Hydrogen Peroxide and Catalase as a way to break dormancy of potato tubers (Solanum tuberosum L.)

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ABSTRACT: The involvement of hydrogen peroxide (H₂O₂) metabolism and CAT activity in breaking dormancy of potato (Solanum tuberosum L.) tubers has been investigated in three cultivars with contrasting dormancy length using two approaches. Sprouting kinetics, H₂O₂ content and CAT activity were measured during tuber storage. Our results showed after 3 years of experimentation (2010, 2011 and 2012), that sprouting occurs after 5, 9 and 12 weeks for Arinda (cv. with short dormancy), spunta (intermediate dormancy) and Atlas (long dormancy) respectively. Biochemical characterization during dormancy and sprouting stages, showed that catalase activity ranged between 20.4 and 35.8 μmol H₂O₂ min⁻¹ mg⁻¹ of protein for ‘Arinda’ and ‘Atlas’ respectively; For H₂O₂ the values ranged between 4.7 and 14.3 mmol (g FW)⁻¹ respectively for ‘Spunta’ and ‘Atlas’.

Sprouting was associated with a significant increase in H₂O₂ content and a decrease of CAT activity. The effect of catalase inhibitor (250 mM, 350 mM and 450 mM) and H₂O₂ application (25 mM, 50 mM and 75 mM) on tuber sprouting behaviour was assessed. Both treatments resulted in shortening dormancy length and synchronised sprouting as compared to the control as well as an increased sprout number per tuber.

Keywords: Antioxidant enzymes, dormancy, potato tuber.

INTRODUCTION

Potato tuber dormancy has been defined as the physiological state in which autonomous sprout growth will not occur, even when the tuber is placed under ideal conditions for sprout growth (Coleman, 1987; Reust, 1986). Dormancy starts at the time of tuber initiation, and its duration is influenced by the age of the tuber and the environmental conditions that prevail during tuber development on the mother plant and after harvest (Struik and Wiersema, 2007; Vreugdenhil, 2007; Burton, 1989; Wurr and Allen, 1976). The length of this dormant period is also dependent on the genotype (Burton, 1989).

This dormancy of the tuber is an advantage for the plant that helps to resist to an unfavourable weather conditions for growth, while for industrial processing it is favourable when tubers can be stored for a certain period of time. However, dormancy is disadvantageous when growth is required soon after tubers have been harvested especially for the late season in the Mediterranean countries. Controlling the length of dormancy period could therefore be of considerable economic importance.

There is evidence that endogenous plant hormones play a pivotal role in the initiation, maintenance and release of potato tuber dormancy (Suttle, 2004; Wiltshire and Cobb, 1996; Coleman et al., 1992). Endogenous ethylene has been shown to play an important role in the induction of tuber dormancy (Suttle, 1998). According to Suttle (2004), cytokinins promote the breakage of dormancy, but this effect depends on the physiological age of the tuber (Fernie and Willmitzer, 2001; Coleman, 1987; Hemberg, 1983). The gibberellins seems to be responsible for the promotion of sprout growth (Suttle, 2004; Van Ittersum and Scholte, 1993). It has been shown that abscisic acid is needed for dormancy induction (Suttle, 1996; Suttle, 1995; Suttle and Hullstrand, 1994). Although auxins are essential for sprout growth, they do not appear to affect dormancy (Wiltshire and Cobb, 1996; Fernie and Willmitzer, 2001; Hemberg, 1983). In contrast to the above-mentioned hormonal regulation, little attention has been given to the possible involvement of reactive oxygen species (ROS) and antioxidants in the control of potato tuber dormancy.

ROS such as superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH) are long considered as causing oxidative damage to lipids, proteins and nucleic acids, increasing evidence indicates that they also function as signalling molecules in plants, notably acting as regulators of growth and development, programmed cell death, hormone signalling and responses to biotic and abiotic stresses (Mittler...
et al., 2004; Bolwell, 1996; Remacle et al., 1995), consequently capable to influence developmental processes like dormancy (Rojas-Beltran et al., 1999).

