Changes in ROS production and antioxidant capacity during tuber sprouting in potato

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A R T I C L E   I N F O

Article info:
Received 31 March 2017
Received in revised form 16 May 2017
Accepted 19 May 2017
Available online 20 May 2017

Keywords:
Solanum tuberosum L.
Tuber
Dormancy
NADPH oxidase
Gene expression

A B S T R A C T

Potato dormancy is a complex process with an extensive release phase. This study investigated involvement of reactive oxygen species during tuber dormancy release. We found that tuber sprouting was delayed by treatment with diphenylene iodonium chloride, an NADPH oxidase inhibitor; NADPH oxidase catalyze the production of ROS. In situ ROS localization and ROS content estimation revealed that dormancy release was associated with an accumulation of superoxide anion and hydrogen peroxide in tuber buds. The antioxidant compounds and enzymes display important changes during the progression of dormancy. The application of Ca2+ induced superoxide anion production and promoted in vitro tuber bud sprouting. Among the seven homologues of NADPH oxidases in potato, the expression of StrbohA and StrbohD were detected in particular when dormancy break. In addition, the expression of key genes that function in the potato dormancy release are discussed in relation to ROS metabolism in other plants.

1. Introduction

The potato (Solanum tuberosum L.) tuber is an underground stem that is formed by the stoppage of longitudinal growth and the subsequent swelling. The meristem activity of apical regions and lateral buds of developing tubers is repressed during tuber swelling. Tuber dormancy is established at the start of tuber formation. This dormancy state lasts for a finite period of time following tuber harvest, even if the tubers are placed under optimal conditions for sprouting. Among the seven homologues of NADPH oxidases in potato, the expression of StrbohA and StrbohD were detected in particular when dormancy break. In addition, the expression of key genes that function in the potato dormancy release are discussed in relation to ROS metabolism in other plants.

It is well known that exogenous ROS application can induce the breaking of dormancy, this has been demonstrated both in plant seeds and in vegetative buds. In planta, ROS produced by NADPH oxidase has been shown to play various important roles in plant development and growth (Foreman et al., 2003). An increasing number of studies have provided evidence that ROS are involved in plant seed germination and dormancy alleviation (Bailly, El-Marrouf-Bouteau, & Corbineau, 2008; Ishibashi et al., 2015; Maarouf-Bouteau, & Corbineau, 2008; Leymarie et al., 2012; Sarath, Hou, Baird, & Mitchell, 2007). In potato tubers, the application both of hydrogen peroxide (H2O2) and inhibitors of catalase (CAT) results in a reduction of the dormancy period and in rapid sprouting (Bajji, Hamdi, Gastiny, Rojas-Beltran, & du Jardin, 2007). H2O2 can easily cross cell membranes, thus it has been proposed as a dormancy breaking signal. The enhanced gene expression of redox regulation has been observed during the release of dormancy in tubers (Liu et al., 2015).

NADPH oxidase, which are also known as respiratory burst oxidase homologues (Rboh), are named owing to their homology with the gp91phox domain of the animal respiratory burst oxidase (Keller et al., 1998). Rboh enzymes are localized to the plasma membrane, where they can transfer electrons from cytosolic NADPH or NADH to apoplastic oxygen, leading to the production of apoplastic superoxide (Sagi et al., 2004). It has been

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http://dx.doi.org/10.1016/j.foodchem.2017.05.107
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demonstrated that rbhB (respiratory burst oxidase homolog B), a typical membrane-bound rbh enzyme that produces superoxide, plays a role in seed germination in Arabidopsis (Müller, Carstens, Linkies, Torres, & Leubner-Metzger, 2009). Superoxide anions (O2•-) produced by NADPH oxidase were reported to regulate seed germination and seedling growth in barley (Ishibashi, Tawaratsumida, Zheng, Yusa, & Iwaya-Inoue, 2010). NADPH oxidases have effect on α-amylase activity during seed germination (Ishibashi et al., 2010). NADPH-dependent O2•-generating activities in plasma membrane fractions were diphenylene iodonium (DPI) sensitive (Kobayashi, Kawakita, Maeshima, Doke, & Yoshioka, 2006; Pakizeh, Kuliev, Mammadov, Ardjmand, & Hasani, 2011). The NADPH oxidase inhibitor DPI retarded germination, stunted root growth, and partially inhibited NADPH oxidase activity in switchgrass seeds (Sarath et al., 2007).

