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Full Length Article

Paclobutrazol Application Effects on Plant Height, Seed Yield and Carbohydrate Metabolism in Canola

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Abstract

The mechanical harvesting of canola seeds requires a suitable plant height. The appropriate time of paclobutrazol application can reduce canola plant height. To evaluate the best application time of paclobutrazol, a field experiment was conducted with the paclobutrazol applied at stalk heights of 10, 20, 30, 40 and 50 cm. Plants without paclobutrazol application were taken as control. The results indicated that the plant height was reduced by 27% with paclobutrazol applied at 10 cm stalk height as compared with the control. The seed yield was significantly improved by mean increment of 21%. The increased branching ability contributed to yield improvement. The total soluble sugar, sucrose, and starch content in the stem, leaf and bud organs were significantly increased by paclobutrazol application at the initial flowering stage. Paclobutrazol enhanced the sucrose synthesis by sucrose phosphate synthase as well as the catalysis via sucrose synthase, neutral invertase, and in particular acid invertase. The amount of carbohydrates during harvesting remained significantly lower in the root after paclobutrazol treatment, thereby indicating the high-efficiency of carbohydrate utilization by the canola plants in comparison with the control plants. Thus, paclobutrazol applied at 10 cm stalk height takes advantages to control plant height and improve seed yield through enhancing carbohydrate utilization efficiency, which is useful in future for canola mechanical harvesting.

Keywords: *Brassica napus* L.; Paclobutrazol; Carbohydrate; Yield

Introduction

Canola is grown worldwide as an important source of edible vegetable oil. Furthermore, canola oil is also an ideal alternative to petroleum, because it is renewable (Karp and Richter, 2011). Thus, canola oil production must be improved to increase the oil supply for daily consumption and industrial applications (Rehman *et al.,* 2013).

Conventional canola production in China has depended on manual practices for decades. However, the mechanization of canola production becomes vital as the labor resources engaged in crop farming are sharply decreased. Canola production depends on mechanical practices by saving labor resources and lowering production costs for farmers (Ahmadi *et al.*, 2008; Shahid *et al.*, 2010). These mechanical practices involve seed harvesting, sowing, transplanting, fertilization and irrigation.

The mechanical harvesting of canola seed requires several optimal agronomic traits, such as the appropriate plant height, lodging and pod shattering resistance. Although breeding for mechanical harvesting in an elite variety is an ideal, combining such traits requires much efforts. Furthermore, traits such as the plant height are environment-dependent, which makes breeding more difficult (Wu *et al.*, 2010). Thus, other agronomical practices, such as the application of plant growth regulator (s) (PGRs) to control canola plant height, could help to meet mechanical seed harvesting demands (Kumar *et al.*, 2012).

Among the PGRs used in agricultural and horticultural systems, paclobutrazol is a widely accepted regulator of plant height. Paclobutrazol is a triazole derivative that inhibits sterol and gibberellin biosynthesis (Hedden and Graebe, 1985; Lee *et al.*, 1985; Khalil and Rahman, 1995; Khan *et al.*, 2009). This compound can markedly affect plant growth and development by altering the photosynthetic rate and modifying the phytohormone levels (Vu and Yelenosky, 1992; Wang and Lin, 1992; Huang *et al.*, 1995; Kim *et al.*, 2012). The effects of paclobutrazol on canola growth have been previously reported by others (Addo-Quaye *et al.*, 1985; Zhou and Xi, 1993). Canola yield could be significantly improved by paclobutrazol application (Zhou and Xi, 1993). However, the influence of paclobutrazol on canola plant growth is dependent on the environment, such as the supplementary soil moisture

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(Scarisbrick *et al.*, 1985). Thus, the inappropriate time and dose of paclobutrazol application could adversely affect the growth and development of canola. Zhou and Xi (1993) previously concluded that the early spraying of paclobutrazol at the three-leaf stage did not significantly affect the plant height of mature canola. A slight increase in the stem elongation was observed when canola was treated by uniconazole, a triazole compound (Zhou and Ye, 1996). Therefore, the appropriate time of paclobutrazol application should be determined for canola to effectively control plant height. Growers in China usually apply paclobutrazol from budding to anthesis as part of the present-day practices of canola farming. To determine the optimum time of paclobutrazol application, this study was conducted with paclobutrazol treatment at various stalk heights in canola. The resulting physiological changes were also assessed.

Materials and Methods

Plant Materials and Crop Husbandry

Field experiments were conducted during 2009-2010 and 2010-2011 for two canola growing seasons at the experimental station of the Zhejiang Academy of Agricultural Sciences, China. A new canola commercially propagated (*Brasscia napus* L.) variety "Zheyou50" with high oil content (50.1%) was used as plant material. The seeds of the variety were sown in respective seed bed on October 3, 2009 and October 5, 2010. Other appropriate management practices such as pesticide and herbicide applications were undertaken to control pests and weeds. One-month-old seedlings of uniform height were selected and transplanted. The soil type of experimental station was loamy clay (loamy, mixed and thermic Aeric Endoaquepts). Before transplanting, 150 kg ha^{-1} of urea was broadcasted as basal fertilizer. 75 kg ha^{-1} of urea was applied by topdressing at the end of January in 2010 and 2011. The field was not irrigated during the canola growth season, because rainfall was sufficient in the Hangzhou region during this period (1041.7 and 1048.3 mm, respectively, for the two growing seasons). The plants were grown in plots spaced 0.35 m apart between rows and 30 m in length, with 0.2 m spaces between plants. Three replications were performed while using a randomized complete design.

