PHOTOSYNTHESIS AND ANTIOXIDANT RESPONSE TO WINTER RAPESEED (*BRASSICA NAPUS* L.) AS AFFECTED BY BORON

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Abstract

Effect of boron on photosynthesis and antioxidant response to rapeseed yield was studied by the field experimentation along with plant analysis during the winter season of 2010 and 2011. The field experimentation was conducted by split plot design with three replications consisting of two factors such as i) two rapeseed cultivars (viz. Xiangzayou 1613 and 09-13581613), assigned in main plots and ii) five boron levels (viz. 0, 4.5, 9.0, 13.5 and 18.0 kgha^{-1}) imposed in the sub-plots. The rate of photosynthesis increased with increasing boron level upto 9.0 kgha^{-1} with simultaneous increase in photosynthetically active radiation, rate of transpiration and stomatal conductance and decrease in intercellular CO_2 concentration in both cultivars, while reverse trend was shown with further increase of B concentration. B @ 9.0 kgha^{-1} improved the activities of antioxidant protective enzyme of SOD and POD and decreased the accumulation of MDA content in the both cultivars. Dry matter translocation increased with increasing B level upto 9.0 kgha^{-1} that resulted the highest seed yield and harvest index of rapeseed in both cultivars. Thus, B @ 9 kgha^{-1} is sufficient for rapeseed cultivation under the subtropical environmental condition of the Southern China. *Brassica napus*

Key words: Rapeseed, *Brassica napus,* Boron, Photosynthesis.

Introduction

The crop yield is the consequence of different physiological and biological processes taking place in plants that manipulate the growth and development of plant parameters, which are generally modified by imposed cultivation regulations. The capacity and effectiveness of those physiological processes (viz. Photosynthesis & its related gas exchange traits) and their consequences formulate the physiological basis of yield variation of crops. On the other hand, antioxidant defense system is one of the most important biological phenomenon in plant which plays a vital role for scavenging harmful reactive oxygen species (ROSs) which caused distress to photosynthetic parts of plants as well as obstruction of nutrient uptake in plants at different stages of plant growth; this defense mechanism also prejudiced by imposed cultivation regulations. Fertilization of boron (B) is an important management practice in rapeseed cultivation (Stangoulis *et al.*, 2000; Mandal & Sinha, 2002) and it is a unique as micronutrient required for the normal growth and development of rapeseed.

Role of B in photosynthesis is unclear and sometimes it is contradictory. There have been no reports on the direct effect of B on the photosynthesis of plant (Gupta & Lipsett, 1981; Dell & Huang, 1997), but some investigators stated indirect association of B with photosynthesis of Soybean (Liu *et al.*, 2005; Liu, 2000). Application of B improved the photosynthesis efficiency of soybean by maintenance of membrane and photosynthates translocation as well as expanding leaf area for photosynthesis (Liu, 2000). Boron excess and deficiency, the both condition decreased the rate of photosynthesis in summer squash (Lovatt & Bates 1984) and citrus (Kastori *et al.*, 1995). Some investigators (Han *et al*., 2009; Ardic *et*

al., 2009; Guidi *et al.*, 2011; Chen *et al*., 2012) reported that photosynthesis was hampered due to high level of B; but in contrary, Sage *et al.* (1989) found that B excess did not affect the photosynthesis in *Streptanthus morrisonii* leaves. B deficiency decreased the CO_2 assimilation in leaves that is closely associated with stomatal activity (Han *et al*., 2009; Sheng *et al*., 2009). On the other hand, Han *et al.* (2009) also reported that B excess reduced the CO_2 assimilation that appears to be correlated to a combination of different reasons viz. oxidative load, reduce in activities of photosynthetic enzymes and impaired electron transport rate. Sotiropoulos *et al.* (2002) investigated that B excess decreased the rate of photosynthesis in plant which is associated with increase in intercellular CO_2 concentration, while stomatal conductance remained unaffected. In contrast, other authors (Lovatt & Bates, 1984; Papadakis *et al.*, 2004) observed a reduction in stomatal conductance.

Boron (B) has been implicated to cause oxidative stress due to occurrence of excess or deficient of B in plants, which is responsible for the over production of reactive oxygen species (ROS). These ROSs (viz. O_2^-) and the radicals derived from ROS (viz. H_2O_2, OH^-) are strongly toxic to plants, may cause damage to lipid peroxidation of cellular membranes, protein denaturation and genotoxic effects i.e. DNA mutation (Zhang *et al.*, 2011; Liu *et.al*., 2009; Rao *et al.*, 2006). Super oxide radical (O_2^-) is extremely bioactive, produced by oxidative metabolism as a byproduct and it converted H_2O_2 into hydroxyl radical (OH^{-1}), which is also responsible for oxygen toxicity in the plant cells (Mittler, 2002; Azevedo-Neto *et al*., 2006). Plants have evolved a well-equipped antioxidant defense mechanism consisting of enzymatic antioxidants and no-enzymatic antioxidants which normally neutralized ROS molecules under steady state condition (Foyer & Noctor, 2005) and consequently reduce cellular damage. In plants, superoxide

dismutase (SOD) and Peroxidase (POD) are two important protective antioxidant enzymes against ROS. SOD is responsible to detoxify the super oxide radical (O_2^-) and produced H_2O_2, which is also toxic to plant, must be removed by converting into H_2O in subsequent reaction. POD is considered as a novel scavenger of H_2O_2 by converting it into H_2O. On the other hand, Malonyldialdehyde (MDA) is the key product of superoxide reaction in the plasma membrane because of attack of ROS to membrane. Thus, the activities of SOD and POD along with MDA accumulation may be able to employ as indices for the ability of a plant to scavenge the hoist level of ROS. Han *et al*. (2009) investigated that B deficiency and excess mediated cellular damage due to production of ROS. Excess supply of B induces ROS production, which stimulated oxidative damage by lipid peroxidation and H_2O_2 accumulation in leaves (Molassiotis *et al*., 2006; Karabal *et al*., 2003). However, there are limited reports, which are a bit conflicting, related to antioxidant response of plants to B-toxicity (Cervilla *et al*., 2007; Gunes *et al*., 2006; Molassiotis *et al*., 2006; Keles *et al*., 2004) and B-deficiency (Han *et al*., 2008; Cakmak, 1997).

