

The *in vitro* effects of CoCl₂ as ethylene synthesis inhibitor on PI based protein pattern of potato plant (*Solanum tuberosum* L.)

Marzieh Taghizadeh and Ali Akbar Ehsanpour*

Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran

Received 10 August 2013

Accepted 14 September 2013

Abstract

The effect of CoCl₂ as an ethylene synthesis inhibitor on changes of protein pattern was investigated in potato (*Solanum tuberosum* L.) plants cultivar White Desiree. In vitro grown plants were subjected to MS medium containing 0, 5, 10, 15, 20, 30, 40 and 60 mg/l CoCl₂ for 4 weeks. Different concentrations of CoCl₂ showed significant effect on the total soluble proteins. Among different concentrations of CoCl₂, using 20 mg/l CoCl₂ was the best concentration to inhibit ethylene formation and induce potato plant growth. Application of CoCl₂ in the culture medium changed the total protein as well as SDS-PAGE and Iso-electric Point Electrophoresis (PI) patterns. Protein pattern in potato tuber did not show any detectable changes.

Keywords: potato, CoCl₂, ethylene, protein pattern

Introduction

The potato (*Solanum tuberosum* L.) is one of the most valuable crop species belonging to the Solanaceae family (Orczyk et al., 2003). The growth and development of potato is sensitive to accumulation of ethylene under *in vitro* culture condition. Ethylene (C₂H₄) is a simple Plant growth regulator which is involved in the regulation of many aspects of plant growth and development and plays a major role in the ripening of climacteric fruits, plant defense, abscission (Dupille et al., 1993). Accumulation of ethylene in *in vitro* culture induces growth abnormalities such as production and development of stoloniferous shoots, small leaves and root generation from stem during short and long-term period tissue culture of potato explants (Sarkar et al., 2002; Sarkar et al., 1999). The negative effects of ethylene on plants under *in vitro* culture can be controlled using cobalt chloride (CoCl₂) as an ethylene inhibitor biosynthesis. The ethylene biosynthesis pathway is often started from methionine (Met) and then produces S-adenosylmethionine (SAM), 1-aminocyclopropane-1-carboxylic acid (ACC) and finally ethylene (Adams and Yang, 1979). The final step in the biosynthesis of ethylene is catalyzed by an ethylene-forming enzyme or ACC oxidase, which is responsible for the conversion of ACC to ethylene

(Yang and Hoffman, 1984). ACC oxidase is a member of the ferrous ion-dependent family of non-haeme oxygenases (Barlow et al., 1997). Ethylene inhibitors can be divided into two categories. The first one refer to those acting on the ethylene receptors, such as AgNO₃ and the second one refers to ethylene biosynthesis, such as CoCl₂. Cobalt is an essential element for humans and animals. In plants, it is not essential but beneficial for their growth. Excess CO is also toxic to plants (Nagpal, 2004).

Proteins are compounds of fundamental importance for all functions in the cell (Dose, 1980). It is well known that alteration of gene expression is always involved in plants under specific culture condition. Protein variation is an essential part of plant response to stress as well as for adaptation to environmental conditions (Hieng et al., 2004). Proteins are final products of informational pathways in cells that produce in response to cellular needs and transfer to proper locations in cells. Previous studies demonstrated that application of STS and Nano silver (Rostami and Ehsanpour, 2009) on potato (*Solanum tuberosum* L.) prevented the ethylene accumulation, and changed the protein pattern were detected by SDS-PAGE. However, so far information about changes in protein pattern of potato plants using SDS-PAGE and PI is not available. The present study was carried out to understand how potato plant cell dose react to inhibition of ethylene biosynthesis and presence of cobalt in the plant.

*Corresponding author E-mail:
ehsanpou@sci.ui.ac.ir

Materials and Methods

Plant material and culture conditions

Potato explants (*Solanum tuberosum* L.) cultivar White Desiree was propagated on MS medium (Murashig and Skoog, 1962) supplemented with silver thiosulfate (STS, 50 μ M), agar (1% w/v), sucrose (3% w/v) and pH 5.8. Auxiliary buds from in vitro propagated plants were transferred to the above mentioned medium containing 0 (control), 5, 10, 15, 20, 30, 40 and 60 mg/L CoCl₂ without STS. All cultures then were kept in the culture room with a 16/8 hour (light/dark) photoperiod at 25 \pm 2°C for 4 weeks.

