



Enhancing Morpho-Physiological and Yield Potential of Rice using Nutrients and Plant Growth Regulators

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the major cereals and the staple food crops which is cultivated all over the world. In order to meet the growing demand of population, it is important to increase the productivity of crop. The productivity of crop is influenced by nutrients and growth hormones. With this background, a field experiment was conducted during the month of January-May 2023 at wetland farms, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India to evaluate the effect of different foliar nutrients and growth regulators on growth, physiology, biochemical and yield attributes in rice. The experimental layout was Factorial Randomized Block Design (FRBD) with two varieties (CO55 and ADT 57). The treatments consisted of foliar spray of T₁- Control (water spray), T₂- Nutrio-hormonal consortia 1, T₃- Nutrio-hormonal consortia 2, T₄- Rice booster 1 and T₅- Rice booster 2 given at the booting stage and 15 days after the first spray. Among the

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treatments, T₅- Rice booster 2 significantly improved leaf area (1813.99 cm², 2059.78 cm²), leaf area index (4.53, 5.15), crop growth rate (32.07 g/m²/day, 37.89 g/m²/day), chlorophyll index (37.60, 41.73), photosynthetic rate (27.15 μmol CO₂ m⁻² s⁻¹, 30.04 μmol CO₂ m⁻² s⁻¹), Transpiration rate (11.83 mmol H₂O m⁻¹ s⁻¹, 14.20 mmol H₂O m⁻¹ s⁻¹), soluble protein content (16.23 mg/g, 17.30 mg/g), nitrate reductase activity (98.15 μg of NO₂/g/h, 112.47 μg of NO₂/g/h) in ADT- 57 variety at both sprays, respectively. Foliar application of rice booster 2 (T₅) resulted in higher number of productive tillers (23), number of spikelets per panicle (165.5), spikelet fertility (95.48%), 1000 grain weight (17.35g), grain yield/ plant (31.40g), grain yield/hectare (6694.32 Kg/ha), harvest index (45.44%) in ADT 57 and resulted in 15% yield increment over the control. Thus the present study concluded that the foliar application of rice booster 2 (T₅) significantly improved the growth, physiology, biochemical and yield attributes in rice variety ADT 57.

Keywords: Rice; foliar spray; nutrients; plant hormones; growth; physiology; yield attributes.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major cereal and the staples food crops which is cultivated all over the world. It is known as “global grain” and has considerable importance on nutritional and food security in the world [1]. Asia is on the top in terms of production and consumption of rice [2]. Global rice production is about 512.8 million tonnes [3]. In 2021-2022 the total area under rice cultivation in India was 463.7 lakh hectares, with the production and productivity of 127.93 million metric tonnes and 2807 kg/hectare [4]. According to Madhukeshwara et al. [5] the population of India during 2050 is expected to be 1.6 billion. In order to meet the demand of the increasing population, additional production of 1.5 tons is needed every year and in order to sustain food security in India, it is necessary to increase the productivity of rice under limited resources. Appropriate crop management strategies are required to improve the efficient use of inputs which is needed to enhance the productivity. Foliar application of nutrients and phytohormones gives immediate results by increasing crop growth and yield. Fertilizer when applied exogenously, not only increased crop yields but also decreased the amount of fertilizer supplied through soil [6].

Macro and Micro nutrients are essential for crops as it plays a vital role in physiology and biochemical properties. Nitrogen increases grain yield and quality by accelerating tillering, leaf area and grain formation. Phosphorus concentration influences grain yield in rice and its application at the maturity stage gradually improves its accumulation in leaves, stem and panicle and thus enhances the grain yield in rice [7]. Potassium is an essential macronutrient which plays a key role in various physiological processes, energy transfer, water uptake,

nutrient transport and enhances the growth and development in plants [8]. Micronutrients such as Zn, B, Fe etc., which are required in trace amounts, influences the metabolism of plants by involving in enzyme activation. Foliar application of nutrients in rice enhanced plant height, number of tillers and significantly increased photosynthetic rate and dry matter production through translocation of carbohydrates to the sink [9,10].

Hormones are key players in activating or inactivating the gene expression, behavior of plants and have added a novel way for modifying the metabolism which influences its growth and development [11]. They play a significant role in manipulating plant growth and development and when exogenously applied, plants have the ability to store them as reversible conjugates and release the active hormone during its growth period, when required [12]. Plant growth hormones enhances grain yield by controlling source and sink size in plants and regulate the movement of accumulated carbohydrate to the sink [13-15]. With this background, the current study was formulated to determine the effect of foliar spray on nutrients and plant growth hormones in rice crops.

