



Effect Of Salt Stress On Morphological And Physiological Characters Of Different Rice Landraces (*Oryza Sativa* L.) From Shirala Tehsil

^{1,2}Shweta Magdum, ¹Sourabh Chavan, ¹Prerana Bhat, ¹Shreya Kamble, ¹Sadhana Kadam and ²Shivram Khadke

¹ Department of Botany, Jaysingpur College Jaysingpur (Affiliated to Shivaji University Kolhapur)
Maharashtra - 416101, India

²Department of Botany, Smt. Kasturbai Walchand College, Sangli, (Affiliated to Shivaji University
Kolhapur) Maharashtra - 416101, India

Abstract: Abiotic stresses such drought, temperature, salinity, metal toxicity, and herbicides are common for plants and can all have an impact on crop yields. Salinity is one of the severe environmental restrictions that have a negative impact on crop loss and productivity globally among all abiotic stresses. In order to produce rice with acceptable characteristics, the landraces of rice are crucial for providing advantageous qualities. These are crucial genetic resources for plant breeding because they fall somewhere between wild relatives and cultivars. The necessity of producing and improving salt-tolerant crops, especially rice, has grown in importance in recent years. The response of the landraces Jondhala, Masad, and Wandar of *Oryza sativa* L. to NaCl at varying salinity levels (0, 50, 100, 150, 200, and 250 mM) was examined with a focus on early seedling stage and seed germination. High salinity reduced germination percentage of seeds and low salinity concentration showed higher germination percentage. The lower concentration increased biomass and seedling height as compare to high concentration in all three rice landraces. Results showed that 50mM and 100mM NaCl in Jondhala and Wandar enhanced the root growth with more roots developed at this salinity. 50mM, 150 mM NaCl is effective for root length in Masad. Abnormal seed germination was found in higher salt concentration due to inhibition of root growth. This study proposes that degree of tolerance of rice landraces to NaCl from morphological result. This study might be useful for further research of salinity effect on growth and physiological processes at advanced stage of these all landraces growth.

Index Terms - Biomass, Germination, Stress, Rice landraces, Salinity, Shirala Tehsil.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most cultivated cereal crop and important staple food all over the world. China was the origin of rice cultivation thousands of years ago, and the practice gradually extended throughout Asia and eventually the rest of the world. With around 640 million tons produced there, or 90% of the world's total, the Asian continent is the primary producer of rice.

India is one of the world's largest producers of rice, grown mostly in the eastern and southern parts of the country. After the spread of rice cultivation in Indian subcontinent, rice became a staple food in the region. Rice production is an important part of Indian economy.

Maharashtra state contributes only 2.8% rice production in India. In Maharashtra, Konkan is well known for rice production. Shirala Tehsil of Sangli district is hotspot of rice cultivation. Shirala Tehsil is located at hilly region of Sahyadri ranges in Sangli district. About 45 rice landraces are cultivated in different localities of Shirala Tehsil. Economy of farmers in Shirala Tehsil is depends on rice cultivation in that region.

One of the most significant environmental stresses affecting plant growth and development is salt stress. Due to their sessile nature, plants must evolve appropriate adaptations to high salinity settings. Salt stress can lead to harmful sodium buildup and a rise in intracellular osmotic pressure. Additionally, it exhibits physiological and metabolic alterations in the way that seeds germinate, photosynthesis, and growth. Various rice landraces react to NaCl concentrations in various ways. Low osmotic potential caused by high soil salinity prevents seeds from germinating.

