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Hydrogen Peroxide and a Catalase, Physiological Regulators of Potato (*Solanum tuberosum* L.) Tuber Dormancy

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ABSTRACT

The involvement of hydrogen peroxide (H_2O_2) metabolism and catalase activity in dormancy release and sprouting of potato (*Solanum tuberosum* L.) tubers has been investigated in nine cultivars with a contrasting dormancy length. Phenotypic characterisation of tubers dormancy was established for all cultivars by following sprouting kenetics (percent of sprouted tubers following time during storage period). Catalase (CAT) activity and H_2O_2 content were measured during two important tuber physiological stages: dormancy and sprouting. Our results revealed three groups of cultivars according to the length of dormancy: 'Arinda', 'Santana', and 'Safrane', with short dormancy (7-8 weeks), 'Mondial', 'Atlas', and 'Liseta', with long dormancy (15-16 weeks) and 'Spunta', 'Tango', and 'Nicola', with intermediate dormancy (12-13 weeks). 80% of cultivars with short dormancy sprouted between the 5th and 6th week after harvest, between the 9th and 10th week for the second group with intermediate dormancy and between the 12th and 13th week for the cultivars with long dormancy. Biochemical characterisation showed CAT activity and low H_2O_2 content during the dormancy stage for all cultivars. CAT activity varied between 30 and 18.57 µmol H_2O_2 min^{-1} mg^{-1} of protein for 'Atlas and 'Nicola, respectively. For H_2O_2, the values ranged between 5.1 and 16.92 mmol $(gFW)^{-1}$ in 'Tango', and 'Liseta', respectively. Sprouting was associated with a significant increase in H_2O_2 content (85.35 in 'Arinda', and 172.5 mmol g^{-1} FW^{-1} in 'Santana') and a decrease of CAT activity (5.09 in Tango and 13.15 µmol H_2O_2 min^{-1} mg^{-1} of protein in 'Satana'.

Keywords: active oxygen species, anti oxidant enzymes, potato tuber, sprouting

INTRODUCTION

At harvest and for a finite period thereafter, potato tubers will not sprout and are considered as dormant and do not have the ability to sprout. After harvesting, tuber dormancy is released progressively. This release is accompanied by numerous physiological and biochemical changes (Burton 1989; Benkeblia *et al.* 2008). Dormancy is defined as "the physiological stage of the tuber in which autonomous sprout growth will not occur, even when placed under ideal conditions for sprouting" (Reust 1986). The length of this dormant period is dependent on the genotype as well as on both pre- and post-harvest conditions (Burton 1989). Tuber dormancy is desirable when potatoes must be stored (industrial processing); however, excessively long dormancy could be a problem in sprouting of seed tubers (early crop installation). Accelerated or delayed sprouting of the harvested tubers may be favoured depending on the intended purpose. Controlling the length of the 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 (Coleman *et al.* 1992; Wiltshire and Cobb 1996; Suttle 2004b). Endogenous ethylene has been shown to play an important role in the induction of tuber dormancy (Suttle 1998). The sustained presence of abscisic acid was found to be essential for both induction and maintenance of tuber dormancy (Suttle and Hultstrand 1994). Cytokinins have been suggested to be involved in the release of tuber dormancy (Hemberg 1985; Coleman 1987) and gibberellins in the regulation of subsequent sprout growth (Suttle 2004a).

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. Generation of ROS, including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), is ubiquitous in biological systems, and occurs either as unavoidable by-products of metabolic reactions or through signal-regulated processes under both normal and stress conditions (Bolwell 1996). Major plant ROS-scavenging mechanisms include superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Bettini *et al*. 2008). SOD regulates the cellular concentration of O_2^- and H_2O_2. H_2O_2 is eliminated by CAT and APX; their different affinities for H_2O_2 (CAT, mM range and APX, µM range) suggest that they belong to two distinct classes of H_2O_2-scavenging enzymes: CAT might be responsible for the removal of excess ROS during stress, whereas APX might be responsible for the fine modulation of ROS for signalling (Mittler 2002). A relationship between ROS metabolism and dormancy breakage in both plant seeds (Hendricks and Taylorson 1975; Fontaine *et al.* 1994) and vegetative buds (Wang *et al.* 1991; Or *et al.* 2002; Pérez and Lira 2005) has been repeatedly reported. In particular, application of H_2O_2 or of 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; Baji *et al.* 2007).

The present work was thus undertaken to evaluate the involvement of CAT and H_2O_2 in the control of potato tuber dormancy. To this end, three groups of potato cultivars with contrasting length of dormancy period were used: long, short and intermediate dormancy period.

