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## Research Article

# Integration of the Nitrogen Fertilization with Manure Influences on Chlorophyll Fluorescence and Photosynthesis Traits in Winter Wheat (*Triticum aestivum* L.) Crop

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## ABSTRACT

Integration of nitrogen fertilizers with organic manures was found to be favorable and has shown a more sustainable way to boost the winter wheat crop's photosynthetic qualities and grain output. A split plot design with multi factor was adopted, wheat varieties were considered as the main factor, and secondary factor was fertilizers doses. The genotype of Zhongmai 175, and Jindong 22 were selected for this study. In the treatments, we applied pure nitrogen as labeled with T1 (Control plot No fertilizer), T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), and combined manure + nitrogen (M+N) as labeled with T5 100 MN (25% Manure + 75% Nitrogen), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 N kg ha<sup>-1</sup> (75% M+ 225% N). Results revealed that the maximum values of total photosynthetic rate 68.12 m mol m<sup>-2</sup> s<sup>-1</sup> and chlorophyll 5.73 mg/g FW was achieved in zhongmai 175 at heading stage in contrast to jindong 22 genotype. However, jindong 22 exhibited greater intercellular CO<sub>2</sub> 1998.47 mol mol<sup>-1</sup>, photosynthetic active radiation 852.14 under T3 at tillering stage. Highest Fv/Fm noted at the heading stage, The FPSII was noted higher in T3 and T7 treatment for the Zhongmai 175 genotype. The highest QP results were obtained at the jointing and flowering stage under T3 and T7 treatments as compared to T1 treatment at the zhongmai 175 genotype in comparison to jindong 22 genotype. In jindong 22 under T7, the maximum LAI results were 5.35 (cm<sup>2</sup>) in compared to Zhongmai 175 at the booting stage. However, the highest Gs and Tr proportions were recorded in zhongmai 175 under T7 as compared to jindong 22 during the flowering stage, were 6.31 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> and 0.90 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, respectively. This study concluded that combine application of nitrogen fertilizer and manure affected photosynthesis parameters of the winter wheat genotypes. This study conveys an innovative insights into the synergistic effects of integrating nitrogen fertilization with manure, exhibiting its potential to enhance chlorophyll fluorescence, photosynthetic efficiency, and physiological characters through various winter wheat genotypes under variable growth stages

**Keywords:** Nitrogen, Manure, Chlorophyll Fluorescence, Winter Wheat.



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## INTRODUCTION

The integration of sustainable nutrient management practices is critical for improving wheat production and mitigating the environmental challenges correlated with intensive chemical fertilizer use (Bargali et al., 2019; Manral et al., 2022). However, it has been noted that inappropriate nitrogen fertilization and long-term nitrogen fertilizer applications reduce the use of nitrogen fertilizer and raise the risk of nitrate leakage into groundwater (Ju et al., et al., 2009; Zhou et al., 2017). It is necessary to limit the use of nitrogen fertilizer and choose an appropriate approach in order to maintain biomass and nitrogen utilization efficiency (NUE). Two significant issues that restrict cereal output in China's dry plains without any additional water are water deficit and soil fertility. Due to little infiltration and little precipitation each event, the Loess Plateau in China experienced significant evaporation on an annual average between 200 and 600 mm (Ranva et al., 2022). Be a result, it is referred to as a typical dry land. Furthermore, more than 90% of this region's agriculture is rained. With a cultivated area of 5 million hectares, winter wheat is one of the key cereal crops farmed on loess plateau and makes up 40% of the entire area under cultivation for food crops (Kubar et al., 2021). Winter wheat grain yields, however, are much lower than normal yields in China, averaging only 2.5 tons  $h^{-1}$  to 3.7 tons  $ha^{-1}$ , have been minimum and unstable for an extended period (Liu et al., 2021). Conversely, local agricultural and fertilizer practices driven by irrationality accelerate the deterioration of soil quality, leading to low soil organic matters (SOMs) levels. Two thirds of the croplands in this area currently have SOMs of less than 1%. On the other side rainfall in this area varies throughout time. For example, between July and September rainfall comes about 60-70%. (Tshikunde et al., 2019; Zhen et al., 2020), additionally, the winter wheat fields are abandoned during that time. On the Loess Plateau, winter wheat's growing season is commonly hampered by drought. This lack of water speeds maturity, reduces grain filling time, and lowers dry matter production, all of which result in low wheat yields (Tong et al., 2021; De et al., 2020).

The nitrogen use efficiency (NUE) evaluation is crucial to comprehending nitrogen fertilizer use and its effect on yield. Reasonable management practices must be utilized to promote nitrogen uptake by crops and limit nitrogen leaching in order to maximize the usage of chemical fertilizer and raise NUE. Nitrogen sources are necessary to regulate nitrogen transport and affect grain production. The use of organic manure in place of chemical fertilizer is a crucial measure that has been well investigated to alleviate the problems caused by random fertilization (Zhang et al., 2021; Xu et al., 2018). According to numerous studies, employing organic manure in place of conventional manure can boost the amount of nutrients and organic matter in the soil, speed up the activity of helpful soil microbes, increase crop yield and soil fertility (Morales et al., 2020; Zhang et al., 2018; Yang et al., 2021). For example, (Ju et al., 2009) discovered that applying organic and inorganic N fertilizer in a ratio of 1:1 or 2:1 greatly increased vegetable production and N uptake. When organic manure was used in place of 25% of chemical N fertilizer, (Liu et al., 2021) found that wheat yields would remain constant, nitrogen uptake would be encouraged, and nitrogen utilization would increase by 20%. (Abebe et al., 2022) showed through a recent meta-analysis that switching to organic manure instead of chemical fertilizer increased agricultural productivity by 6.8%. In order to address crop development requirements and boost crop output in the Loess Plateau, fertilization is becoming a significant strategy to improve soil fertility (Machado et al., 2021). Groundwater contamination, greenhouse gas emissions, and eutrophication have been caused by local farmers' incorrect and excessive application of chemical fertilizer, particularly nitrogen (Ma et al., 2022).

There is more focus on environmental preservation and fertility of soil, building rather than just improving crop yields in order to achieve the sustainability of dry land farming with the rapid development of agriculture. Inorganic fertilizers alone did not significantly increase soil nutrients, crop yields, fertilizer use, or water efficiency, according to multiple studies (Khan et al., 2022; Semenova et al., 2021). Manure combined with inorganic fertilizers did. A maximum proportion of manure nitrogen to inorganic nitrogen, however, does not lead to a high yield or an effective use of nitrogen and water; instead, it causes a significant buildup of soil nitrate residue, which seeps into deeper soil and contaminates subsurface water (Gu et al., 2017). This research's conclusions will provide a theoretical framework for the development of soil fertility in dry land and the production of winter wheat. Studies have shown that frequent applications of chemical and organic fertilizers made in combination over time can successfully reduce nitrogen leaching in the soil and groundwater contamination while increasing soil microbial biomass carbon (Ebert et al., 2022; Zhang et al., 2016; Sharma et al., 2020; Yangen et al, 2019) asserts that organic manure can lessen nitrate accumulation and leaching in the soil profile. (Cao et al., 2022) found that the combination of organic fertilizers may reduce the likelihood of nitrate leaching as well as the apparent nitrogen surplus. But using the wrong proportions of organic manure and inorganic fertilizer can result in nitrate nitrogen accumulation, which contaminates groundwater,

as well as significantly increasing biomass, the efficiency of fertilizer use, and the amount of water used (Yang et al., 2020; Zhang et al., 2022). Keeping these facts in mind the following specific objectives were set: (1) To reveal the effect of the nitrogen fertilization and manure on chlorophyll fluorescence and photosynthesis characteristics of the winter wheat. (2) To identify the relationships of grain yield with chlorophyll fluorescence and photosynthesis characteristics of winter wheat.

