EVALUATION OF RECOMBINANT EXPRESSION OF CODA GENE REGULATED BY RD29A PROMOTER IN TOBACCO

Bui Thi Thu Huong[1], Dong Huy Gioi[1], Ngo Manh Dung[2,3], Pham Bich Ngoc[2], Chu Hoang Ha[2]
[1]*Vietnam National University of Agriculture*
[2]*Vietnam Academy of Science and Technology*
[3]*Thainguyen University*

SUMMARY

There are many evidences showing the fact that some hard conditions from environment, namely abiotic stress, could make several significantly negative impacts on crop production. However, some plant species can overcome these bad situations by complicated mechanisms. The reason is that there are genetically valuable factors in their cells enhancing their stress tolerance. This study has shown the result of the construction the recombinant vector carrying *Cod*A gene which could encode an enzyme, named *choline oxidase,* and is controlled by Rd29A promoter. The vector with Rd29A/CodA cassette was transformed into *Agrobacterium tumefaciens* C58 strain and used to mediate with *K326 Nicotiana tabacum*. The tobacco lines transformed sucessfully the structure could stimulate a biosynthesis of high amount glycinebetaine, the chemical can improve stress tolerance of plants. Specifically, eight transgenic lines (3, 4, 5, 6, 8, 9, 15, and 16) were confirmed the presence of *CodA* gene by PCR using a *CodA* specific primer pair and tested with *in vitro* stress assays. The collected data had confirmed that some Rd29A/*CodA* transgenic tobacco lines illustrating the resistance of them to some stresses and accumulated 6 to 7 times higher amount of glycinbetaine in artificially salty and drought mediums supplemented with NaCl 250mM or 2.5% PEG6000, respectively, than those in normal state.

Keywords: Artificial stress, *CodA*, glycinebetaine, Rd29A promoter, stress tolerance.

1. INTRODUCTION

Nowaday, there were many evidences to show that abiotic stress made a significant negative impact on crop production. As results, the crop yields would be declined in many areas. It was also indicated that environmental factors had limited as much as 70% crop production (Boyer JS, 1982). The roots of the consequence were continuous reduction of arable land, reduction of water resources and increased global warming trends and climate change (Lobell DB *et al.*, 1980). These stresses as drought, high salinity or extreme temperatures are considered ones of the most seriously environmental factors which affected to plant in series pathways of biochemical, physiological, developmental responses, and productivity. It is said that one of the earliest metabolic responses to abiotic stresses and the inhibition of growth was the inhibition of protein synthesis (Vincent D *et al.*, 2007) and an increase in protein folding and processing (Liu JX, and Howell SH, 2010). However, some species can overcome these bad conditions by complicated mechanisms. One crucial mechanism was explored that plant accumulated compatible solutes such as polyols, sugars, amino acids, betaines. And among them, glycinebetaine (GB) was demonstrated the compatible solute accumulating rapidly in many plants exposed to the adverse conditions.

Moreover, in *Arthrobacter globiformis* bacteria, GB can be directly synthesized from choline by *CodA* gene transformation pathway (Rathinasbapathi B. *et al.*, 1997). Therefore, the gene could be used to improve plant stress tolerance, especially when it was controlled by a stress inducible promoter like Rd29A promoter. Derived from *Arabidopsis thaliana*, the Rd29A promoter not only increased the resistance to different stresses such as drought, low temperature (Mie K., *et al.*, 2004; Wu Y., H. *et al.*, 2008), but also minimized the other

negative effects such as dwarfism (Cong L., *et al.*, 2008). Besides that, the survival rates of the transgenic clones (with the Rd29A promoter) had a greater probability of survival after recovery from exposure to stress temperatures. This was compared to the non-transgenic cells that showed damage to the plant with no recovery after stress (Babak B, *et al.*, 2007). This report proved that the Rd29A was a good promoter when it was used to control *CodA* gene in the transgenic plantlets with the resistance to environmental stress.

2. RESEARCH METHODOLOGY

2.1. Materials

Bacterial strains: *Agrobacterium tumefaciens* strain C58*, Escherichia coli* strain; plasmids: pCAMBIA1301 and pBT carrying *Rd29A, CodA* gene*; K326 Nicotiana tabacum* plant were provided by Institute of Biotechnology. Chemicals originated from Fermentas, Thermo Scientific. Equipments were imported from Germany, Switzerland, USA and Japan.

