

Use of Biotechnology to Improve the Tolerance in Rice (*Oryza sativa*) to Drought Stress

Ahsan A. Kadhimi^{1,3*}, Arshad Naji Alhasnawi^{1,4}, Anizan Isahak²,
Mehdi Farshad Ashraf¹, Azhar Mohamad⁵, Febri Doni¹,
Wan Mohtar Wan Yusoff¹ and Che-Radziah CheMohd Zain^{1*}

¹School of Biosciences & Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Malaysia.

²School of Environmental Science and Natural Resources, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Malaysia.

³University of Baghdad, Ministry of Higher Education, Iraq.

⁴University Presidency, AL- Muthanna University, Iraq.

⁵Malaysian Nuclear Agency, Malaysia.

(Received: 10 July 2014; accepted: 20 August 2014)

Abiotic stress factors are the main limitations to plant growth and yield in agriculture. Among them, drought stress, which is caused by water deficit, is probably the most impacting adverse condition and the most widely encounter Rice (*Oryza Sativa* L.) is one of the most important cereal crops that provides a staple diet for almost half of the world's population. However, rice yield and quality are affected by environmental stress. Drought is one of the most common environmental stresses affecting rice growth and productivity. There's the possible development of biotechnological tools to address the critical problems of crop improvement for a sustainable agriculture. Among the available biotechnological tools, the in-vitro culture alone or combined with mutagenesis, which are induced with physicochemical or biological agents by using (PEG) can be exploited to increase genetic variability and mutants, as a potential source of new commercial cultivars, in-vitro culture environments . Following this, it is preferred that there are more studies on finding the varieties of rice tolerant to drought stress by using biotechnological in order to reduce the risk of abiotic stress, which is the most important stresses of drought on agriculture and rice production.

Key words: Tolerance in rice, Drought stress, Biotechnological tools, Environmental stresses.

The second most commonly refined cereals, after wheat, in the world is Rice (*Oryza sativa*) and is a staple food for over half the world's inhabitants. Recently, note experts difficult environmental stresses, for instance waterlogged soil, saline, frost, and high temperature, and drought decrease crop yields and damage quality,

which guide to food insecurity. Under intense stress circumstances, crops totally fail, which in sequence may guide to high food prices, food shortage, migration of inhabitants from rural and villages insecurity.

One of the explanations to have a secure and sustainable food manufacture is to breed diversities that are tolerant to stress circumstances during their development and growth. Such inhabitants can be irradiated in vitro to bring grown, multiplied and mutations in the field for the choice of required genotypes. Applying a mixture of in vitro and mutation methods, new genotypes can be produced in a crop of rice; the instruction of mutations proposes the option to create only a

* To whom all correspondence should be addressed.
E-mail: cradzian@ukm.edu.my

restricted number of the required genetic alterations in varieties and genotypes, which are adjusted to the local eco-climatic situations. Local researches have demonstrated that alterations are able of a direct impact on the genetic material and its factors, particularly DNA (DNA) that is dependable for the constancy of the relocate of characteristics from one generation to another. Therefore, the “Coordinated Research Project on the In-Vitro Techniques for the Selection of Radiation Induced Mutations Adapted to Adverse Environmental Conditions” was commenced and centred mainly on the enhancement of vegetative proliferated plants.

Now a day’s technology of plant tissue culture allocate the construction of large inhabitants of plants in a short period and on a year round foundation in the laboratory. Tissue culture and plant cell have been a practical tool to research stress tolerance instruments under in vitro situations. In vitro culture methods reduce environmental differences because of described controlled conditions, nutrient media, and homogeneity of stress function. Additionally, the simplicity of such managements facilitates studying large plant inhabitants and stress actions in a short period of time and restricted space.

Reproduction of drought stress by Polyethylene Glycol (PEG) brings drought stress on the plant and an important difference from the control carries on to enhance with the rising solute probable (ϕ s). PEG-6000 has long been used as a consistent indicator under laboratory situations for checking the drought tolerant genotypes. This is for the reason that polyethylene glycol performs as a non-penetrating osmotic agent effecting into rising solute probable (ϕ s) and obstruction of a combination of water by the root system. Drought screening applying some seed technological parameters has been set up to be quite functional in a number of crops. Under laboratory situations. This method can be further expanded to test drought tolerance in other genotypes

Alteration methods in mixture with the in-vitro culture have become a significant instrument in improvement locally modified cultivars. Since the mid-twentieth century to this day, many studies were done to identify how to enhance the construction of rice. Under situations of drought stresses it is reviewed in the current study, the

applying of some biotechnology in the improvement tolerance of rice to drought.

