INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–0870/2017/19–4–779–786
DOI: 10.17957/IJAB/15.0359
http://www.fspublishers.org

Full Length Article

Effects of Gibberellin and Gibberellin Biosynthesis Inhibitor (Paclobutrazol) Applications on Radish (*Raphanus sativus*) Taproot Expansion and the Presence of Authentic Hormones

Bashir Mohammed Omer Jabir[1,2], Karanja Benard Kinuthia[1], Mohamed Almahadi Faroug[1,2], Faisal Nureldin Awad[3], Evelyne M. Muleke[1], Zarwali Ahmadzai[1] and Liwang Liu[1*]

[1]*National Key Laboratory of Crop Genetics and Germplasm Enhancement, Key Laboratory of Crop Biology and Genetic Improvement of Horticultural Crops (East China), Ministry of Agriculture of China, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, P.R. China*

[2]*Al- Zaiem Al-Azhari University, P.O. Box 1432, Khartoum North, Sudan,*

[3]*National Food Research Centre, P.O. Box 213, Khartoum North, Sudan*

[*]For correspondence: nauliulw@njau.edu.cn

Abstract

Radish (*Raphanus sativus* L), is a taproot plant that undergoes noticeable organ size modification during plant growth and development. Paclobutrazol (PBZ) inhibits gibberellin biosynthesis and stimulates the proliferation and expansion of plant root cells in growth and developmental stages. Our study aims to examine the effects of PBZ treatment on the storage organ of plant. Four treatments were applied at the pre-cortex splitting stage of "Nau-yh" radish plant. Foliar spray treatments including PBZ (50 mg/L), gibberellic acid (GA_3, 150 mg/L) and a mix of GA_3+PBZ (150+50 mg/L) were applied. The results revealed considerable improvement in taproot growth and decreases development in aerial parts after 48 days of the growth. Paclobutrazol treatment showed enhanced growth parameters, most notably, root weight was 33% higher than GA^3 treatment, 28% higher than the control (H_2O), and 23% higher than the PBZ+GA^3 treatment. PBZ also significantly promoted xylem development in tuberous root of radish. In contrast, the GA_3 treatment inhibited tuberous root growth and enhanced shoot growth in plants. Furthermore, mass spectrometry assay confirmed the absence of gibberellins in paclobutrazol-treated plants, whereas auxin was found in all treatments. Our study provides the first evidence on the applicability of PBZ in root size expansion of radish plant. © 2017 Friends Science Publishers

Keywords: Gibberellic acid; Paclobutrazol; Plant hormone; Radish; Root growth

Introduction

Radish (*Raphanus sativus* L.), is an important vegetable crop grown for fleshy edible roots, cultivated in cold climatic zones of the world. The taproot thickening of radish is being the most critical stage (Yu *et al.*, 2015). The root formation is controlled by several complex interactions between the physiological and environmental factors (Grunewald *et al.*, 2009). The literature reviews that radish cultivation is betters in Asian and African countries as to other continents (Zaki *et al.*, 2010). The optimum temperature for root growth has been reported to be in the range of 14–22°C (Abdel *et al.*, 2016).

The hormone interactions have several mechanisms, which act at both levels of hormone response and biosynthesis in creating a delicate response network (Weiss and Ori, 2007; Hadi *et al.*, 2015). The impact of gibberellins, auxin, cytokinin, and brassinosteroids were considered as essential for plant growth and development process (De Bruyne *et al.*, 2014; Depuydt and Hardtke, 2011). Gibberellins are plant growth hormones which promote cell division and regulate numerous physiological processes including seed germination, stem elongation, leaf, root and reproductive organs expansion (Achard *et al.*, 2009; Schwechheimer and Willige, 2009; Colebrook *et al.*, 2014). Several studies described the inhibition of gibberellin biosynthetic pathway by growth retardants in order control crop production. Growth retardants were employed to reduce the shoot system, thereby lowering the risk of lodging in cereal crops, also used in making ornamental plants more compact with better canopy structure as well as improving the formation of reproductive structures in many other crops. (Liangjiu *et al.*, 1990; Sridharan *et al.*, 2009, 2015; Rademacher, 2016). Expansion in xylem area is essential for growth of radish primary root (Cavusoglu *et al.*, 2009). Xylem expansion is attributed to the formation of procambium cells into vascular tissues, first forming protoxylem, and vascular cambium develops

To cite this paper: Jabir, B.M.O., K.B. Kinuthia, M.A. Faroug, F. NureldinAwad, E.M. Muleke, Z. Ahmadzai and L. Liu, 2017. Effects of gibberellin and gibberellin biosynthesis inhibitor (paclobutrazol) applications on radish (*Raphanus sativus*) taproot expansion and the presence of authentic hormones. *Int. J. Agric. Biol*., 19: 779–786

through secondary growth for formation of secondary xylem (Abdel *et al.*, 2016).

