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REVIEW ON POSITIVE ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN SEED GERMINATION

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ABSTRACT

In many seeds, the process of germination features testa rupture and endosperm rupture are two separate events. Endosperm rupture requires cell wall weakening in the endosperm layer. Puncture force measurements are a useful tool for quantifying this endosperm weakening. Various mechanisms have been proposed to promote endosperm weakening. Endosperm weakening requires cell wall weakening. This process involves cleavage of cell wall polymers or loosening of bonds between the polymers. Several molecular mechanisms have been proposed for endosperm weakening. Most prominent among them is, cleavage of cell wall polymers in the endosperm by reactive oxygen species, or more specifically, by apoplastic hydroxyl radicals ($^{\bullet}OH$) formed when superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) undergo a Fenton reaction in the presence of peroxidases. Regulation of seed germination is quite complex and is further complicated by interaction of hormones like Gibberellin (GA), Abscisic Acid (ABA) and ethylene. Moreover, the involvement of reactive oxygen species (ROS) in hormone signaling for such regulation is still less understood. Positive interactive role of ROS with GA, ABA and ethylene was observed in seed germination of Vignaradiata. ROS production is essential for lignification and cross-linking of cell wall polymers in vascular tissue. Oxidation of the germination inhibitor(s) present in the pericarp by H_2O_2 promotes seed germination. Antioxidants which are derivatives of well-known germination inhibitors suppressed seed germination in a dose-dependent manner. To initiate seed germination, the germination inhibitor(s) should be decomposed by an oxidant such as H_2O_2.

INTRODUCTION

ROS are reactive molecules and Free Radicals derived from molecular oxygen. They are chemically reactive molecules containing oxygen (H_2O_2, O_2^-, $^{\bullet}OH$). These ROS were initially recognized as toxic by-products of aerobic metabolism but it also involved in "Positive" developmental role and a novel direct action during seed endosperm weakening and germination. Seeds are reported to generate ROS either in dry condition or even when imbibed (Bailly, 2004) and ROS generation has been associated with a positive role in the process of germination (Chaudhuri*et al.,* 2008; Garnczarska and Wojtyla, 2008; Ishibashi*et al.,* 2010). Seed germination, an early developmental event, starts with imbibitional water uptake and culminates into radicle protrusion. Seed germination involves activation of embryonic growth following hydration. Underlying this activation is a metabolic up regulation that includes some subtle intangible regulatory components like Reactive Oxygen Species (ROS) which are connected with messengers like hormones through complex signalling chains (Kar, 2007). The highly reactive free radicals, along with hydrogen peroxide (H_2O_2), are collectively termed active/reactive oxygen species (AOS/ROS). While the intracellular glassy state must curtail molecular and AOS mobility, and thus interaction, during the process of desiccation, intracellular structures are highly vulnerable as conditions for radical generation are enhanced (Vertucci and Farrant, 1995; Pammenter and Berjak, 1999; Walters *et al.,* 2005). For an endospermic seed to complete germination, the growth potential of the radicle must be high enough to overcome the tissue resistance of the endosperm (Bewley, 1997). Factors that influence germination, e.g. plant hormones, can thus influence the resistance of the endosperm tissue by promoting or inhibiting endosperm weakening. Increased cellular levels of Reactive Oxygen Species (ROS) are known to occur during seed development and germination (Bailly, 2004). Reactive Oxygen Species (ROS) were initially recognized as toxic by-products of aerobic metabolism, removed by means of antioxidants and antioxidative enzymes.

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So, most studies on ROS and antioxidants have been focused on negative of role in seeds like seed deterioration followed by ageing. In recent years, it has become apparent that ROS play an important positive role like endosperm weakening and seed germination.

