Gibberellin action on growth, development and production of tobacco

ALMEIDA, A. Q. de [1*]; VIEIRA, E. L.[2]

[1*]M.Sc. Crop Science Dept., Botucatu, SP – Brazil. e-mail: adrianaq_almeida@yahoo.com.br.
[2]Dr. Bahia Reconcavo Federal University – UFRB, Cruz das Almas, BA – Brazil. e-mail: elvieira@ufrb.edu.br.

ABSTRACT

This work aimed at evaluating the action of gibberellin on growth, development and production of *Nicotiana tabacum* L., Type Brasil-Bahia. Liquid gibberellin in doses of 0, 1.25, 3.75, 6.75 and 10.0 mL L^{-1} was sprayed on leaves of plants grown in greenhouse. The pelleted seeds were sowed in expanded polystyrene trays containing PlantMax®. Fifteen days after sowing (DAS), the treatments were applied. Six sprayings were made once a day, every five days. After 43 DAS, the number of leaves, length of the stem and root, dry matter of stem, leaves and roots, and leaf area were evaluated. In the field, the plants remained for 64 days and the number of total leaves, number of feasible leaves, plant height, dry matter of stem and leaves, and leaf area were evaluated. The results showed that, in the greenhouse, gibberellin increases efficiently the stem length, leaf dry matter and leaf area. In the field, gibberellin does not increase the leaf dry matter and the leaf area of Brasil-Bahia tobacco.
Keywords: Elongation, GA3, increasing, leaf pulverization, tobacco.

Ação de giberelina no crescimento, desenvolvimento e produção de fumo

RESUMO

O objetivo do presente trabalho foi avaliar a ação da giberelina no crescimento, desenvolvimento e produção de *Nicotiana tabacum* L. Tipo Brasil-Bahia. Giberelina líquida nas doses 0; 1.25; 3.75; 6.75 e 10.0 mL L^{-1} foi aplicada através de pulverizações foliares em casa de vegetação. As sementes peletizadas foram semeadas em bandejas de poliestireno expandido contendo substrato Plantmax®. Aos 15 dias após a semeadura (DAS) os tratamentos foram aplicados. Seis pulverizações foram feitas uma vez ao dia, a cada cinco dias. Aos 43 DAS, o número de folhas, comprimento do caule e raiz, massa de matéria seca dos caules, folhas e raízes, e área foliar foram avaliados. No campo, as plantas permaneceram por 64 dias e o número de folhas totais,

número de folhas viáveis, altura das plantas, massa de matéria seca de folhas e caules, e área foliar foram avaliados. Os resultados revelaram que a giberelina incrementa eficientemente o comprimento do caule, massa de matéria seca das folhas e área foliar em casa de vegetação. No campo, a giberelina não incrementa a massa de matéria seca das folhas e área foliar do fumo Tipo Brasil-Bahia.
Palavras-chave: Alongação, GA_3, incremento, pulverização foliar, fumo.

INTRODUCTION

Brazil is among one of the major worldwide references as far as tobacco (*Nicotiana tabacum* L.) quality is concerned, consolidating the second position as major producer. Furthermore, it is the largest tobacco exporter in the world due mainly to the integrated system and large production values in many tobacco styles. According to Brasil (2010) the national production in 2007/2008 was 1932 kg ha^{-1} and harvested area was 473 thousand hectares.

Verdial et al. (2000) suggests that the seedlings size influences the early development of tobacco, or either, bigger seedlings tends to form bigger plants after transplant.

Therefore, the search for improvement in the current productivity levels with reduction in production costs in tobacco culture in Brazil have led to the incorporation in the seedlings production system of new technologies, such as the application of liquid gibberellin aiming to achieve seedlings in a shorter period of time, more vigorous and healthy, in order to improve their field performance.

The exogenous application of GA_3 causes an excessive elongation, it target is, therefore, the intercalary meristem which is, in the plant, next to the base of the internode (TAIZ & ZEIGER, 2009). The significance of the GA_3 (gibberellic acid) effect becomes clear when showed that the embryos synthesize gibberellin, releasing it into the endosperm during germination (RODRIGUES & LEITE, 2004).