2. MATERIALS AND METHODS

2.1 Study Area

A field experiment was conducted during the month of January –May, 2023 at wetland farms in Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India situated at 11° N latitude and 77° E longitude with an altitude of about 426.72 m above mean sea level with the aim to increase the growth and yield potential of rice by exogenous application of nutrients and plant growth regulators.

2.2 Experimental Design

The total area of the experimental field was 782 m² with the length 34 m and width 23 m was laid out in factorial randomized block design (FRBD), combining two factors such as five treatments and two varieties, each treatment was replicated four times. Single experimental plot consisted of 12.21 m² with total number of 40 plots and in each plot 256 plants are maintained with a spacing of 20 cm x 20 cm. So, three samples were taken from each replication for taking observation.

2.3 Treatment Details

The treatments are T₁- Control (water spray), T₂- Nutrio-hormonal consortia 1(macro and micronutrients and major plant hormones), T₃- Nutrio-hormonal consortia 2 (T₂+ ZnSO₄), T₄- Rice booster 1 (MAP, KCl, ZnSO₄, FeSO₄, Borax and major phytohormones), T₅- Rice booster 2 (T₄+ melatonin and salicylic acid) were given during booting stage and 15 days after first spray. Treatments were prepared as a 1% foliar spray solution, T₂ (50 ml in 1L) T₃ (33.3 ml in 1L) T₄ and T₅ (20 ml in 1L), respectively. Two different rice varieties such as CO 55 and ADT 57 were used in these experiments. A fertile land with good irrigation facility was selected for raising nursery. Transplanting was done in a well puddled plot.

The following growth parameters were recorded on 10 days after the first and the second spray. Leaf area was measured by using leaf area meter (LICOR, Model LI 3000, Lincoln, NE, USA). The plant leaf samples were collected from each replication for all the treatments and inserted into the leaf area meter. The value was expressed as cm² plant⁻¹. The leaf area index (LAI) and crop growth rate (CGR) was calculated from the following formula suggested by Watson [16].

$$LAI = \frac{\text{Total leaf area of a plant}}{\text{Ground area}}$$

$$CGR = \frac{W_2 - W_1}{P(t_2 - t_1)}$$

Where,

W₁ and W₂ – Dry weight of the whole plant at time t₁ and t₂ respectively

t₁ and t₂ – Time interval between two stages

P- Spacing or Ground area on which W₁ and W₂ are recorded

The physiological parameter such as chlorophyll index (SPAD value) was measured by using chlorophyll meter from Minolta (model 502 Minolta Japan). It was measured from the five physiologically active leaves for all replication and the average was computed. The gas exchange parameters were recorded after 10 days of the first and the second spray using portable photosynthesis system (PPS; LI-6400 XT, Licor Inc., Lincoln, NE, USA). Three values were recorded for each replication and average was taken. The photosynthetic rate was expressed as μmol CO₂ m⁻² s⁻¹ and transpiration rate was denoted by mmol H₂O m⁻¹ s⁻¹.

The biochemical observations were taken 10 days after the first and the second spray. Leaf soluble protein was estimated by Lowry et al. [17] and expressed as mg/g. 250 mg of leaf sample was macerated with 10 ml of phosphate buffer and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and made up the volume to 25 ml. from this 1 ml was taken in a test tube to which 5 ml of alkaline copper tartarate and 0.5 ml of folin reagent was added. After 30 minutes absorbance was measured at 660 nm by spectrophotometer. Nitrate reductase enzyme were estimated by the procedure suggested by Silveira et al. [18]. The value was expresses as μg of NO₂ g⁻¹ h⁻¹.

The yield and yield components were recorded before the harvest of the crop. The number of productive tillers per plant, number of spikelets per panicle, spikelet fertility (%), 1000 grain weight (g), grain yield per plant (g plant⁻¹) and harvest index (%) were recorded from the average obtained from the observation of three plants from each replication.

2.4 Statistical Analysis

The data obtained was subjected to statistical analysis by using SPSS software. The two-way analysis of variance (ANOVA) was used to compare means between the five treatments and the two rice varieties at 5% significance level.

