Journal of Plant Physiology 170 (2013) 1251–1258

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

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

Physiology

Seed orientation and magnetic field strength have more influence on tomato seed performance than relative humidity and duration of exposure to non-uniform static magnetic fields

Danny Poinapen[a,b,c,*], Daniel C.W. Brown[a,d], Girish K. Beeharry[c]

[a] Department of Biology, University of Western Ontario, London, Ontario, Canada
[b] Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada
[c] Department of Physics, University of Mauritius, Réduit, Mauritius
[d] Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Hospital Research Centre, Winnipeg, Manitoba, Canada

ARTICLE INFO

Article history:
Received 4 September 2012
Received in revised form 11 April 2013
Accepted 12 April 2013
Available online 10 June 2013

Keywords:
Biomass partitioning
Electrolyte leakage
Germination
Orthogonal array (OA)
S. lycopersicum

ABSTRACT

Different factors (e.g., light, humidity, and temperature) including exposure to static magnetic fields (SMFs), referred here as critical factors, can significantly affect horticultural seed performance. However, the link between magnetic field parameters and other interdependent factors affecting seed viability is unclear. The importance of these critical factors affecting tomato (*Solanum lycopersicum* L.) var. MST/32 seed performance was assessed after performing several treatments based on a L_9 (3^4) (four factors at three levels) orthogonal array (OA) design. The variable factors in the design were magnetic flux density ($R_1 = 332.1 \pm 37.8$ mT; $R_2 = 108.7 \pm 26.9$ mT; and $R_3 = 50.6 \pm 10.5$ mT), exposure time (1, 2, and 24 h), seed orientation (North polarity, South polarity, and control – no magnetic field), and relative humidity (RH) (7.0, 25.5, and 75.5%). After seed moisture content stabilisation at the different chosen RH, seeds were exposed in dark under laboratory conditions to several treatments based on the OA design before performance evaluation. Treatments not employing magnetic field exposure were used as controls. Results indicate that electrolyte leakage rate was reduced by a factor of 1.62 times during seed imbibition when non-uniform SMFs were employed. Higher germination (~11.0%) was observed in magnetically-exposed seeds than in non-exposed ones, although seedlings emerging from SMF treatments did not show a consistent increase in biomass accumulation. The respective influence of the four critical factors tested on seed performance was ranked (in decreasing order) as seed orientation to external magnetic fields, magnetic field strength, RH, and exposure time. This study suggests a significant effect of non-uniform SMFs on seed performance with respect to RH, and more pronounced effects are observed during seed imbibition rather than during later developmental stages.

Introduction

Among many techniques (e.g., light, temperature, and chemical treatments) used to increase seed performance (Bewley and Black, 1994), magnetic fields are usually overlooked despite their potential. Although the effects of static magnetic fields (SMFs) targeting the performance of various seeds and crops have been reported (Belyavskaya, 2004; Galland and Pazur, 2005), many inconsistencies and seemingly contradictory observations exist. These inconsistencies in the literature are linked to the lack of appropriate systematic approaches to isolate the bioeffect of the treatment relative to other factors affecting plant development, the use of different exposure systems, different seed types, and the lack of uniformity in growing conditions. (Phirke et al., 1996; Harris et al., 2009).

Interdependent factors such relative humidity (RH) and temperature, referred here as critical factors causing seed deterioration when not rigorously controlled during seed storage, negatively affect seed viability (Copeland and Mc Donald, 2001). RH is very important as it directly affects seed moisture content (MC) and viability (Copeland and Mc Donald, 2001). At high MC (>14% corresponding to high RH $\geq$ 75%) hydrolytic reactions and respiration are favoured because sugars, free fatty acids, and other substrates increase as more water molecules become loosely bound, thereby promoting seed deterioration (Black et al., 2006). As seed MC is lowered (when RH is reduced to 18–25%), molecular mobility is

Abbreviations: MC, moisture content; RH, relative humidity; SMFs, static magnetic fields.

* Corresponding author at: Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, Agriculture and Agri-Food Canada, 1391 Sandford St, London, Ontario, N5V 4T3, Canada. Tel.: +1 5194571470x252.
E-mail address: dpoinapen@gmail.com (D. Poinapen).

0176-1617/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2013.04.016

Table 1
Four factors at three levels orthogonal array design.

Levels	Factors			
	Magnetic flux density (mT)	Exposure time (h)	Orientation	Relative humidity (%)
1	R_1	1	N	7.0
2	R_2	2	S	22.5
3	R_3	24	C	75.5

reduced within the aqueous matrices of cells. Therefore, diffusion-driven reactions are slowed down and seed longevity is preserved. For maximum seed longevity, the critical MC must be between 3% and 8% as water is weakly bound to polar non-ionic sites. When water content is reduced below 3% (RH < 10%) in cells, oxidation of metals ions causes molecular degradation when seeds become too dry because water molecules are strongly bounded, consequently making seeds less viable (Copeland and Mc Donald, 2001; Black et al., 2006).

