

**GROWTH AND YIELD OF AROMATIC RICE AS INFLUENCED BY
EXOGENOUS APPLICATION OF GAMMA AMINOBUTYRIC ACID
(GABA)**

A THESIS

BY

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Student no. 1601391

Session: 2023-2024

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IN
AGRONOMY**



DEPARTMENT OF AGRONOMY

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR-5200**

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DEPARTMENT OF AGRONOMY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR
JUNE, 2024



*Dedicated to
My
Beloved Parents*

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GROWTH AND YIELD OF AROMATIC RICE AS INFLUENCED BY EXOGENOUS APPLICATION OF GAMMA AMINOBUTYRIC ACID (GABA)

ABSTRACT

Rice (*Oryza sativa* L.) is the most important staple food for over two billion people in Asia. Aromatic rice has become very popular due to its special appeal for aroma and grain quality. GABA is recognized as a non-protein amino acid that is involved in different physio-biochemical processes in plants. A field experiment was carried out at Agronomy Research Field in Hajee Mohammad Danesh Science and Technology University, Dinajpur located at 25.56° N latitude and 88.5° E longitude at an altitude of 37 m above from the mean sea level during the period from July 2023 to December 2023 to study the influence of exogenous application of GABA on growth and yield contributing character of aromatic rice (Tulsimala). The experiment was laid out into Randomized Complete Block Design (RCBD) with three replications. Two factors were taken in this study *viz.* factor A: D₁= Without GABA application, D₂= 50 ppm of GABA, D₃= 100 ppm of GABA and D₄= 150 ppm of GABA; Factor B: T₁= Application time of GABA at 30 DAT, T₂= at 60 DAT and T₃= at 90 DAT. Data were recorded on different growth and yield contributing parameters. Among the yield and yield contributing parameters namely effective tillers hill⁻¹, filled grains panicle⁻¹, grain yield, straw yield and biological yield were significantly varied with application of GABA. The highest number of effective tillers hill⁻¹ (20.03), number of filled grains panicle⁻¹ (231.00) were found in D₃T₂ treatment combination due to application of 100 ppm of GABA at 60 DAT. The highest grain yield (3.04 t ha⁻¹), straw yield (5.45 t ha⁻¹), biological yield (8.49 t ha⁻¹) and harvest index (35.83%) were found in D₃T₂ (100 ppm of GABA+ Application time of GABA at 60 DAT). Besides, lowest grain yield (1.92 t ha⁻¹) was found by D₁T₁ (Without GABA application+ at 30 DAT), lowest straw yield (4.85 t ha⁻¹) was achieved from D₁T₂ treatment (Without GABA application+ At 60 DAT), lowest biological yield (6.83 t ha⁻¹), and finally lowest harvest index (28.17 %) were achieved from D₁T₁ treatment (Without GABA application+ at 30 DAT). From these results it can be concluded that all of the important growth parameters and yield contributing characters were improved due to application of GABA 100 ppm resulting higher yield of Tulsimala variety of aromatic rice.

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LIST OF ABBREVIATIONS AND ACRONYMS

AEZ	Agro- Ecological Zone
ANOVA	Analysis of Variances
BRRRI	Bangladesh Rice Research Institute
cm	Centimeter
DAT	Days After Transplanting
cv.	Cultivar (s)
CV	Coefficient of Variance
<i>et al.,</i>	And others
FAO	Food and Agriculture Organization
g	Gram(s)
LSD	Least Significant Difference
m	Meter
No.	Number
NS	Non-significant
var.	Variety
tha ⁻¹	Ton per hectare
°C	Degree Centigrade
%	Percentage
ppm	Parts Per Million
GABA	Gamma Aminobutyric Acid
RCBD	Randomized Complete Block Design
HSTU	Hajee Mohammad Danesh Science and Technology University

CHAPTER I

INTRODUCTION

Agriculture production systems are mainly threatened by climate change now-a-days and also for other various things like resource shortage and pressure from the ever-increasing food demand and so on. According to FAO (2020), even before COVID-19, 135 million people globally were already struggling with severe food insecurity and the present scenario creates a situation of “a crisis within a crisis”. Cereals occupy more than half of the world’s harvested area and are the most important food source for human consumption. Rice (*Oryza sativa* L.) is one of the major cereals which is consumed by more than 50% of the world population (Muthayya *et al.*, 2014). Rice (*Oryza sativa* L.) belongs to the family Poaceae and is the most important cereal crops dominant over all other crops in respect of economic and social significance in Bangladesh. It provides nearly 48% of rural employment, about two-thirds of the total calorie supply and one-half of the total protein intakes of an average person in the country. The rice sector contributes one-half of the agricultural GDP and one-sixth of the national income in Bangladesh (Cereal Grains of Bangladesh. 97072). The global grain production is needed to be increased substantially to meet the food demands of the growing population (Searchinger *et al.*, 2018).

Rice is the most consumed cereal grain and represents the major pillar of food security among rice eating population of the globe. Rice is an important cereal crop that feeds billions of people around the world (Mahajan *et al.*, 2010). Due to their nutritional status, traditional and indigenous rice varieties are gaining attention in the fast-growing food revival revolution. It includes aromatic, medicinal, and organic rice varieties. Rice in its whole form has more fibre, resistant starch, and a diversity of proteins and lipids compared to its polished form.

Fragrant rice is a unique rice type and world-famous due to its special aroma, appearance, and taste, thus regarded as a superfine grain (Giraud *et al.*, 2013). Rice aroma was regarded as one of the most critical quality characters (Das *et al.*, 2018). Aroma is one of the diagnostic aspects of rice quality that determines the acceptance or rejection of rice before it is tested (Verma *et al.*, 2015). Aroma intensities are closely related to the mixture of aroma compounds in rice (Sansenya *et al.*, 2018). The 2-acetyl-1-pyrroline (2AP) was reported as a crucial volatile compound involved in the intricate volatile chemistry of aroma in aromatic rice.

Aromatic rice has become more popular, and its demand is increasing in international markets worldwide (Hashemi *et al.*, 2013). According to FAO, 2017 report by the Food and Agriculture Organisation, the amount of aromatic rice exported through various private companies in Bangladesh is about 5,998 tonnes per year. However, the Bangladesh Rice Exporters Association says the export of aromatic rice has exceeded 10,000 tonnes annually. On the other hand, demand for quality food is increasing globally, whereas fragrant rice is liked by consumers due to its pleasant smell and better cooking qualities, and thus attracted the attention of the researchers and growers. Generally, fragrant rice is with good flavor, but its poor resistance against biotic and abiotic stresses could lead to substantial yield reduction (Fitzgerald *et al.*, 2010, Mo *et al.*, 2015, Poonlaphdecha *et al.*, 2012). Thus, approaches to improve rice aroma without compromising yield are needed to get higher economic returns (Huang *et al.*, 2012).

According to the Department of Agricultural Extension (DAE), Bangladesh produces 1.8-1.9 million tonnes of aromatic rice, of which it exports only 9,000-10,000 tonnes (Mahmud *et al.*, 2022). Rice is not exported from Bangladesh without special approval from the commerce ministry. However, packaged aromatic rice is regularly exported to different countries, but its export market is still very small. According to the Bangladesh Rice Exporters Association, there is a good demand for aromatic rice in Europe, America and the Middle East. Some private companies such as Ispahani, Square, Pran are exporting packaged aromatic rice to 136 countries. The demand for aromatic rice is higher in countries where there are expatriate Bangladeshis. According to a 2017 report by the Food and Agriculture Organisation, the amount of aromatic rice exported through various private companies in Bangladesh is about 5,998 tonnes per year. However, the Bangladesh Rice Exporters Association says the export of fragrant rice has exceeded 10,000 tonnes annually (Annual Research Review Workshop, 2020-2021).

Bangladesh now has a good volume of fine and premium quality aromatic rice which can be exported after fulfilling national demand. Last year BRRI has sent some samples of premium quality aromatic rice varieties i.e. BRRI dhan34 and BRRI dhan50 to the diplomat's in Dhaka and also to the United Arab Emirates as a gift. After that step, the demand for fragrant rice has increased abroad. The demand is high mainly in the countries where South Asians live. In addition, the demand for fragrant rice has recently increased among the natives of Europe and America. Aromatic rice is now very popular for fast food and various Chinese dishes. Most of the fragrant rice is exported from Bangladesh to Middle Eastern countries. Especially in Europe and America, there has a huge demand for Bangladeshi aromatic rice.

According to the data of some concerned organizations, the export of fragrant rice has been increasing continuously for 10 years.

According to the current export policy (2018-21), there is an opportunity to export 25 varieties of aromatic rice (Daily Sun, 2021). Although there are many types of aromatic rice in the country, the Ministry of Commerce has a list of exportable aromatic rice. To keep the rice market stable in the last few years, the government has decided to export only aromatic rice. Due to the lack of awareness and promotions about the quality of the local aromatic rice, the use of foreign aromatic rice instead of our variants is seen in the reputed hotels and restaurants. It incurs a lot of foreign currency on imports. However, it is possible to save a huge amount of foreign currencies by cultivating commercially more high-yielding aromatic varieties developed by our scientists (Daily Sun, 2021).

The weather of Bangladesh is very suitable for the cultivation of aromatic rice. However, rice is more aromatic in cold-prone areas. Aromatic rice is being cultivated commercially especially in Dinajpur, Thakurgaon, Panchagarh, Rangpur, Naogaon, Rajshahi, and Mymensingh districts. There are many regional aromatic rice varieties in Bangladesh. BRRI's research has shown that at one time about 57 types of aromatic varieties were cultivated in Bangladesh; now more or less 32 types of aromatic varieties were cultivated. In addition, the research for developing high-yielding and anthocyanins and antioxidants enrich rice by pure line selection of locally popular aromatic rice is going on in full swing at BRRI (Daily Sun, 2021).

The aroma biosynthesis in fragrant rice could substantially affected by external environmental factors as well as crop management practices (Mo *et al.*, 2015). However, the grain yield was generally lower in aromatic rice than non-aromatic rice cultivars. Thus, some effective strategies are needed and have also been previously employed to improve grain yield production in aromatic rice (Mo *et al.*, 2017).

Chen *et al.*, (2008) demonstrated that 2-AP biosynthesis in aromatic rice is inhibited by the expression of the BADH₂ gene; this gene encodes Betaine Aldehyde Dehydrogenase (BADH) which catalyzes the conversion of Gamma-Aminobutyl Aldehyde (GABald) into GABA instead of 2-AP. Furthermore, the study of Mo *et al.*, (2016) indicated that the 2-AP in aromatic rice was transformed mainly from proline catalyzed by proline dehydrogenase (PDH). Previous study discovered that 2-AP formation is closely related to proline and gamma-aminobutyric acid (GABA). Hence, it can be concluded that GABA has multiple effects on aromatic rice growth and development.

GABA is recognized as a signaling molecule that is involved in various physio-biochemical processes to regulate plant stress responses (Ramesh *et al.*, 2017; Routray *et al.*, 2018). In general, the endogenous concentration of GABA in plants is relatively low but its levels may be enhanced in response to the various stress conditions (Li *et al.*, 2017). Previous studies confirmed that GABA is endowed with the ability to regulate physio-biochemical metabolism in different plants (Yu *et al.*, 2007; Song *et al.*, 2010). Exogenous GABA application improved the chlorophyll contents and other photosynthetic attributes in rice seedlings (Li *et al.*, 2017). Xie *et al.*, (2019) revealed that GABA application improved the nutrient uptake in rice. GABA, a non-protein amino acid, is an important plant signaling molecule involved in different physiological and biochemical mechanisms in plants (Li *et al.*, 2016a; Li *et al.*, 2016b). Besides, GABA was recently reported to regulate rice yield under different nitrogen application by affecting the growth and yield-related traits in fragrant rice (Li *et al.*, 2020). Moreover, the grain 2AP and GABA contents were enhanced under salt stress (Poonlaphdecha *et al.*, 2012) and shading treatments whereas significant positive correlation was observed between GABA and 2AP content in grains of fragrant rice (Mo *et al.*, 2015). Thus, there might be a feasible approach for GABA application to improve grain yield and regulate the 2AP biosynthesis in fragrant rice under low light conditions.

Gamma amino butyric acid has attracted great attention over the last several decades due to its ubiquity in life. GABA is a four-carbon non-protein amino acid synthesized during brown rice germination via the decarboxylation of L-glutamic acid, a reaction catalyzed by glutamate decarboxylase (Bown *et al.*, 1997). Due to its relevance and biological functions, GABA is becoming recognized as an essential nutrient for a healthy balanced diet. Recognizing the health benefits of GABA, methods for producing GABA-enriched foods, particularly rice, are gaining popularity. Numerous elements, individually or in combination (cultivar, genetic variation, environmental conditions, maturation, temperature, ionic strength, and moisture content), affect rice germination, phenolic content, and synthesis of GABA (Hussain *et al.*, 2020). GBR has a ten-fold higher GABA content than milled white rice and a two-fold higher GABA content than brown rice (Patil *et al.*, 2011).

Gamma-aminobutyric acid (GABA) is often involved in signaling transduction mechanisms in plants under different abiotic stresses such as chilling, heat, osmotic, respiratory stress, and so on (Vijayakumari *et al.*, 2016), as well as modulates numerous physio-biochemical responses that help plants to cope against these stresses. GABA-induced signaling against abiotic stresses is also associated with the activation of the antioxidative defense system, osmoregulation, pH maintenance, and buffering tool for C and N metabolism (Li *et al.*,

2016). To the best of our knowledge, the GABA-induced regulations in physio-biochemical processes and yield of aromatic rice (Ashraf *et al.*, 2022). Foliar application of GABA positively altered several physio-biochemical processes in plants, improved the balance between 2-Acetyl-1-Pyrroline (2AP) content and yield in fragrant rice (Xie *et al.*, 2021). The present study was conducted with the hypothesis that application of GABA could enhance the 2-AP formations in aromatic rice with the objectives:

- The proposed approach would be to find out appropriate dose of GABA for aromatic rice.
- To optimize the application time of GABA for aromatic rice
- This study might be a possible route to increase yield of aromatic rice.

