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Effect of thiourea on potato contents of carotenoids, polyphenols ascorbic acid and nitrates

F. MANI [1] *
A. MANI [2]
T. BETTAIEB [3]
C. HANNACHI [1]

[1] High Agronomic InstituteChott- Mariem. Tunisia.
[2] Laboratory of metabolic biophysics and applied pharmacology, Faculty of Medecine , Sousse, Tunisia
[3] National AgronomicInstituteof Tunisia.

Corresponding author: ferdaousmani78@yahoo.fr

Abstract - Thiourea (250, 500, 750 and 1000 mM) was tested as seed- potato soaking before planting. The results of the field experiment showed that seed soaking with thiourea (500 mM) tended to improve carotenoids content (5,3 mg/kg over control). At 250 and 1000 mM, thiourea increases also significantly polyphenols (433 and 426 mg/kg respectively). Besides, regardless of the level of applied thiourea, the content of ascorbic acid has improved (13,9-18,3 mg/kg, over control = 11,4 mg/kg). It was further noted that application of thiourea at high levels (750 and 1000 mM) decreases significantly nitrates tubers content (191 and 183 mg/kg respectively), while at low levels (250 mM) nitrates content was increasing (252 mg/kg) over control (241 mg/kg).

Key words : Potato / thiourea / ascorbic acid / nitrates / carotenoids.

1. Introduction

Potatoes are the most consumed vegetable and an under estimated source of antioxidant phytonutrients including phenolic com- pounds, anthocyanins, and ascorbic acid that can play a major role in maintaining human health. Antioxidants may protect against degenerative diseases such as diabetes, cardiovascular diseases, and cancer that can be initiated by cellular damage resulting from excessive reactive oxygen species (ROS) that disrupt nucleic acids, proteins, and lipid molecules (Liu *et al.* 2006 ; Hale *et al*. 2008). Potato has been also ranked as the third most important source of phenolics in the American diet after apples and oranges (Hwang *et al.* 2011). Furthermore potatoes are an important source of total phenolics and antioxidants, (Thompson *et al.* 2009; Navarre *et al.* 2011). In fact, phenolics are a large group of small molecular weight compounds that determine organoleptic properties and con-tribute antioxidant activity. Caffeic acid derivatives, particularly chlorogenic acid, are themain phenolic constituents in potatoes and can contribute approximately 90 % of the total phenolic content (Wegener and Jansen 2011 ; Nassar *et al.* 2013). So potatoes contribute to the daily intake of polyphenols and their consumption, thereby, may have positive effects on health (Dusser *et al.* 2012 **;** Miranda *et al.* 2013). Tuber content of antioxidant compounds is affected by genotype, agronomic factors, post-harvest storage, processing conditions, and environmental factors (Reddivari *et al.* 2007 ; Ezekiel *et al.* 2013).

Potato generally has greater amounts of vitamin C on a fresh mass (FM) basis than apple, carrot, cucumber, grape, lettuce, squash, sweet corn, and tomato (Novy *et al.* 2008). Several wild accessions were identified with greater antioxidant potential assessed as ascorbic acid (Blessington *et al*, 2010 ; Tajner – Czopeck *et al.* 2012 ; Love and Pavek 2008). Besides, nitrates content is an important quality characteristic of vegetables. Nitrate itself is relatively non-toxic but its metabolites may produce infantile methaemoglobinaemia, carcinogenesis and possibly even teratogenesis (Santamaria 2006).

Exogenous app lication of various synthetic organic compounds such as Thiourea has great implications in changing plant growth both under normal and stressful conditions (Srivastava *et al.* 2009; Anjum *et al.* 2011).
Applied thiourea positively interacted with N and P in improving net photosynthesis, Chl, starch, proteins contents, nitrate reductase activity, dry matter yield and harvest index in cluster bean (Burman *et al.* 2007).
The external use of thiourea may provide an attractive opportunity to determine varietal responses and roles of thiourea in improving potato quality. Few studies indicated that modulation of plant growth is strongly dependent on the plant growth stage and dose of thiourea applied (Sahu and Singh 1995). This study is based on previous one, where thiourea improved yield and cholrophyll content of potato tubers (Mani 2012 ; Mani *et al.* 2012 ; Mani *et al.* 2013). The objectives of this study were to find out the optimum thiourea concentration and intra-specific response of potato to mother tuber-applied thiourea based on changes in composition of ascorbic acid, nitrates carotenoids and polyphenols.

