CHAPTER 5

DISCUSSION
Rice (*Oryza sativa* L.) is the main food source for more than one third of the world’s population, providing 30–75% of the total calories to more than 3 billion Asians where 90% of the world’s rice is grown and produced. In global rice productivity scenario, irrigated rice ecosystem that comprises 55% of the world’s rice-growing area, provides 75% of global rice production. While, rainfed lowland and flood-prone area constitute 35% of the total rice area, covering 47 million ha in Asia, provides 25% of global rice production due to various biotic and abiotic factors associated with rainfed ecosystems (Naresh *et al.* 2011). It is a major challenge for the plant breeders and biotechnologists to develop new varieties that possess tolerance against different abiotic stresses. To achieve the objectives, it is essential to characterize different rice accessions against to various abiotic stresses so as to identify the genes, mechanisms involved in plant’s response to abiotic stress.

5.1 Phenotypic evaluation of rice genotypes under drought stress

Rice (*Oryza sativa* L.) has evolved as a semi-aquatic plant and therefore is very sensitive to water stress. Water deficit is one of major abiotic stresses to reduce crop production worldwide, limiting the grain yield productivity of crop species, especially in arid and semiarid zones (>1.2 billion hectares) (Kjine 2006; Passioura 2007). Phenotypic characterization and selection of tolerance rice genotypes for water deficit tolerant trait is a fruitful subject to solve the problems of the dry land rice crop (Ndijondjop *et al.* 2010; Guan *et al.* 2010). The phenotypic screening of large numbers of rice genotypes is confronted with high cost involved in the techniques and the procedures to quantify the important physiological responses. The use of visual drought scoring has been the common technique widely practiced in rice (Wamala and Maia Huali 1993).

To identify tolerant rice genotypes, phenotyping experiments against drought stress were conducted under upland field conditions at the experimental farm of Krishi Vigyan Kendra (KVK), Santhapur and later under controlled rainout shelter conditions at Central Rice Research Institute, Cuttack.

In the study conducted at experimental farm of KrishiVigyan Kendra (KVK), Santhapur, a set of 758 BAM rice accessions were evaluated against drought stress at vegetative stage (Fig. 13). Among them, data could be collected only from 408 rice accessions which survived the
stress. Of them, 116 accessions were scored as drought tolerant with a SES score “0-3” while the remaining 292 accessions were scored as susceptible with a SES score of ’5-9’ (Fig. 15). Narrowing down the number further, only twenty three (7 with score ’0’ and 16 with SES score ‘1’) genotypes could be classified as highly tolerant and tolerant against drought at vegetative stage.

5.2 Evaluation of rice accessions under rainout shelter

These 116 genotypes selected from direct seeding experiment conducted at santhapur were screened again under Rainout Shelter conditions at Central Rice Research Institute, Cuttack (Fig. 14). The experiment has the objective of looking for traits that are suitable for screening rice that influence plant water relations under drought conditions (Jha et al. 1997). Of the 116 accessions, 38 rice accessions were scored as drought tolerant (“0-3” score) while 78 were scored as susceptible (’5-9’). Of these 38, twenty five genotypes having score of 0-1 (8 with score ’0’ and 17 with SES score ‘1’) were good against drought at vegetative stage and they also showed early recovery. Based on the physiological parameters, 24 genotypes had more than 75% of RWC% and 4genotypes had >40 SPAD value. Based on the pooled data, (drought score, SPAD and RWC), 13 genotypes (BAM8, BAM28, BAM46, BAM47, BAM715, BAM731, BAM859, BAM860, BAM971, BAM988, BAM1209, BAM1218 and BAM1243) were identified as most promising (Table 10).

A preliminary screening followed by screening under controlled conditions can greatly help in narrowing down the number of genotypes when the numbers of germplasm accessions are more in number. This was followed by four three different phenotyping experiments to study shoot and root traits and germination under drought stress and an experiment to test whether drought tolerance and salinity tolerance mechanisms are related (Fig. 16). After identification and confirmation of the tolerant genotypes, molecular analyses were conducted to understand the genes that are associated with tolerance mechanisms.

