

In recent years, integrative breeding strategies have gained significant attention for enhancing plant stress tolerance, combining traditional and modern methodologies to achieve sustainable agricultural development. Therefore, this chapter is focused on a detailed description of the conventional and modern breeding techniques for developing stress-tolerant varieties of *O. sativa*. Section 2.1 provides an overview of the various breeding methods such as pure line selection, pedigree, back cross and much more. In addition, this section describes the advent of modern plant breeding methods including genomic assisted breeding, genome editing, multi-omics approaches, etc., which has revolutionized our ability to dissect complex stress tolerance traits and expedite improvement in *O. sativa*. Furthermore, complementing these advancements, computational approaches have emerged as powerful tools to explore and analyze stress tolerance mechanisms in *O. sativa*. This is explored in section 2.2 including the aims of this thesis to utilize computational methods for developing stress-resilient *O. sativa* cultivars addressing the challenges of global food security.

2.1 Integrative breeding strategies in plant stress tolerance

2.1.1 Conventional plant breeding approaches

Plant breeding involves the modification or improvement of desired characteristics of plants, to develop superior and new varieties of crops aimed at the betterment of human welfare. These improved varieties typically show better quality, enhanced nutritional value, and maximized yield, due to which they are more economically and agronomically advantageous. Thus, a successful variety is often characterised by a balanced combination of traits that make it more financially advantageous for producers. However, several factors, such as agricultural practices, biotic and abiotic stresses and consumer needs, affect the development of these advanced varieties. Therefore, plant breeders must remain continuously well-versed in these factors and adapt their breeding strategies accordingly. The process of developing new varieties generally follows a series of interconnected steps, including the generation of genetic variation, selection, evaluation, multiplication, and distribution [Akhtar *et al.*, 2023].

Conventional breeding methods do not utilise advanced molecular breeding techniques to create new plant varieties. Thus, the law of inheritance remains intact in conventional breeding. In this approach, the traits of interest are selected based on their greater performance on predetermined traits [Akhtar *et al.*, 2023]. The hybridization technique is commonly utilized to introduce traits of interest from closely related species and integrate them into new varieties; hence, genotype selection is primarily driven by phenotype. The selected plant varieties are then tested and evaluated before being released as new cultivars. Generally, the selected varieties are not error-free, as genotype-by-environment interactions can affect their phenotypic expression. Thus, conventional breeding is an applied discipline that relies extensively on the knowledge, experiences, and observations of plant breeders.

Conventional breeding consists of different methods used for both self and cross-pollinated plants. For self-pollinated plants methods such as pure-line selection, mass selection, pedigree and back cross methods are available. In cross-pollinated plants, recurrent selection and hybridization are the commonly used approaches for conventional plant breeding.

1. Pure line selection

The selection within a single population is one of the earliest techniques used in plant breeding. For instance, this method has been applied to self-pollinated crops such as *O. sativa* [Lamichhane and Thapa, 2022]. The pure lines are the progeny resulting from the self-pollination of a single homozygous parent. This method is unable to generate novel genotypes as the progeny share the same genotype with their parents. Furthermore, only superior homozygous parental genotypes are selected, which are subjected to self-pollination. The offspring derived from each parental genotype are cultivated and evaluated separately for desired traits. Second-generation offspring with superior characteristics undergo initial yield trials. These are followed by multi-location trials, often involving check varieties, to evaluate performance across different environments. After several years of conducting trials in multiple locations, improved genotypes are released as a new variety. The entire process of variety release typically spans approximately six to seven years. The pure-line method is mainly utilized for the improvement of local or older pure line and self-pollinated crop varieties.