## MATERIALS AND METHODS

### Experimental locations

In the Experimental Site of Shanxi Agriculture University, Taigu (N 37°25', E 112°33'), in the Shanxi Province of China, a field trial was carried out in 2020–2021. With an average annual temperature of 12°C, 442 mm of rainfall, 1840.2 mm of potential evapotranspiration and 2672 hours of sunshine, the research region at Taigu Base has a mild climate. The study area is a high dry region with semi-arid weather typical of the northeast loess plateau. The fallow season, which runs from July to August is followed by seasonal months with 60% to 70% of rainfall. An established field's basic soil properties were 7.2 pH, 7.7 mg kg<sup>-1</sup> SOM, and 51.12, 19.34, 243.26 mg kg<sup>-1</sup> of available nitrogen, phosphorus and potassium, respectively.

### Treatments detail

The field experimental were established at the experimental station of Shanxi Agriculture University taigu in the Shanxi province of China (N 37°26', E 112°35'). The plot size were 20 m<sup>2</sup> (4 x 5). The genotype of Zhongmai 175, and Jingdong 22, were selected for this study, which had a crude protein percentage of 17.4%. Both genotypes are high yielding genotypes of Shanxi province. The experiment used a multi-factor split-plot design (pure N), with several wheat types acting as key plot and fertilizers treatment acting as secondary plot. The two types of wheat genotypes were selected as Zhongmai 175 (North) and Jingdong 22 (South). A 25% nitrogen reduction along with the application of organic fertilizer (chicken manure) (1.5% N content) along with total setup of seven treatments (Pure nitrogen and manure + nitrogen). Treatments were labeled as T1 (Control plot No fertilizer used), T2 (100 N kg<sup>-1</sup> ha), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), and combined manure + nitrogen (M+N) as labeled with T5 100 MN (25% Manure + 75% Nitrogen), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 N kg ha<sup>-1</sup> (75% M+ 225% N). Each treatment was repeated three times. 120 kg ha<sup>-1</sup> of urea (46.1% N content), potassium (50% K<sub>2</sub>O content), and phosphorus (16% P<sub>2</sub>O<sub>5</sub> content) were used as a base fertilizer. The management of local wheat production is the same as that of other farming techniques. All other agronomic activities, including watering, insecticide and pesticide application, and weed management, were consistently and accurately carried out in accordance with the plant's growth stage and requirements.

### Photosynthetic active radiation (PAR)

We used measurement devices to evaluate the PAR (photosynthetic active radiation) in varied environments (LI-COR Inc., LI-191SA quantum sensor, Lincoln, NE, USA). The tops of the winter wheat seedlings were seen four times at different growth stages in all treatments under nitrogen and manure while hanging on a horizontal arm of the observation scaffold. During the hours of 11:00 p.m. to 1:00 a.m. on a bright, sunny day, measurement sensors were placed on wheat of both cultivars in various plots (Kubar et al., 2021).

### Photosynthesis parameters

Under different nitrogen ratios, nitrogen levels and nitrogen timings, the winter wheat crops (Pn) photosynthesis rate, (Tr) transpiration rate, (Ci) intercellular CO<sub>2</sub> absorption, and (Gs) stomatal conductance were measured using the LI-6400 convenient photosynthesis method, which was created by LI-COR Inc. in Lincoln, Nebraska, USA. To determine the photosynthetic traits, two fully extended flag leaves were chosen during jointing, flowering, and grain filling from every treatments winter wheat crop. A bright, sunny day with 400 mol mol<sup>-1</sup> CO<sub>2</sub> concentrations, all the dimensions were measured between 10:00 and 11:00 (Kubar et al., 2021).

### Chlorophyll fluorescence measurements

Using a pulse modulated fluorometer (FMS-2, Norfolk, UK, and Hansatech Instruments Led.). The chlorophyll fluorescence characteristics of flag leaves were investigated during the flowering and filling seasons. The light treatments that were used to determine Fm and Fs were far red light, while the light treatments that were used to determine F0 were far red light. S0 treatment involved adjusting light intensity to 1800 mol m<sup>2</sup> s<sup>-1</sup>, s<sup>1</sup> treatment to 1350 mol m<sup>2</sup> s<sup>-1</sup>, s<sup>2</sup> treatment to 900 mol m<sup>2</sup> s<sup>-1</sup>, and S<sup>3</sup> treatment to 450 mol m<sup>2</sup> s<sup>-1</sup>. The maximum photochemical efficiency (Fv/Fm) and additional parameters were then evaluated following a 15-minute dark adaption period. The photochemical quenching coefficient, non-photochemical quenching coefficient, and quantum efficiency of PSII

photochemistry were all calculated using the appropriate formula. A total of 3 leaves from each plot were counted.

### Statistical analysis

The statistics used in this current study has an average outcomes of the three replications. An ANOVA using a randomized complete block design was used to analyze the data. The F-test was used to evaluate each source's significance. We used the LSD at  $p < 0.05$  to compare treatments based on significant variations. Origin 8.5 was used to create the graphics, and Microsoft Excel 2013 was used to process the data. All the analysis were completed using the SPSS 20.0 versions.

## RESULTS

### Photosynthetic active radiation (PAR)

Photosynthetic active radiation of the winter wheat crop demonstrated a substantial variation between cultivars from tillering to blooming phase below progressive nitrogen rate ( $p < 0.05$ ) presented in figure 1. The highest PAR value during the winter wheat growth season was mostly focused during the tillering and heading phases under both T3 and T4 cultivars, but during the jointing and booting phases under T7 inorganic treatment in both Zhongmai 175 and Jindong 22 cultivars in comparison to control treatment. Whole, following the T7 (Inorganic) treatment as compare to the control during the Jointing stage, PAR significantly increased in both jindong22 and zhongmai 175 cultivar. Regarding Jindong 22, in this research, PAR was higher in the T3 and T4 treatments than in the control plots during all winter wheat crop growth stages. Regarding zhongmai, PAR with treatment T7 was considerably greater than control plot during booting period. Zhongmai 175 outperformed Jindong 22 cultivar, although overall T3 at the tillering stage recorded the highest PAR at 852.14 when compared to the control. T5 and T6 (Inorganic) in both cultivars produced less favorable results at the floweringing stage.

