

BANARAS HINDU UNIVERSITY



“MECHANISM OF TOLERANCE TO DROUGHT AND SALT STRESS IN CROP PLANTS”

A DISSERTATION REPORT FOR PARTIAL FULFILMENT OF DEGREE OF
MASTER OF SCIENCE IN PLANT BIOTECHNOLOGY

(2018 – 2020)

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Sir,

I have great pleasure in forwarding the review report entitled “**MECHANISM OF TOLERANCE TO DROUGHT AND SALT STRESS IN CROP PLANTS**” submitted by **Sanjeev Yadav, Enroll.No. 407178** in partial fulfillment of the requirements for the degree of **Master of Science in Plant Biotechnology**, from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation reported herein, was carried out by the candidate under my guidance to the best of my knowledge and belief, the review report presented is genuine and original. No part of the work has been submitted for any degree or distinction.

Thanking you,

Forwarded by

Yours Faithfully

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“MECHANISM OF TOLERANCE TO DROUGHT AND SALT STRESS IN CROP PLANTS”

BY
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Review report submitted in partial fulfillment of the requirements for the degree of

Master of Science In Plant Biotechnology

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ACKNOWLEDGEMENT

I express my deep sense of gratitude and respect to my project supervisor **Dr. Satheeshkumar P.K. (Assoc. Prof.), Department of Botany, Banaras Hindu University**, for providing me the opportunity to do my review under his esteemed supervision. His guidance, enthusiastic attention, constant encouragement, patience helped me in successful pursuance of his endeavor of my interest.

I am grateful to, **Prof. R. S. Upadhyay**, Head of department of Botany and **Prof. R.K. SINGH**, Head of department of Genetics and Plant breeding, for providing all necessary facilities in the Department. I convey my sincere thanks to all the honorable faculty members of Department of Botany and Department of Genetics and Plant Breeding of Banaras Hindu University, who have taught and enriched my knowledge in much more than just academics. I would also like to thanks our respected course- coordinator **Dr. Ravindra Prasad** for his constant support.

I also express my gratitude towards my seniors **Rohini Nayak, Mritunjay Sharma, Preeti kumari** and **Anita kumari** for helping me during my lab works.

I sincerely thank non-teaching staff of Botany and Genetics and Plant Breeding, for the help rendered when needed.

From core of my heart, I owe a deep debt of gratitude to my beloved Parents (**Mr. Amarjeet Yadav** and **Mrs. Umrawati Devi**) and family (Grandfather-**Shree Phoolchand Yadav** and sister **Shreya**) who have been the vital source of inspiration that helped me to set higher for their blessings and inspiring thoughts throughout my work. This review report would not have been possible without the filial affection, obstinate sacrifice, pampered support, and sincere prayers and blessings of the biggest asset of my life.

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1. INTRODUCTION

World population is increasing exponentially and to feed such enormous population there is a need of enough production which seems to be quite tough task in the upcoming days with day to day changing environmental condition. Opposite increasing population there is decline in production worldwide. In this, abiotic stress like drought, salinity etc. play a major role. Studies suggest a reduction up to 50% in the average yield of major crops which results into loss of hundreds of million dollars every year (**Mahajan and Tuteja 2005**). Stress during the reproductive phase cause a high reduction in the grain production and hence yield. The abiotic stresses include various factors like drought, salinity, heat (high temperature), cold and heavy metals toxicity. Here, in this review we have taken drought and salinity into consideration. Salt stress affects the plants in many part of the world, it causes ill effects on the plants either by accumulation in higher concentration in soil hence generating a low water potential in the roots surrounding which compel water to come out from the root. As water maintains the turgor and thus contributes in cell expansion and also in cell size increment this got hindered, excessive irrigation and persistent water logging also causes salinity. According **UNESCO WATER PORTAL 2008**, 62% of the world's land and 30% of the irrigated areas suffers from the problem of salinity. Drought stress is one of the major critical constraints for the growth and production of crops. Migration from arid and semi- arid areas worsen the situation of water scarcity. The increasing temperature by factor of 1⁰C in last decades also an alarming factor to be considered. Four basic approaches to major drought are meteorological, hydrological, agricultural and socioeconomic according to **National Drought Mitigation Center**. In the developing countries like India where till now approx. 60% of the population depends upon the agriculture sector, the impact of drought and salt stresses is equally significant. Like in year of drought the productivity is significantly reduced and thus directly affects the livelihood of farmers and finally economy. About 42% of India's land area is facing drought with 6% exceptionally dry according to **Drought Early Warning System (DEWS)**. Rather considering individual, combined effect is to be considered since is more deleterious. Since plants are immobile so they generate an adoptive mechanism within themselves to cope out from the deleterious effect of salt and drought. At various

levels they develop different mechanism of adaptation like in drought tolerance at physiological and morphological level avoidance and adjustment or adoptive comes into play under this complex network of root system, scoto-active stomata, size Dec easement, leaf waxing, leaf rolling and curling to lessen evapotranspiration (**Taiz and Zeiger;1991**) are some of them. At biochemical level a complex set of signal mechanism like that of ABA mediated and involvement of various factor comes into role while protein molecule, osmoprotectants and molecular breeding like technique comes under molecular strategy of drought tolerance. In the same way salt tolerance mechanism also works viz. at biochemical level with ROS generation, anti-oxidative mechanism and bio-stimulants affects while at the physiological and molecular level with the help of mechanism helpful to control the osmotic stress and ionic toxicity, the basis of which is shoot growth reduction(**Roy et al. 2004**),water potential management by compatible solute and with the management of high K^+/Na^+ cytosolic concentration. In this process pumps like (SOS, NHX and HKTs) play crucial role. Meanwhile, impact of drought and salinity stress on crop productivity is a part of this report. Further, breeding approaches to generate a tolerant line is also discussed. The basis of the review is either avoidance or adaptation to these environmental stresses.

2. ABIOTIC STRESS

As the name suggests these are the stresses of non-living factors on the living ones(e.g. plants). (**Norbarg et al.2004**) suggested non-living factors must affect the surrounding more than its normal range of variation to cause ill effect on the individual physiology. Abiotic stresses are naturally occurring stresses, which are generally intangible and inanimate. These stresses are un- variable. More than 6% worldland and about 20% of the irrigated land got affected by abiotic stress as the studies performed by **Munns and Tester 2008**.Natural crops encounter both type of stresses in their life cycle either it will be in the form of abiotic or biotic. Abiotic stresses can be in the form of cold, heat, drought, salt, toxins etc. These abiotic stresses are interconnected due to the interconnections in their signaling mechanism. For e.g. a disruption in the ion distribution and homeostasis is seen because of the osmotic stress. This in turns results

into the affected growth and productivity of the crop plants(Gao, Ji-Ping; et al. 2007). Moreover, Plants experience different form of abiotic stress during their life cycle (Farroq et al. 2009).

