Cross-talk between plant nutrients under Abiotic stress conditions and the genetic analysis

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Introduction

Whatever is the exploration, discoveries, inventions reach upto, Nature has always been astonishing and important subjects are "**The Plants**". Plants provide the most abundant element on the earth's crust- The Oxygen which is required for life. It is our need to save those life giving ones and provide them resistance against various factors.

Some environmental change disrupts the homeostasis of a plant thus altering its normal physiological functions. The condition is often known as "The Plant Stress". Now the question arises:

(1) Is the plant able to withstand the unfavorable conditions or is it susceptible?
(2) Another thing is that the effect of stress is temporary or does it interfere the metabolic functioning to bring permanent changes?

Energy and mineral nutrients is important for the structure and physiology of the plant. And the interaction between the plant nutrients and various cycles is important to understand its mechanism. Study proves that almost 50% of crop loss is due to abiotic stress. The biochemical, molecular interventions help to overcome abiotic stress in a plant, and depending on it the phenotypic expression is developed.

Types of stress conditions to the plants: Abiotic and Biotic

Biotic stress

The biotic factors involve the micro-organisms or any living beings and pathogens which harm these plants. The pests feed and destroy the crops disrupting the ecology. Various types of Bacteria, Viruses, Fungi have been identified which cause minimum to a great loss in the ecosystem.

Abiotic Stress

As agricultural crops are often exposed to environmental stresses, it is important to save them. For example, the high salinity, high/low temperature, humidity, nutrient levels, adaptive nature of the plant, hormonal changes etc. contribute to the abiotic stress.

Loss of leaf water causes loss of stomatal turgor resulting in its closure. If Reactive Oxygen Species (ROS) is produced, it causes chemical damage to the DNA and proteins and can have lethal effects on cellular metabolism. Oxidative stress can lead to mineral deficiency. Temperature stress can damage enzymes and membranes, thus inhibiting photosynthesis as well as respiration.

Imbalanced availability of soil mineral elements causes salinity stress.

Phenotypically the size and shape of leaf is altered in response to stress. A lot of adverse effects may be observed due to these stress conditions in plant such as growth inhibition, alternate pigmentation etc. The complete genomic sequence of model plant *Arabidopsis thaliana*, microarray studies can provide us sufficient data to study a novel transgene in a plant. Post transcriptional gene regulation is carried out by miRNA's. Plant miRNA's are involved in various abiotic and biotic stress responses [1]. High-throughput facilities allow the identification of these RNA's in a plant. RNAi (miRNA mediated RNA interference) helps to develop resistant transgenic crops.

Review of literature

The plant model system-*Arabidopsis thaliana*

Image source: http://research.unt.edu/research-profiles/mechanisms-defense%E2%80%94studies-plant-communication-and-survival

Arabidopsis thaliana, a Dicot belongs to Brassicaceae family, is an organism of choice for many plant biologists, physiologists, geneticists for several decades. The plant is native to Eurasia, is now grown throughout the world. Its small genomic size makes it advantageous over other plant species. It is the first plant which is completely sequenced in the year 2000 (Genome has ~ 25,500 genes) and can be manipulated using genetic engineering techniques.

TAIR (The Arabidopsis Information Resource) gives description about Arabidopsis. Scientists have been developing new approaches and tests in Arabidopsis before implementing into another species. The novel technologies applied to study Arabidopsis explore the plant research forward efficiently, so that the experimental results may enhance eco-friendly agricultural practices which ultimately lead to increased food production. The classical genetic mapping in Arabidopsis was carried out in the year 1960's and 1970's. High throughput transformation using T-DNA vector is generated mostly to develop activation tagging populations in Arabidopsis [2].

We gain comprehensive knowledge of a complete plant by understanding the biology of *Arabidopsis thaliana*.