Rice (*Oryza sativa*)

The second main crop international fits in the genus *Oryza* and has two cultivated and 22 feral variety. The cultivated variety are *Oryza glaberrima* and *Oryza sativa*. *Oryza sativa* is produced all over the world. Several cultures have proof of early rice cultivation, containing India, China, and the evolutions of Southeast Asia. Conversely, the original archaeological proof appears from eastern and central China and times to 7000–5000 BC (Encyclopædia Britannica, 2010). It is sophisticated in more than 50 countries across Australia, Europe, Africa, South America, North America, and Asia, covering a whole land area of 164 million hectares with a construction level of about 723 million metric tons (FAO 2011). Rice is the basis of 27% of nutritional energy and 20% of nutritional protein in the expanding world (Redoña, 2004). Regarding 90% of the whole rice developed in the world is created by 200 million small cultivators (Tonini and Cabrera, 2011). Rice is fundamental to the lives of billions of people around the world, and producing rice is the main particular applying of land for creating food, covering 9% of the earth’s arable land. Calories from rice are mostly significant in Asia, particularly among the poor, where it reports for 50-80% of daily caloric eating (Gramene Reference ID 8380, 2001). As a consequence of increasing in income and population in main rice-consuming countries, the demand for rice has been progressively rising over the years. Mohanty (2009) assessed that the worldwide demand for rice will enhance from 465 million ton in 2012 to about 487 million ton in 2020. Consequently, a sustainable development in rice construction international is required to make sure maintain human health, food security, and maintain the livelihoods of millions of small cultivators. One of the most severe long-term challenges to attain sustainable development in rice construction is weather change (Vaghefi *et al.*, 2011; Wassmann and Dobermann, 2007; IFPRI, 2010). Rice sustainability and productivity are intimidated by abiotic and biotic stresses, and the outcomes of these stresses can be additional heightened by remarkable changes in worldwide climate. One of the significant ways to make sure food safety and at the same time

present feasible incomes for poor rice cultivators in the future is to expand new rice diversities that are more tolerant of the undesirable effects of a more unstable climate (Mackill *et al.*, 2010; Haefele *et al.*, 2010).

Abiotic stress

Dealing with plant environmental stress is the base of sustainable agriculture. Stress is an experience that limits crop efficiency or devastates biomass. Stress can be biotic, reasoned by diseases and insects, or abiotic, which may contain air pollution, salinity, flooding, mineral deficiency, metal toxicity, adverse temperature, adverse pH, and drought. Among the abiotic stresses having an effect on crop efficiency, drought is considered as most destructive (Borlaug and Dowsell, 2005).

Definition of drought

Droughts happen in all parts of the world counting areas that normally obtain very high rainfall. Droughts reproduce water shortages over an area for extensive periods of time such as a year, a season, or a month. Numerous variables such as humidity winds, temperature, and geographic features, control changeability in the main water source i.e. precipitation over an area [Mishra & Singh, 2010]. Drought can be described as the deficiency of irrigation or rainfall for a period of time enough to reduce soil moisture and damage plants. Drought stress effects when water loss from the plant surpasses the capability of the plant's roots to absorb water and when the plant's water content is decreased enough to obstruct with usual plant procedures. Naturally, water is generally the most restrictive feature for plant growth.

Drought Stress on Rice

Drought is the most important environmental stress on agricultural construction international (Cattivelli *et al.* 2008) and an incredible attempt is being concerned to progress crop yields in the face of rising water dearth. Drought has an effect on photosynthetic activity, yield, pigment content, membrane integrity, osmotic adjustments, and water relations, plant growth (Benjamin and Nielsen 2006). Drought-prone areas and probable agricultural land with no irrigation system in place have been less developed than those with expanded irrigation systems or more consistent rainfall because of problems and

high costs of expanding enhanced technologies. Consequently, rice yields are demonstrating a stable reducing international in unirrigated and drought-prone regions. Drought is extensive in several areas and are supposed to cause, by 2050, severe drought of more than 50% of all arable lands (Vinocur and Altman, 2005). The globe food grain construction requires to be doubled by the year 2050 to meet the food commands of the ever-rising inhabitants (Tilman *et al.*, 2002), which is going to achieve 9 billion by that time (Virmani and Ilyas-Ahmed, 2007). Abiotic stresses show a main challenge in our mission for sustainable food construction, as these may decrease the potential yields by 70% in crop plants (Katiyar-Agarwal *et al.*, 2006). Although, if the crop experiences an early drought, thus influencing germination, afterwards the suboptimal plant inhabitants is the main cause of low grain yield. Early season drought strictly decreases germination and stand organization mainly because of decreased water uptake during the imbibitions phase of germination, decreased energy deliver, and damaged enzyme performances (Okcu *et al.* 2005; Taiz and Zeiger 2010). Development is an permanent enhance in weight, size, or volume, which comprises the stages of cell elongation, cell division, and discrimination. Both cell enlargement and cell division are had an effect on under drought because of damaged enzyme activities, reduced energy supply and loss of turgor, (Kiani *et al.* 2007; Farooq *et al.* 2009; Taiz and Zeiger 2010). Even though roots were less influenced than shoots (Liu *et al.* 2011). Drought as well reduced leaf area because of loss of turgor and decreased leaf numbers (Farooq *et al.* 2010). All these issues supply to decreased dry matter accretion and grain yield under drought. The study of diverse developmental and growth occasions in crop plants with regard to time is named crop phenology. Drought powerfully influences crop phenology by limitation the crop growth cycle with a few exemptions. While drought happens during the vegetative phase of crop development, it may significantly reduce financial yield. Drought stress during grain and reproductive filling stages is more disturbing (Reddy *et al.* 2003; Vijay 2004; Yadav *et al.* 2004; Lafitte *et al.* 2007).