The objective of the present work was to evaluate the effect of the application, by spraying on leaves, of liquid gibberellin on growth, development and production of tobacco Brasil-Bahia Type.

MATERIAL AND METHODS

The experiments, in greenhouse and field, where carried out at the Capivari Farm, 12º37’28,9’’ South Latitude and 39º03’41,1’’ West Longitude, in Governador Mangabeira – Bahia – Brazil and the analysis carried out in the UFRB (Bahia Reconcavo Federal University) Plant Physiology Laboratory, in Cruz das Almas - Bahia – Brazil,

from May to September 2006. Tobacco Brazil – Bahia Type and liquid gibberellins (GA_3) composed of 4% GA_3 and 96% of inert ingredients were used.

The concentrations used were 0 (control with water), 1.25, 3.75, 6.75 and 10.0 mL L^{-1} of gibberellin prepared in water.

In the greenhouse, the peletized seeds were sowed in expanded polystyrene trays containing PlantMax® substrate humidified with water. Peletized seeds are used because besides making the handling of individual tiny tobacco seeds easier, they can be inserted precisely in the determined place, such as in the correct depth, reaching better results compared to the naked seed (Hutchens, 1999).

The trays containing 256 cells were divided in four quadrants corresponding to one repetition each. In order to keep the humidity of substrate these trays were covered with cotton material, according to seedlings production system of company until full germination of seeds (which happens around the seventh day).

After 15 DAS, when the small plants already had enough leaf area, the treatments were applied by spraying the leaves. Six sprayings were made, once a day, every five days using a hand sprayer, applying 100 mL in each treatment, always in the early morning (between five-thirty and six-thirty a.m.). After a period of three hours, the small plants were irrigated with faucet water, in order to keep the humidity of the substrate throughout the experimental period.

After thirty-three DAS, when the six sprayings were finished, the plants were irrigated only with water until the transplantation to the field.

When the seedlings producing phase was concluded (in the greenhouse), which happened on the forty-third DAS, with plants reaching around 15 cm in height, part of them were used to evaluate the growth and development under greenhouse conditions, and the others were transplanted to the field for production evaluation. No fertilization was used during this period.

Growth and development evaluation – a plant of each quadrant was collected corresponding four replicates of each treatment. These were taken from the substrate, washed with faucet water and had the aerial and roots area measured with a millimeter ruler. The number of leaves was determined by direct counting. The determination of leaf area was by means of ratio of disc dry matter and full leaf dry matter. The discs were obtained with the help of a known area perforator, avoiding the central nervure, taking four leaf discs from each plant (replicates).

The roots, leaves, stem and leaf discs had been conditioned separately in identified paper bags and placed in a forced air circulation greenhouse of 65ºC ± 5ºC for

72h in order to determine dry matter content, carried through with aid of an analytical precision scale.

Production evaluation – After growth and development evaluation, twelve plants each treatment were transplanted to the field and planted with a distance of 1,0 m between in line and 0,45 m between plants and the treatments distanced by 1,50 m. The fertilization used was based on NPK following the company production system (non-divulged data).

The plants remained in the field for 64 days (107 DAS) and when the leaves, destined to manufacturing of cigar and cigarettes, covers reached the harvest point four plants of each treatment were selected corresponding to four replications.

The number of leaves was determined by direct counting, and classified with the help of company employees, into full leaves and viable leaves (leaves for the production of covers, without defects, green, big, less fragile, ticker and consistent). The height of plants was defined by using a measuring tape. Twenty leaf discs were taken in order to determine leaf area.

Stems, leaves and leaf discs were separately put into identified paper bags and put into an air circulation forced oven with temperatures of 65ºC ± 5ºC until reaching constant weight for determination of dry matter with the aid of an analytical scale.

To evaluate the growth, development and production, the experiment was completely randomized with five treatments and four replicates. The data collected was submitted to analysis of variance due to the level of significance in F-Test at 5% of probability (FERREIRA, 2000). Polynomial regression analysis was performed in those variables where the test indicated significant discrepancies between the performed treatments (BANZATTO; KRONKA, 1995).

