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# Introductory Chapter: Recent Advances in Rice Biotechnology for Abiotic Stress Tolerance

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## 1. Introduction

The world population is rising gradually, and it would be approximately 9.1 billion in 2050 but the production of agriculture is not escalating with this speed. The global production of agriculture should be increased up to 100-110 percent till 2050 to feed the whole population. Moreover, agricultural production and crop growth are negatively affected by abiotic stresses. So, it is a major hurdle in the way of increasing food production world-wide. The drought, heat, cold, salinity, ultra violet radiations and heavy metal toxicity are the major abiotic stresses that affect the crop yield. The significant source of calories for human beings is cereals. The maize, wheat and rice produce 10%, 17% and 23% respectively. *Oryza sativa* (rice) serve as a staple food and a famous cost-effective cereal. Acceptance of green revolution cultivars lead towards an essential revolution in the production of rice [1]. First green revolution came into being by producing high-yielding semi dwarf rice and wheat cultivars via plant breeding and providing high doses of nitrogen fertilizers. It helped in preventing the famine in semi-arid regions of Southern Asia [2].

The first transgenic rice was developed almost two decades before that initiated the high-throughput transformation protocols for the development of high yielding cultivars and it laid the foundation of transgenic rice biotechnology [3]. After the genome sequencing of rice, the development of transgenic rice varieties for better stress tolerance, high yield and better nutritional qualities became relatively easy and gained more importance. Similarly, the donor genes from the other species (like bacteria, fungi, viruses, animals and insects) can be inserted in the rice for obtaining the improved cultivars for making agriculture more sustainable. Improvement of some other significant traits (like photosynthetic rate, aroma and nutrition of rice grains) with the good stress tolerance has gained attention in past few years [2]. Some examples of these traits will be discussed in the next sections comprehensively.

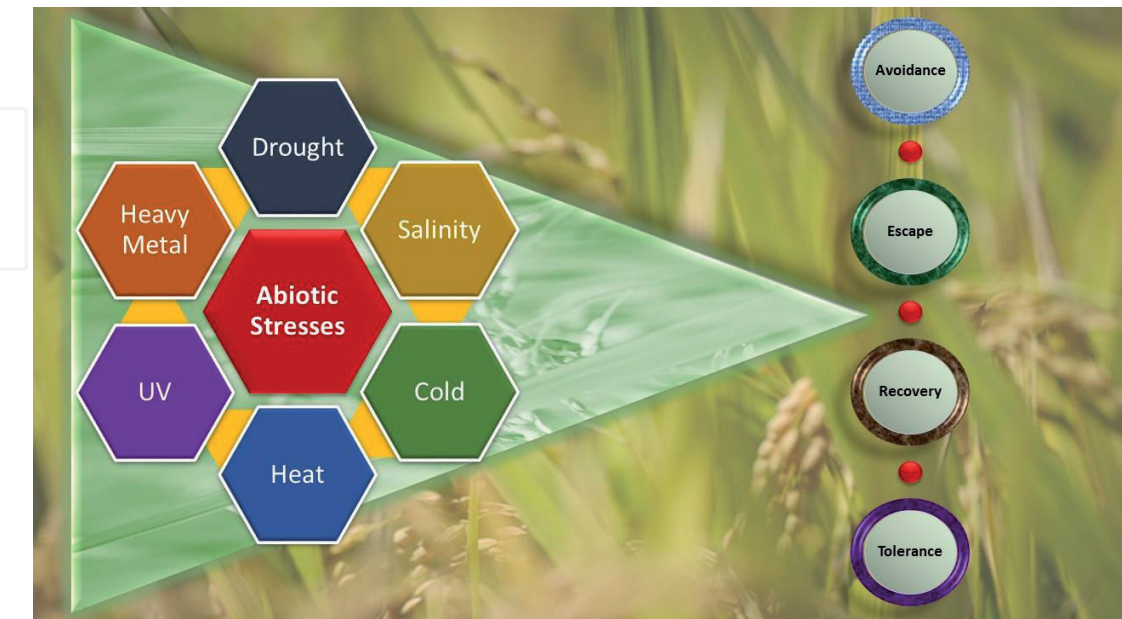
The transgenic or genetically improved crops are an integral part of the agriculture industry in the modern world. The transgenic cereal crops have been commercialized and cultivated by more than twenty-eight countries. In 1996, only 1.7 million hectares of land have been cultivated with the transgenic crop that was increased up to 170 million hectares till 2012 and still it is increasing. Continuous efforts are made to bring the rice yield maximum for developing the large market of it globally [2]. Therefore, it is necessary to develop more cultivars of rice by using

either old plant breeding techniques or new targeted genome editing techniques like zinc finger proteins (ZFNs), transcription activator-like nucleases (TALENs) and clustered regularly interspaced short palindromic repeats associated with Cas9 protein (CRISPR-Cas9) to develop cultivar of rice for improved agronomical traits.