In order to contribute to understanding of the role of ROS in the release of tuber dormancy, ROS production was measured, and the effect of NADPH oxidase inhibitor DPI application on tuber sprouting behavior was evaluated in this study. As the maintenance of the cellular ROS homeostasis requires a fine-tuned balance between ROS generation and scavenging, the activity of the NADPH oxidase, the antioxidant enzymes and compounds, were investigated during the release of dormancy in tubers. Additionally, we examined the expression of Strbhb genes during tuber dormancy release process. Finally, we evaluated the expression of key genes that are known to regulate the release of dormancy in tubers, and we discussed the potential relationships among gene expression and ROS metabolism involved in dormancy release in relation to what we have known in other plant species.

2. Materials and methods

2.1. Plant Materials

Potato (S. tuberosum L. cv. Russet Burbank and cv. QR01) tubers used in the present work were field grown in the experimental farm of Yuling potato breeding station in late April 2015 in the Shaanxi Province of China. Plants of Russet Burbank and QR01 were managed under standard agronomic practices without irrigation and were harvested in late September. Once harvested, healthy and uniform tubers (dia. 35–45 mm) were manually selected and stored under darkness conditions around 15 °C for fully maturity and wound healing for a period of a month. After that, tubers of ‘Russet Burbank’ were placed to release dormancy in the darkness conditions (approximately 22 ± 2 °C, approximately 60% RH). Tubers of ‘QR01’ were stored at 4 °C in the dark until further use. ‘Russet Burbank’ tubers were thoroughly washed with tap water and then rinsed twice with distilled water prior to sampling. For sample collection, the top 1 cm bud end (with major eye of a potato tuber) tissue were excised and flash frozen at day 0 (start storage), day 30, day 45, day 60 (dormancy break) and day 67 (sprouting) of storage tuber respectively. Three biological replicates were assayed for biochemical studies and RNA extraction. The collected tuber tissues were further crushed in a powder under liquid nitrogen, then stored at −80 °C before analysis.

2.2. In vitro tuber sprouting assay

To evaluate the roles of NADPH oxidase in dormancy processes in tubers, in vitro tuber meristem complexes isolated from QR01 tubers after five months storage at 4 °C were treated with inhibitor of NADPH oxidase DPI. Discs (complex of apical bud meristem) of around 1 cm height containing one bud eye were excised from tubers (Dormant ‘QR01’ potato) using a 8 mm diameter cork borer. Discs were washed three times for 15 min in sterile buffer (20 mM MES, 300 mM d-mannitol, and 5 mM ascorbic acid, pH 6.5). Discs were treated by 0, 2.5, 10 and 20 μM DPI for 10 min respectively and subsequently placed in petri dishes (16–18 discs per dish and two replicates) lined with moist filter paper. Petri dishes were sealed and stored in darkness under tissue culture conditions for two weeks. The filter paper was regularly moistened by adding sterile water. Sprouting of tuber discs were considered as ≥3 mm sprouts occurrence.

2.3. Measurements of O2•- production rate and H2O2 content

O2•- was measured by monitoring the nitrite formation from hydroxylamine in the presence of O2•- according to Elstner and Heupel (1976) described. Briefly, 0.5 g of tuber powder was homogenized with 5.0 mL of 50 mM potassium phosphate buffer (pH 7.8) on ice. After centrifugation at 12 000 rpm for 20 min at 4 °C, 1.0 mL of the supernatant was incubated with 1.0 mL of 50 mM phosphate buffer and 1.0 mL of 1 mM hydroxylamine hydrochloride at 25 °C for 60 min, then 1.0 mL of 17 mM sulfanilamide and 7 mL naphthylamine was added to the incubation mixture. After reaction at 25 °C for 20 min, the absorbance was read at 530 nm. A standard curve was developed to calculate the production rate of O2•- from the chemical reaction of KNO2 and hydroxylamine.