2. Mass selection

In the mass selection method, a large plant population with similar phenotypes are selected, and their seeds are combined to produce a novel variety suited for both self and cross-pollinated crops. It is employed to increase the productivity of the base population by enhancing the prevalence of traits of interest. The plants with highly heritable traits are selected for the breeding program, and plants with defective traits are rejected. The phenotype of the progenies is selected based on various traits such as plant height, disease resistance, and early or late maturing [Lamichhane and Thapa, 2022]. Further, after the selection of the individuals, they are sown in the field, and harvested seeds are mixed for sowing for the next generation. These mixed and harvested seeds are then grown in bulk quantity resulting in the preliminary yield trials. Subsequently, the varieties obtained from these trials are tested at different locations over the next three to four years. By the seventh year, the most promising varieties are released as new cultivars. Additionally, this method is used to improve local varieties and purify existing pure-line varieties [Akhtar *et al.*, 2023].

3. Pedigree method

Pedigree breeding involves creating segregating populations by crossing parent plants. These populations are then subjected to generations of self-pollination and selection until a set of derived lines is established that combines the positive traits of both parents [Bressegello and Coelho, 2013]. This method is particularly effective for breeding traits that are easy to classify and of a qualitative nature, such as disease resistance, plant structure, and the color or shape of plant parts. This method is advantageous for breeders as various types of good traits of different plants can be combined in a single plant, and the genealogy of each new variety can be traced. However, the success rate of varieties developed by this method is generally low, as the yield evaluation occurs only when the seeds are ready for the replicated trials. Although there are fewer chances of improvement in the yield potential, plant breeders still utilize this method to enhance specific traits in crop varieties.

4. Back cross method

Back cross is a method that involves crossing between filial 1 (F1) hybrid or segregating generations with one of its homozygous parents to produce progeny that are genetically similar to the parent. For successful backcrossing, breeders require a suitable recurrent parent, a donor parent having traits which are lacking in the recurrent parent, heritable traits governed by one or few genes and multiple backcrosses [Lamichhane and Thapa, 2022]. The plants which contain desired traits are the donor parents, whereas recurrent parents involve plants which receive the selected genes. The backcrossed progeny from the F1 generation are referred to as backcross hybrids (BC1), which are then cultivated in separate fields for evaluation. Afterwards, these BC1 hybrids are again backcrossed with the recurrent parents, and the BC6 generation is grown for seed production. Following this, the new variety undergoes multiple yield trials at various locations, with the recurrent parent serving as a check va-

riety. After several years of testing, the improved variety is released. This breeding method is also characterized by uneven gene contributions from the two parental lines in the newly developed varieties.

5. Recurrent selection

This method includes the reselection of desired traits from successive generations to improve their frequency through crosses between heterozygous recurrent parent and inbred individuals. This method is widely utilized in cross-pollinated crops such as *O. sativa*, maize, millet and others. Moreover, there are three types of recurrent selection methods i.e., simple recurrent selection, recurrent selection for combining ability and reciprocal recurrent selection. Simple recurrent is performed on highly heritable traits until improvement in selected progenies is found. Further, the second type of recurrent selection is applied to the traits governed by incomplete dominance or over-dominance. The reciprocal recurrent selection is applied to the traits directed by both additive and non-additive gene action.

6. Hybridization

Hybridization is a conventional breeding method in which hybrids are generated through the mating of two parents from the same or genetically distinct species. This method helps overcome certain reproductive barriers, such as sexual incompatibility or male/female sterility. The two commonly used hybridization methods are inter-specific and intra-specific hybridization. The inter-specific hybridization involves crossing individuals from two different species that are sexually incompatible. This method is used to introduce traits from one species into another, expanding the genetic diversity of the offspring. Further, intra-specific hybridization occurs when two or more plants from the same species are crossed to combine desirable traits. This approach is commonly used to improve specific characteristics, such as yield or disease resistance, within a single species.

