http://dx.doi.org/10.1590/1678-4499.259

Is the enhancement produced by priming in cottonseeds maintained during storage?

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Received: *Aug. 11, 2014;* ***Accepted:*** *Sept. 10, 2014*

Abstract

The objective of this study was to evaluate: a) whether the effects of hydropriming and osmopriming in manitol on cottonseed germination are maintained during their storage; b) whether the behavior of the treated seeds -in storage- depends on the type of priming substrate used; c) whether seeds from different harvest years [age] have a similar response. The effects of 16 h of hydropriming and 41 h in mannitol were tested at 18 and 25 °C on the velocity of germination, standard germination and the vigor in cottonseeds from the 2007 and 2008 harvests, and within 0, 6 and 12 months of postpriming storage. The enhancement produced by the priming treatments used remained at least for 6 months in the case of hydropriming and 12 months for osmopriming in mannitol. The latter was more effective in increasing velocity of germination and the vigor index (CWVI). Priming enhanced the behavior in both seed lots but was more effective on the younger seeds.

Key words: *Gossypium hirsutum*, priming, germination, vigor, year of harvest, storage.

O aumento produtivo associado ao *priming* em sementes de algodão mantém-se durante o armazenamento?

Resumo

O objetivo do presente trabalho foi avaliar: (a) se os efeitos do ***hidropriming*** e do ***osmopriming*** no manitol sobre a germinação das sementes de algodão mantêm-se durante a sua armazenagem, (b) se o comportamento da armazenagem das sementes tratadas depende do substrato do ***priming*** empregado, e (c) se sementes de um ano diferente de colheita (idade) respondem de igual maneira. Os efeitos de 16 horas de ***hidropriming*** e 41 horas em manitol foram avaliados a 18 °C e 25 °C sobre a velocidade de germinação, a germinação ***standard*** e o vigor das sementes de algodão das colheitas de 2007 e 2008 e nos meses 0, 6 e 12 de armazenagem ***postpriming***. O ***enhancement*** produzido pelos tratamentos de ***priming*** utilizados ficou invariável no mínimo durante 6 meses, no caso do ***hidropriming,*** e 12 meses, para o ***osmopriming*** em manitol. O ***osmopriming*** em manitol foi mais efetivo para aumentar a velocidade de germinação e o índice de vigor (CWVI). O ***priming*** melhorou o comportamento em ambos os lotes de sementes mas foi mais efetivo nas sementes mais novas.

Palavras-chave: *Gossypium hirsutum*, priming, germinação, vigor, ano de colheita, armazenagem.

1. INTRODUCTION

Cotton is one of the most important crops of semiarid regions of Argentina where some stress factors such as lack of water, salinity and inadequate temperatures may affect the germination and emergence of this crop. Priming is a pre-sowing treatment broadly used to enhance germination and the performance of seeds in the above conditions (Parera and Cantliffe, 1994; Welbaum et al., 1998). This treatment can be done in water or in osmotic solutions. Effectiveness of hydropriming has been shown in many species (Bittencourt et al., 2005; Choudhary et al., 2008; Dorna et al., 2013; Fanan and November, 2007; Fujikura et al., 1993;). Mannitol, though in general less tested (Parera and Cantliffe, 1994; Welbaum et al., 1998), has shown promising results in preliminary studies in cotton.

To implement priming on a commercial scale, one of the aspects to be considered is whether the benefits that result from such treatment are maintained over time while seeds are stored. In this sense, there exist variable responses depending on the species, variety, age of the seeds and storage conditions (Dorna et al., 2013; Parera and Cantliffe, 1994). In melon, the positive effect of priming was maintained after a period of storage (Singh et al., 2001) while other species have shown a negative relation between priming and storage, with loss of longevity, germination or vigor (Drew et al., 1997; Nascimento and West, 2000).

On the other hand, some studies have shown that there can exist an interaction between the osmotic solution used in priming and the potential for storage of the seeds

(Dorna et al., 2013; Parera and Cantliffe, 1994) and that priming before storage could control the deterioration of the seeds produced during this period of time (Rahman et al., 2013).

