

A Seminar Paper
On

Drought Resistance in Rice: Conventional to Molecular Breeding

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Abstract

Drought is the most important menace for agricultural food production, particularly in the cultivation of rice, considered as a semi-aquatic plant. Drought resistance is a complex quantitative trait whose effect depends on the action and interaction of different morphological, biochemical and physiological responses. The main objectives of this review are to highlight different responses, indicators, causes, adaptation processes and breeding methods for resistant rice variety against drought stress. Response strategy of rice crop mainly characterized by leaf rolling, speedy stomatal shutting as well as augmented abscisic acid (ABA) synthesis to reduce water shortage stress. Results showed that, increased SOD (superoxide dismutase), POD (peroxidase) activity, MSI (membrane stability index), proline, sucrose, glucose helped rice genotypes to stand better under drought stress. Physiological traits are the yield constancy parameters and could be useful for evaluating drought resistance rice genotypes while biochemical characters play role in osmotic adjustment including stabilization of cell membrane under stress conditions. Developing a durable drought-resistant rice variety through conventional breeding is much more time consuming and more laborious although outcomes are not so precise. Nowadays molecular as well as biotechnological tools such as QTL analysis, gene transformation along with marker-assisted selection has been more preferred by the plant breeders to develop a stable resistant rice variety within shortest time. Overall, this review provides a systemic glimpse of breeding methods from conventional to the latest innovation in molecular development of drought-resistance rice variety development. The information of the review paper might provide as a guideline for researchers as well as rice breeders.

Key words: Rice, Drought resistance, conventional breeding, molecular breeding.

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Chapter 1

Introduction

Rice is the most important staple food, provides about 80% daily calories consumed by more than 35% of the world's population, particularly in Asia (Sahebi *et al.* 2018). Generally, rice plants are susceptible to water deficiency as posse's tiny root system, thinner cuticular layer as well as speedy stomata closure (Singhal *et al.* 2016). In the aim of attaining self sustainability in the rice production next to 2050, it is prerequisite to develop resistant rice varieties against both biotic and abiotic stresses along with consideration of higher yield and superior quality (Chukwu *et al.* 2019). Drought stress is one of the distressing abiotic factors that are assumed to affect above 50% of the total global arable soil by the year of 2050 (Sahebi *et al.* 2018). Drought stress may cause a tremendous yield loss which may increase according the growth stage of rice plant. Severe drought stress can be harmful for growth and development of plant even can lead to death of the plant at any stage of growth. Male sterility as well as embryo abortion is the common effect of drought stress just after pollination at the reproductive stage leads to poor reproductive success (Ozga *et al.* 2016). In the developing countries, it is essential to reduce this yield reduction for achieving self sufficiency for food for the ever increasing populations (Gosal *et al.* 2009).

Drought resistance can be defined as the capacity of a plant to produce its highest economic product under water-deficit environment relative to the water-constraint-free management. Several factors play essential role in the response mechanism of plant to drought stress such as plant species, age of plant, growth stage, plants genotype as well as intensity of drought (Gall *et al.* 2015). Drought resistance scheme involved drought escape, drought avoidance as well as drought tolerance mechanisms. Drought tolerance mechanism comprises cellular adaptation, phenotypic adjustment as well as physiological acclimatization that are governed by heritable factors at various stages. Cellular adaptation causes reduced osmotic potential, decreased turgor pressure and chlorophyll content. Increased stomatal conductance, reduced rate of transpiration to minimize excessive water loss, proper assimilate partitioning, early synchronization of male and female flowering and early maturation for superior production are the main physiological events under drought stress. On the other hand, amplified length and thickness of root, thick cuticular/waxy layer on leaf surface, reduced leaf size and area, lesser epithelial cells, late leaf

senescence are the morphological adaptation process under water-deficit environment (Sahebi *et al.* 2018).

For the better inspection and analysis of drought response in plants, genotype, growth stage, timing and intensity of drought as well as its co-occurrence with other abiotic factors such as salinity and temperature are significant to understand (Hu and Xiong 2014). In the earlier period, traditional breeding methods such as introduction, hybridization, composite breeding, multiline selection, as well as backcross breeding were practiced for developing resistant varieties. However, these methods are slow, expensive, and hectic for developing resistance in crops. Generally, conventional breeding develops new plant varieties by the process of selection, and seeks to accomplish expression of genetic material which is already present within a species. The current breeding methods for developing a durable drought-resistant rice variety encompass advanced biotechnological tools such as marker assisted selection, genetic engineering as well as QTL analysis that require short span of time and offers most precise targeted conclusion.

Objectives-

- To review the available information on several resistant mechanisms of rice under drought stress.
- To describe the present methods for developing durable and stable drought resistant rice variety through conventional breeding as well as modern biotechnological tools.

Chapter 2

Materials and Methods

This seminar paper is solely a review paper which has been prepared by collecting information from various secondary resources. Secondary data were collected from different websites, national and international journals, reports, publications and books available in the library of Bangabandhu Sheikh Mujibur Rahman Agricultural University and consultation with my major professor Dr. A. K. M. Aminul Islam, Dept. of Genetics and Plant Breeding, BSMRAU, Gazipur.

Chapter 3

Review of Findings

3.1 Drought- the major problem for rice:

Water is the most essential restraining factor for rice cultivation (Liu *et al.* 2015). Drought stress causes unfavorable along with unaffordable alterations within plant body affecting growth as well as photosynthetic activity to a great extent, thus badly inhibits yield production (Yordanov *et al.* 2013). Rigorous drought stress inhibits photosynthesis, hampers normal metabolic activity, and even can result in death of plant (Shao *et al.* 2011).

Different researches have indicated that water is the most important for rice production (Moradi, 2016). Water stress can be characterized as decrease of water content, turgor pressure, entire water potential, drooping, closing of stomata, as well as reduction in cell amplification with growth and development ((Fig. 1) (Liu *et al.* 2015; Wei *et al.* 2016).