Paclobutrazol Treatment and Sampling

The Zheyou 50 variety is a semi-winter type of canola that needs a period of low temperature for vernalization. The variety budded on March 10, 2010 and March 12, 2011 during the respective seasons. The stalk height for paclobutrazol application started 10 cm, followed by the advancement in stem elongation before budding at the correspondingly stalk heights of 20, 30, 40 and 50 cm. Plants without paclobutrazol application served as controls. The paclobutrazol concentration used was 100 μmol L^{-1} recommended for canola production systems in China (Zhou and Xi, 1993). Paclobutrazol was sprayed on the field surrounded by an iron shelf with nylon film. The paclobutrazol powder was evenly dispersed in distilled water and applied using an electronic sprayer. During sampling, the whole plant was uprooted and immediately transported to the laboratory for further analysis. Only plants in the core sampling area were selected using a destruction sampling. Twenty plants were randomly chosen in each plot and used for physiological analysis.

Yield and Yield Components Determination

Two-thirds of the plants, except those along the border in each 30 m-long plot were collected for yield determination at the canola harvesting stage. Twenty plants in each plot were randomly selected for yield component measurement. The seed yield of each plant used for yield component determination was added into the total seed yield of each plot. The measured yield components included the numbers of branches per plant, siliques per plant, and seeds per silique, as well as the 1000-seed-weight.

Carbohydrate Content Measurement

After sampling, the plant roots were washed with water as soon as possible with minimum injury. Each plant at the initial flowering stage (siliques and seeds at harvesting stage) was divided into the root, leaf, stem and bud regions using a sharp knife. The samples were first dried at 105°C for 30 min and then at 70°C to constant weight. The dried tissues were well ground into powder by a pulverizer (Wenlin Dalin Machine Com. Ltd, China). The powder was boiled in 30 mL of 800 mL L^{-1} ethanol for 30 min and centrifuged at 10000 ×*g* twice. After sample extraction, the powder was cooled into room temperature. The supernatant was added to 200 mg of active charcoal to remove chlorophyll before it was used for the determination of the total soluble sugar content (TSSC) and sucrose content according to the method described by Hendrix (1993). The pellet was retained for starch analysis. The starch in the pellets was digested with amylo glucosidase for 100 min at 55°C following the protocol demonstrated by Hendrix (1993).

Enzyme Assay

Leaf and bud samples at the initial flowering stage were collected, frozen in liquid nitrogen, and quickly ground into powder. Sucrose synthase (SS), sucrose phosphate synthase (SPS), acid invertase (AI), and neutral invertase (NI) was extracted, as described by Ruan *et al.* (2005). Their activity levels were measured according to the method used by King *et al.* (1997).

Statistics

The means of the collected data from the different treatments during both seasons were compared using Duncan's test, with $P< 0.05$ as significant.

Results

Plant Height, Yield and Yield Components

The canola plant height was significantly affected by paclobutrazol application (Table 1). The plant stem elongation was inhibited by paclobutrazol application at 10 cm stalk height during the initial flowering stage. Thus, dwarf plants were obtained during the harvesting stage, with an average decrease in plant height by 35% and 73% of the control in 2010 and 2011, respectively. The seed yield for both years was significantly increased after paclobutrazol application. Seed yield increased by 21.6% and 21.1% when paclobutrazol was applied at 10 cm stalk height in 2010 and 2011, respectively. The number of siliques per plant was significantly influenced by paclobutrazol application, whereas the seed number per silique and the 1000-seed-weight were less responsive to paclobutrazol treatment. The numbers of siliques per plant with paclobutrazol treatment at 10 cm stalk height were notably 25% and 18% higher than those of the control for the two seasons. The increased number of siliques per plant was due to the increased branching after paclobutrazol application. The number of branches per plant at 10 cm stalk height was increased by 29% and 30% after paclobutrazol treatment, as compared with the control; this indicated an average increment of 30 and 22 siliques per branch for the said treatment (Table 1).

Carbohydrate Content and Enzymatic Activity at the Initial Flowering Stage

Total soluble sugar content (TSSC): The effect of paclobutrazol application at different canola stalk heights on TSSC of the root, stem, leaf, and bud was similar for the two seasons (Fig. 1). Among the four assayed organs, the leaf had the highest TSSC at the initial flowering stage, whereas the bud ranked the last. The TSSC in the root was relatively stable, and no significant differences were found after paclobutrazol application at different stalk heights in both years. The stem accumulated considerable amounts of soluble sugars as paclobutrazol was applied at 10 cm stalk height, which increased by 33.7% and 51.3% in 2010 and 2011, respectively as compared with the control. The TSSC in the leaf and bud followed a similar trend, which exhibited an averagely increment of 54.8% and 51.1%, respectively with paclobutrazol treatment at 10 cm stalk height during the two growth seasons (Fig. 1). Thus, paclobutrazol stimulated the accumulation of total soluble sugars in stem, leaf, and bud.

Sucrose Content

The influence of paclobutrazol application at different stalk heights on the sucrose content of canola tissues was generally similar with that of TSSC (Fig. 2). However, the sucrose content in the root, stem, leaf and bud tissues was relatively lower than TSSC. The sucrose content in the root varied slightly and was not significant among treatments.