RESULTS AND DISCUSSION

The length of roots and root dry matter, were not significantly influenced by the treatments (Table 1).
The number of leaves (Figure 1) was significant with the point of minimum at 4.54 mL L^{-1} of gibberellins and 13.4% inferior then the control. The higher concentrations, from the point of minimum, caused significant increase in the number of leaves. The higher concentration of gibberellin used (10.0 mL L^{-1}) had a behavior similar the control, whereas both treatments achieved an average of 5.75 leaves per plant.

TABLE 1. Analysis of variance of growth and development, under greenhouse conditions, in the response of leaves sprayed with five gibberellin doses. **LN** – leaf number; **SL** - stem length; **RL** – root length; **SDM** – stem dry matter; **RDM** – root dry matter; **LDM** – leaf dry matter; **LA** – leaf area.

DM								
VF	LD	LN	SL (cm)	RL (cm)	SDM (g)	RDM (g)	LDM (g)	LA (cm^2)
Treatment	4	0.57*	359.3**	0.05ns	0.09**	0.03ns	0.05*	7.18*
error	15	0.28	14.0	0.87	0.01	0.02	0.01	1.42
VC (%)		9.95	13.07	15.44	9.72	14.48	7.24	4.73
General mean		5.35	28.63	6.06	0.80	1.07	0.99	25.20

*Significant at 5% of probability by F-Test; **Top significant at 5% of probability; ns non-significant.

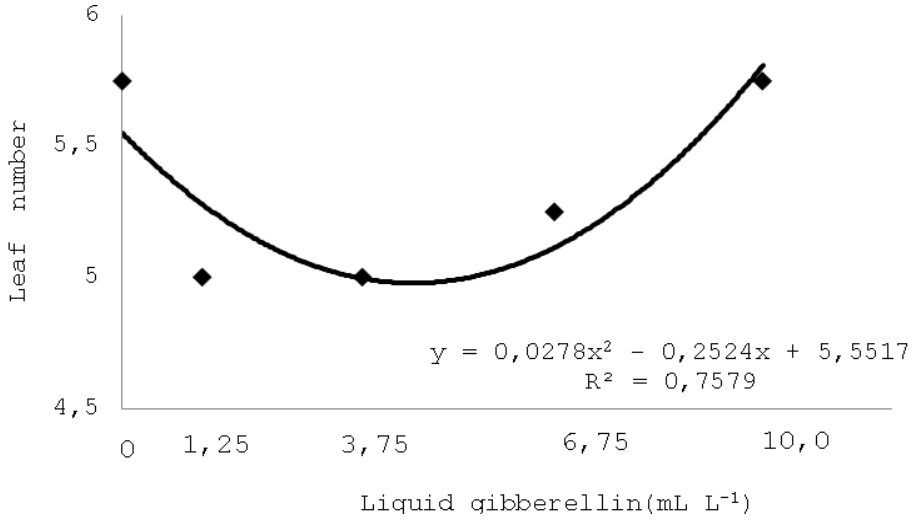

Figure 1. Leaf number in *Nicotiana tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves at greenhouse.

Regarding the control, all of GA_3 concentrations used promoted increase in length of stem. The maximum length found of 38.1cm, was in the concentration 6.6 mL L^{-1} of gibberellin; higher than the control in 202.0% (Figure 2).

The results showed significant effect ($P<0,05$) the GA_3 concentrations in the stem dry matter (Figure 3). The point of minimum with 6.72 mL L^{-1} of GA_3 corresponds to less than 136.5% compared the control.

