Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2018, 46(2):371-375. DOI:10.15835/nbha46210749

AcademicPres

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Original Article

Solid Matrix Priming Treatment with O_2 Enhanced Quality of Leek Seed Lots

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Abstract

This study aims to determine the effect of solid matrix priming (SMP) treatment with an air composition of O_2, N_2, air, vacuum on four leek (*Allium ampeloprasum* L.) seed lots of various ages, in terms of enhancing germination, mean germination time, electrical conductivity of solute leakage and catalase activity. Untreated seeds were used as control. Solid matrix priming at a seed: vermiculite: water ratio of 2.5:1.25:3.75 (w/w/w) was applied at 20 °C for 24 hours in the dark. Solid matrix priming with O_2 was found to give the highest germination, lowest mean germination time, lowest electrical conductivity (reduced solute leakage) and the highest catalase activity among all treatments and lots. SMP treatment with air provided positive response, while N_2 and vacuum treatments were found to be less effective. The advantages of solid matrix priming were more pronounced in lower quality lot than in the higher quality ones. The results indicated that oxygen enrichment in SMP may enhance leek seed quality.

Keywords: catalase; mean germination time; N_2; seed germination; solute leakage

Introduction

The techniques of priming, in which seeds are pre-imbibed in low water potential liquid solutions [e.g. Polyethylene glycol or any low water potential salt solution (i.e. KNO_3, NaCl)], to a stage immediately prior to germination have been used to improve the rate and uniformity of seed germination (Rajjou *et al.*, 2012). Priming extends the second (lag) phase of imbibition. During the treatment, seed water uptake reaches high percentages, but not sufficient to allow radicle emergence and seeds undergo early physiological processes (completed the first two stages of imbibition) that occur during germination (McDonald, 1999; Jisha *et al.*, 2013). The processes involve synthesis of RNA, proteins, and the restoration of the activity of enzymes involved in cell detoxification mechanisms, such as catalase (Parera and Cantliffe, 1991; Khan, 1992; Oge *et al.*, 2008). Various factors are involved in maximizing the benefit of priming. Atmosphere composition during treatment can also play a significant role in the invigoration process of the seeds. Oxygen is a key component in respiration, affecting various biochemical cellular and molecular events that take place during the enhancement process obtained through priming (Halpin-Ingham and Sundstrom, 1992; Halmer, 2004). Providing oxygen allows repair mechanisms (invigoration) in seed to occur more quickly (Ibrahim and Roberts, 1983; Ozbingol *et al.*, 1999). Therefore, oxygenation during osmo-conditioning and hydration treatments in which seeds are kept in water, PEG or any low water potential salt solution (i.e. KNO_3, NaCl) to provide enriched dissolved oxygen (aerated hydration) in the liquid-is a method for increasing the level of benefit in vegetable seeds (Bujalski *et al.*, 1991).

However, the amount of research on the effect of atmosphere composition during solid matrix priming is not found to the best of our knowledge. Seeds in matrix priming studies in general are kept in air which contains about 21% oxygen (Khan, 1992; Pandita *et al.*, 2010; Ermis *et al.*, 2016). However, the percentages of oxygen content during post-priming germination of tomato seed has been shown to affect germination (Ozbingol *et al.*, 1999).

In this study, we tested the effect of solid matrix priming (SMP) treatment performed with atmosphere of different compositions on the germination percentages and rates, and also on electrical conductivity of solute leakage and catalase activity in leek seed lots.

Received: 23 Feb 2017. Received in revised form: 30 May 2017. Accepted: 01 Jun 2017. Published online: 24 Jan 2018.

Materials and Methods

Biological materials

A sample of four leek (*Allium ampeloprasum* L.) seed lots cv. Inegol were obtained from different seed companies. The seeds were stored in water and air proof laminated aluminium foil packets at 5 °C until use. The normal seed germination percentages of the seed lots ranged between 72 and 83%. Seed moisture content was 6.1-6.8% on receipt (ISTA, 2010).

Experimental procedures

The solid matrix priming (SMP) treatment carried out by mixing seed, vermiculite (No.5), larger particle size and water at the ratio of 2.5: 1.25: 3.75 g for 24 hours at 20 °C in the dark in 182 ml aluminum foil plastic packets (13 cm, length, 9 cm wide). As soon as the mentioned materials were put in the packets, they were fully filled (rendered turgid) with pure either O_2 or N_2 or air (21%). A fourth vacuum treatment was performed by sucking the air from the packets with syringes. The air hole made by the syringe was closed using strong tapes in order to keep the air composition inside constant. Packets were placed into the water to check for air loss before the treatment started. Packets were kept firm (i.e. the air is not lost in the packet) to make sure the air composition was the same throughout the treatment. After the treatment, the seeds were separated from the vermiculite and then dried at 25 °C down to initial seed moisture. Relative humidity was 50% during drying and the seeds were weighed frequently to make sure that they are at initial level. Germination and electrical conductivity tests were conducted within three days after the treatment, during which the seeds were kept at 5 °C.

