EFFECT OF HYDROGEN PEROXIDE AND THIOUREA ON DORMANCY BREAKING OF MICROTUBERS AND FIELD-GROWN TUBERS OF POTATO

F. MANI, T. BETTAIEB[1], N. DOUDECH[2] and C. HANNACHI[2]
High Agronomic Institut, , P. O. Box 4042, Chott- Mariem, Tunisia
[1]National Agronomic Institut, P. O. Box 1082, Tunis, Tunisia
[2]High Agronomic Institut, P. O. Box 4042, Chott- Mariem, Tunisia
Corresponding author: ferdaousmani78@yahoo.fr

ABSTRACT

Potato (*Solanum tuberosum* L.) microtubers or field-grown tubers have a dormant apical bud (also called tuber dormancy). They do not readily sprout even if environmental conditions are favorable, including optimum temperature and humidity. The objective of this study was to evaluate the involvement of hydrogen peroxide (by direct or indirect application of thiourea, a catalase inhibitor) in dormancy release and sprouting of potato microtubers and tubers was evaluated using two complementary experiments. First, the kinetics of the sprouting (percentage of sprouted microtubers with time) was examined on microtubers planted in peat and cultivated in a glasshouse after exogenous application of different concentrations of hydrogen peroxide (20, 40 and 60 mM) and thiourea (250, 50 0 and 750 mM). Second, the sprouting kinetics was examined on field-grown tubers during storage, after application of hydrogen peroxide (20, 40, 60 and 80 mM) and thiourea (250, 500, 750 and 1000 mM). Their sprouting capacity was also evaluated. Then, kinetics of field emergence of treated and sprouted tubers was examined after planting them in a field. Direct application of hydrogen peroxide or application of catalase inhibition through thiourea application on the release of dormancy promote sprouting on potato microtubers and field-grown tubers. Results showed that hydrogen peroxide (20 mM) caused rapid and synchronous sprouting of microtubers; while higher concentrations (40 and 60 mM), caused asynchronous sprouting. Thiourea at a concentration of 250 mM was the most effective in reducing the dormancy period and increasing the number of sprouted microtubers. In field experiment, sprouting was optimal when tubers were treated with 60 mM of hydrogen peroxide; whereas at a lower concentration, sprouting was less stimulated. In addition, tubers treated with 250 mM thiourea had maximum sprouting and better sprouting capacity. It is clear that both substances affect hormonal regulation and antioxidant enzymes, leading to dormancy release in both: microtubers and tubers.

Key Words: Antioxidant enzymes, potato, *Solanum tuberosum,* thiourea

RÉSUMÉ

Les microtubercules de la pomme de terre (*Solanum tuberosum* L.) ou bien les tubercules cultivées en champs sont dotées d'une dormance des tubercules. Elle ne poussent pas facilement, même si les conditions environnementales sont favorables, incluant température optimal et l' humidité. L'objectif de cette thiourea, une catalase inhibitrice) dans la libération de la dormance et la germination des microtubercules de pomme de terre était évalué utilisant deux essais complémentaires. D'abord, la cinétique de germination (pourcentage de microtubercules germées avec le temps) était examinee sur des microtubercules plantées sur susbtract tourbeux et cultivées dans une serre après application exogène de différentes concentrations du peroxide d'hydrogène (20, 40 et 60 mM) et thiourea (250, 50 0 et 750 mM). Deuxièmement, la cinétique de germination était examinée pendant le stockage sur des tubercules cultivées en champ après application du peroxide d'hydrogène (20, 40, 60 et 80 mM) et thiourea (250, 500, 750 et 1000 mM). Leur capacité de germination était évaluée. En suite, la cinétique d'émergence au champs des tubercules traitées et germées était examinée après les avoir plantées au champs. L'application

directe du peroxide d'hydrogène ou application de la catalase inhibitrice à travers l' application du thiourea sur la liberation de la dormance promeut la germination des microtbercules ainsi que les tubercules produites en champs. Les résultats ont montré que le peroxide d'hydrogène (20 mM) ont cause un rapide et une germination synchronisée des microtuberculoses; alors que les concentration plus élevées (40 et 60 mM), ont cause une germination non synchronisées. Thiourea avec une concentration de 250 mM était le plus efficace en terme de réduction de la période de dormance et l'augmentation du nombre des microtubercules germées. Dans l'essai en champs, la germination était optimal lorsque les tubercules étaient traitées avec 60 mM du peroxide d' hydrogen; pendant qu' à une basse concentration, la germination était moins stimulées. En plus, les tubercules treatées avec 250 mM de thiourea avaient un maximum de germination et une meilleur capacité de germination. Il est claire que toutes les deux substances affectent la regulation hormonale et les enzymes antioxidants, conduisant ainsi à la libétation de la dormance dans les microtbercules et les tubercules.

Mots Clés: Enzymes antioxidants, potate, *Solanum tuberosum,* thiourea

INTRODUCTION

Potato microtubers or field-grown tubers have a dormant apical bud (also called tuber dormancy). They do not readily sprout even if environmental conditions are favorable, including optimum temperature and humidity. In normal conditions, this dormancy is spontaneously broken after a period of maturation of the microtuber or the tuber, leading to the growth of a new sprout (Ewing *et al.,* 2004). This dormancy has advantages and disadvantages; for the plant it is advantageous to survive a period unfavourable for growth, while for man it is favourable when plant material needs to be stored for a certain period of time. However, dormancy is disadvantageous when growth is required soon after tubers have been harvested. In fact, dormancy period is important when storage is desired. Thus, lack of control over microtuber dormancy duration may detract from their immediate efficient use at any time of the year (Suttle, 2004).

The length of the dormancy period is cultivar-specific; varying from 4 to 5 months in microtubers (Hassan-Pannah *et al*., 2007) and from to 2 to 3 months in field-grown tubers (Suttle, 2004). This dormancy poses a challenge to the economical utilisation of microtubers in seed tuber production systems since they must be cold-stored and cannot be immediately planted. Few workers have succeeded in breaking microtubers and field grown tubers dormancy, but the information on impact of dormancy release treatments on subsequent field emergence is meager. In addition, it has been observed that forcing potato tubers to break dormancy and to sproutearly with some agents (GA3, Rendite) did not lead to better yield upon cultivation (Verhees, 2002).