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MATERIALS AND METHODS

Experiment to follow sprouting kinetics

***Plant material*:** Three groups of potato cultivars with contrasting dormancy period were used in this work: the first group with short dormancy (7-8 weeks) ('Santana', 'Arinda' and 'Safrane') the second group with long dormancy (15-16 weeks) ('Atlas', 'Mondial' and 'Liseta') and the third group with intermediate dormancy (12-13 weeks) ('Spunta', 'Nicola' and 'Tango'). Plants were field grown under the standard cultural conditions of north Tunisia (vegetative growth: Temperature 15-20°C and photoperiod: 10-12 h, tuber growth: Temperature 18-25°C and photoperiod: 13-14 h).

***Sprouting kinetic control*:** For sprouting kinetics following, the percentage of sprouted tubers was recorded at weekly intervals in accordance with established guidelines (Reust 1986). A tuber was considered as having sprouted when it showed at least one sprout 2 mm long. The moment of 80% sprouting was used to characterise the end of dormancy (Van Ittersum *et al.* 1992). After harvest, healthy and uniform tubers (35-45 mm) were manually selected and stored to sprout in the dark under a controlled environment at 20°C and 90% relative humidity (HR). Three hundred tubers per cultivar were used to follow the sprouting kinetics.

Experiment to determine catalase activity and H_2O_2 content

***Protein extraction*:** A sample of 45 tubers by cultivar and by physiological stage (dormancy and sprouting) was ground in liquid nitrogen. Frozen plant tissue (10 g) 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 EDTA, 20% (w/v) sorbitol, 2% (w/v) polyvinylpolypyrrolidone (PVPP) and centrifuged at 12,000 × *g* for 20 min at 4°C. The supernatant was collected and the total soluble protein concentration was determined by the method of Bradford (1976), using the Protein Assay Kit from BioRad.

***Enzyme activity*:** Soluble protein samples were subjected to non-denaturing 10% PAGE basically according to Laemmli (1970) but without SDS. CAT isozymes 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 H_2O_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 a green background). CAT activity was measured spectrophotometrically by monitoring the decline in A_{240} because of H_2O_2 consumption (ξ = 39.4 mM^{-1} cm^{-1}) according to the method of Clairbone (1985). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate buffer (pH 7.5), 50 µg protein extract and 15 mM H_2O_2. CAT activity was expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

***Measurement of H_2O_2 by the luminol method*:** The quantification of H_2O_2 was determined by chemiluminescence (Warm and Laties 1982), with modifications. One gram of plant tissue ground in liquid nitrogen was gently mixed on ice with 250 mg active carbon and 4 ml of 5% w/v trichloroacetic acid (TCA). The crude extracts were centrifuged for 30 min at 12,000 × *g*. A sample of supernatant (2 ml) was passed through a BioRad column AG1*8 (polyprep prefilled chromatography columns). 50 ml of all eluates collected were mixed with 100 ml of 0.5 mM K_3 Fe $(CN)_6$ soluble in 0.2 M NH_4OH (pH 9.5) and 50 ml of 0.5 mM luminal.

CAT activity and H_2O_2 content were quantified at dormancy and sprouting states for the nine cultivars tested in the apical "buds/sprouts" of tubers. The parameters' values were the average of three independent experiments.

Statistical analysis

All experiments were conducted as a randomized complete block design and data obtained were subjected to analysis of variance at 5% level using SAS program version 9.0 (SAS Institute, Cary, NC). When the main effect was significant, differences between means were evaluated for significance by using Duncan's multiple range test ($P < 0.05$).

RESULTS

Kinetics of sprouting tubers showed three groups of cultivars with contrasting length of dormancy: 'Santana', 'Safrane' and 'Arinda' (short), 'Atlas', 'Liseta' and 'Mondial' (long) and 'Spunta', 'Tango' and 'Nicola' (intermediate). The end of dormancy characterised by 80% of sprouting was reached between the 5th and 6th week after harvest for the cultivars with short dormancy, between the 9th and 10th week for cultivars with intermediate dormancy length and

