

Article ID: 188711
DOI: 10.5586/aa/188711

Publication History
Received: 2023-10-25
Accepted: 2024-05-14
Published: 2024-07-11

Handling Editor
Alina Wiszniewska; University of Agriculture in Kraków, Kraków, Poland; <https://orcid.org/0000-0001-7737-819X>

Authors' Contributions
NS: Research concept and design; MN: Collection and/or assembly of data; KM: Data analysis and interpretation; TAY: Writing the article; AR, AW: Critical revision of the article; OF: Final approval of the article






Funding
No funding was received to conduct this study.

Competing Interests
The authors have no competing interests to declare that are relevant to the content of this article.

Copyright Notice
© The Author(s) 2024. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.

ORIGINAL RESEARCH

Elevated auxin levels during the reproductive stage improve rice crop productivity and grain quality

Muhammad Naveed¹, Naeem Sarwar ^{1*}, Khuram Mubeen², Atique-ur Rehman ¹, Omer Farooq ¹, Allah Wasaya ¹, Tauqeer Ahmad Yasir ¹

¹Institute of Agronomy, Bahauddin Zakariya University, Multan 60800, Pakistan

²Department of Agronomy, MNS University of Agriculture, Multan 59070, Pakistan

* To whom correspondence should be addressed. Email: naeemsarwar@bzu.edu.pk

Abstract

A higher sterility percentage is a common characteristic in the rice crop nowadays due to soil moisture and elevated temperature stress. We hypothesized that an improved auxin level during the reproductive stage may overcome this issue in common rice cultivars. Various rice cultivars were grown in pots, and naphthalene acetic acid (NAA) was applied as a source of auxin with a variable concentration (0 (N₀), 20 (N₁), 30 (N₂), 40 (N₃), and 50 (N₄) μmol L⁻¹) at the reproductive stage. The results revealed that all the levels of NAA improved crop productivity in all the cultivars, while its higher level (40 μmol L⁻¹), i.e. N₃ along with cultivar V₂ (Punjab Basmati), gave supreme results. The NAA application elevated the auxin level in the plants and improved the content of antioxidants to overcome the oxidative stress in the rice crop. The improved physiological mechanism resulted in higher crop productivity in terms of grain weight, grain yield, and harvest index under the aforementioned treatment combination. Moreover, it improved the quality of rice grains, and a very low sterility percentage was recorded in the treatment with the NAA application. It was also reported that grain quality was also maintained even after cooking. Therefore, the foliar application of NAA at the reproductive stage may be a useful strategy for improving rice growth, morpho-physiological characteristics, grain yield, and quality attributes.

Keywords

fine rice; naphthalene acetic acid; photosynthesis; antioxidants; quality

1. Introduction

To ensure food security for the rapidly growing world population, crop production should be increased by 50% by 2050 (van Dijk et al., 2021). The global temperature is rising continuously and is expected to rise by 5–7 °C at the end of the century, which may affect crop production, especially cereals (Beena et al., 2013; IPCC, 2021). Reduced production of cereals may create a food security concern, particularly in developing countries like Pakistan (Janni et al., 2020; Sarwar et al., 2023). To combat the situation, special attention is needed for better crop productivity in current climatic conditions (Dinar et al., 2019; Khan et al., 2023).

Cereals are the dietary need for a large portion of the population (Waldamichael et al., 2022) in which the rice crop is ranked at a significant position due to its widespread consumption (Ruan et al., 2023). Rice is a dominant crop in more than 30 countries of the world, with above 90 % production in Asia (Kondamudi et al., 2012). High temperature stress is one of the key factors in current global warming situation. Rice crop yields may decline up to 41 % due to elevated temperature (Shah et al., 2011). The rice crop is suffering in the current weather conditions as it is highly sensitive to abiotic stresses, especially at the reproductive stage (Mujtaba et al., 2022; Neang et al.,

2020). Elevated temperature (≥ 32 °C) causes a significant decline in crop growth, stomatal conductance, and pollen viability, which in turn reduces plant biomass and enhances the proportion of sterile grains (Beena et al., 2018; Fu et al., 2012; Jagadish et al., 2012).

Many strategies are being used to manage abiotic stresses, including nutrient application and the use of hormones or growth regulators. Auxin is a very important growth regulator (Barbier et al., 2019; Matthes et al., 2019) improving plant growth and panicle development in normal and stressed environments (Abou El-ghit, 2015; Li et al., 2019). Similarly, it improves the antioxidant activity, which stabilises plants under abiotic stresses (Khan et al., 2023). Likewise, optimum levels of auxin also improve the plant rooting mechanism and enhance nutrient uptake (Kurpea & Smalle, 2020).

In a stressed environment, especially at the reproductive stage, auxin production in plants is reduced, which causes pollen viability and thus reduced fertility in the rice crop (Fu et al., 2015; Zhang & Peer, 2017). Auxin (Indole-3-acetic acid) is naturally produced in plants, while its concentration can also be enhanced through its exogenous application. It has been reported that the application of naphthalene acetic acid (NAA) improves the auxin level in plants (Sarwar et al., 2019), which strengthens leaf chlorophyll contents and other photosynthetic activities in plants (Hossain, 2023). It also plays a crucial role in optimisation of physiological processes like leaf senescence, leaf & fruit abscission, fruit setting, and development of vascular tissues (Alabadí et al., 2009). NAA also improves pollen viability, thereby enhancing the number of fertile grains, leading toward better crop yield and quality (Bakhsh et al., 2011; Hussain et al., 2021; Sajid et al., 2016).

