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The application of diniconazole and prohydrojasmon as plant growth regulators to induce growth and tuberization of potato

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Summary

The potato is a major vegetable crop around the world, and tuberous hypertrophy is a highly complex developmental process effected by various factors that play central role in potato growth. Tuber growth under hypertrophic conditions is regulated by variations in carbohydrates and endogenous Phytohormones. In the present study, we aimed to establish the basis for the enlargement of potato tubers, including changes in the chemical content of factors related to tuber formation and hypertrophy based on phytohormonal regulation, plants height and tuber biomass and other selected attributes. Our results reveal that the application of plant growth regulators such as diniconazole (Din) and prohydrojasmon (PDJ) significantly impact on potato plants growth and yield, including that of tubers. Plants treated with Din and PDJ effectively showed stunted growth and reduced development but enhance the tuber formation and its weight. Plants treated with Din and PDJ significantly reduced the GAs and ABA accumulation and increase the sucrose level and cause significant increase in tuber development. In conclusion, a higher gibberellin content in potatoes may inhibit tuberization. Diniconazole and PDJ treatment reduced gibberellin accumulation, which in turn regulated abscisic acid content, demonstrating that the abscisic acid content mostly increased as gibberellin content decreased, and thereby promoting tuberization.

Introduction

The potato plant is one of the main food crops with a yield of more than 300 million tons per annum globally; it is the fourth most important food crop after rice, wheat, and corn in terms of production volume and cultivation area (Obidiegwu et al., 2015). Originating in the central Andean Highlands of South America, different varieties of potato are grown in more than 125 countries, and the potato is consumed by more than one billion people worldwide every day (Obidiegwu et al., 2015). Potatoes are cultivated on approximately 80% of the land area in Asia and Europe. From 2000-2010, potato production was highest in Asia (41.1%) and Europe (40.4%), with production in North America, Latin America, and Africa as well (Maeng et al., 2015). However, regardless of geographic area, adverse environmental conditions will result in a decrease in potato production (Obidiegwu et al., 2015). Diverse conditions related to biotic, abiotic, and anthropogenic factors are decreasing the productivity of potato crops. Among other factors, poor growing practices, lack of high-quality planting materials, low use of inputs, poor control of disease and pests, improper timing of planting and harvesting, rigid traditional local food habits, and poor soil management practices represent major constraints in potato growth.

Diniconazole (Din) is triazole-based growth regulator, and triazole generally inhibits plant height (Eum et al., 2012). Diniconazole inhibits Giberillic acid (GA) biosynthesis (Choi et al., 2011) and is an N-containing heterocycle group that inhibits the oxidative demethylation reaction associated with cytochrome P450, which is involved in the conversion of kaurene into kaurenoic acid in the GA biosynthesis process. Diniconazole is widely used as a plant growth inhibitor in different types of plants, including flowering and fruiting plants (Hwang et al., 2016). It is currently used to inhibit plant growth in Chinese cabbage and grasses (Kim and Lee, 2015).

As with many aspects of plant development, plant hormones have played a primary role in the regulation of potato tuber dormancy. The hormone that controls stolon growth is GA. Generally, GA content increases or decreases in potato plant leaves in response to environmental factors such as photoperiod, temperature, and light intensity. The spray application of GA to potato plant leaves promotes stolon growth but inhibits tuber growth. In contrast, abscisic acid (ABA) (Iuchi et al., 2001) has the opposite effect of GA and is largely involved in the induction and maintenance of the vegetative organs (Sohn et al., 2011). Abscisic acid treatment of potato plants is known to inhibit stolon growth but promote tuber growth (Sohn et al., 2011). Prohydrojasmon (n-prophyl dihydrojasmonate; PDJ) is a jasmonic acid (JA) derivative, which is a plant growth regulator composed of cyclopentanone, valeraldehyde, diethyl malonate, and n-propanol. Prohydrojasmon interacts with GA and has been used to regulate the growth of pear fruit (Association, 2014). Prohydrojasmon has also been used to improve fruit quality in Japan, and it has been reported that PDJ use in apples may stimulate anthocyanin biosynthesis genes to promote fruiting and reduce chilling injury, such as splitting and spotting (Kondo, 2009). In this experiment, Din and PDJ inhibited both plant height and stem length compared with the control group. The results were the same for the Din and PDJ plants. In the first experiment, these treatments showed the greatest suppression effect of PDJ in the shoots.

Jasmonic acid induces and promotes organ maturation and aging and inhibits growth-related processes such as plant growth, seed production, and germination (Choi et al., 2011). Jasmonic acid and JA-related substances showed a strong effect in inducing tuberization. One study focused on the effect of a short-day (SD) treatment of potato leaves on tuberization and the isolation of the principle substances from the leaves. A principle substance evidenced was tuberonic acid, which is a compound with an $^{-}$OH group at the 12th carbon position of JA and a glycoside derivative that that was closely related to tuberization (Koda et al., 1991). Jasmonic acid and JA-related substances have been reported to be involved not only in the tuberization of potatoes but also in that of yams, Jerusalem artichokes, sweet potatoes, onions, and garlic. (Kim et al., 2013). Investigated under incubation the effect of the growth regulators the auxin-based NAA and 2, 4-D on the tuberization of *Gastrodia elata* Blume and observed a healthy tuberization. Another experiment addressed the effect of plant endogenous substances (e.g., ABA, JA, and sugars) on garlic bulb enlargement and secondary growth (Sohn et al., 2011). In most plants, sucrose is important for the transport of plant materials, and starch is a major carbohydrate. Many studies have been

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conducted on the conversion of sucrose to starch and the storage of starch in plant tissue over many years. Potatoes, which are relatively large in size and have a dominant metabolism that converts sucrose into starch, are used as a representative research material for starch metabolism (Geigenberger et al., 2004). As such, in the second phase of the experiment, additional experiments were conducted to compare the effect of the growth inhibition regulators on sucrose and starch content and, in turn, on tuberization.

