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Full Length Article

Nitrogen Fertilization Effects on Growth, Leaf Gas Exchange and Chlorophyll Fluorescence of *Brassica juncea*

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Abstract

This study assessed the effect of nitrogen (N) on growth, leaf gas exchange and chlorophyll fluorescence traits of *Brassica* (*Brassica juncea* L.) under greenhouse condition. The experiment was consisted of five N concentrations including 0, 50, 100, 150 and 200% of the standard N concentration in Hoagland's solution equivalent to 0, 7.5, 15, 22.5 and 30 m*M*. Plant response to N nutrition was evaluated at 40, 60 and 80 days after sowing (DAS). Results showed that the N effectively increased the plant biomass ($P < 0.05$) when N increased from 0 to 22.5 mM but there was no significant difference among all growth attributes with further increase in N. The photosynthesis (P_n) rate remarkably improved with the increase in N concentration. However, there was a significant decline of 21.32% ($N_{150\%}$) and 41.56% ($N_{200\%}$) at high N nutrition compared to $N_{100\%}$ at 80 DAS. Stomatal Conductance (g_s) and transpiration rate (T_r) increased with an increase in N nutrition up to 60 DAS however, both factors showed a reduction above $N_{100\%}$ when measured at 80 DAS. Intercellular CO_2 concentration (Ci) was found lower in plants subjected to high N concentration. Nitrogen supply also increased the photochemical efficiency (Fv/Fm), indicating that light energy conversion efficiency and potential photosynthetic reaction center activity was improved. There was no significant difference between 15–30 m*M* N treatments, suggesting that excessive application of N (N>22.5 Mm) could not enhance the growth of *Brassica*. © 2020 Friends Science Publishers

Key words: *Brassica*; Chlorophyll fluorescence; Electron transport rate (ETR); Nitrogen; Photosynthesis; SPAD

Introduction

The green leafy vegetables that provide tremendous health-beneficial vitamins, minerals, antioxidants, and dietary fibers are low in calories, but also cause low sodium content, lack of trans fatty acids and saturated fat (Mazahar *et al.* 2015). These are also enriched with nitrate and nitrite injurious to human health when used in surplus (Additives *et al.* 2017; Roila *et al.* 2018). Nitrogen fertilization plays the most important role in the growth and development of plants being limiting nutrient which affects yields as well (Ozdemir *et al.* 2010; Rehman *et al.* 2013; Rasool *et al.* 2019). Excessive fertilization, however, usually results in the accumulation of nitrate, which is often harmful. High N fertilization also increases the level of carotene and decreases the level of vitamin C in plants (Chenard *et al.* 2005). Moreover, the decreased nitrogen limits the growth, yields and leaf area index (Cheema *et al.* 2001).

Photosynthesis is the primary physiological process of converting solar energy into chemical energy. The obtained chemical energy is used to synthesize organic materials from inorganic substances such as carbon dioxide (CO_2) and water (H_2O), resulting in oxygen release (O_2) (Tkemaladze and Makhashvili 2016; Xiankui and Chuankuan 2018). Photosynthetic pigments are the centroid dependent on the number of processes of photosynthesis, including primary reaction, photophosphorylation, and assimilation of CO_2 (Trebst and Avron 2012). Chlorophyll fluorescence is another tool associated with photosynthesis and its underlying processes. Therefore, chlorophyll fluorescence may be used to research the relationship between stress and photosynthesis. Any stress effect on photosynthesis can be detected by changing the dynamics of the induction of chlorophyll fluorescence (Kong *et al.* 2016). Chlorophyll fluorescence is regarded as a fast and better indicator among other physiological indicators in the detection of stress in plant (Xie *et al.* 2019; Rasool *et al.* 2020). The photosynthesis and fluorescence are significantly affected by N application rates (Stagnari *et al.* 2015; Wu *et al.* 2017). The maximum efficiency of PS II photochemistry under dark adaptation (Fv/Fm) and photochemical quenching (qP) decreased as N deficiency increased and non-photochemical quenching (qN) increased. This fluorescence change affects PS II's photochemical activity and reduces photosynthesis (Yin and Tian *et al.* 2013; Xue *et al.* 2014). It had been reported in maize that higher rate of net photosynthesis rate (P_n), PS II activity, primary chemical conversion efficiency, and photochemical quantum ultimately increased the N use efficiency (Wu *et al.* 2019).