CHAPTER II

REVIEW OF LITERATURE

Rice is the staple food crops in Bangladesh. Our people depend on rice cultivation. Other crops are not as important to them as rice. Aromatic rice is considered an important commodity in the global market because of its strong aroma and eating and cooking quality. Aromatic or fragrant rice is considered the best quality rice because it fulfills all desirable characteristics, and has a global identity (Hinge, 2019). Growth, development and other activities of aromatic rice plants are significantly influenced by different factors such as; environmental factors (abiotic factors), biotic factors, variety and cultural practices etc. GABA is a non-protein amino acid that acts as a signaling molecule that regulates growth and development, metabolism and stress response in plants (Ramesh *et al.*, 2017). The positive effects of GABA on different plants under adverse conditions have also been widely studied. Several numbers of research works on the effect of GABA on different cultivation have been conducted at home and abroad. GABA is nearly unfamiliar to the farmers in producing crops in Bangladesh. The present study showed the effect of application of GABA on the productivity of aromatic rice. Also, the available literature on the effect of GABA on growth, physiological, biochemical changes and yield in plants has been reviewed below. Exogenous application of GABA used to evaluate the different roles of morpho-physiology, yield and yield attributing characters of aromatic rice at university campus. In this case a review is made with the available literature related to the present study. Most of the research reports showed a positive effect of GABA treatment on yield of aromatic rice and other crops. The findings of various authors are cited below:

2.1 Effect of GABA in Rice

Effect of GABA on Grain Yield in Fragrant Rice

Both pot and field experiments were conducted by Xie *et al.*, (2021) in 2017 at the Experimental Research Area, College of Agriculture, South China Agricultural University, Guangzhou, China to investigate exogenous γ -aminobutyric acid (GABA) application could enhance the 2-acetyl-1-pyrroline (2AP) accumulations and yield of fragrant rice under low light conditions. Field and pot experiments were conducted with three fragrant rice cultivars i.e., Basmati and Yuxiangyouzhan (indica), and Yungengyou 14 (japonica) that were grown under three different treatments i.e., normal light + GABA 0 mgL⁻¹ (CK), low light + GABA 0 mgL⁻¹ (T₁), and low light + GABA 250 mgL⁻¹ (T₂). The results revealed that the grain 2AP

contents were increased by 14.67–34.83% and up to 29.34% under T₁ and T₂ treatments in pot and field experiments, respectively, as compared with CK. The T₁ and T₂ treatments improved aroma owing to regulation in the accumulation of micronutrients i.e., Na, Mn, and Fe and enzyme activities involved in 2AP biosynthesis. The grain yield was substantially reduced in T₁ as compared with T₂ treatment for all rice cultivars. On the other hand, GABA application improved the grain yield under low light conditions by regulating the plant growth, and related physiological and biochemical attributes in all rice cultivars.

Gaoa *et al.*, (2020) conducted a pot experiment during 2016–17 at Department of Crop Science and Technology, College of Agriculture, South China Agricultural University, Guangzhou, People's Republic of China to investigate the optimum time for exogenous γ -aminobutyric acid (GABA) application at different growth stages regulates 2-acetyl-1-pyrroline, yield, quality and antioxidant attributes in fragrant rice. They applied with five GABA levels i.e. no GABA application (CK), application of GABA at 250 mg l⁻¹ with 25 ml pot⁻¹ at tillering stage (S1), panicle initiation stage (S2), heading stage (S3), and at tillering, panicle initiation and heading stages (S4). The result showed that a significant increase in grain yield under the GABA treatments and higher grain yield was found at S4 treatment than CK for its antioxidant attributes that can regulate growth and development and metabolism stress.

Effect of GABA on 2-Acetyl-1-Pyrroline (2AP) Biosynthesis in Fragrant Rice

GABA was recently reported to regulate rice yield under different nitrogen application by affecting the growth and yield-related traits in fragrant rice (Li *et al.*, 2020). Moreover, the grain 2AP and GABA contents were enhanced under salt stress (Poonlaphdecha *et al.*, 2012) and shading treatments whereas significant positive correlation was observed between GABA and 2AP content in grains of fragrant rice (Mo *et al.*, 2015). Thus, there might be a feasible approach for GABA application to improve grain yield and regulate the 2AP biosynthesis in fragrant rice under low light conditions.

The application of GABA at panicle initiation stage could regulate the aroma formation for three aromatic rice cultivars (Xie *et al.*, 2019). Xie *et al.*, (2020) indicated that spraying GABA at the initial heading stage could modulate the grain 2AP contents, grain yield and grain quality in fragrant rice.

The growth and productivity of rice varies among different rice genotype (Li *et al.*, 2019b). These results suggested that the interactive effects of GABA and cultivar influenced grain quality attributes, however, the molecular mechanisms about GABA-cultivar interaction need

to be further studied. Overall, exogenous GABA applications during different growth stages of fragrant rice modulated the grain 2AP contents, enzymes involved in its formation and grain yield.

Tang *et al.*, (2006) revealed the differences in proline contents in leaves at MS and the 2AP contents from 14 days after full heading stage to MS under zinc, iron, and lanthanum application. In a pot experiment, a significant increase or relative stability of proline contents in leaves at HS and MS was noted under the GABA treatments. Exogenous GABA improved the 2AP content and regulated grain yield formation in different fragrant rice genotypes under different nitrogen levels (Li *et al.*, 2020, Xie *et al.*, 2019).

Effect of GABA on Total Tillers Plant⁻¹ and Grains Panicle⁻¹ in Fragrant Rice

Ashraf *et al.*, (2022) conducted a pot experiment in a rain-protected net house at Experimental Research Area, College of Agriculture, South China Agricultural University, Guangzhou, China (23°09' N, 113°22' E, and 11 m above the sea level) from April to July 2016 to assess the role of exogenous gamma-aminobutyric acid (GABA) application to modulate the growth, yield, and related physio-biochemical mechanisms in two aromatic rice cultivars, that is, Guixiangzhan (GXZ) and Nongxiang 18 (NX-18), under Pb toxic and normal conditions. The experimental treatments were comprised of Ck: without Pb and GABA (control), GABA: 1 mM GABA is applied under normal conditions (without Pb), Pb + GABA: 1 mM GABA is applied under Pb toxicity (800 mg kg⁻¹ of soil), and Pb= only Pb (800 mg kg⁻¹ of soil) is applied (no GABA). The required concentrations of GABA were applied as a foliar spray. Results revealed that exogenous GABA application improved leaf chlorophyll, proline, protein and GABA contents, photosynthesis and gas exchange, and antioxidant defense under Pb toxicity in both rice cultivars. The yield and related traits, that is, productive tillers/pot, grains/panicle, filled grain %, 1,000-grain weight, and grain yield were 13.64 and 10.29, 0.37% and 2.26%, 3.89 and 19.06%, 7.35 and 12.84%, and 17.92 and 40.56 lower under Pb treatment than Pb + GABA for GXZ and NX-18, respectively. Furthermore, exogenous GABA application in rice reduced Pb contents in shoot, leaves, panicle, and grains compared with Pb-exposed plants without GABA. Overall, GXZ performed better than NX-18 under Pb toxic conditions.

A pot experiment was conducted by Varayanond *et al.*, (2005) during 2004 to investigate the effects of water soaking on the content of gamma-aminobutyric acid (GABA) in 6 Thai rice varieties; namely, Khao Dawk Mali 105, Pathum Thani 1, Chai Nat 1, Suphan Buri 1, Leuang Pratew 123 and Plai Ngahm. The result revealed that Plai Ngahm had the highest

percentage of germ weight while Patum Thani 1 had the lowest. Percentage of germ weight showed no relation to the GABA content. High GABA content of germ was found in 3 rice varieties: Khao Dawk Mali 105 (186.2 mg/kg of germ) Pathum Thani 1 (154.6 mg/kg of germ) and Chai Nat 1 (144.5 mg/kg of germ). Plai Ngahm, on the other hand, contained GABA 116.9 mg/kg of germ. Water soaking can enrich GABA content in the germ of all rice varieties. The GABA accumulation differed among rice varieties and according to soaking time.

Effect of GABA on Plant Growth and Grain Quality in Fragrant rice

Xie *et al.*, (2020) conducted field experiments from June 4 to July 1 (EXP.1) and from June 13 to July 11 (EXP.2) in 2014 at the Agricultural Experiment Farm, South China Agricultural University (SCAU), Guangzhou, China. The effects of exogenous application of γ -aminobutyric acid (GABA) on yield and quality characteristics, especially 2-acetyl-1-pyrroline (2AP) biosynthesis were investigated. Two field experiments, i.e., experiment 1 (EXP.1) and experiment 2 (EXP.2), were conducted, and four GABA levels, i.e., 0, 50, 500, 1000 mg L⁻¹, classified as CK, T₁, T₂, and T₃, respectively, were sprayed at the initial heading stage of a fragrant rice cultivar, Yuxiangyouzhan. The results showed that exogenous GABA application increased the 2AP content in grains 8.58%–19.44% in EXP.1 and 0.01%–6.22% in EXP.2. Exogenous GABA treatments also increased the GABA concentration in the grains and proline content in fragrant rice. Overall, exogenous GABA application increased the 2AP content in the grains and the highest 2AP content in the grains was observed in the T₂ treatment (500 mg L⁻¹). The 2AP content was significantly correlated with the GABA concentration in the grains. Additionally, exogenous GABA application regulated plant growth and grain quality attributes.

Effect of GABA on Nutrients Contents in Fragrant rice

Simla *et al.*, (2023) evaluated a few indigenous rice varieties/cultivars for their potential to enhance GABA content by varying the soaking and germination durations. The result showed that GABA content of rice varieties Jyothi and Chitteni and the cultivar Njavara increased steadily from 19.18 mg/kg, 26.67 mg/kg, and 28.63 mg/kg at 0 h soaking and germination to 116.89 mg/kg, 127.97 mg/kg and 130.29 mg/kg after 72 h soaking and germination. The GABA content in the germinated Njavara was superior or comparable to the GABA content in most of the earlier reported Indian rice varieties. The study explored the possibilities for enhancing GABA content in indigenous rice varieties/cultivars. The research indicated that soaking and germination significantly enhanced the accumulation of GABA.

Xie *et al.*, (2019) conducted a pot experiment in the greenhouse of the Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, China, during the early growing season of 2017 to assess the γ -aminobutyric acid (GABA) and nitrogen (N) application induced regulations in the biochemical basis of rice aroma formation. Four N levels, that is, 0, 0.87, 1.75, and 2.61 g/pot, and two GABA treatments, that is, 0 mg/L (GABA0) and 250 mg/L (GABA 250), were applied to three fragrant rice cultivars, that is, Yuxiangyouzhan, Yungengyou 14, and Basmati-385. Results showed that GABA 250 increased 2AP, Na, Mn, Zn, and Fe contents by 8.44%, 10.95%, 25.70%, 11.14%, and 43.30%, respectively, under N treatments across cultivars. The GABA 250 further enhanced the activities of proline dehydrogenase (PDH), ornithine aminotransferase (OAT) (both at 15 days after heading (dAH), and diamine oxidase (DAO) (at maturity) by 20.36%, 11.24%, and 17.71%, respectively. The result revealed that exogenous GABA and N applications improved the 2AP contents and nutrient uptake in fragrant rice. GABA 250 can increase 2AP, Na, Mn, Zn, and Fe contents, but decrease in protein content in grains as compared to GABA 0. The GABA250 treatment further enhanced the activities of PDH and OAT at 15 d AH and DAO activity at maturity but reduced the proline contents at maturity.

Exogenous GABA applications improved the growth, development, and photosynthesis in plants (Luo *et al.*, 2011; Li *et al.*, 2016a). Malekzadeh *et al.*, (2014) indicated an increase in antioxidant enzyme activities under GABA application. Additionally, GABA also plays an important role in ion absorption, nitrate uptake and nitrogen metabolism in plants (Ma *et al.*, 2016).

Effect of GABA on Leaf Chlorophyll and Proline Contents in Fragrant rice

GABA -induced regulations in physio-biochemical processes and yield of aromatic rice (Ashraf *et al.*, 2022). Foliar application of GABA positively altered several physio-biochemical processes in plants, improved the balance between 2-acetyl-1-pyrroline (2AP) content and yield in fragrant rice (Xie *et al.*, 2021).

Exogenous GABA application improved the chlorophyll contents and other photosynthetic attributes in rice seedlings (Li *et al.*, 2017). Xie *et al.*, (2019) revealed that GABA application improved the nutrient uptake in rice.

The 2AP contents were also associated with the GABA contents in grains but not with the GABA contents in the leaves of aromatic rice (Poonlaphdecha *et al.*, 2012). Mo *et al.*, (2015) reported that the 2AP contents were significantly and positively correlated with grain GABA contents in fragrant rice. The application of exogenous GABA resulted in more GABA

accumulation in grains. In general, the GABA in grains is synthesized via GABAald or by the conversion of glutamate (Bouché *et al.*, 2004; Fait. *et al.*, 2008). Moreover, the biosynthesis and accumulation of 2AP are associated with glutamate and GABAald (Xie *et al.*, 2020).

exogenous GABA application and light intensity affect the carbon and nitrogen metabolism (Bouche *et al.*, 2004, Mo *et al.*, 2015, Mo *et al.*, 2019a, Mo *et al.*, 2019b) that may be related to the balance between grain yield and 2AP, however further study is needed to evaluate the role/involvement of GABA in carbon and nitrogen metabolism in relation to the grain yield and 2AP formation (Sepehri *et al.*, 2018, Sepehri *et al.*, 2019, Sepehri *et al.*, 2020). Thus, GABA being a plant growth regulator could effectively improve the physiological basis of grain yield and aroma biosynthesis in fragrant rice under low light and/or shading effect.

How GABA application affected the 2AP formation in fragrant rice under low light conduction? It could be explained by three ways, i.e., the direct role of GABA in 2AP accumulation (Xie *et al.*, 2019, Xie *et al.*, 2020), the regulation of GABA on the precursors and enzymes involved in 2AP biosynthesis (Li *et al.*, 2016a, Li *et al.*, 2016b, Mo *et al.*, 2019a, Mo *et al.*, 2019b, Xie *et al.*, 2019, Xie *et al.*, 2020), and the role of micro-nutrients in the modulation of 2AP synthesis (Li *et al.*, 2016a, Li *et al.*, 2016b, Tang *et al.*, 2006).

Application of GABA could directly enhance 2AP content, and a relationship between 2AP and GABA content have been reported in fragrant rice (Liu *et al.*, 2020, Mo *et al.*, 2015, Poonlaphdecha *et al.*, 2012, Xie *et al.*, 2019, Xie *et al.*, 2020).

Liu *et al.*, (2020) reported that there is a significant and positive relationship between 2AP in grain and GABA content in leaf. But Xie *et al.*, (2019) reported a negative relationship between 2AP and GABA content after GABA application under different N levels.

Xie *et al.*, (2020) reported enhancement in 2AP content resulted from improving the proline content under GABA application whilst Mo *et al.*, 2019a, Mo *et al.*, 2019b found significant and positive correlations of P5C with grain 2AP contents. Owing to the difference in plant tissues and growth conditions, Xie *et al.*, (2019) reported that 2AP content is related to the proline and P5C content in leaf after GABA application under different N levels.