There has been a steady flow of studies on the formation and tuberization of potatoes. However, studies have rarely been conducted on measures against potato plant aboveground overgrowth and poor potato tuberization and production. Compared to those available for other crops, techniques to improve potato plant tubers, which can be practically used to increase potato yields, are insufficient. In this study, we aimed to investigate the effect of the plant growth regulators Din and PDJ, considered to be superior in improving potato tuberization, by regulating endogenous hormones (GA, ABA, JA). The goal of the study was to improve potato cultivation by establishing a basis for cultivation using the growth regulators Din and PDJ and to understand how these growth regulators contribute to the growth and development of the potato plant and its tubers.

Materials and methods

Experimental conditions and sampling

This experiment was carried out at Kyungpook National University (Daegu, South Korea) using a Su-mi cultivated potato species. Potato tubers were cut into chunks with at least two eyes and weighing between 30-40 g, sterilized, and finally sown in 90 × 35 cm plots for all treatments. The experiment was completely randomized, and for each treatment there were three replicates each of 12 m². At growth stage 3 (25-30 days after sowing when the installation of the tuber started), these plants were exposed to foliar treatment of Diniconazole (Din) and Prohydrojasmon (PDJ) for five and 10 days. The plants were harvested 65-70 days after sowing (30-40 days after treatment). The treatments consisted of [1] control without any treatment, [2] Din 100 mg/l, and [3] PDJ 100 mg/l. The concentrations of Din and PDJ were selected and prepared according to (Jung et al., 2020; Uefune et al., 2014). The foliar treatment was given for 5 and 10 days. After the fifth and tenth days of foliar treatment, plant growth characteristics were investigated by first harvesting the plant and then measuring the characteristics based on three plant parts: shoots, stolon's, and tubers.

Measurement of plant growth characteristics

Plant height and stem length were measured 5 and 10 days after treatment DAT. A plant growth quantitative survey was conducted to determine the effect of the growth regulator on tuber enlargement and yield after harvest. Plant height, stem length, and chlorophyll content were measured at 5 and 10 days after treatment. Measurements were repeated for three random replicates. Chlorophyll content was measured with a CCM-300 chlorophyll content meter (Opti-Sciences, USA).

Extraction and quantification of gibberellin (GA)

The freeze-dried plant samples (0.5 g) were used for the extraction and quantification of GA ions following an established protocol (Lee et al., 1998). First, the sample was converted into a fine powder, a process which was supplemented [$^2H_2$] based on GA internal standards The standards for GAs were 25 ng of [17,17- $^2H_2$] GA_1, GA_{53}, GA_{19}, GA_8, ,GA_{20}, and GA_{44}. A further 25 ng of [17,17-$^2H_2$] GA_{12}, [1,2-$^3H_2$] GA_1, and [1,2-$^3H_2$] GA_4 internal standards were added (all the internal standard were obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia; (Khan et al., 2012). A gas chromatograph (Hewlett-Packard 6890, 5973N mass selective detector) was used to determine the GA. A coupled gas chromatography-mass spectroscopy (GC-MS) method with selected ion monitoring (GC/MS-SIM) was used for the analysis and quantification of the GAs (GA_{12}, GA_9, GA_{24}, and GA_4). The endogenous GA_{12}, GA_9, GA_{24}, and GA_4 contents were calculated from the peak area ratios at 300/302, 298/300, 314/316, and 284/286, respectively.

Extraction and quantification of abscisic acid

The extraction and quantification of endogenous ABA was followed by the standard method of (Qi et al., 1998), with slight modification as per (Shahzad et al., 2017). Briefly, 0.3 g of the freeze-dried plant samples were used for chromatography and extraction by adding an internal standard Me-[2H6] ABA. The resultant extraction was dried by N2 gas, followed by methylation with diazomethane for the quantification of ABA by GC-MS coupled with SIM (5973 Network Mass Selective Detector and 6890 N Network Gas Chromatograph, Agilent Technologies, Santa Clara, CA, United States). The lab-base (ThermoQuset, Manchester, United Kingdom) data software were used to determine the signal ions at *m/z* 162 and 190 for Me-ABA and at *m/z* 166 and 194 for Me-[2H6]-ABA. The experiment was repeated three times.

Extraction and quantification of jasmonic acid (JA)

The endogenous JA content was extracted and quantified according to the protocol of (Bilal et al., 2018; McCloud and Baldwin, 1997). Powdered samples (0.3 g) were extracted in a solution of acetone and 50mM citric acid at the (70:30, v/v) ratio, and [9,10-2H2]-9,10-dihydro-JA (20 ng) was added. The aqueous solution was filtered and extracted three times with 30 mL diethyl ether. The resulting extracts were loaded on a solid-phase extraction cartridge (500 mg of sorbent, aminopropyl). The cartridges were washed using 7.0 mL of trichloromethane and 2-propanol (2:1 v/v). Upon evaporation of the solvents, the samples were methylated and analyzed by GC-MS (6890N network GC system), and a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). The ion fragment was inspected at m/z = 83 AMU, corresponding to the base peaks of JA and [9,10-2H2]-9,10-dihydro-JA. The amount of JA was estimated using the value of endo peak areas compared with the respective standards.

