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PRIMING OF AMMONIUM NITRATE ON BIOCHEMICAL CHANGES IN GERMINATING RICE SEEDS AND SEEDLINGS

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ABSTRACT

Rice (*Oryza sativa* L.) is the staple food for almost half of the world so enhancing rice yield is vital for national food security, environmental sustainability and to mitigate climate change effects. This experiment was conducted at Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP, India in the *kharif* season of year 2023 to study the effect of nutri-priming using ammonium nitrate on biochemical changes in germinating rice seeds and seedlings. Ten treatments including 2mM, 4mM, 6mM, 8mM, 10mM, 15mM, 20mM and 25 mM of ammonium nitrate, hydro-primed and dry seeds, were taken for comparison. These treatments were statistically analysed under complete randomized design with three replications. Seeds treated with ammonium nitrate were found to have better enhanced á-Amylase activity, total soluble sugar and total soluble protein in germinating rice seeds and total chlorophyll and carotenoids, total soluble sugar, total soluble protein and nitrate reductase activity in their seedlings as compared to hydro-primed and dry seeds. Priming of ammonium nitrate @ 15mM and 20mM to rice seeds for 18 hours enhanced all biochemical parameters. The study emphasized the hypothesis that priming with ammonium nitrate salt outperformed dry seeds.

(Key words: Rice, nutri-priming, ammonium nitrate, biochemical changes, germinating seeds, rice leaves)

INTRODUCTION

As a major food crop in Asia, enhancing rice yield and nitrogen use efficiency is vital for national food security, socioeconomic stability, and buffer the effect of climate change. Abiotic stresses such as drought, flood, cold, heat, heavy metal, etc. can cause serious disruptions in the physiological and biochemical processes of plants which affect seed vigour and lead to low productivity in crops (Hussain *et al.*, 2019). To overcome these various strategies have been employed to enhance abiotic stress tolerance in plants. Seed priming is one such method which is a practical and straightforward technique that effectively promotes rapid and uniform seed emergence, increases seedling vigor, and improves yields in many field crops, especially under adverse environmental conditions (Zhang *et al.*, 2023). Madavi *et al*. (2024) in their experiment, found an increase in plant height due to application of Zn, Cu, Mn and B. Nitrogen (N) is part of biomolecules necessary for plant metabolism, such as nucleic acids, amino acids, proteins, hormones, and pigments. In general, nitrate (NO_3^-) and ammonium (NH_4^+) are the main sources of inorganic N available in soils for plants (Sandhu *et al.*, 2021). The objective of this study was to mitigate the impact of climate change and abiotic stress on rice productivity and reduce the use of fertilizers thus reducing pollution and leading to sustainability of environment.

MATERIALS AND METHODS

This experiment was conducted at Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP, India in the *kharif* season of year 2023 to study the effect of nutri-priming using ammonium nitrate on biochemical changes in germinating rice seeds and their seedlings. The research was laid out in CRD with three replications and ten treatments as 2mM, 4mM, 6mM, 8mM, 10mM, 15mM, 20mM, and 25mM of ammonium nitrate, hydro-primed and non-primed seeds with rice genotype Nati Mansoori which were washed and surface sterilized using 0.01% $HgCl_2$ (mercuric chloride) solution for two minutes. Following this, the seeds were thoroughly rinsed 4-5 times with distilled water. Seeds were primed by soaking them in specific solutions at a ratio of 10 seeds to 5 ml of solution in a beaker. This process was carried out for 18 hours at 30°C in a BOD incubator. After soaking, the seeds were air-dried under a fan to return them to their original moisture content.

Sowing of the seeds

Primed seeds from different salt treatments were placed in separate petri plates, covered with germination paper, and spaced evenly. These plates were then placed in a BOD incubator at 28°C for 7 days. For pot culture 30 pots were filled with soil mixed with well-decomposed FYM. Then in each pot, 2-3 primed seeds were sown.

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Studies of biochemical parameters

On 3rd day after germination endosperm of germinated seeds were taken and the enzyme assay of á-Amylase activity was done using the Bernfeld (1955) method. The total soluble sugar content in the endosperm of germinating rice seeds was estimated on the 3rd day after germination using the anthrone method used by Dubois *et al.* (1956). Total soluble protein content was estimated on the 5th day after germination in the endosperm of germinating rice seeds using the Bradford method (1976).

