



Full Length Article

Dry Matter Partitioning and Mineral Constitution Response of Sunflower (*Helianthus annuus*) to Integrated Nitrogen and Boron Nutrition in Calcareous Soils

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Abstract

Abundant plant growth and development prominently rely on adequate supply of macro and micro nutrients. Among various nutrients, nitrogen (N) and boron (B) holds great importance in sustaining and enhancing the productivity of sunflower. In calcareous soils of Pakistan, B deficiency is prevalent that affects the uptake of nutrients. The present field study aimed to assess the impact of B on biomass production and nutrient absorption in calcareous soils by considering different B doses (0–6 kg ha⁻¹) under variable N rates (50–100% RN ‘recommended nitrogen’) for consecutive two years of 2011 and 2012. The growth traits as leaf area index, leaf area duration, crop growth rate, net assimilation rate and dry matter accumulation were significantly affected with B rate of 3.20–3.50 kg ha⁻¹ with 100% RN. In addition, B rate of 3.44 kg ha⁻¹ with 100% RN produced maximum biological yield and harvest index in both years. Leaf and achene N contents were higher by 3.32 and 3.38 kg B ha⁻¹, respectively with 100% RN fertilization as compared to 75% RN and 50% RN. B fertilization at 3.50 kg ha⁻¹ also had highest B contents in leaf and achenes under 100% RN. The lower B contents were found at reduced level of 50% RN in contrast to 100% RN. Chlorophyll pigments (chl *a*, *b* and total) were also affected positively with B rate of 3.46 kg ha⁻¹ under highest level of N nutrition. However, higher biomass production and mineral uptake could be obtained at 3.0–3.50 kg ha⁻¹ B rate with 100% RN compared to 75% RN and 50% RN nutrition. © 2016 Friends Science Publishers

Keywords: Dry matter yield; N-B interaction; Nutrient uptake; Chlorophyll pigments; *Helianthus annuus*

Introduction

At present, there is an acute shortage of edible oil in Pakistan, as its accessibility is reducing every year due to increased human population (Chaudhry *et al.*, 1998). The gap between demand and supply is bridged through imports of edible oil over the years. To fulfill the edible oil requirement, sunflower crop has great potential to confine the gap between supply and demand of edible oil compared to conventional oil seed crops (Shah *et al.*, 2013).

Concerning sunflower growth limiting restraints, imbalanced nutrition is one of the most important limiting factors related to sunflower yield (Wivstad, 2005). The availability of nitrogen (N) in alkaline-calcareous soils is reduced due to high temperature and volatilization losses. Its deficiency causes reduced cell development and stunting growth due to deterioration of chlorophyll pigments which are used for the synthesis of photosynthetic apparatus (Mahmood *et al.*, 2001; Rasheed *et al.*, 2004). The metabolic processes based on protein synthesis, responsible to increase the growth and yield are strongly dependents of N supply. Insufficiency of N persuades physiological and morphological modifications like reduced shoot growth, leaf

numbers, leaf area and chlorosis etc. in crop plants (Du *et al.*, 2015). Its increased supply gives faster leaf expansion, leaf area and photosynthetic rate through uptake of more minerals from the soil rhizosphere and finally improved grain yield. Optimal management of N fertilization has been considered highly interdependent and essential for plant growth and improved crop performance (Pandey *et al.*, 2000; Suo and Wang, 2000).

Boron (B) is an essential micro nutrient necessary for improving sunflower production (Brown *et al.*, 2002). Hellal *et al.* (2009) stated the contribution of B in meristematic growth, cell division, elongation, cell wall stability and binding of nitrogenous synthesizes, which in turn maximize the crop growth rate. Boron is also involved in the formation of pollen tube, seed filling, transportation of sugars, nucleic acid metabolism, lignifications, respiration, protein synthesis, RNA, IAA and carbohydrates metabolism, transportation across the membranes, enzymes, growth regulators and N uptake which finally enhanced the growth of sunflower (Shaaban, 2010). In calcareous soils, there is a problem of nutrient uptake due to low organic matter, high pH and temperature; and B supply in these soils may be helpful to increase nitrogenase enzyme activity by

providing adequate oxygen for N uptake (Bonilla *et al.*, 1997). In view of above considerations, the current study was undertaken to explore the interactive effect of N and B nutrition on growth and mineral uptake of sunflower, and also to determine the adequate N and B application rates for higher sunflower productivity in calcareous soils.

Materials and Methods

Experimental Design and Details

A field trial was planned for the cropping seasons of 2011 and 2012 at the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan (73° 06' E, 31° 26' N and at altitude of 184.4 m). The experimental soil was characterized as sandy clay loam with 58.73% sand, 21.08% silt, 20.19% clay, 7.9 pH, 1.25 dS m⁻¹ EC, 0.85% organic matter, 0.057% N, 6.49 mg kg⁻¹ P, 172 mg kg⁻¹ K and 0.39 mg kg⁻¹ B.

The experimental treatments were randomized in factorial arrangement under randomized complete block design with three replicates and plot's dimension of 7.0 m × 3.0 m. The proposed study was comprised of 50% (56 kg ha⁻¹), 75% (84 kg ha⁻¹) and 100% (112 kg ha⁻¹) recommended nitrogen (RN) as Urea (46% N) source. B rates applied were 0, 2.0, 4.0 and 6.0 kg ha⁻¹ with boric acid source (17% B). For cultivation, ridges were prepared with tractor mounted cultivator by keeping specific planting geometry (75 cm × 20 cm). Hysun-33 sunflower hybrid was sown using hand dibbling method during 1st week of August in both years (2011 and 2012) with 6 kg ha⁻¹ seed rate. P in the form of triple super phosphate (TSP) and K as sulphate of potash (SOP) fertilizers were applied at 57 and 62 kg ha⁻¹, respectively. Whole phosphorus and potash, and one third N were applied to the experimental units as basal dose with band placement. Remaining N was fertilized into two splits at 4-6 leaf and flowering growth stages. B fertilizer was applied as per treatment through mixing into the soil as basal dose and at 4-6 leaf stage. Four irrigations were applied during the whole growing period. Plant protection measures were implemented to keep crop free of weeds, insect pests, and diseases. The crop was harvested on mid-November, 2011 and 2012.