3. RESULTS AND DISCUSSION

3.1 Growth Attributes

3.1.1 Leaf area

Rice growth characters such as leaf area, leaf area index and crop growth rate were significantly influenced by the foliar application of

nutrients and phytohormones. The highest leaf area was recorded in ADT 57 variety at first spray (1813.99 cm²) and second spray (2059.78 cm²) in T₅ (Rice booster 2) when compared with control (water spray) as indicated in Table 1. Plant growth hormones and nutrients plays a crucial role in enhancing cell division and shoot branching which in turn influences the leaf area in rice. Nutrients and growth enhancers plays a key role in chlorophyll synthesis and helps in transfer of solar energy through photosynthesis in leaves. Thus the application of nutrients influences the leaf soluble protein content which leads to enhancement of leaf area in rice [19]. These results are in line with the results reported by Pillai et al. [20] and Goutam et al. [21].

3.1.2 Leaf area index

Among the two different varieties ADT 57 resulted in increased LAI at first spray (4.53) and second spray (5.15) under T₅ (Rice booster 2) than CO 55 variety, respectively, which was significantly higher than the control (Table 2). The LAI is directly correlated with the leaf area and amount of light that is captured by the plants during crop growth [22]. Foliar application of plant growth regulators such as salicylic acid significantly increased LAI in rice [23]. Our finding is in consistent with other studies where Pal et al. [24] observed that the foliar application of growth enhancers significantly increased LAI in rice. The results are in accordance with Sudhagar Rao et al. [25] who reported that application of nutrients during different growth stages in rice increased leaf area index in rice.

3.1.3 Crop growth rate

In the present study, the data (Table 2) showed that ADT 57 recorded highest Crop Growth Rate (CGR) in first spray (32.07 g/m²/day) and second spray (37.89 g/m²/day) in T₅ (Rice booster 2) followed by T₄ (Rice booster 1) than control (water spray). Higher CGR under foliar application of nutrients might be due to increase in LAI and improved chlorophyll synthesis which results in high biomass production. As nutrients helps in activating different physiological and biochemical processes such as stomatal regulation and enzyme activation that leads to increased crop growth rate [26]. Crop growth rate is closely related to the amount of dry matter accumulated and the rate of photosynthetic efficiency of the crops [27]. Ghasal et al. [28] noted that the foliar spray of nutrients given at maximum tillering and panicle initiation increases crop growth rate in aromatic rice.

3.2 Physiological Parameters

3.2.1 Chlorophyll index (SPAD value)

Chlorophyll is an essential photosynthetic pigment which influences the efficiency of photosynthesis process by light absorption thereby it increases the growth and development of plants. As given in Fig. 1 the maximum SPAD value was noted in ADT 57 at foliar application of T₅ at two different stages of spray (37.60 and 41.73), respectively. The minimum SPAD value was observed in control (water spray) at both sprays. Similar finding was reported by Silalert and Pattanagul [29] that the chlorophyll content was higher when melatonin given as a foliar spray. Foliar feeding with different plant hormones increased chlorophyll content in rice [30]. Nutrients plays a key role in chlorophyll synthesis and are an integral part in chlorophyll structure. The buildup of photosynthetic products and enzymes which are involved in numerous physiological metabolisms are facilitated by the exogenous application of plant hormones during anthesis stage in rice. The increase in carbohydrate concentration increased sink activity that delay leaf senescence by enhancing chlorophyll content in rice [31]. Bala et al. [32] also stated that the foliar application of nutrients enhanced chlorophyll content in rice.

3.2.2 Photosynthetic rate

The ADT 57 variety performed better and recorded higher photosynthetic rate under the foliar application of T₅ during first spray (27.15 μmol CO₂ m⁻² s⁻¹) and second spray (30.04 μmol CO₂ m⁻² s⁻¹) as given in Fig. 2. The foliar application of nutrients considerably increases the biochemical activity in the leaf as well as the concentration of chlorophyll and carotenoid, which in turn improves the rate of photosynthesis in plants [33]. Our result is in confirmatory with Raghunath et al. [34] who observed that foliar spray of both plant growth hormones and nutrients increased photosynthetic rate in rice.