The treated and untreated (unprimed) seeds were germinated at 20 °C between wet paper towels (20 x 20 cm, Filtrak, Germany) for 14 days (ISTA, 2010) in the dark. In each germination test, three replications of 50 seeds were used for each primed and unprimed seeds. Paper towels were rolled and placed into plastic bags in order to prevent water loss. Germination was scored as radicle emergence when the radicle had protruded 2 mm. Radicle emergence was counted daily. Normal germination was determined after 14 days (ISTA, 2010).

The mean germination times (MGT) were calculated on the basis of daily radicle protrusion counts using the following formula:

$$MGT = \Sigma (n.D)/\Sigma n$$

n=number of newly-germinated seeds at time D; D=days from sowing, beginning of tests; Σ n=final germination (%)

For the electrical conductivity test, three replicates of 50 seeds (in SMP treated and untreated seed samples were weighed to four decimal places and soaked in 40 ml of distilled water for 24 hours at 20 °C in the dark. The electrical conductivity of the seed soak water was measured using a conductivity meter (Schott-Gerate, GmbH Hofheim) and expressed as per gram of seeds ($\mu S cm^{-1} g^{-1}$). The conductivity test was conducted according to Mathews *et al.*, (2009).

Catalase (CAT) activity was measured according to the method of Cakmak *et al.*, (1993). The measurements were conducted on 60 samples (4 treatment and control x 4 lots = 20 x 3 replicates = 60 samples) using 0.5 g of seeds for each sample. CAT activity was determined by monitoring the rate of disappearance of H_2O_2. All experiments were performed in the dark. Activity was measured within two days after the priming treatment. Seed samples (0.5 g) were homogenized in a Heidolph, Diax 900 homogenizer in 5 ml 100 mM sodium phosphate buffer (pH 7.5) containing 100 mM Na-phosphate, 0.5 mM EDTA-Na_2 and 1 mM ascorbic acid. The homogenized samples were centrifuged at 18,000 g for 30 min. The supernatant was used as a crude enzyme extract in the CAT analysis. Catalase activity (EC 1.11.1.6) was estimated by the decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2. Absorbance was measured using a Shimadzu UV/VIS 1201 spectrophotometer (Cakmak *et al.*, 1993).

Statistical procedures

A statistical analysis was performed using SPSS. Mean values of the treated and untreated seed samples were compared using the Duncan test. The results were converted into percentages prior to analysis in germination values. The comparison of mean values undertaken as part of an analysis of variance (ANOVA). Linear regression analysis was conducted between EC and germination percentages and significance was indicated.

Results

The quality for the four seed lots used in this study, as measured by normal germination differed between 72% (lot 2) and 83% (lot 3) Lots 1 and 4 had 81% and 78% normal germination, respectively. In all lots, seeds that received SMP treatment with O_2 or air were found to be superior to those treated with N_2 or vacuum, as well as the control, with regards to germination percentage, mean germination time, catalase activity and electrical conductivity. SMP treatment in some lots with N_2 or vacuum yielded even lower values than those of the control (Figs. 1 and 3; Tables 1 and 2). The difference between the treated and untreated seeds was greater in the lot 2. In this lot, the SMP treatment with O_2 increased normal germination from 72 to 79%, while reducing mean germination time from 4.9 to 3.6 days (Table 1).

Seeds that received the SMP treatment with O_2 or air started to germinate earlier than the other seeds (Fig. 1). By the day 6 of germination, the advantage from O_2 or air was lost compared with the other treatments. Mean normal germination of treated lots was the highest (84.3%) when O_2 was present during the treatment. This germination percentage was higher ($p < 0.05$) compared with that in the other treatments. In general, treatment with O_2 or air provided faster germinating seeds than the N_2 or vacuum treatment and the control conditions (Table 1). All treatments resulted in faster germinating seeds than the control, but only those treated with O_2 or air were significantly ($p < 0.05$) better to the seeds in the control with regards to normal germination percentage (Table 1). The most negative effect was observed in the SMP treatment with N_2, in which germination was 21% lower (negative) than the control. Germination was 23% higher than control when O_2 was present. The germination percentage values were 10% higher in the air treatment and 9% lower in the vacuum treatment than control (Table 2).