H2O2 was determined as Sergiev, Alexieva, and Karanov (1997) described with some modifications. 0.5 g of tuber powder was homogenized with 5.0 mL 0.1% (w/v) trichloroacetic acid (TCA) on ice. After centrifugation at 12 000 rpm for 20 min, 0.5 mL of the supernatant was incubated with 0.5 mL of 0.1 mM sodium phosphate buffer (pH 7.0) and 2.0 mL of 1 M potassium iodide (KI). The absorbance of incubation mixture was read at 390 nm. The content of H2O2 was given on a standard curve.

2.4. Staining of superoxide ion and hydrogen peroxide

For O2•- and H2O2 detection, around 1 cm length × 8 mm width (1 mm thickness) of hand-cut longitudinal sections of tuber apical bud complex were incubated in 0.1 mg·mL−1 nitroblue tetrazolium (NBT) (in 10 mM Tris-HCl buffer, pH 7.4) and 0.1 mg·mL−1 diaminobenzidine (DAB) (in 50 mM Tris-AC buffer, pH 5.0) at room temperature for 30 min in darkness respectively. The O2•- and H2O2 were visualized as either a blue color at the site of NBT precipitation or a brown color at the site of DAB polymerization. Stained tuber samples were rinsed by 70% ethanol and sterile water, and photographed under natural light. NBT stained sections of apical bud complex were further fixed with 5% formalin, embedded with paraffin, and sectioned to visualize subcellular deposits of superoxide anion by light microscope.

2.5. Total antioxidant capacity assay

The total antioxidant capacity was evaluated by a commercial kit (Cat. No. S0119, Beyotime) following the instructions. 1.0 g of tuber powder was homogenized with 50 mM phosphate buffer and centrifuged at 12 000 rpm for 10 min at 4 °C. Appropriately diluted supernates or standard solution (10 μL) were mixed with ABTS™ working solution (170 μL) and reacted for about 6 min before measuring the absorbance at 405 nm by a microplate reader. The results were calculated as mmol·g−1 TE.

2.6. Ascorbate and glutathione analysis

Both ascorbate (AsA) and dehydroascorbate (DHA) was measured as described by Wang and Jiao (2001). 0.5 g of tuber powder was homogenized with 0.3 M TCA and centrifuged at 12 000 rpm for 20 min at 4 °C. The supernatant was detected by reading
absorbance at 534 nm. The content of AsA was determined from a standard curve prepared with pure AsA (0–60 μg mL⁻¹). The content of DHA was calculated by subtracting the content of AsA from the Total AsA. Both reduced glutathione (GSG) and oxidized glutathione (GSSG) was measured as described by Wang and Jiao (2001). The supernatant was detected by reading absorbance at 412 nm. The content of GSG and GSSG was determined from a standard curve developed with pure GSG and GSSG in the range 0–100/μM. The content of total glutathione was the sum of both GSG and GSSG.

2.7. Total phenolics and flavonoids analysis

Both flavonoid and phenolics were measured as described by Lenucci, Cadinu, Taurino, Piro, and Dallessandro (2006). Briefly, 0.5 g of tuber powder was homogenized with 10.0 mL of 1% HCl-methanol solutions on ice, the homogenate was kept in darkness for 20 min and centrifuged for 10 min at 12,000 rpm at 4 °C. The supernatant was collected and the absorbance was measured separately at 280 nm and 325 nm for phenolics and flavonoids, the concentration of phenolics was expressed as gallic acid equivalents, the content of flavonoid was expressed as A₃₂₅ per gram fresh samples.