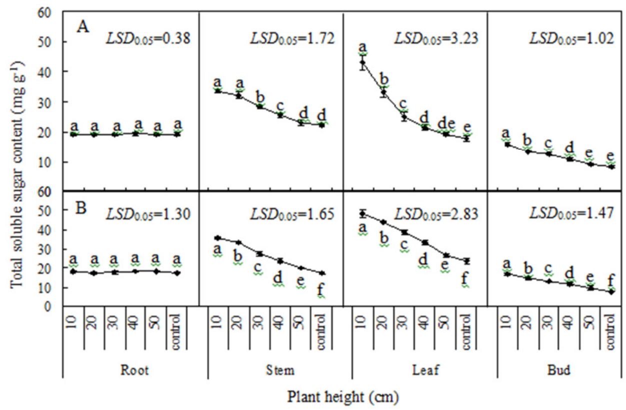

Fig. 1: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on total soluble sugar content (TSSC) in the root, stem, leaf, and bud of canola at the initial flowering stage during 2010 (A) and 2011 (B)

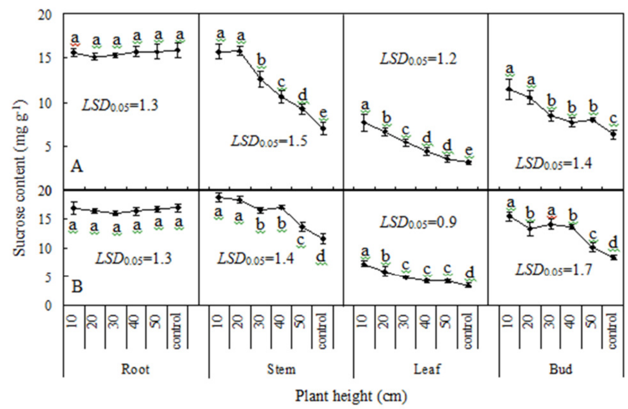

Fig. 2: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on sucrose in the root, stem, leaf, and bud of canola at the initial flowering stage during 2010 (A) and 2011 (B)

The sucrose content in the leaf was the lowest, whereas in stem was the highest after paclobutrazol application. No significant differences in the sucrose content in stems were found between plants after paclobutrazol application at stalk heights of 10 and 20 cm for the two years; however, their values were markedly higher than those of the control, which were increased by 54.9% and 55.2% in 2010 and by 38.2% and 36.7% in 2011, respectively. The leaf sucrose content with paclobutrazol application at 50 cm stalk height and the control was not significantly different, whereas significant differences were observed among those treated at stalk heights of 10, 20 and 30 cm. The bud sucrose content was slightly lower than stem and accumulated significantly after paclobutrazol application. Compared with the control, the bud sucrose content with paclobutrazol application at 10 cm stalk height increased by 44.6% and 45.7% in 2010 and 2011, respectively (Fig. 2).

Table 1: Effect of paclobutrazol application at different stalk heights on plant height, yield, and yield components of canola across two years during 2010 and 2011 in Hangzhou, China

Paclobutrazol application	Plant height (cm) (Initial flowering)	Plant height (cm) (harvesting)	No. of branches per plant	No. of siliques per plant	Seed number per silique	1000-seed weight (g)	Yield (kg ha^{-1})
			2010				
10 cm stalk height	28.7 e	100.8 e	16.00 a	586.9 a	23.08 a	4.06 a	3844 a
20 cm stalk height	44.0 d	112.5 d	14.42 b	540.3 b	24.03 a	3.99 a	3692 b
30 cm stalk height	59.3 c	114.3 d	13.25 c	510.8 c	24.41 a	3.96 a	3587 c
40 cm stalk height	61.3c	126.9c	12.88c	492.6 d	24.26a	4.01a	3426 d
50 cm stalk height	75.0 b	143.9 b	12.69 c	478.3 e	24.62 a	4.07 a	3358 e
Control (No application)	82.3 a	148.3 a	11.36 d	445.9 f	24.24 a	4.07 a	3015 f
			2011				
10 cm stalk height	32.7 f	115.4 f	15.44 a	562.3 a	24.12 a	4.17 a	3961 a
20 cm stalk height	50.2 e	119.6 e	13.96 b	546.4 b	23.74 a	4.28 a	3781 b
30 cm stalk height	60.0 d	131.7 d	12.42 c	518.4 c	23.98 a	4.07 a	3582c
40 cm stalk height	71.6c	135.8c	11.86 cd	490.6 d	24.16a	4.13a	3459 d
50 cm stalk height	84.8 b	142.1 b	11.11 de	475.1 e	23.97 a	4.11 a	3316 e
Control (No application)	94.0 a	147.5 a	10.81 e	459.4 f	24.39 a	4.09 a	3126f

Different lowercase letters after numbers in the same column show a significant difference at 5% probability level

Starch Content

Unlike TSSC and sucrose content, the root starch content in the controls was significantly higher than with paclobutrazol treatment. Moreover, root starch content decreased as the paclobutrazol application time advanced, that is, plants of shorter heights during paclobutrazol application contained less root starch. The root starch content in the control was 2.49 and 2.25-fold higher than plants with paclobutrazol application at 10 cm stem height in 2010 and 2011, respectively. However, the starch content in stem, leaf and bud followed an opposite trend as compared with the root. Their starch content decreased as the plant height during paclobutrazol application was increased. The leaf exhibited the highest starch content for these three tissues. The starch content in the stem, leaf and bud with paclobutrazol application at 10 cm stem height was increased on average by 32.5%, 29.2% and 37.8%, respectively, as compared with the control for the two years (Fig. 3).

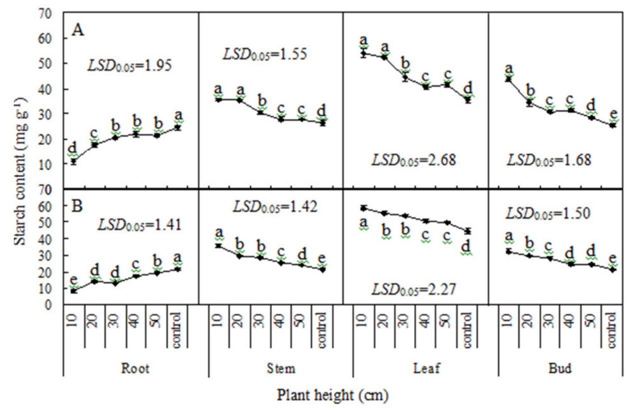

Fig. 3: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on starch in the root, stem, leaf and bud of canola at the initial flowering stage during 2010 (A) and 2011 (B)

Leaf and Bud SS and SPS Activity

The SS and SPS activity was monitored during the two seasons in the leaf and bud tissues after paclobutrazol application at the initial flowering stage. The result showed that the leaf had high SS and SPS activity, whereas their activity was low in the bud (Fig. 4). The SS and SPS activity generally decreased as the height of the stalk during paclobutrazol application was increased, although the difference in some stages was not significant i.e., the leaf SS activity among the plants with paclobutrazol application at stalk heights of 30, 40 and 50 cm in 2010. The SS and SPS activity in leaf and bud with paclobutrazol application at 10 cm stalk height was significantly higher than the control, with an average increment of 41.9% and 66.9% in 2010 and that of 36.9% and 58.8% in 2011, respectively (Fig. 4).

