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Imbibition profile in polyethylene glycol 6000 osmotic solution and physiological potential of soybean seeds

Camilla Atsumi Zanuncio Sediyama-Bhering[1], Carla Quinhones Godoy Soares[1], Deisy Xavier Amora[1], Danúbia Aparecida Costa Nobre[1], Carlos Sigueyuki Sediyama[1], Denise Cunha Fernandes dos Santos Dias[1], Múcio Silva Reis[1]

[1] Universidade Federal de Viçosa, Centro de Ciências Agrárias, Campus Universitário, Av. PH Rolfs, s/n, Centro, CEP 36571-000, Viçosa-MG, Brasil. E-mail: csediyama@gmail.com; carla.quinhones@gmail.com; deisyamora@yahoo.com.br; danubia_nobre@yahoo.com.br; csediyama@ufv.br; dcdias@ufv.br; msreis@ufv.br

ABSTRACT

The objective of this work was to study the imbibition profile in water or in polyethylene glycol 6000 osmotic solution and the effect of the osmoconditioning on the germination and vigor of seeds of six soybean cultivars - Confiança, UFV-16, Splendor, Garantia, UFVS 2005 and UFV-18. The cultivars were grown in the field at Viçosa, Minas Gerais, in a randomized complete block design, the seeds were harvested at the R8 stage and 15 and 30 days later. Seed samples of each cultivar per harvest time and replication were soaked in distilled water (control) or osmoconditioned in -0.8 MPa PEG 6000 solution at 20 °C, for 96 h, in the presence of 0.2% Captan fungicide. Vigor and viability of the seeds were evaluated by the first and final counting in the germination test on paper rolls and speed of seedling emergence on sand seedbed. The imbibition speed and the humidity of the osmoconditioned seeds of all six cultivars and three harvest times were lower than of those seeds soaked in water. The germination and vigor of osmoconditioned seeds were higher for all cultivars at all harvest times, mainly with 30 days harvest delay, indicating the conditioning efficacy to increase the germination of weathered seeds.

Key words: germination, *Glycine max*, osmoconditioning, seed quality, vigor

Perfil de embebição e potencial fisiológico de sementes de soja osmocondicionadas em polietilenoglicol 6000

RESUMO

O objetivo neste trabalho foi estudar o perfil de embebição em água ou em solução osmótica de polietilenoglicol 6000 e o efeito desse na germinação e no vigor de sementes de seis cultivares de soja - Confiança, UFV-16, Splendor, Garantia, UFVS 2005 e UFV-18. Os cultivares foram semeados no campo, em Viçosa, Minas Gerais, no delineamento em blocos completos casualizados, sementes foram colhidas no estádio R8, e 15 e 30 dias após. Amostras de sementes de cada cultivar, época de colheita e repetição foram embebidas em água destilada (controle) ou em PEG 6000, -0.8 MPa, a 20 °C, por 96 h, a 0.2% do fungicida Captan. O vigor e a viabilidade foram avaliados pela primeira e contagem final do teste de germinação e velocidade de emergência em leito de areia. A velocidade de embebição e a umidade das sementes osmocondicionadas dos seis cultivares e três épocas de colheita foram menores do que as embebidas em água. A germinação e o vigor das sementes osmocondicionadas, de todos os cultivares e épocas de colheita foram maiores, principalmente nas colheitas com atraso de 30 dias, indicando a eficácia do condicionamento em incrementar a germinação de sementes intemperizadas.

Palavras-chave: germinação, *Glycine max*, solução osmótica, qualidade da semente, vigor

Introduction

Seed quality characteristics such as dry matter weight and physiological potential are maximal at the physiological maturity, decreasing from this point depending on the environmental condition prior to the harvest and the processes of seed production. Harvest must occur as soon as possible after the seed reaches its physiological maturity, since rainy periods may cause irrecoverable damage to the quality of the seeds (Sediyama et al., 1972). The tolerance level for seed deterioration in the field varies depending on cultivar and the environment, however high temperature and precipitation are more important than the period of time that the seed remains in the field after the physiological maturity. Unfavorable weather conditions have led to the production and rejection of soybean seed lots that do not meet the quality standards, among them the minimum of 80 percent germination rate (Peske & Meneghello, 2013).

