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The Effect of Different Priming Treatments and Storage Conditions on Germination Performance of Lettuce Seeds

A. Korkmaz[1)] and W. G. Pill[2)]
([1)]Kahramanmaras Sutcu Imam University, Department of Horticulture, Kahramanmaras, Turkey and [2)]University of Delaware, Department of Plant and Soil Sciences, Newark, DE, USA)

Summary

The effects of different priming treatments on final germination percentage (FGP), rate (G_{50}), and synchrony ($G_{10–90}$) of the seeds of two lettuce (*Lactuca sativa* L.) cultivars before and after storage were investigated. Seeds of 'Cortina' and 'Greenlakes' were osmotically primed in K_3PO_4, KH_2PO_4 or $NH_4H_2PO_4$ and PEG 8000, having a water potential of –0.49 or –1.5 MPa for 10 or 20 hours at 15 °C in darkness. Following priming, the seeds were subjected to germination tests at 20 °C from which FGP, G_{50}, and $G_{10–90}$ values were calculated. Among all the priming agents, only priming with KH_2PO_4 (–1.5 MPa) for 20 hours improved the germination synchrony of low vigour cultivar, 'Greenlakes' and therefore KH_2PO_4 was selected to be the priming agent for subsequent experiments. Primed (–1.5 MPa KH_2PO_4, for 20 hours) 'Cortina' and 'Greenlakes' seeds were either surface dried for 2 h at 21 °C and 58 % relative humidity or dried back at 20 °C and 20 % RH for 2 h, then they were stored at 4 °C (refrigerator) or 20 °C (incubator) for one month. Following storage, germination tests were performed and it was seen that storing the primed seeds of both cultivars for one month had little or no effect on the viability (FGP) and germination rate (G_{50}), but slightly reduced the germination synchrony ($G_{10–90}$).

Zusammenfassung

Wirkung unterschiedlicher Behandlungsmethoden und Lagerungsbedingungen auf die Keimung von Salatsaatgut. Die Wirkung von unterschiedlichen Behandlungsmethoden mit und ohne anschließender Saatgutlagerung auf die Keimung in Prozent (FGP), die Keimrate (G_{50}) und die Gleichmäßigkeit der Keimung ($G_{10–90}$) wurde an Saatgut zweier Salatsorten (*Lactuca sativa* L.) untersucht. Saatgut der Sorten 'Cortina' and 'Greenlakes' wurde in K_3PO_4, KH_2PO_4 oder $NH_4H_2PO_4$ mit PEG 8000, das ein Wasserpotential von –0.49 oder –1.5 MPa aufwies, 10 oder 20 Stunden bei 15 °C und Dunkelheit osmotisch aufbereitet. Die Keimversuche erfolgten bei 20 °C, aufgrund der ermittelten Werte wurden FGP, G_{50} und $G_{10–90}$ errechnet. Da von den Agenzien nur KH_2PO_4 (–1.5 MPa) mit einer Behandlungsdauer von 20 Stunden die gleichmäßige Keimung der schwachwüchsigen Sorte 'Greenlakes' verbesserte, wurde KH_2PO_4 als das Behandlungsmittel für weitere Versuche ausgewählt. KH_2PO_4-behandeltes (–1.5 MPa, 20 Stunden) Saatgut von 'Cortina' und 'Greenlakes' wurde dabei entweder 2 h bei 21 °C und 58 % relativer Feuchte oberflächengetrocknet oder 2 Stunden bei 20 °C und 20 % RH zurückgetrocknet und anschließend bei 4 °C (Kühlschrank) bzw. 20 °C (Inkubator) ein Monat lang gelagert. Nach dieser Lagerung wurden wiederum Keimversuche durchgeführt, die zeigten, dass die Lagerung der behandelten Samen über einen Monat bei beiden Sorten geringen oder gar keinen Einfluss auf die Keimfähigkeit (FGB) und die Keimrate (G_{50}) hat, dass aber die Gleichmäßigkeit der Keimung ($G_{10–90}$) leicht reduziert wurde.