Although dormancy is thought to be regulated mainly by endogenous abscissic acid present in tubers,it has been suggested that the stimulatory effect of different agents on dormancy breaking in potato acts through the antioxidant system and the reactive oxygen speciesmainly the hydrogen peroxide (H_2O_2) (Claassens and Vreugdenhil, 2000; Suttle, 2004). Hence, the objective of this study was to evaluate the effects of hydrogen peroxide and thiourea on dormancy and sprouting of potato microtubers and field grown tubers is described.

MATERIELS AND METHODS

Production of microtubers. The microtubers (cv. Spunta, 100 mg) were obtained from *in vitro* cultures grown in total darkness in a MS/2 liquid medium containing 5 mg l^{-1} of Benzyl Adenine and 80 g l^{-1} sucrose (Mani, 2012). The dorrnancy-breaking treatments involved soaking microtubers in solutions of hydrogen peroxide (20, 40 and 60 mM) and thiourea (250, 500 and 750 mM) in water (control microtubers) for 2 hours. Treated microtubers were kept in continuous darkness at 20 °C for sprouting on peat.This substrate was characterised by a bulk density of 0.5, a total porosity of 90%, a water retention capacity of 400 to 1500%, and a C / N ratio of 50 (Titouna, 2011). The substrate was placed in plastic containers of 50 cm length and 30 cm wide with a depth of 15 cm. Microtubers were cultivated under glasshouse; the culture lasted four months (September to December).

The sprouting kinetics of treated microtubers and sprouted microtubers were examined. Microtubers were considered sprouted when the length of the germinated sprouts was 1 mm (Delaplace *et al.,*2008). Data are based on the averages of 30 microtubers.

Field experiment. The field-grown tubers (variety Spunta) were obtained from a season crop in the region of Chott-Mariem, in Tunisia. This field experiment was carried out at Chott-Mariem Farm in The Institute of Agricultural Sciences during 2011- 2012 (Longitude 10°38'E, Latitude 35°55'N, altitude 15 m above sea level).

Healthy potato tubers of uniform size (approximately 50 mm) were washed with distilled water immediately after harvesting to remove surface soil. Each group of 21 tubers was immersed in one of various treatment of hydrogen peroxide (20, 40, 60 and 80 mM) and thiourea (0, 250, 500, 750 and 1000 mM) for two hours in laboratory at 24 °C. After dipping, treated and control tubers were dried and kept in astore with 90% relative humidity and total darkness until dormancy release. Three weeks later, tubers sprouted. We considered sprouting of up to 3 cm out of the tuber as end of dormancy period and this time was considered as dormancy breaking time (Delaplace *et al.,* 2008). After breaking dormancy, tubers were planted in the field at a spacing of 80 cm x 30 cm in a Randomised Complete Block Design (RCBD) in three replications. Farm yard manure (30 t ha^{-1}), triple super phosphate (P$_2$O$_5$ 45%:150 kg ha^{-1}), and potassium sulphate (K$_2$O 54%: 400 kg ha^{-1}), were used in this study and were incorporated in soil before planting.

The sprouting kinetics of treated field-grown tubers and the percentage of sprouted tubers were examined. The sprouting capacity was also examined as well as the kinetics of field emergence of treated field-grown tubers. Observations on sprouting were made by examining each microtuber and each tuber under a lens on a daily basis.

Content of H$_2$O$_2$ was estimated using fresh field grown tuber tissues. Fresh tissue tubers were frozen in liquid nitrogen and transformed to homogenous ground powder. Then, 1 g of the frozen powder and 250 mg of activated charcoal were homogenised for 2 min, in 4 ml cold 5% (w/v) trichloroacetic acid. The two substances were centrifuged at 12,100 g for 30 min at 4°C. The amount of H$_2$O$_2$ in the resulting extracts was quantified by the chemiluminescence reaction with luminol as suggested by Warm and Laties (1982).

Data are based on the averages of 30 tubers. All data were analysed by analysis of variance at 5% level using SAS Programme. The measured parameters were:

(i) The kinetics of sprouting of microtubers treated with hydrogen peroxide (20, 40 and 60 mM), with thiourea (250, 500 and 750 mM).

(ii) The kinetics of sprouting of field grown tubers treated with hydrogen peroxide (20, 40, 60 and 80 mM), with thiourea (250, 500, 750 and 1000 mM), their sprouting capacity (number of sprouts, sprout length and sprout diameter), their kinetics of field emergence after planting them on field and finally the hydrogen peroxide content of all produced tubers.

RESULTS

Hydrogen peroxide and kinetics of sprouting of microtubers. Figure 1 shows the effects of different concentrations of hydrogen peroxide on the rate of sprouting of microtubers. Sprouting started after 5 days for control of microtubers, while treated microtubers remained dormant and sprouting was delayed until 35 days (microtubers treated with 40 mM of H2O2) and until 40 days (microtubers treated with 60 mM of H2O2). Concerning microtubers treated with 20 mM of H2O2) sprouting was delayed until 65 days. Sevenity five days after treatments, germination has ended for all microtubers.

The best germination rate was obtained with microtubers soaked in hydrogen peroxide solution of 20 mM (67%), while the rate of germination of control microtubers (soaked in distilled water) did not exceed 53%, implying a difference of 14% (Table 2).