Few abiotic factors with their consequences in short are explained as follows:

- a. **Cold** –It affects the quality and post- harvest life of agricultural crop productivity. Under the process of cold acclimation plants are given a sub-lethal exposure to cold stress, so that they can acquire a chilling and freezing tolerance. Through a signal transducing pathway, the signal of cold stress is transduced, which includes Ca^{+2} , ROS, Protein kinase, Lipid signaling cascades. Against cold stress ABA also mediates response. Depending upon the gene expression plants species tolerate cold stress in different-2 ways.
- b. **Heat**- With the increasing day to day temperature it becomes the matter of global concern. High temperature variation also increases the vulnerability of crop plants in near future. Growth arrest followed by the decrease in production of the crop is the impact to be concern. At different stages of life-cycle of plants it causes different problems like during germination period the percentage of seed germination declines, during reproductive phase tapetal cell lost function followed by anther dys-plasticity. Apart it poor pollen grain and anther indehiscence is also seen as a cause of heat stress.
- c. **Heavy metals**- Toxins got added in the agricultural crop with the addition of chemical fertilizers, sewage, waste water irrigation or with the rapid industrialization. Heavy metals like Pb, Hg and Cd etc. are the main metals which contaminate the soil. These metals get biomagnified and reside in the soil over thousands of years, hence become the main concerns of agricultural system.
- d. **Drought**- Droughts are the results of prolonged period of in-appropriate rainfall. Drought can be severe or intermittent based on the continuity period. Severe drought can be lethal since causes less soil-water availability to the plants while intermittent

drought cannot be lethal and can affect plant growth and development. Tolerancy enables the plant species to withstand and maintain the productivity in harsh condition. This tolerance mechanism can be restricted to the particular generation of a plant or can be transfer from generation to generation which allow such plants to called as self-acclimatized and gene adopted respectively. Growth arrest, low shoot/root by such plants. According **Tanveer et al. 2007; Donohue et al.2013 and Sharma et al. 2019** drought imposes serious threat to plant growth and productivity.

- e. **Salt-** Studies suggests about 30% land loss within next 15 years due to increasing salinization of arable land (**Munns and Tester 2008; Szabolcs 1998**). Salinity results into decrease in soil water availability due to decrease in osmotic potential of soil (**Munns 2005; Agarwal et al. 2006; Chinnusamy et al. 2006**). These osmotic stresses are followed by ionic toxicity. In this intake of essential ions like K^+ and Ca^{+2} got hindered while Na^+ and Cl^- like harmful ions increases results into adverse effect on metabolic activities in the cytosol. Decrease in cytosolic metabolism, increase in ROS production and reduction in cell expansion noticed during salinity stress. To cope out of ill effect of ion toxicity a 3-level mechanism is playing role which is as, reduced Na^+ uptake from soil through roots, loading and un-loading of Na^+ into and from xylem and sequestration of it into the vacuole are the key feature.

2.1 Morphological, Anatomical, Bio-Chemical and Molecular Manifestation against Stress Tolerance

A plant under stress condition shows a variety of responses at different levels like morphological, anatomical, bio-chemical and molecular. These responses are either a result of adaptation or arise as temporary response. For the continuation of generation production of viable seeds are utmost important, this is possible only by developing tolerance in plant species. Susceptibility of the plants is more towards drought stress during the reproductive stage during which growth, flowering and seed development takes place as suggested by the studies. Viability of produced seeds by the plants depends on the combination of short term and long- term responses. Short term responses include root signal recognition, stomata closure, and decrease carbon

assimilation in leaf while inhibition of growth, hydraulic changes, signal transport and cell drought signaling, osmotic adjustment in stem and root respectively. Roots and shoots are the levels, where a long-term response takes place. In the above shoot portion of the plant, inhibition of its growth, reduced area of leaves for transpiration, sensing, grain abortion, anthocyanin accumulation, osmoprotectants intervention, ROS scavenging anti-oxidative enzyme while maintenance of turgor pressure, delayed root growth, increased root/shoot ratio, increased absorption area in below ground portion of the plant is seen.

Likewise, in that of drought a salt stress condition also shows various response and adaptation at different levels which are designated as manifestation of salt stress. At morphological and anatomical level reduction in numbers and size of leaves, fewer stomata number, thickening of leaf cuticle and earlier lignifications of roots are noticed. These adaptations are essential for maintaining tissue water content or succulence. **Heikel 1977** found salinity significantly decreases the leaf water content in radish and sun flower while it's remain constant in case of wheat and thus productivity remains unaffected. According **Jennings 1976** succulence is co-related with increase in total leaf volume. Further, **Gorham et al. 1985** suggested that this increase in leaf-volume is to increase the cell size, to increase the vacuole/cytoplasm ratio, so that a large number of Na^+ and Cl^- ions get sequestered into the vacuole. Apart it some salt glands and bladders or trichomes are also observed in some halophytes and glycophytes as well as salt localizing organ (**Greenway and Munns 1980**). **Bal and Dutt 1986** found salt glands in wild rice *Oryza coarctata* Roxb. While at biochemical and molecular level, a well-established mechanism to minimize and control the osmotic stress and thus ionic toxicity is found. Studies also found mechanism to maintain high K^+/Na^+ cytosolic concentration, loading and unloading of Na^+ into and from xylem and a well-organized Na^+ sequestration in vacuole with the help of pumps on expense of ATP. Osmolytes, Polyamines, LEA proteins and anti-oxidative system is also found in plants system against stress.

3. DROUGHT STRESS:

The presence of low soil and atmospheric humidity with a high ambient air temperature results into drought stress. The explained condition takes place as a result of lack of balance between evapotranspiration flux and the soil water intaked(**Lipiec et al.2013**). A high reduction in the yield has been noticed year to year because of drought stress.

More than 150 published definitions of drought has been proposed by researches which shows differences in regions, needs and disciplinary approaches. **Wilhite and Glantz** categorized the definition in terms of four basic approaches to measure drought viz.meteorological, hydrological, agricultural and Socio-economic according to National Drought Mitigation Center. About 50% of the crop yield reduced by drought and temperature as the studies said.

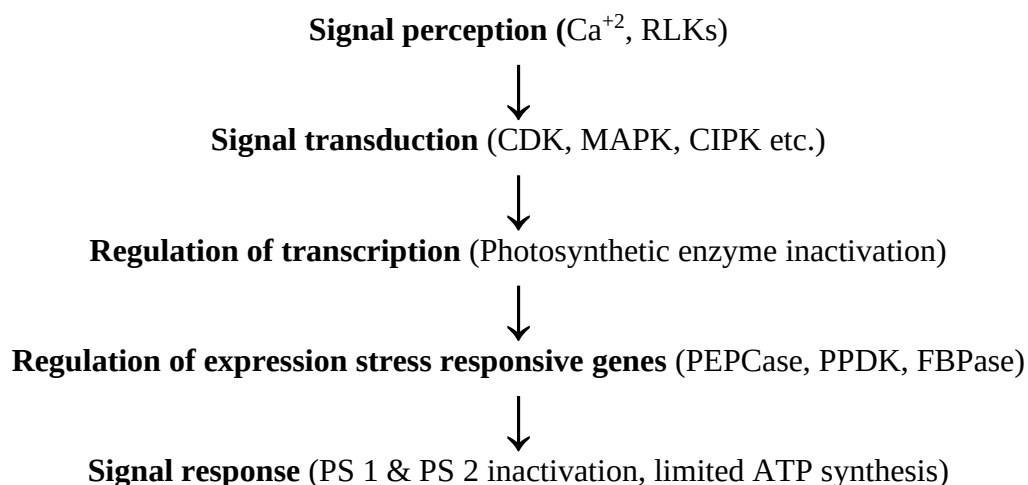
4. TOLERANCE TO DROUGHT STRESS:

To escape or to adopt from unfavorable environmental conditions like drought, temperature, Salt etc. A dynamic response has been developed by plants at different levels viz. physiological, biochemical, molecular etc. (**Huber and Bauerle 2016**).Since these responses are complex so it involves several factors including signaling, transcription factors, hormones and secondary metabolites. Further heat and drought stresses affects more the reproductive phase which ultimately results in the low grain production **F.Bekkaoui et al.;** heat and drought stresses in crops and approaches for its mitigation).Drought stress takes place when soil and atmospheric humidity is low and the ambient air temperature is high. According(**Lipiec et al.2013**), this condition takes place as result of an imbalance between the evapotranspiration flux and the water intake from the soil The mechanism of resistance which are associated with the different plant traits are multigenic and so difficult to control and engineer. Activation and regulation of several stress related transcripts and proteins have been identified based upon transcriptomics, proteomics and gene expression studies, which are generally classified into two major groups. First group involves in signaling cascades and in transcriptional

control whereas second in membrane protection as osmoprotectants, as antioxidants and as ROS scavengers. These two groups can be the target of attempts to produce plants that have enhanced stress tolerance.