2.1.2 Modern plant breeding approaches

Over the past two decades, conventional breeding approaches have been widely utilized for the breeding of desired traits in crops. It has been used for both increasing crop productivity as well as other agronomic traits. However, conventional breeding is time-consuming, less efficient, and cannot create pure gene lines. In addition, issues such as sexual barriers and incompatibilities—both pre- and post-fertilization—pose significant challenges. To overcome these limitations and achieve more precise traits, such as high nutrient absorption, weed resistance, and reduced harvest time, conventional breeding methods have been integrated with advanced scientific disciplines [Anand *et al.*, 2023]. Modern plant breeding approaches such as genomic selection (GS), Marker Assisted Selection (MAS), Genome Wide Association Studies (GWAS), and various multi-omics technologies, are now applied to accelerate genetic improvement in crops [Akhtar *et al.*, 2023]. The shift to modern plant breeding techniques occurred when molecular techniques were incorporated with conventional breeding approaches to enhance genetic gains. One key advantage of modern techniques is genotype-based breeding, which enables the identification of pure gene lines and significantly reduces the time required to develop novel varieties. It uses specific markers such as Restriction Fragment Length Polymorphism (RFLP) for Marker Assisted Breeding and Quantitative Trait Loci (QTL) mapping, helping in rapidly selecting superior plants at the seedling stage. These methods also facilitate the identification of recessive alleles and pinpoint the locations of specific genes. Furthermore, modern breeding techniques tend to produce stable genotypes which are less affected by the genotype-to-environment interactions, making the desired traits in new varieties more reliable and consistent. To develop more stress-tolerant crop varieties, various modern plant breeding, including MAS, genomic assisted breeding, High Throughput Phenotyping (HTP), GWAS, mutation breeding, genome editing, speed breeding and multi-omics methods, are employed. These methods collectively offer a more precise, efficient, and scalable way to enhance crop resilience and productivity.

1. Marker Assisted Selection

MAS is a breeding method which utilizes molecular markers to study genomic regions involved in the expression of the desired traits, including those associated with stress tolerance. Simple sequence repeats (SSRs), RFLP, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP) and SNP are some of the majorly used molecular or DNA markers [Akhtar *et al.*, 2023]. MAS is particularly effective for selecting traits that exhibit low heritability, are expensive to evaluate, or require substantial labor and time. Several studies utilized molecular markers and MAS to select desirable traits, improving efficiency and precision in breeding programs. A recent study on Tainung82, a *Japonica* variety of *O. sativa* was performed to improve its resistance by pyramiding of five known BB-resistant genes i.e., *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* through MAS [Hsu *et al.*, 2020]. Another study on three drought yield QTLs i.e., qDTY_{2.2}, qDTY_{3.1} and qDTY_{12.1} were pyramided by MAS to improve the yield of Malaysian *O. sativa* cultivar MR219 [Shamsudin *et al.*, 2016].

2. Genomic assisted breeding

This is a modern breeding approach that employs molecular markers and GS to accelerate the development of new crop varieties with improved stress tolerance. GS allows effective study of important QTLs, even for genes with small effects, associated with desired traits. These QTLs and genes play a crucial role in understanding how crop species respond to various stresses. This makes genomic assisted breeding a promising method for analysing and improving stress tolerance. Further, this method can be particularly useful for investigating quantitative traits that are difficult or expensive to identify through traditional phenotyping methods, offering a more cost-effective and precise way to enhance crop performance. A recent study was performed on developing a high-yielding, climate-resilient *O. sativa* cultivar against multiple biotic and abiotic stresses. 15 QTLs and genes related to drought, submergence, gall midge, RB and BB diseases were employed using the GS method [Yadav *et al.*, 2021]. Furthermore, pyramiding DT QTLs qDTY_{3.2} and qDTY_{12.1} were utilized to develop near-isogenic lines of drought-susceptible varieties of *O. sativa* [Tyagi *et al.*, 2024].