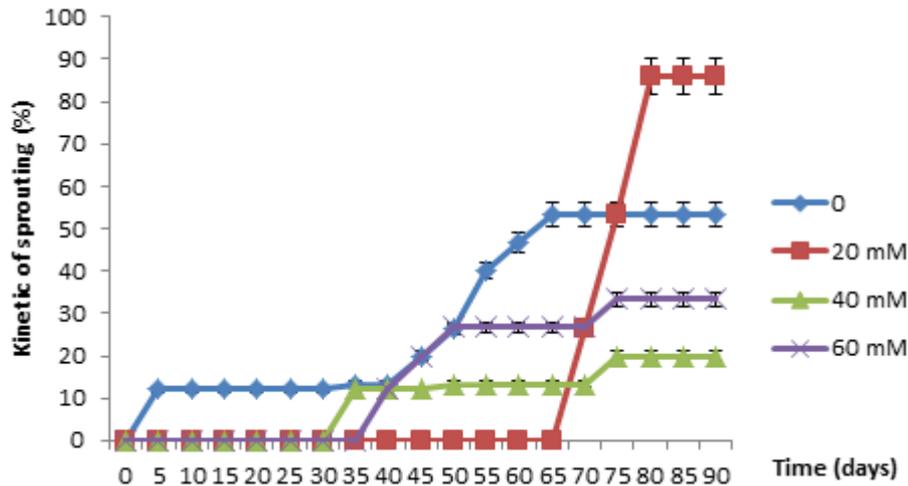**Thiourea and the kinetic of sprouting of microtubers.** Comparing to control microtubers,

Figure 1. Kinetics of sprouting of microtubers (100 mg) (number of sprouted microtubers with time) after soaking in hydrogen peroxide for 2 hr, using Spunta variety. Experimental conditions: darkness, temperature 20 ° C, RH: 90%, 90 days.

thiourea delayed germination of microtubers from 20 (TH = 500 mM) to 35 days (TH = 500 mM (Fig. 2). However, this substance improved the germination. In addition, the higher the concentration of thiourea increases the higher the percentage of germination also increases. Thus, in all cases thiourea breaks dormancy, while its concentration determines the percentage of sprouting. Indeed, the lowest concentration of TH (250 mM) was sufficient to induce maximum microtuber sprouting rate (93%) (Table 1, Figs. 2 and 3), but increasing thiourea concentration did not improve sprouting.

The field experiment

Hydrogen peroxide and kinetics of sprouting of tubers. In tubers treated with hydrogen peroxide (40 mM), sprouting started 5 days after onset of the treatment; whereas in control tubers sprouting started in the 6th day. Moreover, 100% of sprouting was reached later: at day 14 for tubers treated with hydrogen peroxide (20 mM); while it ended on the 17th day for control tubers (Fig. 4). So, hydrogen peroxide shortened dormancy period and accelerated tuber sprouting. In addition, hydrogen peroxide increased the percentage of sprouted tubers, compared with control, whatever the concentration applied (Table 2).

Thiourea effect and kinetics of sprouting of tubers. Sprouting started 3 days after onset of the treatments of thiourea, while in control tubers sprouting started on the 6th day (Fig. 5). Hundred per cent of sprouting was reached at day 15 for tubers treated with thiourea, while it ends on the 17th day for control tubers. Therefore, thiourea shortened dormancy and accelerated tuber sprouting. In addition, application of thiourea improved the percentage of sprouted tubers mainly at 250 mM, where there was almost total sprouting of tubers (97%); while the lowest value was obtained in control tubers (67%).

In summary, hydrogen peroxide (at 60 mM) and thiourea (at 250 mM) shortens the dormancy period of 6 days compared with the control. In addition, these two substances enhance the sprouting rate:by 83% (hydrogen peroxide, 60 mM) to 97% (thiourea, 250 mM) (Table 2).

Hydrogen peroxide and sprouting capacity of tubers. There was a significant difference on sprouting capacity between treated and control tubers (Table 3). Tubers treated with 60 mM of hydrogen peroxide produced the greatest number of sprouts (5 sprouts/per tuber), whereas control tubers produced less sprouts (3.5 sprouts per tuber). Moreover, longer sprouts (88 .8 and 96 mm) were obtained at 60 and 80 mM hydrogen peroxide. While shorter sprouts (66 .81 and 64.76

TABLE 1. Evaluation of sprouting of potato microtubers after soaking (2 hr) in distilled water, hydrogen peroxide or thiourea

	Water	Hydrogen Peroxide (mM)			Thiourea (mM)		
Concentration (mM)	0	20	40	60	250	500	750
Length of dormancy*	5±1.34a	70±4.65c	35±2.73b	40±4.91c	35±4.89c	25±3.48b	45±6.32d
Sprouting (%)	53.33±7.88a	66.66±9.45b	20±5.42c	33.33±6.81c	93.33±9.79d	80±5.44d	60±6.28b

* The length of dormancy is the number of days between the date of harvest and sowing time microtubers

cm) were obtained in tubers treated with 20 and 40 mM of hydrogen peroxide. However, tubers producing long sprouts (with 60 and 80 mM) had also thicker sprouts. Indeed their diameter varied between 5.8 and 5.2 mm respectively, while the diameter of the rest ranged from 4 .5 and 5 mm.

Thiourea and sprouting capacity of tubers. Treating tubers with thiourea had a significant effect on their sprouting capacity (Table 4). In this context, results presented in Table 4 showed that tubers treated with 250 mM thiourea produced more sprouts (6.5 sprouts per tuber), more than twice that produced by control tubers (3.76 sprouts per tuber). The remaining tubers provided between 4.2 and 5.0 sprouts per tuber. Moreover, tubers treated with 250 and 1000 mM thiourea and control tubers, produced longer sprouts: 83, 84 and 80 mm, respectively; while sprouts produced at 500 and 750 mM thiourea measured 79.61 mm and 72.72, respectively. In addition, tubers producing more sprouts (treated by 250 and 1000 mM), had thicker sprouts (5.6 and 5.2 mm, respectively); while control tubers produced thinner sprouts (4.8 mm diameter). Therefore, tubers treated with 250 and 1000 mM thiourea, produced more sprouts, longer and thicker than the rest of tubers. Moreover, it is for these concentrations that the rate of sprouting was maximum. This was not verified for control tubers, where sprouting was less important and the number and the diameter of sprouts were less stimulated, despite the significant length of sprouts. So, thiourea not only increased the number of sprouts per tuber, but also allowed longer and thicker sprouts, especially when applied at 250 mM.

Hydrogen peroxide and emergence of field-grown tubers. Tubers treated with 20 mM of hydrogen peroxide began to emerge from the 13th day after planting, while those treated with 80 mM of thiourea began to rise at the end of the 16th day (Fig. 6.). Moreover, the period required for emergence of all tubers was estimated at 18 days for tubers treated with 20 mM thiourea, and about 23 days for the rest of the tubers.