Knowledge of which environmental factors critically affect seed viability and performance when designing studies to evaluate magnetic field effects is very important to target control of the most influential factors to limit experimental variability. In our opinion, the lack of understanding of the critical effects and control of these factors have led to experimental variability (noise) that masked the apparent more subtle effects of magnetic fields. Evaluating the effect of these critical factors on seed performance is tedious, very time-consuming and overlooked when using conventional full factorial designs. The orthogonal array (OA), also known as the Taguchi's method (Taguchi, 1986, 1987), estimates maximum number of main effects of factors in an unbiased way by relating the signal-to-noise ratio (effects of each variable on the output) to control variables. In molecular biology and biotechnology, the OA method has successfully identified the contribution of factors and their optimum individual combination in many processes (Cobb and Clarkson, 1994; Dabrowski et al., 2003; Prakasham et al., 2005; Rao et al., 2008). The OA method is a robust design that considers and handles variability known as noise factors (especially those due to uncontrollable or difficult to control factors) affecting the systems under study. Among many advantages, OA reduces experimental treatments and keeps all pair-wise combinations. For instance, instead of running a full factorial design containing 4 factors at 3 levels each (3^4 = 81 treatments), experimental treatments are reduced to 9 using the OA approach.

Since no established standard method exists to study the interaction between magnetic fields and plants (Phirke et al., 1996), the orthogonal array represents a systematic approach to limit experimental variability that masks individual factor effects by allowing independent replication of observations to rank the impact of critical factors. Consequently, the OA method allows the investigator to make unbiased decisions on which factors have the most influence on measured outcomes and are most important to control to limit experimental variability. To address the issue of inconsistent and contradictory observations seen in previous approaches, this study aimed at investigating the link between magnetic field parameters and interdependent factors affecting seed viability using an OA design combining different magnetic field parameters (magnetic field strength, exposure time, and polarity) and RH on tomato (*Solanum lycopersicum* L.) seed performance under laboratory conditions. In our approach, light intensity and temperature were most easily controlled and were thus held constant. Seed performance was evaluated in terms of vigour by measuring seed electrolyte leakage, germination, and seedling biomass accumulation.

Materials and methods

Seed materials

Tomato (*Solanum lycopersicum* L.) var. MST/32 seeds of 80% average germination capacity (produced in 2006, Arsenal, Mauritius; stored in dark at 12 ± 1 °C; ~20% relative humidity (RH)), were supplied by the Barkly Plants and Seeds Production Service, Ministry of Agro-Industry, Beau-Bassin, Mauritius. These seeds were used in the experiments performed in 2008–2009.

RH and seed moisture stabilisation

Tomato seed moisture content (MC) was adjusted using saturated salt solutions of NaOH (RH = 7%), KAc (RH = 22.5%), and NaCl (RH = 75.5%) respectively (Windholz et al., 1983; Copeland and Mc Donald, 2001). These RH values produce the lower, middle, and upper limits for seed MC considered as a "safe range" to prevent seeds from losing their viability during storage (Copeland and Mc Donald, 2001). Seed batches (~900 seeds per batch) of same sizes with undamaged seed coats were allowed to equilibrate for 7 d at 7, 22.5, and 75.5% in standard Petri dishes in sealed vacuum desiccators at 25 ± 1 °C in the dark. A calibrated digital hygrometer was placed in each vacuum desiccator to monitor the RH. After 7 d, the

Table 2
A L_9 (3^4) matrix (four factors at three levels) representing different treatments obtained from the orthogonal array design consisting of magnetic flux density (R_1 = 332.1 ± 37.8 mT; R_2 = 108.7 ± 27.8 mT, and R_3 = 50.6 ± 10.5 mT), exposure time (1, 2, and 24 h), seed orientation (N: North polarity, S: South polarity, and C: no field), and relative humidity (RH = 7.0, 22.5, and 75.5%). Treatments 3, 5, and 7 are controls (no field applied).

Treatments	Factors			
	Magnetic flux density (mT)	Exposure time (h)	Orientation	Relative humidity (%)
1	R_1	1	N	7.0
2	R_1	2	S	22.5
3[a]	R_1	24	C	75.5
4	R_2	1	S	75.5
5[a]	R_2	2	C	7.0
6	R_2	24	N	22.5
7[a]	R_3	1	C	22.5
8	R_3	2	N	75.5
9	R_3	24	S	7.0

[a] Treatments 3, 5, and 7 were generated as control from the OA design as no magnetic field treatment was employed because C = no field applied ($R_1 = R_2 = R_3$ = 0 mT).