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EXOGENOUS APPLICATION OF INDOLE ACETIC ACID (IAA) AND GIBERRELIC ACID (GA_3) INDUCES CHANGES IN CARBON AND NITROGEN METABOLISMS THAT AFFECT TOBACCO (*NICOTIANA TABACUM* L.) PRODUCTION

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Abstract

Experiments, both pot and field trial, were conducted to examine the effects of exogenously applied IAA and GA_3 (at 0, 10, 20 and 30 days after topping) individually or in combination, on growth and C and N metabolisms of tobacco (*Nicotiana tabacum* L.). Application of IAA or GA_3, at low concentrations had a promising effect in terms of promoting the yield and quality of tobacco. The key enzymes' activities were also enhanced by low PGRs concentrations, which is known to be involved in the C and N metabolisms. The combined treatment of the two PGRs was more effective than the PGRs applied individually in improving the activities of nitrate reductase (NR), invertase (INV) and amylase (AMY) finally resulting in improved soluble sugars, reducing sugars, starch, total C and N, soluble proteins and nicotine content. The optimum levels for improvement of C and N metabolites were found to be GA_3 at 50 mg/L and IAA at 30 mg/L.

Key word: Yield; Proportion of high quality tobacco; Carbon and nitrogen metabolism.

Introduction

Tobacco (*Nicotiana tabacum* L.), with its leaves representing the primary product, is one of the most important crops of the world (Peedin *et al*., 2011). To achieve maximum leaf production and boost leaf ripening, an appropriate cultivation strategy must be adopted for flue-cured tobacco (Li *et al*., 2016). It is now widely known that topping (young leaves and flowering head removal) can shift the tobacco plant from reproductive to vegetative phase (Guo *et al*., 2011). Topping has a role in signal transduction involved in hormonal homeostasis, such as to uncover the synthesis of indole acetic acid (IAA) and also to increase the content of jasmonic acid (JA) as well as nicotine synthesis (Li *et al*., 2007). Several studies have been conducted to explore a series of physiological and molecular responses of topping, such as modification of hormonal stress tolerance, and sink-source relationships (Fei *et al*., 2016).

Indole acetic acid (IAA) occurs ubiquitously in all plants (Delker *et al*., 2008). IAA is actively involved in cell division, growth and differentiation (Vanneste *et al*., 2009), pollen development (Xu *et al*., 2008), leaf formation (Ke *et al*., 2000), and embryogenesis (Al-Khassawneh *et al*., 2006). For example, Khaled *et al*., (2015) showed that IAA played an important role in altering the physiological phenomena and enhancing the growth and production of vegetable crops. Analogous to IAA, gibberellins (GA_S) also play a critical role in a myriad of physiological processes involved in growth and development such as seed germination, elongation of stem and hypocotyls, development of all plant parts (Swain *et al*., 2005; Al-Khassawneh *et al*.,2006). Of a variety of GAs, GA_3 is the most widely used compound for achieving enhanced crop productivity (Bose *et al*., 2013). It is believed that crop production could be increased through exogenous application of IAA or GA_3 (Cai *et al*., 2014).

Carbon (C) and nitrogen (N) metabolisms have been intensively studied by many researchers as they are the key processes involved in growth and development of plants (Krapp *et al*., 2005). Carbon metabolism mainly includes the transformation of inorganic carbon into organic carbon in chloroplasts through photosynthesis, the transport of phosphotriose into the cytoplasm through the chloroplast membrane to finally transform into monosaccharides (Schlüter *et al*., 2012). Nitrogen metabolism includes the reduction and assimilation of inorganic nitrogen, the conversion and synthesis of organic nitrogen compounds and other metabolic processes (Szal *et al*., 2012). Interactions between CO_2 and NO_3 assimilation are crucial for growth and development and hence crop productivity (Lawlor, 2002; Cho *et al*., 2015). However, relative distribution of these bio-molecules in various sink organs is regulated by different plant hormones to a large extent (Krapp *et al*., 2005), e.g., polyamines (Chatterjee *et al*., 1988) and abscisic acid, gibberellins, IAA and zeatin riboside (Xie *et al*., 2003).

In the current study, our principal objective was to test whether exogenous application of GA_3 or IAA individually or in combination positively influenced the improvement of tobacco yield. At the same time, the relationship between foliar spray with GA_3 or IAA and the regulation of C and N metabolism, especially those related to tobacco quality.