2.2. Methods

2.2.1. Vector construction and transformation to tobacco via Agrobacterium tumefaciens

Recombinant vector pCAMBIA1301 carrying the Rd29A promoter was designed by restriction enzyme reaction, recombinant vector ligation. And then, recombinant vectors were transformed into *E.coli* and *Agrobacterium* strain C58 (Cohen *et al.*, 1972). The recombinant plasmids were checked by PCR with specific primers under thermal cycles as follows: 94°C/4 min, 94°C/20 seconds, 45ºC/20 seconds, 72°C/1 minute 20 seconds, 72°C/4 min 16ºC/30 min, 26 cycles.

2.2.2.Creating CodA transgenic tobacco line

Transfer the recombinant vector to the tobacco leaf pieces through *A. tumefaciens*that was followed by the method of Topping (Topping JS., 1998) with some little modifications. The transferred pieces were cultured in (on) MS (Murashige and Skoog, 1982) medium supplemented with 3% sucrose, 1 mg/l BAP, 500 mg/l cefotaxime, and 5 mg/l hygromycin in 14 days, then in MS + 1 mg/l BAP + 500 mg/l cefotaxime + 10 mg/l hygromycin for shoot regeneration and transgenic shoot selection. The antibiotic resistant shoots were separated from the leaf pieces and transferred to the similar selective medium. After 14 days, the survival shoots were continuously transferred to rooting medium, MS also supplemented with 3% sucrose, 0.1 mg/l IBA, 250 mg/l cefotaxime, and 2.5 mg/l hygromycin.

2.2.3. Determining the presence of CodA gene in putative transgenic lines

Genomic DNA from the regenerated tobacco lines after hygromycin selection were extracted and examined by PCR using a codA specific primer pair.

2.2.4. In vitro stress treatment

The shoots of positive transgenic lines were multiplication and tested stress tolerance by culturing in medium supplemented with 250mM NaCl (Wang *et al.*, 2003; Barunava *et al.*, 2010); 2.5% PEG6000 (La *et al.*, 2012).

GB content of transgenic lines was extracted and quantified as described by Grieveand Grattan, (1983) before and after stress treatment.

3.RESULTS AND DISSCUSTION

3.1. Constructing plant transformation vector carrying Rd29A::CodA

In order to construct recombinant vector, pBTvector carrying Rd29A promoter and pCAMBIA1301/CaMV35S::*CodA-cmyc* vector were digested by *Hind* III, *Xba* I (Fig. 1). The Rd29A promoter fragment and the PCAMBIA1301/*CodA* vector without CAMV35S promoter were purified and ligated. The obtaining recombinant vector had checked by digestion reaction (Fig. 2) and was transformed into *E.coli DH5α* for gene cloning and into *A. tumefaciens* for plant transformation.

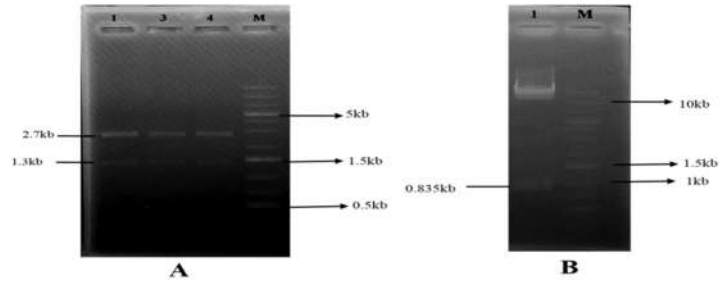


Figure 1. pBTvector carrying Rd29A promoter(A) and PCAMBIA1301/CodA vector (B) digested by HindIII/XbaI

A-1, 3 and 4:PBT/Rd29A vectors were treated by HindIII/XbaI, resulting in 1.3 kp bands, and 2.7 kb bands corresponding to the Rd29A promoter and the rest of the pBT vector. B-1:PCAMBIA1301/ CAMV35S ::CodA vector was treated by HindIII/XbaI , resulting a 0.835 kb band corresponding to CAMV35S promoter separated from the PCAMBIA1301/CaMV35S::CodA

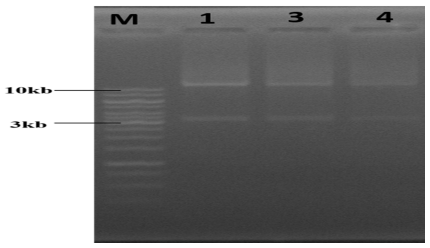


Figure 2. The recombination vector digested byHindIII/SacI

1, 3, 4: recombinant plasmids treated by HinhIII/ XbaI, resulting in the fragment including Rd29A (nearly 3.2 kb bands) and the rest of vector (10kb).