Ma *et al.*, 2016 observed that GABA addition was reported to affect the ions absorption and/or element uptake in rice seedling under ammonium toxicity. Moreover, nitrogen could improve the 2AP content in grain and low light stress conditions could lead towards higher 2AP accumulation (Mo *et al.*, 2015, Mo *et al.*, 2019a, Mo *et al.*, 2019b).

Li *et al.*, (2019) confirmed that exogenous GABA application increased the mean grain yield of fragrant rice by 11.84% as a consequence of the increase in grain weight, spikelet per pot, grain weight per leaf area and dry weight. Moreover, study has reported that GABA alleviates the antioxidant defense system (Li *et al.*, 2017).

The GABA provided partial protection to the photosynthetic pigments, by scavenging the degenerative ROS activities via proline accumulation and/or activation of the antioxidative defense system (Khanna *et al.*, 2021). These inter-linked physiological mechanisms are modulated by GABA to improve rice performance under Pb stress. GABA application-induced improvements in yield and quality attributes of fragrant rice were also reported by (Xie *et al.*, 2020).

GABA, a non-protein amino acid, is an important plant signaling molecule involved in different physiological and biochemical mechanisms in plants (Li *et al.*, 2016a, Li *et al.*, 2016b). GABA is recognized as a signaling molecule that is involved in various physio-biochemical processes to regulate plant stress responses (Ramesh *et al.*, 2017; Routray *et al.*, 2018).

In general, the endogenous concentration of GABA in plants is relatively low but its levels may be enhanced in response to the various stress conditions (Li *et al.*, 2017). Yu *et al.*, 2007; Song *et al.*, 2010 reported that GABA is endowed with the ability to regulate physio-biochemical metabolism in different plants.

Gamma amino butyric acid has attracted great attention over the last several decades due to its ubiquity in life. GABA is a four-carbon non-protein amino acid synthesized during brown rice germination via the decarboxylation of L-glutamic acid, a reaction catalyzed by glutamate decarboxylase (Bown *et al.*, 1997).

Effect of GABA on Filled Grains Panicle⁻¹ and 1000-Grains Weight in Fragrant Rice

Ashraf *et al.*, (2022) conducted a pot experiment in a rain-protected net house at Experimental Research Area, College of Agriculture, South China Agricultural University, Guangzhou, China (23°09' N, 113°22' E, and 11 m above the sea level) from April to July 2016 to assess the role of exogenous Gamma-Aminobutyric Acid (GABA) application to modulate the growth, yield, and related physio-biochemical mechanisms in two aromatic rice cultivars, that is, Guixiangzhan (GXZ) and Nongxiang 18 (NX-18), under Pb toxic and normal conditions. The experimental treatments were comprised of Ck: without Pb and GABA (control), GABA: 1 mM GABA is applied under normal conditions (without Pb), Pb

+ GABA: 1 mM GABA is applied under Pb toxicity (800 mg kg⁻¹ of soil), and Pb= only Pb (800 mg kg⁻¹ of soil) is applied (no GABA). The required concentrations of GABA were applied as a foliar spray. Results revealed that exogenous GABA application improved leaf chlorophyll, proline, protein and GABA contents, photosynthesis and gas exchange, and antioxidant defense under Pb toxicity in both rice cultivars. The yield and related traits, that is, productive tillers/pot, grains/panicle, filled grain %, 1,000-grain weight, and grain yield were 13.64 and 10.29, 0.37% and 2.26%, 3.89 and 19.06%, 7.35 and 12.84%, and 17.92 and 40.56 lower under Pb treatment than Pb + GABA for GXZ and NX-18, respectively. Furthermore, exogenous GABA application in rice reduced Pb contents in shoot, leaves, panicle, and grains compared with Pb-exposed plants without GABA. Overall, GXZ performed better than NX-18 under Pb toxic conditions.

Effect of GABA on Environmental Stress Reduction

Gamma-aminobutyric acid (GABA) is often involved in signaling transduction mechanisms in plants under different abiotic stresses such as chilling, heat, osmotic, respiratory stress, and so on (Vijayakumari *et al.*, 2016), as well as modulates numerous physio-biochemical responses that help plants to cope against these stresses. It has been reported by Li *et al.* (2016) that GABA-induced signaling against abiotic stresses is also associated with the activation of the antioxidative defense system, osmoregulation, pH maintenance, and buffering tool for C and N metabolism. It was found that the GABA concentration tends to be higher in response to environmental stresses such as drought, UV irradiation, mechanical damage, extreme temperature, salinity, and hypoxic conditions (Bouché *et al.*, 2004).

The GABA may have some ROS scavenging and/or regulatory roles in plants (Shi *et al.*, 2010), as the GABA shunt pathway provides succinate and nicotinamide adenine dinucleotide (NADH) during respiration, hence regulations in GABA might have a direct role in regulating the ROS/RO intermediates under oxidative stress (Bouche *et al.*, 2004). The reduced oxidative stress in GABA-treated plants under metal toxicity was also reported previously (Nayyar *et al.*, 2014; Kumar *et al.*, 2019).

Associations of GABA with polyamines and phytohormones could improve the plant performance under stress conditions (Podlešáková *et al.*, 2019). The GABA shunt pathway also regulates C: N fluxes by actively assimilating the glutamate-generated carbons (Bouche *et al.*, 2004).

2.2 Effect of GABA on other crops

Kinnersley *et al.*, (2000) found that the duckweed treated with GABA yielded higher levels of Mn than the untreated plants.

The GABA-induced modulations in photosynthetic machinery, especially “photosystem I and II,” were noted in *Piper nigrum* Linn (Vijayakumari *et al.*, 2016).

Non-significant improvements due to GABA application were noted in chlorophyll contents and chlorophyll fluorescence, except for photochemical quenching coefficient, and actual photochemical efficiency in hydroponically cultured tomato seedlings under salt stress (Luo *et al.*, 2011).

Li *et al.*, (2016) reported substantial enhancements in proline contents in maize under GABA application.

GABA-induced maintenance in energy status and enhanced chilling tolerance due to modulations in enzymatic and non-enzymatic antioxidants were previously noted in peach fruit under chilling stress (Yang *et al.*, 2011).

Shi *et al.*, (2010) found significant modifications in antioxidants to remove ROS in *Caragana intermedia* plants due to GABA application.

Regulations in the activities of NR and GS were also observed in *Arabidopsis thaliana* seedlings under GABA application (Barbosa *et al.*, 2010).

The GABA application improved the Cd tolerance in maize by upregulating the antioxidant enzyme activities and polyamine biosynthesis-responsive genes, that is ornithine decarboxylase and spermidine synthase (Seifikalhor *et al.*, 2020).

Modulation in GS and NR activities in maize (Li *et al.*, 2016; Li *et al.*, 2016) was noted due to GABA, which shows the involvement of GABA in C:N metabolism; however, the mechanism and extent of its involvement in C-N metabolism are still not clear.

Ramesh *et al.*, (2015) found that GABA-induced Al tolerance was due to aluminum-activated malate transporters (ALMT) in wheat; nevertheless, the exact mechanism of GABA involvement to reduce Pb contents in upper plant parts and molecular basis of Pb tolerance due to GABA application are yet to be explored.

The application of GABA could significantly improve the growth and productivity of black cummin under water deficit conditions (Rezaei *et al.*, 2018).

Exogenous application of GABA could transiently increase the tolerance to root hypoxia of *Prunus* rootstock (Salvatierra *et al.*, 2016).

GABA could regulate the nitrate concentration and metabolism in the leaves of Pakchoi (*Brassica campestris* sp. *chinensis* Makino) (Li *et al.*, 2018).

Kinnersley *et al.*, (2000) found that the duckweed treated with GABA yielded higher levels of Mn than the untreated plants.

Li *et al.*, (2016a) suggested that exogenous GABA improved the salt stress tolerance in growing wheat plants due to the enhancement of photosynthesis and antioxidant enzyme activities.

Shang *et al.*, (2011) showed that GABA application played an important role in alleviating chilling injury in cold-stored peach fruit.

Exogenous GABA applications could improve salt stress resistance in wheat seedlings (Li *et al.*, 2016) and enhance photosynthesis capacity in maize seedling (Li *et al.*, 2016).

CHAPTER III

MATERIALS AND METHODS

The materials and methods used for conducting this experiment has been presented in this section. It includes short description of various information as for example location of the experimental site, soil, climatic condition of the experimental area, materials that are used in the experiments, design of the experiments, treatments, data collection parameters and analysis procedure of collected data. The experiments were carried out during the period from July 2023 to December, 2023 at the research field of Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200.

3.1 Description of experimental site

3.1.1 Geographical location

The experimental field (Research Field of HSTU) was located at 25.56° N latitude and 88.5° E longitude at an altitude of 37 m above from the mean sea level. This area is situated at the AEZ-1 which is Old Himalayan Piedmont Plain.

3.1.2 Agro Ecological Zone

The soil of the experimental field belongs to the Agro-ecological zone of “Old Himalayan Piedmont Plain. This was a region of complex relief and soil developed over the Dinajpur is sandy loam. The experimental site was shown in the map of AEZ of Bangladesh.

3.1.3 Climate

The experimental area is under the sub-tropical climate that is characterized by high temperature, high humidity and heavy rainfall with occasional gusty winds in kharif season (April-September) and less rainfall associated with moderately low temperature during the Rabi season (October-March). The average annual precipitation of the site was around 167 mm. The average maximum temperature in summer was 28° C and average minimum temperature was 18° C. Sometimes it goes nearly 10° C. The average mean temperature was 23° C. The experiment was done during the rabi season.

3.1.4 Soil

The experimental field is medium high with sandy loam type soil with a pH value 6.5, low in organic matter and fertile. The soil was well drained and morphological characteristics of soil of the experimental site have been presented in the appendix.

3.2 Experimental details

3.2.1 Planting material

The experiment was conducted with local variety 'Tulshimala' of aromatic rice as test crops.

3.2.2 Experimental Treatments

Two factors experiment was considered for the present study which is as follows:

Factor A: GABA solution

G₁= 0 (Control)

G₂= 50 ppm

G₃= 100 ppm

G₄= 150 ppm

Factor B: Time of application of GABA

T₁= Tillering stage (at 30 DAT)

T₂= Panicle initiation stage (at 60 DAT)

T₃= Booting stage (at 90 DAT)

Treatments were replicated three times.

3.3 Description of treatments

Preparation of GABA solution

0.1 N HCL prepared in 80% ethanol was used to dissolve GABA standard. GABA stock solution (1 mg mL⁻¹) was prepared by dissolving 10 mg of GABA in 10 mL of 0.1 N HCL. GABA has formula C₄H₉NO₂.

3.4 Conduction of experiments

3.4.1 Experimental design

This factorial experiment was arranged in a randomized complete block design with three replications. The total number of unit plots was 36. The size of the unit plot was 3 m × 2 m. The treatments were randomly distributed to each block. The distance between two adjacent

replications (block) was 0.7 m and row-to-row distance was 0.5 m, respectively. The inter block and inter row spaces were used as footpath and irrigation or drainage channel.

3.4.2 Layout of the experiments

Treatment combination

D₁T₁= Without GABA application+ At 30 DAT

D₁T₂= Without GABA application+ At 60 DAT

D₁T₃= Without GABA application+ At 90 DAT

D₂T₁= 50 ppm of GABA+ Application time of GABA at 30 DAT

D₂T₂= 50 ppm of GABA+ Application time of GABA at 60 DAT

D₂T₃= 50 ppm of GABA+ Application time of GABA at 90 DAT

D₃T₁= 100 ppm of GABA+ Application time of GABA at 30 DAT

D₃T₂= 100 ppm of GABA+ Application time of GABA at 60 DAT

D₃T₃= 100 ppm of GABA+ Application time of GABA at 90 DAT

D₄T₁= 150 ppm of GABA+ Application time of GABA at 30 DAT

D₄T₂= 150 ppm of GABA+ Application time of GABA at 60 DAT

D₄T₃= 150 ppm of GABA+ Application time of GABA at 90 DAT

Three equal blocks made up the total area. According to the experiment's design, each block contained 12 plots with 12 randomly assigned treatments for GABA dose and time.

The layout of the study is given below-

Block-1	Block-2	Block-3
4	1	12
7	5	4
10	11	7
9	2	10
3	12	9
8	4	3
6	7	8
1	10	6
5	9	1
11	3	5
2	8	11
12	6	2

Figure 1: Layout of the Experiment

3.4.3 Collection of seeds of Tulshimala

Seeds were immersed in water in a bucket for 24 hours. The immersed seeds were taken out from water and kept in gunny bags. The seeds started sprouting after 48 hours. These seeds were suitable for sowing in the seed bed in 72 hours.

3.4.4 Preparation of seedbed

The seed bed was made by puddling with 3-4 ploughing followed by laddering. Gently irrigation was provided, and weeds were removed from the bed as and when necessary and no fertilizer was used in the nursery bed.

3.4.5 Seed sowing

The sprouted seeds were sown in the seedbeds on 23 June 2023. The beds were irrigated to a depth of 2-3 cm after establishing the roots. Excess water was occasionally drained off to attain water depth of 5 cm that partially controlled weeds.

3.4.6 Preparation of experimental land

The plot selected for the experiment was opened on the 30th July 2023 with a power tiller, and left exposed to the sun for a week. For obtaining good puddle condition, the land was harrowed, ploughed and cross-ploughed several times after one week followed by laddering. Weeds and stubbles were removed from the field. The experimental plot was partitioned into unit plots in accordance with the experimental design.

3.4.7 Fertilizer dose and methods of application

The fertilizers N, P, K, S, Zn and B in the form of urea, TSP, MoP, DAP, gypsum, zinc sulphate and borax, respectively were applied. The entire amount of TSP, MP, gypsum, zinc sulphate and borax were applied during the final preparation of land. The mixture of cowdung and compost was applied during 15 days before transplanting. Urea was applied in three equal installments at seedling establishment, tillering and before panicle initiation.

3.4.8 Uprooting of seedlings

The seedbed was wetted by application water in the morning to make the soil soft so that uprooting became easy. Then uprooting was done in the evening on the previous day of transplanting. Seedlings were uprooted carefully without causing any injury to the roots and stem and kept in water for the whole night.

3.4.9 Transplanting of seedlings

Twenty-eight days old seedlings of the fine rice cultivar were transplanted in the main field on 20 July in 2023 with a spacing 20 cm from row to row and 15 cm from hill to hill.

3.4.10 Intercultural operations

After the establishment of seedlings, various intercultural operations were accomplished for better growth and development of the rice seedlings.

3.4.10.1 Weeding

Weeding was done during the first two top dressings of urea to break the soil crust, to keep the plots free from weeds and to incorporate the urea properly into the soil which reduced the loss of urea through de-nitrification and leaching.