However, there are plentiful reports on the role of B-stress (either B-deficiency or toxicity) on photosynthesis and antioxidant responses in citrus, pears, summer squash, kiwifruit , mandarin etc., whereas only few focused on the effect of B application on those parameters in rapeseed. The effect of B-fertilization on the photosynthesis and antioxidant response to rapeseed is largely unknown as compared to the stress effect of boron. But rapeseed is an important oil crop which is considered to be most susceptible to B element. Therefore, the objective of this experiment was undertaken to study the physiological basis of yield variation in rapeseed through photosynthesis and its related gas exchange traits, antioxidant response, DM translocation and yield in relation to different levels of B-fertilization.

Materials and Methods

Experimental condition and plant materials: The experiment was carried out in the South China Agricultural University (SCAU) experimental farm (23°09' E, 113°22' W, 11 m) located in Tianhe district under Guangzhou during the winter season of 2010 and 2011. The soil of experimental field was well-drained sandy loam acidic (pH=4.88) consisting of organic matter 25.65 gkg^{-1} as well as available nitrogen 85.47, phosphorus 25.14, potassium 153.20 and boron 0.56 $mgkg^{-1}$. The extractable B level of the experimental plot was low or insufficient according the critical levels indicated by some investigators (Hu *et al*., 1994; Keren & Bingham, 1985). The experiment was carried out by split plot design with three replications. There were two factors in this experiment viz. i) rapeseed variety was assigned in main plots as main factor and ii) boron dose was placed in the sub-plots as sub factor. There were two rapeseed varieties viz. V_1 = Xiangzayou 1613 (hybrid) and V_2 = 09-13581613 (inbred) and the five boron levels viz. B_0 = 0, B_1 = 4.5, B_2 = 9.0, B_3 = 13.5 and B_4 = 18.0 kg ha^{-1}. The seeds were sown on last week of November , the row to row distance was 30cm. The plots were fertilized by compound fertilizer (the ratio of N-P-K, 15-15-15) @ 600 kg ha^{-1} as basal dose. The plots were top dressed by urea fertilizer @ 240 kg ha^{-1} during budding stage of the crop. The crop was thinned and transplanted simultaneously at 30 days after sowing (DAS). The plots were irrigated three times, first irrigation was given just after sowing the seeds in plots, 2nd irrigation was applied at seedling stage (30 DAS) and the 3rd irrigation was at pod formation stage (75 DAS). Three insecticides viz. Acetamiprid @ 0.91 g/L water, Chloropyrifos (ANSI, ISO, BSI) @ 0.61 g/L water and Beta cypermethrin @ 0.61 g/L water were sprayed twice in the plots for controlling *thrips, diamondback* moth and *aphids*, respectively, one at 15 DAS and another at 25 DAS. The crop was harvested in the middle of April within 145 DAS.

Calculation of DM accumulation and translocation: Three plants from each plot were sampled at flowering and harvesting stages to measure the DM translocation and translocation efficiency. The plants were cut near the soil surface at the base of the plant during sampling and then the plants were separated into leaf, stem and siliqua and oven dried for 72 h at 70ºC. The following three parameters referring to dry matter translocation during flowering to maturity stage of rapeseed were estimated according to Cox *et al*. (1986); Dordas & Sioulas (2009).

i) Dry matter translocation ($kgha^{-1}$) = Shoot DM (leaf, stem & flower) at flowering - Shoot DM (leaf, stem & vegetative components of siliqua except seed) at maturity

ii) $$\text{Apparent translocation efficiency (ATE) of DM (\%)} = \frac{\text{DM translocation}}{\text{DM at flowering}} \times 100$$

iii) $$\text{Apparent conversion efficiency (ACE) from flowering DM to seed DM (\%)} = \frac{\text{DM translocation}}{\text{Seed DM at maturity}} \times 100$$

Measurement of photosynthetic factors: Photosynthesis (Pn) and its related gas exchange traits were measured at different stages of phenological development of crop *viz*., 65 DAS (stem elongation stage), 80 DAS (flowering initiation stage) and 95 DAS (pod setting stage) which represent the 2.1, 4.0 and 4.7, respectively of the rapeseed development code according to Sylvester-Bradley and Makepeace (1984). Measurement of photosynthesis rate (Pn, $\mu mol\ m^{-2}s^{-1}$) and its related gas exchange traits viz. photosynthetically active radiation (PAR, $\mu mol\ CO_2\ m^{-2}s^{-1}$), rate of transpiration (E, $mmol\ H_2O\ m^{-2}s^{-1}$), inter cellular CO_2 concentration (Icc, $\mu mol\ CO_2\ mol^{-1}$) and stomatal conductance (g_s, $mol\ H_2O\ m^{-2}s^{-1}$) were done directly using a portable photosynthesis system (Li-6400XT). The data were recorded from fully expanded 3rd healthy leaf from the apex of the plant during 9-11 am on some days along with clear sunshine.

Assays of antioxidant enzyme

Sampling and extraction: Five top leaves and 5 siliquas from main branch of the plants were sampled to measure antioxidant activity viz. super-oxide dismutase (SOD) activity, peroxidase (POD) activity and Malondialdehyde (MDA) content at 10 days interval from 100 DAS to 130 DAS (i.e.10 DAF, 20 DAF and 30 DAF) and these growth stages match up to 5.3 (30% potential pods more than 2cm long) 5.6 (60% potential pods more than 2cm long) and 5.9 (almost all potential pods more than 2cm long), respectively in the rapeseed development code of Sylvester-Bradley & Makepeace (1984). After sampling, all samples were immediately transferred to a small thermopore box at 4^0C and were stored at -80ºC until further analyses. Before analysis, the midrib (main vein) and petioles (leaf stalk) of the leaves were discarded and the leaves were chopped into small pieces with a scissor and samples were stored in plastic bags at -20°C in a Sanyo medical freezer VR-L6111W (Sanyo Electric Co; Ltd, Moriguchi, Japan) till further analyses. The enzyme was extracted by grinding 0.3 g sample (i.e., leaf and siliqua) from each treatment to a fine powder with ¼ teaspoon of SiO_2 with sodium phosphate buffer solution (PBS, pH=7.8) by mortar and pestle and then homogenized by adding buffer and transferred into 5ml centrifugal tube. All events were completed at 0-4ºC. The obtained homogenates were centrifuged at 8000 r/min for 15 minutes under 4^0C and optical density (OD) was recorded using spectrophotometer (Shimadzu UV-2450, Japan).