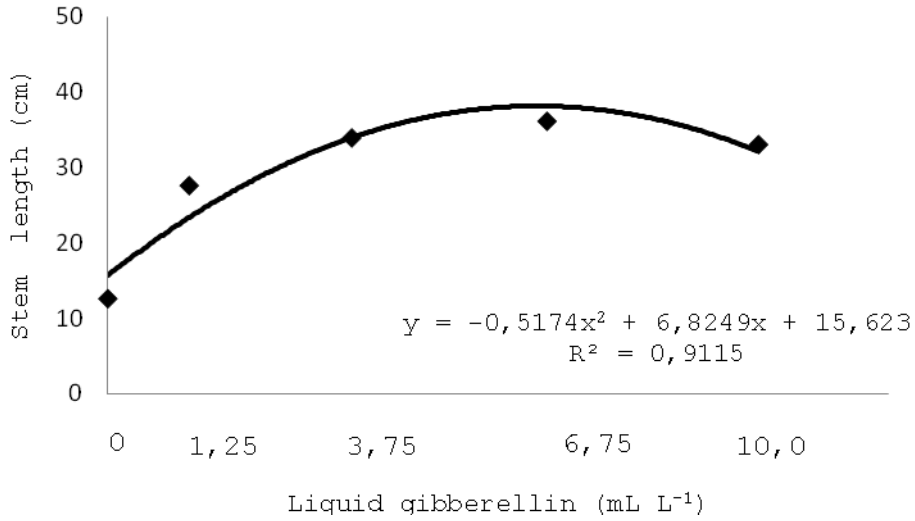

Figure 2. Stem length of *Nicotiana tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves at greenhouse.

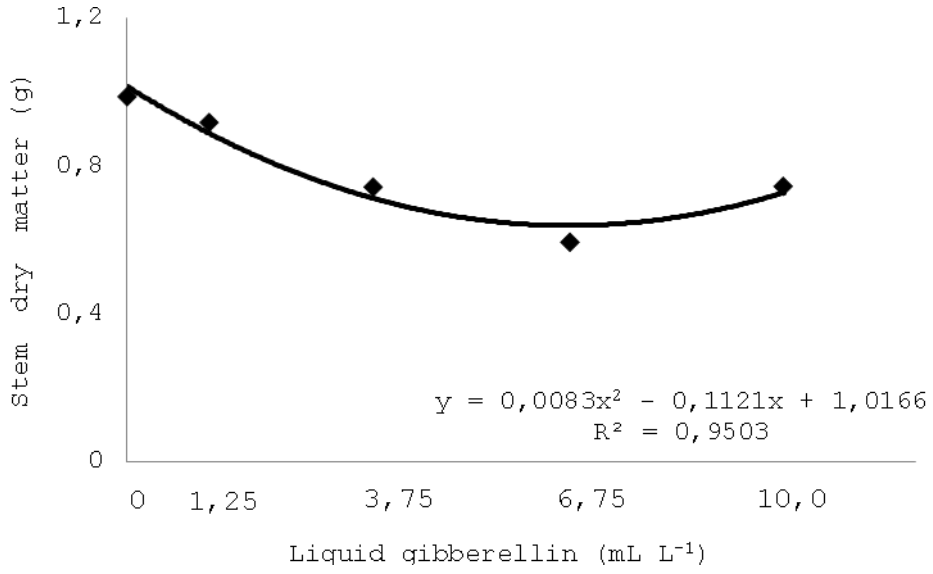

Figure 3. Stem dry matter of *Nicotiana tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves at greenhouse.

Compared to the control, all of concentrations of liquid gibberellin increased leaf dry matter and found a point of maximum (1.12 g) in 3.07 L^{-1} mL and point of minimum in 8.25 mL L^{-1} of GA_3 (Figure 4) and are, respectively, 40.3% and 15.2%, higher than the control treatment.