Arabidopsis genome may be missing some homologs of genes present in rice genome (evidence from rice genome project), because of the polyploidy of crop species genome. The genes present in Arabidopsis represent a reasonable model for plant kingdom.
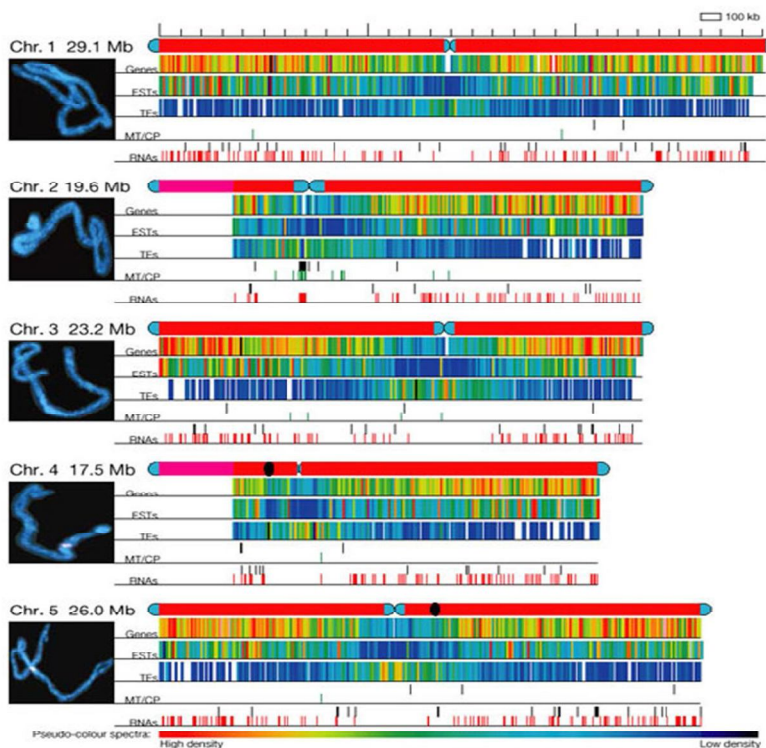
Each chromosome is represented as a coloured bar. Sequenced portions are red, telomeric and centromeric regions are light blue, heterochromatic knobs are shown black and the rDNA repeat regions are magenta. The unsequenced telomeres 2N and 4N are depicted with dashed lines. Telomeres are not drawn to scale. Images of DAPI-stained chromosomes by P. Fransz. The frequency of features was given pseudo-colour assignments, from red (high density) to deep blue (low density). Gene density ('Genes') ranged from 38 per 100 kb to 1 gene per 100 kb; expressed sequence tag matches ('ESTs') ranged from more than 200 per 100 kb to 1 per 100 kb. Transposable element densities ('TEs') ranged from 33 per 100 kb to 1 per 100 kb. Mitochondrial and chloroplast insertions ('MT/CP') were assigned black and green tick marks, respectively. Transfer RNAs and small nucleolar RNAs ('RNAs') were assigned black and red ticks marks, respectively.
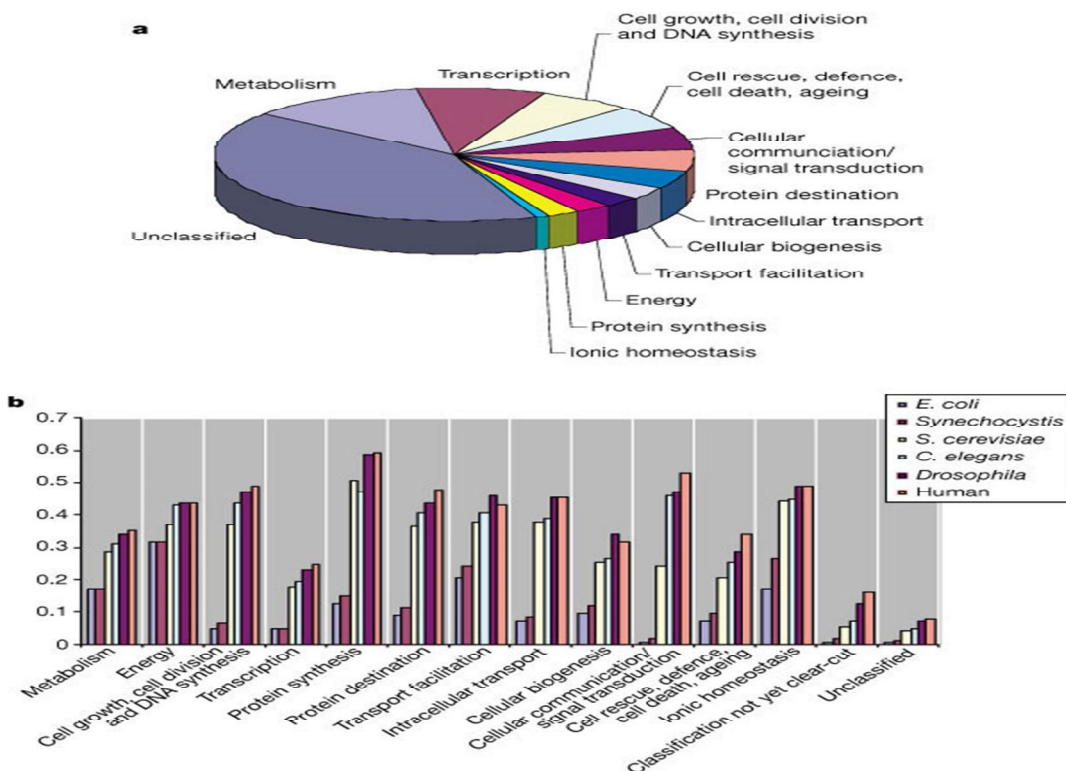
Image source:
http://www.nature.com/nature/journal/v408/n6814/full/408796a0.html