Measurements

Leaf samples from an area of 1.0 m × 1.0 m were randomly collected from five plants of each experimental plot at pre- and post-anthesis growth stages of sunflower. Leaves were separated, fresh weight recorded at each sampling and a sub-sample of 20 g was used to measure leaf area using an automatic leaf area meter and then summed up. Leaf area index (LAI) was computed as the fraction of total leaf area to the land area. Leaf area duration (LAD) was estimated by using the method of Hunt (1978) as; $LAD_1 = [(LAI_1 + LAI_2) \times (t_2 - t_1)]/2$. Where, LAI₁ and LAI₂ are the leaf area indices at time t_1 and t_2 , respectively. At final harvest, cumulative

LAD was calculated by summing up all the values calculated at different harvests. Crop growth rate (CGR) was computed using the formula of Hunt (1978); $CGR (g m^{-2} day^{-1}) = (W_2 - W_1)/(t_2 - t_1)$ where, W_1 and W_2 are the total dry masses per unit land area at time t_1 and t_2 , respectively. Net assimilation rate (NAR) was determined as: $NAR (g m^{-2} day^{-1}) = (TDM/LAD)$ where, TDM is total dry matter and LAD is the leaf area duration. The same samples harvested to calculate LAI were oven dried at 70°C until constant weight providing total dry matter (TDM). Weight of air dried plants (except achenes) was recorded on plant basis and then converted to kg ha⁻¹. The recorded weight was then added to the already calculated achene yield (kg ha⁻¹) to find out biological yield. Harvest index (HI) was computed as the ratio of achene yield to biological yield and expressed in percentage.

$$HI (\%) = (\text{Achene yield} / \text{Biological yield}) \times 100$$

The mineral constitution of N and B in leaves and achenes were estimated by micro-Kjeldahl (N × 5.95) and Azomethine-H methods, respectively (Chapman and Pratt, 1961; Bremner, 1965). The chlorophyll contents (chl. *a*, *b*, and total) were measured following the method cited by Maqsood *et al.* (2013). Fresh leaves (0.05 g) were chopped by using 80% acetone. After dissolving all green portion of leaf, the final volume of 5 mL was made with 80% acetone solution and extracted overnight upon 4°C. The extracts were centrifuged at 10,000 rpm for 5 min. Absorbance of filtered supernatant was noted at 645 nm and 663 nm using a double beam spectrophotometer (Cecil, 7200) and the contents of chlorophyll were estimated using the following equations;

$$\text{Chl } a (\text{mg g}^{-1} \text{ f. wt.}) = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645})] \times V / 1000 \times W$$

$$\text{Chl } b (\text{mg g}^{-1} \text{ f. wt.}) = [22.9 (\text{OD}_{645}) - 4.68 (\text{OD}_{663})] \times V / 1000 \times W$$

Whereas, the total chlorophyll contents were determined by summing chl *a* and *b*.

Statistical Analysis

Preparation of figures and graphs was done by expending Microsoft Excel and regression analysis was performed to determine the best B rate using model with highest determination coefficient at 95% confidence interval. For statistical analysis of collected data, MSTAT statistical package was employed through analysis of variance function (Russel and Eisensmith, 1983). When F-test indicated statistical significance, the treatments' means were divided using the least significance different (LSD) test at $p \leq 0.05$.

Results

Growth Indices

Soil B application of 3.37 and 3.28 kg ha⁻¹ for 50- and 100% RN respectively, significantly improved the leaf area index at pre-anthesis in contrast to post-anthesis stages in 2011.

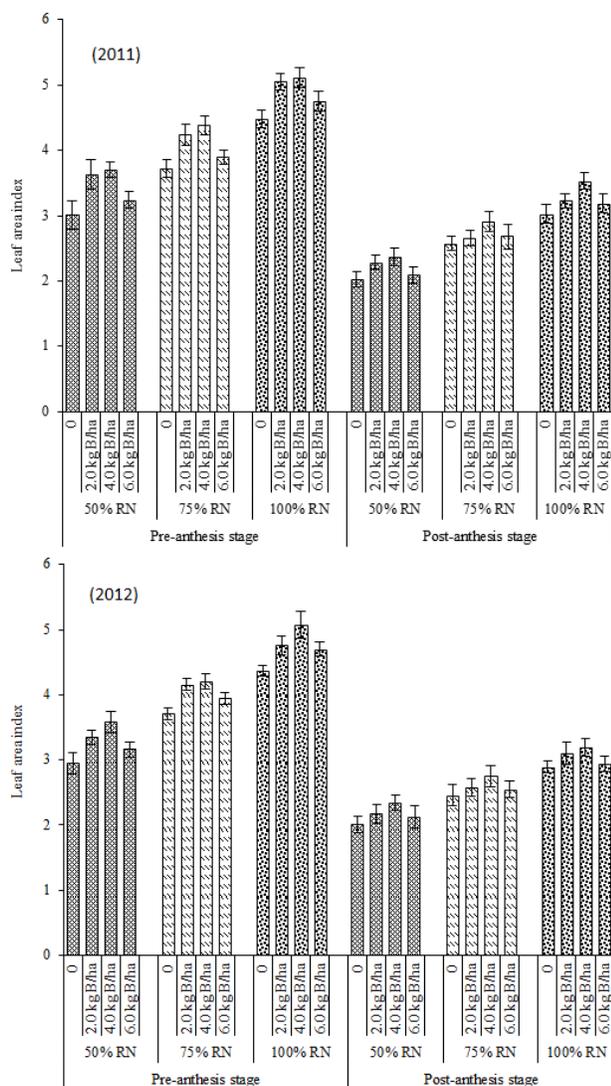


Fig. 1: Leaf area index of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

With 75% RN nutrition, the B application of 3.27 kg ha⁻¹ performed well in giving maximum leaf area index at pre-anthesis stage (Fig. 1). At post-anthesis stage, maximum leaf area index was attained with B application of 3.20, 3.72 and 3.58 kg ha⁻¹ with 100-, 75- and 50% RN levels, respectively. The maximum rate for B considerably reduced the leaf area index under least N level of 50% RN. During 2012, maximum improvement in leaf area index was attained at 3.68 and 3.21 kg ha⁻¹ B rates with 100% RN at both pre- and post-anthesis stages, respectively. Under limited N levels of 75- and 50% RN, 3.43 and 3.42 kg ha⁻¹ B rates, respectively also increased the leaf area index at pre-anthesis stage. At post-anthesis stage, B application rates of 3.21, 3.55 and 3.53 kg ha⁻¹ ensured maximum leaf area index in the order of 100-, 75 and 50% RN (Fig. 1). Use of 100% RN followed by 75% and 50% RN produced

maximum leaf area index. The best B rate for growth related attributes of sunflower was verified by regression model equations (Table 1).

