



Research Article

Influence of Homobrassinolide on Photosynthetic Traits during Mid-Grain Filling Stage and Its Impact on Yield of Wheat Cultivars

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ABSTRACT

Photosynthesis is the cornerstone of physiological activities and it is the primary source of dry matter production in plants. Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds that offers unique possibility of augmenting crop yield by playing vital role in various metabolic activities of plants and or protecting plants from environmental extremities. Thus, a field experiment was conducted during *rabi* season at the University Research Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar to investigate the effect of homobrassinolide (HBL) foliar spray on photosynthetic traits during mid-grain filling stage and its impact on yield of three wheat cultivars *i.e.*, K0307, UP262 and HD2967. Two different doses of homobrassinolide @ 0.2 ppm (T_2) and 0.4 ppm (T_3) were applied as foliar spray both at pre-heading stage *i.e.* at 55 DAS and at completion of heading *i.e.* at 80 DAS, and distilled water was sprayed in control plots (T_1). Results revealed that photosynthetic traits like net photosynthesis (P_n), transpiration rate (E), stomatal conductance (C) varied significantly among the wheat cultivars. However, HBL application was found to have significant effect on traits like stomatal conductance (C), stomatal limitation (L_s), intrinsic water use efficiency ($iWUE$), intercellular CO_2 concentration and SPAD value of flag leaves. Though wheat cultivars were varied significantly in most of the yield and yield parameters but HBL application was found to have significant influence on grain yield only. The results also indicated that net photosynthetic rate during mid-grain filling was highly correlated with number of filled grain ear⁻¹, test weight and biological yield of the wheat cultivars. Most interesting finding was a positive correlation between SPAD value and grain yield which might indicate that HBL application would improve grain yield due to cumulative increase in photo-assimilate production by retaining greenness of leaves.

Key words: Homobrassinolide, Photosynthetic traits, SPAD value, Wheat, Yield

Introduction

Wheat is one of the world's largest cereal crops, and most important staple food for about 36% of world population. Photosynthesis is the primary source of dry matter production and photosynthesis reflects the efficiency as it is a determinant of light-use efficiency, biomass production, and crop yields. Total biomass

production is the integration of metabolic reaction of the plant and as such any factor influencing the metabolic activity at any period of plant growth can affect yield of a crop. Higher grain yield largely results from optimum integration of the processes like production of photoassimilates and its partitioning efficiency (Tollenaar and Lee, 2006; Fischer, 2007). It is suggested that from 70% to more than 90% of the grain yield is derived from photosynthates produced during post-anthesis and flag leaf blade contributes to as

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much as 50% of the canopy photosynthates during early and mid-grain falling (Austin *et al.*, 1977; Bidinger *et al.*, 1977; Rawson *et al.*, 1983). It had been reported that winter wheat productivity was limited by a number of factors and the most important factor was the ability of plants to absorb photosynthetically active radiation (Slapakauskas and Ruzgas, 2005). So studying the photosynthesis during the senescence of wheat leaves is important since it contributes greatly to grain yield. However, a comprehensive approach for studying the cumulative influence of post-anthesis photosynthesis on yield of wheat is very rare.

Increases either in photosynthetic efficiency or the total photosynthetic capacity of plants would lead to enhanced photo-assimilate production (Van Camp, 2005). Thus, yield potential of wheat can be regulated either through alternation of and reconstitution of genetic make-up or by modification of environment through improved cultural treatment. A possible and eco-friendly approach to increasing plant growth and yield is foliar application of plant growth regulators. Among plant growth regulators, brassinosteroids (BRs) are important group of naturally occurring plant hormones essential for growth and development of plants. BRs are the first steroidal hormones reported in plants. It was first isolated from pollen extract of *Brassica napus* (Grove *et al.*, 1979). Brassinosteroid (BRs) represent a new sixth class of plant hormones with wide occurrence in the plant kingdom. Chemically they are polyhydroxy steroids and all BRs characterised from plants have a common α -cholestane skeleton. Application of BRs has been reported to activate cell elongation, cell division, proton pump (Rao *et al.*, 2002; Hayat *et al.*, 2003; Nomura *et al.*, 2005). BRs are known to elicit a wide range of physiological processes in plants (Behnamnia *et al.*, 2009; Hayat *et al.*, 2010) and have an economic impact on plant metabolism, growth and productivity (Ali *et al.*, 2007). BRs are also reported to influence seed germination, photo-morphogenesis, crop establishment and growth and ultimately yield in a number of crops (Sairam, 1994; Fariduddin *et al.*, 2004; Sharma *et al.*, 2015; Upreity and Murti, 2004). BRs have been reported to be very promising in improving