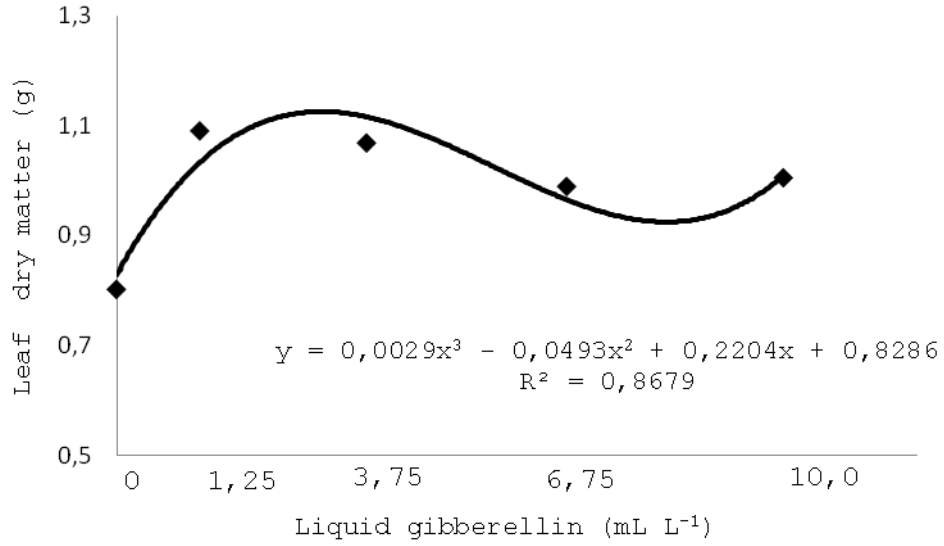

Figure 4. Leaf dry matter of Nicotiana *tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves at greenhouse.

Greater leaf area was achieved in concentration of 2.75 mL L^{-1} of gibberellin and represents an area of 26.8 cm^2. This amount was higher than control's (which achieved an amount of 23.20 cm^2) in 15.7% with increase of 3.6 cm^2. The point of minimum was found in the 7.93 mL of gibberellin concentration with an amount corresponding to a leaf area of 23.92 cm^2. This amount was even higher than control, which presented average leaf area of 23.20 cm^2, in 3.15% (Figure 5).

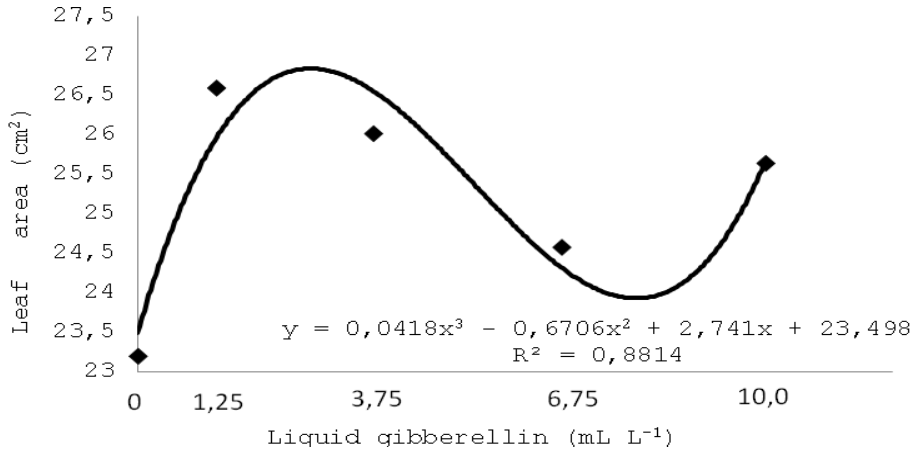

Figure 5. Leaf area of *Nicotiana tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves at greenhouse.

Regarding the parameters evaluated in the field, the number of total leaves, number of viable leaves and plant height were not significantly influenced by liquid

gibberellin (Table 2). In other hand, dry matter of leaves, stem dry matter and leaf area were strongly affected.

TABLE 2. Analysis of variance of production, in the field, in the response of leaves sprayed with five gibberellin doses. **LN** – leaf number; **NVL** – number of viable leaves; **PH** – plant height; **LDM** – leaf dry matter; **SDM** – stem dry matter; **LA** – leaf area.

		DM					
VF	LD	LN	NVL (cm)	PH (cm)	LDM (g)	SDM (g)	LA (cm^2)
Treatment	4	7.92^{ns}	2.62^{ns}	135.72^{ns}	122.09^{*}	325.74^{*}	96246.1^{*}
error	15	13.37	2.22	100.57	31.53	157.03	23149.4
VC (%)		31.25	19.21	12.36	14.60	36.99	19.94
General mean		11.70	7.75	81.12	38.48	33.88	1018.40

*Significant at 5% of probability by F-Test; **Top significant at 5% of probability; ^{ns} non-significant.