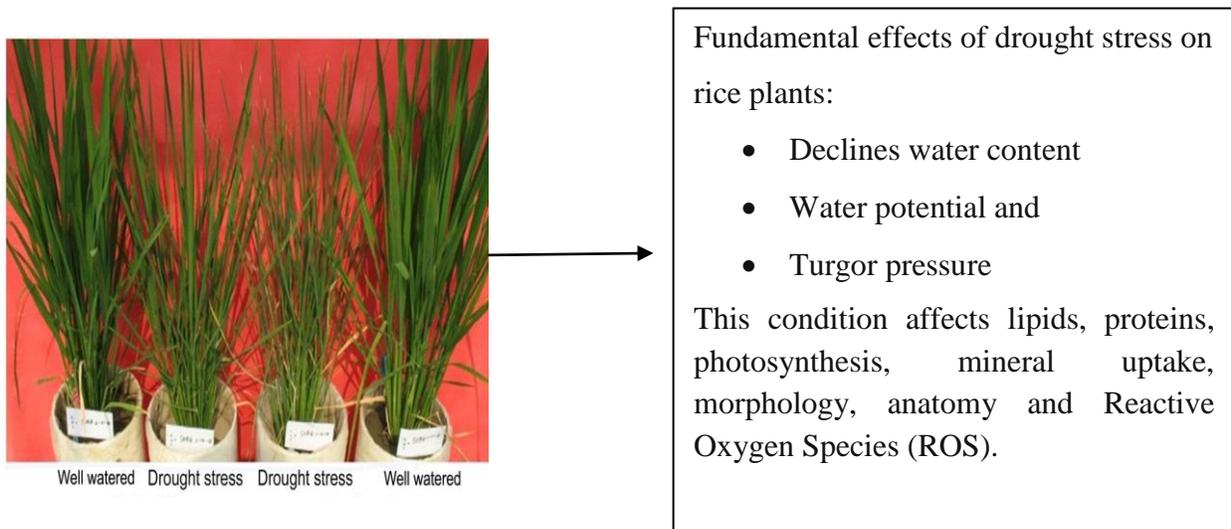


Figure 1: Fundamental effects of drought stress on rice (Source: modified from Liu *et al.* 2015 and Wei *et al.* 2016).

An experiment was conducted with rice cultivar by Yang *et al.* (2019), where main plots were divided into 4 levels of treatments: I₁- drought stress at the start of stem elongation stage, I₂ - drought stress at the start of boot stage, I₃- drought stress at the start of grain filling stage and I₄- full irrigation or control. The results showed that (Fig. 2), relative water content at I₁ is significantly lower than other treatments including full irrigation stage (Yang *et al.* 2019).

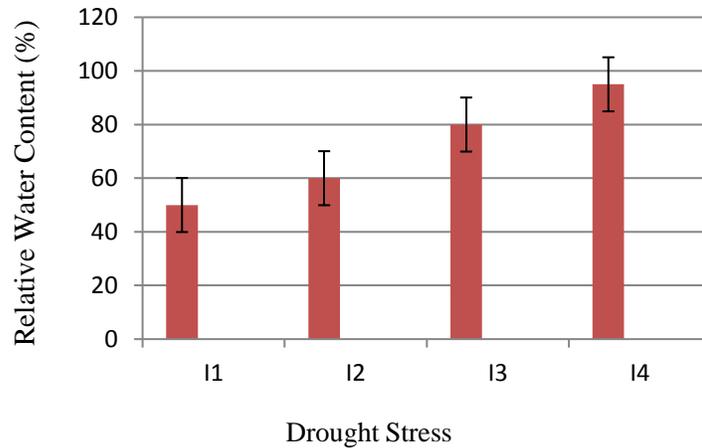
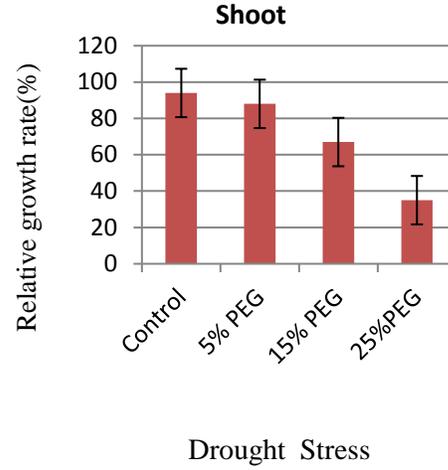
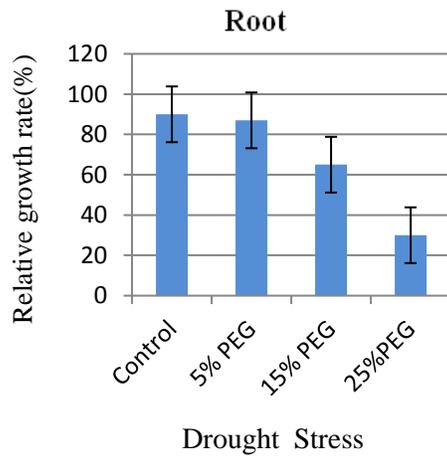


Figure 2: Relative water content (%) of rice under drought stress (Source: Yang *et al.* 2019).

Another study reveals that, the relative growth rate (RGR) and relative water content (RWC) of rice gradually decreased with the increasing drought stress which was imposed by several PEG (Poly Ethylene Glycol) concentrations (0%, 5%, 15% and 25%) both for shoot and root (Fig. 3) (Guo *et al.* 2013). PEG is an osmotic agent, which play an important role in the regulation of mineral elements, hormone, protein metabolism and signal transduction. The main function of PEG is to slow down the moisture rate of import and export, which benefit to reduce membrane system injury in process of imbibitions and repair impaired membrane system. RGR value of a plant reflects its vigour and is considered a good index of its exposure to stresses of all sorts (Ashraf, 2013).

(A)



(B)

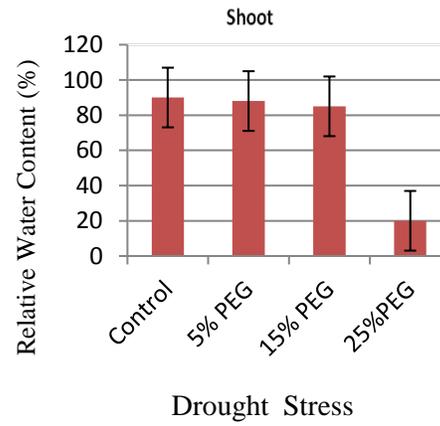
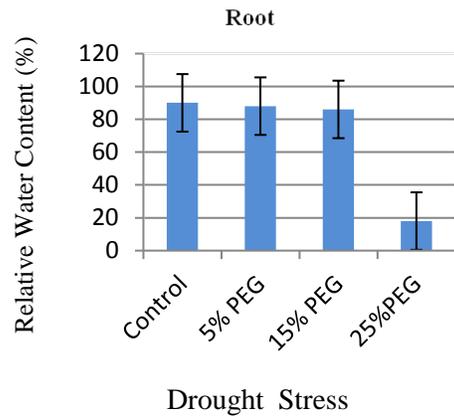


Figure 3: Relative growth rate (RGR) (A) and water content (WC) (B) of rice shoot and root under drought stress (Source: Guo *et al.* 2013).