Paclobutrazol, a triazole type inhibitor of GA biosynthesis, and one of the most potent plant growth retardants. Previously, its effects were reported in decreasing the stem length and also was involved in vascular formation (Solé Gil, 2016). It is also utilized in boosting the growth of agronomic and horticultural crops (Wang *et al.*, 1986; Kuai *et al.*, 2015). The Paclobutrazol mode of action is reported by holding up energy transport to mitochondria and preventing the biosynthesis gibberellin, thus, cell elongation will reduce, accompanied with shortened above-ground bone, while contributing in the enhancement of photosynthesis and minerals uptake, leading to major incresae in the final yield of rooty plants (Bai and Chaney, 2001; Nouriyani *et al.*, 2012). Earlier, the positive effects of PBZ treatment for improvement of various rooty plants were reported such as for, carrot and potato, in terms of increasing the yield as well as the overall quality of plants (Wang *et al.*, 2015; Mabvongwe *et al.*, 2016). The main objective of present study was to assess the paclobutrazol amelioratory effects on radish growth.

Materials and Methods

Plant Material and Treatments with Plant Growth Regulators

The Seeds of ('Nau-yh') radish advanced inbred line in this study was obtained from the National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, China. The seed were late bolting cultivar with early mature and relatively tolerant to high temperature. The seeds were sterilized by calcium hypochlorite solution for 30 min before being washed with distilled water. All the Seeds were germinated on moist filter paper in dark room for three days at 25°C (Schopfer and Plachy, 1993). The seedlings germinated after 48 h, and then transferred immediately to trays with commercial media (petmos soil) in greenhouse enviroment (25±2°C/18±2°C day/night). After seven dayes the seedlings were transplanted to plastic pots (size 20×20 cm), containing the mixed media of soil and peat moss (1:1, V/V), in permanent places (greenhouse). The experiment was conducted by using split plot design with the hormone treatment as the main plot and the stages representing the sub-plots. Each treatment in the sub-plot was replicated three times (3 pots per treatment). The temperature was maintained at 35±2/27±2°C day/night. After transplanting, juvenile plants were divided into four groups, each group was treated by spraying an aqueous solution of either GA_3 (150 mg/L) for the first group, PBZ (50 mg/L) for the second group, a combined mixture of GA_3+PBZ (150 mg/L+50 mg/L respectivly) for the third group and water for the control group, the total volume of each treatment was 250 mL per group. The GA_3+PBZ treatments were conducted by sprying GA_3 first, then PBZ was sprayed after 12 h. Treatments were conducted at the pre-cortex splitting stage (10 days after transplanting into plastic pots), in the course of three days' intervals (overall treatment for 9 days). Water was withheld for one day before each treatment, in order to enhance exogenous hormone absorption. The Samples of taproots were collected at three different developmental stages including cortex splitting stage (Stage 1, 24 days), expanding stage (Stage 2, 36 days), and mature stage (Stage 3, 48 days). Harvesting of the whole remaining plants was done after 48 days from the transplanting date. Data and observations were collected and recorded after each stage until the final harvesting time.

Growth and Photosynthesis Net Rate Determination

After harvesting, fresh weight of roots and shoots were weighed using digital balance (Sartorius-BSA124S), root diameter measured using a digital Vernier (MNT-150, CHINA), and the plant height was measured from the first true leaf up to youngest leaf. At the beginning of root expansion, the net photosynthesis rate was measured in fully expanded top leaves during mature stage. Measurements were conducted at 11:00 am on a sunny day using a portable photosynthesis system (LI-COR 6400 XT.), following the manufacturer's instruction. Net photosynthesis rate was measured from youngest leaves, on the main stem of two plants per treatment. During measurements, the greenhouse temperature and an average relative humidity were approximately 35°C and 40%, respectively. Steady-state net photosynthetic rates at light saturation (Amax) and leaf intercellular CO_2 concentration (Ci) were measured in mature leaf taken from the upper part of the canopy. For Amax and Ci determination, light and Co^2 in the chamber were maintained at 1500 μmol/m^2/s photosynthetic active radiation (PAR) and 360 ppm, respectively, while leaf temperature was set at 25°C. After the measurements, leaves were scanned for photosynthesis data.