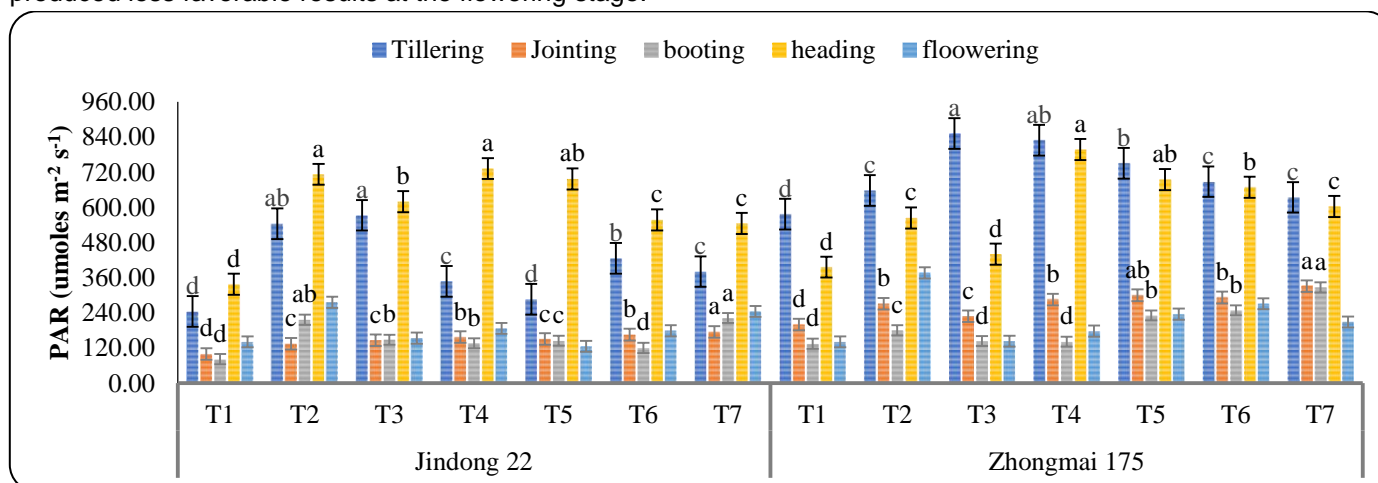


Figure 1. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on photosynthetic active radiation (PAR) of winter wheat. T1 (Control plot), T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE ( $n=3$ ).

### Photosynthetic parameters

Winter wheat plants' and nitrogen's photosynthetic traits are accurately reflected by the photosynthetic light response curve. The trend in the jindong 22 and zhongmai 175's photosynthetic light response curves during various growth stage is presented in figure. 2. Within a particular range, increasing nitrogen application led to an increase in photosynthetic (Pn) activity. The photosynthetic Pn of winter wheat was generally higher at heading phases than at the tillering and flowering ones. Regarding Jindong 17, over the course of the full growing season, the PN under treatment T4 (inorganic) at heading stages reached minimal 68.13 mmol m<sup>-2</sup> s<sup>-1</sup> value. It performed 12.87% better than the reference plot. When it comes to zhongmai 175, both cultivars recorded highest values of 70.90 mmol m<sup>-2</sup> s<sup>-1</sup> in the heading phase under treatment T4 throughout the growing season of winter wheat, while zhongmai 175 is greater than jindong 22 when comparison to treatment control. The lowest results under treatment T4 (Inorganic) during the floweringing stages were 11.36 and 12.33 when compared to control and other all growing phases.

At the floweringing phases of the T7 (Inorganic) treatment, Jindong 17 and zhongmai 175 achieved the maximum

stomatal conductance (GS) values of  $0.87 \text{ H}_2\text{O mol m}^{-2} \text{ s}^{-1}$  and  $0.90 \text{ H}_2\text{O mol m}^{-2} \text{ s}^{-1}$ , respectively presented in figure 3. Comparing the two cultivars to the control Zhongmai 175 performed better than Jindong22. The GS in T7 and T4 both improved by 82.54% and 28.21%, respectively. During the stages of heading and under Jindong 22 and by 78.95% and 27.67% during the stages of tillering and heading under Zhongmai 175 respectively. While under T4 (Pure Nitrogen) the tillering and jointing phases performed better than control, under T7 (Inorganic) the booting, flowering, and heading phases performed better. The minimum values were 0.21 and 0.25 under T6 (Organic) and T2 when compared to control plot (pure N).

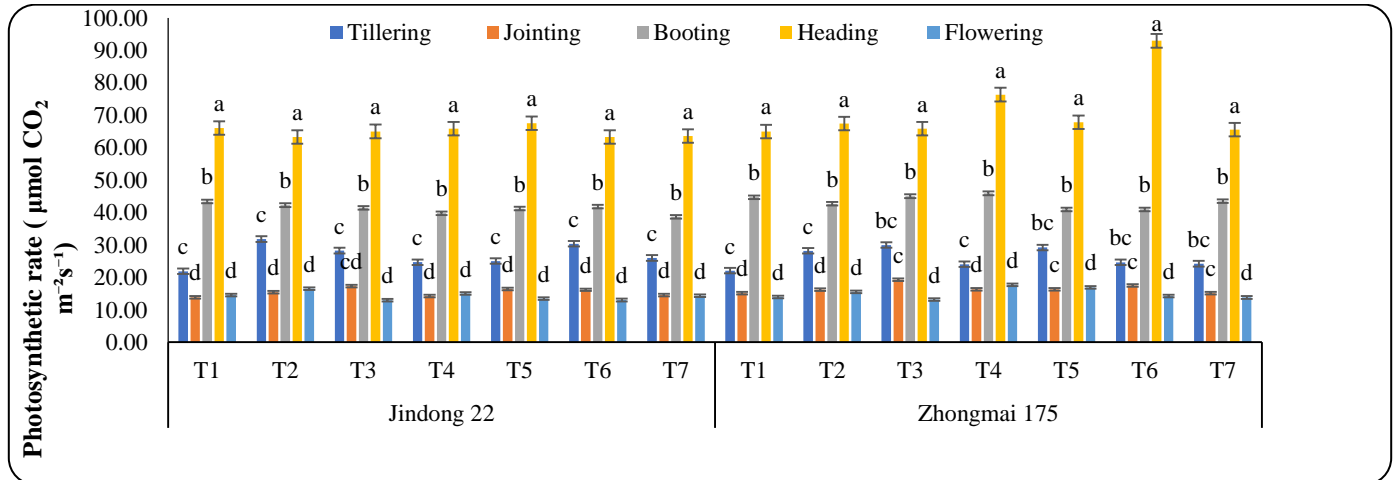


Figure 2. Effect of combined application of nitrogen (N) and manure + nitrogen (MN) on photosynthetic rate of winter wheat. T1 (Control plot.) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

Winter wheat's intercellular (Ci) response to nitrogen was presented in figure 4. With rise of application of nitrogen, Ci went up. In the winter wheat growing season the highest Ci values were  $1998.47 \text{ mol mol}^{-1}$  and  $\text{mol mol}^{-1}$ , with the majority of these values concentrated in the Tillerin stages. Both cultivars' Ci under the T3 therapy considerably increased when compared to the treatment control at all growth phases, with Jindong 22 outperforming Zhongmai 175 while rising by 109.27% and 137.23%. Comparing the maximum results under T2 to the control, they were 340.14 and 346.20.