a. **Proportion of predicted *Arabidopsis* genes in different functional categories.**
b. **Comparison of functional categories between organisms.**

Nutrient uptake by plants

Essential elements required by plant include: C,H,O,K,Ca,Mg, N,S,P,Cl,B,Cu,Fe, Mn,Mo,Ni,Zn. If any of these elements is insufficient to plant, it may cause physiological disorders in plants. Plant nutrients are linked to many metabolic pathways. Advances in the molecular techniques enabled the identification of pathways assimilating these elements and proving that there is cross-talk in between these pathways.

Macro elements - These are required in large amounts for plant growth and development. Nitrogen, phosphorous, potassium, magnesium, calcium and sulphur are usually regarded as macro elements. These elements usually comprise at least 0.1% of the dry weight of plants. Nitrogen is supplied as a mixture of nitrate/ammonium ions.

Phosphorous is usually supplied as the phosphate ion of ammonium, sodium or potassium salts.

Micro elements - These elements are required in trace amounts for plant growth and development they have many and diverse roles. Manganese, iodine, copper, cobalt, boron, molybdenum, iron and zinc usually comprise the micro elements, although other elements such as nickel and aluminium are frequently found in some formulations. Iron is added as iron sulphate, Ethylene-Di-amine-Tetra-Acetic acid (EDTA) is used in conjunction with iron sulphate.

Table 1: Importance and deficiencies of few essential elements in plants

ELEMENT	FUNCTION	DEFICIENCY	VISUAL SYMPTOMS
Nitrogen	A component of nucleic acids, proteins etc., most required; induces plant to produce more food	Chlorosis, light green foliage, stalks become slender	Image source: http://gardentia.net/manures-and-fertilizers/
Carbon	Basic component of lipids carbohydrates, hormones, enzymes, nucleic acids etc., structural element; obtained as CO_2 from atmosphere	Retarded plant growth, white deposits on leaves, plants die	Image source: http://www.wholehomenews.com/blog/Whats-Wrong-With-This-Plant/346
Potassium	Regulates osmotic potential, principal inorganic cation, proper functioning of enzymes required for various processes (photosynthesis etc.,)	Interveinal chlorosis,old leaves wilt	Image source: http://www.omafra.gov.on.ca/IPM/images/apples/plant-nutrition/apples_potassium_01_zoom.jpg

Calcium	Cell wall synthesis, membrane function, cell signaling	Blossom-end rot, new leaves are irregularly shaped,stunted plant growth,necrosis around leaf,	Image source: http://5e.plantphys.net/article.php?id=289
Magnesium	Enzyme cofactor, component of chlorophyll	Interveinal chlorosis of older leaves	Image source: http://5e.plantphys.net/article.php?id=289
Phosphorous	Component of nucleic acids, energy transfer, component of intermediates in respiration & photosynthesis; required for cell division & growth	Leaf tips look burnt; stunted growth,dark green leaves	Image source: http://asktheplant.com/?p=67
Sulphur	Component of some amino acids(methionine,cysteine) and some cofactors	Young leaves turn yellow	Image source: http://5e.plantphys.net/article.php?id=289
Chlorine	Required for photosynthesis	Wilting due to high branched roots,leaflet blade tip wilting with chlorosis	Image source: http://5e.plantphys.net/article.php?id=289