Extraction and quantification of carbohydrates (sucrose)

The sucrose content of the potato plants in their respective treatments was sampled five and 10 days after treatment. The sucrose of the powdered samples (0.1 g) was extracted by shaking at 80 °C for 20 min with 80% methanol. The residue was centrifuged at 12,000 rpm, and the supernatant was added to distilled water and filtered (Whitman 0.45 μm). The solution was analyzed by an HPLC (Alliancee2695, Waters Co.) and the refractive index (RI) was used. Columns were analyzed using a Sugar Pak I (6.5 × 300 mm, Waters, CO.) at a temperature of 90 °C with a mobile phase of 0.01M Ca-EDTA (50 mg/L distilled H2O) and a flow rate of 0.5 mL/min.

Statistical analysis

The experimental treatments were independently performed in triplicate. The values for the means were determined using Duncan's multiple range test (DMRT) with significance at $P < 0.05$. The Statistical Analysis System (SAS 9.1) was used for DMRT analysis. GraphPad Prism software (version 6.0, San Diego, CA, USA) was used to graphically present the results.

Results and discussion

Plant phenotypic variation in response to Din and PDJ treatment

In the current study, we evaluated the possible effects of Din and PDJ on potato plant growth and development. The current results show that at 5 days after treatment (Duwayri and Van Tran), plants treated with Din showed significantly reduced plant height compared with the control and PDJ plants. In contrast, the PDJ plants showed an increase in plant height from the Din and 10 DAT (Fig. 1). Stem length showed the same trend as plant height. Five DAT, stem length was significantly reduced in plants treated with Din, while the PDJ and control plants showed no difference 5 DAT. Ten DAT, the Din and PDJ plants showed significantly decreased stem length compared to control plants (see Fig. 1 and Supplementary Fig. 1). (Bandara et al., 1995) stated that the application of plant growth regulators at the tuber initiation stage can alter photosynthetic partition in favor of tuber production and increase yields. Moreover, the chlorophyll content was significantly enhanced in the plants treated with PDJ compared to control and Din-treated plants, while control and Din plants showed no difference 5 DAT. Ten DAT, the PDJ plants showed enhanced chlorophyll content compared to control and Din plants, while the Din plants had slightly decreased chlorophyll content compared to control plants 10 DAT (Fig. 2). In one experiment, it was found that a plant growth inhibitor decreased plant GA content as well as cell differentiation and expansion, which in turn increased chlorophyll content (Kim and Lee, 2015).

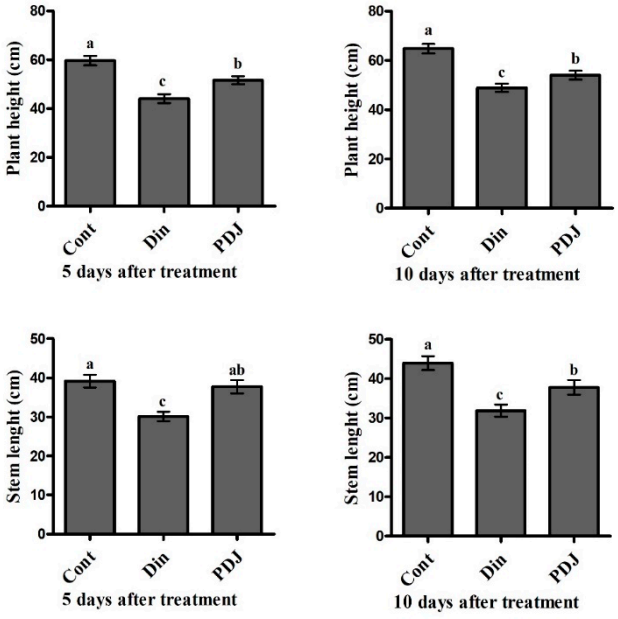

Fig. 1: Effects of different treatments on the inhibition of plant height and stem length per each treatment 60 and 90 days after sowing (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between the mean values were determined using a Duncan's multiple range test (DMRT) at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

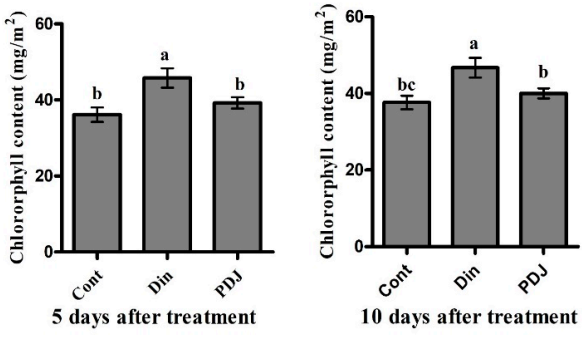

Fig. 2: Chlorophyll content of leaves per each treatment (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between the mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

Diniconazole and prohydrojasmon regulate potato yield

Tubers that have the greatest weight are usually produced by the lowest stolon's production and formed at the beginning of tuber development (Levy and Veilleux, 2007). Tuber weight was found to show significant differences among the treated and control plants. The tuber weight of the Din- and PDJ-treated plants was significantly higher than in the control plants. Furthermore, the Din-treated plants show significantly enhanced tuber weight compared to the PDJ and control plants 5 and 10 DAT (Fig. 3 and Supplementary Fig. 2). The increase in yield may be caused by the reduction of stem length that was as more assimilated were channel to tuber growth (Balamani and Poovaiah, 1985). The increase in yield in plants where Din was applied may also be explained by early tuberization due to low levels of GA. Application of Din and PDJ coinciding with tuber initiation resulted in higher yields compared to the control group with later tuber initiation (Levy and Veilleux, 2007).