3.2 Drought resistance mechanisms of rice:

Drought resistance can be considered as the capacity of a crop to resist numerous harmful changes with production of expected economic yield with minimum loss in a water-deficit environmental condition relevant with water restraint free management. Actually, drought resistance is a complicated phenomenon comprising several integrated factors such as action and interaction of different morphological, physiological as well as biochemical uniqueness (Gall *et al.* 2015). According to Luo 2010, plant resistance to drought can be characterized into drought tolerance along with drought avoidance mechanisms (Fig. 4).

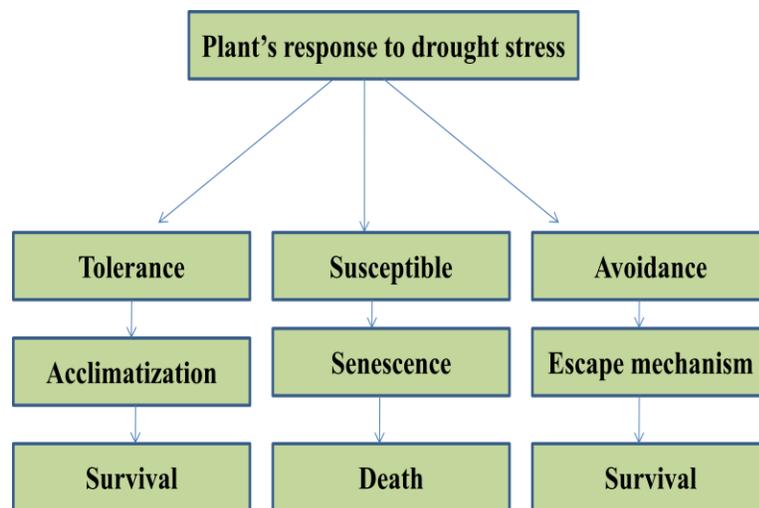


Figure 4: Response strategy of plant to drought stress (Source: Luo, 2010).

Drought avoidance is defined as the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture. Rice varieties which can evade drought stress typically encompass deep and coarse roots with a better capability for high branching and soil penetration, high root to shoot ratio, flexibility in leaf rolling, premature stomatal closure as well as elevated cuticular resistance (Luo, 2010).

Drought escape can be defined as the capability of a plant to complete its total life cycle previous to severe soil and plant water deficits development. This mechanism comprises quick phenological growth (early flowering plus early maturity), developmental flexibility (deviation in the time of growth phase depending on the degree of water deficit) as well as remobilization of pre-anthesis assimilates to the grain (Kumar *et al.* 2014).

Morphological traits of rice have been significantly impacted by drought resulting from phenotypic adaptation processes. Study results showed that vegetative and reproductive stages of crops are seriously affected by drought stress. Yield losses of rice under drought stress are accompanied with reduced plant height, number of tiller/plant, number of panicle/plant, number of spike/panicle, number of grains, grain weight etc (Dencic *et al.* 2015) as a consequence of phenotypic adaptation. An experiment was conducted on pot with four treatments (Duan *et al.* 2016) where most of the yield contributing characters shows significant reduction at both tillering+booting (T₂) stage as well as booting stage (T₃) comparing to control(T₀) and drought at the tillering stage(T₁) (Table 1).

Table 1: Different yield components of rice under drought stress

Treatments	Number of panicles/p	Grains/spike	Filled grain (%)	Thousand grain weight(g)	Yield (g/pot)
Control, T ₀	21.34	192.84	87.47	25.50	90.6
Drought at tillering stage (T ₁)	20.00	180.58	75.40	25.75	68.03
At tillering+booting stage(T ₂)	17.67	176.05	65.75	22.43	64.18
At booting stage (T ₃)	17.67	179.58	66.13	23.72	52.37

Source: (Duan *et al.* 2016)

In general, rice respond to drought stress with a wide range of internal modifications leading to changes at cellular, physiological, biochemical and also molecular level. (Fig. 5) for survival under drought stress condition that are generally indications of tolerance or resistance mechanisms (Reddy *et al.* 2014; Prasad *et al.* 2015).

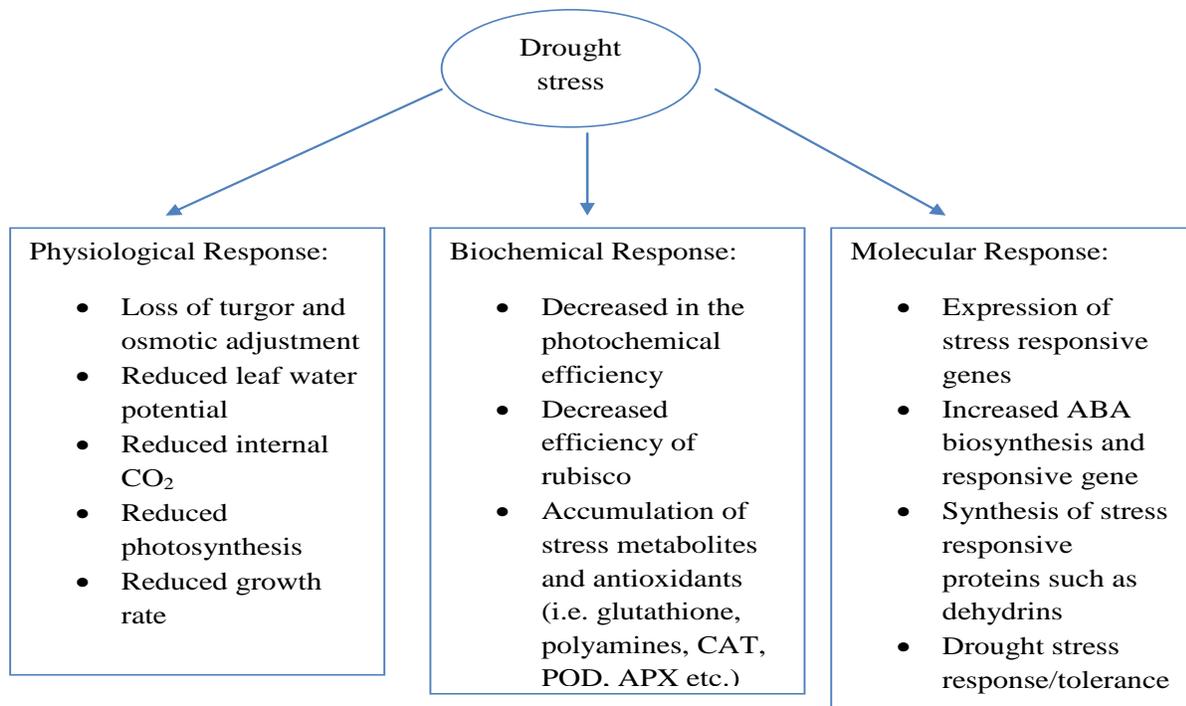


Figure 5: Physiological and molecular basis of rice resistance to drought stress (Source: modified from Reddy *et al.* 2014 and Prasad *et al.* 2015).

