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The role of Gibberellic acid on some physiological responses of transgenic tobacco (*Nicotiana tabacum* L.) plant carrying Ri T-DNA

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Abstract

Transgenic and non transgenic *Nicotiana tabacum* L (cultivar Wisconsin) containing Ri T-DNA were treated with 0, 0.2 and 0.4 mgL^{-1} GA_3 in Murashig and Skoog medium. Some physiological parameters including shoot length, leaf area, number of auxiliary bud, fresh and dry weight, number and length of trichomes were measured. Shoot length, fresh weight and dry weight were increased but number of trichome did not change by GA_3 treatment. Chlorophyll, carotenoid and anthocyanin pigments of leaf were decreased. Auxin and gibberellic acid content of leaf and root were also measured. Exogenous GA_3 increased root auxin in the transgenic plants while it did not change in shoot. GA_3 treatment increased gibberellin content in both of root and shoot.

***Keywords*:** auxin, gibberellic acid, growth parameters, *Nicotiana tabacum*

Introduction

Tissue culture provides a useful system to investigate plant hormone responses and their growth and developmental processes (Zhang et al., 2008). Gibberellins (GAs) constitute a group of tetracyclic diterpenes that best known for their influence on leaf expansion, stem elongation, flower, fruit development and plant morphology (Yamaguchi, 2008; Chauhan et al., 2010). To date, 136 GAs from higher plants (128 species) have been identified (MacMillan, 2002). GA_3 is the first widely available active form of commercial gibberellins which is economically an important secondary metabolite (Martin, 1983).

GA_s promote cell elongation by induction of enzymes involved in cell wall loosening and expansion, such as xyloglucan endotransglycosylase (XET), expansin and pectic methylesterase (PME). Several studies on different plant species have shown that the exogenous application of GA_3 can enhance the productivity of crops affecting the vital physiological process (Rahman et al., 2004; Bora and Sarma, 2006). The vegetative growth characteristics of Gladiolus and *Zantideschia aethiopica* plants were improved as a result of GA_3 treatment (Kirad et al., 2001; Prasad et al., 2002; Brooking and Cohen, 2002). GA_3 increases shoot length by increasing its rate of elongation in majority of plants, including *Brassica campestris* (Pressman and Shaked, 1991). Root length was also observed to increase by GA_3 treatment in *Lupinus albus* (Sidiras and Karsioti, 1996). GA_3 increased dry matter and leaf-area index in mustard plant (Khan, 1996), and photosynthetic rate in leaves of bean (Khan et al., 2002).

Nicotiana tabacum is a model system for tissue culture and plant science investigations (Lang, 1989) as well as useful tool for genetic transformation and expression studies (Bate and Twell, 1998; Holmberg et al., 1997). In this study, transgenic tobacco plants carrying Ri T-DNA containing auxin biosynthesis genes (AUX1 and AUX2 genes) was used (Zamanzadeh and Ehsanpour, 2011). Based on the data released by Zamanzadeh and Ehsanpour, (2011), transgenic tobacco carrying Ri-TDNA has been reported to have short shoot length with compacted auxiliary buds. The reason for this change of growth pattern and morphology of this transgenic plant has not been well understood. It is speculated that might be due to gibberellic acid and auxin interaction. Based on these data, the objectives of the present study was to understand some physiological responses of transgenic tobacco plants after treatment with gibberellic acid (GA3).

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Materials and Methods

In this study *in vitro* propagated of transgenic (T) plants originated from T1 seeds and non transgenic (NT) tobacco plants (cultivar Wisconsin) were grown in MS (Murashige and Skoog, 1962) medium supplemented with 0, 0.2 and 0.4 mgL^{-1} GA_3. After four weeks, their growth parameters including shoot length, leaf area, number of auxiliary buds, fresh and dry weight, number and length of trichomes were measured. Chlorophyll was also extracted from the leaves and measured based on method of Arnon (1949). Carotenoid content was estimated using Kirk and Allen method (1965) and illustrated as milligrams per gram fresh weight. Anthocyanin was extracted and estimated by the method of Laby (2000). The amount of IAA produced in the root and leaves were determined by the method described by Mandal et al. (2007) and Gibberellin contents was extracted and measured by Berríos (2004) method.

Results

Growth parameters

GA_3 treatments in concentrations of 0.2 and 0.4 mgL^{-1} significantly increased the length of shoots in both transgenic and non transgenic tobacco plants when compared to untreated plants, but both concentrations of GA_3 had similar effect on length of shoots. Length of shoots in the transgenic plants was significantly shorter than those of untreated non transgenic plants (table 1)

The fresh and dry weight of the non transgenic plants were increased with GA_3 treatment significantly. The fresh weight in the transgenic plants decreased, while the dry weight did not change.