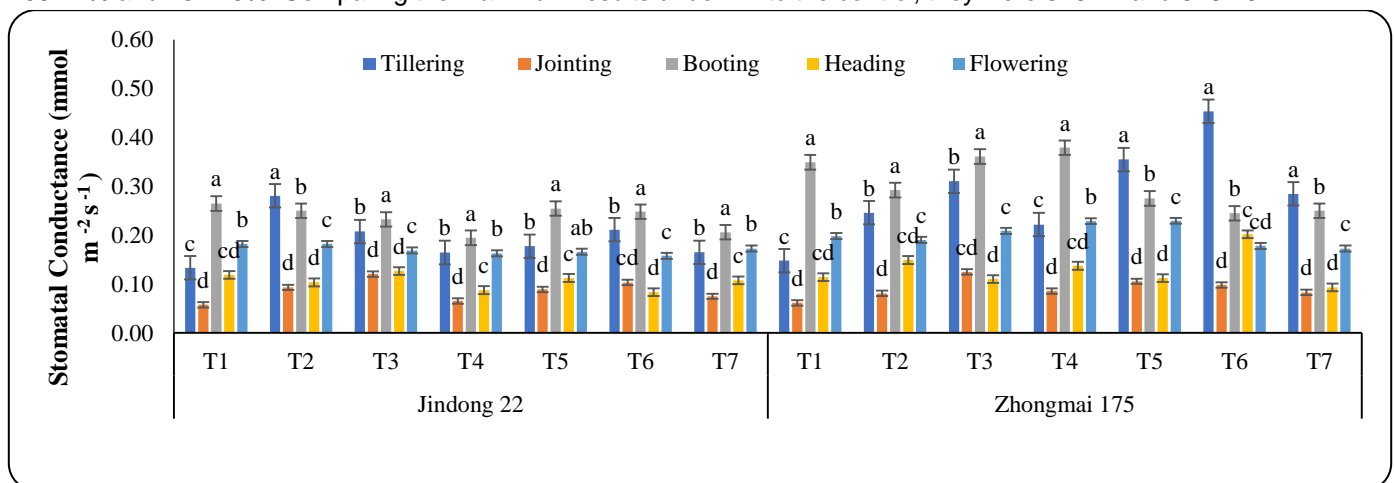


Figure 3. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on stomatal conductance of winter wheat. T1 (Control plot.) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

During the tillering, heading, and blooming periods of the T7 (Inorganic) treatment, jindong 17 and zhongmai 175 respectively, reported the greatest transpiration rates (Tr). A summary of the winter wheat crop's whole growing

season was published in figure 5. In comparison to the control plot, transpiration rates under T7 Treatment increased by 113.96% under Jindong22, by 53.12% at Zhongmai 175, and by 294.79% overall. When compared to control, Zhongmai 175 produced more than Jindong 22 during the heading and blooming phases of the plant's life cycle (T7). However, during the jointing phases, Jindong 17 and Zhongmai 175 recorded the minimum values of 2.22 and 2.01, respectively, under T4 and T5 (Inorganic).

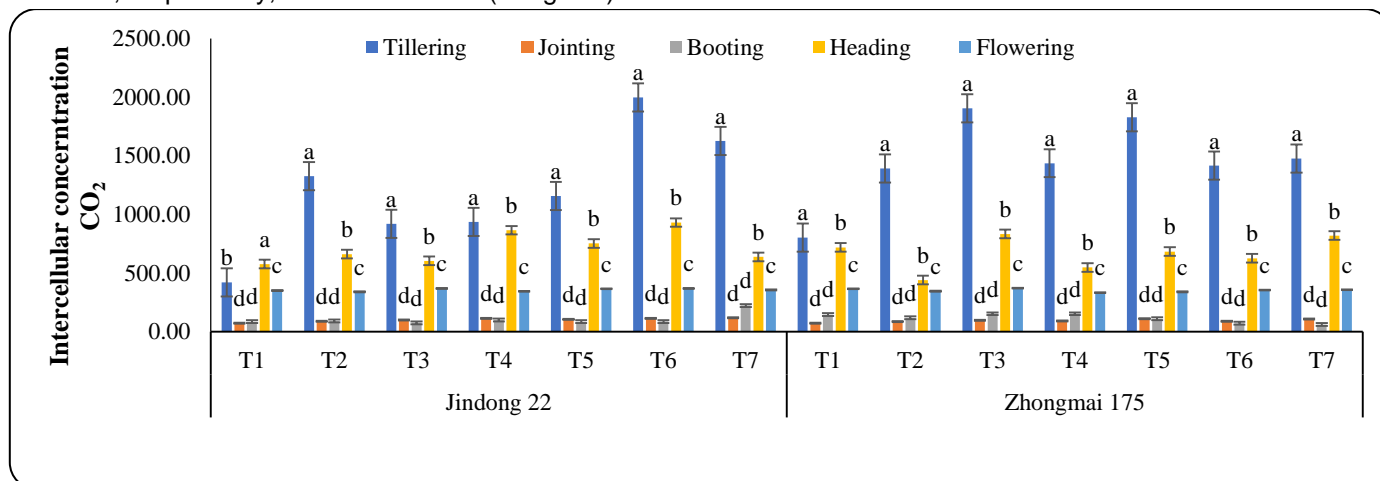


Figure 4. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on intercellular CO<sub>2</sub> concentration of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

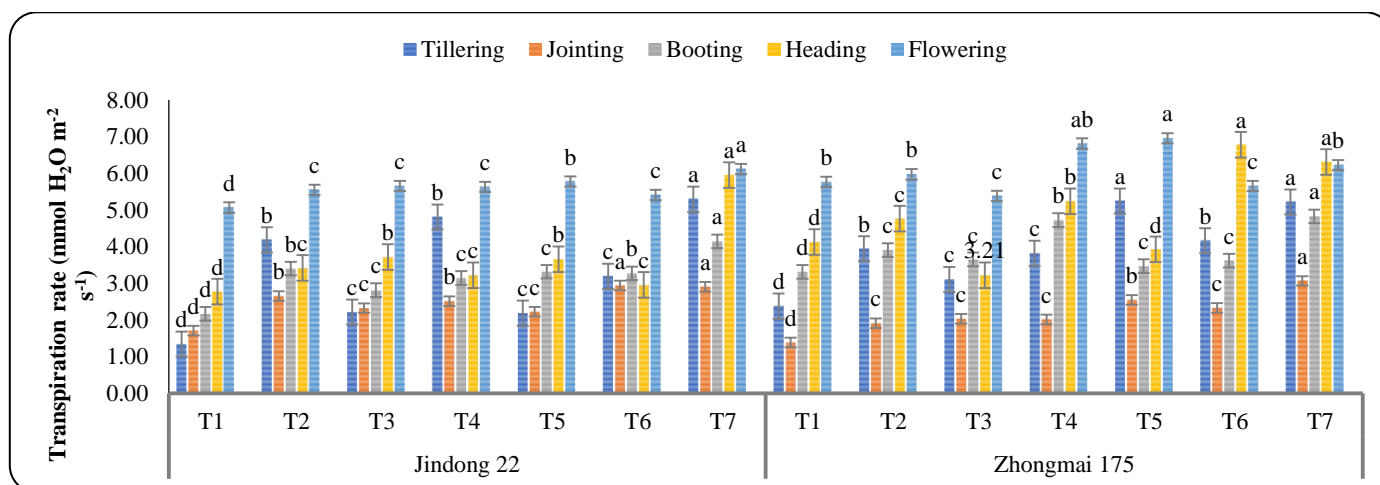


Figure 5. Effect of combine application of nitrogen (N) and manure + nitrogen (MN) on transpiration rate of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg<sup>-1</sup> ha (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

### Chlorophyll Fluorescence Parameters

The ratio Fv/Fm shows the PSII's highest photochemical efficiency. The experiment showed that there was a significant change in Fv/Fm between different growth stages and treatments presented in figure 6, proving that the applications of nitrogen and organic manure had no effects on the winter wheat's PSII's maximum photochemical efficiency. With different therapies, T3 and T7 levels are higher than T1 levels. The jindong 22 cultivar produced the best results at the heading stage when compared to the zhongmai 175 cultivar.