3.2.3 Transpiration rate

Transpiration rate is directly linked to the amount of water taken up by the crop for its physiological and biochemical process. It was observed that among the two varieties used in this study, maximum transpiration rate was found in ADT 57 (11.83, 14.20 mmol H₂O m⁻¹ s⁻¹) than in CO 55 (10.72, 12.44 mmol H₂O m⁻¹ s⁻¹) under T₅ at first

and second spray (Fig. 3). Similar finding was observed when nutrients and plant growth hormones given as foliar spray enhanced transpiration rate in rice [35-36].

Table 1. Effect of different nutrients and plant growth hormones on leaf area of rice genotypes

Treatments	Leaf Area (cm ²)			
	1 st Spray		2 nd Spray	
	CO 55	ADT 57	CO 55	ADT 57
T ₁ - Control (water spray)	1306.28	1396.40	1579.89	1626.70
T ₂ - Nutrio-hormonal consortia 1	1403.93	1500.72	1652.60	1703.88
T ₃ - Nutrio-hormonal consortia 2	1559.93	1603.98	1756.42	1817.60
T ₄ - Rice booster 1	1617.68	1710.28	1810.78	1893.29
T ₅ - Rice booster 2	1695.47	1813.99	1932.53	2059.78
	SEd	CD (P=0.05)	SEd	CD (P=0.05)
V	21.39	43.89**	31.97	65.61*
T	33.82	69.41**	50.55	103.73**
VxT	47.84	98.07NS	71.50	146.57NS

V= Variety, T= treatment, VxT= Variety and Treatment interaction, NS= Not Significant, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, SEd= Standard error difference, CD= Critical Difference

Table 2. Effect of different nutrients and plant growth hormones on leaf area index and crop growth rate of rice genotypes

Treatments	Leaf Area Index				Crop Growth Rate (g/m ² /day)			
	1 st Spray		2 nd Spray		1 st Spray		2 nd Spray	
	CO 55	ADT 57	CO 55	ADT 57	CO 55	ADT 57	CO 55	ADT 57
T ₁ - Control (water spray)	3.27	3.49	3.95	4.07	23.91	23.91	27.60	27.97
T ₂ - Nutrio-hormonal consortia 1	3.51	3.75	4.13	4.26	24.68	26.72	29.47	30.73
T ₃ - Nutrio-hormonal consortia 2	3.90	4.01	4.39	4.54	27.00	28.83	30.08	33.35
T ₄ - Rice booster 1	4.04	4.28	4.53	4.73	28.14	31.72	32.08	35.33
T ₅ - Rice booster 2	4.24	4.53	4.83	5.15	30.91	32.07	34.32	37.89
	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
V	0.054	0.110**	0.080	0.164*	0.356	0.731**	0.264	0.542**
T	0.085	0.174**	0.127	0.260**	0.563	1.156**	0.418	0.857**
VxT	0.120	0.246NS	0.179	0.366NS	0.796	1.631*	0.591	1.212**

V= Variety, T= treatment, VxT= Variety and Treatment interaction, NS= Not Significant, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, SEd= Standard error difference, CD= Critical Difference

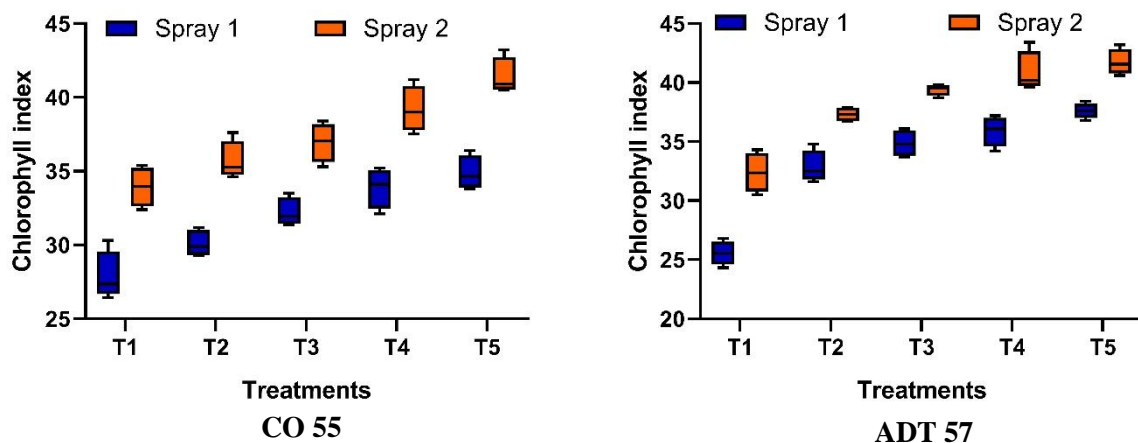


Fig. 1. Effect of different nutrients and plant growth hormones on chlorophyll index of rice genotypes