Another controversial aspect of priming is its effectiveness according to the initial quality of the seeds (Nascimento, 1998). In melon, priming in healthy and high quality seeds is recommended (Nascimento, 2002). Nevertheless, in other cases, better responses were found in lots of seeds of low quality (Bittencourt et al., 2005; Choudhary et al., 2008; McDonald, 1999; Nascimento and Souza de Aragão, 2004).

Previous studies in cotton showed encouraging results in the use of this treatment. In low temperature conditions, to which this species is very sensitive, hydropriming and osmopriming in mannitol allowed acceleration of germination (Casenave and Toselli, 2007; Ghaderi et al., 2008) but it is not known whether these effects remain during seed storage and if they depend on the age of the seed used. Under the hypothesis that the benefits of priming in cotton are maintained from one year to the next during storage of seeds of different age and that they depend on the used substrate, the aim of this study was to evaluate: a) if the effects of hydropriming and osmopriming in mannitol on the germination of cottonseeds are maintained during their storage, b) whether the behavior of the treated seeds in storage depend on the substrate of priming used and c) whether seeds from different harvest years (age) have a similar response.

2. MATERIALS AND METHODS

The tests were conducted during 2009, with cottonseeds of the Guazuncho III INTA variety, harvested in 2007 and 2008, commercially provided and chemically delinted and cured.

The following treatments were applied: 16 hours of priming in water (H16), 41 hours of priming in mannitol – 1 MPa (M41) and seeds without priming (Control). To do the priming, 100 seeds were uniformly distributed on rolls of paper (4 towels), watered with 44 ml of water or mannitol to obtain the relation between volume of solution: weight of paper of 3:1. The paper rolls were placed in plastic bags to avoid evaporation and were incubated at a constant temperature of 25 °C. After 16 hours of incubation in water and 41 hours in mannitol, to reach the same state of hydration (Casenave and Toselli, 2007), the seeds were removed and dried in the air until they returned to the initial water content (7-8%) and were stored in laboratory conditions until the time of their evaluation.

To determine if the effects of the treatment are maintained during the time, the seeds newly treated were evaluated (time 0 of storage) and 6 and 12 months after priming has been completed. Treated seeds were kept in laboratory conditions and untreated seeds (Control) were kept refrigerated at 5 to 7 °C to preserve its initial quality. Priming was performed in a staggered fashion, 6 and 12 months before the evaluation of seed behavior that was completed as a whole on the same date. Ten days before this date, the priming corresponding to time 0 of storage was done.

The effects of priming on germination were evaluated at the optimal temperature of 25 °C and under thermal stress, at 18 °C (AOSA, 1980).

For germination tests, four repetitions of 25 seeds were placed in paper rolls soaked in water in a relation volume: paper weight of 3:1. The rolls were then placed in plastic bags to prevent evaporation and were incubated at 25 °C or 18 °C with 8 hours of photoperiod (ISTA, 2012).

Mean time to germination (T_{50}) was calculated following Brar and Stewart (1994) for which the number of germinated seeds (radicles longer than 1 mm with evidence of geotropic curvature), was recorded daily. At the end of the test (12 days), standard germination was determined and expressed as percentage in number of the normal seedlings (ISTA, 2012).

Vigor was determined by recording the percentage of normal seedlings 4 cm or more of total length on the 4th day after seeding, at 25 °C or on the 7th day when the temperature of germination was 18 °C. The added percentages of seedlings in the warm and cold tests resulted in the vigor index (CWVI = cool warm vigor index), which allows classifying cottonseeds as seeds of low vigor (less than 120), regular vigor (between 120 and 139), good vigor (between 140 and 159) and excellent vigor (above 160) (Baughman et al., 1994).

The tests were repeated twice and completed following a randomized statistics design. Data were analyzed as a factorial experiment (2 years of harvest × 2 temperatures × 3 priming treatments × 3 storage periods). Data transformed to the arcsin of the square root of the germination proportion were analyzed using ANOVA and the average Newman-Keuls test using the statistics software Infostat (2004) at the 5% significance level.