Key words. *Lactuca sativa* – lettuce – seed priming – seed storage

Introduction

In lettuce, yield and timing of harvest are affected by the time taken for seedlings to emerge (KORKMAZ et al. 1999). To improve crop earliness and uniformity, seedling emergence should be fast and uniform. Seed vigor has been enhanced by osmotic priming, defined as the enhancement of physiological and biochemical events in seeds during suspension of germination by low osmotic potential and negligible matric potential of imbibing medium (KHAN 1992), so that rate, synchrony and percentage of seedling emergence from such seeds were greater than from control seeds especially under such adverse seed-bed conditions as low temperature, high temperature or salinity (PILL 1995). Osmotic priming improved seedling emergence or yield in many crops including asparagus (PILL et al. 1991), leek (DEARMAN et al. 1987), and lettuce (CANTLIFFE 1991).

Since the response to a given priming treatment can vary between seed lots of the same cultivar (BROCKLEHURST et al. 1984), the optimal priming treatment for a seedlot is determined by experimentation (WELBAUM et

al. 1998). Factors that affect the response of a seedlot to priming include the nature of the priming agent, the duration of seed exposure to the priming agent, and the temperature and osmotic potential of the priming agent (KHAN 1992).

Primed seeds can be stored following drying back and upon rehydration, they may still exhibit faster germination rates, greater tolerance to environmental stress, and reduced dormancy (KHAN 1992). However, there are conflicting results on the effect of priming on the storage life of the seeds. Some studies have reported that priming did not affect the storage life of seeds. For example, DEARMEN et al. (1987) reported that primed carrot and leek seeds retained their improved vigor after a storage period of 450 days at 10 °C. ATHERTON and FAROOQUE (1983) also reported that primed spinach seeds exhibited faster and more uniform germination than non-primed seeds after 30 days of storage at 5 °C. On the contrary, tomato (OWEN and PILL 1994) and muskmelon (OLUOCH and WELBAUM 1996) seeds exhibited reduced storage life following priming. Therefore, our objectives in this study were to 1: find the best priming agent, temperature, osmotic potential, and time combination for two cultivars of lettuce and 2: determine germination percentage, rate, and synchrony of primed seeds that were surface dried or dried-back and then stored at 4 or 20 °C for one month.

Materials and Methods

Seedlot characterization

Seeds of 'Cortina' lettuce (*Lactuca sativa* L.) were acquired from Leen de Mos (Gravenzande, The Netherlands) in 1994. Seeds of 'Greenlakes' lettuce, harvested in 1974, were acquired from Dr. Anwar Khan of Cornell University, Itacha, NY, USA. All the experiments reported in this study were carried out in the fall of 1995 at University of Delaware, Newark, DE, USA.

A preliminary experiment established that light was unnecessary for germination of 'Cortina' or 'Greenlakes' seeds. Thus, seeds of both cultivars were subjected to continuous 20 °C (ANONYMOUS 1985) or continuous 5 °C in darkness in an incubator. The 5 °C sub-optimal temperature trial represented a cold germination test that should exaggerate vigor differences between the seed lots. Five replications of 50 seeds of each cultivar were placed in transparent polystyrene boxes (Stewart Plastics, Croydon, UK) (12 cm x 8 cm x 2 cm) on double layers of germination paper (Seed Germination Blotters No.385; Seed Bureau, Chicago, IL, USA) that were moistened with 5 ml of deionised water. Each day, germinated seeds (2 mm long radicle protrusion) were counted then removed from the boxes. From the total number of seeds germinated, angular transformation of final germination percentage (FGP), days to 10, 50 and 90 % of FGP were calculated. Time to 50 % FGP (G_{50}) is an inverse measure of germination rate, while time between 10 % and 90 % of germination ($G_{10–90}$) is considered to be an estimate of the spread of germination, the inverse of germination synchrony. This method of data collection was used for the following experiments.

Determination of priming agent, osmotic potential, and duration

Seeds of 'Cortina' and 'Greenlakes' lettuce were primed in water potentials of –0.49 and –1.5 MPa provided by K_3PO_4, KH_2PO_4 or $NH_4H_2PO_4$ for 10 or 20 hours. The seeds also were primed in PEG, with nominal concentrations of 181 and 342 g PEG 8000 kg^{-1} water to yield –0.49 and –1.5 MPa, respectively (MICHEL 1983). The experiment was a 2 (cultivar) X 4 (priming agent) X 2 (water potential) X 2 (priming duration) factorial. The treatments were replicated four times with 50 seeds per replication. Non-factorial untreated control seeds of both cultivars were also included.

