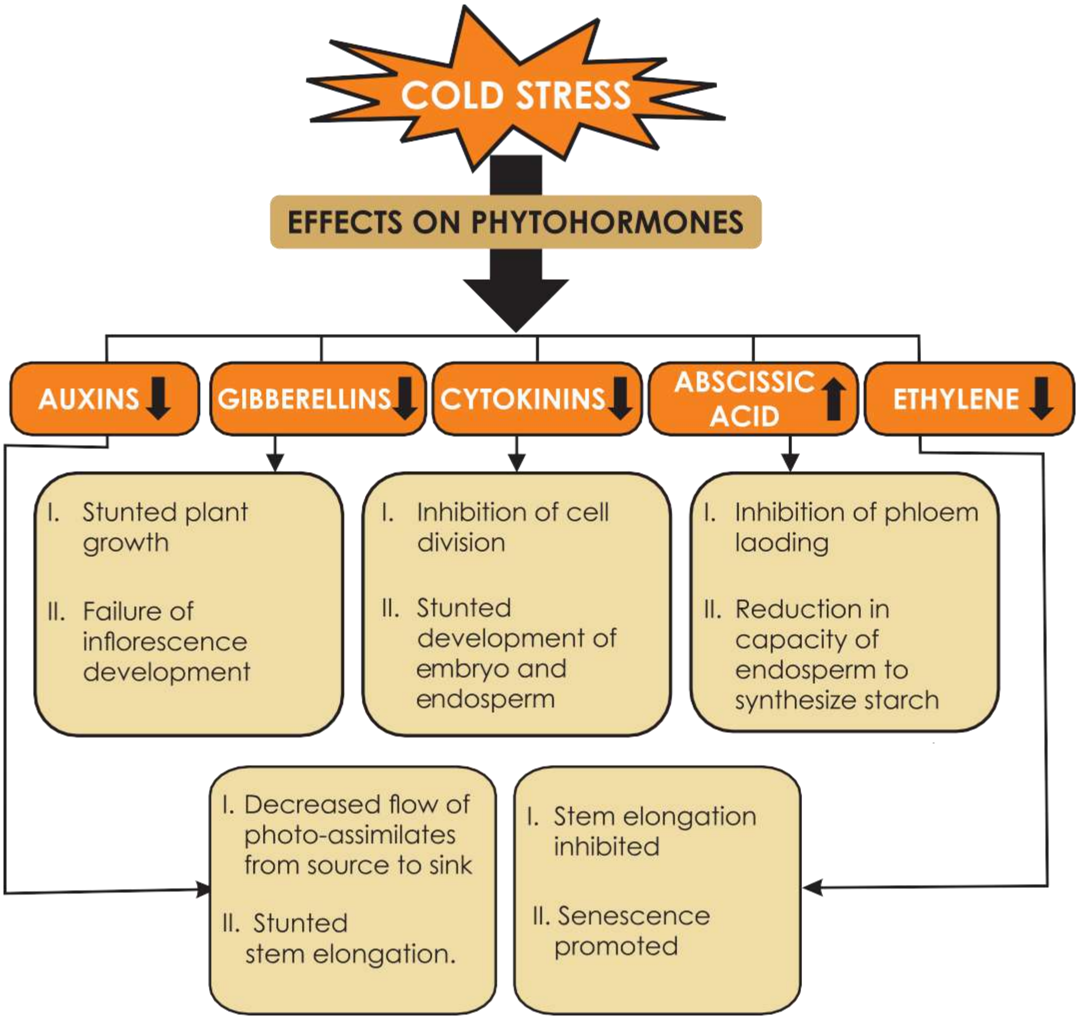
Plants also exhibit some morpho-anotomical alterations to cope with high-temperature stress. These alterations include reduction in cell size, closure of stomata, increased stomatal and trichomes densities and greater xylem vessels. The third mechanism to cope with high temperatures is biochemical alterations. Plants increase the production of stress-related proteins, which enhance the activities of such antioxidants as superoxide dismutase (SOD),catalase (CAT) and peroxidise (POD) in plant cells. These antioxidants scavenge the ROS and reduce photo-oxidation and maintain the integrity of the chloroplast membrane and increase the rate of photosynthesis.

Among the various methods to induce high-temperature stress in plants, the more common ones are foliar application of or pre-sowing seed treatment with, low-concentrations of inorganic salts, osmoprotectants, signalling molecules (e.g., growth hormones) and oxidants (e.g., H_2O_2) and preconditioning of plants. Similarly, sugarcane leaves manifest higher thermostability, lower lipid peroxidation the product is melondialdehyde, or MDA and lower damage to chloroplast upon exposure to high-temperature stress in heat-acclimated as compared to non-acclimated plants. It is observed that exogenous application of PSAP promotes a plant's tolerance to heat. PSAP application prior to stress treatment may increase the MDA content and stimulate the activity of guaiacol peroxidase, SOD and catalase, which may be the reasons for the induction of heat tolerance. PSAP successfully applied to induce heat tolerance in various plant species.

It was observed that PSAP-treated plants has been recorded lower membrane damage, higher rate of photosynthesis and improved leaf water potential and had greater shoot dry mass than that in untreated plants. In many other crop plants, exogenous application of PSAP improved heat resistance by improving chlorophyll fluorescence properties, hardening and higher resistance to thermal damage of the pigment-protein complexes structure and greater activity of PSII during the linear increase in temperature. Under heat stress PSAP is required for maintenance of antioxidant activity which is why greater amount of PSAP are required for growth to mitigate the adverse effects of stress.

15% to 20% Reduction in Sugarcane Yield

- Observed at temperatures of 15°C or lower
- Poor tiller growth
- Tissues are damaged
- Progressive decrease in water absorption through roots
- Discolouration in cholorophyll
- Decreased rate of biochemical reactions
- Decreased CO_2 fixation
- In autumn, suppressed sugarcane development
- In freezing temperatures, reverse sucrose accumulation



Impact of Cold Stress on Sugarcane

The optimal temperatures for sugarcane are between 25°C and 35°C.

The lowering of root temperature from 28°C to 21°C and from 15°C to 10°C, causes a progressive decrease in water absorption.

High Temperature Stress

Early shoot growth is sensitive to cold, which significantly affects crop yield and is therefore particularly important to breeding programmes in some subtropical sugarcane-growing areas. A major factor determining the commercial success of a clone is its ability to tiller well following adverse winters.

In subtropical regions, freezing temperatures may terminate or even reverse sucrose accumulation in autumn or early winter. Freezing further reduces yields by delaying and suppressing crop development in spring, resulting in a shortened growth season and producing poor crop stands. Resistance to stress from freezing is required for different tissues at different stage of crop development.

Moderate pre-harvest freezes cause insignificant yield losses whereas severe freezes can result in total loss. Due to poor tillering following a severe winter, shoot population decreases by 78% and yield by 87%, when the underground buds are not protected from freezing. Levels of a greater number of metabolites change specifically in response to cold than to heat, pointing towards the strong impact of cold on plant metabolism.

Low temperature stress on sugarcane plants

The rate of metabolic and biochemical processes decreases gradually with decrease in temperature and may cease under severe cold. Cold-temperature stress (0 to -10°C) has broad-spectrum affects on cellular components and metabolic processes of sugarcane plants. Extreme cold temperatures impose stress of variable severity depending on the intensity and duration of stress. Several studies indicate that the membrane systems of the cell are the primary site of freezing injury in plants, and freeze-induced membrane damage results primarily from the severe dehydration associated with freezing. As temperatures drops below 0°C, ice formation is generally initiated in intercellular spaces in the extracellular fluid,which has a higher freezing point (lower solute concentration) than the intracellular fluid.

Because the chemical potential of ice is less than that of liquid water at a given temperature, the formation of extracellular ice results in a drop in water potential outside the cell. Consequently, there is movement of unfrozen water down the chemical potential gradient from inside the cell to the intercellular spaces. At 10°C, more that 90% of the osmotically active water typically moves out of the cells, and the osmotic potential of the remaining unfrozen intracellular and intercellular fluid is greater.

Multiple forms of membrane damage can occur as a consequence of freeze-induced cellular dehydration including expansion-inducedlysis, lamellar-to-hexagonal- II phase transitions, and fracture jump lesions. Low-temperature-induced change in membrane fluidity is one of the immediate consequences in plants during low-temperature stress and might represent a potential site of perception and/or injury.

Freeze-induced production of ROS contributes to membrane damage, and intercellular ice can form adhesions with cell walls and membranes and cause cell rupture. There is also evidence that protein denaturation occurs in plants at low temperatures which could potentially result in cellular damage.

Approaches to induce tolerance to low temperature stress

The importance of proper membrane fluidity in tolerance to low temperature has been delineated by mutation analysis, transgenic and physiological studies. At low temperature, greater unsaturation of membrane lipids appears to be crucial to optimum membrane function. Plants have several mechanisms or approaches to cope with low temperature stress. Cold acclimation is a key approach to stabilize membranes against freezing injury.

Tolerance to cold prevents expansion-induced lyses and the formation of hexagonal II phase lipids in plants. Multiple mechanisms appear to be involved in this stabilization. The best documented are changes in lipid composition.

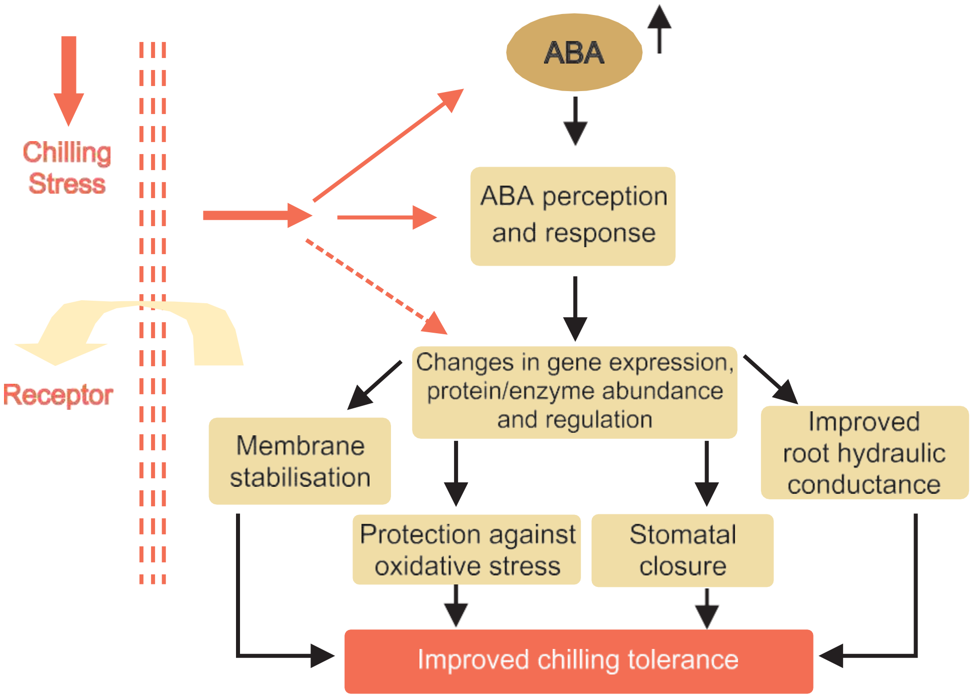
Similarly, the accumulation of sucrose and other simple sugars that typically occurs with cold acclimation also seems likely to contribute to the stabilization of membranes as these molecules can protect membranes against freeze-induced damage in vitro.

In addition, there is emerging evidence that certain novel hydrophilic and late embryogenesis abundant (LEA) proteins also participate in the stabilization of membranes as these molecules can protect membranes against freeze-induced damage in vitro.

These hydrophilic and LEA proteins are predicted to contain regions capable of forming amphipathic α-helices, which are shown to have a strong effect on intrinsic curvature of the monolayer and their propensity to form hexagonal II phase. They are said to defer their formation at lower temperatures.

Role of Abscisic Acid (ABA) in chilling tolerance.

Upon exposure to chilling stress, ABA level increases in tolerant genotypes, which results in changes in gene expression, protein/enzyme abundance, and regulation.



As a consequence, membranes stabilize, root hydraulic conductance increases, stomata close, and oxidative stress is ameliorated. All these changes contribute to greater tolerance to cold. The accumulation of ABA to chilling-sensitive plants before, during, or even shortly after a low-temperature treatment has been shown to protect the plants from chilling injury. When chilling-sensitive plants are exposed to chilling temperatures, endogenous ABA levels increase. The comparative effectiveness of ABA and calcium chloride during cold stress in sugarcane in terms of antioxidant enzyme activity suggested that ABA at lower temperatures was more protective against cold stress.

Another mechanism through which plants can cope with low-temperature stress might be the extensive water-binding capacity of hydrophilic proteins, which provide a protective environment in the proximity of stabilization. Although freezing injury is thought to result primarily from membrane lesions caused by cellular dehydration, additional factors may also contribute to freezing-induced cellular damage.The enhancement of antioxidative mechanisms, increased levels of sugars in the apoplastic space, and the induction of genes encoding molecular chaperones could have protective effects to reduce freez-induced cellular damage to promote growth and development, and also to improve water-use efficiency.

10% to 20% Reduction in Sugarcane Yield

The terms salt and ion stress, refer only to excess. Excess salt is due to its ions. In ion stress the soluble mineral concentration is not high enough to lower the water potential appreciably. Whereas in salt stress higher mineral concentration lowers the water potential appreciably (0.5 to 1.0 bar).

Sugarcane is a very salt sensitive non halophyte (Group III) crop and belongs to the glycophyte group. Glycophytes cannot grow in the presence high concentration of Na salts. Halophytes tolerate high salt concentrations.

Soil salinity reduces plant growth by disturbing different biochemical / physiological processes. Inhibits the anabolic and stimulate catabolic processes.Salinity in the root zone of sugarcane decreases sucrose yield, also affects biomass and juice quality. Saline soil reduces mill able stocks per hectare, stalk length and stalk weight.

NaCl and KCl induce conformational changes in the enzymes either by direct interaction with the protein or indirectly through interaction with the lipid component of the membrane.

High concentrations of Al, Fe, Mg and Mn are generally considered toxic for sugarcane. The toxic concentrations are lower in acid soils and soils deficient in ‘K’. The injuries due to ions involved specific effect initially on the plasma membrane and then on the protoplasm.

Ion toxicity has greater impact under nutrient imbalance.

Ion stresses are alleviated by increasing ‘K’ and ‘Ca’ ions, neutralizing soil acidity and complexing the soil with Silica. Salinity tolerance is a complex process and is controlled by many genes. These genes are expressed only when plant is exposed to salinity.