3. RESULTS AND DISCUSSION

When comparing the general means, velocity of germination increased significantly as a consequence of priming treatments, especially in mannitol. T_{50} for untreated seeds was 3.30 days, significantly decreasing to 2.93 and 2.63 days for hydropriming and osmopriming in mannitol respectively. Both priming treatments enhanced the behavior of seeds, both at 25 °C and 18 °C (Table 1), already observed in this species (Casenave and Toselli, 2007; Ghaderi et al., 2008). At a suboptimal temperature, M41 was more effective than H16 producing a greater reduction of T_{50}. The effects of priming on velocity of germination can be explained by the reduction in the base temperature or thermal time (Welbaum et al., 1998); in cotton, this effect has been

attributed to a reduction in thermal time (Casenave and Toselli, 2007). Reductions in T_{50} from 0.4 to 0.8 days induced by priming are important considering that during the sowing period of the species, one expects a reduction of 5mm/day of soil available water. At such evaporation rate, available water in the first 10 cm of depth would be exhausted in only 3 days under the current climatic conditions in the semiarid cultivation areas of Argentina.

Some studies suggest that the sensitivity to the temperature of germination after priming could be modified by the conditions of seed drying. In lettuce, velocity of germination at suboptimal temperature did not register any difference from control seeds after a process of quick drying, while slow drying evidenced the effects of priming (Schwember and Bradford, 2005). In this study, the seeds went through a process of natural drying associated with slow drying, at room temperature and humidity until they reached the initial water content, thus probably allowing for the observed response.

The effect of storage time for each priming treatment used is shown in figure 1. Seeds treated and planted immediately after priming (time 0), germinated more quickly than untreated seeds. After 6 months both treatments, H16 and M41 continued to be equally effective. At 12 months of storage the favorable effects of H16 on velocity of germination were lost, while the positive effects of M41 remained at a level comparable to the initial level. This interaction was also observed by Fanan and Novembre (2007) in eggplant seeds treated with water or PEG, in which osmopriming was better than hydropriming after 4 months of storage.

It has been suggested that conditions and duration of storage can affect germination after such period (Dorna et al., 2013; Parera and Cantliffe, 1994). In our case, post priming storage in laboratory conditions did not produce adverse effects since T_{50} maintained values lower than or equal to those of control seeds, which remained stored in conditions such that metabolic activity and deterioration processes are reduced to the minimum, which allows to maintain the quality of the seeds over time (5 to 7 °C).

With relation to the year of harvest (age), the comparison of the general means showed significantly slower germination in 2007 harvest seeds (T_{50} = 3.18 days) than 2008 harvest seeds (T_{50} = 2.72 days). The statistics analysis indicated a significant interaction for treatment, storage and harvest year. Hydropriming during 16 h (H16) accelerated germination with respect to that of control seeds, namely, 0.4 to 0.6 days for 2008 seeds, and 0.48 days for 2007 seeds (Table 2). In the latter, the effects disappeared after 12 months of storage. M41 was the most effective treatment regardless of the harvest year, with reductions in the T_{50} that reached 0.7 to 0.8 days in 2008 seeds and even surpassed, in 2007 seeds, the nontreated 2008 seeds. Seeds harvested in 2008 had a better behavior due to the benefits of the osmopriming in mannitol were maintained during 12 months, while in 2007 seeds, effects disappeared after 6 months.

There is controversy on how the initial quality of seeds influences the response to priming, partly due to the used methodology, since in order to evaluate this aspect of the response to *priming*, in some studies, seeds are artificially aged by exposing them to conditions of controlled deterioration before treating them. In cauliflower seeds submitted to controlled deterioration, effects of hydropriming and osmopriming in PEG, were compared showing that these treatments were