3.4.10.2 Application of irrigation water

Irrigation water was added to each plot as and when necessary. All the plots were kept irrigated maintaining 3-5 cm stagnant water throughout the entire period up to 15 days before harvesting. Irrigation had applied before the water level was fell down to the root of the crop plants.

3.4.10.3 Plant protection measures

There were some incidences of insects specially stem borer which was controlled by Furadan 5G @ 10 kg ha⁻¹ at 30 days after transplanting. Brown spot of rice was controlled by spraying tilth. Rifit was used as herbicide @ 1.0 L. ha⁻¹. And ripcord was used as insecticide @ 200 ml ha⁻¹. Batir was applied as insecticides @ 12.54 g ha⁻¹.

3.4.10.4 Preparation and application of GABA solution

The doses of GABA application were 50 ppm, 100 ppm, 150 ppm. To make it's recommended doses GABA solution was mixed in 500 ml water. That was the working solution. Again, this solution was diluted to make up the desired concentration for application in each plot. GABA solution was applied at tillering stage (at 30 DAT), panicle initiation stage (at 60 DAT) and booting stage (at 90 DAT).

3.5 General observation of the experimental field

The field was observed time to time to detect visual differences among the treatments and any kind of infestation by weeds, insects and diseases so that considerable losses by pest was minimized.

3.6 Harvesting, threshing and cleaning

The rice plant was harvested depending upon the maturity of the plant and harvesting was done manually from each plot. The harvested crop of each plot was bundled separately, properly tagged and brought to threshing floor. Enough care was taken for harvesting, threshing and also cleaning of rice seed. Fresh weight of grain and straw were recorded plot wise. The grains were cleaned and finally the weight was adjusted to a moisture content of

12%. The straw was sun dried and the yields of grain and straw plot⁻¹ were recorded and converted to ton ha⁻¹.

3.7 Recording of data

The following data was recorded during the experiment.

A. Crop growth parameters

1. Plant height (cm)
2. Number of tillers hill⁻¹

B. Yield and yield contributing parameters

1. Total number of tillers hill⁻¹
2. Number of effective tillers hill⁻¹
3. Number of non-effective tillers hill⁻¹
4. Panicle length (cm)
5. Number of filled grains panicle⁻¹
6. Number of unfilled grains panicle⁻¹
7. Weight of 1000-grain (g)
8. Grain yield (t ha⁻¹)
9. Straw yield (t ha⁻¹)
10. Biological yield (t ha⁻¹)
11. Harvest index (%)

3.8 Description of the recorded data

A. Crop growth parameters

1. Plant height (cm)

The height of plant was recorded in centimeters (cm) at the time of 25, 50, 75 DAT and at harvest. The height was measured from the ground level to the tip of the plant. Plant height of 5 hills were measured and averaged for each plot.

2. No. of total tillers hill⁻¹

Total tillers which had at least one leaf visible were counted. It includes both productive and unproductive tillers. It was counted from the average of the same 5 hills pre-selected at random from the inner rows of each plot.

B. Yield and yield contributing parameters

1. Total no. of tillers hill⁻¹

The total tiller was calculated by averaging the number of effective tillers and non-effective tiller of 5 hill of each plot.

2. No. of effective tillers hill⁻¹

The total number of effective tillers per hill was counted as the number of panicles tillersg tiller during flowering which had at least one grain. The number of effective tillers per hill was counted from five selected hills and finally averaged for counting effective tillers number per hill.

3. No. of non-effective tillers hill⁻¹

The total number of non-effective tillers was counted as tillers which have no panicle on the head. The total number of non-effective tillers hill-1 was counted from 5 selected plants at harvest and average value was recorded.

4. Panicle length (cm)

The length of the panicle was measured with a meter scale from 10 selected panicles and the average value was recorded. The length of the panicle was measured from basal node of the rachis to apex of each panicle.

5. No. of filled grains panicle⁻¹

If any kernel was present in grain, the grain was considered to be filled. The total number of filled grains were recorded on ten panicles and finally averaged.

6. No. of unfilled grains panicle⁻¹

Unfilled grains mean the absence of any kernel inside the grain and such grains present on each of ten panicles were counted and finally averaged for each plot.

7. Weight of 1000 grain (g)

One thousand cleaned dried grains were counted randomly from each plot and weighed by using a digital electric balance when the grains retained 12% moisture and the mean weight was expressed in grams.

8. Grain yield (t ha⁻¹)

Grain from each plot area was thoroughly sun dried till constant weight was attained. Then yield per hectare was determined based on net plot area. Weight of grains of each plot was converted into t ha⁻¹.

9. Straw yield (t ha⁻¹)

After separation of grains from plants of each plot the straw was sun dried till a constant weight is obtained and expressed as ton ha⁻¹.

10. Biological yield (t ha⁻¹)

Grain yield together with straw yield was calculated as biological yield with the following formula:

$$\text{Biological yield (t ha}^{-1}\text{)} = \text{Grain yield (t ha}^{-1}\text{)} + \text{Straw yield (t ha}^{-1}\text{)}$$

11. Harvest index (%)

It denotes the ratio of grain yield to biological yield and was calculated with the following formula.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield (t ha}^{-1}\text{)}}{\text{Biological yield (t ha}^{-1}\text{)}} \times 100$$

3.9 Statistical Analysis

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique and the mean difference were adjudged by LSD test using the statistical computer package program, Statistix-10. The significant difference among treatment means were estimated by Duncan's Multiple Test Range at 5% level of probability (Gomez and Gomez, 19884).

CHAPTER IV

RESULT AND DISCUSSION

This chapter comprises of the presentation and discussions of the research obtained due to different application of GABA on growth and yield contributing characters of aromatic rice (Tulsimala). The results of studies such as plant height, effective tiller, grain yield, straw yield, biological yield etc. of rice plants are discussed in this chapter. The results of the present investigation have been presented, discussed and compared with the help of table and graphs as far as available with the results of the researchers.

4.1 Effect of GABA on growth and yield contributing characters

4.1.1 Plant height (cm)

The plant height at different intervals was taken but results showed that except 30 DAT all data statistical significantly varied due to different concentrations of GABA solution at 60 DAT, 90 DAT and at harvesting time (Appendix VI and Table 1). There is an effect of GABA solution to increase the plant height of aromatic rice at all sampling dates. At 30 DAT, the tallest plant height (38.59 cm) was observed at treatment D₄ (150 ppm of GABA). At 60 and 90 DAT, the tallest plant height (86.89 cm and 120.29 cm) was found at treatment D₄ (150 ppm of GABA). At harvest the highest plant height (150.24 cm) was observed at D₄ (150 ppm of GABA). But the shortest plant height from 30 DAT (37.08 cm) was observed at D₂ (50 ppm of GABA) and 60, 90 DAT and at harvest (78.02 cm, 105.18 cm and 136.93 cm respectively) was observed at D₁ treatment (0 ppm of GABA). These results indicate that GABA has a positive effect on plant height of aromatic rice (Tulsimala) and has no negative effect on plant height. The results obtained from the study were conformity with the findings of Xie *et al.*, (2020). They found that the application of GABA increased plant height.

Table 1: Effect of different dose of GABA on plant height (cm) at different days intervals

GABA Dose	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	at harvest
D ₁	38.29	78.02c	105.18c	136.93d
D ₂	37.08	81.95b	115.89b	143.55c
D ₃	37.92	83.74b	117.02b	147.18b
D ₄	38.59	86.89a	120.29a	150.24a
CV%	8.92	3.39	2.00	1.64
LSD _(0.05)	3.31	2.74	2.23	2.32
LS	NS	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁= Without GABA application (Control)

D₂= 50 ppm of GABA

D₃= 100 ppm of GABA

D₄= 150 ppm of GABA

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

*= Significant at 5% level of significance

NS= Non-significant

Plant height had a significant effect on 60 DAT, 90 DAT and at harvest, whereas GABA had no significant effect on 30 DAT plant height (Appendix VI and Table 2). At 30 DAT and 60 DAT the tallest plant height (38.93 cm and 89.61 cm respectively) was observed at T₁ treatment (at tillering stage). But at 90 DAT and at harvesting the tallest plant height (122.67 cm and 148.59 cm) was found at T₂ treatment (at panicle initiation stage). The shortest plant height at 30 and 60 DAT (37.35cm and 79.13 cm) was observed at T₂ treatment (at panicle initiation stage). But at 90 DAT and at harvesting the shortest plant height (107.57 cm and 141.33 cm) was found at T₃ and T₁ treatment respectively. Plant height was increased significantly for the application of GABA.

Table 2: Effect of different time of application of GABA on plant height (cm) at different days intervals

Time of application	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	at harvest
T ₁	38.93	89.61a	113.54b	141.33c
T ₂	37.36	79.14b	122.67a	148.59a
T ₃	37.63	79.21b	107.57c	143.50b
CV%	8.92	3.39	2.00	1.64
LSD _(0.05)	2.87	2.37	1.94	2.00
LS	NS	**	**	**

In a column, figures having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

T₁= Application time of GABA at 30 DAT

T₂= Application time of GABA at 60 DAT

T₃= Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

NS= Non-significant

The foliar application of GABA at various DAT combined with different doses caused a significant increase in plant height up to a certain period (Appendix VI and Table 3). The highest plant height at 30 DAT and 60 DAT (40.75 cm and 95.58 cm) was found at D₄T₁ treatment (150 ppm of GABA+ Application time of GABA at 30 DAT). But at 90 DAT and at harvest the highest plant height (131.01 cm and 155.54 cm) was observed at D₄T₂ treatment (150 ppm of GABA+ Application time of GABA at 60 DAT). On the other hand, the shortest plant height at 30, 60, 90 DAT and at harvest (35.66 cm, 76.95 cm, 104.28 cm and 136.83 cm) were found at D₃T₂, D₁T₃, D₁T₁, D₁T₃ treatment respectively. GABA has different rules in plant during growth and development and reducing abiotic stress that's why higher antioxidant enzyme activity can better maintain the stability of cells and ensure the progress of various physiological activities (Xie *et al.*, 2020). Our result also agreed with this statement.

Table 3: Effect of interaction of different dose and application time of GABA on plant height (cm) at different days intervals

Interaction of dose and time	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	at harvest
D ₁ T ₁	37.71	80.03cd	104.28f	136.93gh
D ₁ T ₂	38.93	77.10d	105.31ef	137.03gh
D ₁ T ₃	38.24	76.95d	105.96ef	136.83h
D ₂ T ₁	37.20	88.25b	113.10d	141.14f
D ₂ T ₂	37.50	78.51cd	126.01b	148.58cd
D ₂ T ₃	36.53	79.10cd	108.55e	140.92fg
D ₃ T ₁	40.06	94.58a	115.77d	142.51ef
D ₃ T ₂	35.66	78.47cd	128.34ab	153.22ab
D ₃ T ₃	38.03	78.17cd	106.95ef	145.81de
D ₄ T ₁	40.75	95.58a	121.01c	144.74def
D ₄ T ₂	37.34	82.47c	131.01a	155.54a
D ₄ T ₃	37.71	82.62c	108.84e	150.45bc
CV%	8.92	3.39	2.00	1.64
LSD _(0.05)	5.74	4.75	3.87	4.02
LS	NS	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁T₁= Without GABA application+ At 30 DAT

D₁T₂= Without GABA application+ At 60 DAT

D₁T₃= Without GABA application+ At 90 DAT

D₂T₁= 50 ppm of GABA+ Application time of GABA at 30 DAT

D₂T₂= 50 ppm of GABA+ Application time of GABA at 60 DAT

D₂T₃= 50 ppm of GABA+ Application time of GABA at 90 DAT

D₃T₁= 100 ppm of GABA+ Application time of GABA at 30 DAT

D₃T₂= 100 ppm of GABA+ Application time of GABA at 60 DAT

D₃T₃= 100 ppm of GABA+ Application time of GABA at 90 DAT

D₄T₁= 150 ppm of GABA+ Application time of GABA at 30 DAT

D₄T₂= 150 ppm of GABA+ Application time of GABA at 60 DAT

D₄T₃= 150 ppm of GABA+ Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

NS= Non-significant

4.1.2 Number of total tillers hill⁻¹

The number of total tillers hill⁻¹ at different interval was taken but results showed that all data statistical significantly varied due to different concentrations of GABA solution at 30 DAT, 60 DAT, 90 DAT and at harvesting time (Appendix VII and Table 4). There is an effect of GABA solution to increase the number of total tillers hill⁻¹ of aromatic rice at all sampling dates. At 30 DAT, the highest number of total tillers hill⁻¹ (8.56) was observed at treatment D₁ (0 ppm of GABA). At 60 DAT the highest number of total tillers hill⁻¹ (14.52) was observed at treatment D₄ (150 ppm of GABA). At 90 and at harvest, the highest number of total tillers hill⁻¹ (20.09 and 20.52) was found at treatment D₃ (100 ppm of GABA). But the lowest number of total tillers hill⁻¹ from 30 DAT (8.38) was observed at D₄ (150 ppm of GABA) and 60 DAT the lowest number of total tillers hill⁻¹ (13.52) was observed at D₁ (0 ppm of GABA) and 90 DAT and at harvest (20.09 and 20.52 respectively) was observed at D₃ treatment (100 ppm of GABA). These results express that GABA has significant effect on total tillers hill⁻¹.

Table 4: Effect of different dose of GABA on number of total tillers hill⁻¹ at different days intervals

GABA Dose	No. of total tillers hill ⁻¹			
	30 DAT	60 DAT	90 DAT	at harvest
D ₁	8.56	13.52b	15.77c	16.39c
D ₂	8.51	13.96ab	18.25b	18.48b
D ₃	8.47	14.26ab	20.08a	20.52a
D ₄	8.39	14.52a	19.82a	19.83a
CV%	2.51	7.23	5.51	5.76
LSD _(0.05)	0.21	0.99	0.99	1.05
LS	NS	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁= Without GABA application (Control)

D₂= 50 ppm of GABA

D₃= 100 ppm of GABA

D₄= 150 ppm of GABA

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

NS= Non-significant

The number of total tillers hill⁻¹ had significant effect on 30 DAT, 60 DAT, 90 DAT and at harvest (Appendix VII and Table 5). At 30 DAT, 60 DAT, 90 DAT and at harvest, the highest number of total tillers hill⁻¹ (8.51, 15.04, 19.32 and 20.10 respectively) was observed at T₃, T₁, T₃, T₂ treatment. The lowest number of total tillers hill⁻¹ at 30 and 60 DAT (8.44 and 13.42) was observed at T₁ and T₃ treatment. But at 90 DAT and at harvesting the lowest number of total tillers hill⁻¹ (16.87, 18.24) was found at T₁ treatment. So, Application time of GABA at proper stage increases total tillers hill⁻¹ significantly.

