International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 5
ISSN: 2088-5334

Growth and Physiological Study of Gamma-Induced M4 Black Rice in Stress Condition

Anjakasi Mar'atul Ula [a], Edi Purwanto [a,*], Parjanto [a]

[a] *Agronomy Department, Faculty of Agriculture, Sebelas Maret University Surakarta, Surakarta, Solo, 57126, Indonesia*
*Corresponding author: *edipurwanto@staff.uns.ac.id*

***Abstract*—Increased rice productivity can be achieved by producing high-yielding black rice seeds. One of the productions of superior seeds is by mutation induction using gamma-ray irradiation. Black rice has a useful content and will be produced when the plant grows in drought stress. This research aims to study the growth and physiological characteristics of black rice induced by gamma rays of 100 gray and 200 gray, as well as know the effect of drought stress on proline content, to obtain superior black rice seeds. The design was researched in the form of an experiment in the field without using a repeat sample. Black rice was planted as a screen house in Jati Village, Karanganyar. The treatment used 24 plant genotypes, namely, 10 genotypes of 100 gamma-ray irradiation and 2 control genotypes, as well as 10 genotypes of 200 gamma-ray irradiation and 2 genotypes of control. Physiological observation variables were plant height, plant dry weight, root dry weight, proline content, and leaf temperature. The results of the research on plant height growth with gamma ray irradiation resulted in shorter plant postures. Leaf temperature and proline content in plants treated with irradiation and drought stress had higher values than in control plants. M4-By-C and M4-Bt-C lines are prospective to be released as new high-yielding varieties because these lines have short stature and high proline content.**

***Keywords*— Irradiation; mutation seeds; drought stress.**

Manuscript received 1 Dec. 2021; revised 19 Jan. 2023; accepted 28 Aug. 2023. Date of publication 31 Oct. 2023.

I. INTRODUCTION

Black rice cultivation continues to increase in Indonesia. Many people are starting to become aware of the importance of food nutrition. Quality and safe food are important factors for health and the environment [1]. Rice is the main cereal crop in Indonesia, and black rice is one of the varieties of species Oryza sativa L. with the highest nutrition. Pujiasmanto et al. [2] state in Black Rice's chapter that Black Rice is super nutritious with high fiber, anthocyanin, antioxidants, and B and E vitamins. Also, black rice is gluten-free, cholesterol-free, and low-sugar rice. Black rice's roots and shoots contain proline [3]. Proline is an amino acid synthesized from the phosphorylation of glutamate. Prolines have important functions in metabolic and physiological health [4].

The presence of proline content to maintain cell turgor and root growth in conditions of low osmotic potential. The proline content in cells, tissues, or plants is believed to tolerate drought stress. So, in black rice, there is a turgor defense mechanism to stay above zero, to keep the tissue water potential low compared to the external water potential, so that plasmolysis does not occur in a drought stress condition. Plants' growth under drought conditions exists its self-defense mechanism to protect from damaging impacts by stress. The drought stress mostly leads to the accumulation of reactive oxygen in chloroplast and mitochondria [5].

Black rice in Indonesia is experiencing a decline and difficulties obtained by small farmers. Black rice generally has a long-term planting of up to 150 days, high plant habitus, and low productivity [6]. The alternative effort that can be made to manage the deficiencies is to produce superior black rice seeds. A seed per planting hole related to the plant population will affect growth and production [7]. Many studies have been conducted to obtain superior rice seeds, including mutation induction through gamma-ray irradiation. Several studies have shown gamma rays effective in varietal assembly programs [8]. Gamma-ray radiation will change the characteristics of morphology, anatomy, and genetics [9]. Gamma rays change the chromosomes' number and structure. Also, the cell system was disrupted, altering the gene activity [10].

Based on the descriptions, it is necessary to research black rice's seed M4 mutation. The research aims to examine the growth and physiology of black rice induced by gamma rays in 100 grays and 200 grays and determine the effect of drought stress on proline content to obtain superior black rice seeds. Information on the results of gamma rays' effect and drought treatment on M4 black rice (mutation seeds and control) might be beneficial to farmers and stakeholders to start cultivating the black rice.

