Environmental and Experimental Botany 150 (2018) 260–271

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Biological mechanisms of a novel hydro-electro hybrid priming recovers potential vigor of onion seeds

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ARTICLE INFO

Keywords:
Electric field
Hydro
Hybrid
Priming
Seed

ABSTRACT

Onion (*Allium cepa*) seeds gradually lose vigor during storage, even under optimal conditions. Some of the loss in vigor is reversible and can be recovered by priming. Electrostatic field priming can instantly increase seed vigor and accelerate germination, though the effects are short-lived. Hydro-priming is most widely used as it does not use chemicals and is a clean process, but requires a long priming time and precise seed moisture control. Here, we investigated the potential of combined hydro-electrostatic hybrid priming on the recovery of vigor in onion seeds. Hydro-electrostatic hybrid priming lead to improved seed vigor recovery. Circular dichroism spectroscopy and electron paramagnetic resonance revealed exposure to an electrostatic field enhanced SOD activity in seeds by changing the secondary structure of the enzyme and led to more efficient free radical scavenging; scanning and transmission microscopy revealed these effects promoted more complete and rapid healing of embryo cell organelles and plasma membranes during the incubation stage of hydro-priming. Hydro-electrostatic hybrid priming has the advantages of greater recovery of potential vigor and a shorter processing time than single hydro-priming, leads to longer lasting effects than single electro-priming, and does not require any chemicals.

1. Introduction

Most agricultural crops are grown from seed, and large quantities of seeds are produced, transported and stored every year. According to the International Seed Federation, China is the fourth largest seed importer in the world; 10.04 kilo tones were imported in 2014 (ISF, 2014). Some species, such as onion, are purchased in massive quantities for multi-season sales. Imported onion seeds are generally stored for up to 4–5 years before sale in China. However, onion seeds have a poor longevity and their vigor is reduced by storage (Schwember and Bradford, 2011). Even under good storage conditions, onion seeds lose vigor more rapidly than other species (Sijbring, 1963). Loss of seed vigor directly delays establishment in the field, and leads to a lack of germination, seedling abnormalities, reduced agricultural production and economic losses. Therefore, seeds with germination rates lower than the legal requirements must be identified and removed from sale.

Loss of seed vigor has been widely studied and is influenced by a variety of mechanisms. Some factors–such as mild membrane deterioration or enzyme inactivation–are reversible, meaning some loss of vigor can be recovered by physical or chemical treatments (Yan and Chen, 2016). Seed priming is used to describe treatments that trigger ‘pre-germination’ but not radicle emergence (Paparella et al., 2015). When dry seeds are sown, germination occurs in three phases: (I) imbibition, (II) pre-germination and (III) emergence (Appendix Fig. 1 in Supplementary material). During seed priming, the supply of water to the seed is controlled at a level below that necessary for actual emergence, but at level sufficient to initiate many physiological processes associated with pre-germination (e.g., free radical scavenging, enzyme activation, membrane recovery). The low water supply prevents initiation of phase III (emergence) by extending and holding the seeds within phase II (pre-germination), before the seeds are dehydrated again and stored. Priming can reduce the duration of phases I and II when the seed is sown (Appendix Fig. 1 in Supplementary material) and increase germination and improve field performance, such as the percentage of established seedlings, plant biomass and grain yield (Ibrahim, 2016; Ghassemigolezani et al., 2010).

Various seed priming techniques have been developed and commercially applied (Singh et al., 2015). Non-chemical methods such as hydro-priming and chemical methods such as osmo-priming are widely used (Paparella et al., 2015). Several priming methods have been

Abbreviations: HEHP, hydro-electrostatic hybrid priming; PCA, principal component analysis; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CD, circular dichroism; EPR, electron paramagnetic resonance; MDA, malondialdehyde; SOD, superoxide dismutase; SEM, scanning electron microscopy; TEM, transmission electron microscopy

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https://doi.org/10.1016/j.envexpbot.2018.04.002
Received 17 November 2017; Received in revised form 2 April 2018; Accepted 2 April 2018
Available online 04 April 2018
0098-8472/

shown to increase onion seed (*Allium cepa* L.) germination (Heydecker, 1974; Dearman et al., 1988; Basra et al., 1994).

Seed priming is routinely used by commercial seed companies to improve the vigor of their products. However, due to the difficulty and complexity of priming procedures, proprietary commercial priming overlooks species-specific parameters and techniques (Taylor et al., 1998; Black and Bewley, 2000). The correct osmotic agents must be chosen based on the permeability of the seeds; for example, oxygen transfer within the osmo-priming solution is limited unless the optimal concentration of PEG is used. Ions released from PEG penetrate and accumulate within seeds, resulting in cytotoxicity and nutritional imbalances (Bujalski and Nienow, 1991). The priming agents are also costly and must be used in massive amounts. Conventional hydro-priming techniques generally require at least 5 days of precise humidity control, which increases the possibility of microorganism proliferation and non-homogeneous control of seed moisture (Singh et al., 2015; Paparella et al., 2015).