Most plants produce antioxidants in stress conditions to manage the situation, while this process can also be triggered by exogenous application of some growth regulators and essential nutrients. Auxin can also work as a stimulator to produce some antioxidants under drought, heat, or nutritional stress (Al-Duraid et al., 2019; Khan et al., 2023). Eminent results have been achieved with NAA application in horticultural and field crops (Basuchaudhuri, 2016; Fatima et al., 2008; Hossain, 2023; Sarwar et al., 2019). Studies have also explored that NAA application improves rice grain quality in terms of its protein contents, fineness, and moisture contents (Jahan & Adam, 2014). Moreover, exogenous application of auxin mitigates heat stress and improves crop yield and grain quality (Aryan et al., 2023). Therefore, we hypothesised that application of NAA at the flowering stage may mitigate the elevated temperature effect, resulting in improvement of grain quality as well as crop yield and its component. Thus, different available genotypes were grown and observed under various levels of NAA. Our major objective was to determine the optimum level of NAA application at the reproductive stage along with the best responsive cultivar in the current climatic conditions. This study will provide valuable information for farmers to maintain rice yield and quality standards in the current climatic condition.

2. Materials and methods

This experiment was conducted in pots placed in a wire house during the 2017 kharif season at the agronomic research area of the Department of Agronomy, Bahauddin Zakariya University Multan, Pakistan. Pots (25 cm × 40 cm × 30 cm) were filled with fertile soil collected from the surrounding field area. Before experimentation, soil samples were collected for the analysis of different physico-chemical properties. The soil was determined as silty clay following the hydrometer technique. Similarly, soil pH (7.8) and EC (1.73 dS m^{-1}) were determined using lab instruments. Moreover, the soil contained 0.46% of organic matter, total Nitrogen (1.8%), Phosphorus (6.52 mg/kg), and available Potassium (175 mg/kg).

The seeds of rice cultivars were collected from the rice research station Kala Shah Kaku Lahore Punjab, Pakistan. Four (4) fine rice genotypes, i.e. V₁ (Kisan Basmati), V₂ (Punjab Basmati), V₃ (Chenab Basmati), and V₄ (Super Basmati), were selected and exposed to four levels of NAA. The experiment was arranged under a completely randomised design (CRD) having three replications. There were 16 pots in one replication, which were replicated thrice. The seeds were water soaked for 24 hours and

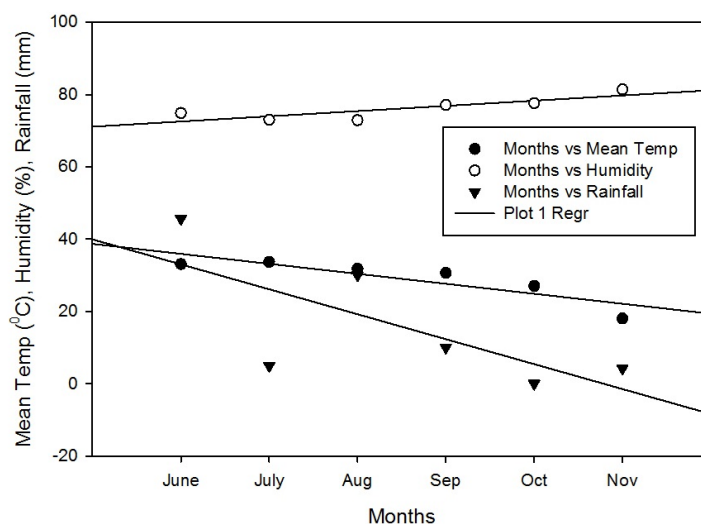


Figure 1 Climatic variations in the growing period.

then sown in respective pots. After successful germination and seedling emergence, the plants were thinned to 3 plants/pot and were allowed to grow in normal conditions up to maturity. NAA was foliarly applied using a hand pump with different concentrations, i.e. N_0 (control), N_1 (20), N_2 (30), N_3 (40), and N_4 (50) $\mu\text{mol/L}$, right before the flowering stage. Irrigation was applied by visual observation to keep the pots moist. No weeds were allowed to grow in the pots. Similarly, Furadan was also applied at the tillering stage to control the root or stem borer attack. Moreover, a fungicide was also applied at the time of flowering for the control of leaf blight. The irrigation was stopped one week before harvesting. The crop span was about 155 days (last week of June – last week of November) starting from sowing to harvesting. All the plants were harvested from each pot, tagged, and placed in the open air in sunshine for one week. Data regarding yield and quality traits were observed afterwards. Climate data were recorded for the growth period of the whole summer season (2017) i.e. June–Nov. It was revealed that June and July were recorded as the hottest months, while October and November were found as the coolest months. The maximum rainfall was recorded in the month of June and compared with others (Figure 1).

2.1. Yield parameters

The pots were harvested and tagged for recording the data of different yield parameters. All the samples were weighed and noted for biological yield (BY) per pot, which were converted to BY per hectare numerically. Similarly, 1,000 grains were counted from each treatment and weighed. Likewise, all the grains were separated manually and weighed for each pot or treatment, which was further converted to grain yield per hectare etc. The harvest index (%) was calculated from the following formula.

$$\text{Harvest Index} = \frac{\text{Grain yield}}{\text{Biological Yield}} \times 100$$

2.2. Quality parameters

Regarding kernel quality, normal kernels (filled grains) were separated from sterile kernels (un-filled) of each treatment and their percentage was determined. These parameters were measured manually using a light lamp and noted. For the determination of amylose contents, the method described by Juliano (1971) was used. Using a spectrophotometer, the intensity of blue colour was studied at 620 nm wavelength. Protein contents were determined with the Bradford method (1976). Grain length, breadth, and thickness (mm) were measured with a vernier caliper, whereas the

elongation ratio (E/R ratio) was calculated by the following formula

$$ER = \frac{\text{Average length of cooked kernel}}{\text{Average length of uncooked kernel}}$$