II. Materials and Method

The research was conducted in July-November 2020 in the screen house at Medicinae Street, UNS Residence No.6, Jati Village, Jaten District, Karanganyar Regency. The main materials used in this study were Black Rice (*Oryza sativa* L.) seeds of Bantul assess and Boyolali mutant M4 induced by gamma-ray irradiation of 100 gray and 200 grays. Black rice in 24 genotypes, by ten genotypes of 100 gray gamma irradiation with two control genotypes, and ten genotypes of 200 gray gamma irradiation with two control genotypes. Each genotype contained ten plant populations for observation. In total, there were 240 plants. Boyolali Black Rice with 100 gray irradiation treatment is written with the code M4-By-100, and the 200 gray irradiation is written with the code M4-By-200. Meanwhile, Bantul Black Rice with 100 gray irradiation treatment was written with the code M4-Bt-100, and the 200 gray irradiation treatment was written with the code M4-Bt-200.

The field experiment given is drought stress. Drought stress treatment will affect the growth of rice plants during the generative phase [11]. The drought stress used in the research plant media was 50% water stress and no stress. Drought stress (watering 50%) is carried out by giving water as much as 50% of the water needs of the rice plant, while not drought stress (watering 100%) is giving water according to the needs of the rice plant. Both will be compared for their effect on the yield of the proline content of black rice plants. Based on gravimetric calculations, the need for 100% water for rice plants grown in polybag media is 2 liters of planting media water. Treatment without stress (watering 100%) was applied to control plants. Control plants were black rice plants without gamma irradiation and stress treatment.

Other materials used in this study were: 80% acetone, filter paper, 2:1 manure, urea 250 kg/ha as a nitrogen source, SP36 156 kg/ha as a source of phosphorus, and KCl 100 kg/ha and pesticides. The research implementation in the field test was carried out in several stages: media preparation (using 2:1 manure, urea 1.25 gr per polybag, SP36 0.75 gr per polybag, and KCl 0.5 g per polybag, seed nursery, planting, drought treatment, maintenance, and harvesting. Drought stress treatment with 100% water requirement for control and 50% for all gamma irradiation treatments. Laboratory analysis conducted was the proline level using the method Bates [12] method, extracted by 200 mg leaf's sample and ninhydrin reagent, and proline concentration determined using the calibration of fresh weight sample in mg/g.

Variable observation of the agronomy characteristics is plant height, shoot dry weight, root dry weight, and leaf temperature. Data analysis was carried out descriptively to assess the characteristics of the research data and continued with the T-test to compare the mutant and control lines data.

III. Results and Discussion

A. Plant Height, Dry Weight Plants, and Dry Weight Roots

Plant height is a variable that can indicate plant vegetative growth activity. With plant height growth, the plant will undergo cell division. Dry-weight plants are a parameter used to decide seedling growth that describes the efficiency of plants' physiological processes.

TABLE I
OBSERVATION RESULT OF 100 GAMMA-RAY IRRADIATION OF BOYOLALI AND BANTUL BLACK RICE

Strain	Treatment	Height Plants	Dry Weight Plants	Dry Weight Roots
Control-01	nds	89.50± 3.96	14.44± 4.31	1.55± 0.20
M4-By-100-A	ds	86.23± 14.40	20.03± 9.17	7.19± 3.66
M4-By-100-B	ds	84.40± 4.78	18.18± 6.64	14.49± 6.15
M4-By-100-C	ds	82.46± 6.34	19.63± 6.04	12.23± 4.30
M4-By-100-D	ds	77.16± 11.62	14.19± 0.69	1.84± 0.25
M4-By-100-E	ds	82.53± 10.07	19.36± 7.63	6.40± 4.17
Control-01	nds	91.30± 9.11	27.88± 5.28	16.26± 10.34
M4-Bt-100-A	ds	83.26± 5.02	16.25± 7.87	6.48± 8.79
M4-Bt-100-B	ds	81.86± 6.34	29.35± 13.90	10.88± 5.38
M4-Bt-100-C	ds	84.26± 9.08	17.59± 7.76	11.23± 6.37
M4-Bt-100-D	ds	89.56± 8.05	27.01± 4.30	4.74± 3.09
M4-Bt-100-E	ds	88.63± 7.38	16.87± 9.68	6.79± 3.06

Remarks:
ds = drought stress, means treatment watering 50% water needed in 100 gray irradiations; nds = not drought stress, means treatment watering 100% water needed in control plant.