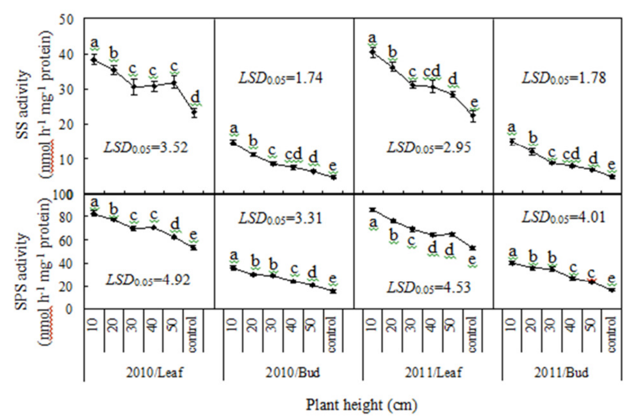

Fig. 4: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on sucrose synthase (SS) and sucrose phosphate synthase (SPS) in the leaf and bud of canola at the initial flowering stage during 2010 and 2011

Leaf and Bud AI and NI Activity

Both AI and NI are important enzymes that cleave sucrose into glucose and fructose (Hajirezaei *et al.*, 2000; Roitsch *et al.*, 2003).Thus, the activities of these two enzymes were measured. Both the leaf and bud NI activity were unexpectedly much less than the AI activity for the two seasons; their difference was higher than tenfold (Fig. 5). Furthermore, the NI activity in the leaf and bud; were distinctly different, whereas the difference between the AI activity of the leaf and bud was relatively small. This indicates that AI had a major function in the cleavage of sucrose in leaf and bud cells with or without paclobutrazol treatment. The effect of paclobutrazol treatment at different stalk heights, the leaf and bud NI activity was only found with paclobutrazol application at 10 cm stalk height; these levels were significantly enhanced during the two years. However, AI activity in the leaf and bud for shorter stalks during treatment (i.e., 10, 20 and 30 cm height) was significantly higher than the control. An average increment of 44.1% and 35.2% in the leaf and bud AI activity during the two years were observed with paclobutrazol application at 10 cm stalk height, as compared with the control (Fig. 5).

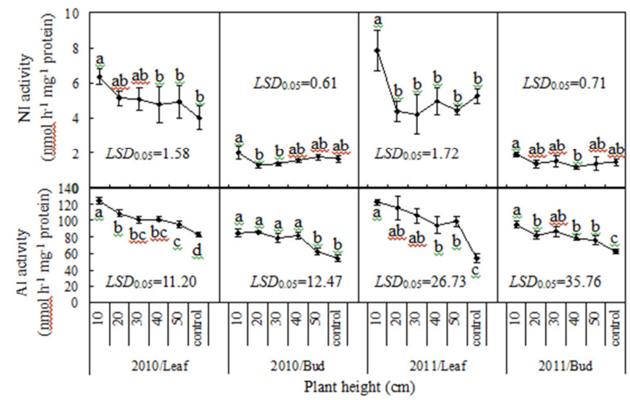

Fig. 5: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on neutral invertase (NI) and acid invertase (AI) in the leaf and bud of canola at the initial flowering stage during 2010 and 2011

Carbohydrate Content at Harvesting Stage

Total soluble sugar content (TSSC): TSSC was determined at the harvesting stage to reveal whether TSSC would be affected by paclobutrazol application. After paclobutrazol treatment, all tissues showed that TSSC was lowest with paclobutrazol application at 10 cm stalk height (Fig. 6). The delayed application of paclobutrazol generally increased the remaining amount of TSSC. The TSSC in the root, stem, silique, and seed were reduced on average by 55.2%, 55.0%, 39.5% and 46.0%, respectively with; paclobutrazol treatment at 10 cm stalk height during the two years (Fig. 6).

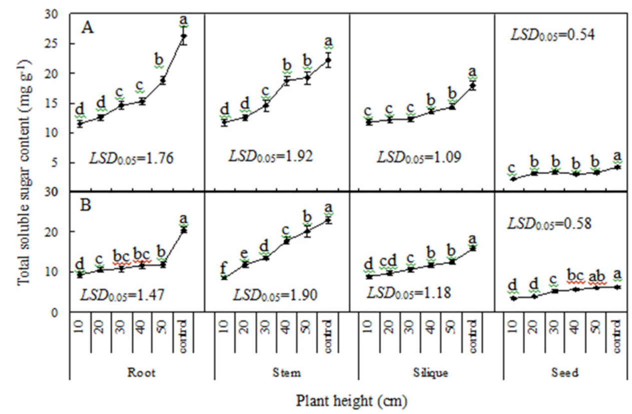

Fig. 6: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on total soluble sugar content (TSSC) in the root, stem, leaf, and bud of canola at the harvesting stage during 2010 (A) and 2011 (B)

Sucrose Content

Paclobutrazol application had a similar effect on the sucrose content in different tissues, as compared with TSSC at the harvesting stage (Fig. 7). However, the remaining sucrose in mature canola tissues was distinctly higher than that of TSSC. The root showed the highest sucrose content among these tissues, whereas the seed had the lowest sucrose content (Fig. 7). The sucrose content in the root, stem, silique, and seed of the controls were 51.6%, 52.9%, 41.2%, and 54.4% higher on the average, respectively, than plants with paclobutrazol application at 10 cm stalk height over two years (Fig. 7).