photosynthesis that ultimately results in higher growth and yield of many crop plants (Braun and Wild, 1984; Vriet *et al.*, 2012). But, the yield improvement due to BRs has been reported to be inconsistent under field conditions (Kamuro and Takatsuto, 1991) and is highly influenced by crop species, concentration and frequency of application (Ramraj *et al.*, 1997). Cumulative treatments with BRs at different stages of growth have been reported to significantly increase yield of crops (Chatterjee *et al.*, 2006; Prakash *et al.*, 2007). However, many workers also reported that applications of 28-homobrassinolide twice in various crops caused yield enhancement (Jangid *et al.*, 2017). In view of the above facts, the present experiment was conducted with four cultivars of wheat to investigate the effects of foliar spray of homobrassinolide on photosynthetic traits during mid-grain filling stage and yield of wheat cultivars.

Materials and Methods

Plant materials and crop grown

The present investigation was conducted at the University Research Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar (at 26°19'86" N, 89°23'53" E with an altitude of 43m) during the year 2016-17. The experimental site falls under sub-Himalayan *Terai* agro-climatic zone of India. The average annual rainfall is 3000 mm and most of which is received during June to September. The soil of the experimental field was sandy loam in texture with low water holding capacity, moderate fertility status and acidic in nature (pH 5.3). Three cultivars of wheat *i.e.* K0307 (V₁), UP 262 (V₂) and HD 2967 (V₃) were planted in a multi-row plot (04 rows) of 2.5 meters length with three replications under timely sown condition. Standard package of practices as recommended for the *Terai* agro-climatic -zone for cultivation of wheat were employed. The crop was irrigated as and when required following the regular recommendations. During the experimental period, the average mean temperature was higher at the beginning and lower at the middle stage of growth followed by gradually increased towards the maturity (Fig. 1).

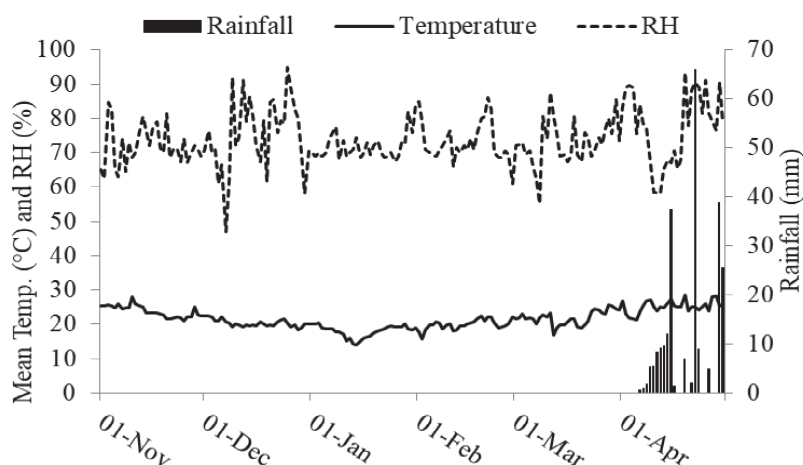


Fig. 1. Mean air temperature (°C), relative humidity (%) and rainfall (mm) during crop growing season of 2016-17

Treatment pattern

The plants were sprayed with the 28-homobrassinolide (HBL) @ 0.2 ppm (T_2) and 0.4 ppm (T_3). Blank spray only with distilled water was made in case of control *i.e.* T_1 . All the plants were treated twice *i.e.* first foliar application was made at pre-heading stage (55 DAS) and the second application was at completion of heading (80 DAS). Tiptop (0.1%) was added to HBL solutions as a surfactant to ensure the better penetration of brassinosteroids into the leaf tissue.