Physiological parameters are yield constancy characters, plays essential role for the evaluation of drought resistance rice genotypes (Dong *et al.* 2018). Several studies reported that drought stress reduces leaf water potential and causes stomatal closure resulting inhibition of photosynthetic genes, shows direct association with rice grain reduction, also low amount of CO₂ fixation lead to decline in photosynthetic amount (Fig. 6). Lowered photosynthetic amount is an outcome of inhibition of RuBisCO (ribulose-1, 5-bisphosphate carboxylase) enzyme activity and ATP synthesis under water deficit environmental condition (Dulai *et al.* 2016).

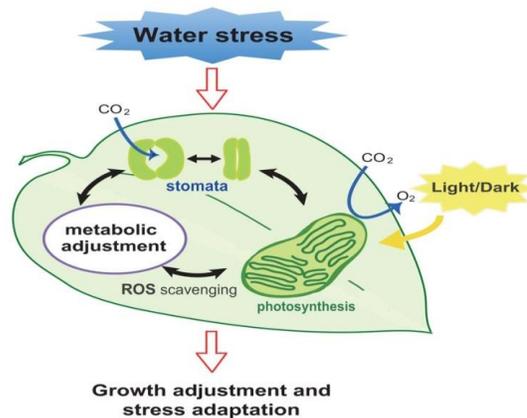


Figure 6: Stomatal resistance of rice to drought stress (Source: Dulai *et al.* 2016).

An experiment was conducted with rice cultivar by Chourasia *et al.* 2017, included drought stress treatments with PEG at 4 different concentrations: 25% (maximum), 20%, 15% and 0% (control). The results showed that, there is very little change in the soluble carbohydrates among the distinguished treatments (Fig. 7) (Yang *et al.* 2019). This indicates that soluble carbohydrate content is almost unaffected by drought stress. Even it shows an increasing trend at 15% (moderate drought stress) PEG concentration in comparison to control condition.

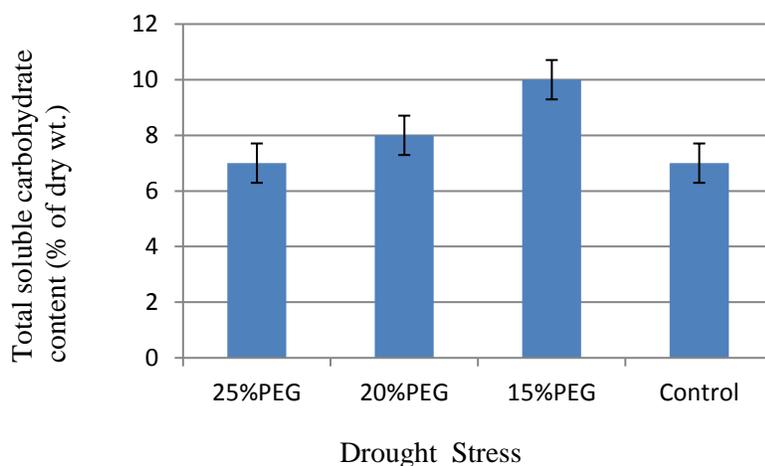


Figure 7: Total soluble carbohydrate content (% of dry wt.) of rice under drought stress (Source: Chourasia *et al.* 2017).

Generally, abiotic stresses results accumulation of more reactive oxygen species (ROS) that triggers the commencement of several scavenging events (You and Chan 2015; Moradi *et al.* 2016). During osmotic stresses, accumulation of several compatible solutes (i.e. proline, sucrose, glycine betaine etc.) increases to a higher concentration in the cytoplasm that promotes water uptake and thus assists in the process of osmotic adjustment. Among the osmolytes, proline possesses prominent ability in the considerable injury reduction attributed by osmotic stress and thus it is considered as the most important osmoprotectant under drought stress (Chourasia *et al.* 2017). Antioxidants that falls in the enzymatic category includes monodehydro ascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and ascorbate-glutathione (Sahebi *et al.* 2018). On the other hand, ascorbate (AsA), carotenoid along with glutathione (GSH) serves as non-enzymatic antioxidants inside the cell. Activity of Proline, Catalase (CAT) and Super Oxide Dismutase (SOD) of two upland rice varieties increases sharply up to maximum drought level (Table 2) (Lum *et al.* 2014).

Table 2: Proline content (mg g⁻¹ fresh weight), Catalase content (μmol min⁻¹ g⁻¹ protein) and Superoxide dismutase (U g⁻¹ protein) activity of local upland rice varieties under drought stress

Drought stresses	Proline content (mg g ⁻¹ fresh weight)		Catalase content (μmol min ⁻¹ g ⁻¹ protein)		Superoxide dismutase (U g ⁻¹ protein)	
	Becor	Kusam	Becor	Kusam	Becor	Kusam
0	19.34d	26.68 d	0.02 b	0.04 b	15.01 e	14.28 e
-2	53.95 cd	29.00 d	0.08 b	0.13 b	47.03 d	62.71 d
-4	103.86 bc	59.07 c	0.13 b	0.26 b	75.17 c	116.30 c
-6	145.52 b	76.21 b	0.16 b	0.87 a	292.44 b	116.30 c
-8	416.20 a	249.61 a	0.95 a	1.10 a	893.91 a	129.16 a

(Source: Lum *et al.* 2014)

An experiment was set up by Dong *et al.* (2018), with two rice cultivars i.e. Jinmai47 and Shiluan02 to demonstrate the antioxidant enzyme activity of superoxide dismutase (SOD) and peroxidase (POD) under drought stress condition. Here, also PEG was used to stimulate drought condition with two different concentrations (i.e. 15% and 30%). Under the 15% PEG treatment, the superoxide dismutase (SOD) and peroxidase (POD) activities increased in both cultivars when compared with the control (except for SOD activity in Shiluan02) (Fig. 7), while the activity of SOD (Fig. 8) in Jinmai47 was significantly higher than that in Shiluan02. Under the 30% PEG treatment, the POD activity in both cultivars was increased when compared with the control, while lower POD activity was detected in Shiluan02 when compared to the Jinmai47 (Fig. 8)