Paraffin Sections and Microtome Histology

Aleica microtomy (Germany) was used for histological examination (HE) as previously described by Ogawa *et al.* (2003). In order to examine the radish taproot growth in different stages at 24, 36 and 48 days correspondingly and changes in cell structure during radish growth and development were observed. The clean and fresh pieces of the root were taken immediately stored overnight in formalin acetic acid (FAA), at ambient temperature. Slices were dehydrated in a series of increasing concentrations of ethanol (70, 85, 95 and 100% v/v), at 2 h intervals per step, then the specimen hyalinized using "clearing agent" mixtures of xylene and ethanol (1:1/ratio v/v) for 2 h, followed by the use of 100% pure xylene for 10 min. Specimens were then immersed in dissolved paraffin at 58-65°C in an oven followed by immersing in xylene/paraffin mixture (1:1/ ratio v/v for 20 min), twice and finally in 100%

paraffin wax for 30 min under vacuum. Samples were soaked and embedded in Spurr resin. A Leica ultramicrotome-cutting machine was used to cut samples into thin sections (5 µm). Slices were placed on glass slides; and stained with Johansen's SafraninO and Fast Green stains (Bryan, 1955). Five slides for each treatment were prepared and analyzed for morphological observation using Nikon ECLIPSE 80i light microscope equipped with a computer assisted morphometric system (Nikon Corporation, Tokyo, Japan). Cells sizes were analyzed with ImageJ software version 1.50i, available online from the National Institute of Health, http://rsbweb.nih.gov/ijm, (Sibout *et al.*, 2008). All tissue sections were subjected to histological analsysis in the second growth stage (36 days), at 0.04 mm^2 of total area, and analysis included the cell count in xylem, cortex, and vessel. Moreover, the cell diameter was measured in the vessels.

Preparation and Extraction of Endogenous Hormones

Extraction of endogenous hormones was performed as previously described by Pan *et al.* (2010), with slight modifications. briefly, frozen root tissues was crushed in liquid nitrogen, then, 50 milligrams of each sample were transferred into 2 mL Eppendorf tubes, followed by the addition of 500 µL of extraction solvent (2-propanol, distilled H2O and concentrated HCl, 2:1:0.002, vol/vol/vol). Samples were shaken in ZQTX-70 shaker (Zhichu, Yi Qi-Shanghai) at 100 r.p.m for 30 min at 4°C, then 1 mL of dichloromethane was added, and further shacked for 30 min. After which, samples were centrifuged for 5 min (10,000×g, 4°C), then, 900 µL aliquots from the lower phase were transferred into new tubes, and concentrated by evaporation under nitrogen flow. The final concentrated samples were dissolved in 0.1 mL of methanol before injection into HPLC.

Mass Spectrometry Analysis of Plant Hormones

Chromatographic separation of hormones was done on a reversed-phase HPLC (Agilent 1200 series) at a constant flow rate of 0.8 mL/min. The mobile phases used were 0.1% formate in water for solvent A, and 0.1% formate in methanol for solvent B. After injecting a volume of 20 µL sample, an HPLC separation method described by Awad *et al.* (2016) was followed with some modifications. In brief, a linear gradient of 10-30% solvent B was applied from 0 to 2 min, followed by an increase to 100% of solvent B from 2 to 11 min, and held at 100% for another 2 min. Then, solvent B was decreased to 30% over 3 min, and finally, the column was equilibrated under the initial conditions for 2 min. Detection of hormones masses was carried out by ESI-MS mass spectrophotometer (Agilent 6410 triple quad LC/MS).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS software (version 19, SPSS Inc., Chicago, IL, USA). The significance among radish cultivars was assessed at $p<0.05$ and means were separated by Least Significant Difference (LSD) test.