Thiourea and emergence of field-grown tubers. Tubers treated with 250 mM thiourea emerged

TABLE 2. Effects of hydrogen peroxide and thiourea on the lenght of dormancy and sprouting of potato tubers

	Water	Hydrogen peroxide (mM)				Thiourea (mM)			
Concentrations	0	20	40	60	80	250	500	750	1000
Length of dormancy	6 ±0.98a	5 ±1.13b	6 ±0.71a	5 ±1.13b	5±1.98b	4 ±0.94c	5 ±1.27b	4 ±1.56c	3 ±0.86a
Sprouting (%)	66.7 ±9.53a	76.7±8.43b	70.0±8.72a	83.3 ±9.76b	76.7 ±7.92b	96.7±10.16c	86.7±8.11c	73.3±8.32a	90.0±9.13c

early (11 days after planting) (Fig. 7). In contrast, control tubers emerged after 14 days, whereas, the remaining tubers on the 23th day after planting.

H_2O_2 content of field-grown tubers. The amounts of hydrogen peroxide recorded in parenchyma of field-grown tissues varied significantly according to concentrations of hydrogen peroxide applied on tuber mother (Fig. 8). Otherwise, there was considerable increase in H_2O_2 content and this increase coincided with their respective sprouting periods. Maximum amount was recorded in tubers treated with 40 mM of hydrogen peroxide, equal to 146.3 nmol. (g FW)$^{-1}$; while lowest value was recorded in control tubers.

Thiourea and H_2O_2 content of field-grown tubers. Figure 9 shows an increase in hydrogen peroxide content in tubers corresponding to their sprouting period. Maximum content of hydrogen peroxide was recorded in tubers treated with 750 mM of thiourea, in this case, it was equal to of 142.1 nmol.(g FW)$^{-1}$.

DISCUSSION

Microtuber experiment. Low concentration of hydrogen peroxide (20 mM) caused rapid and synchronous sprouting of microtubers; while higher concentration (40 and 60 mM), caused asynchronous sprouting (Fig. 1). This suggests that the concentration of 20 mM hydrogen peroxide is sufficient to inhibit catalase and to stimulate ascorbate peroxidase leading to sprouting. But when higher concentrations (40 and 60 mM) are applied, the excess hydrogen peroxide is metabolised by the ascorbate-glutathione cycle. This may explain the transition phase of dormancy in microtubers treated with these concentrations.

Thiourea, at a concentration of 250 mM was the most effective in reducing the dormancy period and increasing the number of sprouted microtubers (Fig. 2). In this case, sprouting was rapid, synchron and almost total. Whereas when higher concentrations were applied (500 and 750 mM), the dormancy period was longer and the percentage of sprouted microtubers was lower. These findings are consistent with similar
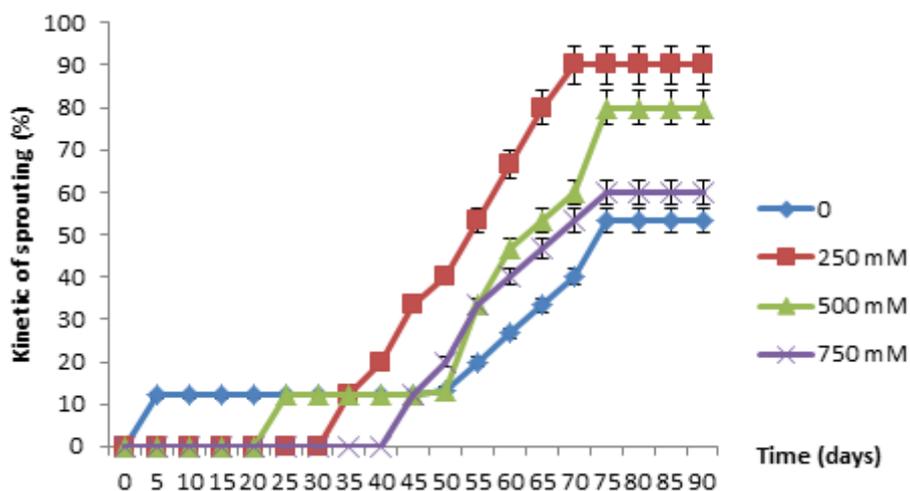
Figure 2. Kinetics of sprouting of potato microtubers (100 mg) (number of sprouted microtubers with time) after soaking in thiourea for 2 hr, Spunta variety. Experimental conditions: darkness, temperature 20 ° C, RH: 90%, 90 days.

Figure 3. Samples of potato plants derived from microtubers treated with thiourea cultured in plastic containers. Variety Spunta (microtubers 30 microtubers/treatment). Experimental conditions: Maximum temperature: 23 ° C Low: 14 ° C, RH = 70% Illuminance: 212 ìmol.m^{-2}.s^{-1}.

research conducted by Bajji *et al.* (2007), where application of thiourea (250 mM) stimulated sprouting and shortened the period of dormancy. These two phenomena are accompanied by irreversible inhibition of catalase activity and an increase in peroxidase activity. Indeed, thiourea inhibits catalase, so surplus intracellular hydrogen peroxide stimulates superoxide dismutase which stimulates germination (Bajji *et al.,* 2007).