The electrical conductivity (EC) of solute leakage after treatment contained O_2 was the lowest in among all the treatments and control. In the treatment with O_2, EC varied between 104.2 and 133.4 μScm^{-1}g^{-1}. In all seed lots, SMP treatment reduced the amount of solute leakage from the seeds in comparison with the control (Fig. 2).

Catalase activity was the highest in SMP treatment with O_2, ranging between 0.055 and 0.088 μmol/fresh weight (Fig. 3). The lowest concentrations were observed in the treatment with N_2, and ranged between 0.034 and 0.046 μmol/fresh weight. In three lots (Lots 1, 2 and 3), catalase activity of the control seeds were higher compared with the seeds subjected to SMP with N_2 or vacuum (Fig. 3). The normal (R^2=0.74, $p < 0.001$) and total (R^2=0.51, $p < 0.05$) germination values of the treated lots were significantly associated with the EC levels in the treated leek seeds (Fig. 4). Seeds with higher levels of

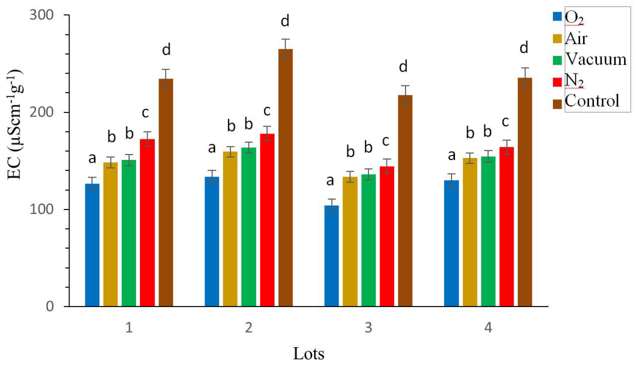

Fig. 2. The effect of SMP treatment combined with oxygen or air or N_2 or vacuum on electrical conductivity of 4 leek seed lots. Bars indicates SEM. Values with the different letter in the same lot are significantly different at 5% probability

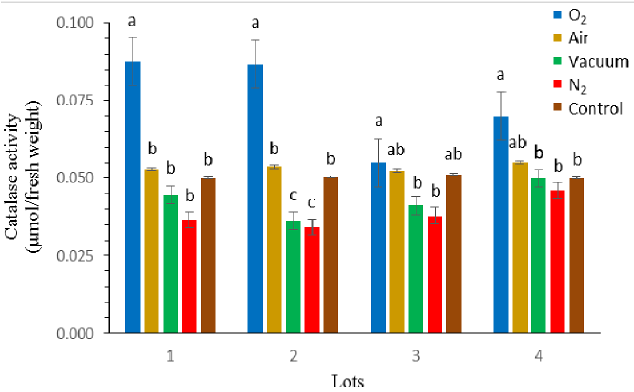

Fig. 3. Changes in catalase activity for SMP treatment combined with oxygen or air or N_2 or vacuum and control leek seed lots. Bars indicates SEM. Values with the different letter in the same lot are significantly different at 5% probability

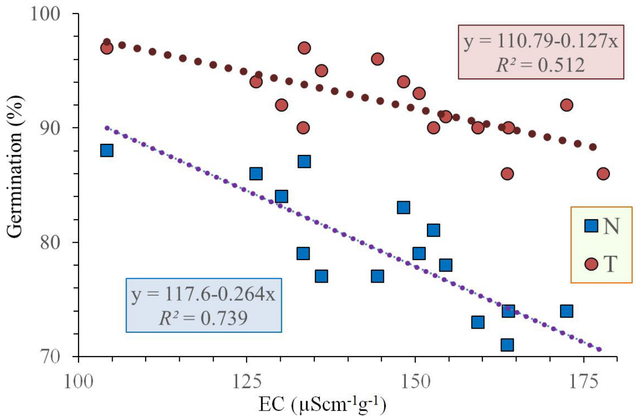

Fig. 4. The relationship between EC and total (T) radicle emergence and normal (N) germination percentages of 16 leek seed lots (4 treatment × 4 lots) after SMP treatment combined with oxygen or air or N_2 or vacuum.