In this study, the ability of chlorophyll fluorescence to investigate the effects of deficit and N surplus on PS II in *Brassica juncea L.* was assessed. The specific aims of this work were: (i) to measure the effect N concentration on biomass and plant physiological traits; (ii) to assess the effects of deficient and surplus N on chlorophyll fluorescence in *Brassica* leaves (iii) to analyze the relationship between photosynthetic parameters and SPAD values and how they affect each other in *Brassica* when subjected to different N fertilization concentration. The results can provide a practical basis for optimal N fertilizer application in *Brassica*.

Materials and Methods

Growth conditions and materials

The experiment was conducted in a greenhouse located at Jiangsu University, Zhenjiang, Jiangsu, China (32.20N, 119.45 E) from October 1 to December 20, 2018, during fall-winter. The average air temperature and relative humidity in the greenhouse were 20.20°C and 77.89%, respectively. *Brassica* crop was grown pots filled with perlite substrate up to 2.5 cm below from the top. The size of the used pots was 25 cm in height and 19 cm in diameter. The same level of water and nutrients were applied during the first 20 DAS for their proper establishment after which experimental treatments were imposed. All the measurements were taken at 40 DAS (rosette stage), 60 DAS (late vegetative stage) and 80 DAS (harvesting stage).

Experimental details and measurements

The treatments consisted of four levels of nitrogen (N): 0% (N_0), 50% ($N_{50\%}$), 100% ($N_{100\%}$), 150% ($N_{150\%}$) and 200% ($N_{200\%}$) of the standard N concentration in Hoagland's solution which were equivalent to concentrations of N as 0, 7.5, 15, 22.5, 30 m*M*, respectively. Where N_0 was kept as control (CK). A completely randomized block design with three replications for each treatment was designed. At the end of the experimental period, the total leaf area per plant was measured by a leaf area meter (Handheld Laser Leaf Area Meter, CI-203, CID Bio-Science, Camas, Washington, U.S.A.). The shoot fresh weight (SFW) and shoot dry weight (SDW) and dry matter content (DMC%) were determined.

Leaf gas exchange measurements

The leaf gas exchange parameters of *Brassica* leaves during its growth and development leaves were measured at 9:00–11:00 a.m. The measured leaf gas exchange parameters were net photosynthetic rate (P_n, μmol $m^{-2}s^{-1}$), leaf stomatal conductance (g_s, mol$m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci, µmol mol^{-1}) and transpiration rate (T_r, mmol $m^{-2}s^{-1}$). During the measurements the basic conditions of photosynthetic active radiation (PAR), temperature and CO_2 concentration were maintained at 800 μmol m^{-2} s^{-1}, 28°C and 500 μmol mol^{-1}, respectively. The data were measured using a portable LI-6400XT photosynthesis measurement system (LI-COR, Lincoln, NE, U.S.A.). The water use efficiency (WUE, μmol $mmol^{-1}$) was calculated from the measured values of P_n and T_r using the following equation:

$$WUE = \frac{Pn}{Tr} \quad (1)$$

Where P_n is the net photosynthetic rate and T_r is the transpiration rate.

Leaf chlorophyll content and chlorophyll fluorescence

The leaf chlorophyll content (Chl) was measured using the 502 SPAD chlorophyll-measuring (Minolta, Japan). Chlorophyll content was determined at 40 DAS (rosette stage), 60 DAS (late vegetative stage) and 80 DAS (harvesting stage).