2.3. Antioxidants

One week after the application of NAA, leaf samples were taken from each treatment and antioxidant activities were determined with the methods suggested by Wu et al. (2014), which are described as follows: Catalase (CAT) was determined with the procedure proposed by Hu et al. (2009). A 3.0 ml mixture was formed from 50 mM phosphate buffer, enzyme extract, and 15 mM H₂O₂. The activity of CAT was determined at 240 nm. Superoxidase dismutase (SOD) was determined as in Stewart and Bewley (1980). A 3.0 ml mixture was prepared using 50 mM phosphate buffer, 13 mM methionine, 0.1 μM EDTA, 75 μM NBT, enzyme extract and 2 μM riboflavin, which was further processed for 15 min and illuminated with 20 W florescent tubes, and reading was taken at 560 nm wavelength. Peroxidase (POD) activity was determined as in Shi et al. (2010). A 3.0 ml mixture was prepared by using 100 μl guaiacol (1.5%) v/v, 100 μl enzyme extract, 100 μl H₂O₂, 2 mM EDTA, and 2.7 ml potassium sulphate buffer. The mixture then underwent through processing and absorbance was read at 470 nm.

Auxin contents were determined using the method described by Hu et al. (2011). To this end, 0.5 g of fresh plant tissue was taken and homogenised in 50 ml of methanol/water (80/20, v/v), which contained 0.01 butylated hydroxytoluene (BHT), and blended in a high speed blender. After that, the solution was placed in –20 °C in a refrigerator for 1 day then centrifuged to collect the supernatant. The samples were analysed for Auxin contents using high-performance liquid chromatography (HPLC).

2.4. Statistical analysis

Statistical analysis of compiled data was done using Fisher's analysis of variance in Statistix software, and significance of treatments was analysed by using the least significant difference (LSD) test at 5% probability (Steel et al., 1997).

3. Results

3.1. Grain yield and quality

The yield parameters showed that Punjab Basmati (V₂) and Kisan Basmati (V₁) produced heavier grains (57%) under 40 μmol/L (N₃), thereby enhancing the grain yield, and a 15.42% higher yield was recorded, compared with the control treatment. Similarly, V₂N₃ had a higher biological yield (14.77 t ha⁻¹) and the harvest index (36.42%), which was almost 9.73% and 4.59% higher than in the control treatment, i.e. Super Basmati without NAA application (V₄N₀). The treatments without NAA resulted in the lowest performance in all the cultivars, with the lowest values observed in V₄N₀ (Table 1).

In terms of grain quality, the plants in the V₂N₃ treatment produced 80.64% of normal kernels, i.e. about 24% more than in the control treatment (V₄N₀). Similarly, V₂N₃ provided 53% less sterile kernels, compared to the control treatment (V₄N₀). As far as protein contents are concerned, both V₂N₃ and V₁N₃ expressed higher protein contents (11.85% and 11.74%, respectively), but the lowest protein contents were observed in V₄N₀ (10.43%). Likewise, the application of NAA decreased the amylose contents to increase the quality of grains. The V₁N₃ treatment combination contributed to production of 23.67% of amylose, which proved to be more reliable in decreasing the amylose level because it produced almost 15% less amylose contents in grains, compared to the control treatment (V₄N₀) (Table 2).

3.2. Grain cooking quality

Cooking quality is the major concern for fine rice. The results revealed that the NAA application improved the grain quality after cooking as well. The V₁N₃ treatment

Table 1 Effect of naphthalene acetic acid (NAA) on yield parameters of various fine rice genotypes.

Treatment	1000-grain weight (g)	Grain yield (t ha ⁻¹)	Biological yield (t ha ⁻¹)	Harvest index (%)
V ₁ N ₀	24.56de	4.66o	13.33op	34.95m
V ₁ N ₁	25.79b-e	4.84lm	13.69lm	35.35jk
V ₁ N ₂	28.18b-e	5.12ef	14.25de	35.92de
V ₁ N ₃	29.40ab	5.24ab	14.49b	36.16b
V ₁ N ₄	26.99b-e	4.97i	13.95hi	35.62gh
V ₂ N ₀	24.88cde	4.70o	13.41o	35.04m
V ₂ N ₁	26.11b-e	4.87kl	13.75kl	35.41jk
V ₂ N ₂	28.49b-e	5.15de	14.31cd	35.98cd
V ₂ N ₃	32.95a	5.38a	14.77a	36.42a
V ₂ N ₄	27.27b-e	5.02h	14.05gh	35.72fg
V ₃ N ₀	24.27e	4.62p	13.25p	34.77n
V ₃ N ₁	25.48b-e	4.81mn	13.63mn	35.28kl
V ₃ N ₂	27.87b-e	5.09fg	14.19ef	35.83ef
V ₃ N ₃	29.11abc	5.20bc	14.41bc	36.08bc
V ₃ N ₄	26.68b-e	4.94ij	13.89ij	35.56hi
V ₄ N ₀	13.96f	4.55q	13.08q	34.50o
V ₄ N ₁	25.19b-e	4.77n	13.55n	35.20l
V ₄ N ₂	27.56b-e	5.05gh	14.11fg	35.78f
V ₄ N ₃	28.80a-d	5.17cd	14.35cd	36.02cd
V ₄ N ₄	26.40b-e	4.90jk	13.81jk	35.47ij
LSD	4.30	0.04	0.10	0.13

V₁ = Kisan Basmati, V₂ = Punjab Basmati, V₃ = Chenab Basmati, V₄ = Super Basmati, N₀ = Control, N₁ = NAA (20 µmol L⁻¹), N₂ = NAA (30 µmol L⁻¹), N₃ = NAA (40 µmol L⁻¹), N₄ = NAA (50 µmol L⁻¹).

Table 2 Effect of naphthalene acetic acid (NAA) on quality parameters of various fine rice genotypes.