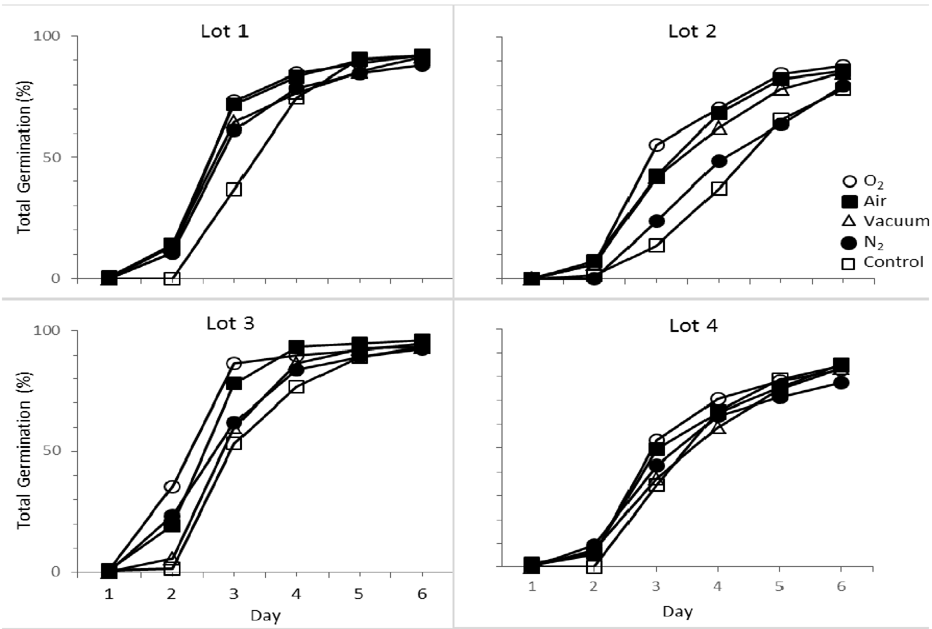

Fig. 1. The effect of Solid Matrix Priming (SMP) treatment combined with oxygen or air or N_2 or vacuum and control on cumulative germination percentages of leek seed lots

Table 1. Normal germination percentages and mean germination time of 4 leek seed lots after SMP treatment combined with oxygen or air or N_2 or vacuum and control. Values with the different letter in the same column are significantly different at 5% probability

	Lots / Normal germination (%)				Mean
	1	2	3	4	(%)
O_2	86a	79a	88a	84a	84.3a
Air	83b	73b	87a	81b	81.0ab
Vacuum	79c	71b	77c	78c	76.3bc
N_2	74d	68c	77c	74d	73.3c
Control	81bc	72b	83b	78c	78.5b
	Mean germination time (d)			Days	
O_2	3.3ab	3.6a	2.9a	3.8a	3.4^{ns}
Air	3.2a	3.8a	3.0a	3.9a	3.5^{ns}
Vacuum	3.5b	3.8a	3.5bc	4.2b	3.8^{ns}
N_2	3.5b	4.5b	3.4b	4.3b	3.9^{ns}
Control	3.8c	4.9c	3.7c	4.2b	4.2^{ns}

Note: in the same criterion and same column are significantly different (Duncan test, $p < 0.05$)
^{ns}: Means of mean germination time values are not significant

Table 2. Advantage of SMP treatment combined with oxygen or air or, N_2 or vacuum compared with the control (untreated) with regard to normal germination and mean germination time in leek seed lots. Values were obtained based on differences between the treated and control seed lots

		Lots				Total advantage
		1	2	3	4	
Normal germination (%)	O_2	5	7	5	6	23
	Air	2	1	4	3	10
	Vacuum	-2	-1	-6	0	-9
	N_2	-7	-4	-6	-4	-21
MGT (d)	O_2	0.5	1.3	0.8	0.4	3.0
	Air	0.6	1.1	0.7	0.3	2.7
	Vacuum	0.3	1.1	0.2	0	1.6
	N_2	0.3	0.4	0.3	0.1	1.1

normal and total (radicle emergence) germination after treatment also had less solute leakage.