A portable fluorimeter (MiniPAM; Walz, Effeltrich, Germany) was used to measure Leaf chlorophyll fluorescence. The targeted plants were covered with black plastics and held in dark 30 min before chlorophyll fluorescence traits measured. The system was made to run according to the manufacturer's instructions after the dark adaptation of 30 min. Fluorescence data was collected containing the following parameters as Fm, dark-adapted minimum fluorescence (Fo), maximum fluorescence (Fm), light adapted maximum fluorescence (Fm′), maximal PS II quantum yield (Fv/Fm), Quantum yield of non-regulated heat dissipation in PSII Y(NO), the nonphotochemical quenching (NPQ), and the quantum efficiency of open PSII reaction centres (Fv'/Fm') was determined. Brassica juncea L. plants were sampled at 40, 60, and 80 DAS in each pot to measure the fluorescence induction kinetics parameters. In each procedure, three regions of concern were selected for calculation in a single leaf. The calculated values were estimated on an average. The measurement of fluorescence was computer-controlled and the procedures were as follows; at initial, switched on the measuring light and actinic light then switched on saturation pulse light after an adaptation of the 20 s and increase kept on increasing the actinic. The saturation pulse light was kept on switching after each 20 s adaptation and the same procedure was repeated 12 times under the photosynthetically active radiation intensity (PAR) as 1, 21, 41, 76, 134, 205, 249, 298, 371, 456, 581 and 726 μmol m^{-2} s^{-1}, respectively.

Statistical analysis

All the data obtained were subjected to variance analysis (ANOVA) to differentiate significant differences ($P < 0.05$). Using Statistix 8.1 software, these mean data were statistically analyzed using a randomized complete block design, and mean results were compared at ($P < 0.05$) through the Tukey test. Regression and correlation coefficients were calculated by standard methods with S.P.S.S. software (v. 13.0, S.P.S.S. Inc.).

Results

Effect of N concentration on growth of *Brassica*

At the final harvest, (80 DAS), the *Brassica* plants were evaluated under different N rates (Fig. 1). N treatments showed a significant effect on the above-ground biomass of *Brassica* when measured at 80 DAS (Fig. 2). Shoot fresh weight (SFW) increased significantly ($P < 0.05$) with an increased concentration of N, but there was no significant increase above 22.5 m*M* concentration (Fig. 2). Shoot dry weight (SDW) ranged from 93.67 to 188.0 g per pot at 0 ($N_{0\%}$) -30 m*M* ($N_{200\%}$) N rate. The maximum SDW was found at 30 m*M* but there was no significant difference compared to SDW when measured at N concentration of 22.5 m*M*. A significant decrease ($P < 0.05$) was observed when the N concentration application reduced from 22.5 to 15.0 m*M*. A similar trend was observed in the leaf area which significantly lower in plants grown under control ($N_{0\%}$) and low N ($N_{50\%}$) supply than $N_{100\%}$, $N_{150\%}$ and $N_{200\%}$. However, $N_{100\%}$, $N_{150\%}$ and $N_{200\%}$ which remained insignificant with each other. Leaf area ranged from 0.144 to 0.260 $m^2 plant^{-1}$ at 0 m*M* N to 22.5 m*M* N concentration, respectively (Fig. 2). Based on SDW, at the harvest stage, 22.5 m*M* N rate was optimum for plant growth. At the final harvest, the maximum amount of shoot fresh and dry weight, dry matter content and leaf area per plant increased 100.70, 86.33 and 80.56% of the control treatment respectively.

Effects on chlorophyll contents (Chl)

Comparing with control (N_0), the leaf chlorophyll contents significantly increased ($P < 0.05$) in the other treatments with the increase in N rates (Fig. 3). While comparing the effect of N concentration on leaf chlorophyll contents, the highest and lowest increase was observed under $N_{200\%}$ (30.9, 23.4 and 51.53) and $N_{50\%}$ (9.0, 7.24 and 27.65) at 40, 60 and 80 DAS respectively. Among SPAD values, the significant difference between $N_{150\%}$ and $N_{200\%}$ was observed at 60 DAS as compared to measurements taken on 40 and 80 DAS under the same treatments. Overall SPAD values increased during the first two stages of measurements while decreased in all treatments during stage near to harvest.