5.3 Morphological and physiological indicators of tolerance to drought, salinity

5.3.1 Leaf rolling and leaf drying

The leaf rolling was used as a visual score for selecting for drought tolerance in rice earlier (Loreo et al. 1976; Steponkus et al. 1982). Leaf rolling is induced by the loss of turgor and poor osmotic adjustment in rice and delayed leaf rolling is an indication of turgor maintenance and dehydration avoidance (Blum 1989). The mechanism of leaf rolling, smaller
degree of leaf rolling is a greater indicative of a degree of dehydration avoidance by the
development of deep roots. Mackill (1991) reported that delayed leaf rolling was positively
related to drought resistance. Jone (1979) showed that degree of leaf rolling at a particular leaf
water potential depends on the cultivars to adjust osmotic balance i.e. tolerance at low water
potential. This implies that screening for drought tolerance on the basis of leaf rolling can also
lead to selection of a range of mechanisms. It also suggests that the variations observed among
the genotypes for leaf rolling and leaf drying traits are under genetic control and selection based
on these traits will be of help in identification of tolerant genotypes. The selection of 13
genotypes (BAM50, BAM61, BAM251, BAM295, BAM731, BAM859, BAM2635, BAM3160,
BAM3252, BAM3414, BAM3625, BAM3414, and BAM4060) that showed less leaf rolling and
leaf drying in all the treatments is an indicative of the wide variation existing in rice. It also
suggests that the variations observed among the genotypes for leaf rolling and leaf drying traits
are under genetic control and selection based on these traits will be of help in identification of
tolerant genotypes.

5. 3. 2 Physiological traits (RWC, Chlorophyll, Photosynthetic rate and Proline
accumulation)

The primary criteria for evaluation of drought resistance of cultivated rice varieties are
the characterization of morphological and physiological traits and grain yield under drought
al. (2008), and Basu et al (2010) reported that, drastic effect of water stress on physiological
traits as chlorophyll, proline, relative water content, anti oxidant enzyme activities and
photosynthetic efficiency was observed in different crops. Decreasing of Relative water content
is indicated that, loss of turgidity, which leads to stomatal closure and reduced photosynthetic
rates (Lv et al. 2007). The ability to maintain higher value of RWC% under drought stress
condition has been suggested as a possible water scarcity tolerance mechanism in rice (O'Toole
and Moya 1978). Gupta and Guhey (2011) and Jongdee et al. (1998) also reported similar
findings. Altinkut et al. (2001), Colom and Vazzana (2003) also reported that maintenance of a
higher relative water content (RWC %) during the drought stress is an indicative of drought
tolerance. In the present study, out of the genotypes studied, six (BAM245, BAM295, BAM859,
BAM3252, BAM3625, BAM4060 and N22 and CR-143-2-2) tolerant genotypes had higher
amounts of relative water content suggesting that these results are in agreement with the earlier
findings of Mostajean and Eichi (2009), Suriyan et al. (2010), Chaum et al. (2010), Wang et al. (2010), Akram et al. (2013) and Kumar et al. (2014) who also analyzed the physiological traits under drought stress (Fig. 19) and (Table 11).

Chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments and a way of measuring of integrity of membrane or heat stability under drought situation (Ananthi et al. 2013). Madhan et al. (2000) reported that high amount of chlorophyll stability indicates their ability to survive under stress conditions with better availability of chlorophyll. Presence of high chlorophyll content (i.e stay green trait) contributes to regulation of photosynthesis mechanism and maintain of relative water content (Guo and Li 1996). Many studies indicated that stay-green is associated with drought tolerance under water-limited conditions and reported several crops as rice (Gholizadeh et al. 2011; Haefele et al. 2010; Hussain et al. 2011), sorghum (Benbella and Paulsen 1998; Borrell et al. 2000), maize (Haussmann et al. 2002) and wheat (Verma et al. 2004).

From the pot based drought experiment, 12 rice genotypes (BAM8, BAM47, BAM50, BAM61, BAM249, BAM295, BAM731, BAM859, BAM2813, BAM3164, BAM3252 and BAM3414) that have higher amount of chlorophyll were identified (Fig. 21) and (Table 11).

