World Applied Sciences Journal 14 (Special Issue of Food and Environment): 67-71, 2011
ISSN 1818-4952

Effect of Ethylene on Potato Sprouts and NADPH Activity

[1]M. Pakizeh, [2]A.A. Kuliev, [3]Z.M. Mammadov, [4]M. Ardjmand and [5]M. Hasani

[1]Department of Biochemistry and Biotechnology, Baku State University, Baku Azerbaijan
[2]Biochemistry and Biotechnology, Baku State University, Baku Azerbaijan
[3]Department of Biochemistry and Biotechnology, Baku State University, Baku Azerbaijan
[4]Department of Chemical Engineering, Tehran South Branch, Islamic Azad University, Tehran, Iran
[5]Department of Chemical Engineering, Faculty of Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract: Treatment of potato tubers *(Agria*) with ethylene gas causes a rapid rise in their respiration rate, reaching 5 to 10 times the rate of untreated tubers over 24 hours of treatment and then falling slowly. The response shows a lag of 8 hours and more than 24 hours of exposure is required for the maximum effect. The optimum temperature is near 25°C. Rapid generation of reactive oxygen species (ROS) at the cell surface has been implicated in plant defense responses. Genetic evidence indicates that a plant NADPH-oxidase is associated with oxidative burst. Additionally, NADPH-dependent O_2-generating activities in plasma membrane fractions were diphenylene iodonium-sensitive and NaN3-in sensitive.

Key words: Agria · Diphenylene iodonium · Ethylene · Carbon dioxide · NADPH · Activity

INTRODUCTION

For many years ethylene has been known to regulate plant growth and development. This response is also induced by other compounds such as propene, 1-butene, carbon monoxide, acetylene and isocyanides [1] reported the ability of many different compounds to induce an ethylene response in plants. Ethylene in plant organic matter can be produced L - methionine [2]. Methionine is activated by ATP and chemical interactions through proximity effect (action synthetase) adnuzy- L - methionine (EC 2.5.1.6) element S - adnuzy - L - methionine is formed. The first step in a protein free amino acid, 1-amino cyclo Propane - 1 - carboxylic acid (ACC) is produced [3]. This element synthesis ACC action pyroksidal phosphate as catalyst is a factor. ACC element formation rate limiting step is the ethylene biosynthesis [4]. Ethylene is a plant hormone having diverse effects on a wide range of plant tissues. In spite of its apparent importance As a regulator of plant growth, the site and mechanisms of its actions have not yet been elucidated [5, 6]. Ethylene evolution is observed under many situations, including mechanical wounding, hypoxia, environmental pollution and invasion by pathogens. The unique properties of ethylene have been discovered to naturally inhibit sprouting in potatoes. The careful introduction of trace elements of ethylene into the refrigerated potato store can delay the sprouting process significantly.

Glucose-6-phosphate dehydrogenase (G-6-PDH) (EC1.1.1.49) is the first enzyme in the pentose phosphate pathway. Several lines of evidence indicate that the NADPH oxidase seems to play a pivotal role in defense responses. Diphenylene iodonium (DPI), an inhibitor of the neutrophil NADPH oxidase, blocks the oxidative burst in plant cells. In the presence of the coenzyme-nicotinamide adenine dinucleotidephosphate (NADP+) and/ or NAD+ it catalyses the oxidation of glucose-6-phosphate (G-6-P). The determination of molecular weight and dimension of G-6-PDH from leuconostoc mesenteroides is well reported in the literature [7, 8, 9].

The aim of the present study was to consider the effect of ethylene concentration and temperature on germination of potatoes. The effect of repeated treatments and recovery of tubers from initial ethylene treatments has also been studied.

Corresponding Author: M. Pakizeh, Department of Biochemistry and Biotechnology, Baku State University, Baku, Azerbaijan. Tel: +982166975124.