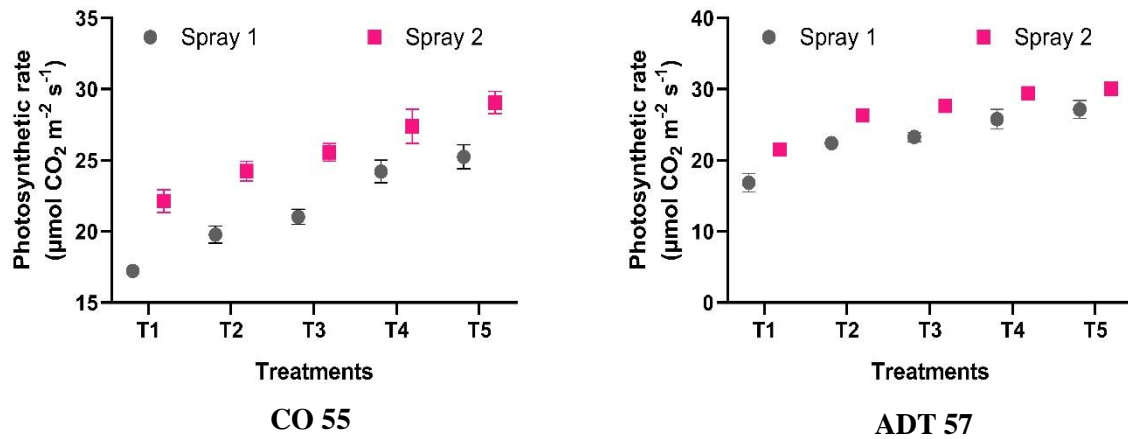


Fig. 2. Effect of different nutrients and plant growth hormones on photosynthetic rate of rice genotypes

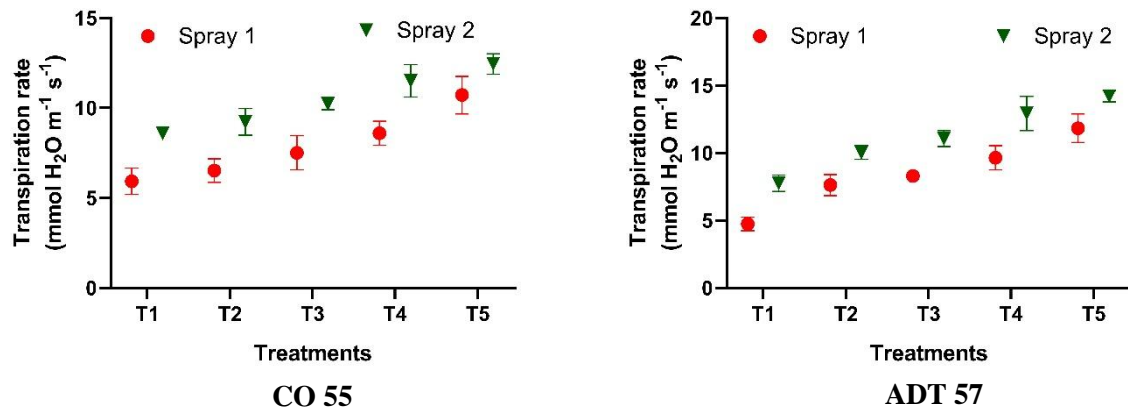


Fig. 3. Effect of different nutrients and plant growth hormones on transpiration rate of rice genotypes

T₁- Control (Water Spray); T₃- Nutrio-hormonal Consortia 2; T₅- Rice Booster 2; T₂- Nutrio-hormonal Consortia 1; T₄- Rice Booster 1

3.3 Biochemical Parameters

3.3.1 Leaf soluble protein

Leaf soluble protein is influenced by the photosynthetic process in plants. Increase in soluble protein increases the leaf area and improves the plant growth and development. Among five treatments T₅ recorded increased leaf soluble protein content in ADT 57 (16.23 mg/g, 17.30 mg/g) at both stages of foliar application than T₁ control (Fig 4). Increase in soluble protein content is probably by the higher amount of chlorophyll and photosynthetic rate. Application of macronutrients increases RuBisCO enzyme activity which accounts for major portion of protein part in plants, enhanced the photosynthetic rate thereby the leaf soluble protein content [37]. Plant growth regulators also

plays a vital role in increasing the leaf soluble protein content in rice. Li et al. [38] studied that application of growth hormones such as melatonin significantly increased soluble protein content in rice. Further Chen et al. [31] found that the foliar feeding of Salicylic acid at 200 mg L⁻¹ enhanced leaf soluble protein content in rice. Our findings are in agreement with the above results.