Results

Plant Features and Growth Parameters

The plant height exhibited significant variations among all studied treatments (Fig. 1E). Leaf size and apex part had different colors, while shoots were greenish in color for PBZ-treated plants. A decrease in shoot growth was observed on plants treated with PBZ from the first stage compared to other treatments (Fig. 1), on the contrary, a higher degree of radish taproot expansion was observed in both PBZ and GA_3+PBZ treatments in comparison to GA_3 treatment and control. Analysis of root diameter, shoot and root fresh weights displayed significant differences as shown in Fig. 2. The PBZ-treated plants exhibited higher values of taproot diameter and fresh weight. However, the plants treated with PBZ were noticeably lower in shoot fresh weight when compared to other treatments. Results of root weight ratio to the total plant biomass in radish treated with PBZ was apparently higher (76%) than other treatments including, 23, 42 and 34% for GA_3, GA_3+PBZ and control, respectively (Fig. 3). In contrast, stem weight ratio to the total plant biomass was drastically lower in the PBZ treatment which showed only 3% compared to other treatments which showed 46, 25 and 32% for GA_3, GA_3+PBZ and control, respectively (Fig. 3). Whereas, leaves ratio to the total plant biomass was 21% in the PBZ treatment, which was lower when compared with other treatments, which were 31, 33 and 34% for the GA_3, GA_3+PBZ and control respectively (Fig. 3).

Photosynthesis Activity

Photosynthesis rate values of PBZ-treatment were significantly higher (9.1 µmol. m^{-2}. sec^{-1}) when compared to other treatments, which showed 7.5, 6.8 and 5.7 µmol/m^2/sec for GA_3, GA_3+PBZ and control respectively, however, no significant difference between GA_3 and GA_3+PBZ treatments were observed (Fig. 4).

Histological Analysis

The histological results showed in Table 1 was significantly higher for xylem cell number in with PBZ treatment, recording 267.8±9.1 cells/0.04 mm^2 compared to other treatments, which had cell counts of 219.6±9.6, 169.0±26 and 158.8±8.5 cells/0.04 mm^2 for GA_3+PBZ, control and GA_3 treatments, respectively. Analysis of cortex cell number showed less value of 7.6±1.6 in the PBZ-treated plants, accompanied with relatively larger cell areas (Fig. 5) as compared to other treatments. Contrarily, GA_3-treated

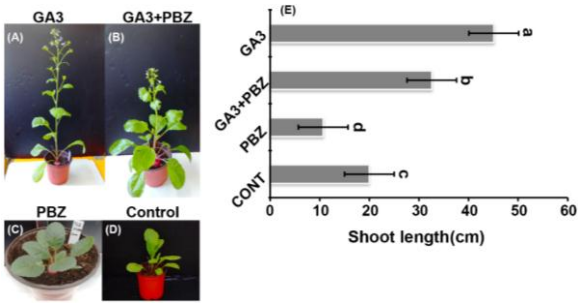

Fig. 1: Radish plant growth effects by response to GA_3/PBZ application

The effects of PBZ and GA_3, on radish growth, the A, B, C and D are represented the PBZ, GA_3, GA_3+PBZ and control respectively for 48 days. (E) is plant length at 48 days; the error bars represent mean ± SD of three independent replicates

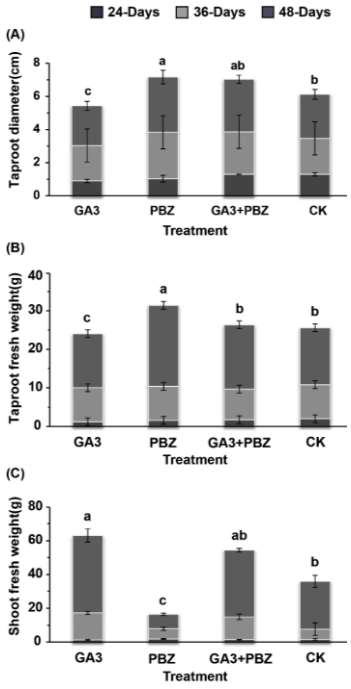

Fig. 2: Comparison between radish root diameters (A), root weight (B) and shoot weight (C) on three different stages (24,36 and 48 days) treated by GA_3/PBZ compared with untreated plants

Description of diameter, fresh and shoot weight during radish growth and development at 24, 36 and 48 days after sowing were harvested. Values are given as mean and SD of three individual biological replicates in each group. Bar values are not sharing a common superscript (a and b) differ significantly at ($p<0.05$), the level of LSD test

plants exhibited relatively larger cell number up to 20.0±1.5 cells/0.04 mm^2 followed by the control. which revealed about 16.2 ± 0.8 cells/0.04 mm^2 (Table 1, Fig. 5). Furthermore, vessel cell diameters in plants treated with PBZ and GA_3+PBZ were observed to be larger (Fig. 6A, B) when compared to GA_3 treatment and control respectively (Fig. 6C, D). Additionally, observations