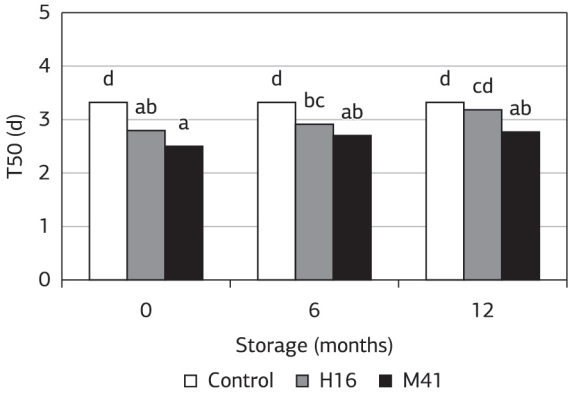

Figure 1. Mean germination time (T_{50}) for cottonseeds as a function of storage and priming treatment: non-treated seeds (Control), hydropriming 16 hours (H16), priming in mannitol (M41). Different letters indicate significant differences (p<0.05).

Table 1. Mean time to germination (T_{50}) in days (d) for cottonseeds, as a function of priming treatments and germination temperature (25 °C and 18 °C). Untreated seeds (Control); 16 hours of priming in water (H16) and 41 hours of priming in mannitol (M41)

Treatment	Temperature (°C)	
	25	18
Control	2,63 b	3,97 e
H16	2,17 a	3,68 d
M41	2,17 a	3,09 c

Different letters in rows and columns indicate significant differences (p<0.05).

Table 2. Mean time to germination (T_{50}) in days (d) based on storage time (0, 6 and 12 months) for cottonseeds harvested in different years (2007 and 2008). Non-treated seeds (Control), hydroprimed 16 hours in water (H16) and osmoprimed 41 hours in mannitol (M41)

Treatment	Storage (months)	T_{50} (d)	
		Harvest year	
		2007	2008
Control	0	3,44 (de)	3,15 (cde)
H16	0	2,96 (bcd)	2,55 (abc)
	6	2,96 (bcd)	2,81 (abcd)
	12	3,69 (e)	2,6 (abc)
M41	0	2,66 (abc)	2,33 (a)
	6	3,06 (bcd)	2,28 (a)
	12	2,98 (bcd)	2,48 (ab)

Different letters in rows and columns mean significant differences (p<0.05).

less effective in aged seeds (Fujikura et al., 1993). On the contrary, in melon seeds artificially aged by different periods of time, the effect of osmopriming in KNO_3 was greater in more deteriorated seeds (Nascimento and Souza de Aragão, 2004). It has been suggested that the processes conducive to seed deterioration are related to the type of aging produced, natural or accelerated, and that priming could include some repair mechanisms (McDonald, 1999). In this study, the differences in initial quality of the seeds in both harvest years are due only to natural deterioration occurred over time starting at the moment of harvest. The activated repair mechanisms during priming, or a reduced degree of deterioration, could explain the better response observed in younger seeds.

Standard germination expressed as percentage of normal seedlings was modified neither by priming nor by temperature or storage. Only seed age affected this variable, significantly less for 2007 seeds (60-70%) with relation to 80% reached by 2008 seeds. In other species (Bittencourt et al., 2005; Choudhary et al., 2008*),* different treatments of osmopriming increased the percentage of normal seedlings in seed lots of different initial quality, while hydropriming was only effective in seed lots of lower or medium physiological quality. Dorna et al. (2013) report a decrease in the percentage of normal seedlings after 6 to 12 months of storage in seeds hydro or osmoprimed in PEG, related to the increase of fungal infections during such period, which was not observed in the current study.

Regarding the vigor, both 2007 and 2008 seeds without priming treatments were classified as seed of low vigor, according to the combined vigor index (CWVI) used. In the 2007 harvest, though M41 had a significant effect, the vigor of the seeds remained in the same category even after treatment. In the 2008 harvest, both H16 and M41 significantly increased this index, which moved up to regular or good respectively (Table 3).

