



Evaluation of Nitrogen Uptake, Growth, and Yield of Rice Affected by Green Manure and Chemical Nitrogen Fertilizer

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Authors' contributions

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

Article Information

DOI: <https://doi.org/10.9734/ajsspn/2024/v10i3318>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/117462>

Original Research Article

Received: 27/03/2024

Accepted: 03/06/2024

Published: 06/06/2024

ABSTRACT

To investigate the combined effect of green manures (GMs) and chemical nitrogen (N) fertilizer on N uptake, growth, and yield of rice, a pot experiment was conducted at the Department of Agronomy, Yezin Agricultural University (YAU). The experiment was conducted from December to June (the summer season) of 2023. The study used a split-plot design with three replications.

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Cite as: Hlaing, Thidar, Kyi Moe, Ei Han Kyaw, Kyaw Ngwe, Myat Moe Hlaing, and Htay Htay Oo. 2024. "Evaluation of Nitrogen Uptake, Growth, and Yield of Rice Affected by Green Manure and Chemical Nitrogen Fertilizer". *Asian Journal of Soil Science and Plant Nutrition* 10 (3):37-56. <https://doi.org/10.9734/ajsspn/2024/v10i3318>.

Chemical N fertilizer was assigned as the main plot factor and different levels of N0%, N25%, N50%, N75%, and N100% were applied based on the N recommended rate (102 kg ha⁻¹) (1.55 g pot⁻¹) of the Sinn-thu-kha rice variety. GM crops were the subplot factor, including no GM (G0), rice bean (GRB), sunn hemp (GSH), and dahincha (GDC). According to the results, N75 and N100 produced the higher dry matter, N uptake, yield, and yield components of rice. The highest growth characters, N uptake, and yield were found in N100 for N levels, GSH for GM levels, and N100GSH as the combined effect. However, similar values were found in the treatment N75GSH. Statistically, the GSH + chemical N treatment produced higher N uptake and yields than those of GRB or GDC + chemical N treatment. When chemical N fertilizers were applied without the use of GMs, the rice yields were lower in comparison to the treatments GMs + N25 or N50 or N75 or N100. In conclusion, combining GSH with either 75% or 100% chemical N resulted in optimal performance for soil nutrient content, growth, N uptake, and rice yield. Additionally, it was observed that the use of chemical N fertilizers was reduced by 25%, which can help mitigate environmental pollution and improve soil health and rice production in the long run.

Keywords: Chemical N fertilizer; green manure; N uptake; plant growth; soil properties; and yield.

1. INTRODUCTION

Rice is a crucial crop for half of the world's population, providing around 35-60% of dietary calories for over three billion individuals worldwide [1]. As the global population continues to grow, farmers need to produce better-quality rice to meet the increasing demands of consumers [2]. However, the excessive use of nitrogen (N) fertilizers in modern rice farming practices can reduce yield and soil fertility [3]. The overuse of chemical fertilizers, particularly inorganic N fertilizers, can result in soil acidification and structural damage, water depletion, and changes in the soil microbe population and their activities [4]. In modern rice cropping systems, unbalanced N fertilizer usage leads to low productivity [5,6] and soil fertility.

Farmers need to maintain high productivity levels and find alternative methods such as animal manure, green manure (GM), compost, vermicompost, and biofertilizers. GM is an excellent option because it's easy to cultivate, and its biomass can be incorporated into the soil. It is a specific species of plant, typically a legume, which can be a tree, a bush, a vine, a crawling plant, or algae. Farmers grow them to maintain or improve soil fertility or control weeds [7]. The residues of GM are rich in N, which can be supplied to the succeeding crops. GM with nitrogen-fixing legume crops can provide a substantial portion of the N requirement for rice and also add organic matter to maintain soil fertility [8].

It is widely recognized that GM residues can improve the chemical and biological properties of soil. However, it is necessary to explore their

effects on the soil properties and growth and yield of rice, as the N effect of GM varies depending on the legume species, agricultural management, and site. Leguminous crops such as pigeon peas, green gram, soybean, or groundnut can be used as GMs. Additionally, perennial woody multipurpose legume trees such as *Leucaena leucocephala* (Lam.) de Wit, *Gliricidia sepium* Kunth., *Cassia siamea* Lam., or non-grain legumes like sunn hemp (*Crotalaria* spp), dhaincha (*Sesbania* spp), Centrosema, Stylosanthes, and Desmodium can also be utilized [9]. In agriculture, legume crops are commonly used to increase the nitrogen content in the soil [10]. This is because legumes have the potential to fix atmospheric nitrogen [11] through a symbiotic relationship with Rhizobium bacteria [12], as well as associations with cyanobacteria [13] and non-symbiotic associations with free-living diazotrophic bacteria that interact with the plant roots [14]. The use of sunn hemp as green manure has been found to result in higher nitrogen use efficiency (NUE), as well as improved growth and yield of rice compared to using green manure with millet [15,16].

In our research, we examined three types of GM crops that have been shown to have a higher rate of N mineralization and a higher biomass in the previous study conducted by [17]. They are rice bean (GRB), sunn hemp (GSH), and dahincha (GDC). The fertility of soil in Myanmar has been decreasing, leading to a decline in rice production. GM crops can help restore soil properties, maintain organic matter, reclaim degraded soils, and provide plant nutrients [18]. They can also reduce the need for excessive chemical N fertilizer application [19] and improve rice production [20,21]. However, GM plants

alone may not provide sufficient nutrients to rice crops. When GM is combined with chemical N fertilizer, the grain yield is equal to or higher than that of conventional cultivation with chemical fertilizer [22]. Incorporating GM has been shown to improve soil organic matter in rice fields by 0.1–0.9%, according to [23]. Using chemical N fertilizer and GM, rice plant growth and yield can be improved [24,25,26]. This study aims to investigate the impact of different GM crops, including rice bean, sunn hemp, and dhaincha, on soil properties, N uptake, growth, and rice yield. It also explores the optimal dosage of chemical N fertilizer when combined with suitable GM crops to achieve sustainable soil properties and rice yields.

2. MATERIALS AND METHODS

2.1 Experimental Site, Design and Treatments

A pot experiment was carried out at the Department of Agronomy, Yezin Agricultural University (YAU) in Nay Pyi Taw, Myanmar, from April to October 2023 during the summer season. The experiment used a split-plot design with three replications under natural conditions. The

main plot factor involved four different percentages of the recommended chemical N fertilizer rate, while the subplot factor involved the cultivation of three GM varieties: rice bean (GRB), sunn hemp (GSH), and dhaincha (GDC). A control treatment was included for the two factors where no chemical N fertilizer was applied and no GM crops were grown. The recommended chemical N fertilizer rate for the tested rice variety (Sinn-thu-kha), a Myanmar rice variety, was 102 kg N ha⁻¹, 27 kg P₂O₅ ha⁻¹, and 117 kg K₂O ha⁻¹ [27]. In this pot experiment, the application of N, P₂O₅, and K₂O (g pot⁻¹) and the weight of GMs (g pot⁻¹) were shown in Table 1.