2. Rice biotechnology for abiotic stress tolerance

Rice is an economically important crop and serious efforts are needed to increase its production. The production of rice can be increased in various ways like use of the available agricultural land at its maximum, utilization of marginal lands for growing the rice crop and using the lands effected by various abiotic stresses (e.g. drought stress, heat stress, cold stress, salt stress, UV radiation stress and metal toxicity) by growing the transgenic rice cultivars that are developed with the help of biotechnology. This is necessary because the agricultural land is decreasing and demand for food is increasing with the increasing world population and it is becoming very difficult to meet the food demands of this exponentially growing population with the same efficiency of the present day cultivars of rice [4].

The production of rice is greatly influenced by various abiotic stresses and it can be compensated by the various genes and regulatory networks present in different stress tolerant cultivars of rice and these genes and networks can be taken from the other plant species that can be used for developing new cultivars of rice (**Figure 1**). Abiotic stresses can induce the expression of many genes. The complex transcriptional networks are responsible for regulating the induction of stress tolerant genes. The important genes that are involved in transcriptional networks are studied by molecular techniques and these genes are useful in developing abiotic stress tolerant transgenic rice [5]. So, various biotechnology methods are used to develop rice cultivars that can combat with abiotic stresses (**Table 1**).



**Figure 1.** Abiotic stresses (e.g. drought, cold, heat, salinity, UV radiations, heavy metals) follow a same mechanism to survive and retain their growth and development. It has four main steps that are (a) avoidance: avoiding the contact with stresses, (b) escape: changing the life-cycle, (c) recovery: vegetative growth potency and (d) tolerance: nullifying the impacts of stresses.

S. No.	Transgene(s)	Improved Trait(s)	References
1.	<i>OsHsp101, AtHsp101</i>	Heat stress tolerance enhanced	[6]
2.	<i>OsMAPK44</i>	Drought and salinity stress tolerance enhanced	[7]
3.	<i>OsPIP1;3</i>	Cold stress tolerance ability enhanced	[8]
4.	<i>Choline mono-oxygenase</i>	Heat and salinity stress tolerance enhanced	[9]
5.	<i>AVP1, SsNHX1</i>	Improved ROS and salinity stress tolerance	[10]
6.	<i>OsSBPase</i>	Improved photosynthetic efficiency and heat tolerance ability	[11]
7.	<i>HvCBF4</i>	Salinity, drought and cold tolerance capacity enhanced	[12]
8.	<i>SUB1A</i>	Enhanced submergence tolerance	[13]
9.	<i>OsDREB1F</i>	Salinity, drought and cold tolerance capacity enhanced	[14]
10.	<i>STAR1, STAR2</i>	Enhanced tolerance to Aluminum toxicity	[15]
11.	<i>ZFP245</i>	Improved ROS, cold and drought stress tolerance	[16]
12.	<i>P5CSF129A</i>	Salinity stress tolerance enhanced	[17]
13.	<i>Isoflavone reductase</i>	Salinity stress tolerance enhanced	[18]
14.	<i>OsHMA3</i>	Enhanced drought and submergence stress tolerance	[19]
15.	<i>OsMAPK2</i>	Tolerance to phosphate deficiency	[20]
16.	<i>OsNAC5</i>	Salinity, drought and cold tolerance capacity enhanced	[21]
17.	<i>OsLEA3-2</i>	Salinity and drought tolerance capacity enhanced	[22]
18.	<i>OsMYB55</i>	Heat stress tolerance enhanced	[23]
19.	<i>PCK, PPDK</i>	Improved ROS stress tolerance	[24]
20.	<i>OsETOL1</i>	Enhanced submergence stress tolerance	[25]
21.	<i>OsMYB48-1</i>	Salinity and drought tolerance capacity enhanced	[26]
22.	<i>VrDREB2A</i>	Increased tolerance to salinity and drought stress	[27]
23.	<i>TaMYB3R1</i>	Enhanced drought and salt stress tolerance	[28]
24.	<i>CaPUB1</i>	Enhanced cold stress tolerance	[29]
25.	<i>OsLEA4</i>	Enhanced drought, salt and heavy metal stress tolerance	[30]
26.	<i>OsNAC2</i>	Enhanced drought and salt stress tolerance	[31]
27.	<i>OsGS</i>	Improved ROS and drought stress tolerance	[32]
28.	<i>TsPIP1;1</i>	Enhanced salinity stress tolerance	[33]
29.	<i>RhMYB96</i>	Enhanced salt tolerance	[34]

**Table 1.**  
*Some transgenes inserted for improving the abiotic stresses.*

### 2.1 Drought stress

Drought is affecting agricultural land worldwide from few past years. Many molecular, physiological and metabolic changes occur in plants due to drought stress that damages their growth and development [35]. During drought stress, plants respond variously and express change in their physiology and morphology.