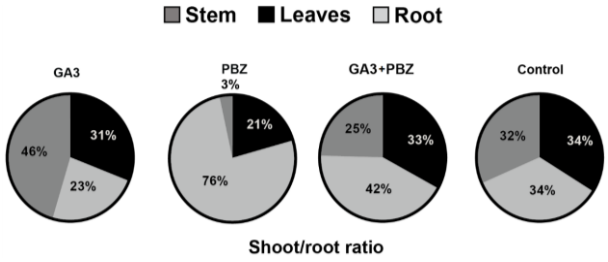

Fig. 3: Comparison between roots and aboveground plant material ratio

Percentage of total biomass allocated to root, leaves and aboveground woody material (shoot + stem) in radish plant treated with paclobutrazol and/or gibberellic acid

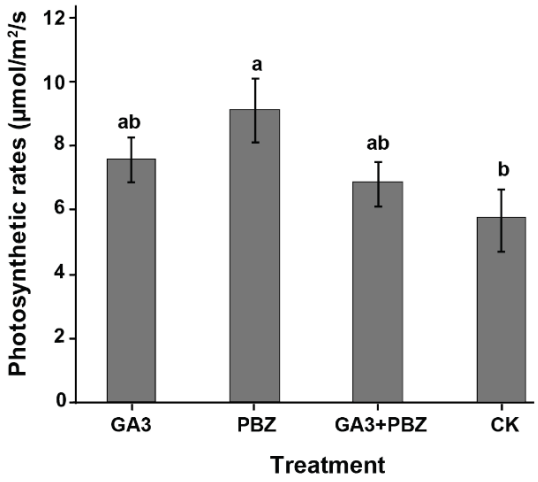

Fig. 4: Photosynthesis net rate affected by PBZ application

Effects of paclobutrazol and gibberellic acid on photosynthetic rate of radish, values are given as mean ± SD of three replicates in each treatment. Bars values are not sharing superscript a and b differ significantly at ($P<0.05$), the level of LSD test

on PBZ-treated plants revealed an apparent enlargement of the secondary xylem area, as well as an increase in starch accumulation (Fig. 7).

Analysis of Hormones in Root and Leaves by LC-MS

Detection of endogenous hormones in radish root tissues from all three treatments and control was carried out by LC-MS, in order to examine the effect of PBZ treatment on the presence of gibberellic acid (GA_3). As presented in figure 8, GA_3 was not detected in the PBZ-treated plant sample, whereas, the GA_3 mass signal was detected in a relatively higher intensity than both of control and GA_3+PBZ treated samples. In addition, auxin (IAA) hormone was monitored in all treatments, due to its direct involvement in inducing plant root growth. However, the mass signal of IAA was detected in all samples at approximately 100 fold the intensity of GA_3, whereas, there were no significant differences regarding signal intensities of IAA except for the control sample which is showed relatively lower m/z value.

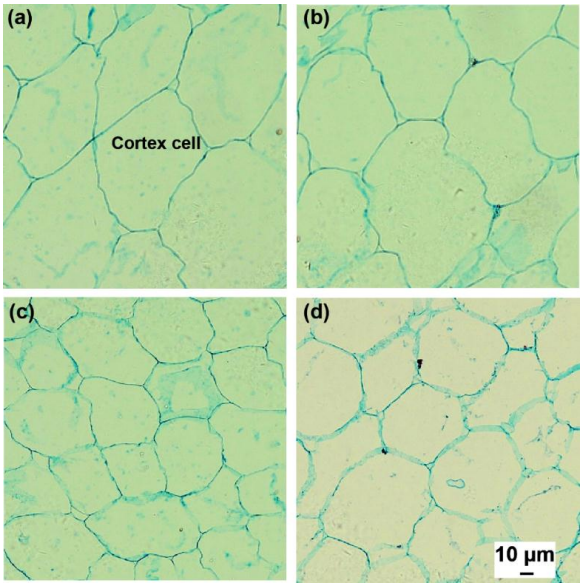

Fig. 5: Effects of GA_3/PBZ on radish taproot cortex cells number and size

Transverse sections of radish root of control, GA_3 and/or paclobutrazol treatments. (a) is paclobutrazol-treated plants had a large root diameter due to the widening of cortex compared with control, GA_3, and GA_3+PBZ (d, c and b) respectively, scale bar 10 µM