Table 5: Effect of different time of application of GABA on number of total tillers hill⁻¹ at different days intervals

Time of application	No. of total tillers hill ⁻¹			
	30 DAT	60 DAT	90 DAT	at harvest
T ₁	8.44	15.05a	16.88b	18.24b
T ₂	8.49	13.73b	19.28a	20.10a
T ₃	8.51	13.42b	19.32a	19.09ab
CV%	2.51	7.23	5.51	5.76
LSD _(0.05)	0.18	0.86	0.86	0.92
LS	NS	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

T₁= Application time of GABA at 30 DAT

T₂= Application time of GABA at 60 DAT

T₃= Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

NS= Non-significant

The foliar application of GABA at various DAT combined with different doses caused a significant increase in the number of total tillers hill⁻¹ up to a certain period (Appendix VII and Table 6). The highest number of total tillers hill⁻¹ at 30 DAT and 60 DAT (8.62 and 15.78 cm) was found at D₁T₂ and D₄T₁ treatment (150 ppm of GABA+ Application time of GABA at 30 DAT). But at 90 DAT and at harvest the highest number of total tillers hill⁻¹ (21.20 and 21.00) was observed at D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT). On the other hand, the lowest number of total tillers hill⁻¹ at 30, 60, 90 DAT and at harvest (8.32, 13.02, 15.50, 16.10) were found at D₄T₂, D₁T₃, D₁T₁, D₁T₁ treatment respectively. Ashraf *et al.*, (2022) also showed that GABA increases total tillers hill⁻¹ significantly.

Table 6: Effect of interaction of different dose and application time of GABA on number of total tillers hill⁻¹ at different days intervals

Interaction of dose and time		No. of total tillers hill ⁻¹			
		30 DAT	60 DAT	90 DAT	at harvest
D ₁	T ₁	8.46	14.25abc	15.50f	16.10e
	T ₂	8.63	13.28c	15.87f	16.55e
	T ₃	8.58	13.02c	15.97f	16.55e
D ₂	T ₁	8.49	14.54abc	16.26ef	17.60de
	T ₂	8.50	14.12abc	19.43bcd	19.06bcd
	T ₃	8.54	13.23c	19.07cd	18.80cd
D ₃	T ₁	8.37	15.62ab	17.90de	19.68abc
	T ₂	8.53	13.58c	21.20a	21.00a
	T ₃	8.50	13.58c	21.17a	20.90ab
D ₄	T ₁	8.43	15.78a	17.80de	19.60abc
	T ₂	8.32	13.95bc	20.60abc	19.73abc
	T ₃	8.42	13.83c	21.07ab	20.17abc
CV%		2.51	7.23	5.51	5.76
LSD _(0.05)		0.36	1.72	1.73	1.83
LS		NS	*	*	*

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁T₁= Without GABA application+ At 30 DAT

D₁T₂= Without GABA application+ At 60 DAT

D₁T₃= Without GABA application+ At 90 DAT

D₂T₁= 50 ppm of GABA+ Application time of GABA at 30 DAT

D₂T₂= 50 ppm of GABA+ Application time of GABA at 60 DAT

D₂T₃= 50 ppm of GABA+ Application time of GABA at 90 DAT

D₃T₁= 100 ppm of GABA+ Application time of GABA at 30 DAT

D₃T₂= 100 ppm of GABA+ Application time of GABA at 60 DAT

D₃T₃= 100 ppm of GABA+ Application time of GABA at 90 DAT

D₄T₁= 150 ppm of GABA+ Application time of GABA at 30 DAT

D₄T₂= 150 ppm of GABA+ Application time of GABA at 60 DAT

D₄T₃= 150 ppm of GABA+ Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

*= Significant at 5% level of significance

NS= Non-significant

4.1.3 Number of effective tillers hill⁻¹

Exogenous application of GABA had a significant effect on number of effective tillers per hill (Table 7). The number of effective tillers increased with the application rate of GABA (0 ppm, 50 ppm, 100 ppm, 150ppm respectively). The highest number of effective tillers (18.64) were found in D₃ treatment and application with 100 ppm GABA (D₄ treatment) also gives effective results (17.79) which varied significantly from the control D₁ (0 ppm of GABA) treatment. The lowest number of effective tillers per hill⁻¹ (11.18) were found in the control D₁ treatment. In producing effective number of tillers hill⁻¹ the treatments may be arranged as D₃>D₄>D₂>D₁. From the result it has been shown that increasing doses GABA increases the vegetative characteristics such as plant height, number of tillers etc. These results might be due to the application of GABA and foliar application reduces the transpiration water losses and increase the total chlorophyll levels in the leaves. Li *et al.*, (2017) found that foliar application of GABA significantly increases number of effective tillers hill⁻¹ of fragrant rice.

4.1.4 Non-effective tillers hill⁻¹ (no.)

The non-effective tillers hill⁻¹ varied significantly due to the effects of different treatment of GABA (Table 7). The highest non-effective tillers hill⁻¹ was produced (5.22) by the D₁ treatment (0 ppm GABA) and lowest number of non-effective tillers per hill⁻¹ produced (1.47) by the D₂ treatment (50 ppm of GABA). In producing non-effective number of tillers hill⁻¹ the treatments may be arranged as D₁>D₄>D₃>D₂.

4.1.5 Panicle length (cm)

Panicle length was significantly influenced by different GABA treatment on aromatic rice (Tulsimala) (Table 7). Maximum panicle length (26.43 cm) was attained in D₃ treatment (100 pm of GABA) and minimum panicle length (24.11 cm) was attained in control treatment (0 ppm GABA). Exogenous application of GABA (150 ppm) also gives an effective result on panicle length (26.32 cm).

Table 7: Effect of GABA on different yield contributing characteristics of aromatic rice (Tulsimala)

Dose	Effective tillers hill ⁻¹ (No.)	Non-effective tillers hill ⁻¹ (No.)	Panicle length (cm)	Filled grain panicle ⁻¹ (No.)	Unfilled grain panicle ⁻¹ (No.)	1000-grain weight (g)
D ₁	11.18c	5.22a	24.10c	189.06c	10.17a	11.08b
D ₂	17.01b	1.48b	25.08b	206.53b	7.63b	11.36b
D ₃	18.64a	1.88b	26.43a	218.22a	4.83c	11.91a
D ₄	17.79ab	2.04b	26.32a	207.77b	5.70c	11.87a
CV%	9.03	28.35	2.26	3.22	18.16	3.28
LSD _(0.05)	1.43	0.74	0.56	6.47	1.26	0.37
LS	**	**	**	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁= Without GABA application (Control)

D₂= 50 ppm of GABA

D₃= 100 ppm of GABA

D₄= 150 ppm of GABA

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

4.1.6 Filled grain panicle⁻¹ (no.)

Table 7 shows the different types of application of GABA had a significant effect on filled grain per panicle. The highest number of filled grains per panicle (218.22) was found in D₃ treatment (100 ppm of GABA). The lowest number of filled grains per panicle (189.06) was found in D₁ treatment (0 ppm of GABA). According to the filled grain per panicle the treatments may be arranged as D₃>D₄>D₂>D₁. This result suggests that exogenous application of GABA could help to increase the filled grain per panicle of Tulsimala variety of aromatic rice. Foliar application also could help the increase of filled grains panicle⁻¹. Ashraf *et al.*, (2022) and Li *et al.*, (2019) also showed that increasing dose of GABA increases filled grain panicle⁻¹ (no.).

4.1.7 Unfilled grain panicle⁻¹ (no.)

Table 7 shows the different types of application of GABA had a significant effect on unfilled grain per panicle. The highest number of unfilled grains per panicle (10.16) was found in D₁ treatment (0 ppm of GABA). The lowest number of unfilled grains per panicle (4.83) was found in D₃ treatment (100 ppm of GABA). According to the unfilled grain per panicle the treatments may be arranged as D₁>D₂>D₄>D₃.

4.1.8 Thousand grains weight (g)

Table 7 shows the effect of different treatment on 1000-grain weight. The maximum 1000-grain weight (11.91 g) was attained at D₃ treatment (100 ppm of GABA). The minimum 1000-grain weight (11.08 g) was attained at control treatment D₁. In case of 1000-grain weight the treatment may be arranged as D₃>D₄>D₂>D₁. So, increasing doses of GABA increase thousand grains weight (g). It was observed that foliar application of GABA give better results over no foliar application of GABA on fragrant rice. Gaoa *et al.*, (2020) also showed thousand rains weight increased due to the application of GABA.

Table 8: Effect of application time of GABA on different yield contributing characteristics of aromatic rice (Tulsimala)

Time	Effective tillers hill ⁻¹ (No.)	Non- effective tillers hill ⁻¹ (No.)	Panicle length (cm)	Filled grain panicle ⁻¹ (No.)	Unfilled grain panicle ⁻¹ (No.)	1000 grain weight (g)
T ₁	14.92b	3.32a	25.07b	195.02b	8.63a	11.21b
T ₂	17.14a	1.95b	25.91a	212.58a	5.53c	11.79a
T ₃	16.40a	2.70a	25.47ab	208.57a	7.10b	11.68a
CV%	9.03	28.35	2.26	3.22	18.16	3.28
LSD _(0.05)	1.24	0.64	0.49	5.61	1.09	0.32
LS	**	**	**	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

T₁= Application time of GABA at 30 DAT

T₂= Application time of GABA at 60 DAT

T₃= Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

4.2 Effect of application time of GABA on growth and yield contributing characters

4.2.1 Number of effective tillers hill⁻¹

Exogenous application time of GABA had a significant effect on number of effective tillers per hill (Table 8). Number of effective tillers increased with the application time of GABA days after transplanting (30 DAT, 60 DAT and 90 DAT respectively). The highest number of effective tillers (17.14) were found in T₂ treatment and application of GABA 90 DAT also gives effective results (16.40) which varied significantly from the T₁ (30 DAT) treatment. The lowest number of effective tillers per hill⁻¹ (14.93) was found in the T₁ treatment. In producing an effective number of tillers hill⁻¹ the treatments may be arranged as T₂>T₃>T₁. So, application of GABA at panicle initiation stage increases the number of effective tillers hill⁻¹ most significantly.

4.2.2 Non-effective tillers hill⁻¹ (no.)

The non-effective tillers hill⁻¹ varied significantly due to the effects of different application time of GABA (Table 8). The highest non-effective tillers hill⁻¹ was produced (3.31) by the T₁ treatment (application of GABA at 30 DAT) and lowest non-effective tillers per hill⁻¹ produced (1.95) by the T₂ treatment (application of GABA at 60 DAT). In producing non-effective number of tillers hill⁻¹ the treatments may be arranged as T₁>T₃>T₂. So, application of GABA at tillering stage effect less on effective tillers hill⁻¹ (no.)

4.2.3 Panicle length (cm)

Panicle length was significantly influenced by different GABA treatments on aromatic rice (Tulsimala) shown in Table 8. Maximum panicle length (25.91 cm) was attained in T₂ treatment (application of GABA at 60 DAT) and minimum panicle length (25.07 cm) was attained in T₁ treatment (application of GABA at 30 DAT). Exogenous application of GABA (at 90 DAT) also gives the effective result on panicle length (25.46 cm).

4.2.4 Filled grain per panicle (no.)

Table 8 shows the different types of application time of GABA had a significant effect on filled grain per panicle. The highest number of filled grain per panicle (212.58) was found in T₂ treatment (application of GABA at 60 DAT). The lowest number of filled grain per panicle (195.02) was found in T₁ treatment (application of GABA at 30 DAT). According to the filled grain per panicle the treatments may be arranged as T₂>T₃>T₁. This result suggests that

exogenous application time of GABA could help to increase the filled grain per panicle of Tulsimala variety of aromatic rice.

4.2.5 Unfilled grain per panicle (no.)

Table 8 shows the different types of application time of GABA had a significant effect on unfilled grain per panicle. The highest number of unfilled grains per panicle (8.63) was found in T₁ treatment (application of GABA at 30 DAT). The lowest number of unfilled grains per panicle (5.53) was found in T₂ treatment (application of GABA at 60 DAT). According to the unfilled grain per panicle the treatments may be arranged as T₁>T₃>T₂.

4.2.6 Thousand grain weight (g)

Table 8 showed the effect of different treatment on 1000-grain weight. The maximum 1000-grain weight (11.79 g) was attained at T₂ treatment (application of GABA at 60 DAT). The minimum 1000-grain weight (11.21 g) was attained at treatment T₁. In the case of 1000-grain weight the treatment may be arranged as T₂>T₃>T₁. It is observed that application of GABA at panicle initiation stage increases Thousand grain weight (g) significantly. Luo *et al.*, (2011) also showed the same result.

Table 9: Interaction effect of application time and rate of GABA on different yield contributing characteristics of aromatic rice (Tulsimala)

Interaction of Dose and Time	Effective tillers hill ⁻¹ (No.)	Non-effective tillers hill ⁻¹ (No.)	Panicle length (cm)	Filled grains panicle ⁻¹ (No.)	Unfilled grains panicle ⁻¹ (No.)	1000-grain weight (g)	
D ₁	T ₁	10.77d	5.33a	23.97g	186.93f	11.27a	11.13ef
	T ₂	11.52d	5.03a	24.18fg	187.17f	8.87bcd	10.92f
	T ₃	11.25d	5.30a	24.17fg	193.07ef	10.37ab	11.20ef
D ₂	T ₁	15.20c	2.40bc	24.67efg	197.73ef	9.67abc	10.98f
	T ₂	18.30ab	0.77e	25.45cde	212.67bcd	5.40fgh	11.64bcde
	T ₃	17.53bc	1.27cde	25.12def	209.20cd	7.83cde	11.47cdef
D ₃	T ₁	17.07bc	2.60b	25.77cd	202.00de	6.33efg	11.36def
	T ₂	20.03a	0.97de	27.17a	231.00a	3.50h	12.37a
	T ₃	18.83ab	2.07bcd	26.35abc	221.67ab	4.66gh	12.00abcd
D ₄	T ₁	16.67bc	2.93b	25.88bcd	193.43ef	7.23def	11.35ef
	T ₂	18.70ab	1.03de	26.85ab	219.50bc	4.33gh	12.23ab
	T ₃	18.00ab	2.17bcd	26.23abc	210.37cd	5.53fgh	12.03abc
CV%	9.03	28.35	2.26	3.22	18.16	3.28	
LSD _(0.05)	2.47	1.27	0.97	11.21	2.18	0.64	
LS	*	*	*	*	*	**	

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁T₁= Without GABA application+ At 30 DAT

D₁T₂= Without GABA application+ At 60 DAT

D₁T₃= Without GABA application+ At 90 DAT

D₂T₁= 50 ppm of GABA+ Application time of GABA at 30 DAT

D₂T₂= 50 ppm of GABA+ Application time of GABA at 60 DAT

D₂T₃= 50 ppm of GABA+ Application time of GABA at 90 DAT

D₃T₁= 100 ppm of GABA+ Application time of GABA at 30 DAT

D₃T₂= 100 ppm of GABA+ Application time of GABA at 60 DAT

D₃T₃= 100 ppm of GABA+ Application time of GABA at 90 DAT

D₄T₁= 150 ppm of GABA+ Application time of GABA at 30 DAT

D₄T₂= 150 ppm of GABA+ Application time of GABA at 60 DAT

D₄T₃= 150 ppm of GABA+ Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

*= Significant at 5% level of significance

4.3 Effect of interaction of GABA dose and application time on growth and yield contributing characters

4.3.1 Number of effective tillers hill⁻¹

The combined effect of application rate and time of GABA influenced significantly the number of effective tillers per hill (Table 9). Result revealed that number of effective tillers increased with the application rate and time of GABA. Highest numbers of effective tillers hill⁻¹ (20.03) were found in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT). The lowest numbers of effective tillers per hill⁻¹ (10.77) were found in the D₁T₁ treatment (Without GABA application+ At 30 DAT). The results showed that T₂ and T₃ stage gave the highest result with the increasing level of GABA over without application of GABA.