2.2 Soil Sampling and Preparation of Pot

A composite surface soil sample (0-20 cm) was collected from 10 points in the rice field. The collected soil samples were processed by bulking, drying at room temperature, removing rocks and coarse organic materials, and grinding to pass through a 2-cm sieve. Approximately 17 kg of soil was then added to each plastic pot. The size of a pot was 1 foot in width and 1 foot in height (0.28 m²). The soil in each pot was moistened with water for two days before planting the seeds.

Table 1. Treatments and application of chemical N fertilizer and green manures

| Treatments | Recommended rate (g pot ⁻¹) of chemical fertilizer | | | Applied green manure (g pot ⁻¹) | |
|------------|--|-------------------------------|------------------|---|------------|
| | N | P ₂ O ₅ | K ₂ O | Fresh weight | Dry weight |
| N0G0 | 0.0 | 0.42 | 1.37 | 0.0 | 0.0 |
| N0GRB | 0.0 | 0.42 | 1.37 | 29.0 | 11.0 |
| N0GSH | 0.0 | 0.42 | 1.37 | 30.0 | 14.0 |
| N0GDC | 0.0 | 0.42 | 1.37 | 27.0 | 6.0 |
| N25G0 | 0.39 | 0.42 | 1.37 | 0.0 | 0.0 |
| N25GRB | 0.39 | 0.42 | 1.37 | 29.0 | 11.0 |
| N25GSH | 0.39 | 0.42 | 1.37 | 30.0 | 14.0 |
| N25GDC | 0.39 | 0.42 | 1.37 | 27.0 | 6.0 |
| N50G0 | 0.78 | 0.42 | 1.37 | 0.0 | 0.0 |
| N50GRB | 0.78 | 0.42 | 1.37 | 29.0 | 11.0 |
| N50GSH | 0.78 | 0.42 | 1.37 | 30.0 | 14.0 |
| N50GDC | 0.78 | 0.42 | 1.37 | 27.0 | 6.0 |
| N75G0 | 1.16 | 0.42 | 1.37 | 0.0 | 0.0 |
| N75GRB | 1.16 | 0.42 | 1.37 | 29.0 | 11.0 |
| N75GSH | 1.16 | 0.42 | 1.37 | 30.0 | 14.0 |
| N75GDC | 1.16 | 0.42 | 1.37 | 27.0 | 6.0 |
| N100G0 | 1.55 | 0.42 | 1.37 | 0.0 | 0.0 |
| N100GRB | 1.55 | 0.42 | 1.37 | 29.0 | 11.0 |
| N100GSH | 1.55 | 0.42 | 1.37 | 30.0 | 14.0 |
| N100GDC | 1.55 | 0.42 | 1.37 | 27.0 | 6.0 |

N0= N omission, N25 = 25% of chemical N rate, N50 = 50% of chemical N rate, N75 = 75% of chemical N rate, N100 = 100% of chemical N rate, G0= no GM, GRB = rice bean, GSH = sunn hemp, GDC = dhaincha

2.3 Analysis of Experimental Soil

Soil samples collected were sub-sampled, mixed, and filtered through a 2-mm sieve for analysis. The soil's physicochemical properties were measured before and after the experiment. Soil bulk density, porosity, and electrical conductivity were analyzed using core sampling and ECe method. Sand, silt, clay, and texture classes were analyzed using the international pipette method. Soil pH was analyzed using a glass electrode [28], and organic carbon by wet digestion [29]. Available N was analyzed by extraction and distillation [30], available P by Olsen bicarbonate method [31], available K by ammonium acetate extraction method [32], and cation exchange capacity (CEC) by leaching method [33].

2.4 Analysis and Application of Green Manures

Seeds of three GM plants (1 g pot⁻¹) were sown in the individual treatment pots, while the pot of G0 (control) was left unsown. No chemical fertilizers were used during cultivation to obtain the actual N content of the GM plants and their growth behavior. Necessary tasks such as watering, weeding, and earthing up were performed to ensure optimal plant growth.

During the flowering stage (50 days after planting), three GM crops (GRB, GSH, and GDC) were uprooted, and their plant parts were cut down to equal size (1 inch) using a knife. The fresh and dry weight of the plant parts was measured immediately and incorporated into the soil for rice cultivation. A small amount of plant parts (2 g) was used to measure the chemical composition of each GM. N was measured using the Kjeldahl distillation method, and carbon (C) by a CHN coder (MT-5, Yanaco). To measure the amount of P₂O₅ and K₂O, the samples were digested separately using the Molybdivanado phosphoric acid and Wet digestion with HNO₃ methods, respectively [34].

2.5 Application of Chemical Fertilizers

In this study, a chemical N fertilizer with an equivalent rate of 102 kg ha⁻¹ (1.55 g pot⁻¹) was applied to pots labeled as N100% in the form of urea. The control pots (N0) did not receive any N fertilizers, but all pots were given 27 kg P₂O₅ ha⁻¹ (0.42 g pot⁻¹) (as triple superphosphate) and 117 kg K₂O ha⁻¹ (1.37 g pot⁻¹) (as muriate of potash). The application of urea and muriate of potash

was done in three doses: 60% was incorporated into the soil one day before transplanting, 20% during the active-tillering stage, and the remaining 20% during the panicle-initiation stage. All triple superphosphate was applied one day before transplanting.

2.6 Measurement of Growth Characters

After four weeks of incorporating GMs, 21-day-old seedlings of the Sinn-thu-kha rice variety (*Oryza sativa* L.), which is a high-yielding Myanmar variety of Indica type and matures in 140 days (medium duration variety), were transplanted into all pots. The plant height and the number of tillers per pot were measured weekly from 10 days after transplanting (DAT) until 50% flowering and then at 2-week intervals until the harvesting stages. Before panicle initiation, the Soil Plant Analysis Development SPAD value was measured using the uppermost fully expanded leaf by the SPAD-502 chlorophyll meter (Konica Minolta, Inc., Osaka, Japan), and afterward, the flag leaf was measured.

2.7 Determination of Dry Matter and N Uptake

The rice plants from each pot were cut 2 cm above the ground at harvest and were divided into sheaths, leaves, panicles, and seeds. They were then dried in an oven at 70°C for 48 hours and weighed instantly. The total weight of all plant parts was added to calculate the dry matter (DM) accumulation, expressed in tons per hectare (t ha⁻¹). The dried samples were then ground into a fine powder using a Cyclotec 1093 Sample Mill (100–120 mesh; Tecator AB, Hoedanaes, Sweden). To measure the N accumulation, all plant parts were thoroughly mixed to get a homogeneous sample. It was then sub-sampled again to measure the N accumulation. The homogenized samples from each replication were pooled. The sample was then digested using the salicylic acid–H₂SO₄–hydrogen peroxide (H₂O₂) method. Total N was analyzed using the method mentioned in the soil analysis. The N uptake for each treatment was determined by multiplying the N content with the dry matter of the sample.