Imbibition is the absorption of a fluid by a solid or colloid that results in swelling and it is the first stage of a sequence of events for the retake of the embryo development and growth in the germination process. Seeds generally present a lower water potential than the substrate in which they germinate, which causes a fast entrance of water into the cotyledons, death of the superficial cells and, in certain situations, damage due to the leakage of solutes and resulting on decrease of the seedlings emergence (Matthews & Powell, 1986).

The speed how the water is absorbed seems to be decisive for the success of germination. Furthermore, osmoconditioning with PEG or NaCl has also been identified to be an effective way to reduce physiological and biochemical damage induced by imbibitions at chilling stress (Posmyk et al., 2001). During osmoconditioning, many genes are induced, which are beneficial for plant to survive the subsequent chilling stress (Farooq et al., 2010).

Soybean seeds harvested at three times from the R8 stage, when 95% of the pods have reached their mature colour, showed greater percentage of imbibition and lower vigor, germination and index of resistance to seed coat shrinking with harvest delay after the 21st day after physiological maturity (R8) (Rocha et al., 1984). The rate of water absorption by the seeds increased with the harvest delay, indicating greater permeability of the membranes caused by the deterioration process.

Reduction of problems caused by fast water uptake by osmoconditioning the seed, which consists in its controlled hydration, that activates the pre-germinative metabolic processes (Nascimento, 2005), but stops just before the root protrusion (Bradford, 1990), has been reported. This process improves germination and seed vigor, besides allowing a faster and more uniform seedling emergence (Del Giúdice et al., 1998; Nascimento, 2005; Khalil et al., 2010; Yadav et al., 2011).

The objective in this work was to evaluate the imbibition in water or in osmotic solution of polyethylene glycol 6000 - PEG 6000, and the effect of the osmoconditioning on the germination and vigor of seeds of six soybean cultivars, harvested at three different times.

Material and Methods

Soybean seeds were multiplied at the Prof. Diogo Alves de Mello Experimental Field and analyzed at the Soybean Breeding and Seed Research laboratories of the Plant Sciences Department at the main campus of the Federal University of Viçosa, Minas Gerais. Seeds of six soybean cultivars of different maturity periods - Confiança (semi-early), UFV-16 (medium), Splendor (medium), Garantia (semi-late), UFVS 2005 (late) and UFV-18 (late) - were produced in the agricultural year of 2005/2006, in a randomized complete block experimental design with four replications. Each plot had 12 rows of plants with five meters length and a spacing of 50 cm between them. The soil was prepared for planting with one ploughing and two disking; the fertilization, the cultural techniques and the phytosanitary control were carried out according to recommendations for the crop (Embrapa Soja, 2011). The daily climatic data of rain precipitation and minimum, average and maximum temperatures were registered during the period of seed development and harvesting (Figure 1).

Four rows of plants of each cultivar were harvested at the R8 reproductive stage (when 95% of pods have the typical coloration of mature pods), and three rows each at 15 and 30 days after this maturation stage, to distinguish the seed vigor levels.

The plants were threshed with a stationary machine and the seeds were submitted to sun drying, until they presented 11 to 12% of moisture content on wet basis. The seeds were packed in cotton cloth bags and kept in a chamber at 10 °C and 70% of air relative humidity until they were evaluated in a laboratory, when they were cleaned and size uniformized with sieve number 13 (6.5 mm of diameter).