Experimental studies of the effect of electricity on plant growth began in 1746. Early researchers explored the use of electricity for different agricultural purposes, such as seed treatment, plant growth acceleration and insect control (Gui et al., 2013). Electricity was found to affect seed germination in the 1960s (Sidaway, 1966). Subsequent research reported a variety of seed biological processes could be accelerated by electrostatic fields (Moon and Chung, 2000; Zhu et al., 2000; Chiabrera and Bianco, 1987; Morar et al., 2002; Huang et al., 2006). Electric field treatment is considered to enhance seed vigor; this technique can be as simple as exposing seeds to an electric field of a given strength for a predetermined time (Moon and Chung, 2000). Electro-priming was first recognized as a priming technique by Leong (Leong et al., 2016). However, electro-priming has limited potential for commercial use, as its effects are significantly more short-lived compared to conventional priming. For example, the effects of electro-priming in sunflower, caragana and wheat seeds are lost after 3 weeks, 7 days and 20 days, respectively (Yang and Yuan, 2013; Liu et al., 2014; Bao et al., 2010).

Commercial horticulture requires smart, precise procedures such as industrial seedling supply and mechanical operations, and high-quality seeds are demanded by industry. In this paper, we report the ability of hydro-priming (in the absence of chemicals) to increase onion (*Allium cepa.*) seed vigor is enhanced by exposure to an electrostatic field. Moreover, we describe this novel priming technique, hydro-electrostatic hybrid priming (HEHP). Furthermore, the differences in the mechanism of action of hydro-, electro- and hybrid priming were investigated and discussed.

2. Materials and methods

2.1. Preparation of seeds

Onion seeds (*Allium cepa.*, Dihuang) were purchased from Wells Seeds (Jiading, Shanghai, China). Seeds were harvested and processed in 2014, and stored under proper conditions (5 °C and 50% relative humidity).

2.2. Treatments and processing steps

Priming treatments were conducted at the Protected Horticulture Laboratory of Shanghai Jiaotong University, China, as shown in Table 1 (Bujalski et al., 1989). Each sample contained 50 well sharp seeds with five samples and three replicates for each treatment.

The various priming treatment groups are illustrated in Table 1. For hydro-priming (Hyd), the seeds were soaked in distilled water at 22 °C for 5 h. For electro-priming, the positive and negative electrodes of a BM-201 electrostatic field generator (Jiangsu, City, China) were connected to two 10 cm × 10 cm copper plates placed 1 cm apart (Appendix Fig. 2 in Supplementary material); the seeds were placed on the lower plate (cathode) and exposed to a 10 kv/cm EF for 40 s (Appendix Fig. 3 in Supplementary material). The direction of the generated EF was identical to that of the atmospheric EF. Groups of seeds exposed to hydro-priming without EF (i.e. Hyd) or hydro-priming with EF (i.e. hydro-electro hybrid priming [HEHP]) were incubated in a climate-controlled chamber (QHX-300BSH-III, made in Shanghai, China) set at 22 °C at 98% humidity for 24, 48 or 96 h in dark, then desiccated by air-drying at 30 °C for 48 h.

Table 1
Priming treatments and processing protocols.

Treatment	Processing steps			
	Step 1: Soaking	Step 2: Electrostatic field irradiation	Step 3: Incubation	Step 4: Desiccation
CK	×	×	×	×
EF	×	√	×	×
Hyd24	√	×	24 h	√
Hyd48	√	×	48 h	√
Hyd96	√	×	96 h	√
HEHP24	√	√	24 h	√
HEHP48	√	√	48 h	√
HEHP96	√	√	96 h	√

Note: HEHP, Hydro-Electrostatic Hybrid Priming; Hyd, Hydro Priming; EF, Electrostatic Field Irradiation.

2.3. Determination of seed germination

All 50 seeds from each sample were sown on damp peat (peat and water, $w/w = 3{:}1$) in germination boxes in a climate-controlled chamber (QHX-300BSH-III, made in Shanghai, China) set at 22 °C and 80% relative humidity; an artificial day-night regime of 12 h of daylight/12 h of darkness was obtained by using TL-lights (TLD 30W/865 cool daylight, PHILIPS, made in Tailand), set at 16 000 lx; three samples and three replicates were assessed for each treatment.

The number of germinated seeds was recorded every 48 h after sowing. Germination percentage (GP, %) was recorded on day 4. Final germination rate (GR, %), average length of radicle (S, mm) and fresh weight (FW, g) were recorded on day 6.

Mean emergence time (MET) was calculated using:

$$\bar{T} = \frac{\Sigma Dt \times n}{\Sigma n} \quad (1)$$

Where: Dt is the number of days after sowing, and n is the number of newly emerged seeds on day Dt (Farooq et al., 2008; Ellis and Roberts, 1980).

Germination index (GI) was calculated as described by the Association of Official Seed Analysts using:

$$\mathrm{GI} = \Sigma\frac{\mathrm{Gt}}{\mathrm{Dt}} \quad (2)$$

where Gt is the number of newly emerged seeds on day Dt (Dezfuli et al., 2008).

Vigor index (VI) was calculated as described by the International Seed Testing Association using:

$$\mathrm{VI} = \mathrm{GI} \times \mathrm{S} \quad (3)$$

2.4. Determination of electrical conductivity

Seeds (0.19 g; about 50 seeds) were washed, soaked in 40 mL of deionized water at 25 °C for 5 h. As the Hyd and HEHP samples were soaked for 5 h during the priming process, some electrolyte leakage may have occurred. Therefore, the EF and CK samples were soaked for an extra 5 h before proceeding to the EC determination protocols. The electrical conductivity of the soaking solution was determined as a2,