The higher leaf area duration was observed with 100% RN as compared to 75- and 50% RN. B application of 3.31 and 3.47 kg ha⁻¹ for 100% RN at pre- and post-anthesis stages caused a significant enhancement in leaf area duration during 2011 (Fig. 2). Application of B at 3.45 kg ha⁻¹ followed by 3.44 kg ha⁻¹ under 75% RN increased the leaf area duration at both stages. Under least N level of 50% RN, maximum increase in leaf area duration was recorded with B application of 3.25 and 3.39 kg ha⁻¹ at both stages of pre- and post-anthesis (Fig. 2). In 2012, the maximum leaf area duration at pre-anthesis stage was observed with 3.48, 3.55 and 3.54 kg ha⁻¹ B applications following N levels of 100-, 75- and 50% RN. At post-anthesis stage, 3.52 and 3.42 kg ha⁻¹ B provided maximum leaf area duration with 100- and 75% RN levels, while 3.32 kg ha⁻¹ B responded superior for 75% RN (Fig. 2). At pre- and post-anthesis stages, B soil application of 3.35 and 3.18 kg ha⁻¹ respectively, had maximum crop growth rate at 100% RN during first year. Higher improvement in crop growth rate was found with B application of 3.38 and 3.43 kg ha⁻¹ with 75% RN, while 3.34 and 3.78 kg ha⁻¹ B rates with 50% RN ensured maximum crop growth rate at pre- and post-anthesis stages, respectively (Fig. 3). In 2012, the maximum increase in crop growth rate at pre-anthesis stage was recorded with 3.49 and 3.65 kg ha⁻¹ soil applied B following the order of 100- and 75% RN, while the B rate of 3.56 kg ha⁻¹ performed better with 50% RN (Fig. 3). Soil applied B at 3.27 kg ha⁻¹ with 100% RN at post-anthesis stage provided best response regarding maximum crop growth rate. Under reduced N levels of 75- and 50% RN, B applications of 3.16 and 3.76 kg ha⁻¹, respectively had higher crop growth rate (Fig. 3). The maximum leaf area duration was recorded with 100% RN, while minimum was with 50% RN.

Maximum net assimilation rate of sunflower plants was showed by 3.47 and 3.07 kg ha⁻¹ B concentrations with 100% RN at pre- and post-anthesis stages respectively during 2011. Soil applied B at 3.46 kg ha⁻¹ provided better response for increased net assimilation rate with 75% RN at pre-anthesis, while B application of 3.63 kg ha⁻¹ behaved well at post-anthesis stage with same N level. B application of 3.44 kg ha⁻¹ exhibited maximum improvement in net assimilation rate at pre-anthesis with 50% RN, while B rate of 3.74 kg ha⁻¹ remained dominant at post-anthesis stage during first year (Fig. 4). B soil application at 3.59 kg ha⁻¹ with 100% RN remained best at pre-anthesis stage in improving the maximum net assimilation rate in 2012. Under 75% RN, B fertilization at 3.53 kg ha⁻¹ had greater net assimilation rate, while 3.75 kg ha⁻¹ provided maximum response for achieving higher net assimilation rate at pre-anthesis stage under 50% RN. Among various soil B rates, 3.38, 3.51 and 3.20 kg ha⁻¹ had maximum net assimilation rate at post-anthesis stage with 100-, 75- and 50% RN

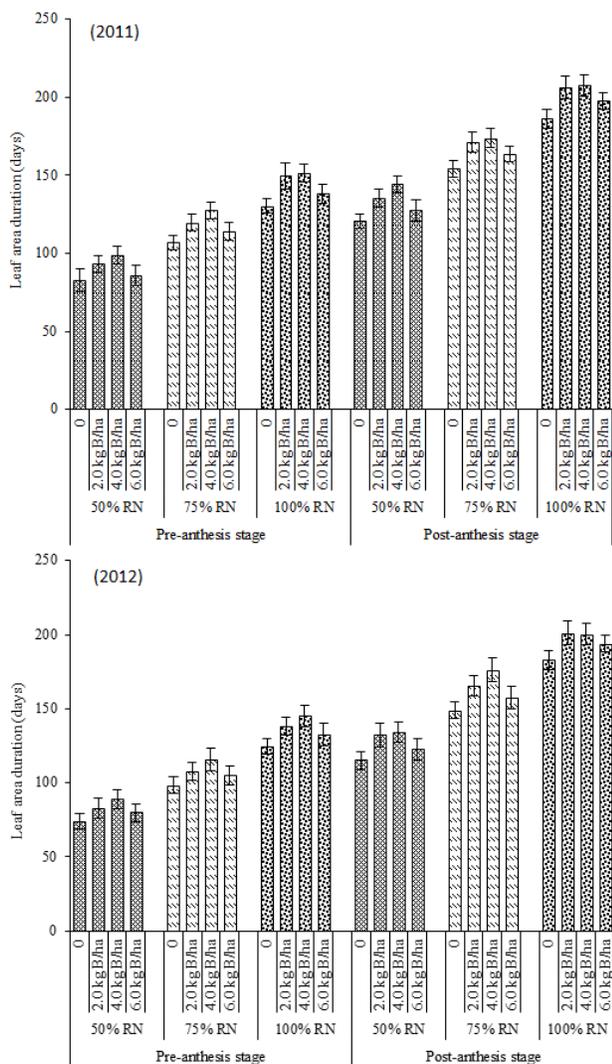


Fig. 2: Leaf area duration of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

levels, respectively. Increased level for N nutrition up to 100% RN resulted in considerable increase of net assimilation rate in contrast to 75- and 50% RN (Fig. 4).