Materials and Methods

Pot experiment: A pot experiment was set up in a glasshouse at the Northwest A&F University (Yangling, P.R. China). The soil used for the experiment was taken from a farmland in Yangling area, Shaanxi province. Detailed soil characteristics and cropping practices at the site are described in Table 1. Pots measuring 45×80 cm

(each) were filled with soil, each containing 35 kg clay soil. Before transplantation, nitrogen (urea) in the soils determined was 8.5 g , phosphorus (P_2O_5) 5.84 g and potassium as K_2O 6.53 g. Tobacco seedlings were transplanted on April 29 and topping was done on July 15, After topping, the tobacco seedlings were divided into nine treatment groups and then foliar applied corresponding concentrations of IAA and GA_3 on leaves (15~20 from the bottom to up) were: (control) deionized water; (T1) 30 mg L^{-1} IAA; (T2) 60 mg L^{-1} IAA; (T3) 50 mg L^{-1} GA_3; (T4) 100 mg L^{-1} GA_3; (T5) 50 mg L^{-1} GA_3 and 30 mg L^{-1} IAA; (T6) 50 mg L^{-1} GA_3 and 60 mg L^{-1} IAA; (T7) 100 mg L^{-1} GA_3 and 30 mg L^{-1} IAA; (T8) 100 mg L^{-1} GA_3 and 60 mg L^{-1} IAA. The treatment solutions were sprayed three times during the experiment. The spray was done at 0, 7, and 14 d after topping (before 9 am.and after 4 pm. every day). The experimental had a completed randomized design with three replications. Seedling samples were collected at 0, 10, 20 and 30 d after topping, soon wrapped in an aluminum foil, and placed in liquid nitrogen. Then the samples were transferred to sealed plastic bags and stored at −80ºC to measure the relevant index.

Determination of carbon metabolites: The oven-dried leaf samples (1.0 g each) were triturated for 30 min using 80% ethanol (v/v) at 80ºC. The extract was subjected to centrifugation for 10 min at 12000 *rpm*. The supernatant was reacted with activated charcoal, and evaporated to dryness using a vacuum evaporator. The dried sample was re-dissolved in distilled water, and used for the measurement of soluble sugars using the anthrone method. The reducing sugars were determined using the method by Gao (2003). The starch content was measured from the ethanol-insoluble residue using the anthrone-H_2SO_4 protocol (Gao, 2003). For measuring total C content through the combustion method described by Yan *et al*., (2015) was employed.

Determination of nitrogen metabolites: Soluble proteins were determined from the frozen leaf samples (each 0.2 g) by homogenizing them in 2 mL of 50 mM cold sodium phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP). The residue was subjected to centrifugation for 20 min at 12000×*g*. The total soluble proteins in the supernatant were quantified using the Bradford G-250 reagent (Bradford 1976). The method described by Mazzoncini *et al*., (2011) was employed for appraising total N content. Nicotine content was quantified using the method following Wang *et al*., (2003).

Enzyme extraction and assays: Leaf samples (each 0.2 g) were triturated well in chilled distilled water, and kept for 3 h at 4ºC. The residue was subjected for 20 min to centrifugation at 12,000 × *g* at 4ºC. Amylase activity (AMY) was assessed following Joel & Bhimba (2012). Invertase activity (INV) was appraised following Cuadrado *et al*., (2001). Nitrate reductase activity (NR) was measured following Liu *et al.*, (2014). Leaf samples (0.2 g each sample) were homogenized in 25 mM phosphate buffered saline (pH 8.7) containing 10 mM cysteine and 1 mM EDTA in a chilled pestle and mortar. The residue was subjected to centrifugation for 15 min at 12,000 × *g* at 4 ºC, and the supernatant was assayed for NR activity. To the enzyme extract 100 mM KNO_3 and 2% (w/v) NADH were added and the mixture was incubated at 25 ºC for 30 min. A control without NADH was also processed concurrently. The reaction was terminated by adding 1% (w/v) sulfanilamide (prepared in 3M HCl) and 0.02% (w/v) of *N*-(1-naphthyl) ethylene diamine dihydrochloride. After color reflection for 15 min, the sample mixture was centrifuged for 5 min at 12000 *g*, and the absorbance was read at 540 nm.

Field experiment: The field experiment was carried out in Luonan (34º 03' N, 110º 06' W) situated in northwest China. The region received 750 mm of rainfall annually. The soil physio-chemical characteristics and cropping practices of the site are shown in Table 1. The size of every plot was 4.6 m × 7.0 m (32.2 cm^2) with 4 rows. Line spacing of tobacco plants was 1.15 m and 0.53 m, respectively. Leave 20 leaves after the topping. The treatment solutions were sprayed three times during the whole experiment life. The spray was done at 0 d, 7 d, 14 d after topping before 9 am.and after 4 pm every day. The spray was applied to the upper leaves (15 to 20 leaves from the bottom up).The upper, middle and lower leaves of tobacco were harvested and weighed after flue-cured. The economic traits including yield and proportion of upper-quality tobacco were calculated.

Statistical analyses: All data were recorded and graphs were prepared using the Excel 2003 (Microsoft, USA), and results are presented as mean±standard error. Statistical analyses were performed with the SPSS version 20 software.

Table 1. Detailed soil characteristics and cropping practices of field and pot experiments.