Photosynthetic gas-exchange measurements

Gas exchange (GE) measurements were carried out by means of an open gas exchange system (CI-340 Hand-held Photosynthesis System, CID-Bioscience, USA) by taking fully grown flag leaves on the main stem. Gas exchange (GE) parameters were measured in the middle of flag leaf blade of the main tiller. Measurements were performed on pre-selected five plants from each replication during mid-grain filling stage *i.e.* twice at weekly interval after second spray and observations were taken between 8.00 am to 11.00 am by holding the chamber perpendicular to the incident light from sun. Parameters like net photosynthesis rate (P_n), rate of transpiration (E), stomatal conductance (C), intercellular CO_2 concentration (C_i), ambient CO_2 concentration (C_a), vapour pressure deficit (VPD), leaf temperature (T_{leaf}) were taken directly from GE

instrument. All GE measurements were taken directly under full sunshine in field condition following the techniques as described by Long and Bernacchi (2003) to overcome the common pitfalls of GE systems. The SPAD value of chlorophyll in the flag leaf was measured by using the chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan). SPAD values were recorded on the same pre-selected plant where photosynthesis data were taken. Stomatal limitation value (L_s) was calculated according to the formula, $L_s = 1 - C_i/C_a$ as given by Larocque (2002). Intrinsic water use efficiency ($iWUE$) was calculated as the ratio of net photosynthesis rate (P_n) to transpiration rate (E) and expressed as μmol of CO_2 fixed per mmol of H_2O transpired.

Harvesting and recording yield data

Pre-selected plants, upon which gas exchange measurements were taken, were harvested at maturity for recording grain and biological yield. Dry weights were recorded after the plant material oven dried at 70°C for 72-h. The grain yield was taken as per plant basis and for test weight, 1000-grains were counted and their average weight (g) was recorded. Harvest index was calculated as the ratio of grain yield to total aboveground biomass.

The experiment was laid out in factorial based on Randomized Block Design with three replications. Data were analyzed by ANOVA

using the SPSS software, version 17.0 and Analysis of variance was carried out using SPSS Statistics 17.0 and test for significant differences among variables were calculated at $P < 0.05$ level of significance.

Results and Discussion

The data as shown in Table 1 revealed that wheat cultivars varied significantly in net photosynthesis rate (P_n), transpiration rate (E) and stomatal conductance (C). significantly higher values of cumulative net photosynthesis rate (P_n), transpiration rate (E) and stomatal conductance (C) during mid-grain filling were observed in K 0307 (V_1) as compared to others wheat cultivars. Though HBL treatments showed significant influence on traits like transpiration rate (E),

stomatal limitation (L_s), intrinsic water use efficiency ($iWUE$) and SPAD values but it did not showed any significant effect on cumulative net photosynthesis during mid-grain filling stage of wheat cultivars. None of photosynthetic traits were found to vary significantly except L_s under variety and treatment interaction. The results indicated that wheat cultivars varied differentially in stomatal limitation (L_s) values and intrinsic water use efficiency ($iWUE$) under the influence of brassinolide (Fig. 2 & 3).

While wheat cultivar UP 262 showed minimum variations both in L_s and $iWUE$, other two cultivars were recorded higher values of both parameters particularly at higher doses of HBL. Data presented in Table 2 showed that all the yield parameters viz. grain yield, test weight,