These findings suggest that releasing microtber dormancy through hydrogen peroxide or thiourea treatment is associated with the involvement of these molecules in the functioning of the antioxidant system and more specifically in the ascorbate-glutathione cycle. In fact, thiourea inhibits catalase activity, without affecting the ascorbate peroxidase. Therefore, following the inhibition of catalase by thiourea, hydrogen peroxide will be available for ascorbate
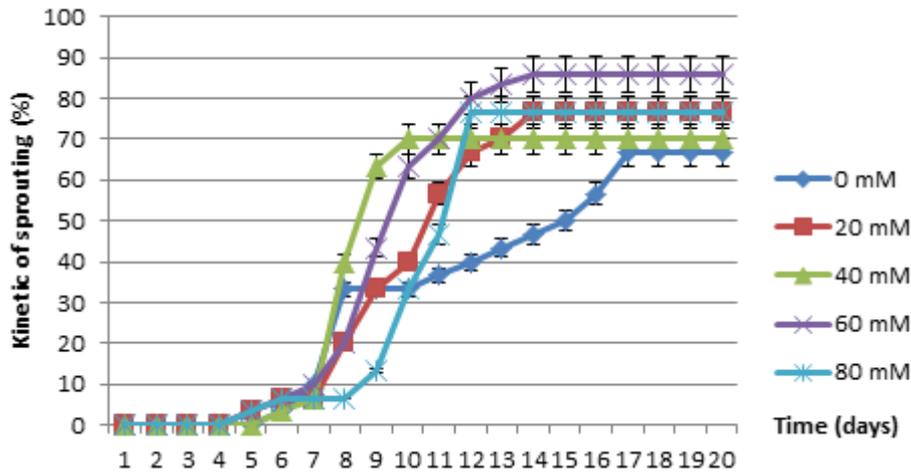
Figure 4. Kinetics of sprouting of potato tubers treated with hydrogen peroxide, variety Spunta (∅ : 50 mm) for 2 hr. Experimental conditions: darkness, temperature 20 ° C, RH = 90%.
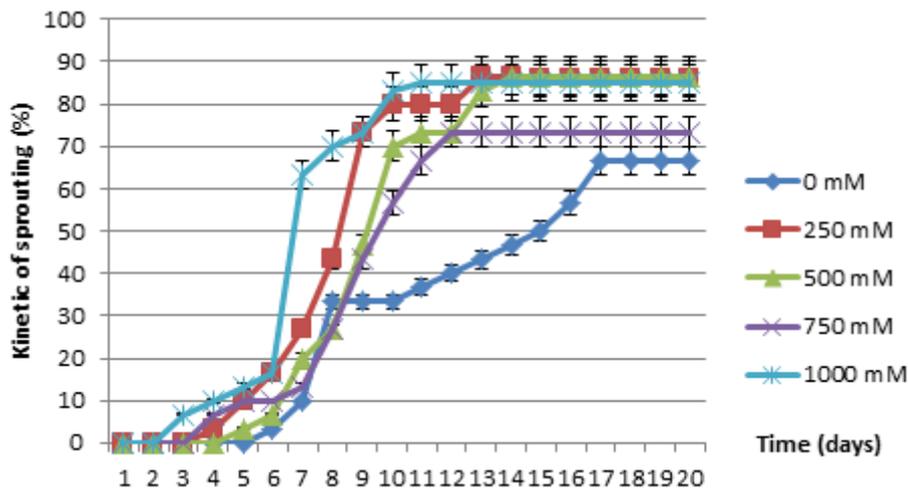

Figure 5. Kinetics of sprouting of potato tubers treated with thiourea, variety Spunta (∅ : 50 mm) for 2 hr. Experimental conditions: darkness, temperature 20 ° C, RH = 90%.

peroxidase, and this activates the glutathione cycle and the pentose phosphate pathways and subsequently release dormancy (Hendrix and Taylor, 2003). In the same vision, Suttle (2012) indicated that hydrogen peroxide applied on microtubers converts to oxygen by monooxygenases involved in cellular respiration which leads to the synthesis of gibberellins involved in breaking dormancy.

Nevertheless, sprouting is possible even for control microtubers (treated with water). In fact, in normal conditions, the metabolism of hydrogen peroxide is active during the early stages of

germination in the mitochondria. Indeed, 0.9 to 1.5% of the oxygen present in the mitochondria is converted into hydrogen peroxide by superoxide dismutase. Therefore, mitochondrial membranes are important source of intracellular hydrogen peroxide, which ensures the breaking of dormancy of microtubers in normal conditions (Bhate and Ramasarma, 2009). Under these conditions, catalase uses 65% of the intracellular hydrogen peroxide; the rest (35%) is used by APX and glutathione peroxidases.

Repression of catalase by applying thiourea or hydrogen peroxide makes the intracellular

TABLE 3.   Number of sprouts, sprout length (mm) and sprout diameter (mm) of potato tubers (Ø: 50 mm), treated with hydrogen peroxide for 2 hr. Experimental conditions: darkness, temperature 20 ° C, RH = 90%

Concentrations (mM)	Number of sprouts	Sprout length (mm)	Sprout diameter (mm)
0	3.50b ± 0.43	82.00a ± 7.67	4.80b ± 0.40
20	4.64a ± 0.39	66.81b ± 7.21	4.68b ± 0.33
40	4.81a ± 0.40	64.76b ± 7.38	4. 62b ± 0.34
60	5.00a ± 0.36	88.80a ± 6.77	5.80a ± 0.31
80	4.20a ± 0.36	96.00a ± 6.77	5.28a ± 0.31

TABLE 4.   Number of sprouts, sprout length (mm) and sprout diameter (mm) of potato tubers (Ø: 50 mm) treated with thiourea for 2 hr. Experimental conditions: darkness, temperature 20 ° C, RH = 90%

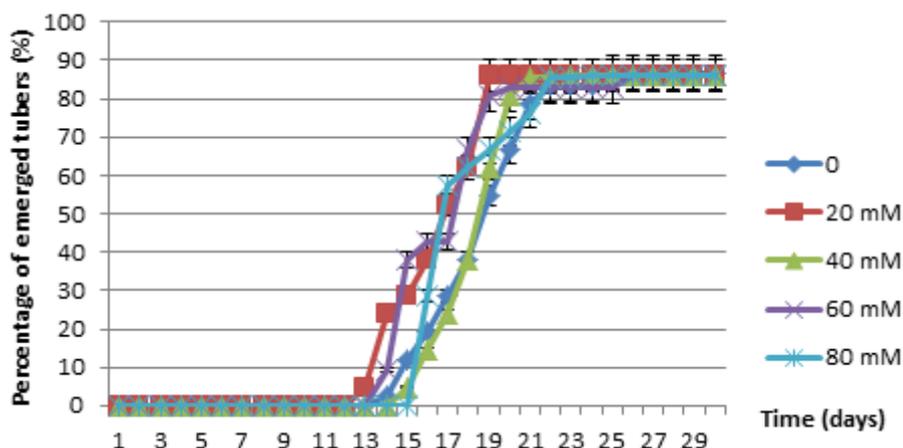| Concentrations (mM)	Number of sprouts	Sprout lenght (mm)	Sprout diameter (mm)
0	3.76 c ± 0.47	80.58 a ± 0.78	4.80 b ± 0.40
250	6.57 a ± 0.36	83.57 a ± 0.61	5.68 a ± 0.36
500	5.08 b ± 0.37	79.61a ± 0.63	5.15 a ± 0.37
750	4.68 c ± 0.41	72.72a ± 0.68	4.82 b ± 0.41
1000	4.29 c ± 0.37	84.44 a ± 0.62	5.30 a ± 0.37

Figure 6.   Kinetics of field emergence of tubers (Ø = 50 mm), treated with hydrogen peroxide and water for 2 hours, variety Spunta.

hydrogen peroxide longer available for ascorbate peroxidase and glutathione peroxidases than for catalase, consequently these enzymes will take over to ensure the metabolism of hydrogen peroxide and, therefore, releasing dormancy (Bhate and Ramasarma, 2009). Figure 10 summarises the cellular reactions involved in breaking dormancy of potato microtubers.