Soil and crop information	Field experiment	Pot experiment
Soil pH	6.78	7.56
Organic matter (g/kg)	15.17	8.74
Available N (mg/kg)	42.36	43.67
Available P (mg/kg)	26.84	10.41
Available K (mg/kg)	135.06	79.7
Transplanting date	29-April	3-May
Toping date	30-June	15-July
Harvesting/Sampling date	13-Oct	15-July and 14-August
Cultivar	Yunyan 99	Yunyan 99

Table 2. Effect of exogenous IAA and GA_3 on yield and proportion of high quality of tobacco in field experiments.

Treatment	Yield (kg /hm^2)	Proportion of high quality tobacco (%)
CK	2370.15 ± 58.25b	44.25 ± 0.61bc
T1	2396.7 ± 56.77b	43.81 ± 0.45c
T2	2504.85 ± 58.22ab	44.31 ± 0.64bc
T3	2500.8 ± 60.75ab	44.78 ± 0.83abc
T4	2403.15 ± 57.26ab	43.11 ± 0.612c
T5	2584.65 ± 58.76a	46.78 ± 0.55a
T6	2547.6 ± 58.26ab	46.12 ± 0.52ab
T7	2520.45 ± 55.89ab	46.23 ± 0.84ab
T8	2407.95 ± 55.37ab	39.82 ± 0.50d

Data are means of three biological replicates. Statistical difference (p<0.05) between the treatments are indicated by different lowercase letters

Results

Estimation of optimal treatment for maximum economic trait: To examine whether GA_3 or IAA could improve production of tobacco, we investigated yield and proportion of high quality tobacco in the mature period. Spraying with either GA_3 or IAA could improve the yield and the proportion of high quality tobacco (Table 2). The yield increased with T2 or T3 were 5.68% and 5.51%, respectively, and the proportions of high quality tobacco at T2 and T3 were 0.14% and 2.19% compared to control, respectively. Of the four treatments (interaction with GA_3 and IAA), The optimal treatment for attaining highest yield and proportion of high quality tobacco was found to be T5 at which a significant increase in yield by 9.05% and quality by 5.71% respectively was observed. No marked difference was observed between the other treatments and control in yield and quality of tobacco.

Carbon metabolites: To explore the possibility of enhancing productivity of tobacco by elevating the C metabolism capacity, we monitored the changes in C metabolism.

Sugars and starch are the main carbon compounds in leaves. No marked difference was observed in C metabolites at 0 day after topping. However, reducing sugars, starch and total carbon content at 30 days after topping were generally higher than those recorded at 0 day. However, soluble sugars generally showed a decline with time after spraying (Fig. 1). As shown in Fig. 1A, there was a significant increase in reducing sugars at T5 at 10d, 20d and 30d after topping, which had been 38.64%, 69.55% and 50.66% relative to CK, respectively. As shown in Fig. 1B, there was a significant increase in soluble sugars at T3, T5, and T6 at the 10d after topping. At 20d after topping, no significant change was found in the soluble sugars at T4 and T8. The optimal value of soluble sugars was recorded in T5. As shown in Fig. 1C, all spraying treatments significantly increased the starch content of tobacco particularly at 10d and 20d after topping. Low concentration of single application of IAA or GA_3 (T1 or T3) had better effect on the starch content than did the high concentration (T4 or T5). As shown in Fig. 1D, there was significant difference between T5 and CK in total C content at 10d, 20d and 30d after topping, which was found to be increased by 13.25%, 32.74% and 40.67% with respect to CK, respectively.

N metabolites: N metabolites include various N-containing compounds, including soluble proteins, total N, and nicotine. It can not only serve as a key product of N metabolism of tobacco, but also as the key process involved in smoking and flavor of tobacco. So, foliar application of IAA or GA_3 (T1 to T4) caused a remarkable effect on soluble protein content compared with CK (Fig. 2A). Treatment of single application of IAA had a better effect on the degradation of nicotine, however, T5 still showed the best effect of all treatments used (Fig. 2B). As shown in Fig. 2C, single application of IAA or GA3 was not so effective in altering the total N content at 30d after topping. However, the optimal effect was recorded in T5, because it caused a significant increase of 8.06% and 19.57 relative to the CK at 20d and 30d, respectively.

Amylase (AMY) and Invertase (INV) activities: The AMY activity showed first an increasing trend and then a decreasing one reaching the maximum value on the 20d after topping (Fig. 3A). Application of low concentrations of IAA or GA_3 markedly improved the AMY activity, however, T1 and T3 increased AMY activity up to 46.02% and 13.64% relative to CK. Combined treatment of foliar spray with IAA and GA_3 caused maximal activity of AMY even after topping. At T5 the AMY activity recorded was 1.38, 1.31 and 1.49 times that of the CK. Invertase (INV) is an enzyme that hydrolyses sucrose into hexose monomers (Cuadrado *et al*., 2001; Roitsch & Gonzalez, 2004). In our study, the INV activity gradually decreased from 0 to 10 days after topping, then slowly increased from 10 to 20 days, and remained stable from 20 to 30 days (Fig. 3B). Combined spray of IAA and GA3, increased the INV activity substantially after topping. Especially, at T5 the increase in INV activity was 10.35, 1.86 and 1.37 times that of the CK.