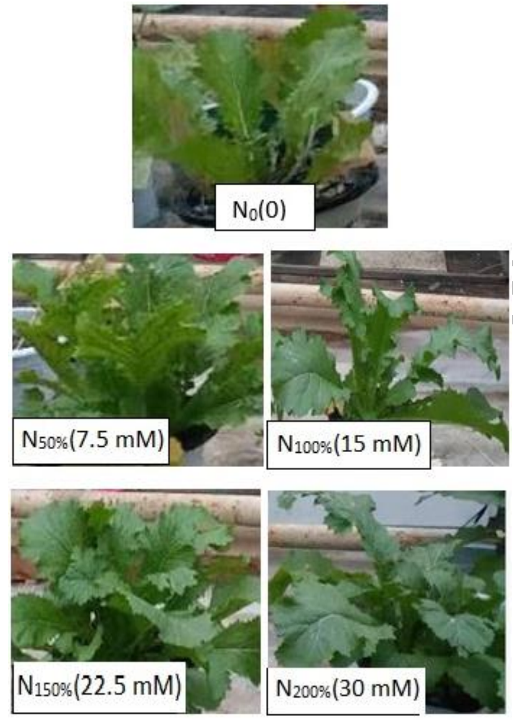

Fig. 1: Growth of *Brassica* under different Nitrogen application at 80 DAS (days after sowing) prior to harvest

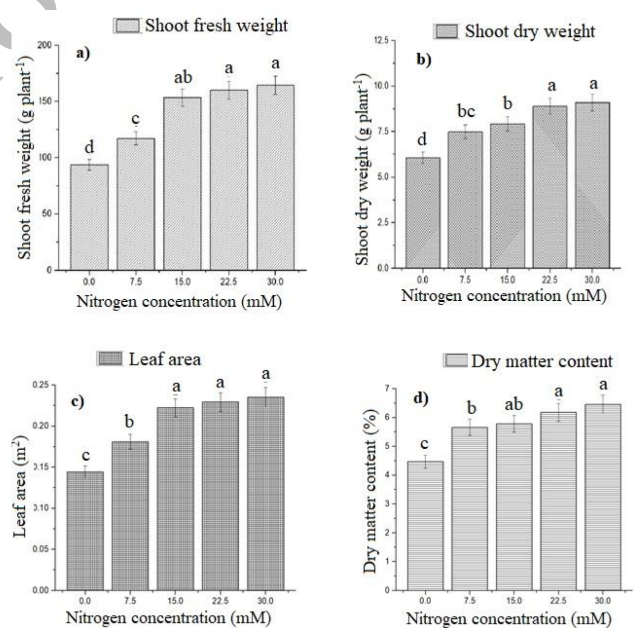

Fig. 2: Growth response of *Brassica* under nitrogen application on harvesting at 80 DAS. (a) Shoot fresh weight (SFW); (b) Shoot dry weight (SDW); (c) Leaf area (LA); (d) Dry matter content (DMC)

Leaf gas exchange traits

Leaf gas exchange parameters comprising P_n, g_s and T_r showed different response under lower to higher N

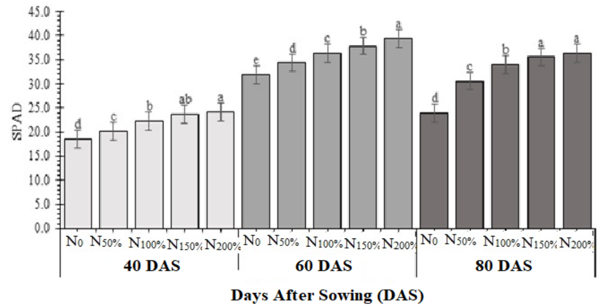

Fig. 3: Effect of different Nitrogen concentrations on SPAD values. *

*Values within the same measured day followed with different letters are significantly different at p < 0.05 according to Tukey's test.

application when compared to control (N_0) at different stages.

No significant difference was found for P_n at $N_{100\%}$, $N_{150\%}$, and $N_{200\%}$ when measured at 40 DAS. Similar trends were observed in stomatal conductance showing no significant increase with the increase in N concentration above $N_{100\%}$ (15 m*M*) at the 40 DAS.