4.1 Biochemical Response to Drought Stress:

Signal perception through cell surface receptors is a common feature of all living organism, once the signal gets perceived it starts a signaling cascade in response (Shulae et al. 2008). There are various receptor proteins found for stress signal reception one of which is RLKs being the oldest one (He et al. 1996). Here, also occurs WAK (Wall associated kinases) which were found to be the subfamily of RLKs. In vascular plants systematic signal cascade is activated as a result of stress perception. Aquaporin proteins which form water channels mainly, here acts as a key factor as a contributor to hydraulic conductivity which are regulated at biochemical level by environmental stimuli with changes such as phosphorylation (Johansson et al. 1998), cytoplasmic pH and calcium (Gerbeau et al. 2002) or re-localization of intercellular compartments. ABA is the most critical hormone involved in regulating tolerance (Lata and Prasad, 2011). It induces inhibition of leaf expansion and response to stomata closure. Several other growth regulators like auxin, cytokinin, ethylene, gibberellic acid, brassinosteroids including other factors like nitrogen and pH have been shown to be involved in the regulation of physiological process through their action as signal molecules in signaling network (Verma et al. 2016). Due to increase in stress level, there is generation of several factors including ROS which in turns increases the level of calcium that leads to the activation of CDPK which results into phosphorylation or de-phosphorylation of TFs, thus regulate the expression level of stress responsive genes (Reddy et al. 2011). Activated Ca^{+2} sensors can bind to cis-elements or can interact with DNA binding proteins resulting in their activation or suppression (Kundla et al. 2010). Because of the interconnection between different stresses they ubiquitously produce ROS which results in oxidative stress so can cause damage to the Protein, Lipid, DNA etc. The antioxidants either enzymatic or non-enzymatic play a crucial role in the scavenging mechanism of these ROS and thus prevent plants from its toxic effects (Gill and Tuteja, 2010). Apart from this many stress tolerant genes and TFs have been isolated and over expressed in transgenic plants to improve stress tolerance such as over expression of cycling Dof factor in *Arabidopsis* enhanced drought tolerance (Corrales et al. 2014, 2017), and stress tolerance TFs includes members of DREB, DOF, NAC families, MYB etc. (Shinozaki et al. 2003).



Flow chart: Showing a simplified signaling pathway mechanism during the drought stress which is responsible for the less ROS generation during stress period. After the perception of signal through successive pathway it results into de- activation of both the Photosystem.

4.2 Physiological response to Drought stress:

Physiological response to drought can be classified to work under two distinct mechanisms. First one is the avoidance that is mainly morphological whereas second is adjustment or adaptation. Increased root systems, reduction in the leaf stomata and number and conductance, increase- in thickness of leaf, decrease- in size of leaf, leaf rolling or folding to lessen evapotranspiration (**Taiz and Zeiger; Sicher et al.2012**). To show adaptive response some plants species also synthesize cuticular wax on their aerial surface (**Lee and Suh 2013**). Based upon the adaptation plants can be categorized into 4 categories. Drought escaping plants (germinate and grow during times of sufficient moisture to complete their life cycle), Drought evading plants (these are non-succulent perennials which restrict their growth only to the period of moisture availability), Drought enduring plants (also known as xerophytes in which there is extensive root system with high level of morphological and physiological adaptations) and Drought resisting plants (also called as succulent perennials, they have water storage in their leaves and stems for sparing uses). Some other strategy to minimize loss of water is ABA mediated closing of stomata (**Blum, 1996**). As a result of these large amounts of CO_2 accumulated which diminish Photosynthesis (**Bohnert and Sheveleva, 1998**). As a result the net flow of electrons occurs towards oxygen because of energy imbalance which ultimately giving rise to ROS (**Leving, 1998**). Hygrotropism is shown by roots in which with a perception of water gradient it redirect its growth towards that (**Lambers et al. 2000**). Apart all, the best adaptation is shown by plants with CAM metabolism viz. C_4 plants in which with the help of Scoto-active stomata and the Compartmentalization between mesophyll and bundle sheath cell, on one hand they remain open during night and on the other hand Compartmentalization helps temporary storage of CO_2 in the form of Malic acid (C_4 species) in Vacuoles during night which in day time once stomata closes will provide abundant CO_2 to RuBisCO for Photosynthesis.

4.3 Molecular events conferring Drought tolerance:

In addition to plant breeding several other droughts induced proteins also play role in tolerance viz. LEA, AQP, HSPs. Plant response against abiotic stress can also be regulated at the translational level. In response to drought stress proteins synthesize proteins that has direct work on membrane and protein protection, acquisition of water and ions along with transportation and homeostasis maintenance are some of the other function of

these stress induced proteins. LEA are expressed at basal level but during osmotic and drought stress they induced to high levels (**Ingram and Bartels, 1996; Barrera -Figuroa et al. 2007**). Five groups of LEA proteins have been described in which gr3 and gr5 forms dimers and co-ordinate ions during stress. Another family of stress protecting proteins called as aquaporins are also identified, facilitating water uptake and allocation by forming cellular water pores. They can either be located on plasma membrane or tonoplast surrounding vacuoles (**Johansson et al. 2000**). HSPs are other group of stress inducing proteins which induce during drought and salinity and are also known as molecular chaperones. They work in protein folding and assembly, removal and disposal of non-functional proteins (**Wang et al. 2004**) and also prevent protein thermal aggregation(**Lee et al. 1995**), thus facilitating the recovery of cell after abiotic stress. HSPs have different family based on Molecular weight like HSP60, HSP70, and HSP100 etc. Other than this a chaperon protein cyclophilin have systemic properties are protein folding. These are highly induced during drought stress. Over expression of cyclophilin encoding genes confers multiple abiotic stress tolerance (**Gottschalk et al, 2008; Sekhar et al, 2010**). Because some of the important role also play by glycine betaine, although mechanism of action of glycine is unclear yet using a transgenic system (**Su et al, 2006**) observed that the apparent effect of its biosynthesis on stress tolerance may be attenuated to protective effects other than changes to the cellular Osmotic balance.

5. SALT STRESS

A soil with electrical conductivity 40mM NaCl is considered as saline. High level of salt concentration causes both hypo ionic and hyper osmotic stress and thus causes various detrimental effect to plant .Studies suggests about 30% land loss within next 15 years due to increasing salinization of arable land which is expected to increase upto 50% by half of the 21st century (**Tuteja and Mahajan 2005**). Salinity results into decrease in soil water availability due to decrease in osmotic potential of soil (**Munns 2005, Agarwal et al. 2006, Chinnusamy et al. 2006**). These osmotic stresses are followed by ionic toxicity. In this intake of essential ions like K⁺ and Ca⁺² got hindered while Na⁺ and Cl⁻ like harmful ions increases results into adverse effect on metabolic activities in the cytosol.