Leaf dry matter showed that the effect of gibberellin concentration differentiated, and the point of minimum, 5.25 mL of gibberellin L^{-1} in watery solution with 31.9 g of mass, 30.8% lower compared to the control.

Stem dry matter registered the smaller value (point of minimum) of 22.75g in the concentration of 5.05 mL of gibberellin with a decrease of 30.48% compared to the control (Figure 7), when in the higher concentration evaluated of 10.0 mL of this plant regulator was higher than the control by 15.6%.

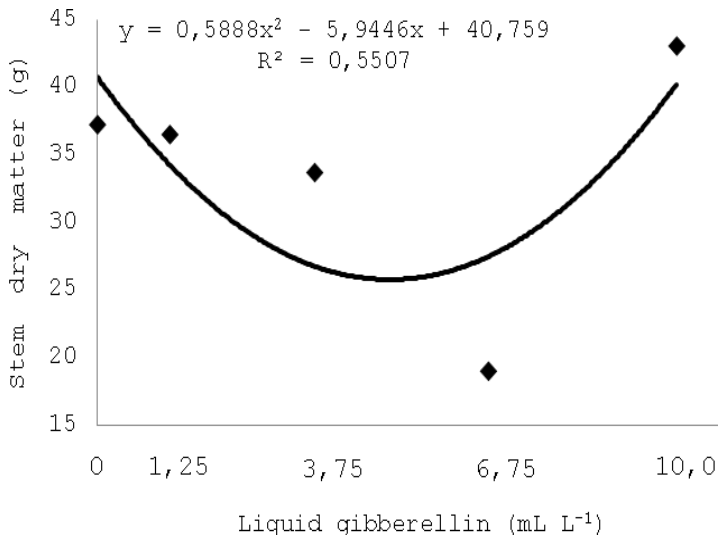

Figure 7. Stem dry matter of *Nicotiana tabacum* L., Brasil-Bahia Type in response of five gibberellin doses by spraying the leaves in the field.

For leaf area, in the field, for the point of minimum of 5.6 mL L^{-1} of liquid gibberellins smaller area with 838.2 cm^2 was found; this was 33% less than control (Figure 8).

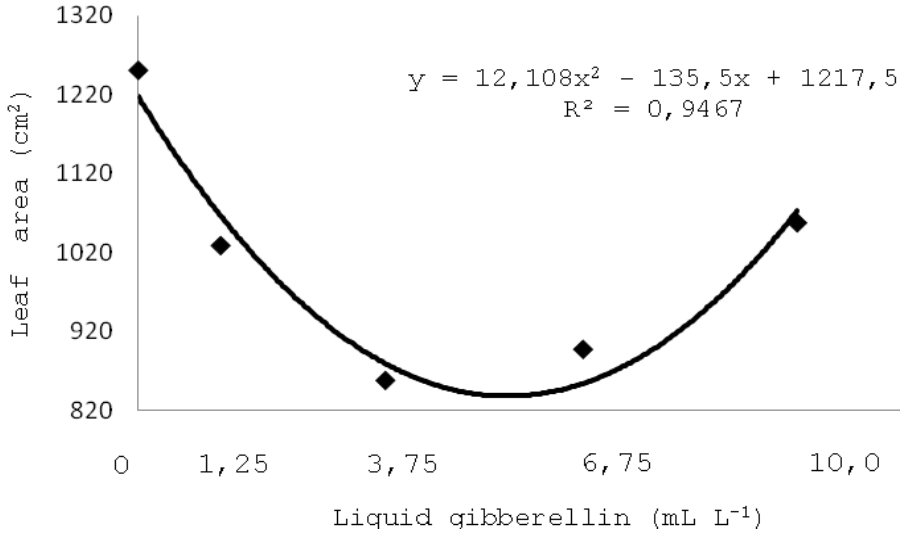

Figure 8. Leaf area of *Nicotiana tabacum* L., Brasil-Bahia Type, in response of five gibberellin doses by spraying the leaves in the field.