Sugarcane is moderately sensitive to salinity. Sugarcane exhibits stunted or no growth under saline conditions, with its yield falling to 50% or even more to its true potential.

Sugarcane sprouting and early growth are considerably more resistant to salinity then at later development stages.

Salt interferes with sugar production in two ways; first by affecting growth rate and yield of the cane and secondly by affecting the sucrose content of the stalk.

Accumulation of sucrose takes place to balance salt concentration in juice and maintain tissue water potential.

Soil Salinity

Saline soil can be defined as soils with in which the electrical conductivity of solution extracted from water-saturated soil paste is 4 dm^{-1} (decisiemens per meter) 40 mM NaCl or more. Sugarcane-glycophytes are severely inhibited in^{+} 100 – 200 mMNaCl.

Soil salinity increases due to

- Chloride (Cl^{-}) content in irrigated water in the form of various salt such as Na and Ca.
- Frequent applications of chloride-based potash fertilizers as (K^{+}) (Cl^{-}), i.e. MoP.
- The amount of chloride apply to sugarcane is 25 times to 100 time more than require 3 to 4 kg (Cl^{-}) per hectare for sugarcane growth in a balance nutrient program.
- The critical tissue (Cl^{-}) concentration for toxicity is about 4 to 7 mg/g and 15 to 50 mg/g of dry weight in Cl^{-}-sensitive and Cl^{-}-tolerant plants respectively.

Improper management of salinity leads to

- Hard soil
- Poor soil structure
- Na+ ions occupy the cation exchange complex of clay Particles causing soil aggregates to break down
- Increased bulk density
- More compact soil
- Decreased total porosity
- Poor soil aeration

Plants in saline soils suffer not only from Na+ levels but are also affected by some degree of hypoxia- deprived of supply of oxygen.

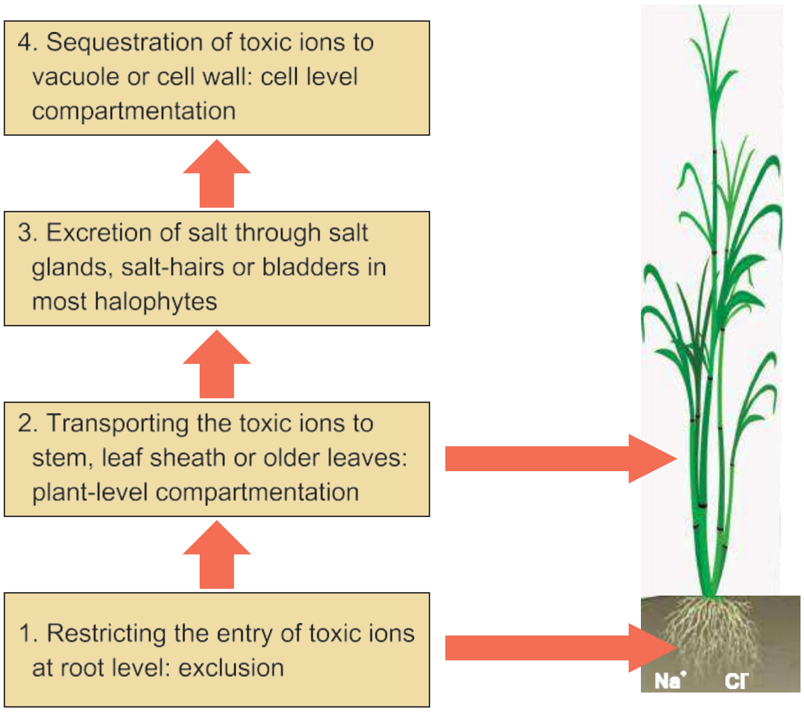
Salinity Stress

Salinization affects many irrigated areas mainly due to the use of brackish (salty) water. Worldwide, more than 45 million hectares of irrigated land has been damaged by salt and 1.5 million hectares taken out of production each year as a result of high salinity.

There are three types of plant response to acclimatize to salinity

- Tolerance to osmotic stress
- Na+ exclusion from leaf blades
- Tissue tolerance

Mechanisms of Salt Tolerance
Predominant salt-tolerance mechanisms operating in plants



4. Sequestration of toxic ions to vacuole or cell wall: cell level compartmentation

3. Excretion of salt through salt glands, salt-hairs or bladders in most halophytes

2. Transporting the toxic ions to stem, leaf sheath or older leaves: plant-level compartmentation

1. Restricting the entry of toxic ions at root level: exclusion

High salinity affects plants in several ways;

- water stress
- ion toxicity
- nutritional disorder
- oxidative stress
- alternation of metabolic processes
- membrane disorganization
- reduction of cell division and expansion
- genotoxicity.

Together, these effects reduce plant growth, development and survival. During the onset and development of salt stress within a plant, all major processes such as photosynthesis, protein synthesis and energy as well as lipid metabolism are affected. Excess Na+ and more important chloride, has the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other form of physiological change.

To improve stress tolerance: exploitation of natural genetic variation and generation of transgenic plants with novel genes or altered expression levels of existing genes.

Among the various sources of soil salinity, irrigation combined with poor drainage is the most serious because it represents losses of once productive agricultural land.

Irrigation water contains calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^{+}) . When water evaporates, Ca^{2+} and Mg^{2+} often precipitate into carbonate, leaving Na^{+} dominant in the soil. As a result, Na^{+} concentrations often exceed those of most micronutrients and sometimes even those of macronutrients.

Increase in cations and their salt, NaCl in particular, in soil generates external osmotic potential that can prevent or reduce the influx of water into roots. The resulting water deficit is similar to drought and is compounded by the presence of Na^{+} ions.

Effect of higher concentration of chloride in sugarcane ecology

1. Cl^{-} is considered a micronutrient but its actual concentration in sugarcane plants is 25 to 100 times the requirement for optimal plant growth and such high concentration in soil affects plant growth and quality.

   Cl^{-} is always highlighted as a problem in terms of salt stress. It is well known that salinity in soil and irrigation water containing considerable amount of Cl^{-} is harmful to both cane yield and quality.

   Close correlations between K^{+} and Cl^{-} have been confirmed in many reports and Cl^{-} is considered to be the counter ion of K^{+}. All these findings taken together suggest that Cl^{-} has a strong effect on sugarcane quality and that K^{+} indirectly influences the quality by increasing Cl^{-} concentration in the juice.

2. Under server salinity, root growth is more adversely affected than shoot growth.

Chlorophyll contents show a decreasing trend and dry matter yield of plants is decreased. Salinity still remains a major source of abiotic stress that limits and threatens agriculture production in many parts of the world. Although a number of mechanisms relating to greater adoption to stress have been suggested, the fact remains that their association with genetic traits for high gains for the yield and their relative importance in different salinity-prone environments are still only pratly defined. Therefore, a well-focussed approach combining molecular, physiological and metabolic aspects of tolerance to abiotic forms of stress is required.

3. An increase in chloride concentration reduces the rate of nitrification. If ammonium fertilizers are used high ammonium concentration in the soil and consistent availability of ammonia to the roots delay cane maturity.

4. An increase in nitrate uptake increases the pH of soil in the rhizosphere. The increased pH soil reduces phosphate uptake leading to leaching and fixation of applied phosphorus.

5. An increase in chloride concentration induces an increase in EC near the roots. As a result, the rate of root elongation is reduced and plant growth declines under saline conditions. The uptake of P & Fe is also influenced by the reduction in root elongation.

6. The rate of transpiration generally tends to decline with increasing rhizospheric salinity in both sensitive and tolerant plants, probably because of salt accumulation in the mesophyll which, in closes the stomatal aperture.

For references and further studies refer 17 to 26 reports from the reference list.

Viral infected Papaya plant

After treatment

25% to 30% reduction in Sugarcane Yield

Stress due to Water Deficit

In severe to moderate drought stress, cane yield decreases by 20% to 30%, respectively, compared to irrigated controls.

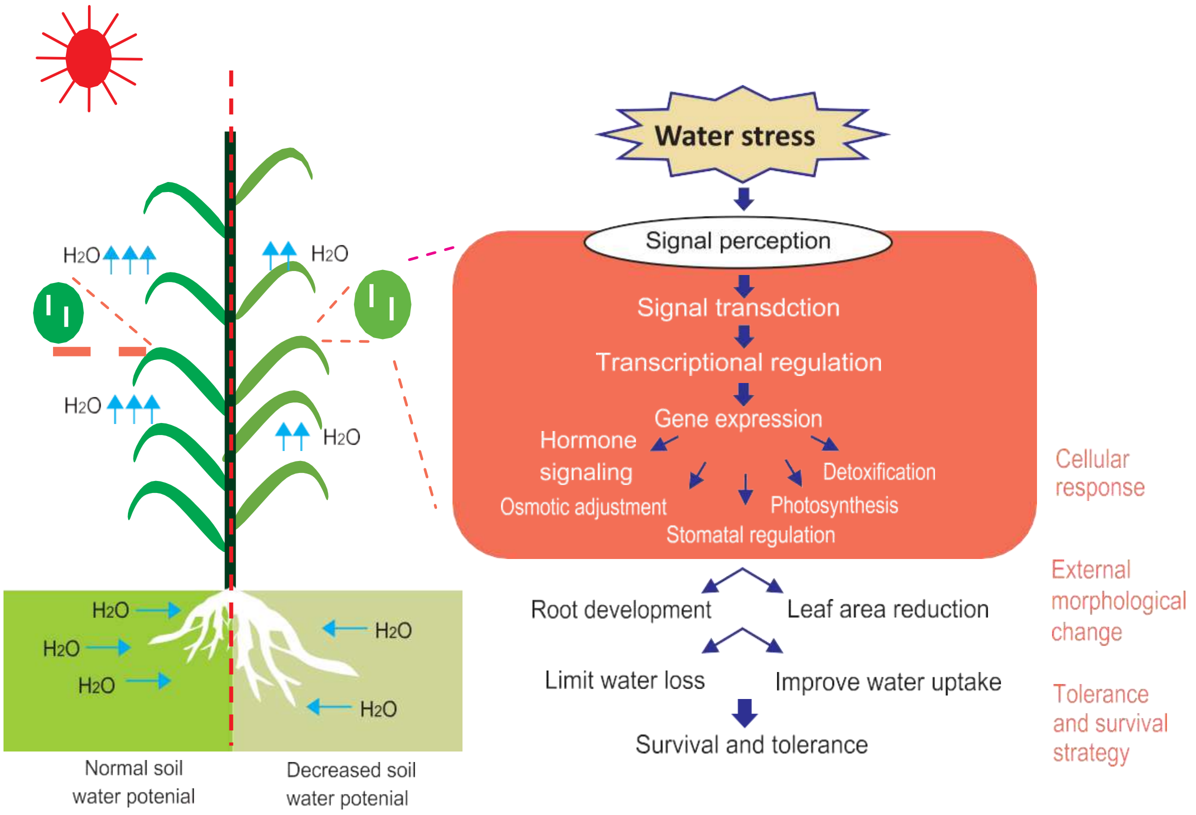
Morphological changes brought on by drought to acclimate plants are

- Reduced leaf area
- Thicker leaves the most prevalent and earliest appearing anatomical acclimation
- Less responsive stomata
- Increased ratio of roots to shoots
- Reduction in cell size

Biochemical Changes in Drought Stress

- Acclimation includes changes in enzyme activity
- Carbohydrates and nitrogen pools
- Accumulation of stress indicators such abscisic acid, glycine betaine (GB), Proline and the metabolites of these compounds.

Each type of drought stress acclimation consists of characters that are present even without drought to various degrees among sugarcane clones.



Root Systems Character

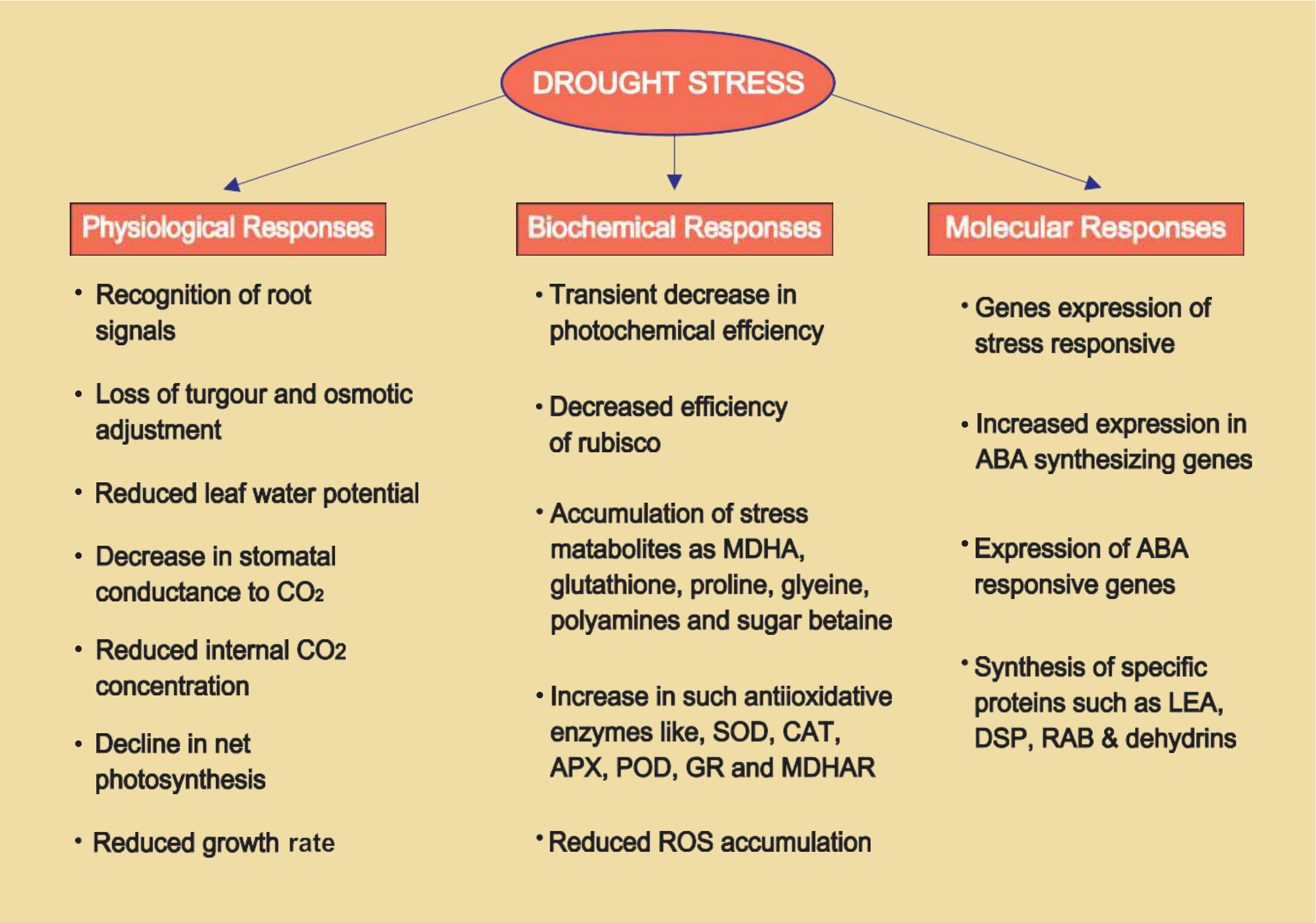
The larger the root system, the greater the drought tolerance of sugarcane.