2.8 Determination of Yield and Yield Components of Rice

The number of panicles per pot, the number of spikelets per panicle, the percentage of filled grains, and the thousand-grain weight (in grams)

were determined from the harvested plants. The rice yield was calculated based on the weight of the heavy seeds, which was adjusted to 14% moisture content. The harvest index (HI) was calculated as the ratio of economic yield (total seed weight) to biological yield (total dry matter weight) [35].

2.9 Statistical Analysis

The data were summarized, and an Analysis of Variance (ANOVA) was performed using the statistical software Statistix (Version 8.0). Treatment means were compared using the Least Significant Difference (LSD) test at a 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Experimental Soil

The soil used in the experiment was sandy loam, with a moderately acidic pH of 5.78. It had very low electrical conductivity (0.07 mS/cm), very low organic carbon content (0.76%), and very low total nitrogen (0.09%). The exchangeable cations were as follows: low Ca⁺⁺ (9.62 meq/100 g), medium Mg⁺⁺ (3.43 meq/100 g), high Na⁺ (1.25 meq/100 g), and low K⁺ (0.13 meq/100 g). The soil had a medium cation exchange capacity of 14.43 meq/100 g, low available phosphorus (2.8 ppm), low available potassium (6.25 ppm), and a bulk density of 1.19 (Table 2).

3.2 Chemical Composition of Green Manure

Based on the analysis data, GSH had the highest total N content (2.42%), the highest organic carbon (63.38%), and the lowest C:N ratio (22.06) among all GM varieties. GDC showed higher results in total P₂O₅ (0.21%) and total K₂O (2.26%) (Table 3). All tested GM crops had a higher N content and lower C:N ratio.

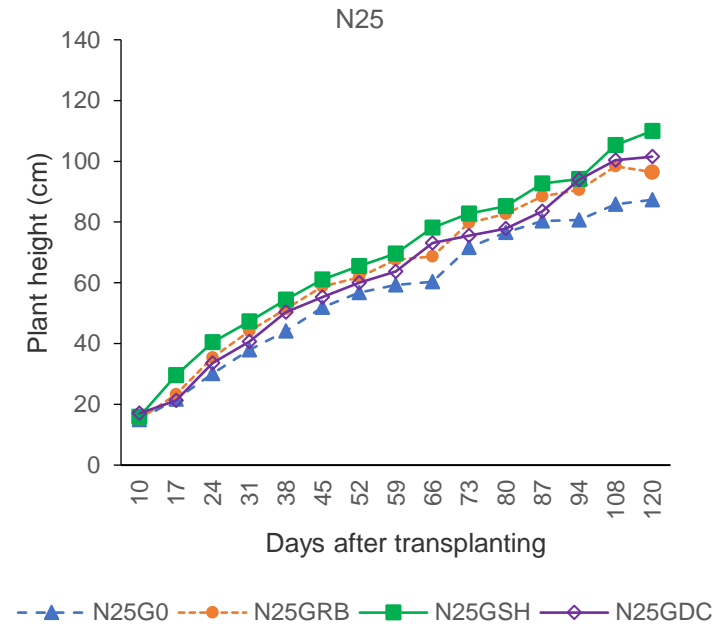
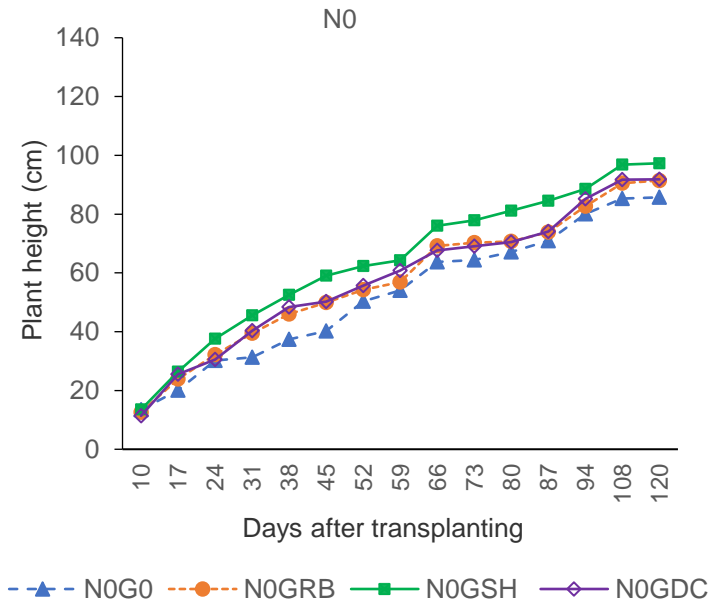
3.3 Plant Growth Characters

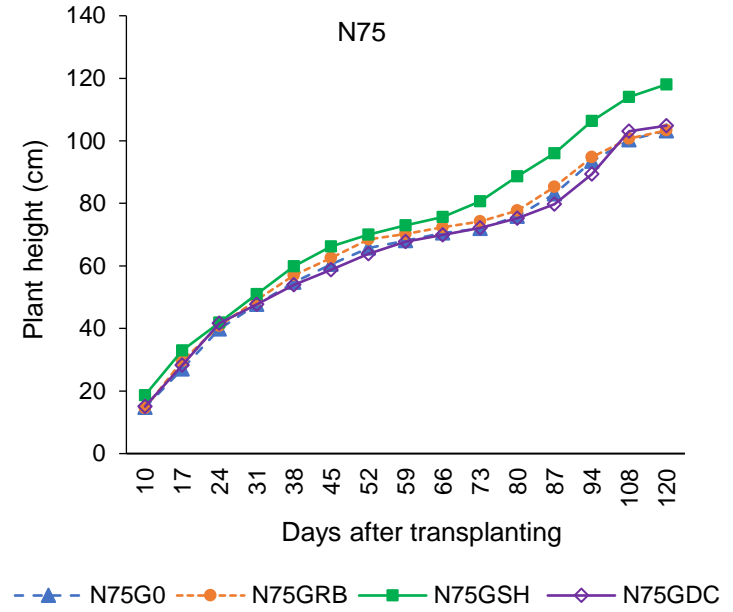
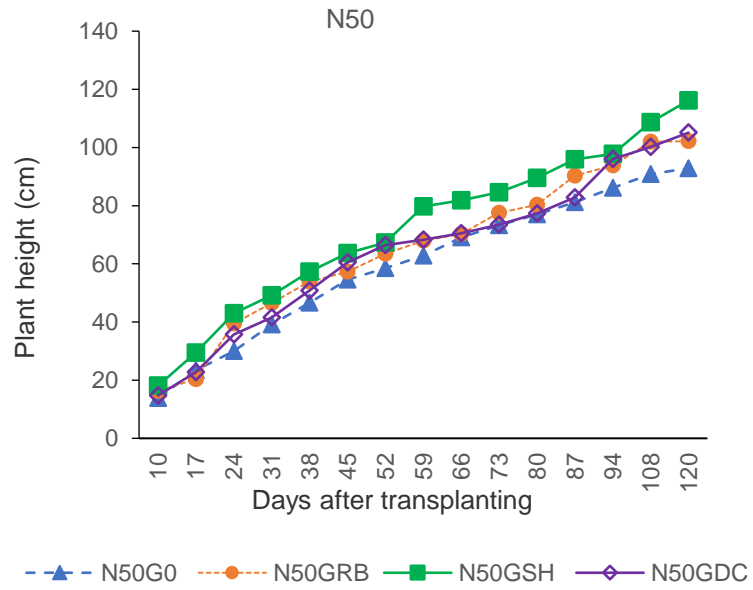
3.3.1 Plant height (cm)