Treatment	Normal kernel (%)	Sterile kernel (%)	Protein contents (%)	Amylose contents (%)
V ₁ N ₀	70.51ef	9.75b	10.96no	25.77bcd
V ₁ N ₁	71.75b-f	9.17b-f	11.15j-m	25.37b-h
V ₁ N ₂	74.16b-e	8.01e-h	11.57b-e	24.56j-m
V ₁ N ₃	75.37b	7.50h	11.74ab	23.67n
V ₁ N ₄	72.94b-f	8.56b-h	11.38f-i	24.96f-l
V ₂ N ₀	70.86def	9.61bc	11.01mno	25.88bc
V ₂ N ₁	72.06b-f	9.03b-g	11.21i-l	25.47b-g
V ₂ N ₂	74.45b-e	7.86fgh	11.61bcd	24.67i-m
V ₂ N ₃	80.64a	5.99i	11.85a	24.25mn
V ₂ N ₄	73.22b-f	8.41c-h	11.42e-h	25.07e-k
V ₃ N ₀	69.19f	9.79b	10.93o	25.97b
V ₃ N ₁	71.43b-f	9.31b-e	11.11k-n	25.58b-f
V ₃ N ₂	73.84b-e	8.13e-h	11.52c-f	24.77h-m
V ₃ N ₃	75.08bc	7.54h	11.70ab	24.36lm
V ₃ N ₄	72.65b-f	8.74b-h	11.32g-j	25.17d-j
V ₄ N ₀	61.33g	12.86a	10.43p	27.61a
V ₄ N ₁	71.17c-f	9.45bcd	11.06l-o	25.67b-e
V ₄ N ₂	73.53b-e	8.26d-h	11.47d-g	24.87g-m
V ₄ N ₃	74.76bcd	7.72gh	11.66bc	24.45klm
V ₄ N ₄	72.34b-f	8.89b-g	11.27h-k	25.28c-i
LSD	4.13	1.31	0.17	0.63

V₁ = Kisan Basmati, V₂ = Punjab Basmati, V₃ = Chenab Basmati, V₄ = Super Basmati, N₀ = Control, N₁ = NAA (20 µmol L⁻¹), N₂ = NAA (30 µmol L⁻¹), N₃ = NAA (40 µmol L⁻¹), N₄ = NAA (50 µmol L⁻¹).

Table 3 Effect of naphthalene acetic acid (NAA) on grain quality of various fine rice genotypes after cooking.

Treatment	Grain length (mm)	Grain breadth (mm)	Grain elongation ratio (E/R)	Grain thickness (mm)
V ₁ N ₀	7.59h	1.34lmn	1.68jkl	1.18lmn
V ₁ N ₁	7.74bc	1.50bc	1.85bcd	1.34a-d
V ₁ N ₂	7.76b	1.53b	1.87ab	1.36ab
V ₁ N ₃	7.84a	1.60a	1.93a	1.38a
V ₁ N ₄	7.75bc	1.52b	1.86abc	1.35abc
V ₂ N ₀	7.58h	1.33mn	1.67kl	1.17mn
V ₂ N ₁	7.69de	1.45d-g	1.80b-h	1.29d-h
V ₂ N ₂	7.73bc	1.47cde	1.83b-f	1.31b-f
V ₂ N ₃	7.74bc	1.49bcd	1.84b-e	1.33a-e
V ₂ N ₄	7.72cd	1.46c-f	1.82b-g	1.30c-g
V ₃ N ₀	7.56h	1.32n	1.65l	1.16n
V ₃ N ₁	7.66efg	1.40h-k	1.76f-i	1.24h-k
V ₃ N ₂	7.68ef	1.42f-i	1.78d-i	1.26f-j
V ₃ N ₃	7.68ef	1.43e-h	1.79c-h	1.28e-i
V ₃ N ₄	7.67efg	1.41g-j	1.77e-i	1.25g-j
V ₄ N ₀	7.50i	1.15o	1.40m	0.95o
V ₄ N ₁	7.64g	1.35lmn	1.71i-l	1.19k-n
V ₄ N ₂	7.65fg	1.37j-m	1.74h-k	1.22j-m
V ₄ N ₃	7.65fg	1.38i-l	1.75g-j	1.23i-l
V ₄ N ₄	7.64g	1.36k-n	1.73h-k	1.21j-n
LSD	0.03	0.04	0.07	0.05

V₁ = Kisan Basmati, V₂ = Punjab Basmati, V₃ = Chenab Basmati, V₄ = Super Basmati, N₀ = Control, N₁ = NAA (20 µmol L⁻¹), N₂ = NAA (30 µmol L⁻¹), N₃ = NAA (40 µmol L⁻¹), N₄ = NAA (50 µmol L⁻¹).

combination (Kisan Basmati with 40 µmol/L NAA) contributed to statistically higher grain cooking quality parameters, including grain length (7.84 mm), grain breadth (1.60 mm), grain elongation ratio (1.93), and grain thickness (1.38 mm), which were almost 4.5%, 28.12%, 27%, and 31% higher, respectively, than in the control treatment (V₄N₀). In turn, the lowest values of grain cooking quality parameters (7.50 mm, 1.15 mm, 1.40 mm, 0.95 mm of grain length, grain breadth, elongation ratio, and grain thickness, respectively) were observed in the control treatment (V₄N₀) (Table 3).