Research results in a plant height of Boyolali M4-By-100-D showed an average yield lower than control-01 plants (Table I). Plants with shorter stature are obtained from mutation breeding than the original plants, so plants with gamma ray irradiation will be more resistant to fall. Short-trunked plants are expected to be able to produce a large number of tillers to produce more grain. According to Syafi [13] black rice is a variety with a high plant posture, so it is very easy to fall. The gamma-ray irradiation treatment in this study was to improve the falling characteristics of rice plants, namely by creating rice plants with shorter stems. The results of gamma irradiation at 100 grays showed results that were under the breeding objectives of this plant.

Bantul Black Rice showed that M4-Bt-100-B had the lowest means value of 81.86, while control plant-01 showed the highest value of 91.30 (Table I). This also happened to the Boyolali 200 gray assessment plant (Table II). This follows the opinion of Francis and Rajasekaran [14]. The highest plant height was found in the control plant (watering), so cell division, enlargement, and elongation went well. However, the Bantul assessment showed that the control plants had lower values than those induced by gamma rays.

TABLE II
OBSERVATION RESULT OF 200 GAMMA-RAY IRRADIATION OF BOYOLALI AND BANTUL BLACK RICE

Strain	Treatment	Height Plants	Dry Weight Plants	Dry Weight Roots
Control-01	nds	74,66± 7,21	35,12± 15,88	16,48± 7,22
M4-By-200-A	ds	78,66± 8,33	35,21± 4,44	10,55± 2,49
M4-By-200-B	ds	78,90± 4,78	32,57± 22,07	9,58± 4,89
M4-By-200-C	ds	81,16± 6,60	42,70± 8,31	17,29± 5,35
M4-By-200-D	ds	76,46± 10,04*	29,44± 7,90	13,83± 2,76
M4-By-200-E	ds	85,50± 6,76*	27,43± 17,73	24,62± 14,10
Control-01	nds	67,40± 8,55	43,10± 11,93	16,14± 0,66
M4-Bt-200-A	ds	78,66± 8,33	25,53± 18,42	14,64± 2,92
M4-Bt-200-B	ds	78,90± 4,78	19,13± 13,15	13,39± 8,81
M4-Bt-200-C	ds	81,16± 6,60	20,60± 13,41	10,28±8,71
M4-Bt-200-D	ds	76,46± 10,04*	34,23± 14,72	11,52± 5,24
M4-Bt-200-E	ds	85,50± 6,76*	13,66± 16,93	12,36± 17,28

Remarks:
ds = drought stress, which means treatment watering 50% water needed in 200 gray irradiations; nds = not drought stress, means treatment watering 100% water needed in control plant; The number followed by (*) sign is significantly different from control with ∝= 0,05

Observation results of dry weight variables in 200 gamma-ray irradiations of Bantul Black Rice showed the lowest values were gamma-induced plants under drought stress, while Control-01 plants showed higher values (shown in Table II). Gamma-ray radiation can produce diversity in many plant species. Changes in traits in mutants reach 95-98%, generally, from dominant to recessive traits. There are differences in radiation doses that cause morphological changes [15]. This study showed drought stress treatment to provide differences in plant dry weight yields.

The availability of nutrients plays an important role as an energy source, so the level of nutrient adequacy plays a role in influencing a plant's biomass. A decrease in soil water content causes a decrease in nutrient uptake during drought stress, thereby reducing the number of nutrients that diffuse from the soil matrix to the root uptake surface [16]. Drought stress can occur at conditions <60% field capacity. Associated with a decrease in plant height due to drought stress, plants reduce the number of leaves to reduce water evaporation as a plant response to drought [17].