The PG 6000 concentration, for the achievement of the osmotic potential of the conditioning solution of -0.8MPa at the temperature of 20 °C, was 251.028 g L^{-1} of demineralised water, according to the equation of Michel & Kaufmann

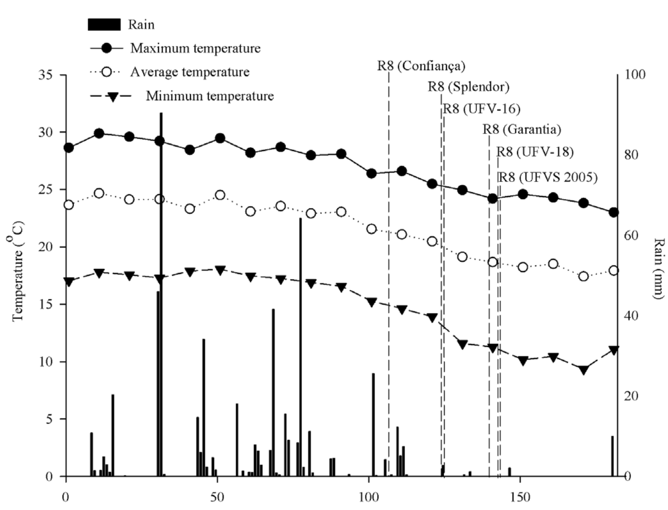

Figure 1. Rain (mm), maximum, average and minimum air temperatures (°C) during the period of seed multiplication in the field counted from the sowing day, and the R8 stages of the six soybean cultivars. Viçosa, Minas Gerais, 2006

(1973): Ψos (atm) = $(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10\text{-}4)CT + (8.39 \times 107)C^2T$, in which: Ψos (atm) = osmotic potential; C = concentration (g/L); T = temperature (°C); and 0.1MPa = 1atm. After the osmoconditioning period, the seeds were superficially washed under tap water to remove PEG 6000, than were dried at room temperature for 48 hours to the initial seed moisture content (10-11%) and were stored until the beginning of the assays.

The seed moisture content, measured on samples dried at 105 ± 1 °C for 24 h (Brasil, 2009), was determined after 0, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 h of imbibition in distilled water or in PEG 6000 solution. The last humidity evaluation was carried out when about 50% of the seeds showing radical protrusion (about 1 mm), which corresponded to the period of 48 h for those imbibed in distilled water. A sample of 100 seeds per cultivar, period of harvest and replication in the field was put into a gerbox, transparent 11 x 11 x 3.5 cm acrylic boxes with lid, with four sheets of germitest paper towel soaked with 30 mL of distilled water or with the same amount of PEG 6000 solution, with the osmotic potential adjusted at -0.8 MPa, in the presence of 0.2% of Captan fungicide. The gerbox with the seeds were placed in a Biochemical Oxygen Demand (B.O.D.) chamber at 20 ± 1 °C (Del Giúdice et al., 1998), set at 12 h photofase.

The germination, with the final counting on the eighth day of the test, according to the Rules for Seed Analysis (Brasil, 2009), was determined for seeds of each cultivar, harvest time and replication in the field, for those which were not conditioned and those which were conditioned in PEG 6000 solution during 96 h (Del Giúdice et al., 1998), the results were presented in percentage of normal seedlings. The seedling emergence test on sand seedbed were carried out in a greenhouse on samples of 50 seeds per cultivar, harvest time, conditioning and field replication sown on plastic trays with sand substrate, the air temperature in the greenhouse ranged from 14 to 34 °C. Daily countings of emerged seedlings were performed up to the 15^{th} day after sowing to allow the estimation of the seedling emergence speed index, according to Maguire (1962).

The statistical model was the split plot design, with cultivars in the plots and harvest times in the subplots, in a randomized complete block experiment, with four replications. The data were submitted to the analyses of variance and regression, and the averages of the qualitative factor were compared with the Tukey´s test, at 5% of probability, when the F test was significant. The regression models were chosen according to the regression coefficient significance by the t test at 5% of probability and also selecting those more suited to the biological phenomenon to be described. The percentage data of the germination test were transformed to arcsine $\sqrt{x/100}$ for the statistical analysis and the means were de-transformed for table presentation. The data processing was carried out with the SAS software (Delwiche & Slaughter, 2013).