3.3. Antioxidant activities and auxin contents

The V₂N₃ treatment combination (Punjab Basmati with 40 µmol/L NAA) exhibited the highest enzymatic activities, including Superoxidase dismutase (118.34 unit g⁻¹ FW), peroxide (4.04 µmol min⁻¹ mg⁻¹ FW), and catalase (2.76 mmol min⁻¹ mg⁻¹ FW), which were 29%, 50%, and 22% higher than in the control treatment, i.e. Super Basmati without NAA application (V₄N₀), respectively. On the other hand, the control treatment (V₄N₀) displayed the lowest enzymatic activity (SOD 83.26 unit g⁻¹ FW, POD 2.00 µmol min⁻¹ mg⁻¹ FW, CAT 2.14 mmol min⁻¹ mg⁻¹ FW). In contrast, the V₂N₃ treatment combination exhibited the maximum auxin content (26.06 ng/g), which was about 44% higher than in the control treatment (V₄N₀), as the control treatment had the lowest auxin contents (13.42 ng/g) during the study (Figure 2).

4. Discussion

The data clearly indicates that the application of naphthalene acetic acid at the panicle stage proved beneficial in enhancing the growth, yield, and quality traits of the fine rice genotypes. Among the fine rice genotypes, V₂ (Punjab basmati) produced higher results in various studied parameters. Vigorous growth was observed in the pots where NAA was applied, whereas decreased growth was noted in the pots without NAA application. This might be due to the improved level of auxin in the rice plants, which mitigated the heat or soil moisture stress and allowed the plants to grow

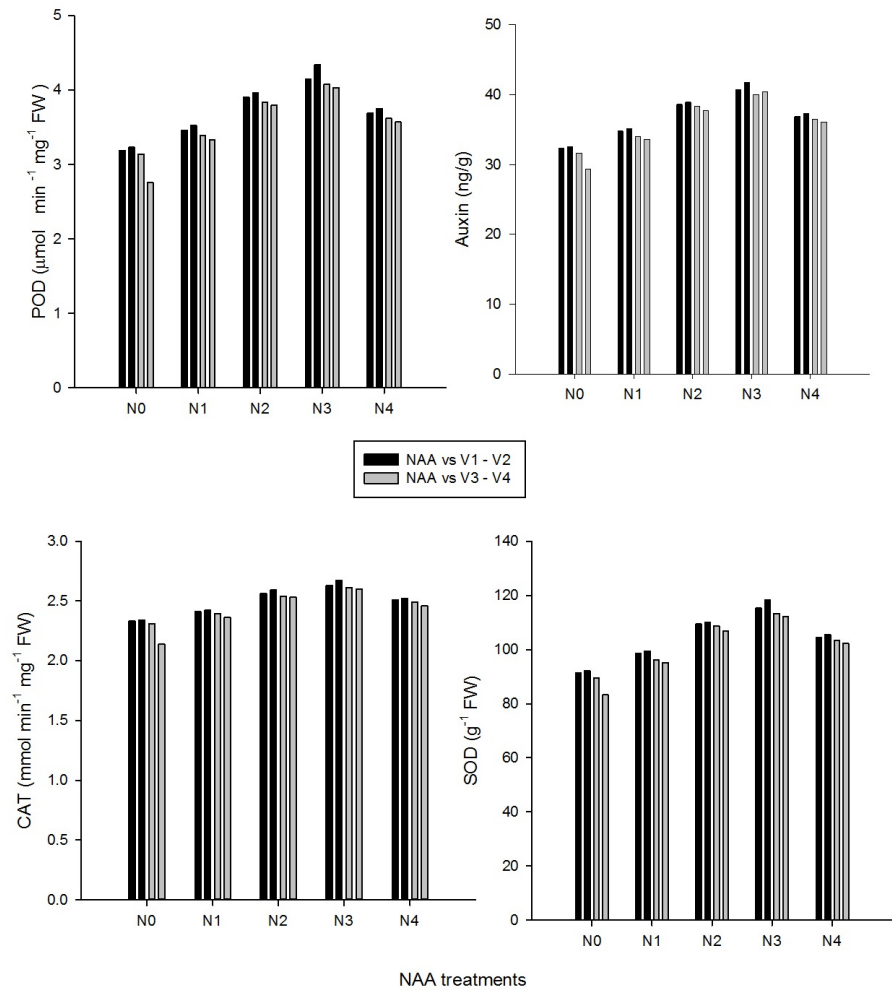


Figure 2 Enzymatic activities, POD ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{FW}$), auxin (ng/g), CAT ($\text{mmol min}^{-1} \text{mg}^{-1} \text{FW}$), and SOD ($\text{g}^{-1} \text{FW}$) levels altered by the NAA application in the rice crop.

normally, as auxin is an excellent growth regulator compound (Abou El-ghit, 2015; Li et al., 2019). Similarly, an optimum level of auxin in the plant enhances the rooting mechanism and improves the nutrient uptake (Kurpea & Smalle, 2020), potentially leading to improved crop productivity. Moreover, it has also been reported that the application of naphthalene acetic acid regulates plant growth, improves cell division, and development of associated organs (Basuchaudhuri, 2016; Hussain et al., 2021).

Abiotic stresses induce the production of ROS (reactive oxygen species), which suppress plant growth (Chauhan et al., 2022; Rehman et al., 2022). Plants have a mechanism to produce antioxidant enzymes, which improve homeostasis ROS and stress tolerance (Laxa et al., 2019). Abiotic stress causes many disabilities within plants, while a strong antioxidant system of plant acts as a scavenger of ROS (Kaya et al., 2020; Meirimler et al., 2014). The current experiment revealed that the rice plants faced stress in the control or at a low dose application of NAA, resulting in reduced crop performance. Conversely, a higher dose of NAA improved plant stress tolerance to maintain the normal growth and reproductive process, ultimately leading to better crop yield. The results exhibited that antioxidant activities, including SOD, POD, and CAT, were enhanced by the application of naphthalene acetic acid ($40 \mu\text{mol L}^{-1}$), potentially mitigating the effects of aerobic conditions or elevated temperatures. Earlier reports also revealed that the application of NAA had beneficial effects on increasing crop yield and antioxidants (Al-Duraid et al., 2019; Ullah et al., 2021). Similarly, plants treated with NAA ($40 \mu\text{mol L}^{-1}$) also enhanced auxin contents, contributing to

increased tolerance to withstand in unfavourable conditions, as auxin regulates many physiological functions in plants (Basuchaudhuri, 2016; Hu et al., 2011).

