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Full Length Research Paper

Exogenous application of plant growth regulators increased the total flavonoid content in *Taraxacum officinale* Wigg

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The effects of plant growth regulators (PGRs) were studied on growth, total flavonoid, gibberellins (GA) and salicylic acid (SA) contents of *Taraxacum officinale* (dandelion), a widely used medicinal plant in Korea. All the four PGRs used; gibberellic acid (GA_3), kinetin (Kn), salicylic acid (SA) and ethephon (2-chloroethylphosphonic acid) were applied at the rates of 0.5 and 1.0 mM. GA_3 markedly enhanced fresh shoot weight, while 0.5 mM of kinetin application significantly enhanced dry root mass as compared to control. SA enhanced both shoot and root attributes, while ethephon decreased plant growth. Endogenous bioactive GA_1 and GA_4 content and SA content enhanced with the application of GA_3, SA and kinetin, but declined with ethephon. The flavonoid content of dandelion significantly increased with SA treatment, but was not altered with the application of other PGRs. The current study demonstrated the favorable effect of GA_3, kinetin and SA on growth, bioactive GAs, SA and flavonoid contents of dandelion. These investigations offered interesting information as PGRs were never tested for plant growth and development of dandelion. It also reports the presence of both early C-13 hydroxylation and non C-13 hydroxylation pathways of GA biosynthesis in dandelion for the first time.

Key words: Plant growth regulators, *Taraxacum officinale*, growth, total flavonoid contents.

INTRODUCTION

Dandelion (*Taraxacum officinale* Wigg.) is known for its medicinal importance for a long time now (Ahmad et al., 2000) and widely used as choleretic, diuretic and anti-carcinogenic. Dandelion was repor-ted to possess guaianolide, desacetylmatricarin, germacranolides, taraxinic acid, β-glucopyranosyl ester and sonchuside A in its roots (Kisiel and Barszcz, 2000). Flavonoids and sesquiterpeniods isolated from the whole plant have been known to carry anti-oxidant and anti-cancerous activity (Hansel et al., 1980; Williams et al., 1996). Flavonoids metabolism in plant plays substantial role in the formation of red and purple anthocyanin pigments. While the non-pigmented flavonoid compounds also play central roles in the biology of plants, serving as signals for pollinators and for other beneficial organisms, participating in plant hormone signaling, facilitating pollen-tube germination, protecting plants from UV-B and functioning as phytoalexins and allelopathic compounds (Taylor and Grotewold, 2005; Cushnie and Lamb, 2005). However, the exogenous application of flavonoids reports plant growth regulation (Yoshiokaa et al., 2004; Peer and Murphy, 2007).

Plant growth regulators especially gibberellins (GAs) are known to promote plant growth, germinate seed and response toward environmental stresses (Hedden and Kamiya, 1997). GAs has also been reported to promote synthesis of flavonoids, as studies had shown that an increase in anthocyanin synthesis by GA_3 promoted levels of flavonoid-specific mRNAs (Weiss et al., 1990). Salicylic acid plays an important role in defense mechanism against plant pathogens. Similarly, kinetin is repor-ted to promote germination, growth development and

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enhances cell division (Allen et al., 2002). The function of SA is somewhat different from GAs and kinetin as they are related to systemic acquired resistance (SAR) signaling in plants (Malamy et al., 1990) and SA indispensability as SAR ingredient was confirmed by using plant over expressing salicylate hydroxylase (Gaffney et al., 1993). However, there are reports that SA signaling affects plant physiology and reproductive development (Raskin, 1995). Contrary to the above mentioned plant growth promoters, ethephon, an ethylene-releasing compound, also can be used to retard stem elongation, promote lateral branching and manipulate flowering date. Ethylene play a role in seed germination, leaf expansion, the initiation and progressing of fruit abscission and ripening and the expression of a number of stress-related responses in plants (Anthony and Schaller, 1996).