Leaf protein extraction

Approximately 0.2 gram of fresh stem-leaf from 4-week-old plants and potato tuber were homogenized in liquid nitrogen, then protein was extracted using extraction buffer (50mM Tris- HCl, 1mM DTT, 2mM EDTA, 2mM 2-Mercaptoethanol, pH 7.5. For extraction of proteinsm buffer was modified as 1mM PMSF at pH 7.2 according to the method described by Amini et al., (2007). For separation of proteins based on their PI, total extracted proteins then were precipitated and purified in a buffer with single pH ranging from 2 to 10, based on Patent No. 89/4217, Tehran, Iran.

The concentration of total soluble proteins from leaf samples were determined according to modified Bradford (1976) method, using bovin serum albumin (BSA) as standard. SDS-PAGE and PI were performed using 12% separating and 5% stacking gels. After electrophoresis at 100V, protein bands were stained using Coomassie brilliant blue and silver nitrate and finally relative density of protein bands with remarkable changes was analyzed.

All experiments were carried out in three replications. Data were subjected to ANOVA and the mean differences were compared by Duncan test at P<0.05.

Results

The effect of CoCl₂ on total protein level

Increase at protein level of leaf-stem at concentrations of 20, 30, 40 and 60 mg/L CoCl₂, compared to the control samples, was observed (Fig 1). It was shown that concentration of 20 mg/L CoCl₂ increased protein content compared to the other treatments.

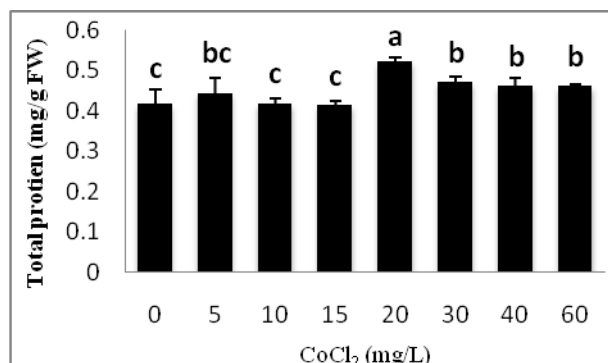


Fig. 1. The effect of CoCl₂ on the total protein level of leaf-stem of potato plants. Uncommon letters indicate the significant differences (P<0.05).

The effect of CoCl₂ on protein patterns production in leaf-stem of potato by PI and SDS-PAGE:

The total protein of explants in leaf-stem parts and potato plants in concentrations of 0 and 20 mg/L CoCl₂ were extracted and used for electrophoresis by SDS-PAGE and PI.

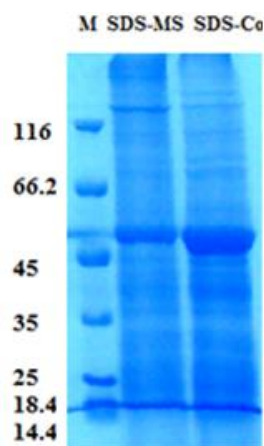


Figure 2. The SDS-PAGE (pH 7) protein pattern extracted from stem-leaf treated with CoCl₂ (+Co) and untreated (-Co), M: Marker

Proteins pattern from stem-leaf using SDS-PAGE revealed no difference between treated and untreated plants. However, there were obvious differences in either severity of some protein expressions (both increase and decrease) or expression of some proteins under experimental conditions. In electrophoresis by PI method, the protein solutions resulted from two optimized concentration of 0 and 20 mg CoCl₂ (data not shown) at pH ranging from 2 to 12, were separated and protein precipitated at a specific pH was loaded

on the SDS-PAGE gel. The intensity of protein bands in acidic and some neutral pH were more than that of protein bands in alkaline pH. As shown in figure 2, the intensity of protein bands in pH 2 was lower compared to the other pH. There was no counterparts for band 1 (Approx.116 KD), band 2 (Approx. 70KD) and band 3 (Approx. 65KD) in pH 2 in the control as well as 20 mg/L CoCl₂. However, the expression intensity of band 4 (Approx. 50KD) at 20 mg/L CoCl₂ was increased compared to the untreated plants as shown in Figure 2. When proteins were separated using PI, at pH 3, the intensity of protein bands compared to pH 2 was increased. Moreover, band 2 and band 3 were observed in cobalt treated plants compared to the control plants (Figure 3 and 4).