Plant cells have evolved enzymatic and non-enzymatic defences to hazard caused by the presence of ROS. Major plant ROS-scavenging mechanisms include superoxide dismutase (SOD, EC 1.15.1.1), ascorbic acid peroxidase (APX, EC 1.11.1.11) and catalase (CAT, 1.11.1.6) (Mittler, 2002). Superoxide dismutases (SOD) are considered key players within the antioxidant defence system, as they regulate the cellular concentration of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ (Van Breusegem et al., 2001). $\text{H}_2\text{O}_2$ is eliminated by catalase and peroxidase. Catalase removes the bulk of $\text{H}_2\text{O}_2$, whereas ascorbic acid peroxidase (APX) can scavenge $\text{H}_2\text{O}_2$ that is inaccessible for catalase because of their higher affinity for $\text{H}_2\text{O}_2$ and their presence in different subcellular locations (Creissen et al., 1994; Scandalios, 1994).

A relationship between ROS metabolism and dormancy breakage in both plant seeds (Whitaker and Beckett, 2010; Wang et al., 1991; Fontaine et al., 1994; Hendricks and Taylorson, 1975) and vegetative buds (Pérez and Lira, 2010; Or et al., 2002; Wang et al., 1991) has been reported in many works. In particular, application of $\text{H}_2\text{O}_2$ or CAT inhibitors releases dormancy in these plant tissues. In potato, however, little is known about the involvement of ROS metabolism in tuber dormancy release (Rojas-Beltran et al., 2000).

The present work was thus undertaken to evaluate the involvement of CAT and $\text{H}_2\text{O}_2$ in the control of potato tuber dormancy and sprouting. The direct application of $\text{H}_2\text{O}_2$ and of a chemical inhibitor of CAT (thiourea) to harvested tubers and assessment of their impact on sprouting has also been studied. To this end, three potato varieties with contrasting length of dormancy period were used: long, short and intermediate dormancy period.

**MATERIALS AND METHODS**

**Experiment 1: Analysis of potato tuber sprouting, $\text{H}_2\text{O}_2$ content and CAT activity in potato tuber**

**Plant material**

Three potato cultivars (Solanum tuberosum L.) with contrasting dormancy length period were used in the present work. 'Aninda' is the variety with short dormancy (5 weeks), 'Spunta' is with intermediate dormancy (9 weeks) and 'Atlas' with long dormancy (12 to 13 weeks). Plant were field grown under the standard cultural conditions of north Tunisia (plantation: late August; harvest: late November; vegetative growth: 15-20°C and 10-12 h of photoperiod). After harvest, healthy and uniform tubers (45-50 mm) were selected and placed in the dark for 1 month at 20°C, then placed in storage to sprout in the dark under constant temperature (20°C) and relative humidity (90%). Three hundred tubers per cultivar were used to follow the sprouting kinetics. The experiment was conducted for 3 consecutive years 2010, 2011 and 2012.

**The kinetics of sprouting**

the percentage of tuber sprouted was recorded at weekly intervals in accordance with established guidelines (Reust, 1986). A tuber was considered as sprouted when it had at least one sprout 2 mm length. The moment of 80% sprouting was used to characterise the end of dormancy (Van Littersum et al., 1992).

**Determination of CAT activity and $\text{H}_2\text{O}_2$ content**

**Protein extraction**

A sample of 45 tubers per cultivar and by physiological stage (dormancy and sprouting) was ground in liquid nitrogen. Frozen plant tissue (10g) was homogenized in 10 ml of extraction buffer: 50 mM potassium phosphate pH 7.6, 10 mM sodium metabisulfite, 1 mM ascorbic acid, 1 mM ethylenediamine-tetraacetic acid (EDTA), 20% (w/v) sorbitol, and 2% (w/v) polyvinylpyrrolidone (PVP) and centrifuged at 12,000 g for 20 min at 4°C. The supernatant was collected and the protein content was determined according to Bradford (1976) using the Bio-Rad Protein Assay. Kit from BioRad, according to manufacturer's instructions.