In this study, the plant heights were increased along with the increment of N level from 0 to 100% (Fig. 1). The treatment N100 among chemical N levels produced the maximum plant height during the crop period. Regarding GM crops, GSH produced higher plant height than other GMs throughout the crop period. For the combined effect, the treatments N100GSH (120 cm) and N75GSH (118 cm) provided the maximum plant height among all treatments, but their values were almost similar (Fig. 1). GSH + chemical N fertilizer can provide higher plant height due to their N supplement to the soil, resulting in higher nitrogen use efficiency (NUE), growth, and yield of rice compared to other GMs [36,37].

Table 2. Physicochemical properties of experimental soil before experiment

| No. | Parameter | Value | Rating |
|-----|----------------------------------|--------------|-----------------|
| 1 | pH (1:2.5) | 5.78 | Moderately acid |
| 2 | EC (mS/cm) | 0.07 | Very Low |
| 3 | Organic carbon (%) | 0.76 | Very Low |
| 4 | Total N (%) | 0.09 | Very Low |
| 5 | Exchangeable Cations (meq/100g) | | |
| | Ca ⁺⁺ | 9.62 | Low |
| | Mg ⁺⁺ | 3.43 | Medium |
| | Na ⁺ | 1.25 | High |
| | K ⁺ | 0.13 | Low |
| 6 | CEC (meq/100g) | 14.43 | Medium |
| 7 | Available P (ppm) | 2.8 | Low |
| 8 | Available K ₂ O (ppm) | 6.25 | Low |
| 9 | Bulk density | 1.19 | - |
| 10 | Texture | | Sandy Loam |
| | Sand (%) | 63.28 | |
| | Silt (%) | 20.89 | |
| | Clay (%) | 15.83 | |





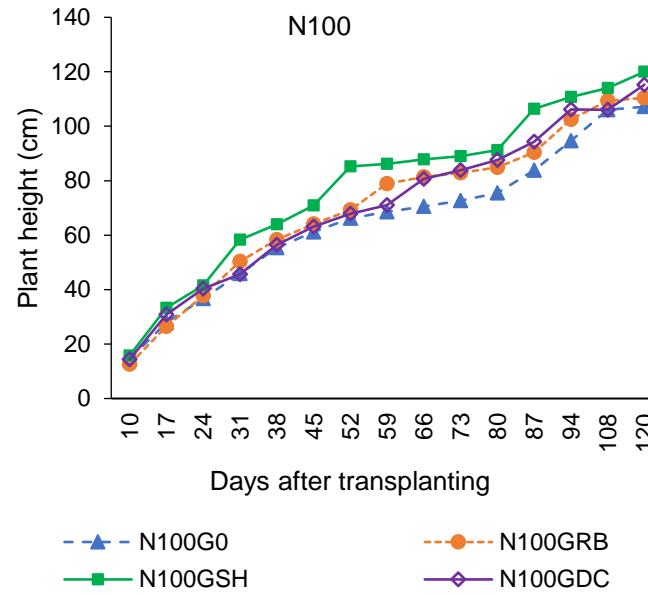
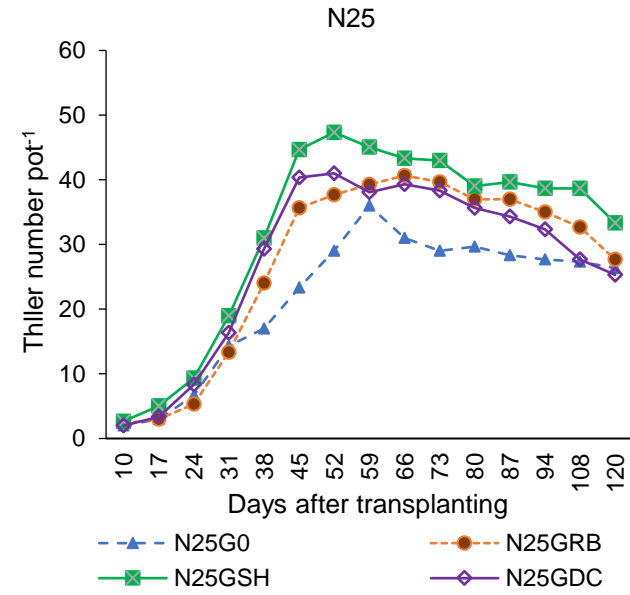
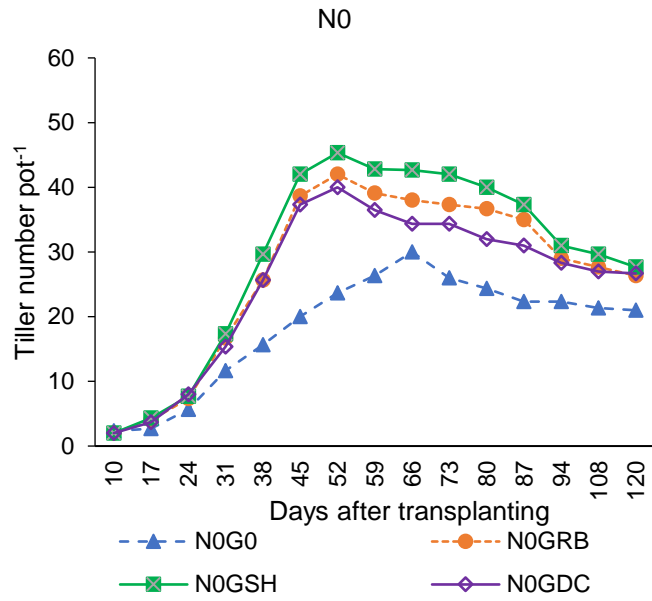
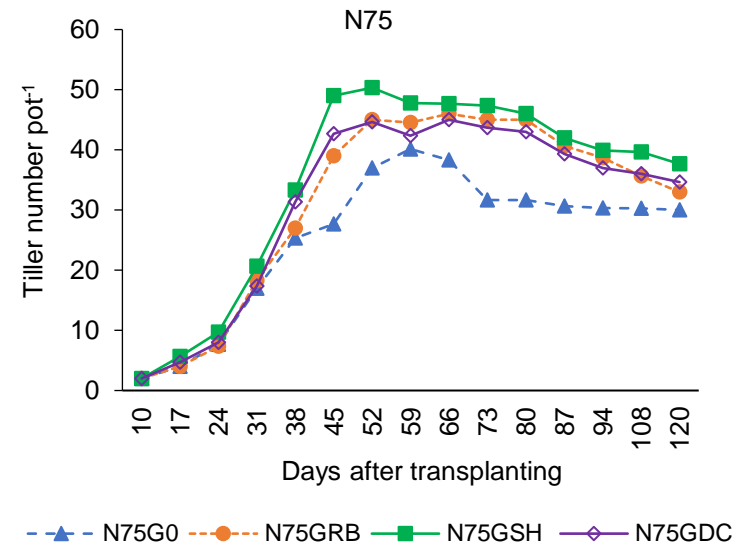
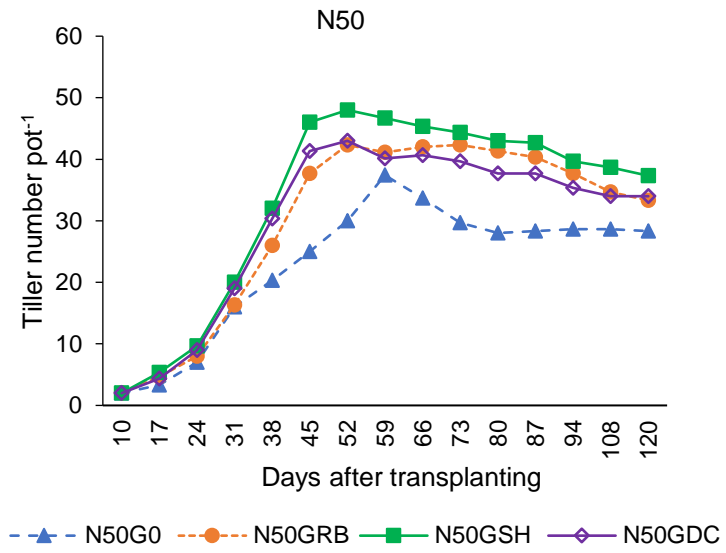