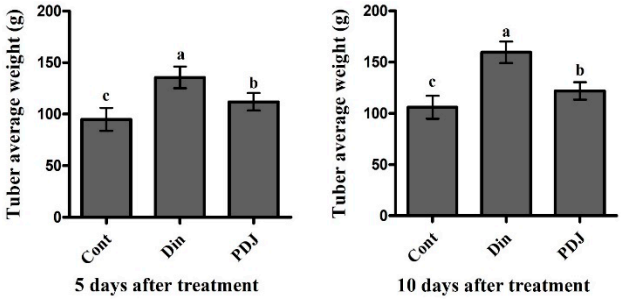

Fig. 3: Effect of each treatment on the average weight of tubers (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between the mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

Gibberellin regulation and effects on tuberization after Diniconazole and prohydrojasmon

A higher GA content is known to inhibit potato tuber formation and maintaining vegetative growth. In this experiment, the GA_{12}, GA_9, GA_{24}, and GA_4 content of the non C-13 hydroxylation pathway (Martinez et al., 2018), which is considered the main GA biosynthesis pathway of tubers, was measured in the shoots. The GA_{12}, GA_9, GA_{24}, and GA_4 contents of the non C-13 hydroxylation pathway NCH of the shoot and tuber parts were measured. At 5 days after treatment, the GA_{12} content in the shoots of the Din- and PDJ-treated plants showed no difference compared to each other; however, the GA_{12} content was significantly decreased in Din-treated plants when compared to control and PDJ plants 10 DAT. Moreover, GA_{12} content was significantly decreased in the tubers of Din-treated plants compared to control and PDJ plants both 5 and 10 DAT. Regarding GA_{24} and GA_9, content in the shoots was significantly decreased in Din-treated plants and slightly decreased in PDJ-treated plants compared to the control plants after 5 and 10 DAT (Fig. 4). In the tubers, GA_9

and GA_{24} content were significantly reduced in Din-treated plants compared to control and PDJ plants 5 and 10 DAT. The inhibitory effects of GA on induction of tuberization has already been demonstrated in earlier studies. In the case of GA_4, content was significantly decreased in Din-treated plants compared to control and PDJ plants 5 and 10 DAT (Fig. 4). The phytohormon involved in the formation and enlargement of tubers is GA; however, when potatoes are cultured in vitro, the synthesis of GA promotes plant growth but inhibits tuber formation. Thus, it has been reported that when potatoes are cultured in vitro, tuber formation can be achieved by inhibiting the synthesis of GA (Park et al., 1992). Therefore, if an increase in GA content is closely related to the inhibition of potato growth, it stands that a reduction in GA content may ultimately have a positive effect on potato tuber enlargement and produce an increase in potato yield. The GA content inhibits tuber formation by restricting the starch accumulation and protein synthesis required for tuber formation (Vreugdenhil and Sergeeva, 1999). In this experiment, the total GA content decreased based on measurement date. These results are consistent with evidence that GA content at the end of the stolon decreases prior to the formation and enlargement of the tuber and suggests that GA content decreases continuously during tuber enlargement.

Effects of diniconazole and prohydrojasmon on Abscisic acid content

Abscisic acid (ABA) is an endogenous hormone known to play a contrasting role to GA in tuberization and is largely involved in the induction and maintenance of vegetative organs. As such, ABA has been reported to promote tuberization. In this experiment, ABA content was measured in plant shoots, stolons, and tubers. 5 DAT, the ABA content in the shoots was significantly increase in plants treated with Din compared with the control and PDJ plants, while the PDJ plants significantly modulated the ABA content of shoots. Ten DAT, the plants treated with Din showed significantly enhanced ABA content compared to PDJ and control plants (Fig. 5). In contrast, plants treated with Din and PDJ showed enhanced ABA content compared to control plants but had slightly decreased ABA in the PDJ-treated plant stolons compared to Din plants. Moreover, ABA content was significantly enhanced in Din-treated plant tubers but slightly decreased in PDJ-treated plants (Fig. 5). ABA (Iuchi et al., 2001) is one of the factors that controls stolon growth. The cessation of stolon growth in potatoes is accompanied by an increase in the ABA to GA ratio (Mokronosov, 1990). The endogenous content of ABA in *andigena* potatoes increased under SD conditions; under these conditions, the ABA/GA ratio increased, and stolon growth was delayed (Macháčková et al., 1998). In addition, ABA content in tubers was significantly enhanced in plants treated with Din compared to PDJ and control plants 5 DAT, while 10 DAT ABA content was significantly increased in both Din and PDJ treated plants compared to control plants but showed non-significant differences to with each other. Overall, ABA content mostly increased as GA content decreased. When potatoes are cultivated in vitro, ABA inhibits the growth of the stolon but promotes the formation of the tuber. Thus, ABA acts as a minor factor in tuberization, stimulating tuberization by inhibiting stolon growth through antagonism of GA (Xu et al., 1998).