The importance of PGRs in plant growth and development is well understood. Efforts have been made to analyze the phytochemical and pharmacological constituents of dandelion but little has been known on the role of PGRs on growth and development of dandelion. Being an important medicinal plant, the effect of PGRs especially GAs, kinetin, salicylic acid and ethephon on growth, flavonoid, bioactive GAs and SA contents of dandelion is yet to be studied and clarified. The main thrust behind the study was to increase the commercial and medicinal values of the plant and also to improve the plant growth by plant management practices through application of phytohormones. Therefore, an effort was made to investigate the influence of PGRs on growth attributes and how the endogenous GA, SA and flavonoid contents of dandelion are affected by them.

MATERIALS AND METHODS

Plant material and PGRs application

Seeds of *Taraxacum officinale* were obtained from Daegu Catholic University, Kyeongsan, South Korea. Seeds were then thoroughly washed with sterilized double distilled water and stored for 60 days at 4°C. Seeds were surface sterilized with 5% sodium hypochlorite for 15 min and sown in pots (62 × 48 cm) under greenhouse conditions at Kyungpook National University, South Korea. 50 ml of each PGRs were applied at the rates of 0.5 and 1.0 mM after 25 days of sowing. Growth attributes were measured after one week of PGRs application. The dry weight was measured after drying the samples at 72°C for 48 h.

Gibberellin analysis

Plant sample was harvested after 7 days of PGRs application and immediately frozen in liquid nitrogen and then stored at -80ºC. The method used for extraction and quantification of endogenous gibberellins was based on the already established procedure of Lee et al. (1998). A 0.5 gram lyophilized sample was used for GA analysis each time. The GC (Hewlett-Packard 6890, 5973N Mass Selective Detector) with HA-1 capillary column (30 × 0.25 mm i.d. 0.25 µm film thickness) oven temperature was programmed for 1 min at 60°C, then a rise of 15°C min^{-1} to 200°C, followed by 5°C min^{-1} to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 ms. Full scan mode (the first trial) and three major ions of the supplemented [$^2H_2$] GAs internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously (standard GAs were purchased from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). The endogenous GA contents of GA_1, GA_4, GA_9 and GA_{20} were calculated from the peak area ratios of 506/508, 284/286, 298/300 and 418/420 respectively. The data was calculated in nano-grams per gram dry weight and the analysis was repeated three times, using different sample each time.

Salicylic acid analysis

The extraction and quantification of endogenous SA was carried out following the procedure of Enyedi et al. (1992) and Seskar et al. (1998). Dry leaf tissues were grinded into powder form and 0.1 g of sample was sequentially extracted with 90 and 100% methanol by centrifuging at 10000 g. The combined methanol extracts was vacuum dried. Dry pellets were re-suspended in 2.5 ml of 5% trichloroacetic acid and the supernatant was partitioned with ethyl acetate, cyclopentane and isopropanol (100:99:1, v/v/v). The top organic layer containing free SA was transferred to a 4 ml vial and dried with nitrogen gas. The dry SA was again suspended in 1 ml of 70% methanol. HPLC condition was maintained at fluorescence detector (Shimdzu RF-10AXL, with excitation 305 nm and emission 365 nm) while separation was done on a C_{18} reverse-phase HPLC column (Waters, Japan). An elution of 0.5% of acetic acid in MeOH and water was done. The gradient solutions were 5 min (A:30%, B:70%), 2.5 min (A:40%, B:60%), 4.5 min (A:60%, B:60%), 5 min (A:30%, B:70%), 3 min (A:30%, B:70%) and the flow rate was 1.0 ml/min.

Total flavonoid assay

The sample was harvested after 7 days of PGRs application and immediately frozen in liquid nitrogen and then stored at minus 80ºC. A 0.5 g of low temperature dried plant sample was grinded and added to 100 ml of methanol. The extract was then added to diethylene glycol and 1 N-NaOH. The Spectrophotometer value was measured in 427 nm. Naringin was used as standard with concentration range of 0 to 1 mg/mL (AOAC 1990).