**Enzyme activity**

Soluble protein samples were subjected to non denaturing 10% PAGE basically according to Laemmli (1970) but without SDS. CAT isoforms were detected on the gel as follows: the gel was washed 3 times (15 min each) with distilled water, then incubated for 10 min in 0.88 mM $\text{H}_2\text{O}_2$ solution, rinsed again with distilled water, and finally incubated with 1% (w/v) ferric chloride and potassium ferricyanide solution until bands appeared (yellow bands on green background). CAT activity was measured spectrophotometrically by monitoring the decline an A$_{340}$ because of $\text{H}_2\text{O}_2$ consumption ($\xi = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) according to the method of Clairborne (1985). The reaction mixture (1ml) consisted of 50 mM potassium phosphate buffer (pH 7.5), 50 µg protein extract and 15 mM $\text{H}_2\text{O}_2$. CAT activity was expressed as µ mol $\text{H}_2\text{O}_2$ min$^{-1}$ mg$^{-1}$ protein.
**Measurement of H$_2$O$_2$ by the luminol method**

The quantification of H$_2$O$_2$ was determined by chemiluminescence according to the protocol of Warm and Laties (1982) with modifications. One gram of plant tissue ground in liquid nitrogen was gently mixed on ice with 250 mg of activate carbon and 4 ml of 5% (w/v) trichloroacetic acid (TCA). The crude extracts were centrifuged for 30 min at 12,000 x g. A sample of supernatant (2 ml) was passed through a BioRad column AG1*8 (poly Prep prefilled chromatography columns). 50 ml of all eluates collected were mixed with 100 ml of 0.5 mM K$_2$Fe (CN)$_6$ soluble in 0.2 M NH$_4$OH (pH 9.5) and 50 ml of 0.5 mM luminol.

CAT activity and H$_2$O$_2$ content were quantified at dormancy and sprouting states for the 3 cultivars tested in the apical "buds/sprouts" of tubers. The parameter's values were the average of three independent experiments.

**Experiment 2: Direct application of H$_2$O$_2$ and of thiourea to harvested tubers and assessment of their impact on sprouting**

Potato tubers were carefully harvested graded (35–45 mm) and stored in the dark at 20°C and 90% RH for 1 day before treatments. Tubers were then treated with 3 doses of thiourea 250, 350 and 450 mM and 3 doses of H$_2$O$_2$: 25, 50 and 75 mM or with water by dipping them into the corresponding solutions for 2 h, after exposing the parenchyma around the apical bud by a limited cutting (8 mm diameter × 8 mm depth).

For each treatment (thiourea, H$_2$O$_2$ and control), 45 tubers were used. Once treated, tubers were stored under sprouting conditions (dark, 20°C, and 90% RH) and examined daily. For this experiment, the quantitative parameters were the sprouting kinetics and the sprout number per sprouted tuber.

**Data analysis**

The data obtained was subjected to ANOVA using SPSS program.

**RESULTS**

Three to seven weeks after harvest, all tubers analysed were still dormant dependent on the variety (Figure 1). Indeed, potato tubers exhibit all types of dormancy (endo-, para-, and ecodormancy) as defined by Lang et al. (1987). At harvest and during a certain period thereafter, all buds (eyes) of the tuber are endodormant. After that, endodormancy is lost (sprouting of typically the most apical bud) and paradormancy (apical dominance) of the lateral buds (including the proximal one) keeps them at rest. When stored under low temperatures, bud growth is then prevented by ecodormancy.

Kinetics of sprouting tubers showed that dormancy length differs from one variety to other. The end of dormancy characterised by 80% of sprouting was reached at the 6th week after harvest for the cultivar ‘Arinda’ (short dormancy), at the 9th week for the cultivar ‘Spunta’ (intermediate dormancy length) and at the 12th week for the cultivar ‘Atlas’ (long dormancy length) (Figure 1).