6. SALT TOLERANCE:

Salt plays a major role in creating stress to the glycophytes. High soil salinity is a condition with a high concentration of soluble salt, in which NaCl is considered to be the most soluble and widespread one. A high level of salinity in soil causes various detrimental effects in plant at different level in the response of which plant develops adoptive mechanism at different levels viz. at biochemical, physiological and at molecular level as well. At biochemical level ROS and RNS generation is seen which in low concentration acts as a signaling molecule but once its concentration exceeds to a threshold level causes a variety of lethal responses which ends with the cell death ultimately, anti-oxidative mechanism and with the help of biostimulants as well plant exhibit responses at biochemical level. At physiological level the effect of osmotic stress and ionic toxicity is dealt with the help of various pumps which either resisting the harmful ions like Na^+ and Cl^- to come inside the cell or sequestering it to vacuole. Maintenance of higher K^+ concentration is also a work of these pumps. While with the help of production of compatible solute the plant respond at molecular level.

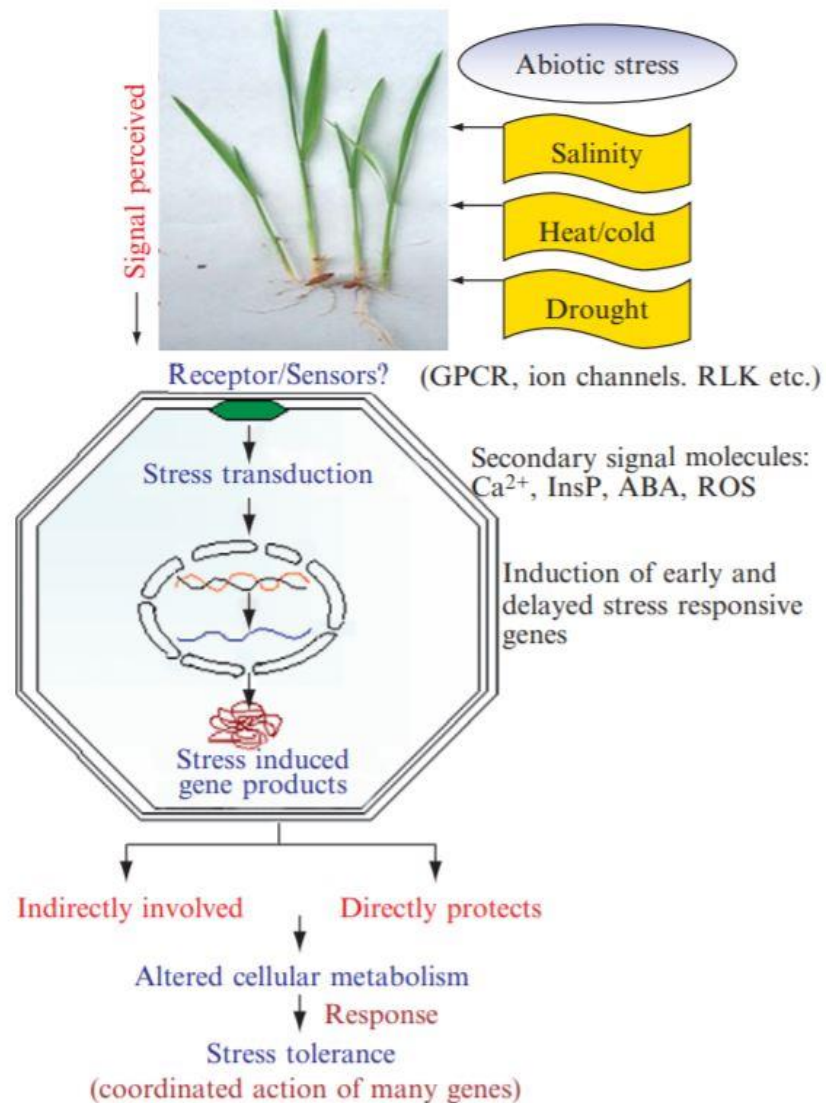


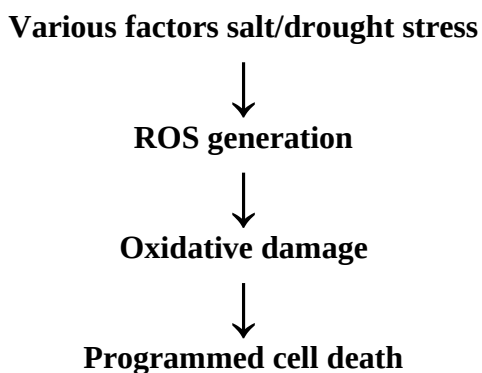
Figure: Showing response pathway to stress. Initially, receptors present at membrane receive the stress signal and transmit it inside the cell through secondary signal molecules, this result in multiple stress responsive gene generation which in turns provides tolerance directly or indirectly. Adopted from book chapter of Tuteja et al.

6.1 Biochemical response:

A plant exhibit biochemical adaptation under the processes viz. ROS and RNS generation, Antioxidative mechanism and with the activity of bio stimulants as well.

a. ROS and RNS generation: Major site of production of RNS is thylakoid membrane bound primary in light the chloroplast and the peroxisome are the main source of ROS generation while in dark mitochondria appears to be the main ROS producer. Studies suggest that 1-6% of the O₂ consumption of isolated mitochondria results

in ROS production. An elevation in the H_2O_2 content was reported under salt stress condition in two pea cultivars where it was found the amount of H_2O_2 is much higher in salt sensitive cultivar rather than salt tolerant one (**Hernandez et al.1995**). Plant mitochondria have two different sites for superoxide generation located at the flavoprotein NADH dehydrogenase and at the ubiquinone cytochrome b segments. Between NaCl resistant and NaCl tolerant cultivar, the succinate dependent O_2^- generation was much higher in latter. But against it isoenzymes like MnSOD and Cu,ZnSOD only decreased in mitochondria from NaCl sensitive cultivar and increases in tolerant one, as a result of which O_2^- scavenging activity decreases from mitochondria. The presence of 110mM of NaCl increases the mitochondrial H_2O_2 up to 3 folds, which results into lipid peroxidation and protein oxidation due to decrease in defense imparts by mitochondrial antioxidants(**Hernandez et al.1993;Gomez et al.1999**). In peroxisome there are mainly two sites where O_2^- is generated. First one is in the matrix by the reaction of enzyme xanthenes oxidase and other one in the peroxisomal membrane (**del Rio et al.1998**). H_2O_2 also produces in higher ratio by a photo respiratory enzyme GOX which coverts flavin oxidases into H_2O_2 and other byproducts. Studies suggested that in root peroxisome of tomato plant the action of APX and CAT is higher than that of SOD results in very less amount of H_2O_2 at this site (**Mittova et al.2004**). Using xylenol orange method (**Bellicampie et al.2000**),the effect of salt stress on ROS accumulation has been analyzed initially by (**Barboa-Espia et al.**).And further by (**Garcia de la Garma et al.; 2010**),in pea leaves and tobacco BY-2 cells respectively. Inspite all chloroplast also produces ROS. By staining of plant tissue with DAB(locate H_2O_2) and NBT(locate O_2^-) ROS accumulation under salt stress can be monitored. In pea leaves and in grapevine plantlet,90 and 100mM NaCl is sufficient for the accumulation of ROS, which can be appeared in the form of small spots on staining like that of H_2O_2 .Beside ROS several other organic/ in-organic compounds acts as player to generate response of plant to salinity like NO. Pre-treatment with SNP (NO donor) found to protect soya-bean plants and sunflower cotyledons from stress caused by NaCl. NO also works in increment RWC, level of chlorophyll and ABA and in better stomatal control (**Dinler et al. 2014**).