The dry matter accumulation varied highly and maximum was recorded with 100% RN at post-anthesis in contrast to pre-anthesis growth stage. At pre-anthesis stage, the maximum increase in biomass production resulted with B rate of 3.40 kg ha⁻¹ with 100% RN during 2011, while in 2012, B application of 3.49 kg ha⁻¹ provided highest biomass accumulation with same N rate. Soil applied B at 3.71 and 3.39 kg ha⁻¹ provided better biomass production with reduced N level of 75% RN (Table 2). Application of B at 3.71 and 3.29 kg ha⁻¹ improved the dry matter production with 50% RN during 2011 and 2012 accordingly. At post-anthesis stage, B application of 3.39 kg ha⁻¹ performed best in producing maximum dry matter

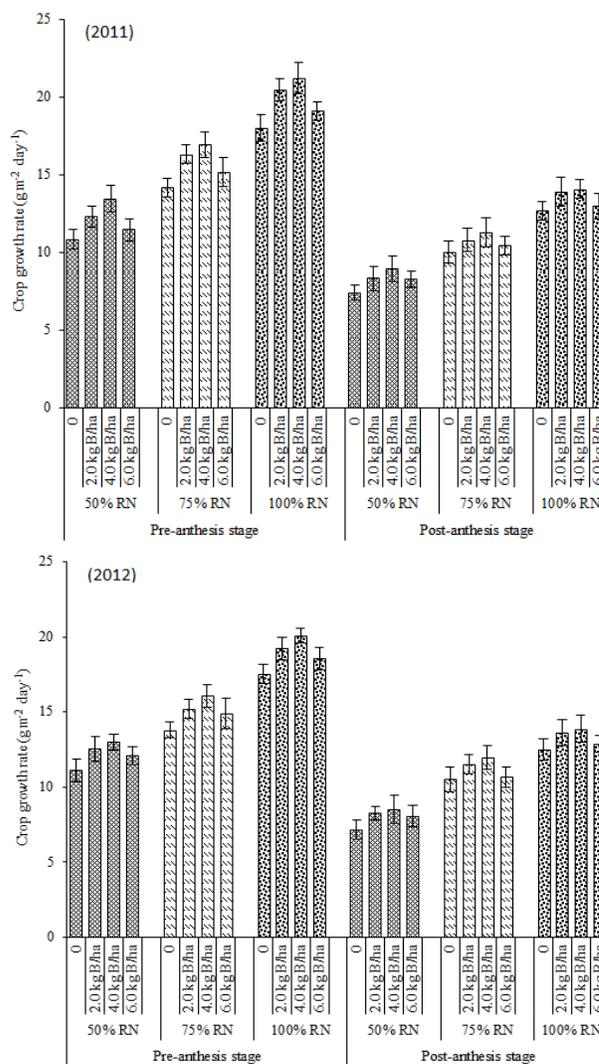


Fig. 3: Crop growth rate of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

during first year; while 3.42 kg B ha⁻¹ ensured highest dry matter in second year with 100% RN. In 2011, maximum dry matter yield under 75% RN was achieved with B application of 3.50 kg ha⁻¹; while in 2012, the maximum was with 3.41 kg ha⁻¹. The higher dry matter yield was attained by 3.29 and 3.28 kg B ha⁻¹ with 50% RN during both years (Table 2). In 2011, maximum biological yield was attained with B application of 3.62, 3.43 and 3.20 kg ha⁻¹ with 100-, 75- and 50% RN, respectively. The trend was diverse in 2012 as 3.27 kg ha⁻¹ B rate had maximum biological yield at 100% RN. Considerable increase in biological yield was exhibited by 3.36 and 3.22 kg ha⁻¹ B with 75- and 50% RN respectively (Table 3). Fertilization with 100% RN produced highest biological yield, followed by 75- and 50% RN, respectively. Highest harvest index was attained with B application of 3.69, 3.75 and 3.21 kg ha⁻¹

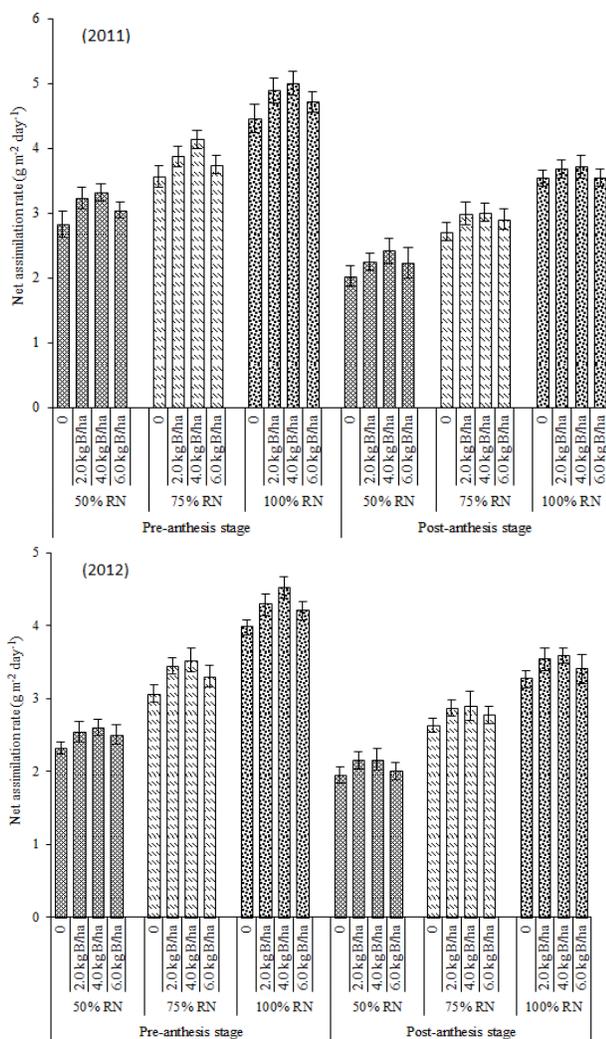


Fig. 4: Crop growth rate of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

during first year at 100-, 75- and 50% RN, respectively. While in 2012, the maximum improvement in harvest index was observed by 3.19, 3.67 and 3.41 kg ha⁻¹ B application following the N levels of 100 > 75 > 50% RN. Use of 100% RN ensured highest harvest index in contrast to 75- and 50% RN (Table 3).