Rice drought resistance is achieved by four procedures that are (a) avoidance: avoiding the contact with stress, (b) escape: changing life-cycle, (c) recovery: vegetative growth potency and (d) tolerance: nullifying the impacts of stress. These procedures are fulfilled by variable mechanisms like reduced leaf area, leaf rolling, senescence of older leaves, increased root proliferation, dense root system, scavenging reactive oxygen species (ROS), early flowering, osmotic adjustment, stomatal closure that minimize the water loss, changes in elasticity of cell wall and maximum uptake of deep water allow plants to survive during extended periods of drought and even allow them to reproduce in limited water supply by maintaining physiological activities [36]. Osmotic potential is kept lower inside the plant cells than outside the cells by an important mechanism known as osmotic adjustment. So, it allows the plants to retain its turgidity and prevents the water loss. Drought stress tolerance in plants can be achieved by the accumulation of inorganic and organic substances like proline, potassium ions, glucose and sucrose.

This mechanism participates in osmotic adjustment and maintains the turgor pressure. Plants exposed to drought stress produce high level of ROS that are extremely toxic and damages the DNA, carbohydrates, proteins and lipid. In drought tolerant plants, non-enzymatic antioxidants and antioxidant enzymes are produced that protect plants from the deleterious effects of ROS. Currently, the production system of rice depends upon the excessive supply of water and therefore, it is more susceptible to drought stress. The drought stress is the most significant restrictive factor in the production of rice and it is becoming a very severe problem. The varieties of rice favored by farmers in the tropical and sub-tropical regions are vulnerable to drought stress [35]. Rice is a significant economic crop of Asia that is mainly cultivated in lowland regions, where agriculture depends on the seasonal rainfall. Thus, rice crop in these areas is very susceptible to drought stress but this problem can be rectified by introducing some drought tolerant genes in the economically important cultivars of rice. For example, jasmine rice or 'Khao Dawk Mali 105' (KDML105) is a world-famous cultivar of rice exported from Thailand, had suffered from drought stress because of limited irrigation and unpredictable rainfalls. Drought tolerance can be achieved after keeping genetic background of KDML105 conserved by chromosome-segment substitution lines (CSSL) which have drought-tolerant quantitative-trait loci (DT-QTL) obtained from the back crossing between drought tolerant donor IR58586-F2-CA-143 (DH212) and KDML105. For more understanding, the drought tolerance related physiological responses consider another CSSL named as CSSL1-16. This line has properties like high proline, high water status, good membrane stability and great osmotic adjustment. Furthermore, it can recommence growth after recovery from stress. So, it can be used as a potential candidate for developing further drought tolerant cultivars while keeping their own genetic background preserved [37].

Another example of producing drought tolerant rice plants is introduction of pea DNA Helicase-47 (PDH47) from the plant *Pisum sativum* by using a constitutive promoter of cauliflower mosaic virus 35S- CaMV had been introduced in the ASD16 a cultivar of Indica rice via *Agrobacterium tumefaciens*. The transcripts of PDH47 are upregulated during drought stress that is correlated with increased water status, hydrogen peroxide accumulation and proline accumulation. It also regulates many stress responsive genes present endogenously during drought stress in transgenic rice [38]. There are many more genes that are expressed during the drought stress in different plants and are also potential candidates for producing transgenic rice that are drought tolerant.