MATERIALS AND METHODS

In one experiment potato tubers *Agria* were harvested at Hamedan-Razan, Iran, 120 days after planting and cleaned with a soft brush. Triplicate samples, averaging 2.7 kg in weight and consisting of 15 carefully selected tubers, were placed in 25-liter metal chambers, which were ventilated continuously with about 12 liters air/hr. All operations were conducted in rooms controlled at 20°C The relative humidity in the chambers was 58 to 63%. After 3 days storage (during which time the respiration rate stabilized), the tubers were treated for 72 hr with ethylene or ethylene chlorhydrin. The ethylene, in concentrations of 0, 0.15, 0.3, 3.0 and 15 µl/L, was supplied continuously in a gas stream from a pressure cylinder through a reduction valve and appropriate capillary flow-meters. The final concentrations of the gas were verified with flame-ionization gas chromatography. The rate of sprouting (Table 1), derived from the data of Figure 1, was determined according to the method of Harrington [10]. It was calculated as follows:

$$Rate = \frac{m_1t_1 + m_2t_2 + m_3t_3 + ... + m_nt_n}{m_1 + m_2 + m_3 + ... + m_n}$$

Where m_1 equals to the number of tubers sprouted at time t_1 and m_2 equals to the increase in number of sprouted tubers observed between t_1 and t_2. This formula gives a value for rate of sprouting in mean days and is the reciprocal X- 100 of Kotowski's formula [11]. In another experiment *Ajiba* potato tubers from the same region were used. After harvest the tubers were washed and dried in air. Later handling procedures were similar to those of the first experiment; 8 tubers weighing about 1.9 kg were used per sample. The effects of treatment for 72 hr with concentrations of 0, 1.5, 7.5 and 15 µl/L of ethylene were tested , as was a concentration of 2.2 µl/L ethylene applied for 0, 12, 36 and 72 hr. In addition, the effect of a continuous treatment with ethylene for 35 days was determined.

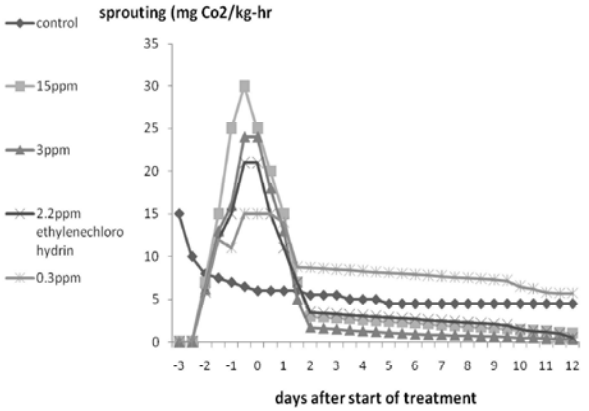

Fig. 1: Effect of various ethylene treatments for 72 hr on the respiration of Hamedan-Razan potatoes

For subcellular localization of NADPH oxidase, contained 0.2 mM carboxanilide-2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT), 15 µM ATP, 2.5 µM GTP (c) S, 0.02% (w/v)Triton X-110, 15.5 mM Tris-Hcl (pH 7.5) and a 20µl fraction with or without various inhibitors in a total volume of 0.5 ml. The reaction was initiated in a microcuvette at room temperature by addition of 75 µM NADPH or buffer for NADPH minus control and scanned by the change in A470 over 7 min in a dual-beam spectrophotometer.

RESULTS

The Effect of Ethylene Concentration: The respiration of potato tubers under continuous treatment in a static system at different concentrations of ethylene are shown in Figure 2.

Table 1: Effect of differinig concenttratiolis of ethylenie on rate of sprouttintg of 'Hamedan-Razan-Iran'Potato tubers (The values are averages of three samples)

		Sprouted		
Treatment (2days)	Days to 50% of Sprouting	After 15 days	Final	Rate Mean days
Air	27.5	8.5	67	29.6
Ethylene, 0.15, µl/l	19.5	18.3	60.5	24.5
Ethylene, 0.3, µl/l	14	26.9	81.7	28.3
Ethylene, 3, µl/l	12	68	90.6	19.6
Ethylene, 15, µl/l	18	45.25	80.3	22
Ethylenechlorhydrin	2.2	98.5	99	16.5

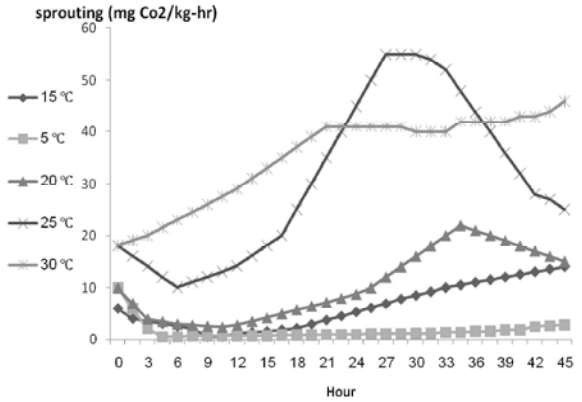

Fig. 2: Respiration of single potato tubers treated from zero time with 15 µl/l of ethylene for various numbers of hours at 20°C. Four other treatment times gave expected intermediate results, but the curves have been omitted for clarity