While taking the measurements at 60 DAS, the maximum P_n was found in $N_{200\%}$ with an increase of 64.25% as compared to control treatment and the maximum stomatal conductance was observed in $N_{150\%}$. The transpiration rate increased with an increase in N concentration during the first two growth stages, however, it showed a decline when the N concentration crossed the $N_{100\%}$.

Afterward, higher N concentration showed a decline in photosynthesis rate when the concentration increased above $N_{100\%}$ (15 m*M*). The P_n decreased up to 30.62 and 50.89% at $N_{150\%}$ and $N_{200\%}$, respectively (Table 1) at the harvesting stage. At 80 DAS, the maximum stomatal conductance was observed in $N_{100\%}$.

Irrespective of treatments, photosynthesis along with associated leaf gas exchange traits showed decline under all N treatments at 80 DAS when compared with the measurements taken 60 DAS. This decline in photosynthetic parameters can be explained due to the leaf senescence. However, Ci did not follow the same trend as of P_n, g_s, and T_r (Table 1). Thus, the fall in P_n is not completely due to leaf senescence but also due to stomatal control that underwent the effect of N concentration. The calculated water use efficiency was highest in $N_{100\%}$ (2.80, 2.39) and $N_{150\%}$ (2.54, 2.44) without causing a significant difference between subsequent measured stages of 60 and 80 DAS, respectively.

Effects of N fertilizer on chlorophyll fluorescence parameters

The ANOVA showed that Fm and Fv/Fm significantly ($P <$ 0.05) increased with increasing N application rate until $N_{150\%}$, where it reached to its peak value followed by a significant decrease in $N_{200\%}$ compared to $N_{150\%}$. Similar response was observed for Fm' and Fv'/Fm' with increase in N concentration up to 22.5 m*M* but further increase in N up to 30 mM decreased only when measured at 80 DAS. While Y(NO) and NPQ showed the opposite trend compared to Fm and Fv/Fm as NPQ values decreased with the increase in N application rates. An increment was found in chlorophyll fluorescence parameters as the N application rate increased for *Brassica*. Compared to control (N_0), maximal PS II quantum yield (Fv/Fm) increased by 9.81, 9.96,10.10 and 11.12% at 40 DAS as N rates increased from 7.5, 15, 22.5, to 30 mM and by 4.63, 7.19, 20.56% and 2.06%, as N rates increased from 7.5, 15, 22.5, to 30 m*M*. However, the increasing rate became redundant at 30 m*M* ($N_{200\%}$) N rate at 80 DAS (Table 2).

Responses of ETR under different N concentration

The effect of N concentration increased with the development in the growth period. The ETR varied with N concentration, and $N_{100\%}$ (15 m*M*) resulted in a peak curve. With the increase in PAR initially, the ETR of the *Brassica* leaves increased initially but after a certain value of PAR, a decrease in ETR occurred at each stage of measurement. At 40 DAS, the N concentration had not significant effect on ETR (Fig. 4a). The ETR increased primarily as PAR increased. N_0, the control treatment resulted in the lowest ETR curve while the largest values were found in $N_{100\%}$ (15 m*M*). Moreover, the reduction in ETR became apparent when increased beyond 400 μmol m^{-2} s^{-1}. The results suggested that ETR was mainly affected by PAR, while less affected by N concentration at the 40 DAS.

ETR remained highest in $N_{100\%}$ when measured at 60 DAS (Fig. 4b). According to results, $N_{100\%}$ showed the highest values of ETR while there was no significant difference between N_0(0), $N_{150\%}$ (22.5 m*M*) and $N_{200\%}$ (30 m*M*) which in turn shows that the ETR decreased with the excessive application of N and with excessive deficiency in N application. The difference among treatments reached a maximum at 60 DAS. ETR curves showed decline under all N treatments at 80 DAS (Fig. 4c), when compared with the measurements taken 60 DAS. The decreasing trend of ETR was ordered as $N_{100\%}>N_{50\%}>N_0>N_{150\%}>N_{200\%}$, suggesting that adequately low N could accelerate the transportation of the photosynthetic electron, whereas extremely low or high concentration of N had no positive effect on ETR.