Determination of SOD activity: Superoxide dismutase (SOD) activity was determined by its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) as described by Beauchamp & Fridovich (1971). The reaction liquids viz. 1.5 ml of 0.1mol/L phosphate buffer solution (PBS, pH=7.5), 0.3 ml of 1.3 mol/L Methionine, 0.3 ml of 750 umol/L NBT, 0.3 ml of 100 umol/L EDTA-Na$_2$ and 0.3 ml of Riboflavin were used to determine to SOD activity. Except Riboflavin all these reaction liquids were mixed together and then 0.25 ml distilled water was added into reaction mixture. A total volume of reaction mixture was reached at 3.0 ml by adding 0.05 ml of enzyme extract, where as 0.05 ml PBS was added in the blank test as control. Riboflavin was added just before incubation of reaction mixture under a light source of 4000 lux at 25ºC for 20 minutes. Finally reaction mixture was measured at 560 nm compared with blank sample and it was expressed as units per g of fresh sample (ug^{-1}).

Determination of POD activity: The peroxidase (POD) activity was assayed spectrophotometrically according to Cai *et al*. (2008). It was measured by using a reaction mixture comprising 1.0 ml of 0.05 ml/L PBS (pH=7.0), 0.95 ml of 0.2% Guaiacol solution and 1.0 ml of 0.3% (v/v) H_2O_2 solution. All these reaction liquid were mixed together and incubated at 25ºC for 2 minutes. A total volume of assay mixture was prepared at 3.0 ml by adding 0.05 ml of enzyme extract. The reaction was started just after adding enzyme extract to assay mixture. POD activity was measured by monitoring the degradation of H_2O_2 at 470 nm over 2 minutes against extract-free blank reaction and it was expressed as units per g of fresh sample per minute (ug^{-1}min^{-1}).

Determination of MDA content: Malonyldialdehyde (MDA) accumulation was measured by the method described by De Vos *et al*. (1991). Thiobarbituric acid (TBA) reaction was used to determine the accumulation MDA in leaf and siliqua, which is an end product of lipid peroxidation in leaf and siliqua, respectively. For reaction, 1.5 ml enzyme liquid, 1.5ml PBS solution (pH=7.0) and 2ml 0.5%TBA solution was taken together in a 10 ml test tube and the assay mixture was heated at 98ºC for 30 minute in a boiling water bath and then it was cooled rapidly in a ice bath. After cooling the assay mixture was centrifuged at 3000 r/min for 20 min at 25ºC and OD was measured at 532 nm and it was expressed as units as µmol per g of fresh sample (µmolg^{-1}).

Statistical analysis: Results were statistically analyzed by analysis of variance (ANOVA) technique as applicable to split plot design (Gomez & Gomez, 1984). Results' trend was similar in both year (2010 & 2011) study and thus, average of two year results were analyzed using a computerized statistical software package (Statistix 8, Tallahassee, FL, USA). Least significant differences (LSD) were calculated at 5% probability level and Duncan's Multiple Range Test (DMRT) was used.
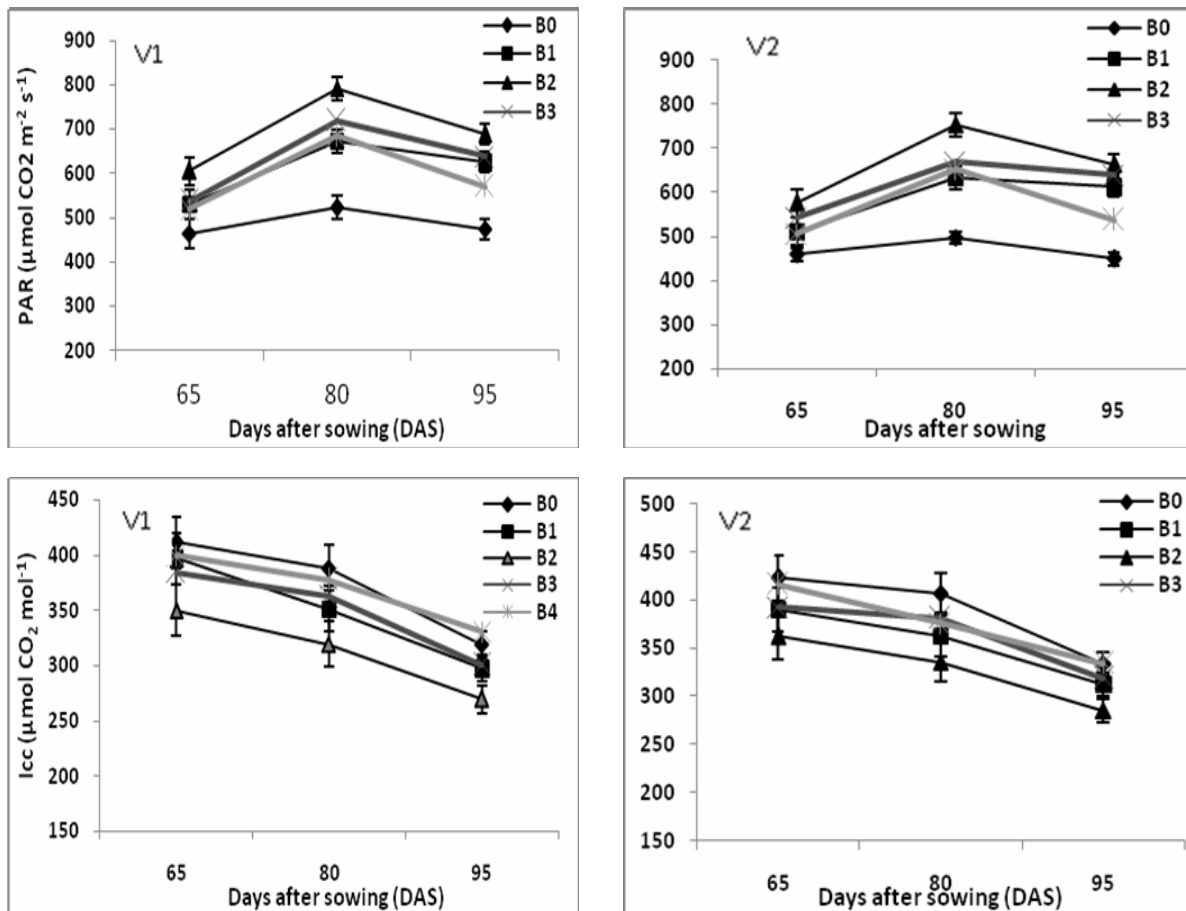
Results

