HYDRATION OF CARROT SEEDS IN RELATION TO OSMOTIC POTENTIAL OF SOLUTION AND CONDITIONING METHOD[1]

MÁRCIO DIAS PEREIRA[2], DENISE CUNHA FERNANDES DOS SANTOS DIAS[3], LUIZ ANTONIO DOS SANTOS DIAS[3], EDUARDO FONTES ARAÚJO[3]

HIDRATAÇÃO DE SEMENTES DE CENOURA EM FUNÇÃO DO POTENCIAL HÍDRICO DA SOLUÇÃO E DO MÉTODO DE CONDICIONAMENTO

ABSTRACT – The objective of this study was to monitor carrot seed hydration in water and osmotic solutions to define appropriate conditions for priming treatment. Two Brasília cultivar carrot seed lots were used. Seeds were imbibed in -1.0 and -1.2 MPa PEG 6000 osmotic solutions and in distilled water, in an incubator BOD at 20ºC, using two different hydration methods: imbibition in moistened paper towel sheets and in aerated solutions. The imbibition curves for each seed lot were drawn after determining seed moisture content at 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 hours hydration in water and after 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264, 312 hours hydration in PEG 6000 solutions. Seed hydration in distilled water was faster than in PEG 6000 solutions; the primary root protrusion occurred at 48 hours imbibition as seeds reached 54% moisture content. Osmotic conditioning of carrot seeds should be performed by imbibition in PEG 6000 -1.0 or -1.2 MPa solutions to attain 40-45% moisture content (moistened paper) or 40-45% (aerated solutions).

Index terms: Carrot, priming, imbibition, *Daucus carota* L.

RESUMO – Este trabalho teve como objetivo monitorar a hidratação de sementes de cenoura em água e em soluções osmóticas, de modo a definir condições adequadas para o condicionamento osmótico. Dois lotes de sementes de cenoura dacultivar Brasília, embebidos em solução osmótica de PEG 6000 a -1,0 e -1,2MPa e em água destilada, em incubadora BOD a 20ºC. Utilizaram-se dois métodos de embebição: em papel toalha umedecido e em soluções aeradas, realizada em frascos contendo as respectivas soluções, acoplados a uma bomba de ar. Para a obtenção das curvas de embebição, determinou-se o teor de água das sementes após 2, 4, 6, 8, 10, 12, 24, 48, 72 e 96 horas em contato com água e após 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264 e 312 horas em soluções de PEG 6000. Os dados foram submetidos à análise de variância e de regressão. Sementes embebidas em água absorveram água mais rapidamente do que as embebidas em PEG 6000, iniciando a protrusão da raiz primária após 48h de embebição, com teor de água de 54%. Para o condicionamento osmótico, recomenda-se a embebição das sementes de cenoura em soluções de PEG 6000 a -1,0 e -1,2 MPa até serem obtidos teores de água entre 40 e 45%, em papel umedecido, e entre 45 e 50%, em solução aerada.

Termos para indexação: cenoura, condicionamento osmótico, embebição, *Daucus carota* L.

[1] Submetido em: 19/04/2007. Aceito para publicação em: 29/06/2007. Parte da Dissertação de Mestrado do primeiro autor apresentada ao Programa de Pós-graduação em Fitotecnia/Produção vegetal, UFV. Órgão financiador: Capes

[2] Eng. Agrônomo, doutorando do Programa de Pós-graduação em Fitotecnia/Produção vegetal da Universidade Federal de Viçosa, marcioagron@yahoo.com.br;

[3] Prof. do Departamento de Fitotecnia da Universidade Federal de Viçosa – UFV, 36570-000 – Viçosa – MG.

INTRODUCTION

In short cycle crops, such as vegetables, fast and uniform establishment of seedlings in the field is fundamental to obtain adequate stand, with possible reflections on product yield and quality. Thus the use of high quality seeds is an important factor within the production process.