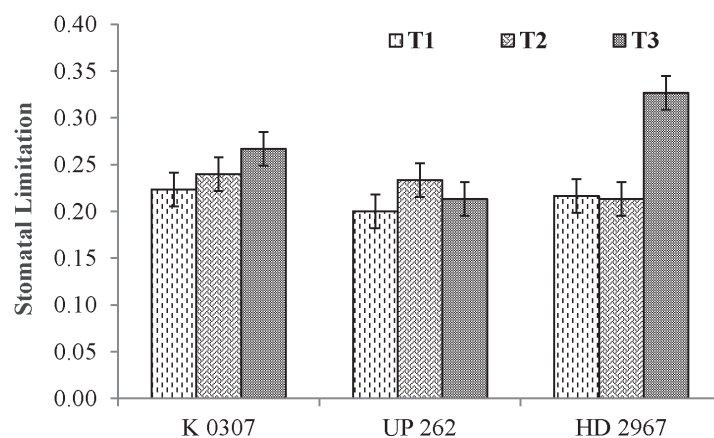


Fig. 2. Effect of homobrassinolide on stomatal limitation (L_s) of mid-grain filling stage of wheat cultivars, error bars indicates $\pm$ SEM

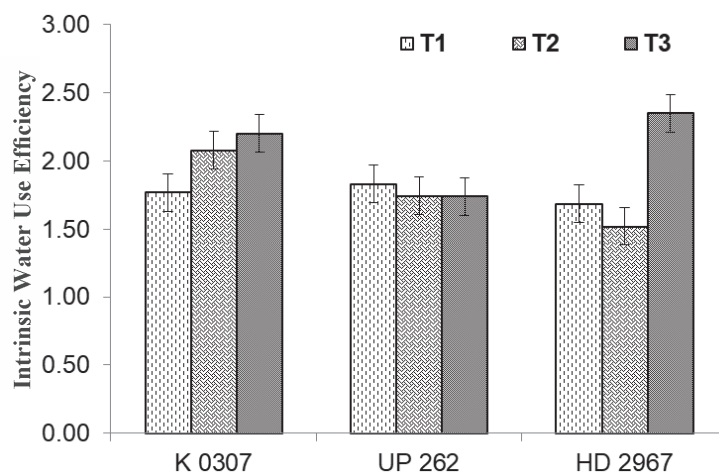


Fig. 3. Effect of homobrassinolide on intrinsic water use efficiency ($iWUE$) of mid-grain filling stage of wheat cultivars, error bars indicates $\pm$ SEM

Table 1. Effect of homobrassinolide on leaf photosynthetic traits (data expressed as combined means of two measurements taken at weekly intervals after second spray) in wheat cultivars

Factors	P _n ($\mu\text{mol}/\text{m}^2/\text{s}$)	E ($\text{mmol}/\text{m}^2/\text{s}$)	C ($\text{mmol}/\text{m}^2/\text{s}$)	T _{leaf} (°C)	C _i (ppm)	VPD (kPa)	C _a (ppm)	Stomatal limitation (L _s)	iWUE	SPAD
Variety										
V ₁	7.44 ^a	3.73 ^a	249.5 ^a	35.33 ^a	181.1 ^a	1.90 ^a	239.9 ^a	0.243 ^{ab}	2.02 ^a	39.29 ^a
V ₂	6.34 ^b	3.60 ^a	234.9 ^a	35.41 ^a	187.7 ^a	2.01 ^a	239.1 ^a	0.216 ^b	1.77 ^a	37.55 ^a
V ₃	5.45 ^c	3.10 ^b	167.3 ^b	35.29 ^a	179.2 ^a	2.19 ^a	239.1 ^a	0.252 ^a	1.85 ^a	38.76 ^a
SEm (±)	0.280	0.119	14.71	0.364	3.34	0.14	3.49	0.010	0.080	1.08
LSD ($P \leq 0.05$)	0.839	0.356	44.11	NS	NS	NS	NS	NS	NS	NS
Treatment										
T ₁	6.82 ^a	3.86 ^a	245.2 ^a	35.20 ^a	190.6 ^a	1.94 ^a	242.4 ^a	0.213 ^b	1.76 ^b	33.91 ^b
T ₂	6.18 ^a	3.47 ^b	213.6 ^{ab}	35.37 ^a	186.1 ^a	2.05 ^a	241.8 ^a	0.229 ^b	1.78 ^b	41.42 ^a
T ₃	6.24 ^a	3.11 ^b	192.9 ^b	35.45 ^a	171.3 ^a	2.12 ^a	233.9 ^a	0.269 ^a	2.10 ^a	40.27 ^a
SEm (±)	0.280	0.119	14.71	0.364	3.34	0.114	3.49	0.010	0.080	1.08
LSD ($P \leq 0.05$)	NS	NS	NS	NS	10.02	NS	NS	0.031	0.239	3.25
Variety × Treatment										
SEm (±)	0.485	0.206	25.48	0.630	5.79	0.197	6.05	0.018	0.138	1.87
LSD ($P \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	0.053	0.415	NS