Fig. 1. Plant height (cm) of Sinn-thu-kha rice variety as affected by GMs and levels of chemical N fertilizer, 2023

N_0 = N omission G_0 = No green manure
 N_{25} = 25% N GRB = Rice bean
 N_{50} = 50% N GSH = Sunn hemp
 N_{75} = 75% N GDC = Dhaincha
 N_{100} = 100%





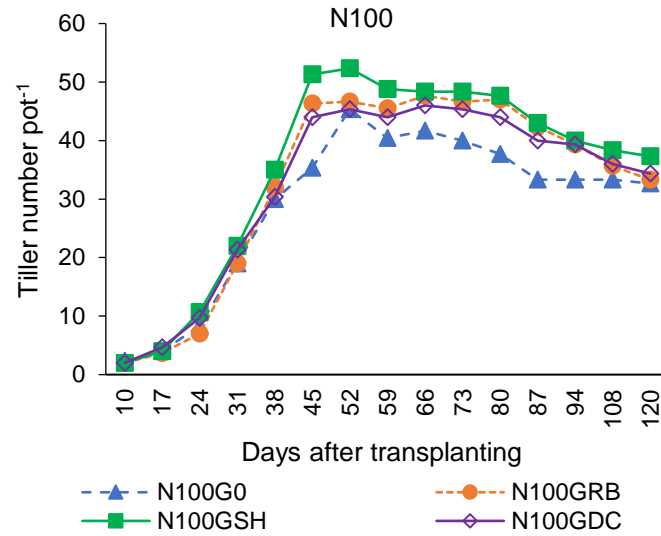
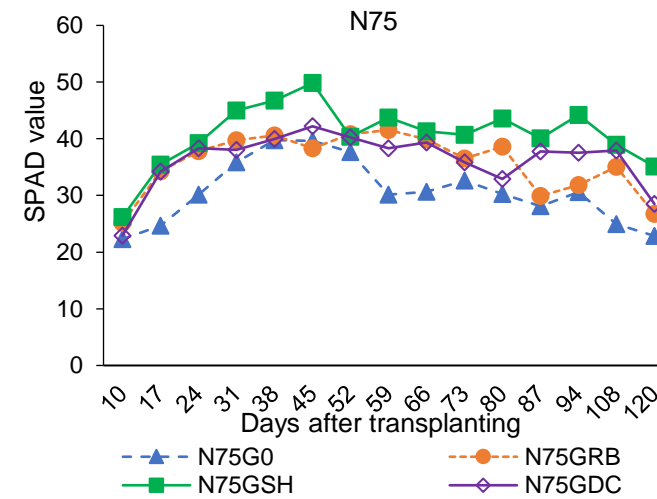
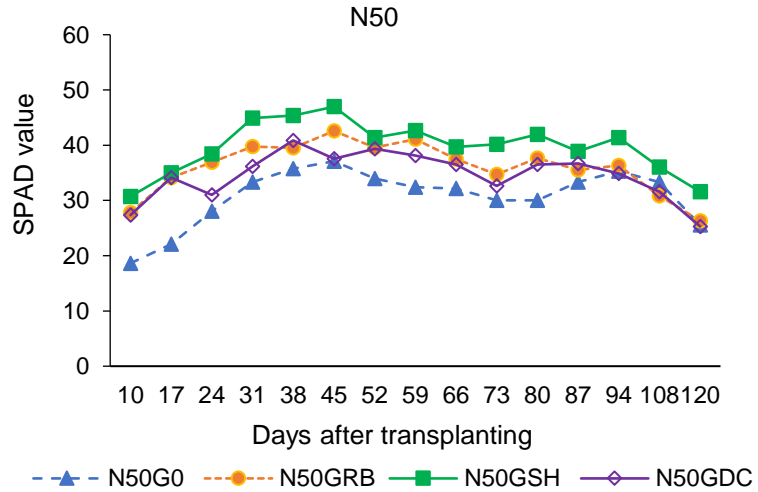
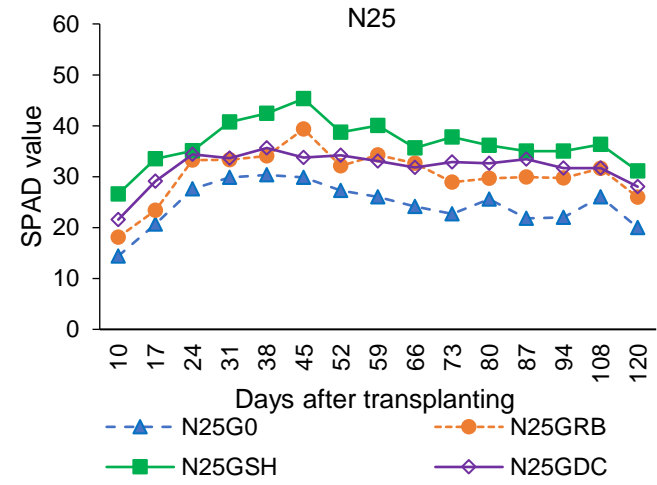
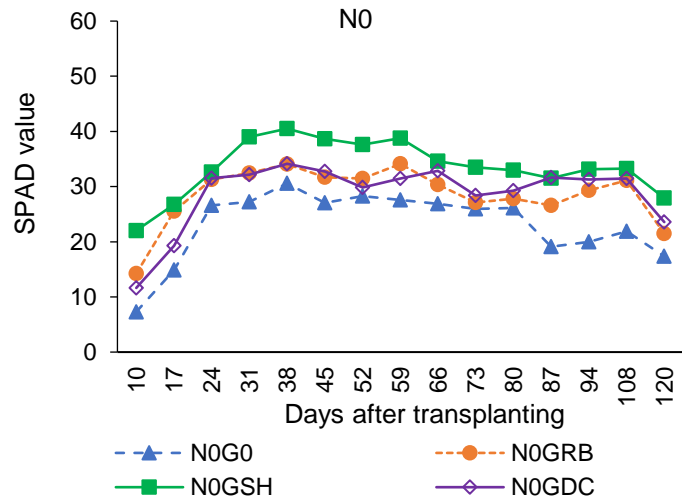


