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Effect of foliar application of brassinolide on photosynthesis and chlorophyll fluorescence traits of *Leymus chinensis* under varying levels of shade

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Abstract

The present study was conducted to determine the effect of exogenous application of brassinolide (BR) on *Leymus chinensis* grown under shade, *i.e.*, control (100% natural light), mild shade (70% natural light), and moderate shade (50% natural light). Shade substantially enhanced the plant growth, synthesis of photosynthetic pigments, photosynthetic efficiency, and chlorophyll (Chl) fluorescence attributes of *L. chinensis* as compared with control. The order of increase was mild shade > moderate shade > natural light except Chl content, where the order of increase was moderate shade > mild shade > natural light. Likewise, application of BR resulted in further exacerbation of plant height, plant fresh and dry mass, but less in case of Chl and carotenoids contents, gas-exchange characteristics, and Chl fluorescence attributes. The results conclude that shade significantly enhanced plant growth through alterations in physiological attributes of *L. chinensis*, while, application of BR may not further improve the plant growth under shade.

Additional key words: chlorophyll pigment; gas exchange; photosynthetic diurnal variation; plant height; plant mass.

Introduction

Leymus chinensis (Trin.) Tzvel. is a perennial plant native to China with good palatability and high forage value. The grassland, where it dominates, spreads widely from the southern Chinese loess plateau to the northern Russian Baikal, and from the Sanjiang plain of eastern China to Ulan Bator in Mongolia. The dominance of *L. chinensis* in semiarid grasslands usually varies along the gradients of water and nutrient availability (Wang and Li 1996). Herbivores can affect plants directly by removing biomass and indirectly by modifying resource availability (*e.g.*, enhancing light penetration, altering water uptake, redistribution of nutrients, and/or reducing carbon assimilation due to leaf removal) (van der Wal *et al*. 2000, Augustine 2003, Gassmann 2004, Hodgkinson and Müller 2005) or differential grazing on neighboring plants (Augner *et al*. 1997, Haag *et al*. 2004). The importance of resource availability and disturbances as well as their interactions on plant community structure and species diversity has been widely reported (Wilson and Tilman 2002, Fynn *et al*. 2005). However, the increase in grazing pressure, coupled with abiotic stresses such as drought, salinity, temperature, and light regimes, has led to a substantial reduction in canopy cover throughout much of the grassland.

Light is the main environmental factor for plants to provide energy for plant photosynthesis, light intensity is not conducive to plant growth. Although severely low light conditions can reduce the photosynthetic efficiency of

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Abbreviations: APX – ascorbate peroxidase; BR – brassinolide; C_i – intercellular CO_2 concentration; Car – carotenoids; CAT – catalase; Chl – chlorophyll; E – transpiration rate; CUE – carboxylation utilization; DAT – days after treatment; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_s – steady-state fluorescence; F_m' – maximal fluorescence yield of the light-adapted state; F_v/F_m – optimal/maximal quantum yield of PSII photochemistry; F_v/F_0 – optimal/minimal quantum yield of PSII or photochemical efficiency of PSII; F_v – variable fluorescence; g_s – stomatal conductance; GR – glutathione reductase; L_s – stomatal limitation; P_N – net photosynthetic rate; PGRs – plant growth regulators; POD – peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; SUE – sunlight-utilization efficiency; TChl – total chlorophyll content; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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plants leading to limited plant growth, an excess light can also hinder the plant growth and development. Under low light conditions the plants may experience reduced activity of Rubisco resulting in lowered CO_2 assimilation rate (Stitt and Schulze 1994), ultimately causing a decreased growth. On the other hand, exposure of plants to excessive light can cause photoinhibition through energy imbalance. This process is associated with rapidly reversible down-regulation of PSII photochemical efficiency or with maintenance of the slow reversible energy-dissipating mechanisms, with repair processes or with the eternal destruction of photosynthetic apparatus (Allen and Ort 2001). However, reasonable shading can not only prevent the plant from excessive light but also improves the micro-meteorological conditions of crop growth and photo-synthetic capacity of plants (Tang *et al*. 2015). This indicates that determination of optimum level of irradiance is important for better plant growth and regulation of plant growth under different light regimes. However, since fifties of the 20th century, it is expected that the amount of short-wave solar radiation reaching the Earth is reduced by 27% every ten years leading to global diming of sunlight. Studying the effects of light intensity on *L. chinensis* can unveil the possible impacts of light intensity and global diming conditions on the productivity of *L. chinesis* and other crop plants (Zhu *et al*. 2010). Chl fluorescence has always been used in studies of the photosynthetic regulation and the plant responses to environment because of its sensitivity, suitability, and nondestructive features (Dai *et al*. 2009). Photoinhibition of PSII can be easily sensed *in vivo* by reduction in 'dark-adapted' ratio of variable to maximum Chl *a* fluorescence, *i.e.,* F_v/F_m (Krause and Weis 1991).

Plant growth regulators (PGRs) are increasingly used for the improvement of plant growth and stress resistance. Brassinolide (BR) is a kind of hormone, which is recognized as the most active, highly efficient, broad-spectrum, and nontoxic plant growth hormone. The main function of BR is to promote the plant growth and increase the yield of crops. In addition, BR improves the photo-synthetic efficiency of plants mainly through enhanced biosynthesis of photosynthetic pigments and protection of photosynthetic machinery from damaging effects of reactive oxygen species (ROS) through enhanced accumu-lation of osmolytes and improved activity of antioxidant enzymes under both normal and stressed conditions as well (Cao *et al.* 2009, Wang *et al*. 2016). Niu *et al*. (2016) reported that exogenous application of BR on *L. chinensis* enhanced the plant height, leaf area, dry mass accumulation, biosynthesis of photosynthetic pigments, stimulated the accumulation of osmolytes, and increased the activity of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), while lowering down the production and activity of ROS under both normal and high temperature stress conditions. Similarly, Wang *et al*. (2016) perceived increased growth, biosynthesis of photosynthetic pig-ments, and exaggerated activity of enzymatic antioxidants (SOD, POD, CAT, GR, APX, *etc*.) in *L. chinensis* plants in response to exogenous application of BR under chilling stress. Considering the promising effects of shade and BR on plant morphological, physiological, and biochemical attributes, the present study was conducted with the aim to evaluate the effect of BR application on growth and photosynthetic efficiency of *L. chinensis* under normal and different levels of shade.