The results of the dry weight of all treatments showed no significant difference. The results data in Table 1 of Bantul on M4-Bt-100-A had the lowest value of 16.25, and Control-01 plants had the highest value of 27.88. Dry weight indicates the weight of the plant without the water content. Control plants without stress treatment had the highest dry weight compared to other plants that were irradiated with 100 gray gamma rays and 50% water stress treatment. This is due to water limitations under stress conditions so that during growth, the results of their metabolism are more focused on roots to survive drought stress [18]. The research results by Pipai [19] treatments without stress and drought stress gave significant and significantly different results on the weight of plant parts, including grain. Grain weight without stress has a higher weight than stress because the supply of photosynthesis needs is not disturbed in normal water conditions, while under stress conditions, the formation of assimilated substances to fill the grain is inhibited.

Giving high doses will inhibit cell division, which causes cell death and affects the growth process, decreasing growth power and plant morphology. The growth of the canopy shows the potential for photosynthesis, so a lack of water will decrease the photosynthetic process, inhibiting plant growth and development [20].

Dry root weight showed no significant difference in all treatments (Table I and Table II). Table I shows the control and 100 gamma rays irradiation mean value is almost the same, while the control mean value is higher than the 200 gamma-ray irradiation plant (shown in Table I and Table II). It can be seen in Table 200gy that Boyolali control-01 has an average value of 16.37, while the M4-Bt-200-A irradiation has an average value of 14.64 (Table II).

The mechanism of root properties related to drought resistance, among others, deep roots can affect water absorption by the size of the groundwater reservoir. The amount of penetration (penetration) of roots in hard soil layers increases water absorption in conditions of deep groundwater storage. Also, it adjusts to root osmotic stress and increases groundwater availability for plants in water shortage conditions [21].

Predominantly dry soil conditions can cause a decrease in dry root weight, causing the soil strength to become greater, making compacting the soil easier [22]. Drought stress is a condition where the soil experiences a decrease in moisture in the root area, which can directly interfere with physiological functions so that, at a certain level, it can cause root growth disruption and reduce plant dry root weight [23].

Aerenchyma is an oxygen transport pathway in rice plants, thus allowing rapid air movement from the crown to the roots. The roots of rice plants and other aquatic plant species contain *Aerenchyma*, facilitating the movement of oxygen and other gases in the roots. This is reinforced by the opinion of Rea [24], which states that the lower the given field capacity, the less groundwater availability, so water absorption by roots is inhibited, and photosynthesis produced is reduced so that the resulting root weight is low. The decrease in root dry weight in plants induced by gamma rays was thought to have occurred due to mutations due to exposure to gamma irradiation, which caused changes in the chromosomal and DNA composition that affected plant metabolism [25].

In this study, irradiation treatment and drought stress affected plant height, dry weight, and canopy dry weight. The comparison can be seen in Table I and Table II. The characteristics of plants with irradiation had shorter plant heights, and plants irradiated with 100 grays had shorter plant heights than 200 gray plants. The dry weight of plants without stress had a higher mean value than 50% drought stress, and the dry weight of plant crowns with 100 gray irradiations also had a lower value than plants with 200 gray irradiations. The root dry weight of plants with stress treatment was higher than without stress, and the root dry weight of plants irradiated with 200 grays showed a higher average than plants irradiated with 100 grays. Changes in traits in mutants reach 95-98%, generally from dominant to recessive traits. There are differences in radiation doses that cause morphological changes.

B. Proline Content

Proline measurement was carried out using the ninhydrin method. The data was calculated from the regression equation with proline content (x) and absorbance (y) and obtained the equation y = 6.903x +0.0558. 21 The proline content obtained is still in the form of M. To make it in gram/ml, it must be multiplied by BMP (Proline Molecular Weight) 115.13 gram/mol. The research results on proline content in 100 gray and 200 irradiated gray plants are presented in Figure 1 (Boyolali variety) and Figure 2 (Bantul variety).