4.3.2 Non-effective tillers hill⁻¹ (no.)

The non-effective tillers hill⁻¹ varied significantly due to the effects of different application time of GABA (Table 9). The highest number of non-effective tillers hill⁻¹ was produced (5.33) by the D₁T₁ treatment (Without GABA application+ At 30 DAT) and lowest non-effective tillers per hill⁻¹ produced (0.77) by the D₂T₂ treatment (50 ppm of GABA+ Application time of GABA at 60 DAT). So application of GABA gave the superior result than without application of GABA.

4.3.3 Panicle length (cm)

Panicle length was significantly influenced by different GABA treatment on aromatic rice (Tulsimala) (Table 9). Maximum panicle length (27.17 cm) was attained in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT) and minimum panicle length (23.97 cm) was attained in D₁T₁ treatment (Without GABA application+ At 30 DAT). Application of GABA gave the superior result than without application of GABA.

4.3.4 Filled grains panicle⁻¹ (no.)

Table 9 shows the different types of application rate and time of GABA had a significant effect on filled grain per panicle. The highest number of filled grain per panicle (231.00) was found in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT). The lowest number of filled grain per panicle (186.93) was found in D₁T₁ treatment (Without GABA application+ At 30 DAT) followed by D₁T₂ and D₁T₃. This result suggests that

exogenous application of GABA could help to increase the filled grain per panicle of Tulsimala variety of aromatic rice.

4.3.5 Unfilled grain panicle⁻¹ (no.)

Table 9 shows the different types of application rate and time of GABA had a significant effect on unfilled grain per panicle. The highest number of unfilled grains per panicle (11.27) was found in D₁T₁ treatment (Without GABA application+ At 30 DAT). The lowest number of unfilled grains per panicle (3.50) was found in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT).

4.3.6 Thousand grains weight (g)

Table 9 shows the effect of different treatment of GABA on 1000-grain weight. The maximum 1000-grain weight (12.37 g) was attained at D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT). The minimum 1000-grain weight (10.92 g) was achieved by treatment D₁T₂.

Table 10: Effect of GABA on the different yields and harvest index of aromatic rice (Tulsimala)

Dose	Grain Yield (t ha ⁻¹)	Straw Yield (t ha ⁻¹)	Biological Yield (t ha ⁻¹)	Harvest Index (%)
D ₁	1.98c	4.88b	6.86c	29.09c
D ₂	2.43b	5.24a	7.66b	31.69b
D ₃	2.76a	5.28a	8.04a	34.34a
D ₄	2.71a	5.23a	7.94a	34.20a
CV%	3.87	3.97	3.54	2.58
LSD _(0.05)	0.09	0.20	0.26	0.82
LS	**	**	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁= Without GABA application

D₂= 50 ppm of GABA

D₃= 100 ppm of GABA

D₄= 150 ppm of GABA

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

*= Significant at 5% level of significance

4.4 Effect of GABA dose on yield parameters

4.4.1 Grain yield (t ha^{-1})

The experimental results showed significant variation in grain yield by application of different GABA levels in Tulsimala aromatic rice (Table 10). Results showed that the highest grain yield (2.76 t ha^{-1}) was recorded from D₃ (100 ppm of GABA) followed by D₄ (150 ppm of GABA) where the lowest grain weight (1.99 t ha^{-1}) was achieved from D₁ (0 ppm of GABA). Li *et al.*, (2020) showed that exogenous GABA improved the 2AP content and regulated grain yield formation in different fragrant rice genotypes under different nitrogen levels.

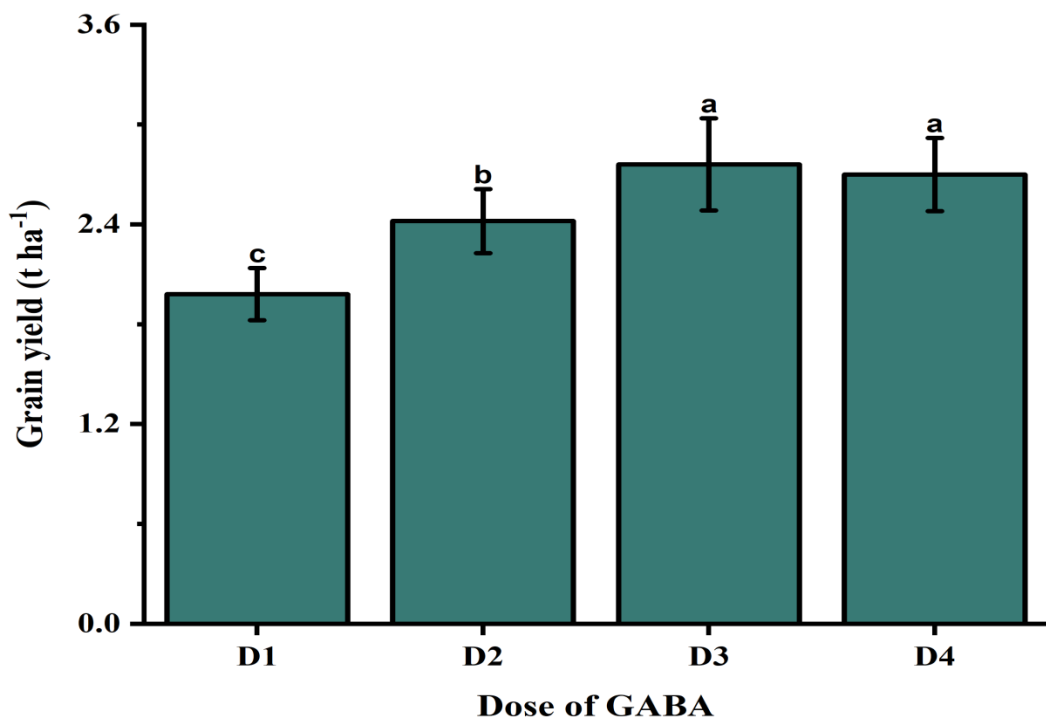


Figure 2. Effect of different doses of GABA on grain yield (t ha^{-1})

Here,

D₁= 0 ppm of GABA (control)

D₂= 50 ppm of GABA

D₃= 100 ppm of GABA

D₄= 150 ppm of GABA

The GABA dose has a significant impact on rice seed production (Figure 2). However, applying GABA @ 100 ppm (D₃) to rice plant resulted in the highest grain yield (2.76 t ha^{-1}) and was statistically similar to D₄ (150 ppm of GABA) treatment. On the other hand, the lowest grain yield (1.98 t ha^{-1}) was found when no GABA was used (D₁). The high yield of rice that GABA contributes together with the fact that yield varies at different doses, may be attributed to a combination of management techniques, genetic factors and environmental factors.

4.4.2 Straw yield (t ha⁻¹)

Table 10 showed that application of GABA in different level influenced the straw yield significantly per hectare of aromatic rice (Tulsimala). Result showed that the highest straw weight (5.28 t ha⁻¹) was recorded under D₃ (100 ppm of GABA) followed by D₂ and D₄ (50 ppm and 150 ppm of GABA respectively) while the lowest straw yield (4.87 t ha⁻¹) was achieved from D₁ treatment (0 ppm of GABA).

4.4.3 Biological yield (t ha⁻¹)

Application of GABA influenced significantly the biological yield per hectare of aromatic rice (Tulsimala) (table 10). Result showed that the highest biological yield (8.04 t ha⁻¹) was recorded under D₃ (100 ppm of GABA) followed by D₂ and D₄ (50 ppm and 150 ppm of GABA respectively) while the lowest biological yield (6.87 t ha⁻¹) was achieved from D₁ treatment (0 ppm of GABA). Xie *et al.*, (2020) showed that GABA application-induced improvements in yield and quality attributes of fragrant rice.

4.4.4 Harvest index (%)

From the table 10, it confirmed that application of GABA in different doses influenced the harvest index (%) of aromatic rice (Tulsimala) significantly. Result showed that the highest harvest index (34.34%) was recorded from D₃ (100 ppm of GABA) followed by D₄ (150 ppm of GABA) while the lowest harvest index (29.09 %) was achieved from D₁ treatment (0 ppm of GABA). Sepehri *et al.*, (2020) expressed that foliar application of GABA increased harvest index significantly.

Table 11: Effect of application time of GABA on the different yields and harvest index of aromatic rice (Tulsimala)

Time	Grain Yield (t ha ⁻¹)	Straw Yield (t ha ⁻¹)	Biological Yield (t ha ⁻¹)	Harvest Index (%)
T ₁	2.28c	5.03b	7.31b	31.20c
T ₂	2.63a	5.23a	7.86a	33.34a
T ₃	2.51b	5.21a	7.71a	32.46b
CV%	3.87	3.97	3.54	2.58
LSD _(0.05)	0.08	0.17	0.23	0.71
LS	**	*	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

T₁= Application time of GABA at 30 DAT

T₂= Application time of GABA at 60 DAT

T₃= Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

*= Significant at 5% level of significance

4.5 Effect of application time of GABA on yield parameters

4.5.1 Grain yield (t ha⁻¹)

The experimental results showed significant variation in grain yield by different application time of GABA in Tulsimala aromatic rice (Table 11). Results exposed that the highest grain yield (2.63 t ha⁻¹) was recorded from T₂ (Application time of GABA at 60 DAT) followed by T₃ (Application time of GABA at 90 DAT) where the lowest grain weight (2.28 t ha⁻¹) was achieved from T₁ (Application time of GABA at 30 DAT).

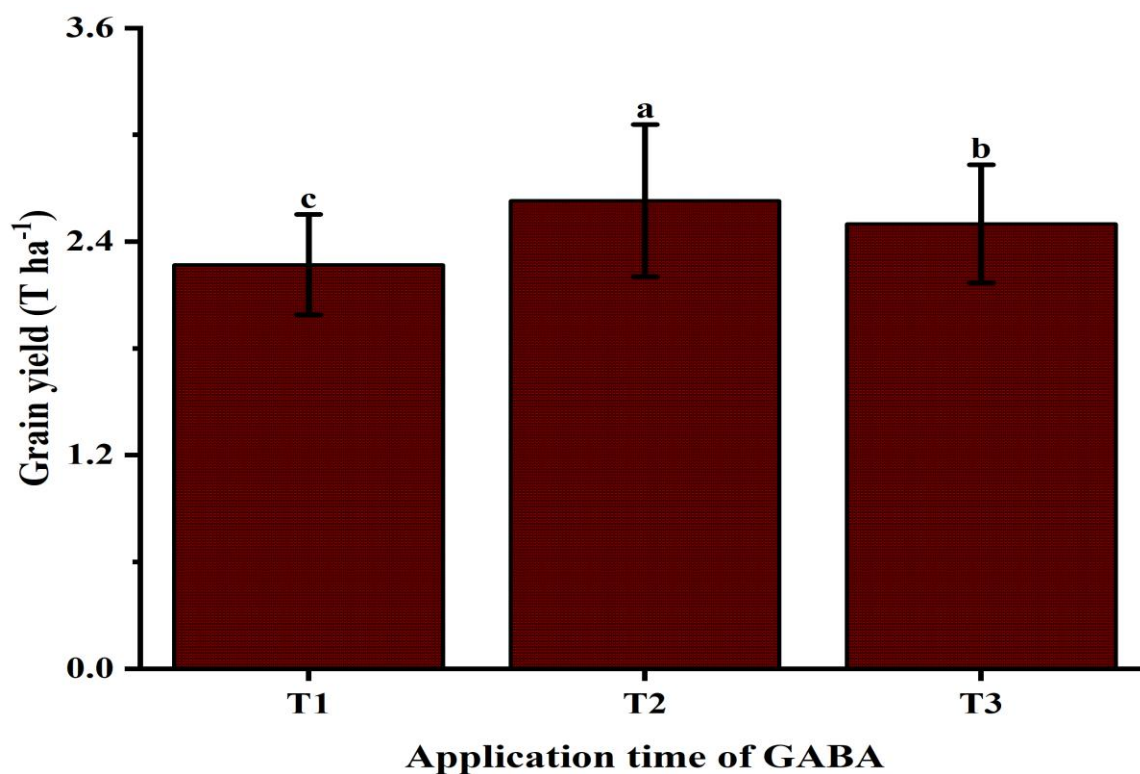


Figure 3. Effect of different application time of GABA on grain yield (t ha⁻¹)

Here,

T₁= Tillering stage (at 30 DAT)

T₂= Panicle initiation stage (at 60 DAT)

T₃= Booting stage (at 90 DAT)

Rice grain yield varied significantly depending on the time when GABA was applied (Figure 3). The results showed that T₁ had the lowest grain yield (2.27 t ha⁻¹) (30 DAT) and it was identical to T₃ (Booting stage (at 90 DAT)) that T₂ (Panicle initiation stage (at 60 DAT)) had the highest grain yield (2.63 t ha⁻¹), which was statistically significant.

4.5.2 Straw yield (t ha⁻¹)

Table 11 showed that different application time of GABA influenced the straw yield significantly per hectare of aromatic rice (Tulsimala). Result showed that the highest straw weight (5.23 t ha⁻¹) was recorded under T₂ (Application time of GABA at 60 DAT) followed by T₃ (Application time of GABA at 90 DAT) while the lowest straw yield (5.03 t ha⁻¹) was achieved from T₁ treatment (Application time of GABA at 30 DAT).

4.5.3 Biological yield (t ha⁻¹)

Application of GABA at different stages of growth and development influenced significantly the biological yield per hectare of aromatic rice (Tulsimala) (table 11). Result showed that the

highest biological yield (7.86 t ha^{-1}) was recorded under T_2 (Application time of GABA at 60 DAT) followed by T_3 (Application time of GABA at 90 DAT) while the lowest biological yield (7.31 t ha^{-1}) was achieved from T_1 treatment (Application time of GABA at 30 DAT).