Regression relationship between g_s, Chl (SPAD) and P_n

The significant linear relationship was obtained between g_s, Chl (SPAD), and P_n ($P < 0.001$) at 40, 60 and 80 DAS. The coefficients (R^2) between g_s and P_n were 0.92 and 0.93 and 0.92 at 40, 60 and 80 DAS, respectively. The R^2 of the linear regression between SPAD and P_n was 0.85, 0.78 and 0.34 at 40, 60 and 80 DAS, respectively (Fig. 5).

Discussion

Several studies have elucidated the sensitivity of leaf growth

Table 1: Effect of different nutrient concentration on leaf gas exchange parameters

DAS	Treatments	P_n	g_s	Ci	Tr	WUE
		(μmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(μmol mol^{-1})	(mmol m^{-2} s^{-1})	(μmol mol^{-1})
40	N_0	13.22d	0.407c	299.2a	7.39d	1.77b
	$N_{50\%}$	18.57c	0.573b	292.3a	8.84c	1.79b
	$N_{100\%}$	23.88ab	0.643a	277.8b	10.57b	2.67a
	$N_{150\%}$	25.04a	0.687a	271.8b	12.39a	2.02b
	$N_{200\%}$	25.24a	0.690a	256.1c	12.40a	2.04b
60	N_0	9.15d	0.160d	249.5d	3.63b	2.52ab
	$N_{50\%}$	16.19c	0.507c	294.6a	9.83a	1.66c
	$N_{100\%}$	23.57b	0.650b	259.4c	8.82a	2.80a
	$N_{150\%}$	24.73ab	0.760a	262.8c	10.10a	2.54ab
	$N_{200\%}$	25.60a	0.630b	278.6b	10.39a	2.28b
80	N_0	6.51d	0.233c	308.9a	5.50c	1.18b
	$N_{50\%}$	12.00c	0.370b	296.2abc	6.76b	1.78ab
	$N_{100\%}$	21.44a	0.657a	287.7bc	8.98a	2.39a
	$N_{150\%}$	16.87b	0.423b	284.3c	6.19b	2.44a
	$N_{200\%}$	12.53c	0.387b	304.5ab	6.39b	1.65b

Values within the same columns followed with different letters are significantly different at $p < 0.05$ according to Tukey's test. DAS = days after sowing; Pn net photosynthetic rate; gs, leaf stomatal conductance; Ci, intercellular CO2 concentration and Tr, transpiration rate.

Table 2: Effect of nutrient concentration on chlorophyll florescence parameters in *Brassica*

DAS	Treatment	Fm	Fv/Fm	Y(NO)	Fm'	Fv'/Fm'	NPQ
40	N_0	0.482bc	0.693c	0.239b	0.459c	0.691c	0.066a
	$N_{50\%}$	0.489bc	0.761b	0.237bc	0.459c	0.750b	0.051ab
	$N_{100\%}$	0.498ab	0.762b	0.230c	0.484b	0.752b	0.028ab
	$N_{150\%}$	0.514a	0.763ab	0.307a	0.508a	0.756ab	0.026ab
	$N_{200\%}$	0.479c	0.770a	0.239b	0.467bc	0.765a	0.011b
80	N_0	0.389b	0.720b	0.239b	0.4587a	.750a	0.017b
	$N_{50\%}$	0.407b	0.723b	0.250b	0.3827b	0.747a	0.045a
	$N_{100\%}$	0.417b	0.750a	0.277a	0.3992b	0.715b	0.017b
	$N_{150\%}$	0.469a	0.759a	0.241b	0.4634a	0.757a	0.017b
	$N_{200\%}$	0.397b	0.720b	0.284a	0.3907b	0.713b	0.012b

Values within the same columns followed with different letters are significantly different at $p < 0.05$ according to Tukey's test. DAS, days after sowing; Fm, maximum fluorescence; Fv/Fm, maximal PS II quantum yield; Y(NO), Quantum yield of non-regulated heat dissipation in PS II, Fm′, light adapted maximum fluorescence; Fv′/Fm′, the quantum efficiency of open PSII reaction centres and NPQ, the nonphotochemical quenching.