3.2. Creating *CodA* transgenic tobacco lines

After transformation, the leaf explants were cultured in MS (Murashige and Skoog, 1982) medium supplemented with hygromycin for shoot selection. The table 1 showed the result of survivability and shooting ability of transformed explants. In first selection, the survival rate of explants was 83.78% in which leaf explants of negative control were had signs of dying as leaf explants and shoots turned yellow gradually. Until the end of second selection, survival rate of transformed explants reduced to 53.23% and wild-type were completely dead. Resistant shoots were separated and transferred to the similar selective medium. After 14 days, survival rate of shoots were 51.16%. Survival shoots were continuously transferred to rooting medium, MS added 250 mg/l cefotaxime, and 2.5 mg/l hygromycin. Rooting rate of shoots were 59.09% after 10 days culturing (table 2).

Table 1. Survivability and shooting ability of tobacco transformation

Period of selection	Samples	Survival rate (%)	Shooting rate(%)
The 1st selection	Transformation	83.78	83.78
(after 14 days)	Negative	100.00	100.00
(*1)	Wild-type	100.00	100.00
The 2nd selection	Transformation	53.23	53.23
(after 21 days)	Negative	0.00	0.00
(*2)	Wild-type	100.00	100.00

*Note: *1: Selective medium 1- MS + 1 mg/l BAP + 500 mg/l cefotaxime + 5 mg/l hygromycin;*
**2: Selective medium 2- MS + 1 mg/l BAP + 500 mg/l cefotaxime + 10 mg/l hygromycin;*
Transformation- Leaf explants were transformed with PCAMBIA1301/Rd29A::CodA vector via A.tumefaciens; Negative -leaf explants cultured in medium containing hygromycin; Wild-type-leaf explants cultured in medium without hygromycin.

Table 2. Result of survival ability of shoots and rooting of selected shoots

Explants	Shooting rate (%)	Rooting rate (%)
Transgenic shoot	51.16	59.09
Negative control	23.33	26.67
Wild-type	100.00	100.00

Note: Transgenic shoot: Shoot formed from transferred leaf pieces; Negative control: Shoot formed from non-transferred leaf pieces; Wild-type: Shoot formed from non-transferred leaf pieces cultured medium without antibiotic.

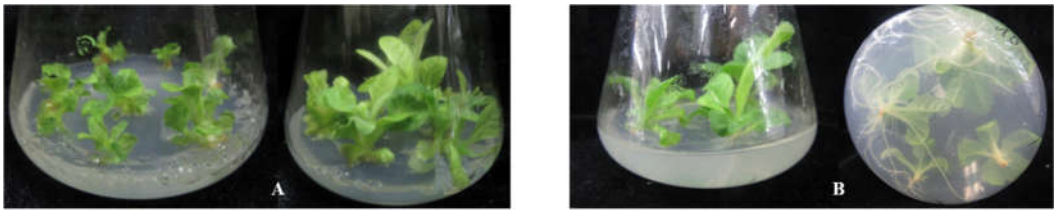


Figure 3. Transferred shoot in selective medium (A) and rooting medium (B)

Shooting culturing medium: MS + 1 mg/l BAP + 500 mg/l cefotaxime + 10 mg/l hygromycin; Rooting culturing medium: MS + 0.1mg/l IBA+ 250mg/l cefotaxime + 2.5 mg/l hygromycin.

4.3. Expression target gene of transgenic plants

Total DNA of 10 transgenic tobacco lines and wild-type were extracted from leaves. 100-200 ng DNA from each sample were used as a template to amplify *CodA* gene with specific primers. Results showed that there were 8 out 10 transgenic tobacco lines appeared band with size about 1.9 kb of the *CodA* gene (Fig. 4) and nearly 1.3 kb lands of Rd29a promoter (Fig. 5).

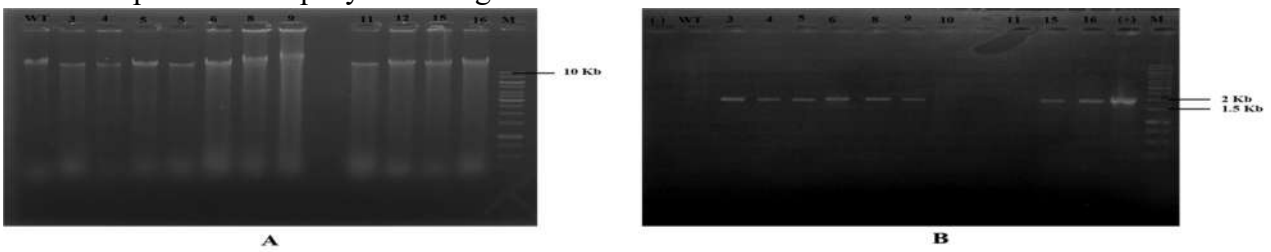


Figure 4. Gel electrophoresis of DNA extraction (A) and PCR products using *CodA* specific primers (B)

M: generuler 1kb DNA ladder; 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 16: transgenic tobacco lines; (-) and (+) were negative control and positive control, respectively.