Regulation of Jasmonic acid content after Diniconazole and prohydrojasmon treatment

Jasmonic acid has a strong effect on the induction of potato tubers. Potato tuber enlargement begins with cell expansion, which is reported to induce JA (Takahashi et al., 1994). It is also reported that JA and JA-related substances are involved in potato, onion, and garlic tuberization (Koda, 1997). In this experiment, JA content was measured in the shoots, stolons, and tubers of the potato plants. The

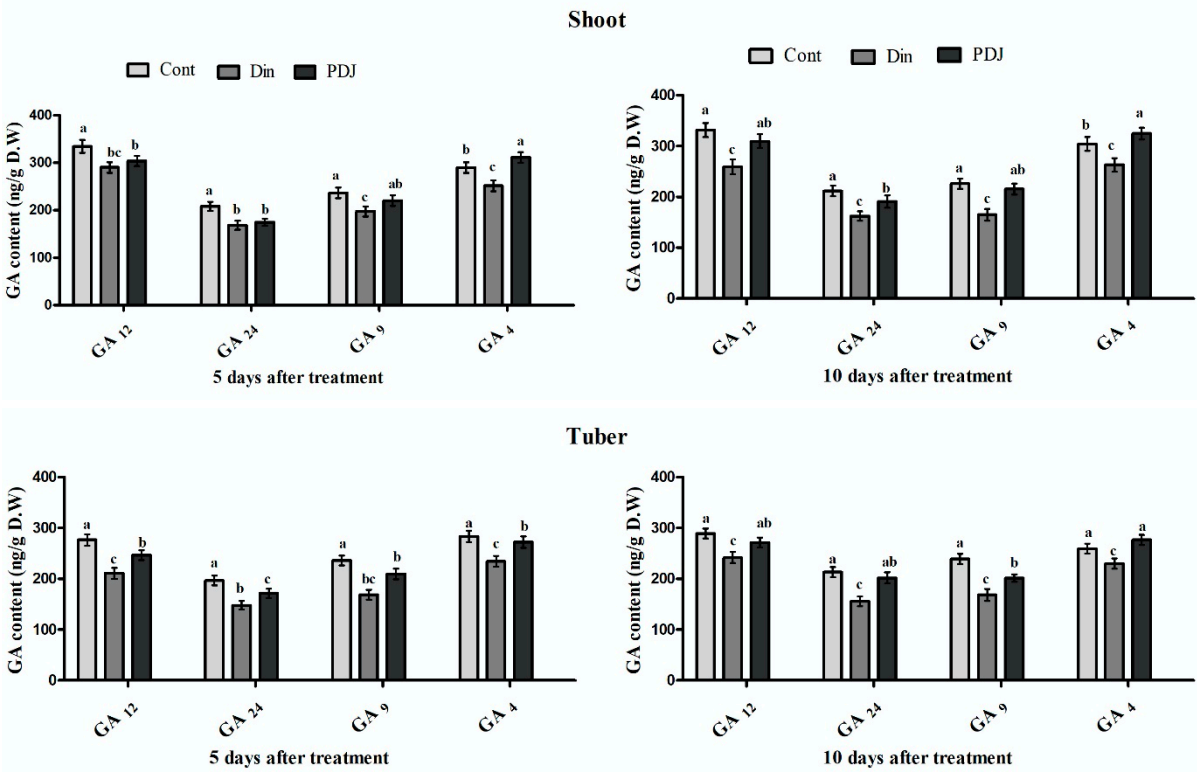

Fig. 4: Effect of treatments on GA content of shoots, stolons, and tubers per each treatment after 5 and 10 days after sowing (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

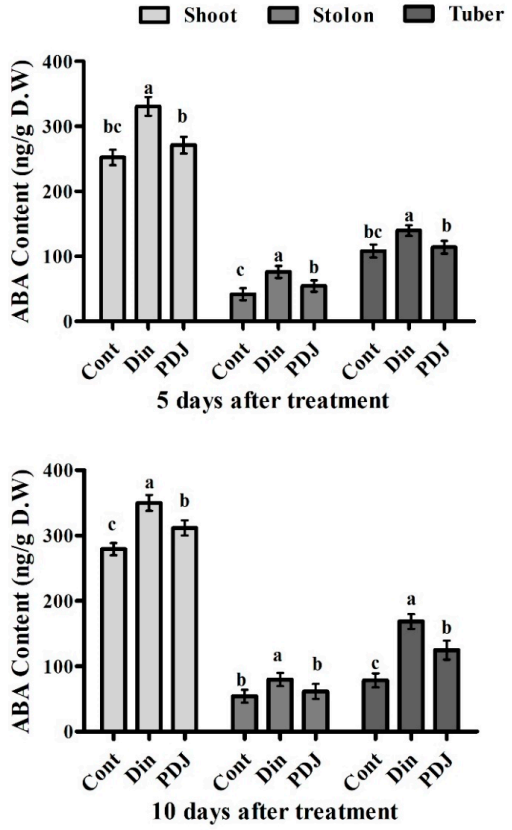

Fig. 5: Abscisic acid contents of shoot per each treatment (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between the mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