3. High Throughput Phenotyping

HTP is an emerging and sophisticated approach for obtaining precise phenotypic data, which is a challenge with GS-assisted plant breeding. When combined with genomic analysis, HTP enables plant breeders to efficiently identify desired traits such as stress tolerance and high crop yield [Aziz and Masmoudi, 2024]. This method includes evaluation of the stress tolerance, yield, and plant growth through automated sensing, data acquisition, and processing. Thus, the phenotypic information obtained from the HTP method helps in trait mapping to determine the QTLs, candidate genes, and associated markers contributing to the desirable genotypes. Furthermore, the integration of genotyping and phenotyping techniques enhances the precision and effectiveness of GS, providing breeders with more opportunities to improve the efficiency and accuracy of plant breeding efforts. In addition, various HTP methods including red–green–blue images, near-infrared imaging and droughtSpotter technology were utilized to determine plant area, water content and transpiration rate to establish screening platforms for drought tolerance in *O. sativa* [Kim *et al.*, 2020]. Another study showed the detection of diseases and pests i.e., sheath blight, rice brown spot, and rice stem borer symptoms in *O. sativa* through a video detection system based on a deep convolutional neural network [Li *et al.*, 2020].

4. Genome Wide Association Studies

GWAS involves mapping large plant populations at high resolution to identify statistically significant associations between phenotypes and genotypes through linkage disequilibrium [Prabhu *et al.*, 2023]. Several studies have shown that GWAS efficiently identify genes and alleles associated with complex traits, including biotic and abiotic stress tolerance. The method benefits from a high recombination rate, substantial allelic diversity, and molecular markers, making it particularly effective for uncover-

ing novel genes that can be used in plant breeding. Recent advancements in GWAS have expanded its application to the epigenome, transcriptome, proteome, and metabolome, providing a more comprehensive view of the genetic and molecular mechanisms underlying stress tolerance. These innovations help breeders identify beneficial genes and alleles for developing stress-tolerant crop varieties [Mahmood *et al.*, 2022]. A recent study revealed 23 marker-trait associations corresponding to 107 and 210 candidate genes for BB and RB diseases through GWAS providing a broad-spectrum resistance [Alam *et al.*, 2024]. Further, several QTLs were identified through GWAS which were associated with drought-related phenotypes such as plant height, grain yield per plant, etc., thus, improving drought resistance in *O. sativa* [Wang *et al.*, 2023a].

5. Mutation breeding

This method of improving crops is performed by inducing mutations in plant DNA by applying insertional, physical, and chemical mutagens. The mutant plant having desired traits is then inspected and selected from the plant population. This method is successfully achieved for both biotic and abiotic stresses. The plant breeders utilize this method for the production of elite breeding lines and novel cultivars with desired traits. The key steps in mutational breeding include the introduction of mutations, screening for desired mutants, testing those mutants, and eventually releasing the improved varieties [Udage, 2021]. A different mutant line i.e., XM14, showed resistance to six Philippine and Japanese *Xoo* races in which the *Xa42* gene was responsible for resistance responses was identified [Viana *et al.*, 2019]. Similarly, in Indonesia, researchers developed a high-yielding, drought-tolerant *O. sativa* mutant that thrives under conditions of low fertilizer use and water deficiency through induced mutation [Oladosu *et al.*, 2019].

6. Genome editing

Genome editing, along with the MAS and GS methods, offers an innovative approach to performing plant breeding with high precision, effectiveness and cost-efficiency. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) is the widely used genome editing method of modern plant breeding approaches. It is composed of a single-guide RNA (sgRNA), Cas9 endonuclease and a protospacer adjacent motif (PAM) (5'NGG3') for creating double-stranded breaks at the targeted genomic sites [Aziz and Masmoudi, 2024]. CRISPR-Cas9 is a powerful tool for improving desired traits, i.e., tolerance against biotic and abiotic stresses in crops. Moreover, it has been reported that CRISPR-Cas9 sequence-specific nucleases engineered *OsERF922* of *O. sativa* showed improvement in RB resistance [Wang *et al.*, 2016]. Further, CRISPR-Cas9-mediated mutagenesis was utilized to target *OsERA1* in *O. sativa* has been shown to improve responses to abscisic acid and drought stress by promoting increased primary root growth under normal conditions [Ogata *et al.*, 2020].