2. Materials and methods

2.1. Vegetal Material and crop management

The experiments were conducted in automn season (Ocober–January) of 2011 and 2012. Potato seeds (*Solanum tuberosum* L.) varietie (Spunta) were obtained from the High Agronomic Institut of Chott-Mariem (Sousse, Tunisia) (ISA Chott-Mariem).
Selection of treatments was made based on the information from previous studies involving the exogenous application of thiourea. For this purpose, two preliminary experiments were performed using low range (50–500 mM) and high range (1500–2000 mM) of thiourea (Bajji *et al.* 2007). No plant survival was found in the high range, and these rates appeared to be growth inhibitory. However, lower levels (50–500 mM) of thiourea in low range improved growth; while 1500 mM thiourea was lethal to most of the potato tubers. Thus, 250–1000 mM thiourea levels were selected for the current experiments.
Potato tubers (variety Spunta) are dipped in solutions of thiourea (0, 250, 500, 750 and 1000 mM) before planting in soil. Experimental design was completely randomized block with three replications for each treatment and each concentration. Inter

row and inter plant spacing were 0,80 and 0,30 m, respectively. Data were collected from twenty one plants for each treatment and each concentration. The irrigation water has a conductivity of 1,4 mS. cm-1 and a pH of 6,2. The chemical composition of water, expressed in meq.l^{-1}, is as follows: Ca 2^+ (7,4), K^+ (0,1), Na^+ (4,9) and Cl^- (5,9). The soil is composed of clay (11,5%), silt (22,5%), sand (61%) and organic matter (1%). Its pH is of 7,6. The amounts of mineral fertilizers and organic, recommended in the area of Chott-Mariem for culture of potato (Hannachi *et al.* 2004; Chehaibi *et al.* 2008) and used in our tests, are farmyard manure (30 $t.ha^{-1}$), triple super phosphate (P_2O_5 45% :150 $kg.ha^{-1}$), and potassium sulphate (K_2O 54% : 400 $kg.ha^{-1}$), they were used as P and K sources respectively. Theses fertilizers are incorporated in soil before planting. One month after planting, potassium sulphate (K_2O 54%: 400 $kg.ha^{-1}$) and ammonium nitrate (NH_4 NO_3); (N 33% :100 $kg.ha^{-1}$) are also incorporated. During the culture period, from October 10 to January 26, monthly weather records set each day give minimum of 9°C and maximum of 21 ° C maxima of temperature regime.

2.2. Measured parameters

Measured parameters are carotenoids, polyphenols, ascorbic acid and nitrates content.

2.2.1. Determination of carotenoids

Potato chips were prepared from 15 g of average sample (four potato tubers) and left to freeze in closed Petri dishes in a freezer for approximately 10-12 h. The frozen samples were then freeze-dried for approximately 12h. The samples were then sligghtly disintegrated in narrower beakers with a glass rod and 10-15 ml acetone was added. The beakers containing the samples were covered with tinfoil to prevent light activity and stored for 2-3 days in a refrigerator. The beakers were then put in a ultrasound bath and sonicated for 20 min, and the samples were filtered through a glass frit. The yellowish filter cake was washed three times with 5 ml acetone until the cake acquired white colour. The filtrates were cantitatively transferred to 25 ml volumetric flasks and made up to the mark (if resulting extract volume was over 25 ml, the redundant acetone was evaporated under nitrogen flow). In the case of turbidity, the acetone extract was filtered through a fold paper filter of medium density. The absorbance of the acetone extracts was the measured in 1 cm cuvettes at λ = 444 nm against acetone and the total carotenoid content in mg/kg of sample was expressed as lutein equivalent from the equation : $(K+X)_L = A_{444}. 25. 15 / 0. 259. m$ (mg /kg), (Brown *et al.*, 1993)

$(K+X)_L$: Total carotenoid content (carotenes and xanthophylls)
A_{444} : Absorbance of acetone extract at λ = 444 nm
m: sample weight

2.2.2. Determination of total polyphenols

Four round-shape tubers of 4-5 cm diameter were selected for every charaecteristic sample. Peeled potato tubers were homogenised in the shortest time using a mixer and for the determination 10 g was weighed into 100 ml volumetric flask. The flask was filled up with 80% ethanol to the mark and after a vigourous agitation for 5 min and homogenisation, the solution was left to settle for 5 min. After sedimentation, 5 ml aliquots were pipetted for the determination. After dilution with distilled water to aproximately 30 ml, 2.5 ml of folin-ciocalteau reagent p.a were added. After agitation and 3 min standing, 7.5 ml 20% Na_2CO_3 p.a. solution was added and the volume was made up to the mark with distilled water. After a vigourous agitation and two hours standing at laboratory temperature, the absorbance of the blue solution was measured against blank in cuvettes of 0.5 cm thickness at λ= 765 nm on spectrophotometer. Polyphenol compounds were expresse as gallic acid content on dry matter (DM) basis. Two parallel determinations with each sample were performed (Chang, 2011).