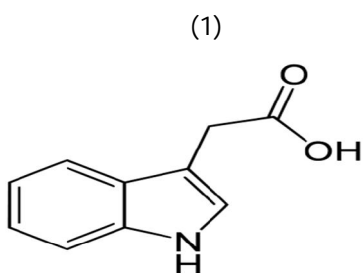| Iron	Synthesis of chlorophyll. Electron transfer component of cytochromes	Chlorosis in interveinal areas,stunted growth	Image source: http://www.organicrosecare.org/articles/nutrients_iron_and_clay.php
Manganese	Enzyme cofactor,enhance root growth,development of fruit,synthesis of chlorophyll, assimilation of nitrate	Reduced plant size ;symptoms vary with species,seed disorders may occur	Image source: http://nutrient-deficiency.blogspot.com/
Copper	Enzyme co-factor, electron-transfer reactions	Dark green leaves,stunted plant	Image source: http://nutrient-deficiency.blogspot.com/
Zinc	Enzyme-co-factor, chlorophyll biosynthesis, catalyst for plant growth regulator,auxin	shortened internodes,stunted growth and necrosis of older leaves,rosette terminal leaves	Image source: http://www.advancednutrients.com/articles/know-about-fixing-nutrient-problems/
Molybdenum	Enzyme co-factor, component of nitrate reductase, required in protein synthesis	Older leaf yellowing,iregular leaf blade formation,chlorosis,necrotic spots at leaf tips	Image source: http://5e.plantphys.net/article.php?id=289

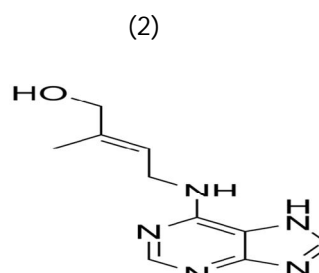| Boron	Essential for root tip,pollen tube and shoot growth;synthesis of DNA & RNA	Leaf blades are distorted, malformation of fruit; retarded growth, necrosis of leaves.	 Image source: http://nutrient-deficiency.blogspot.com/

Plant growth regulators

There are five main classes of plant growth regulators, namely Auxins, cytokines, gibberellins, abscisic acid, and ethylene. Auxins promote both cell division and cell growth. The most naturally occurring auxin is IAA (indole-3-acetic acid), commonly used and extremely effective auxin is 2,4-D. cytokinins promote cell division. Naturally occurring cytokinins include Zeatin and 2iP (2-isopentyl adenine).

Gibberellins are involved in regulating cell elongation and are agronomically important in determining plant height and fruit-set (GA3 being the most common). Abscisic acid (ABA) inhibits cell division and is used in plant tissue culture to promote distinct developmental pathways such as somatic embryogenesis. Ethylene is gaseous naturally occurring plant growth regulator most commonly associated with fruit ripening.

(1)

(2)

Chemical Structure of IAA

chemical Structure of Zeatin

Image source: (1) http://en.wikipedia.org/wiki/Auxin
(2) http://commons.wikimedia.org/wiki/File:Zeatin.png

Nitrogen use efficiency (NUE)

Various internal and external factors affect the Nitrogen uptake and its metabolism by plants. Studies prove the metabolic links between Nitrogen to Carbon, Sulphur etc. Nitrogen is one of the most essential element that has a direct impact on the crop production and yield. Only 33% N2 is available to crop and the remaining 67% is lost in the environment [3], hence it is additionally supplied as fertilizer. NUE is given as the ratio of output (total plant N, yield)

to input (soil N). Nitrate is taken up by the plant and most of it is reduced by NR enzyme and finally to NH4+ ion by NiR. Nitrate signals many metabolizing enzymes. Nitrate responsive genes require the presence of both N/C. Nitrate deficiency enhances the level of

carbohydrates, phosphoesters & secondary metabolites but decreases amino acid synthesis.

PNUE : Plants possessing C4 photosynthesis have a greater PNUE (photosynthetic nitrogen use efficiency) than C3 plants.
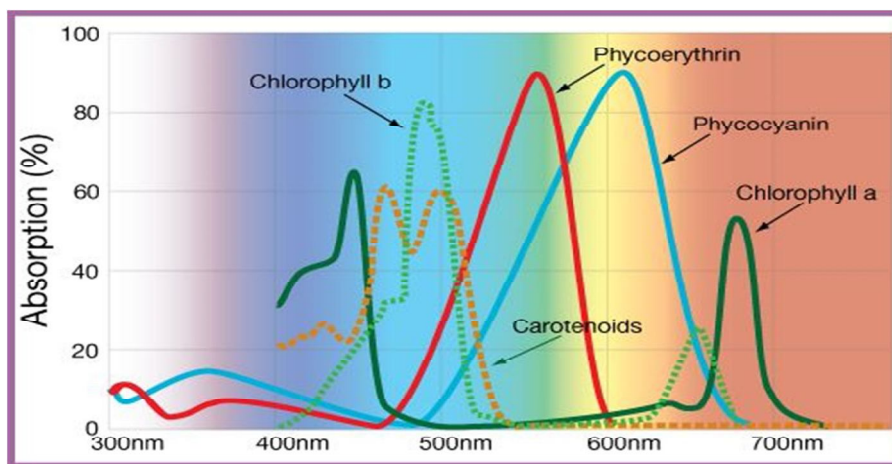
Light spectrum