The perception of drought stress at plant level is indicated by decline in photosynthesis rate and plant growth (Cornic and Massacci 1996; Mwanamwenge et al. 1999). Another reason of decreased photosynthetic rate may be the decreased leaf water potential and relative water content under water stress due to limited irrigation, which has a pronounced effect on photosynthetic rate. In the study, gentoypes like BAM1243 (34.278 μmole/m²/sec), BAM253 (29.58 μmole/m²/sec), BAM4939 (17.59 μmole/m²/sec) and BAM2635 (14.023 μmole/m²/sec) were identified as they possess very high photosynthetic rates when compared to susceptible control IR20 (2.439 μmole/m²/sec) (Fig. 22).

Reduction in stomatal conductance could reduce photosynthetic rate by lowering CO₂ concentration (Downtown et al. 1985) and most of the rice genotypes decreased stomatal conductance significantly under water stressed conditions. This observation confirmed the findings of Subrahmanyan et al. (2006) and Shah and Paulsen (2003). Stomatal conductance and uptake of CO₂ are reduced by water deficit which ultimately affects the growth of crop plants (Scheuermann et al. 1991). The decline in photosynthesis due to water stress can resultin variations in stomatal and non-stomatal response of the plant (Ort et al. 1994; Shangguan et al.
Plants show stomatal limitations to overcome short term reduction in water availability, while the long term stress may result in non-stomatal limitation (Cornic and Briantais 1991). Minimum stomatal conductance was observed in genotypes like BAM46 (0.024 mole/m²/sec), BAM1243 (0.011 mole/m²/sec) and BAM2635 (0.011 mole/m²/sec) (Fig. 23).

5. 4 Root traits

Significant variation was observed for traits like root length, shoot length, fresh shoot weight, dry shoot weight, fresh root weight, and dry root weight. It was found that degree of leaf rolling is highly correlated with maintenance of leaf water potential (O, Toole and Moya 1987) and in the present study, leaf rolling score (LR) is considered as an alternative way to show leaf water status. Only three rice genotypes i.e. BAM47, BAM859 and N22 were scored as highly tolerant while 9 genotypes (BAM8, BAM50, BAM83, BAM295, BAM3160, BAM3164, BAM4060, CR-143-2-2, and Vandana) were tolerant (Fig. 25).

Root and shoot length is an important component of the adaptation of rice under drought-prone environments (Kondo et al. 2003; Matsui and Singh 2003) and shoot length reduction is due to the lower turgor pressure in the water stress condition (Amin et al. 2009). A total of nine genotypes had high root lengths [(BAM47 (48.5 cm), BAM1209 (47.5 cm), BAM971 (47 cm), BAM3160 (46 cm), tolerant control N22 (44.4 cm), BAM4060 (42 cm), BAM3164 (38 cm), BAM251 (37 cm) and BAM3613 (35 cm)] while the susceptible control IR20 had a root length of 12.6 cm suggesting the role of root length in conferring tolerance to drought (Fig. 26) and (Table 12).

5. 5 Germination under osmotic stress

Simulation of drought stress by polyethylene glycol (PEG) induces drought stress on plants (Turkan et al. 2005; Landjeva et al. 2008) and it can be utilized to test genotypes to isolate drought tolerant genotypes (Lawlor 1970) and this approach was used earlier (Jing and Chang 2003): Germination percentage under induced stress has been accepted as an alternate method to testing the genotypes under field conditions (Shari and Mohsen 2008). At higher level of stress, six genotypes i.e. BAM8, BAM47, BAM61, BAM3252 showed >35% germination, the values similar to N22 and Vandana, the tolerant control (Fig. 30). The present result has corroborated the earlier findings in rice (Jiang and Lafitte 2007), maize (Ibrahim et al.

5. 6 Identification of genotypes against Salinity stress

As expected, just like under drought stress, wide variation observed among the genotypes ranging from score 1 (highly tolerant) to score 9 (highly susceptible) was observed under salinity stress. Of the tested ones, only two i.e. BAM 47, BAM 971 along with controls (Pokkali, FL 479) showed high tolerance.