3.3.2 Nitrate reductase

Nitrate reductase is a key enzyme involved in the nitrogen assimilation process which leads to amino acid synthesis. On comparison with the T₁ (control), all the treatment significantly increased nitrate reductase activity. Within the treatments, the higher value was found under the T₅ application in ADT 57 (98.15, 112.47 µg of NO₂/g/h) than CO 55 variety (90.43, 103.19 µg of

NO₂/g/h) at both stages of foliar application (Fig. 5). Asma et al. [39] observed that exogenous application of salicylic acid enhanced nitrate reductase activity in rice.

3.4 Yield and Yield Components

3.4.1 Number of productive tillers

Foliar application of T₅ at booting stage and 15 days after first spray increased number of productive tillers (23) in ADT 57 rice variety, when compared with that of CO 55 (20). Control plots resulted in lesser number of productive tillers in both the varieties (Fig. 6). The increase in number of productive tillers was mainly by the foliar spray of nutrients which contributed to the activation of hormones that triggers the growth of primordial reproductive organ and the photosynthates partitioning in plants [40]. The results of the present study are in accordance to the finding of Ramesh et al. [41] who reported that the increase in the number of effective tillers

and panicle per m⁻² was mainly by the application of plant growth hormones. Jagathjothi et al. [9] also observed that the number of productive tillers m⁻² enhanced by the foliar spray of nutrients.

3.4.2 Total number of spikelets per panicle

The data on number of spikelets per panicle (Table 3) revealed the significant effect of T₅ foliar application than other treatments and recorded higher value than control. ADT 57 (165.5) showed higher values than CO 55 (147.50) variety. Application of micronutrients facilitate protein and carbohydrate synthesis that led to bolder seeds and thus enhanced number of spikelets/panicle [42]. Grain filling is a crucial stage that affects crop yield [43]. However exogenous spray of growth enhancers increases grain filling in rice. Pan et al. [44] also reported that application of growth hormones increased number of spikelet per panicle in rice.

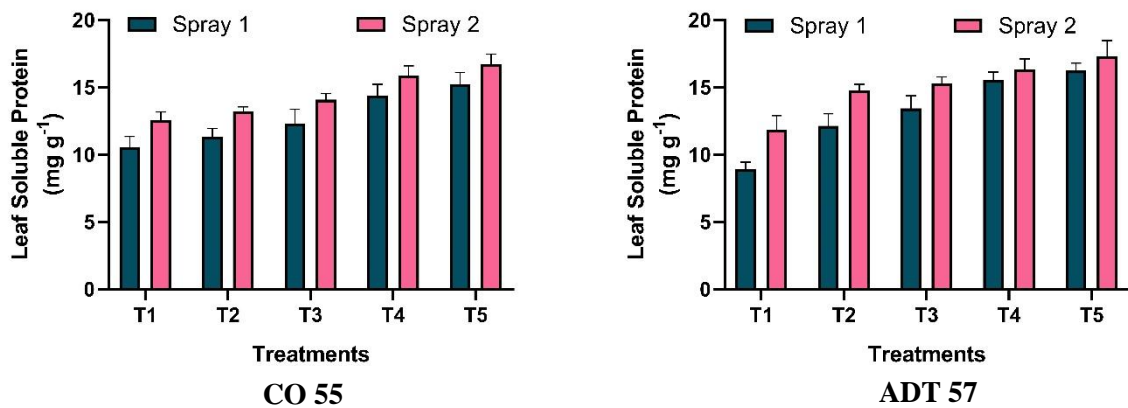


Fig. 4. Effect of different nutrients and plant growth hormones on leaf soluble protein of rice genotypes