Field experiment carried out with tubers.
Sprouting was optimal when tubers were treated with 60 mM of hydrogen peroxide, whereas at a lower concentration, sprouting is less stimulated (Fig. 3). Moreover, tubers treated with 250 mM thiourea had a maximum sprouting percentage and better sprouting capacity. Yet application of hydrogen peroxide caused an increase in the intracellular hydrogen peroxide and the application of the thiourea inhibited catalase, which also increased the rate of intracellular hydrogen peroxide. Consequently, hydrogen peroxide applied directly or indirectly (through
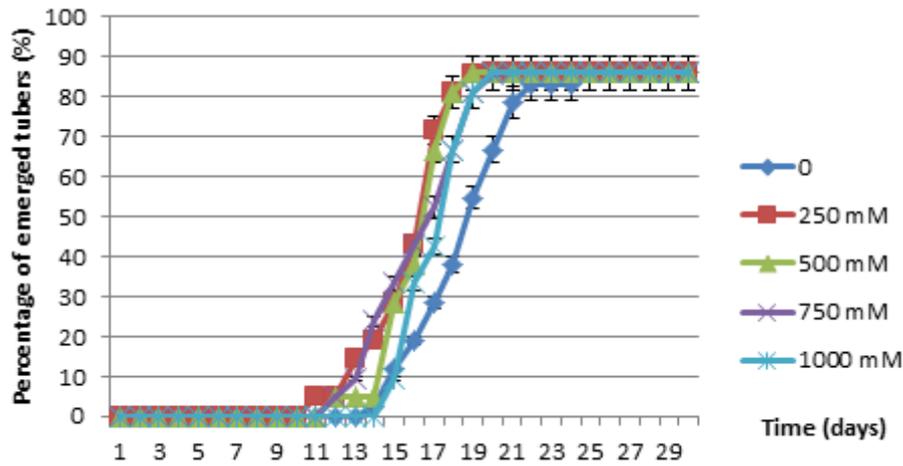
Figure 7. Kinetics of field emergence of potato tubers (Ø = 50 mm), treated with thiourea and water for 2 hours, variety Spunta.
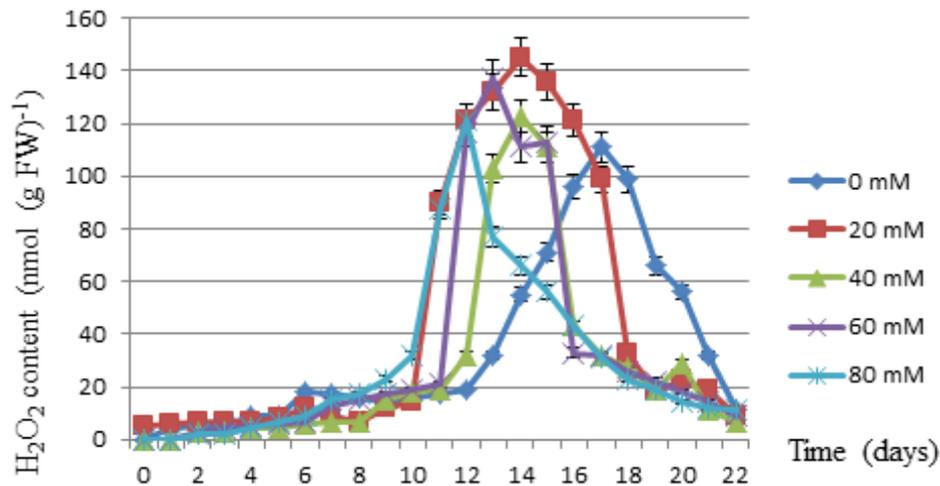

Figure 8. H_2O_2 content in nmol. (g FW)$^{-1}$ of field grown potato tubers after application of hydrogen peroxide.

application of thiourea) stimulates germination only when in low concentrations.

Hydrogen peroxide content was analysed during storage in the parenchyma tissues of field-grown tubers treated with hydrogen peroxide and thiourea. This experiment indicates that the activity of antioxidant enzymes such as: superoxide dismutase, ascorbate peroxydase and catalase is increased when hydrogen peroxide or thiourea are applied. Moreover, application of hydrogen peroxide or thiourea did not show any alteration in the activity of the antioxidant system in the corresponding parenchyma tissues of tubers.