Results and Discussion

The imbibition profiles in water and in PEG 6000 solution of the seeds from the six soybean cultivars, Confiança (semi-early), UFV-16 (medium), Splendor (medium), Garantia (semi-late), UFVS 2005 (late) and UFV-18 (late), were similar in all three harvest times (Figures 2, 3 and 4). The lower imbibition speed and moisture content of the seeds in PEG 6000 solution demonstrates the effectiveness of this product in restraining water absorption, as reported for conditioned seeds of scarlet eggplant (Gomes et al., 2012); of wheat seeds in osmopriming solution (Jafar et al., 2012) and carrots in PEG 6000 solution at -1.2 MPa at 20 °C (Pereira et al., 2009).

The initial moisture content of soybean seeds varied from 11 to 13% (Figures 2, 3 and 4), with faster water absorption in the first 12 h, but with lower imbibition intensity in the PEG 6000 solution. The seed moisture content on paper soaked with distilled water was 54% after 12 h (Figures 2, 3 and 4), higher

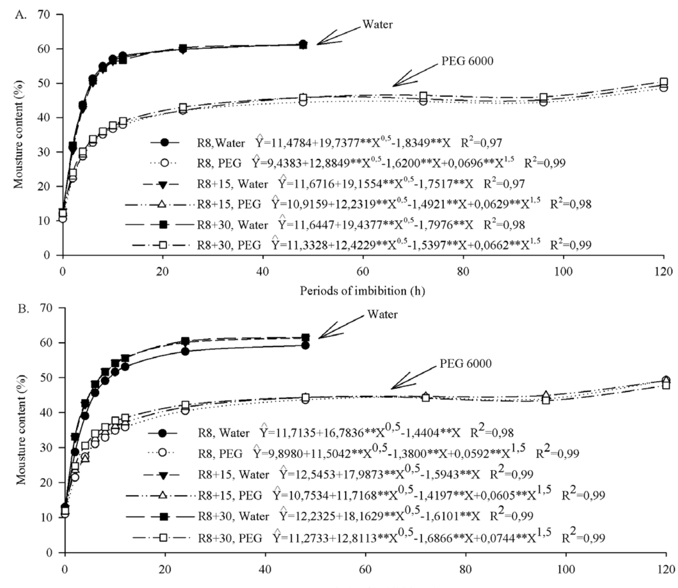

Figure 2. Moisture content (%) of soybean seeds, harvested at the R8, R8+15 days and R8+30 days stages, after different periods of imbibition in distilled water and in PEG 6000 solution. A) Confiança cultivar. B) Splendor cultivar

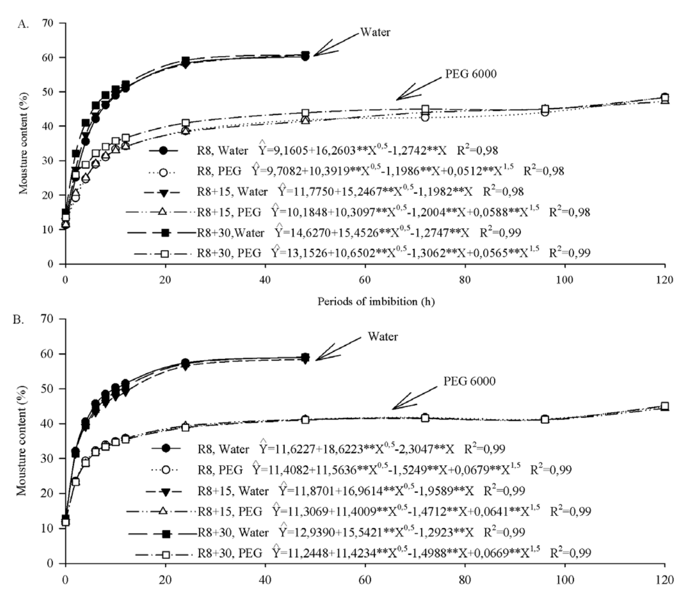

Figure 3. Moisture Content (%) of soybean seeds harvested at the R8, R8+15 days and R8+30 days stages after different periods of imbibition in distilled water and in PEG 6000 solution. A) UFV-16 cultivar. B) Garantia cultivar