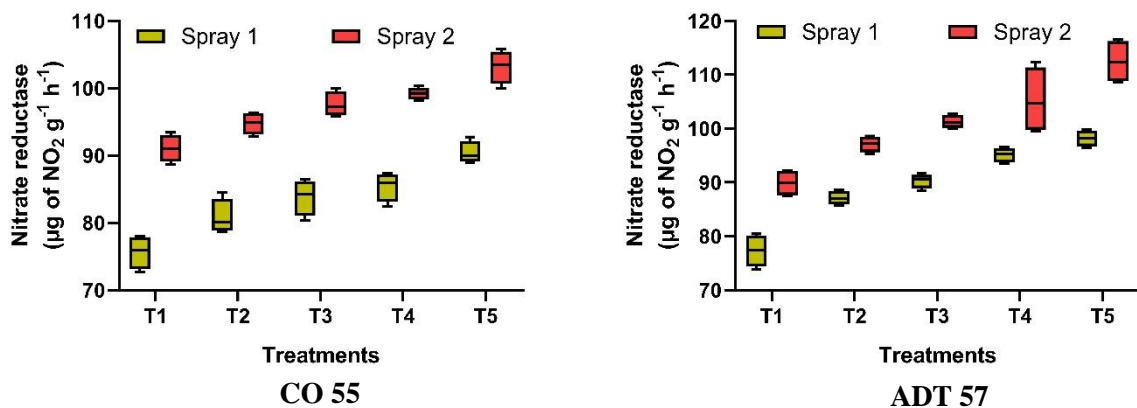


Fig. 5. Effect of different nutrients and plant growth hormones on nitrate reductase of rice genotypes

T₁- Control (Water Spray); T₃- Nutrio-hormonal Consortia 2; T₅- Rice Booster 2; T₂- Nutrio-hormonal Consortia 1; T₄- Rice Booster 1

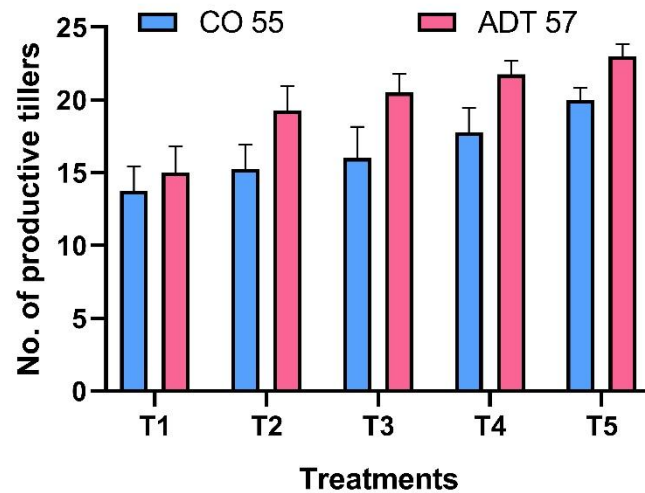


Fig. 6. Effect of different nutrients and plant growth hormones on number of productive tillers of rice genotypes

Table 3. Effect of nutrients and plant growth hormones on number of spikelet/panicle and spikelet fertility of rice genotypes

Treatments	Number of spikelet per panicle		Spikelet fertility (%)	
	CO 55	ADT 57	CO 55	ADT 57
T ₁ - Control (water spray)	124.75	128.50	85.58	86.64
T ₂ - Nutrio-hormonal consortia 1	128.25	135.25	87.67	89.30
T ₃ - Nutrio-hormonal consortia 2	134.50	147.25	88.63	91.50
T ₄ - Rice booster 1	142.50	157.25	90.88	93.32
T ₅ - Rice booster 2	147.50	165.50	93.57	95.48
	SEd	CD (P=0.05)	SEd	CD (P=0.05)
V	1.525	3.130**	0.670	1.375**
T	2.412	4.949**	1.060	2.174**
VxT	3.411	6.999*	1.499	3.072NS

V= Variety, T= treatment, VxT= Variety and Treatment interaction, NS= Not Significant, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, SEd= Standard deviation, CD= Critical Difference

3.4.3 Spikelet fertility and 1000 grain weight

The data presented (Table 3 and Table 4) indicated that the maximum spikelet fertility (95.48%) and 1000 grain weight (17.35g) was observed on ADT 57 by the foliar application of T₅ (rice booster 2). Next higher result was obtained from T₄. The least value was recorded in T₁ (control). Nutrients and plant growth hormones enhanced spikelet fertility. Our findings are supported by the result of Golada et al. [45] that hormones such as salicylic acid increased the cellular membrane integrity which may have increased the transport of photoassimilation to grains, which in turn spikelet fertility in rice. Haifaa et al. [15] also stated that enhanced amount of spikelet fertility was observed by the exogenous spray of phytohormones in rice.