Only four genotypes i.e.BAM3625, BAM83, BAM47 and BAM3414along with controls i.e. Pokkali, FL 479 showed high shoot lengths under salinity stress. Interestingly, inBAM971, BAM1218 and BAM3160, the shoot growth gradually increased in both treatments while in Pokkali and FL 479, a reduction was seen in shoot length (Fig. 31). Increased root length may possibly be resulted from reallocation of photosynthesis into the root, instead of the shoot, thus causing a reduction in shoot growth. On the other hand, root length of salt-tolerant cultivar was reduced by salinity stress. While an increase in root growth in order to increase water influx is usually documented as a general response to salinity, experimental evidence indicates that reduced root and increased shoot growth may improve salinity tolerance by restricting the flux of toxic ions to the shoot and consequently by delaying the onset of the tolerance threshold (Moya et al. 1999; Dalton et al. 2000; Maggio et al. 2007). This factor may possibly contribute to salinity tolerance in the salt-tolerant rice cultivar. Similar results of increase in shoot and decrease in root growth was also observed earlier (Rodriguez et al. 2006; Purnendu et al. 2004; Maiti et al. 2006), but Munns and Tester (2008) and Maiti et al. (2006) reported that salinity may directly or indirectly inhibit. Roy et al. (2002) reported that reduction of dry biomass increased with the increased of salinity level.

5. 7 Promising genotype against both drought and salinity

In the present study, the characterization of genotypes for both morphological and physiological traits under, resulted in identification of seven rice genotypes i.e.BAM47, BAM731, BAM859, BAM971, BAM3160, BAM3252, and BAM 4060 showed high levels of tolerance for both drought and salinity stresses. These rice genotypes can be utilized in the breeding programs to develop varieties with tolerance to both drought and salinity. These genotypes also can be used in discovery of new genes that function in protection of the cells
(e.g., enzymes for generating protective metabolites and proteins), and regulatory genes that
regulate stress response (e.g., transcription factors and protein kinases).

5. 8 Molecular analyses

5. 8.1 Microsatellite profiling

Molecular marker technology serves as a tool for selecting such complex traits and
allows breeders to track genetic loci controlling abiotic stress tolerance traits in rice, without
having to measure the phenotype, thus reducing the need for extensive field testing over space
and time (Nguyen et al. 1997).

In the present study, molecular profiling was done using microsatellite markers linked to
QTLs related to drought tolerance traits (Chandrababu et al. 2003; Akihiko et al. 2008;
Kanagaraj et al. 2010; Li et al. 2011; Temnykh et al. 2011). The molecular markers such as
RM219, RM212 (McCouch et al. 2002, Boopathi 2008; Bernier et al. 2008; Yue et al. 2006),
RM440 and RM289 (Cheng et al. 2001; Thomson et al. 2003; Yun et al. 2013), RM545, RM81
(Xing et al. 2002; Shuxian et al. 2013; Jonaliza et al. 2004), RM256 (Venuprasad et al. 2009),
RM208, RM324, RM250 (Nguyen et al. 2006; Zhou et al. 2011; Shalabh et al. 2012; Isaac et al.
2011) and RM314 and RM276 (Bernier et al. 2008) has been found to be linked with drought
resistance traits such as plant height, biomass, proline content deep root mass, leaf drying,
relative water content, osmotic adjustment, basal root thickness, tiller number and deep root to
shoot ratio, grain yield and panicle length, canopy temperature.

Out of the 33 markers tested, 12 SSR markers (RM208, RM212, RM81, RM256,
RM250, RM324, RM314, RM289, RM545, RM276, RM440 and RM219) showed high
polymorphism (Fig. 38) and these markers were located on different chromosomes (1, 2, 3, 5, 6,
8 and 9) while the remaining 21 markers were found to be monomorphic.

The more number of amplified alleles at the same locus, the more informative locus it
becomes to estimate variation at genotype level. From the study, it can be noted that markers RM
256, RM314, RM289 and RM545 had highest polymorphism information content value as the
PIC values for the 12 markers varied from 0.129 (RM250) to 0.493 (RM545) with an average
PIC value of 0.37 (Fig. 39).

5. 8.2 Genetic relationships of rice genotypes

The SSR markers provide adequate power of resolution to discriminate between different
rice genotypes and it could serve as a potential tool in the identification and characterization of
genetically distant cultivars. The pair wise genetic dissimilarity of the genotypes indicates that highest genetic dissimilarity was between BAM971 and BAM253 is (85.61%), BAM3252 and BAM2813 is (85.41%). The lowest genetic dissimilarity among the 43 rice genotypes tested was between BAM290 and Vandana (0.04%) (Table 14).