Mechanism on Formation of Reactive Oxygen Species

NADH oxidases located in the plasma membrane catalyse the formation of apoplastic $O_2^{-\bullet}$anions. $O_2^{-\bullet}$is dismutated by the antioxidant enzyme Superoxide Dismutase (SOD), leading to the formation of $H_2O_2$and molecular oxygen. Thus $O_2-\bullet$and $H_2O_2$are both present in the apoplast. In the presence of bivalent cations (e.g. Fe2+or Cu2+), •OH can be formed from H_2O_2in the so-called Fenton reaction, and regeneration of these catalytic bivalent cations can be achieved by the oxidation of $O_2.-\bullet$ (Vianello and Macri, 1991). The generation of •OH in the cell wall by a Fenton-type reaction can also take place in the presence of peroxidases, which are abundant in the plant cell wall (Chen and Schopfer, 1999), or in the presence of ascorbate and bivalent cations (Fry, 1998). •OH areable to cleave hemicelluloses and have been shown to cause invitrocleavage of cell wall polysaccharides (Fry, 1998; Schweikert *et al.,* 2002).

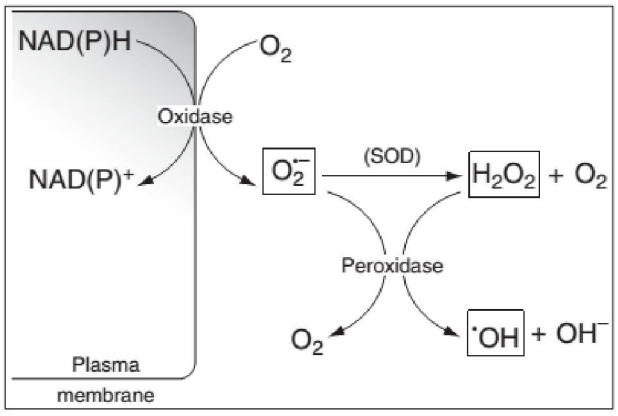

Fig. 1.Qualitative model for the generation of apoplastic hydroxyl radicals. NADH oxidases located in the plasma membrane catalyse the formation of apoplastic $O_2.-\bullet$anions. $O_2.-\bullet$ is dismutated to H_2O_2 and molecular oxygen by superoxide dismutase (SOD). A Fenton-type reaction can take place in the presence of peroxidases, leading to the formation of •OH. (From Schopfer *et al.,* 2001.)

H_2O_2reacts with O−• in the presence of cell wall peroxidases, leading to the formation of •OH (Chen and Schopfer, 1999). These •OH then act on the cell wall by causing polysaccharide cleavage resulting in endosperm weakening. Peroxidases are generally present in cell walls of growing tissues in great abundance, OH can potentially be generated there whenever H_2O_2 and O_2 are available. Peroxidase activity increases in the micropylar endosperm of tomato seeds prior to endosperm rupture (Morohashi, 2002). Ascorbate peroxidase scavenging H_2O_2 is localized at the site of H_2O_2 generation in the plant cell (Asada, 1999), and is likely to be micro compartmentalized by organizing scavenging complex of enzymes such as superoxide dismutase and monodehydroascorbatereductase (Ogawa *et al.,* 1995, Asada *et al.,* 1996, Asada,1999). During the biosynthesis of lignin, H_2O_2 is produced at the site of lignification (Ogawa *et al.,* 1996, Ogawa *et al.,* 1997). Thereby, H_2O_2 generated is scavenged or utilized at its generation site in plants. If H_2O_2 is generated at an unexpected site or reaches a level that exceeds the scavenging capacity, it causes oxidative damage to plants. H_2O_2 impairs the photosynthetic activity of isolated chloroplasts when applied exogenously even at 1µM which is a level having little effect on the activity when generated in chloroplasts (Asada and Takahashi, 1987).

Role of Ros in Cell wall Loosening

Endosperm weakening followed by seed germination requires cell wall weakening. This process involves cleavage of cell wall polymers or loosening of bonds between the polymers. Several molecular mechanisms have been proposed for endosperm weakening. Digestion of cell wall polysaccharides by hydrolytic enzymes is one mechanism. The main focus so far has been on β-1,4-mannanase (Bewley, 1997) and β-1,3-glucanase (Leubner-Metzger, 2003). Reactive Oxygen Species (ROS) produced in the cell wall play a role in endosperm weakening, which takes place before endosperm rupture. Peroxidase activity increases in the micropylar endosperm of tomato seeds prior to endosperm rupture (i.e. during endosperm weakening) (Morohashi, 2002).The cleavage of cell wall polymers by •OH not only takes place in the endosperm, but also plays a role in radicle elongation. The cell walls have to be loosened in order to allow cell elongation, caused by water uptake, which takes place when the water potential in the embryo is lower than that of the surrounding medium (Muller *et al.,* 2007).