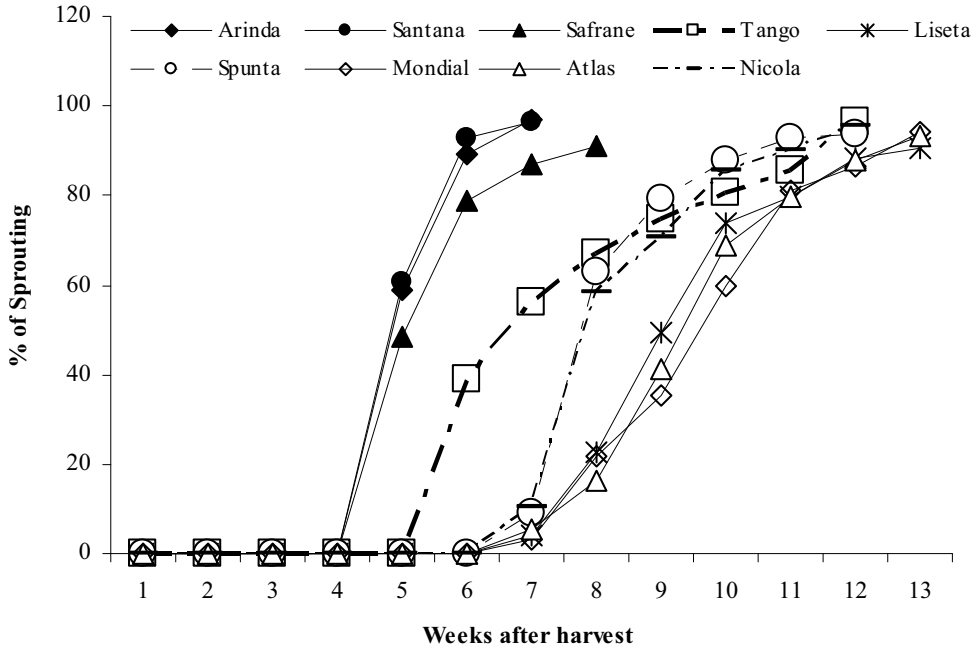

Fig. 1 Kinetics of sprouting (%) during storage conditions (dark, 20°C, 90% relative humidity) of nine cultivars potato tubers with contrasting dormancy length: ('Arinda', 'Santana' and 'Safrane' with short dormancy, 'Spunta', 'Tango' and 'Nicola' with medium dormancy and 'Atlas', 'Mondial' and 'Liseta' with long dormancy). The sprouting rate is the ratio of sprouted tubers and the total number of tubers use. Three hundred tubers per cultivar were used to follow the sprouting kinetics.

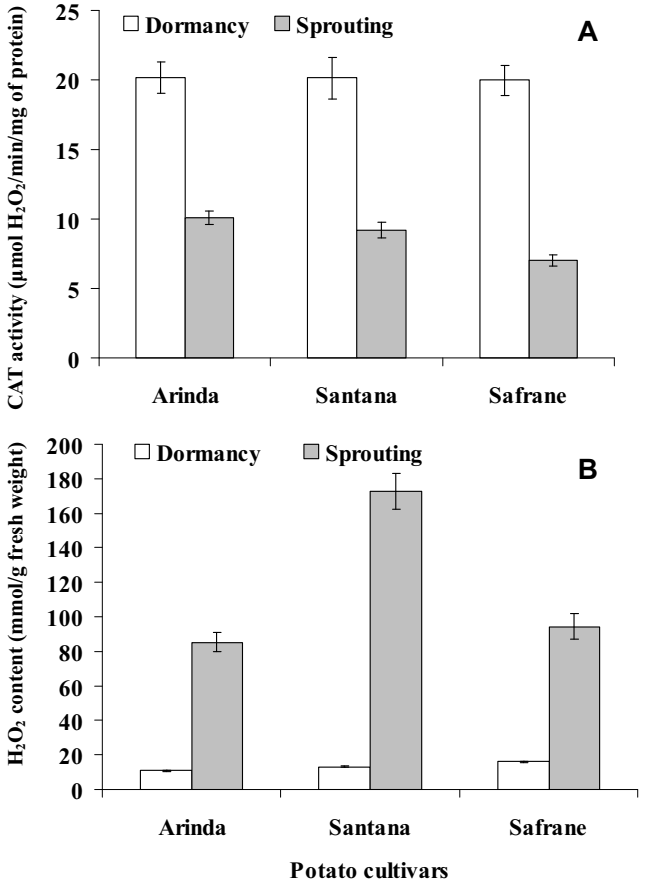

Fig. 2 Catalase activity (**A**) and hydrogen peroxide content (**B**) in tubers of three potato cultivars with short dormancy length at two physiological stages: dormancy and sprouting. Indicated values represent the averages of three repetitions. 45 tubers per cultivar and physiological stage were used. Bars represent the average of their estimated standard deviation. Means with the same letters are not significant at $p = 0.05$ probability level according to DMRT.