Starch Content

The difference in the starch content among the various organs was relatively small (Fig. 8). The root always maintained a higher starch content after paclobutrazol application at different stalk heights in both years. However, the silique had the highest starch content in the control. The starch content in the silique was not significant between plants with paclobutrazol application at stalk heights of 10 and 20cm during both years. The starch content in the root, stem, silique, and seed with paclobutrazol application at 10 cm stalk height was reduced on average by 25.5%, 46.6%, 57.0% and 46.6%, respectively as compared with the control (Fig. 8).

Discussion

Paclobutrazol is a useful PGR that has been applied in agricultural systems for decades. PGRs are used on crops such as rice and wheat to inhibit plant height as well as increase the number of tillers and consequently yield (Assuero *et al.*, 2012). Similar practices are being adopted in canola production system, including the regulation of plant height, enhancement of branches and improvement of seed yield, especially with the rapid expansion of mechanized harvesting. The main goal of this study was to control plant height using paclobutrazol without any yield loss. The results were positive and suggested that paclobutrazol application after budding was suitable (Table I). Zhou and Ye (1996) reported that canola applied with foliar spraying of uniconazole (which is functionally similar with paclobutrazol) or paclobutrazol at the seedling stage will have reduced the seedling height; however, the effect on the eventual plant height almost disappeared because no significant differences in plant height were observed at the maturation stage among treatments (Zhou and Xi, 1993; Zhou and Ye, 1996). Consequently, their findings suggested that paclobutrazol had a temporal effect on plant height, and the advantageous benefits from paclobutrazol could be obtained when application was postponed to the seedling stage. Compared with the control, all treatments in the present study exhibited trend of decreasing plant height and increasing seed yield. The increased seed yield of canola was mainly caused by the increased number of branches and siliques per plant. This finding is partially agrees with previous reports on canola (Zhou and Xi, 1993). The principle of increased seed yield was similar to other crops such as rice, to a certain extent. The increased seed yield was caused by the promotion of tillage (branches) production after paclobutrazol induction. From agronomic perspective, the number of branches in canola will affect the yield by directly influencing the number of siliques expressed per unit (Angadi *et al.*, 2003). Branching is an intricate agronomic trait that can be regulated by numerous factors, such as developmental cues, the planting date, and nitrogen supplements (Sorefan *et al.*, 2003; Zhu and Kranz, 2012). Previous studies showed that the differentiation of the apical meristem can be inhibited by paclobutrazol (Serrani *et al.*, 2010). Thus PGR can stimulate axillary bud emergence and improve effective branch formation. However, further evidence is needed because phytohormones such as auxin and gibberellin have significant effects on stem elongation (Dietz *et al.*, 1990; Dahanayake and Galwey, 1999; Dayan *et al.*, 2012). Moreover, the detailed mechanism by which paclobutrazol influences branch formation is not within the scope of the present investigation.

Yield improvement after paclobutrazol application was attributed to the enhanced leaf photosynthesis and accumulation of photosynthetic pigments (mainly chlorophyll) (Zhou and Xi, 1993). However, a detailed discussion regarding the influence of paclobutrazol on carbohydrate metabolism in canola has not yet been reported. Therefore, this study focused on unveiling the modified carbohydrate metabolism caused by paclobutrazol application to further understand the yield improvement. The leaf is the dominant organ during the initial flowering stage in canola and produces most of the biomass and photoassimilates for the various organs (Hua *et al.*, 2012; Fig. 1 & 3). The photoassimilates in leaves are not in static;

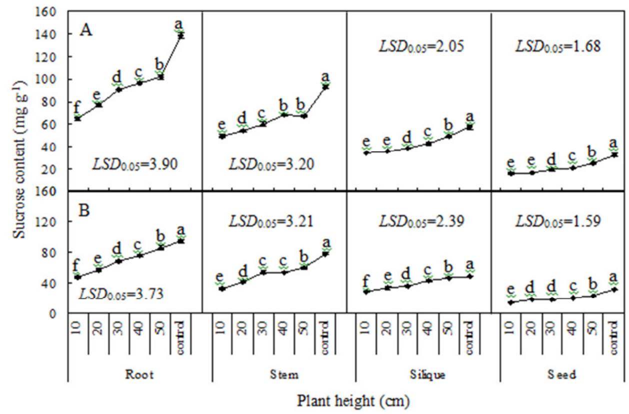

Fig. 7: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40 and 50 cm) on sucrose content in the root, stem, leaf, and bud of canola at the harvesting stage during 2010 (A) and 2011 (B)

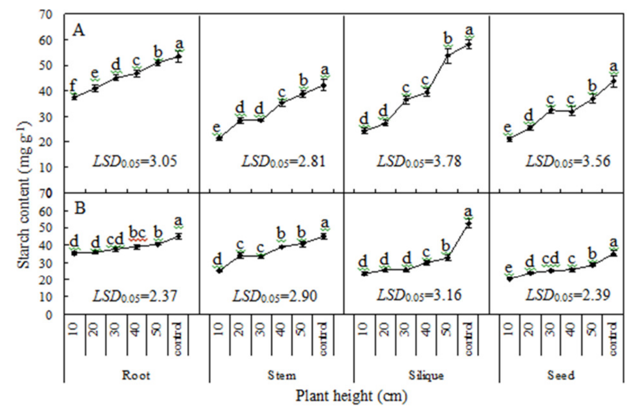

Fig. 8: Influence of paclobutrazol applied at different stalk heights (10, 20, 30, 40, and 50 cm) on starch content in the root, stem, leaf, and bud of canola at the harvesting stage during 2010 (A) and 2011 (B)