Nitrate reductase (NR) activity: NR activity gradually decreased over time (Fig. 3C). Single application of IAA or GA_3 treatment (T1, T2, T3, T4) had no significant effect on NR activity measured after 10 d of topping. However, NR activity at T5 was 1.8 and 2.56 times that of the CK at 20 and 30d after topping, while other treatments showed no significant difference compared with the CK.

Discussion

The optimal treatment for maximum yield and quality of tobacco: Plant growth regulators could involve in the improvement of plant growth and yield (Hadi *et al*., 2010). They can optimize the plant morphology and biomass, and improve crop quality by regulating a variety of metabolic activities (Tassi *et al*., 2008; Zeng *et al*., 2012). For instance, Ren *et al*., (2016) reported increased accumulation of photosynthetic products, increased grain filling rate, and improved ear character, thereby leading to increased grain yield of summer maize by spraying exogenous ethephon and diethyl aminoethyl hexanoate (DA-6).

Many studies have been earlier conducted on the interaction of different hormones, such as that of ABA and GAs (Chiang *et al*., 2015), of ABA, GAS and Eth (Liang *et al*., 2013), and of SA and ABA (Hisano *et al*., 2015). IAA is known to play a vital role in higher plants as a plant

growth promoter and in signal transduction. In addition, it plays an effective role in stress tolerance (Kai *et al*., 2007; Agami *et al*., 2013). In addition, GA_3 is believed to be involved in improving crop productivity under normal or stressful regimes (Bose *et al*., 2013), because it is linked with several growth and development processes (Swain *et al*., 2005; Al-Khassawneh, 2006). However, there is a little information available whether IAA and GA_3 in combination are involved in the improvement of productivity, especially in tobacco. In our study, although both IAA and GA_3 applied individually improved the productivity and quality of tobacco, their combination proved to be more effective in productivity and quality improvement. The optimum level of both hormones applied in combination was T5 (GA_3 50 + IAA 30 mg/L).

C and N metabolism processes respond differentially to IAA and GA_3: The hormone-induced increase in crop production is believed to be linked to a coordinated relationship between the source and sink. So we conducted a critical analysis of the C and N metabolites and key enzyme activities involved in C and N metabolism under each treatment. It is also known that metabolism of C and N in plants is intensively affected by stressful enviroments (Myers *et al*., 2007).

Nitrogen assimilation has been under intensive study by the researchers for a long span of time because of its considerable role in plant growth and development (Davenport *et al*., 2015). Nitrate reductase (NR) plays key role in in nitrate assimilation (Zhao *et al*., 2013). Our data show that NR activity under T5 (GA_3 50 + IAA 30) was significantly higher than that under CK at 20 and 30d after topping. Nicotine, a key alkaloid of tobacco, accounting nearly 0.6%-3% of the leaf dry weight (Shoji *et al*., 2010). In our study, single application of IAA had a marked effect on the degradation of nicotine, however, T5 still showed the best results compared with the other treatments applied. Biomass production and grain yield of crops were directly resulted by the assimilation of nitrogen (Zhang *et al*., 2017). Foliar-applied IAA or GA_3 after topping increased the total nitrogen content in the upper leaves of tobacco. However, a prominent synergistic effect of IAA and GA_3 applied in combination was observed on total N content. Also, catabolism of organic matter was found to be increased by the application of IAA and GA_3 to give rise carbohydrates and proteins. For example, GA_3 or IAA applied separately significantly increased the soluble protein content in tobacco, but the combination of GA_3 and IAA was found to be more effective in the degradation of soluble proteins. However, the conversion of free amino acids during protein degradation may have a positive effect on the quality of tobacco.