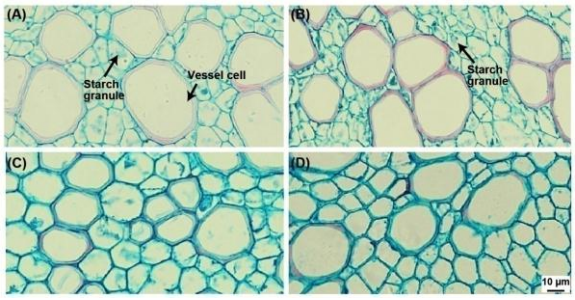

Fig. 6: Transverse sections of radish taproot vessel cell diameter treated by GA^3 and/or paclobutrazol

Effects of GA^3/PBZ on the radish root anatomical structure. A, B, C and D represents the cross sections of roots under PBZ, GA^3 + PBZ, GA^3, and control treatments, respectively. The arrow indicates to starch granule and vessel cells size of GA^3/PBZ-treated plants compared with control. Scale bars (10 µm) in length

Discussion

The effect of PBZ treatment on radish root growth and development was studied, our study analyzed variations among some morphological, physiological and biochemical parameters during growth rates. It is necessary to highlight the well-studied biosynthesis pathway of gibberellins in higher plants in order to understand the concept of PBZ inhibition. GAs formation occurs primarily through the methylerythritol phosphate (MEP) pathway (Kasahara *et al*., 2002), where the hydrocarbon intermediate *ent*-kaurene is synthesized from *trans*-geranylgeranyl diphosphate (GGPP)

Table 1: Gibberellicacid/Paclobutrazol induced cells number of secondary xylem cortex and vessel cells on *R. sativus* L. taproot (in 0.04 mm^2 area)

Treatment	Xylem cell	Cortex cell	Vessel cell
PBZ	267.8 ± 9.1^a	7.6 ± 1.6^d	25.6 ± 2.6^a
GA_3	158.8 ± 8.5^c	20.0 ± 1.5^a	9.0 ± 2.6^c
GA_3 + PBZ	219.6 ± 9.6^b	10.4 ± 1.5^c	22.8 ± 3.1^a
Control	169.0 ± 26.5^c	16.2 ± 0.8^b	17.4 ± 3.9^b

Data are expressed as a mean of five replications ± standard deviation (SD unshared letters in a single column of the same parameter significantly differ at ($p<0.05$), the level of LSD test

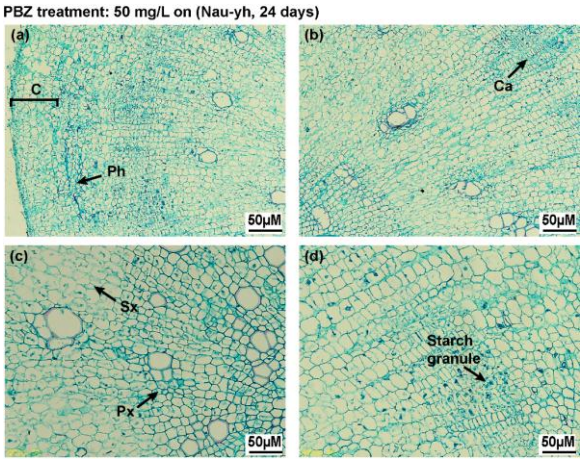

Fig. 7: Cross sections of radish taproot anatomy effected by GA3 and/or paclobutrazol

Effects of pacloputrazol on radish taproot cross sections represent cortex cells (Co), cambium (Ca), phloem (Ph), primary xylem (Px), and secondary xylem (Sx). A and B are wideness of xylem area at 24-DAS and 36-DAS respectively. Scale bar 50 µM. C, D are starch granules contains at 24-DAS, and 3-DAS respectively, scale bar 50 µM

in two sequential enzymatic conversions catalyzed by terpene cyclases. Then, cytochrome P450-dependent mono-oxygenases would be involved in the following steps leading to GA_{12}-aldehyde and further to GA_{12}. The following conversions are taken by the dioxygenases, which use the 2-oxoglutarate as a co-substrate and catalyze the subsequent hydroxylations into different GAs including the GA_3 (Hedden and Thomas, 2012). As presented in Fig. 9, paclobutrazol inhibition of gibberellins occurs through the competitive binding block of *ent*-kaurene oxidation from forming the GA_{12}-aldehyde (Rademacher, 2016). This blockage is achieved, because of the structural similarities between the PBZ and *ent*-kaurene, which is clearly demonstrated by computer-assisted molecular modelling method (Katagi *et al.*, 1987).