Leaf Character

The potential rate of transpirational water loss is regulated by leaf size, exposure, structural modifications in the stomata, bulliform cells and cuticle. Low density of stomata, narrow band bulliform cells and thick cuticle check transpiration.

Stomata Character

Many stomatal characters which restrict water loss, such as low frequency and small size will also restrict assimilation of carbon and subsequently limit growth.



DROUGHT STRESS

Physiological Responses

- Recognition of root signals
- Loss of turgour and osmotic adjustment
- Reduced leaf water potential
- Decrease in stomatal conductance to CO_2
- Reduced internal CO_2 concentration
- Decline in net photosynthesis
- Reduced growth rate

Biochemical Responses

- Transient decrease in photochemical effciency
- Decreased efficiency of rubisco
- Accumulation of stress matabolites as MDHA, glutathione, proline, glyeine, polyamines and sugar betaine
- Increase in such antiioxidative enzymes like, SOD, CAT, APX, POD, GR and MDHAR
- Reduced ROS accumulation

Molecular Responses

- Genes expression of stress responsive
- Increased expression in ABA synthesizing genes
- Expression of ABA responsive genes
- Synthesis of specific proteins such as LEA, DSP, RAB & dehydrins

Osmotic Adjustment in Drought Stress

A leaf increases its resistance to dehydration by lowering its cellular osmotic potential through the accumulation of cellular solutes.

Metabolic Adaptation

Water deficit if severe and prolonged, will affect most of the functions of a plant.

- Proline accumulates significantly in stressed sugarcane leaves.
- Abscisic acid, known as a stress hormone, increase about 75-fold in stressed sugarcane leaves.
- Drought triggers the production of ABA and thus both ABA-dependent and ABA-independent pathways involved in the plant drought response.
- Among a total of 64 dehydration-increased metabolites, 16 are regulated by ABA - dependent pathways, including some amino acids, ethanolamine, glucose and fructose. 35 are regulated by ABA- independent pathways, such as raffinose and galactinol, metabolites belonging to TCA cycle and GABA shunt, and 13 other are regulated by both ABA- dependent and ABA- independent pathways, including proline, agmatine, methionine, lysine, saccharopine and pheynylalanine.

Heavy Metal Stress

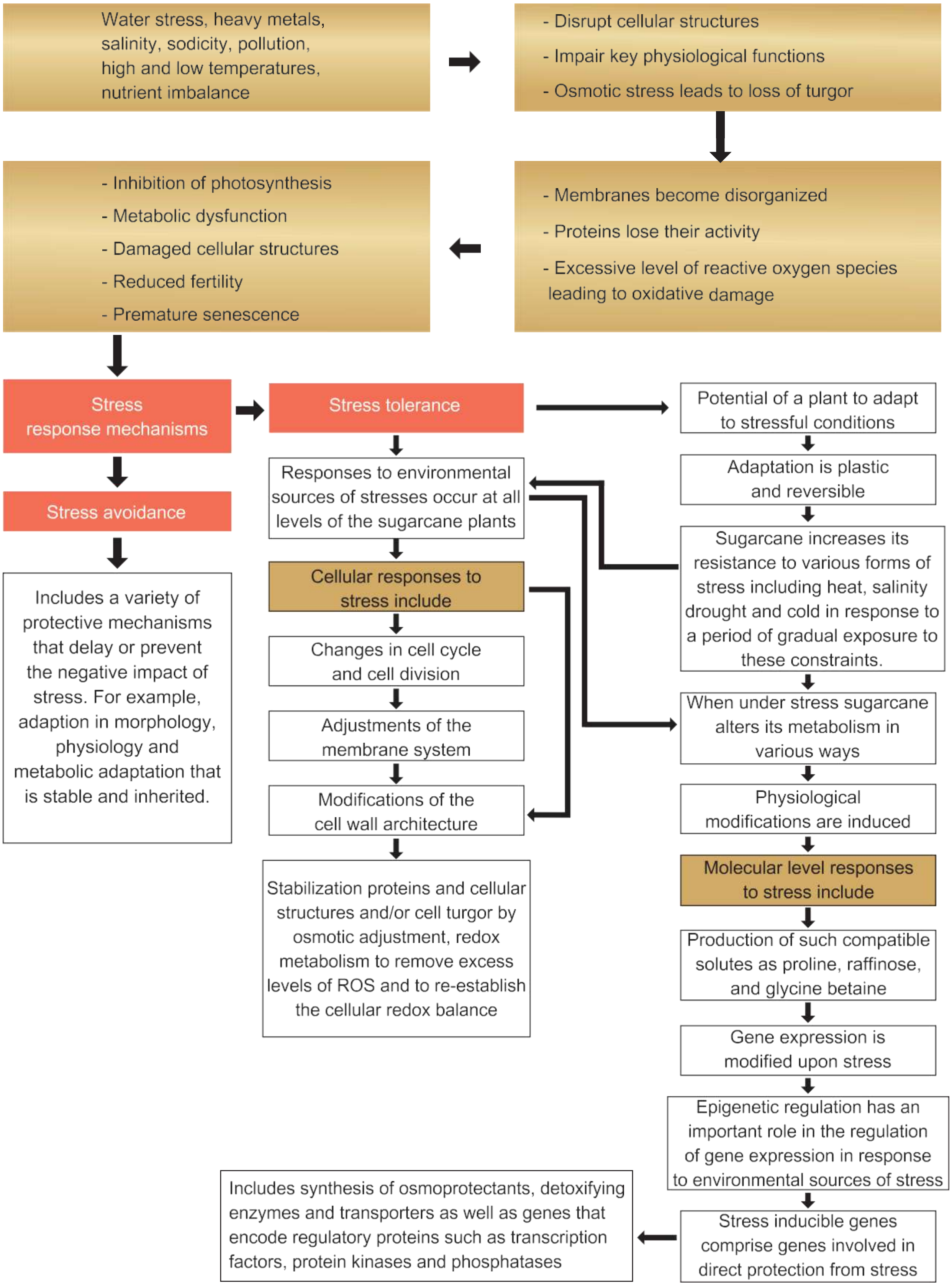
Such metals as arsenic, mercury, cadmium and lead are non-essential and potentially highly toxic.

Phytotoxicity of heavy metals

- Inhibit transpiration and photosynthesis
- Disturb carbohydrates metabolism
- Drive such secondary sources of stress as nutrition stress and oxidative stress

Abiotic Stress and Yield Losses

High Temperature Stress



Water stress, heavy metals, salinity, sodicity, pollution, high and low temperatures, nutrient imbalance

- Disrupt cellular structures
- Impair key physiological functions
- Osmotic stress leads to loss of turgor

- Membranes become disorganized
- Proteins lose their activity
- Excessive level of reactive oxygen species leading to oxidative damage

- Inhibition of photosynthesis
- Metabolic dysfunction
- Damaged cellular structures
- Reduced fertility
- Premature senescence

Stress response mechanisms

Stress avoidance

Includes a variety of protective mechanisms that delay or prevent the negative impact of stress. For example, adaption in morphology, physiology and metabolic adaptation that is stable and inherited.

Stress tolerance

Responses to environmental sources of stresses occur at all levels of the sugarcane plants

Cellular responses to stress include

Changes in cell cycle and cell division

Adjustments of the membrane system

Modifications of the cell wall architecture

Stabilization proteins and cellular structures and/or cell turgor by osmotic adjustment, redox metabolism to remove excess levels of ROS and to re-establish the cellular redox balance

Potential of a plant to adapt to stressful conditions

Adaptation is plastic and reversible

Sugarcane increases its resistance to various forms of stress including heat, salinity drought and cold in response to a period of gradual exposure to these constraints.

When under stress sugarcane alters its metabolism in various ways

Physiological modifications are induced

Molecular level responses to stress include

Production of such compatible solutes as proline, raffinose, and glycine betaine

Gene expression is modified upon stress

Epigenetic regulation has an important role in the regulation of gene expression in response to environmental sources of stress

Stress inducible genes comprise genes involved in direct protection from stress

Includes synthesis of osmoprotectants, detoxifying enzymes and transporters as well as genes that encode regulatory proteins such as transcription factors, protein kinases and phosphatases

Abiotic Stress and Yield Losses

High Temperature Stress

Plants are unable to move away from their environment and therefore they have evolved to deal with abiotic and biotic sources of stress with internal mechanisms of tolerance and resistance. Consequently the genes must evolve to perform and adapt quickly to environmental changes.

Nutrient management approaches to alleviate temperature stresses

Inadequate and unbalanced supply of mineral nutrients and impoverished soil are particular problems that lower global food production, especially in the developing countries. It is estimated that about 60% of the cultivated area has growth-limiting problems associated with nutrient deficiencies and toxicities.

Adequate nutrition is essential for the integrity of plant structure and key physiological processes. Nitrogen and magnesium are structural parts of chlorophyll needed for photosynthesis; Phosphorus is needed for energy production and storage, and is a structural part of nucleic acids; and potassium is needed for osmotic regulation and activation of enzymes. Therefore, a well-nourished plant is expected to produce more biomass per unit of transpired water than a poorly nourished one. It was also found that in N and P-deficient plants, markedly reduced hydraulic conductivity of the root cells. Research suggests that plant nutrients are also required for in alleviating various forms of abiotic stress: for example P, Si and K helps in greater tolerance to salinity in sugarcane. It seems that in the coming decades PSAP-related research will be a high-priority research area contributing to crop production and sustaining soil fertility. Survival and productivity of crop plants exposed to environmental sources of stress are dependent on the plants ability to develop adaptive mechanisms to avoid or tolerate stress.

Accumulating evidence suggests that the nutritional status of plants greatly affects their ability to adapt to adverse environmental conditions. This review is an effort to highlight the role of essential mineral nutrients in increasing the stress tolerance of crop plants.

Enhancement of Nutrients Uptake under Stress

1. Poor nutrition exacerbates the adverse effects of abiotic sources of stress and exogenous addition of high levels of macronutrients can alleviate such adverse effect of stress on plant growth.

2. After nitrogen, phosphorous is the second major nutrient for plant growth beause it is an integral part of biochemicals as such nucleic acids, nucleotides and phospholipids.

3. In most cases, salinity lowers the level of phosphorus accumulation in plants, which develop P deficiency. Concentration of soluble P in soil is usually very low (1 ppm or less). Phosphorous exists in two forms in soil, as organic and inorganic phosphate. Like other nutrient elements such as potassium, iron, zinc and copper possesses limited mobility in the soil.

Approaches for Crop Improvement under Abiotic Forms of Stress Conditions

- Crop rotation
- Intercropping
- Lower planting density
- Mulching
- Application of organic manure
- Application of bio fertilizers

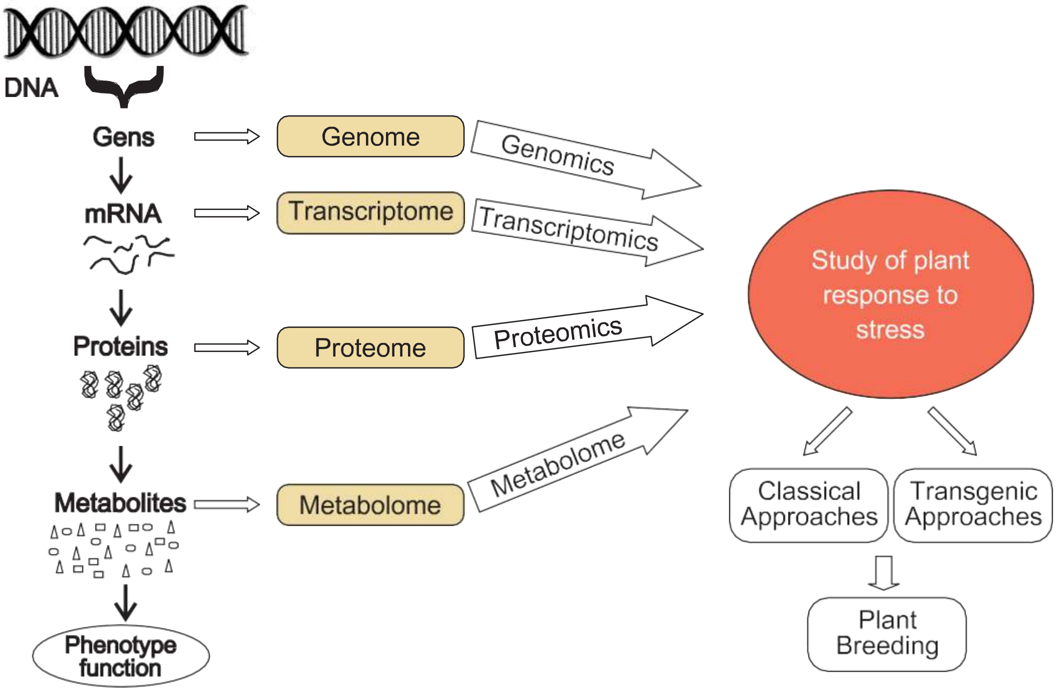
The Importance of Genetic Diversity in Managing Abiotic Forms of Stress

The main victims of increasing stress from abiotic sources are the fauna and flora, closely followed by the poor populations living in marginal areas. All living organisms are frequently exposed to many kinds of abiotic stress during their life. However, today it is observed that the majority of wild species are not able to adapt as quickly as necessary to abrupt changes that are occurring in the environment. Agriculture has been undergoing huge shifts from the paradigms introduced by the 'Green Revolution' friendly to agriculture and now it is beginning to be replaced by the concept of sustainable agriculture, where crops and livestock must be designed by breeders for better adaptation to the environment so that they perform better despite abiotic and biotic sources of stresses.

Presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids. This can be achieved through phenotypic and molecular characterization of PGR. Sometimes, large size of germplasm may limit their use in breeding. This may be overcome by developing and using subsets like core and minicore collection representing the diversity of the entire collection of the species. Molecular markers are indispensable tools for measuring the diversity of plant species. Low assay cost, affordable hardware, throughput, convenience, and ease of assay development and automation are important factors when choosing a technology.