## 2.2 Heat stress

Heat stress is a major limiting factor in the production of crops across the world because of global warming. A negative correlation is present between the increased temperature and crop yield especially in case of rice, wheat, barley and maize [39]. The heat stress can damage rice plants severely by inhibiting the metabolic activities, seed setting, plant growth and pollen fertility; therefore production of rice is reduced [40]. Excessive heat can also decrease the photosynthetic ability of plant, reduce water use efficiency, seed weight and grain mass and shorten the leaf area. Heat stress can damage both at vegetative and reproductive stage from sprouting to maturity. But, flowering and booting are the two more critical stages that can cause complete sterility in rice cultivars [41]. Heat tolerance belongs to plants that can lessen the effects of stress and give enough economic yields even at high temperature. Like other plant species, rice also has variations in germplasm to combat with heat stress. Tolerance is achieved by adjusting different molecular, morphological and physiological traits in rice cultivars. High temperature enhances the expression of stress tolerating genes and increases the metabolite production which is beneficial for achieving stress tolerance in plants [42]. During heat stress plants adopt multiple mechanisms like avoidance, survival and escape. These mechanisms impose avoidance for short term and develop resistance for long term survival. At the cellular level, effects of stress can be neutralized by certain factors and mechanisms; such as transcriptional control, antioxidant defense, osmolytes, late embryogenesis abundant (LEA) proteins and the factors that participate in signaling cascades. In hot environments, yield is reduced because of early maturity comes under the domain of avoidance strategies while suffering from heat stress [41].

Several morphological markers like long anthers, high pollen fertility, long basal pores and large basal dehiscence can be used for determining heat tolerant rice. As well as grain yield, number of spikelet, weight of thousand grains and seed setting percentage can be used for screening rice germplasm against high temperature stress; a great reduction is found in all these factors during heat stress. So, these parameters can be used to screen heat tolerant rice. Opening of the rice spikelet in early morning can be another beneficial criteria for the selection of heat tolerant rice cultivars [41]. A thermo-tolerant wild cultivar known as *Oryza meridionalis* can maintain high photosynthesis rate at high temperature. This high rate of photosynthesis is due to good stability and activity of Rubisco and it can be used as a significant physiological marker to determine the heat tolerance in rice [43]. The chlorophyll content and electrolyte leakage from leaves and roots can be increased during heat stress; it can also be used as a marker to analyze the heat tolerance. The fluid content of the membrane, lipid molecule present in membranes, cooling effect of transpiration and leaf position are some other beneficial physiological marker to determine the heat tolerance in rice.

Plant breeding efficacy has been increased by using marker assisted selection (MAS). Numerous molecular markers are used in MAS, but, single nucleotide polymorphism (SNPs) and simple sequence repeats (SSRs) are extensively used. This procedure is used to gather the information about the genes involved in providing resistance against biotic and abiotic stresses [44]. Still, there are very limited numbers of rice cultivars that are engineered to tolerate heat stress. Because the SNPs are present more than the SSRs in the rice genome, so SNPs are more prone to use for developing heat tolerance. A few SNPs related with heat tolerance have been known. Due to the very complex nature of this trait, each marker can contribute very less towards the variance. Therefore, several markers that are linked

with various quantitative trait loci (QTLs) are used to improve a cultivar for heat tolerance [45]. Many genes are responsible for heat tolerance at various stages; for example, ZFP is a gene that is related with heat tolerance at seedling stage [46] and OsWRKY11 is another gene that is responsible for heat tolerance in rice [47]. The heat tolerant genes are needed to be identified in rice cultivars and the genes from the other plant species can also be transferred to the rice cultivars to improve the heat tolerance. Other approaches like use of mutagens and a recent technology like CRISPR-Cas9 should be applied for further development of heat tolerant rice cultivars.

### 2.3 Cold stress

The cold stress is a significant environmental factor that affects the development and growth of rice crop. At the stage of seedling development, sudden decrease in temperature can influence the development of chlorophyll [48]. The damage of rice seedling due to cold stress ultimately decreases the grain yield. So, the cold stress is a major limitation which can be overcome by using cold tolerant rice varieties [49]. Because rice crop is evolved in tropical region, so it has limited adaptability to chilling stress. Improving rice varieties to make them cold tolerant, enable the cultivation of rice in northerly latitudes. Different signal transduction pathways and genetic networks are involved in controlling chilling tolerance [50]. In japonica rice, chilling tolerance is achieved by interactions between rice G-protein  $\alpha$ -subunit 1 (RGA1) and chilling tolerance divergence 1 (COLD1) followed by the calcium signaling initiated in the response of downstream network of stress response belongs to C repeat binding factor (CBF) that is a transcription factor [51]. Still, there is limited information available about the stress response and adaptation. The plant show response to abnormal surrounding temperature by changing their gene expression and adapt the suitable architecture due to the developmental plasticity. In SAM (shoot apical meristem) intrinsic signals can be disrupted by cold stress and stress tolerance is enhanced by setting the dormancy cycling at the SAM [52]. For the survival mechanism against cold stress, protection of niche forms of root stem cell is a sacrifice [53]. The differentiated cells are properly organized, and meristematic activity is maintained by this rehabilitated development in response to the cold temperature. During cold stress, many specific genes like OsMYB3R-2 are activated by the various transcription factors to maintain the mitotic cell and their cold tolerance. The survival and growth are enhanced by keeping maintained the cellular activity and cell behavior during and after the cold stress.