In this study, PBZ treatment resulted in a significant decrease in shoot length and shoot weight, which is in agreement with the previous study of (Lolaei *et al.*, 2013). Other studies revealed that, early paclobutrazol application could clearly reduce the stem length in potato, accompanied with a significant increase in the number of tubers per plant,

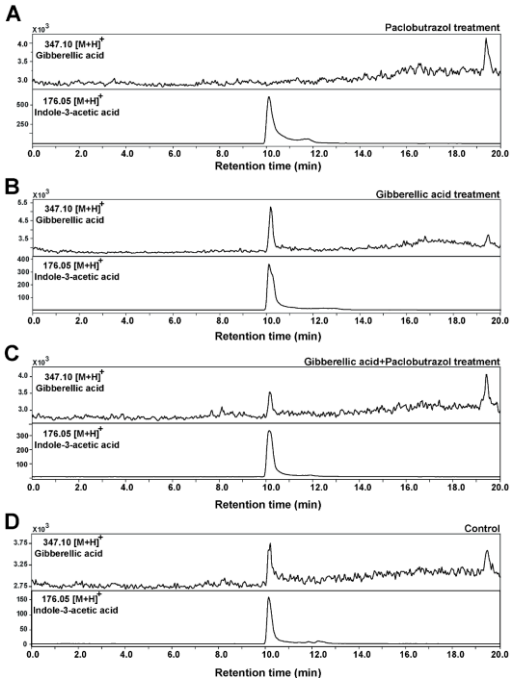

Fig. 8: GA_3 and IAA authentic hormones detected by HPLC-MS Chromatographic separation

A representative chromatogram is showing LC separation and MS detection with ESI mode of gibberellic acid and indole-3-acetic acid in *R. sativus* L. A (Paclobutrazol), B (gibberellic acid), C (Paclobutrazol + gibberellic acid) and D (Control)

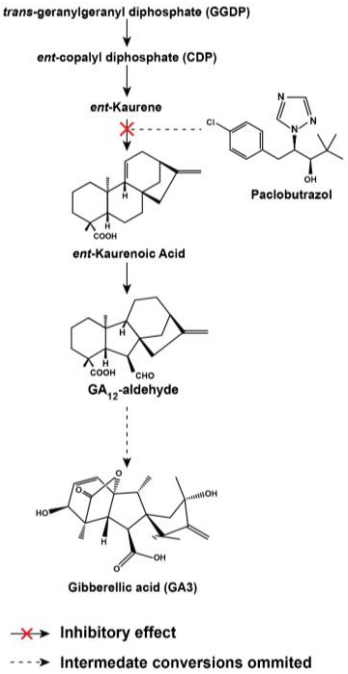

Fig. 9: Gibberellin biosynthesis pathway and the effect of PBZ

The multi-step enzymatic biosynthesis pathway of GA_3 starting from the *trans*-geranylgeranyl diphosphate (GGDP), and the inhibitory role of PBZ

as well as the reduction in plant height and modification in the aboveground structure (Rodriguez-Falcon *et al.*, 2006; Mariana and Hamdani, 2016). However, in this study, we showed that, the application of paclobutrazol has significant influence on increasing taproot weight and diameter, this outcome is supported by the findings of Bidadi *et al.* (2010) in *Arabidopsis*, Wang *et al.* (2015) in carrot and Mariana and Hamdani (2016) in potato. In contrast, our result showed that, GA_3 treatment revealed a clear increase in shoot weight (Fig. 2C) and stem ratio (Fig. 3). It has been reported that the GAs greatly enhance internode elongation and it capable of promoting rapid growth of whole plants in different species (Suge and Rappaport, 1968; Salisbury and Ross, 1992).