The crop growth was summed up in the grain yield; hence, the optimum growth ultimately had better results. The experiment results revealed optimised growth under the highest dose of NAA application, i.e. N₃ (NAA @ 40 µmol L⁻¹), as it improved the contents of auxin and antioxidants that scavenges ROS produced in a stressed environment. Naphthalene acetic acid N₂ (NAA @ 20 µmol L⁻¹) was slightly more prominent after N₃, but the other doses did not give considerable results. The application of naphthalene acetic acid provides better growth, which makes plant gain additional total dry biomass and enhance yield traits (Alam et al., 2002; Bakhsh et al., 2011).

Grain quality is a major concern while growing fine rice, as its export and local consumption largely depend on this parameter. The results revealed that the crop grown under the NAA application improved grain quality, especially at its highest level of application. This may be attributed to the optimum growth of the rice crop, wherein the plants produced better dry matter, which may have been transferred towards grain for its optimum size. Similarly, the crop also completed the reproductive phase in normal conditions, as NAA boosted the auxin contents, improved defensive mechanisms, and reduced pollen sterility. It was also reported that auxin improves plant growth and panicle development in both normal and stress conditions (Abou El-ghit, 2015; Li et al., 2019). Due to this fact, the crop grown under NAA had reduced grain sterility and enhanced normal kernels. Moreover, this treatment also optimised other quality parameters like amylose and protein contents, which are a result of a better growing environment. The naphthalene acetic acid foliar application maximises protein production (Jahan & Adam, 2014).

Grain cooking quality traits are generally controlled by the genetic makeup of genotypes; however, a significant trend was observed with NAA spraying. The NAA dose of 40 µmol L⁻¹ contributed to a better length, breadth, thickness, and elongation ratio of the fine rice genotype grains, whereas reduced values were observed in the control treatments. This may be attributed to the application of naphthalene acetic acid, as it promotes better plant growth and increased cell division, which may result in better yield and grain quality (Basuchaudhuri, 2016).

5. Conclusion

The rice crop exhibited improvements in growth, yield, and quality parameters under the NAA application, with maximum results observed in the V₂N₃ (Punjab Basmati with 40 µmol/L NAA) combination. These increments may be due to the combined effect of an improved physiological mechanism in the rice plants, as evidenced by the auxin level and photosynthetic efficiency. The NAA application also improved the defence mechanism by enhancing antioxidant enzymes. More importantly, it also improved the rice grain quality at harvest and after cooking, which may further enhance the international trade. The results also revealed that the auxin application improved the pollen development and reduced the negative impact of elevated temperature on fertility and grain quality.

References

- Abou El-ghit, H. M. (2015). Effect of naphthalene acetic acid (NAA) on growth and yield of rosemary (*Rosemarinus officinalis* L.) under salinity stress. *Egyptian Journal of Botany*, 56(2), 303–317. <https://doi.org/10.21608/EJBO.2016.390>
- Alabadí, D., Blázquez, M. A., Carbonell, J., Ferrándiz, C., & Pérez-Amador, M. A. (2009). Instructive roles for hormones in plant development. *International Journal of Developmental*, 53(8), Article 1597. <https://doi.org/10.1387/ijdb.072423da>
- Alam, S. M., Shereen, A., & Khan, M. A. (2002). Growth response of wheat cultivars to naphthalene acetic acid (NAA) and ethrel. *Pakistan Journal of Botany*, 34(2), 135–137.
- Al-Duraid, M. H., Al-Taey, K. A., & Al-Kikhani, A. H. J. (2019). Effect of phenylalanine and naphthalene acetic acid on growth, yield and antioxidant activity of fenugreek *Trigonella foenum-graecum*. *IOP Conference Series: Earth and Environmental Science*, 388(1), Article 012073. <https://doi.org/10.1088/1755-1315/388/1/012073>
- Aryan, S., Gulab, G., Kakar, K., Habibi, N., Amin, M. W., Sadat, M. I., Zahid, T., Durani, A., Baber, B. M., Safi, Z., & Zerak, A. (2023). Auxin application at the flowering stage of