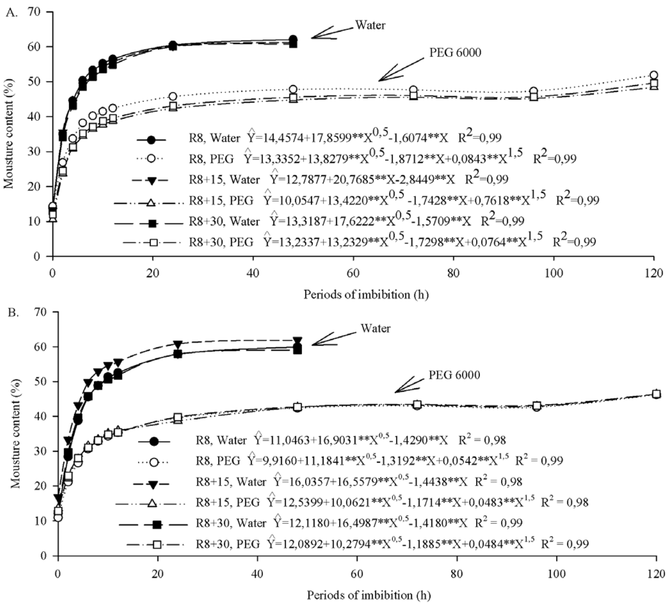

Figure 4. Moisture Content (%) of soybean seeds harvested at the R8, R8+15 days and R8+30 days stages after different periods of imbibition in distilled water and in PEG 6000 solution. A) UFV-18 cultivar. B) UFVS 2005 cultivar

than those in PEG 6000 solution, which, after achieving 37%, had an extension of the phase II of hydration. It occurs when the pre-germinative repair-mechanism of the macromolecules and other cellular structures takes place (Varier et al., 2010).

The fast imbibition in the germination process is called phase I. It is a consequence of the reduced matrix potential of dry seeds and can reach up to -100MPa, which explains their fast hydration even in osmotic solutions. The phase II is characterized by a dramatic reduction in the hydration speed and the respiratory intensity, which depend on the water potential of the substrate and the seed species. The primary root protrusion characterizes the phase III of imbibition (Bewley, 1997). The duration of priming should be taken into account, since the effects of priming can be altered by the duration of treatment, as already observed for carrot (Lopes et al., 2011) and scarlet eggplant (Gomes et al., 2012) whose conditioning reduced germination and seed performance under the conditions evaluated.

The moisture content of soybean seeds at the end of the osmotic treatment in PEG 6000 solution for 96 hours was, in average, 44% (Figures 2, 3 and 4). For osmotic conditioning, carrot seed imbibition is recommended in the PEG 6000 solutions at -1.0 and -1.2 PMa until moisture contents are obtained between 40% and 45% in moistened paper and 45 to 50% in aerated solution (Pereira et al., 2009).

The final seed moisture content in distilled water was 60% (Figures 2, 3 and 4), corresponding to the beginning of phase III, when 50% of the seeds showed root with about 1 mm. Sweet pepper seeds conditioned in water also had a progressive increase in the degree of moisture in the first 12 h of imbibition, reaching 55% (Posse et al., 2001). The phase III of seed imbibition in distilled water or in PEG 6000 solution was achieved after 48 and 100 h, respectively, as reported for soybean seeds of the UFV-10 (Uberaba), IAC-8, Doko RC and Savana cultivars (Del Giúdice et al., 1998).

The best adjustments of the regression curves, for the seeds imbibed in water and in the PEG 6000 solution, were achieved with quadratic and cubic root equations. The imbibition behaviour of soybean seeds is in accordance with the mentioned three phases pattern, characterized by a phase of fast water absorption (phase I), followed by a stationary phase (phase II), and finishing with the increase in the absorption rate, which coincides with the root protrusion and the seedling growth (phase III) (Bewley, 1997).