Fig. 2. Tiller number pot⁻¹ of Sinn-thu-kha rice variety as affected by GMs and levels of chemical N fertilizer, 2023

*N*₀ = N omission *G*₀ = No green manure
*N*₂₅ = 25% N *G*_{RB} = Rice bean
*N*₅₀ = 50% N *G*_{SH} = Sunn hemp
*N*₇₅ = 75% N *G*_D = Dhaincha
*N*₁₀₀ = 100%



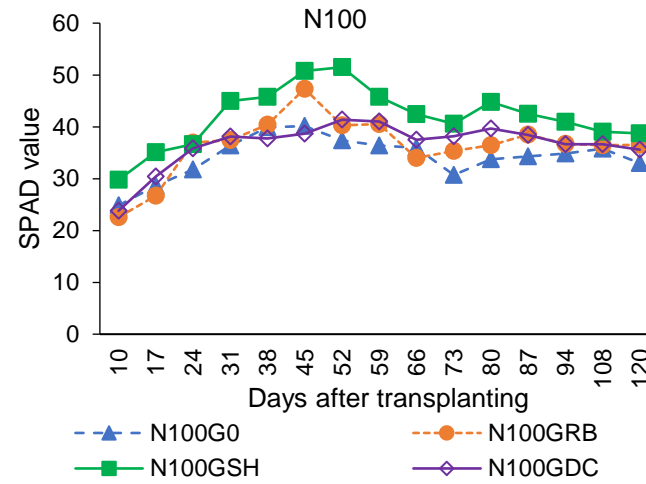


Fig. 3. SPAD value of Sinn-thu-kha rice variety as affected by GMs and levels of chemical N fertilizer, 2023

N_0 = N omission G_0 = No green manure
 N_{25} = 25% N GRB = Rice bean
 N_{50} = 50% N GSH = Sunn hemp
 N_{75} = 75% N GDC = Dhaincha
 N_{100} = 100%

Table 3. Chemical composition of green manure crops

| No. | Green manure | Percentage | | | | | C:N |
|-----|--------------|------------|---------|-------------------------------------|------------------------|----------------|-------|
| | | Moisture | Total N | Total P ₂ O ₅ | Total K ₂ O | Organic carbon | |
| 1. | Rice Bean | 60.67 | 2.02 | 0.13 | 1.89 | 50.71 | 25.10 |
| 2. | Sunn hemp | 65.51 | 2.42 | 0.14 | 1.37 | 63.38 | 22.06 |
| 3. | Dhaincha | 57.64 | 2.52 | 0.21 | 2.26 | 51.09 | 25.10 |

3.3.2 Tiller number per pot

The resulting pattern of tiller number was similar to that of plant height. As the N level increased from 0 to 100%, the tiller number also increased. The maximum number of tillers was found at 52 DAT, after which the number of tillers decreased. The highest value was found in N100, which was similar to that of N75. The treatment GSH showed the highest number of tillers compared to GDC and GRB at all growth stages. At the harvesting stage, the maximum tiller numbers were observed in N100GSH (37.3), N75GSH (37.7), and N50GSH (37.3), but the values were not significantly different (Figure 2). According to [38], GSH can provide rice plants with a higher tiller number due to their increased nitrogen supply. On the other hand, the tiller numbers in treatment N100G0 were lower when GMs were not incorporated, compared to the treatment combined with GMs.

3.3.3 SPAD value

The SPAD values varied depending on the chemical N level and GMs (Fig. 3). The higher application of N resulted in an increase in SPAD values for the sinn-thu-kha rice variety. The maximum values were observed in treatment N100. The peak point for SPAD values was observed between 38 and 45 DAT in all treatments, after which the values gradually decreased. All GM crops improved SPAD values, especially GSH, which maintained the highest value throughout the crop period, followed by GDC and GRB. Treatment N100GSH maintained higher SPAD values (38.8) until the harvesting stage, which is similar to N75GSH (35.1) (Fig. 3). GSH contains a higher nitrogen percentage and provides greater biomass to the soil. These higher N doses from GSH significantly increased SPAD values at different growth stages of rice [39]. However, lower SPAD values were observed at all N levels without GMs, in contrast to the treatments that included GMs.

3.4 Dry Matter

The supply of N level positively affected the DM as its concentration increased from 0% to 100%,

resulting in an increase in DM (Fig. 4). The highest value for chemical N level was recorded in N100. In the case of GM crops, GSH resulted in the maximum DM at each N level. All GM crops showed higher DM production than the control treatment (N0G0). When the combined effect of different factors was analyzed, N100GSH exhibited the highest DM (112.90 g pot⁻¹), which was also observed in N75GSH (106.12). However, the treatment N100G0 resulted in lower DM, even though N100% was applied to the rice plants, but no GM cultivation was carried out in that treatment. On the other hand, using only GM incorporation wasn't enough to provide the optimum DM for rice plants. This was evident in the treatments N0GRB, N0GSH, and N0GDC. The GSH had a better N supply than GRB and GDC, resulting in similar DM among treatments: N75GSH, N100GRB, and N100GDC, even though the chemical N levels were differently combined. Under sunn hemp incorporation, both rice grain and straw biomass yields exceeded those of millet or no green manure application [40].