Statistical analysis

Duncan Multiple Range Test (DMRT) was carried out to determine whether significant ($P < 0.05$) differences occurred between individual treatments. To analyze the data SAS version 9.1 (SPSS Inc) was used.

RESULTS

Growth attributes

All growth parameters were promoted by GAs, kinetin and SA, while ethephon showed an opposite effect on the growth attributes of dandelion. Maximum shoot length and shoot fresh biomass was recorded in GA applied plants. SA treatment enhanced root length and fresh root biomass as compared to other treatments. The growth attributes were promoted favorably by 1.0 mM of PGRs as compared to 0.5 mM concentration, although the

Table 1. Effect of GA_3, Kinetin, SA and Ethephon on length and weight of shoot and root of plant.

Treatment	Concentration (mM)	SL(cm)	RL(cm)	SFW (g/plant)	SDW (g/plant)	RFW (g/plant)	RDW (g/plant)
Control	0	12.3 ± 0.5bc	20.5 ± 0.7a	2.8 ± 0.1d	0.3 ± 0b	1.1 ± 0c	0.1 ± 0ab
GA_3	0.5	20.0 ± 0.8a	18.4 ± 0.6a	4.8 ± 0.2b	0.4 ± 0ab	0.8 ± 0.1cd	0.1 ± 0b
	1.0	21.4 ± 1.2a	19.4 ± 1.1a	5.7 ± 0.3a	0.4 ± 0ab	0.7 ± 0.1d	0.1 ± 0b
Kinetin	0.5	13.1 ± 0.9b	11.4 ± 1.5c	3.5 ± 0.2c	0.4 ± 0ab	1.7 ± 0.1b	0.2 ± 0a
	1.0	13.3 ± 0.4b	14.8 ± 0.2b	3.3 ± 0.3c	0.3 ± 0.1b	1.7 ± 0.2ab	0.1 ± 0a
SA	0.5	13.3 ± 0.5b	19.8 ± 1.9a	3.8 ± 0.5c	0.5 ± 0.1a	2.0 ± 0.2a	0.1 ± 0a
	1.0	12.4 ± 0.3bc	20.7 ± 0.6a	3.4 ± 0.4c	0.3 ± 0b	2.0 ± 0.1a	0.1 ± 0a
Ethephon	0.5	10.5 ± 0.2c	13.4 ± 0.8c	2.0 ± 0.1e	0.2 ± 0c	1.0 ± 0c	0.1 ± 0ab
	1.0	9.6 ± 0.3d	14.7 ± 1.2b	1.8 ± 0.1e	0.2 ± 0c	1.0 ± 0.1c	0.1 ± 0ab

SL: shoot length, RL: root length, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight and RDW: root dry weight. In a column, treatment means having a common letter(s) are not significantly different at the 5% level by DMRT. Values in the table refer to mean ± SD.

resulting increase was insignificant (Table 1). The results revealed that the dry weight parameter was least affected by the application of PGRs.

Endogenous gibberellin and salicylic acid contents

Endogenous bioactive GA_1 content was significantly enhanced by exogenous GA_3, Kinetin (Kn) and SA, while decreased by ethephon application. However, maximum increase in GA_1 content was observed in dandelion plants treated with elevated level (1.0 mM) of GA_3 (Figure 1). Almost similar results were obtained for endogenous GA_4 contents, although endogenous GA_4 level was lower than GA_1. Elevated SA application reduced GA_4 content as compared to basic SA applied treatments (Figure 1). Ethephon significantly decreased the endogenous GA_1 and GA_4 contents.

Likewise bioactive GAs, the endogenous SA content significantly increased with exogenous SA, GA3 and Kn application, while drastically reduced by ethephon. The elevated concentrations of PGRs provided better results than 0.5 mM applied treatments (Figure 2). SA analysis revealed that plants treated with exogenous SA contained much higher amounts of endogenous SA as compared to GA_3 and Kn. The later produced almost similar increase in the level of endogenous SA (Figure 2).