Image source: http://www.instructables.com/id/High-Brightness-LED-Grow-Light/ step1/Light-Spectrum-Requirements/

Chloroplast is the site of photosynthesis. Photosystem I and II form structural units of protein complexes help in absorption of light and transfer of energy. Light dependent (Z scheme in thylakoid membrane of chloroplast) and light independent reactions (C3 /C4 Cycle) play a major role in the process. The light independent reactions are enhanced due to increased CO_2 uptake.

Genetic approach

Few genes have been identified which are involved in the common signaling pathway such as SYMRK (Symbiosis

receptor like kinase) [4], ALMT1 (Aluminium Activate Malate Transporter in wheat (Sasaki et.al, 2005). MATE (Multidrug and Toxic compound Extrusion- Type citrate transporter identified in Barley (Furukawa et.al, 2007). APETALA2 gene family, G proteins, DREB- like proteins increase crop yield and help the plant to overcome environmental stress. Dehydration-responsive element-Binding protein (DREB2) often interacts with abscisic acid (ABA) in plant growth and adaptive mechanism of stress tolerance. The functional analysis of Homeobox and

AP2TFs identifies novel pathways to better understand the molecular mechanisms of plants.

Environmental stress inducible genes are of two types: (a) Provide resistance to plant; (b) Regulate Gene expression and signal transduction. The transcriptome which supports the stress response may depend on the post transcriptional mechanism and alternate splicing and RNA processing. Also the post translational changes might be regulated by the stress response. The functional analysis of a gene can be done using cDNA microarrays, prepared under different stress conditions. Other factors such as genome editing, marker assisted selection can also be considered. Due to multi-genes and complex genetic mechanisms, it might be difficult to develop these stress tolerants.

Image source: http://www.cell.com/action/showImagesData?pii=S1360-1385%2812%2900151-3
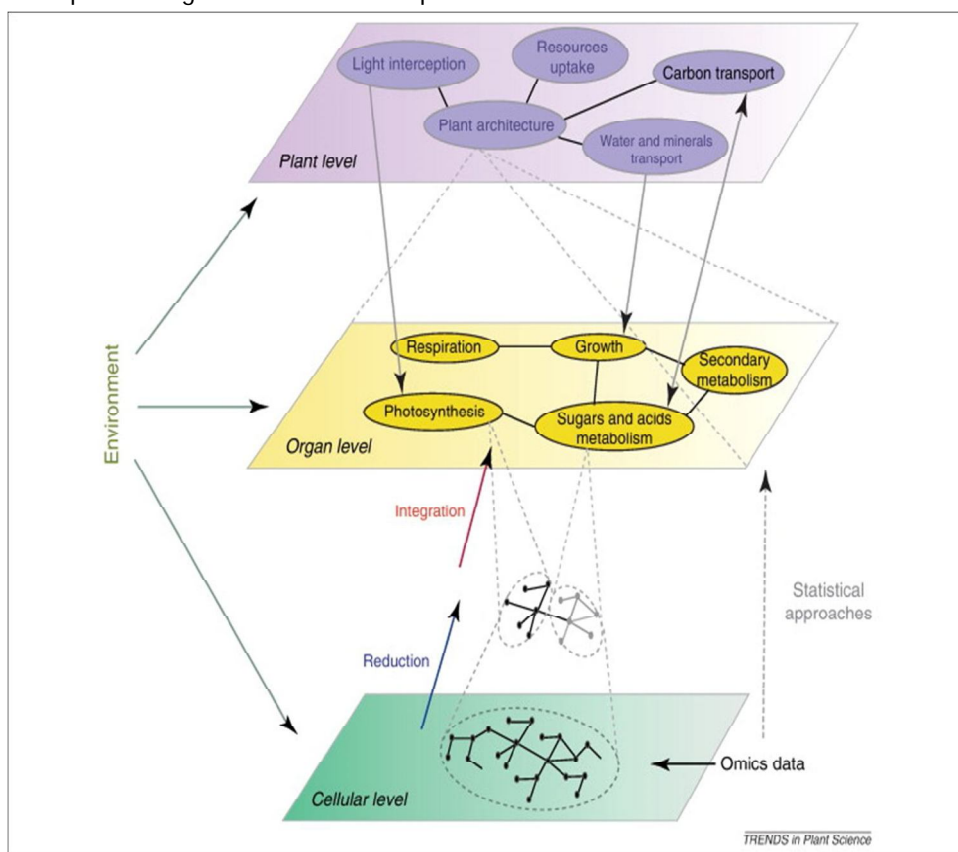
Complex plant stress response involves many genes and biochemical-molecular mechanisms. Other factors include the HSPs or chaperones, LEA (Late Embryogenesis Abundant) proteins (Bray et.al 2007), antifreeze proteins (Bray