The formation of axillary bud following by its outgrowth in axils of primordia of leaf controls the shoot branching. The axillary bud initiation is controlled by MOC1 (Monoclum 1) and LAX 1 (Lax Panicle 1). The signaling pathways involved in the axillary bud outgrowth formation and biosynthesis of strigolactone are important for tillering in rice crop and are controlled by the dwarf genes, that are dwarf 53 (D53), dwarf 27 (D27), dwarf 17 (D17), dwarf 14 (D14), dwarf 10 (D10) and dwarf 3 (D3) [54]. In rice the outgrowth of axillary buds is repressed by the OsTB1 that acts in the downstream region of the dwarf genes. The interaction between the OsMADS57 and OsTB1 is responsible for reducing the inhibitory effect on the D14, a gene responsible for the organogenesis of rice tillers by producing a receptor for the hormone strigolactone [55], and it enables D14 to regulate the initiation of axillary bud [56]. The plant endogenous conditions and environmental situations control the development of axillary buds that are basically indeterminate structures [57]. To adapt the cold environment a network has been identified having core gene OsMADS57. The rice tiller growth is maintained by the overexpression of OsMADS57 during cold stress. OsMADS57 directly binds with the promoter of the



OsWRKY94 gene and enhances its expression in response to chilling stress while retarding its expression during normal temperature. Moreover, during normal temperature and cold stress, OsTB1 directly target and suppress the OsWRKY94 gene and under the cold stress OsMADS57 is responsible for promoting the transcription of D14 and suppress the tillering while during normal conditions expression of D14 was repressed to enhance the tillering. It shows that OsTB1 and OsMADS57 equally contribute to develop the cold tolerance in rice by targeting OsWRKY94 [52]. So, they can be used as potential candidates for developing cold tolerant rice cultivars.

Cold tolerance can also be achieved by the biogenesis of chloroplast in rice crop. In plant development and growth, chloroplast plays an important role and its development is affected by the chilling temperature. The genes and regulators that are involved in the biogenesis of chloroplast are identified and characterized. The mutant of WSL5 (white strip leaf 5) in rice has been characterized. The white stripped leaves are developed by this mutant during early leaf development stage and show albino phenotype during chilling stress. A unique chloroplast-targeted-pentatricopeptide repeat protein is encoded by the WSL5; the molecular and genetic analysis has revealed it. The RNA sequence analysis revealed that in the mutant, expression of the nuclear-encoded-photosynthetic genes was significantly repressed and the genes responsible for the chloroplast formation were also changed significantly. WSL5 gene is required for the development of chloroplast during cold stress [58]. A transcription factor OsMYB3R-2 from rice having a DNA binding domain is involved in enhancing the cold tolerance [59]. Another transcription factor MYBS3 of rice having a DNA binding repeat also play a significant role in adapting the cold environment [60].

The progress in developing the genetically modified plants by introducing and overexpressing the novel genes appears to be a very good practically possible option to speed up the process of breeding in plants. Instinctively, the faster way of inserting the genes with beneficial traits is genetic engineering instead of molecular or conventional breeding; and it would be the only possible option if the gene of interest belongs to some other species of plants, distant relatives, or from any non-plant source. The utilization of gene knockout strategies and genomic approaches are developing to enhance the efforts to measure thoroughly and make it easy to completely understand the complicated quantitative traits like tolerance to different temperature fluctuations and extremes. Many relevant genes have been identified by using molecular and genetic techniques to understand the plant response for the cold stress and these genes can be used further for developing cold tolerant varieties [61].

## **2.4 Salt stress**

Rice crop is susceptible to salt stress and 1/3 of total world agricultural land is affected with it. The salinity of both water and soil has negative impact on the production of rice. The increasing level of sodium ions in the agricultural lands is becoming a severe threat for the agriculture worldwide. The plants suffer an osmotic stress due to the accumulation of salts at the outer side of the roots and suffer an ionic stress due to the accumulation of salts at the inner side of the plants [4]. The increase in food supply must be equal to the rate of increasing population and this demand can only be satisfied via utilizing all the available resources of land at their maximum potential. So, it is also necessary to use the saline soils at their fullest production potential. Different methods like agronomic adjustments, reclamation and different biological amendments are carried out in combination for increasing the production of saline soils. The use of salinity tolerant genetically improved crop varieties is the most suitable option for the sustainable crop



production in these areas [62]. The genetic diversity of crops regarding salinity tolerance must be evaluated for developing the salt tolerant crop varieties. The molecular mapping approaches have made it possible to identify the genomic regions responsible for the salt tolerance and assessment of the genetic diversity of different crops and varieties is becoming very easy [63]. The chromosomal regions (QTLs) responsible for the tolerance of salt stress in rice crop can be identified through various molecular mapping approaches.