World rice construction applies about 1,578 km³ of water which is 30% of the fresh water

applied international (Trijatmiko, 2005). Rice, as a paddy field crop, is mainly vulnerable to water stress and its most vulnerable to harm from water shortage, consistent with the available statistics, the proportion of drought influenced land region in the world more than doubled from the 1970s to the early 2000s, timing in relation to plant growth phase and concentration of the stress can all differ significantly (Witcombe *et al.*, 2008 and Wani *et al.*, 2010). Of all the cereals, rice (*Oryza sativa*) is most vulnerable to harm from water shortage (Lafitte and Bennet, 2003). Consistent with available statistics, the proportion of drought influenced land area in the world more than doubled from the 1970s to the early 2000s (Isendahl and Schmidt, 2006). Rice, as a paddy field crop, is mainly vulnerable to water stress (Tao *et al.*, 2006; Yang *et al.*, 2008). It is assessed that 50% of world rice construction is influenced by drought (Bouman, *et al.* 2005). Water shortage is becoming progressively more common in irrigated regions because of falling water tables. Genetic development of rice for drought tolerance through predictable breeding is slow as a result of the low heritability of yield under stress, low inherent difference in the field and the restriction that there is typically only one experimentally droughted crop yearly (Ribaut *et al.*, 1997). Drought can delay transplanting, seeding, and/or crop organization, drought typically communicate with the tillering phase which can originate decline in rooting and the tillering capabilities, leaf senescence, root function or even death, and effect finally in diminish of efficiency yield and heads loss. Leaf gas exchange and leaf expansion are two such responsive procedures that can be reserved by drought stress. At the plant level, decreased leaf region is possibly the clear mechanism by which plants and crops limit their water loss in reaction to drought (Sadras and Milroy, 1996). Rahman *et al.* (2002) stated that tiller number, plant height, panicle length, panicle number, 1000-grain weight, number of filled grains per panicle, total dry matter (TDM), harvest index (HI), and yield were reduced with stress. Grain yield was decreased noticeably in all cultivars with drought beginning at panicle beginning or at pinnacle. Water stress at pinnacle decreased grain yield more than other stress actions. The diminution in yield mainly effected from the decreased in productive

panicle number and overflowing grain proportion. Kumar *et al.* (2006) set up that the proportion of untaken grain was considerably higher in sites that were influenced by drought at reproductive phase. Rahimi and Mostajeran, (2009) stated that one of the major difficulties of rice production and cultivation is the shortage of water sources, particularly during phases of low precipitation which have an effect on the vegetative development rate and the amount of yield.

Consequently, expanding drought-tolerant rice diversities and decreasing drought expenditure during rice construction is critical to enhanced rice yield. Because of the multifaceted polygenic nature of drought tolerance, tries to develop this feature through predictable breeding have met with little achievement. On the other hand, the classification and relocate of genes that present tolerance / resistance to drought stress through transgenic machinery is frequently planned as one resolution for defending crops beside a water stress environment and rising crop yields international, mainly in less expanded regions that are intimidated by food shortage and low crop efficiency (Nelson *et al.* 2007).

Use some of biotechnology in rice tolerance to drought

Mutation

Mutation breeding is an instrument applied to revise the nature and function of genes which are the basis of plant growth and building blocks and improvement, thus generating raw materials for economic crops and genetic improvement (Adamu, and Aliyu, 2007). Mutation could be described as a relatively and permanent rare modify in the number or series of nucleotides in a genome. Mutation happens naturally (unplanned mutation) or it can be unnaturally encouraged by different mutagenic agents which is consequently named inducible mutations (Singh, 1996). Mutation has been applied to create several cultivars with enhanced financial value and to revise plant and genetic developmental events (Van *et al.*, 1990; Bertagen-Sagnard *et al.*, 1996). Diverse mutagenic agents are applied to induce favourable mutation at high occurrence that comprises chemical mutagens and ionizing radiation (Ahloowalia and Maluszynski 2001). Mutations are the instrument applied to revise the character and function of genes which are the basis