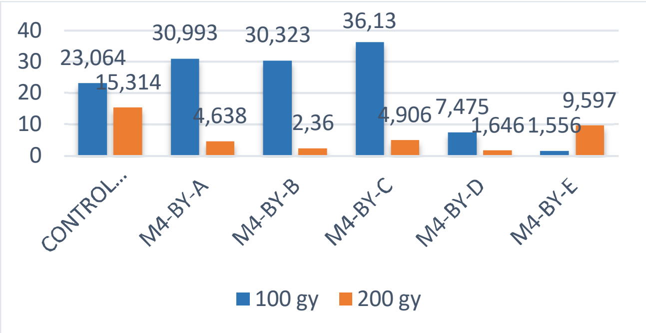

Fig. 1 Proline content in 100 and 200 gray gamma-ray irradiation of Boyolali seeds M4 generation by different soil water treatments

The proline content of the M4-By-C line, which was 36.13, showed the highest value, with a watering drought stress factor of 50%, when compared to control plants, Control By-01 had a lower value of 23.064 (Figure 1). This is presumably because proline in plants with low water availability is synthesized because of cell osmotic regulation by increasing levels of dissolved compounds in cells so that the intracellular osmotic potential is lower or at least comparable to the osmotic potential of the medium around the cell [26].

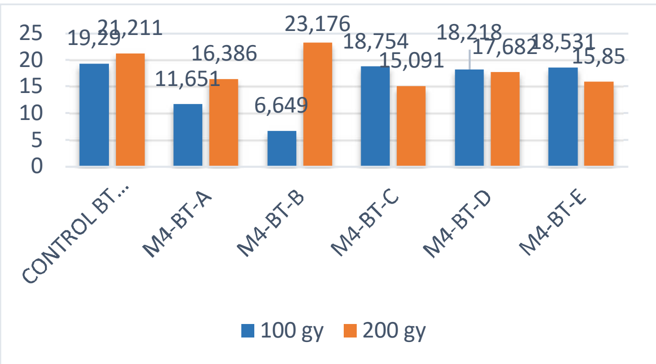

Fig. 2 Proline content in 100 and 200 gray gamma-ray irradiation of Bantul seeds M4 generation by different soil water treatments

The 200 gy plants showed that the M4-Bt-C line had the highest proline content of 23,176, while the control plant Bt-01 showed a lower value of 10,736 (Figure 2). Proline accumulation is an attempt by plants to maintain cell turgidity. The accumulation of compatible osmolytes can reduce the water potential in the cells, thereby allowing additional water uptake from the environment and protecting the mechanism from the effects of water deprivation. So, adjusting plant osmosis can help deal with water stress.

However, higher proline content did not occur in all stressed plants. It is suspected that the amount of proline accumulation produced by plants will differ according to their genetic characteristics. The difference between the two irradiation factors is that they also have different proline content. The proline content of irradiated 100 gy has a higher value than irradiated 200 gy (Figures 1 and 2). This is due to the deterministic effect of gamma-ray irradiation, resulting in cell death due to exposure to the irradiation. The higher the irradiation dose, the lower the maximum growth potential, germination, vigor index, growth speed, and plant growth simultaneously.

C. Leaf Temperature

This M4 generation of black rice plant breeding aims to study the growth of black rice and the physiological characteristics of irradiated plants. Temperature is one of the important variables in research on stress because the higher the drought stress, the higher the leaf temperature. Leaf temperatures for each Boyolali and Bantul variety are presented in Figures 3 and 4, respectively.

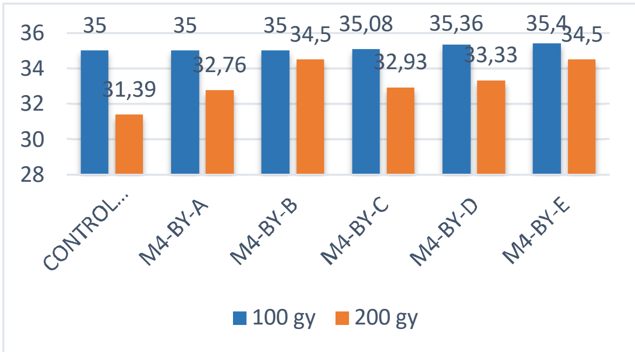

Fig. 3 Leaf temperature in 100 and 200 gray gamma-ray irradiation of Boyolali seeds M4 generation by different soil water treatments