The salt stress badly effects the physiological, morphological and biochemical features of the rice. It has the negative impact on plant height, shoot dry weight, total tillers, total dry matter and root dry weight. The various physiological attributes that are affected by the salt stress are senescence, calcium ion uptake, sodium ion uptake potassium ion uptake, total cations uptake, osmotic potential, transcription efficiency and relative growth rate [64]. The biochemical features of rice that are effected by the salt stress are proline content, anthocyanins, peroxidase (POX) activity, calcium content, sodium content, potassium content, chlorophyll content and hydrogen peroxide content [4]. Various levels of salinity tolerance are observed at the whole plant level and leaves of rice [65]. Likewise, the rice plant behavior against the salinity stress may vary at reproductive and vegetative phase and this may not be related to the net relative tolerance of the plant. It is mandatory to know the stage that is more susceptible to the salt stress because it is important for comparing the performance of different cultivars during stress. The process of photosynthesis is necessary for the good vegetative and reproductive growth. In the leaf tissues, increased sodium concentration adversely affects the essential cellular metabolism and net photosynthesis. In the process of photosynthesis, chlorophyll content is significantly important but during salinity stress, there is no relationship found between the photosynthesis and chlorophyll content because net photosynthesis rate is decreased by the sodium ion concentration which does not have any connection with the chlorophyll content. It shows disturbance in some other cellular processes of photosynthesis due to salinity stress. The carbon dioxide fixation and stomatal aperture are affected by the sodium ion accumulation in the leaf at the same time, so it can be a reason for the decrease in photosynthesis during salt stress [4]. Different mechanisms have been evolved in rice plant to cope with salt stress conditions. An example of this type of mechanism is compartmenting of salts within the plant.

Numerous genes and QTLs are activated during the salt stress, which could be determined by the different molecular mapping approaches. Many types of molecular marker are present to identify different QTLs. SNP (Single nucleotide polymorphism), SSLP (simple sequence length polymorphism), RFLP (restriction fragment length polymorphism), AFLP (Amplified fragment length polymorphism), STS (sequence tagged sites) and SSRs (simple sequence repeats) are various markers of DNA that are used for genotyping in the studies of molecular mapping [66]. For example: the QTLs of pollen fertility, sodium ion concentration and calcium, sodium and potassium accumulation have been identified in F<sub>2</sub> population of rice by using SSR marker [67]. The QTLs for yield related traits and morphological traits are identified by F<sub>2</sub> population of rice as plant material and SSR as DNA marker [66] and the QTLs of potassium and sodium uptake for increasing the salinity tolerance in rice are determined by the AFLP, RFLP and SSR [68]. QTLs of salt stress responsive genes have been identified by the SNP in the rice crop [69]. The rice germplasm and these identified QTLs are found salt stress tolerant and are useful for three main reasons: (1) salt tolerance can be understood by the molecular genetics in rice, (2) rice germplasm that is tolerant to salt stress can be introduced to make them salt tolerant and (3) for the screening of rice germplasm, identified QTLs are used against salinity stress [4]. Moreover, new

genes responsible for the salt tolerance can be identified and incorporated in the rice cultivars to make them salt tolerant.

## 2.5 UV radiation stress

The ultraviolet (UV) radiations are present in the region of solar electromagnetic spectrum that has the wavelength ( $\lambda$ ) from 200 nm to 400 nm. The UV radiations have shorter wavelength as compared to photosynthetically active radiation that has the wavelength ranges from 700 nm to 400 nm. The UV radiations are composed of three different types that are UV-A, UV-B and UV-C. The UV-C radiations have a very smaller wavelength that is 200-280 nm and it emits photons with high energy which are absorbed totally by ozone layer and are not be able to reach the Earth surface [70]. The UV-A radiations ranges from 315 nm to 415 nm and are more constant. The UV-A radiations causes a little harm to the plants, so the major source of damage are UV-B radiations that have wavelength between 280 and 315 nm [71]. UV-B can cause mutations, reduction in photosynthetic activity, reduction in chlorophyll content, lower electron transfer rate, damage genetic material, decrease the biomass, reduce leaf size, lessen leaf number and eventually decrease the plant productivity. At the cell level, it initiates the oxidative stress through enhancing the level of ROS that ultimately damages the DNA, lipids and proteins; so, the integrity and functionality of cell membrane and enzymes is compromised. The light for photosynthesis can be maximally captured in higher plant by the exposure with UV-B. It is important for increasing the secondary metabolites, enzymatic and non-enzymatic antioxidants, bioactive compounds and cyto-solutes (sugars, glycine, betane, proline) for the survival of plants. The adverse effects of the radiation can be mitigated by the growth regulators present in the plants. The specific signaling pathways are present in plants that are involved in regulating protective gene expression responses against UV-B are vital for survival of plants in sunlight. The identification of the genes responsible for the UV-B radiation, survival is necessary to develop the crop varieties resistant to UV radiation stress [72].