of plants growth, the building blocks and development, thus generating raw materials for economic crops and genetic improvement (Adamu *et al.*, 2004). Mutation origins different structural adjusts with DNA, such as a alteration in a single nucleotide foundation of a gene (point mutation), substitute of one nucleotide foundation by another, cutting of one or more foundation couples in the DNA series (frame modify mutation), chromosomal reorganization, duplication, or loss of a chromosome sections (Poehlman and Sleper, 1995). The initiation of mutation with compound mutagens relies on chemical mutagen attentiveness, period of action and other features (Alcantara *et al.*, 1996). Induced mutation is achieved by the applying of chemical or physical mutagens. The rate of impulsive mutations is too low to be considered for useful purposes. Consequently, chemical or physical mutagens might be applied with in vitro or in vivo methods to enhance the mutation incidence (Lyakh and lagron, 2005). Mutation methods in mixture with tissue culture techniques present influential technology to develop plants. It is probable to improve deep-rooted plants by changing particular characteristics by inducing. Recent studies stated that plant introduction to diverse doses of gamma (50pP)-irradiation may develop the tolerance to abiotic stress circumstances, for instance, drought and salt (Moussa, 2011 and Song *et al.* 2012). Mutation method has been applied to generate many cultivars with enhanced financial value and study of plant and genetic developmental facts (Bertagen-Sagnard *et al.*, 1996). Induced mutation has enormous serves and potentials as a flattering approach in genetic development of crops. A variety of mutagenic agents are applied to induce favourable mutation at high occurrence that comprises chemical mutagens and ionizing radiation (Ahloowalia, and Maluszynski, 2001). Gamma radiation, arranged of high energy photons, is a kind of ionizing radiation, capable to break through and cooperate with living tissues. It origins reduced growth rate and imitation ability in company with DNA injure and morphological alterations (Kovalchuk *et al.*, 2004 and Wiat *et al.*, 2007). Although, irradiation with low doses is identified to have stimulatory results on plant development, a

perception consigned to as hormesis (Calabrese, 2002). Information with reference to the applying of Gamma-ray as an instrument to develop seed dynamism is still insufficient. The procedure of seed priming achieved with osmotic agents persuades the pregermi-native metabolism, mainly the antioxidant reaction and DNA mend purposes, guiding to improved germination effectiveness, an attribute highly considered for agricultural functions (Ventura, *et al.* 2012). Priming actions might as well progress stress tolerance in developing seeds, leaving a type of "stress memory" (Chen and Arora 2012). On the contrary with its high applicability, modest information is accessible on physical priming techniques (Vasilevski, 2003).

In vitro Tissue Culture

Plant tissue culture methods are critical to several kinds of academic examination, in addition to many functional features of plant science. Presently, having become an established technology, the methods are not only applied for the studies in plant gene regulation and molecular biology but as well functional to molecular breeding and plant biotechnology. The comparisons of the results persuaded by the stress in the plant cultured in-vivo and in-vitro situations propose that the in-vitro system can be applied as substitute to field valuations for revising the common consequence of water-stress on plant development and growth. The most extensively applied technique for the choice of genotypes tolerant to abiotic stress is the in-vitro choice pressure method. This is derived from the in-vitro culture of plant cells organs or tissues on a medium complemented with choosy agents, allocating regenerating and selecting plants with advantageous features (Rosa and Aurelio, 2012). Callus, as commenced material in plant tissue culture, is described as an unorganized tissue accumulation producing on solid substrate by applying tissue culture skill, which can shape from numerous elements of large intact plants (Mineo 1990). During callus discrimination, only those highest and tangential cells in callus were motivated into energetically dividing cells, and the level of general separation generally relies on the hormone equilibrium of the support medium and the physiological situation of the tissue. Dissimilarities in callus introduction among rice diversities were observed in some of the initial studies in rice tissue

culture gained maximum callus arrangement in basmati rice cv.370 on MS medium complemented with 2.0mg/l of 2,4-D. Although, somatic embryogenesis was attained by means of MS medium complemented with 2mg/l from each of 2,4-D and Kin. It has been stated that the subsequent features outcome plant regeneration occurrence in genotype, rice; developmental phase of calli in the explants, carbohydrates source, hormonal composition of the medium, biased drought or water stress inducing managements and other medium complements (Saharan *et al.*, 2004). Ilahi *et al.* (2005) stated that callus of a local diversity of rice (*Oryza sativa* L. cv. Swat-II) was provoked and the occurrence of callus introduction was studied on customized MS medium by means of a diversity of mixtures of 2,4-D and Kin, they as well stated that addition of tryptophan to diverse mixtures of cytokinins and auxins enhanced the calli and embryogenic callus accumulation have been productively reproduced on MS complemented with 1.0 mg/l of Kin and 0.5 mg/l of NAA. Khatun *et al* (2010) accounted that genotype dependence still plays a significant role for any plant tissue culture work and studied on callus regeneration and induction possible of twenty five rice cultivars through in vitro micro propagation and another culture find Pazuki. A and Sohani, M.M. (2013) that the most appropriate and responsive rice cultivar in callus beginning is in reducing order: Gb > Hm >> Hn e” Gr. consequently can be used in tissue culture refereed breeding program .