Materials and methods

Experimental material: Pot experiment was carried out to determine the effect of BR on one-year-old *L. chinensis* plants grown under various levels of shade at the College of Agronomy and Biotechnology, Southwest University, Chongqing, China. The experimental area is located between latitudes of 29° 49′ 32″ N, longitudes 106°26′ 02″ E, and altitude of 220 m. Irradiance is about 1,200 annual sunshine hours. The average PAR was 1,135 μmol(photon) m^{-2} s^{-1} for May and June 2015. The average temperature was 18.2°C and the annual precipitation was 1,200 mm. Seeds of *L. chinensis* plants were sown in dishes containing sand for germination in laboratory in July 2014, followed by transplanting of seedlings in pots (diameter of 24.5 cm and height of 20 cm). Plants were allowed to grow until April, 2015. Consistently growing uniform seedlings were selected as experimental material.

Treatments: The experiment consisted of six treatments with spraying of 0.1 mg(BR) L^{-1} solution on plants exposed to three different levels of shade:

Treatment		
T_0	Control	100% natural light
T_1	Mild shade	70% natural light
T_2	Moderate shade	50% natural light
BT_0	Control + BR	100% natural light + 0.1 mg(BR) L^{-1}
BT_1	Mild shade + BR	70% natural light + 0.1 mg(BR) L^{-1}
BT_2	Moderate shade + BR	50% natural light + 0.1 mg(BR) L^{-1}

Brassinolide (*Sigma-Aldrich Company*, USA) was dissolved with ethanol and then diluted with distilled water to 0.1 mg L^{-1}. For creating different levels of shade, a shade shed was built on 18 May, 2015. The area of the shade shed was 400 cm × 200 cm, and the height from ground was 1 m. Two treatments were placed under the same shade conditions, *i.e.*, T_0 and BT_0, T_1 and BT_1, and T_2 and BT_2. Each treatment was replicated five times. BR was applied three times, *i.e.*, 19 May, 26 May, and 2 June, in evening at 18:00 h to avoid the effect of light. Each pot was supplied with 25 ml of Hoagland's nutrient solution every 5 d to ensure suitable nutrient supply. Water was applied to each pot every 2 d.

Measurements: After the treatments, data were recorded regarding growth, photosynthetic pigments, gas-exchange characteristics, and Chl fluorescence parameters three times at different durations. Growth, photosynthetic pigments, and gas-exchange characteristics were measured 20, 40, and 60 d after the imposition of treatments (DAT). Furthermore, photosynthetic diurnal variation was determined on 20 July with an interval of 2 h from 6:00–18:00 h. Chl fluorescence attributes were measured 6, 12, and 18 DAT.

Growth attributes: Plants were uprooted and plant were rinsed with tap water followed by 2–3 times rinsing with distilled water. The plant height was measured from the tip of the stem to parietal lobe at the base of plants. Adhered water was absorbed from the seedlings by using filter paper. Fresh mass (FM) of seedlings was determined by weighing the seedlings and then drying by placing the seedlings in oven at 105°C for 30 min followed by drying at 65°C till constant mass in order to obtain the seedling dry mass (DM).

Photosynthetic pigments: Chl *a*, Chl *b*, total Chl (TChl), and carotenoid (Car) contents were measured by Wellburn (1994) method. A leaf sample of 0.1 g was ground and placed in 15-mL centrifuge tube along with 10 mL of miscible liquids by 95% acetone and absolute ethyl alcohol (1:1, v/v). Then it was covered with black plastic bag and kept at dark place until the samples changed into white color. The absorbance was measured at 665, 649, 470, and 652 nm, respectively, by a spectrophotometer (*UV-6000, Shanghai Metash Instrument Co. Ltd*, China).

Gas exchange: The gas-exchange characteristics of *L. chinensis* leaves were measured in the morning (10:00–11:30 h) each time using the *LI-6400* portable measuring instrument under synthetic PAR of 1,000 μmol(photon) m^{-2} s^{-1} for different treatments. The ecophysiological indexes measured included the net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), transpiration rate (E), sunlight-utilization efficiency (SUE), water-use efficiency (WUE), carboxylation utilization (CUE), and stomatal limitation (L_s). Each index was measured three times, and the average value was taken. Similarly, P_N, g_s, and E were recorded every two h (6:00–18:00 h) on 20 July in order to undermine the photosynthetic diurnal variation.

Chl fluorescence parameters were determined using an open gas-exchange system (*LI-6400*; *LI-COR, Inc.*, Lincoln, NE, USA) with an integrated fluorescence chamber (*LI-6400-40* leaf chamber fluorometer). Five plants were selected and their leaves were dark-adapted for 20 min, and then irradiated with weak light [0.1 μmol (photon) m^{-2} s^{-1}]. The minimal fluorescence yield of the dark-adapted state (F_0) was measured, and then 0.8-s saturation pulse light [3,000 μmol(photon) m^{-2} s^{-1}] was used to determine the maximal fluorescence yield of the dark-adapted state (F_m). In leaves, the steady-state fluorescence (F_s) was measured after 30-min light adaptation and the maximal fluorescence yield of the light-adapted state (F_m') was measured by 0.8-s saturation pulse light [3,000 μmol(photon) m^{-2} s^{-1}]. Optimal/maximal quantum yield of PSII or photochemical efficiency of PSII was calculated as: $F_v/F_m = (F_m - F_0)/F_m$, optimal/minimal quantum yield of PSII was calculated as: $F_v/F_0 = (F_m - F_0)/F_0$, and photosynthetic quantum yield of PSII was calculated as: $\Phi_{PSII} = (F_m' - F_s)/F_m'$.

Statistical analysis: The data were analyzed using *Microsoft Excel* and *SPSS* statistical software. The single factor analysis of variance (*ANOVA*) was used for analysis of data and *Duncan*'s multiple range test was used to compare the means at 5% probability level.

Results

Shade increased plant growth and development of *L. chinensis* at all levels which was further enhanced by exogenous application of BR. The plant height increased from 20 to 40 DAT, but then declined at 60 DAT. However, plant FM and DM increased linearly from 20 to 60 DAT. Shade enhanced the plant growth as compared to natural light (control) and the pattern of increase in growth was in the order of mild shade > moderate shade > natural light. Application of BR enhanced the plant height, plant FM and DM under natural light by 4–8%, 13–29%, and 10–15%, respectively; under mild shade by 2–6%, 14–16%, and 4–17%, respectively, and under moderate shade conditions by 2–3%, 8%, and 8%, respectively, as compared with their respective controls (Table 1, Fig. 1).