In the dendrogram drawn from the data, the genotypes were grouped into two major groups: group-A and group-B. Group-A consisted of different subgroups (Fig. 40). The subgroup AIV was largest comprising of 10 genotypes, second subgroup AI contained 8 genotypes, third subgroup AV consisted of 6 genotypes, fourth sub group AIII comprised of 4 genotypes, and other sub groups of AII, AVI, AVII and AVIII contain 3 genotypes. Finally ninth sub group comprised 2 genotypes. Group B consisted of only one genotype which showed 21% genetic similarity with other 42 genotypes.

5. 9 Abiotic stress tolerance genes

The genes related to drought and Salinity tolerance can be identified by thorough understanding of abiotic stress responses in plants using gene expression profiling tools. In rice, several studies on whole-genome gene expression in response to drought and salinity have been conducted. Saijo et al. (2000) studied the response of rice to drought, salinity, cold and other stresses and found major differences between two plant species and also suggested that the downstream pathways leading to drought, salinity and cold tolerance are different from each other. Hazen et al. (2005) measured the expression of approximately half of the genes in rice (21,000 genes) in phenotypically divergent accessions and their transgressive segregants to associate stress regulated gene expression changes with quantitative trait loci (QTLs) for osmotic adjustment (OA), a trait associated with drought tolerance. Water deficit and salinity elicits complex responses at cellular, physiological, biochemical and molecular levels (Bray 1993). Such response involves the activation or suppression of many genes, triggering a variety of biochemical-molecular mechanisms. Accumulation of compatible, low molecular weight osmolytes such as sugar alcohols, amino acids and increased activity of certain enzymes has been suggested as a major mechanism that may underlie the adaptation or tolerance of plants to abiotic stresses (Yancey et al. 1982).

Genes expressed during abiotic stress are anticipated to promote cellular tolerance of dehydration through various functions and such protective functions of these gene products are predicted from the nucleotide/amino acid sequence of the genes (Bray 1993). In addition, genes
induced during drought stress conditions are thought to function in the regulation of genes for signal transduction to mediate drought stress response.

The mechanism of stress perception and cell signaling plays an important role in altering the ability of the plant to survive under abiotic stress conditions (Iba 2002; Zhu 2002). Transcription factors and protein kinases are important components of signal amplification and transduction networks conveying diverse signals to specific responses. The DREBs (dehydration responsive element binding) are members of the ERF family of transcription factors and follow ABA-independent signal transduction pathway. The two subclasses of DREBs, DREB1 and DREB2 are separately involved in cold and dehydration stress, respectively (Agarwal et al. 2006). Ras/Rho family of Ras-related plant-specific signaling molecules plays important roles in plant growth, development and acting as key regulators in responses to environmental changes in plants (Gu et al. 2004; Nibau et al. 2006). OsRacB encoded a putative protein of 197 amino acids and transcription unit was 2930 bp in length, consisting of seven exons and six introns. Gu et al. (2004) and Berken (2006) revealed that RopGTPase are master regulator involved in the negative regulation of abscisic acid (ABA) signaling and influencing to adaptation of plants to various environmental situations. The expressions of Ras/Rho genes occurred in whole leaf and single cells of Arabidopsis (Park et al. 2004), Auxin signaling pathways (Tao et al. 2005) and Salt tolerance (Luo et al. 2006).