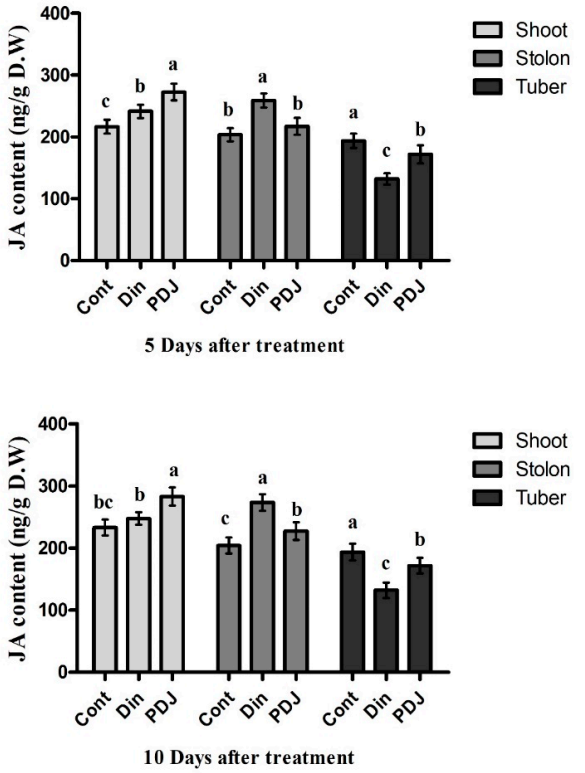

Fig. 6: Total JA contents by site per each treatment (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

results show that JA content in the shoots was significantly increased in plants treated with 5 and 10 DAT compared to Din and control plants, while JA content was slightly reduced in Din plants compared to PDJ plants 5 and 10 DAT. However, JA content in the tubers was significantly decreased in plants treated with Din 5 and 10 DAT (Fig. 6). Jasmonic acid and MeJA were reported to stop stolon growth and induce expansion of potato cells (Sohn et al., 2011). In contrast, JA content in the stolons was significantly enhanced in the plants treated with Din and PDJ compared to control plants, while PDJ plants featured slightly reduced JA levels compared to Din plants five and 10 DAT (Fig. 6). When potatoes are cultured in vitro, the JA and JA derivatives stimulate tuber formation and potato enlargement as the JA content of the stolon increases and tuberization begins. Jasmonic acid and its derivatives cause a spindle-shaped cell division in the opposite direction of the GA in the stolon (Aksenova et al., 2012).

Quantitative measurement of sucrose and its role in tuberization

A high sucrose level is necessary for the induction of tubers, suggesting that sucrose content is important for tuberization when potatoes are cultivated in vitro. In addition, during tuberization, changes in cell division direction, increases in cell number and size, and relocation of carbohydrate metabolism occur simultaneously (Aksenova et al., 2012). In this study, sucrose content was measured in the shoots, stolons, and tubers of the potato plants. The results show that sucrose content in the shoots was more decreased in plants treated with Din compared to control and PDJ plants, while the PDJ slightly increased sucrose content compared to Din 5 and 10 DAT (Fig. 7). It has been hypothesized that carbohydrate levels in the stolon regulate tuberization (Ševčíková et al., 2017). Treatment of plants with Din increases the number of enzymes responsible for starch biosynthesis and therefore increased the starch content of the tuber (Appeldoorn et al., 1997). The application of paclobutrazol increased starch synthesis and accumulation in tubers; in contrast, in the plant stolon, plants treated with Din and PDJ significantly regulated sucrose content compared to control plants and showed non-significant differences 5 and 10 DAT. However, the Din-treated plant significantly enhance sucrose content in the stolon compared to control and PDJ plants. In the tubers, plants treated with Din significantly reduced sucrose content five and 10 DAT, whereas PDJ plants did not show significant differences from control plants (Fig. 7). These findings indicate that an increase in sucrose content acts to decrease GA content; because GA content is the most important regulator in tuber formation, sucrose content has been reported to regulate tuberization because it affects GA content. Sucrose content was decreased in all plant parts in accordance with measurement date. However, this result does not clearly explain the interaction between sucrose and GA in terms of tuberization due to inconsistent findings in previous reports. Further studies are needed to understand contradictions about the role of sucrose in vitro; indeed, the nature of the interaction between sucrose and GA remains controversial (Ševčíková et al., 2017).

Discussion

Potato tuberization is a complex developmental process that relies on interactions between biochemical, genetic, and environmental factors (Kolomiets et al., 2001). It comprises the induction of the growth and initiation of stolons (Sarkar, 2008). Diniconazole is a triazole-based growth regulator, and triazole generally inhibits plant height