2.8. Enzymes activity

All samples were prepared for enzyme activity by homogenizing 1.0 g of tuber powder in a solution of 50 mM phosphate buffer containing 1 mM EDTA and 1% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 12000 rpm for 10 min at 4 °C. The activity of NADPH oxidase, SOD, CAT and APX were measured separately by using NADPH oxidase assay kit (Cat. No. A116), SOD assay kit (Cat. No. A003-1), CAT assay kit (Cat. No. A007-1), and APX assay kit (Cat. No. A123) produced by Nanjing Jianchen Bioengineering Institute according to manufacturer’s directions.

2.9. RNA extraction and RT-PCR, qRT-PCR

Frozen samples from five time points of storage, apical and lateral buds were used for RNA extraction respectively. RNA was isolated using RNA simple Total RNA Kit (Cat. No. DP419, TIANGEN) according to the manufacturer’s directions. Extracted RNA was stored at -80°C until use. The potato ubi3 gene was used as the PCR control. The gene-specific primers were designed using Primer Premier 5.0 (Table S1). The first-strand cDNAs were synthesized from 500 ng of total RNA with the PrimeScript®RT reagent Kit (Cat. No. RR047A, Takara) including gDNA Eraser in 20 μL reaction volume. The RT-PCR was performed in a 50 μL reaction volume using the Premix Taq™ kit (Cat. No. RR902Q, Takara), by mixing 1 μL of a five-fold diluted cDNA, 25 μL of Premix Taq and 20 μM of each primer. The PCR conditions were 10 s at 98 °C, followed by 30 cycles of 30 s at 53 °C and 30 s at 72 °C. The qRT-PCR was performed on a Step One Plus™ Real-Time PCR System using the Quantitect®SYBR® Green PCR Kit (Cat. No. 204054, QIAGEN) as described by Liu et al. (2016). The relative expression levels for each gene was normalized using an ubi3 as the internal control and calculated using 2⁻ΔΔCT method in comparison with the initial time point gene. Three biological replicate samples were each subjected to qRT-PCR measurement.

2.10. Statistical analysis

Data represent the means ± SD of three replicates. The statistical analyses were performed with IBM SPSS Statistics 20. Data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan’s multiple-range tests. Differences at P < 0.05 were considered as significant.

3. Results

3.1. Inhibition of NADPH oxidase delays tuber sprouting in vitro

The effect of the NADPH-oxidase inhibitor DPI on tuber sprouting are shown in Fig. 1. After two weeks of incubation, in vitro tuber meristem complexes had a sprouting rate (with ≥3 mm sprouts) of 52% and 43% following 2.5 μM and 5 μM DPI treatment, respectively, while the controls had a sprouting rate of 85%. Of note, the data of 10 μM and 20 μM DPI treatment was not shown because high doses of this inhibitor damaged in vitro meristem complexes.

3.2. ROS production during the release of dormancy in tubers

NADPH oxidase catalyzes the production of O₂⁻ and H₂O₂. We stained both dormant and sprouting tuber meristem complexes with NBT and DBA to monitor the accumulation of ROS. As shown in Fig. 2, for O₂⁻ accumulation, hand-cut sections and transverse paraffin sections through the apical bud confirmed the presence of formazan deposits at the tissue and cellular level in sprouting tuber. In contrast, the extent of NBT staining apparent in sprouting bud of dormant tuber was only very slight (Fig. 2A, B, E, F). H₂O₂ accumulation could be detected in dormant apical buds, but there was much stronger staining in sprouting buds (Fig. 2C, D). The dark precipitates that resulted from the staining indicated the presence of O₂⁻ and H₂O₂ in the cortex and parenchyma tissues during the progression of the release of dormancy in tubers. To further characterize the ROS production during the release of dormancy in tubers, the amounts of both O₂⁻ and H₂O₂ in bud end of the tuber were also evaluated, and we observed a marked increase in O₂⁻ release rate which coincided with dormancy break at day 60 in storage; Meanwhile, the H₂O₂ content increased at all time points over day 0 except for day 60 of storage (Fig. 3), revealing that endogenous ROS accumulation is associated with the release of dormancy in tubers.