Calmodulin is a part of the network of signal transduction pathways centered on calcium ions as second messenger in eliciting responses to different signals, including many biotic and abiotic stress signals (Epstein 1998; DeFalco et al. 2010). It's playing important roles in the structural integrity of the cell wall and intracellular regulator in plant growth and development during the stress responses (Reddy 2011). There is ample evidence for the involvement of Ca$^{2+}$ signaling in abiotic stress responses including, Mechanical stimulation (Monshausen et al. 2009; Walley and Dehesh 2010), Drought (Zhang et al. 2001; Rizhsky et al. 2004; Kaplan et al. 2006; Zong et al.2008), Osmotic (Nayyar 2003) and Salinity (Plieth 2005;Chinnusamy et al. 2004; Mahajan et al. 2005), Cold (Liu et al. 2009; Doherty et al. 2009), Flooding (Subbaiah and Sachs 2003; Lee et al. 2001) and Heat shocks (Rizhsky et al. 2004;Yoo et al. 2005; Zhang et al. 2009). OsCDPK is a class of plant protein kinases that contain a kinase domain and a Ca$^{2+}$-binding domain has been shown to be involved in multiple cellular responses to a wide range of environmental stimuli, including abiotic stresses such as cold, high salinity, and drought.
(Sanders et al. 1999). Based on the above evidences, attempts were made in this study to identify candidate genes involved in drought/salinity tolerance in rice and also sequencing analysis of these genes in phenotypic tolerant rice accessions under drought and salinity stress condition. Putative candidate genes/transcription factors as OsCDPK7 (Saijo et al. 2000-Calcium dependant protein kinase), OsDREBl (Fukao et al. 2011-Dehydration-responsive binding element), OsCam (Latha et al. 2004-Calmodulin), OsERF3 (Wan et al. 2011-Plant-specific ethylene response factors), OsGRF8 (Choi et al. 2004- Growth regulating factor) and OsRacB (Luo et al. 2004-Small guanosine triphosphate (GTP)-binding proteins) involved in salinity and dehydration tolerance in plants were chosen for analysis in this study.

5. 9. 1 Sequence alignment analysis

PCR amplification was performed in 14 selected genotypes (including tolerant, moderate tolerant and susceptible) and the PCR products were sequenced and the nucleotide sequences were aligned using Nippon bare genome as reference (Table 15) and Fig. 42 to 48).

The sequence alignment results had indicated nucleotide changes i.e. InDels and SNPs in different positions in known abiotic stress tolerance genes such as OsCDPK7, OsDREBl, OsCam, OsERF3, OsGRF8 and OsRacB. Among the six genes studied, allelic variation was observed in four genes (OsCDPK7, OsERF3, OsGRF8 and OsDREBl) in the exon sequences while for the remaining two genes i.e. OsCam, and OsRacB, the changes (allelic variation) were in the intronic region of nucleotide sequences.

5. 9. 1. 1 Calmodulin

The SNPs were located in the intron region of the nucleotide sequence of the gene. Oscam gene does not affect in the tolerance genotypes, but these SNPs only located in these two genotypes only (Fig. 42).

5. 9. 1. 2 OsERF3:

These variations in the form of SNPs and InDels in different positions. In the exon regions has generated interest. It appears that the change at the 588th position, no clear conclusion is evident from this change as it could not distinguish tolerant and susceptible genotypes. However, In the 615th position, tolerant controls N22, CR-143-2-2, and reference genome Nippon bare, BAM859 (tolerant), BAM731 (tolerant), BAM3252 (tolerant), BAM4060 (tolerant) and BAM47(tolerant) having “A “nucleotide changing to “G” allele nucleotide in susceptible genotypes as IR20, BAM8(susceptible), BAM295(susceptible), BAM28(susceptible),
BAM1243(susceptible), BAM83(susceptible) and BAM290(susceptible) (Fig. 43). This change appears to confer tolerance as A is related to tolerance while G is related to susceptibility.

5.9.1.3 OsGRF8:

In IR20, the susceptible control and BAM8 (susceptible), BAM83 (susceptible), BAM295 (susceptible) had “TC”. In BAM47, BAM731, BAM4060, BAM3252, BAM290, BAM1243, Nippon bare and including tolerant controls N22, CR-143-2-2 had “CA”. In IR20, the susceptible control and BAM8 (susceptible), BAM83 (susceptible), BAM295 (susceptible) had “TTG” while in BAM47, BAM731, BAM4060, BAM3252, BAM290, BAM1243, Nippon bare and tolerant controls N22, CR-143-2-2 had “GAT” (Fig. 44). This alleles having TC and TTG in these positions is related to susceptibility while change to CA and GAT leads to tolerance. SNPs showing “A” at the position 1456 in the susceptible controls IR20 and BAM1243 (susceptible), BAM28 (susceptible), BAM8 (susceptible), BAM83 (susceptible) and BAM295 (susceptible). It appears that the change observed at 1456 position to A confer susceptibility.