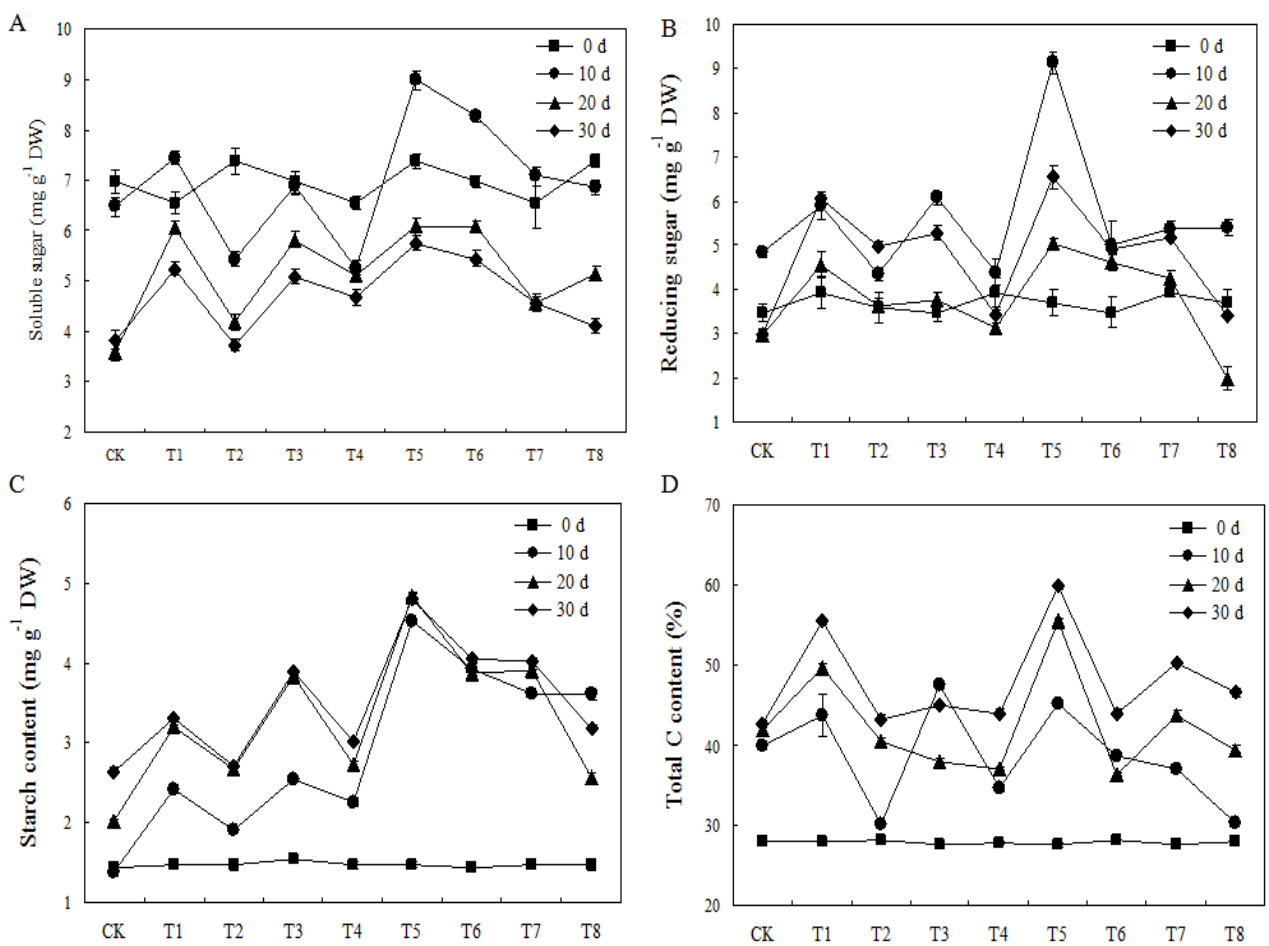

Fig. 1. Effect of exogenous IAA and GA_3 on C metabolism of tobacco in pot experiment. (A) Soluble sugar, (B) Reducing sugars, (C) Starch content, (D) Total C content. CK (control, deionised water); T1 (30 mg L^{-1} IAA); T2 (60 mg L^{-1} IAA); T3 (50 mg L^{-1} GA_3); T4 (100 mg L^{-1} GA_3); T5 (50 mg L^{-1} GA_3 and 30 mg L^{-1} IAA); T6 (50 mg L^{-1} GA_3 and 60 mg L^{-1} IAA); T7 (100 mg L^{-1} GA_3 and 30 mg L^{-1} IAA); T8 (100 mg L^{-1} GA_3 and 60 mg^{-1} IAA). Data are means±SD of three replicates.