The number of auxiliary buds was not affected in the non transgenic plants by GA_3 treatment, while it was significantly increased in the transgenic plants. GA_3 treatments significantly decreased the leaf area in both T and NT plants when compared to the untreated plants. Concentration of 0.2 and 0.4 mgL-1 of GA_3 had similar effect on leaf area in the transgenic plants. In non transgenic plants, the minimum value of leaf area was obtained in plant treated with 0.2 mgL^{-1} GA3. The number and the length of leaf trichomes in transgenic plants were significantly higher than those in the non transgenic plants.

Photosynthetic and non photosynthetic pigments

The effects of GA_3 on photosynthetic and non photosynthetic pigments of tobacco leaves are illustrated in table 2. GA_3 treatments with 0.2 and 0.4 mgL^{-1} equally decreased chlorophyll a, b and total chlorophyll in transgenic and non transgenic plants. Carotenoid content in the non transgenic plants increased significantly with increasing of GA_3 concentration, but decreased in the transgenic plants, especially in concentration of 0.2 mgL^{-1} GA_3. While anthocyanin content was not affected by treatment with GA_3 at 0.2 mgL^{-1} but it was significantly decreased at 0.4 mgL^{-1} of GA3 in the NT plants. The anthocyanin content of transgenic plants was decreased with increasing of GA_3 concentration too.

Table 1. Effects of GA_3 on growth parameters of tobacco (NT=non transgenic, T=transgenic). Similar letters represent no significant differences ($P<0.05$).

plant	NT	NT	NT	T	T	T
GA_3 (mg/L)	0	0.2	0.4	0	0.2	0.4
Shoot Length (cm)	28.7 ± 2.51b	75 ± 5.56a	80.7 ± 7.09a	24 ± 1.73c	66.7 ±5.68a	73.4 ± 8.62a
Shoot Fresh weight (g)	4.1 ±0.25a	4.6 ±0.7a	5.76 ±0.703b	3.68 ±0.107b	2.39 ± 0.215bc	2.2 ± 0.58c
Shoot Dry weight (g)	0.16 ± 0.019a	0.24 ±0.009b	0.28 ± .035b	0.16 ± 0.02a	0.13 ± 0.022 a	0.13 ± 0.02a
Auxiliary bud Number	0.67 ± 0.57b	0.67 ± 0.57b	0.67 ± 0.57b	1 ± 0.81b	2 ± 1a	2.67 ± 1.15a
Leaf Area (mm^2/plant)	3996.7± 255.21 a	2603.4 ± 200.4 c	3085 ± 235.16 b	3091.4±259.48 b	1468.4 ± 85.78 d	1455 ± 217.02 d
Trichome Number/mm^2	49.4 ± 5.99b	47.7 ±6.19b	50.8 ±4.25b	154.7 ± 13.29 a	155.7 ± 10.74 a	158.3 ± 7.53 a
Trichome Length (mm)	0.1437± 0.014 b	0.142 ± 0.022 b	0.1433 ± 0.016 b	0.3156 ± 0.023 a	0.309 ± 0.024 a	0.32 ± 0.029 a

Table 2. Effect of GA_3 on photosynthetic and non photosynthetic pigments of tobacco leaves (NT= non transgenic, T= transgenic).
Similar letters represent no significant differences (P<0.05).

Plant	NT	NT	NT	T	T	T
GA_3(mg/L)	0	0.2	0.4	0	0.2	0.4
Chla(mg/g FW)	1.23±0.117	0.99±0.74ab	0.86±0.09b	1.17±0.128a	0.53±0.119c	0.48±0.031c
Chlb(mg/g FW)	0.47±0.366a	0.30±0.067b	0.29±0.029b	0.43±0.053a	0.20±0.008c	0.19±0.001c
Total(mg/g FW)	1.69±0.135a	1.29±0.141b	1.16±0.119b	1.60±0.188a	0.73±0.124c	0.68±0.029c
Carotenoid (mg/g FW)	3.15±0.113d	3.50±0.060c	4.65±0.098a	3.85±0.068b	1.64±0.101f	2.08±0.106e
Anthocyanin (µg/g)	4.05±0.055a	4.14±0.134a	2.55±0.087d	4.20±0.086a	3.81±0.099b	3.33±0.088c

Auxin content

Effect of GA_3 treatments on levels of shoot and root auxin is shown in figure1. Auxin level in shoots was similar in both untreated transgenic and non transgenic plants. However, auxin level in shoots was not affected by GA_3. Data recorded on auxin level of roots revealed that GA_3 enhanced the auxin content in both plants. Auxin content of root in the transgenic plants was significantly higher than that of non transgenic plants.