The different salt concentrations have an impact on the germination and growth of the landraces of Jondhala, Masad, and Wander rice. The landraces selected for the project came from the Shirala Tehsil in the Sangli District of Maharashtra, which is renowned for its rice cultivation and has a long history of producing a wide range of indigenous varieties. While some landraces of rice have a positive effect on crop output, others have the opposite effect. Numerous studies have been conducted to assess the salt tolerance of various rice varieties (Hakim et al., 2010; Jamil et al., 2012). The research of the seed response to different salts is truly important since the effects of different salt types on *Oryza sativa* L. seed germination and early seedling growth are not fully understood. In recent years, the importance of creating and enhancing salt-tolerant crops—particularly rice—has increased. To ensure food security for the growing population, much research needs to be focused on understanding how this important crop responds to environmental stress, especially salt (Amirjani, 2010). Additionally, it is usually costly and time-consuming to reclaim land that has been affected by salt (Tsegay & Gebreslassie, 2014). This circumstance has led some scientists to become interested in developing salt-tolerant rice cultivars in an effort to prevent unnecessary losses in agriculture while also reducing the problems related to food scarcity (Hakim et al., 2014). Therefore, expanding agriculture into areas affected by salt requires developing high-yielding, salt-tolerant rice cultivars.

II. RESEARCH METHODOLOGY

2.1 Plant materials and seed sterilization

We gathered Wandar, Jondhala, and Masad seeds from Shirala Tehsil farmers. With a few minor adjustments, seed sterilization was carried out in accordance with the study of Htwe et al. (2011). After choosing seeds that were robust, healthy, and consistent in size, they were surface sterilized for 30 seconds using a 70% ethanol solution. Fungicide was used to wash the seeds for an additional twenty minutes. After being carefully cleaned five times with autoclaved distilled water, the seeds were allowed to air dry on tissue paper.

2.2 Experimental design and salinity treatments

Ten sterile seeds were spread out and allowed to develop on Whatman No. 1 filter paper in a sterile Petri dish in order to examine the impact of salt on seed germination. Salt solutions with concentrations of 0 (deionized water) as a reference, 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM, depending on the kind of salt, were used to wet each filter paper (Khan et al., 1997). Each Petri dish was filled with 5 ml of the corresponding concentrations of each type of salt. For two weeks, the treatments were kept in a growth chamber at a room temperature of $25 \pm 1^\circ\text{C}$ and 12 hours of sunshine (Hakim et al., 2010). To make sure the results were consistent, the experiment was conducted three times.

2.3 Observation and data collection

Up to fourteen days, the number of seeds that sprouted and germinated was counted and observed every day. According to M. S. Rahman et al. (2001), seeds that have developed the capacity to form at least one discernible plumule or radicle are referred to be sprouted seeds. When a radicle of at least 2 mm emerged from the seed coat, the seed was said to have germinated. Following the administration of the medication for fourteen days, parameters were measured and computed. According to Tsegay and Gebreslassie (2014), the experiment's metrics were water absorption %, germination percentage, germination index, relative injury rate, seed vigor, mean germination time, biomass, and salt tolerance. Three seedlings were chosen at random for each treatment of each type of salt in order to measure the morphological traits of the seedlings, such as height, shoot length, root length, and leaf length (Zhang et al., 2014). Using a dissecting microscope, the morphological traits of the leaves and roots for each treatment were noted at the early seedling stage.

2.3.1 Measurement of Water Uptake Percentage

The maximum, minimum, standard deviation, mean, and normally distributed values for each of the study's variables were determined using descriptive statistics. Data with a normal distribution demonstrates how sensitive the variables are to cyclical fluctuations and conjecture. When data is not regularly distributed, it indicates that it is susceptible to cyclical fluctuations and speculations, which can lead to arbitrage opportunities and provide investors with the opportunity to make more than the typical profit. However, the APT makes the premise that investors can only make regular profits and that there shouldn't be arbitrage in the market. Data normality is tested using the Jarque-Bera test.

2.3.2 Measurement of Germination Percentage (GP)

In research, germination percentage—the actual proportion of seeds in a sample that sprout in an experiment—is crucial for comparing the quality of seed collections (FAO, 1983). Using (Kandil et al., 2012), this parameter was calculated.

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

2.3.3 Relative Injury Rate (RIR)

Relative injury rate is the rate of injured or damaged seeds as compared to the healthy germinated seeds.

$$\text{Relative Injury Rate} = \frac{\text{GP in control} - \text{GP in salt treatment seeds}}{\text{GP in Control}}$$

2.3.4 Survival Percentage

Following seed germination, some of the plantlets that have sprouted suffer harm from outside sources, which leads to their eventual demise. The number of plantlets that remain after damage is used to determine the survival percentage.