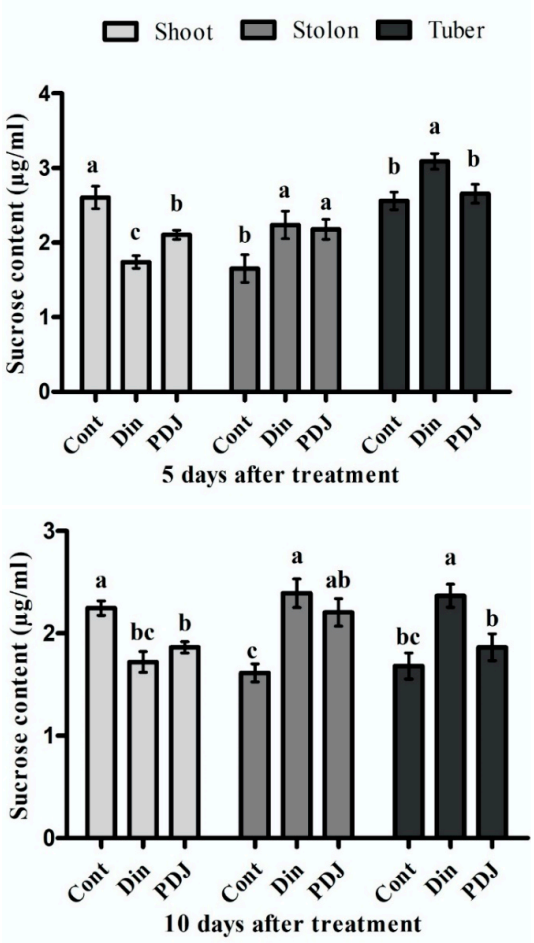

Fig. 7: Sucrose content by site per each treatment (Cont; Control, Din; Diniconazole, PDJ; Prohydrojasmon). The differences between the mean values were determined using a DMRT at $P < 0.05$. The results were graphically presented using GraphPad Prism (version 5.0, San Diego, CA, USA). The SAS 9.1 was used for DMRT analysis.

(Eum et al., 2012). Diniconazole also inhibits GA biosynthesis (Choi et al., 2011). Moreover, Din is an N-containing heterocycle group that inhibits the oxidative demethylation reaction associated with cytochrome P450 and is involved in the conversion of kaurene into kaurenoic acid in the GA biosynthesis process (Kim et al., 2013). It is likely that the application of Din quickly arrests the activity of GA in the plant cells responsible for stem elongation, thereby reducing stem length and increasing tuberization (Davis et al., 1991). Our results show that plants treated with Din showed a significant decrease in plant height, stem length, and chlorophyll content 5 and 10 DAT (Fig. 1). Moreover, PDJ (n-prophyl dihydrojasmonate) is a JA derivative, which is a plant growth regulator composed of cyclopentanone, valeraldehyde, diethyl malonate, and n-propanol. Prohydrojasmon interacts with GA and has been used to regulate the growth of pears (Association, 2014). Our results show that PDJ-treated plants featured increased plant height, stem length, and chlorophyll content (Figs. 1 and 2), revealing that PDJ as a growth regulator increased plants' phenotypical expression. Conversely, Din treatment limited plant height, stem length, and chlorophyll content by inhibiting GA accumulation. Diniconazole reduced stem length and plant height; this may be because of low GA levels following Din application as high levels of GA limit the radial expansion of plant organs (Wenzel et al., 2000).

Tuber initiation on different stolons is not a synchronous process, and tubers may appear earlier or later on different stolons (Vreugdenhil and Struik, 1989). The highest potato yield was observed in Din treatments five and 10 DAT, with an approximately 40% increase in yield for Din-treated plants versus control and PDJ plants (Fig. 3 and Supplementary Fig. 1). The increase in yield may have been caused by the reduction in stem length as more assimilates were channeled to tuber growth, thereby increasing yield (Balamani and Poovaiah, 1985). Increased yield following the application of Din may also be explained by early tuberization due to low GA levels. Application of Din coinciding with tuber initiation resulted in higher yield compared to the PDJ and control plants (Bandara et al., 1998; Levy and Veilleux, 2007). Stolon formation and branching is stimulated by GA3, and potato tuber growth is increased while tuberization is delayed (Ewing, 1995).

Gibberellin enhances stem elongation and stimulates growth, branching, and potato stolon initiation. High GA levels favor stolon initiation (Vreugdenhil and Struik, 1989). The conditions that favor stolon growth are mostly unfavorable for tuber initiation because tuber initiation is related to ceasing stolon growth and cell divisions in its apex. Cessation of stolon elongation is accompanied by a decrease in GA content (Davis et al., 1991). Similarly, the current results show a reduction in GA12, GA24 and GA4 5 and 10 DAT in Din-treated plants, whereas PDJ plants had increased GA content (Fig. 4). This difference may result from Din's inhibitory effects on GA accumulation, which increases potato yield and tuberization by decreasing plant height and stem length. The inhibitory effect of GA on tuberization induction has been demonstrated in earlier studies.

One of the experiments with mutant *gal*, a dwarf potato deficient in hydroxylation of GA_{12} aldehyde at the formation of GA_{53}, confirmed the inhibitory effects of GA on the photoperiodic induction of tuber formation. A decline in the endogenous GA levels permitted the mutant to produce tubers under the long day (LD) (Vreugdenhil and Sergeeva, 1999).

To our knowledge, our study is the first to use GC-MS to quantify endogenous GAs during various stages of stolon elongation and tuber formation under tuber-inducing and non-tuber inducing conditions. GA_1, GA_{20}, GA_4, and GA_9 were detected in small tissue samples. GA_4 and GA_9 concentrations did not change significantly during the development of stolons and tubers, whereas the content of GA_{20} was too low to observe possible changes (Xu et al., 1998).

The dynamic functions of GA forms in potatoes include the complex role in the photoperiodic induction of tuber formation, as (Levy and Veilleux, 2007; Macháčková et al., 1998) reported. Their experiments with the transgenic *andigena* potato lines observed enhanced activity of GA_3 oxidase in catalyzing the conversion of GA_{20} into GA_1, substantial accumulation of GA_1 in shoots, and a decrease in the content of GA_{20} in shoots and stolon's. Such transgenic plants were characterized by accelerating tuber formation under Short day (SD) conditions and increasing tuber yield. In this study, spraying control plant leaves with GA_1 solution resulted in increased rather than reduced tuber yield under SD conditions (Macháčková et al., 1998).