On 15th days after germination the chlorophyll and carotenoid content of the leaf sample was determined by the non-destructive method given by Hiscox and Israelstam (1979) known as DMSO method, total soluble sugar content was estimated in the leaves of ammonium nitrate primed seedlings using anthrone method used by Dubois *et al.* (1956) and total soluble protein content was estimated using the Bradford method (1976). The nitrate reductase enzyme activity of green leaves was determined by using the method of Srivastava *et al.* (1974) at 20th day after sowing.

All the data collected were analysed by OPSTAT statistics software (Sheoran and Vinay Kumar, CCS HAU, HISAR). To test the significance of treatments, one-way analysis of variance (ANOVA) was carried out for biochemical parameters at 5% level of significance using Completely Randomised Design.

RESULTS AND DISCUSSION

Seeds

á-Amylase activity on 3rd DAG (µM maltose ml^{-1} min^{-1})

The data from the table indicates that ammonium nitrate priming influenced significantly á-amylase activity on the 3rd day of germination. Non-primed dry seeds exhibited significantly lower (2.56 µM maltose ml^{-1} min^{-1}) á-amylase activity compared to both hydro-primed (3.28 µM maltose ml^{-1} min^{-1}) and ammonium nitrate primed seeds. Additionally, when comparing hydro-primed seeds to ammonium nitrate seeds, the latter show notably higher á-amylase activity. However, á-amylase activity due to ammonium nitrate priming was highest (5.53 µM maltose ml^{-1} min^{-1}) in 15 mM NH_4NO_3 across different ammonium nitrate concentrations. There was increase in á-amylase activity on increasing the concentration of NH_4NO_3 up to 15mM and then decreased at higher concentrations. Similarly Sathish and Sundareswaran (2010) found an increase in á-amylase activity when treated with 1% KH_2PO_4. Wang *et al.* (2016) found a strong correlation between enhanced á-amylase activity and higher seed germination rates, improving seedling growth, and stress tolerance during the establishment phase of the seedlings.

Total soluble sugar content on 3rd DAG (mg g^{-1} FW)

The effect of ammonium nitrate (NH_4NO_3) priming on total soluble sugar in the endosperm of the rice variety Nati Mansoori on the 3rd day of germination was found to influence significantly. Non-primed dry seeds exhibited significantly lower (3.48 mg g^{-1} FW) total soluble sugar compared to both hydro-primed (3.69 mg g^{-1} FW) and ammonium nitrate primed seeds. Ammonium nitrate seeds on comparison with hydro-primed seeds were found to have significantly higher total soluble sugar content which was highest (7.82 mg g^{-1} FW) in 15 mM NH_4NO_3 across different ammonium nitrate concentrations. Total soluble sugar content increased with increasing concentrations of ammonium nitrate up to 15mM and then decreased with further higher concentrations. According to Mukasa *et al.* (2003) there is an increase in metabolism in endosperm which in turn increases á-amylase activity and enhances total soluble sugar content. Pereira *et al.* (2021) also found in their study that there was a high level of total soluble sugar present in the roots of seeds which were treated with ammonium nitrate.

Total soluble protein content on 5th DAG (mg g^{-1} FW)

On the 5th day of germination, the effect of ammonium nitrate (NH_4NO_3) priming on total soluble protein content in the endosperm of the rice influenced significantly. Dry seeds exhibited significantly lower (0.56 mg g^{-1} FW) total soluble protein content compared to both hydro-primed (0.63 mg g^{-1} FW) and ammonium nitrate-primed seeds. Ammonium nitrate primed seeds showed higher total soluble protein content compared to hydro-primed seeds which was highest (1.68 mg g^{-1} FW) in 20 mM NH_4NO_3 across different ammonium nitrate concentrations. Total soluble protein content was increased up to 20mM of NH_4NO_3 and then decreased. Seed priming enhanced plants ability to counteract oxidative stress by regulating processes such as scavenging reactive oxygen species (ROS), enhancing antioxidant defence, accumulating metabolites like soluble proteins, and upregulating genes involved in secondary metabolism (Khan *et al.*,2019).