2.3.5 Measurement of Seed vigour

The entire collection of seed characteristics that serves as a gauge of seed activity and performance during germination and seedling growth is known as seed vigor. It also shows that the seed's capacity to perform all physiological functions has diminished. The Abdul-Baki and Anderson (1973) approach was used to calculate the value of seed vigor.

$$\text{Seed vigour} = \frac{\text{Length of hypocotyl} + \text{length of radical}}{100} \times GP$$

2.3.6 Measurement of Salt Tolerance (ST)

Salt tolerance can be calculated by referring to (Tsegay and Gebreslassie, 2014).

$$\text{Salt Tolerance} = \frac{\text{Seedling dry weight of salt treatment}}{\text{Seedling dry weight in control}} \times 100$$

2.3.7 Measurement of Biomass

To determine biomass, seedlings must be weighed both fresh and dried before and after treatments. On the day of harvest (Day 15), the fresh weight of the seedlings in each treatment was measured. For weight standardization, fresh seedling samples were then dried for 48 hours at 78°C. The dry weight of the seedlings was then determined by weighing them once again. (Carpicy et al., 2009).

2.3.8 Proline estimation

The dried plant material was broken down to produce sulphosalicylic acid. A crushed plant sample was transferred to a centrifuge tube, and sulphosalicylic acid was added to bring the amount down to 10 ml. The material was centrifuged for ten minutes at 5000 RPM. The next step was using 1 milliliter of the centrifuged sample's supernatant. To the supernatant, two milliliters of glacial acetic acid and ninhydrin reagent were added. The sample was chilled in an ice bath after being cooked for an hour in a water bath. Add 4 milliliters of toluene to the sample. To separate the two layers, the test tube was shaken vigorously and let to rest. The top layer was removed for proline spectrophotometric measurement at a wavelength of 520 nm.

III. RESULTS AND DISCUSSION

3.1 Water Uptake Percentage:

In Wandar 250mM concentration shows high water uptake percentage than other concentrations in Wandar. In Masad 250mM concentration shows high water uptake % than other concentrations in Masad. In Jondhala 150mM concentrations shows high water uptake % than other concentrations in Jondhala.

Table:1- Water Uptake Percentage of rice landraces at different salt concentrations

Landraces	Control	50mM	100mM	150mM	200mM	250mM
Wandar	22.89%	23.61%	18.65%	19.25%	24.67%	46.74%
Masad	22.13%	23.58%	25.65%	22.68%	21.76%	27.30%
Jondhala	18.50%	19.70%	15.67%	23.90%	22.82%	16.00%

3.2 Germination percentage:

In Wandar germination % is 100% in control, 50mM, 100mM concentrations, while germination % is 90% in 150mM, 200mM and 250mM concentrations. In Masad germination % is 60% in control, 50mM, 100mM and 250mM while in 150mM it is 20% and in 200mM it is 50%. In jondhala germination % is 100% in control and 200mM concentrations while it is 90% in 50mM and 100mM concentrations and 80% in 150mM concentrations.

Table:2- Germination percentage of rice landraces at different salt concentrations

Landraces	Control	50mM	100mM	150mM	200mM	250mM
Wandar	100%	100%	100%	90%	90%	90%
Masad	60%	60%	60%	20%	50%	60%
Jondhala	100%	90%	90%	80%	100%	80%

3.2 Relative Injury Rate:

In Wander injury rate of 50mM and 100mM concentrations is 0% while the injury rate of 150mM, 200mM and 250mM concentrations is 0.1%. The injury rate in Masad 50mM, 100mM and 250mM is 0% while in 150mM is 0.66% and 200mM is 0.16%. Injury rate in Jondhala 50mM and 100mM concentrations is 0.1%, while in 150mM and 250mM concentrations is 0.2% and 200mM concentration has 0%.