2.2.3. Determination of ascorbic acid

Four round shape potato tubers of 4-5 cm diameter were washed, weighed, and homogenised with a weighed amount of oxalic acid solution (28 g of (COOH) 2. 2 H_2H in 1l). The homogenate was filtered and ascorbic acid was determined in the filtrate polarographically by the method of standard addition on the polarograph under the following parameters : initial potential = 250 mV, final potential 300 mV, rate 20 Mv/s, bubble period : 120 s, number of scans 1 , static period 1 s, height of pulse 50 mV, witdth

80 mV. Two parallel determinations with each sample were performed (Lachman *et al.*, 2000).

2.2.4. Determination of nitrates

An extract of potato tubers was prepared (after the addition of Cu $S0_4$, Al_2 $SO_4)_3$ and Ag_2 SO_4 and analysed using ion selective electrode method according to Davideck *et al.* (1997).

All data of these parameters are analyzed by variance at 5% level using SAS program.

3. Results

3.1. Carotenoids

Higher values of carotenoids were found in tubers treated with 500 mM of thiourea, and this increase was statically different (P = 0.0399) (Table 1). While low values were found in control tubers (carotenoids = 9,4 mg/kg), and this value was also statically dignificant (P = 0.0031). The rest of tubers exceeded this everage, thus confirming the fact that tuber colour and carotenoids content are affected by the amount of thiourea applied on tuber mother.

Table 1 : Carotenoids content

Thiourea (mM)	0	250	500	750	1000
Carotenoids (mg/kg)	9.4 $^{0.0031*}$	12.9 $^{0.7122}$	14.7 $^{0.0399*}$	13.3 $^{0.0425}$	10.7 $^{0.0672}$

*statically significant at level P < 0 .05

3.2. Polyphenols

Higher values were found in treated tubers comparing to control (carotenoids= 407 mg/kg) altough this value was statically significant (P = 0.0145) (Table 2). But the increase was not statically significant except for tubers treated with 250 mM of thiourea (carotenoids = 433 mg/kg, P = 0.0315). On the average, tubers treated with 250 mM of thiourea exceeded the control ones by 26 mg/kg.

Table 2 : Polyphenols content

Thiourea (mM)	0	250	500	750	1000
Polyphenols (mg/kg)	407 $^{0.0145*}$	433 $^{0.0315*}$	441 $^{0.2980}$	479 $^{0.0667}$	426 $^{0.087*}$

*statically significant at level P < 0 .05

3.3. Ascorbic Acid

In all tubers treated, a tendancy was observed to an increased ascorbic acid content as compared with the control tubers (Ascorbic acid = 11.4 mg/kg), although this increas was not statically significant (Table 3). The tendancy was more apparent with higher levels of thiourea. So the effect of thiourea on ascorbic acid content of tubers was affected by the amount of thiourea applied on moyher tuber. In fact, more the concentrations of thiourea is high, more the content of ascorbic acid is important. Thus, most increase of ascorbic acid content were found at 750 and 1000 mM of thiourea (18.5 and 18.3 mg/kg respectively).

Table 3 : Ascorbic acid content

Thiourea (mM)	0	250	500	750	1000
Ascorbic acid (mg/kg)	11.4 $^{0.1819}$	13.9 $^{0.0664}$	15.6 $^{0.1834}$	18.5 $^{0.0912}$	18.3 $^{0.5108}$

*statically significant at level P < 0 .05

3.4. Nitrates

The highest value of nitrates was found in control tubers (nitrates =241 mg/kg) and this value was statically significant (P = 0.0489) (Table 4). A slight increase of nitrate content (11 mg/kg) was measured in tubers treated with 250 of thiourea, but this was statically significant. Yet, there was a tendancy to decreased nitrates content with high amount of thiourea, high and statically significant value was found with 750 mM of thiourea (nitrates = 191 mg/kg) (P = 0.0541).

Table 4 : Nitrates content					
Thiourea (mM)	0	250	500	750	1000
Nitrates (mg/kg)	241 $^{0.0489*}$	252 $^{0.1176}$	189 $^{0.1982}$	191 $^{0.0541*}$	183 $^{0.3121}$

*statically significant at level P < 0 .05

4. Discussion

The available literature suggests that thiourea can effectively promote plant growth when applied as seed treatment under optimal (Garg *et al.* 2006; Jagetiya and Kaur 2006) and suboptimal conditions (Anjum *et al.* 2008; Srivastava *et al.* 2009). However, soaking potato tubers in thiourea solutions has not been the subject of intensive studies.