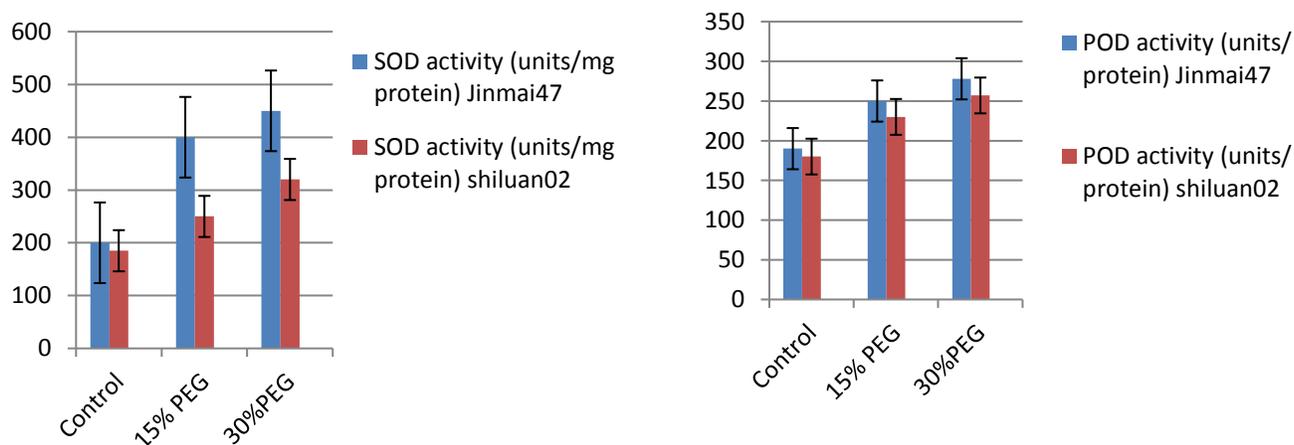


Figure 8: Effect of PEG/drought stress on antioxidant activity (i.e. SOD & POD) (Source: Dong *et al.* 2018)

Fangjun *et al.* (2012) conducted an experiment with five wild rice genotypes to identify tolerance one under drought stress condition. Among non enzymatic osmo-protectants both carotenoid and glutathione content has showed an increasing trend with the rising of drought stress but after a certain period carotenoid content declines (above 20% PEG concentration) as further enhancement of drought stress (Fig. 9). But Ac002 shows smaller reduction than others and this magnitude of reduction trend indicates better adaptable ability under stress.

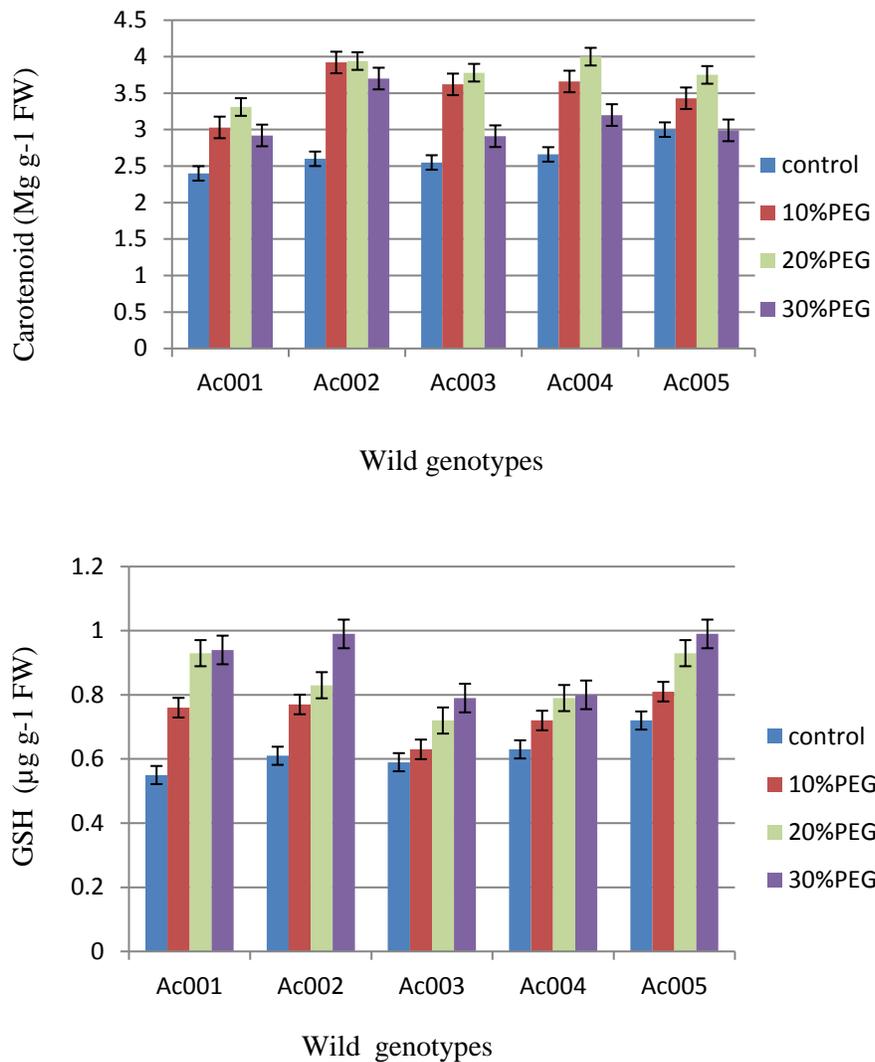


Figure 9: Carotenoid and Glutathione (GSH) content of some wild rice genotypes under drought stress (Source: Fangjun *et al.* 2012).

Table 3 showed combined correlation study under drought stress for total soluble sugars, proline content and membrane stability index (MSI) of rice. Results indicated that all biochemical parameters are highly significant with each other (Kadam *et al.* 2017) under water deficit environment.

Table 3: Combined correlation study under drought stress for total soluble sugars, proline content and membrane stability index (MSI) of rice

	Sucrose	Glucose	Proline	MSI
Sucrose	1.000			
Glucose	0.806**	1.000		
Proline	0.928**	0.761**	1.000	
MSI	0.671**	0.432**	0.738**	1.000

(Source: Kadam *et al.* 2017)

Abscisic acid (ABA), cytokinins (CK), Jasmonic acid and ethylene are most prominent and vital plant hormones in plants, plays essential role in the drought resistance mechanism (Ozga *et al.* 2016). Normally, rate of ABA synthesis increases under drought stress to convey stress transduction signaling pathway and causes reduction in the CKs as the results of decreasing the rate and extent normal photosynthetic gene expression regulation (Fig. 10) (Li *et al.* 2017; Raghavendra *et al.* 2010).