Seedling

Total chlorophyll and carotenoids on 15th DAG (mg g^{-1} FW)

The total chlorophyll and carotenoid content which was measured from the leaves of rice seedlings obtained from sowing ammonium nitrate (NH_4NO_3) primed seeds at 15th days after germination was found significantly higher from dry seeds and hydro-primed seeds. Leaves from dry seeds exhibited lower chlorophyll (3.39 mg g^{-1} FW) and carotenoid (0.18 mg g^{-1} FW) content. Hydro-primed treatment also showed a significant influence on non-primed dry seeds with 4.40 mg g^{-1} FW chlorophyll and 0.21 mg g^{-1} FW carotenoids. Among the ammonium nitrate treatments, it was found that 25 mM NH_4NO_3 treatment resulted in significantly higher (6.69 mg g^{-1} FW) chlorophyll and (0.39 mg g^{-1} FW) carotenoid content compared to other treatments. There was an increase in the total chlorophyll and carotenoid content on increase in concentrations. Dhawne *et al.* (2024) found in their experiment an increase in the total chlorophyll content of flag leaf of rice on application of inorganic nitrogen fertilizers.

Total soluble sugar content on 15th DAG (mg g^{-1} FW)

The data on the effect of ammonium nitrate (NH_4NO_3) priming on total soluble sugar in the leaves of the rice on the 15th day of germination indicate a significant influence on total soluble sugar. Dry seeds exhibit significantly lower (15.11 mg g^{-1}) total soluble sugar compared to both hydro-primed (15.71 mg g^{-1}) and ammonium nitrate primed seeds. Additionally, when comparing hydro primed seeds to ammonium nitrate seeds, the latter showed notably higher on total soluble sugar content which was found similar as in seeds. However, on total soluble sugar content due to ammonium nitrate priming was highest (17.36 mg g^{-1}) in 25 mM NH_4NO_3 across different ammonium nitrate concentrations. It showed an increase in total soluble sugar content on increasing concentrations of ammonium nitrate. According to Zrig *et al.* (2022) potassium nitrate primed linseed was having good amount of soluble sugar compared to non-primed seeds. It increased fructose from 0.23 to 1.6 mg g^{-1} FW, glucose and sucrose was increased by 3 folds from non-prime.

Total soluble protein content on 15th DAG (mg g^{-1}FW)

From the data on the effect of ammonium nitrate (NH_4NO_3) priming on total soluble protein content in the leaves of the rice variety Nati Mansoori on the 15th day of germination, it was found to influence significantly on total soluble protein content as dry seeds exhibited significantly lower (6.36 mg g^{-1} FW) total soluble protein content compared to both hydro-primed (6.89 mg g^{-1} FW) and ammonium nitrate primed seeds. On comparing hydro-primed seeds to ammonium nitrate-primed seeds, the latter was found higher on total soluble protein content and highest (8.20 mg g^{-1} FW) in 25 mM NH_4NO_3 across different ammonium nitrate concentrations which was found to increase with increasing concentrations. Seed priming enhances plants' ability to counteract stress by regulating processes such as enhancing antioxidant defence, accumulating metabolites like soluble proteins, and upregulating genes involved in secondary metabolism (Zulfiqar and Muhammad, 2021). Total soluble protein content was found to be enhanced in the leaves of wheat seedling when compared with hydro-primed.

Nitrate Reductase Activity content on 20th DAG (n mol g^{-1} FW hr^{-1})

The data reveal that ammonium nitrate priming of rice seeds significantly influenced nitrate reductase activity as dry seeds exhibited significantly lower (156.28 n mol g^{-1} FW hr^{-1}) content of nitrate reductase activity compared to ammonium nitrate primed and hydro primed (168.48 n mol g^{-1} FW hr^{-1}). Ammonium nitrate treated seeds were found to perform better than hydro-primed. Among the treatments with different salts, at 20th days of germination, nitrate reductase activity imposed by ammonium nitrate priming significantly increased with concentrations with 25 mM NH_4NO_3 (210 n mol g^{-1} FW hr^{-1}). Srivastava *et al.* (2017) in their experiment with rice seeds found that seeds treated with ammonium nitrate have significantly better amounts of nitrate reductase activity.