Photosynthesis and related gas exchange traits: The rate of Photosynthesis (Pn), photosynthetically active radiation (PAR), intercellular CO_2 concentration (Icc), the rate of transpiration (E) and Stomatal conductance (Gs) at 65 DAS, 80 DAS and 95 DAS of plant growth were significantly varied due to various level of boron fertilization in both cultivars (Table 1 & Fig. 1). Irrespective of treatments and cultivars, Pn increased upto 80 DAS; then it was declined because of defoliation of leaves. The same trend was observed in PAR, whereas Icc, E and Gs decreased almost linearly with passage of time. In both variety, Pn increased with increasing B level upto B_2 and the decreased with further increasing of B level and maximum Pn was recorded in B_2 treatment followed by B_1 and B_3 and the lowest rate was found when no boron (B_o) was used in the crop. Similar tendency was observed in case of PAR. Icc showed a declining tendency, where the Pn rates were higher in all the treatments of both the cultivars. The maximum rate of transpiration (E) occurred when the crop was fertilized with B_2 level which was followed by B_3 level at all stages and lowest rate was found when the crop was not fertilized by boron (B_0) in both the cultivars. Similar observation was found for stomatal conductance (Gs).

Table 1. Effects of boron on the transpiration rate (E) and stomatal conductance (Gs) at different growth stage of rapeseed cultivars (averaged across two years).
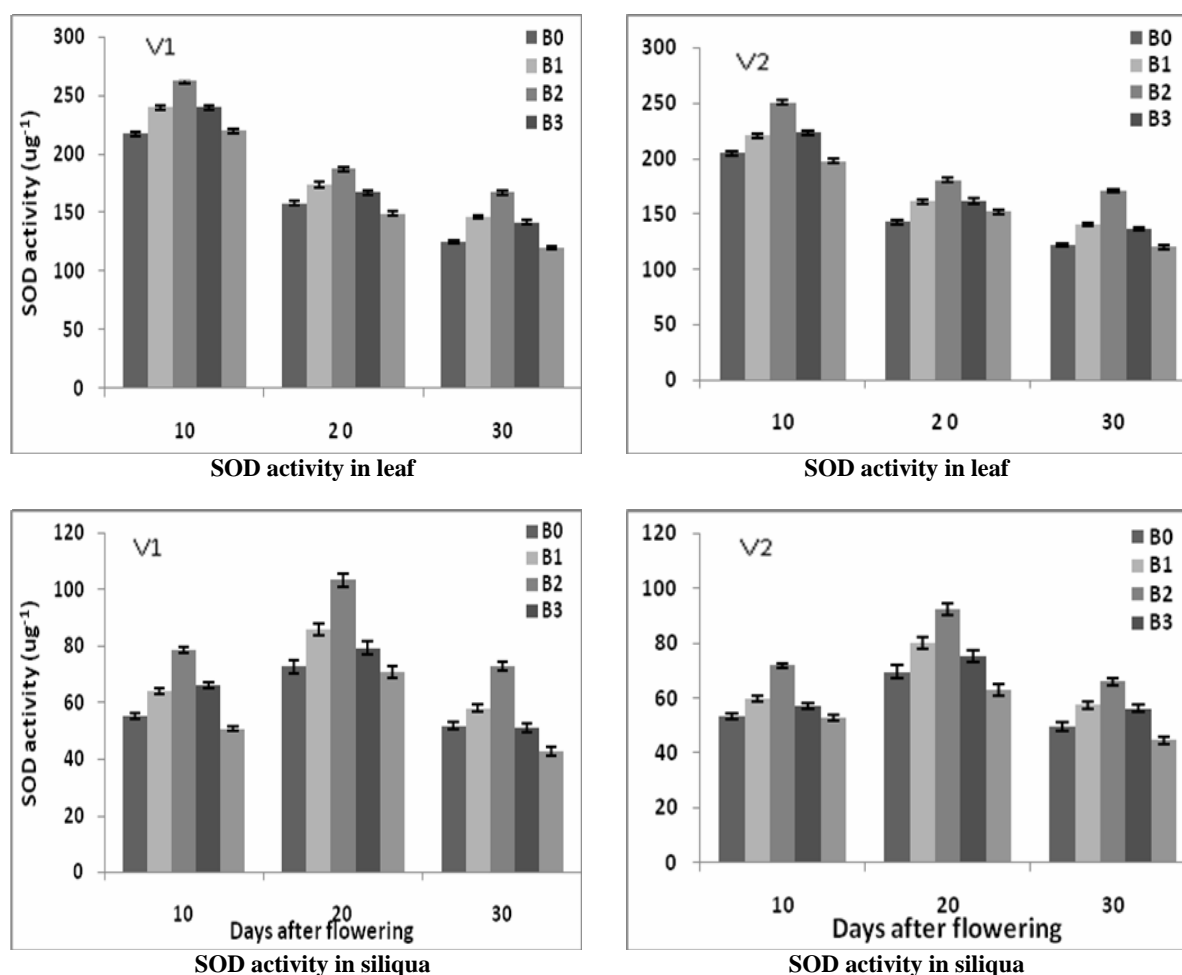
Treatments		Transpiration rate (mmol H_2O m^{-2}s^{-1})			Stomatal conductance (mol H_2O m^{-2}s^{-1})		
		65 DAS	80 DAS	95 DAS	65 DAS	80 DAS	95 DAS
	B_0	6.03 e	5.57 e	0.59 ef	0.53 de	0.34 de	0.34 de
	B_1	7.99 bc	7.29 bc	0.71 cd	0.59 ce	0.50 b	0.50 b
V_1	B_2	10.14 a	9.10 a	0.90 a	0.76 a	0.58 a	0.58 a
	B_3	8.41 b	7.79 b	0.76 bc	0.61 bd	0.44 bc	0.44 bc
	B_4	7.39 cd	6.59 d	0.64 df	0.55 ce	0.37 d	0.37 d
3							
	B_0	5.71 e	5.40 e	0.57 f	0.48 e	0.31 e	0.31 e
	B_1	8.10 b	6.90 cd	0.70 cd	0.66 ac	0.47 bc	0.47 bc
V_2	B_2	9.57 a	8.53 a	0.85 ab	0.72 ab	0.56 a	0.56 a
	B_3	8.17 b	7.68 b	0.72 cd	0.59 ce	0.43 c	0.43 c
	B_4	7.05 d	6.28 d	0.65 de	0.55 ce	0.36 de	0.36 de
SE (±)		0.67	0.64	0.55	0.10	0.13	0.05