3.3. Changes in NADPH oxidase activity

NADPH oxidase catalyze the production of O₂⁻ from oxygen and NADPH. The activity of NADPH oxidase was investigated during tuber dormancy release process. As shown in Fig. 3, the activity of NADPH oxidase gradually increased, reaching a maximum at day 60 of storage which coincided with the time point of dormancy break, although there was a slight decrease at day 45 of storage. The observation of the high activity of NADPH oxidase at day 60 of storage was also consistent with the marked accumulation of ROS (Fig. 3).

3.4. Expression of Strboh genes during the release of dormancy in tubers

The expression of seven Strboh genes of the NADPH oxidase family of potato were examined with semiquantitative PCR. We observed that only StrbohA and StrbohB was expressed in particular in sprouting tubers (at day 67 of storage), in apical buds and in lateral buds. Expression of StrbohC and StrbohD was found to be constitutive in all sampling time examined. Neither StrbohE nor StrbohF exhibited expression in tubers during dormancy progression, as well as in apical and lateral buds (Fig. 4). This points to a major role of StrbohA and StrbohB in the release of dormancy in tubers.
3.5. Changes in antioxidant compounds

Ascorbate and glutathione are known to be important players in helping plants to manage ROS metabolism. The cellular redox balance is regulated by these compounds. Both total AsA content and the reduced AsA content decreased significantly in the period from the first day of storage to day 60 of storage, then increased when tuber sprouting. The DHA content did not differ in this process (Fig. 5). Meanwhile, the content of total glutathione content and the GSG content increased from the first day of storage to day 60 of storage, then both of them start to decrease from the day 60 to day 67 of storage. No accumulation of GSSG was observed during dormancy release process (Fig. 5).

Both phenolics and flavonoids are free or cell wall-bound secondary metabolites that are, like ascorbate and glutathione, able to inactivate ROS. As shown in Fig. 5, following a similar change with glutathione, the content of phenolics increased from the first day of storage to day 60 of storage, then it begins to decrease with the occurrence of dormancy break (Fig. 5). A decrease in flavonoids content was also observed during the tuber dormancy break at day 60 of storage, although this decrease was not significant (Fig. 5).
is possible that phenolics and flavonoids levels were associated with ROS signaling when dormancy break at 60 days.

3.6. Changes in antioxidant enzymatic activity

The antioxidant enzymes SOD, APX, and CAT display important changes during seed and bud germination. We also evaluated the activities of these enzymes during the release of tuber dormancy. As shown in Fig. 5, the change in SOD and CAT activity occurred following dormancy break. SOD activity increased significantly from day 1 to day 45 of storage, then decreased by day 60 of storage when tuber dormancy break. Likewise, CAT activity also significantly increased at day 45 of storage, then progressively decreased from day 45 to day 67 of storage, these changes correspond to the time points of tuber dormancy break and sprouting. APX activity was relatively steady during tuber storage. In addition, total antioxidant capacity was investigated by ABTS assay, the results showed that there was no significant change in total antioxidant capacity during tuber dormancy release (Fig. 5).

3.7. Calcium ions dependent NADPH oxidase promotes tuber sprouting

Given what is known about the role of Ca^{2+} in regulating NADPH oxidase, we investigated the effects of Ca^{2+} on ROS generation and tuber dormancy. As the periderm of potato tubers is nearly impermeable to chemicals, gases, and liquids, the in vitro tuber discs of apical bud complex isolated from QR01 tubers after two months storage at 4°C were exposed to CaCl_2 solutions. As shown in Fig. 6, application of Ca^{2+} to in vitro tuber discs caused a rapid elevation in O_2·^- production. Moreover, increasing ROS levels led to about 69% of tuber discs sprouting than tuber discs sprouted at 35% as control. These results provide indirect evidence that activation of ROS production by Ca^{2+} might have effects on tuber sprouting.