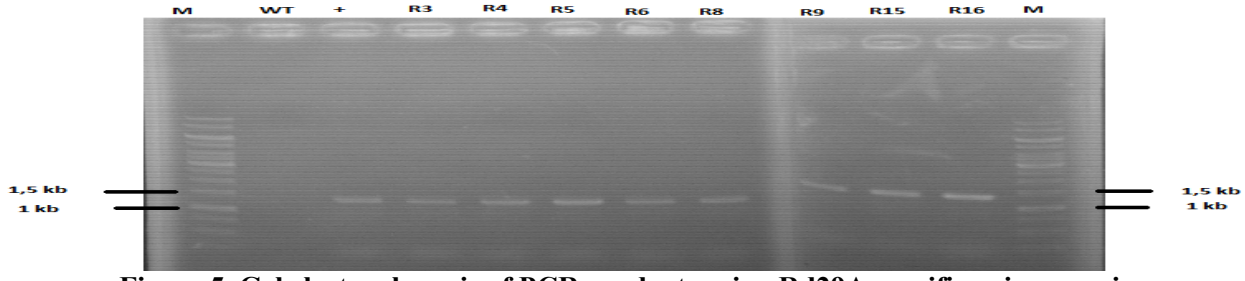


Figure 5. Gel electrophoresis of PCR products using Rd29A specific primers pair

Note:nearly 1.3 kb bands were the Rd29A promoter fragments.

In order to evaluate drought and salinity tolerance of transgenic lines, the transgenic and wild-type tobacco shoots were cultured in rooting medium supplemented with 2.5% PEG6000 (drought condition) or 250 mMNaCl (salinity condition). Results showed that the rooting rates of 8 transgenic lines (72.22-75.56%) were higher than that of WT (46.67%) after 14 days of culturing. In addition, leaves of WT plants were yellow and had a less number of roots; where as the transgenic lines showed remained green leaves and well-developed roots after *in vitro* drought treatment.

The transgenic tobacco lines also showed higher salinity tolerance than WT in salinity medium. Especially, transgenic lines 4, 6, 8, 15, 16 had rooting rate were around 72.22, 60.00, 72.22, 72.22, and 58.89% respectively after 14 day culturing and their roots were also bigger and longer, meanwhile, the rooting rate of WT was about 17.78% and their roots were short. Moreover, the leaves of transformed lines were greener than that of WT (Fig. 6, 7). It demonstrated that the tissue or organ of the plant could response to stress that affected to. Specially, root- cells were sensitive in responding to stress and different with specific type of stress (Dinneny JR *et al.*, 2008). The

data demonstrated that they almost expressed the desired tolerances (Fig. 6, 7). On the contrast, the non-transferred line, WT, had not the tolerances. The results provided that all the transgenic lines had ability in facing to extreme conditions *in vitro* such as drought, salinity. It was expressed in the survivability and rooting rate of the shoots when they exposed to these conditions.