Major Challenges to Breeding Programmes;

The complex nature of traits that confer to tolerance to abiotic stress. It is difficult to sort the traits that govern tolerance into manageable genetic components that can be modified by molecular approaches. In crop breeding, advances in molecular biology and genomics have a large impact on the speed of identification and characterization of genes and genetic regions associated with quantitative and qualitative traits. High-throughput marker systems are currently being used extensively in breeding programme to improve selection efficiency and accuracy and to focus on traits of great importance of adaptation. New tools and approaches such as genetic modification, gene knockout, RNA interference, genomics, proteomics, metabolomics and meta-genomics offer fresh insights in this field and have enable us to make many advances in elucidating the role of genetics in controlling complex traits such as those involved in the response to abiotic stress. Although in the "Green Revolution" the main focus of genetic improvement has been on large and uniform production, currently features such as disease resistance, nutritional quality and abiotic stress tolerance have assumed a prominent space of modern breeding programme. Recent discoveries about the mechanics of gene silencing ruled by RNA interference explain how plants can fight viral infections and genetic disturbances caused by uncontrolled gene expression, working as a very fine tuning process inside the cell.



Molecular approaches to plant breeding

Sugarcane is now an extremely important crop, particularly in tropical countries. Its use for sugar and ethanol production is critical and cultivated area and biomass yield are increasing. Environmental pollution by anthropogenic activities are perhaps some of the most disturbing problems the world is facing, and crops are subjected to pollution, which may have drastic effects on crop production. Curiously, literature on the study of abiotic stresses on sugarcane is very limited, indicating that far more research needs to be done and investigations on this topic need to be prioritized.

Grown mainly for sugar and ethanol in both tropical and subtropical parts of the world, sugarcane plays a significant role in world economy. Thus, the impact of sugarcane on the economy is crystal clear and its use in several different ways cannot be regarded as potential, but has been show to be real. It seems that the current cultivars are the result of interspecific crosses between S. officinarum, a species exhibiting high sugar content, and S. spontaneum, which exhibits higher plant vigor and resistance to several pests and abiotic forms of stress. In situ hybridization has revealed that S. officinarum has a basic chromosome number of $x = 10$ and that S. spontaneum has a chromosome number of $x = 8$. Thus two distinct chromosome organizations coexist in modern cultivars. As a perennial crop, sugarcane generally allows four to six harvests before replanting is necessary.

Prior to harvest, a sugarcane plantation can be set on fire to facilitate manual harvest and transport and to remove leaves and other non-sucrose-containing material. However, this process release particles in the atmosphere that can lead to respiratory problems in populations exposed to them. Due to social pressure and economic reasons, this system has been gradually replaced by green harvest. This change has many advantages. The presence of postharvest residues in soil can reduce CO_2 emission form soil, soil erosion, phosphorus availability and delay early leaf development. The increase in productivity has been ascribed to the development and widespread use of improved cultivars with increased resistance to stress better water management, nutrients and other resources, and the availability of relatively cheap chemical fertilizers and pesticides.

Any quick literature search will reveal the astonishing number of papers that are published weekly on the effect of distinct types of abiotic stress on plant species. Such a boom in this topic basically started in the late 1990's and today there are groups in every part of the world working on this topic. Yet, as far as sugarcane is concerned, the number of papers published is limited when compared to other crops, which is probably due to the historical and worldwide importance of these other crops. Another relevant aspect is that sugarcane is quite an unusual type of plant, with peculiarities such as multiple copies of the genome, which are hurdles to genetical, physiological and biochemical approaches. Climate change, the impact of bio-fuels on the world economy, and the successful use of sugarcane, particularly in countries such as India and Brazil, has changed the focus dramatically and has identified the urgent need for large-scale research into exotic plant species, particularly those related to sugarcane.

Plants and other living organisms have developed strategies to deal with conditions that may lead to stressful situations. The mechanisms of combating the detrimental effects of adverse environmental conditions, which in turn may limit sugarcane productivity, such as scarcity of water, salt and excess of metals, and extreme low and high temperatures, demand research on stress alleviation, particularly following the application of PSAP. Here we have focused our attention on the biochemical responses triggered by some key abiotic forms of stress in sugarcane, based on the manipulation of metabolic pathways towards adaptation responses observed in PSAP-treated sugarcane plants.

Redox Balance and Antioxidant Mechanisms

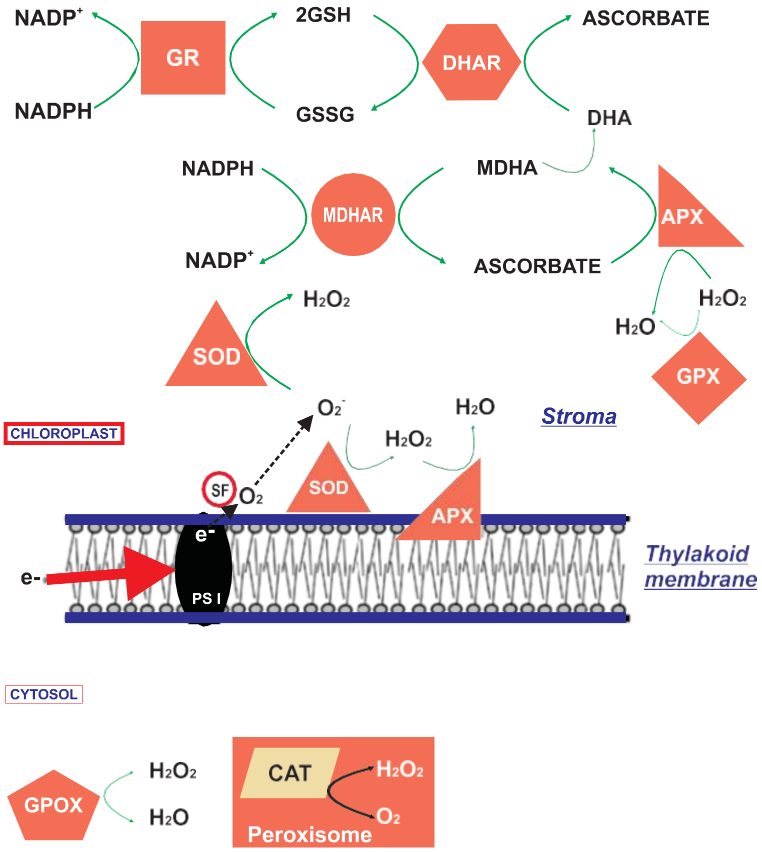
Independent of the type of stress discussed here, an imbalanced oxidative status can be maintained. This fact can be related to the reduction of molecular oxygen within cells, favouring production or increasing the availability of ROS, in particular the superoxide free radical anion (O_2^-) and hydrogen peroxide (H_2O_2), which react with several cellular components leading to damage to the cell system and even cell death.

In plant cells, ROS are generated in large amounts by both constitutive and inducible routes, but under normal situations, the redox balance of the cell is maintained via the constitutive action of a wide range of antioxidant mechanisms capable of preventing the cascades of uncontrolled oxidation by removal of ROS. Such mechanisms can involve enzymatic or non-enzymatic systems. Plant ROS-scavenging enzymatic mechanisms include the action, for example, of superoxide dismutase (SOD), which dismutates O_2^- to H_2O_2. Subsequently, H_2O_2 may be detoxified to H_2O by ascorbate peroxidase (APX), glutathione peroxidase (GPX), catalase (CAT) and other peroxidases. In addition, for the detoxification of H_2O_2, phenolic compounds can act as antioxidants by donating electrons to guaiacol-type peroxidases, such as guaiacol peroxidase (GPOX). Non-enzymatic antioxidants include ascorbate and glutathione (GSH), as well as vitamins, flavonoids, alkaloids and carotenoids. Some of these metabolites also have a prominent role in biotic stress. For example, GSH is oxidized by ROS, forming oxidized glutathione (GSSG) and ascorbate is oxidized to mono dehydro ascorbate (MDHA) and DHA (dehydro ascorbate).

The ascorbate-glutathione cycle, GSSG, MDHA and DHA can be reduced, reforming GSH and ascorbate, so that during stress, plants increase the activity of GSH biosynthetic enzymes and GSH levels. A more comprehensive study may be required to understand the enzymatic or non-enzymatic systems and the responses of sugarcane plants to abiotic form of stress when treated with PSAP. The measurements of antioxidants as stress markers will remain an essential aspect in assessing the plant response to stress, being a powerful tool to aid our understanding of the cellular and molecular mechanisms involved in responses to stress at the level of cell.

Salt or Salinity Stress

Although plants have evolved complex mechanisms for adaptation to osmotic and ionic stress caused by high salt, such as osmotic adjustment through an accumulation of compatible solutes, the extreme sensitivity of sugarcane to salinity at various growth stages is manifest in considerable reduction in growth rate. Salt stress severely impairs productivity and quality of the product, mainly due to a reduction in photosynthetic efficiency, which consequently reduces sucrose concentration in the stalk. This is triggered mainly by a disruption in the homeostasis of plant water potential and ion distribution at the cellular level and the whole plant level.



Antioxidant system involving enzymatic and non-enzymatic mechanisms.

SF : stromal factor
PS 1 : Photosystem I
GR : glutathione reductase
APX : ascorbate peroxidase
GPX : glutathione peroxidase
SOD : superoxide dismutase
CAT : catalase
MDHAR : mono de hydro ascorbate reductase
DHAR : de hydro ascorbate reductase

Consequently, new strategies to enhance crop yield stability on saline soils by treating sugarcane plants with PSAP represent a major research priority in sugarcane cultivation, besides selection and physiological characterization of salt-tolerant sugarcane clones which may have been reported.

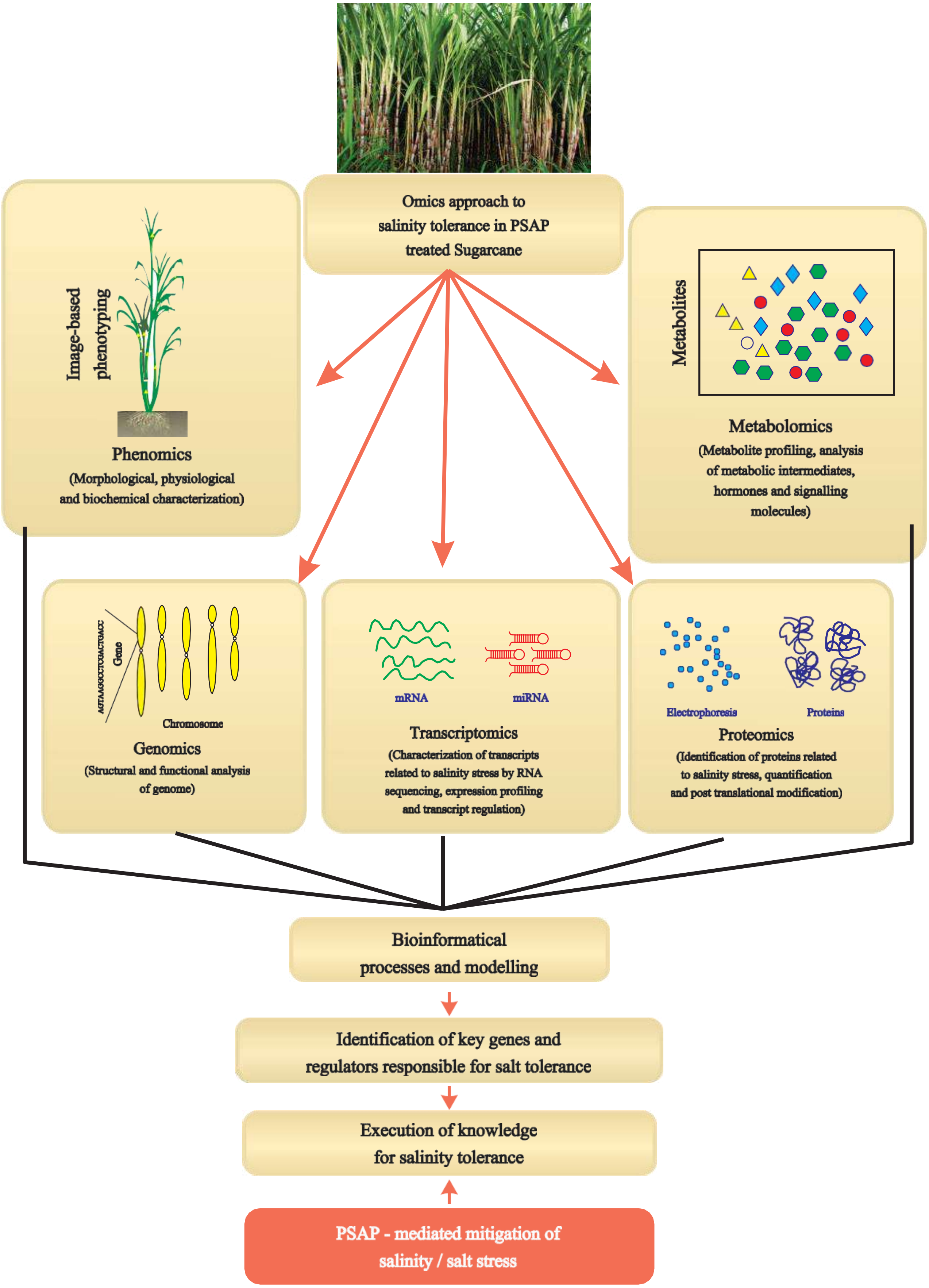
Biochemical studies have explored several aspects of sugarcane cell metabolism, which have improved our understanding of the adaptation mechanisms to osmotic and ionic stress caused by high salt. For example, larger amounts of leached-out and retained Na+, K+ were observed in NaCl$^-$ / KCl-treated calli, compared to the control calli, implying that sugarcane can be considered as a Na+-excluding plant species. On the other hand, the retained K+ content was significantly higher in the control than in the NaCl$^-$ treated calli.

It is noteworthy that growth retardation and reduced cell viability are associated with a conspicuous increase in Na+ and a corresponding decline in K+ concentrations, demonstrating the typical glyeophyte character of the plant.

PSAP-treated sugarcane also needs to be studied to ascertain whether it is a salinity-tolerant clone of sugarcane and therefore accumulates less Na+ and more K+ as compared to its sensitive counterpart, and consequently exhibits a higher K+: Na + ratio.

The levels of flavonoids, which appear to be important antioxidants in tolerance to environmental forms of stress, was higher in tolerant clones than in sensitive clones, confirming that these substances can also protect cane from ion-induced oxidative stress during salinity stress. Moreover, this result could further explain the greater tolerance of sugarcane seedlings to salt stress when primed with PSAP. It is well known that priming improves several aspects of plant growth under stress.