Seeds were placed in germination boxes on double layers of germination papers saturated with one of priming agents at –0.49 or –1.5 MPa, and kept for 10 or 20 h at 15 °C in darkness. Priming for 10 hours was started 10 hours after the start of the 20-hour priming so that the germination assay of all treatments would start at the same time. Following priming, the seeds from each box were washed into a sieve and rinsed under demineralised water for 1 minute. The seeds then were transferred to boxes containing double layers of germination paper moistened with half-strength Hoagland solution (HOAGLAND and ARNON 1938; KORKMAZ et al. 1999). Germination was conducted at 20 °C in darkness.

Determination of different drying and storage regimes of primed seeds

The salt, KH_2PO_4, was chosen as a priming agent because in Experiment 2, KH_2PO_4 (–1.5 MPa) for 20 h was the only priming treatment that gave a lower $G_{10–90}$ than non-primed 'Greenlakes' control seeds, improving the synchrony of its germination (see Table 2).

Fifty seeds from each cultivar were placed in germination boxes on double layers of germination paper saturated with KH_2PO_4 (–1.5 MPa) and kept at 15 °C for 20 hours. Following priming, seeds were rinsed (as described in Expt. 2), then surface-dried for 2 h at 21 °C and 58 % relative humidity (RH), or dried-back at 20 °C and 20 % RH for 2 h. This low RH was created by placing the seeds in cheesecloth-lined 38 x 10 mm propylene tissue capsules (Histoprep, Fisher Scientific, Malvern, PA, USA). The capsules then were placed on a porous platform above sulfuric acid with a specific gravity of 1.4983 (899 g H_2SO_4 per liter) within a sealed desiccator.

The surface-dried and dried-back seeds were placed in 40 mm diameter x 14 mm tall seamless metal cans with tightly fitting lids (Ellisco Inc., Pennsauken, NY, USA), and stored at 4 °C (refrigerator) or 20 °C (incubator) for one month.

The priming also was repeated as described above, but this time after surface-drying or drying-back, the seeds were subjected immediately to germination testing (without storage), along with those that had been stored for one month.

The starting time of each treatment was adjusted so that seeds from all treatments could begin the germination assay and moisture determination at the same time. The experiment was a 2 (cultivar) x 2 (surface-dried vs. dried-back) x 2 (storage vs. no storage) x 2 (storage at 4 °C vs. 20 °C) factorial. There were 10 replications of 50 seeds of each treatment. Four of

these replications were used for germination testing while the remaining 6 replications were used for seed moisture determination. Non-factorial control seeds also were included both for the germination assay and moisture determination.

Moisture determination was performed according to ISTA rules (ANONYMOUS 1985). Six replications of seeds from all treatments were weighed, then placed in metal cans (the same cans used for storage previously) and oven-dried at 130 °C for 1 hour. At the end of 1 hour, seeds were weighed again, and their moisture contents expressed as percentages of oven-dry weight were calculated and subjected to analysis of variance.

Results and Discussion

Seedlot characterization

'Cortina' seeds were of higher vigor than 'Greenlakes' seeds. At both 5 and 20 °C, 'Cortina' had 100 % germination, compared to 91 % and 87 %, respectively, for 'Greenlakes' (Table 1). G_{50} and $G_{10–90}$ values for both cultivars were much lower at 20 °C than at 5 °C, but 'Cortina' had lower values than 'Greenlakes' at both germination temperatures. From these results, it was concluded that 'Cortina' was a high vigor seedlot while the 20-year old 'Greenlakes', although of high viability, was of low vigor.