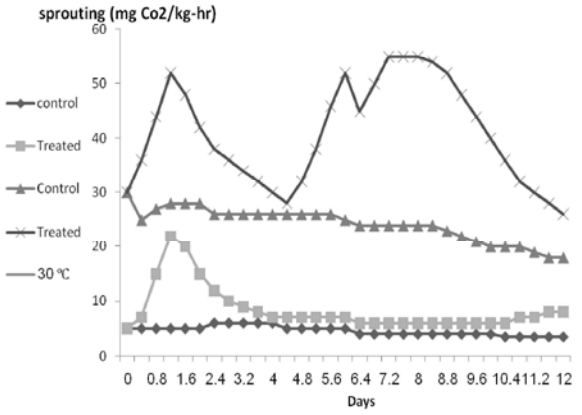

Fig. 3: Respiration of single potato tubers treated continuously with differing concentrations of ethylene at 20°C. The treatment "0.002 , µl/l " is the untreated control but reflects air pollution plus endogenous ethylene

Partial stimulation of respiration was observed in potatoes treated with 0.2 µl/L and full stimulation was observed with any concentration above 2.5 µl/L. The respiration maximum occurred at the same time for all concentrations of ethylene.

The Effect of Storage Temperature: The respiration results of potato tubers in static systems containing 15 µl/l ethylene at different temperatures are shown in Figure 3. Temperature had a striking effect on both the magnitude and the timing of the respiratory rise. The peak respiration increased with temperature to 25°C but was lower at temperatures above 25°C. The interval between the start of treatment and the respiration peak was

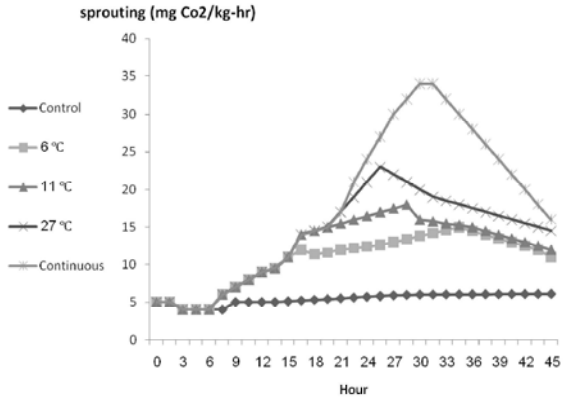

Fig. 4: Response of single potato tubers to 10 µl/l ethylene at different temperatures

reduced by increasing temperature. The respiration of tubers treated at 35°C did not fall markedly following the respiration peak.

The Effect of Repeated Treatments: The respiration response of potato tubers to alternate treatments with air and ethylene is compared with the climacteric-like induced response of oranges in Figure 4. The initial response was parallel for both tissues, but subsequent treatments had little effect on the respiration of potato tubers, whereas oranges responded to each reapplication of ethylene. The respiration rate of oranges was higher than that of potatoes at all times.

The Recovery of Tubers from an Initial Ethylene Treatment: The respiration rate of a large lot of potatoes treated with ethylene for 1 day and then returned to air. The respiration rate of the treated tubers returned to that of the control by about 4 to 5 days after the removal of ethylene. The respiration rate of individual tubers taken at intervals from the above lot and retreated with ethylene is shown also. Renewed application of ethylene slightly stimulated respiration of once-treated tubers as little as 3 days after cessation of the initial treatment. The respiration response to the second treatment gradually increased with extension of the recovery period, reaching about 67% of the initial response 21days after termination of the first treatment.

Intracellular Distribution of NADPH-Dependent O_2-generating Activities: Because treatment of potato tubers with HWC elicitor caused a massive oxidative burst 8-12 hr after treatment, intracellular distribution of NADPH-dependent O_2-generating activities from potato tubers treated with HWC elicitor for 8 hr were examined.