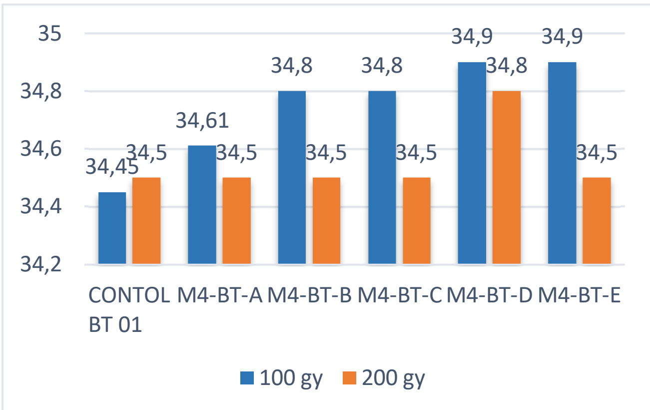

Fig. 4 Leaf temperature in 100 and 200 gray gamma-ray irradiation of Bantul seeds M4 generation by different soil water treatments

Leaf temperature in Boyolali black rice with 100 gray irradiation treatment had higher temperature than the 200 gray irradiation treatment (Figure 3). The average temperature of Boyolali black rice leaves is 100 gray and 200 gray, respectively, 35.14 degrees Celsius and 33.23 degrees

Celsius. In addition, leaf temperature in control plants was lower than in plants treated with drought stress. Under conditions of strong solar radiation, the average leaf temperature is around 35 degrees Celsius [27].

The leaf temperature of Bantul black rice with 100 gray irradiation treatment had an average of 34.74 Celsius, while plants with 200 gray irradiation treatment had a lower average of 34.55 Celsius, but the two did not have much difference. Water is an absolute necessity for plants' various metabolic activities [28]. Leaf temperature will increase when plants are exposed to high temperatures. High environmental temperatures are also affected by watering capacity, affecting the temperature of the root area and an increase in leaf temperature. This is the result of leaf temperature; lower leaf temperatures are needed for plants to reduce transpiration rates and maintain normal physiological functions in leaves [29]; when warmer temperatures encourage higher plant transpiration, physiologically, the leaf temperature increases [30].

IV. Conclusion

Plant breeding with gamma ray irradiation affects the characteristics of crop yields, and water stress affects the proline content (plant physiology) of M4 generation Bantul and Boyolali black rice. The height of the black rice plant with the treatment has a shorter posture, so it is not easy to fall. The dry weight of the plant crown was lower with treatment than without treatment, and on the contrary, the dry weight of plant roots was higher with treatment than without treatment. The physiological variables of leaf temperature and proline content in treated plants had higher values than in control plants. The M4-By-C and M4-Bt-C lines are prospective to be cultivated as new superior varieties because these lines have the advantage of shorter plant height so that they are not easy to fall and have high proline content.

Acknowledgments

Grant of P2M UNS 2021 funds this research. The authors thank the observation team, Rizhal Akbar Jaya and Wiwin Anjar Mustikasari, for their participation.

References

[1] M.S. Farooq, X. Wang, M. Uzair, H. Fatima, S. Fiaz, Z. Maqbool, O.U. Rehman," Recent trends in nitrogen cycle and eco-efficient nitrogen management strategies in aerobic rice system", *J. Frontiers in Plant Science,* vol.13, no.36, pp. 24, 2022, doi: 10.3389/fpls.2022.960641.

[2] E. Abascal, L. Gómez-Coma, Ortiz, "Global diagnosis of nitrate pollution in groundwater and review of removal technologies", *J. Sci Total Environ, vol.*810, pp. 152233, 2022, doi: 10.1016/J.SCITOTENV.2021.15223.

[3] M.A. Haque, R. Mia, S.T. Mahmud, M.A. Bakar, "Sustainable dyeing and functionalization of wool fabrics with black rice extract", *J. Science Direct,* vol. 7, no.1, pp. 10334, 2022, doi: 10.1016/j.resenv.2021.100045.

[4] T.A. Urmi, M.M. Islam, K.N. Zumur, M.A. Abedin, M.M. Haque, "Combined Effect of Salicylic Acid and Proline Mitigates Drought Stress in Rice (*Oryza sativa* L.) through the Modulation of Physiological Attributes and Antioxidant Enzymes", *J. MDPI*, vol. 12, no.7, pp. 1-9, 2023. doi: 10.3390/antiox12071438.