Flow chart: -Showing effect of ROS generation due to the in-balance between AOX and ROS.

b. Effect of Bio stimulants in salt stress response:

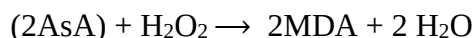
This mechanism depends on somewhat generating memory likewise vaccination in animals. In this ROS (H_2O_2) is exogenously applied assumes to be induce an improved salt tolerance in different plants species viz. wheat, rice, maize (**Hossain et al.2013**). An improvement in salinity effect of sunflower cotyledon on application of exogenous melatonin with the modulation of GSH metabolism or by increasing the activity of H_2O_2 scavenging enzyme (APX), improving the photosynthetic rate and increasing the K^+/Na^+ ratio in *M.hupehensis*(**Kaur and Bhatla 2016; Li et al.2012**). Polyamines also play a key role in plant protection from stress. The mung bean seedling stress response has been increases on application of PAs. As on one hand it increases the level of glutathione, ascorbate and on the other hand also increases the activity of antioxidants enzyme(**Nahar et al 2016**), (DHAR, GR, CAT, GPX).

c. Antioxidative mechanism in Salt stress response:

To cope with the increased stress generated by ROS and also excess level of ROS developed in various cell organelle, plants have developed a well-established antioxidative mechanism (AOX), so that its proteins, lipid, DNA and ultimately cell can be rescued from the damage. AOX can be either enzymatic (SOD, CAT, APX GR) or non-enzymatic (ascorbic acid, alpha tocopherol, reduced glutathione). A 52% reduction is produced by NaCl stress in the total ascorbate pool in soluble fraction in NaCl sensitive pea genotype after a stress of 15 days. It was due to the loss of both oxidized (DHA) and reduced (ASC) forms. In sensitive plants the level of ascorbate and glutathione is affected than that of tolerant one. In *Oryza sativa cv.Pokkali* the enzyme concentration of CAT was found to be decreased in leaf crude extract with the application of 100mM NaCl(**Lee et al. 2013**). In *Triticum aestivum L.cv.Bezostaya*, the SOD concentration increases in leaf apoplast with the application of 250-500mM of NaCl(**Mutlu et al. 2009**).

Significant role plays by Ascorbate and Glutathione:

Ascorbate:



In tobacco over expression of APX1 results in enhanced tolerance to oxidative stress (**Yabuta et al. 2002**).According (**Mittler and Poulos, 2005**)APX and GPX have high affinity towards H_2O_2 in comparison to other anti -oxidants.

Glutathione:

According **(Sunder et al., 2007)** it is a tripeptide and is found in cytosol, chloroplast, ER, vacuoles and mitochondria. It is distributed universally in animals, plants, micro-organisms and plays an essential role as free radical scavenger **(Monneveux et al., 2006)**. Action of glutathione decides the level of H_2O_2 **(Meyer et al., 2005)**. Apart working as main non-enzymatic antioxidants, it also contributes in several other biological functions like regulation of protein, DNA synthesis, protein activity and maintaining membrane integrity **(Cobuslay et al., 2002)**.

6.2 Physiological aspects of Salt tolerance:

Based on the ability to grow in high salt medium, plants can be classified as glycophytes and halophytes. Majority of plants can- not tolerate soil with high salt concentration so cannot be grown in these areas **(Parvaiz and Satyawati)** termed as glycophytes or non-halophytes **(Xiong and Zhu, 2002)**. 2% terrestrial plants known to be halophytes **(Flower et al., 1986)**, which can grow in this area. When salt like NaCl got incorporated into the plants, very initially it causes osmotic effect which creates a dramatic situation initially with the reducing soil water potential which on one hand creates hampering in water uptake by root and on the other hand also movement of water from root to soil. It causes intercellular turgor reduction hence decrease cell expansion **(Tester and Munns, 2008)**. Its result into initiating signals to produce ABA which causes stomatal closure and hence lower conductance **(Roy et al., 2014)**. The second phase is ion toxicity (because of Na^+ and Cl^- accumulation) inducing tissue necrosis and early leaf senescence **(Roy et al., 2014)**. An induction of ROS and disturb aerobic metabolism is noticed by osmotic and ionic effect. K^+ comes under the category of essential micronutrient which play a crucial role in many of functions viz. turgor generation, enzyme activation, pH homeostasis etc. **(Barragon et al., 2012)**. It has to compete with Na^+ because of similar ionic radii and hydration energy. Studies suggest high K^+/Na^+ ratio is to be maintained by the plant to survive under high salt stress. A deleterious effect of Na^+ in many of the processes like in photosynthesis, nutrient transport etc. demands a mechanism to combat from its harmful effect.

Control of the Osmotic stress and Control of reaching Ion toxicity are the two mechanisms developed in plant to control toxic level of Na^+ . Shoot growth reduction **(Ray et al., 2014)** and water potential maintenance by compatible solutes are the two responses seen at the time of osmotic stress while Na^+ loading control in xylem, Na^+ retrieval from xylem before reaching leaves and Na^+ compartmentization at cellular and sub cellular level (in vacuoles) to control ion toxicity are seen.

In plants proton motive force is necessary to drive Na^+ across membrane which are provided by H^+ -ATPases and H^+ pyrophosphatases (PPases). Two Na^+/H^+ antiporters found in the plants which uses energy generated by pmf. First one is the SOS1 found on plasma membrane for the exclusion of Na^+ back to soil **(Ji et al., 2013)** and

second one is the NHX1 found at tonoplast having role in Na^+ compartmentization in Vacuoles(Fukuda et al., 2011). Apart, it Na^+ is re-absorbed in root cell from sap xylem by HKT preventing accumulation of Na^+ in shoot(Rus et al., 2004).

SOS1 and the plant response to salt stress: Study suggested nearly 95% of absorbed Na^+ is extruded back in apoplast for growth and survival under salinity condition. Because of Na^+ transport a negative impact created in surrounding neighbor cell so is catalyzed by Na^+/H^+ antiporter encoded by SOS and present at plasma membrane (AtNHX7) identified in rice (Martinez-Atienza et al. 2007). It uses the proton gradient created by P-ATPase or PPase for exchange of Na^+ and H^+ . SOS promoter activity found in all tissue but most active at root epidermal cell as root tip requires special protection because of small vacuole resist vacuolar Na^+ compartmentalization and at root parenchyma cell living vasculature (Kronzucker and Britto 2011).