these compounds, mainly sucrose and starch, are in a metabolic state. The carbohydrate are either transported into other non-photosynthetic organs or cleaved into smaller molecules (Tschaplinski and Blake, 1994; Spann *et al.*, 2008). Sucrose is always cleaved by invertase, SS, or both into single sugar molecules, such as fructose and glucose (Nguyen-Quoc and Foyer, 2001; Tomlinson *et al.,* 2004). The results of this study clearly suggested that paclobutrazol can enhance the production of carbohydrates in the leaf and other tissues, which is in agreement with other reports (Wieland and Wample, 1985; Zheng *et al.*, 2012). The increased of TSSC and sucrose content could be partially accounted for by the activating effect of paclobutrazol on enzymes related to sucrose synthesis and catalysis. The enzymatic activity of the major enzymes that cleave sucrose, namely, SS and invertase, is not evenly allocated in cells of distinct organs at different developmental stages (Figs. 4 &

5). In this study, the SS activity in the leaf and bud were not sufficiently high regardless of paclobutrazol treatment. By contrast, SPS in the bud exhibited only half of its activity in the leaf. This observation indicated that the leaf was a strong site of enzyme activity. By contrast, both the leaf and bud AI activity were very high, particularly with paclobutrazol application at10 cm stalk height. Consequently, strong sucrose cleavage probably occurs in the leaf and bud after paclobutrazol treatment by combining the effects of AI, NI, and SS. Higher SPS activity was detected in the leaf at the initial flowering stage after paclobutrazol induction. Generally, SPS promotes sucrose synthesis, whereas SS causes sucrose breakdown (Baxter *et al.*, 2003; Fallahi *et al.*, 2008). Thus, a homoeostasis between sucrose synthesis and catalysis can be formed in leaf, which could be a key reason for the low sucrose content in the leaf at the initial flowering stage after paclobutrazol treatment. The TSSC at the initial flowering stage in the stem ranked second after paclobutrazol application. Given that the stem is physiologically large channel for the transport of substances including carbohydrates to other tissues (Ho and Nichols, 1975; Oparka and Davies, 1985), transient deposition of TSSC could be observed. Furthermore, the accumulation of soluble sugars and sucrose with paclobutrazol treatment at a10 cm stalk height might be used as a substrate for stem lignification (Amthor, 2003), however this hypothesis was not tested in this study. Regardless of the positive effect of paclobutrazol application on the leaf, stem, and bud starch content. The leaf starch content was the highest at the initial flowering stage. Only one growth stage was monitored in this study, and the status of the succeeding stages remains unknown. An assumption is that significant starch metabolism, such as the conversion into glucose occurs during the latter stages. The root starch content was likewise significantly reduced after paclobutrazol treatment. This decrease may be explained by the transient increase in growth of above-ground tissues and the lagging of root growth. Therefore, the carbohydrate content of above-ground organs, including the stem, leaf, and bud were significantly enhanced by paclobutrazol application. This effect is partially due to the increased activity of carbohydrate-related metabolic enzymes at the initial flowering stage.

The leaves of canola plants drop off the plants during the harvesting stage (Hua *et al.*, 2012).The observed TSSC and sucrose content were mainly concentrated in the roots. Although the stem TSSC and sucrose content can be stimulated by paclobutrazol application, especially at a 10 cm stalk height during the initial flowering stage, the stem contained the least amount at the harvesting stage at the same treatment. This observation indicates the highly efficient transformation of starch into other polysaccharides. Previous studies have reported that paclobutrazol increased the cytokine in levels, thereby producing wider but shorter stems. Thus, no significant reductions in the stem dry matter were observed (Berova and Zlatev, 2000). Maintaining the stem dry weight in this context required the consumption of a considerable amount of carbohydrates. The silique and seed are the centers of growth after flowering (Hua *et al.*, 2012). The canola plant canopy structure is established by developing siliques, which produce most carbohydrates (King *et al.*, 1997; Hua *et al.*, 2012). Whether paclobutrazol can promote silique photosynthesis remains unknown because such experiments are never performed during silique development. However, we noted that the measured amount of carbohydrates, including TSSC, sucrose content, and starch content, were significantly reduced in the silique after paclobutrazol spraying. This reduction could be accounted for the following mechanisms: (1)carbohydrate transport from the silique to other tissue, especially to the seeds, which would utilize them as substrates for lipid biosynthesis (Focks and Benning, 1998; Hernádez *et al.*, 2012); (2) the different transformation efficiencies for converting carbohydrates in the stem into other metabolites, such as cellulose or lignin (Coleman *et al.*, 2009; Richet *et al.*, 2011); (3) carbohydrate supply to the additional branches to obtain higher seed yield. The first mechanism can be explained by the plant structure, particularly the very short distance between the developing silique and seed by their direct connection with helium (Bennett *et al.*, 2011). For the second mechanism, other metabolites in the stem were not measured, such as the lignin content. Thus, an exact explanation is difficult to obtain. However, we found that the seed weight and oil content had no remarkable changes (data not shown). We deduced that the third mechanism was more probable than the previous ones. Given that silique carbohydrates were increased by paclobutrazol, these carbohydrates could be diluted by the mean increment in the number of siliques. Thus, only a low level of carbohydrates could be observed in the tissues under paclobutrazol treatment.

In conclusion, paclobutrazol can significantly reduce canola plant height and improve seed yield when applied at 10 cm stalk height. The increased seed yield was attributed to enhancement of branch differentiation and, consequently, silique numbers; and paclobutrazol enhanced the enzyme activity for sucrose synthesis and catalysis.