3.8. Changes in expression of dormancy related genes

To investigate expression of key genes during tuber dormancy release process, qRT-PCR was conducted in this study. As shown in Fig. S1, the expression of DELAY OF GERMINATION 1 (DOG1), which is known to be a major regulator of dormancy, decreased during dormancy release process. The expression of gene involved in gibberellic acid (GA) biosynthesis (GA3ox2), increased gradually during dormancy release process, with the maximal expression occurring at day 67 of storage. The expression of gene involved in abscisic acid (ABA) catabolism (CYP707A1), below initial level across storage but rose dramatically at day 67 of storage. The expression of SLP, which is known to be involved in gibberellic acid (GA) signaling, was detected across storage, their expression was highest at day 30 and day 60 of storage. The expression of ABIS,
which is known to be involved in abscisic acid (ABA) signaling, was detected only at the start of storage and absent or below the limits of detection throughout dormancy progression.

4. Discussion

In this study, we investigated ROS metabolism during the dormancy release process in tubers. ROS produced by NADPH oxidase is known to play important roles in plant development (Bailly et al., 2008). NADPH oxidase localized at the plasma membrane produce $\text{O}_2^{-}$ by transferring electrons from cytoplasmic NADPH to oxygen, this oxygen subsequently dismutates to $\text{H}_2\text{O}_2$ and $\text{O}_2$. Studies of ROS metabolism and dormancy release have been reported for seeds of soybean (Puntarulo, Sánchez, & Boveris, 1988), maize (Hite, Auh, & Scandalios, 1999), wheat (Caliskan & Cuming, 1998), Arabidopsis thaliana (Leymarie et al., 2012), and barley (Ishibashi et al., 2015), as well as for vegetative buds of apple and grape (Pérez & Lira, 2005; Wang, Jiao, & Faust, 1991). It also has been suggested that NADPH oxidase plays a role in seed germination in rice (Liu, Xing, Li, & Zhang, 2007) and in warm-season C4-grasses (Sarath et al., 2007). Although the role of ROS and NADPH oxidase is now quite well documented in plants, there exist few studies on the influence of ROS on tuber dormancy. Subcellular distribution of NADPH oxidase activity in potato tissues was also examined (Pakizeh et al., 2011). Thus, it is necessary to explore the role of ROS metabolism during potato dormancy release.

In agreement with previous studies which established that inhibitor (DPI) of NADPH oxidase, inhibits ROS generation and delays seed germination (Ishibashi et al., 2015; Müller et al., 2009), our results showed that the sprouting of tuber apical bud meristem can be suppressed by DPI. In plants, and more especially in seeds, ROS can originate from the mitochondrial respiratory chain or can be produced through the action of enzymes like NADPH oxidase (Bailly, 2004). The role of ROS in tuber dormancy release is also highlighted by the data of histochemical stain. $\text{O}_2^{-}$ and $\text{H}_2\text{O}_2$ appeared as intensely dark precipitates mainly at the sprouting tuber bud. The most apparent evidence is a transient but significant increase in $\text{O}_2^{-}$ and $\text{H}_2\text{O}_2$ content at day 60 and day 67 of storage, which corresponds to dormancy break and sprouting. On the other hand, as was observed in the activity of NADPH oxidase, the increased enzyme activities at day 60 of storage corresponds to the transit accumulation of $\text{O}_2^{-}$. This demonstrates that ROS production is tightly associated with the breaking of tuber bud, and that, in agreement with previous proposals that ROS can act as cell messengers (Bailly, 2004). ROS generated by NADPH oxidase in signaling result in GA biosynthesis in plant seeds, which further promote germination (Ishibashi et al., 2015). We could speculate that the increased activity of NADPH oxidase and consequently the rise
In ROS content may be one of the early and relevant events leading to the termination of potato dormancy.