Mineral Profile

Uptake of N through leaves and achenes was significantly improved with 100% RN as compared to 75- and 50% RN. Maximum uptake of N in leaves was observed with 3.38, 3.84 and 3.48 kg ha⁻¹ B for 100% RN in 2011. Soil application of B with 3.26, 3.56 and 3.29 kg ha⁻¹ significantly improved the leaf N contents following the 100-, 75- and 50% RN levels during 2012 (Table 4). In 2011, achene N contents were considerably increased when 3.40 kg ha⁻¹ B was applied in combination with 100% RN.

Soil application of B at 3.78 kg ha⁻¹ with 75% RN ensured more achene N contents. Likewise, with reduced level of 50% RN, B application of 3.53 kg ha⁻¹ notably improved the achene N contents. Exogenous application of B with 3.36, 3.48 and 3.21 kg ha⁻¹ under 100-, 75- and 50% RN, respectively had an increasing impact on achene N contents during 2012 (Table 4).

In 2011, B contents in leaves were higher by B application of 3.48 kg ha⁻¹ with 100- and 50% RN. The highest uptake of B contents through leaves was recorded with B concentration of 3.51 kg ha⁻¹ for 75% RN. Application of B at 3.75, 3.50 and 3.15 kg ha⁻¹ had highest B contents in leaves with 100-, 75- and 50% RN, respectively in 2012. Leaf N contents decreased as N level shifted towards 50% RN (Table 4). The maximum achene B contents with 100% RN were observed by soil application of 3.60 kg B ha⁻¹. At 75% RN, achene B contents were higher with 3.40 and 3.38 kg ha⁻¹ B concentrations when plants grown with 75- and 50% RN levels, respectively during 2011. Uptake of B by achenes was significantly improved by 3.44, 3.83 and 3.14 kg B ha⁻¹ in reduced and recommended N levels of 100-, 75- and 50% RN, respectively during 2012 (Table 4).

Maximum chl. *a* contents in 2011 were found for 3.42 kg ha⁻¹ B with 100% RN; while in 2012, B concentration of 3.55 kg ha⁻¹ had maximum chl. *a* contents at same N level. Under reduced N level of 75% RN, soil applied B at 3.95 kg ha⁻¹ significantly improved chl. *a* contents during 2011, while maximum was with 3.48 kg B ha⁻¹ for 2012. The B application of 3.66 and 3.46 kg ha⁻¹ observed higher chl. *a* contents with 50% RN during 2011 and 2012 years (Table 5). The contents of chl. *b* were maintained higher by B application of 3.45 kg ha⁻¹ with 100% RN. B application at 3.44 and 3.46 kg ha⁻¹ improved higher chl. *b* contents for 75- and 50% RN during 2011. Likewise in 2012, B application of 3.45, 3.25 and 3.53 kg ha⁻¹ ensured significant improvement in chl. *b* contents with 100-, 75- and 50% RN, respectively (Table 5). In 2011, B application of 3.43 kg ha⁻¹ provided higher total chlorophyll contents with 100% RN, while soil applied B at 3.38 kg ha⁻¹ under same N level observed maximum total chlorophyll contents during 2012. Soil application of B at 3.72 and 3.64 kg ha⁻¹ exposed major increase in total chlorophyll contents with 75% RN during both years. A considerable increase in total chlorophyll contents was evident with 3.57 and 3.48 kg ha⁻¹ B for reduced level of 50% RN (Table 5).

Discussion

All growth traits such as leaf area index and duration, crop growth and net assimilation rates were considerably improved with integrated N and B fertilization during the pre-anthesis in parallel to post-anthesis stage. A higher growth was due to more accumulation of soluble carbohydrates like glucose, sucrose and fructose by sunflower plants. This accumulation might be due to