The winter wheat's PSII (PSII) quantum efficiency was shown in Figure 7. To be influenced by light and nitrogen. It shows that PSII increased significantly along with the rise in the nitrogen and organic manure range. The PSII of the T3, and T7 treatments was greater than that of the T1 treatment by 10.8 and 50.3% and 33 and 112.4%,

respectively. PSII originally dropped during numerous growth stages before climbing when more nitrogen and organic manure were applied. With differences of 6.7-22.3%, 0.8-46.0%, and 38.9-87.5%, respectively, between the PSII of Zhongmai 175 cultivar and Jindong 22 cultivar under the T3 and T7 treatments. In addition, Zhongmai 175 had the highest jointing stage of the FPSII of T7 treatment relative to Jindong 22 cultivar throughout the various growth phases.

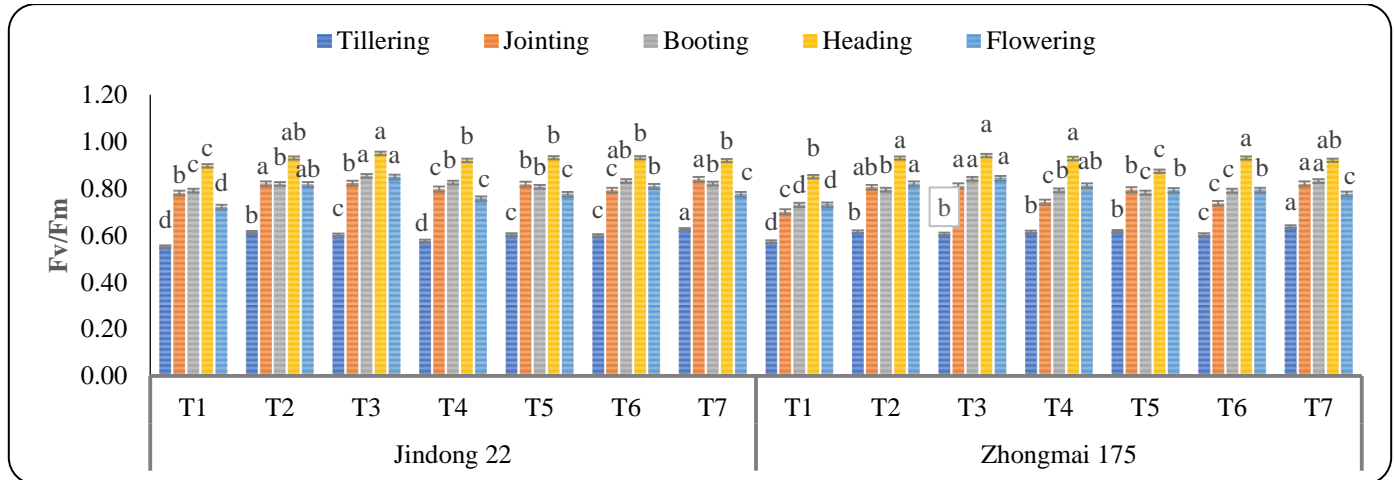


Figure 6. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on Fv/Fm of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

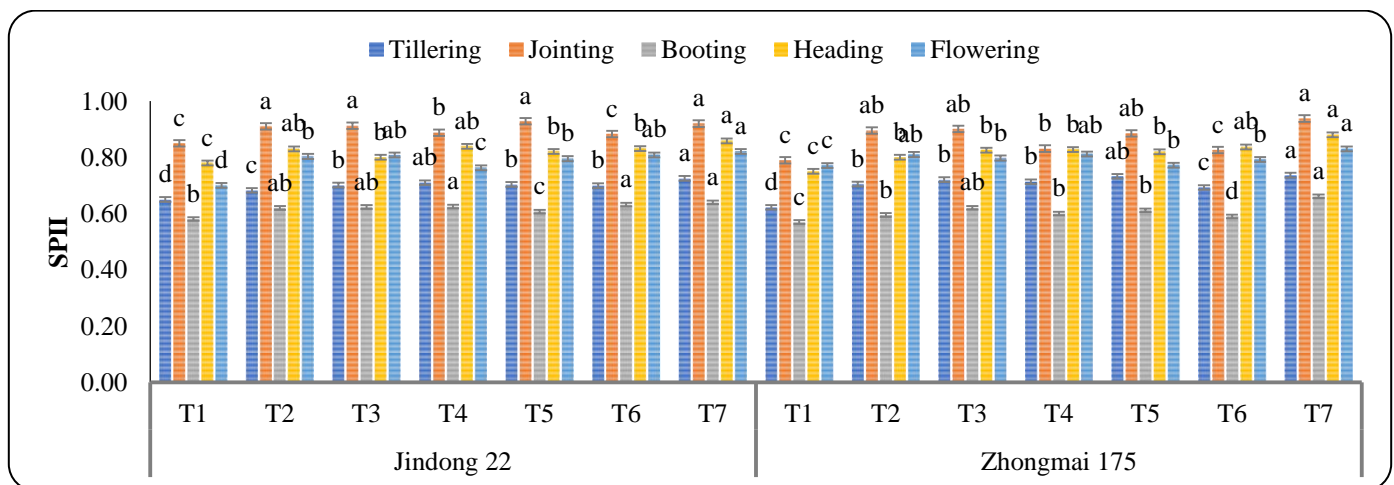


Figure 7. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on SPii of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg<sup>-1</sup> ha (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

The Q P characterizes the effectiveness with which the light quantum caught by QPSII was changed into chemical energy as well as the openness of the PSII reactions center. With increasing QP size, PSII's electron transfer activity rises. The QP's altering pattern roughly matches that of PSII presented in figure 8. Zhongmai 175 cultivar outperformed the jindong 22 cultivar during the flowering and jointing stage under T3 and T7 treatment as compared to T1 treatment.

The PSII reaction center's non radiative energy dissipation is reflected in the QN. The research indicated in figure 9 that at the jointing phase, QN grew significantly as nitrogen rate was raised, and that the QN of the T4 and T7 treatments were higher than those of the T1 treatment, respectively, by 21.8-23.5% and 40.2-41.3%. The QN of T4 treatment was significantly greater than other treatments at the heading stage, being 16.3-72.0% higher than those of the other treatments. The QN increased initially before falling. The jindong 22 cultivar treatment's QN was lowest under T3 and T5, being 2.2-20.0% and 9.6-29.1% lower than those under T5 and T6, respectively. When compared

to T1 treatments, T4 and T7 had QNs that were 5.5-71.6% and 14.0-75.5% higher, respectively. QN also increased as nitrogen administration increased when T4 and T7 were applied.