7. Speed breeding

Speed breeding includes the manipulation of the growth and environmental conditions of crops to accelerate the breeding process by reducing the generation time in crops. It saves breeding time and resources by producing ~ 3 to 9 generations per year as compared to 1 to 2 generations created by conventional breeding [Wanga *et al.*, 2021]. The genotypes produced by this method are more stable and homozygous resulting in the rapid development of novel crop varieties. In addition, the combination of speed breeding with MAS and genome-edited breeding methods helps in the selection of high-yielding novel varieties resistant to various stresses. A recent study identified a novel pi21 haplotype for RB disease of *O. sativa* through speed breeding combining bulk sergeant analysis and whole genome sequencing [Shendekar *et al.*, 2023]. Further, the speed breeding was coupled with an SNP-assisted selection method to develop the "YNU31-2-4", a salt-tolerant cultivar of *O. sativa Japonica* [Zainuddin *et al.*, 2024].

8. Multi-omics approaches

The plant breeding process is further enhanced through multi-omics approaches, integrating genomics, transcriptomics, proteomics, metabolomics, and phenomics. These multi-omics studies are particularly effective in identifying and interpreting stress-tolerant gene functions, mechanisms, and pathways under various environmental stresses. By combining multi-omics with genome editing techniques, breeders can gain detailed insights into the molecular mechanism governing plant responses to stress, offering new strategies for improving crop resilience. This integrated approach allows for a more comprehensive understanding of how plants cope with stress, ultimately developing more robust and adaptable crop varieties. In addition, a recent study revealed that by performing RNA-Seq analysis on Aus, drought, and heat-tolerant cultivars of *O. sativa*, 56 drought tolerance differentially expressed genes (DEGs) were discovered [Ahmad, 2022]. Further, the molecular mechanism of *Xa5*, a candidate gene of the QTL qBlr5a for bacterial leaf streak disease was studied suggesting high resistance by *Xa5* [Xie *et al.*, 2020].

2.2 Advanced computational approaches to uncovering stress tolerance mechanisms in *Oryza sativa*

Approaches such as genome editing, MAS, and speed breeding rely heavily on advanced computational methods for performing plant breeding. These computational approaches enable the precise identification and selection of stress-tolerant genes, accelerating the development of new varieties of *O. sativa*. By utilizing these methods, breeders can more efficiently transfer desirable traits for stress resistance, resulting in elite varieties with improved resilience to various environmental challenges. This section explores various *in silico* methods developed to identify, characterize, and analyze stress-tolerant genes in *O. sativa*.

The first set of methods includes proteomics and structural bioinformatics analyses to identify disease-resistance proteins in *O. sativa*, specifically against *Xoo* and *M. oryzae*. In addition, a DL-based model has been developed to predict disease-resistance proteins in *O. sativa* and its related species. To assess the performance of this model, it is compared to several major ML models.

Further, transcriptomic and genome-wide analyses have been performed on *P. cineraria*, a DT species native to the Indian Thar Desert, to identify drought tolerance genes that could potentially be incorporated into *O. sativa*. These studies explore the functional characteristics, expression patterns, evolutionary relationships, and protein structures of the identified drought tolerance genes. A variant analysis between the Indian and Arabian cultivars of *P. cineraria* was also performed to further investigate the drought tolerance genes. Finally, structural and evolutionary differences in these genes were analyzed and compared between *P. cineraria* and both *O. sativa Japonica* and *Indica*, providing insights into how drought tolerance genes can be successfully integrated into *O. sativa* to develop DT varieties of *O. sativa*.