Native gel assays of CAT activity, showed an important CAT activity at dormancy stage and a significant reduction at sprouting stage for the 3 cultivars (Figure 2).

Biochemical characterization of tubers demonstrated that dormancy was associated with high catalase activity and low hydrogen peroxide content and sprouting was marked by a significant decrease in CAT activity and increase in H$_2$O$_2$ content (Figure 3). Statistical analysis for CAT activity and H$_2$O$_2$ content revealed highly significant differences between dormancy and sprouting, and between cultivars. The same findings were reported by M’Hamdi et al. (2009). The highest values of CAT activity (35.8 µmol H$_2$O$_2$ min$^{-1}$ mg$^{-1}$ of protein) were obtained on ‘Atlas’ tubers at dormancy stage and the lowest value (7.9 µmol H$_2$O$_2$ min$^{-1}$ mg$^{-1}$ of protein) on ‘Spunta’ tubers at sprouting (Figure 3A). Unlike catalase activity, the content of H$_2$O$_2$ was highest at sprouting stage (122.8 mmol g$^{-1}$ FW) on ‘Atlas’ tubers and the minimum content (4.7 mmol g$^{-1}$ FW) on ‘Spunta’ tubers (Figure 3B). Based on these results, we can suggest that the accumulation of H$_2$O$_2$ may result, at least partially, from a reduction in CAT activity and that this enzyme may play an important role in the dormancy and spraying of potato tubers.

The effect of 3 doses of catalase inhibitor (Thiourea) (250 mM, 350 mM and 450 mM) or of exogenous H$_2$O$_2$ application (25 mM, 50 mM and 75 mM) on tuber spraying behaviour was assessed. Both treatments resulted in a reduction of the dormancy period and a rapid and synchronised spraying of the treated tubers when compared to the control. Thiourea application resulted in spraying acceleration of the treated tubers when compared to tubers treated with water (Figure 4) and to the control. Using thiourea makes spraying 20, 23 and 18 days faster respectively for Arinda, Spunta and Atlas, compared to the non treated tubers (Figures 4 and 1).

Same results were obtained for the tubers treated with H$_2$O$_2$ (Figure 5), an accelerated spraying compared to the control of an average of 17, 20 and 17 days respectively for Arinda, Spunta and Atlas. The 80% of spraying was reached only under the treatment of 250 and 350 mM of Thiourea and 50 mM of H$_2$O$_2$. It seems
that high dose of Thiourea (450mM) and H$_2$O$_2$ (75mM) which leads to more accumulation of H$_2$O$_2$ in the tissues are not benefits for plants.

Furthermore, thiourea or H$_2$O$_2$ application resulted in an increase of the sprout number per tuber (Figure 6).

**DISCUSSION**

In a previous study performed on nine potato cultivars, the activity of antioxidant enzymes during tuber dormancy has been analysed (M’Hamdi et al., 2009). It was found that, during tuber dormancy CAT activity was decreased and H$_2$O$_2$ increased from the stage of dormancy to sprouting. Other studies on plant seeds and tree buds (Bajji et al, 2007; Pérez and Lira, 2005; Or et., 2002; Fontaine et al., 1994; Nir G and Lavee, 1993; Hendricks and Taylorson, 1975) indicate that H$_2$O$_2$ accumulation may play a crucial role in the mechanism of dormancy breakage. However, the relationship between these two processes is still not well understood. The present work has been particularly focused on the implication of H$_2$O$_2$ metabolism and a CAT inhibitor (thiourea) in the release of potato tuber dormancy.