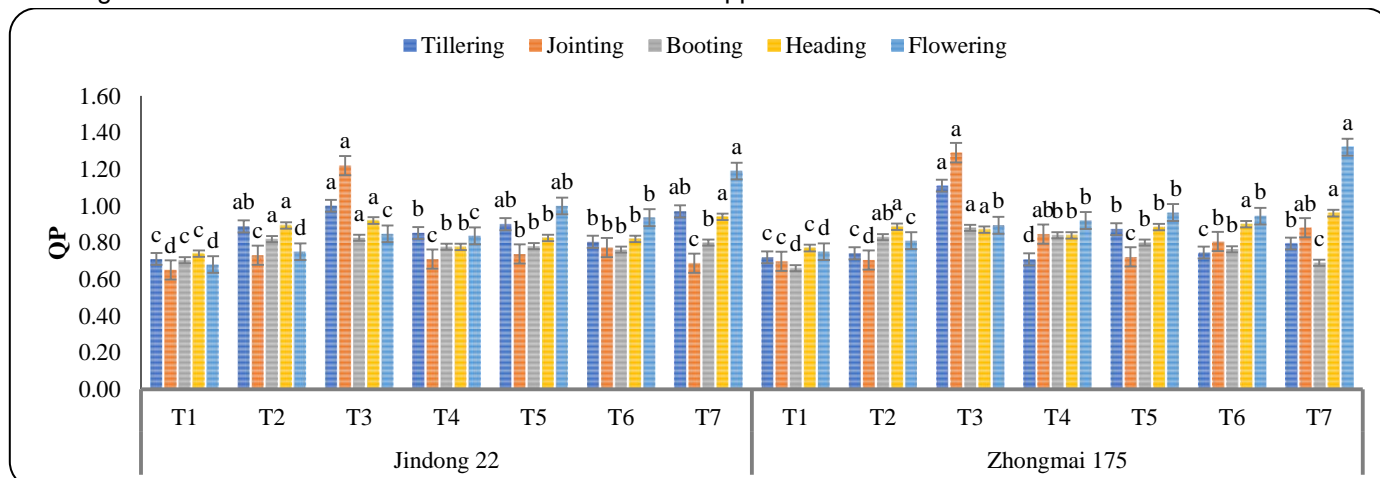


Figure 8. Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on QP of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg ha<sup>-1</sup> (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

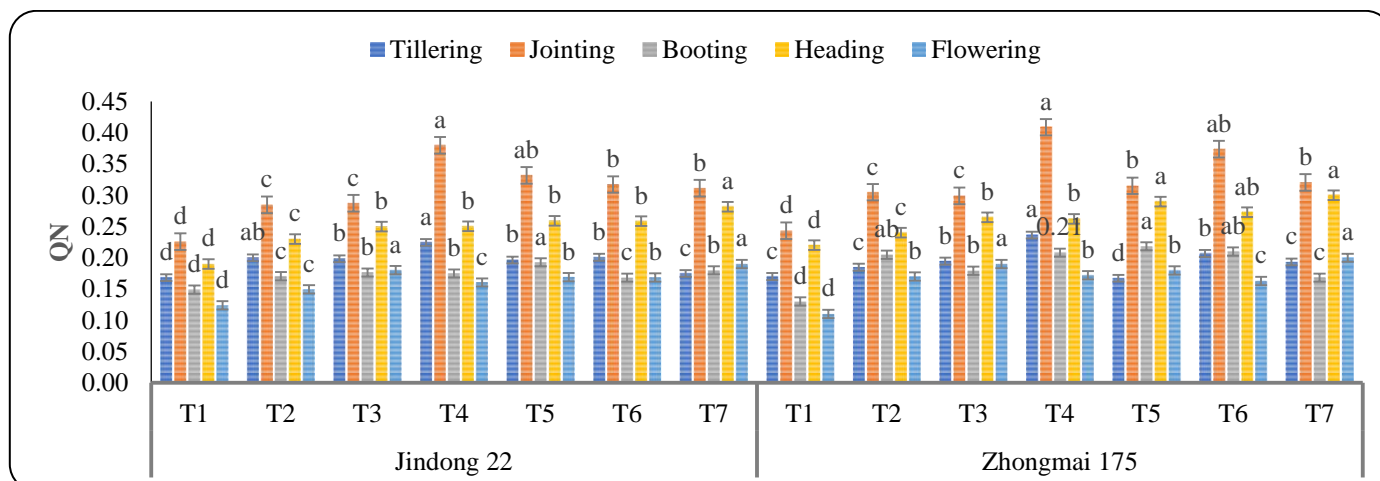


Figure 9 Effect of combined applications of nitrogen (N) and manure + nitrogen (MN) on QN of winter wheat. T1 (Control plot,) T2 (100 N kg ha<sup>-1</sup>), T3 (200 N kg ha<sup>-1</sup>), T4 (300 N kg ha<sup>-1</sup>), T5 100 NM kg<sup>-1</sup> ha (25% M+ 75% N), T6 200 MN kg ha<sup>-1</sup> (50% M +150% N and T7 300 MN kg ha<sup>-1</sup> (75% M+ 225% N). Means with separate bars and the same letters are not significantly different at a significance level of  $p < 0.05$ . The mean values are represented as  $\pm$  SE (n=3).

### Relationship of grain yield with photosynthetic and fluorescence traits

The relationship between photosynthetic and fluorescence factors and grain yield was significant and positively affected shown in figure.10. Moreover, the fitted equations (Pn)  $y = 0.0009x + 9.6304$   $R^2 = 0.4594$ , (Gs)  $y = 5E-05x + 0.4554$   $R^2 = 0.7932$ , (Ci)  $y = 0.2941x - 651.54$   $R^2 = 0.7523$ , and (Tr)  $y = 0.0003x + 0.1586$   $R^2 = 0.4876$  boosted grain yield by raising the photosynthetic parameters and fluorescence parameters. The relationship between grain yields and certain fluorescence characteristics (Figure. 6.1). The correlation coefficient varies between  $y = 3E-05x + 0.0852$   $R^2 = 0.3641$  and  $y = 9E-05x + 0.1766$   $R^2 = 0.2877$ ,  $y = 1E-05x + 0.7381$   $R^2 = 0.2108$ , and a weak association between yield with QN, QN, and Fv/Fm. In our investigation, grain yield and yield attributes were also revealed to have positive, significant associations.

### Correlation among grains yields and the other studied parameters

According to Pearson correlations study, the photosynthetic rate, intercellular CO<sub>2</sub> concentration, stomatal conductance, QP, and QN all showed significant positive connections with yield presented in figure 11. There was, however, a poor correlation between PAR and Tr, Fv/Fm. Additionally, the relationship between yield and transpiration rate was only marginally positive.