In the greenhouse, similar results of significant behavior of stem length and non-significant to the root length and dry matter, were found by Scalon et al. (2006) evaluating the initial growth of "orelha-de-macaco" seedlings (*Enterolobium contortisiliqunn* (Vell.) Morong). These authors observed a significant behavior in the growth of the aerial parts due to the application of gibberellin, but the results were not significant for roots dry matter and length when the same regulator was used. Oliveira et al. (2005) evaluating the *Passiflora alata* Curtis seedlings production verified that the root dry matter and length were not significantly altered by application of same plant regulator. According to Taiz & Zeiger (2009), gibberellin influences root growth in a non-accented way.

Gibberellin concentration caused significant increase in number of leaves. However, the higher gibberellin concentration used (10.0 mL L^{-1}) induced similar responses in the control achieving in both treatments an average of 5.75 leaves per plant. Leonel & Pedroso (2005) also found expressive effects regarding the increase of leaf quantity in the sweet passion-fruit tree; the measure that had increased the GA3 concentration up to 300 mg L^{-1} of solution.

Increase in stem length can be, according to Taiz & Zeiger (2009), due to the action of gibberellin promoting the cell division elongation, or either, to higher number of cells and higher elongation in plants after application

this regulator. Similar results were found by Barros (2004) when the increase in height of corn was smaller from control concentration until 400 mL L^{-1} of GA_3, were observed. This behavior is characteristic of the GA_3 action in the plant, or either, the plants showed to be higher and lighter due to cells elongation caused by gibberellins.

All of amounts found in the leaf dry matter, mainly in the 1.25 and 3.75 mL L^{-1} of gibberellin, were higher than control values. Similar results were found by Carvalho et al. (2005) when gibberellin was applied in banana culture. A significant decrease from the 120 µmol L^{-1} solution concentration was noted and it was concluded that gibberellin from this concentration, presented inverse action or either, instead of stimulating, inhibited the production of assimilates in the leaves. Magalhães et al. (2002), noticed that the presence of high concentrations of gibberellic acid in genipap trees, few were the leaves, or, when present, they were abnormal, indicating that even the gibberellin keeping the cells competence, an excess of this plant regulator can change the plant´s metabolism.

All the concentrations of gibberellin used promoted increase in leaf area compared to the control, over all in the 1.25 and 3.75 mL of gibberellin. These are the wanted results for tobacco culture, in which the main commercial part is the leaf. Leonel & Rodrigues (1996) found similar values in their experiments with “Cravo” lemon tree, where there was an increase in leaf area using 25, 50 and 75 ppm of gibberellins.

In field, the results achieved for leaf number, number of viable leaves and plant height is in agreement with the ones achieved by Alleoni et al. (2000). They explain that plant regulators are more active on vegetative phase and therefore, their influence may have been reduced or diluted in the field. Furthermore, gibberellin promotes the elongation and not the appearance of new leaves. These results disagree from Sachs et al. (1960) cited by Vichiato (2007), which reported that gibberellin application in stems promotes the increase of cells division in apical meristem promoting the formation of high number of cells and the individual elongation each one, favoring growth increase.

All the concentrations of GA_3 provided smaller leaf area in field, compared to control, although Modesto et al. (2006) notice that the leaves growing can be increased in several species with the use of gibberellin. An increase in the amount this variable can be noticed in Figure 8; therefore, new experiments would be needed, using higher concentrations of gibberellin to corroborate the results.

No estimated amount of leaf dry matter was higher than control. According Alleoni (2000) plant regulators are more active in production of dry matter on vegetative phase. The

same happened with the stem dry mass, but in a higher concentration there was a discrete addition with more concentrated applications of GA_3. To corroborating effects, new experiments would be needed, using higher concentrations this regulator.

CONCLUSIONS

- Liquid gibberellin (4% de GA_3) is efficient for increasing stem length, dry matter and leaf area, under greenhouse conditions, for *Nicotiana tabacum* L., Brasil-Bahia type;
- In the field, the gibberellin applied under greenhouse conditions, did not increase leaf dry matter and the leaf area of Brasil-Bahia Type Tobacco.