## 2.6 Heavy metal toxicity

Many solutes are present in the rhizosphere required for the growth and development of plants. Plants uptake these solutes by roots and distribute them in the whole plant body. The successful plant life is ensured by up taking the other components with water from the rhizospheric soil by roots. The developmental plasticity and physiological activity in the plant roots is carried out by the water uptake with soluble elements. The distribution and uptake of these inorganic materials inside the plants is an intrinsic property of energy and material fluidity. In plant cells, a plethora of physiological and structural functions is supported by these essential ions but if these essential ions are present in the non-physiological concentrations, they can turn out as limiting factors. The cellular homeostasis is affected by their availability to plants, Inequalities in comparative abundance of these elements in soil and their rate of uptake. The defense system and adaption of plants is dependent on the developmental and physiological changes triggered by ion toxicity; but it can cause the permanent damage to the plant. In the rhizospheric soil, the ions of heavy metals are also present that can be absorbed by the roots with the water and nutrients and can be incorporated into the plant tissues. The zinc, iron, manganese, copper, aluminum, chromium, cadmium, cobalt, lead, arsenic, nickel and molybdenum are some toxic metals for plants [73].

The concentration of metal ions is excessive in polluted areas and plants growing in those areas suffer from metal toxicity. Some soils have high level of heavy metals

naturally like serpentine soils and the mining areas also have high heavy metal content due to activities of human beings. The environmental pollutants including high concentrations of heavy metals are becoming a major challenge for all organisms (plants, animals, microbes, etc.) across the globe. The ionic homeostasis is achieved in plants by maintaining the levels of essential and non-essential ions. Furthermore, this homeostasis in cells and tissues must be maintained in an organ specific manner. The risk of heavy metals can be mitigated by minimizing their uptake, avoiding their exposure and intracellular sequestering in the plants. The cellular structures, membranes structures, basic metabolism and transport processes are affected, if plant is not able to combat with the harmful effects and internalization of heavy metals. The stress reactions are triggered by the high levels of non-toxic and toxic metal ions that disturb the development at molecular, structural and physiological level. The defense mechanism is started in the plants upon the beginning of heavy metal toxicity [74]. The oxidative stress is caused by the heavy metal toxicity, so cells and sub-cellular compartments are protected by plant defense system. The plant growth and development are continued if the heavy metal ions are present in low or moderate concentrations. But, plants cannot survive during the long-term exposure of high level of heavy metal toxicity. The metal tolerance is a unique feature of some plant species in stress biology. The compartmentalization and of heavy metals, restriction of heavy metals in apoplast, extracellular attenuation and chelation of heavy metals and exclusion strategies are some mechanisms of achieving stress tolerance in the plants.

The most practical approach adopted by the plants to combat with the heavy metal toxicity is hyper accumulation and hyper tolerance of heavy metals inside the plant without effecting its viability [75]. The hyper accumulation of metal ions protects the plants from pathogens and herbivores in some conditions (Hörger et al. 2013). The contamination of heavy metals is present in the wide range of terrestrial habitats. In food chain, plants have vital importance, so heavy metal accumulation in plants causes severe contamination in the food chain. So, it is significantly important to know that how plants combat with the heavy metal exposure. The knowledge of these mechanisms is used in developing the practical biological methods to develop the tolerance against heavy metal toxicity [74]. Many promising and existing biotechnological approaches are used are used for this purpose [75]. Before concluding the major efforts used for making plant to heavy metal stress, it is necessary to understand the key factors involved in adjusting the heavy metal tolerance. The total cell protein study is called proteomics; it is a most valuable tool for understanding the basic procedures of development and stress responses in plants [73]. The sensing, transport and uptake of heavy metal, their sequestration in the vacuole and formation of phyto-chelating-heavy metal complex are some main procedures for influencing the plant susceptibility for the metal ions. These processes are controlled at transcriptional and post-transcriptional levels and the secondary process responsible for maintaining the plant during metal toxicity includes the defense reaction derived by the production of antioxidants to preserve the membrane lipid integrity. Diverse genes are responsible for controlling these processes. The heavy metal tolerance of the plants is achieved by the various biotechnological, molecular and genetic tools [74].