Table:1- Water Uptake Percentage of rice landraces at different salt concentrations

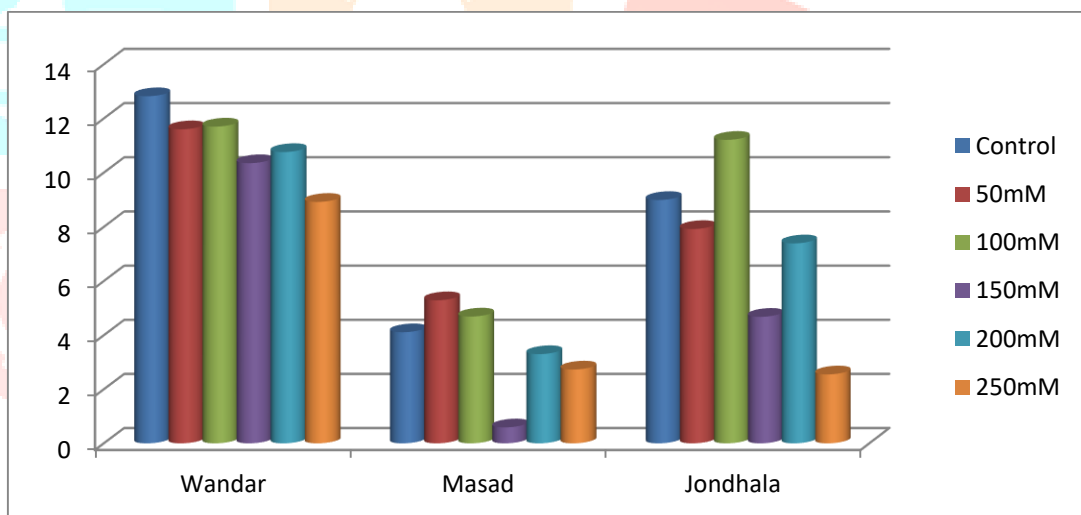
Landraces	Control	50mM	100mM	150mM	200mM	250mM
Wandar	-	0%	0%	0.1%	0.1%	0.1%
Masad	-	0%	0%	0.66%	0.16	0%
Jondhala	-	0.1%	0.1%	0.2%	0%	0.2%

3.3 Seed Vigor:

In Wandar seed vigor of control is more than all other concentrations and the seed vigor of 250mM concentration is less than all concentrations. In Masad Seed vigor of 50mM is more than all other concentrations and the seed vigor of 150mM is less than that of all the concentrations. In Jondhala seed vigor of 100mM concentration is more than all other concentrations, while the seed vigor of 250mM is less than all other concentrations.

Table:3- Seed Vigor of rice landraces at different salt concentrations

Landraces	Control	50mM	100mM	150mM	200mM	250mM
Wandar	12.82	11.6	11.7	10.35	10.76	8.92
Masad	4.11	5.29	4.69	0.59	3.30	2.73
Jondhala	8.99	7.92	11.21	4.68	7.39	2.55