[5] S.M.N. Tourky, W.M. Shukry, M.A. Hossain, "Cobalt enhanced the drought-stress tolerance of rice (*Oryza sativa* L.) by mitigating the oxidative damage and enhancing yield attributes", *J. Science Direct*, vol. 159, no.5, pp. 197-207, 2023. doi: 10.1016/j.sajb.2023.05.035.

[6] M. Sharma, M. Chaudhary, S.N. Raina, D. Sahoo, "Comparative analysis of the efficiency of seed protein profiles in assessing genetic variation and population structure among indigenous Manipur black rice cultivars", *J. Springer*, vol. 50, pp. 3365–3378, 2023, doi: 10.5897/AJB11.2178.

[7] O. Herliana, I. Widiyawati, and S. N. Hadi, "The Effect of Stable Manure and Seedling Number on Growth and Yield of Black Rice (Oryza sativa L. Indica)," *Caraka Tani J. Sustain. Agric.*, vol. 34, no. 1, pp. 13–21, 2019. doi: 10.20961/carakatani.v34i1.27098.

[8] E.Beyramizadeh, A. Arminian, A. Fazeli, "Evaluating the effect of gamma rays on Zamiifolia (Zamioculcas zamiifolia) plant in vitro and genetic diversity of the resulting genotypes using the ISSR marker", *J. S Nature*. vol. 13, no. 8308, pp 522-534, 2023, doi: 10.1038/s41598-023-35618-2.

[9] S.N. Mizil, E.A.J. ElKaaby, M.Z.F. Al-Shammary, "In Vitro Effect of Mannitol Stress and γ-ray on Secondary Products From Golden Sunrise Cherry Tomato Callus", *J. Ibn Al-Haitham Journal for Pure and Applied Sciences,* vol.36, no. 3, 2023, doi: 10.30526/36.3.3117.

[10] İ.H. Çerçi, Ö.F. Durusoy, K.N. Çerçi, "The Effects of Shriveling up Waste Watermelons in Different Solar Drying Systems on Withering and Relevant Microbial Parameters", *J. Scielo Brazil*, vol. 1, no. 66, 2023, doi: 10.1590/1678-4324-2023220861.

[11] B. Adhikari, O.J. Olorunwa , S. Brazel , T.C. Barickman, "Impact of salt stress on physiology, leaf mass, and nutrient accumulation in romaine lettuce", J. Photosynthetica, vol. 61, no.3, pp. 342-353, 2023, doi: 10.32615/ps.2023.027.

[12] L S Bates, RP Waldren, ID Teare, "Rapid Determination of Free Proline for Water-stress Studies". Plant and Soil, Vol 39(1), pp.205-207, 1973. doi: 10.1016/j.jinsphys .2022.104363.

[13] S. Syafi, V. Suryanti, B. Pujiasmanto, and E. Purwanto, “Morphological Response of Takka Plant (Tacca leontopetaloides L.) as Traditional Medicine for Drought Stress,” in *5th International Conference on Food, Agriculture and Natural Resources (FANRes 2019)*, 2020, pp. 121–125. doi: 10.2991/aer.k.200325.024

[14] N. Francis, R. Rajasekaran, "Gamma irradiation to induce beneficial mutants in proso millet (*Panicum miliaceum* L.): an underutilized food crop", *J. Radiation Biology vol.1, pp. 1277-1288, 2022,* doi: 10.1090/09553002,2022,20224292.

[15] A.J.S. Abaka, M.H.S. Al-Asadi, N.Y. Abdel, "Effect of Cultivar and Spraying With The Amino Acid Phenylalanine on The Yield of Black Rice Grains and Their Content of Some Anthocyanin Pigments", *J.Annals of Forest Research, vol.66. no.1, pp.3278-3290, 2023,* doi: 10.3390/jof9060611.