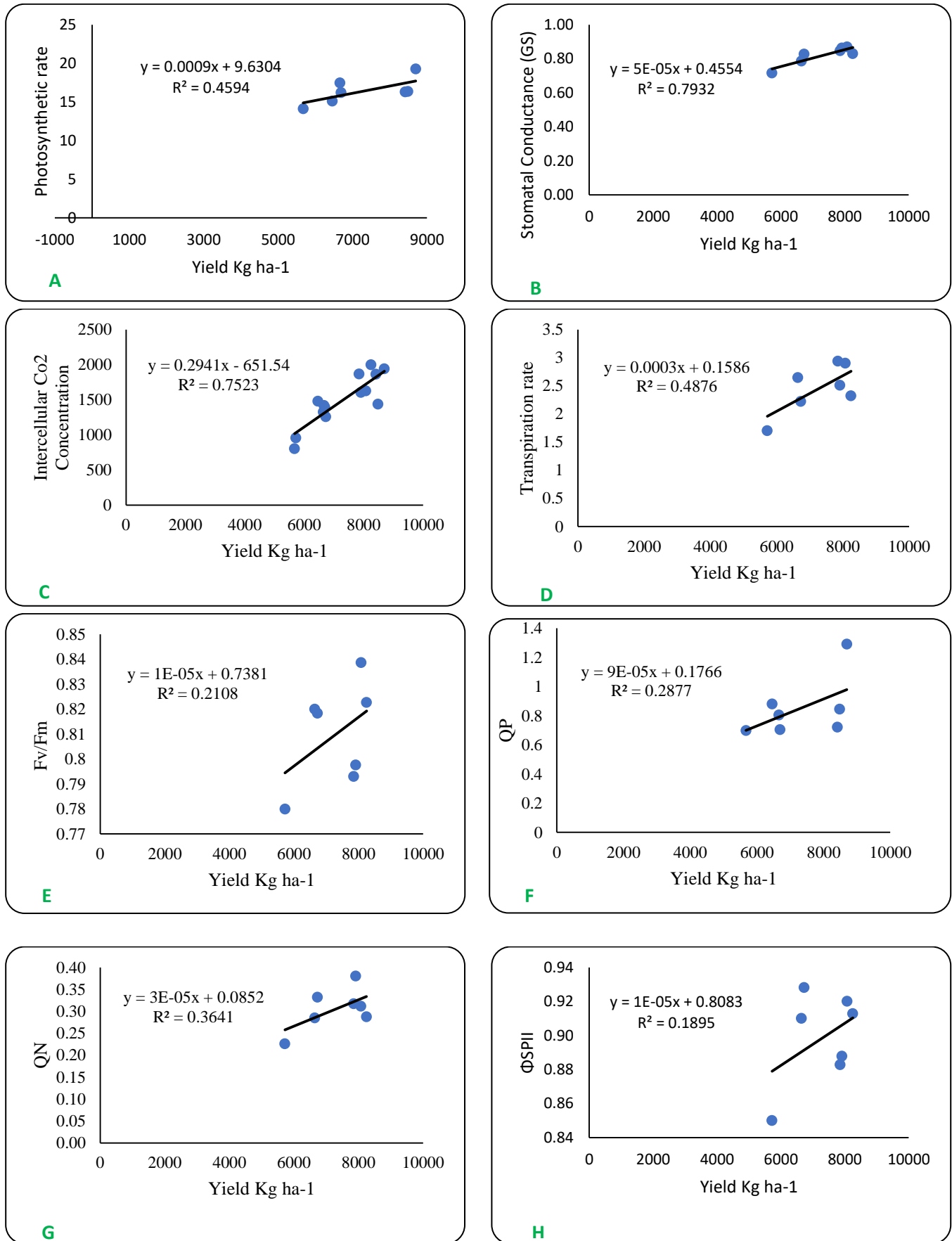


Figure 10 (A, B, C, D, E, F, G, H). Relationship of grain yield with PAR, Photosynth parameters and chlorophyll fluorescence traits.

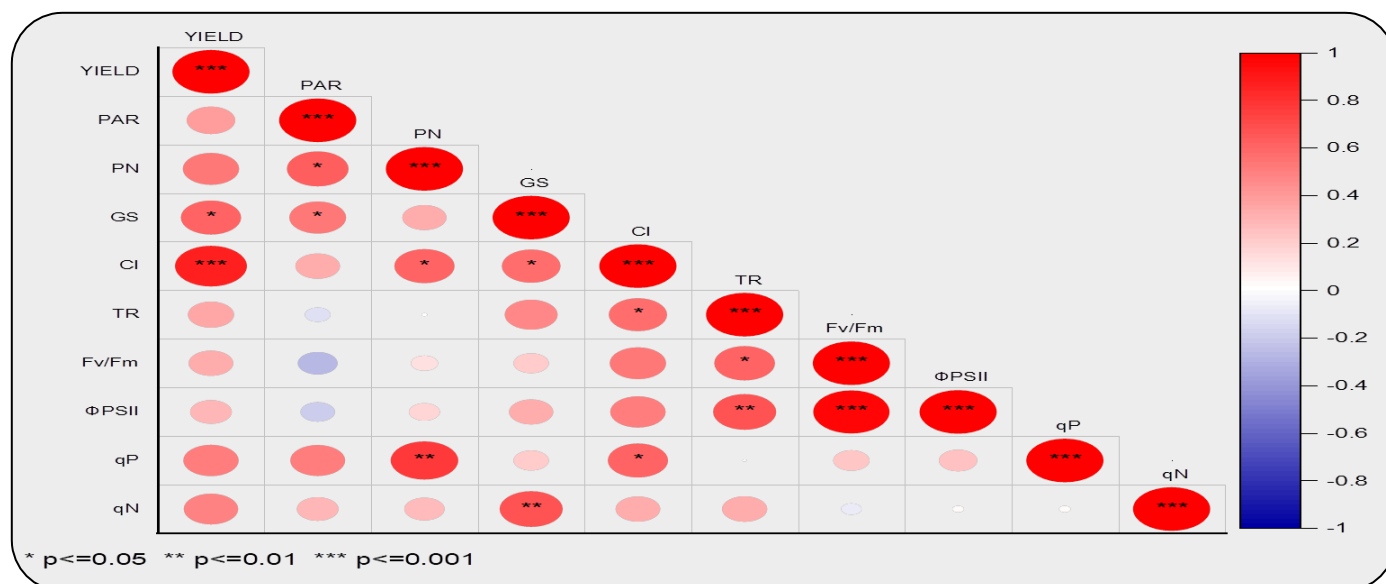


Figure 11. Relationship of grain yield with several indicators of wheat as affected by integrative effect of the manure and nitrogen applications of winter wheat.

## DISCUSSION

Growing environments is one of the key variations in winter wheat development and growth between cultivars and nitrogen + organic manure (Figure.1). This may result in enhanced crop growths and lower formation of photo integrates due to the shadow effects or reduced sunlight's acquisition (Li et al., 2015; Ju et al., 2009; Islam et al., 2014). The amount of received Photosynthetic Active Radiation (PAR) transmittance at the top of winter wheat altered and decreased with the application of nitrogen fertilizer and manure, although shadow intensity increased with the addition of more nitrogen and organic manure. Fertilizer input frequently decreased as it increased, and the amount of shadow grew more intense (Sainju et al., 2019; Lv et al., 2013). In this experiment, the beginning of the flowering stage (flowering started application of nitrogen in different treatments) was when winter wheat plants in the T3 and T4 treatments received the most PAR transmittance, whereas plants in the organic manure treatment received the least PAR transmittance both before and during the flowering stage. Thus, under  $200 \text{ kg N ha}^{-1}$ , the zhongmai 175 cultivar produced better outcomes during the winter wheat crop's flowering stage. This resulted in a much higher flower count, which may be attributed to the increased PAR transmittance brought on by the absence of the shadowing effects as well as the efficient use of soil resources ( water and nutrients), which promoted the creation of more carbohydrates during flowering. Winter wheat seedlings raised in nitrogen and organic manure typically have shaded early growth, which is unfavorable to yield and yield components. Similar to (Zhang et al., 2021; Ionut et al., 2018), our results show that winter wheat cultivars that received combined nitrogen + manure applications for longer than 60 days under T3 had decreased PAR transmittance at the top of the plant (70 to 75%) throughout the blooming stage (Mahmud et al., 2021).