However, further study of PSAP-treated plants may answer many questions about salt stress in sugarcane that remain to be answered. Which antioxidant systems are mainly involved in the defence against to salt stress and what are the signalling molecules involved in the process? How can water and nutrient use efficiency affect or be involved in the response or adaptation mechanisms to salt stress? Which salts can alter or produce changes in the general metabolism of the cell and which are the main metabolic routes affected, and when and how? To answer to such questions, genomic research involving the development of EST library is required, and the metabolic profile may give some insight into the interactions.



Omics approach to
salinity tolerance in PSAP
treated Sugarcane

Phenomics
(Morphological, physiological
and biochemical characterization)

Metabolomics
(Metabolite profiling, analysis
of metabolic intermediates,
hormones and signalling
molecules)

Genomics
(Structural and functional analysis
of genome)

Transcriptomics
(Characterization of transcripts
related to salinity stress by RNA
sequencing, expression profiling
and transcript regulation)

Proteomics
(Identification of proteins related
to salinity stress, quantification
and post translational modification)

Bioinformatical
processes and modelling

Identification of key genes and
regulators responsible for salt tolerance

Execution of knowledge
for salinity tolerance

PSAP - mediated mitigation of
salinity / salt stress

In addition, transcriptome, metabolome and proteome studies using sugarcane lines tolerant and sensitive to salt stress may reveal the important steps from gene expression towards mechanisms of salt tolerance in PSAP-treated sugarcane.

Temperature Stress

Contrary to some other abiotic factors, the effect of temperature on sugarcane growth has been described since the mid 1960's and numerous studies have shown that temperature affects the development of the plant in various ways. Sugarcane responds to chilling temperatures with dramatic alterations in photosynthesis, which is by far the most explored process in sugarcane for obvious reasons.

For instance, under chilling temperatures, the rate of photosynthesis is severely decreased. Furthermore, earlier studies carried out on leaves of a cold-sensitive cultivar revealed that important photosynthetic enzymes affected by chilling temperatures (10^0C) include sucrose phosphate synthase, NADP-malate dehydrogenase and pyruvate orthophosphate dikinase, indicating a fundamental role of these enzymes in sugarcane subjected to low temperatures. It was also observed that chilling temperatures increased aspartate and alanine levels in the leaves of the cane plants, raising questions about the biochemical modifications involved in cold injury.

The protective mechanisms in PSAP-treated sugarcane plants against chilling injury still need to be explored, but they may well be dependent on a complex antioxidant system. Associated with biochemical unveiling, further molecular and genomic studies are required to elucidate how sugarcane responds to low temperature when treated with PSAP.

So far, the expression profiles of cold-inducible genes have revealed proteins that are directly involved in chilling and freezing tolerance. For instance, one sugarcane EST encoding a putative xanthine dehydrogenase (XDH) was significantly induced after exposure to cold. XDH is a gene encoding a putative NAD-dependent dehydrogenase that might be involved in protection against oxidative stress due to such exposure.

On the other hand, markedly higher temperatures also seem to affect sugarcane development in various ways. For example, sugarcane grown under high temperatures (40^0C) showed a significant decline in shoot dry mass, increased tillering, early senescence and smaller internodes, probably due to a reduction in carbon partitioned to stored sucrose.

Sugarcane Leaves Showing Stress

Photosynthesis Activity Reduced

Furthermore, it has been shown that elevated temperature affects metabolic pathways mainly through oxidative damage to cells, thereby affecting the levels of both primary and secondary metabolites.

For example, the synthesis of free proline, glycinebetaine, soluble sugars, carotenoids and flavonoids was shown to be enhanced after heat-stress (40^{0}C), and such changes in metabolite levels were crucial to improving heat tolerance of sugarcane .

It is noted, using biophysical and biochemical approaches, that in sugarcane the effects of heat stress are reversible through small heat-shock proteins (sHsp), which constitute an important chaperone family. This indicates a mechanism to compensate for the damage caused by high-temperature stress, thereby pointing to a potential source of improved tolerance to heat stress.

The global increase in ambient temperature will be a critical factor for plant growth in the future. Renewed scientific interest will hopefully lead to a better understanding of the physiological responses of plants to high temperatures, mechanisms of heat tolerance

and thus open up new possibilities for greater thermo-tolerance of sugarcane in PSAP-treated sugarcane plants.

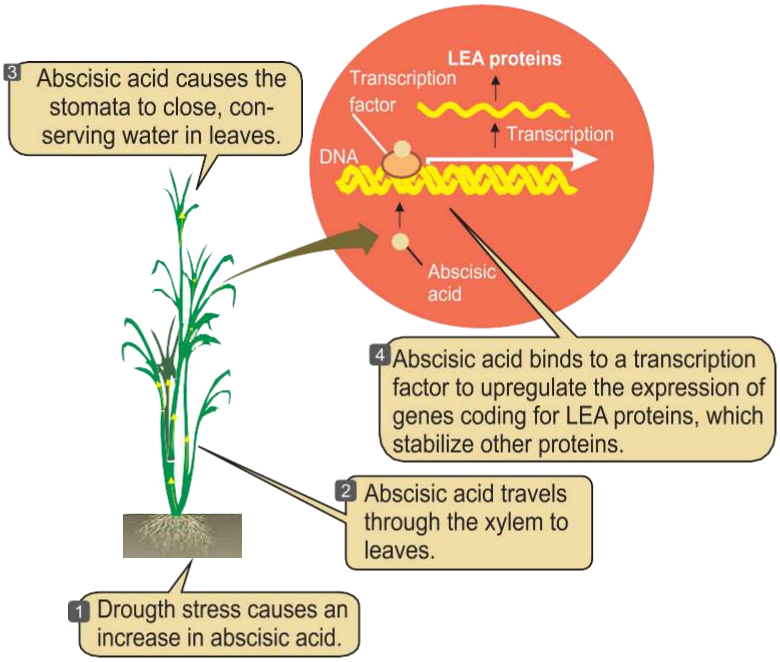
Drought Stress

Sugarcane is grown in almost all tropical and subtropical areas of the globe, including regions where water availability is limited or highly variable. Additionally, an increase in the frequency of a different kind of El Nino, which is characterized by an anomalous increase in sea surface temperature (SST) in the central tropical pacific, flanked by colder regions in the west and east, may alter the distribution pattern of precipitation in important sugarcane-producing countries such as Australia, Brazil, India and China.

Thus, an understanding of how PSAP-treated sugarcane plants respond to drought is fundamental to improving crop management and water use efficiency and ensuring the viability of sugarcane cultivation. The first response of a plant to water deficit is reduction in growth. As the plant water potential decreases, photosynthetic rate also decreases. A key priority is to understand the biochemical changes that occur in photosynthesis when sugarcane is exposed to drought stress.

Some preliminary studies showed decreased activity of such enzymes such as ribulose-1,5-bisphosphate carboxylase (Rubisco), phosphoenol- pyruvate carboxylase (PEPC), NADP malic enzyme (NADP-ME) and PPDK, with the decline in leaf water potential, the impact of drought being more prominent on PPDK activity. However, available literature about the response of PEPC to drought stress is not consistent, with other studies reporting no changes in its activity or even a small increase in its activity. Sugarcane is a C4 plant and as such has a CO_2-concentrating mechanism, which provides, among other advantages, a reduction in photorespiration and higher water use efficiency when compared to C3 plant species. There is evidence that sugarcane utilizes two distinct forms of C4 metabolism, identified by the decarboxylation enzymes used: (NADP-ME) and phosphoenolpyruvate carboxykinase (PEPCK), with PEPCK decarboxylation predominating over NADP-ME.

During drought stress, plants usually have a lower carbon assimilation rate, which provides an insufficient sink for electrons generated in the electron transport chain (ETC) and consequently leads to overproduction of ROS. Up-regulation of genes encoding for polyamine oxidase, cytochrome-c-oxidase, S-adenosylmethionine (SAM), decarboxylase and thioredoxins, which directly or indirectly participate in the regulation of intracellular redox status, has been demonstrated in sugarcane under drought stress and may contribute to the plants tolerance to water deficit. In a similar manner to catalase (CAT), this enzyme is responsible for the reduction of H_2O_2 to H_2O and O_2, and a decline in peroxidase activity is considered a limiting step to ROS neutralization in sugarcane. The accumulation of the osmolytes trehalose and proline also contributes to the reduction in the damage caused by the accumulation of ROS and is associated with drought tolerance in sugarcane. Another point that deserves attention is the response mediated by ABA, the plant hormone related to water stress signalling and regulating water balance.



Drought responses in sugarcane were found to be analogous to those induced by exogenous ABA application. Both drought and ABA induced the expression of genes encoding a PP2C-like protein phosphatase, a S-adenosylmethionine decarboxylase and two delta-12 oleate desaturases. It is also reported that SodERF3, a sugarcane ERF, (ethylene responsive factor) is induced by ABA under drought stress, and the factor may also be involved in salt and drought tolerance. However, plant response to drought is a complex phenomenon, especially with a polyploid genome like sugarcane, besides the fact that drought stress involves biochemical networks that are still being elucidated. For example, phosphorus and potash supply through PSAP improved the acclimation capacity of sugarcane by affecting plant characteristics related to water status and photosynthetic performance and causing network modulation under water deficit.

Toxic Metal Stress

The contamination of water, soil and sediments with toxic metals has been and will continue to be a major environmental problem that needs to be dealt with. Nowadays, it is being realized that apart from the intensive programmes and continuous efforts in plant breeding to increase sugarcane productivity, it is also necessary to deal with pollution caused by contaminated water, pesticides, fertilizers, sewage sludge, industrial residues and herbicides, which contain different concentrations of toxic metals. These metals can be taken up by the growing sugarcane, severely affecting plant development. In the past few years, a number of reports have focused on the effects of toxic metals on a wide range of plant species.

In one study, high concentrations of zinc (65 and 130 ppm) were shown to increase lipid peroxidation in sugarcane, thereby affecting membrane integrity of leaves, root growth and mitotic efficiency. The interference of Zn in normal mitosis could be related to inhibition of DNA synthesis. Moreover, excess Zn increased the content of photosynthetic pigments in leaf tissues of sugarcane, which could be related to a disturbed synthesis of proteins and nucleic acids. In metal stress due to excess cadmium, the antioxidant enzymes of sugarcane seedlings were affected in different ways showing a decrease in CAT activity and an increase in glutathione redutase (GR). Aluminium is a very commonly occurring metal and, due to its world wide distribution in soils and in the earth's crust, deserves special attention, particularly if sugarcane is cultivated in Al-contaminated soils. When Al toxicity is considered, the compatible solutes trehalose, glycinebetaine and proline can be indicators of the interaction between drought and acidity stress in soil.

However, the accumulation of these compatible solutes in plant leaf tissue cannot prevent losses in dry matter production of sugarcane. Along with biochemical data, molecular analyses are required for understanding the mechanisms of Al toxicity in the specific case of sugarcane treated with PSAP. For this purpose, a sugarcane expressed sequence tag (SUCEST) data bank can be used (http://www.sucest-fun.org) to study biochemical pathways related to Al toxicity.

A more comprehensive view has to be taken and must necessarily include studies on gene expression, protein translation and enzyme activity. These are essential tools for generating a wide range of information on the responses of PSAP-treated sugarcane to in a form of heavy metals stresses. A molecular genetic approach may help to identify the expression of genes associated with nutrient uptake and metal tolerance.

Nutrient Stress

Nutrient status is an environmental factor that can influence growth rate, number of green leaves per mother shoot, leaf area and tiller density of sugarcane. Therefore, nutrient imbalance is one of the oldest subjects in sugarcane science. Ion stress caused by excess aluminum (Al) and iron (Fe) on sugarcane could be alleviated with additions of phosphorus (P) and potassium (K) instantly made available with application of PSAP. Hence the necessity of having adequate K available to utilize unassimilated nitrogen (N) in sugarcane to bring about a stage of maturity where the reducing sugars are converted to sucrose. Nutrient deficiency is detrimental to sugarcane growth and development, and can reduce yields, a phenomenon that continues to be the subject of extensive research. The quantum yield for CO_2 uptake decreased linearly with decreasing leaf nitrogen (N) content and the rate of photosynthesis decreased with increased severity of K deficiency.

Therefore, the application of PSAP along with K fertilizers to a soil deficient in K could improve sucrose recovery through the reduction in fiber content. It has been shown that balanced use of all the needed nutrients can help in improving cane productivity and enhance sugar recovery by making the plant resistant to abiotic as well as biotic form of stress, and through better synthesis and storage of sugar. For example, P supply alleviated the negative effects of water deficit on sugarcane photosynthesis, possibly by increasing proline content. Although drought-tolerant sugarcane genotypes exhibited higher free-proline content than drought-sensitive plants, more studies are needed to confirm that the above response is more efficiently modulated by PSAP in sugarcane.

Another example of resistance to abiotic form of stress is, P^{-}, K^{-} and Si-enhanced salt tolerance in salt-sensitive sugarcane genotypes resulting in decreased Na+ concentration and increased K+ with improvement in K+/Na+ ratio.

It is also interesting to note that the application of PSAP at the time of planting sugarcane under water stress significantly increased the stomatal diffusive resistance, thereby decreasing transpiration rate and increasing the leaf water potential, length of the cane, sucrose content of the juice and sugarcane yield.

Excess nutrients can trigger extreme stress responses in sugarcane.