Table 1: Regression equations relating to growth traits of sunflower

N rates (kg ha ⁻¹)	Leaf area index		Leaf area duration	
	Pre-anthesis stage	Post-anthesis stage	Pre-anthesis stage	Post-anthesis stage
2011				
50% RN	$y = -0.06x^2 + 0.39x + 4.48$, R ² = 0.99	$y = -0.03x^2 + 0.21x + 2.02$, R ² = 0.97	$y = -1.48x^2 + 9.63x + 81.93$, R ² = 0.93	$y = -1.97x^2 + 13.41x + 119.45$, R ² = 0.93
75% RN	$y = -0.06x^2 + 0.41x + 3.70$, R ² = 0.98	$y = -0.02x^2 + 0.15x + 2.54$, R ² = 0.67	$y = -1.64x^2 + 11.23x + 106.2$, R ² = 0.94	$y = -1.73x^2 + 11.93x + 154.3$, R ² = 0.99
100% RN	$y = -0.07x^2 + 0.44x + 3.01$, R ² = 0.99	$y = -0.03x^2 + 0.25x + 2.98$, R ² = 0.80	$y = -2.05x^2 + 13.62x + 130.36$, R ² = 0.99	$y = -1.89x^2 + 13.15x + 186.6$, R ² = 0.99
2012				
50% RN	$y = -0.05x^2 + 0.35x + 2.93$, R ² = 0.94	$y = -0.02x^2 + 0.16x + 1.99$, R ² = 0.85	$y = -1.13x^2 + 7.99x + 73.23$, R ² = 0.92	$y = -1.79x^2 + 11.96x + 115.36$, R ² = 0.99
75% RN	$y = -0.04x^2 + 0.31x + 3.72$, R ² = 0.99	$y = -0.02x^2 + 0.14x + 2.44$, R ² = 0.80	$y = -1.25x^2 + 8.87x + 97.56$, R ² = 0.89	$y = -2.22x^2 + 15.18x + 147.63$, R ² = 0.94
100% RN	$y = -0.05x^2 + 0.35x + 4.34$, R ² = 0.92	$y = -0.03x^2 + 0.18x + 2.87$, R ² = 0.95	$y = -1.64x^2 + 11.38x + 123.96$, R ² = 0.96	$y = -1.52x^2 + 10.72x + 183.75$, R ² = 0.95
2011				
Crop growth rate		Net assimilation rate		
	Pre-anthesis stage	Post-anthesis stage	Pre-anthesis stage	Post-anthesis stage
50% RN	$y = -0.22x^2 + 1.46x + 10.72$, R ² = 0.89	$y = -0.19x^2 + 0.77x + 7.38$, R ² = 0.95	$y = -0.04x^2 + 0.28x + 2.83$, R ² = 0.99	$y = -0.03x^2 + 0.19x + 2.01$, R ² = 0.94
75% RN	$y = -0.24x^2 + 1.64x + 14.17$, R ² = 0.99	$y = -0.1x^2 + 0.68x + 9.98$, R ² = 0.93	$y = -0.04x^2 + 0.30x + 3.54$, R ² = 0.89	$y = -0.02x^2 + 0.17x + 2.72$, R ² = 0.97
100% RN	$y = -0.28x^2 + 1.93x + 17.96$, R ² = 0.99	$y = -0.15x^2 + 0.92x + 12.69$, R ² = 0.99	$y = -0.05x^2 + 0.31x + 4.46$, R ² = 0.99	$y = -0.02x^2 + 0.12x + 3.53$, R ² = 0.98
2012				
50% RN	$y = -0.14x^2 + 1.03x + 11.13$, R ² = 0.99	$y = -0.09x^2 + 0.73x + 7.16$, R ² = 0.99	$y = -0.02x^2 + 0.15x + 2.33$, R ² = 1	$y = -0.02x^2 + 0.15x + 1.95$, R ² = 0.99
75% RN	$y = -0.16x^2 + 1.17x + 13.74$, R ² = 0.95	$y = -0.14x^2 + 0.91x + 10.46$, R ² = 0.95	$y = -0.04x^2 + 0.26x + 3.07$, R ² = 1	$y = -0.02x^2 + 0.16x + 2.64$, R ² = 0.99
100% RN	$y = -0.2x^2 + 1.39x + 17.48$, R ² = 0.96	$y = -0.13x^2 + 0.85x + 12.50$, R ² = 0.99	$y = -0.04x^2 + 0.28x + 3.96$, R ² = 0.92	$y = -0.03x^2 + 0.19x + 3.27$, R ² = 0.99

Table 2: Dry matter yield (g m⁻²) of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

Boron application rates (kg ha ⁻¹)	Pre-anthesis stage			Post-anthesis stage		
	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN
2011						
0	272.49 c	393.06 b	572.07 a ^b	351.51 c	514.63 b	646.84 a ^b
2.0	347.85	459.52	688.51	425.37	610.43	762.31
4.0	361.35	518.78	723.15	477.32	636.77	811.36
6.0	331.13	452.56	631.25	399.22	573.32	703.54
Regression	$y = -6.59x^2 + 49.06x + 273.4$, R ² = 0.99	$y = -8.29x^2 + 61.64x + 387.15$, R ² = 0.91	$y = -13.02x^2 + 88.74x + 569.83$, R ² = 0.99	$y = -9.49x^2 + 66.74x + 346.1$, R ² = 0.92	$y = -9.95x^2 + 69.84x + 513.61$, R ² = 0.99	$y = -13.96x^2 + 94.69x + 642.32$, R ² = 0.97
2012						
0	214.23 c	357.13 b	516.38 a ^b	313.82 c	499.48 b	606.94 a ^b
2.0	290.16	455.75	624.14	399.79	575.56	732.25
4.0	309.75	472.76	672.15	451.35	628.82	759.47
6.0	243.11	406.28	581.78	343.32	538.99	673.50
Regression	$y = -8.91x^2 + 58.77x + 212.74$, R ² = 0.99	$y = -10.32x^2 + 70.14x + 357.04$, R ² = 1	$y = -12.38x^2 + 86.51x + 512.45$, R ² = 0.9764	$y = -12.13x^2 + 79.75x + 307.56$, R ² = 0.92	$y = -10.37x^2 + 70.81x + 493.47$, R ² = 0.92	$y = -13.21x^2 + 90.57x + 606.18$, R ² = 0.99

^bValues in horizontal rows (a, b, c) for varying N levels not sharing common letter(s) differ significantly ($p \leq 0.05$)

positive effects of B on translocation of these soluble carbohydrates, as well as stimulating effects on N and carbon fixation rates (Perica *et al.*, 2001; Bellaloui and Mengistu, 2015). The early emergence and faster growth of sunflower leaves at both growth stages was due to positive impact of proper N and B nutrition and might be the reason of increased leaf area index (Allam, 2003). Increasing application rate for N showed encouraging effects on leaf area index owing to its role in development of new leaves

and shoots through production of proteins, chlorophyll pigments and nucleic acids (Rakhsh and Golchin, 2012; Mahmood *et al.*, 2001). Maximum sunflower growth with B application in present study might be due to its critical role to support cell wall (Goldbach *et al.*, 2001). Indeed, B nutrient cross links two monomers that stable the cell wall matrix, finally improve the plant growth through higher cell wall stability and by formation of borate link (Mouhtaridou *et al.*, 2004).

Table 3: Biological yield and harvest index of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

Boron application rates (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)			Harvest index (%)		
	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN
2011						
0	7591 c	9130 b	10609 a ^b	22.65 c	25.20 b	27.38 a ^b
2.0	8156	9489	10947	23.51	25.92	28.92
4.0	8360	10009	10985	24.19	26.61	29.91
6.0	7727	9331	10844	22.82	25.88	28.66
Regression	$y = -74.87x^2 + 479.85x + 7567.2, R^2 = 0.97$	$y = -64.81x^2 + 445.03x + 90, R^2 = 0.78$	$y = -29.94x^2 + 216.78x + 10615, R^2 = 0.99$	$y = -0.14x^2 + 0.89x + 22.56, R^2 = 0.88$	$y = -0.09x^2 + 0.68x + 25.13, R^2 = 0.90$	$y = -0.17x^2 + 1.28x + 27.29, R^2 = 0.95$
2012						
0	7450 c	8866 b	10458 a ^b	21.21 c	24.44 b	27.02 a ^b
2.0	7900	9189	10858	23.03	25.34	28.84
4.0	8099	9885	10967	23.20	26.21	29.06
6.0	7563	9000	10599	22.14	25.21	27.50
Regression	$y = -61.62x^2 + 396.65x + 7425.8, R^2 = 0.95$	$y = -75.5x^2 + 507.9x + 8768.3, R^2 = 0.69$	$y = -48x^2 + 314.6x + 10449, R^2 = 0.98$	$y = -0.18x^2 + 1.23x + 21.23, R^2 = 0.99$	$y = -0.12x^2 + 0.87x + 24.35, R^2 = 0.89$	$y = -0.21x^2 + 1.35x + 27.01, R^2 = 0.99$