Total flavonoid content

The total flavonoid contents were significantly increased by SA, GA_3 and Kn, while it decreased with ethephon application. Highest flavonoid content (134.64 ug/g) was recorded in dandelion plants treated with basic SA level, while GA_3 and Kn provided an identical increase in dandelion flavonoids (Figure 3).

DISCUSSION

The role and function of PGRs have been substantially studies in different species; however, this is the first ever report on dandelion. In current study, exogenous GA_3, Kn and SA prompted growth attributes, while ethephon suppressed it. Similar observations were recorded earlier as various phytohormones (like GA, Kn and SA) had increased the plant growth and biomass in strawberry, maize and apple and in some cynobacteria as well (Sharma and Singh, 2009; Gunes et al., 2007; Pan et al., 2008). Application of an aqueous solution of SA on shoots of soybean significantly increased growth of the plant (Eraslan et al., 2007). Kinetin and SA may regulate plant growth and development by enhancing GA metabolism of the plants (Mukharjee and Kumar, 2007). Thus current investigations not only confirm previous reports, but also showed that dandelion responded in a similar fashion to PGRs application like other plants. However, ethephon had significantly decreased growth attributes of dandelion which may imputes to the role of ethylene as an antigibberellin (Lieberman, 1979). Similar findings of plant's height reduction by ethephon were reported in daffodils as well (Moe, 1980).

Current investigation demonstrated that both bioactive GA_1 and GA_4 are present in dandelion. We haven't found any report regarding the presence of GA biosynthesis in dandelion. However, presence of GA_1 and GA_4 in dandelion shows that both GA biosynthesis pathways are functional in this plant, as GA_1 is produced through early C-13 hydroxylation pathway and GA_4 through non C-13 hydroxylation pathway (Kim et al., 2007). An increase in endogenous bioactive GA content in response to exogenous GA_3, Kn and SA application suggested the favorable role of these PGRs in GA metabolism. However, a decline in GA content in response to ethephon application may further strengthen the inhibitory role of