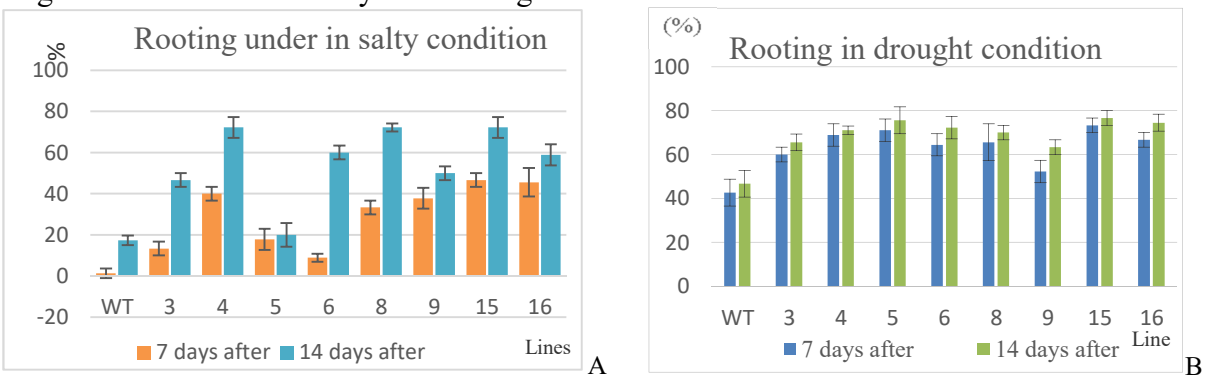
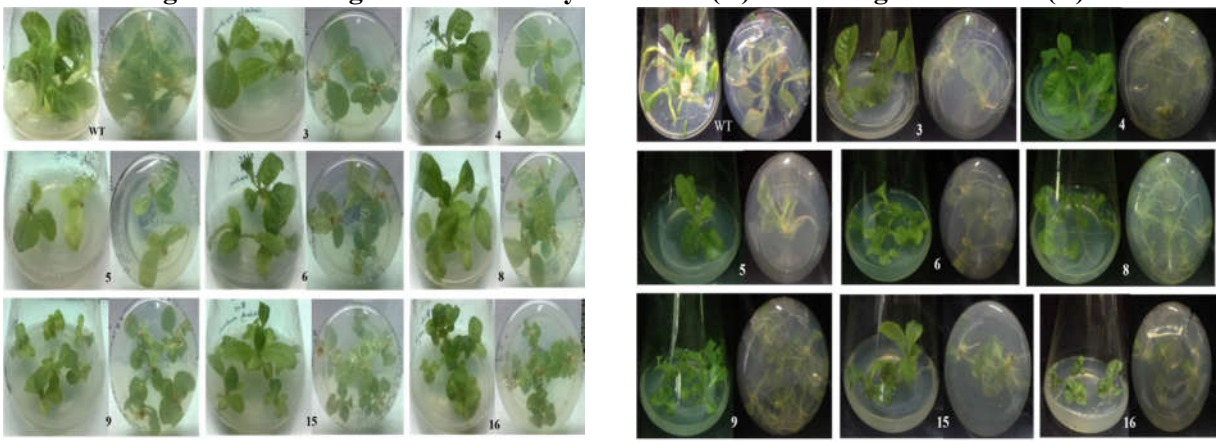


Figure 6. Rooting of shoots in salty condition (A) and drought condition (B)



A B

Figure 7. Transferred shoots in rooting medium added 250 mMNaCl(A) and 2.5% PEG6000 (B)

Base medium: MS + 3% sucrose + 0,1mg/l IBA;

WT-wild type tobacco; 3, 4, 5, 6, 8, 9, 10, 11, 15, 16 were transgenic tobacco lines

Assesment of glycine betaine (GB) content of transgenic lines

GB was one of the most common compatible organic solutes produced at high concentrations in the cytosol which supports plants get over stress strategies (Serraj and Sinclair, 2002). Compatible solutes are low-molecular- weight, highly soluble compounds that are usually nontoxic. Generally, they protected plants from stress through different means such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, stabilization of membranes, and native structures of enzymes and proteins. Therefore, glycinebetaine (N, N, N-trimethyl glycine) was one of the most extensively studied quaternary ammonium compounds and compatible solutes in plants, animals and bacteria (Wahid *et al.*, 2007). Many other studies demonstrated that glycinebetaine plays an important role in enhancing plant tolerance under a range of abiotic stresses (Quan *et al.*, 2004). In addition to direct protective roles of glycinebetaine either through positive effects on enzyme and membrane integrity or as an osmo-protectant, glycinebetaine may also protects cells from environmental stresses indirectly by participating in signal transduction pathways (Subbarao *et al.*, 2000). Furthermore, GB is much more effective than other compatible solutes in the stabilization *in vitro* of the quaternary structure of enzymes and complex proteins, as well as the highly ordered state of membranes, at high concentrations of salts and

extreme temperatures. These properties of GB were deduced for the most part from studies based on comparative physiology and genetics, as well as from experiments *in vitro*.

The introduction of genes synthesizing glycinebetaine into non-accumulators of glycinebetaine proved to be effective in increasing tolerance to various abiotic stresses (Sakamoto A and Murata N, 2002). Similarly, *CodA* transgenic approaches had proved in some plants such as *Arabidopsis*, rice (*Oryza sativa*), and tobacco (*Nicotianatabacum*), none of which naturally synthesized GB (Sakamoto A and Murata N, 2000). However, the result of this research showed that there was a little GB appeared in the WT that was to be a critical determinant of stress tolerance of plants. It was an extremely efficient compatible solute in the transgenic lines and its presence is strongly associated with the growth of plants in dry and/or saline environments. It was similar to declaration of Rhodes D and Hanson A.D (1993).