Significant differences ($P \leq 0.05$) between varieties as well as between treatments are indicated by different letters according to Duncan's test, NS- Non significant

Table 2. Effect of homobrassinolide on grain yield and yield parameters in wheat cultivars

Factors	Filled grains ear ⁻¹	Yield (g plant ⁻¹)	Test wt. (g 1000 grain ⁻¹)	Biological yield (g plant ⁻¹)	HI
Variety					
V ₁	46.79 ^a	16.36 ^a	44.44 ^a	47.76 ^a	0.348 ^b
V ₂	44.86 ^a	16.20 ^a	44.52 ^a	47.38 ^a	0.344 ^b
V ₃	45.91 ^a	14.68 ^b	41.29 ^b	35.69 ^b	0.414 ^a
SEm (±)	1.34	0.495	0.197	1.36	0.016
LSD ($P \leq 0.05$)	NS	1.484	0.589	4.08	0.048
Treatment					
T ₁	48.57 ^a	13.99 ^b	43.34 ^a	41.46 ^a	0.347 ^a
T ₂	44.65 ^a	16.69 ^a	43.46 ^a	45.23 ^a	0.380 ^a
T ₃	44.34 ^a	16.56 ^a	43.46 ^a	44.14 ^a	0.379 ^a
SEm (±)	1.34	0.495	0.197	1.36	0.016
LSD ($P \leq 0.05$)	NS	1.484	NS	NS	NS
Variety × Treatment					
SEm (±)	2.33	0.857	0.340	2.36	0.027
LSD ($P \leq 0.05$)	NS	NS	1.019	NS	NS

Significant differences ($P \leq 0.05$) between varieties as well as between treatments are indicated by different letters according to Duncan's test, NS- Non significant

biological yield and harvest index (HI) except numbers of filled grain ear⁻¹ were varied significantly among the wheat cultivars. Wheat cultivar, K 0307 showed maximum grain yield and biological yield whereas minimum was recorded in HD 2967 (Table 2). However, higher value of harvest index was recorded in HD 2967 as compared to other wheat cultivars. But HBL application was found only to have significant effect on grain yield of wheat cultivars under investigation. Higher grain yield was recorded in T₂ i.e. under HBL application @ 0.2 ppm.

Correlation studies between photosynthetic traits of mid-grain filling stage and yield parameters revealed that net photosynthesis rate (P_n) had maximum numbers significant associations with yield attributes (Table 3). P_n showed significant associations with numbers of filled grains ear⁻¹, test weight and biological yield. Among the flag leaf photosynthetic traits, SPAD values during mid-grain stage showed positive correlation with grain yield.

The variation in photosynthetic traits and yield parameters among the wheat cultivars might be attributed to the differences in genetic

composition resulting in alterations in their photosynthetic capacities and partitioning of photo-assimilate. It might be due to genotypic variation in greenness of leaf and stomatal conductance which ultimately regulate photosynthesis. These findings are in agreement with observation of many workers (Ahmed *et al.*, 2012; Baz *et al.*, 2009). Higher stomatal limitation (L_s) along with corresponding lower values of intrinsic water use efficiency (iWUE) under HBL application might be attributed to its significant role in conferring tolerance to water stress (Fariduddin *et al.*, 2014).