Gibberellin content

Gibberellin content of roots and shoots in NT plants were significantly higher than those of transgenic plants. GA_3 at 0.2 and 0.4 mg/L showed similar effect by increasing of gibberellin content in shoots of the transgenic plant. GA_3 treatments increased the gibberellin content of the roots in both transgenic and non transgenic plants, in particular at 0.4 mg/L^{-1} concentration (figure 2).

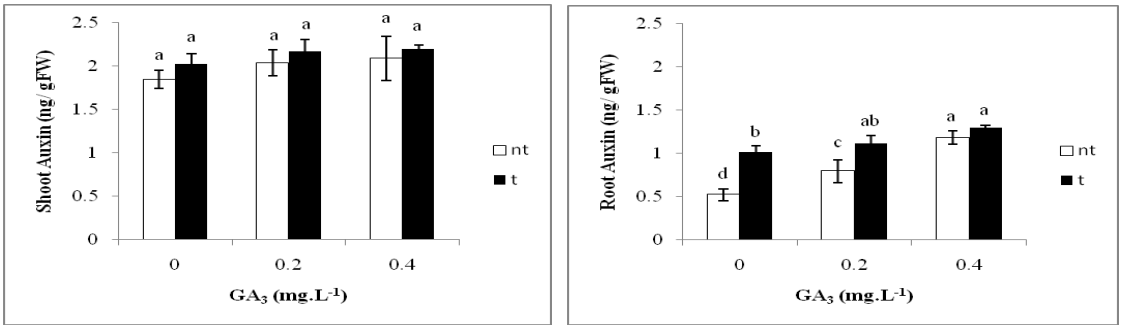

Figure 1. Effect of GA_3 on auxin content of root and shoot in tobacco plants (nt= non transgenic, t= transgenic). Similar letters represent no significant differences (P<0.05).

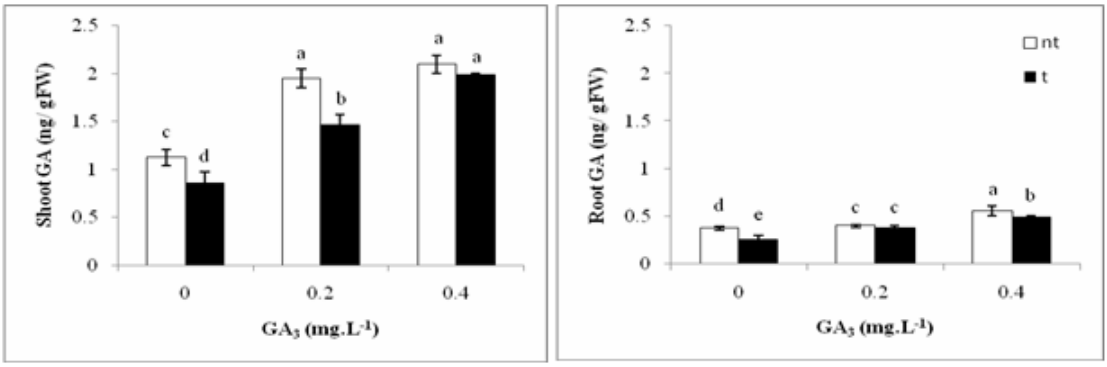

Figure 2. Effect of GA_3 on Gibberellin content of root and shoot in tobacco plant (nt= non transgenic, t= transgenic). Similar letters represent no significant differences (P<0.05).

Discussion

GA is a phytohormone which affects plant morphology as well as its physiological responses (Chauhan et al., 2010). In this study two different concentrations of GA_3 (0.2 and 0.4 mg/L) showed a dramatic effect on plant length. The plant growth consists of two steps, cell divisions and subsequent cell elongation. GA_3 has been reported to increase cell wall extensibility leading to elongation (Rahman et al., 2004). It also activates cell division in the intercalary meristem, assisting in the change of rosette plants in long stem (Iqbal et al., 2011). The increase in plant height due to GA_3 application might be due to its effect on elongation of internodes. Hully and Phillips (1995) suggested that GA_3 can increase the cell number and size by a subsequence affect on plant growth. Application of gibberellin could also promote shoot elongation. Similar observation was also reported by Xu et al. (1997). Freedborg et al. (2001) reported that exogenous application of GA_3 leads to elongation of shoots. This data supports our finding in the transgenic and non transgenic plants after treatment with gibberellin.

Plant growth regulators are chemicals with influence on plant growth when they are applied in very little quantities. It is known that the developmental processes in plants are regulated by the action and balance of the different group of growth regulators, which may act as activators or inhibitors of the metabolic processes (Ortuno et al., 1999). The increasing of fresh and dry weight in non transgenic plants may be due to an increase of protein and carbohydrate contents are reported by others (Gehan et al., 2011). Similar response was observed in our experiments when tobacco plants were exposed to GA3. Decreasing of fresh weight in the transgenic plants can be explained by some internal hormonal disequilibrium. The action of these substances depends on environmental conditions and plant characteristics and genetic potential (Vieira and Almeida, 2010).