[16] O. Ahluwalia, P. C. Singh, and R. Bhatia, "A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria," *Resour. Environ. Sustain.*, vol. 5, p. 100032, 2021. doi: 10.1016/j.resenv.2021.100032

[17] R.K. Salgotra, B.S. Chauhan, "Ecophysiological Responses of Rice (*Oryza sativa* L.) to Drought and High Temperature", *J. MDPI Agronomy,* vol.13, no.7.pp. 1877, 2023, doi: 10.3390/agronomy13071877.

[18] M.J. Zaghum, K. Ali, S. Teng, "Integrated Genetic and Omics Approaches for the Regulation of Nutritional Activities in Rice (*Oryza sativa* L.)", *J. MDPI Agriculture, vol. 1,. No.11, pp. 1757, 2022,* doi: 10.3390/agriculture12111757.

[19] S.A.F. El-Okkiah, M.M. El-Afry, "Foliar spray of silica improved water stress tolerance in rice (*Oryza sativa* L.) cultivars", *J.Frontiers in Plant Science,* vol.13, no.1, 2022, doi: 10.3389/fpls.2022.935090.

[20] R. Pipai, A. McNeill, M. Unkovich, M. Banabas," Biological nitrogen fixation by legume cover plants in oil palm plantations: calibration of the ureide technique and effects of plantation age and soil nitrat", *J. Springer,* vol.8, no.1, pp. 1-16, 2023, doi: 10.1007/s11104-023-06147-8.

[21] B. Sharma, S. Tiwari, K.C. Kumawat," Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations", *J. Science Direct,* vol. 860, 2023, doi: 10.1016/j.scitotenv.2022.160476.

[22] N.S. Shimoyama, "Targeted Mapping of Oryza sativa Chilling Tolerance Candidate Genes Using Multiple Stress-Relevant Quantitative Traits", Ph.D. dissertation, Faculty of the Graduate School, Marquette University, 2023.

[23] H.F. Alharby,” Combined Role of Fe Nanoparticles (Fe NPs) and *Staphylococcus aureus* L. in the Alleviation of Chromium Stress in Rice Plants”, *J.MDPI Life,* vol.12, no.3, pp. 338, 2022, doi: 10.1016/j.scitotenv.2022.160476.

[24] R.S. Rea, M.R. Islam, M.M. Rahman, B. Nath, K. Mix, "Growth, Nutrient Accumulation, and Drought Tolerance in Crop Plants with

Silicon Application: A Review", *J.MDPI Sustainability,* vol.14, no.8, pp.4525, 2022, doi: 10.3390/su14084525.

[25] S. Shrestha, "Bioconversion of agricultural residues into value-added products by pectinase-producing bacteria and expression of pectinase gene in E. coli for biomass valorization", Ph.D. dissertation Department of Biotechnology, Lakehead University, 2023.

[26] A. Thomas, R. Beena, G. Laksmi, K.B. Soni, S. Alex, "Changes in sucrose metabolic enzymes to water stress in contrasting rice genotypes", *J. Science Direct,* vol.5, 2022, doi: 10.1016/j.stress.2022.100088.

[27] A. He, T. Zhao, Q. Yin, Y. Mu, Y. Wang," Effects of shading at different growth stages with various shading intensities on the grain yield and anthocyanin content of colored rice (*Oryza sativa L.*)", *J. Science Direct,*vol.283, no.108555, 2022, doi: 10.1016/j.fcr.2022.108555.

[28] J. Singh, S. Kumar, "Evaluation of the DNDCv.CAN model for simulating greenhouse gas emissions under crop rotations that include winter cover crops", *J.Csiro,* vol.60, no.6, pp.534-546, 2021, doi; 10.1071/SR21075.

[29] F. Sarsu, S. Penna, G.C. Nikalje, "Strategies for Screening Induced Mutants for Stress Tolerance", *J.Springer,* pp.151-176, 2023, doi; 10.1079/9781789249095.000.

[30] W. Sadok, J. R. Lopez, and K. P. Smith, "Transpiration increases under high-temperature stress: Potential mechanisms, trade-offs and prospects for crop resilience in a warming world," *Plant, Cell \& Environ.*, vol. 44, no. 7, pp. 2102–2116, 2021. doi:10.1111/pce.13970.