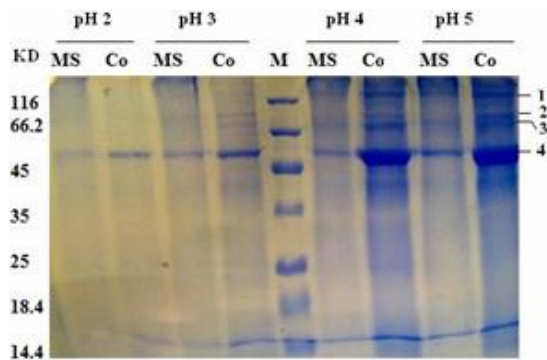


Fig. 3. The effect of CoCl₂ on protein pattern at pH 2, 3, 4, and 5 (MS: control, Co: 20 mg/l CoCl₂)

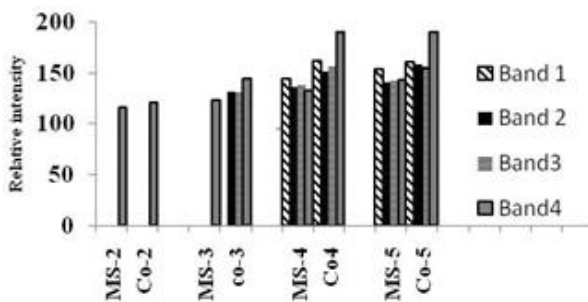


Fig. 4. The effect of CoCl₂ on relative intensity of protein bands at pH: 2, 3, 4 and 5

There was an increase in intensity of protein bands at pH 4 and in particular pH 5 compared to the other pH. Band 1, band 2, band 3, and band 4, had more intensity in treated plants with cobalt. In this pH, also, more bands were observed compared to the control and other pH. At pH 6 and pH 7 (Figure 5 and 6), band 1 and band 4 in cobalt treated plants had higher expression levels and bands 2 and 3 were absent (Fig. 4 and 5). At pH 10-12 (Figure 7), there

was a considerable decrease in protein bands. At higher alkaline pH, the intensity of protein bands decreased more. Bands 2 and 3 were absent and the intensity of band 1 did not change significantly

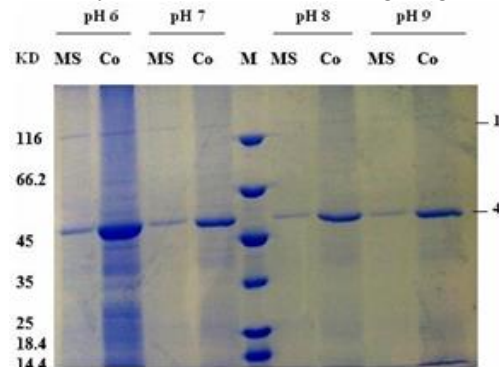


Fig. 5: The effect of CoCl₂ on protein patterns pH: 6, 7, 8 and 9 (MS: control, Co: 20 mg/l CoCl₂).

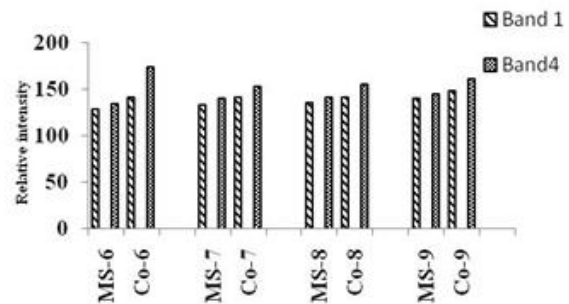


Fig.6. The effect of CoCl₂ on relative intensity of protein bands at pH 6, 7, 8 and 9.

compared to the controls (Figure 8). However, the intensity of band 2 at 20 mg/l CoCl₂ increased much higher compared to the controls. As figure 9 shows when protein pattern of the tuber were analyzed by SDS PAGE, no obvious changes were observed either in treated or untreated plants with cobalt.