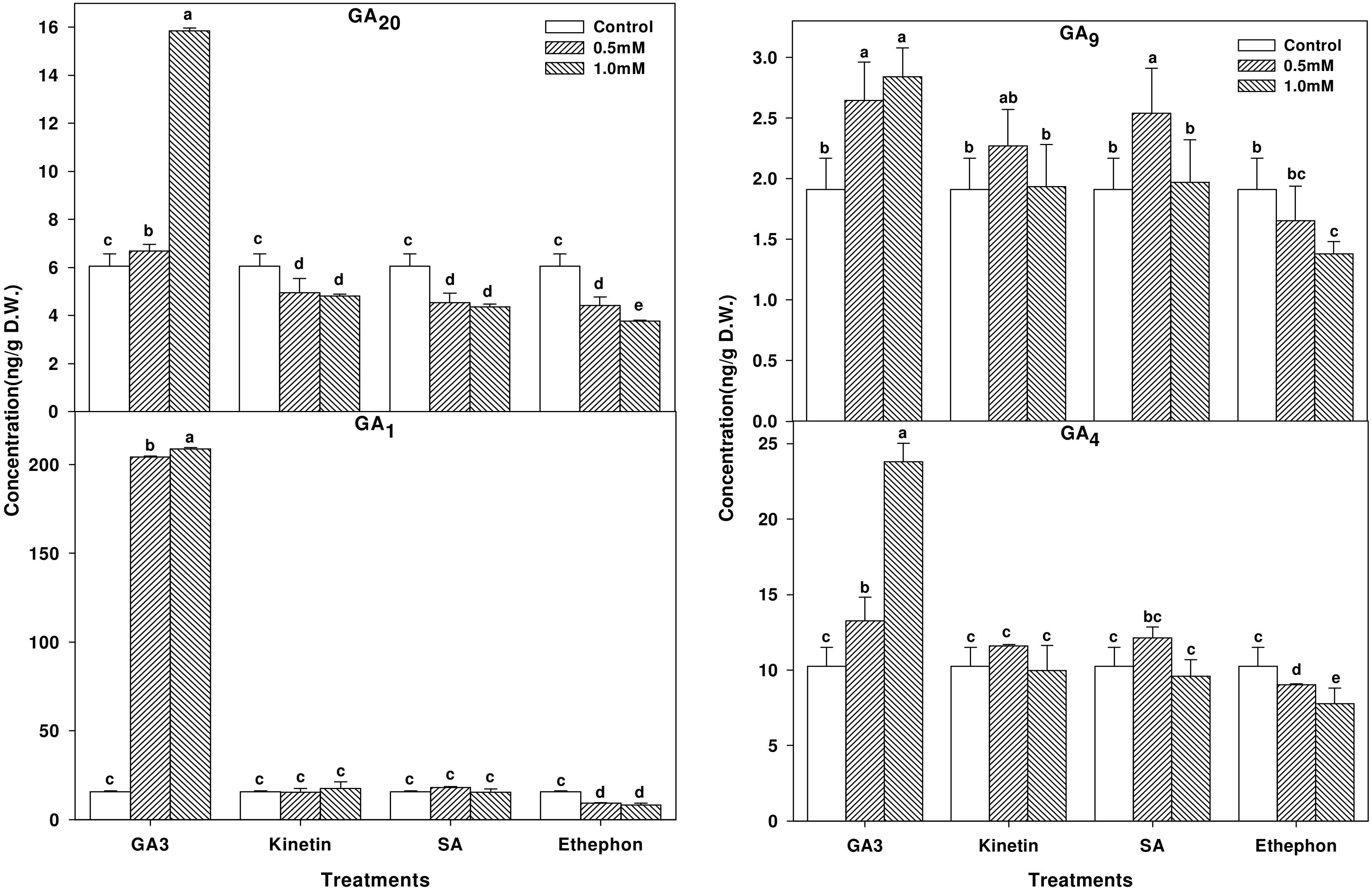

Figure 1. Explains the effects of PGRs on endogenous GA_{20}, GA_1 and GA_9, GA_4 contents of dandelion. The error bars shows the standard error. Treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