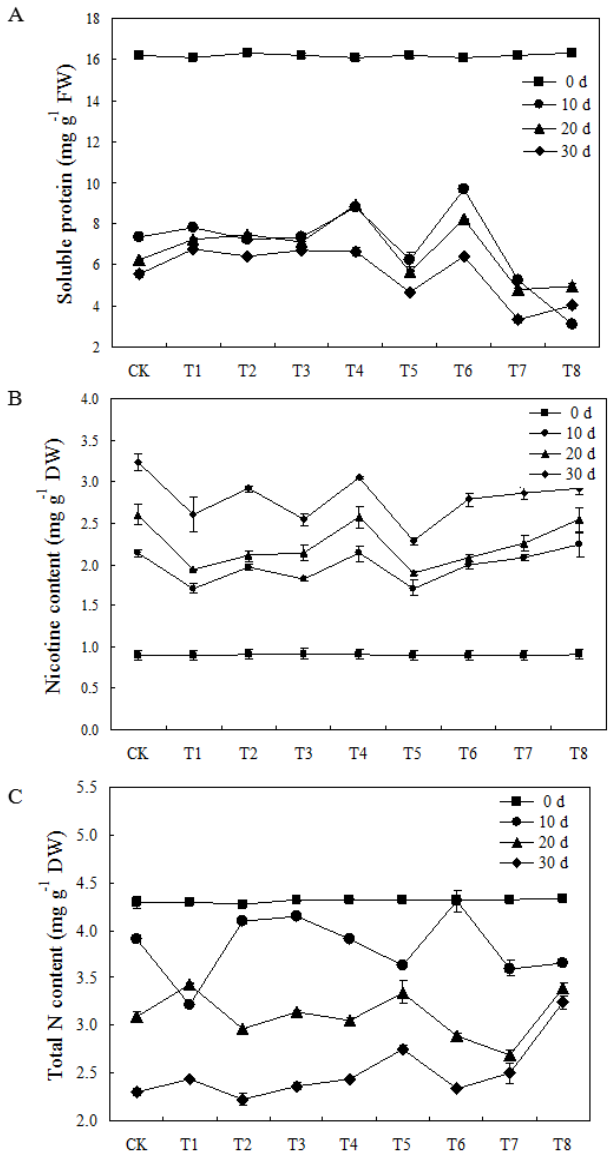

Fig. 2. Effect of exogenous IAA and GA_3 on N metabolism of tobacco. (A) Soluble proteins, (B) Nicotine, (C) Total N content. The same should be followed in all following figures.

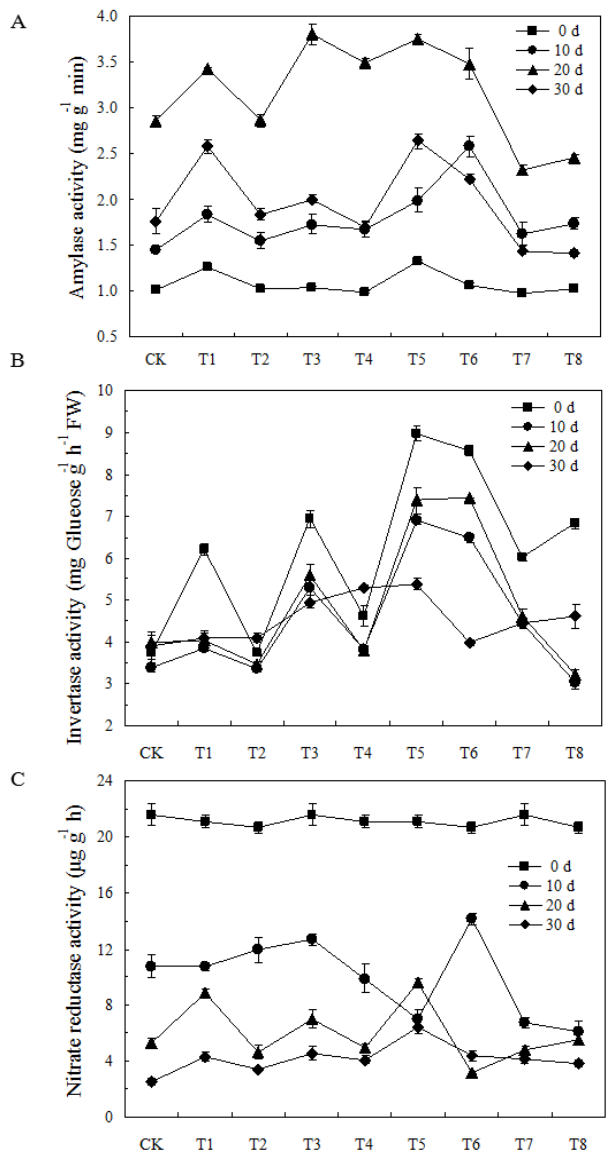

Fig. 3. Effect of exogenous IAA and GA_3 on key enzymes involved in C and N metabolisms of tobacco. (A) AMY activity, (B) INV activity, (C) Nitrate reductase activity.

N assimilation is closely linked to C metabolism and photosynthesis (Abadie *et al*., 2018; Saiz *et al*., 2017). Starch is a key C reserve in higher plants. It accumulates during day, but its hydrolysis takes place during night to yield reduced C to plants (Pokhilko *et al*., 2014). Total carbon content can be used as a comprehensive representation of carbon metabolites, which have an important impact on improving the quality of tobacco. Reducing sugars are the main chemical ingredients that affect the taste of tobacco. The activities of AMY and INV are important indicators of carbon metabolism. Our results confirmed that there was no significant difference in C metabolites at 0 day after topping. However, reducing sugars, starch and total carbon content at 30 days after topping were generally higher than those at 0 day. While soluble sugar content generally showed a downward trend with time after spraying (Fig. 1). In this study, the activities of AMY and INV in tobacco plants in T5 at 30d after topping were significantly higher than those in CK (Fig. 3). Thus, our results suggested that low concentrations of of IAA or GA_3 (T1 or T3) had better effect on the starch content than the high concentrations (T4 or T5).

Conclusion

Our study showed that the positive role of two types plant growth regulators (IAA or GA_3) in improving the tobacco productivity can be ascribed to balanced C and N metabolisms. It can be concluded that exogenously applied low concentrations of GA_3 or IAA applied individually after topping effectively improved yield and enhanced quality of tobacco by increasing the activities of some key enzymes involved in C and N metabolisms. However, both hormones applied in combination (GA_3 50 mg/L and IAA 30 mg/L) were found to be more effective than their individual application in improving the productivity, quality and N and C metabolisms.