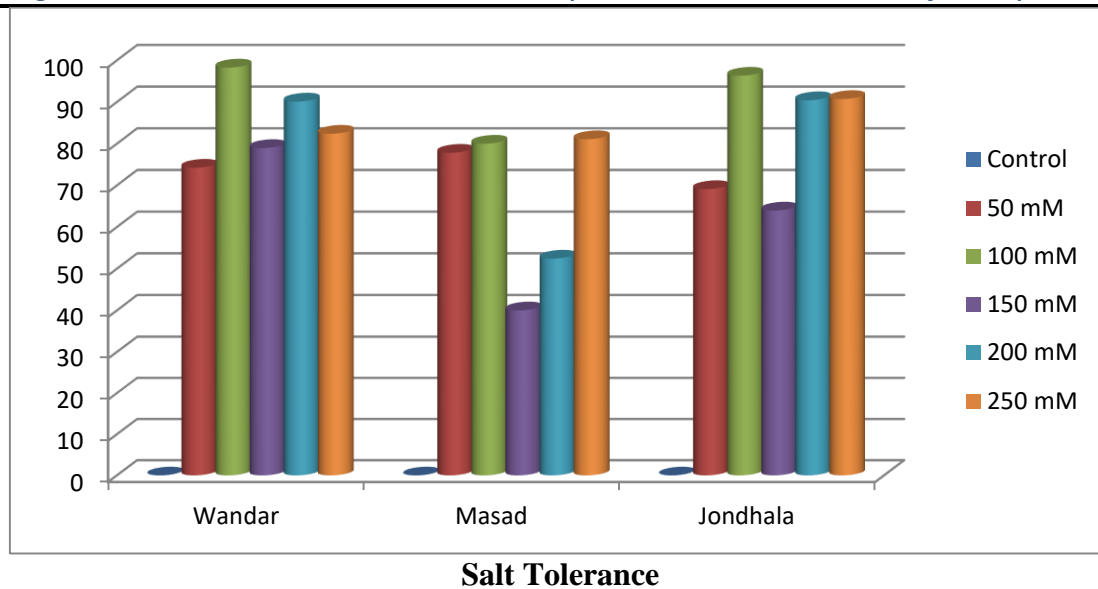
Seed Vigor

3.4 Salt Tolerance:

In Wandar Salt tolerance is more in 100mM concentration and less in 50mM concentration. In Masad Salt tolerance is more in 250mM concentration and less in 150mM concentration. In Jondhala Salt tolerance is more in 250mM concentration and less in 150mM concentration.

Table:4- Salt Tolerance of rice landraces at different salt concentrations

Landraces	Control	50 mM	100 mM	150 mM	200 mM	250 mM
Wandar	-	74.24	98.28	78.96	90.12	82.40
Masad	-	77.83	80	40	52.43	81.08
Jondhala	-	69.11	96.32	63.97	90.44	90.73

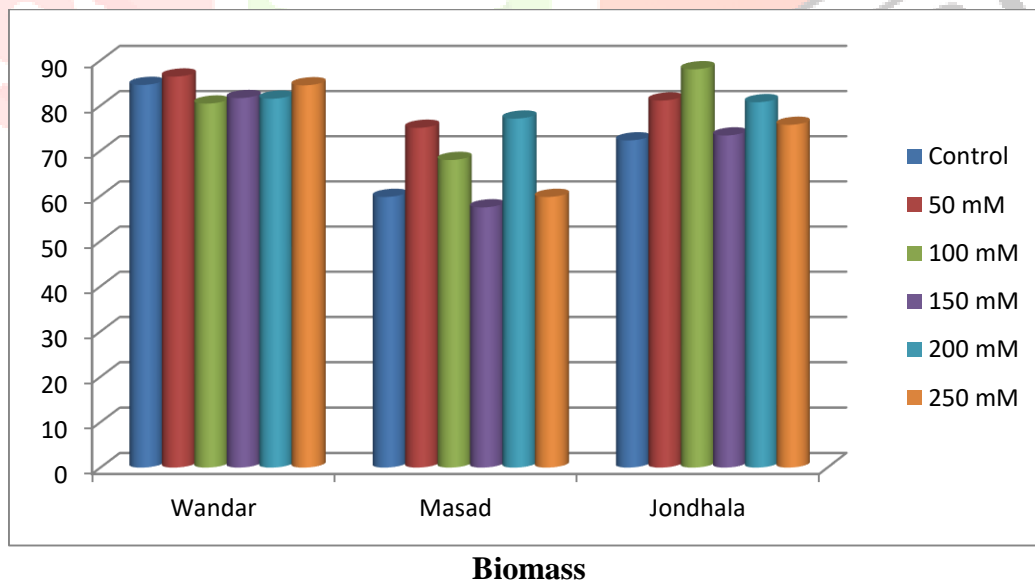


3.5 Biomass (mg):

In Wandar Measurement of biomass is more in 50mM concentration and less in 100mM concentration. In Masad measurement of biomass is more in 200mM concentration and less in 150mM concentration. In Jondhala measurement of biomass is more in 100mM concentration and less in control.