V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}. Within a column for two group of cultivars, different letters indicate significant differences according to Duncan's multiple range test (P=0.05)

V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}.
Fig. 1. The rate of photosynthesis (Pn), photosynthetically active radiation (PAR) and internal cellular CO_2 concentration (Icc) at different growth stages of rapeseed cultivars as affected by boron. Data are the means and the error bars indicate ±SE (n=3).

V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}.
Fig. 2. SOD activity in leaf and siliqua at different growth stages of rapeseed cultivars as affected by boron. Data are the means and the error bars indicate ±SE (n=3).

Anti-oxidant enzyme activity

SOD activity: Significant variation was observed in SOD activity of leaves and siliqua at different stage of plant growth due to different levels of boron in both the cultivars (Fig. 2). In leaves, the SOD activity was decreased almost linearly with advancement of time, whereas in siliqua, it was increased up to 20 DAF and then drastically decreased during the next growth period. Considering SOD activity, boron is more sensitive to V_1 as compared to V_2. Among the five levels of boron, B_2 showed significantly highest activity of SOD and it was followed by B_3 and B_2 and significantly the lowest activity was observed either in B_0 or B_4 in both leaves and silliqua at different growth stages of both rapeseed cultivars.

POD activity: In Fig. 3, different levels of boron treatments caused significant changes in POD activity of both leaves and siliqua at different growth stage of plant. In leaves, the POD activity was decreased almost linearly with passage of time, whereas in siliqua, it was increased up to 20 DAF and then slightly decreased at later stages.

In both leaf and siliqua, the highest POD activity was observed in B_2 followed by B_1 and the lowest activity was found when boron level was maximum (B_4) except in siliqua at 10 DAF for cultivar V_1. The trend was almost identical in other cultivar (V_2).

MDA activity: MDA content significantly varied due to different levels of boron in both the leaves and siliqua at different growth stages of plant (Fig. 4). Irrespective of treatments and cultivars, MDA content in leaves and siliqua was increased markedly with the progressive of growth period. B fertilizer significantly decreased the MDA content of leaf and siliqua upto B_2 level and after that it was increased with further application of B. Irrespective of treatments and growth stage, B_4 produced the highest content of MDA followed by B_0, while moderate level of B_2 produced the lowest content of MDA in both leaf and siliqua of both the cultivars.

Dry matter (DM), DM translocation and seed yield: The different levels of boron showed significant variation in the dry matter content of vegetative tissues and its translocation from sources to sink at both of flowering

and maturity stages of plant growth (Table 2). DM accumulation during flowering and maturity stage increased with increasing boron application up to B_2 and then decreased slowly in both the cultivars. Consequently DM translocation, apparent translocation efficiency (ATE) and apparent conversion efficiency (ACE) of pre-flowering assimilates to seed was increased with increasing boron level up to B_2 and then decreased with further increase of boron level. Seed yield and harvest index (HI) were influenced significantly due to different levels of boron. In V_1 cultivar, significantly the highest seed yield produced by B_2 treatment was followed by B_3 and the lowest seed yield was observed when no boron was used and the similar trend was found in V_2 cultivar.