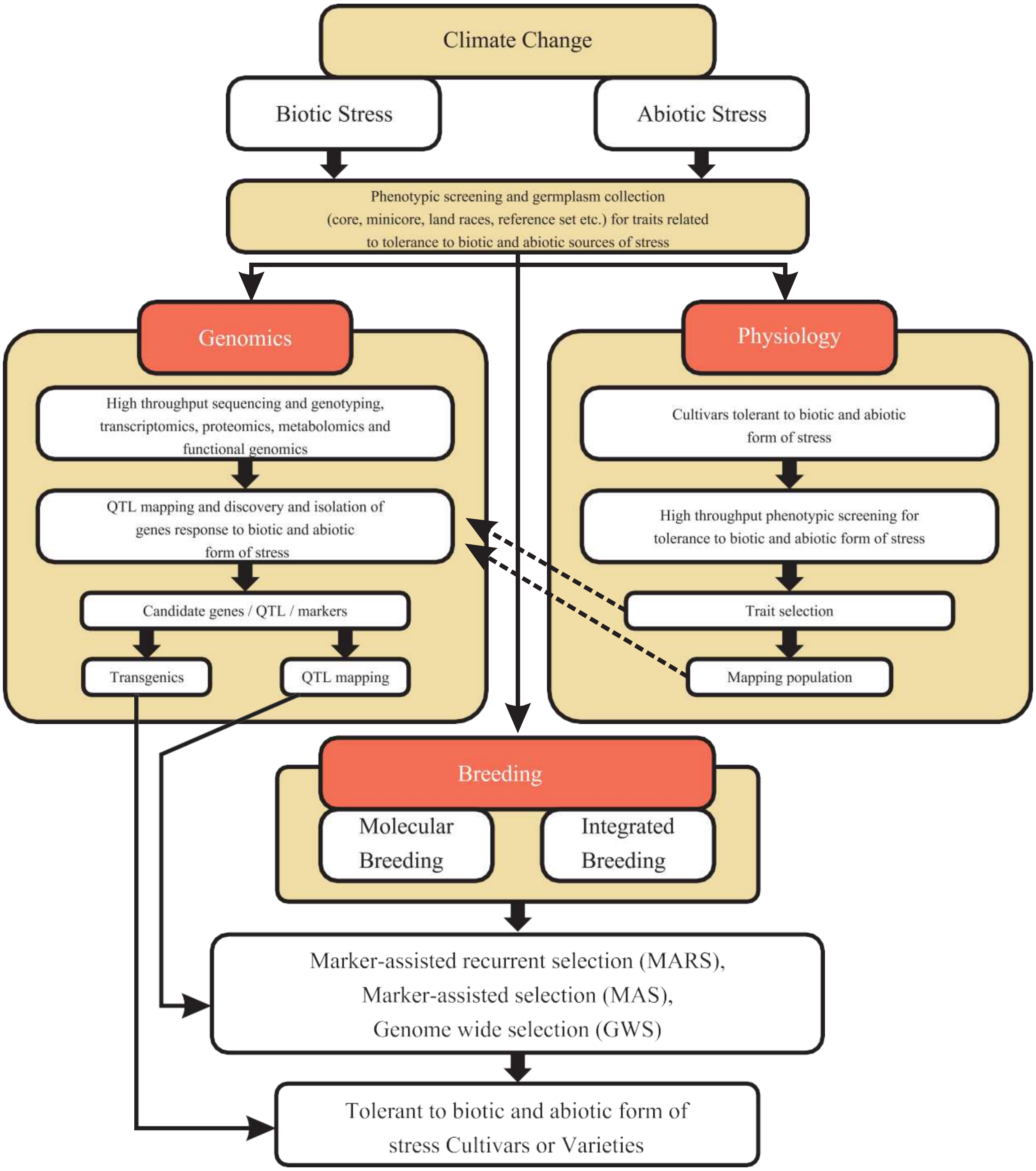
Stress responses to both deficiency and excess of nutrients appear to involve complex mechanisms that modulate the uptake and accumulation of ions. Therefore, identifying and understanding, in PSAP-treated sugarcane plants, the expression of genes responsible for or associated with nutrient uptake and distribution may lead to efficient nutrient management in sugarcane, controlling the application of fertilizers to sugarcane crops and consequently the environmental impact of fertilizer production and application.

However, when sugarcane is compared to other crops, some of which are perhaps less economically important, the contribution is still quite limited and much more has to be done. In particular, research is required on the molecular and biochemical modifications that are involved in adaptation responses to drought, salt, extreme temperature and excess nutrients and metals in PSAP-treated sugarcane plants.

Independent of the source and nature of the abiotic agent, natural or anthropogenic, we do believe that such studies should be placed on the agenda with high priority, since sugarcane is the most important cash crop cultivated for the extraction of sugar and energy derivatives produced worldwide.

Studies on high temperatures are also scarce in sugarcane, but that is possibly because sugarcane is a tropical and subtropical plant species. Yet, research on metabolic pathway regulation, plant development and yield of sugarcane under heat stress is very important, considering the potential risk of global warming and its effect on this crop and as a consequence, on sugar and ethanol production. The application of PSAP can counter the losses from stress including heat.

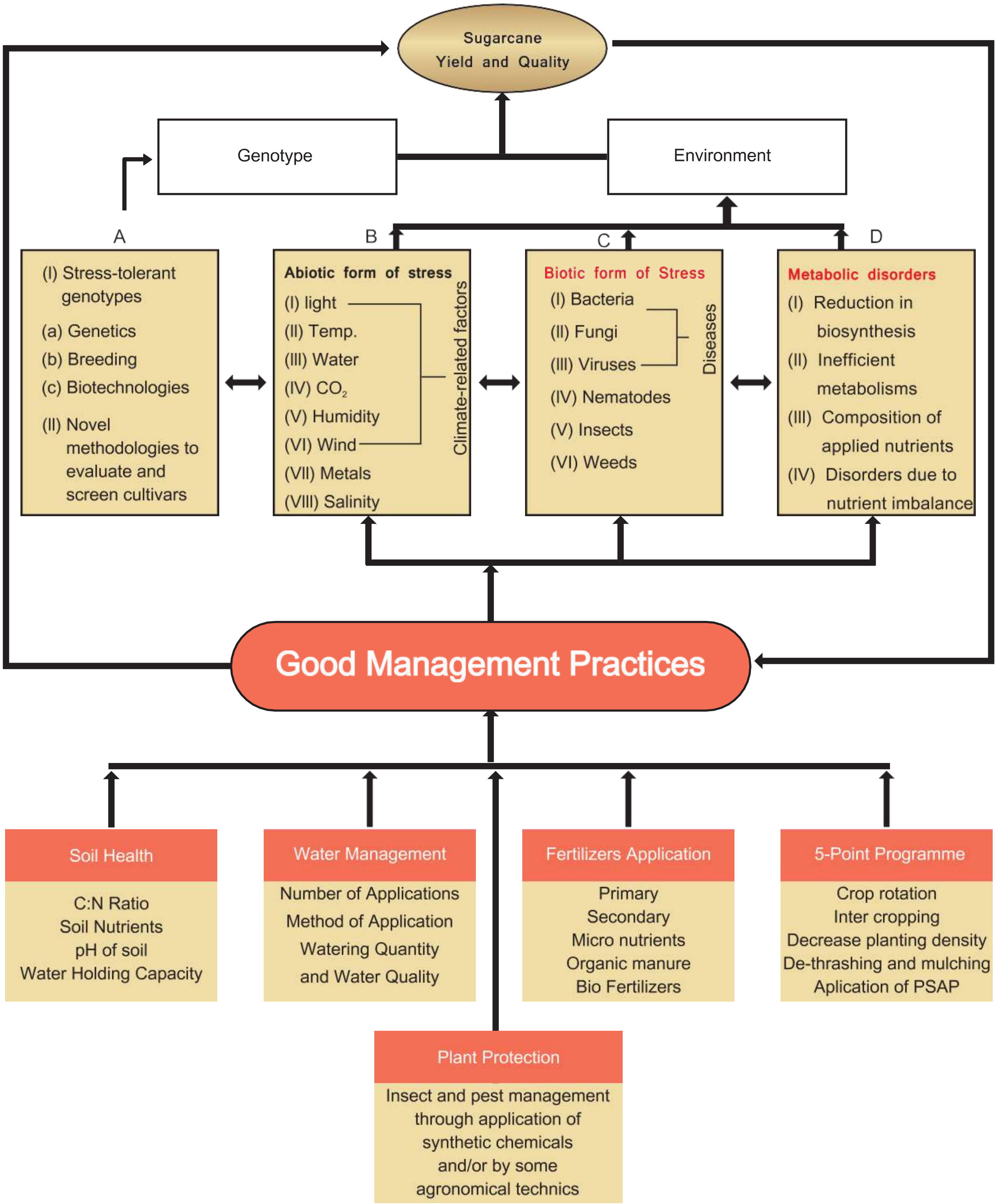
PSAP Technology, Genetic Science, Soil health and Omics Combined Together can endorse Sustainable Agriculture



Integrating genomics, physiology, and breeding techniques for developing improved cultivars with enhanced tolerance to abiotic and biotic form of stress are required to study such traits in PSAP-treated plants.

Abiotic Stress and Yield Losses

High Temperature Stress

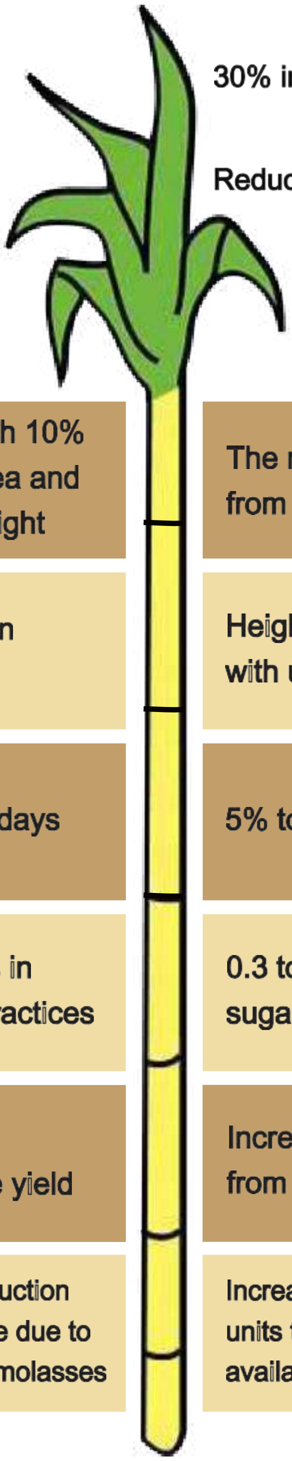


Abiotic Stress and Yield Losses

High Temperature Stress

Growth Observations in Treated Sugarcane

Application of 5 kg PSAP / acre



- 30% Increase in yield
- 30% Increase in sugar production
- 30% Increase in co-generation

- 0.3% improvement in sugar recovery in ccs
- 30% increase in ethanol production
- Reduction in unit cost of sugar production

- Dark green leaves with 10% to 20% higher leaf area and 20% to 30% more weight
- 20 to 30% increases in mature and millable
- Early maturity by few days
- 30 to 100% increases in yield base on other practices
- 10 to 20 tonnes / acre increase in sugarcane yield
- Increase in ethanol production from 90 to 110 liter / acre due to availability of additional molasses

- The number of tillers increases from 20 to 30%
- Height increases by 10% to 20% with uniform internodal length
- 5% to 10% increase in girth
- 0.3 to 1.0 % improvement in sugar recovery in CCS %
- Increases in sugar production from 1.3 to 1.7 tonnes / acre
- Increase in co-generation 400 units to 500 units / acre. Due to availability of additional bagasse

Abiotic Stress and Yield Losses

High Temperature Stress

Application of PSAP at the rate of 5 Kg. per acre in three to four sprays to the sugarcane enhances cane yield by 30% with improvement of 0.5% sugar recovery in CCS unit.

PSAP IMPROVES METABOLIC CAPACITY OF PLANTS

★ Most of the crops in general have genetic tolerance to diseases and pest, however, the nutrient balance, favourable soil and atmospheric conditions support luxuriant growth of crop plants.

★ The disease and pest tolerance as well as yield & quality reduces due to following three causes.

1) Imbalance in major nutrients, particularly N, P and K. Nitrogen, being a basic component of protoplasm, proteins, enzymes etc., helps to produce more vegetative growth whereas Potash works as radar while inducing various stress tolerances and Phosphate is most useful for sugar and carbohydrate synthesis and its interconversion. Phosphate is also required as energy source of various growth processes. Yield & quality is govern by P & K nutrients.

2) The balanced nutrition is require to be maintained throughout growth phases of crop ontogeny. In practice, farmers generally apply more Nitrogen and less P & K leading to succulence and poor tolerance to biological (diseases, pest), physiological (water, osmotic potential) and environmental (temperature, humidity, frost etc.) stresses.

3) Besides N, P & K micro nutrients and secondary nutrients also plays great role in crop growth, vigour and built up of tolerance to various stresses.

Due to above facts, overall crop growth is affected, crops looks unhealthy and weak which in turn get further affected due to diseases and pest. The ultimate effect is higher spending for disease and pest management, low yield and poor quality of crop produce.

The Research Organisations and Government Departments recommends balanced proportion of N, P & K. Whatever P is applied, hardly 10 to 12 % quantity is taken up by plants and remaining get fixed in the soil. K uptake is also poor. Hence, foliar spray of P & K through PSAP gives fantastic improvement in crop growth, vigour, quality & yield of sugarcane. With use of PSAP in lesser cultivation cost, farmer can get higher quality & yields.

PSAP

TECHNOLOGY

Abiotic Stress and Yield Losses

High Temperature Stress

1. Formulated after 6 years of untiring and in depth rigorous research efforts. PSAP tried and tested on farmers' fields. PSAP technology has been proved that it spectacularly increases cane yield and improves sugarcane quality. PSAP also induces diseases, pest and various types of stress tolerance in sugarcane. Besides, this product is nontoxic, environment friendly having wide range of crops applicability. Hence can be instrumental in bringing most needed all round next agriculture revolution in our country.

2. PSAP applications are easy to handle and can be used without much changes in the agricultural practices in vogue. Applications of PSAP are flexible and can be adopted at given situation

3. Application of PSAP is complementary to the existing agricultural production technology as well as emerging technologies such as precision agriculture. Sustainable agriculture can be endorsed with PSAP.

4. PSAP is very effective in all most all the crops in improving plant health, inducing stress tolerance, higher yield (30-100%), quality of produce (sweetness, keeping quality, lustre). Ultimately farmers and customers are benefited.

5 By spraying 5 kg of PSAP in 4 sprays with interval of 15-20 days on 50-60 days sugarcane after emergence definitely results in ;

★ Cane yield improvement to the tune of 100 - 200 Quintal per acre (around 30% higher than unsprayed). This fetches additional income to cane growers. Even after deducting the cost of product and spraying cost, farmers gets B:C Ratio of Rs 1.0 : 4.0.

★ Overall sugar recovery increases by 0.3% which helps to improve balance sheet of sugar mills.

★ Per acre sugarcane yield improvement as well as sugar recovery enhancement helps to reduce cane area requirement to fulfil the crushing needs of sugar mill and also helps to increase the production of side products like ethanol about 30%, co-generation 30% (due to additional bagasse availability), bio manures etc. All this together add to the income and profit of sugar mills.

6. PSAP being eco-friendly, nontoxic and having no residual effect, the agricultural produce is very safe for human or animal or birds consumption.