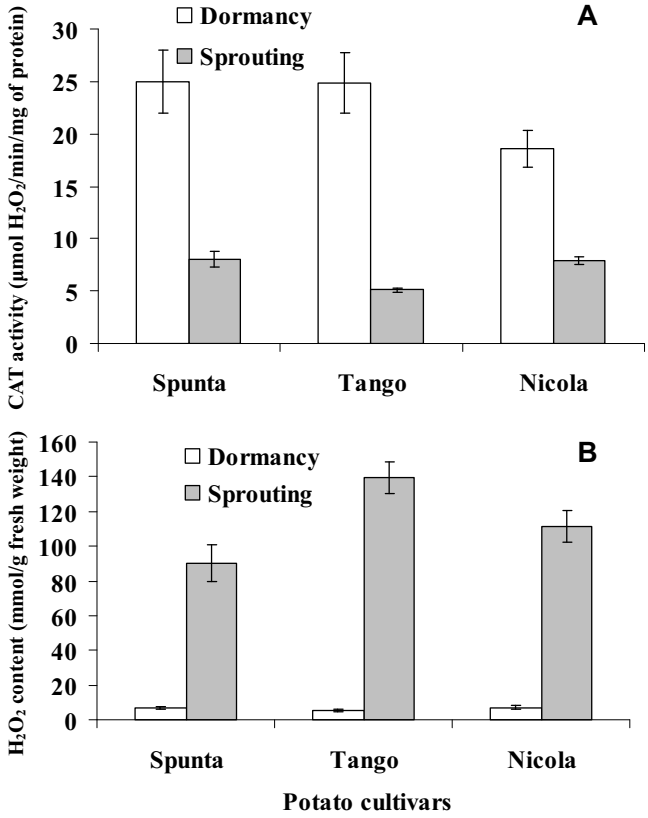

Fig. 3 Catalase activity (**A**) and hydrogen peroxide content (**B**) in tubers of three potato cultivars with medium dormancy length at two physiological stages: dormancy and sprouting. Indicated values represent the averages of three repetitions. 45 tubers per cultivar and physiological stage were used. Bars represent the average of their estimated standard deviation. Means with the same letters are not significant at $p = 0.05$ probability level according to DMRT.

between the 12th and 13th week for the cultivars with long dormancy length (**Fig. 1**).

Biochemical characterization of tubers demonstrated that dormancy was associated with CAT activity and low hydrogen peroxide content and sprouting was marked by a significant decrease in CAT activity and increase in H_2O_2 content (**Figs. 2-4**).

Statistical analysis for activity and H_2O_2 content revealed highly significant differences between dormancy and germination, and between groups of varieties. The highest values of CAT activity (30.25 µmol H_2O_2 min^{-1} mg^{-1} of protein) and weakest (5.09 µmol H_2O_2 min^{-1} mg^{-1} of protein) were measured in the tubers of 'Liseta' at the dormancy stage (**Fig. 4A**) and 'Tango' in germination, respectively (**Fig. 3A**). Unlike CAT activity, the highest content of H_2O_2 (172.5 mmol g^{-1} FW) was found in 'Santana' tubers in germination (**Fig. 2B**) and the lowest (5.1 mmol g^{-1} FW) for 'Tango' in dormancy.

Based on these results, we suggest that the accumulation of H_2O_2 may result, at least partially, from a reduction in CAT activity and that this enzyme and this compound may play an important role in the dormancy and sprouting of potato tubers.

Native gel analyses confirm resulting from spectrophotometric one and showed an important reduction of CAT activity at the three groups of cultivars (**Fig. 5**).

DISCUSSION

Our results on potato tubers and previous ones on other plant seeds and tree buds (Hendricks and Taylorson 1975; Nir and Lavee 1993; Fontaine *et al.* 1994; Or *et al.* 2002; Perez and Lira 2005) indicate that H_2O_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.

It has been suggested that CAT inhibitor or exogenous H_2O_2 treatments induce dormancy breakage by favoring the oxidative pentose phosphate pathway (OPPP) (Hendricks and Taylorson 1975; Nir and Lavee 1993; Fontaine *et al.* 1994). In the present work, it seems, as in seeds and fruit tree buds, sprouting was associated with CAT inhibition and H_2O_2 accumulation which might activate the OPPP and thus leads to dormancy breakage and initiation of sprouting. Additional experiments with OPPP inhibitors are required in order to clearly elucidate the role played by this important pathway.