In tobacco seeds, b-1,3-glucanase is induced in the micropylar part of the endosperm prior to radicle protrusion suggested that the enzyme contributes to the weakening of the cell walls of the micropylar endosperm by helping to hydrolyse cell wall b-1,3-glucans and thereby contributes to facilitating penetration of the radicle (Vogeli-Lange *et al*., 1994 and Leubner-Metzger *et al*., 1995). Non-enzymatic cleavage of wall polymers by the hydroxyl radical (OH). This extremely reactive, short-lived intermediate of O_2 reduction is principally capable of splitting covalent bonds in all kinds of organic molecules in a diffusion-limited reaction, i.e. within a few nanometers of its site of formation. As their range of action is limited by diffusion, they must be produced directly in the cell wall in order to cleave cell wall polymers (Halliwell and Gutteridge, 1989; Schopfer, 2001). Cell-wall polysaccharides such as pectin and xyloglucan can be broken down in vitro by OH generated in a Fenton-type reaction, e.g.by the reduction of O_2 with ascorbate in the presence of Cu ions.

OH produced in the apoplastic space of plant tissues, could act as a site-specific oxidant targeted to play a useful physiological role in cell-wall loosening processes underlying cell expansion, fruit ripening and organ abscission. (Fry, 1998). Radicle penetration through the micropylar portion of the endosperm may accompany the breakdown of the cell wall, that is, wounding (Wu *et al.,*2001). Many studies have shown that peroxidase is induced by wounding in plants (Desbiez and Boyer, 1981; Espelie *et al.,* 1986; Kawaoka *et al.,,* 1994; Kato *et al.,* 2000). On the other hand, peroxidase has been reported to be involved in generation of H_2O_2 from NADH (Maeder *et al.,* 1980). Inhibition of catalase in lettuce seeds led to higher concentrations of H_2O_2in the seeds and to faster germination (Hendricks and Taylorson, 1975).

Interaction of Hormones with Ros

Regulation of seed germination is quite complex and is further complicated by interaction of hormones like Gibberellin (GA), Abscisic Acid (ABA) and ethylene. Moreover, the involvement of Reactive Oxygen Species (ROS) in hormone signaling for such regulation is still less understood. Among phytohormones, GA and ABA are well known for their antagonistic action for seed dormancy and germination. They play a contrasting role GA breaks dormancy and promotes germination while ABA maintains dormancy and inhibits germination (Bentsink and Kooenneef, 2002). Recently, Reactive Oxygen Species (ROS) have been demonstrated to play roles in growth and development either directly by participating in the process of cellular growth or differentiation or indirectly by signaling for induction of processes or reactions related to growth and differentiation (Bailly, 2004).

ROS may act downstream of plant hormones (Kwak, 2003). H2O2 rescued germination almost fully from the inhibition imposed by ABA and ABA might have affected ROS metabolism either directly or by interfering with ethylene action on ROS metabolism (Chaudhuri *et al.,* 2013). Sarath *et al*. (2007) also demonstrated a reversal of ABA-induced inhibition of germination by H2O2 and they explained this as due to interference of H2O2 in ABA signalling. On the other hand, action of GA on seed germination of V. radiatais in line with H_2O_2, as H2O2 can partially overcome the inhibition by paclobutrazole on germination. Roles of ROS in GAsignalling in the aleurone layer and Programmed Cell Death (PCD) in Hordeumvulgare has already been established, where GA initiates cell death of aleurone cells, whereas ABA inhibits cell death (Ishibashi *et al.,* 2013). GA may have some other way of signalling in use for germination as H_2O_2 could not fully rescue when GA synthesis was blocked by paclobutrazole (Chaudhuri *et al.,* 2013). Liu *et al*. (2010) demonstrated that H_2O_2upregulates ABA catabolism through NO signalling while promotes GA synthesis thus favouring germination. On the other hand, Bahin *et al*. (2011) proposed that H_2O_2alleviates dormancy by activating GA signalling and synthesis, not by repressing ABA signalling.