In the field, seeds are normally exposed to unfavorable soil and climate conditions that can damage emergence speed and percentage, and this fact is more expressive when direct sowing is adopted, as in carrot cropping. In this case the use of priming treatments is interesting, such as osmotic conditioning or priming to improve seed performance in the field (Frett et al., 1991; Pill et al., 1991) and tolerance to adverse conditions, such as water stress and unsuitable temperatures (Bradford, 1986; Khan, 1992; Parera and Cantliffe, 1994, Bittencourt et al., 2004). In carrot, many authors have also reported the positive effects of osmotic conditioning (Cantliffe and Elballa, 1994; Copeland and McDonald, 1995 and Balbinot and Lopes, 2006).

This treatment consists of controlled hydration of the seeds in water or in solution with known osmotic potential, to activate the germination preparation processes without permitting the protrusion of the primary root (Heydecker et al., 1975; Pill, 1995). The osmotic solution can be obtained by using salts (Taylor, 1997) or substances with high molecular weight that are chemically inert, such as glycol polyethylene – PEG 6000 or PEG 8000 (Heydecker and Coolbear, 1977). This is the most used osmotic agent, although it has the disadvantage of needing an artificial aeration system on most occasions because oxygen solubility is inversely proportional to the PEG concentration. The low oxygen level induces anaerobiosis that favors ethanol production which is toxic to the seeds (Brocklehurst and Dearman, 1984).

Imbibition is the first step of a sequence of events that culminates with the primary root emission. Water absorption by the seed ocurr according to the triphasic pattern reported by Bewley and Black (1994). In phase I, a fast transference of water from the substrate to the seeds is observed, which is the consequence of the matrix forces, followed by phase II, where water absorption by the seeds is established. The start of phase III is characterized by primary root protrusion and new expressive increase in the seed moisture content; only viable seeds reach this phase (Bewley and Black, 1994).

Primary root emission (phase III) indicates the moment when the seeds lose the tolerance to dehydration, because as they imbibe water this tolerance decreases (Bewley and Black, 1994; Leprince et al., 2000). Thus seeds hydrated up to imbibition phase II will not lose viability if they dehydrate, so that germination will continue when the seed is rehydrated (Senaratna and McKersie, 1983; Koster and Leopold, 1988). However, drying the seed after primary root protrusion usually results in viability loss (Taylor 1997). In the osmotic conditioning technique, the seeds are kept hydrated for a determined time period that varies with factors that include the species, imbibition temperature and osmotic solution concentration (Bradford, 1986; Nascimento, 2004). If the interruption of the water supply to the seeds is premature, the metabolism activation may be insufficient to promote the expected benefits; when late, it may contribute to intensifying the possibility of reversing the conditioning effects (Marcos Filho, 2005).

To define the most suitable conditions for seed conditioning, it is necessary to understand the pattern of imbibition of these seeds and the influence of the main factors involved in this process until the start of the primary root emission and especially the best combination of osmotic potential and agent, temperature, imbibition period and conditioning method. The treatment duration should be defined based on the imbibition curve of the seed lot, as already demonstrated by Bittencourt et al. (2004) and Caseiro (2003). The imbibition period necessary for primary root emission varies according to the species, cultivar and physiological potential of a lot, and is longer for lots with lower physiological quality (Caseiro, 2003). Thus the objective of this study was to determine the imbibition pattern of carrot seed in water and osmotic solutions, with and without aeration system, to define suitable conditions for the osmotic conditioning of the seeds.

MATERIAL AND METHODS

The study was carried out in the Seed Research Laboratory in the Crop Science Departments at the Federal University of Viçosa, from January to December 2006. Two commercial lots of Brasilia cultivar carrot seeds (*Daucus carota*) were used, with initial germination of 70% (lot 1) and 76% (lot 2) and initial water content of 12% (lot 1) and

10% (lot 2). The seeds from each lot were placed in plastic bags and stored in a refrigerator at approximately 10°C throughout the experimental phase.