Polyethylene glycol (PEG)

PEG is nontoxic, non-ionic and inert and of high molecular weight. It is very soluble in water, and is accessible at a extensive range of molecular weights (e.g., PEG-4000, PEG-4500, PEG-6000, and PEG-8000), it reproduces water shortfalls situations in cultured cells in a comparable method to that practical in the cells of intact plants subjected to genuine drought circumstances, high molecular weight PEG (PEG-6000) persuades water stress in plants by diminishing the water possible of the nutrient explanation without being taken up and with no proof of toxicity (Wani *et al.*, 2010) . PEG is a polymer compound with several functions from manufacturing industrialized to remedy, it persuades morphological alterations of delighted plantlets, counting considerable statement of epicuticular

wax and customized leaf outside structure, it is applied to change the osmotic probable of nutrient explanation and therefore persuades plant water shortage in a comparatively managed method, it was supposed that PEG of large molecular weight does not infiltrate plant tissues and therefore is a perfect osmoticum for applying in hydroponics root medium (Michel and Kaufmann, 1973; Money, 1989). Al-Bahrany (2002) studied the reaction of Hassawi rice (*Oryza sativa*) callus to differing amounts of PEG persuaded water stress counting callus growth, water substance and proline accretion. In recent years, PEG has been extensively applied to persuade water stress and drought tolerant cultivars have been recognized in several crops by expanding approaches derived from the applying PEG (Badiane *et al* 2004).

CONCLUSIONS

Therefore, we could feel the danger of abiotic stress and most importantly of the drought on the growth and production of strategic crops including the rice that needs to be high water requirements. The advantage of biotechnology can be explored, such as tissue culture and mutation induction, and the use of (PEG) in breeding and the development of rice varieties tolerant to drought stresses can grow and give production under the conditions of drought stresses.

ACKNOWLEDGMENTS

To Ministry of Higher Education and Scientific Research IRAQ, for financial supports and moral support. To complete this search

REFERENCES

1. Adamu, A. K. and Aliyu, H. Morphological effect of sodium azide on tomato (*Lycopersicon esculentum* Mill). *Sci. World J.*, 2007; **2**(4): 9-12.
2. Adamu, A. K., Clung, S. S. and Abubakar, S. Effects of ionizing radiation (gamma-rays) on tomato (*Lycopersicon esculentum* L.). *Nigeria J. of Exp. and Appl. Biol.*, 2004; **5**(2): 185-193.
3. Ahloowalia, B. S. and Maluszynski, M. Induced mutation. A new paradigm in plant breeding. *Euphytica*. 2001; **118**(2):167-173.