- rice alleviates the negative impact of heat stress on spikelet fertility and yield attributes. *Agriculture*, 13, Article 866. <https://doi.org/10.3390/agriculture13040866>
- Bakhsh, I., Khan, H. U., Khan, M. Q., & Javaria, S. (2011). Effect of naphthalene acetic acid and phosphorus levels on yield potential of transplanted coarse rice. *Sarhad Journal of Agriculture*, 27(2), 161–165.
- Barbier, F. F., Dun, E. A., Kerr, S. C., Chabikwa, T. G., & Beveridge, C. A. (2019). An update on the signals controlling shoot branching. *Trends Plant Science*, 24(3), 220–236. <https://doi.org/10.1016/j.tplants.2018.12.001>
- Basuchaudhuri, P. (2016). 1-Naphthaleneacetic acid in rice cultivation. *Current Science*, 110(1), 52–56.
- Beena, R. (2013). Research paradigm and inference of studies on high temperature stress in rice (*Oryza sativa* L.). In A. Hemantaranjan (Ed.), *Advances in plant physiology, an international treatise series* (Vol. 14, pp. 497–511). Scientific Publishers.
- Beena, R., Vighneswaran, V., Sindhumole, P., Narayankutty, M. C., & Voleti, S. R. (2018). Impact of high temperature stress during reproductive and grain filling stage in rice. *Oryza-An International Journal on Rice*, 55(1), 126–133. <https://doi.org/10.5958/2249-5266.2018.00015.2>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chauhan, P. K., Upadhyay, S. K., Tripathi, M., Singh, R., Krishna, D., Singh, S. K., & Dwivedi, P. (2022). Understanding the salinity stress on plant and developing sustainable management strategies mediated salt-tolerant plant growth-promoting rhizobacteria and CRISPR/Cas9. *Biotechnology and Genetic Engineering Reviews*, 19, 1–37. <https://doi.org/10.1080/02648725.2022.2131958>
- Dinar, A., Tieu, A., & Huynh, H. (2019). Water scarcity impacts on global food production. *Global Food Security*, 23, 212–226. <https://doi.org/10.1016/j.gfs.2019.07.007>
- Fatima, Z., Bano, A., Sial, R., & Aslam, M. (2008). Response of chickpea to plant growth regulators on nitrogen fixation and yield. *Pakistan Journal of Botany*, 40(5), 2005–2013.
- Fu, G., Song, J., Xiong, J., Lia, X., Zhang, X., Wang, X., Le, M., & Tao, L. (2012). Thermal resistance of common rice maintainer and restorer lines to high temperature during flowering and early grain filling stage. *Rice Science*, 19, 309–314. [https://doi.org/10.1016/S1672-6308\(12\)60055-9](https://doi.org/10.1016/S1672-6308(12)60055-9)
- Fu, S. F., Wei, J. Y., Chen, H. W., Liu, Y. Y., Lu, H. Y., & Chou, J. Y. (2015). Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signaling & Behavior*, 10(8), Article e1048052. <https://doi.org/10.1080/15592324.2015.1048052>
- Hossain, S. A. (2023). Influence of naphthalene acetic acid on the fruit growth, chlorophyll, ph and total soluble solid content in rose apple. *International Journal of Life Science and Agriculture Research*, 2(1), 1–8. <https://doi.org/10.55677/ijlsar/V02I01Y2023-01>
- Hu, Y., Ge, Y., Zhang, C., Ju, T., & Cheng, W. (2009). Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regulation*, 59, 51–61. <https://doi.org/10.1007/s10725-009-9387-7>
- Hu, Y., Li, Y., Zhang, Y., Li, G., & Chen, Y. (2011). Development of sample preparation method for auxin analysis in plants by vacuum microwave-assisted extraction combined with molecularly imprinted clean-up procedure. *Analytical and Bioanalytical Chemistry*, 399, 3367–3374. <https://doi.org/10.1007/s00216-010-4257-8>
- Hussain, I., Khakwani, A. A., Bakhsh, I., Khan, A., & Shehryar, A. (2021). Effect of naphthalene acetic acid (NAA) on grain yield and bioeconomic efficiency of coarse rice (*Oryza sativa* L.). *Pakistan Journal of Botany*, 53(6), 2017–2023. [https://doi.org/10.30848/PJB2021-6\(21\)](https://doi.org/10.30848/PJB2021-6(21))
- IPPC. (2021). *Plant health and climate change*. FAO. <http://www.fao.org/3/cb3764en/cb3764en.pdf>
- Jagadish, S. V. K., Septiningsih, E. M., Kohli, A., Thomson, M. J., Ye, C., Redona, E., Kumar, A., Gregorio, G. B., Wassmann, R., Ismail, A. M., & Singh, R. K. (2012). Genetic advances in adapting rice to a rapidly changing climate. *Journal of Agronomy and Crop Science*, 198(5), 360–373. <https://doi.org/10.1111/j.1439-037X.2012.00525.x>
- Jahan, N., & Adam, A. M. M. G. (2014). Changes in biochemical components of rice following NAA application. *Journal of the Asiatic Society of Bangladesh*, 40(2), 173–178.
- Janni, M., Gulli, M., Maestri, E., Marmiroli, M., Valliyodan, B., Nguyen, H. T., & Marmiroli, N. (2020). Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. *Journal of Experimental Botany*, 71(13), 3780–3802. <https://doi.org/10.1093/jxb/eraa034>
- Juliano, B. O. (1971). A simplified assay for milled rice amylase. *Cereal Science Today*, 12, 334–340.