3.5 N uptake

In this study, we analyzed the N content of the pooled samples from each replication. Due to this, we could not calculate the mean comparison among the treatments. However, we observed that rice plants responded differently in terms of N uptake based on the chemical N levels and GM crops. As shown in Fig. 5, N uptake increased with an increase in chemical N application. Among GMs, GSH provided higher N uptake than GRB and GDC. At harvest time, N100GSH showed the highest N uptake of 1.04 g pot⁻¹, while N75GSH achieved a similar N uptake of 0.96 g pot⁻¹. However, treatments with GRB and GDC resulted in lower values. Without GMs, treatments N25G0, N50G0, and N100G0 did not accumulate optimum N uptake. The plots N0G0 achieved the lowest N uptake. According to the chemical composition of GMs (Table 3), the GSH contains a higher total amount of nitrogen than the GRB and GDC. In the other studies, the greater N content in both shoot and root was obtained from sunn hemp than millet,

possibly due to biological N fixation by sunn hemp [41,42,43]. Additionally, the GSH has achieved higher biomass and applied more nitrogen than GRB and GDC (Table 1). Therefore, it can be inferred that the total nitrogen supply from GSH is possibly higher than those of GRB and GDC. As a result, the total nitrogen uptake of rice is high in the treatments

that are incorporated with GSH. This result proves that GSH possesses higher biomass, higher N content, and higher decomposition rate than other GMs and can release N faster than others. Studies with three legumes, Odhiambo [44], showed that sunn hemp tends to release N at a faster rate, followed by lablab and velvet bean.

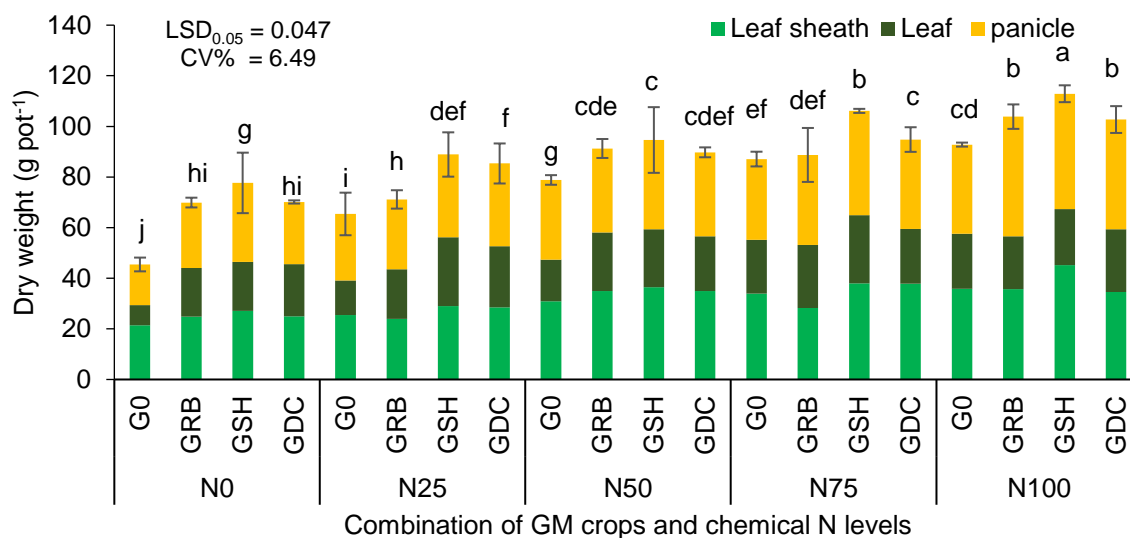


Fig. 4. Dry matter (g pot⁻¹) of Sinn-thu-kha rice variety affected by GMs and levels of chemical N fertilizer at harvesting stage, 2023

The bar graph with the same letter is significantly different by the LSD test ($p < 0.05$). The error bar represents the standard error

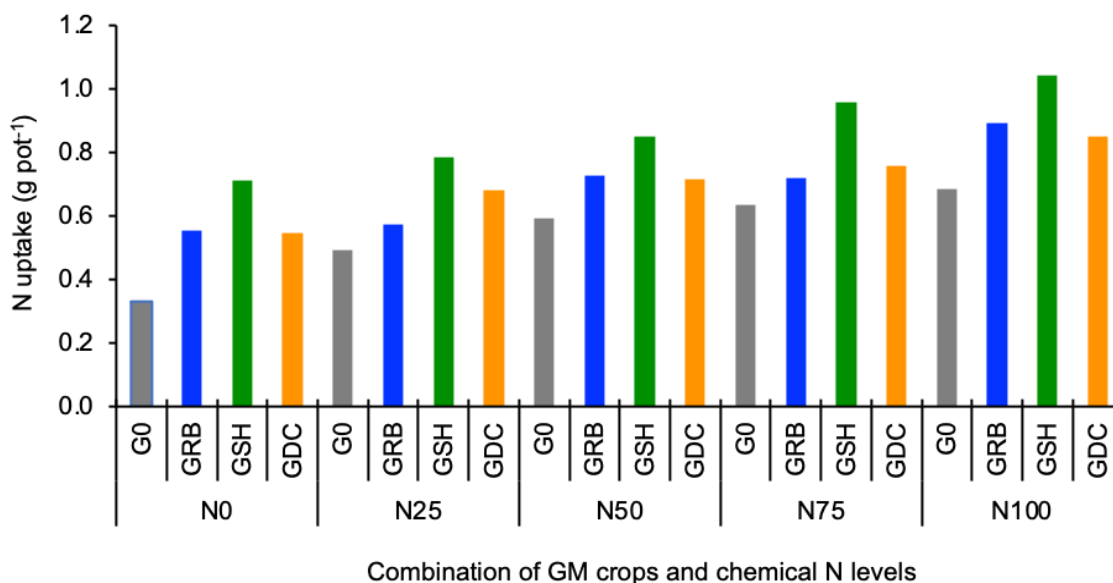


Fig. 5. N uptake (g pot⁻¹) of Sinn-thu-kha rice variety affected by GMs and levels of chemical N fertilizer at harvesting stage, 2023

Table 4. Harvest index, yield, and yield components of Sinn-thu-kha rice variety affected by GMs and levels of chemical N fertilizer, 2023

| Treatments | Harvest Index | No. of panicles pot ⁻¹ | No. of spikelet panicle ⁻¹ | Filled grain (%) | 1000 grain weight (g) | Yield (g pot ⁻¹) |
|--------------------------|---------------|-----------------------------------|---------------------------------------|------------------|-----------------------|------------------------------|
| Nitrogen (N) | | | | | | |
| N0 | 0.47 a | 25.42 c | 146.67 d | 58.48 d | 18.45 ab | 30.67 c |
| N25 | 0.43 b | 28.00 bc | 160.17 c | 71.53 c | 17.98 b | 33.03 c |
| N50 | 0.42 b | 33.25 ab | 173.75 b | 75.80 b | 18.57 a | 37.28 b |
| N75 | 0.41 b | 33.92 a | 183.42 ab | 79.93 a | 18.37 ab | 39.13 ab |
| N100 | 0.40 b | 34.33 a | 192.83 a | 82.83 a | 18.40 ab | 41.48 a |
| LSD _{0.05} | 0.03 | 5.60 | 11.66 | 3.90 | 0.54 | 4.08 |
| Green manure (GM) | | | | | | |
| G0 | 0.38 b | 27.87 c | 157.07 c | 68.07 c | 17.85 b | 27.50 c |
| GRB | 0.45 a | 30.53 b | 171.00 b | 75.76 b | 18.21 b | 37.21 b |
| GSH | 0.45 a | 34.47 a | 183.60 a | 80.70 a | 19.07 a | 42.39 a |
| GDC | 0.43 a | 31.07 b | 173.80 ab | 70.33 c | 18.29 b | 38.18 b |
| LSD _{0.05} | 0.03 | 2.51 | 10.2 | 3.04 | 0.50 | 1.72 |
| Pr>F | | | | | | |
| Nitrogen | ** | * | ** | ** | ns | ** |
| Green manure | ** | ** | ** | ** | ** | ** |
| N x GM | ** | ns | ns | ** | ns | ** |
| CV% (a) | 7.89 | 19.21 | 7.23 | 5.62 | 3.10 | 11.92 |
| CV% (b) | 9.74 | 10.84 | 7.98 | 5.52 | 3.68 | 6.36 |