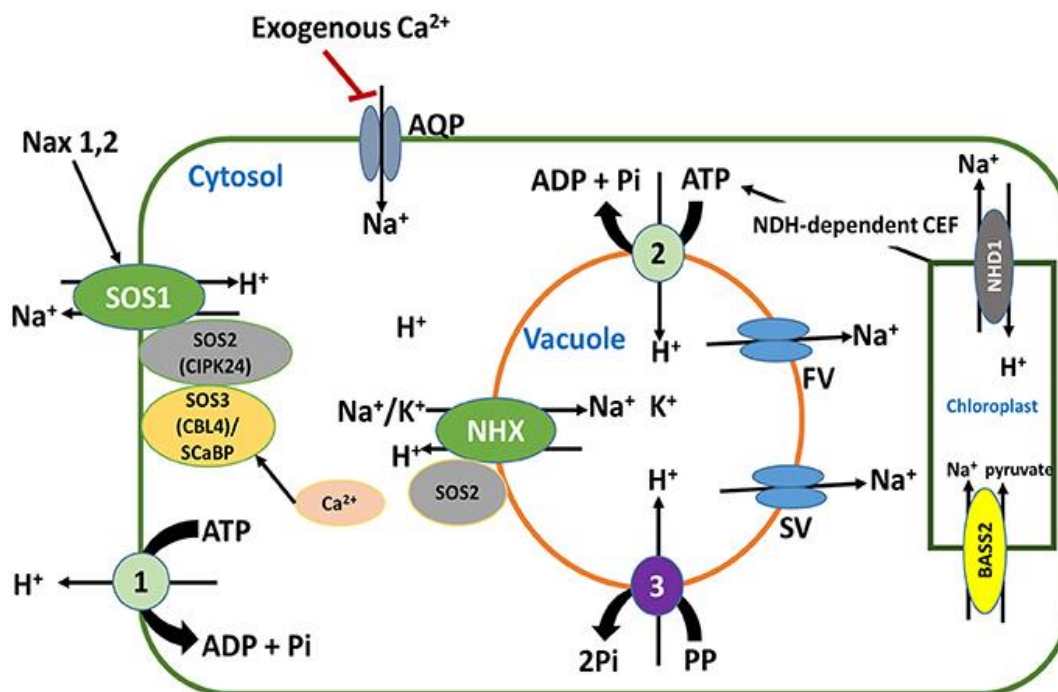


Figure: Showing the regulation of ion homeostasis by various pumps. Initially, salinity stress signal is perceived by receptors at plasma membrane which results into activation of various ion pumps. SOS is also activated under signal whose component regulates some of other pumps. NHX and HKTs are the other pumps which get activated. Adopted from research paper of Dekoum et al. ; 2017

Role of SOS1: Efflux of Na^+ from roots, to gain time for Na^+ storage in vacuole, it slowdown the Na^+ accumulation in cytoplasm and loading and unloading of Na^+ into and from xylem are some of the function of SOS1. SOS1 also maintains cellular homeostasis by Na^+ exclusion(Coin et al. 2011). High salinity regulation of SOS1 transcript level is partly under control of SOS2 and SOS3(Shi et al. 2000). It is notable that SOS2/CIPK24 is protein kinase and CBL4/SOS3 is calcium sensor. SOS2 and SOS3 together with SOS1

comprising SOS signaling pathway. Apart SOS2 and SOS3, SOS1 also interact with other stress proteins like SCABP8/CBL10 (SOS3) homolog thus interact with SOS2 hence form complex and regulates SOS1 activity against salt stress. At last SOS also plays a key role in K^+ homeostasis (protein CBL10 directly interact with AKT1 and negatively regulate activity in roots **(Ren et al. 2013)**).

HKTs and plant response to salt stress: - High affinity to potassium transporters (HKTs) belongs to integral membrane protein class and facilitates cation transfer across the membrane. This family on the basis of transport characteristic divided into two classes. HKT 1 has a S forming S-G-G-G motif whereas G is occupied at the position of S in class 2nd**(Maser et al. 2002)**. Here it is notable that due to the presence of S it favors Na^+ transport while HKT2 can transport both Na^+ and K^+ depending on their external concentration. Na^+ retrieval from the transpiration stream to avoid Na^+ accumulation is a key role of HKT1. **Ren et al. 2005; Wu et al. 2009** and others suggest about other four HKT1 members in the genome of rice viz. OsHKT1:1, OsHKT1:2, OsHKT1:4. Studies also suggest K^+ protects plant cell from Na^+ stress. A reduction in the level of accumulation of K^+ in xylem sap, root and shoot of hkt mutants results into increase in accumulation of Na^+ in same tissue. This causes Na^+ toxicity in the xylem sap and other tissues. However, the effect of athkt1:1 disruption and point mutation found in OsHKT1:5 proteins on K^+ accumulation is smaller than the inverse effect of Na^+ accumulation**(Ren ZH, et al.2005; Surnarpi 2005)**. The genes thus indirectly propel K^+ release from xylem parenchyma cell into xylem vessel. KOR (K^+ outward rectify channel) and NOR(non- selective outward rectifying channel are the two- independent depolarization activated outward K^+ rectifying channels have been identified in xylem parenchyma of barley using patch clamping analysis- which are found largely K^+ selectively and predominantly permeable K^+ and Ca^{+2} respectively**(Wegner LH, et al.1997)**. By inverted control of level Na^+ and K^+ xylem sap of hkt1 mutant plant suggest two essential mechanism towards mediating salt tolerance by AtHKT1:1 and OsHKT1:5 that are prevention of Na^+ over- accumulation in photosynthetic tissues and promotion of K^+ accumulation in the photosynthetic tissue as well. In rice OsHKT2:1 catalyzes high affinity Na^+ influx in low salt roots, condition where Na^+ influx is beneficial**(Horie T.et al.2007)**, opposite it activity down regulated when outer concentration of Na^+ increases to reduce Na^+ influx further. Subfamily 2 is less characterized than that of 1 although it was first identified**(Schroeder JI et al.1994)**.A down regulation in the function of TaHKT2;1 is noticed acts as Na^+/K^+ symporter when expressed in *Xenopus* oocytes**(Rubio F et al. 1995)** to improve growth in saline conditions by reducing Na^+ accumulation.

NHX and plant response to salt stress: - NHX proteins belongs a large subfamily of monovalent cation/proton antiporter (CPAs) made up of CPA1 and CP2 subgroups. CPA1 family present ubiquitously in all eukaryotes includes member NHX type **(Bassil et al.2012)**. NHX mainly present at the tonoplast and helps in sequestration

of Na^+/H^+ into the vacuole mainly but in normal condition transport K^+/H^+ using H^+ -ATPase generated potential. An enhance in the level of salt tolerancy is noticed on the over-expression of (AtNHX) in some of the cereals crops support the notion of being a major part of Na^+/H^+ exchange activity in the tonoplast(**Blumwald et al. 2005**). Six AtNHX are known out of which AtNHX1 and AtNHX2 are pre-dominant and functional redundant and with throughout tissue expression in *Arabidopsis*(**Andres Z et al. 2012; Bassil E et al. 2011**). Experiment with double mutated nhx results into more acidification and approx 72% K^+ concentration reduction in vacuoles. Recently (GmSALT3) *Glycine max* salt tolerate associated gene on chromosome 3 has been isolated (**Guan R et al.2014**) by using QTL mapping with the product located at endoplasmic reticulum. The gene GmSALT3 expresses strongly in root xylem and phloem of salt tolerate cultivar. Salt hypersensitivity and Na^+ over-accumulation in leaves and stem of soya bean is shown by defect of GmSALT3.

Other function of NHX: - NHX also play role in K^+ homeostasis, pH homeostasis and in vesicular trafficking as well.