In this context, several studies confirm that the effect of hydrogen peroxide is perceptible at concentrations above 10 µmol; but at lower concentrations, it does not affect the cellular balance (Pei *et al.*, 2000). So hydrogen peroxide applied directly (20 mM) or indirect (250 mM thiourea) would be sufficient to affect the cellular balance, causing a cascade of biochemical reactions that are: calcium ion migration to the meristematic cells (Zabrouskov *et al.,* 2002), increased respiration and increasing production of adenosine triphosphate (ATP) leading to the sprouting of tubers. These reactions can explain the emergence of proximal apical buds and the
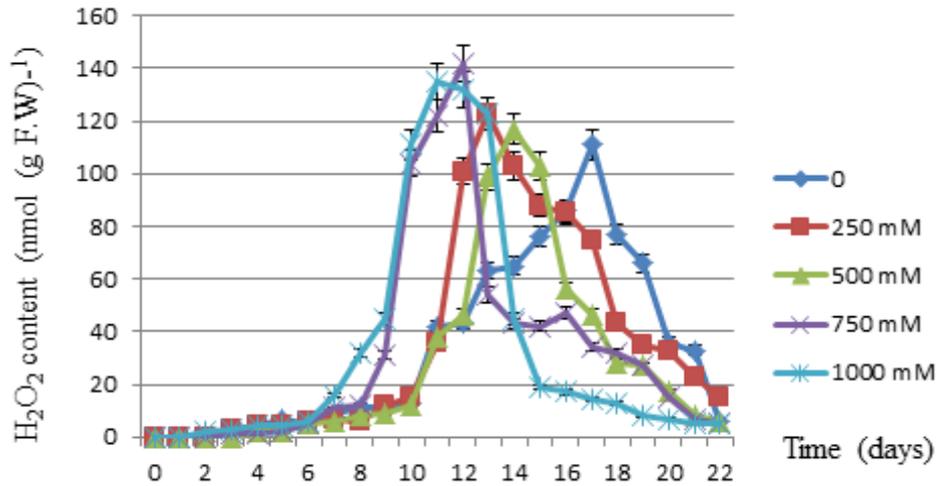
Figure 9. H_2O_2 content in nmol.(g FW)$^{-1}$ of field grown potato tubers after application of thiourea.
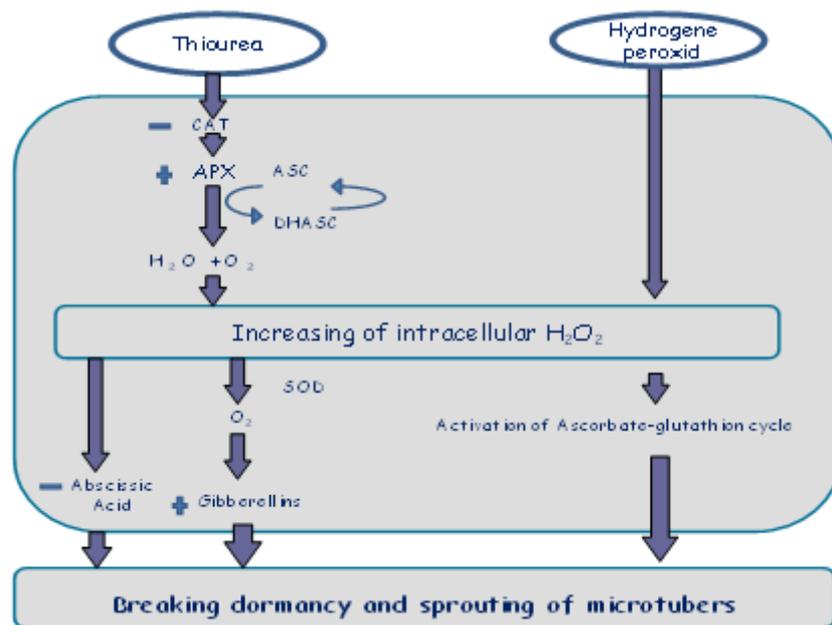

Figure 10. Cellular reactions involved in the dormancy breaking and germination of microtubers. (CAT: Catalase, APX: Ascorbateperoxydase, ASC: Ascorbate, DHASC: Dehydroascorbate, H_2O_2: Hydrogen peroxide, SOD: Superoxyde dismutase) (My own creation accordind to results of discussion).

decrease of the membrane integrity in tuber at the time of sprouting after treatment with hydrogen peroxide.

These findings are consistent with recent studies confirming that moderate oxygenation of cells through hydrogen peroxide causes an increase in intracellular Ca^{2+} and an increase in the concentration of iron, and an active input in mitosis causing significant sprouting (Jones and Smirnoff, 2005). In addition, some studies suggest that hydrogen peroxide oxidises sprouting inhibitors naturally present in the plant, and that the hydrogen peroxide is itself a stimulator of sprouting rather than oxygen.

Therefore, to stimulate sprouting, sprouting inhibitors such as chlorogenic acid and caffeic acid must be decomposed by an oxidant such as hydrogen peroxide (Macheix*et al.,* 2005).

However, we note that when the concentration of hydrogen peroxide increases (hydrogen peroxide (40 mM) or thiourea (500 and 750 mM), germination decreases. In fact, hydrogen peroxide is toxic to plants at high concentrations; it consequently activates lipid peroxidation and causes damage in the cell wall, and a decrease of membrane integrity. In this context, it was suggested that the accumulation of reactive forms of oxygen, specifically hydrogen peroxide is a major factor responsible for oxidative stress and functional decline in older cells (Halliwell, 2006).

This accumulation of hydrogen peroxide causes oxidation and several protein glycation: glycoxidation, desamidation, ubiquitation and conjugation with lipid peroxidation products (Desikan *et al.,* 2005) which may lead to decrease in sprouting capacity of tubers and may even cause theirphysiological death. However, we note that when the concentrations of hydrogen peroxide are high (80 mM hydrogen peroxide or 1000 mM thiourea), sprouting increases while remaining below the maximum value recorded.

So, it is possible at these conditions that potato cells can adapt to high concentrations of exogenous hydrogen peroxide in regulating the production of intracellular hydrogen peroxide, which makes the cells temporarily insensitive to an increased intake of hydrogen peroxide. In fact, the potato tuber can regulate excessive intake of hydrogen peroxide using phenolic compounds localised in the epidermis of the tuber. These deactivate hydrogen peroxide through coupling with ascorbate and monodehydroascorbate (Grace, 2005).

Several studies have been carried to explain the mechanism by which hydrogen peroxide breaks dormancy. Some of these suggest that hydrogen peroxide regulates the expression of a number of genes whose expression products are involved in dormancy (Kazuhiro *et al.,* 2005). Others show that hydrogen peroxide acts at the genomic level, thereby inducing the expression of genes for the transition from the dormant phase to the sprouting phase (El-Maarouf Bouteau, 2007, Leymarie *et al.*, 2007).On the one hand, genetic studies have established a relationship between application of hydrogen peroxide and activation of genes(ox GA 1, ox GA 2 and ox GA 3) involved in the biosynthesis of gibberellins (Kloosterman *et al.,* 2007). On the other hand, biochemical studies performed *in vitro* have shown that treatment with hydrogen peroxide results in a decrease in the level of endogenous abscisic acid, due to deactivation of protein phosphatases 1 and ABI 1, ABI 2, 2C, involved in the biosynthesis of abscisic acid (Nicolas *et al.,* 2003).