4. Ahloowalia, B. S. and Maluszynski, M. Induced mutation. A new paradigm in plant breeding. *Euphyt.* 2001; **118**(2):167-173
5. Al-Bahrany, A. M. Callus growth and proline accumulation in response to polyethylene glycol induced osmotic stress in rice *Oryzasativa* L. *Pak. J. Biol. Sci.*,2002; **15**: 1294–1296.
6. Alcantara, T. P., Bosland, P. W. and Smith, D. W. Ethyl methane sulfonate induced seed mutagenesis of Capsicum annum. *J. Heredity*, 1996; **87**(3): 239-241.
7. Aroca, R. Plant Responses to Drought Stress ISBN 978-3-642-32652-3 ISBN 978-3-642-32653-0 (eBook) 2012; Springer Heidelberg New York Dordrecht London.
8. Badiane, F. A., Diouf, D., San, D., Diouf, O., Goudiaby, V. and Diallo, N. Screening cowpea *Vignaunguiculata*(L.) Walp. Varieties by inducing water deficit and RAPD analyses. *Afric. J. Biotech.*, 2004; **3**: 174- 178.
9. Benjamin, J.G., Nielsen, D.C.). Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Res*, 2006; **97**: 248–253.
10. Bertagen-Sagnard, B., Fouilloux, G. and Chupeau, Y. Induced albino mutations as a tool for genetic analysis and cell biology in flax (*Linumusatssimum*). *J. Exp. Bot.*, 1996; **47**: 189-194.
11. Bertagen-Sagnard, B.,Fouilloux, G. and Chupeau, Y. Induced albino mutations as a tool for genetic analysis and cell biology in flax (*Linumusatssimum*). *J. Experimental Botany*. 1996; **47**: 189-194.
12. Borlaug, N.E., and C. R. Dowswell. Feeding a world of ten billion people: A 21st century challenge. In proc. of “In the wake of double helix: From the green revolution to the gene revolution” 27–31st May 2003 Bologna, Italy.
13. Bouman, B.A.M., Peng, S., Castaoeda, A.R., and Visperas, R.M. Yield and water use of irrigated tropical rice system. *Agricultural water Management*, 2005; **74**, 2, 87-105. doi:10.1016/j.agwat.2004.11.007.
14. Calabrese, E. J. “Hormesis: changing view of the dose-response, a personal account of the history and current status,” *MutationResearch*, 2002; **511**(3), pp. 181–189.
15. Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo. A.M., Francia. E., Mare, C., Tondelli, A., Stanca, A.M. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Res*, 2008; **105**: 1–14.
16. Chen, R., and Arora, R. “Priming memory invokes seed stresstolerance,” *Environmental and Experimental Botany*, 2012; **94**: 33–45. Delhi, India.Edition. Chicago: Encyclopædia Britannica.
17. Encyclopædia Britannica (2010). Encyclopaedia Britannica Student and Home.
18. FAO. FAOSTAT. <http://faostat.fao.org/site/567/default.aspx# ancor>. Assessed 16 Jan 2013.
19. Farooq, M., Basra, S.M.A., Wahid, A., Ahmad, N., Saleem, B.A. Improving the drought tolerance in rice (*Oryzasativa* L.) by exogenous application of salicylic acid. *J Agron Crop Sci*, 2009; **195**: 237–246.
20. Farooq, M., Kobayashi, N., Ito, O., Wahid, A., Serraj, R. Broader leaves result in better performance of indica rice under drought stress. *J Plant Physiol*, 2010; **167**:1066–1075.
21. Haefele, S.M., Ismail, A.M. Johnson, D.E., Vera Cruz, C. and Samson, B. (2010). “Crop and Natural Resource Management for Climate-Ready Rice in Unfavorable Environments: Coping with Adverse Conditions and Creating Opportunities”. Paper from the CURE Workshop on Climate Change, May 4, 2010, Siem Reap, Cambodia. Online: http://irri.org/climatedocs/presentation_Lists/Docs/2_Haefele.pdf, accessed on November 15, 2011.
22. Hongbo, S., Zongsuo, L. and Mingan, S. Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (*Triticumaestivum* L.) genotypes at maturation stage. *Colloids and Surfaces B: Biointerfases*. 2005; **45**: 7–13.
23. IFPRI. (International Food Policy Research Institute).(2010). Food Security, Farming and Climate Change to 2050, Scenarios, Results and Policy Options. Washington, D.C.:IFPRI. Online:www.ifpri.org/sites/default/files/publications/ib66.pdf, accessed on November 30, 2011.
24. International Rice Research Institute. (IRRI). (2001).Rice Research and Production in the 21st Century. (Gramene Reference ID 8380).
25. Isendahl, N. and Schmidt, G. (2006). Drought in the Mediterranean-WWF policy proposals. World Wide Fund for Nature, Adena, Madrid.
26. Jones, N., Ougham, H., Thomas, H., Pasakinskiene, I. Markers and mapping revisited: finding your gene. *New Phytol*, 2009; **183**: 935–966.
27. Katiyar-Agarwal, P., Agarwal, P., Reddy, M.K. and Sopory, S.K. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant CellRep*. 2006; **25**: 1263–1274.
28. Khatun, R. S. M., Islam, S. and Miah, M. A. B. Studies on plant regeneration efficiency through *in vitro* micropropagation and another culture