The speed of water uptake by seed tissues is decisive for the germination success, but fast imbibition may damage them, when in contact with pure water (Matthews & Powell, 1986). The damage caused by fast seed imbibition may occur due to the reduction in the integrity of the cell membranes, with loss of essential nutrients, increase of microorganism activity, leaking of solutes or low oxygen availability, leading to the anaerobic respiratory process (Armstrong & McDonald, 1992).

The cultivar, the harvest time and the osmotic conditioning affected the percentage of normal seedlings in the first and final countings of the germination test, and the speed of seedling emergence on sandbed (Table 1). No significant interactions were observed, therefore, the harvest time and the osmotic

Table 1. Analysis of variance of the first (FC) and final counting in the germination test (GT) and seedling emergence speed index on sand seedbed (ESI) of the seeds of six soybean cultivars harvested at three different times, osmoconditioned or not with PEG 6000. Viçosa, Minas Gerais, 2007[1/]

Source of variation	df	Mean square		
		FC	GT	ESI
Block	3	573.5977	319.3352	3.2894
Cultivar	5	2401.7133 **	2118.1836 **	5.9027 *
Error a	15	263.0066	193.6555	1.9907
Harvest time	2	5500.0329 **	4292.8633 **	47.9473 **
Cultivar x Harvest time	10	140.5652	102.6371	1.3089
Error b	36	87.2888	67.9875	0.6688
Conditioning	1	14723.1370 **	9605.0955 **	52.4117 **
Cultivar x Conditioning	5	146.2626	105.6115	1.1252
Harvest time x Conditioning	2	260.5189	100.2667	0.0697
Cultivar x Harvest time x Conditioning	10	24.8002	18.5528	0.4628
Error c	54	84.4255	58.7285	0.5783
Overall mean		66.65	68.76	7.071
CV (%)	Error a	24.33	20.24	19.97
	Error b	14.02	11.99	11.58
	Error c	13.79	11.15	8.81

[1/] Percentage values of germination were transformed to arcsine √x/100 for statistical analysis.
**,* F significant at 1 and 5% probability, respectively.

Table 2. Estimated means of the first (FC) and final counting (GT) in the germination test and seedling emergence speed index on sand seedbed (ESI) of the seeds of six soybean cultivars harvested at three different times, osmoconditioned or not with PEG 6000. Viçosa, Minas Gerais, 2007[1/]

Cultivar	Cond	Harvest time			Mean	Harvest time			Mean	Harvest time			Mean
		R8	R8+15	R8+30		R8	R8+15	R8+30		R8	R8+15	R8+30	
		FC (%)				GT (%)				ESI			
Confiança	No	98	96	88	95	98	97	92	96	7.440	7.060	6.420	6.973
	Yes	99	100	95	98	99	100	95	98	8.470	8.030	7.280	7.927
	Mean	99	98	92	97 a	99	98	94	97 a	7.955	7.545	6.850	7.450 a
UFV-16	No	87	88	76	84	91	91	83	88	7.180	6.960	5.980	6.707
	Yes	97	98	95	97	97	98	95	97	8.988	8.260	6.730	7.993
	Mean	93	94	87	91 abc	95	95	90	93 ab	8.084	7.610	6.355	7.350 ab
Splendor	No	94	94	80	90	95	96	86	93	7.270	7.160	5.680	6.703
	Yes	98	99	95	98	98	99	95	98	8.160	8.220	6.596	7.659
	Mean	97	97	88	95 ab	97	98	91	96 ab	7.715	7.690	6.138	7.181 ab
Garantia	No	86	75	57	74	88	80	64	78	7.250	6.320	5.340	6.303
	Yes	96	90	78	89	96	91	81	90	7.776	7.110	5.410	6.765
	Mean	92	84	68	82 c	93	86	73	85 c	7.513	6.715	5.375	6.534 b
UFVS 2005	No	94	90	79	88	95	93	84	91	6.800	6.690	6.080	6.523
	Yes	98	97	95	97	98	98	95	98	7.400	7.290	6.930	7.207
	Mean	97	94	88	93 ab	97	96	90	95 ab	7.100	6.990	6.505	6.865 ab
UFV-18	No	87	84	70	81	89	87	74	84	7.110	7.010	5.730	6.617
	Yes	97	95	91	95	97	95	91	95	7.820	7.600	7.030	7.483
	Mean	93	90	82	89 bc	94	92	84	90 bc	7.465	7.305	6.380	7.050 ab
Overall Mean	No	92	89	76	86 b	93	91	81	89 b	7.175	6.867	5.872	6.638 b
	Yes	98	97	92	96 a	98	98	93	96 a	8.102	7.752	6.663	7.506 a
	Mean	95 A	94 A	85 B	92	96 A	95 A	88 B	93	7.639 A	7.309 B	6.267 C	7.071