In this report, the result demonstrated that in the normal condition, glycine betaine (GB) was acummulated on the transgenic lines about 0.276 mM/g to 2.758 mM/g but almost none in non transgenic lines. However, the stress had stimulated GB synethsis in both transgenic and non transgenic lines. The accumulation of GB is induced under stress conditions, and the level of GB is correlated with the degree of enhanced tolerance to stress (Saneoka H *et al.*, 1995). The result showed the plant synthesized GB as exposing the stress higher than that in casual condition and all transgenic lines accumulated a6 to 7 times higher mount of GB than that of WT plant. Specicalilly, amount of GB in lines gained in drough circumstance higher than that in sality state.

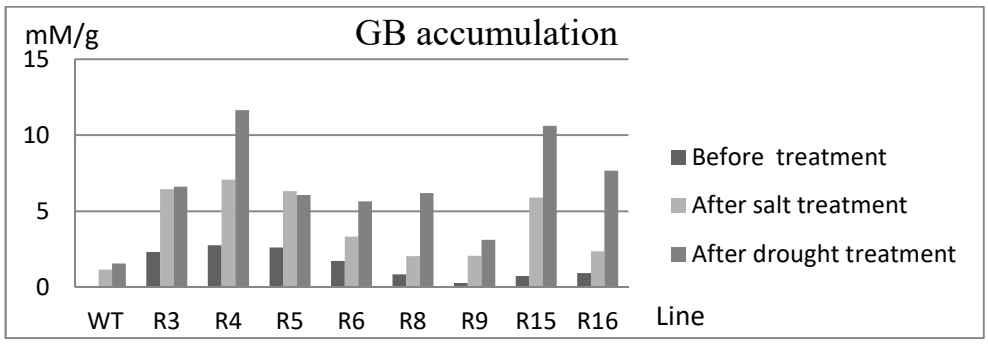


Figure 8. The GB accomulation of transgenic lines before and after stress

The level and duration of stress really had a significant effect on the complexity of the response (Pinheiro C and Chaves MM, 2011). The data showed the similarity with some other publications such as the results of Bui Van Thang *et al.* (2013) and Bui Thi Thu Huong *et al*. (2016). They had originally studied *CodA*transgenic tobacco lines which partly showed *in vitro* salt, drought and heat tolerance as culturing inmedium supplemented NaCl 250 mM, 2.5% PEG6000 or in high temperate conditions, 42°C. However, aGBamount of transgenic lines was only accumulatedfrom 0.73 to 2.91 mM/g. Therefore, it would be illutrated that Rd29A could effectively stimulate *CodA* gene expreess the function. For example, some Rd29A/*codA* transgenic lines such as R4, R15 and R16treated by artifical drought in 14 days, get 11.654, 10.625 and 7.674 mM/g GB, respectively.

Under stress conditions, the stress-inducible promoter Rd29A would increase glycinebetaine concentration in leaves, leading to stomata closure, reducing the osmotic potential and enhancing drought resistance in *Nicotiana tabacum*, however, under non-stress conditions, there would be no negative effects on plant growth and development (Alejandro C E-M *et al.*, 2015). In 1994, the *Rd29A* gene of *Arabidopsis* were already described and analyzed by Yamaguchi-Shinozaki and Shinozaki as induced promoter which regulated the response to dehydration, low

temperature, high salt or treatment with exogenous abscisic acid. The transcription of genes that hybridize to Rd29 cDNA was induced very rapidly and with a high rate at 20 min after the start of dehydration, and this transcription is followed by a second induction phase that begins after 3 - 4 hours of dehydration (Yamaguchi-Shinozaki *et al.* 1992). The levels of Rd29 mRNA change differently in response to dehydration, low temperature, salt stress, or exposure to ABA (Yamaguchi-Shinozaki and Shinozaki, 1993). Dehydration induces Rd29A mRNA with two-step kinetics. Rd29A promoter hadbeen studied that it could improve the activity Gus gene in droughtstress *in vitro* (Bui Van Thang *et al.*, 2012). The results of this research had illustrated that Rd29A promoter had increased the express of *CodA* gene with the higher number of GB accumulated in transgenic lines.

4. CONCLUSIONS

1. Recombinant PCAMBIA1301 vector carrying *CodA* gene regulated by Rd29A promoter that was successfully constructed and used to transfer to model plant, tobacco.

2. There were eight transgenic tobacco lines which had been demonstrated the presence of *CodA* gene, Rd29A promoter by PCR.