Biosynthesis of photosynthetic pigments significantly increased due to shade; BR sprayed on *L. chinensis* resulted in further enhancement at each level of shade, but the increase was not statistically significant. Chl *a*, *b*,

Table 1. Effect of BR on morphological attributes and photosynthetic pigments of *Leymus chinensis* under different light intensities after 60 d of treatments. Car – carotenoids, Chl – chlorophyll, TChl – total chlorophyll content, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}]. Values are means ± SE (n = 5). Values followed by *the same letter* within columns are not significantly different according to *Duncan*'s multiple range test (p<0.05).

Treatment	Plant height [cm]	Fresh mass [mg per plant]	Dry mass [mg per plant]	Chl *a* [mg g^{-1}]	Chl *b* [mg g^{-1}]	Chl *a*/*b*	TChl [mg g^{-1}]	Car [mg g^{-1}]
T_0	32.50 ± 0.38^{e}	533.1 ± 9.2^{d}	183.7 ± 3.1^{d}	1.57 ± 0.01^{b}	0.77 ± 0.04^{c}	2.02 ± 0.09^{a}	2.18 ± 0.01^{cd}	0.219 ± 0.013^{c}
BT_0	33.73 ± 0.22^{d}	612.6 ± 4.3^{c}	204.0 ± 1.7^{cd}	1.72 ± 0.09^{ab}	0.85 ± 0.05^{c}	2.03 ± 0.03^{a}	2.07 ± 0.33^{d}	0.220 ± 0.000^{c}
T_1	38.15 ± 0.56^{b}	733.7 ± 16.2^{b}	264.1 ± 3.1^{ab}	1.82 ± 0.04^{a}	1.29 ± 0.05^{b}	1.41 ± 0.08^{bc}	2.60 ± 0.11^{cd}	0.257 ± 0.011^{b}
BT_1	40.43 ± 0.22^{a}	851.3 ± 8.1^{a}	274.0 ± 4.2^{a}	1.80 ± 0.04^{a}	1.26 ± 0.03^{b}	1.43 ± 0.00^{b}	2.73 ± 0.00^{bc}	0.269 ± 0.000^{b}
T_2	34.53 ± 0.09^{cd}	642.0 ± 3.2^{c}	212.7 ± 2.4^{cd}	1.88 ± 0.09^{a}	1.63 ± 0.01^{a}	1.16 ± 0.06^{d}	3.26 ± 0.12^{ab}	0.274 ± 0.007^{ab}
BT_2	35.52 ± 0.58^{c}	690.7 ± 5.7^{bc}	230.6 ± 4.6^{bc}	1.92 ± 0.05^{a}	1.62 ± 0.01^{a}	1.19 ± 0.03^{cd}	3.35 ± 0.21^{a}	0.299 ± 0.003^{a}

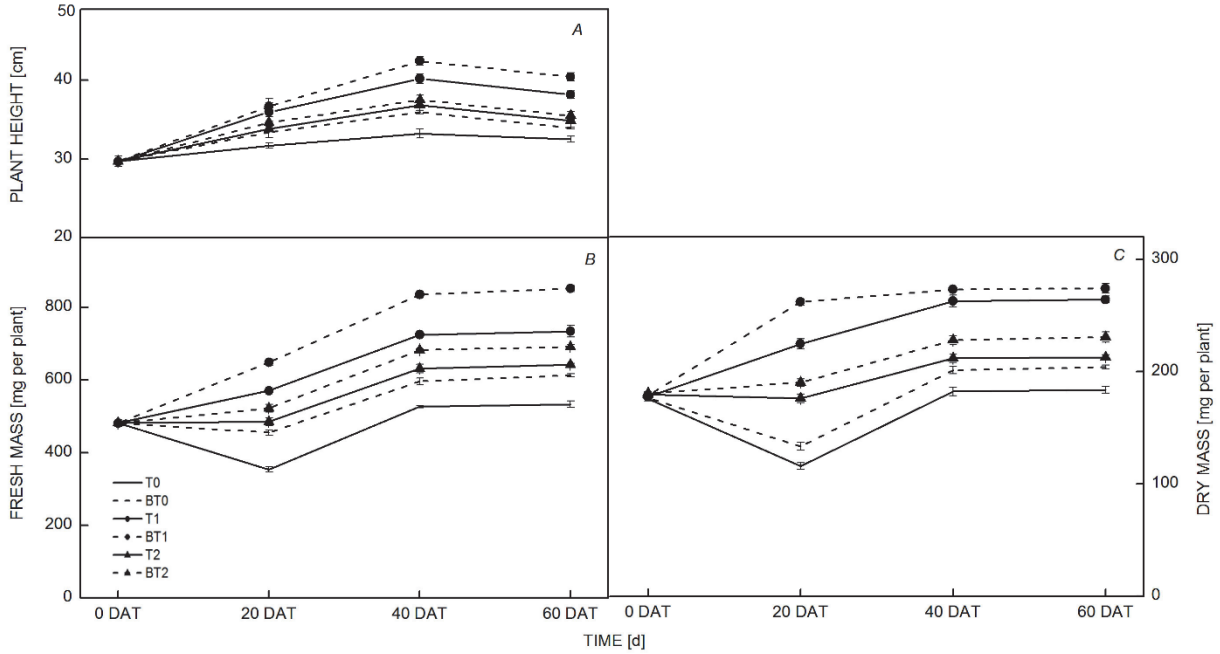

Fig. 1. Dynamic changes after BR treatment on plant height, fresh and dry mass of *Leymus chinensis* under different light intensities. Mean values ± SE (n = 5), DAT – days after treatment, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}].

TChl, and Car increased from 20 to 40 DAT and then tended to decrease at 60 DAT; however, Chl *a*/*b* ratio decreased linearly from 20 to 60 DAT. An increase in the biosynthesis of photosynthetic pigments was perceived by the effect of shade on *L. chinensis* as compared to natural light conditions (control). The increase in photosynthetic pigments followed the order: moderate shade > mild shade > natural light, except for the Chl *a*/*b* ratio, where the order was natural light > mild shade > moderate shade (Table 1, Fig. 2).