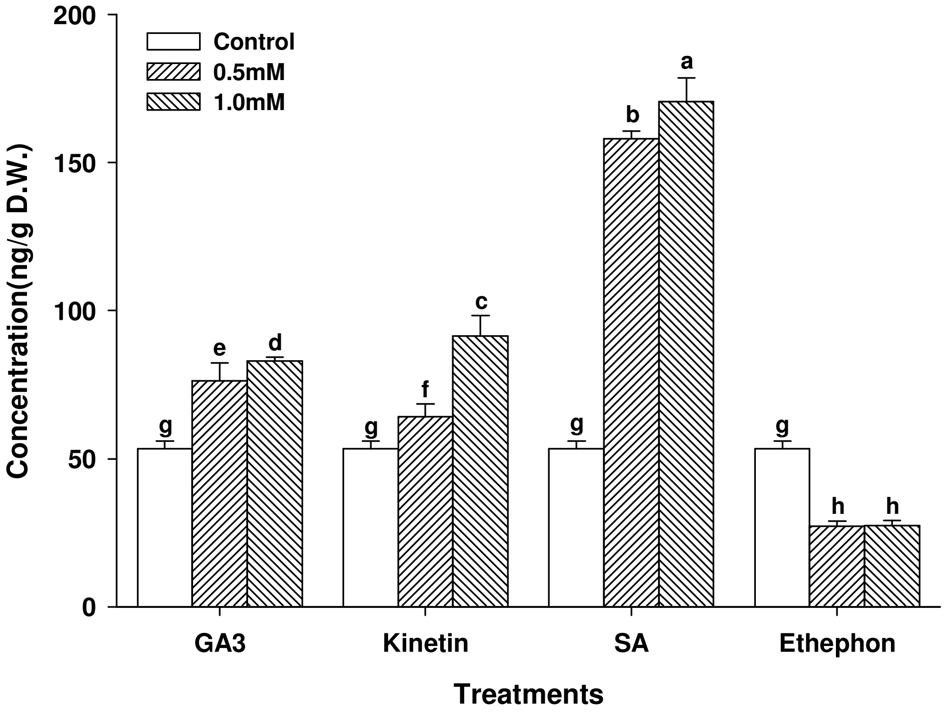

Figure 2. Effect of PGRs on total SA content of dandelion. The error bars shows the standard error. Treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

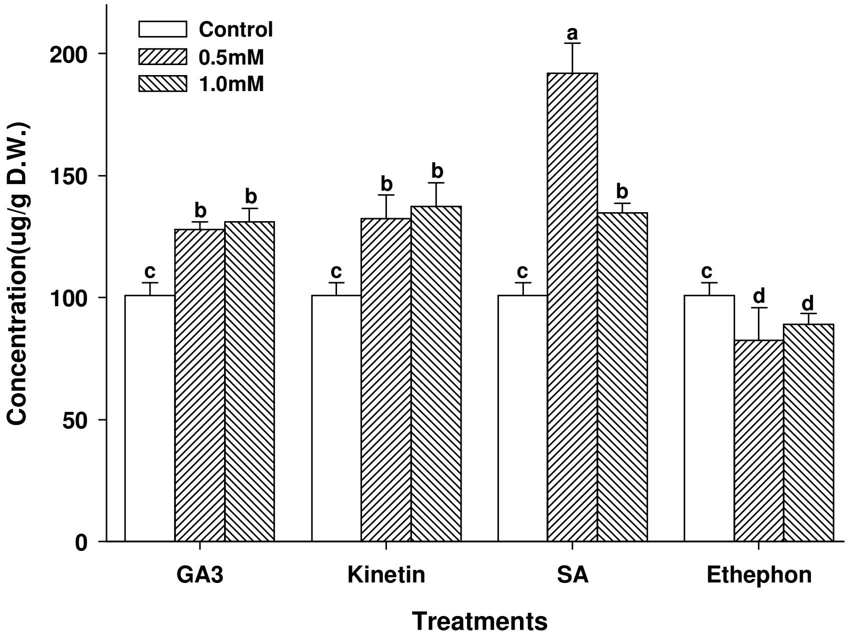

Figure 3. Effect of PGRs on total flavonoid content of dandelion. The error bars shows the standard error. Treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

ethephon during GA metabolism.

The endogenous SA contents significantly increased with exogenous SA, GA_3 and Kn application, while drastically reduced in response to ethephon application. SA may influence a wide range of developmental and physiological processes, including seed germination and fruit yield, transpiration rate, stomatal closure membrane permeability (Barkosky and Einhellig, 1993; Elaleem et al., 2009), growth and photosynthesis (Khodary, 2004). It showed that PGRs especially plant growth promoters aid the plant through enhancing SA metabolism, while plant growth retardants such as ethephon inhibit SA biosynthesis. An increase in endogenous SA contents thus enhances both SAR capacity and growth and development of treated plants. However, the effect of plant growth promoters on endogenous SA content varied and a similar observation was reported in an earlier study (Berhow, 2000).

Flavonoids play a vital role in the physiology of plants by producing the red and purple anthocyanin pigments (Taylor and Grotewold, 2005). The function of flavonoids was study in auxin transport but in case of other PGRs limited information is available. Current study suggests that flavonoid levels of plants were significantly affected by PGRs. SA, Kn and GA_3 significantly promoted these secondary metabolites, which shows the significance of these PGRs in the biosynthesis of flavonoids (Klessig and Malamy, 1994). In addition to their anti-oxidant properties, flavonoids have anti-proliferative, anti-tumor and pro-apoptotic activities and being used as medicinal plant, the relationship of PGRs on total flavonoids contents may be of great economic value.

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