K^+ homeostasis: Being important nutrient component and most abundant cation in plant it comprises about 10% of plant dry matter. In vacuoles it plays a key role in cell volume regulation (**Andreas et al. 2014**). The double mutation displayed K^+/H^+ activity, in guard cell disrupted K^+ accumulation which results in affected stomatal movement (**Andreas et al. 2014**). Study suggested NHX1 and NHX2 be the main transporter to mediate K^+ uptake to the vacuole.

pH homeostasis: pH is an important factor to enzyme for their action, a small change in the pH can denature the enzyme hence proteins(pumps) and is regulated by H^+ pumps. With the colour change in *Ipomea* petals from red to blue as the pH increases by the factor 1, the involvement of NHX transporters to pH regulation is best illustrated. Endomembrane pH homeostasis mediated by NHX5 and NHX6 transporters (**Reguera et al.**). NHX5 and NHX6 transporters are crucial for association of vacuolar sorting receptor and their cargo in *Arabidopsis*.

Vesicular trafficking: AtNHX5 and AtNHX6 are the major player in the process of vesicular trafficking. Studies found due to lack of both AtNHX5 and AtNHX6 plants show a much reduced growth. It is because where on one hand it plays a role in cell expansion; on the other hand also protect plant from higher salt stress. According **Reguera et al. 2015** AtNHX5 and AtNHX6 resides in golgi, trans golgi network and on pre-vacuolar compartment. Apart AtNHX5 and AtNHX6 also contribute in maintaining endomembrane pH homeostasis. According **Sottosanto et al. 2004** apart all AtNHX5, AtNHX6, NHX1 also play a crucial role in protein trafficking and targeting by regulating inter-vesicular pH properly.

6.3 Molecular events conferring tolerance:

At molecular level during osmotic stress in response a lot of organic solutes got accumulated for e.g. proline, free amino acid, sugars and quaternary ammonium compounds, these molecules are collectively known as compatible solute. According, **Ashrafjou et al. 2010; Nabati et al. 2011** these compatible solutes although in higher concentration do not interfere with the plant enzymatic activities. **Bohnert et al. 1995** suggests the presence of these chemicals in cytoplasm which assists the plant in turgor maintenance during osmotic stress by sequestering Na^+ and Cl^- like harmful ions into the vacuole. Under salt stress condition plant accumulate sucrose (**Nabati et al. 2011**) as well as starch (**Parida et al. 2002; Kerepesi and Galiba 2000**) which play a major role in osmotic adjustment, carbon storage and radical scavenging. Experimental analysis of shoots of four *Brassica* sp. Such as *B.carinata*, *B.juncea*, *B.campestris* and *B.napus* reveals presence of higher level of soluble sugars under salinity stress condition (**Ashraf and Naqvi**). An accumulation of nitrogen containing compound (NCC) such as amino acid, amides, proteins, polyamines is also noticed on exposure to salinity stress and according (**Mansour 2000**), these accumulation is frequently correlated with the salt tolerance. These increased levels of NCCs helps the susceptible plants in osmotic adjustments. Moreover, **Quasim 2000** found a increased level of total free amino acid on increasing the level of Salt dose in the growth medium of 8 cultivars of *conola* lines. In Rice sub-cellular compartmentation of Glycine betane biosynthesis is important for increasing salt tolerance (**Sakamoto et al. 1998**). In plant cell these Glycine betane play a crucial role in osmotic adjustment, protection of cellular macromolecule, storage of nitrogen compounds, pH maintenance, detoxification and scavenging of free radicals from the cell. According, **Gadallah et al. 1999** Proline play a vital role in membrane stabilization in plant cells and since higher plants are more prone to salinity stress so proline concentration is already found at high level which is added by increasing the level under stress condition (**Dogan et al. 2010; Nabati et al. 2011**). **Alia et al. 1993** observed a significant role of proline in decreasing lipid per-oxidation in *B.juncea*. **Bohnert et al. 1985** reported Polyols present in considerable percentage of compatible solutes and known to be play a significant role in ROS scavenging, osmotic adjustment and osmoprotection. With the advancement in technology, transgenic approaches with the help of modern technology have been used to improve stress tolerance in plants (**Allen 1995**). A number of transgenes have been identified, isolated and cloned and expressed in plants which are potential sources of resistance to abiotic stresses. These genes includes *Rab*(response to abscisic acid) and *Salt* (induced in response to salt); genes for enzymes involved in proline and betane biosynthesis in bacteria and plants.

7. DROUGHT AND SALT STRESS IN CROP PRODUCTIVITY:

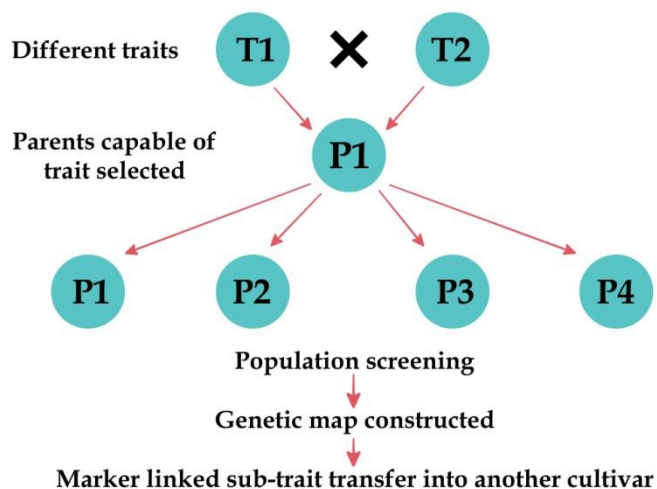
Being a major part of abiotic stresses contribute a lot in year by year degrading yield of crop plants. A zero hunger target is fixed by UN and promoting it as one of the sustainable development goal to transform our world. It is aimed to double the agricultural production by 2030 targeting sustainable and resilient agricultural production system that strengthens the capacity for adaptation to drought and other disasters. Drought is one of the major causes of yield losses globally. Study conducted on the four crops viz. wheat, rice, maize and soy and it was found that around three-fourth (approx. 454 million hectare) global harvested areas experienced drought. These yield losses cost around 166 billion U.S. dollars. An estimation of agricultural G.D.P. loss by 0.8% on each drought globally. Out of 454 Mha, contribution of each crop in order of 161 Mha for wheat, 124 Mha for maize, 102 Mha for rice and 67 Mha for soy. Using the superposed epoch analysis **Lesk et al. 2016** reported that the loss of cereal is 4.9%-5.2% for the period of 1964-2007. Study find the vulnerability level of these crops which suggests wheat is most vulnerable to drought because of proportion of irrigated areas for this crop is lower hence experience higher field losses while remarkably, rice is found to be more insensitive crop to drought because of its larger irrigation area. The scenario of losses (166 billion USD) comprises in order of \$58 B for maize the highest one, \$57 B for wheat, \$37 B for rice and \$24 B for soy respectively between 1983-2009. 25 countries around the world are listed as most vulnerable countries and are associated with approx. 93% of the aggregated loss. In the list China and United States are at the top most position and itself accounts for 56% of losses because of their large areas of cropland (**Acc. CRED 2017**).