The P_N was elevated during the day time from 6:00 until 10:00 h and then declined up to 14:00 h followed by an increase at 16:00 h and again decline at 18:00 at each level of shade. Day mean values indicated that shade enhanced P_N and BR spray further boosted up P_N, but this increase and effect was not statistically significant. At 10:00 h, when P_N was the highest one, shade significantly stimulated P_N over normal natural light conditions and maximum P_N was observed under mild shade, which was statistically the same as moderate shade. At peak sunshine hours, *i.e.,*12:00 and 14:00 h, shade again increased P_N significantly. At 8:00 h, when light was weak, shade significantly decreased the P_N and BR did not help mitigate negative effects of shade at that light intensity. The maximum g_s occurred at 8:00 h, after which it declined and increased again at 18:00 h at each level of shade. Shade and BR treatments did not influence the g_s significantly, however, an increase was observed in day mean values under shade. The maximum E was observed at 10:00 h, but then decreased at 12:00 h and again decreased up to 18:00 h

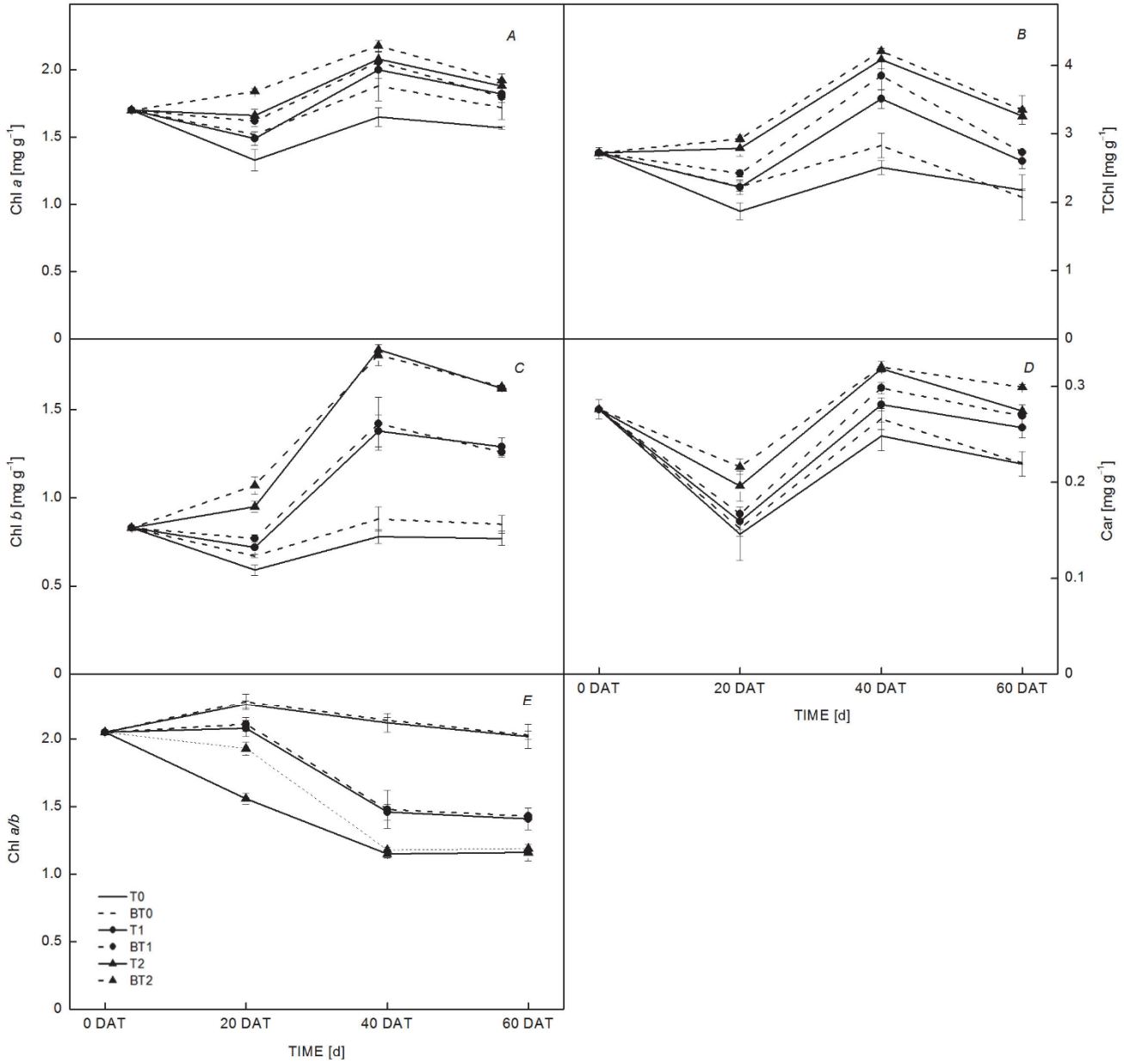

Fig. 2. Dynamic changes after BR treatment on Chl *a,* Chl *b,* Chl *a*/*b,* TChl, and Car of *Leymus chinensis* under different light intensities. Mean value ± SE (n = 5), DAT – days after treatments. Car – carotenoids, Chl – chlorophyll, TChl – total chlorophyll content, T_0 – 100% natural light, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}].

at each level of shade. Day mean values as well as different day time values did not suggest any significant effect of shade and BR spray on *E*. However, under natural light and moderate shade conditions, higher *E* was found at 10:00 h, while under mild shade, maximum increase in *E* occurred at 8:00 h. Overall, an increase in P_N and g_s, and decrease in *E* were noticed in their day mean values under shade, but this effect was not statistically significant (Table 2). Shade enhanced the most of the gas-exchange parameters in the order: mild shade > moderate shade > natural light, while it resulted in reduced *E* and C_i as compared with control. The shade significantly increased P_N compared with natural light conditions and BR boosted up the shade effect insignificantly. The maximum P_N was observed at mild shade followed by moderate shade and natural light which were statistically same with each other. The g_s was not much affected by shade and BR spray, but stomatal limitation (L_s) was maximum under both shade conditions. The *E* significantly decreased under shade compared with control; it was minimum at moderate shade and

Table 2. Effect of BR on photosynthetic diurnal variation of *Leymus chinensis* under different light intensities. P_N – net photosynthetic rate, g_s – stomatal conductance, E – transpiration rate, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}]. Values in the table are means ± SE (n = 5). Values followed by *the same letter* within columns (for the same index) are not significantly different according to *Duncan*'s multiple range test (p<0.05).