[1/] Means of cultivars followed by the same lower case letter in the column or by the same capital letter in the horizontal line, do not differ by the Tukey´s test at 5% probability. Percentage data were transformed to arcsine √x/100 for analysis and, later, the averages were de-transformed for presentation. Coefficients of variation: Error a (FC, GT and ESI) = 24.33, 20.24 and 19.97%; Error b (FC, GT and ESI) = 14.02, 11.99 and 11.58%; Error c (FC, GT and ESI) = 13.79, 11.15 and 8.81%.

conditioning additively affected the germination and vigor of the seeds of all soybean cultivars.

Higher germination averages (Table 2) were observed for the seeds of Confiança (97%), Splendor (96%), UFVS 2005 (95%) and UFV-16 (93%) cultivars; and lower for the seeds of Garantia (85%) and UFV-18 (90%) cultivars. Correspondingly, the same rank was observed in the first counting results, seedling emergence speed index, it was observed higher speed for Confiança (7.450) and lower for Garantia (6.534) seeds. The germination was higher at the R8 and R8+15 harvest times than at the R8+30, which is in accordance with the decrease of seed germination with the harvest delay (Sediyama et al., 1972). The maximal longevity potential of the soybean seeds is attained close to the full maturity stage after which the seed humidity content naturally declines to 14-15%. For best quality of soybean seeds, it is recommended to harvest between 12 and 15% water (Embrapa Soja, 2011).

Conditioning the seeds with PEG 6000 improved the germination (96%) when compared to nonconditioned seeds (89%) (Table 2). The same results were obtained for seed vigor, evaluated by the first count in the germination test (96 for conditioned seeds against 86% for nonconditioned) and the seedling emergence speed index on sand seedbed (7.506 against 6.638). Priming in PEG 6000 for four days increased the percentage and speed of germination and seedling emergence of carrot (Pereira et al., 2009). Aymen et al. (2012) also found beneficial effects of priming when evaluating the growth and yield of safflower under saline condition. However, the greatest positive effect of the conditioning on the germination was observed at the R8+30 harvest, which demonstrates the efficacy of the treatment with PEG 6000, as reported for soybean seeds of the cultivars UFV-10 (Uberaba), IAC-8, Doko RC and Savana (Del Giúdice et al., 1998).

Comparing the embibition profiles of the cultivars (Figures 2, 3 and 4) and the physiological quality of their seeds (Table 2), there is no evidence of relationship between imbibition profile in water or in PEG 6000 and the different seed quality of the six cultivars.

Conclusions

Higher quality seeds were produced by Confiança, Splendor, UFVS-2005 and UFV-16 soybean cultivars, while Garantia and UFV-18 cultivars yielded seeds of lower quality.

The imbibition speed and the humidity of the osmoconditioned seeds of all six cultivars and three harvest times were lower than of those seeds soaked in water.

The germination and vigor of osmoconditioned seeds were higher for all cultivars at all harvest times, mainly with 30 days harvest delay, indicating the conditioning efficacy to increase the germination of weathered seeds.

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