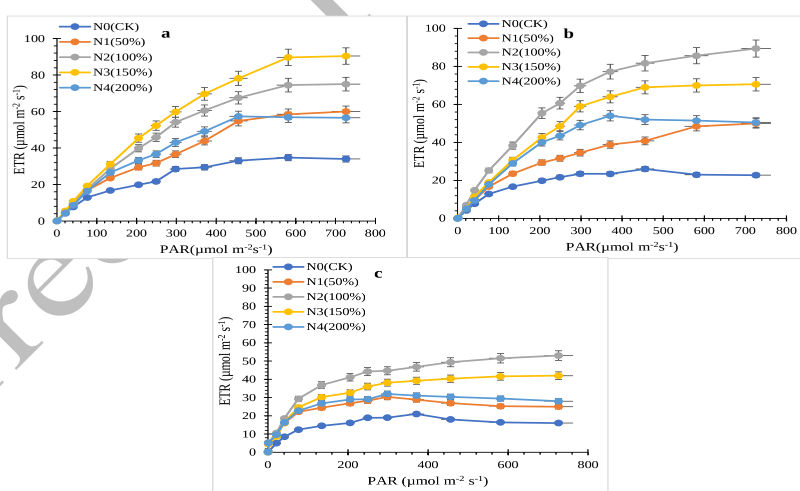

Fig. 4: Effect of nutrient concentration on electron transport rate (ETR) curves. Graph (a) represent ETR curves at 40 DAS, (b) at 60 DAS, and (c) at 80 DAS

to N availability. Studies on leaf size variation in response to N supply are based on cell production and expansion which actually are the contributors to leaf size distribution (Sorin *et al.* 2016). However, the contribution of N supply at different developmental stages of the crop stimulates the leaf growth differently. Reduction in leaf size is more prominently when the plant is subjected to N deficiency at the early stages of leaf development when cell division is still continued (Roggatz *et al.* 1999). The application of N above a certain level could not promote the growth of *Brassica*. Non-

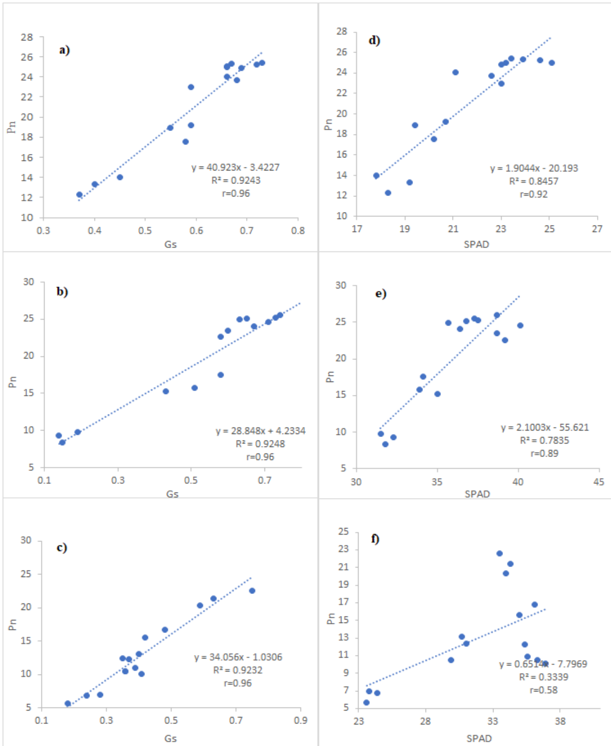

Fig. 5: Linear regression relationship between g_s, SPAD and P_n. Graphs (**a**, **d**) refer measurements at 40 DAS, (**b**, **e**) refer measurements taken at 60 DAS and (**c**, **f**) to measurements conducted at 80 DAS

significant difference in leaf area of *Brassica* with N application above 15 m*M* could be explained with excessive N application contributed to nitrate accumulation in root zone resulting in non-significant difference in growth traits with increase in N application often correlated with the decrease in photosynthetic ability (Ullah *et al.* 2017). Similar findings were achieved that a moderate dose of N resulted in maximum leaf area and dry matter as compared to a high dose of N in sunflower (Zeng *et al.* 2014).