Index	Treatment	Time [h] 6:00	8:00	10:00	12:00	14:00	16:00	18:00	Day mean value
P_N	T_0	1.510 ± 0.357^{a}	2.816 ± 0.358^{a}	8.619 ± 0.359^{b}	0.712 ± 0.123^{c}	0.075 ± 0.003^{c}	4.907 ± 0.365^{b}	1.214 ± 0.081^{b}	2.836
[μmol m^{-2} s^{-1}]	BT_0	1..781 ± 0.432^{a}	2.748 ± 0.385^{a}	9.581 ± 0.701^{b}	0.802 ± 0.323^{c}	0.080 ± 0.022^{c}	5.872 ± 0.641^{ab}	1.259 ± 0.139^{ab}	3.160
	T_1	0.812 ± 0.107^{a}	1.523 ± 0.138^{b}	14.873 ± 0.092^{a}	1.246 ± 0.009^{ab}	0.524 ± 0.123^{a}	6.354 ± 0.499^{ab}	1.236 ± 0.142^{ab}	3.795
	BT_1	2.182 ± 0.388^{a}	2.135 ± 0.086^{ab}	15.023 ± 0.879^{a}	1.493 ± 0.359^{ab}	0.558 ± 0.031^{a}	7.122 ± 0.523^{a}	1.417 ± 0.139^{a}	4.283
	T_2	1.262 ± 0.028^{a}	1.426 ± 0.039^{b}	13.116 ± 1.13^{a}	0.923 ± 0.082^{bc}	0.253 ± 0.024^{b}	5.463 ± 0.644^{ab}	1.348 ± 0.169^{a}	3.398
	BT_2	1.687 ± 0.367^{a}	1.511 ± 0.158^{b}	13.225 ± 0.835^{a}	0.933 ± 0.03^{bc}	0.329 ± 0.143^{b}	6.909 ± 0.311^{a}	1.280 ± 0.173^{b}	3.696
g_s	T_0	0.0280 ± 0.0010^{a}	0.0391 ± 0.0037^{a}	0.0177 ± 0.0017^{a}	0.0116 ± 0.0004^{a}	0.0133 ± 0.0015^{a}	0.0088 ± 0.0015^{a}	0.0100 ± 0.0014^{ab}	0.0184
[mmol(H_2O) m^{-2} s^{-1}]	BT_0	0.0313 ± 0.0001^{a}	0.0362 ± 0.0018^{a}	0.0174 ± 0.0029^{a}	0.0164 ± 0.0025^{a}	0.0115 ± 0.0003^{a}	0.0094 ± 0.0010^{a}	0.0109 ± 0.0006^{ab}	0.0190
	T_1	0.0283 ± 0.0045^{a}	0.0433 ± 0.0055^{a}	0.0141 ± 0.0078^{a}	0.0127 ± 0.0008^{a}	0.0151 ± 0.0020^{a}	0.0100 ± 0.0029^{a}	0.0132 ± 0.0018^{a}	0.0205
	BT_1	0.0268 ± 0.0021^{a}	0.0504 ± 0.0075^{a}	0.0211 ± 0.0022^{a}	0.0122 ± 0.0005^{a}	0.0155 ± 0.0013^{a}	0.0066 ± 0.0004^{a}	0.0121 ± 0.0005^{ab}	0.0207
	T_2	0.0314 ± 0.0020^{a}	0.0372 ± 0.0051^{a}	0.0147 ± 0.0074^{a}	0.0134 ± 0.0024^{a}	0.0138 ± 0.0018^{a}	0.0074 ± 0.0011^{a}	0.0084 ± 0.0016^{b}	0.0191
	BT_2	0.0310 ± 0.0007^{a}	0.0439 ± 0.0038^{a}	0.0229 ± 0.0031^{a}	0.0145 ± 0.0002^{a}	0.0145 ± 0.0023^{a}	0.0056 ± 0.0008^{a}	0.0107 ± 0.0009^{ab}	0.0204
E	T_0	0.370 ± 0.010^{a}	0.962 ± 0.074^{a}	0.994 ± 0.123^{a}	0.664 ± 0.123^{a}	0.912 ± 0.090^{a}	0.644 ± 0.054^{a}	0.561 ± 0.046^{ab}	0.730
[mmol(H_2O) m^{-2} s^{-1}]	BT_0	0.354 ± 0.052^{a}	0.852 ± 0.090^{a}	0.903 ± 0.153^{a}	0.699 ± 0.048^{a}	0.835 ± 0.162^{a}	0.715 ± 0.207^{a}	0.677 ± 0.090^{a}	0.719
	T_1	0.358 ± 0.011^{a}	0.889 ± 0.016^{a}	0.795 ± 0.127^{a}	0.669 ± 0.127^{a}	0.738 ± 0.011^{a}	0.634 ± 0.066^{a}	0.551 ± 0.034^{ab}	0.662
	BT_1	0.347 ± 0.009^{a}	0.835 ± 0.091^{a}	0.800 ± 0.070^{a}	0.647 ± 0.019^{a}	0.766 ± 0.080^{a}	0.581 ± 0.094^{a}	0.568 ± 0.064^{ab}	0.649
	T_2	0.345 ± 0.021^{a}	0.918 ± 0.114^{a}	0.963 ± 0.089^{a}	0.599 ± 0.057^{a}	0.661 ± 0.086^{a}	0.495 ± 0.027^{a}	0.557 ± 0.027^{ab}	0.648
	BT_2	0.363 ± 0.018^{a}	0.824 ± 0.108^{a}	0.779 ± 0.140^{a}	0.714 ± 0.128^{a}	0.818 ± 0.098^{a}	0.495 ± 0.070^{a}	0.442 ± 0.083^{b}	0.634

Table 3. Effect of BR on gas exchange characteristics of *Leymus chinensis* under different light intensities at 60 d after treatments. C_i – intercellular CO_2 concentration, CUE – carboxylation utilization, E – transpiration rate, g_s – stomatal conductance, L_s – stomatal limitation, P_N – net photosynthetic rate, SUE – sunlight-utilization efficiency, WUE – water-use efficiency, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}].Values in the table are means ± SE (n = 5). Values followed by *the same letter* within columns are not significantly different according to *Duncan*'s multiple range test (p < 0.05).