The physical characteristics of plants include their rate of photosynthetic activity, stomatal conductance, transpiration rate, intercellular  $\text{CO}_2$  concentrations, and chlorophyll content. An important nutrient, nitrogen is also a crucial part of the production of proteins (Figure. 2 to 5). It is a fundamental part of grains and is directly related to how plants function. As a result, nitrogen has been generously added to agricultural products in recent seasons to increase winter wheat grain yields. However, the use of large amounts of N fertilizer in agriculture production directly affected the dangers of fertilizers, contributed to soil pollution, and brought about a malicious cycle in the agricultural environment, despite the fact that specific researches have shown that complex nitrogen application reduced the rate of leaves' photosynthetic activity (Ebert et al., 2022; Yangen et al., 2019).

The light transference of winter wheat's high nitrogen content, which was demonstrated to be noticeably superior to that of the crop's lower nitrogen content, may be significantly governed by the crop's high nitrogen content, according to (Luo et al., 2021; Cao et al., 2022). This research demonstrated that when  $200 \text{ kg N ha}^{-1}$  was sprayed after blooming, winter wheat's net photosynthetic rate, stomatal conductance, and transpiration rate all significantly increased. Additionally, it demonstrated a significant drop in the intercellular  $\text{CO}_2$  concentration. This might be

because diverse techniques enhanced the light sites for agricultural output while simultaneously applying a high rate of nitrogen. As a result of both of these factors acting simultaneously, photo thermal resources were more efficient because there was adequate nitrogen on hand. To prevent the leaves' initial matting during the later stages of winter wheat, the ageing period of all plants was postponed, and the function period was extended. Additionally, the individual plants thrived and had a sizable amount of green leaf area (Machado et al., 2021; Khan et al., 2022; Fan et al., 2011). The results of this study revealed that, despite nitrogen fertilizer's potential to increase plants' capacity to produce chlorophyll and to be one of the most significant active factors in modifying plants' leaf photosynthetic capacity (Manik et al., 2019; Deng et al., 2006), photosynthetic activity was directly related to leaf photosynthetic rate and crop yield was an important factor in crop yield increases (Vibhuti et al., 2020; Voltr et al., 2011).

This research study demonstrates that the photosynthetic rate of winter wheat often increased initially after the water, soil and air contents were similar, then stabilized with the enhancement of nitrogen application (Figure. 2 to 5). The intercellular CO<sub>2</sub> concentration, transpiration rate, and net photosynthetic rate all showed similar trends, suggesting that winter wheat can increase its photosynthetic rate given a sufficient supply of nitrogen. Appropriate nitrogen fertilizer application can increase the above-ground photosynthetic rate of winter wheat, enhance the transfer and development of dry matter production, and promote plant yield and growth (Figures 2 to 5). This study's results showed that winter wheat leaves of the Zhongmai 175 cultivar with N T3 and T7 (200 kg/ha<sup>-1</sup> and 300/kg ha<sup>-1</sup>) fertilizers all extended to the optimal level for photosynthetic traits. This may be because the right amount of nitrogen fertilizer encouraged the synthesis of chlorophyll, which in turn allowed the winter wheat plants to maintain active defense enzymes at the same time. A few studies have also demonstrated the value of using better nitrogen fertilizer to increase winter wheat yield and enhance canopy atmosphere. However, an abnormal canopy structure brought on by an abundance of leaves would decrease the yields of winter wheat grains Duan et al., 2014; Davidar et al., 2010). The distribution, dissipation, and transmission of light energy by leaves can be systematically reflected by the dynamic properties of chlorophyll fluorescence (Doe et al., 2018). According to this study, as the degree of shade increases, wheat flag leaves' PSII and QP greatly rise while their QN significantly falls. This work shows that the lights energy received by the antenna pigments in PSII loses less heat and that the partial stimulated light energy is used extra efficiently in shaded settings (Deng et al., 2006; Schjonning et al., 2018). Wheat benefits from increased nitrogen fertilizer because it increases the plant's capacity to absorb light energy, convert light energy more efficiently, and successfully use that light energy for photosynthesis (Narula et al., 2001). This study found that enough nitrogen (N<sub>2</sub>) improved PSII, QP, and decreased QN in wheat under no shading and light shading. (Padalia et al., 2022) claim that while nitrogen fertilizer has a weaker regulating impact on PSII when exposed to shadow than it does when exposed to normal light, it has a higher regulating impact on QP and QN. Additionally, our results showed that the Fv/Fm of the Jindong 22 and Zhongmai 175 cultivars were not significantly altered by any treatment. The decrease in Fv/Fm is noticeable in Kaifeng, though, and might be blocking PSII from taking part in photochemical reactions and possible photosynthetic activity. In the course of our research, we found that the control plot treatment significantly reduced PSII, indicating a decline in PSII's electron transport activities (Figure 6 to 9). These results suggest that winter wheat leaves may be less photochemical active during the grain filling and blooming stages due to N deficiency. Furthermore, a surplus of organic manure and nitrogen is not conducive to the effective utilization of solar energy captured.

## CONCLUSIONS

The integration of nitrogen fertilization with manure presents a promising approach to improving the photosynthetic traits and productivity of winter wheat under sustainable agricultural practices. Integrated application of 300 kg M+N ha<sup>-1</sup> with (75% manure + 225% nitrogen fertilizer at the flowering, jointing, and heading stages successfully increased the winter wheat's photosynthetic active radiation (PAR), photosynthetic characteristics, and chlorophyll fluorescence traits of the Zhongmai 175 genotype. integrated application of inorganic fertilizer with manures influenced on chlorophyll fluorescence and photosynthesis traits of the winter wheat crop. Combined application of nitrogen + manure improved the PSII quantum efficiency (fv/fm and QP), reflecting higher light energy conversion into chemical energy. Moreover Zhongmai 175 genotype exhibited superior photosynthetic and fluorescence characters under integrated fertilization compared to Jindong 22. However strong positive correlation between grain yield and photosynthetic traits (Pn, Ci and Gs) highlight the potential of integrated fertilization in boosting winter wheat productivity. This study recommended that use of combine application of nitrogen and manures fertilizers could be an effective strategy to enhance grain yield and photosynthetic traits of winter wheat crop.

## AUTHOR CONTRIBUTIONS

M.S. Kubar: Conceptualization, Methodology, Writing - Original Draft, YW: Supervision, Reviewing and Editing, K.A. Kubar: Data Curation, Formal Analysis, Q. Baloch: Investigation, Resources, M. Narejo: Writing - Review & Editing, G.B. Bugti: Validation, Visualization, K.L. Khattri: Software, Formal Analysis, H.R. Lakho: Investigation, Methodology. S. Ahmed: Data Curation, Investigation, Mahazullah: Resources, editing, A.A.Kubar: Conceptualization, Funding Acquisition

## COMPETING OF INTEREST

The authors declare no competing interests.

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