Germination Promoting Mechanism of Ros

It is known that H_2O_2 promotes germination in various species. H_2O_2 promoted seed germination in a dose-dependent manner as did respiratory inhibitors, indicating that H_2O_2 itself possibly promotes seed germination rather than O_2. H_2O_2 is a toxic molecule due to its highly oxidative reactivity and long life. In the presence of catalytic metal ions such as iron and copper, it produces hydroxyl radical (·OH) which strongly oxidizes cell components such as membrane lipids and enzymes.H_2O_2 can be used in high concentrations to promote germination of seeds with hard seed coats by scarification, it also has a germination-promoting effect in lower concentrations (Naredo *et al.,* 1998; Ogawa and Iwabuchi, 2001). Exogenously applied H_2O_2 ameliorates seed germination in many plants (Fontaine *et al.,* 1994, Chien and Lin, 1994). This has been explained by the fact that the scavenging activity for H_2O_2 is high enough, resulting in the production of O_2 for mitochondrial respiration. However, an early step of seed germination (dormancy breakage) dispenses with mitochondrial O_2 respiration and requires the activation of the oxidative pentose phosphate pathway. Thioredoxin reduction by NADPH produced via the oxidative pentose phosphate pathway allows the mobilization of storage proteins of cereals, leading to germination (Fontaine *et al.,* 1994). The pericarp and seedcoat often contain phenolic compounds and alkaloids which inhibit seed germination (Bhattacharyya *et al*., 1999, Tao andButa 1986). Oxidation of germination inhibitor(s) is likely to exclude a reaction that is mediated by peroxidases because strong inhibitors of peroxidases that have micromolar Ki value promoted seed germination. It is likely that H_2O_2oxidatively denatures germination inhibitor(s) such as ferulic and coumaric acids. The germination frequency of Z. elegans seeds was enhanced by H_2O_2.The concentration of H_2O_2 giving the maximal promotional effect varied depending on the time after imbibition of the seeds. The concentrations of H_2O_2 that gave half the maximal germination frequency achieved 24 h and 48 h after imbibition were obtained between 2 and 5 mM, whereas it was between 5 and 20 mMH_2O_2 at 36 h and 48 h after imbibition.

These suggest that H_2O_2 influences more than two steps in the process of germination and/or is continuously consumed at a certain rate to promote seed germination. The disproportionation of H_2O_2 resulting in an increased O2 level is considered to enhance the oxidative respiration, which can be the reason for seed germination promotion (Ogawa, 2001).
Imbibition of dry seeds is associated with a rapid increase in oxygen uptake and mitochondrial respiration supporting ATP synthesis (Bewley and Black, 1985). It is estimated that up to 2% of mitochondrial O_2 consumption in seeds is involved in the generation of H_2O_2 (Cakmak *et al.,* 1993). High concentrations of O_2 generally enhance the germination of plant seeds, and such promotion has also been considered to be due to the enhancement of mitochondrial oxidative respiration. The $K_m$value for mitochondrial Cytcoxidase which participates in the ATP production metabolism accompanied with O_2 consumption is estimated to be approximately 140 nM O_2, whereas that for alternative oxidase whose reaction is not accompanied with ATP production is approximately 1.7 μM O_2 (Millar *et al.,* 1994).

Germination inhibitor(s) may block other processes involved in germination aside from mitochondrial respiration. Activation of the oxidative pentose phosphate pathway generally leads to seed germination, and has been proposed to be promoted by H_2O_2 (Fontaine *et al.,* 1994). The oxidative pentose phosphate pathway provides NADPH which is used for the reduction of redox-regulating proteins such as thioredoxin. Such proteins regulate mobilization of storage proteins and the modulation of activities of enzymes and transcriptional factors by their reduction of disulfide bond in the target molecules (Kobrehel *et al.,* 1992). So, the activation of the pentose phosphate pathway leads to germination. Antioxidant germination inhibitors such as phenolic compounds may block activation of the pathway. Catalase and ascorbate peroxidase activities increase and dehydroascorbatereductase activity decreases with germination of wheat seeds. So, there is low scavenging activity of H_2O_2 in seeds at the initial stage of germination and the temporal oxidized state of the seed embryo that is induced by H_2O_2 might initiate germination and that antioxidant germination inhibitor(s) might prevent the induction of the oxidized state in seeds (Cakmak*et al.,* 1993).

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