Treatment	P_N [μmol m^{-2} s^{-1}]	g_s [mmol(H_2O) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	C_i [μmol (CO_2)mol^{-1}]	SUE	WUE [μmol(CO_2) mmol(H_2O)$^{-1}$]	CUE [mol m^{-2} s^{-1}]	L_s [%]
T_0	1.377 ± 0.158^{b}	0.057 ± 0.002^{a}	1.544 ± 0.208^{a}	395.73 ± 6.26^{a}	0.0014 ± 0.0002^{b}	0.908 ± 0.109^{c}	0.0035 ± 0.0004^{b}	0.006 ± 0.016^{c}
BT_0	1.441 ± 0.005^{b}	0.063 ± 0.007^{a}	1.483 ± 0.080^{ab}	386.91 ± 3.96^{ab}	0.0014 ± 0.0000^{b}	0.976 ± 0.048^{c}	0.0037 ± 0.0000^{b}	0.016 ± 0.010^{bc}
T_1	2.571 ± 0.139^{a}	0.098 ± 0.009^{a}	1.315 ± 0.026^{ab}	328.01 ± 1.39^{c}	0.0026 ± 0.0001^{a}	1.958 ± 0.135^{ab}	0.0078 ± 0.0004^{a}	0.165 ± 0.004^{a}
BT_1	2.861 ± 0.060^{a}	0.117 ± 0.010^{a}	1.220 ± 0.046^{ab}	320.77 ± 6.93^{c}	0.0029 ± 0.0001^{a}	2.355 ± 0.141^{a}	0.0089 ± 0.0003^{a}	0.180 ± 0.018^{a}
T_2	1.591 ± 0.204^{b}	0.064 ± 0.002^{a}	1.153 ± 0.066^{b}	375.09 ± 6.00^{b}	0.0016 ± 0.0002^{b}	1.370 ± 0.096^{bc}	0.0043±0.0006^{b}	0.047 ± 0.015^{b}
BT_2	1.967 ± 0.340^{b}	0.071 ± 0.003^{a}	1.283 ± 0.093^{ab}	335.31 ± 3.65^{c}	0.0020 ± 0.0003^{b}	1.582 ± 0.354^{bc}	0.0059±0.0011^{b}	0.148 ± 0.009^{a}

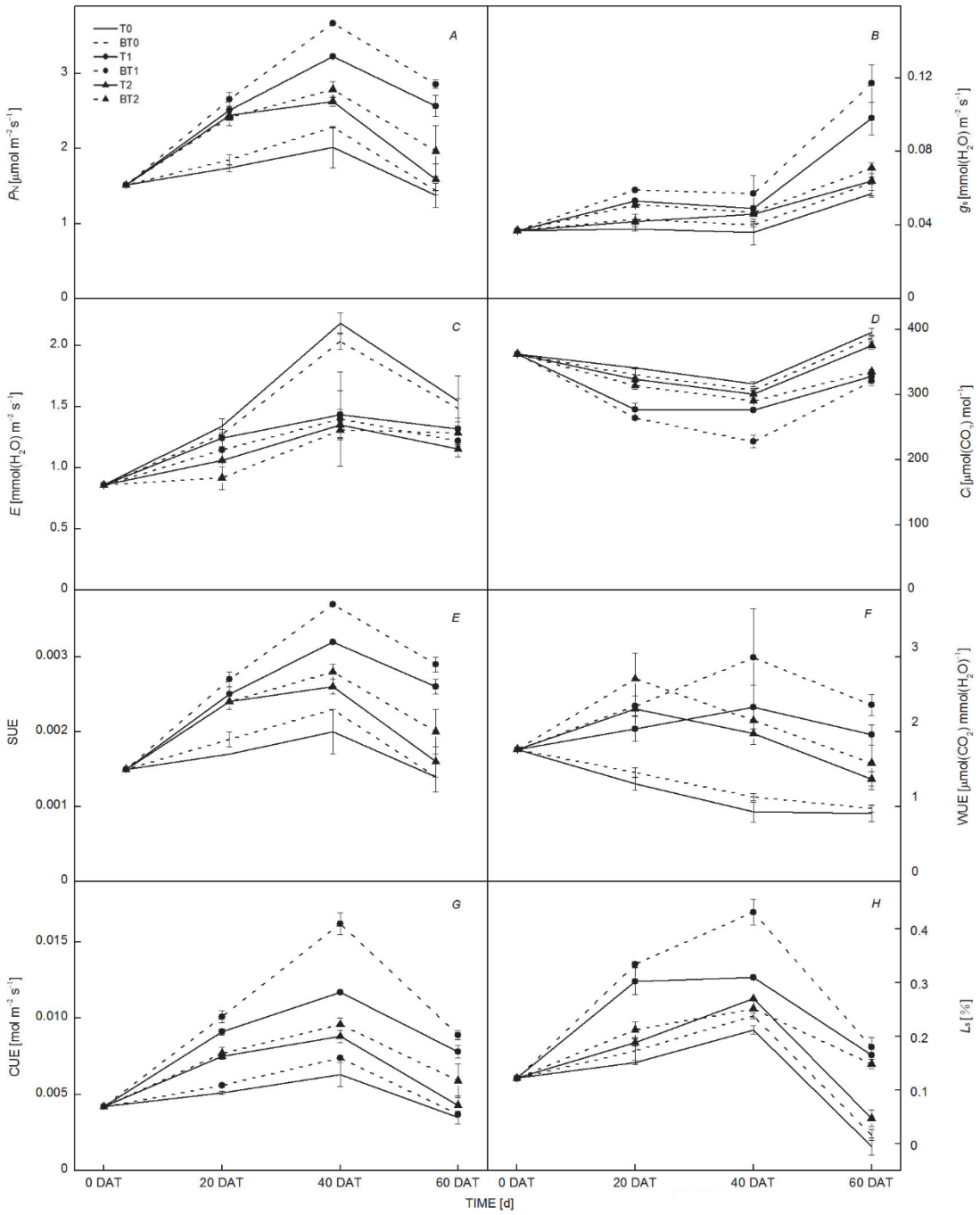

Fig. 3. Dynamic changes after BR treatment on gas-exchange characteristics of *Leymus chinensis* under different light intensities. Mean value ± SE (n = 5). DAT – days after treatments. C_i – intercellular CO_2 concentration, CUE – carboxylation utilization, E – transpiration rate, g_s – stomatal conductance, L_s – stomatal limitation, P_N – net photosynthetic rate, SUE – sunlight-utilization efficiency, WUE – water-use efficiency, T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}].

statistically different from control, *i.e.*, normal natural light conditions. Shade significantly decreased C_i compared with natural light conditions. The minimum C_i values were observed at mild shade followed by moderate shade. Shade substantially increased sunlight-utilization efficiency (SUE), water-use efficiency (WUE), and carboxylation efficiency (CUE) compared with normal light conditions. However, BR spray did not boost shade effect for SUE, WUE, and CUE significantly. Maximum SUE, WUE, and CUE were observed at mild shade

Table 4. Effect of BR on chlorophyll fluorescence traits of *Leymus chinensis* under different light intensities after 18 days of treatments. DAT – days after treatments, F_0 – minimal fluorescence yield of the dark-adapted state, F_m – maximal fluorescence yield of the dark-adapted state, F_v/F_m – optimal/maximal quantum yield of PSII photochemistry, F_v/F_0 – optimal/minimal quantum yield of PSII or photochemical efficiency of PSII, Φ_{PSII} – photosynthetic quantum yield of PSII. T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}]. Values in the table are means ± SE (n = 5). Values followed by *the same letter* within columns are not significantly different according to *Duncan*'s multiple range test (p<0.05).