3. SomeRd29A/*CodA* transgenic tobacco lines expressed the tolerance to drought and salinity stresses and accumulated a high mount of GB after drought treatment.

Acknowledgment

This report was conducted under financial support by the project "Study on improvement of abiotic stress tolerance in soybean via genetic transformation", code VAST02.02/16-17.

REFERENCES

1. Alejandro C E-M, Chao, Ma, Michael S Reid, and Cai-Zhong Jiang (2015). Over expression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. Horticulture research, 2: 1-9.

2. Babak B, Akira K, Fevziye C, Mie K, Kazuko Y, Kazuo W. (2007). *Arabidopsis* Rd29A::DREB1A enhances freezing tolerance in transgenic potato. Plant Cell Reports [serial online]. 26(8):1275-1282.

3. Barunava P., Sudipta R., Andreas R., Arun L. M. (2010). Enhanced salt tolerance of transgenic tobacco plants by co - expression of PcINO1and McIMT1 is accompanied by increased level of myoinositol and methylated inositol, Protoplasma. 245(1-4):143-152.

4. Boyer JS (1982). Plant productivity and environment. Science, 218 (4571):443-448.

5. Bui Thi Thu Huong, Dong Huy Gioi, Bui Van Thang, Le Van Son, Chu Hoang Ha (2016). Development of *Arthrobacter globiformisCodA* gene in improving plant resistances to disadvantageous conditions. International conference on Agriculture development in the context of international integration-Opportunities and challenges: 203- 215.

6. Bui Van Thang, Le Van Son, Chu Hoang Ha (2013). Study on creating tobacco (*Nicotiana tabacum L.*) transgenic lines with *CodA*gene encoding *choline oxidase* which increased salt tolerance. National conference of Biotechnology: 1059-1063.

7. Bui Van Thang, Pham Thi Hang, Do Xuan Dong, Le Van Son, Chu Hoang Ha (2012). Study on the activity of the drought-resistant Rd29A promoter in transgenic ostriches (*Melia azedarach* L.). Journal of Science and Technology, VAST 3: 504-510.

8. Cong L, Zheng H, Zhang Y, Chai T. (2008). Arabidopsis DREB1A confers high salinity tolerance and regulates the expression of GA dioxygenases in Tobacco. Plant Science. February. 174(2):156-164.

9. Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN (2008). Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. Science. 320(5878):942-945.

10. Grieve CM. and Grattan SR. (1983). Rapid assay for the determination of water soluble quaternary ammonium compounds. Plant Soil, 70: 303 - 307.

11. La O A., Bambang S., Purwoko, Ahygad J., Oteng HIS.(2012). Pendugaan toleransi padi hibrida terhadap kekeringan dengan polyetilen glikol (PEG) 6000. J. Agrivigor, 11(2): 292 - 299.

12. Liu JX, Howell SH (2010). Endoplasmic reticulum protein quality control and its relationship to environmental stress responses in plants. Plant Cell. 22(9):2930–2942.

13. Lobell DB, Schlenker W, Costa-Roberts J (1980). Climate trends and global crop production since. Science. 2011; 333 (6042):616-620.

14. Mie K., Setsuko M., Kazuo S., Kazuko Y-S., (2004). A Combination of the *Arabidopsis* DREB1A Gene and Stress-Inducible Rd29A Promoter Improved Drought- and Low-Temperature Stress Tolerance in Tobacco by Gene Transfer. Plant Cell Physiol. 45(3): 346-350.

15. Pinheiro C, Chaves MM (2011). Photosynthesis and drought: can we make metabolic connections from available data. J Exp Bot. 62(3):869-882.

16. Quan R.D., Shang M., Zhang H., Zhang J. (2004). Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize, Plant Sci. 166, 141-149.

17. Rathinasbapathi B, Burnet M, Russell BL, Gage DA, Liao PC, Nye GJ, Scott P, Golbeck JH, Hanson AD (1997) Choline monooxygenase, an unusual iron-sulfur

enzyme catalyzing the first step of glycine betaine synthesis in plants: Prosthetic group characterization and cDNA cloning. Proc Natl Acad Sci USA. 94 (7):3454-3458.

18. Rhodes D, Hanson AD (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. Annu Rev Plant Physiol Plant MolBiol 44:357–384.

19. Sakamoto A, Murata N (2000). Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. J Exp Bot 51:81-88.

20. Sakamoto A., Murata N. (2002). The role of glycine betaine in the protection of plants from stress: clues from transgenic plants, Plant Cell Environ. 25: 163-171.