- of twenty five rice cultivars in Bangladesh. *J. Appl. sci. res.*, 2010; **6**(11): 1705-1711.
29. Kiani, S.P., Talia, P., Maury, P., Grieu, P., Heinz, R., Perrault, A., Nishinakamasu, V., Hopp, E., Gentzbitte, I. L., Paniego, N., Sarrafi, A. Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. *Plant Sci*, 2007; **172**: 773–787.
 30. Knox, G. Drought-Tolerant Plants for North and Central Florida. 2005. Copyright by University of Florida Cooperative Extension Service. IFAS Disaster Handbook Web site <http://disaster.ifas.ufl.edu>
 31. Kovalchuk, I., Abramov, V., Pogribny, I. and Kovalchuk, O. "Molecular aspects of plant adaptation to life in the Chernobyl zone," *Plant Physiology*, 2004; **135**(1), pp. 357–363.
 32. Kumar, R., Sarawgi, A. K., Ramos, C., Amarante, S. T., Ismail, A. M., Wade, W. J. Partitioning of dry matter during drought stress in rainfed lowland rice. *Field Crops Res.*, 2006; **96**: 455-465.
 33. Lafitte, H. and Bennet, J. Requirement for aerobic rice. Physiological and molecular considerations. In: Bouman, B. A. M.; Hengsdijk, H.; Hardy, B. (Eds.) *Water - Wise Rice Production*. IRRI, Los Baños, Philippines 2003.
 34. Lafitte, H.R., Yongsheng, G., Yan, S., Li, Z.K. Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *J Exp Bot*, 2007; **58**:169–175.
 35. Li, S., Wang, S., Deng, Q., Zheng, A., Zhu, J., Liu, H., Wang, L., GAO, F., Zou, T., Huang, B., Cao, X., Xu, L., Yu, C., Ai, P. and Li P (2012). Identification of genome-wide variations among three elite restorer lines for hybrid-rice. *PLoS ONE* **7**(2): e30952
 36. Liu, H., Wang, X., Wang, D., Zou, Z., Liang, Z. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge. *Ind Crops Prod*, 2011; **33**:84–88
 37. Lyakh, V. A. and lagron, V. A. Induced mutation variability in *Linum gradiflorum* Desp. In: *Mutation Breeding Newsletter and Review*. JointFAO/IAEA Division of Nuclear Techniques in Food and Agriculture andFAO/IAEA Agriculture and Biotechnology Laboratory. 4-5, IAEA, 2005; Vienna.
 38. Mackill, D. J., Abdelbagi, M., Ismail, A. M., Pamplona, D.L.Sanchez, J. J., Carandang and Endang M. S. "Stress-Tolerant Rice Varieties for Adaptation to a Changing Climate". *Crop, Environment & Bioinformatics*, 2010; **7**: 250259. Online: [www.tari.gov.tw/csam/CEB/member/publication/7\(4\)/004.pdf](http://www.tari.gov.tw/csam/CEB/member/publication/7(4)/004.pdf), accessed on November 20, 2011
 39. Michel, B. E. and Kaufmann, M. R. The osmotic potential of polyethylene glycol 6000. *Plant Physiol*. 1973; **51**:914-917.
 40. Mineo, L. Plant tissue culture techniques. In: *Proceedings of the eleventh workshop/conference of the Association for Biology Laboratory Education (ABLE)*, 1990; 195 p.
 41. Mishra, A. K. and Singh, V. P. 'A review of drought concepts', *Journal of Hydrology*, 2010; **391** (1-2), 202–216. <http://www.sciencedirect.com/science/article/pii/S0022169410004257>
 42. Mohanty, S. "Rice and the Global Financial Crisis." *Rice Today*, 2009; **8**(1): 40.
 43. Money, N. P. Osmotic pressure of aqueous polyethylene glycols. Relationship between molecular weight and vapor pressure deficit. *Plant Physiol.*, 1989; **91**: 766-769.
 44. Moussa. H. R. "Low dose of gamma irradiation enhanced drought tolerance in soybean," *Bulgarian Journal of Agricultural Science*, 2011; **17**(1), pp. 63–72.
 45. Nelson, D.E., Repetti, P.P., Adams, T.R., Creelman, R.A., Wu, J., Warner, D.C., Anstrom, D.C., Bensen, R.J., Castiglioni, P.P., Donnarummo, M.G., Hinchey, B.S., Kumimoto, R.W., Maszle, D.R., Canales, R.D., Krolikowski, K.A., Dotson, S.B., Gutterson, N., Ratcliffe, O.J., Heard, J.E. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc Natl Acad Sci USA*. 2007; **104**:16450–16455.
 46. Okcu, G., Kaya, M.D., Atak, M. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turk J Agric For*, 2005; **29**:237–242.
 47. Pazuki, A. and Sohani, M.M. Phenotypic evaluation of scutellum-derived calluses in 'Indica' rice cultivars. *Acta agriculturae Slovenica*, 2013; **101** - 2, str. 239 – 247.
 48. Poehlman, J. M. and Sleper, D. A (1995). *Breeding Field Crops*. Fourth edition, Iowa State University Press, Ames, Iowa.
 49. Rahimi, V. and Mostajeran, A. Effects of Drought Stress on Growth and Yield of Rice (*Oryza sativa* L.) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves. *Ameri. J. Agric. and Environ. Sci.*, 2009; **5** (2): 264-272.
 50. Rahman, M. T., Islam, M. T., Islam, M. O. Effect of water stress at different growth stages on yield and yield contributing characters of transplanted Aman rice. *Pak. J. Biol. Sci.*, 2002; **5**: 2. 169-172.