However, other studies suggest that hydrogen peroxide activates directly the production cytosolic calcium, protein kinases and phosphatases, which triggers the breaking of dormancy (Desikan*et al.,* 2005). Otherwise, it was proposed that hydrogen peroxide may play a mediation role, and at a certain concentration, it stimulates abscisic acid and ethylene to induce sprouting (Bailly, 2004). Furthermore, hydrogen peroxide acts on the protein metabolism of the tuber. Indeed, sprouting is accompanied by carbonylation of reserve proteins, which makes them more susceptible to proteases and proteolysis and a decrease in the activity of the pentose phosphate pathway (Buchman and Palmer, 2005). This explains the depolymerisation of the starch into reducing sugars (glucose and fructose) found in the potato tuber at the end of dormancy (Fauconnier *et al.,* 2002).

REFERENCES

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* 14: 93 -107.

Bajji, M., Mhamdi ,M.,Castiny, F., Rojas-Beltran, J. and Du Jardin, P. 2007. Catalase inhibition accelerates dormancy release and sprouting

in potato (*Solanum tuberosum* L.). *Biotechnologie Agronomie Société et Environnnement* 11(2):121–131.

Bhate, B. and Ramasarma, T. 2009. Evidence for H_2O_2 as the product of reduction of oxygen by alternative oxydase in mitochondria from potato tubers. *Archives of Biochemistry and Biophysics* 486 (2):165-169.

Claassens, M. and Vreugdenhil, D. 2000. Is dormancy breaking of potato tubers the reverse of tuber initiation. *Potato Research* 43:347-369.

Delaplace, P., Fauconnier, M. and Dujardin, P. 2008. Méthodes de mesure de l'âge physiologique des tubercules semences de pomme de terre (*Solanum tuberosum* L.). *Biotechnologie Agronomie Société et Environnement* 12(2):171-184.

Desikan, R., Hankock, J. and Neill, S. 2005. Reactive oxygen species as signalling molecules. pp. 169 -196. In: Smirnoff, N. (Ed.). Antioxidants and reactive oxygene species in plants. Oxford, UK:Blackwell Publishing.

Ewing, E., Simko, I., Omer, E. and Davies, P. 2004. Polygene mapping as a tool to study the physiology of potato tuberization and dormancy. *American Potato Journal* 81:281-289.

Fauconnier, M., Rojas-Beltran, J., Delcarte, J., Dejaeghère, F., Marlier, M. and Du Jardin, P. 2002. Lipooxygenease pathway and membrane permeability and composition during storage of potato tubers (*Solanum tuberosum* L. cv. Bintje and Desirée) in different conditions. *Plant Biology* 4:77-85.

Grace, S. 2005. Phenolics as antioxidants. pp. 141-168. In: Smirnoff, N. (Ed.). Antioxidants and reactive oxygene species in plants. Oxford, UK : Blackwell Publishing.

Halliwell, B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology* 141:312 - 322.

Hassan-Panah, D., Shahryari, R., Shamel, A., and Fathi, L. 2007. Effect of thiourea and GA on Agria's mini tuber dormancy breaking. *Proceeding of 5th Iranian Horticultural science Research Center*. Shiraz University, 1-4 sep. Shiraz, Iran. p. 100.

Jones, M. and Smirnoff, N. 2005. Reactive oxygen species in plant development and pathogen defence. pp. 197 - 214. In: Smirnoff , N. (Ed.). Antioxidants and reactive oxygene species in plants. Oxford, UK : Blackwell Publishing.

Kloosterman, B., Navarro, C., Bijsterbosch, G., Lange, T., Prat, S., Visser, R. and Bachem, C. 2007. StGA2ox1 is induced prior to stolon swelling and controls GA levels during potato tuber development. *Plant Journal* 52:362 - 373.

Leymarie, J., Bruneaux, E., Gibot Leclerc, S. and Corbineau, F. 2007. Identification of transcripts potentially involved in barley seed germination and dormancy using cDNA AFLP. *Journal of Experimental Botany* 58:425-437.

Macheix, J., Fleuriet, A. and Jay-Allemand, C. 2005. Les composés phénoliques des végétaux. Un exemple de métabolites secondaires d'importance économique. Lausanne : Presses polytechniques et universitaires romandes (Collection : Biologie).

Mani, F. 2012. Contrôle de la dormance de microtubercules et de tubercules de pomme de terre (*Solanum tuberosum* L.). PhD. p. 43. ISA Chott Mariem. Tunisia.

Nicolas, G., Bradford, K., Côme, C. and Pritchard, H. 2003. The biology of seeds: Recent research advances. Wallingford: CABI Publishing. pp. 211 - 216.

Pei, Z., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G., Grill, E. and Schroeder, J. 2000. Calcium channels activated by hydrogen peroxide mediate abscisicsignalling in guard cells. *Nature* 406:731 -734.

Suttle, J. 2004. Pysiological regulation of potato tuber dormancy. *American Journal of Potato Research* 81:253-262.

Suttle, J., Abrams, S., De Stefano, L. and Huckle, L. 2012. Chemical inhibition of potato ABA-8'-hydroxylase activity alters in vitro and in vivo ABA metabolism and endogenous ABA levels but does not affect potato microtuber dormancy duration. J*ournal of Experimental Botany*. doi: 10.1093/jxb/ers146. First published online: June 3.

Titouna, D. 2011. Etude numérique de la solution nutritive dans un milieu poreux. Cas de laine

de roche floriculture et export. PhD, p. 4. Université de Hadj Lakhdar Batna, Algeria.

Verhees, J. 2002. Cell cycle and storage related gene expression in potato tubers (these de doctorat). Wageningen Agricultural University, the Netherlands. p. 33.

Warm, E. and Laties, G. 1982. Quantification of hydrogen peroxide in plant extracts by the chemiluminescence reaction with luminal. *Phytochemistry* 21:827-831.

Zabrouskov, V., Kumar, G., Sychalla, J. and Knowles, N. 2002. Oxidative metabolism and the physiological age of seed potatoes are affected by increased α-linolenate content. *Physiologia Plantarum* 116:172 - 185.