4.5.4 Harvest index (%)

From table 11 it confirmed that application of GABA in different time influenced the harvest index (%) of aromatic rice (Tulsimala) significantly. Result showed that the highest harvest index (33.34%) was recorded from T_2 (Application time of GABA at 60 DAT) while the lowest harvest index (31.20 %) was achieved from T_1 treatment (Application time of GABA at 30 DAT).

Table 12: Interaction effect of application time and rate of GABA on the different yields and harvest index of aromatic rice (Tulsimala)

Interaction of Dose and Time		Grain Yield (t ha ⁻¹)	Straw Yield (t ha ⁻¹)	Biological Yield (t ha ⁻¹)	Harvest Index (%)
D ₁	T ₁	1.92e	4.91c	6.84f	28.17g
	T ₂	2.02e	4.85c	6.87f	29.53fg
	T ₃	2.03e	4.86c	6.89ef	29.58f
D ₂	T ₁	2.24d	5.10bc	7.34de	30.59ef
	T ₂	2.57c	5.30ab	7.87bc	32.73d
	T ₃	2.46c	5.32ab	7.78bcd	31.76de
D ₃	T ₁	2.47c	5.04bc	7.50cd	33.01cd
	T ₂	3.04a	5.45a	8.49a	35.83a
	T ₃	2.78b	5.35ab	8.13ab	34.18bc
D ₄	T ₁	2.49c	5.07bc	7.55cd	33.03cd
	T ₂	2.89ab	5.32ab	8.21ab	35.28ab
	T ₃	2.75b	5.29ab	8.05ab	34.29bc
CV%		3.87	3.97	3.54	2.58
LSD _(0.05)		0.16	0.34	0.46	1.41
LS		**	*	**	**

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

D₁T₁= Without GABA application+ At 30 DAT

D₁T₂= Without GABA application+ At 60 DAT

D₁T₃= Without GABA application+ At 90 DAT

D₂T₁= 50 ppm of GABA+ Application time of GABA at 30 DAT

D₂T₂= 50 ppm of GABA+ Application time of GABA at 60 DAT

D₂T₃= 50 ppm of GABA+ Application time of GABA at 90 DAT

D₃T₁= 100 ppm of GABA+ Application time of GABA at 30 DAT

D₃T₂= 100 ppm of GABA+ Application time of GABA at 60 DAT

D₃T₃= 100 ppm of GABA+ Application time of GABA at 90 DAT

D₄T₁= 150 ppm of GABA+ Application time of GABA at 30 DAT

D₄T₂= 150 ppm of GABA+ Application time of GABA at 60 DAT

D₄T₃= 150 ppm of GABA+ Application time of GABA at 90 DAT

LS= Level of significance

CV= Co-efficient of variance

LSD= Least Significant Difference

** = Significant at 1% level of significance

*= Significant at 5% level of significance

4.6 Effect of interaction of GABA dose and application time on yield parameters

4.6.1 Grain yield ($t\ ha^{-1}$)

The combined effect of application time and rate of GABA treatment influenced significantly the grain yield ha^{-1} of Tulsimala aromatic rice (Table 12). Result showed that the highest grain yield ($3.04\ t\ ha^{-1}$) was recorded from D_3T_2 (100 ppm of GABA+ Application time of GABA at 60 DAT) while the lowest grain weight ($1.92\ t\ ha^{-1}$) was achieved by D_1T_1 (Without GABA application+ At 30 DAT). Malekzadeh *et al.*, (2014) observed that application of GABA with proper timing of application significantly increased grain yield ($t\ ha^{-1}$) in fragrant rice.

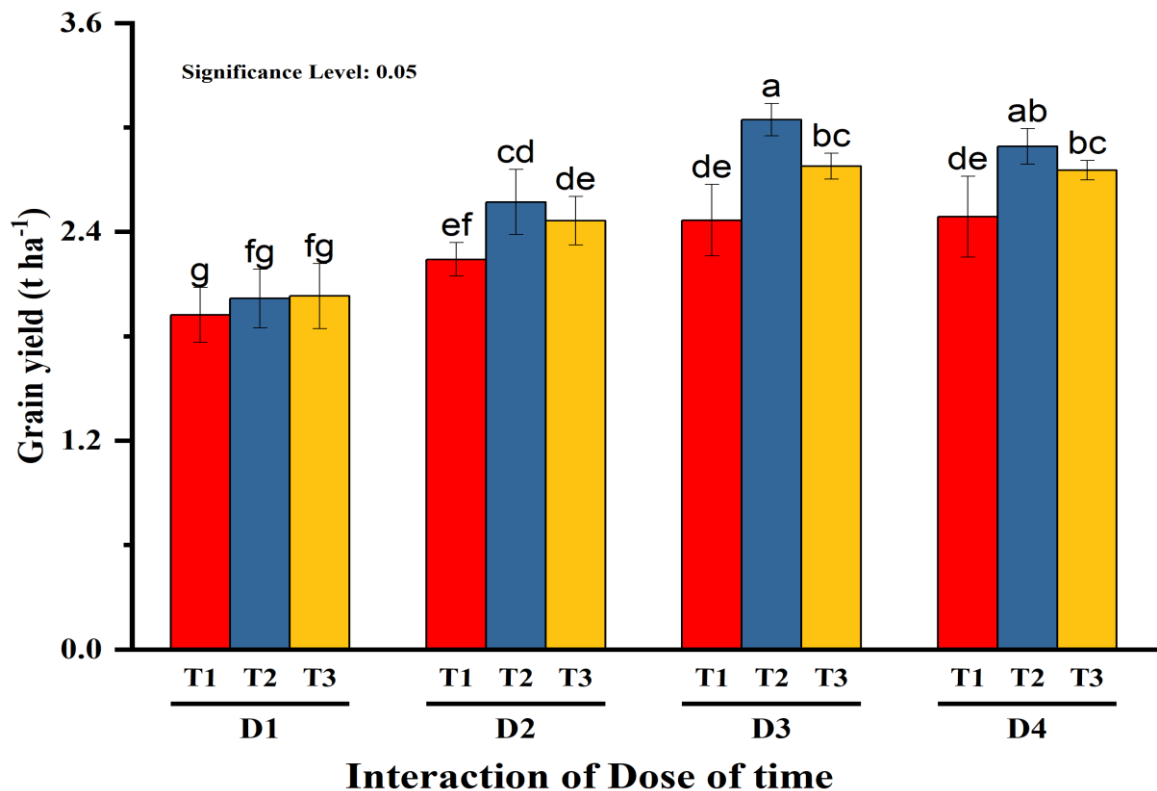


Figure 4. Interaction effect of different dose and application time of GABA on grain yield ($t\ ha^{-1}$)

Here,

D_1T_1 = Without GABA application+ At 30 DAT

D_1T_2 = Without GABA application+ At 60 DAT

D_1T_3 = Without GABA application+ At 90 DAT

D_2T_1 = 50 ppm of GABA+ Application time of GABA at 30 DAT

D_2T_2 = 50 ppm of GABA+ Application time of GABA at 60 DAT

D_2T_3 = 50 ppm of GABA+ Application time of GABA at 90 DAT

D_3T_1 = 100 ppm of GABA+ Application time of GABA at 30 DAT

D_3T_2 = 100 ppm of GABA+ Application time of GABA at 60 DAT

D_3T_3 = 100 ppm of GABA+ Application time of GABA at 90 DAT

D_4T_1 = 150 ppm of GABA+ Application time of GABA at 30 DAT

D_4T_2 = 150 ppm of GABA+ Application time of GABA at 60 DAT

D_4T_3 = 150 ppm of GABA+ Application time of GABA at 90 DAT

An interaction between dose and different application time of GABA was observed in respect of grain yield of rice (Figure 4). The maximum grain yield (3.04 t ha^{-1}) was found from D_3T_2 (100 ppm of GABA+ Application time of GABA at 60 DAT) treatment combinations. Again the minimum grain yield (1.92 t ha^{-1}) was observed from D_1T_1 [(0 ppm of GABA (control) At 30 DAT)] which was statistically different from other treatment combinations.

4.6.2 Straw yield (t ha^{-1})

Application time and levels of GABA interaction influenced significantly the straw yield per hectare of aromatic rice (Tulsimala) (Table 12). Result revealed the highest straw yield (5.45 t ha^{-1}) was recorded from D_3T_2 (100 ppm of GABA+ Application time of GABA at 60 DAT) followed by D_3T_3 (100 ppm of GABA+ Application time of GABA at 90 DAT) and D_4T_2 (150 ppm of GABA+ Application time of GABA at 60 DAT) while the lowest straw yield (4.85 t ha^{-1}) was achieved from D_1T_2 treatment (Without GABA application+ At 60 DAT).

4.6.3 Biological yield (t ha^{-1})

From table 12 it recited that combination of application time and levels of GABA treatment influenced significantly the biological yield per hectare of aromatic rice (Tulsimala). Biological yield varied according to the treatment combination. Result showed the highest biological yield (8.49 t ha^{-1}) was recorded from D_3T_2 (100 ppm of GABA+ Application time of GABA at 60 DAT) where the lowest biological yield (6.83 t ha^{-1}) was achieved by D_1T_1 treatment (Without GABA application+ At 30 DAT).

4.6.4 Harvest index (%)

The interaction effect of GABA rate and application time influenced the harvest index (%) of aromatic rice (Tulsimala) significantly (Table 12). Result revealed that the highest harvest index (35.83%) was recorded from D_3T_2 (100 ppm of GABA+ Application time of GABA at 60 DAT) while the lowest harvest index (28.17%) was achieved from D_1T_1 treatment (Without GABA application+ At 30 DAT).

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in an ideal type of rice growing soil on southwest corner of research field of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from July, 2023 to December, 2023. The experiment was laid out into Randomized Complete Block Design (RCBD) with three replications. Two factors were taken in this study *viz.* factor A: D₁= Without GABA application, D₂= 50 ppm of GABA, D₃= 100 ppm of GABA and D₄= 150 ppm of GABA; Factor B: T₁= Application time of GABA at 30 DAT, T₂= at 60 DAT and T₃= at 90 DAT. Data were recorded on different growth and yield contributing parameters. Nearly all parameters are showed significant in this study.

In the case of dose treatment, significant variation was found for the parameters. Results indicated that the highest number of effective tillers hill⁻¹ (18.64), highest panicle length (26.43 cm), highest number of filled grain panicle⁻¹ (218.22) were found in D₃ (100 ppm of GABA) and highest number of non- effective tillers hill⁻¹ (5.22), highest number of unfilled grain per panicle (10.16) was found in D₁ treatment (without application of GABA). Again thousand grain weight (11.91 g), grain yield (2.76 t ha⁻¹), straw yield (5.28 t ha⁻¹), biological yield (8.04 t ha⁻¹), harvest index (34.34%) were found in D₃ (100 ppm of GABA). On the other hand, the lowest number of effective tillers hill⁻¹ (11.18), lowest panicle length (24.11 cm), lowest number of filled grain per panicle (189.06) were found in D₁ treatment (0 ppm of GABA) and lowest number of non-effective tillers per hill⁻¹ (1.47) was found in D₂ treatment (50 ppm of GABA), lowest number of unfilled grain per panicle (4.83) was found in D₃ treatment (100 ppm of GABA). Again lowest thousand grain weight (11.08 g), grain yield (1.99 t ha⁻¹), straw yield (4.87 t ha⁻¹), biological yield (6.87 t ha⁻¹), harvest index (29.09 %) were achieved from D₁ treatment (without application of GABA).

On the other hand, in case of application time of GABA to aromatic rice variety Tulsimala, significant influence was observed according to treatment. Results showed that the highest number of effective tillers hill⁻¹ (17.14), highest panicle length (25.91 cm), highest number of filled grain panicle⁻¹ (212.58) were found in T₂ treatment (application of GABA at 60 DAT) and highest number of non- effective tillers hill⁻¹ (3.31), highest number of unfilled grain per panicle (8.63) was found in T₁ treatment (application of GABA at 30 DAT). Again thousand grain weight (11.79 g), grain yield (2.76 t ha⁻¹), straw yield (5.23 t ha⁻¹), biological yield

(7.86 t ha⁻¹), harvest index (33.34%) were found in T₂ treatment (application of GABA at 60 DAT). On the other hand, the lowest number of effective tillers hill⁻¹ (14.93), lowest panicle length (25.07 cm), lowest number of filled grain per panicle (195.02) were found in T₁ treatment (application of GABA at 30 DAT) and lowest number of non-effective tillers per hill⁻¹ (1.95), lowest number of unfilled grain per panicle (5.53) were found in T₂ treatment (application of GABA at 60 DAT). Again lowest thousand grain weight (11.21 g), grain yield (2.28 t ha⁻¹), straw yield (5.03 t ha⁻¹), biological yield (7.31 t ha⁻¹), harvest index (31.20 %) were recorded from T₁ treatment (application of GABA at 30 DAT).

In case of combined effect of application dose and time of GABA, the highest number of effective tillers hill⁻¹ (20.03), highest panicle length (27.17 cm), highest number of filled grain panicle⁻¹ (231.00) were found in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT) and highest number of non-effective tillers hill⁻¹ (5.33), highest number of unfilled grain per panicle (11.27) were found in D₁T₁ treatment (Without GABA application+ At 30 DAT). Again 1000-grain weight (12.37 g), grain yield (3.04 t ha⁻¹), straw yield (5.45 t ha⁻¹), biological yield (8.49 t ha⁻¹), harvest index (35.83%) was recorded from D₃T₂ (100 ppm of GABA+ Application time of GABA at 60 DAT). On the other hand, the lowest number of effective tillers hill⁻¹ (10.77), lowest panicle length (23.97 cm), lowest number of filled grain per panicle (186.93) were found in D₁T₁ treatment (Without GABA application+ At 30 DAT) and lowest number of non-effective tillers per hill⁻¹ (0.77) was found in D₂T₂ treatment (50 ppm of GABA+ Application time of GABA at 60 DAT), lowest number of unfilled grain per panicle (3.50) was found in D₃T₂ treatment (100 ppm of GABA+ Application time of GABA at 60 DAT). Again lowest thousand grain weight (10.92 g) was achieved by treatment D₁T₂ (Without GABA application+ At 60 DAT), lowest grain yield (1.92 t ha⁻¹) was found by D₁T₁ (Without GABA application+ At 30 DAT), lowest straw yield (4.85 t ha⁻¹) was achieved from D₁T₂ treatment (Without GABA application+ At 60 DAT), lowest biological yield (6.83 t ha⁻¹), and finally lowest harvest index (28.17 %) were achieved from D₁T₁ treatment (Without GABA application+ At 30 DAT).