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References

Abadie, C., C. Bathellier and G. Tcherkez. 2018. Carbon allocation to major metabolites in illuminated leaves is not just proportional to photosynthesis when gaseous conditions (CO_2 and O_2) vary. *New Phytologist*, 218: 94-106.

Agami, R.A. and G.F. Mohamed. 2013. Exogenous treatment with indole-3-acetic acid and salicylic acid alleviates cadmium toxicity in wheat seedlings. *Ecotox. Environ. Safe*, 94: 164-171.

Al-Khassawneh, N.M., N.S. Karam and R.A. Shibli. 2006. Growth and flowering of black iris (*Iris nigricans* Dinsm.) following treatment with plant growth regulators. *Sci. Hortic-Amsterdam*., 107: 187-193.

Bose, S.K., R.K. Yadav, S. Mishra, R.S. Sangwan, A.K. Singh and B. Mishra. 2013. Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in *Mentha Arvensis* L. *Plant Physiol Bioch*., 66: 150-158.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Cai, T., H. Xu, D. Peng, Y. Yin, W. Yang, Y. Ni, X. Chen, C. Xu, D. Yang, Z. Cui and Z. Wang. 2014. Exogenous hormonal application improves grain yield of wheat by optimizing tiller productivity. *Field Crop Res*., 155: 172-183.

Chatterjee, S., N. Maitra, B. Ghosh and S.P. Sen 1988. Effect of polyamines on photosynthesis of source and sink organs in rice (*Oryza sativa* L.). *Plant Cell Physiol.*, 29: 1207-1213.

Chiang, M.H., H.L. Shen and W.H. Cheng. 2015. Genetic analyses of the interaction between abscisic acid and gibberellins in the control of leaf development in *Arabidopsis thaliana*. *Plant Sci*., 236: 260-271.

Cho, M.H., H.L. Park and T.R. Hahn. 2015. Engineering leaf carbon metabolism to improve plant productivity. *Plant Biotechnol. Rep*., 9: 1-10.

Cuadrado, Y., H. Guerra, A.B. Martin, P. Gallego, O. Hita, A. Dorado and N. Villalobos. 2001. Differences in invertase activity in embryogenic and non-embryogenic calli from *Medicago arborea*. *Plant Cell*, 67: 145-151.

Davenport, S., L.L. Pascaline and J.P. Sanchez-Tamburrrino. 2015. Nitrate metabolism in tobacco leaves overexpressing Arabidopsis nitrite reductase. *Plant Physiol. Bioch*., 97: 96-107.

Delker, C. and A.M. Raschke. 2008. Auxin dynamics: the dazzling complexity of a small molecule's message. *Planta*., 227: 929-941.

Fei, L., H. Zhang, S. Wang, W. Xiao, D. Chao, W. Liu and H. Guo. 2016. Identification of topping responsive proteins in Tobacco roots. *Front. Plant Sci*., 7: 1-11.

Gao, J.F. 2003. The experimental guidance of plant physiology. *Higher Education Press*, Beijing.

Guo, H., Y. Kan and W. Liu. 2011. Differential expression of mirnas in response to topping in flue-cured tobacco (*Nicotiana tabacum*) roots. *Plos One*, 6: 1-15.

Hadi, F., A. Bano and M.P. Fuller. 2010. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3, and IAA) and EDTA alone and in combinations. *Chemosphere*, 80: 457-462.

Hisano, H., T. Matsuura, I.C. Mori, M. Yamane and K. Sato. 2015. Endogenous hormone levels affect the regeneration ability of callus derived from different organs in barley. *Plant Physiol. Bioch*., 99: 66-72.

Joel, E.L. and B.V. Bhimba. 2012. Production of alpha amylase by mangrove associated fungi *Pestalotiopsis microspore* strain VB5 and *Aspergillus oryzae* strain VB6. *Indian J. Marine Sci*., 41: 279-283.

Kai, K., K. Wakasa and H. Miyagawa. 2007. Metabolism of indole-3-acetic acid in rice: Identification and characterization of N-*β*-D-glucopyranosyl indole-3-acetic acid and its conjugates. *Phytochemistry*, 68: 2512-22.

Ke, H., W. Xue, Y. Sun, X. Yu and P. Liu. 2000. Leafy head formation of the progenies of transgenic plants of Chinese cabbage with exogenous auxin genes. *Cell Res*., 10: 151-160.

Khaled, A.M., S. Sikder, M.R. Islam, M.A. Hasan and M.M. Bahadur. 2015. Growth yield and yield attributes of tomato (*Lycopersicon esculentum* Mill.) as influenced by indole acetic acid. *J. Environ. Sci-China*., 8: 139-145.

Krapp, A., V. Saliba-Colombani and F. Daniel-Vedele. 2005. Analysis of C and N metabolisms and of C/N interactions using quantitative genetics. *Photosynth. Res*., 83: 251-263.

Lawlor, D.W. 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J. Exp. Bot*., 53: 773-787.