51. Reddy, G.K.M., Dangi, K.S., Kumar, S.S., Reddy, A.V. Effect of moisture stress on seed yield and quality in sunflower (*Helianthus annuus* L.). *J Oilseeds Res*, 2003; **20**: 282–283.
52. RedoñaEdilberto, D. “Rice Biotechnology for Developing Countries in Asia” in *Agricultural Biotechnology: Finding Common International Goals*, A. Eaglesham (Ed.). National Agricultural Biotechnology Council (NABC) Report No. 16. Ithaca (New York): 2004; National Agricultural Biotechnology Council. Online: http://nabc.cals.cornell.edu/pubs/nabc_16/nabc_16.pdf, accessed on December 20, 2011.
53. Ribaut, J. M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G. O., & Hoisington, D. A. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics*, 1997; **94**(6-7), 887-896.
54. Rosa, M. P. and Aurelio, G. In vitro Tissue Culture, a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions. <http://dx.doi.org/10.5772/506712012>
55. Sadras, V. O. and Milory, S. P. (1996). Soil-water thresholds for the responses of leaf expansion and gas exchange: A review. *Field Crops Res.*, 47: 253-266. Cited from Davatgara, N., Neishabouria, M. R., Sepaskhabb, A. R. and Soltanic, A. (2009). Physiological and morphological responses of rice (*Oryzasativa*L.) to varying water stress management strategies. *International J. of Plant Produc.*, 3 (4): 1735-6814.
56. Saharan, V., Yadav, R. C., Yadav, R. N. and Chapagain, P. B. High frequency plant regeneration from desiccated calli of indica rice (*Oryzasativa*L.). *African J. Biotech.*, 3(5): 256-259. Iahi, I., Bano, S., Jabeen, M and Rahim, F. (2005). Micropropagation of rice (*Oryzasativa*L. CV SWAT-II) through somatic embryogenesis. *Pak. J. Bot.*, 2004; **37**(2): 237-242.
57. Singh, P (1996). *Essential of Plant Breeding*, First edition, Kalyani Publisher, NewSong, J. Y., Kim, D. S., Lee *et al*, M.-C. “Physiological characterization of gamma-ray induced salt tolerant rice mutants,” *AustralianJournal of Crop Science*, 2012; **6**,(3), 421–429.
58. Taiz, L., Zeiger, E. (2010). *Plant Physiology*, 5th edn. Sinauer Associates Inc. Publishers, Massachusetts.
59. Tao, H., Brueck, H., Dittert, K., Kreye, C., Lin, S., Sattelmacher, B. Growth and yield formation for rice (*Oryzasativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Res*, 2006; **95**: 1–12.
60. Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. Agricultural sustainability and intensive production practices. *Nature*, 2002 **418**: 671–677.
61. Tonini, A. and Cabrera, E. Opportunities for Global Rice Research in a Changing World. Technical Bulletin No. 15. Los Baños (Philippines): International Rice Research Institute (IRRI) 2011.
62. Trijatmiko, K. R. Comparative analysis of drought resistance genes in Arabidopsis and rice. *Ph.D. thesis*, Wageningen University, Wageningen, The Netherlands. 2005.
63. Vaghefi, N., Nasir Samsudin, M., Makmom, A. and Bagheri, M. “The Economic Impact of Climate Change on the Rice Production in Malaysia”. *International Journal of Agricultural Research*, 2011; **6**(1):67-74.
64. Van, R. W., Den-Bulk, H. J. M., Loffer, W. H. and Koornneef, M. Somaclonal variation in tomato: effect of explants source and a comparison with chemical mutagenesis. *Theor. Appl. Genet.*, 1990; **80**: 817-825.
65. Vasilevski, G. “Perspectives of the application of biophysical methods in sustainable agriculture,” *Bulgarian Journal of Plant Physiology*, 2003; pp. 179–186.
66. Ventura, L., Dona, M., Macovei, A. *et al*. “Understanding the molecular pathways associated with seed vigor: role of DNA repair mechanisms,” *Plant Physiology and Biochemistry*, 2012; **60**, pp. 196–206.
67. Vijay, K.L. Irrigation strategies for crop production under water scarcity. International Commission on Irrigation and Drainage New Delhi 110–021:89–109 2004.
68. Vinocur, B., and A. Altman. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotechnol.*2005; **16**: 123–132.
69. Virmani, S.S., and M. Ilyas-Ahmed. Rice breeding for sustainable production. In *Breeding Major Food Staples*, eds. M. S. Kang and P.M. Priyadarshan, 141–191. 2007; Malden, MA: Blackwell.
70. Wani, S. H., Sofi, P. A., Gosal, S. S. and Singh, N. B. *In vitro* screening of rice (*Oryzasativa*L) callus for drought tolerance. *Communic. In Biomet and Crop Sci.*,2010; **5** (2): 108–115.
71. Wassmann, R. and Dobermann, A. (2007). “Climate Change Adoption through Rice Production in Regions with High Poverty Levels.” ICRISAT and CGIAR 35th Anniversary Symposium “Climate-Proofing Innovation for Poverty Reduction and Food Security,” November 22-24, 2007. SATeJournal,

- 4(1):1-24. Online: <http://ejournal.icrisat.org/SpecialProject/sp8.pdf>, accessed on January 1, 2012.
72. Wi, S. G., Chung, B. Y., Kimet, et al J. "Effect of gamma irradiation on morphological changes and biological responses in plants," *Micron*, 2007; **38**(6), pp. 553–564.
73. Witcombe, J.R., Hollington, P.A., Howarth, C.J., Reader, S., Steele, K.A. Breeding for abiotic stresses for sustainable agriculture. *Philos Trans R Soc Lon B BiolSci*, 2008; **363**:703–716.
74. Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M., Howarth, C.J. Genomic regions associated with grain yield and aspects of post flowering drought tolerance in pearl millet across environments and tester background. *Euphytica*, 2004; **136**: 265–277.
75. Yang, X., Liang, Z., Wen, X., Lu, C. Genetic engineering of the biosynthesis of glycinebetaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. *Plant Mol Biol*, 2008; **66**: 73–86.