The production of ROS by plant has often been regarded as a cause of stress that might affect the dormancy release. Therefore, antioxidant compounds and enzymes have been widely considered as being of particular importance for the completion of sprouting (Bailly, 2004). Ascorbate and glutathione are known to contribute to ROS homeostasis. In agreement with previous work on tuber aging (Delaplace et al., 2009), we found that the content of ascorbate gradually decreased before dormancy break, however, the decrease of glutathione content started after dormancy break, indicating ascorbate precedes glutathione in response to ROS signaling. These compounds likely also contribute to the control of cellular redox balance (Tommasi, Paciolla, de Pinto, & De Gara, 2001). As another ROS homeostasis moderator, the activity of SOD showed a decrease at the time of dormancy break. This is inconsistent with previous reports that SOD activity increased or was relatively constant during tuber dormancy (Bajji et al., 2007; Rojas-Beltran, Dejaeghere, Abd Alla Kotb, & du Jardin, 2000), indicating that the change of SOD activity does not seem to correlate with tuber dormancy release. The reduction in CAT activity started from the day 45 of storage to day of 67 of storage, indicating the transient accumulation of ROS which may partially result in a reduction in CAT activity. This was also supported by the evidence that CAT inhibitor (thiourea) can lead to rapid sprouting (Bajji et al., 2007). Similar change of CAT activity has also been reported (Rojas-Beltran et al., 2000). The enhancement of germination was associated with stimulation of CAT activity in plant seeds (Bailly, 2004). H2O2 accumulation was observed in grapevine buds following the inhibition of CAT (Pérez & Lira, 2005). Furthermore, the total antioxidant capacity didn’t change significantly within the given time frame, which probably because tuber dormancy break is not concomitant with the enhanced oxidative stress, this was supported by a previous reports that overall antioxidant capacity was not statistically significant change during tuber ageing (Delaplace et al., 2009).

In the cytoplasm of plant cells, ROS modulate the redox status and additionally trigger oxidative modifications of proteins and stored mRNAs (Bazin et al., 2011; Oracz et al., 2009), which in turn regulates cell signaling to trigger dormancy break. Therefore, comparative analysis of changes in antioxidant enzymes or compounds support a role for ROS which is involved in dormancy release in tuber and may, in turn, be involved in free radical formation.

It has been suggested that NADPH oxidase plays a role in seed germination in switchgrass (Sarath et al., 2007), sunflower (Oracz et al., 2009) and barley (Ishibashi et al., 2010). Consequently, we propose that the increase in cellular ROS content in conjunction with the stimulation of ROS generating enzymes, such as NADPH oxidase. The activation of NADPH oxidase is known to depend on the influx of Ca2+ into the cytoplasm (Suzuki et al., 2011). CaCl2 treatment on in vitro tuber apical buds clearly promoted superoxide production, and finally resulted in bud sprouting, indicating that NADPH oxidase activation might contribute to this early sprouting. Ca2+ can induce and restore the superoxide-forming at the wound surface of potato tuber (Kumar, Iyer, & Knowles, 2007). Ten respiratory burst oxidase homologues (rboh A–J) are reported in Arabidopsis (Kwak et al., 2003) and seven (Strboh A–F and H) have been identified in potato (Yoshioka et al., 2001). Among the Strboh genes of potato, StrbohA and StrbohD was expressed in particular at day 67 of storage when tubers start sprouting. Müller et al. (2009) proposed that AtrbohD is a major producer of superoxide in germinating Arabidopsis seeds, and inhibition of superoxide production by DPI leads to a delay in Arabidopsis seed germination and causes endosperm weakening. StrbohA and StrbohD are also required for H2O2 accumulation and for resistance to Phytophthora pathogens in Nicotiana benthamiana (Yoshioka et al., 2003). Transcripts of StrbohC, StrbohD and StrbohH were constitutively present, and likely accounted for the basal activity of NADPH oxidase in potato. AtrbohD is known to mediate ABA-induced ROS production (Lightfoot, Boettcher, Little, Shirley, & Able, 2008). Neither StrbohC nor StrbohH expression was detected in our experiments. This suggest that StrbohA and StrbohD is critically important for superoxide production during tuber dormancy release.