REFERENCES

ALLEONI, B.; BOSQUEIRO, M.; ROSSI, M. Efeito dos reguladores vegetais de Stimulate® no desenvolvimeto e produtividade do feijoeiro (*Phaseolus vulgaris* L.). **Ciências Exatas e da Terra, Ciências Agrárias e Engenharia**, v. 6, p. 23-35, 2000.

BANZATTO, D.A.; KRONKA, S. do N. **Experimentação Agrícola**. Jaboticabal: Fundação de Estudos e Pesquisa em Agronomia, Medicina Veterinária e Zootecnia. 1995.

BARROS, T. F. **Ação de giberelina líquida na germinação de sementes, vigor de plântulas e crescimento inicial de plantas cultivadas.** Cruz das Almas. 2004. 53f. Tese (Mestrado em Ciências Agrárias). Universidade Federal da Bahia, Escola de Agronomia, Cruz das Almas.

CARVALHO, J. A. B. S. de. Uso de giberelina GA_3 na seleção do porte de bananeira das cultivares Prata e Prata Anã. **Rev. Bras. Frutic.**, v. 27, p. 449-453, 2005.

HUTCHENS, T, W. [S.l]: **Tobacco: Production, Chemistry and Technology**. DAVIS, D. L. (Ed), NIELSEN, M. T. (Ed): Physiology and Agronomy. Blackwell Science, 1999.

LEONEL, S.; PEDROSO, C. J. Produção de mudas de maracujazeiro doce com uso de biorregulador. **Rev. Bras. Frutic.**, v. 27, p. 107-09, 2005.

LEONEL, S.; RODRIGUES, J. D. Efeitos de giberelinas, citocininas e do nitrato de potássio no crescimento e desenvolvimento do porta-enxerto do limoeiro ‘cravo’. **Sci. agric.**, v. 53, p. 2-3, 1996.

MAGALHÃES, G. L.; CARMO, D. O.; COSTA, M. A. P. C.; SOUZA, F. V. D.; HANSEN, D. S. Efeito de diferentes concentrações de GA_3 (ácido giberelico) no alongamento de brotações in vitro de jenipapo (Genipa americana L.). **In**: Anais, XVII Congresso Brasileiro de Fruticultura; 2002.

BRASIL. Ministério da Agricultura e do Abastecimento. Safras - grãos. Disponível: <http://www.conab.gov.br/conabweb/download/safra/FeijaoTotalSerieHist.xls> Acesso em: 04 jun 2010.

MODESTO, J. C.; RODRIGUES, J. D.; ONO, E. O.; HABERMANN, G. Aplicação de ácido giberélico (GA_3) em pré-colheita de tangerina ‘Poncã’ (*Citrus reticulata* blanco). **Acta Sci. Agron**., v. 28, p. 34-70, 2006.

OLIVEIRA, A. de; GISELA FERREIRA, G.; RODRIGUES, J. D.; FERRARI, T. B.; KUNZ, V.; PRIMO, M. A.; POLETTI, L. D. Efeito de reguladores vegetais no desenvolvimento de mudas de *Passiflora alata* Curtis. **Rev. Bras. Frutic.**, v. 27, p. 9-13, 2005.

RODRIGUES, T de J. D. , LEITE, I. C. **Fisiologia vegetal – hormônios das plantas**. Jaboticabal: Funep, 2004.

SCALON, S. de P. Q.; MUSSURY, R. M.; GOMES, A. A.; SILVA, K. A.; WATJIER, F.; SCALON FILHO, H. Germinação e crescimento inicial da muda de orelha-de-macaco (*Enterolobium contortisiliqunn* (Vell.) Morong): efeitos de tratamentos químicos e luminosidade. **Revista Árvore,** v. 30, p. 529-536, 2006.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 3.ed. Porto Alegre: Artmed, 2009.

VERDIAL, M. F. Métodos de formação de mudas de maracujazeiro amarelo. **Scientia Agrícola,** v. 57, p. 795-798, 2000.

VICHIATO, M. R. de M. Alongamento de plantas de *Dendrobium nobile* Lindl. com pulverização de ácido giberélico. **Ciência e Agrotecnologia,** v. 31, p. 7-10, 2007.