2.2.1 *In silico* characterization of novel disease-resistance proteins in *Oryza sativa Japonica* against bacterial leaf blight and rice blast diseases

O. sativa is a vital staple crop that sustains nearly half of the global population. However, its production is severely affected by both biotic and abiotic stresses. *Xoo* and *M. oryzae* is the major bacterial and fungal pathogens leading to BB and RB diseases, respectively, both of which significantly reduce *O. sativa* productivity. Although the use of pesticides has reduced the impact of pathogenic diseases, it also causes significant environmental damage and poses health risks to humans. Furthermore, excessive pesticide application has led to the development of resistance among pathogens, threatening the long-term sustainability of *O. sativa* production. Thus, to reduce the dependency on pesticides, developing disease-resistant varieties of *O. sativa* is regarded as an effective approach. In addition, *O. sativa* has its well-established defense system against *Xoo* and *M. oryzae* which is governed by various disease-resistance genes or proteins. The structural and functional information of such genes or proteins is still lacking. Thus, the current research has focused on identifying novel disease-resistance proteins in *O. sativa Japonica* and understanding the signaling mechanism accountable for the recognition of innate immunity.

In the present thesis work, computational approaches were employed to identify and characterize disease-resistance proteins against BB and RB diseases in *O. sativa*. Gene network analysis revealed five novel disease-resistance proteins against BB and RB diseases. Structural, functional, phylogenetic studies and *in silico* gene expression analysis demonstrated the presence of the identified proteins in disease resistance mechanisms. This study provides valuable insights for developing disease-resistant *O. sativa* varieties by predicting novel candidate resistance proteins, which could assist breeders in improving crop yield and enhancing food security through molecular breeding and biotechnology.

2.2.2 Deep learning-based identification of disease-resistance proteins in *Oryza sativa* and its related species

O. sativa has a well-established immune system involving disease-resistance proteins and genes, such as R proteins and PRRs. The knowledge gained from basic plant biology research has greatly contributed to plant breeding and crop improvement. However, conventional experimental methods for identifying disease-resistance proteins are time-consuming and often lack the specificity needed to enhance varieties of *O. sativa*. This limitation can delay breeding programs aimed at developing elite varieties with improved tolerance to biotic stress in *O. sativa*. In addition, various image-based detection methods of diseases in *O. sativa* are utilized. These detection methods are also time-consuming and provide a high false detection rate. Therefore, novel efficient and accurate methods are required for the rapid identification of disease-resistance proteins. In this context, computational approaches, including ML and DL, have great potential in identifying disease-resistance proteins by integrating omics data. Therefore, the next objective of this thesis work is focussed on the utilization of DL and ML models for the identification of disease-resistance proteins in *O. sativa* and its closely related species. This approach involves a proteomics dataset to predict disease-resistance proteins thus, advancing breeding goals in *O. sativa*.

This thesis work compares the DL-based multilayer perceptron (MLP) model with five existing well-established ML models using protein datasets from *O. sativa* and its related species. The proposed MLP model outperformed the ML models in identifying disease-resistance proteins from a set of *O. sativa* proteins that were involved in various biological processes, including disease resistance, morphological development, and flowering. In addition, the MLP model consists of higher performance metrics than ML models. The proposed model could be utilized by geneticists and breeders in developing disease-resistant *O. sativa* varieties and contribute to the transition of traditional *O. sativa* farming practices into a new era of smart farming.