Treatment	F_0	F_m	F_v/F_m	F_v/F_0	Φ_{PSII}
T_0	29.932 ± 1.389^{b}	135.175 ± 6.82^{c}	0.778 ± 0.004^{b}	3.527 ± 0.252^{c}	0.214 ± 0.023^{b}
BT_0	30.216 ± 0.791^{b}	143.642 ± 2.214^{bc}	0.790 ± 0.002^{ab}	3.757 ± 0.055^{bc}	0.239 ± 0.025^{b}
T_1	41.098 ± 1.463^{a}	217.285 ± 8.585^{a}	0.811 ± 0.001^{a}	4.286 ± 0.025^{a}	0.367 ± 0.024^{a}
BT_1	41.106 ± 1.523^{a}	218.371 ± 11.172^{a}	0.811 ± 0.003^{a}	4.307 ± 0.09^{a}	0.378 ± 0.028^{a}
T_2	31.464 ± 2.425^{b}	150.642 ± 8.787^{bc}	0.792 ± 0.006^{ab}	3.804 ± 0.139^{bc}	0.282 ± 0.023^{ab}
BT_2	33.418 ± 0.968^{b}	168.681 ± 3.561^{b}	0.802 ± 0.002^{ab}	4.05 ± 0.054^{ab}	0.292 ± 0.031^{ab}

followed by moderate shade (Table 3). The P_N, E, SUE, CUE, and L_s were elevated from 20 to 40 DAT followed by a decrease, while g_s and C_i declined from 20 DAT onwards until 40 DAT and then again increased until 60 DAT. However, a differential response was noticed in WUE, where under natural light and moderate shade condition, the maximum value was obtained at 20 DAT, while at mild shade, the maximum value was observed at 40 DAT. The E followed the order: natural light > mild shade > moderate shade, while C_i at 20 and 40 DAT was in the order: natural light > moderate shade > mild shade, and at 60 DAT, the order was natural light > mild shade > moderate shade (Fig. 3).Chl fluorescence attributes of *L. chinensis* were significantly affected by shade, while BR application did not change Chl fluorescence attributes significantly at each level of shade. Shade increased the F_0 and F_m over normal natural light conditions. Maximum values for F_0 and F_m were observed under mild shade followed by moderate shade which was statistically same with normal natural light conditions. Shade also increased the optimal/minimal quantum yield of PSII or photochemical efficiency of PSII (F_v/F_0) and optimal/maximal quantum yield of PSII (F_v/F_m) and photosynthetic quantum yield of PSII (Φ_{PSII}) significantly over control. Maximum values for F_v/F_0, F_v/F_m, and Φ_{PSII} were observed at mild shade followed by moderate shade which was statistically as under natural light conditions. Overall, shade exaggerated all the Chl fluorescence attiributes of *L. chinensis* as compared to natural light and the order of increase was: mild shade > moderate shade > natural light (Table 4). F_0 and F_m decreased first from 6–12 DAT and again increased until 18 DAT. The F_v/F_0 and F_v/F_m continued decreasing from 6 until 18 DAT. Φ_{PSII} increased from 6 to 12 DAT and then decreased until 18 DAT. Improvement in Chl fluorescence attributes under shade was evident at each stage of growth (Fig. 4).

Discussion

The increased growth of *L. chinensis* under shade was attributable to the enhanced biosynthesis of photosynthetic pigments and photosynthetic activity. Similar to our results, it has been reported that the growth of European yew improved with increased shade (Perrin and Mitchell 2013). Light is one of the primary factors affecting the plant growth and productivity through modulation of plant physiological processes and the light intensity has profound position in this context (Wang *et al*. 2016). The increase in *L. chinensis* growth by BR spraying under normal and shade conditions may be attributed to the positive modifications in the cell division and elongation (Fujii and Saka 2001, Vriet *et al*. 2012). Nassar (2004) reported an increase in height and biomass of banana plants by application of homobrassinolide under heat stress. Application of BR to plants can improve plant growth and development, but in the present study, BR did not influence photosynthetic pigments and photosynthetic activity significantly.

Photosynthetic pigments, such as Chl *a,* Chl *b*, TChl, and Car, increased linearly with the increase in shade, *i.e.*, from 100% of natural light to 50% natural light. The increase in biosynthesis of photosynthetic pigments under shade indicated the acclimation of *L. chinensis* to low light conditions for capturing and utilizing maximum amount of light energy and enhancing photosynthetic efficiency (French and Moore 2003). Similar results were reported by Chaves *et al*. (2008) who observed an increase in Chl concentrations in coffee plants under low light conditions as compared to high light conditions. In our study, application of BR further increased the biosynthesis of photosynthetic pigments at each level of shade as observed in *Lycopersicon esculentum* due to BR spray (Hayat *et al*. 2011). Our results are in line with Janeczko *et al*. (2007)