Table:5- Biomass of rice landraces at different salt concentrations

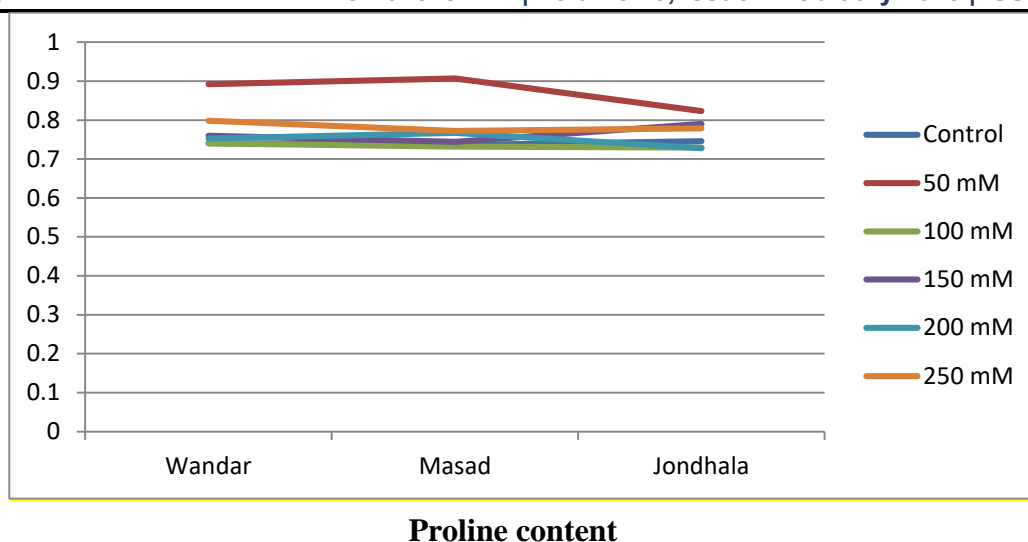
Landraces	Control	50 mM	100 mM	150 mM	200 mM	250 mM
Wandar	84.58	86.43	80.45	81.67	81.53	84.49
Masad	59.78	75.08	67.96	57.47	77.12	59.78
Jondhala	72.30	81.12	88.04	73.39	80.75	75.74



3.6 Proline content (mg):

Table:6- Water Proline content (mg) of rice landraces at different salt concentrations

Landraces	Control	50 mM	100 mM	150 mM	200 mM	250 mM
Wandar	0.741	0.892	0.740	0.760	0.752	0.798
Masad	0.736	0.907	0.732	0.744	0.767	0.772
Jondhala	0.746	0.823	0.729	0.790	0.728	0.779



The amount of plant stress is directly indicated by the proline concentration. The rice seedlings of Wandar, Masad and Jondhala landraces treated with different salt concentration has the highest proline content at 50 mM and 250 mM salt concentration. Whereas the seedling treated with 100mM salt concentration has the lowest proline content.

IV. CONCLUSION

According to the study, three rice landraces treated with 50 mM and 100 mM NaCl concentrations show enhanced germination, survival rates, and seed vigor, although greater concentrations often result in lower germination percentages. Across the three landraces, lower concentrations of NaCl often perform better at encouraging seedling development and vigor. Furthermore, different levels of plant stress are indicated by proline concentration, with lower proline levels being linked to more successful therapies. Here, the Jondhala, Masad, and Wandar landraces of rice have decreased proline content at a concentration of 100 mM NaCl. Thus, among all the treatments, a concentration of 100 mM NaCl is the most effective.

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VI. REFERENCES

- [1] Abdul-Baki, A. A., & Anderson, J. D. 1973. Vigor determination in soybean seed by multiple criteria. *Crop Science*, 13(6), 630-633.
- [2] Almeida, D. M., Almadanim, M. C., Lourenço, T., Abreu, I. A., Saibo, N. J., and Oliveira, M. M. 2016. Screening for abiotic stress tolerance in Rice: salt, cold, and drought. *Environmental Responses in Plants: Methods and Protocols*, 155-182.
- [3] Amirjani, M. R. 2010. Effect of NaCl on some physiological parameters of rice. *Eur J Biol Sci*, 3(1), 6-16.
- [4] Carpycy, E., Celyk, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. *African Journal of Biotechnology*, 8(19).
- [5] Hakim, M., Juraimi, A. S., Begum, M., Hanafi, M., Ismail, M. R., & Selamat, A. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, 9(13), 1911-1918.