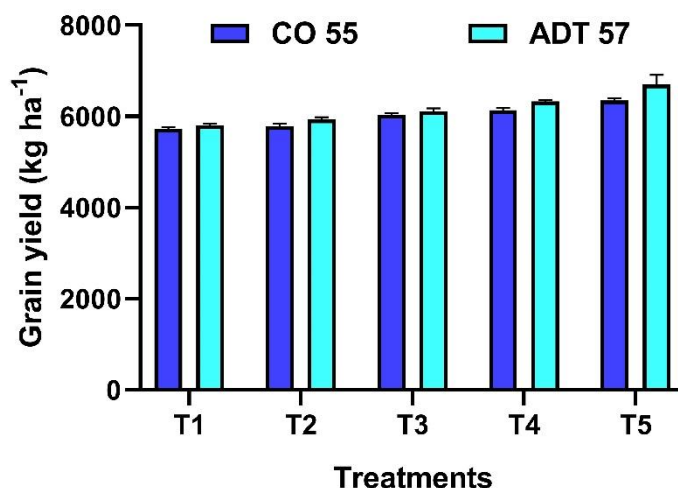
3.4.4 Grain yield and harvest index

Grain yield and harvest index were significantly enhanced by the foliar application of nutrients and growth enhancers. Among the two different varieties ADT 57 showed increased grain yield (31.40 g/plant, 6694.32 Kg/ha) and harvest index (45.44%) compared to CO 55 (Fig.7 and Table 4) under the effect of foliar application of T₅ followed, by T₄ on comparison with control (water spray). Higher grain production may be attributed by greater vegetative development which improved light absorption and enhanced dry matter production towards the economic part. Nutrients, generally influence starch production and their allocation to grain production [46]. The nutrients facilitated source to sink transport and improved partitioning efficiency in reproductive growth than vegetative biomass [47]. Increase in

Table 4. Effect of nutrients and plant growth hormones on 1000 grain weight and harvest index of rice genotypes

Treatments	1000 Grain weight (g)		Harvest index (%)	
	CO 55	ADT 57	CO 55	ADT 57
T ₁ - Control (water spray)	18.84	18.14	32.98	35.07
T ₂ - Nutrio-hormonal consortia 1	19.01	19.50	34.78	40.73
T ₃ - Nutrio-hormonal consortia 2	19.55	20.06	36.57	42.55
T ₄ - Rice booster 1	19.98	20.50	38.22	44.34
T ₅ - Rice booster 2	20.50	21.35	42.52	45.44
	SEd	CD (P=0.05)	SEd	CD (P=0.05)
V	0.161	0.331*	0.462	0.948**
T	0.255	0.523**	0.730	1.499**
VxT	0.360	0.740*	1.033	2.119*

V= Variety, T= treatment, VxT= Variety and Treatment interaction, * = Significant at 5% level of probability, ** = Significant at 1% level of probability, SEd= Standard deviation, CD= Critical Difference

**Fig. 7. Effect of different nutrients and plant growth hormones on grain yield per hectare of rice genotypes**

T₁- Control (Water Spray); T₃- Nutrio-hormonal Consortia 2; T₅- Rice Booster 2; T₂- Nutrio-hormonal Consortia 1; T₄- Rice Booster 1

harvest index was mainly due to the better mobilization of reserve food material that resulted in higher grain yield and lesser straw production. Our result was in consistent with Singh et al. [48] and Elankavi et al. [14]. Pal et al. [49] also reported that the extent of available form of photosynthates and the photosynthetic area directly linked with the grain formation.

4. CONCLUSION

In our study, foliar nutrition helps to overcome the nutritional and hormonal deficiencies in rice. Hence, use of foliar formulations in an appropriate and comprehensive manner is essential to improve growth and development of rice crop. Thus, the present study concludes that the foliar application of rice booster 2 (T₅) at booting stage and 15 days after first spray

significantly improved growth, physiology, biochemical, and yield attributes in ADT 57 rice variety which performed better for most of the traits and produced higher yield (6694.32 Kg/ha) than that of CO 55 (6353.26 Kg/ha) and resulted in 15% yield enhancement over the control.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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