It is possible that the activation of OPPP is only part of the metabolic pathways induced by H_2O_2 and leading to the termination of dormancy. In fact, two other mechanisms have been suggested for seeds, and may occur in tubers, to explain the promotion of their germination by H_2O_2. In the first mechanism, a build up of H_2O_2 would yield oxygen, used by respiration and other oxidation processes (Roberts 1969) and by monooxygenases implicated in gibberellins biosynthesis (Fontaine-Roux *et al.* 1997). The second suggested mechanism may involve a peroxidase interacting with H_2O_2 to oxidize germination inhibitors (Ching 1959). Other effects of H_2O_2 in cellular mechanisms involved in germination cannot, however, be excluded. H_2O_2 is indeed an important molecule in plants since, on the one hand, its production is dependent on the rate of several major physiological processes and environmental factors, and, on the other hand, the endogenous level of H_2O_2 modulates the expression of many genes and is involved in the control of growth and differentiation (Penel 1997). Although there has been rapid progress in recent years on how ROS control

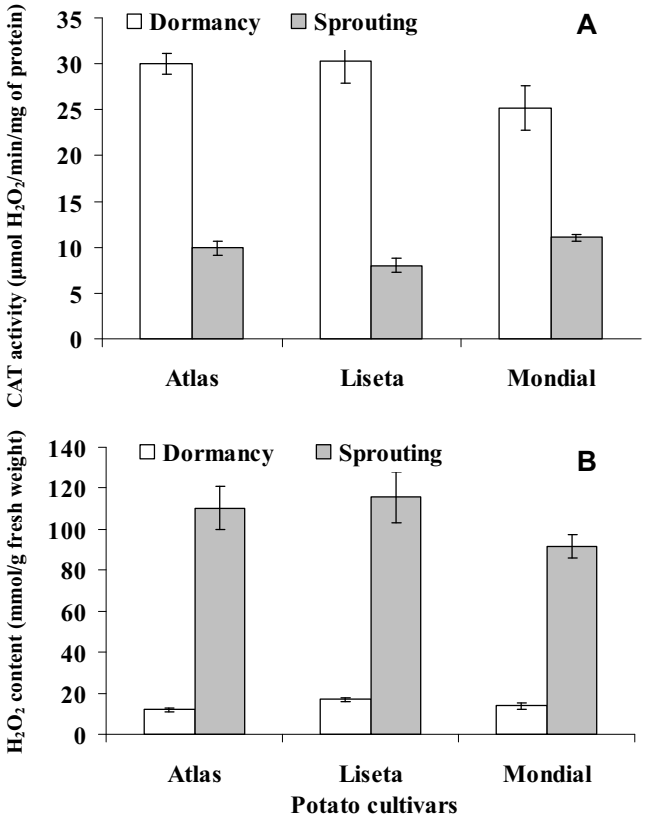

Fig. 4 Catalase activity (**A**) and hydrogen peroxide content (**B**) in tubers of three potato cultivars with long dormancy length at two physiological stages: dormancy and sprouting. Indicated values represent the averages of three repetitions. 45 tubers per cultivar and physiological stage were used. Bars represent the average of their estimated standard deviation. Means with the same letters are not significant at $p = 0.05$ probability level of Duncan test.

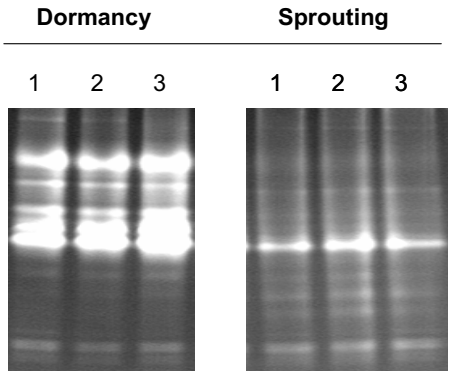

Fig. 5 Native gel assays of the catalase activity in the tubers at two physiological stage (dormancy and sprouting) in three groups of potato cultivars: lanes 1 and 2 (cultivars with long dormancy length, lanes 3 and 4: cultivars with intermediate dormancy length and lines 5 and 6: cultivars with a short dormancy length.

various plant processes, there are still many uncertainties and gaps in our understanding of how H_2O_2 interacts with hormones during dormancy/sprouting. In fact, Bailly (2004) reported that the control of dormancy by hormones such abscisic acid and ethylene could be connected to H_2O_2 signalling and such kind of interplay constitutes a challenge for future researchers in this area.

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