The yield of plants depends upon each of the processes involved in the life cycle of particular plant. The adverse causes at molecular level results in the detrimental effect at plant system level. There are various stages during which salt stress can cause detrimental effect in processes like germination, growth, photosynthesis pigment and photosynthetic water relation, nutrient imbalance, oxidative stress and yield. The effect of salt stress on any of the above process can cause reduction in the yield of crop which is most countable effect of salt stress in agriculture. Yield depends upon the number of pods per plant, seeds per pods, seed weight etc. which are negatively co-related with salinity levels. It has been noticed a decrease in the number of pods with increasing level of salinity of *V. radiata*. According **Nahar and Hasanuzzanan 2009** reduction in yield of *V. radiata* cv. BAR1 Mung2, BARI Mung5 and BARI Mung6 by 77, 73 and 63% respectively on application of 250mM NaCl. According, **Zeng, L. and Shannon M.C. 2000** yield of rice depends upon the length of panicle, number of spikelet's per panicle, amount of grain per spikelet. Except, test weight salt treatment significantly affects the grain yields. According, **Shereen et al 2005** Sterility was considered as one of the major cause of reduction in yield under salt stress. According, **Khatun et al. 1995**, a decreased flower and pollen viability due

to delay in panicle emergence and flowering results in reduced seed set. Shereen et al. 2005 observed 77% reduction in grain yield at very lower level of salinity viz at 50mM NaCl concentration. **Hasanuzzanan et al. 2009** reported a reduction of 50, 38, 44 and 36% in grain yield of BRI1, BRR1 Dhan41, BRR1 Dhan44 and BRR1 Dhan46 respectively on application of 150 mM salinity. At global level 20% of the cultivated land and 33% of irrigated land are salt affected and degraded. About 10-million-hectare world's agricultural land is destroyed by salt accumulation each year. It is estimated that 1/3-rd of the world's food production from 45 million hectares of irrigated land is also salt affected; about half of the arable land viz. 50% will be affected by salinity by 2050.

8. PLANT BREEDING FOR DROUGHT AND SALT STRESS:

8.1 Molecular breeding for Drought tolerance: -



Flow chart: showing the molecular pathway to transfer the salt tolerancy mechanism from one crop to other.

Water stress tolerant traits are mainly quantitative and are controlled by multiple genes. In the process of breeding with desired traits, first two traits showing extreme phenotype are selected and those parents were selected which are capable of segregation of trait and progenies were obtained. Once population were screened using molecular markers like RFLP, RAPD, SNPs. genetic map is constructed and finally marker associated with

trait are identified using computer software. With the identification of molecular markers linked to specific sub-traits it is possible to transfer these various traits into other adopted cultivars with various agronomic backgrounds. A more promising approach is the use of molecular MAS which enable us to dissect quantitative traits into their single genetic components thereby selecting and breeding plants that are resistant to water stress (Chinnusamy et al. 2005; Hussain 2006).

8.2 Molecular breeding for Salt tolerance:

At molecular level it comes to be a major promising approach. Using this approach molecular map of different crops like rice, wheat, barley etc. have been developed. The tracking of genetic loci controlling stress resistance without phenotype measurement is possible using these molecular genetic markers. This on one hand reduces the extensive field testing over time and space and on the other hand also offers a strategy of molecular assisted selection (MAS). Stress responsive genes can be analyzed by following targeted and non-targeted strategy. The availability of relevant biochemical information is the basis of targeted approach. While differential hybridization and short-gun cloning like indirect methods are the basis of non-targeted strategy. Understanding the mechanism of stress responsive upregulated gene and its transfer from organism to plants will also expand the ways in which plants can be utilized. Based upon the studies the QTLs associated with salt stress were identified in many of the crop plants such as in rice with having major effects (Thomson MJ et al. 2007; Saibo N, et al.2011; Rahman MR et al 2010; Tiwari S et al. 2016). For example, at chromosome 1 found a locus that contains major QTLs *saltol* (derived from *Pokkali* landrace). QTL of SKC1 was later identified as OsHKT1:5 located with Saltol region (Rahman MR et al.2010) and found to be associated with the shoot K^+ concentration. Through molecular assisted breeding markers were developed and used to transfer the saltol locus (recently fine mapped and known to be involved in Na^+/K^+ homeostasis under salt stress(Ismail AM 2013) in several popular varieties (Sack M 2016; Xuan T et al. 2012). Moreover, saltol locus 14 transcript factors were identified based upon the expression analyses of transcriptional factors in which 8 were strictly found to be co-related with salinity tolerance (Kushwaha HR et al. 2017). Example – With the help of breeding approach CR dhan was released in India in year 2009 with percentage of Mashuri/Ormundakan and Mashuri/Chakrakanda, in both of this mashuri acts as donor which furnish a tolerancy level of (5-8 dS/M) with the designation of breeding line CR2095-181-I and CR2096-71-2 respectively. Likewise, several other varieties like BRRI Dhan 53, NSIC Rc294 also released in Bangladesh and Philippines in which BR23 and RSB Rc 50 acts as the donor variety which also furnishes a salt tolerancy to rice species respectively.

9. CONCLUSION AND FUTURE PERSPECTIVE:

In arid and semi- arid regions, abiotic stress more specifically osmotic and ionic stress are responsible for decrease in the yield. 47% of the world agricultural land is estimated to experience drought and approx. 20% of the irrigated land are affected by salinity. The problem got further aided by global climate change. Continued environmental stresses are responsible for the production of ROS in different cell compartment like chloroplast, peroxisome, mitochondria etc. On one hand where ROS in small concentration causes beneficial functions like signaling regulation etc. on other hand if the concentration goes above a threshold level causes protein oxidation, lipid peroxidation, DNA damage and ultimately initiates programmed cell death (PCD). Yet there are inherent physical, molecular and morphological limitation to the plant ability to respond to stress. Overproduction of ROS causes oxidative stress a condition where ROS or free Radicals are generated extra or intra cellularly which can exert their toxic effect to the cell. The increases in the amount of generation of ROS activates a chain antioxidants defense mechanism which could be either enzymatic (SOD, CAT, GPX, GR) or non- enzymatic (Glutathione, Alpha- Tocopherols, Carotenoids and Flavonoids). Increased atmospheric concentration of different stressing agent causes elevation in the oxidative stress and hence an increased accumulation of compatible substances, so it can enhance drought and salt tolerance and their suitability as crops in a future world of climate change. Yet a lot work has been done in this field yet a lot to be done which provide a scope in near future. Likewise, to identify master regulator that control stress response activation(e.g. **Baena- Gonzalez et al. 2007; Baena-Gonzalez and Sheen 2008**) will provide promising avenues to enhance crop tolerance to abiotic stress apart from affected ROS homeostasis and signaling pathway by these alternations are the exciting aspect of these studies. Combining effect of more than one factor like drought along with salinity or salinity with high atmospheric carbon-dioxide level will also provide a scope. Its effects on the ROS metabolism and signaling are subject of active research (**Keles and Oncell 2002; Rizhsky et al. 2002, 2004; Hawezi Leger and Gentzbittel 2008; Kaussevizky et al. 2008**). ROS metabolism can be tracked by after involvement of biotic and abiotic factors viz. heat and pathogens etc. Pyramiding of ROS scavenging enzymes may also use to be obtaining abiotic stress plants. A lot of work to be done to search isoforms of enzyme to develop stress tolerant plant without compromising the yield is the biggest challenge for researchers. A defined set of markers is to be discovered to accurately predict tolerance towards a particular type of stress. It should be noted while manipulating a gene of interest (GOI) that it should contribute not only at few stages but preferably for whole life of plant. Genomic, Proteomics, Ion omics and Metabolomics are some of the modern technique which is helpful to study the plant response against abiotic stress. Finally, it is utmost important in day to day changing environment to develop such a model plant which not only provide a sufficient yield by

standing in the harsh stress condition but also can interact with these stresses like elevated atmospheric CO₂ concentration in order to assess the suitability of plants as crops in future.

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