Means followed by the same letter in each factor in each column are not significantly different in LSD tests ($p < 0.05$). * Significant at 5% level, ** significant at 1% level

Numbers in N treatments show the amount of N applied as a percentage based on 1.55 g pot⁻¹

G0 = no GM, GRB = rice bean, GSH = sunn hemp, GDC = dhaincha

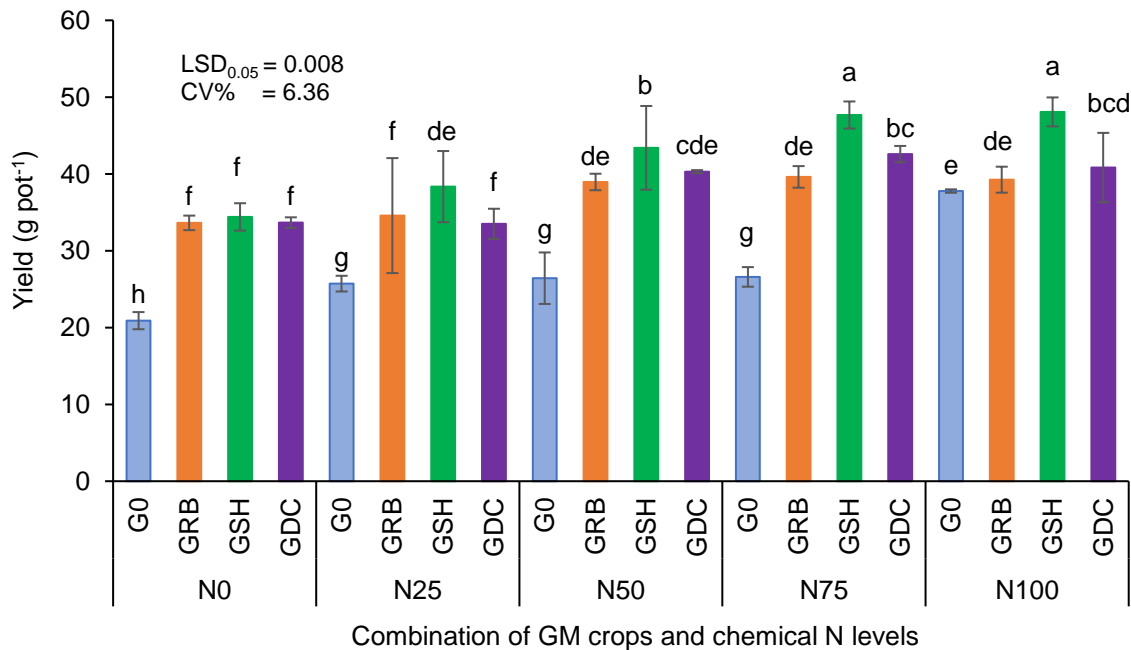


Fig. 6. Yield (g pot⁻¹) of Sinn-thu-kha rice variety by the combined effect of GMs and levels of chemical N fertilizer, 2023

The bar graph with the same letter is significantly different by the LSD test ($p < 0.05$). The error bar represents the standard error



Image 1. GMs cultivation as treatments in the pot experiment



Image 2. Rice cultivation after the incorporation of GMs in the pot experiment

3.6 Yield and Yield Components

Yield and yield components were significantly different among different N levels and GMs, except for the 1000 grain weight ($P \leq 0.01$) (Table 4). An interaction effect of N and GMs was also observed on the yield of the sinn-thu-kha rice variety ($P \leq 0.01$). For the chemical N level, the maximum yield (g pot^{-1}) was obtained by the treatment N100 (41.48) due to higher yield components. However, similar yields were also achieved from treatments: N75 (39.13) and N50 (37.28).

Regarding GM crops, it was found that GSH produced the highest yield (42.39 g pot^{-1}) due to having a higher number of panicles and filled grain percentage. GDC (38.18) and GRB (37.21) yielded less than GSH but more than G0. When chemical N level and GMs were combined, N100+GSH resulted in the highest yield (48.07 g pot^{-1}), which was similar to that (47.68) of N75GSH (Fig. 6). This means that using 25% less chemical N fertilizer would not decrease rice yield.

In this study, rice yields were significantly lower when chemical N levels were applied without incorporating GMs. The recommended chemical nitrogen fertilizer N100% + no GM provided a lower yield than the yields obtained by treatments N100GRB, N100GSH, and N100GDC. Therefore, incorporating GMs effectively improves the yield components and yield of rice. Among GMs, GSH was more effective in supplying N to rice plants, resulting in higher grain yield when 75% and 100% of the recommended chemical N rate were applied. Our findings prove that GMs, when combined with chemical N fertilizer, can lead to higher rice yield. Several researchers also reported that biological N fixation by leguminous GMs can reduce the need for inorganic N fertilizers for the succeeding crop [45,46].

4. CONCLUSION

Our study showed that combining GMs with chemical N fertilizer effectively promotes N uptake, growth, yield components, and yield of the Sinn-thu-kha rice variety, especially when GSH (sunn hemp) is used. This is because sunn hemp provides the necessary N demands for rice, but it needs to combine with 75% of the recommended chemical N fertilizer rate, 1.55 g pot^{-1} (102 kg N ha^{-1}). When a 100% chemical N fertilizer rate is used, GRB (rice beans) and GDC

(dahincha) can also provide satisfactory rice yields. By using GSH, it is possible to reduce the chemical N fertilizer rate by 25% without decreasing the rice yield. Therefore, incorporating GMs, particularly GSH, not only increases rice yield but also helps alleviate the pollution caused by chemical fertilizers, leading to sustainable agriculture.

ACKNOWLEDGEMENT

This study received advice and guidance from my supervisory committee and financial support from the Yuntianhua scholarship of Yuntianhua Co., Ltd, Yunnan Province, China. I gratefully acknowledge all the supporters of my research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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