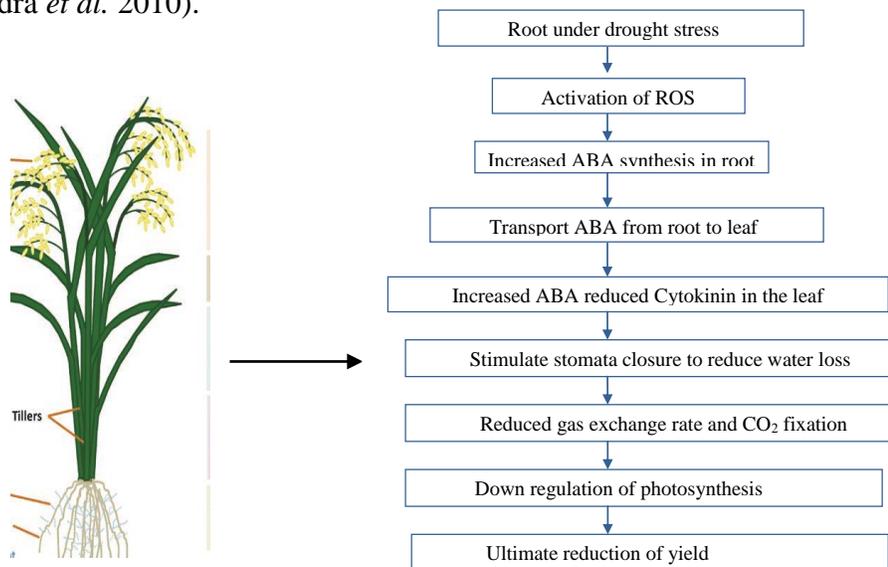


Figure 10: A possible flow chart illustrating the mechanism of root-sourced signaling strategies in rice under drought stress (Source: modified from Li *et al.* 2017 and Raghavendra *et al.* 2010).

There are a number of genes that regulates stress tolerance mechanism by producing diverse enzymes as well as proteins such as, late embryogenesis abundant (lea), responsible for abscisic acid (ABA) synthesis, rubisco (Rubp), helicase, proline, glutathione-S transferase (GST), along with carbohydrates under drought stress (Zheng *et al.* 2013). Gene(s) associated with drought tolerance improve our perceptive of this complicated phenomenon. In rice, a number of practical genomic studies have been undertaken for detection and identification of drought responsible gene expression.

3.3 Breeding methods of rice for drought resistance

3.3.1 Conventional breeding:

In recent times, pedigree selection, recurrent selection, backcrossing, along with induced mutation are the main methods in conventional breeding. The foremost trouble of abiotic stress tolerance is its complications ensuing from numerous factors such as the limitation in the efficient selection criterion, little genetic diversity under water-deficit condition in favor of yield in addition to yield component (Atkinson and Urwin, 2012). However, selection of high yielding variety is the chief target in plant breeding program under well watered condition as in several cases, high yielding variety is able to still produce moderate to high yield under water-deficit environment (Dixit *et al.* 2014). Fundamentally, conventional breeding methods plays important role in the conservation of germplasm and hybridization among sexually divergent parent, as well as in the improvement of idle genetic characters.

Many genes can be combined together through pedigree selection governing both biotic and abiotic traits, one of the most important advantages of pedigree selection (Posadas *et al.* 2014). However, the main drawback of pedigree selection is that it consumes long period plus need to evaluate many lines occasionally over the planting seasons and also need to keep a record on selection a major criterion. (Posadas *et al.* 2014).

Recurrent selection is usually used for the varietal perfection in self pollinating crops that includes multiple crosses to assemble favorable genes maintaining the genetic diversity. It requires less time, more accurate genetic gains can be achieved, as well as exceedingly diverse breeding lines can be developed of. The method has been broadly studied in maize (Bolaños and Edmeades, 2013), rice (Pang *et al.* 2017), wheat (Wiersma *et al.* 2001) in addition to soybean (Posadas *et al.* 2014).The overall selection procedure for the development of drought tolerant in rice is presented in Figure 11.

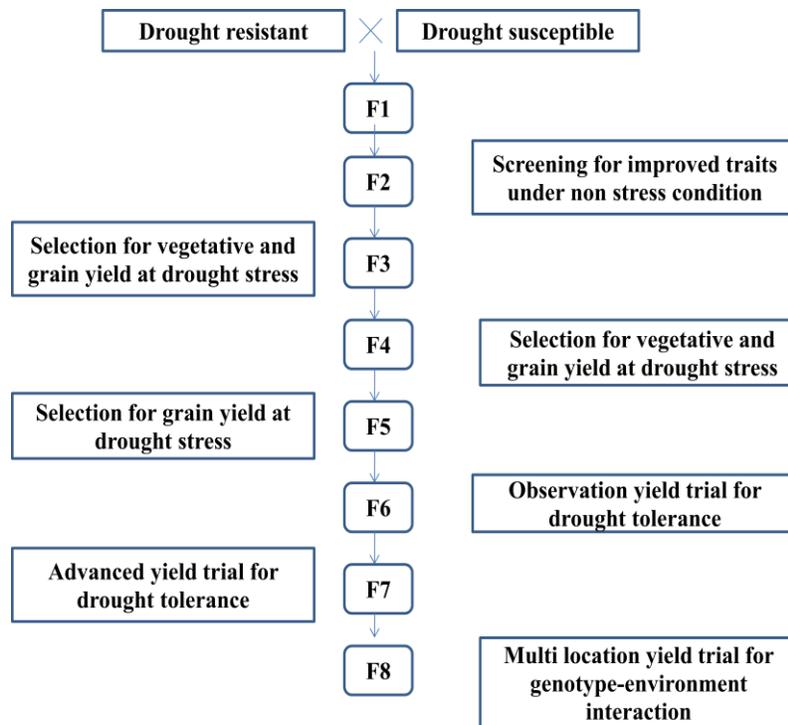


Figure 11: The overall selection procedure for the development of drought tolerance (Source: Miah *et al.* 2013)

Generally, conventional backcrossing is used for the transfer of a particular gene controlling a most wanted trait from a donor parent to a recipient parent that lacks that gene or trait for the aim of recovery of the recipient parent. This method triggers a perfect as well as accurate means of developing an enormous number of advanced breeding lines (Chukwu *et al.* 2019). The exploit of backcrossing breeding has lead to the improvement of rice with drought-tolerant strategy (Lafitte *et al.* 2006).

A high yielding super green rice variety including drought tolerant trait even under low fertilizer-water-deficit condition has been developed in Indonesia through the exploitation of induced mutation (Effendi *et al.* 2017). Two superior lines namely MR219-9 and MR219-4 have been developed from well known MR219 rice variety in Malaysia, possessing higher yield potential as well as drought resistance characters (Rahim *et al.* 2012). The international rice research institute (IRRI) has generated wide range rice varieties with disease resistance and drought tolerance over the past three decades by means of conventional breeding techniques (Table 4) (Kumar *et al.* 2014).