Li, C., W. Teng, Q. Shi and F. Zhang. 2007. Multiple signals regulate nicotine synthesis in tobacco plant. *Plant Signaling & Behavior*., 2: 280-281.

Li, F., H.Z. Zhang, S.X. Wang, W.F. Xiao, C. Ding, W.Q. Liu and H.X. Guo. 2016. Identification of topping responsive proteins in tobacco roots. *Front. Plant Sci*., 7: 1-11.

Liang, Z., Y. Ma, T. Xu, B. Cui, Y. Liu, Z. Guo and D. Yang. 2013. Effects of abscisic acid, gibberellin, ethylene and their interactions on production of phenolic acids in *Salvia miltiorrhiza* Bunge hairy roots. *Plos One*, 8: e72806.

Liu, C., Y. Wang, K. Pan, T. Zhu, W. Li and L. Zhang. 2014. Carbon and nitrogen metabolism in leaves and roots of dwarf bamboo (*Fargesia denudata* Yi) subjected to drought for two consecutive years during sprouting period. *J Plant Growth Regul*., 33: 243-255.

Mazzoncini, M., T.B. Sapkota, P. Bàrberi, D. Antichi and R. Risaliti. 2011. Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. *Soil Till Res*., 114: 165-174.

Myers, J.A., and K. Kitajima. 2007. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J. Ecol*., 95: 383-395.

Peedin, G.F. 2011. Tobacco cultivation, in: M.L. Myers (Ed.), Specialty Crops, International Labor Organization.

Pokhilko, A., A. Flis, R. Sulpice, M. Stitt and O. Ebenhöh. 2014. Adjustment of carbon fluxes to light conditions regulates the daily turnover of starch in plants: a computational model. *Mol. Biosyst*., 103: 613-627.

Ren, B., Y. Zhu, J. Zhang, S. Dong, P. Liu and B. Zhao. 2016. Effects of spraying exogenous hormone 6-benzyladenine (6-BA) after waterlogging on grain yield and growth of summer maize. *Field Crop Res*., 188: 96-104.

Saiz-Fernã, n. I., D. N. De., B. Brzobohatã½, A. Muã±Oz-Rueda and M. Lacuesta. 2017. The imbalance between c and n metabolism during high nitrate supply inhibits photosynthesis and overall growth in maize (*Zea mays* L.). *Plant Physiol. Biochem.*, 120: 213-222.

Schlüter, U., M. Mascher, C. Colmsee, U. Scholz, A. Bräutigam, H. Fahnenstich and U. Sonnewald. 2012. Maize source leaf adaptation to nitrogen deficiency affects not only nitrogen and carbon metabolism but also control of phosphate homeostasis. *Plant Physiol*., 160: 1384-1406.

Shoji, T., M. Kajikawa and T. Hashimoto. 2010. Clustered transcription factor genes regulate nicotine biosynthesis in tobacco. *Plant Cell*., 22: 3390-3049.

Swain, S.M. and D.P. Singh. 2005. Tall tales from sly dwarves: novel functions of gibberellins in plant development. *Trends Plant Sci*., 10: 123-129.

Szal, B. and A. Podgorska. 2012. The role of mitochondria in leaf nitrogen metabolism. *Plant Cell Environ*., 35: 1756-1768.

Tassi, E., J. Pouget, G. Petruzzelli and M. Barbafieri. 2008. The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. *Chemosphere*, 71: 66-73.

Vanneste, S. and J. Friml. 2009. Auxin: a trigger for change in plant development. *Cell*., 136: 1005-1016.

Wang, R. 2003. Tobacco Chemistry. *China Agriculture Press*. Beijing.

Xie, Z., D. Jiang, W. Cao, T. Dai and Q. Jing. 2003. Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statusses. *Plant Growth Regul*., 41: 117-127.

Xu, Y. and Y. Niimi. 2008. Cold treatment affects microspore development and induces IAA production in pollen sacs in tulip. *Sci. Horti. Asmterdam*., 115: 168-175.

Yan, K., Z. Chen, J. Wang, S. Tan and X. Wu. 2015. Stable carbon isotope composition of tobacco leaves in different ecological regions. *Acta Ecologica Sinica*., 35: 3846-3853.

Zeng, Q., L. Jiang, D. Wang, S. Huang and D. Yang. 2012. Camptothecin and 10-hydroxyc amptothecin accumulation in tender leaves of *Camptotheca acuminata* saplings after treatment with plant growth regulators. *Plant Growth Regul*., 68: 467-473.

Zhang, R., Y. Sun, Z. Liu, J. Wen and S. Yan. 2017. Effects of melatonin on seedling growth, mineral nutrition, and nitrogen metabolism in cucumber under nitrate stress. *J. Pineal. Res*., 62: 1-13.

Zhao, X.Q., X.L. Nie and X.G. Xiao. 2013. Over-expression of a tobacco nitrate reductase gene in wheat (*Triticum aestivum* L.) increases seed protein content and weight without augmenting nitrogen supplying. *Plos One*, 8: 1-11.

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