Determination of priming agent, osmotic potential, and duration

None of the priming treatments had any effect on the FGP of 'Cortina' seeds (all had 100 % germination) compared to untreated seeds (Table 2 and 3). This was not an unexpected result because it was known from the results of Expt. 1 (Table 1) that 'Cortina' is a highly vigorous cultivar. However, priming increased the FGP of low vigor 'Greenlakes' when KH_2PO_4 was used as a priming agent irrespective of its water potential or the length of the priming period. In addition to KH_2PO_4, other priming treatments of 'Greenlakes' seeds that increased FGP over that of untreated seeds were: 10 h or 20 h with K_3PO_4 at –1.5 MPa, 10 h with $NH_4H_2PO_4$ at –0.49 or –1.5 MPa, and 10 h or 20 h with PEG at –0.49 MPa or 10 h with PEG at –1.5 MPa.

The germination of 'Cortina' seeds was hastened by 20 h of priming with KH_2PO_4 or K_3PO_4 at –0.49 or –1.5 MPa. The germination rate of 'Greenlakes' was improved by all priming treatments compared to untreated seeds except KH_2PO_4 at –0.49 MPa for 10 h, which had no effect on G_{50}, and with $NH_4H_2PO_4$ at –0.49 MPa for 20 h, which increased the G_{50} from 0.96 days (untreated seeds) to 1.06 days.

All treatments failed to improve the germination synchrony of 'Cortina' seeds. However, priming 'Greenlakes' seeds with KH_2PO_4 at –1.5 MPa for 20 h was the only treatment that improved the synchrony of 'Greenlakes' seeds. The same priming treatment ($NH_4H_2PO_4$ at –0.49 MPa for 20 h) that reduced the germination rate of 'Greenlakes' seeds also reduced germination synchrony, with the $G_{10–90}$ value increasing from 0.95 days (untreated seeds) to 1.30 days. Although there are several reports of priming lettuce in 1 % K_3PO_4 at 15 °C (CANTLIFFE 1991; SEALE et al. 1994), we found that only KH_2PO_4 (–1.5 MPa, 20 h, 15 °C) positively influenced all germination characteristics (increased germination percentage and rate, but primarily germination synchrony) of 'Greenlakes', a low vigor cultivar. Consequently, this priming treatment was chosen for subsequent experiment.

Determination of different drying and storage regimes of primed seeds

Priming had no effect on FGP of either cultivar irrespective of whether the primed seeds were stored or not, the storage temperature, and the drying regime following priming compared to untreated seeds, although 'Cortina' had higher FGP than 'Greenlakes' (Table 4).

Primed 'Cortina' seeds stored at 4°C, had a higher germination rate than primed seeds stored at 20 °C, irrespective of the drying regime. Compared to untreated 'Cortina' seeds (G_{50}=0.76 days), the lowest G_{50} values of primed and stored seeds were obtained with dried-back seeds stored at 4°C (G_{50}=0.45 days). When primed 'Cortina' seeds were not stored, surface-dried seeds (G_{50}=0.33 days) gave faster germination than dried-back seeds (G_{50}=0.73 days). Only when primed-surface-dried seeds of 'Greenlakes' were not stored, was germination rate improved (G_{50}=0.73 days) compared to untreated seeds (G_{50}=0.87 days). Storage of primed 'Greenlakes' seeds raesulted in G_{50} values either not different or higher than that of untreated seeds.

Germination synchrony of seeds of all priming treatments, including non-stored primed seeds, was the same or less than that of untreated seeds. Storage of surface-dried 'Cortina' seeds at 20 °C resulted in higher $G_{10–90}$ than those that were stored at 4°C. When seeds were stored at 20 °C, drying back resulted in similar

Table 1. Final germination percentage (FGP) and its angular transformation [FGP.], days to 50 % FGP (G_{50}) and days between 10 % and 90 % germination ($G_{10–90}$) of 'Cortina' and 'Greenlakes' lettuce seed germination in darkness at 5 °C or 20 °C

Cultivar	FGP %	[FGP]	G_{50} (days)	$G_{10–90}$ (days)
Germination at 20 °C				
'Cortina'	100	[88]	0.5	0.8
'Greenlakes'	91	[72]	0.9	1.6
Germination at 5 °C				
'Cortina'	100	[88]	6.4	1.9
'Greenlakes'	87	[69]	8.2	4.3
$LSD_{0.05}$	[12]		0.3	0.6
Significances[z]				
Cultivar (cv)	[***]		***	***
Temperature (t)	[**]		***	***
cv X t	NS		*	*

[z] ***, **, *, NS Significant at P ≤ 0.001, 0.01, 0.05, or not significant, respectively.