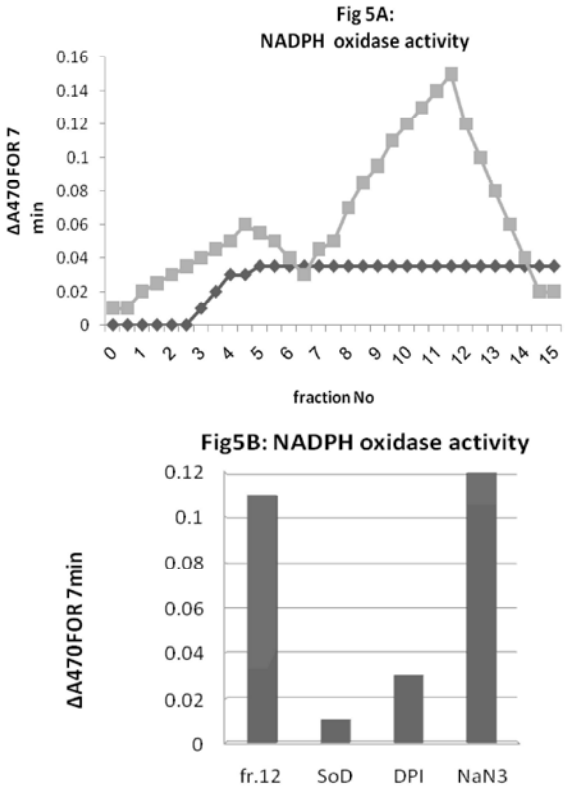

Fig. 5a,b: Subcellular distribution of NADPH oxidase activity in potato tissues. Microsomal proteins prepared from potato tuber tissues treated with HWC elicitor for 8 h were fractionated by sucrose density-gradient centrifugation. (A) Upper panel: O_2 -generating activities, with (closed circles) or without (open circles) NADPH, were assayed as XTT-reducing activity as described in the Materials and methods. Lower panels: the distribution of StrbohB proteins and marker proteins, such as PM aquaporin for the plasma membrane, Bip for ER and vacuolar membrane ATPase A subunit, was assayed by immunoblotting using specific antibodies. (B) Effects of SOD (100 U/ml), DPI (40 µM) and NaN3 (10 mM) on XTT-reducing activity in fraction no. 12.

O_2-generation was detected by XTT-reducing activities. The distribution of NADPH oxidase activities was paralleled by the distribution of immune stained bands of StrbohB and PM aquaporin (Fig. 5A). NADPH oxidaseis DPI - sensitive and NaN3 (a general inhibitor of haemprotein) -insensitive. The effects of these inhibitors on the activity were confirmed using fraction no 12. According to Kobayashi *et al*. [12] and Sang *et al*. [13] ddition of 120 U/ml of superoxide dismutase (SOD) or 40 µM of DPI resulted in suppression of NADPH oxidase activity; on the other hand, 15 mM of NaN3 did not change the activity (Fig. 5B).

DISCUSSION

It is therefore unlikely that the respiration increase is due to a simple physical effect of ethylene on gaseous exchange. The fact that a short treatment with ethylene will lead to a later partial rise in respiration also indicates that the lag phase is an active period during which the mechanism of the subsequent respiratory rises set in motion. The shape of the actual respiration curve suggests that following the lag period , there is an ethylene-induced but respiration-linked formation of some product which causes the eventual decrease in respiration when the product "feeds back" on its own synthesis. Alternatively, there may be a requirement for a preformed substrate and respiration decreases when the substrate is depleted. These hypotheses are supported by the apparent insensitivity of treated tubers to a closely subsequent ethylene treatment. In a resting tuber, the metabolic rate is so low that one could infer that the above postulated product or required substrate would be used or reformed slowly, thus explaining the long period required for recovery of the response to ethylene. The physiological observations reported here appear pertinent to studies on the role of ethylene in the climacteric rise in fruit respiration and in the phenomena associated with injury in plants such as the induced respiration of tissue slices. Based on the shape and timing of the respiratory curve which follows ethylene treatment of tissues, we have already suggested [14] that the climacteric rise in fruit respiration may be a respiratory response to ethylene common to many parenchymatous tissues in the presence of ethylene . The more detailed studies of the potato response reported here give further support to this hypothesis. The effect of temperature on the respiratory response of potato tubers under the influence of ethylene (Fig. 4) is strikingly similar to that reported by Biale and Young [15] for the respiration of ripening avocados, with a sharp optimum at 25°C and a reduced and modified response at higher temperatures. It is well known that plant tissues produce abundant ethylene when damaged [5]. Subsequently the respiration of the tissue increases, a phenomena on dubbed the wound response. As referred to above, Ca2+ in flux across the plasma membrane may contribute to the activation process of NADPH oxidase in the plasma membrane. Furthermore, it was confirmed that