The analysis of paclobutrazol effects on the photosynthesis activity of radish displayed significantly higher rate in PBZ-treated plants than other treatments (Fig. 4), this was supported by the phenotypic observations of plants, as PBZ-treated plant showed darker green colored leaves compared to other treatments (Fig. 1), and this is attributed to higher chlorophyll accumulation in leaves. Previous studies in different plants cultivars had shown leaves exhibited higher chlorophyll content when treated with PBZ (Setia *et al.*, 1995; Bañón *et al.*, 2002). A Study on *Catharanthus roseus* plant indicated that, the gibberellin biosynthesis inhibitor is capable of enhancing photosynthesis net assimilation rate and chlorophyll content (Abdul Jaleel *et al.*, 2007).

Cells number and cell sizes are important aspects to evaluate the improvements in crop yield and quality after specific treatments. In this study, the histological analysis revealed an increase in root xylem cell number and vessel cell size with PBZ and GA_3+PBZ treatments compared to control and GA_3 treatments, and these changes are revealed in the last two stages (36 and 48 days). Another indicator was the cortex cells number, in PBZ-treated plants, which was significantly lower compared to the control and GA_3-treated plants; this was accompanied by significant increase in cells size. The variation in xylem, cortex and vessel cells in radish root were associated with changes, which lead to increase the thickness of the radish taproot. In general, root thickening occurred by a combination of cell division and cell enlargement (Bidadi *et al.*, 2010; Guo, *et al.*, 2015; Wang *et al.*, 2015; Mabvongwe *et al.*, 2016).

Furthermore, an increase in starch granules accumulation was observed seen in taproot cross sections of radish seedlings treated with PBZ compared to GA_3 treatment (Tsegaw *et al.*, 2005), these was similarly observed in our study. From the results found in this study, it can be demonstrated that, elevated photosynthesis at maturity may contribute to accumulation of starch granule and subsequently, taproot extension. Early flowering occurred in the GA_3 treated seedlings at 35 days after transplanting, followed by GA_3+PBZ treatment. Whereas,

no flowering initiated in the control and the PBZ treatment up to 48 days (Fig. 1), this observation was similar to the previous studies of Kamuro *et al*. (2001), Mutasa-Gottgens and Hedden (2009), and Abbasi *et al*. (2012), they reported that, GA_3 hormone promoted plants floral bud initiation and flowering in both long and short-day conditions. They also mentioned that, spraying of GA_3 on radish plants results in a significant increase and acceleration of flowering. PBZ-treated plants exhibited better insect attacks resistance, particularly, to butterfly larva, compare to their untreated counterparts; this may be due to effects of paclobutrazol spray on plant latex which makes it unpalatable for insects.

Liquid chromatography-tandem mass spectrometry (LC–MS/MS) detection of plant hormones revealed the absence of GA_3 mass signal in PBZ-treated plants, which indicates the inhibitory effect of PBZ on the plant capability to produce the GA_3. It was reported that, the application of uniconazole, (structural analog of PBZ) could decrease the endogenous GA_3 and IAA (Zhou and Leul, 1999). The auxin mass signal was detected in all samples with no evidence of any inhibitory effect from PBZ, GA_3+PBZ or GA_3 treatments. Auxin signaling was pointed out to have direct role in regulating the GA levels (Hedden and Thomas, 2012) with the up-regulation of GA 20-oxidase (*GA20ox*) and/or GA 3-oxidase (*GA3ox*) however, the presence of auxin in the PBZ-treated plant may indicate that, the inhibition is specifically targeted towards the GA_3 biosynthesis.

Conclusion

Results of this study confirmed the responses of radish 'Nau-yh' cultivar to paclobutrazol in enhancing the taproot growth and reducing the plant height. PBZ treatment results showed a significant increase of the primary roots and a decrease of plant shoots. Additionally the xylem area, vessel cells size and cortex cell number were increased considerably accompanied with higher photosynthesis rate in PBZ-treated plants, while the GA_3 treatment showed an opposite effect. GA_3 was inhibition by the PBZ was proved by LC-MS analysis. This outcome can suggest the application of PBZ as a potential tool for the enhancement of radish growth, thus opens the possibility for increasing its production.

Acknowledgments

This research was supported by grants from the Natural Science Foundation of China (31372064, 31501759, 31601766), NSF of Jiangsu Province (BK20140706), National Key Technology Research and Development Program of China (2016YFD0100204-25), Key Technology R & D Program of Jiangsu Province (BE2016379) and Jiangsu Agricultural Science and Technology Innovation Fund (JASTIF, CX (16) 2012).

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(Received 17 December 2016; Accetped 10 April 2017)