Discussion

Treatment of potato plants with cobalt induced some changes in total soluble proteins of stem-leaf explants of potato cultivar White Desiree. In our experiments 20, 30, 40, 60 mg/L CoCl₂ increased protein content of the potato leaf and stem. The protein content changes might be due to the ethylene biosynthesis inhibition or as a result of changes in physiology and metabolism of potato plant cells responded to cobalt as a heavy metal (Clemens, 2006). We need to confirm the possible cobalt function in details in the future.

If looking at the cobalt as a heavy metal, cobalt can change the total protein by altering the expression level of some proteins to protect plant

cells against toxicity effects. Normally, organisms apply several ways to detoxify heavy metals. One of

expression in details in the plant cells exposed to heavy metals. In the present study for the first time

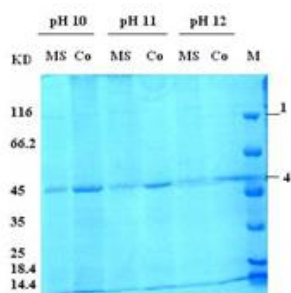


Fig. 7. The effect of CoCl₂ on protein patterns at pH 10, 11 and 12 (MS: control, Co: 20 mg/l CoCl₂).

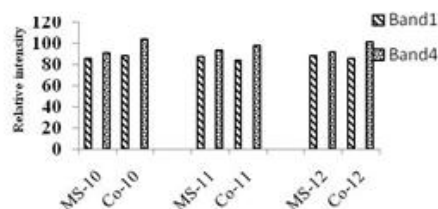


Fig. 8. The effect of CoCl₂ on relative intensity of protein bands at pH 10, 11 and 12.

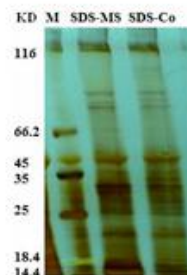


Fig. 9. The effect of CoCl₂ on potato tuber protein pattern using SDS-PAGE. (MS: control, Co: 20 mg/l CoCl₂).

the more general ways is to synthesize cyctein-rich proteins and peptides known as phytoaktines (Clemens, 2006). Whether the increased proteins in the present study include phytoaktines is increased needs to be studied in the future. Furthermore, the production of reactive oxygen species (ROS) is one the biochemical changes due to the heavy metals (e.g. cobalt) responses to plant (Cho and Park, 2000). Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase and catalase (CAT) play an important role in defending these toxic compounds (Gupta et al., 2002).

When proteins are separated on the basis of molecular weight using SDS PAGE, distinctive protein bands were revealed on the gel. It has been reported that, heavy metals including (cobalt) are able to change the plant protein production patterns (Ewais, 1997). The bands obtained by this way may be representative of a series of proteins with more or less similar molecular weight only. In other words, SDS PAGE does not show any other information from the separated proteins on the gel. In contrast, two dimensional electrophoresis (2DE) method illustrates more details of the proteins. In fact by using 2DE it is possible to identify a single protein spot and discuss the actual changes of protein

we are presenting some data in which proteins first separated based on the specific PI and the isolated proteins were loaded on the SDS-PAGE gel. In fact this method of isolation is simple 2DE with some differences with the actual 2DE, protein bands in this way show 2 characters including molecular weight as well as PI. In the present study protein bands were separated within ranges of pH from 2 to 10. In this study a few protein bands in particular those with approximately 55 kD, were low in acidic PI while much higher in basic PI. Also the molecular weight of particular this protein almost was constant in the pH 2-10 but whether this is a single protein, separated in different ranges of pH or different proteins with similar molecular weight is unknown. Since, large subunit of RuBisCo enzyme is about 55 KD (Dhingra et al., 2004), it can be suggested that protein band with 55 KD might be RuBisCo enzyme.

The protein pattern of the potato tuber on SDS-PAGE did not reveal any change, suggesting no effect or very low effect of cobalt on tubers. In our previous data, we found that no cobalt was detectable in potato tuber tissue (data not shown). Consequently, it is acceptable to assume that cobalt has no effect on protein changes in the tuber.