Discussion

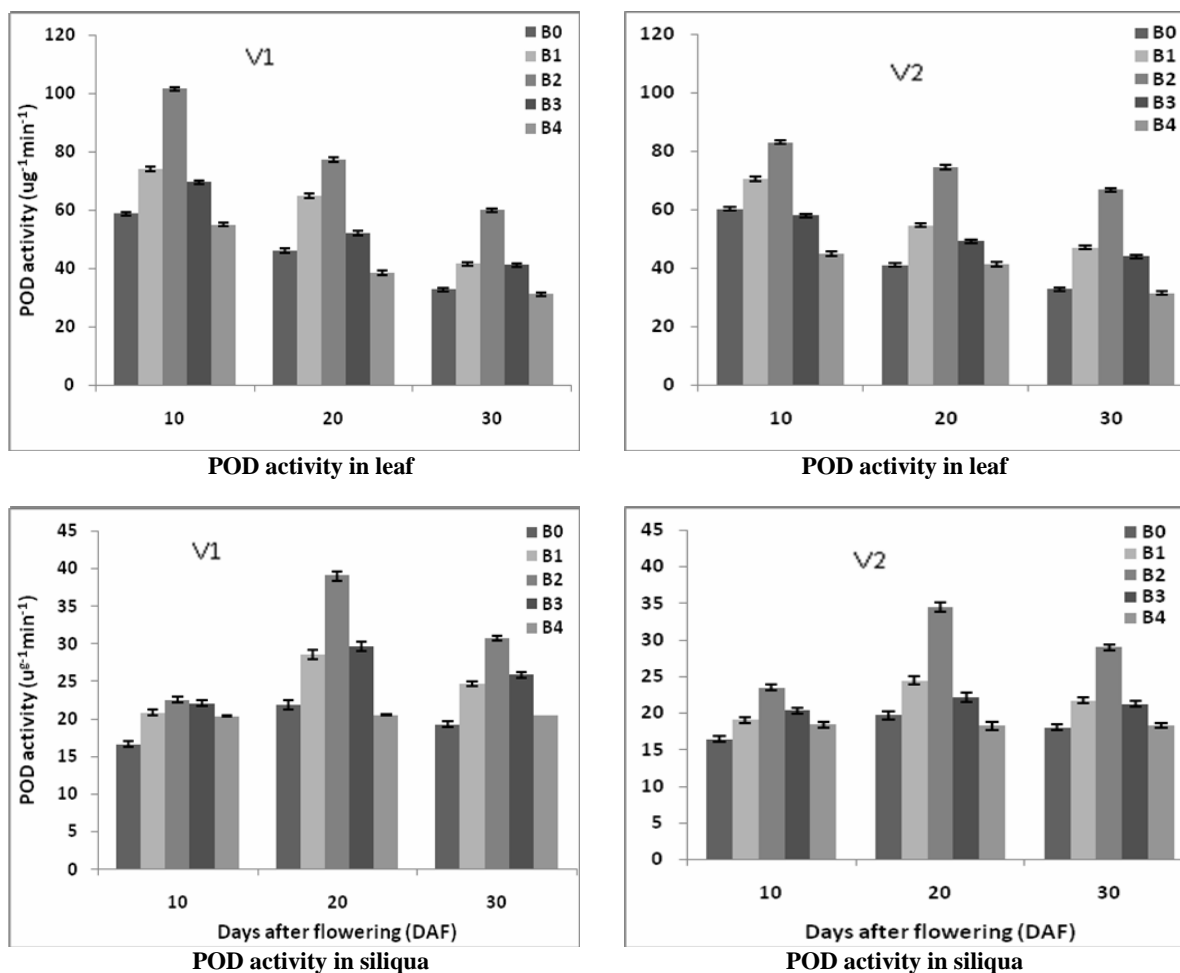**Photosynthesis and related gas exchange traits:** According to review search, there have been no reports on the direct effect of B on the photosynthesis of plant (Gupta & Lipsett, 1981; Dell & Huang, 1997), but some investigators stated indirect association of B with photosynthesis of Soybean (Liu *et al*., 2005; Liu, 2000). Application of B improved the photosynthesis efficiency of soybean by maintenance of membrane and photosynthates translocation as well as expanding leaf area for photosynthesis (Liu, 2000). Consistent with this previous results, our study showed that the rate of photosynthesis (Pn), Photosyntheticallly active radiation (PAR), transpiration rate (E) and Stomatal conductance (Gs) increased with increasing boron level up to B_2 and then decreased slowly with further increasing of B rate at all stages of plant growth. On the other hand, intercellular CO_2 concentration (Icc) showed a reverse trend under these treatments and the Icc appeared to be reduced with the increase in the Pn rate. This phenomenon might be due to boron stress (excess or deficiency), that reduced the photosynthetic

capacity of rapeseed leaves, consistent with the study by Han *et al*. (2009) in citrus plants. Compared with control and other treatments, treatment B_2 significantly increased the Pn probably due to adequate supply of B, which induces higher utilization of Icc as well as higher interception of PAR in the leaves of rapeseed. In this study, Icc appeared to be lower as compared to the rate of photosynthesis and other gas exchange traits might be because of higher utilization of Icc by rapeseed plants, which is consistent with Austin (1989) and he stated that lower Icc is the indicative of higher CO_2 assimilation at the sub-cellular, cellular and tissues level of organization as a result of higher nutrient availability and more nutrient flux to meet the metabolic demand of crop. Further increase in B level tended to depress the Pn and related gas exchange traits possibly due to B becoming unavailable to plants or might have created the toxic effect on rapeseed. This result agreed with the earlier finding of some investigators, who reported that B excess and deficiency caused decreased rate of photosynthesis in *Cucurbita pepo* (Lovatt & Bates, 1984), sunflower (Kastori *et al*., 1995) and citrus (Papadakis *et al*., 2004) leaves. Similarly significant variation was observed in transpiration rate (E) due to variation of B concentration and B_2 transpired at higher rate as compared to control and other treatments. The significance of B in stomatal conductance (GS) has not been investigated yet, but Han *et al*. (2008) reported on impaired stomatal conductance under B-deficient condition in citrus plants. In our study significant variation in Gs was found due to application of different levels of boron and Gs increased with the increase B level upto B_2 and the significantly lowest Gs was found when no B-fertilizer was used: these results correlates well with the B induced changes in the rate of transpiration.

Table 2. Effect of boron on DM translocation, apparent translocation efficiency (ATE) and apparent conversion efficiency (ACE) and seed yield of rapeseed cultivars (averaged across two years).

Treatments		DM at flowering (g/m^2)	DM at harvest (g/m^2)	Seed DM (g/m^2)	DM translocation (g/m^2)	ATE (%)	ACE (%)	Seed Yield (tha^{-1})	HI (%)
	B_0	365.06f	344.27f	142.29e	20.79cd	5.70d	14.64bc	1.42e	29.36c
	B_1	476.39c	448.09c	225.99c	28.30c	5.94d	12.57c	2.26c	33.53b
V_1	B_2	683.41a	606.40a	335.81a	77.01a	11.29a	22.93a	3.36a	35.65a
	B_3	538.52b	493.36b	239.86b	45.16b	8.42bc	18.80ab	2.40b	32.74b
	B_4	444.70d	414.59d	175.22d	30.10c	6.72bd	17.08b	1.75d	29.69c
	B_0	277.99g	261.65g	105.06g	16.34cd	5.88d	15.71bc	1.05g	28.61c
	B_1	412.71e	386.54e	166.37d	26.17c	6.35cd	15.75bc	1.67d	30.09c
V_2	B_2	558.87b	509.38b	250.29b	49.48b	8.85b	19.77ab	2.50b	32.96b
	B_3	455.89cd	429.51cd	170.05d	26.38c	5.79d	15.48bc	1.70d	28.35c
	B_4	351.23f	332.52f	120.29f	18.72cd	5.33d	15.56bc	1.20f	26.57d
SE (±)		12.60	11.39	4.20	4.42	0.86	2.23	0.04	0.75