Abiotic Stress and Yield Losses

High Temperature Stress

Application of 5 kg PSAP per acre

Observations in PSAP-treated Sugarcane

- Increases stress tolerance
- Induces diseases and pest resistance
- Improves vigour and growth
- Increases yield and quality
- Non toxic and environmental-friendly
- Complements emerging technologies
- Certified in organic farming

Additional Benefits to Sugar Mills by Crushing PSAP Sugarcane

30 % Increase Sugarcane yield

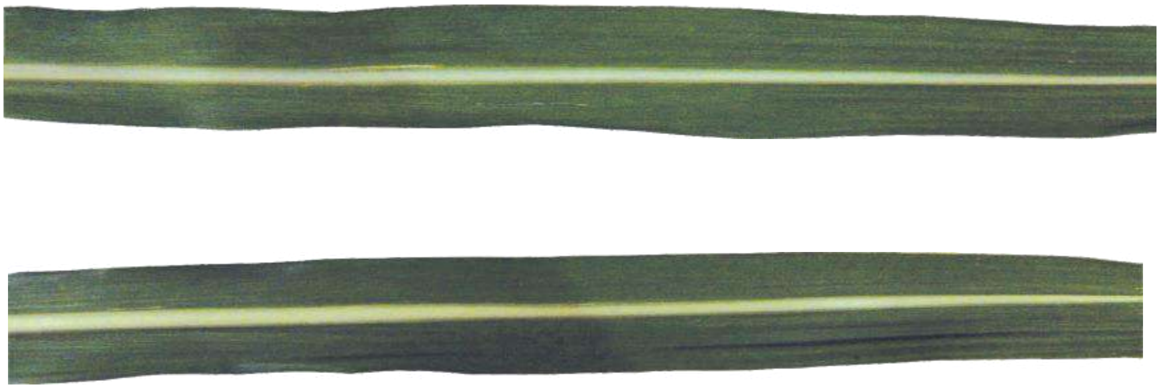
Additional sugar production 30 %

30 % Increase in ethanol production.

Increase in co-generation. 30%

Sugarcane Leaves

Cross Section



PSAP Treated

Control - Unsprayed

Sugarcane Leaves

Acclimations in Strees

PSAP Treated

Control - Unsprayed

Sugarcane Leaves

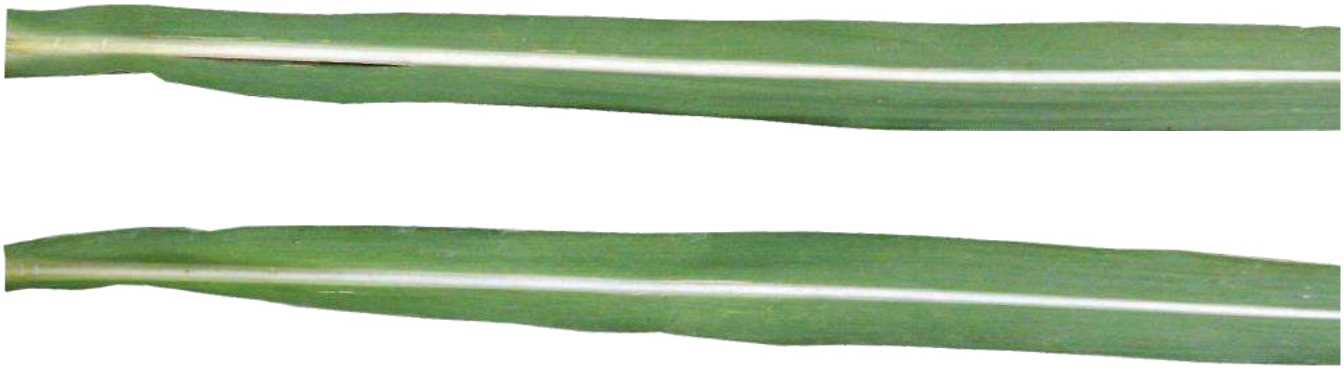
Response to Temperature Stress

PSAP Treated

Control - Unsprayed

Sugarcane Leaves

Alleviate Stress



PSAP Treated

Control - Unsprayed

Sugarcane Leaves

Weight Comparison

PSAP Treated

Control - Unsprayed

PSAP

Spray Schedule for Sugarcane

Dose ☛ **Dissolve 200 grams of PSAP in 30 liters of water for spray.**

Apply 5 kg of PSAP per acre for sugarcane in 3 to 4 sprays

Spraying method for new plantations and ratoon crops

- Spray sugarcane leaves throughly from inside out.
- No other chemical should be mixed with the PSAP solution.
- Spray PSAP 4-5 days after irrigation appears to be the most effective.
- Apply 120-330 liters of solution per acre for spray
- Start PSAP spray in sugarcane at 6 to 7 leaves Stage.

Schedule for 4 sprays

5 kg PSAP per acre given in 4 sprays
Mix 200 grams (one PSAP pouch) in 30 liters of water (2 pumps) i.e. 6.5 gm/liter of water

Spray	Age of the cane crop	PSAP solution to be sprayed
1	40 to 50 days	120 liters
2	55 to 65 days	180 liters
3	75 to 85 days	210 liters
4	95 to 105 days	240 liters

Schedule for 3 sprays

5 kg PSAP per acre given in 3 sprays
Mix 200 grams (one PSAP pouch) in 30 liters of water (2 pumps) i.e. 6.5 gm/liter of water

Spray	Age of the cane crop	PSAP solution to be sprayed
1	55 to 65 days	180 liters
2	75 to 85 days	240 liters
3	95 to 105 days	330 liters

Sugarcane 50-60 Days

Sugarcane 75-85 Days

List of References

1) C.J.Lovatt and R.L.Mikkelsen, 2006. Phosphite Fertilizers : What are they? Can you use them? What can they do?Better crops/Vol 90

Abstract

Interest is growing in phosphite as part of a total production program. Phosphite contains one less oxygen (O) than phosphate, making its chemistry and behavior quite different. Phosphite is more soluble than phosphate, making leaf and root uptake more efficient, thus high concentrations can be toxic for plants. Phosphite also has unique effects on plant metabolism.

Phosphite supplied through the soil or foliage is slowly converted to phosphate. Soil and foliar applications are made at relatively low rates to prevent nutrition problems. For some plant species, phosphite may offer some unique benefits not seen with phosphate applications.

2) Hoang Thi Bich Thao and Takeo Yamakawa : Phosphite (Phosphorus acid): Fungicide, Fertilizer or Bio-Stimulator? Soil Science Plant Nutrition 55(5):228-234 Kyushu University, Japan.

Abstract

Phosphite (Phi), a reduced form of phosphate (Pi), is widely marketed as either a fungicide or fertilizer or sometimes as a bio-stimulant. This is confusing for both distributors and growers. The present paper explores data from various studies to clarify that Phi does not provide plant P nutrition and thus cannot complement or substitute Pi at any rate. In addition, Phi itself does not have any beneficial effect on the growth of healthy plants, regardless of whether it is applied alone or in combination with Pi at different ratios or different rates. Plants fertilized with Pi allowing for approximately 80–90% of its maximum growth might still be at risk of the effect. This negative effect becomes more pronounced under more seriously Pi-deficient conditions. Although a number of studies have shown positive crop responses to Phi, these responses are likely to be attributable to the suppression of plant diseases by Phi and/or to Pi formed from oxidation of Phi by microbes. In addition, indirectly providing P by Phi-to-Pi oxidation is not an effective means of supplying P to plants compared with Pi fertilizer. An understanding of these issues will aid the right selection of fertilizer as well as minimize the harmful effects of Phi use on crops.

3) Nyoman Pugeg Aryantha and David I Guest : 2004, Phosphonate (PO_3^-) effectiveness against Phytophthora Cinnamomi Rands on Thryptomene Calycina, Banksia grandis and Banksia Spinulosa. Plant Pathology Journal 3(1) 19:25-2004. School of Botany. The University of Melbourne.

Abstract

The present study shows that Potassium phosphonate has been proven to slow down the growth rate of P. cinnamomi in in vitro . Phosphonate drench as low as 1 g L⁻¹was effective in protecting Thryptomene calycina , Banksia grandis and B. spinulosa in pot and field trials. In glass house trials, concentrations as low as 1 g L⁻¹ (drench) significantly suppressed the P. cinnamomi population. Concentrations over 2 1/2 g L⁻¹ were phytotoxic to all plant species tested. The most sensitive species was B. spinulosa . Phosphonate (5 g L⁻¹) killed all B. spinulosa plants in seven weeks, therefore it must be used with a great care. Phosphonate treatment alone was effective protecting plants from disease in the field, but did not result in high plant health.

Despite new root growth in pot trials after seven weeks, poor growth was commonly observed on T. calycina after 14 months in field trials. This suggests that phosphonate is not suitable as sole application particularly for the long term. A combination of phosphonate with compost as well as antagonist as an integrated management will be a good alternative for P. cinnamomi management in the future.

4) Varadarajan D.K., Karthikeyan A.S., Matilda P.D. (2002) Phosphite, an analog of Phosphate, Suppresses the coordinated expression of genes under Phosphate starvation. Plant Physiology 129 : 1232-1240.

Abstract

Phosphate (Pi) and its analog phosphite (Phi) are acquired by plants via Pi transporters. Although the uptake and mobility of Phi and Pi are similar, there is no evidence suggesting that plants can utilize Phi as a sole source of phosphorus. Phi is also known to interfere with many of the Pi starvation responses in plants and yeast (Saccharomyces cerevisiae). In this study, effects of Phi on plant growth and coordinated expression of genes induced by Pi starvation were analyzed. Phi suppressed many of Pi starvation responses that are commonly observed in plants.

Enhanced root growth and root to shoot ratio, a hallmark of Pi stress response, was strongly inhibited by Phi. The negative effects of Phi were not obvious in plants supplemented with Pi. The expression of Pi starvation-induced genes such as LePT1, LePT2, AtPT1, and AtPT2 (high-affinity Pi transporters); LePS2 (a novel acid phosphatase); LePS3 and TPSI1 (novel genes); andPAP1 (purple acid phosphatase) was suppressed by Phi in plants and cell cultures. Expression of luciferase reporter gene driven by the Pi starvation-induced AtPT2 promoter was also suppressed by Phi. These analyses showed that suppression of Pi starvation-induced genes is an early response to addition of Phi. These data also provide evidence that Phi interferes with gene expression at the level of transcription. Synchronized suppression of multiple Pi starvation-induced genes by Phi points to its action on the early molecular events, probably signal transduction, in Pi starvation response.

5) Avila F.W., Faquin V., Araujo J.L., Margues D.J., Ribeiro P.M., Ramos S.J. and others (2011) Phosphite Supply affects Phosphorous nutrition and biochemical responses in maize plants. Australian Journal of Crop Science 5 : 646 - 653

Abstract

Phosphate (Pi) is the major phosphorus (P) form used for plant nutrition, whereas phosphite (Phi) is effective in controlling important plant diseases caused by Oomycetes pathogens. However, Phi-based products also have been widely marketed as either P fertilizer or biostimulant, such as elicitor of biochemical responses to abiotic and biotic stress agents, although these effects are not as yet well understood. This investigation has aimed to evaluate the effect of Phi supply as part of the P fertilization, and its influence on the guaiacol peroxidase activity and contents of total fenolics and lignin in maize plants.

This study was conducted in an experimental design completely randomized, with 2 P concentrations (52 μ M = low P concentration, and 644 μM = adequate P concentration) and 2 P forms (100% phosphate, and 75/25% as Pi/Phi, respectively). Based on studies of uptake kinetics of the 31P, it was shown Phi inhibits Pi uptake competitively in maize, regardless of the plant Pi status. Replacement of 1/4 of Pi by Phi decreased the biomass production of the plants grown under low Pi supply, but no effect was observed in the plants grown under adequate Pi supply, with the advantage of eliciting biochemical responses to stress agents, such as stimulation of the guaiacol peroxidase activity and lignin biosynthesis.

6) Mc Donald, A.E., B. Grant, and W.C. Plaxton. 2001. Phosphite (Phosphorous acid) : Its relevance in the environment and agriculture and influence on plant phosphate starvation response. Journal Plant Nutrition 24:1505-1519.

Abstract

Phosphites (H2PO3 −; Phi) are alkali metal salts of phosphorous acid [HPO(OH)2] that are being widely marketed either as an agricultural fungicide or as a superior source of plant phosphorus (P) nutrition. Published research conclusively indicates that Phi functions as an effective control agent for a number of crop diseases caused by various species of pathogenic pseudo fungi belonging to the genus Phytophthora. However, evidence that Phi can be directly used by plants as a sole source of nutritional P is lacking. When Phi is administered in such as way as to allow it to come into contact with bacteria, either associated with plant root systems or in the soil, then the oxidation of Phi to phosphate (HPO4 2−; Pi) may take place. By this indirect method Phi could thus become available to the plant as a P nutrient.

The rates at which this occurs are slow, taking months or as much as a year, depending on the soil type. Phi is not without direct effects on plants itself, as Phi concentrations comparable to those required to control plant infection by pathogenic Phytophthora, or to restrict Phytophthora growth in sterile culture, are extremely phytotoxic to Pi-deprived, but not Pi-fertilized, plants. This is because Phi treatment negates the acclimation of plants to Pi deficiency by disrupting the induction of enzymes (e.g., acid phosphatase) and transporters (e.g., high-affinity plasmalemma Pi translocator) characteristic of their Pi starvation response. Thus, Phi intensifies the deleterious effects of P-deficiency by 'tricking' Pi-deprived plant cells into sensing that they are Pi-sufficient, when, in fact, their cellular Pi content is extremely low. The Phi anion appears to effectively obstruct the signal transduction pathway by which plants (and yeast) perceive and respond to Pi deprivation at the molecular level. The review concludes by citing concerns and recommendations regarding the significant input of Phi into food products and the environment that arises from its extensive use in agriculture and industry.

7) RALITZA DANOVA-ALT1 , COR DIJKEMA2 , PIETER DE WAARD2 & MARGRET KÖCK ; Martin Luther University of Halle-Wittenberg, Biocenter, 06120 Halle, Germany and 2 Wageningen NMR Center, Dreijenlaan 3, 6703 HA Wageningen, the Netherlands ; Transport and compartmentation of phosphite in higher plant cells – kinetic and 31P nuclear magnetic resonance studies Plant, Cell and Environment (2008) 31, 1510–1521

Abstract

Phosphite (Phi, H2PO3 -), being the active part of several fungicides, has been shown to influence not only the fungal metabolism but also the development of phosphatedeficient plants. However, the mechanism of phosphite effects on plants is still widely unknown. In this paper we analysed uptake, subcellular distribution and metabolic effects of Phi in tobacco BY-2 cells using in vivo 31P nuclear magnetic resonance (31P-NMR) spectroscopy. Based on the kinetic properties of the phosphate transport system of tobacco BY-2 cells, it was demonstrated that phosphite inhibited phosphate uptake in a competitive manner. To directly follow the fate of phosphate and phosphite in cytoplasmic and vacuolar pools of tobacco cells, we took advantage of the pH-sensitive chemical shift of the Phi anion. The NMR studies showed a distinct cytoplasmic accumulation of Phi in Pi-deprived cells, whereas Pi resupply resulted in a rapid efflux of Phi. Pi-preloaded cells shifted Phi directly into vacuoles. These studies allowed for the first time to follow Phi flux processes in an in vivo setting in plants. On the other hand, the external Pi nutrition status and the metabolic state of the cells had a strong influence on the intracellular compartmentalization of xenobiotic Phi.