the distribution of NADPH oxidase activities in the fractions from sucrose density-gradient centrifugation was paralleled by the distribution of immune stained bands of StrbohB and PM aquaporin (Fig. 5). These lines of evidence indicate tha tboth StrbohA and StrbohB proteins are localized on the plasma membrane. NADPH oxidase in phagocytic cells makes an enzymatic complex and comprises two membrane-associated proteins (p22phox and gp91phox, also known as Nox2) and cytosolic components (p67phox, p47phox and Rac2, a small G protein) [16]. When discs are cut from potato tubers, the respiration rate of these slices immediately increases to three to five times that of the intact tuber [17]. It is therefore possible that in this situation ethylene may be a "wound hormone;" the initial response of tissues to damage is production of ethylene which in turn induces the wound respiration and associated processes of repair. Thus the respiratory response of potato tubers to ethylene may well represent a normal physiological response of the tissue to a supposed injury signaled by ethylene treatment. The elucidation of the signal mechanism in this comparatively simple ethylene response may well be a useful tool for investigating the control that ethylene exercises in plant tissues.

REFERENCES

1. Martun M. Willams, 2002. Effect of Shoot Removal During Tuberization on Volunteer Potato (*Solanum tuberosum*) Tuber Production-Weed Technol., 16: 617-619.
2. Haase, T., C. Sch¨uler and J. He, 2006. The effect of different N and K sources on tuber nutrient uptake, total and graded yield of potatoes (*Solanum tuberosum* L.) for Processing, 24666: 113-117.
3. Kloosterman, B, and R.G.F. Visser, 2006. Isolation and characterization of a novel potato Auxin/Indole-3-Acetic Acid family member (StIAA2) that is involved in petiole hyponasty and shoot morphogenesis-Plant Physiology and Biochemistry.
4. Coleman, W.K. and J. McInerney, 1997. Enhanced dormancy release and emergence from potato tubers after exposure to a controlled atmosphere. American Potato J., 74: 173-182.
5. Streck, N.A. *et al*., 2006. Simulating the development of field grown potato (*Solanum tuberosum* L.) Agmet, 3633: 11-18.
6. Patel, N. and T.B.S. Rajput, 2006. Effect of drip tape placement depth and irrigation level on yield of potato Agwat, 2370: 15-19.
7. Qin, L., Y. Liu, T. Wang, S.J. Wei, M.L. Block, B. Wilson, B. Liu and J.S. Hong, 2004. Nadph oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J. Biol. Chem., 279: 1415-1421.
8. Ishaque, A., M. Milhausem and R.H. Levy, 1974. Biochem. Biophys. Res. Comm., 59: 894.
9. Olive, C. and R.H. Levy, 1979. The effect of temperature on the germination of sexveral kinds of vegetable seeds. XVIth Int. Hort. Congr., 2: 435-441.
10. Harrinton, J.F., 1962. The effect of temperature on the germination of sexveral kinds of vegetable seeds. XVIth Int. Hort. Congr., 2: 475-484.
11. Irena Rylski, Lawrence Rappaport and K. Harlan, 1974. Dual Effects of Ethylene on Potato Dormancy and Sprout Growth. Plant Physiol., 53: 658-662.
12. Kobayashi, M., K. Kawakita, M. Maeshima, N. Doke and H. Yoshioka, 2006. Subcellular localization of Strboh proteins and NADPH-dependent O_2-generating activity in potato tuber tissues. J. Experimental Botany, 57: 1373-1379.
13. Sang, Y., D. Cui and X. Wang, 2001. Phospholipase D and phosphatidicacid-mediated generation of superoxide in Arabidopsis. Plant Physiol., 126: 1449-1458.
14. Reid, M.S. and H.K. Pratt, 1970. Ethylene and the respiration climacteric. Nature, 226: 976-977.
15. Biale, J.B. and R.E. Young, 1962. The biochemistry of fruit maturation. Endeavour, 21: 164-174.
16. Ono, E., H.L. Wong, T. Kawasaki, M. Hasegawa, O. Kodama and K. Shimamoto, 2001. Essential role of the small GTPase Rac indisease resistance of rice. Proceedings of the National Academy of Sciences, USA, 98: 759-764.
17. Kawasaki, T., K. Henmi, E. Ono, S. Hatakeyama, M. Iwano, H. Satoh and K. Shimamoto, 1999. The small GTP-binding protein Rac isa regulator of cell death in plants. Proceedings of the National Academy of Sciences, USA, 96: 10922-10926.