### **3. Rice biotechnology under climate change conditions**

Due to climate change, the food availability is affected in 21st century to feed the population which is increasing day by day. Globally, climate change have bad effects on the human health, food security and water resources [76]. About 30-60% of rice



income is decreased per year because of different types of stresses [77]. The rice fields are susceptible due to climate change, expansion of urban area and ruined of cultivation field. Due to some cases, 100% rice production is destroyed [78]. The rice yield is reduced due to increase in temperature by accumulation of carbon dioxide in environment. The production and quality of rice (*Oryza sativa* L.) is badly affected by climate change which is mainly abiotic stresses (heat, flood, salinity, cold and drought) and biotic stresses (pest, weeds and pathogens). Climate change causes the formation of unripe grains, white grains, and fractured grains. The grain damage is reduced by use of thermo-stable varieties which are formed through conventional breeding and genetic engineering. Thermo tolerant 1 (TT1) is the most important (QTL) present in *Oryza glaberrima*, it codes  $\alpha 2$  which is the subunit of 26S proteasome and is involved in destruction of ubiquitinated protein.

The removal of cytotoxic degraded protein is done by OgTT1 (*Oryza glaberrima* thermo tolerant 1) maintain the cell from high temperature. Over expression of TT1 increases the ability of heat tolerant stress in rice. It helps to grow the rice in global warming [79]. To protect the food, production of heat tolerant varieties which can be grown in high temperature is a major challenge. In *Arabidopsis thaliana*, Dehydration responsive element binding protein 2A (DREB2A) is regulated by DNA polymerase subunit B3-1 (DPB3-1), is a transcriptional controller which regulates the growth of plant and increases the thermostability [80]. If temperature is around about 35°C in chamber trial at the time of flowering, then spikelet infertility occurs due to heat stress. When the temperature in Japan cities (Tokai, Kanto) was reached at 40°C in 2007, it induced extensive unproductiveness. The increase in world temperature is expected, that is why we need to develop techniques to protect the grain quality and production from heat damage [81]. During the ripening of rice, the high temperature causes the poor-quality grain production. Late transplanting and thermo-stable varieties are used in preventive measure. Deep-flood irrigation is a process which reduces the rate of milky white seeds when the temperature is high and low. Adequate quantity of nitrogen is necessary to protect the grain from disease at reproductive stage [81]. In future, if water availability is less, yield of rice will badly be affected.

Due to climate change, the crop rotation time can be reduced. There are many positive and negative effects of climate change on crop, so scientists try to develop the varieties which can be cultivated in harsh conditions to provide enough food to global population [82]. Galactinol synthase 2 gene from *Arabidopsis* is introduced in two rice varieties and have ability to produce the good quality in waterless field environment. The biotechnologically advanced varieties have greater intensity of galactinol as compared to non-transgenic varieties. The drought resistance rice has greater ability of photosynthesis, recovering and plant growth. It is clear that AtGOLS2 gene is beneficial which provides higher quantity of rice in drought stress [83]. Rice varieties which are better grown in anaerobic environment produce less yield in aerobic situation. To accurately measure the expression of different genes in a condition, it is necessary to find out the genes which have same output in aerobic and anaerobic systems [84]. Transcriptional reaction and ecological variation is affected by macroclimate and meadow type, then we identify the genes which are involved in production and photosynthesis [82].

#### 4. Future prospects

In modern-day agriculture, biotechnology has a wide range of applications. It helps in understanding the genetics of important traits as well as in developing new cultivars. The transgenic crop deployment and the marker assisted breeding



are the two main areas. The remarkable progress has been made by the marker assisted rice breeding. Modern tools of biotechnology help in studying the functions of genes and mechanism of resistance through improved varieties via MAS. Exploitation of tissue culture techniques used as a vital mean or work as an assistant to other procedures together with recombinant DNA methods is at its frontline in plant improvement and modification for agriculture. The targeted genome editing methods like ZFNs, TALENs, CRISPR/Cas9 are implanted for the modification of non-operational alleles of the useful genes or to repress or knock out the non-useful alleles in plants without making the plant transgenic. The techniques and tools of biotechnology are a very sophisticated option for the rice breeders to develop the useful crop varieties.

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