Abscisic acid (ABA), which is considered distinct from GA and which retards plant growth, has a positive impact on tuberization induction. Many reports have shown that ABA content increases under inductive conditions (Ewing, 1995). Potato leaf treatment with ABA stimulates tuber formation and counteracts GA's inhibitory influence (Xu et al., 1998). At the same time, the process of tuber formation is not directly related to ABA accumulation in the leaves, as evident from the *droopy* mutant of the *S. phureja* potato deficient in ABA synthesis Under SD conditions and despite blockage of ABA synthesis, this mutant transits to tuber formation (Parry et al., 1991); this suggests that ABA does not play a direct role in tuberization induction and that its stimulatory effect is due to its antagonism of GA (Chailakhyan et al., 1984; Xu et al., 1998).

In potato tubers, ABA has been shown to be involved in the regulation of tuber dormancy and wound healing (Lulai et al., 2008; Suttle and Hultstrand, 1994). We conclude that ABA is not only

the main regulator of tuber formation, but the effect of the exogenous application of ABA may be due to the antagonistic effects between ABA and GA (Krauss and Marschner, 1982).

As (Koda, 1997) reported, JA and its derivatives JA methyl ester (tuberonic acid [TA]) and glucoside of TA (Sohn et al.) are compounds stimulating tuber formation and growth on potato explants cultivated in vitro. In this experiment, JA content was measured in the shoots, stolons, and tubers of potato plants. The results show that JA content in the shoots was significantly increased by Din and reduced in PDJ plants (Fig. 6). Jasmonic acid is synthesized in the leaves along with the 13C hydroperoxide pathway and is then metabolized into TA and GTA. The accumulation of JA in the potato leaves and aboveground shoots promotes tuber formation under certain conditions (Gao et al., 2005; Kolomiets et al., 2000).

The high sucrose level necessary for the induction of tubers suggests that sucrose content is important in tuberization when potatoes are cultivated in vitro. In addition, during tuberization, changes in cell division direction, increases in cell number and size, and relocation of carbohydrate metabolism occur simultaneously (Aksenova et al., 2012). As a result of all these structural and metabolic changes in the potato, a high sink capacity storage and tuber are formed. The induction of tubers vis-a-vis an increase in sucrose content decreases GA content (Vreugdenhil and Sergeeva, 1999; Xu et al., 1998).

Conclusion

Many Phytohormones and substances are involved in potato tuberization, the most important of which is GA. ABA stimulates tuber enlargement by acting in opposition to GA. Sucrose also stimulates tuberization by affecting GA content. Jasmonic acid and JA-biosynthesis-related enzymes affect tuberization through cell division, and the tuber enlargement effect of JA and JA derivatives was reported to oppose the tuber inhibition effect of GA. In the first phase, the plant growth regulators Din and PDJ, which induced small plant height, high tuber yields, low GA content, and high ABA content, were the most important regulators. Phytohormones and carbohydrates involved in potato tuberization were analyzed by treatment with Din and PDJ. Diniconazole and PDJ, which generally result in plants with low GA content, tend to induce tuber enlargement. Plant height, ABA content, JA content, and sucrose content were different in these plants, and tubers in plants treated with Din and PDJ were enlarged versus the tubers of control plants. Further studies must investigate the relationship between different Phytohormones (e.g. GA, ABA, and JA) and starch and sucrose to identify the effects of plant growth regulators on tuberization.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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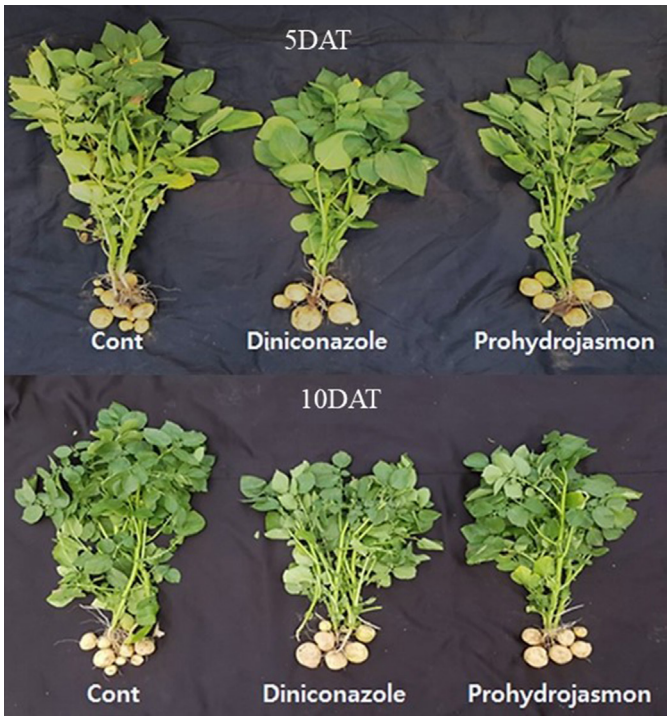
Supplementary material



Supplementary Fig. 1: phenotypical visualization of potato plants height after 5 and 10 days of Din and PDJ treatments.

Supplementary Fig. 2: phenotypical visualization of potato tuber average weight after 5 and 10 days of DIN and PDJ treatments.