- Kaya, C., Ashraf, M., Alyemeni, M. N., & Ahmad, P. (2020). Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. *Physiologia Plantarum*, 168(2), 345–360. <https://doi.org/10.1111/ppl.13012>
- Khan, M., Ali, S., Al Azzawi, T. N. I., Saqib, S., Ullah, F., Ayaz, A., & Zaman, W. (2023). The key role of ROS and RNS as signalling molecule in plant-microbe interactions. *Antioxidants*, 12(2), Article 268. <https://doi.org/10.3390/antiox12020268>
- Khan, M. I. R., Kumari, S., Nazir, F., Khanna, R. R., Gupta, R., & Chhillar, H. (2023). Defensive role of plant hormones in advancing abiotic stress-resistant rice plants. *Rice Science*, 30(1), 15–35. <https://doi.org/10.1016/j.rsci.2022.08.002>
- Kondamudi, R., Swamy, K. N., Chakravarthy, D. V. N., Vishnuprasanth, V., Rao, Y. V., Rao, P. R., Sarla, N., Subrahmanyam, D., & Voleti, S. R. (2012). Heat stress in rice—physiological mechanisms and adaptation strategies. In B. Venkateswarlu, A. Shanker, C. Shanker, & M. Maheswari (Eds.), *Crop stress and its management: Perspectives and strategies* (pp. 193–224). Springer. https://doi.org/10.1007/978-94-007-2220-0_6
- Kurpea, J., & Smalle, J. A. (2020). Auxin/cytokinin antagonistic control of the shoot/root growth ratio and its relevance for the adaptation to drought and nutrient deficiency stresses. *International Journal of Molecular Sciences*, 23, Article 1993. <https://doi.org/10.3390/ijms23041933>
- Laxa, M., Liebthal, M., Telman, W., Chibani, K., & Dietz, K. J. (2019). The role of the plant antioxidant system in drought tolerance. *Antioxidants*, 8(4), Article 94. <https://doi.org/10.3390/antiox8040094>
- Li, Y., Zhu, J., Wu, L., Shao, Y., Wu, Y., & Mao, C. (2019). Functional divergence of PIN1 paralogous genes in rice. *Plant and Cell Physiology*, 60, 2720–2732. <https://doi.org/10.1093/pcp/pcz159>
- Matthes, M. S., Best, N. B., Robil, J. M., Malcomber, S., Gallavotti, A., & McSteen, P. (2019). Auxin EvoDevo: Conservation and diversification of genes regulating auxin biosynthesis, transport, and signalling. *Molecular Plant*, 12, 298–320. <https://doi.org/10.1016/j.molp.2018.12.012>
- Meisrimler, C. N., Buck, F., & Lüthje, S. (2014). Alterations in soluble Class III peroxidases of maize shoots by flooding stress. *Proteomes*, 2(3), 303–322. <https://doi.org/10.3390/proteomes2030303>
- Mujtaba, A., Nabi, G., Masood, M., Iqbal, M., Asfahan, H. M., Sultan, M., Majeed, F., Hensel, O., & Nasairahmdi, A. (2022). Impact of cropping pattern and climatic parameters in lower Chenab canal system — A case study from Punjab Pakistan. *Agriculture*, 12(5), Article 708. <https://doi.org/10.3390/agriculture12050708>
- Neang, S., de Ocampo, M., Egdane, J. A., Platten, J. D., Ismail, A. M., Seki, M., Suzuki, Y., Skoulding, N. S., Kano-Nakata, M., Yamauchi, A., & Mitsuya, S. (2020). A GWAS approach to find SNPs associated with salt removal in rice leaf sheath. *Annals of Botany*, 126(7), 1193–1202. <https://doi.org/10.1093/aob/mcaa139>
- Rehman, R. S., Ali, M., Ali Zafar, S., Hussain, M., Pasha, A., Saqib Naveed, M., Ahmad, M., & Waseem, M. (2022). Abscisic acid mediated abiotic stress tolerance in plants. *Asian Journal of Research in Crop Science*, 7(1), 1–7. <https://doi.org/10.9734/ajrcs/2022/v7i130128>
- Ruan, S., Qi, J., Wu, F., Lai, R., & Tang, X. (2023). Response of yield, grain quality and volatile organic compounds of aromatic rice to vermicompost application. *Journal of Cereal Science*, 109, Article 103620. <https://doi.org/10.1016/j.jcs.2022.103620>
- Sajid, M., Amin, N., Ahmad, H. A. B. I. B., & Khan, K. (2016). Effect of gibberellic acid on enhancing flowering time in *Chrysanthemum morifolium*. *Pakistan Journal of Botany*, 48(2), 477–483.
- Sarwar, N., Farooq, O., Wasaya, A., Saliq, S., & Mubeen, K. (2019). Improved auxin level at panicle initiation stage enhance the heat stress tolerance in rice plants. In *Proceedings of the Agronomy Australia Conference, Wagga Wagga, NSW, Australia*.
- Sarwar, N., Mubeen, K., Farooq, O., Wasaya, A., Yasir, T. A., Shahzad, M., Javed, M., Hussain, A., Awan, M. I., Dawood, M., & Ahmad, S. (2023). Impact of heat stress on cereal crops and its mitigation strategies. In M. Ahmed & S. Ahmad (Eds.), *Disaster risk reduction in agriculture* (pp. 191–210). Springer. https://doi.org/10.1007/978-981-99-1763-1_10
- Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Chen, C., & Wang, K. (2011). Impact of high-temperature stress on rice plant and its traits related to tolerance. *The Journal of Agricultural Science*, 149, 545–556. <https://doi.org/10.1017/S0021859611000360>
- Shi, G., Cai, Q., Liu, C., & Wu, L. (2010). Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes. *Plant Growth Regulation*, 61, 45–52. <https://doi.org/10.1007/s10725-010-9447-z>
- Steel, R. G. D., & Torrie, J. H. (1997). *Principles and procedures of statistics, a biometrical approach*. McGraw-Hill.

- Stewart, R. R. C., & Bewley, J. D. (1980). Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiology*, 65(2), 245–248. <https://doi.org/10.1104/pp.65.2.245>
- Ullah, S., Afzal, I., Shumaila, S., & Shah, W. (2021). Effect of naphthyl acetic acid foliar spray on the physiological mechanism of drought stress tolerance in maize (*Zea mays* L.). *Plant Stress*, 2, Article 100035. <https://doi.org/10.1016/j.stress.2021.100035>
- van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, 2, 494–501. <https://doi.org/10.1038/s43016-021-00322-9>
- Waldamichael, F. G., Debelee, T. G., Schwenker, F., Ayano, Y. M., & Kebede, S. R. (2022). Machine learning in cereal crops disease detection: A review. *Algorithms*, 15(3), Article 75. <https://doi.org/10.3390/a15030075>
- Wu, M., Wang, P. Y., Sun, L. G., Zhang, J. J., Yu, J., Wang, Y. W., & Chen, G. X. (2014). Alleviation of cadmium toxicity by cerium in rice seedling is related to improved photosynthesis, elevated antioxidant enzymes and decreased oxidative stress. *Plant Growth Regulation*, 74, 251–260. <https://doi.org/10.1007/s10725-014-9916-x>
- Zhang, J., & Peer, W. A. (2017). Auxin homeostasis: The DAO of catabolism. *Journal of Experimental Botany*, 68(12), 3145–3154. <https://doi.org/10.1093/jxb/erx221>