Zhang et al. (2008) showed that gibberellin and its signaling pathway inhibit shoot bud regeneration of *Arabidopsis*. Bora and Sarma (2006) reported that in pea, GA_3 up to 250 µg/mL, was highly stimulatory on number of branches per plant, and its effect declined at higher concentrations. Based on present data, GA_3 application enhanced the auxiliary buds only in the transgenic plants. This finding might be linked to the genetic potential of the transgenic plants that is different from that in the non transgenic tobacco plants. Decreasing the leaf area in both transgenic and non transgenic plants, tested with GA_3 compared with the untreated plants, can be due to the fact that GA_3 stimulates the growth by increasing cell size and division (Jupe et al., 1988).

In our experiments when GA3 was applied to the culture medium, the number of trichomes did not change either in transgenic or non trangenic plants. This was against the report indicating that, in GA-deficient mutant (ga1-3), GA-response mutant (spy-5), and uniconazol treated tobacco plants (a GA-biosynthesis inhibitor), the trichome numbers were reduced (XueYing et al., 2007). The difference might be due to the difference between plant genotypes or experimental conditions, such as concentration of GA3 used (Bekheta et al., 2008). Application of GA_3 resulted in a decrease in the chlorophyll content in both plants in the present study. Similar results were observed in pea. It was suggested that the increase in cell volume, caused by GA_3, was not correlated with an increase in synthesis of chlorophyll content. It might however be due to dilution of the chlorophyll content in the leaves (Bora and Sarma, 2006).

Exogenous GA_3 has a negative feedback regulation effect on gibberellin biosynthesis pathway (Hedden and Phillips, 2000). Exogenous GA_3 treatment might cause the geranylgeranyl pyrophosphate precursor to enter into carotenoid synthesis pathway and increase the carotenoid content in the non transgenic tobacco plants. The result of carotenoid content of non transgenic plant is supported by findings of Munjal and Guswami (1995), the fact that when Paclobutrazol (a GA-biosynthesis inhibitor) was applied to potato the carotenoids in the leaf were increased (Tekalign et al., 2005). Decreasing of carotenoid content in the transgenic plants in the present study, shows that different responses of these plants to GA_3 might be due to existence of Ri T-DNA in these plants.

Anthocyanins are secondary metabolites, which play an important role in the physiology of plants. GAs, jasmonate and ABA, but not 2,4-D, ethylene and cytokinins, may interact or crosstalk with sucrose to form a complex web of overlapping signaling pathways that coordinate anthocyanin accumulation.

Yang et al. (1996), suggested that both auxin and GA, are indispensable factors for normal stem elongation in intact peas. GA and IAA appear to have different roles in cooperatively promoting the stem growth, with GA largely conferring increased elongation potential, principally by stimulating the cell division, and with auxin leading to the promotion of cell elongation. Our results revealed that GA_3 induced of auxin biosynthesis in the roots of both T and TN tobacco plants. Also, Law (1987)

showed that in *Pisum sativum* L. the process of elongation happened in the presence of indole-3-acetic acid and its precursors, except for L-tryptophan, which required the addition of gibberellin, for induction of growth. It is proposed that gibberellin increases the biosynthesis of indole-3-acetic acid by regulating the conversion of L-tryptophan to D-tryptophan, which is then converted to the auxin. Furthermore, Li et al. (2003) indicated that GA_3, during flower-bud induction, significantly inhibited the activities of PAL, PPO, POD and IAA-oxidase, delaying the biosynthesis of lignin and raising the level of IAA in leaves of current shoots. Treatment with 0.2 and 0.4 mgL^{-1} of GA_3 could not change significantly the IAA content of tobacco shoots, because the effects of this hormone on plants vary depending on the plant organ. More production of auxin in roots of the transgenic plants might be a response of auxin biosynthesis in the transgenic plants (Zamanzadeh and Ehsanpour, 2011).

GA_3 treatment increased the gibberellin content in roots and shoots in the both tested plants. When the grapevine was treated with GAs, a substantial increase in the GA content in the apical bud and tendril was observed (Yao et al., 2010). Exogenous application of GA_3 on *Paris polyphylla* was also reported (Li et al., 2010).

In conclusion, our data revealed that GA_3 treatment resulted in morphological change of the transgenic and non transgenic tobacco plants and alter the pigment and hormone contents. These changes under the influence of growth regulators might be due to activation of mechanisms related to the GA and IAA action as a consequence of Ri T-DND transformation.

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