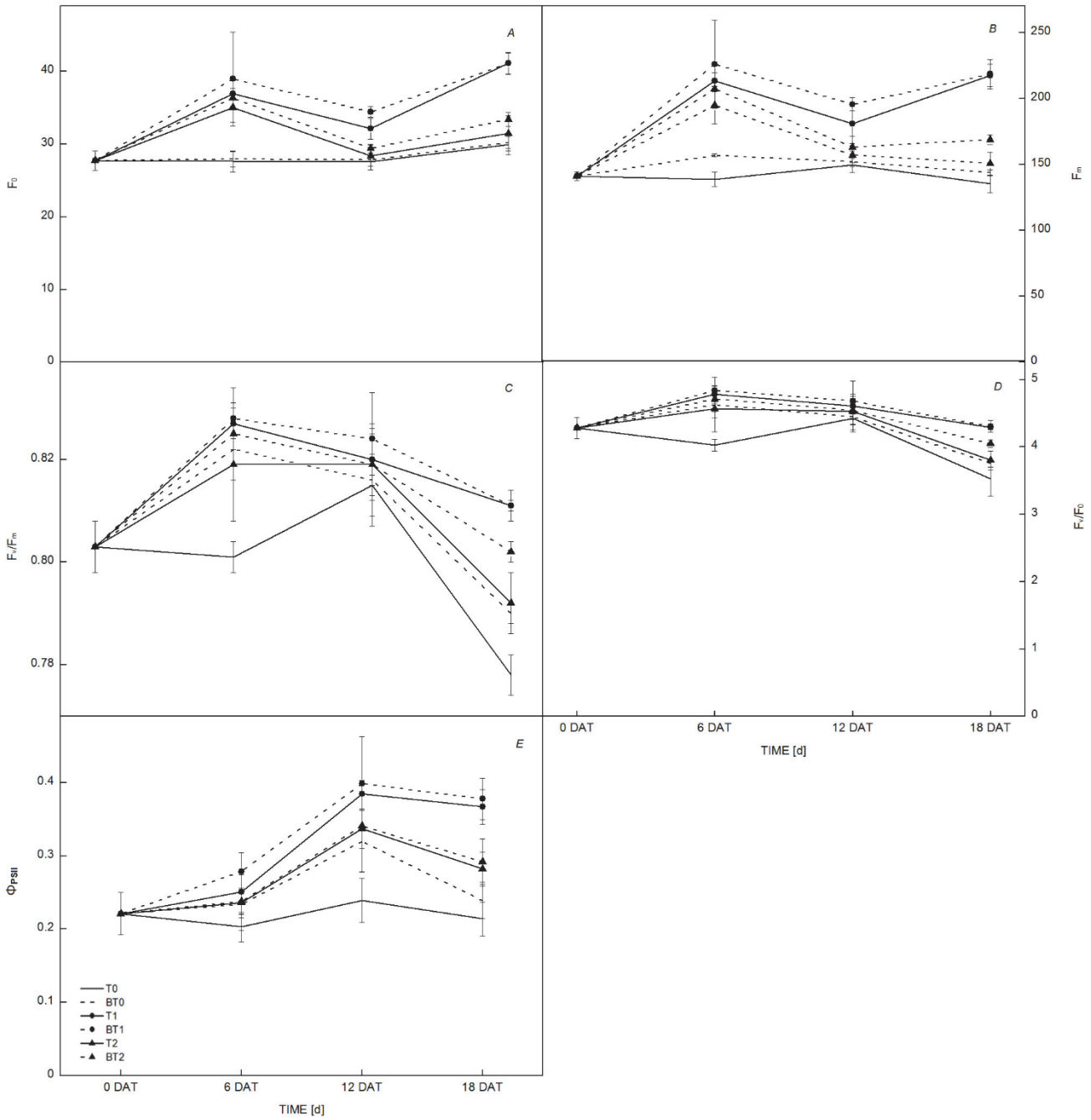

Fig. 4. Dynamic changes after BR treatment on chlorophyll fluorescence traits of *Leymus chinensis* under different light intensities. Mean value ± SE (n = 5). DAT – days after treatments, F_0 – minimal fluorescence yield of the dark-adapted state, F_m – maximal fluorescence yield of the dark-adapted state, F_v/F_m – optimal/maximal quantum yield of PSII photochemistry, F_v/F_0 – optimal/minimal quantum yield of PSII or photochemical efficiency of PSII, F_v – variable fluorescence, Φ_{PSII} – photosynthetic quantum yield of PSII. T_0 – 100% natural light, T_1 – mild shade (70% natural light), T_2 – moderate shade (50% natural light), BT_0 – 100% natural light + 0.1 mg(BR) L^{-1}, BT_1 – mild shade + BR [70% natural light + 0.1 mg(BR) L^{-1}], BT_2 – moderate shade + BR [50% natural light + 0.1 mg(BR) L^{-1}].

who reported higher concentrations of photosynthetic pigments in rape plants in response to BR application under low temperature stress.

The highest values of P_N at 10:00 h indicated that at this time, the light intensity was optimum for *L. chinensis* to have maximum photosynthetic efficiency, and mild shade (70% natural light) further favored this increase. However, it was also observed that at peak sunshine hours, *i.e.*, 12:00 and 14:00 h, P_N drastically dropped down. The reason behind this might be reduced efficiency of

photosynthetic apparatus due to absorbance of excessive light energy, which causes inactivation of photosynthetic machinery through photoinhibition (Bertamini *et al*. 2006, Dai *et al*. 2009). Shade significantly increased the P_N during peak sunshine hours by reducing the photoinhibition phenomenon. Reduction in P_N at 8:00 (morning) was observed because at this time, PAR level is generally very low and shade treatment further blocked the sun light. The insignificant change in g_s and E under shade and BR treatment might be due to the fact that these are normally temperature- and water-dependent.

Improvement in gas-exchange parameters under shade might be through exacerbation of antioxidants activity, and stomatal regulation under shade that resulted in better utilization of light energy and increased CO_2 assimilation rate leading to enhanced photosynthetic efficiency (Chaves *et al*. 2008). Tang *et al*. (2015) reported an increase in photosynthetic rate of *Torreya grandis* seedlings under shade due to enhanced biosynthesis of photosynthetic pigments, better protection of photosynthetic machinery, and also due to ultrastructural changes in chloroplasts, such as increased number of grana, grana lamellae, and osmiophilic globule number and size.

Our results showed that the shade increased the Chl fluorescence attributes, *i.e.*, F_0, F_v, F_v/F_m, F_v/F_0, and Φ_{PSII} with maximum increase occurring under 70% natural light. In plants exposed to continuous high light, Chl fluorescence declines due to combination of photochemical quenching (q_P) and nonphotochemical quenching (NPQ) in order to avoid photodamage (Müller *et al.* 2001; Ensminger *et al*. 2006). Shade protects the plants from continuous high light. Plants subjected to high irradiances show usually lower values of F_v/F_m than those, which are subjected to low irradiances (Björkman and Demmig 1987, Baker 2008). Demmig-Adams and Adams III (1992) reported that F_v/F_m values were lower in Pacific yew foliage exposed to sun as compared to foliage grown in shade. Higher values of quantum yield of PSII under mild shade (70% natural light) revealed that shade substantially nullified the effects of 100% natural light. Between 6–20 DAT, concentrations of photosynthetic pigments, such as Chl *a,* Chl *b*, and Car, were the lowest under 100% natural light conditions but their concentration substantially increased under shade. Chl fluorescence attributes also increased, thus our results confirmed that shade significantly mitigated the effects of photoinhibition on plants.

Conclusion: Our results revealed that shade substantially enhanced the plant growth and biosynthesis of photosynthetic pigments by increasing the photosynthetic efficiency and chlorophyll fluorescence attributes of *L. chinensis*. Based on the present data, we cannot conclude that brassinolide correlated positively with shade regarding increase in growth and photosynthetic efficiency.

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