Studies have been conducted to elucidate the mechanism by which ROS breaks dormancy in plant (Bailly et al., 2008). Such effect of ROS on dormancy release does not seem to be a peculiar characteristic of potato since several studies have shown similar responses in both seeds and fruit tree buds, providing additional evidence that common regulatory mechanisms of dormancy could occur in these different plant tissues. Some of these have suggested that H2O2 regulates the expression of genes that are involved in dormancy. We therefore studied the expression of genes known to be important in the potato dormancy release process in parallel to ROS metabolism. The relationships between GA and ABA levels and sensitivity responses are known to be major regulatory aspects controlling dormancy. GAs are associated with the release of dormancy and sprouting, while ABA is mainly related to the onset and maintenance of tuber dormancy (Sonnemwald, 2000). We found that the expression of GA3ox2, which is involved in GA biosynthesis, significantly increased at the dormancy release time point; Meanwhile, the expression of CYP707A1, which is related to ABA catabolism, exhibited transient maxima during late storage. The enhanced expression of CYP707A1 suggests that decreased ABA content might contribute to dormancy release. The increased biosynthesis of GA and catabolism of ABA coincided with the
accumulation of ROS as well as tuber sprouting. Liu, Ye, Liu, Chen, and Zhang (2010) proposed that H2O2 affects the regulation of ABA catabolism and GA biosynthesis during seed germination. Interestingly, we found that GAs signaling might be involved in the induction of the release of dormancy, as the expression of SLP, a protease with expression known to be induced by GAs, is increasing following dormancy release process, even before the dormancy break. It has been reported that ROS can trigger barley and Arabidopsis seed germination via the GA signaling (Bahn et al., 2011; Leymarie et al., 2012). ROS produced by NADPH oxidase in embryos is also specifically involved in GA biosynthesis upon seed germination (Ishibashi et al., 2015). However, the expression of ABIS, which is involved in ABA signaling downstream of its perception (Finkelstein, Reeves, Ariizumi, & Steber, 2008), is decreasing during dormancy release process, this suggests that dormancy release might not be associated with ABA signaling. DOG1 has been identified as a major regulator of dormancy in various plant species. We found that the expression of DOG1 declined gradually during the dormancy release process. In Arabidopsis, deeper dormancy has been associated with the accumulation of DOG1 mRNA, which then disappears during seed imbibitions (Bentsink, Jowett, Hanhart, & Koornneef, 2006). The relationship between ABA, gibberellin and ROS in the regulation of dormancy is almost certainly highly complex, and although our results do not provide direct evidence, our findings relating to ROS metabolism in parallel to the expression of dormancy related genes are in agreement with previous studies that ROS, as mediated by NADPH oxidase, is involved in dormancy release by regulating GA/ABA levels.

In summary, we demonstrated that ROS metabolism is involved in the dormancy release process in tubers. We speculate that the increased activity of NADPH oxidase and consequently the rise in ROS content may be one of the early events leading to potato dormancy break. We found that StrbohA and StrbohB are key genes that mediate ROS production during the dormancy release process. This work also underlines the potential mechanisms of dormancy release in tubers by comparing between dormancy in potato with what is known about the dormancy release mechanisms of dormancy release in tuber by comparing between dormancy in potato and what is known about the dormancy release process in other plant species.

Conflict of interest statement
The authors declare no conflict of interest.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 31601358; No. 31560413), the Fund for Young Scholars of Northwest A&F University (No. Z109021610), and the Fund for Hundred-Talent Program of Shaanxi Province (No. A289021401).

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.05.107.

References