The combined effects of the priming treatments, the age of seeds and storage time are shown in figure 2. Vigor expressed by CWVI, was enhanced with priming, especially with osmopriming in mannitol. In other species such as asparagus, chickpeas and carrots, also osmopriming (PEG) was more effective than hydropriming in increasing vigor expressed as percentage of germination at first count (Bittencourt et al., 2005; Choudhary et al., 2008). Nascimento and West (2000) observed a decrease of vigor in melon seeds osmoprimed in KNO_3 after a storage period of 12 months. These researchers attributed this negative effect on vigor to the drying at high temperatures and of short duration, conditions different from the ones used in this study, which probably explain why in this case vigor was not affected by storage.

The initial quality of seeds was different for the 2007 and 2008 lots, however priming enhanced vigor in both cases. These results coincide with those of Parera and Cantliffe (1994), who reported that higher quality lots respond better to priming treatments. The positive effects of osmopriming in PEG and mannitol over the physiological quality of

Table 3. Vigor index (CWVI)* for different priming treatment in cottonseeds from different harvest year. Non-treated (Control), hydroprimed 16 hours (H16) and osmoprimed in mannitol 41 hours (M41)

Treatment	Harvest year	
	2007	2008
Control	89,5 a	118 c
H16	93,67 a	126,5 d
M41	101,5 b	140,83 e

Different letters in columns and rows indicate significant differences (p<0.05). *(CWVI < 120, low vigour; 120-139, regular; 140-159, good; CWVI > 160, excellent).

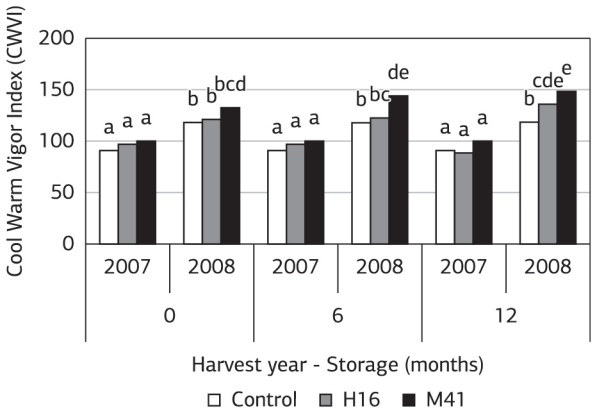

Figure 2. Cool Warm Vigour index (CWVI) as a function of priming treatment in cottonseeds from different harvest year (2007 and 2008), stored during 0, 6 and 12 months. Non-treated (Control), hydroprimed 16 hours (H_{16}) and osmoprimed in mannitol 41 hours (M_{41}). Different letters indicate significant differences (p<0.05).

stored seeds have also been reported by Rahman et al. (2013), who showed that priming reduces the peroxidation of unsaturated acids. This allows controlling deterioration during storage, which is especially important in seeds with high lipid content such as cottonseeds, and that could explain the results obtained in this study.

4. CONCLUSION

The enhancement produced by treatments of priming used is maintained at least 6 months in the case of hydropriming and 12 months for osmopriming in mannitol, making it possible to store primed cottonseeds under ambient conditions from one year to the next until sowing date, which could encourage the use of priming in commercial scale for cottonseeds.

Behavior during the storage of treated cottonseeds varies according to the substrate. Osmopriming in mannitol is the most effective treatment to increase velocity of germination and vigor index (CWVI).

Priming improves cottonseed behavior in both harvests though it is more effective in younger seeds.

REFERENCES

ASSOCIATION OF OFFICIAL SEED ANALYSTS - AOSA. Seed Vigor Testing Handbook. Part I. Seed vigor: its meaning and application. Washington, 1980. 88p.

BAUGHMAN, T.; BOMAN, R.; LEMON, R. Cool warm test. Texas Cooperative Extension. The Texas A&M University System, 1994.