- [6] Jamil, M., Bashir, S., Anwar, S., Bibi, S., Bangash, A., Ullah, F., & Rha, E. S. 2012. Effect of salinity on physiological and biochemical characteristics of different varieties of rice. *Pak J Bot*, 44, 7-13.
- [7] Kalhori N., Ying T., Nulit R., Sahebi M., Abiri R. and Atabaki N. 2018. Effect of Four Different Salts on Seed Germination and Morphological Characteristics of *Oryza sativa* L. cv. MR219. *International Journal of Advanced Research in Botany*, 4(1): 29-45.
- [8] Kandil, A., Sharief, A., & Ahmed, S. 2012. Germination and seedling growth of some chickpea cultivars (*Cicer arietinum* L.) under salinity stress. *Journal of Basic & Applied Sciences*, 8, 561-571.
- [9] Khan, M., Hamid, A., & Karim, M. 1997. Effect of sodium chloride on germination and seedling characters of different types of rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*, 179(3), 163-169.
- [10] Luan, Z., Xiao, M., Zhou, D., Zhang, H., Tian, Y., Wu, Y., Guan, B., & Song, Y. 2014. Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (*Helianthus annuus* L.). *The Scientific World Journal*, 2014.
- Nozulaidi, M., Khairi, M., & Jahan, M. S. (2015). Effects of different salinity levels on rice production. *Aust J Basic Appl Sci*, 9, 524-530.
- [11] Pradheeban, L., Nissanka, N., & Suriyagoda, L. 2015. Clustering of Rice (*Oryza sativa* L.) Varieties Cultivated in Jaffna District of Sri Lanka based on Salt Tolerance during Germination and Seedling Stages. *Tropical Agricultural Research*, 25(3).
- [12] Tobe, K., Li, X., & Omasa, K. (2004). Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (Chenopodiaceae). *Seed Science Research*, 14(04), 345-353.
- [13] Rajpar, I., Sultan, M., Depar, N., & Kharal, M. 2016. Tolerance of barley (*Hordium vulgare* L.) to chlorides of sodium, calcium and magnesium. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences (Pakistan)*.
- [14] Singh, J., Sastry, E. V. D., & Singh, V. 2012. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. *Physiology and molecular biology of plants : an international journal of functional plant biology*, 18(1), 45-50. doi: 10.1007/s12298-011-0097-z
- [15] Sudharani, M., Reddy, P. R., & Jayalakshmi, V. 2012. A Comprehensive Review on "Genetic Components Of Salinity Tolerance in Rice (*Oryza sativa* L.)". *Int J Appl Biol Pharm Technol*, 3, 312-322.
- [16] Teh, C. Y., Mahmood, M., Shaharuddin, N. A., & Ho, C. L. 2014. In vitro rice shoot apices as simple model to study the effect of NaCl and the potential of exogenous proline and glutathione in mitigating salinity stress. *Plant Growth Regulation*, 75(3), 771-781.
- [17] Tsegay, B. A., and Gebreslassie, B. 2014. The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. abyssinicum. *African Journal of Plant Science*, 8(5), 225-231.
- [18] Zehra, A., Gul, B., Ansari, R., Alatar, A., Hegazy, A., & Khan, M. A. 2013. Interactive effect of salt, light and temperature on seed germination and recovery of a halophytic grass-*Phragmites karka*. *Pak. J. Bot*, 45(3), 725-736.
- [19] Zhang, D., Wang, Z., Wang, N., Gao, Y., Liu, Y., Wu, Y., Bai, Y., Zhang, Z., Lin, X., & Dong, Y. 2014. Tissue culture-induced heritable genomic variation in rice, and their phenotypic implications. *PLoS one*, 9(5), e96879.