Table 4: High-yielding drought-tolerant varieties developed from IRRI's drought breeding programme and released in different countries of South and Southeast Asia and Africa

Name	Designation	Days to maturity	Plant height	Country of release, release year
Sahod Ulan 1	IR74371-54-1-1	110	104	Philippines, 2009
Hardinath 1	IR80411-B-49-1-1	115	100	Nepal, 2009
Sahbhagi dhan	IR74371-70-1-1	110	104	India, 2010
BRR1 dhan56	IR74371-70-1-1	110	108	Bangladesh, 2011
BRR1 dhan57	IR74371-70-1-1	110	108	Bangladesh, 2011
Sookha dhan 3	IR74371-70-1-1	110	108	Nepal, 2011
Sookha dhan 1	IR74371-46-1-1	110	101	Nepal, 2011
Sookha dhan 2	IR74371-54-1-1	110	104	Nepal, 2011
Katihian 1	IR79913-B-176-B-4	105	90	Philippines, 2011
Sahod Ulan 3	IR81412-B-B-82-1	120	107	Philippines, 2011
Inpago LIPI Go 1	IR79971-B-191-B-B	105	119	Indonesia, 2011
Inpago LIPI Go 2	IR79971-B-227-B-B	113	114	Indonesia, 2011
M'ZIVA	IR77080-B-B-34-3	120	130	Mozambique, 2013
UPIA3	IR74371-54-1-1	110	104	Nigeria, 2013

(Source: Kumar *et al.* 2014)

With the commencement of advanced biotechnological approaches, our knowledge and understanding related to plant responses under drought inside the whole plant body as well as at molecular level has amplified promptly. Modern methods, such as proteomics, genome-wide selection, stable isotopes, in addition to fluorescence or thermal imaging, found to have contributed in order to filling the gap between genotype–phenotype. The major biotechnological tools are molecular technology and genetic engineering that has led to the development of drought tolerance in rice.

3.3.2 Biotechnological and molecular approaches for drought resistance:

With the improvement of plant physiology, our understanding is also rising regarding the complex drought-tolerant mechanisms as well as its correlation with diverse traits, screening efficacy using molecular markers and several genomic tools for successful detection of QTLs as well as alleles related to drought tolerant traits. The discovery of the targeted genes accountable for plant tolerance ability at diverse abiotic stress is important for developing genetically modified crops with improved drought stress tolerance (Gosal *et al.* 2009).

3.3.2.1 QTLs for drought resistance (list of QTL genes for drought):

The mapping technique involves five major steps: (i) development of mapping population (b) detection of polymorphism; (c) high-density sequencing of interesting candidate genes or the panel's genotyping markers; (d) studying the level of genetic differentiation amongst the groups within the particular population (population structure) and the relatedness coefficient between individual pairs within the population (kinship); and (e) analyzing the association mapping according to data obtained on population structure, kinship, and the correlation of genotypic and phenotypic data (Sahebi *et al.* 2018). In précis, several QTLs associated to drought tolerance mechanism in rice have already been identified (Table 5).

Table 5: Identified QTLs for shoot and root responses under drought stress condition in rice

Trait	Population	Marker	Type	QTL	References
Seedling drought resistance	Indica ×Azucena	RFLP, AFLP & SSR	Recombinant inbred line	7	Zheng <i>et al.</i> 2013
Cellular membrane stability	IR62266 ×CT9993	RFLP, AFLP & SSR	Doubled haploid line	9	Tripathy <i>et al.</i> 2000
Leaf water relations and rolling	Azucena ×Bala	RFLP, AFLP & SSR	Recombinant inbred line	13	Khowaja <i>et al.</i> 2008
Seed fertility, spikelet per panicle and grain yield	Teqing ×Lemont	SNP	Introgression lines	5	Wang <i>et al.</i> 2014

Root number, thickness, length, and penetration index	IR58821 ×IR52561	AFLP & RFLP	Recombinant inbred line	28	Ali <i>et al.</i> 2000
Root architecture and distribution	IR64 ×Azucena	RFLP	Bulk- segregant and Selective genotyping	39	Zheng <i>et al.</i> 2013
Root traits	IR1552 ×Azucena	SSR	Recombinant inbred line	23	Lou <i>et al.</i> 2015
Deep roots	3 populations	SSR, SNP	Recombinant inbred line	6	Vikram <i>et al.</i> 2012
Root penetration, root number, and tiller number	CO39 ×Moroberekan	RFLP	Recombinant inbred line	39	Venuprasad <i>et al.</i> 2012
Root-penetration	Azucena ×Bala	RFLP & AFLP	Recombinant inbred line	18	Yadav <i>et al.</i> 2013
Grain yield under drought	Two population	SSR		1	Palanog <i>et al.</i> 2014
Grain yield in aerobic environments	Three populations	SSR	Recombinant inbred lines	1	Hemamalini <i>et al.</i> 2000
Dehydration avoidance	Bala ×Azucena	RFLP, AFLP & SSR	Recombinant inbred line	15	Price <i>et al.</i> 2002
Osmotic adjustment and Dehydration tolerance	CO39 ×Moroberekan	RFLP	Recombinant inbred line	17	Lilley <i>et al.</i> 1996
Osmotic adjustment	CT9993 × IR62266	RFLP, AFLP & SSR	Doubled haploid line	5	Zhang <i>et al.</i> 2001

3.3.2.2 Genetic engineering for drought resistance:

Plants comprise constant pathways including signal transduction process under drought stress by accumulating diverse groups of genes that include transcriptional factors, molecular chaperones, enzymes as well as other useful proteins (Usman *et al.* 2017). In reality, hundreds or even thousands of these genes (regulatory element and protein) have been identified by means of various genomic tools and techniques. In case of rice genome, identified drought tolerance genes have been incorporated in order to observe their effect on drought perfection either by suppression or over expression as indicated in Table 6.

Table 6: Drought resistance gene that has been identified in rice

Gene action	Gene	Transformation	Phenotype	References
Arginine decarboxylase	ADC	Biolistic	Reduction in chlorophyll under water deficiency	Capell <i>et al.</i> 2004
Polyamine synthesis	ADC	Agrobacterium	Improved drought tolerance by producing higher levels of putrescine and spermine synthesis	Du <i>et al.</i> 2010
abscisic acid Metabolism	CaMV35SP	Agrobacterium	Oxidative and drought stress resistance and increase of the xanthophylls	You <i>et al.</i> 2012
Amino acid metabolism	OsOAT	Agrobacterium	Improve drought tolerance and increase seed setting	You <i>et al.</i> 2013; Li <i>et al.</i> 2011
Reactive oxygen species scavenging	OsSRO1c	Agrobacterium	Oxidative stress tolerance and stomata closure regulation	Phung <i>et al.</i> 2011
Trehalose synthesis	OsTPS1 TPSP	Agrobacterium	Tolerance of rice seedling to drought, cold, and high salinity	Duan <i>et al.</i> 2012