We found that low levels of thiourea stimulates carotenoids content while high levels of thiourea inhibits carotenoids tuber content. This inhibition was probably due to overall yellowing of leaves and constriction and browning of roots. Such reductions are observed as a result of aberrant metabolism due to applied thiourea (Gunther and Pestemer 1990) and of a loss of leaf pigments, especially the Chl *b* (Pratab and Sharma 2010; Shah *et al.* 2011). Although exogenous application of growth regulators such thiourea in appropriate concentrations has been reported to promote photosynthetic pigments (Burman *et al.*, 2007; Al-Whaibi *et al.* 2012), high levels are inhibitory (Kavina *et al.*, 2011). In our study, low concentration (250–750 mM) of applied thiourea improved carotenoids content. Increase in carotenoids is yet another important attribute of stress tolerance in plants due to having roles in light harvesting at photosystems and scavengers of reactive oxygen species via xanthophyll cycle in chloroplast (Triantaphylidès and Havaux 2009).

In the current study, 250-750 mM thiourea level appeared to have improved the Carotenoids, while at higher thiourea level (1000 mM), tubers contains lowest contents of Carotenoids. This revealed that thiourea toxicity was offset by lowest concentration of Carotenoids most likely due to their antioxidative properties (Havaux *et al.* 2007). Available literature suggests that loss of chlorophylls and carotenoids contents is due to deficiency of nutrients (Tejada-Zarco *et al.* 2004). This suggests that higher level of thiourea probably suppressed the water and minerals absorption by roots, thereby causing their deficiency within the plants and yellowing of leaves. However, no such effects were evident at lower thiourea levels. These findings further supported the notion that thiourea is a bioregulator of growth. A critical analysis of results revealed that growth stimulating role of thiourea at low levels can be attributed to its multiple roles. It may act either as a nutritional supplement due to having nitrogen and sulfur or as biostimulator of cell growth; a role typical of plant bioregulator. The former role of thiourea appears to be less likely at such low concentrations, while its latter role is more plausible. The increase of carotenoids content with tuber applied thiourea might be due to its property of inhibiting urease and reduction in the volatilization of NH_3, which is otherwise toxic to the roots, and enhanced the urea use efficiency (Bayrakli 1990). While using low levels of thiourea as seed treatment, reported that it signaled the expression of numerous genes in *Brassica juncea*, most of which were declared as markers of salinity tolerance. In the present case, as evident from quantitative plant attributes at low levels of thiourea, we infer that at low levels, thiourea facilitated nutrient acquisition and transport (Garg *et al.* 2006; Burman *et al.* 2007; Anjum *et al.* 2008, 2011). Nonetheless, in-depth studies are imperative to figure out such a role of thiourea. So it's clear that improvement in potato carotenoids with thiourea treatments appeared to have resulted from increased photosynthetic efficiency and canopy photosynthesis on account of the biological activity of -SH group. It was also apparent that leaf senescence was delayed under the influence of this chemical. It is therefore suggested that thiourea is the potential bioregulator for improving photosynthetic efficiency and yield of potato (Mani *et al.* 2012) and possibly other cultures, and that thiourea, a sulphydryl compound holds considerable promise in this context. Taken together these results indicate that low levels of thiourea increases nitrates content without developing major stress symptoms. These data sustain the hypothesis that thiourea could be involved in lipid metabolism of the chloroplast that is strictly depending on photosynthetic activity (Zhao *et al.* 2007). Further analyses are needed to unravel this possible intriguing role of thiourea.

High levels of thiourea increased polyphenols and ascorbic acid content in potato tubers. The data reported are in agreement with similar results undertaken by Camire and Kubow (2009) and Lachman and Hamouz (2008). This increase is may be due to an increasing of CO_2 content and therafter a decrease of glycoalcaloids and citric acid decrease in tubers. Thus, thiourea inhibits polyphenols according to Güllçin *et al.* (2005). Although action of thiourea, envisaged here, points to its role in increasing or decreasing polyphenols and ascorbic acid, further studies are needed to explore biological basis of these findings. Otherwise, in our study, it's has been showed that thiourea improve ascorbic acid content in tubers. These findings are in agree with works of Zhu *et al.* (2002). They indicates that thiourea provided protection against protein oxidation and also significantly inhibited oxidation of ascorbate. Consequently they conclude that the protection by thiourea against protein oxidation is not through scavenging of hydroxyl radicals, but rather through the chelation and the formation of a redox-inactive thiourea- complex.