Many researchers have reported the positive influences of BRs on photosynthetic parameters and biomass production (Braun and Wild, 1984; Sharma *et al.*, 2015). It has also been reported that brassinosteroids cause enhancement in grain yield in various crops through improvement in photosynthesis efficiency which can further be caused by stomatal or non-stomatal factors or by their combination (Prakash *et al.*, 2007; Rao and Rani, 2013). Many workers also observed significant influence of BRs on a conductivity of stomas (Hayat *et al.*, 2010). Though our results

Table 3. Simple correlation analysis of yield parameters with post-anthesis photosynthetic traits

Parameters	No. of grain	Yield	Test wt.	Biomass	HI
P _n	0.476*	0.289	0.606**	0.482*	-0.285
E	0.378	-0.045	0.421*	0.245	-0.263
C	0.286	0.053	0.501**	0.382*	-0.330
C _i	0.102	-0.159	-0.040	-0.184	0.077
VPD	-0.180	-0.103	-0.208	-0.103	0.041
T _{leaf}	-0.200	-0.040	0.165	0.136	-0.168
C _a	0.094	-0.013	-0.170	-0.151	0.135
L _s	-0.073	0.194	-0.079	0.131	-0.011
iWUE	0.173	0.367	0.234	0.287	-0.066
SPAD	-0.358	0.419*	-0.020	0.233	0.121

*Significant at $P \leq 0.05$ and ** significant at $P \leq 0.01$

indicated that HBL did not have any significant influence on net photosynthetic rate but HBL application showed significant impact on grain yield which could be attributed to retaining greenness of leaves as reflected by higher SPAD value in HBL treated plants as compared to control. The increase in SPAD value in HBL treated plants indicates the increase in chlorophyll contents which might be attributed to the cumulative effect of this hormone on the leaf metabolism, expression of regulatory genes involved in chlorophyll biosynthesis or less degradation of this pigment due to better defense mechanisms under HBL treatment (Takahasi *et al.*, 1994; Bajguz, 2000; Xia *et al.*, 2009). Horton (2000) reported that delayed leaf senescence or a stay-green phenotype was a desired characteristic in crops and could constitute a target for improvement of crop productivity. Enhancement of chlorophyll content by BRs treatment has been reported and it has also been attributed to several factors including inhibition of senescence and enhanced uptake of minerals (Bhatia and Kaur, 1997; Ananthi and Vanagamudi, 2013).

Thus, the positive correlation between SPAD values and grain yield might be attributed to enhanced photo-assimilate production by increasing the plant cumulative photosynthetic capacity by delaying leaf senescence or by introduction of stay-green characteristic during grain filling period (Van Camp, 2005). This might indicate that HBL application would result in

delayed leaf senescence or stay-green phenotype. Since an enhancement of leaf chlorophyll content or delayed leaf senescence might be considered as a desired characteristic in crops and targeted for improvement of crop productivity. Green leaves and stem are the sites for photosynthesis and its rate also depends on chlorophyll bearing surface area (Edwards and Walkers, 1983). Though photosynthesis increases with the increase in chlorophyll content which is a manifestation of delayed leaf senescence under brassinosteroids treatment (Shen *et al.*, 1990), but a non-significant and significant correlation of P_n with grain yield and biological yield, respectively, offers clear indication that HBL application caused enhanced biomass production resulted from more photosynthesis due to prolonged maintenance of leaf chlorophyll but that was not redirected in grain yield because of sink limitations. Since it is generally accepted that the genotypes which are able to photosynthesis in flag leaf for longer time, tend to yield more (Gouth *et al.*, 2009) but delayed plant senescence during grain filling actually results in much non-structural carbohydrate left in the straw and that leads to a low HI (Yang and Zhang, 2010).

Conclusions

From the present discussion it would be concluded that application of HBL resulted in differential response in photosynthetic traits during mid-grain filling stage of wheat cultivars

used under this study. However, HBL applications significantly altered the SPAD value of the wheat cultivars. Applications of HBL significantly increased the grain yield of wheat cultivars but such response was specific to variety and dose of HBL treatment. A significant correlation between SPAD values and grain yield would lead to establish the role of HBL in retaining greenness of leaves that might be considered as a desired characteristic in crops and targeted for improvement of crop productivity to meet up the future demand.

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