et.al, 2000), free-radical scavengers (Wang et.al, 2000); MAPK (Mitogen Activated Protein Kinase) involved in signaling cascades and in transcriptional control; (Calcium Dependent Protein Kinase) CDPK (Ludwig et. al, 2004); SOS Kinase (Zhu et.al, 2001); Phospholipases (Frank et.al, 2000); ZnHD (Zinc finger Homeodomain regulon) etc.

Regulatory circuit includes stress sensors, signaling pathways, a network comprising of protein-protein interactions, different proteins or metabolites. Functional genomics can thus provide a few detecting and signal transduction tools for obtaining cellular networks. This could also be achieved by modifying plant hormones such as Gibberellin (GA) class of growth hormones. Systems biology approach helps in deep understanding and the advanced biotechnology techniques provide a quick report for this post genomic era [5].

Databases

A few stress related genes and available information of different genes are being saved into different databases for the Bioinformatics study, such as the Plant Stress Gene Database [6]. GenBank Database provides genomic sequences of target genes. PDB (Protein Data Bank) gives structural classification of proteins. Protein and DNA are compared by BLAST to sequences in GenBank. FASTA, STIFDB2 [7], Clustal-w and several algorithms and tools are being used to target genomic comparisons.

Nutrient acquisition

The acquisition and distribution of nutrients is important for the proper growth of plant. Nutrient dynamics explains the interaction between soil microorganisms and plant growth-promoting rhizobacteria. Modification of primary metabolism affects the interaction among nutrients such as the supply of carbon skeletons required for N assimilation.

Genomic sequences and molecular approaches of the model plant enable its extension to crop plant for sustainable agriculture. The cross talk between the elements probably occurs in the molecular interactions during nutrient uptake. Studies prove the metabolic links between Nitrogen to Carbon, Sulphur etc. Nitrogen is one of the most essential element that has a direct impact on the crop production and yield.
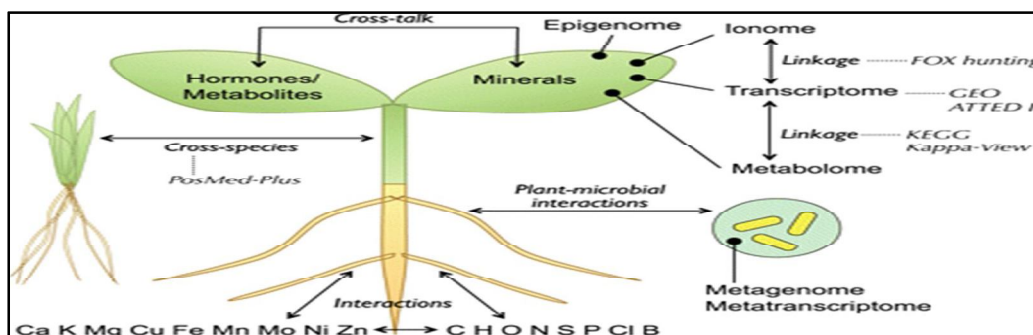
The combination of omics and bioinformatics provides powerful tools for investigating cross-talk between internal/external environments and plant nutrients. Various transcription factors enable plants to withstand stress condition and the modern "OMICS" analysis provides another tool to target the associated genes [8]. Next Generation sequencing allows transcriptomic analysis of various plant species [9].

Conclusion

Although plants are often exposed to various environmental stresses, intensive research provides an eye to the problem and a few solutions are known today. Apart from plant physiology, alterations in the genome are being identified. It is thus important to understand the biochemical and molecular responses to target the stress tolerance by the plant.

Advancement in technology has helped us to save time and resources. Plant engineering helps us to regulate those stress emerging sites and develop resistant ones.Cross-talk between many different nutrients and physiological pathways is still unknown. Computational analysis may help in better understanding to find out these unknown molecular interactions.

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