The N contents in leaves are distributed mainly in the complex of photosynthetic proteins, thus affecting photosynthesis. Photosynthesis intensity may reflect plant growth potential and stress tolerance intensity (Han 2011; Wei *et al.* 2016). Photosynthesis demonstrates the N supply influenced by the target leaves in reaction to the leaf dry matter. The N deficiency affected leaf N content, which then decreased the P_n (Hiratsuka *et al.* 2015). The P_n and g_s decreased at lower and higher N nutrition while higher values of Ci can be interpreted due to higher mesophyll resistance. The present study findings are found to be consistent with the findings of N effect on sunflower where high-N grown plants had lower intercellular CO_2 concentration (Ci) when compared with low-N grown plants (Cechin and Fumis 2004). This reduction in P_n may be possible due to a carboxylation efficiency depression followed by a decrease in Rubisco leaves concentration and activity (Nakaji *et al.* 2001). The increased P_n with increase in N resulted in increased total assimilatory area to a certain extent of N application followed by a decreasing trend of P_n. In order to sustain better growth, the importance of N as a stimulator component of photosynthetic apparatus, an optimal amount of N application is required varying according to development growth.

The maximal PS II quantum yield and Fv/Fm increased up to $N_{150\%}$. Similar findings were evident from the study conducted on cotton that excessive N decreased that Fv/Fm due to photoinhibition (Wu *et al.* 2019). The complex of photosystem II (PS II) appears to be associated with a significant inhibition of photosynthesis by high salt accumulation caused due to high doses of N. High nitrate or nitrite accumulation significantly reduces PS II activity and inhibits the quantity of PS II electron transport and CO_2 assimilation in maize, suggesting that high application of N causes salt accumulation in the rootzone (Foyer *et al.* 1994).

The higher the N concentration above the $N_{100\%}$, the higher the decrease in ETR showing the blockage in ETR and P_n. Our results are consistent with the findings on rice studied under N application where ETR increased initially and then decreased as N application amount increased (Long *et al.* 2013), suggesting that ETR and P_n increase with the increase in N nutrition up to a specific level while further increment shows adverse effects and cause photosynthesis inhibition and lower photochemical quantum yield. Hence at too low or at over N fertilization, the stomatal closure is therefore correlated with ETR down-regulation, which is offset by increased thermal dissipation (NPQ). This rise (NPQ) would dissipate some excitation energy at the cost of photochemical usage, resulting in a reduction of PS II control and a decrease in electron transport quantities (Zribi *et al.* 2009).

Conclusion

The application of N had a major impact on *Brassica* growth and physiological features. Prior to harvesting, P_n and g_s peaked in $N_{100\%}$, while both P_n, g_s and Fv/Fm decreased as the N rate increased from 22.7 to 30 m*M*. These findings suggested that both low and high levels of N blocked the transportation of photosynthetic electrons and reduced the photosynthetic rate, and also reduced the degree of openness of the *Brassica* PS II reaction. The N increase in *Brassica* assists to improve the ETR and the degree of the openness of PS II reaction center, achieving higher photochemical quantum yield. It was concluded that 15 m*M* to 22.5 m*M* N concentration in liquid nutrients solution is more suitable for practical application. It was thus possible to verify the potential of fluorescence sensing to detect the differences among N rates.

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Author Contributions

Ikram Ullah, Mao Hanping, and Qaiser Javed designed the research; Ikram Ullah and Muhammad Saif Ullah conducted the experiments and collected data; Ghulam Rasool and Muddassir Ali contributed to data analysis; Ikram Ullah wrote the original manuscript; Mao Hanping and Ahmad Azeem contributed to review and editing the manuscript. All authors approved the final manuscript.

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