The present study was conducted to find out whether the application of GABA on aromatic rice has any significant effect or not. Our results indicated beneficial effects of GABA on aromatic rice production. Also, application time of GABA has a profound effect too. Application of GABA has a significant effect on effective tillers hill⁻¹, 1000- grain weight, filled grain panicle⁻¹, grain yield, straw yield & biological yield.

From the above findings, it can be concluded that the highest performance regarding yield and yield contributing parameters the effect of D₃T₂ (100 ppm of GABA+ Application time of GABA at 60 DAT) gave the highest grain yield. So, this treatment combination can be treated as the best compared to others.

Recommendations:

From the above experimental findings, it is apparent that 100 ppm of GABA treatment at 60 DAT (Panicle initiation stage) performed better on yield and yield parameters of aromatic rice cv. Tulsimala.

In order to recommend the practices for the aromatic rice growers, the following aspects would be considered in future:

- i. Similar experiments need to be conducted in different location and season of Bangladesh to draw a final conclusion regarding the effect of GABA combined with application time on aromatic rice.
- ii. Such study is needed in different Agro-Ecological Zones (AEZ) of Bangladesh for regional adaptability and other performance.
- iii. Varietal trials need to be investigated.

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APPENDICES

Appendix I: Mean temperature, rainfall and humidity during research period (June 2023-November 2023)

Months	Temperature (°c)	Rainfall (mm)	Humidity (%)
June	29	361.9	83
July	29	425.2	84
August	29	372.9	84
September	29	440.0	85
October	26	122.7	82
November	23	9.2	76

Appendix II: Fertilizer used in research, their doses and percentage of active ingredient

Fertilizer	Doses (kg ha ⁻¹)	Active ingredient (%)
Urea	260	46 (N)
TSP	75	44-46 (P ₂ O ₅)
MOP	90	60 (K ₂ O)
Gypsum	4	18 (S)

Appendix III: Morphological, physical and chemical characteristics of soil of experimental field

A. Morphological characteristics of soil

Morphological parameters	characteristics
Location	Agronomy research field, HSTU, Dinajpur
Agro Ecological Zone (AEZ-1)	Old Himalayan Piedmont Plain
Geographical position	25°38 N latitude and 88°41 E lonitudes
General soil types	Non-calcareous dark gray floodplain soil
Parent materials	Old Brahmaputrs River borne Deposit
Land type	Medium high land
Elevation	37 meter above the mean sea level
Drainage	Well drained
Cropping Pattern	Rice crop grown year round
Topography	Fairly leveled

B. Physical characteristics of soil

Characteristics	Value (%)
Sand	58
Silt	28
Clay	14
Soil textural class	Sandy loam

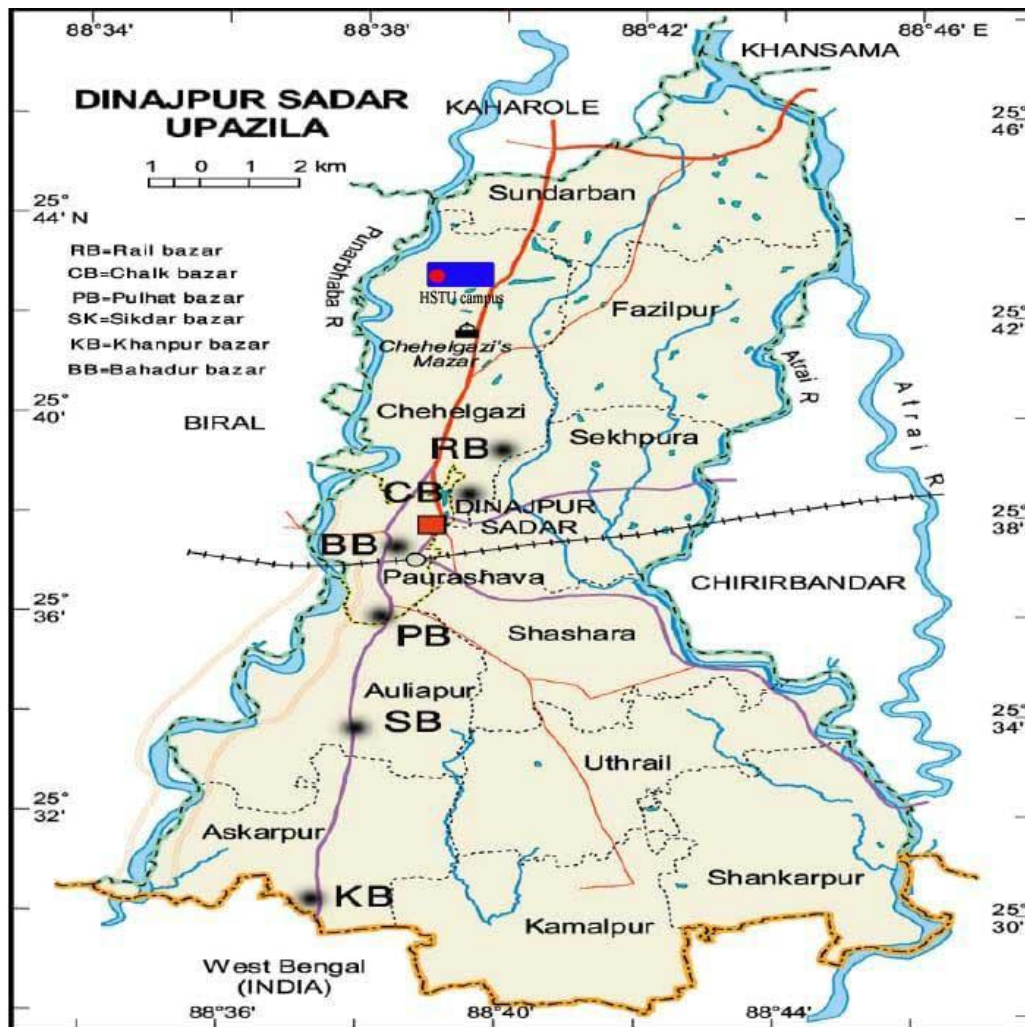
C. Chemical properties of soil

Characteristics	Value (%)
pH (soil : water = 1: 1.25)	5.41
Organic matter	1.48
Organic Carbon	0.72
Total N	0.08
Available P (ppm)	11.20
Exchangeable P (meq)	0.10
Exchangeable Ca (meq)	2.48
Exchangeable Mg (meq)	2.29
Available S (ppm)	17.29
Available B (ppm)	0.13
Available Zn (ppm)	0.90
Available Fe (ppm)	51.90
Available Mn (ppm)	12.13

Appendix IV: Properties of GABA

Characteristics	Unit
Formula	$C_4H_9NO_2$
Boiling point	247.9 °C (478.2 °F; 521.0 K)
Molar Mass	103.12 g/mol
Density	1.11 g/mL
Appearance	White microcrystalline powder
Solubility	Freely soluble in Water; Insoluble in Acetor Slightly soluble in Alcohol

Appendix V: Location of the experimental site



Appendix VI: Analysis of variance (mean square) of the data for plant height of aromatic rice

Source of variation	Degrees of freedom	Mean Squares			
		30 DAT	60 DAT	90 DAT	At harvest
Factor A	3	3.8867*	123.211**	385.524**	295.230**
Factor B	2	8.4843*	435.712**	693.240**	166.785**
Factor A×B	6	6.1490*	32.426**	90.523**	22.702**
Error	22	11.4842	7.867	5.228	5.625
CV (%)		8.92	3.39	2.00	1.64

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

**= Significant at 1% level of significance

*= Significant at 5% level of significance

CV= Co-efficient of variance

df= Degrees of freedom

Factor A= GABA dose

Factor B= Application time

Factor AB= GABA dose and Application time

Appendix VII: Analysis of variance (mean square) of the data for total tillers of aromatic rice

Source of variation	Degrees of freedom	Mean Squares			
		30 DAT	60 DAT	90 DAT	At harvest
Factor A	3	0.046*	1.67**	35.23**	29.69**
Factor B	2	0.018*	8.99**	23.61**	2.92**
Factor A×B	6	0.013*	0.45**	1.91**	0.34**
Error	22	0.05	1.03	1.04	1.17
CV (%)		2.51	7.23	5.51	5.76

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

**= Significant at 1% level of significance

*= Significant at 5% level of significance

CV= Co-efficient of variance

df= Degrees of freedom,

Factor A= GABA dose

Factor B= Application time

Factor AB= GABA dose and Application time

Appendix VIII: Analysis of variance (mean square) of the data for yield and other crop characters of aromatic rice

Source of variation	Degrees of freedom	Mean Squares			
		Effective Tillers (no.)	Non-effective Tillers (no.)	Panicle Length (cm)	Grains per Panicle (no.)
Factor A	3	103.15**	26.86**	10.98**	1315.31**
Factor B	2	15.24**	5.62**	2.13**	1015.93**
Factor A×B	6	0.97**	0.46**	0.19**	128.61**
Error	22	2.13	0.57	0.33	43.84
CV (%)		9.03	28.35	2.26	3.22

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

**= Significant at 1% level of significance

*= Significant at 5% level of significance

CV= Co-efficient of variance,

df= Degrees of freedom,

Factor A= GABA dose,

Factor B= Application time,

Factor AB= GABA dose and Application time

Appendix IX: Analysis of variance (mean square) of the data for yield and other crop characters of aromatic rice

Source of variation	Degrees of freedom	Mean Squares					
		Sterile Grains/Panicle (no.)	1000-Grain Weight (g)	Grain Yield (t ha ⁻¹)	Straw Yield (t ha ⁻¹)	Biological Yield (t ha ⁻¹)	Harvest Index (%)
Factor A	3	50.36**	1.45**	1.13**	0.32**	2.55**	55.28**
Factor B	2	28.83**	1.15**	0.38**	0.14*	0.98**	13.89**
Factor A×B	6	0.59*	0.23**	0.03**	0.03*	0.12**	0.43**
Error	22	1.66	0.14	0.01	0.04	0.07	0.69
CV (%)		18.16	3.28	3.87	3.97	3.54	2.58

In a column, figure having common letter(s) bearing same do not differ significantly at 5% level of significance by Duncan's Multiple Range Test.

Legend:

**= Significant at 1% level of significance

*= Significant at 5% level of significance

CV= Co-efficient of variance

df= Degrees of freedom

Factor A= GABA dose

Factor B= Application time

Factor AB= GABA dose and Application time

Appendix X: Some photos of my research work

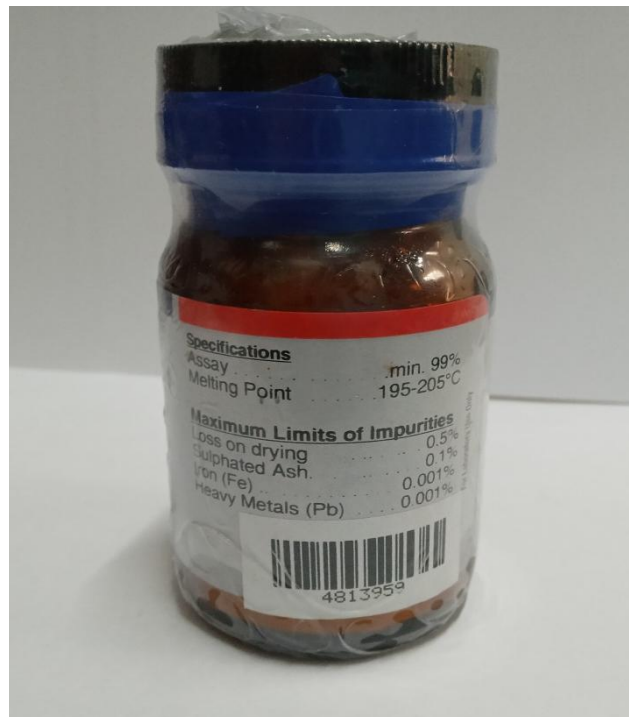
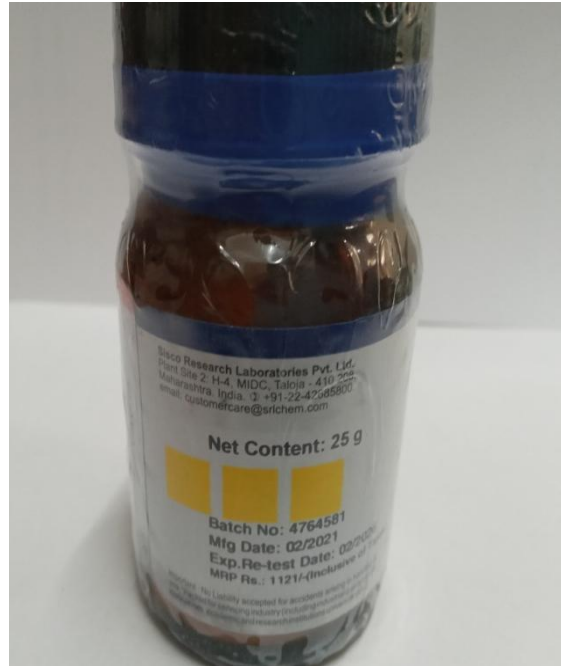
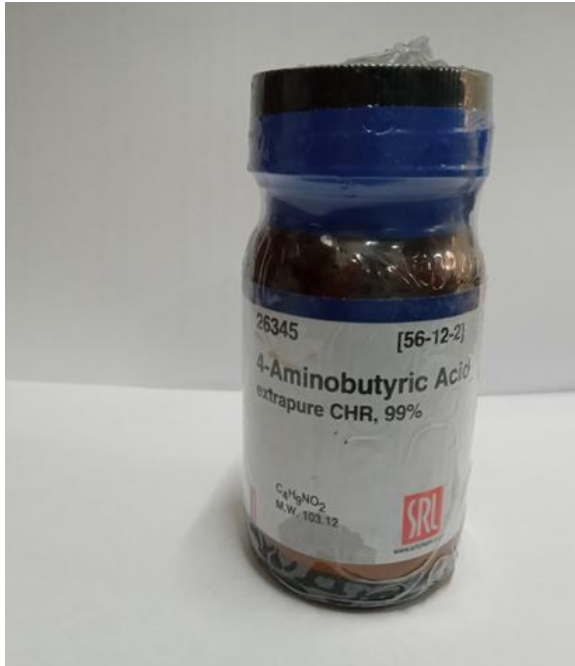


Figure: 4-Aminobutyric Acid



Figure: Thirty-six research plots were divided into three rows



Figure: Well growth and development of rice seeds



Figure: Development of maturing of rice seeds



Figure: Maturity of rice fields



Figure: Time of taking different types of data after harvesting



Figure: Packets of thousand seeds of thirty-six plots