8) Stehmann, C. and B.R. Grant. 2000. Inhibition of the glycolytic pathway and hexose monophophate bypass by phosphonate. Pesticide Biochemistry and Physiology 67:13-24.

Abstract

Previous studies have suggested that the phosphonate ion (Phi), an isostere of phosphate, might be a general inhibitor of enzymes which are allosterically regulated by phosphate or which have a requirement for divalent cations. In this paper, the capacity of Phi to inhibit selected enzymes of this type from Phytophthora palmivora is compared with its effects on the same enzymes isolated from other organisms, in particular from Saccharomyces cerevisiae, which are widely used in linked assays of many enzymes. It was found that Phi inhibited the activity of the enzymes from P. palmivora investigated, though to different degrees. IC50 values (concentration required to inhibit enzyme activity by 50%) for Phi ranged from 0.74 ± 0.07 mM (NAD-dependent glyceraldehyde-3-phosphate dehydrogenase) to 116.1 ± 7.3 mM (6-phosphogluconate dehydrogenase).

Among the activities tested glucose-6-phosphate dehydrogenase activity from P. palmivora was significantly more sensitive to Phi than the same enzyme from yeast, although its absolute IC50 value (29.0 ± 3.4 mM) was high in comparison to most fungicides. It was also found that the auxiliary enzymes from rabbit muscle (aldolase, glycerophosphate dehydrogenase, and triosephosphate isomerase) and yeast (glucose-6-phosphate dehydrogenase) used in enzyme-linked assays were all sensitive to Phi, giving IC50 values between 7.7 ± 0.4 and 73.6 ± 2.0 mM, a sensitivity comparable to the other enzymes under investigation. Inorganic phosphate also inhibited the activity of the enzyme glucose-6-phosphate dehydrogenase and the aldolase/triosephosphate isomerase/glycerophosphate dehydrogenase mixture with IC50 values of 108.3 ± 7.7 and 13.0 ± 0.6 mM, respectively. In conclusion, Phi inhibition was found to be widespread, supporting the hypothesis that Phi may inhibit several enzymes rather than acting at a single unique site. It was also found that the coupling enzymes used in many of the assays for these enzymes were themselves susceptible to Phi and phosphate inhibition, which must be taken into account in the interpretation of the results obtained with this type of assay.

9) Carla A. Ticconi, Carla A. Delatorre, and Steffen Abel. Attenuation of Phosphate Starvation Reponses by Phosphite in Arabidopsis. Plant Physiol, November 2001, Vol. 127, pp. 963-972.

Abstract

When inorganic phosphate is limiting, Arabidopsis has the facultative ability to metabolize exogenous nucleic acid substrates, which we utilized previously to identify insensitive phosphate starvation response mutants in a conditional genetic screen. In this study, we examined the effect of the phosphate analog, phosphite (Phi), on molecular and morphological responses to phosphate starvation.

Phi significantly inhibited plant growth on phosphate-sufficient (2 mm) and nucleic acid-containing (2 mm phosphorus) media at concentrations higher than 2.5 mm. However, with respect to suppressing typical responses to phosphate limitation, Phi effects were very similar to those of phosphate.

Phosphate starvation responses, which we examined and found to be almost identically affected by both anions, included changes in: (a) the root-to-shoot ratio; (b) root hair formation; (c) anthocyanin accumulation; (d) the activities of phosphate starvationinducible nucleolytic enzymes, including ribonuclease, phosphodiesterase, and acid phosphatase; and (e) steady-state mRNA levels of phosphate starvation-inducible genes. It is important that induction of primary auxin response genes by indole-3-acetic acid in the presence of growth-inhibitory Phi concentrations suggests that Phi selectively inhibits phosphate starvation responses. Thus, the use of Phi may allow further dissection of phosphate signaling by genetic selection for constitutive phosphate starvation response mutants on media containing organophosphates as the only source of phosphorus.

10) Arne M. Ratjenl, Joska Gerendas. A critical assessment of the suitability of phosphite as a source of phosphorous. Journal of Plant Nutrition and Soil Science, Volume 172, Issue 6, Pages 821-828, December, 2009.

Abstract

Marketing of phosphite-containing preparations for foliar application, together with recent reports of positive yield responses, has revived the question as to whether phosphite (HPO3-) is a suitable P source for plants. Two experiments using zucchini (Cucurbita pepo L. convar. giromontina) have been conducted to evaluate the P-nutritional effect of phosphite either provided via the substrate or as a foliar spray. Plants grown in a P-deficient substrate were severely damaged when phosphite was applied as foliar fertiliser and more drastically when provided via the substrate. Growth of P-deficient plants receiving phosphite as a foliar spray was impaired in a dose-dependent manner after foliar P application (concentrations 0.0, 0.9, 2.7, and 4.5 g P L^{-1}), while foliar provision of phosphate improved plant growth and yield. In the youngest leaves of phosphite-treated plants, which had developed after foliar spray, phosphite accumulated to considerable extent, reaching a similar concentration as phosphate at tissue level.

These results confirm that P-deficient plants are very sensitive to phosphite, which represents a nutritionally ineffective form of P. It should thus not be considered as a form of P suitable for fertiliser manufacture.

11) Central Sugarcane Research Station, Padegaon

All-India Coordinated Research project on Sugarcane [AICRP (S)], Zonal Research Centre, Peninsular Zone CSRS Padegaon, Mahatma Phule Agricultural University - Rahuri, Ahamadnagar district, Maharashtra.

Tested the impact of PSAP on yield and quality of sugarcane in a tropical region. Reports confirmed 18.75 t/ha. increase in sugarcane yield and 0.26% improvement in CCS recovery leading to 3.3 t/ha. additional sugar production with the sprays of PSAP. Studies also revealed that even after 50% reduction in recommended P and K fertilizers, application of 3 kg PSAP per acre given in split sprays increased sugarcane yield and improved sugar recovery.

12) ICAR- National Research Center for Grapes, Pune

PSAP (ProPhite) tested by ICAR- NRCG based on the request and samples submitted by MRDBS, Maharashtra Rajya Draksha Bagayatdar Sangh, grape growers Association, on the contents of pesticides and P and K percentage in ProPhite (PSAP).

Test reports revealed no content of pesticides when tested for 996 chemicals. The content of P and K in PSAP was found to be 16.27% and 30.23% respectively, which was different the contents of P and K in mono and/or di potassium phosphate P: 25.8% and K : 32% and P : 19.6% and K : 49%. Based on the content of P and K in PSAP, it was certified that PSAP is not analogous to potassium phosphate or mono and / or di potassium salt of phosphorous acid.

13) UPCSR- Uttar Pradesh Council of Sugarcane Research, Shahjanpur

Tested the impact of PSAP further for two years at two locations in central as well as easten Uttar Pradesh, i.e. in a sub-tropical region. The test report revealed very impressive impact of PSAP on yield and quality in the local sugarcane variety and even in under different geo conditions.

Sugarcane yield increased by as much as 24.4 tonnes per hectare and recovery improved in CCS percentage by 0.5% unit. Improvement in yield and CCS percentage meant that sugar production increased by 3.47 tonnes per hectare with an estimated increase in alcohol production by 275 liter per hectare and a co-generation of 1220 units per hectare with additional bagasse availability.

14) **ICAR- National Research Center for Grapes, Pune**

Tested ProPhite (PSAP) in comparison with potassium phosphite for the control various diseases grapes. PSAP proved far superior in controlling various diseases like downy mildew and anthracnose in grape. It was also observed that split sprays of PSAP can reduce fungicide sprays by 50 %. PSAP also improved the yield by 34.80% to 68.66% which applied singly in combination with fungicides. PSAP was also shown as analogous to potassium phospite either as mono-di potassium salt of phosphorous Acid.

15) **Research Article on Management of Downy Mildew of Cucumber by Lowering Toxic Fungicide Applications**

The experiment was conducted to decrease fungicide applications for the management of downy mildew of cucumber. Different combinations of PSAP, fungicides and micronutrients were tested. The combination containing a fungicide and PSAP were more effective than fungicides, micronutrients applied singly or in combinations. PSAP and fungicide controled the downy mildew disease most effectively and increase the yield by 40 %. PSAP applied singly also effectively controlled the downy mildew of cucumber and increased the yield of cucumber by 25% the results clearly showed that, yield can be increased by 40% with 50% reduction in fungicide applications or fungicides can be replaced with PSAP. None of the attempted treatments showed any kind of phytotoxicity on cucumber crop.

16) **RVKVV – Gwalior – College of Horticulture – Mandsaur**

The field trials were conducted to evaluate the PSAP against Downy mildew and Powdery mildew. PSAP tested at AICRP M & AP research filed, Mandsaur for evaluation of bio-efficacy of PSAP on Opium Poppy (Papaver somniferum).

The Field trials have been conducted during 2018- 2019, 2019-2020 and 2020 – 2021 for three years. From above experiment it is evident that the foliar spray of 50% reduction of recommended spray for the crop + with PSAP @ 6 gm / liter shows maximum reduction in disease incidences and maximum increase in seed yield, latex and husk yield without any symptom of phytotoxicity.

17) **NSI – National Sugar Institute Kanpur**

Collaborative Research Project was conducted for testing of PSAP - bio-efficacy on sugarcane for two years in two plantations and one ratoon crop. On the basis of above study, it is concluded that application of PSAP only through foliar sprays (four prays at 60, 75, 90 and 120 DAP) gave significantly better results with all doses of PSAP than control (without PSAP application treatment). Foliar application of PSAP @ 12.5 kg per hectare at different periods after planting along with 100 per cent recommended dose of NPK (180:80:80) applied in sugarcane cultivation is helpful in improved growth, juice purity and higher net return with improved benefit cost ratio.

18) **CSAUT - Chandra Shekhar Azad University of Agriculture & Technology, Kanpur**

Testing of PSAP – "Potassium salt of active phosphorus" a research molecule on sugarcane for 2019-20 and 2020 – 2021 two plantations and one ratoon crop season. For both yield and quality aspects of sugarcane by the application of various dosed of PSAP, it can be concluded that application of PSAP @ 12.5 Kg/ha with 180: 80:80 Kg N: P: K as foliar spray on sugarcane crop at 60, 75, 90 and 120 Days After Planting gave significant higher yield of cane along with best quality of juices and higher Brix.

19) **ICAR - IISR – On AICRP in Soybean, Khandwa Road, Indore (M.P.)**

"Bio-efficacy evaluation of potassium salt of active phosphorus (PSAP) on soybean" Trial conducted at ten (10) location/centers of AICRP Soybean (Agronomy) during kharif 2020 and 2021.

20) **Guangxi Academy of Agricultural Sciences - China**

Predication of Photosynthetic Leaf Gas Exchange of Sugarcane (Saccharum spp) Leaves in Response to Leaf Positions to Foliar Spray of Potassium Salt of Active Phosphorus under Limited Water Irrigation. PSAP role in limited water condition is accessed.

In conclusion, overall, the present results revealed that the PSAP application might be an efficient technique for improving the tolerance of sugarcane plants subjected to limited water irrigation. It also up regulated the photosynthetic capacity by protecting the negative impacts of sugarcane plants during limited irrigation. Taken together, PSAP has a significant role in sugarcane cultivation under insufficient water availability for irrigation and its optimum dose will be supportive in mitigation of limited irrigation in a variety of crops for sugar and bio-energy sectors. This combination also greatly improved the photo-synthetic activities and plant growth. However, to suggest an optimum dose of PSAP concentration, a large-scale demonstration under field conditions should be assessed in later studies. Krishan K. Verma, Xiu-Peng Song, Chhedi Lal Verma, Mukesh Kumar Malviya, Dao-Jun Guo, Vishnu D. Rajput, Anjney Sharma, Kai-Jun Wei, Gan-Lin Chen, Sushil Solomon, and Yang-Rui Li

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PSAP

Spray Schedule for Sugarcane

At GBL Sammerwadi

Year 2019

Staff Promoting PSAP to its Cane farmers

At GBL Sammerwadi

Year 2019

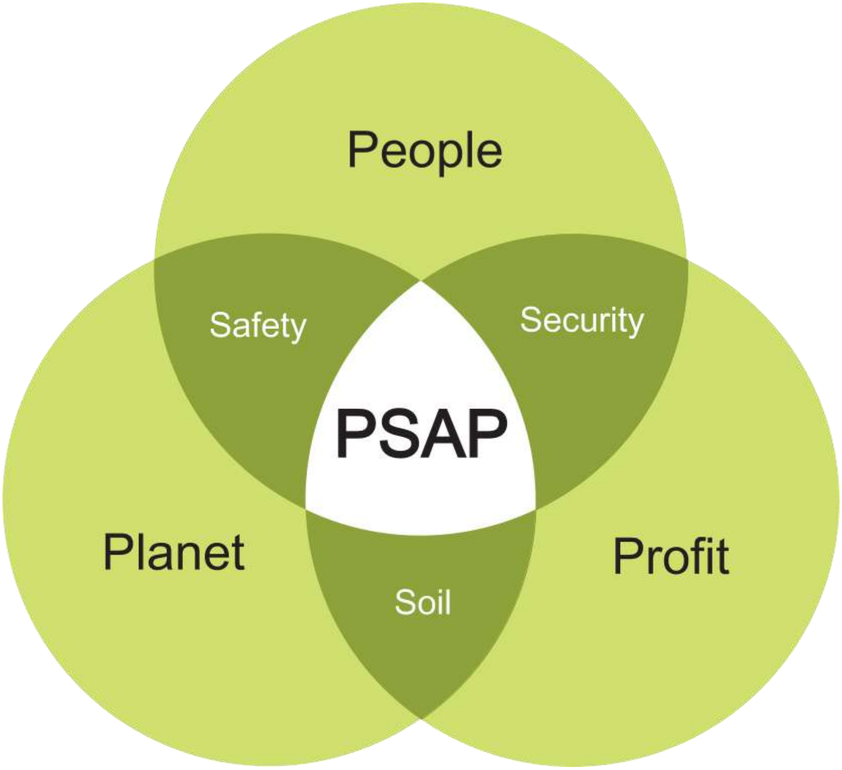
Staff Promoting PSAP to its Cane farmers

PSAP at Farmers Field

PSAP in Export Cultivation

Grower farmers harvested on an average 50 kg bunches in saline soil of Kolhapur District, Maharashtra state in India by applying PSAP

|| वसुधैव कुटुम्बकम ||



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