Our results showed that the treatment of dormant potato tubers with CAT inhibitor or exogenous H$_2$O$_2$ application allowed a breaking dormancy, an accelerated sprouting and an increase in sprout number. This indicates that CAT inhibition may break dormancy (dormancy, apical dominance) and increase the number of sprouts (apical dominance, multiple sprouting) by accelerating tuber ageing. It is known that ageing of tubers is accompanied by a progressive increase in oxidative stress (Kumar and Knowles, 1996) and the use of CAT inhibitors or exogenous H$_2$O$_2$ may accelerate the induction of oxidative stress and consequently advances the physiological age of the tuber. In seeds, accelerated ageing was found to be closely related to a decrease in the activities of detoxifying enzymes, including SOD and CAT (Bailly et al, 1996).

Some studies suggested that CAT inhibitor or exogenous H$_2$O$_2$ treatments induce dormancy breakage by favouring the oxidative pentose phosphate pathway (OPPPP) (Fontaine et al., 1994; Nir and Lavee, 1993; Hendricks and Taylorson, 1975). In the present work, as in seeds and fruit tree buds, CAT inhibition or exogenous H$_2$O$_2$ application on potato tubers could result in an increase in the level of endogenous H$_2$O$_2$ in bud tissues which might activate the OPPP and thus leads to dormancy breakage and initiation of sprouting.

Our results showed also that a high dose of Thiourea didn’t improve neither the sprouting percentage nor the sprout number per sprouted tuber. Additional experiments with Thiourea as CAT inhibitors are thus required in order to clearly elucidate their role. The same observation has been noted when we used high dose of H$_2$O$_2$ which leads to more accumulation of H$_2$O$_2$ in the tissues that could affect the cellular metabolism.

Although there has been rapid progress in recent years on how ROS control various plant processes, there are still many uncertainties and gaps in our understanding of how H$_2$O$_2$ interacts with hormones during dormancy/sprouting. Bailly (2004) reported that the control of dormancy by hormones such as ABA and ethylene could be connected to H$_2$O$_2$ signalling and such kind of interplay constitutes a challenge for future research in this area.

**REFERENCES**


Figure 1. Kinetics of sprouting (%) during storage conditions (dark, 20°C, 90% relative humidity) of 3 cultivars of potato tubers with contrasting dormancy length (‘Arinda’ with short dormancy, ‘Spunta’ with medium dormancy and ‘Atlas’ with long dormancy) during the years 2010, 2011 and 2012. The sprouting rate is the ratio of sprouted tubers and the total number of tubers used. Three hundred tubers per cultivar were used to follow the sprouting kinetics.
Figure 2. Native gel assays of the catalase activity in the tubers at two physiological stage: dormancy and sprouting in three potato cultivars. Lane 1 (cultivars with short dormancy length), lane 2 (cultivars with medium dormancy length), lane 3 (cultivars with long dormancy length).

Figure 3. (A) Catalase activity in tubers of three potato cultivars with contrasting dormancy length ('Arinda' with short dormancy, 'Spunta' with medium dormancy and 'Atlas' with long dormancy) at two physiological stage: dormancy and sprouting.
(B) Hydrogen peroxide content in tubers of three potato cultivars with contrasting dormancy length at two physiological stages: dormancy and sprouting.

Values represent the averages of three repetitions. 45 tubers per cultivar and physiological stage were used. Bars represent the average of their estimated standard deviation for 2 years 2010 and 2011.
Figure 4. Sprouting (%) of potato tubers for the 3 cultivars (Arinda, Spunta and Atlas) during storage after different treatments with thiourea (250 mM, 350 mM and 450 mM) and control tuber treated with water (H\textsubscript{2}O).
Figure 5. Sprouting (%) of potato tubers during storage of 3 cultivars (Arinda, Spunta and Atlas) after different treatments with hydrogen peroxide H$_2$O$_2$ (25mM, 50 mM and 75 mM) and tuber treated with water (H$_2$O).

Figure 6. (A) Mean sprout number per sprouted tuber of stored potato after different treatments with thiourea (0, 250, 350 and 450 mM).

(B) Mean sprout number per sprouted tuber of stored potato after different treatments with Hydrogen peroxide H$_2$O$_2$ (0, 25, 50 and 75 mM).