2.2.3 AP2/ERF transcription factor orthologs of the desert tree *Prosopis cineraria* show higher copy number and DNA-binding affinity than drought-sensitive species

O. sativa, a staple crop, is highly vulnerable to abiotic stresses such as drought. It negatively impacts the yield and overall growth of *O. sativa*. It contains several drought tolerance genes such as *ERF*, *AP2*, *RAV*, *DREB*, etc. improving productivity under drought conditions. The structural and functional information on such genes is still unknown. Thus, the ectopic expression of several drought tolerance genes from DT plants is utilized to improve drought tolerance in *O. sativa*. Further, several approaches have identified drought tolerance genes from DT plants, but still, the characterization of such genes is lacking in *O. sativa*. Therefore, to identify and study robust orthologs conferring tolerance to extremophile DT plants naturally adapted to extreme drought-prone environments, the current work explores *P. cineraria*. It is a highly DT legume tree of the Indian Thar Desert due to its leguminous nature. In addition, Table 2.1 depicts detailed information on the drought tolerance genes or proteins isolated from various DT species and incorporated in *O. sativa* to improve tolerance against drought conditions.

Table 2.1 : Show the drought tolerance genes (or proteins) incorporated in *O. sativa* from drought-tolerant species.

Gene/protein	Resistance mechanism	Function in drought tolerance in <i>O. sativa</i>	Reference
<i>EcNAC67</i>	Salinity and drought tolerance in <i>O. sativa</i> induced by EcNAC67 from finger millet (<i>Eleusine coracana</i> L.)	Delayed leaf rolling, higher root and shoot mass, and increase relative water content	[Rahman <i>et al.</i> , 2016]
<i>TlOsm</i>	Tolerance against cold, drought and salinity in <i>O. sativa</i> through osmotin protein by <i>Tripogon loliiformis</i>	Improved plant growth, tillering and survival rate	[Le <i>et al.</i> , 2018]
<i>HvCBF4</i>	Enhanced drought and salinity tolerance in <i>O. sativa</i> by C-repeat/dehydration-responsive element binding factors (CBF/DREBs) from <i>Hordeum vulgare</i> (Barley)	Increased survival rates, less wilting and rolling of leaves, high photochemical efficiency of photosystem II in the dark-adapted state	[Oh <i>et al.</i> , 2007]
<i>SiMYB56</i>	Drought tolerance in <i>O. sativa</i> through R2R3-MYB transcription factor SiMYB56 from foxtail millet	Lower Malondialdehyde (MDA) content, higher lignin content and upregulated expression of Abscissic acid (ABA) synthesis	[Sahu <i>et al.</i> , 2023]

In this regard, the current work explored *AP2/ERF* genes through drought-responsive profiling and several genome-wide studies such as phylogenetic classification, *cis*-element and gene structure analysis. Further, CNV and structural analysis between DT and DS species revealed significant differences in copy numbers and binding of the AP2 domain with the AP2/ERF proteins. The nucleotide variations such as SNPs, and MNPs, were investigated among the Indian and Arabian cultivars of *P. cineraria*. Further, to validate the utilization of *APE/ERF* genes from *P. cineraria* into *O. sativa*, a genome-wide analysis including amino acid variation and hydrogen binding analysis was performed. Therefore, this study demonstrated that *AP2/ERF* genes can be a potential candidate for developing drought tolerance in *O. sativa*.

2.3 Conclusion

Climatic change has significantly intensified both biotic and abiotic stresses on *O. sativa*, adversely affecting its health and reducing productivity by up to 100%. These stresses contribute to the decline in stable production of *O. sativa*, threatening global food security. While several approaches, such as the application of pesticides and the development of novel stress-tolerant varieties, have been performed, additional research is needed to effectively mitigate these stresses. In this context, computational approaches integrated with *O. sativa* breeding programs present a valuable opportunity to enhance *O. sativa* production under stress conditions. Analyzing multi-omics data facilitates a better understanding of the structural, molecular and functional aspects of the stress-tolerant genes and proteins which act as candidates for developing novel varieties. Further, the combination of DL algorithms with multi-omics data can also help in finding suitable genes or proteins that can lead to advancement in *O. sativa* breeding programs. In addition, transferring stress-tolerant genes from DT species to *O. sativa* provides a novel approach for developing DT varieties of *O. sativa*. In this regard, this thesis addresses three important biological questions related to the development of stress-tolerant varieties of *O. sativa*.