Table 2. Final germination percentage (FGP) and angular transformation of FGP [FGP], days to 50 % of FGP (G_{50}) and days between 10 and 90 % of FGP ($G_{10–90}$) of 'Cortina' (C) and 'Greenlakes' (G) lettuce at 20 °C following priming for 10 or 20 hours at 15 °C in –0.49 or –1.5 MPa salts (KH_2PO_4, K_3PO_4, $NH_4H_2PO_4$) or PEG

Priming conditions										
	Water	Duration	FGP	[FGP]	FGP	[FGP]	G_{50} (days)		$G_{10–90}$ (days)	
Agent	potential (–MPa)	(hours)	'C'		'G'		'C'	'G'	'C'	'G'
KH_2PO_4	0.49	10	100	[90]	93	[75]	0.75	0.90	0.46	1.15
	0.49	20	100	[90]	95	[77]	0.68	0.75	0.65	1.01
	1.50	10	100	[90]	93	[74]	0.75	0.81	0.41	0.91
	1.50	20	100	[90]	92	[73]	0.69	0.78	0.65	0.67
K_3PO_4	0.49	10	100	[90]	88	[70]	0.72	0.85	0.60	0.97
	0.49	20	100	[90]	91	[72]	0.63	0.90	0.70	0.94
	1.50	10	100	[90]	95	[77]	0.72	0.82	0.52	0.89
	1.50	20	100	[90]	95	[81]	0.46	0.83	0.78	0.99
$NH_4H_2PO_4$	0.49	10	100	[90]	92	[73]	0.75	0.85	0.42	0.91
	0.49	20	100	[90]	91	[72]	0.74	1.06	0.57	1.30
	1.50	10	100	[90]	92	[74]	0.75	0.84	0.42	0.91
	1.50	20	100	[90]	88	[70]	0.71	0.86	0.58	0.98
PEG	0.49	10	100	[90]	92	[74]	0.74	0.85	0.43	0.86
	0.49	20	100	[90]	97	[79]	0.74	0.85	0.49	1.06
	1.50	10	100	[90]	94	[76]	0.75	0.84	0.41	0.84
	1.50	20	100	[90]	90	[72]	0.75	0.86	0.43	0.79
Untreated seeds			100	[90]	85	[67]	0.79	0.96	0.57	0.99
$LSD_{0.05}$ (4-way interaction)				[5]			0.07		0.26	

Table 3. Statistical significances of factors given in Table 2.

Significances[z]	FGP	G_{50} (days)	$G_{10–90}$ (days)
Cultivar (cv)	[***]	***	***
Priming agent (pa)	[NS]	***	*
cv X pa	[NS]	***	NS
Water potential (wp)	[NS]	***	**
cv X wp	[NS]	NS	*
pa X wp	[*]	**	NS
cv X pa X wp	[**]	NS	NS
priming duration (pd)	[NS]	**	**
cv X pd	[NS]	***	NS
pa X pd	[NS]	***	NS
cv X pa X pd	[NS]	***	NS
wp X pd	[NS]	*	NS
cv X wp X pd	[NS]	NS	NS
pa X wp X pd	[NS]	**	NS
cv X pa X wp X pd	[NS]	*	NS

[z] ***, **, *, NS Significant at $P \leq 0.001$, 0.01, 0.05, or not significant, respectively.

germination synchrony ($G_{10–90}$=0.83 days) compared to surface drying ($G_{10–90}$=0.76 days). For 'Greenlakes', except for the higher $G_{10–90}$ of surface-dried seeds stored at 20 °C ($G_{10–90}$=1.23 days), all treatments resulted in germination synchronies not different from untreated seeds ($G_{10–90}$=0.90 days).

The moisture content of primed 'Cortina' seeds was higher with surface-drying compared to drying-back, and was higher following storage at 4 °C than at 20 °C (Table 4). Seed moisture content of primed 'Greenlakes' seeds was the same or lower than that of untreated seeds, except for surface-dried seeds that were not stored.