V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}. Within a column for two group of cultivars, different letters indicate significant differences according to Duncan's multiple range test (p=0.05)
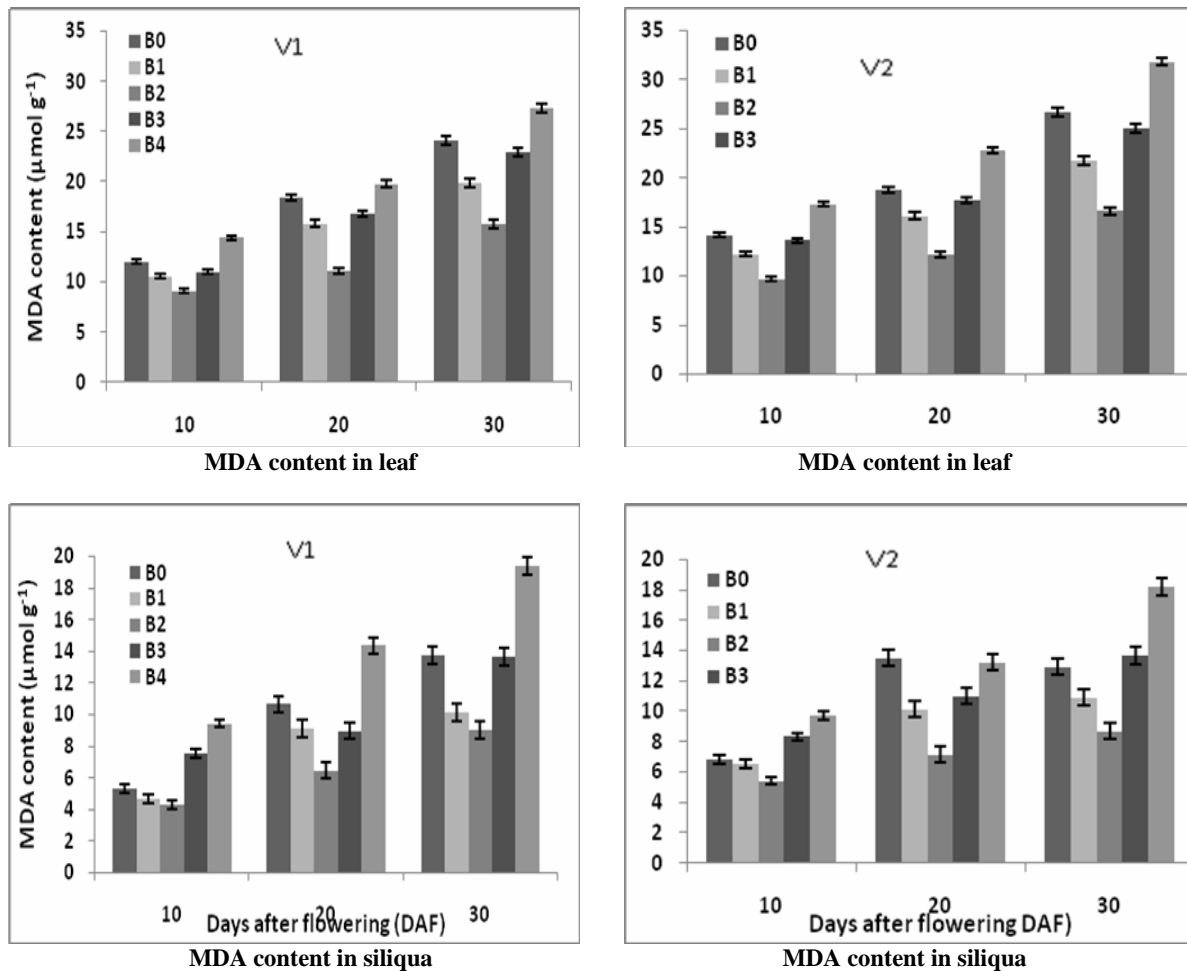
V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}.
Fig. 3. POD activity in leaf and siliqua at different growth stages of rapeseed cultivars as affected by boron. Data are the means and the error bars indicate ± SE (n=3).

Anti-oxidant enzymes: Production of excess level of reactive oxygen species (ROS) is a general phenomenon in a plant under stress condition during the normal course of metabolism. Oxidative stress has been reported under B-deficiency (Giusti & Wrolstad, 2001; Han *et al*., 2008) and B-excess (Gunes *et al*., 2006; Molassiotis *et al*., 2006; Sortiropoulos *et al*., 2002) in different plant species. The ROS and the radicals derived from ROS are highly bioactive and cause cellular damages in plants (Rao *et al*., 2006; Liu *et al*., 2009; Zhang *et al*., 2011). SOD and POD are considered as the front line defense antioxidant enzymes that detoxify the ROS and consequently reduce the cellular damage in plants. It is evident from this study that under increasing concentration of boron level, the activity of SOD and POD in both leaf and siliqua first increase and then decrease chronologically in both cultivars. As compared with the control (B_0) and other treatments, SOD and POD activity were significantly highest in B_2 level and it might be due to enhanced generation of ROS (Keles *et al*., 2004) under this treatment. In this study, B_0 and B_4 compared to other treatments induced oxidative

damage in leaves and siliqua of both cultivars, as investigated by the higher accumulation of MDA reactive compound, which is in agreement with earlier results obtained for B-deficient sunflower (El-Shintinawy, 1999) and sweet orange (Han *et al*., 2008) as well as B-excess apple rootstock (Molassiotis *et al*., 2006), grape (Gunes *et al*., 2006) and tomato (Cervilla *et al*., 2007). The oxidative stress because of B-deficiency as well as B-excess leads to huge generation of ROS (Han *et al*., 2008) in plants and consequently some of protective antioxidant and metabolites are seriously affected by these ROS molecules. In our study, B deficient (B_0) and excess (B_4) leaves and siliqua showed lower activity of SOD and POD probably because of scavenging excess amount of ROS due to higher accumulation of MDA under those boron levels. These results revealed that adequate level of B fertilization partially improved the harmful effects of rapeseed senescence by modulating the capacity of antioxidant enzymes, alleviating antioxidant system, which supported in sustaining plant growth and yield of rapeseed.