Acknowledgment

The authors gratefully acknowledge the University of Isfahan for their support.

References

1-Adams D. O. and Yang S. F. (1979) Ethylene

biosynthesis – identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Sciences* 76: 170-174.
 2- Amini F., Ehsanpour A. A., Hoang Q. T. and Shin J. Sh. (2007) Protein pattern changes in tomato under *in vitro* salt stress. *Russian Journal*

- of *Plant Physiology* 54: 464-471.
- 3- Barlow J. N., Zhang Z., John P., Baldwin J. and Schofield C. J. (1997) Inactivation of 1-aminocyclopropane-1-carboxylic acid involves oxidative modifications. *Biochemistry* 36: 3563–3569.
 - 4- Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal of Biochemistry* 72: 248-254.
 - 5- Brooks S. C., Herman J. S., Hornberger G. M. and Mills A. L. (1998) Biodegradation of cobalt–citrate complexes: Implications for cobalt mobility in groundwater. *Journal of contaminant hydrology* 32: 99-115.
 - 6- Cho U. H. and Park J. O. (2000) Mercury-induced oxidative stress in tomato seedlings. *Plant Science* 156: 1-9.
 - 7- Clemens S. (2006) Evolution and function of phytochelatin synthases. *Journal of plant physiology* 163: 319-332.
 - 8- Dhingra A., Portis A. R, Daniell H. (2004). Enhanced translation of a chloroplast-expressed RbcS gene restores small subunit levels and photosynthesis in nuclear RbcS antisense plants. *Proc. Natl. Acad. Sci. U.S.A.* **101** (16): 6315–20.
 - 8- Dose K. (1980) *Biochemie*. Springer Berlin, Heidelberg, New York.
 - 9- Dupille E., Rombaldi C., Lelievre J. M., Cleyet-Marel, J. C., Pech J. C. and Latche A. (1993) Purification, properties and partial amino-acid sequence of 1-aminocyclopropane-1-carboxylic acid oxidase from apple fruits. *Planta* 190: 65–70.
 - 10- Ewais E. (1997) Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. *Biologia Plantarum* 39: 403-410.
 - 11- Gupta M., Cuypers A., Vangronsveld J. and Clijsters H. (2002) Copper affects the enzymes of the ascorbate-glutathione cycle and its related metabolites in the roots of *Phaseolus vulgaris*. *Physiologia Plantarum* 106: 262-267.
 - 12- Hieng B., Ugrinovich K., Sustar-Vozlich J. and Kidric M. (2004) Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* cultivars differing in sensitivity. *Journal of Plant Physiology* 161: 519-530.
 - 13- Murashige T. and Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.
 - 14- Orczyk W., Przetakiewicz J. and Nadolska-Orczyk A. (2003) Somatic hybrids of *Solanum tuberosum*—application to genetics and breeding. *Plant Cell, Tissue and Organ Culture* 74: 1-13.
 - 15- Reeves R. D., Baker A. J. M., Borhid A. and Berazain R. (1999) Nickle hyperaccumulation in the serpentine flora of Cuba. *Annals of Botany* 83:29-38.
 - 16- Rostami F. and Ehsanpour A. (2009) Application of Silver Thiosulphate (STS) on silver Accumulation and protein pattern of potato under *in vitro* culture. *Malaysia Application of Biology* 38: 49-54.
 - 17- Sarkar D., Kaushik S. and Naik P. (1999) Minimal growth conservation of potato microplants: silver thiosulfate reduces ethylene-induced growth abnormalities during prolonged storage *in vitro*. *Plant Cell Reports* 18: 89-93.
 - 18- Sarkar D., Sud K. C., Chakrabarti S. K. and Naik P. S. (2002) Growing of potato microplants in the presence of alginate- silver thiosulphate capsules reduces ethylene- induced culture abnormalities during minimal growth conservation *in vitro*. *Plant Cell, Tissue and Organ Culture* 68: 79-89.
 - 19- Yang S. F. and Hoffman N. E. (1984) Ethylene biosynthesis and its regulation in higher plants, *Annu. Rev. Plant Physiol* 135: 155–189.