BITTENCOURT, M.L.C.; DIAS, D.C.F.S.; DIAS, L.A.S.; ARAÚJO, E.F. Germination and vigour of primed asparagus seeds. Scientia Agricola, v.62, p.319-324, 2005. http://dx.doi.org/10.1590/S0103-90162005000400003

BRAR, G.S.; STEWART, B.A. Germination under controlled temperature and field emergence of 13 *Sorghum* cultivars. Crop Science, v.34, p.1336-1340, 1994. http://dx.doi.org/10.2135/cropsci1994.0011183X003400050036x

CASENAVE, E.C.; TOSELLI, M.E. Hydropriming as a pre- treatment for cotton germination under thermal and water stress conditions. Seed Science & Technology, v.35, p.88-98, 2007. http://dx.doi.org/10.15258/sst.2007.35.1.08

CHOUDHARY, V.K.; CAURASIA A.K.; GUPA, A.; NASSEM, M.; MAITI, R.K. Effect of priming on germination and vigour of chickipea seeds. International Journal of Agriculture Environment & Biotechnology, v.1, p.196-198, 2008.

DORNA, H.; JAROSZ, M.; SZOPINSKA, D.; SZULC, I.; ROSINSKA, A. Germination, vigour and health of primed *Allium cepa* L. seeds after storage. Acta Scientiarum Pololonorum, Hortorum Cultus, v.12, p.43-58, 2013.

DREW, R.L.K.; HANDS, L.J.; GRAY, D. Relating the effects of priming to germination of unprimed seeds. Seed Science & Technology, v.25, p.537-548, 1997.

FANAN, S.; NOVEMBRE, A.D.L.C. Tecnologia de sementes e fibras: condicionamento fisiológico de sementes de berinjela. Bragantia, v.66, p.675-683, 2007. http://dx.doi.org/10.1590/S0006-87052007000400018

FUJIKURA, Y.; KRAAK, H.L.; BASRA, A.S.; KARSSEN, C.M. Hydropriming, a simple and inexpensive priming method. Seed Science & Technology, v.21, p.639-642, 1993.

GHADERI, F.A.; SOLTANI, E.; AFSHIN, S.; ASGHAR, M.A. Effect of priming on response of germination to temperature in cotton. Journal of Agricultural Sciences and Natural Resources, v.15, p.44-51, 2008.

INFOSTAT. InfoStat version 2004. Argentina: Universidad Nacional de Córdoba, 2004.

INTERNATIONAL SEED TESTING ASSOCIATION - ISTA. International rules for seed testing. Bassersdorf, 2012.

MCDONALD, M.B. Seed deterioration: physiology, repair and assessment. Seed Science & Technology, v.27, p.177-237, 1999.

NASCIMENTO, W.M. Condicionamento osmótico de sementes de hortaliças: potencialidades e implicações. Horticultura Brasileira, v.16, p.106-109, 1998.

NASCIMENTO, W.M. Sementes de melão osmoticamente condicionadas: vale a pena utilizá-las? Horticultura Brasileira, v.20, p.133-135, 2002. http://dx.doi.org/10.1590/S0102-05362002000200003

NASCIMENTO, W.M.; WEST, S.H. Drying during muskmelon seed priming and its effects on seed germination and deterioration. Seed Science & Technology, v.28, p.211-215, 2000.

NASCIMENTO, W.M.; SOUZA DE ARAGÃO, F.A. Muskmelon seed priming in relation to seed vigor. Scientia Agricicola, v.61, p.114-117, 2004. http://dx.doi.org/10.1590/S0103-90162004000100019

PARERA, C.A.; CANTILIFFE, D.J. Presowing seed priming. Horticultural Reviews, v.16, p.109-141, 1994.

RAHMAN, I.U.; ALI, N.; RAB, A.; SHAH, Z. Role of pre storage seed priming in controlling seed deterioration during storage. Sarhad Journal of Agriculture, v.29, p.379-386, 2013.

SINGH, G.; GILL, S.S.; SANDHU, K.K. Storage of Primed seeds of muskmelon. Seed Research, v.29, p.235-237, 2001.

SCHWEMBER, A.R.; BRADFORD, K.J. Drying rates following priming affect temperature sensitivity of germination and longevity of lettuce seeds. HortScience, v.40, p.778-781, 2005.

WELBAUM, G.E.; SHEN, Z.; OLUOCH, M.O.; JETT, L.W. The evolution and effects of priming vegetable seeds. Seed Technology, v.20, p.209-235, 1998.