^bValues in horizontal rows (a, b, c) for varying N levels not sharing common letter(s) differ significantly ($p \leq 0.05$)

Prolonged leaf area duration was attained at post-anthesis stage in contrast to pre-anthesis. This suggests that seed filling is directly correlated with maximum plant growth at any growth stage either pre- or post-anthesis. In general, photosynthates are blocked in vegetative plant parts due to low sink demand. Therefore, keeping higher leaf area duration during post anthesis is essential for higher productivity (Hall *et al.*, 1989). An improvement in leaf area duration was directly correlated with increment in leaf area might be due to positive effect of N and B on cell expansion by retaining higher water contents (Abbadi *et al.*, 2008). In the present study, combined N and B use was also liable for increasing growth and net assimilation rates of sunflower crop because of high dependency on B supply for segregation of N into ammonium, nitrate and amino acid compounds (Shelp, 1990). Soil B application with appropriate N availability resulted in considerable increase in crop growth and net assimilation rates due to promoting appropriate nutrient balancing and assimilation plus synthesizes transport. Increase in crop growth rate because B has great contribution in meristematic growth, cell division, elongation, cell wall stability and binding of nitrogenous synthesizes. Proper N management in sunflower crop improved the light interception, photosynthetic efficiency, superior transfer of assimilates that led towards higher crop growth and net assimilation rates (Shekhawat and Shivay, 2012). Also, reduced chlorophyll contents, photosynthesis, leaf stomatal conductance, accumulation of starch and sugars indicated a decrease in export of non-structural carbohydrates and photosynthates from leaves to the yield attributed sink due to B deficiency which leads to decline in crop growth (Osterhuis and Zhao, 2006). Accumulation of dry biomass might change with varying N and B nutrition rates as biomass production increased three times with B application due to its promising effect on biological N fixation and plant metabolism compared to the B deficient plants (Asad *et al.*, 2003). However, better root development and N

assimilation (Ali and Mishra, 2001) with integrated N and B nutrition in sunflower ensured higher growth in present study.

Improvement in biological yield with N and B nutrients because of favorable impact on biological N fixation, chlorophyll contents, assimilates production, plant metabolism and on plant growth owing to its role in development of new leaves through production of nucleic acids (Rahman *et al.*, 2009). The higher harvest index with combined N and B nutrition might be due to its significant role in amino acids, chlorophyll pigments, carbohydrates transport and permeability of cell membranes, pollen viability (Verma *et al.*, 2004) and pollen tube growth. Hence, plants treated with B had more light interception, biomass production and crop growth resulting in greater harvest index (Shekhawat and Shivay, 2012).

The higher contents for N in leaf and achenes were also observed with N and B amendments due to increased enzymatic activity of nitrate reductase which regulates NO₃-N to amino acids (Fazli *et al.*, 2008). Conversion of N to nitrate by means of B application in the soil and its absorption charges the roots negatively that promotes more cations absorption, eventually increased N uptake (Uygun and Rimmer, 2000; Wojcik, 2000). Several studies depicted that B is involved in increasing the absorption of N through fixation in heterocyst cells. Reduced nitrogenase enzyme activity under prevailing conditions of B deficiency was elucidated with nitrogenase destruction by altered status of O₂ (Bonilla *et al.*, 1990; Garcia-Gonzales, 1991). The positive correlation between N and B fertilizers on higher B contents in leaf and achenes might be due to more roots and shoot growth, which released more root exudates and finally enhanced B availability in the soil rhizosphere.

Integration of N in calcareous soils had synergistic influence on B uptake through plant roots (Lefebvre *et al.*, 2002) by increasing nitrate contents in the cell sap, which promotes growth of roots with better accessibility in the soil rhizosphere for nutrients absorption. Sufficient availability

Table 4: Mineral constitution of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

Boron application rates (kg ha ⁻¹)	Leaf nitrogen (%)			Achene nitrogen (%)			Leaf boron (mg kg ⁻¹)			Achene boron (mg kg ⁻¹)		
	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN
2011												
0	1.24 c	1.65 b	2.15 a ^b	1.76 c	2.19 b	2.78 a ^b	1.93 c	2.81 b	3.62 a ^b	1.54 c	2.42 b	3.18 a ^b
2.0	1.32	1.82	2.44	1.84	2.29	2.86	2.27	3.11	4.23	1.99	2.78	3.34
4.0	1.41	1.86	2.45	1.86	2.35	2.99	2.39	3.25	4.36	2.08	2.81	3.55
6.0	1.29	1.80	2.29	1.81	2.28	2.82	2.13	3.00	3.98	1.76	2.60	3.31
Regression	$y = -0.013x^2 + 0.08x + 1.23, R^2 = 0.84$	$y = -0.01x^2 + 0.11x + 1.65, R^2 = 0.99$	$y = -0.03x^2 + 0.19x + 2.15, R^2 = 0.99$	$y = -0.01x^2 + 0.06x + 1.76, R^2 = 0.99$	$y = -0.01x^2 + 0.08x + 2.18, R^2 = 0.96$	$y = -0.01x^2 + 0.11x + 2.76, R^2 = 0.75$	$y = -0.037x^2 + 0.26x + 1.92, R^2 = 0.98$	$y = -0.03x^2 + 0.24x + 2.79, R^2 = 0.94$	$y = -0.06x^2 + 0.43x + 3.62, R^2 = 0.99$	$y = -0.05x^2 + 0.33x + 1.54, R^2 = 0.99$	$y = -0.04x^2 + 0.24x + 2.43, R^2 = 0.99$	$y = -0.03x^2 + 0.18x + 3.15, R^2 = 0.82$
2012												
0	1.02 c	1.43 b	1.93 a ^b	1.44 c	1.92 b	2.38 a ^b	1.56 c	2.52 b	3.43 a ^b	1.27 c	2.08 b	2.94 a ^b
2.0	1.10	1.57	2.09	1.58	2.04	2.64	2.04	2.89	3.85	1.68	2.40	3.18
4.0	1.15	1.58	2.27	1.61	2.09	2.67	2.13	3.08	4.05	1.77	2.53	3.19
6.0	1.05	1.52	1.97	1.48	1.99	2.50	1.65	2.75	3.79	1.34	2.37	3.07
Regression	$y = -0.01x^2 + 0.07x + 1.01, R^2 = 0.92$	$y = -0.01x^2 + 0.09x + 1.43, R^2 = 0.98$	$y = -0.03x^2 + 0.18x + 1.91, R^2 = 0.82$	$y = -0.02x^2 + 0.11x + 1.44, R^2 = 0.99$	$y = -0.01x^2 + 0.09x + 1.92, R^2 = 0.97$	$y = -0.03x^2 + 0.18x + 2.38, R^2 = 0.99$	$y = -0.06x^2 + 0.37x + 1.55, R^2 = 0.99$	$y = -0.04x^2 + 0.31x + 2.50, R^2 = 0.96$	$y = -0.04x^2 + 0.32x + 3.42, R^2 = 0.98$	$y = -0.02x^2 + 0.15x + 2.95, R^2 = 0.98$	$y = -0.03x^2 + 0.23x + 2.07, R^2 = 0.99$	$y = -0.02x^2 + 0.15x + 2.95, R^2 = 0.98$