The extent of seed drying, after priming, and subsequent storage conditions (temperature and relative humidity) can affect germination performance. In seed priming studies, seeds usually are transferred directly from the priming solution to the germination media, generally resulting in very rapid germination compared to seeds imbibed at the same time. When there is no seed drying, this response represents the release of seeds from osmotic inhibition. The freshly primed seeds have completed phase I (hydration) and II (lag phase) of germination, and only require a favorable water potential gradient for water uptake in order to begin radicle growth. Primed seeds have the advantage of entering immediately into phase III of inhibition (growth), while seeds, which are dried after priming must repeat phase I and often at least a short phase II before growth occurs (PILL 1995).

Typically, seed germination or seedling emergence is delayed in proportion to the degree of seed drying after

Table 4. Final germination percentage (FGP) and angular transformation of FGP [FGP], days to 50% of FGP (G_{50}) and days between 10 and 90 % ($G_{10–90}$) of FGP at 20 °C of primed 'Cortina' (C) and 'Greenlakes' (G) lettuce seeds following two drying regimes and one month storage at 4 or 20 °C.

		FGP	[FGP]	FGP	[FGP]	G_{50} (days)		$G_{10–90}$ (days)		Seed water (%)[x]	
Drying regime	Storage conditions	'C'		'G'		'C'	'G'	'C'	'G'	'C'	'G'
Surface dried	4 °C	99	[86]	88	[70]	0.66	0.83	0.46	0.81	8.16	7.03
Surface dried	20 °C	100	[90]	93	[74]	0.75	1.04	0.76	1.23	5.28	6.64
Dried back	4 °C	100	[88]	92	[74]	0.45	0.85	0.81	0.96	7.09	6.68
Dried back	20 °C	100	[90]	90	[72]	0.66	0.89	0.83	1.01	5.35	5.45
Surface dried	None	100	[90]	90	[71]	0.33	0.73	0.65	0.87	9.84	10.18
Dried back	None	99	[87]	89	[72]	0.73	1.00	0.54	0.99	4.32	4.55
Control seeds[z]	None	100	[90]	87	[70]	0.76	0.87	0.52	0.98	6.58	6.37
$LSD_{0.05}$			[4]			0.06		0.19		1.72	
Significances[y]											
Cultivar (cv)			[***]			***		***		NS	
Seed treatment (st)			[NS]			***		NS		***	
cv X st			[NS]			***		**		***	

[z] Non-primed seeds
[y] ***, **, NS Significant at $P \leq 0.001$, 0.01, or not significant, respectively.
[x] Wet weight basis, oven-dried (130 °C, 1 h).

priming but before sowing. We noted little difference in G_{50} of both lettuce cultivars following surface-drying or drying-back (Table 4). Slower or less synchronous emergence from primed and dried-back seeds (compared with primed seeds which have not been dried-back) has been reported for carrot, celery, and onion (BROCKLEHURST and DEARMAN 1983), parsley (PILL 1986), and tomato (PILL et al. 1991). Delayed emergence of primed and dried-back seeds may result from the increased time needed for seeds to re-imbibe.

ARGERICH et al. (1989) reported that primed tomato seeds stored at 4 °C retained viability and germination rate after one year, but at 30 °C viability and vigor were markedly reduced within six months. These authors concluded that primed tomato seeds must be considered to be vigorous with a reduced shelf life. KRAAK and WEGES (1989) documented that the storage life of lettuce seeds is shortened following priming. TARQUIS and BRADFORD (1992) also reported that although priming accelerated the germination rates of lettuce, the re-dried seeds were highly susceptible to deterioration in storage. On the contrary, our results demonstrated that storing the primed seeds of both cultivars for one month had little or no effect on the viability (FGP) and rate (G_{50}), but slightly reduced the germination synchrony ($G_{10–90}$). It would appear that primed seeds of 'Cortina' and 'Greenlakes' can be stored for one month at 4 or 20 °C following either surface-drying or drying-back with little or no negative effect on subsequent germination.

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Addresses of authors: Ahmet Korkmaz, Department of Horticulture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey, 46100, e-mail: akorkmaz@ksu.edu.tr and Wallace. G. Pill, Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA, 19717.