Hence, it was found that á-amylase activity and total soluble sugar content on 3rd day after germination were found highest in treatment 15 mM NH_4NO_3 whereas dry seeds and hydro-primed exhibit significantly lower á-amylase activity and total soluble sugar content. Total soluble protein content was found highest in treatment 20 mM NH_4NO_3 on the 5th day after germination in the endosperm of the germinating seed compared to hydro-primed and dry seeds. Similar results were found in leaves of ammonium nitrate primed seedlings. Chlorophyll and carotenoid content was tested on 15th day after germination and found highest in 25 mM NH_4NO_3 treatment. There was significant increase in total soluble sugar content, total soluble protein content on 15th day after germination and nitrate reductase activity on 20th day after germination. It can be concluded that seed priming with ammonium nitrate enhanced primary metabolites in the germinating seeds and their leaves.

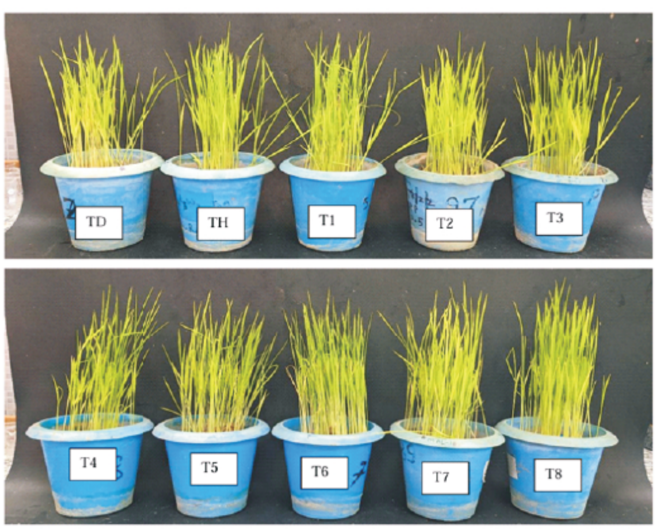

Plate 1. Effect of priming on rice seedlings germination using ammonium nitrate

TD = dry seed; TH = hydro-primed; T1 = 2mM; T2 = 4mM, T3 = 6mM; T4 = 8mM; T5 = 10mM; T6 = 15mM; T7 = 20mM; T8 = 25mM NH_4NO_3

Table 1. Effect of seed priming using ammonium nitrate salts on á-amylase activity and total soluble sugar on 3rd day after germination and total soluble protein on 5th day of germination of rice variety Nati Mansoori

Treatments	á-amylase activity (µM maltose ml^{-1} min^{-1})	Soluble sugar (mg g^{-1} FW)	Protein content (mg g^{-1} FW)
Dry seed	2.56	3.48	0.56
Hydro-primed	3.28	3.69	0.63
2mM NH_4NO_3	3.58	4.23	0.64
4mMNH_4NO_3	3.76	4.58	0.71
6mM NH_4NO_3	4.39	5.63	0.84
8mM NH_4NO_3	4.73	6.46	1.28
10mM NH_4NO_3	4.96	6.97	1.34
15mM NH_4NO_3	5.53	7.82	1.45
20mM NH_4NO_3	5.42	7.65	1.68
25mM NH_4NO_3	4.68	6.36	1.65
SEm±	0.14	0.16	0.16
CD at 5%	0.41	0.47	0.48

Table 2. Effect of seed priming using ammonium nitrate salts on Total chlorophyll and carotenoid, total soluble sugar, total soluble protein on 15th day of germination and nitrate reductase activity on 20th day after germination of rice variety Nati Mansoori

Treatments	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total soluble sugar (mg g^{-1} FW)	Total soluble protein content (mg g^{-1} FW)	Nitrate reductase activity (n mol g^{-1} FW hr^{-1})
Dry seed	3.39	0.18	15.11	6.36	156.28
Hydro-primed	4.40	0.21	15.71	6.89	168.48
2mM NH_4NO_3	4.94	0.23	15.74	6.93	172.36
4mMNH_4NO_3	5.16	0.26	15.92	7.12	181.25
6mM NH_4NO_3	5.39	0.30	16.31	7.24	183.28
8mM NH_4NO_3	5.56	0.32	16.74	7.39	187.24
10mM NH_4NO_3	5.61	0.34	16.82	7.56	193.29
15mM NH_4NO_3	5.92	0.35	16.93	7.71	196.92
20mM NH_4NO_3	6.67	0.36	17.24	8.11	206.27
25mM NH_4NO_3	6.69	0.39	17.36	8.20	210.24
SEm±	0.15	0.03	0.16	0.07	0.16
CD at 5%	0.44	0.09	0.47	0.20	0.47

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