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References

Addo-Quaye, A.A., R.W. Daniels and D.H. Scarisbrick, 1985. The influence of paclobutrazol on the distribution and utilization of ^{14}C-labelled assimilate fixed at anthesis in oil-seed rape (*Brassica napus* L.). *J. Agric. Sci.,* 105: 365–373

Ahmadi, E., H.R. Ghassemzadeh, M. Moghaddam and K.U. Kim, 2008. Development of a precision seed drill for oilseed rape. *Turk. J. Agric. For.,* 32: 451–458

Amthor, J.S., 2003. Efficiency of lignin biosynthesis: a quantitative analysis. *Ann. Bot.,* 91: 673–695

Angadi, S.V., H.W. Cutforth, B.G. McConkey and Y. Gan, 2003. Yield adjustment by canola growth at different plant populations under semiarid conditions. *Crop Sci.,* 43: 1358–1366

Assuero, S.G., M. Lorenzo, N.M.P. Ramírez, L.M. Velázquez and J.A. Tognetti, 2012. Tillering promotion by paclobutrzol in wheat and its relationship with plant carbohydrate status. *N.Z. J. Agric. Res.,* 55: 347–358

Baxter, C.J., C.H. Foyer, J. Turner, S.A. Rolfe and W.P. Quick, 2003. Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development. *J. Exp. Bot.,* 54: 1813–1820

Bennett, E.J., J.A. Roberts and C. Wagstaff, 2011. The role of pod in seed development: strategies for manipulating yield. *New Phytol*., 190: 838–853

Berova, M., and Z. Zlatev., 2000. Physiological response and yield of paclobutrazol-treated tomato plants (*Lycopersicon esculentum* Mill). *Plant Growth Regul.,* 30: 117- 123

Coleman, H.D., J. Yan and S.D. Mansfield, 2009. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall structure. *Proc. Natl. Acad. Sci. USA,* 106: 13118–13123

Dahanayake, S.R. and N.W. Galwey, 1999. Effects of interactions between low-temperature treatments, gibberellin (GA_3) and photoperiod on flowering and stem height of spring rape (*Brassica napus* var. *annua*). *Ann. Bot.,* 84: 321–327

Dayan, J., N. Voronin, F. Gong, T. Sun, P. Hedden, H. Fromm and R. Aloni, 2012. Leaf-induced gibberellin signaling is essential for internode elongation, cambial activity, and fiber differentiation in tobacco stems. *Plant Cell,* 24: 66–79

Dietz. A., U. Kutschera and P.M. Ray, 1990. Auxin enhancement of mRNAs in epidermis and internal tissue of the pea stem and its significance for control of elongation. *Plant Physiol.,* 93: 432–438

Fallahi. H., G.N. Scofield, M.R. Badger, W.S. Chow, R.T. Furbank and Y. Ruan, 2008. Localization of sucrose synthase in developing seed and siliques of *Arabidopsis thaliana* reveals diverse roles for SUS during development. *J. Exp. Bot.,* 59: 3283–3295

Focks, N. and C. Benning, 1998. *Wrinkled1*: a novel low-seed-oil mutant of Arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. *Plant Physiol*., 118: 91–101

Hajirezaei, M., Y. Takahata, R.N. Trethewey, L. Willmitzer and U. Sonnewald, 2000. Impact of elevated cytosolic and apoplasticinvertase activity on carbon metabolism during potato tube development. *J. Exp. Bot.,* 51: 439–445

Hedden, P. and J.E. Graebe, 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Maluspumila* embryos. *J. Plant Growth Regul.,* 4: 111–122

Hernádez, M.L., L. Whitehead, Z. He, V. Gazda, A. Gilday, E. Kozhevnikova, F.E. Vaistij, R.T. Larson and I. Graham, 2012. A cytosolic acyl transferase contributes to triacylglycerol synthesis in sucrose-rescued Arabidopsis seed oil catabolism mutants. *Plant Physiol.,* 160: 215–225

Hendrix, D.L., 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Sci.,* 33: 1306–1311

Ho, L.C. and R. Nichols, 1975. The role of phloem transport in the translocation of sucrose along the stem of carnation cut flowers. *Ann. Bot.,* 39: 439–446

Huang, W.D., T. Shen, Z.H. Han and S. Liu, 1995. Influence of paclobutrazol on photosynthesis rate and dry matter partitioning in the apple tree. *J. Plant Nutr.,* 18: 901–910

Hua, W., Li, R.J., Zhan, G.M., Liu, J., Li, J., Wang, X.F., Liu, G.H., Wang, H.Z., 2012. Maternal control of seed oil content in *Brassica napus*: the role of silique wall photosynthesis. *Plant J.,* 69: 432-44

Karp, A. and G.M. Richter, 2011. Meeting the challenge of food and energy security. *J. Exp. Bot.*, 62: 3263–3271

Khalil, I.A. and H.U. Rahman, 1995. Effect of paclobutrazol on growth, chloroplast pigments and sterol biosynthesis of maize (*Zea mays* L.). *Plant Sci.,* 105: 15–21

Khan, M.S.H., T. Wagatsuma, A. Akhter and K. Tawaraya, 2009. Sterol biosynthesis inhibition by paclobutrazol induces greater aluminum (Al) sensitivity in Al-tolerant rice. *Amer. J. Plant Physiol.,*4: 89–99

Kim, J., R.L. Wilson, J.B. Case and B.M. Binder, 2012. A comparative study of ethylene growth response kinetics in eudicots and monocots reveals a role for gibberellin in growth inhibition and recovery. *Plant Physiol.,* 160: 1567–1580

King, S.P., J.E. Lunn and R.T. Furbank, 1997. Carbohydrate content and enzyme metabolism in developing siliques. *Plant Physiol.,* 114: 153–160