Discussion

The results of present study showed that solid matrix priming increased germination and mean germination times, reduced solute leakage, and increased catalase activity in leek seed lots. The extent of the positive effects associated with the SMP was related to the atmosphere composition used during the treatment. The presence of oxygen increased the advantage and effectiveness of the SMP treatment to a greater extent than air, N_2 or vacuum. The results of this study are in agreement with previous reports showing that solid matrix priming is beneficial in enhancing germination percentages and germination rate in a range of crop seeds, cultivated either in the greenhouse or under field conditions (Khan, 1992; Jett *et al.*, 1996; Pandita *et al.*, 2010; Jisha *et al.*, 2013). The difference in this study compared with earlier ones is that SMP was combined with different atmosphere composition. The treatment with oxygen was found to be superior to the other types of treatment. The respiration of seeds during the priming process plays an important role in the metabolic activity taking place (Halpin-Ingham and Sundstrom, 1992; Jisha *et al.*, 2013). In priming methods the availability of oxygen can be limited when PEG or water are used. To prevent this, in many priming treatments oxygen enrichment is performed by applying air bubbles through the osmotic solution during the osmo-conditioning period (Bujalski *et al.*, 1991). The effect of atmosphere composition (i.e. oxygen enrichment) and its effect on the invigoration of seeds have not been investigated in solid matrix priming. In SMP free air movement is possible during priming because of the large particle size of the carrier (i.e. vermiculite or perlite) used (Parera and Cantliffe, 1991).

However, the results from this study indicate the benefit of the priming treatment can be extended with pure oxygen supply into the medium compared to that with air (Table 1), i.e. more than 21% oxygen during priming can be advantageous for extending the enhancement. This effect is most likely due to the enhancement of seed respiration activity as a result of oxygen transfer across the seed coat towards the respiratory systems (energy mechanism, i.e. ATP), which increases the rate of the metabolic processes occurring during priming. Respiration and energy metabolism (such as faster ATP use and oxygen uptake) have also been shown to be a physiological seed quality parameter in other studies (Venter and Grabe, 1989; Mc Donald, 1999, Edelstein and Welbaum, 2011).

Rapid germination following SMP will likely lead to larger seedlings and well-developed transplants in the modules within a shorter time-frame. Fast emergence can be due to priming allowing the completion of the first two imbibition phases (Wang *et al.*, 2003). The results of this study showed that higher oxygen contents than air, along with high seed moisture content, in the first two phases of imbibition affects rate of germination.

The application of SMP or other priming treatments may trigger various biochemical processes, including repair of damage and enzymatic activity (Powell *et al.*, 2000; Sung and Chiu, 2001; Chiu *et al.*, 2005). In the present study, SMP increased catalase activity and reduced EC levels in SMP-treated seeds - particularly of those primed in oxygen - in comparison with the control and the N_2 and vacuum treatments. The reduced solute leakage with the oxygen-treatment suggests oxygen may be helping repair to cell structure thereby reducing leakage from the cell membranes. The increase after SMP in anti-oxidative scavenging enzymes such as catalase (CAT) \ removal of reactive oxygen species is occurring during priming which can help reduce the amount of damaging reactive oxygen species and harmful products such as H_2O_2 (Kibinza *et al.*, 2011). Catalase activity was higher in SMP with O_2 and the low quality lot (lot 2) compared with the other air compositions and lots (Table 1, Figs. 2 and 3). This suggests more detoxification activity with oxygen and in aged seeds (Wang *et al.*, 2003).

Electrical conductivity measures the leakage of electrolytes from seeds into soak water, which is an indicator of cellular integrity and is used as a seed vigor test (Matthews *et al.*, 2012). The basis of the electrical conductivity (EC) test is the observation that low vigor seeds leak higher levels of solutes into the soak water, which can be readily measured through the EC of the soak solution. Among other physiological changes, the effect of

SMP is primarily based on reducing seed leakage as results of repair of damage (Kepczynska *et al*., 2007; Matthews *et al*., 2009; Ermis *et al*., 2016). The significant relationship in this study between EC and total and normal germination after priming (Fig. 4) showed that SMP enhanced seed quality most likely by enabling cell repair mechanisms to operate. In different vegetable seeds, EC is related with seed germination and vigor (Matthews *et al*., 2012). Lower EC values are an indication of higher quality and potentially better seedling emergence. Along with the treatment with N_2, the vacuum condition also provided lower seed quality values than that of the control. This indicates that oxygen (i.e. respiration) is necessary for benefits from the priming treatment to be obtained. Bujalski *et al*. (1991) and Ozbingol *et al*. (1998, 1999) reported that oxygen suppression in the treatment reduced seed quality, and that approximately 10% oxygen was considered as the lower threshold (minimum percentage) for ensuring the improvement of seed quality parameters. In the absence of oxygen, some seed quality parameters in this study were determined to be lower than that of the control.

In conclusion, the positive effects of SMP treatment on leek seed quality can be maximized by using oxygen during the treatment. Without oxygen, SMP with N_2 and vacuum resulted in lower values than the control. The greater positive effect observed in the lower quality lot implies that using oxygen can also be good option for enhancing the quality of aged seeds.

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