21. Saneoka H, Nagasaka C, Hahn DT, Yang W-J, Premachandra GS, Joly RJ, Rhodes D (1995). Salt tolerance of glycinebetaine-deficient and -containing maize lines. Plant Physiol 107:631-638.

22. Serraj R., Sinclair T.R (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions. Plant Cell Environ. 25: 333-341.

23. Subbarao G.V., Nam N.H., Chauhan Y.S., Johansen C. (2000). Osmotic adjustment, water relations and carbohydrate remobilization in pi- geonpea under water deficits, J. Plant Physiol. 157: 651-659.

24. Topping J. S. (1998). Tobacco transformation. Methods of molecular biology, Plant virology protocols, 81: 365 - 372.

25. Vincent D, Ergul A, Bohlman MC, Tattersall EA, Tillett RL, Wheatley MD, Woolsey R, Quilici DR, Joets J, Schlauch K, Schooley DA, Cushman JC, Cramer GR (2007). Proteomic analysis reveals differences between *Vitisvinifera* L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. J Exp Bot. 58(7):1873-1892.

26. Wahid A., Gelani S., Ashraf M., Foolad M.R. (2007) Heat tolerance in plants: an overview, Environ. Exp. Bot. 61: 199- 223.

27. Wang XY., Shan X Y., Yang A F., Zhang J Z. (2003). Construction of plant expression vector and analysis herbicide resistance and salt tolerance of transgenic tobacco. China Journal of Agricultural Biotechnology, 1(2): 85 - 91.

28. Wu Y., H. Zhou, Y.-X. Que, R.-K. Chen, M.-Q. Zhang. (2008). Cloning and identification of promoter Rd29A and its application in sugarcane drought resistance. Sugar Tech (2008) 10(1): 36-41.

29. Yamaguchi-Shinozaki, K., and Shinozaki, K. (1993). Characterization of the expression of a desiccation-responsive Rd29 gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. MOI. Gen. Genet. 236: 331-340.

30. Yamaguchi-Shinozaki, K., Koizumi, M., Urao S., and Shinozaki, K. (1992). Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis thaliana*: Sequence analysis of one cDNA clone that encodes a putative trans-membrane channel protein. Plant Cell Physiol. 33: 217-224.

ĐÁNH GIÁ SỰ BIỂU HIỆN CỦA GEN *CODA* ĐƯỢC ĐIỀU KHIỂN BỞI PROMOTER RD29A TRONG CÂY THUỐC LÁ

Bùi Thị Thu Hương[1], Đồng Huy Giới[1], Ngô Mạnh Dũng[2,3], Phạm Bích Ngọc[2], Chu Hoàng Hà[2]

[1]Học viện Nông nghiệp Việt Nam

[2]Viện Hàn lâm Khoa học và Công nghệ Việt Nam

[3]Đại học Thái Nguyên

TÓM TẮT

Những yếu tố bất lợi của môi trường ảnh hưởng nghiêm trọng đến sinh trưởng phát triển và năng suất cây trồng. Tuy nhiên, một số thực vật có khả năng chống chịu điều kiện bất lợi này do chúng có chứa một số yếu tố có thể tăng cường khả năng chống chịu của thực vật. Nghiên cứu này nhằm đánh giá sự biểu hiện của gen *CodA* mã hóa *choline oxidaza*, dưới sự kiểm soát của promoter cảm ứng hạn Rd29A. Kết quả cho thấy, vector chuyển gen *CodA* điều khiển bởi promoter Rd29A đã được tạo ra thành công và chuyển vào cây mô hình thuốc lá qua chủng vi khuẩn *C58 Agrobacterium tumefaciens*. Có 8 dòng chuyển gen (3, 4, 5, 6, 8, 9, 15, 16) đã xác nhận thành công sự hiện diện của gen *CodA*, Rd29A bằng phương pháp PCR sử dụng mồi cặp đặc hiệu và thực hiện khảo nghiệm khả năng chịu điều kiện bất lợi *in vitro*. Một số dòng thuốc lá chuyển gen Rd29A/*CodA* cho thấy có khả năng chống chịu muối và hạn *in vitro* (trong môi trường nhân tạo có bổ sung NaCl 250 mM hoặc 2,5% PEG6000) và đã tích lũy lượng Glycine betain cao hơn gấp 6-7 lần so với chúng khi ở trạng thái bình thường.

Từ khoá: CodA, glycinebetaine, khả năng chống chịu bất lợi, promoter rd29A, stress nhân tạo.

Received : 02/6/2019
Revised : 20/9/2019
Accepted : 27/9/2019