Concerning nitrates content, in the present study, plants are generally less sensitive to higher levels of thiourea since tubers treated with high level of thiourea accumulates less nitrates but the exact physiological mechanism of thiourea toxicity to plants is not known yet. Nevertheless, tubers being directly exposed to thiourea may pose itself as a weakened sink in utilizing assimilates for cellular growth (Herbers and Sonnewald 1999; Who 2003; Ge *et al.* 2006). Otherwise, the low levels of nitrate in plants treated with of thiourea are probably due to an decrease in nitrate reductase activity. These results well describe the induction trend of nitrate assimilation pathway, as suggested by the increase of nitrate reductase activity and amino acids accompanied by the consequent decrease of reducing sugars, the main source of carbon skeletons (Crawford 1995). Among nitrogen inorganic molecules, nitrate is the predominant form in agricultural soils, where it can reach concentrations three or more orders of magnitude higher than in natural soils (Hagedorn *et al.* 2001 ; Owen and Jones 2001). In root cells, the uptake of this mineral nutrient involves inducible and constitutive transport systems (Orsel *et al.* 2002). Both systems mediate the transport of the anion by H^+ symport mechanisms (Ulrich and Novacky 1990 ; Espen *et al.* 2004) sustained by H^+-ATPase (Palmgren 2001 ; Sondergaard *et al.* 2004). The first step of nitrate assimilation, that occurs in both roots and shoots, involves its reduction to ammonia by nitrate reductase (NR) and nitrite reductase (NiR) enzymes, followed by transfer of ammonia to α-chetoglutaric acid by the action of glutamine synthetase (GS) and glutamate synthase (GOGAT) (Oaks, 1985). The pathway is induced in the presence of nitrate and shows many connections with other cellular traits, among which carbohydrate and amino acid metabolism, redox status and pH homeostasis (Hirel and Lea 2001). Hence, nitrate and carbon metabolisms appear strictly linked and co- regulated.

Taken together, in roots where photosynthesis cannot satisfy this request and/or the demand of carbon skeleton is high, sucrose pool was also affected. The decrease of nitrate due to an decrease in nitrate reductase activity observed in plants treated with high levels of thiourea may provoke changes in carbohydrate availability and the increase of amino acid level. In fact, these data are in agreement with the inhibitory effect on nitrate reductase evocated by an increase of some amino acids, mainly asparagine and glutamine (Paul and Foyer 2001). Moreover, it is know that nitrate reductase activity increases after sucrose addition whilst the low sugar content (Harris *et al.* 2000 ; Klein *et al.* 2000 ; Paul and Foyer 2001; Rockel *et al.* 2002 ; Lamattina *et al.* 2003). The results suggested that this feedback mechanism was activated in plants treated with low levels of thiourea.

From another point of view, since thiourea affects nitrates content, it may have a genetic effects on plants, since in a previous work on Arabidopsis, it was found that high concentrations of nitrate in plants induced *AtHB1* and *AtHB2*, two genes that encode for hemoglobins (Hb2) and a monodehydroascorbate reductase (MDHAR) (Wang *et al.* 2000). Other-workers (Barchman *et al.* 2005) suggested that these proteins could change their abundance in relation to the redox status, whereas other workers (Foyer *et al.* 2001) speculated on the possibility that the induction of hemoglobin could aim at reducing oxygen concentration during nitrate reductase synthesis, since molybdenium can be sensitive to oxygen. Besides, hemoglobin and MDHAR are known to be involved in the scavenging of nitrate that can be produced by cytosolic and/or plasmamembrane nitrate reductase when nitrite is used as substrate (Djennane *et al.* 2002, Scavasankar *et al.* 2005 ; Stohr and Shremalu 2006). This supports the hypothesis that thiourea may control Hb2 and MDHAR synthesis leading to controlling nitrate reductase activity and consequently nitrate levels in potato tissues.

5. Conclusion

In potato tubers, carotenoids, polyphenols, ascorbic acid and nitrates found to change in accumulation in response to thiourea application. Moreover, the results underline the strict relationship between nitrate accumulation and carbon metabolisms in potato tuber in response to thiourea. Besides, a dramatic increase of nitrates content, and consequently an increase in nitrates assimilation pathway, the exposure to a low level of thiourea (250-500 mM) seems to induce an increase in carotenoids, polyphenols and ascorbic acid tuber content. So we suggest that thiourea is a signaling molecule which is involved in many biochemical and physiological processes. Nonetheless, use of thiourea at the lower levels is economical, and likely to have great physiological implications in potato plant biology.

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