Proline synthesis	P5CS P5CS	Biolistic	Increased biomass production under salinity and drought stresses	Zhang <i>et al.</i> 2010
LEA (Late Embryogenes is Abundant) protein gene	HVA1 HVA1 HVA1 OsLEA3-1 OsLEA3-2	Agrobacterium Agrobacterium Agrobacterium Agrobacterium Agrobacterium Biolistic	Cell membrane stability, higher leaf relative water content (RWC), Drought and salinity tolerance, Increased drought tolerance and salinity stress	Bae <i>et al.</i> 2011
Various Regulatory Genes Transcription factor Jasmonate and ethylene-responsive factor 1	ABF3, AP37 OsZIP23 OsZIP72 JERF1 OsRDCP1	Agrobacterium Agrobacterium Agrobacterium Agrobacterium Agrobacterium	Improved salinity and drought tolerance, Drought resistance and ABA sensitivity, Enhances the osmotic and drought tolerance, Stomata regulation under drought stress	Gao <i>et al.</i> 2011 You <i>et al.</i> 2013

3.3.2.3 Marker-Assisted Selection (MAS) for drought resistance:

The common vocabulary used in current breeding methods comprise marker-assisted selection (MAS), marker-assisted pedigree selection (MAPS), genomic selection (GS) or genome-wide selection (GWS), marker-assisted recurrent selection (MARS) as well as marker-assisted backcrossing (MABC). Among the methods listed here, MABC is the most preferred as most effective and extensively used method (Miah *et al.* 2017; Usman *et al.* 2018, Chukwu *et al.* 2019). DNA based markers offer the doorway for the discovery of better genotypes in early generations (Usman *et al.* 2018).

An outline of the approach exploited in improving drought tolerance rice varieties by using marker-assisted backcrossing as well as pedigree selection is showed in Figure 12.

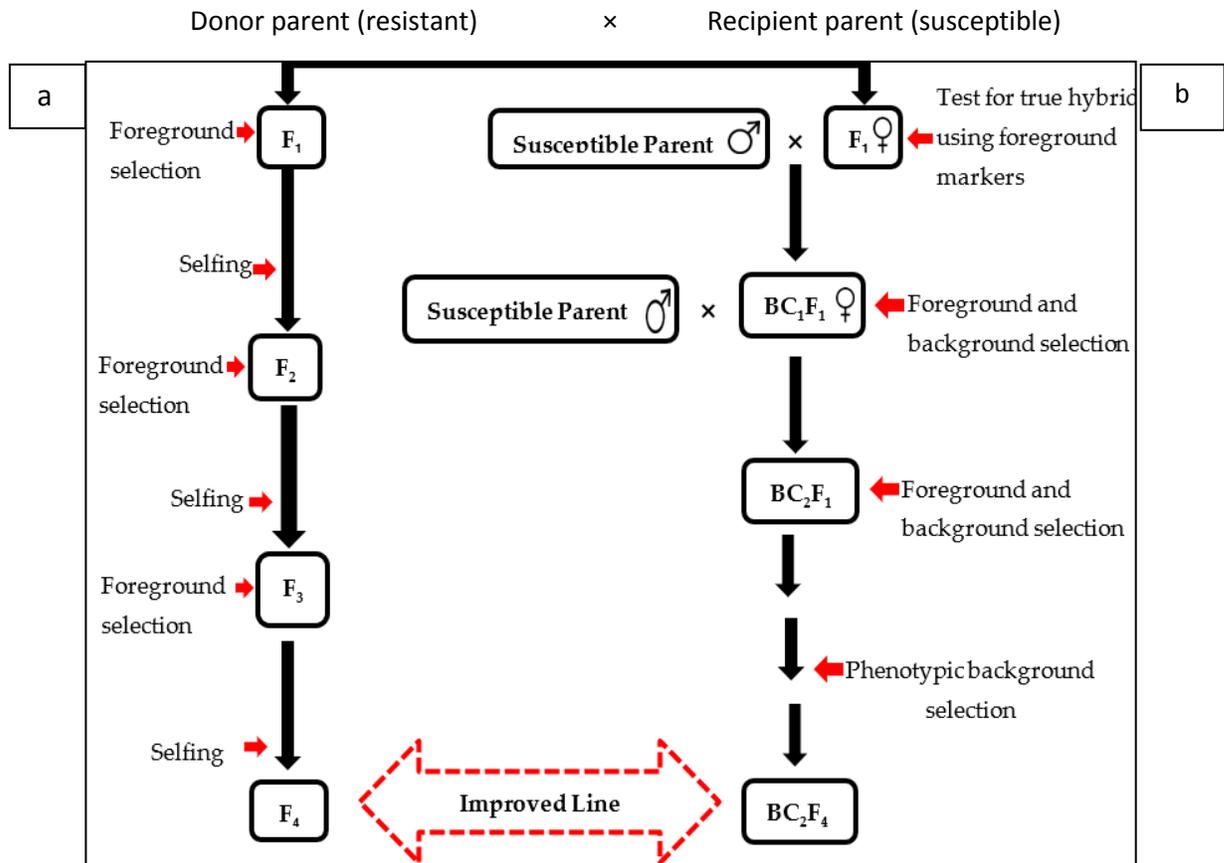


Figure 12: Method of development of drought resistance in rice through (a) marker-assisted pedigree selection; (b) marker-assisted backcrossing (Source: Usman *et al.* 2018).

Nowadays, marker assisted selection is drawing more attention to the plant breeders as well as rice breeders to develop a stable resistance rice cultivar against several abiotic stresses such as drought stress that confers more accuracy with shortest time consumption. It also provides exact selection of desired trait for incorporation to a new genotype that lacks such trait or gene.

Chapter 4

Summary and Conclusions

1. Physiological and biochemical characters such as upholding of water potential through deep root system, synchronized modulation of metabolic processes and the level of osmolytes and antioxidants are key factors in the selection for drought tolerance or resistance. Among osmolytes proline, Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POD), Glutathione (GSH) have been found to be increased significantly with the extent of drought stress and highest at 25-30% PEG (-8 to -10 water potential) concentration and provides osmotic functions inside plants body under water deficit condition.

2. To pact with the upcoming challenge for food demand and to augment crop selection effectiveness, marker-assisted breeding, along with transgenic approaches have been adopted, generating preferred traits via exogenous transformation into elite varieties. Several QTLs for drought resistance have been identified in rice; consequently, lots of efforts have been exploited using QTLs. The current progress in functional genomics has been made it promising to accomplish high throughput genotyping that aids in isolating candidate QTL controlling drought tolerance traits. Therefore, prominent cloning of such QTL for drought tolerance traits would aid for better understanding of the genetic origin of drought resistance.

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