^bValues in horizontal rows (a, b, c) for varying N levels not sharing common letter(s) differ significantly ($p \leq 0.05$)

Table 5: Chlorophyll contents of sunflower as affected by B soil application at basal and after 32 d of sowing with 50-, 75- and 100% RN

Boron application rates (kg ha ⁻¹)	Chl. a (mg g ⁻¹ f. wt.)			Chl. b (mg g ⁻¹ f. wt.)			Total Chl. (mg g ⁻¹ f. wt.)		
	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN	50% RN	75% RN	100% RN
2011									
0	0.81 c	1.21 b	1.62 a ^b	0.42 c	0.58 b	0.80 a ^b	1.23 c	1.79 b	2.42 a ^b
2.0	0.96	1.29	1.79	0.49	0.67	0.89	1.45	1.96	2.68
4.0	0.99	1.41	1.82	0.51	0.69	0.93	1.50	2.10	2.75
6.0	0.92	1.31	1.71	0.46	0.63	0.85	1.38	1.94	2.56
Regression	$y = -0.01x^2 + 0.10x + 0.81, R^2 = 0.99$	$y = -0.01x^2 + 0.08x + 1.19, R^2 = 0.83$	$y = -0.02x^2 + 0.12x + 1.62, R^2 = 1$	$y = -0.01x^2 + 0.05x + 0.42, R^2 = 0.99$	$y = -0.01x^2 + 0.06x + 0.58, R^2 = 0.99$	$y = -0.01x^2 + 0.07x + 0.79, R^2 = 0.97$	$y = -0.02x^2 + 0.15x + 1.23, R^2 = 1$	$y = -0.02x^2 + 0.15x + 1.77, R^2 = 0.92$	$y = -0.03x^2 + 0.19x + 2.42, R^2 = 0.99$
2012									
0	0.74 c	1.14 b	1.57 a ^b	0.35 c	0.52 b	0.74 a ^b	1.09 c	1.66 b	2.33 a ^b
2.0	0.88	1.31	1.67	0.41	0.59	0.85	1.29	1.85	2.56
4.0	0.91	1.34	1.75	0.45	0.63	0.87	1.36	1.97	2.62
6.0	0.82	1.24	1.64	0.39	0.54	0.80	1.21	1.81	2.44
Regression	$y = -0.01x^2 + 0.09x + 0.74, R^2 = 0.99$	$y = -0.02x^2 + 0.12x + 1.14, R^2 = 0.99$	$y = -0.01x^2 + 0.09x + 1.56, R^2 = 0.91$	$y = -0.01x^2 + 0.05x + 0.35, R^2 = 0.93$	$y = -0.01x^2 + 0.06x + 0.52, R^2 = 0.93$	$y = -0.01x^2 + 0.07x + 0.74, R^2 = 1$	$y = -0.02x^2 + 0.15x + 1.08, R^2 = 0.98$	$y = -0.022x^2 + 0.16x + 1.65, R^2 = 0.95$	$y = -0.03x^2 + 0.17x + 2.33, R^2 = 0.99$

^bValues in horizontal rows (a, b, c) for varying N levels not sharing common letter(s) differ significantly ($p \leq 0.05$)

of B with N promotes the activity of RNase, membrane integrity (Sharma *et al.*, 1999) and accumulation of auxins and phenols which allows the membrane to pass out more nutrients and metabolites. In present study, sufficient B supply might enhance the N absorption by its fixation, which is directly involved in the production of amino acids and proteins and finally chlorophyll pigments (Rakhsh and Golchin, 2012). Superior impact of integrated N and B with higher chlorophyll contents was observed in present study due to synthesizing more chloroplast and carotenoids pigments. The activity of Hill reaction is declined in B deficient plants because of higher chlorophyll degradation and reducing chlorophyll production (Wang *et al.*, 2007; Maqsood *et al.*, 2013). These findings are also reinforced by

Tepe and Aydemir (2011) where substantial increase in chlorophyll contents with combined N and B application in barley and lentil plants was observed.

Conclusion

Integrated use of N with adequate B supply was found better for the enhancement of growth, mineral absorption and production of chlorophyll pigments versus their individual use. Sunflower 'Hysun-33' hybrid responded well in terms of growth, dry matter accumulation and nutrients absorption under 100% RN nutrition with best B rate close to 3.0–3.50 kg ha⁻¹. On the basis of highest dry biomass production and nutrients uptake, the adaptation of interactive use of N and

B nutrients may be suggested as appropriate approach for the farmers in calcareous soils of Pakistan.

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