5.9.1.4 OsCDPK7:

These variations were located in the exon regions. At position of 1185, SNPs (A/G) was observed in tolerant rice accessions as BAM47 (tolerant), BAM859 (tolerant), BAM4060 (tolerant), and BAM731 (tolerant) and tolerant control N22 followed “A” allele. The remaining genotypes having “G” allele including one of the control CR-143-2-2, IR20, BAM8, BAM28, BAM83, BAM290, BAM295, BAM1243, BAM3252 and reference genome Nippon bare (Fig. 45). The change at position appears to be inconclusive as CR 143-2-2 also have the G nucleotide at 1185.

5.9.1.5 OsDREB1:

These variations were located in the exon regions. At the position of 927 (G/A) and 947 (A/T) SNPs were observed in only in susceptible control IR20 and phenotypic susceptible rice accessions as BAM83 (susceptible), BAM290 (susceptible), BAM295 (susceptible). It appears that the change at positions 927 (to G) and 947 (to A) confer susceptibility to the genotype (Fig. 46).

The change at 904-907 (AGA/ CAG) was observed in only BAM83 (susceptible) and BAM290 (susceptible) susceptible rice accessions having “AGA” and remaining all BAM accession including tolerant controls N22, CR-143-2-2, and susceptible control IR20 having
“CAG” nucleotide sequence was observed. The change is does not appear to be related to either tolerance or susceptibility.

5. 9. 1. 6 OsRacB:

These variations were located in the intron regions. A single SNPs was identified at the position of 1974 (G/C) in tolerance rice accessions as BAM47 (tolerant), BAM83 (susceptible), BAM731 (tolerant), BAM859 (tolerant), BAM4060 (tolerant), and tolerant controls such as CR-143-2-2, N22 and Nippon bare having “G” allele while all susceptible BAM accessions and IR20 has “C” at the position (Fig. 47). The sequence of Nippon bare has similar sequence pattern as that of the tolerant controls and tolerant BAM accessions. Though a relationship could be established, since the change is at the intron region, it may not be of practical value. From the analysis it is possible to differentiate tolerant accessions from the susceptible rice accessions by following the alleles of three genes OsCDPK7, OsERF3 and OsGRF8 that are known confer tolerance to drought and salinity.

From the sequence pattern, six genotypes i.e. BAM47, BAM290, BAM731, BAM859, BAM3252 and BAM4060 were similar to N22, CR-143-2-2, the tolerant controls. But, all phenotyping data suggest that four rice accessions i.e. BAM47, BAM731, BAM859, and BAM4060 showed tolerance similar to N22, CR-143-2-2, the tolerant controls indicating that some of these studied genes, may not be involved in conferring tolerance. This evident from data obtained from accessions like BAM 290 and BAM3252 which possess sequences similar to the tolerant controls, they are not highly tolerant to stress.

5. 9. 2 Sequence similarity and identity

Similarity and identity each have a distinct meaning. Identity is defined by the extent to which two nucleotide sequences are invariant. Therefore, percent identity was given in terms of the fraction of nucleotides according to base-to-base level within an alignment between two sequences. Similarity is the extent to which the nucleotide sequences are related and were calculated by including sequence gaps and mismatches. The sequence similarity between the controls and promising accessions for different genes:

Among the total genotypes, CR143-2-2, the tolerant control was close to BAM245 (tolerant), BAM3613 (tolerant) and BAM731 (tolerant), Vandana was close to BAM859 (tolerant), BAM46 (susceptible) and BAM50 (tolerant) and another tolerant control N22 was closed to BAM 28 (susceptible), BAM47 (tolerant) and BAM271 (susceptible) (Table 16).
5.9.3 Phylogenetic tree analyses

The phylogenetic tree analysis of OsDREB1 gene results suggest that susceptible control IR20 is closely associated with BAM83, BAM290 and BAM295 and this group of tree also includes BAM47, BAM8 and N22 and CR-143-2-2. In case of OsCDPK7 and OsCam genes, susceptible genotypes like BAM8, BAM28, BAM83, BAM295 and BAM1243 are similar to Nippon bare while tolerant ones are in a separate group. In case of OsRacB and OsGRF8, the Nippon bare sequence is similar tolerant genotypes while susceptible genotypes had another tree structure. In case of OsERF3, Nippon bare is similar to tolerant genotypes like BAM290, BAM731, BAM3252 and BAM4060, while susceptible genotypes like BAM8 followed the IR20 pattern (Fig. 48).