Kumar, S., S. Ghatty, J. Satyanarayana, A. Guha, B. Chaitanya and A.R. Reddy, 2012. Paclobutrazol treatment as a potential strategy for higher seed oil yield in field-grown *Camelina sativa* L. Crantz. *BMC Res. Notes*, 5:137–149

Lee, E.H., J.K. Byun and S.J. Wilding, 1985, A new gibberellin biosynthesis inhibitor, paclobutrazol (PP_{333}) confers increased SO_2 tolerance on snap bean plants. *Environ. Exp. Bot.,* 25: 265–275

Nguyen-Quoc, B. and C.H. Foyer, 2001. A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. *J. Exp. Bot.,* 52: 881–889

Oparka, K.J. and H.V. Davies, 1985. Translocation of assimilates within and between potato stems. *Ann. Bot.,* 56: 45–54

Rehman, H., Q. Iqbal, M. Farooq, A. Wahid, I. Afzal and S.M.A. Basra, 2013. Sulphur application improves the growth, seed yield and oil quality of canola. *Acta Physiol. Plant.,* 35: 2999–3006

Richet, N., D. Afif, F. Huber, B. Pollet, J. Banvoy, R.E. Zein, C. Lapierre, P. Dizengremel, P. Perré and M. Cabané, 2011. Cellulose and lignin biosynthesis is altered by ozone in wood of hybrid poplar (*Populus tremulaxalba*). *J. Exp. Bot.,* 62: 3575–3586

Roitsch, T., M.E. Balibrea, M. Hofmann, R. Proels and A.K. Sinha, 2003. Extracellar invertase: key metabolic enzyme and PR protein. *J. Exp. Bot.,* 54: 513–524

Ruan, Y.L., D.J. Llewellyn, R.T. Furbank and P.S. Chourey, 2005. The delayed initiation and slow elongation of fuzz-like short fiber cells in relation to altered patterns of sucrose synthase expression and plasmodesmata gating in a lintless mutant of cotton. *J. Exp. Bot.,* 56: 977–984

Scarisbrick, D.H., A.A. Addo-Quaye, R.W. Daniels and S. Mahamud, 1985. The effect of paclobutrazol on plant height and yield of oil-seed rape (*Brassica napus* L.). *J. Agric. Sci.,* 105: 605–612

Serrani, J.C., E. Carrera, O. Ruiz-Rivero, L. Gallego Giraldo, E.P. Peres and J.L. García-Martínez, 2010. Inhibition of auxin transport from the ovary or from the apical shoot induces parthenocarpic fruit-set in tomato mediated by gibberellins. *Plant Physiol.,* 153: 851–862

Shahid, L.A., M.A. Saeed and N. Amjad, 2010. Present status and future prospects of mechanized production of oilseed crops in Pakistan-a review. *Pak. J. Agric. Res.,* 23: 83–93

Sorefan, K., J. Booker, K. Haurogné, M. Goussot, K. Bainbridge, E. Foo, S. Chatfield, S. Ward, C. Beveridge, C. Rameau and O. Leyser, 2003. *MAX4* and *RMS1* are orthoogousdioxygenase-like genes that regulate root branching in *Arabidopsis* and pea. *Gene Dev.,* 17: 1469–1474

Spann, T.M., R.H. Beede and T.M. DeJong, 2008. Seasonal carbohydrate storage and mobilization in bearing and non-bearing pistachio (*Pistaciavera*) trees. *Tree Physiol.,* 28: 207–213

Tomlinson, K.L., S. McHugh, H. Labbe, J.L. Grainger, L.E. James, K.M. Pomeroy, J.W. Mullin, S.S. Miller, D.T. Dennis and B.L.A. Miki, 2004. Evidence that hexose-to-sucrose ratio does not control the switch to storage product and accumulation in oilseeds: analysis of tobacco seed development and effects of overexpressing apoplastic invertase. *J. Exp. Bot.,* 55: 2291–2303

Tschaplinski, T.J. and T.J. Blake, 1994. Carbohydrate mobilization following shoot defoliation and decapitation in hybrid poplar. *Tree Physiol.,* 14: 141–151

Vu, J.C.V. and G. Yelenosky, 1992. Growth and photosynthesis of sweet orange plants treated with paclobutrazol. *J. Plant Growth Regul.,* 11: 85–89

Wang, L. and C. Lin, 1992. The effect of paclobutrazol on physiological and biochemical changes in the primary roots of pea. *J. Exp. Bot.,* 43: 1367–172

Wieland, W.F. and R.L. Wample, 1985. Effects of paclobutrazol on growth, photosynthesis and carbohydrate content of 'Dlicious' apple. *Sci. Hortic.,* 26: 139–147

Wu, X., Z. Wang, X. Chang and R. Jin, 2010. Genetic dissection of the developmental behaviours of plant height in wheat under diverse water regimes. *J. Exp. Bot.*, 61: 2923–2937

Zheng, R., Y. Wu and Y. Xia, 2012.Chlorocholine chloride and paclobutrazol treatments promote carbohydrate accumulation in bulbs of *Lilium* Oriental hybrids 'Sorbonne'. *J. Zhejiang Univ. Sci. B,* 13: 136–144

Zhou, W. and Q. Ye, 1996. Physiological and yield effects of uniconazole on winter rape (*Brassica napus* L.). *J. Plant Growth Regul.,* 15: 69–73

Zhou, W.J. and H.F. Xi, 1993. Effects of mixtalol and paclobutrazol on photosynthesis and yield of rape (*Brassica napus*). *J. Plant Growth Regul.,* 12: 157–161

Zhu, H. and R.G. Kranz, 2012. A nitrogen-regulated glutamine aminotransferase (GAT1_2.1) represses shoot branching in Arabidopsis. *Plant Physiol.,* 160: 1770–1780

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