V1 = Xiangzayou1613 and V2 = 09-13581613, while B0= 0, B1=4.5, B2=9, B3=13.5 and B4=18 kg B ha^{-1}.
Fig. 4. MDA content in leaf and siliqua at different growth stages of rapeseed cultivars as affected by boron. Data are the means and the error bars indicate ± SE (n=3).

DM translocation and seed yield: The DM of crop seed partly comes from the non-structural carbohydrates which are stored in the stem and leaves before flowering and transferred to the fruiting organ after flowering and partly from the photosynthesis products of leaves after flowering. Irrespective of treatments and variety, the DM stored in vegetative organs at flowering stage was higher in B_2 treatment than those of control and other treatments and consequently it showed the higher DM translocation as well as higher apparent translocation efficiency (ATE) of DM than others treatments in the present study. The apparent conversion efficiency (ACE) of DM which was produced before heading and translocated into seeds was 22.93% and 19.77% in V_1 and V_2 cultivars respectively at B_2 boron level and those are higher contribution of pre-flowering assimilates to seed than control and other treatments. This might be evident that the seed yield of rapeseed mostly comes from the accumulated DM at vegetative organs before flowering and partly from the products of photosynthesis after flowering under adequate level of boron concentration. However, it is suggested that the application of boron likely enhanced the translocation of assimilates and photosynthate from source (leaves,

stems) to sink (seed) of rapeseed. Brown *et al*. (2002) reported that B uptake accelerate the transport process of DM from source to sink in plants. Application of boron caused significant changes in the physiology and biochemistry of plants during seed development, which is attributed to the synthesis and transformation of carbohydrate in seed (Singal *et al*., 1992; Singal *et al*., 1995). Irrespective of treatments and variety, significant variation was observed in seed yield of rapeseed due to fertilization of boron. Seed yield increased with increasing boron concentration up to B_2 and then decreased gradually with increasing boron concentration. It might be due to adequate supply of available B in the experimental plots due to supplementary application of B fertilizer. In the experimental plot, the available concentration of B was 0.568 mg kg^{-1}, which is not sufficient for plant growth under subtropical environment condition of Southern China (Bolanos *et al*., 2004); moreover it is near to critical level of 0.5 mgkg^{-1} (Zhang, 2001; Lu, 1998; Keren & Bingham, 1985). Noteworthy, plant showed B deficiency symptom in absence of B application, even though seed yield increased with increasing B level up to B_2 in the present experiment. One possible justification for the decline in seed yield of

rapeseed at higher level of B application is the antagonism relationship of B with other nutrients. Asad *et al.* (1997) observed that the relative uptake rates of calcium significantly decreased in both shoot and root of canola as B concentration increased. An increase of rapeseed yield by applying B fertilizer has already been reported by different researchers (Grant & Bailey, 1993; Moradi-Telavat *et al.*, 2008; Malhi *et al.*, 2003). In contrast, it was investigated that the seed yield of rapeseed decreased as B fertilizer was increased (Karamanos *et al.*, 2003). The harvest index (HI) is the ratio of seed yield to total plant biological yield, which usually expressed as percentage. The higher HI ensures higher allocation of assimilates from vegetative parts to seed (Kumar *et al.*, 2001). The HI showed an improvement following the application of B fertilizer up to B_2 level in both the cultivars against control treatment subsequently resulting in higher seed yield in these treatments.

Conclusion

Boron is one of the most vital micronutrient for rapeseed growth and development. Two rapeseed varieties viz. V_1 = Xiangzayou 1613 (hybrid) and V_2 = 09-13581613 (inbred) and the five level of boron viz. $B_0 = 0$, $B_1 = 4.5$, $B_2 = 9.0$, $B_3 = 13.5$ and $B_4 = 18.0$ kg ha^{-1} were used. Irrespective of cultivars, B_2 (9.0 kg ha^{-1}) ensure better translocation of photosynthetic products from source (i.e. leaves, stem etc) to sink (i.e., seed and siliqua) have been suggested to overcome the frequently source limited yield in rapeseed. Photosynthesis and its related gas exchange traits were significantly influenced by B application @ 9.0 kg ha^{-1}. It was also found that the DM translocation, apparent translocation efficiency (ATE) and apparent conversion efficiency (ACE) of DM were also affected by increment application of boron up to B_2 which indicate the higher remobilization of B and assimilates from source to sink. Furthermore, SOD and POD activity was increased and MDA accumulation was decreased with application B @ 9.0 kg ha^{-1} in the leaves and siliqua of rapeseed in both cultivars, which partially improved the harmful effects of rapeseed senescence by modulating the capacity of antioxidant enzymes, alleviating antioxidant system, which supported in sustaining plant growth and yield of rapeseed. Supplementary applications of B @ 9.0 kg ha^{-1} in rapeseed partially increased its availability, acquisition, mobilization and influx into the plant tissue and thus, improve the rate of photosynthesis and its related gas exchange traits, antioxidant enzyme activity and dry matter translocation and finally all these together contributed to a significant increase in seed yield and harvest index of *Brassica napus* L. under subtropical environment of Southern China. Therefore, adequate level of available B in soil enhanced the capacity of source (leaves, stem and pericarp) and for seed filling, which resulted in the improved sink (seed) in the present study. Thus, it is inferred that boron is an important micro nutrient, its deficiency and excess influenced the rapeseed cultivation and 9 kg B ha^{-1} was an optimum concentration for the growth of *Brassica napus* L. under the subtropical environmental condition of Southern China.

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