The seeds from each lot were imbibed in an osmotic solution of glycol polyethylene 6000 (PEG 6000), at the osmotic potentials of 0.0 (distilled water), -1.0 and -1.2 PMa, determined according to Vilella et al. (1991). Addition was made to each solution of 0.15% active ingredient of the commercial product Captan 750 TS 24. The following conditioning methods were used:

- Imbibition in moistened paper: in gerbox boxes, 4.0g seed from each lot, divided into two 2.0g subsamples, were distributed on the two sheets of paper towel moistened with 4.5 ml distilled water and each PEG solution, and this volume was sufficient to cover half the thickness of the seeds, and part of their surface was exposed to the atmosphere of the inside of the boxes. The boxes were closed, wrapped in transparent plastic bags to prevent loss by evaporation, and kept in a BOD incubator at 20ºC.

- Immersion in aerated solution: a system was used (Figure 1) developed to condition seeds in solutions with external aeration, produced by an aquarium pump. In this method, 4.0g seed were placed in 250.0 mL Erlenmeyers containing 40.0mL each conditioning solution, at the ratio of 1:10 (seeds: solution). The Erlenmeyers were closed with a rubber cork and coupled to a pump (aquarium pump) to aerate the solutions. The system was kept in a BOD at 20°C.

In both the conditioning methods, the seed water content was determined after 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hours of imbibition in distilled water (0.0MPa) and after 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264 and 2 hours of imbibition in PEG 6000 solutions at -1.0 and -1.2MPa. For this, seed subsamples were removed from each one of the two subsamples from each treatment that were placed on paper towel for superficial drying. Next, the water content was determined in a oven at 105º ± 3ºC for 24 hours and the results were expressed in percentage (moisture base), as prescribed in the Rules for Seed Analysis (Brasil 1992).

The experiment was carried out in a complete randomized block design, with four replications. The data regarding the water content obtained after each imbibition period, for each lot and treatment, was submitted to analyses of variance and regression, and the imbibition curves were obtained of the seeds in distilled water (0.0 PMa) and PEG solution at the potentials of -1.0 and - 1.2 PMa. The curves were drawn using the regression equations calculated by the Excel program and fitted on the Sigma plot.

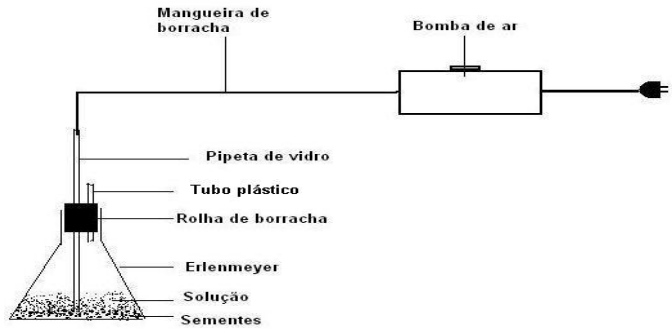

FIGURE 1. System used to condition seeds by the aerated solution method.

RESULTS AND DISCUSSION

Figures 2, 3, 4 and 5 show that generally the carrot seed imbibition curves were similar for the two lots, in both the imbibition treatments using moistened paper towel and in aerated solutions. Faster water absorption by the seeds was observed in the first 12 hours and there was greater imbibition speed in water compared to that in PEG solutions. Thus, after 12 hours of imbibition, the seeds imbibition in moistened paper with distilled water (Figures 2 and 3), whose initial water content was 12% (lot 1)and 10% (lot 2), attained about 50% water, a value greater than that observed in the seeds imbibed in PEG solutions (about 40%). Sweet pepper seeds conditioned in water also had a progressive increase in the degree of moisture in the first 12 hours of imbibition, when they reached 55% water (Posse et al., 2001). There was also fast water absorption for the seeds imbibed in aerated solutions (Figures 4 and5), mainly in the first six hours, when they reached 48% water, reaching 50% (lot 1) and 52% (lot 2) water with 12 hours imbibition. This rapid imbibition characterizes phase I of

the seed germination process, according to Bewley and Black (1994) and was a consequence of the reduced matrix potential verified in dry seeds, that can reach values of up to -100 PMa, that justifies seed hydration even in osmotic solutions.

After 12 hours (Figures 1 to 4) there was no expressive increase in the imbibition rates in water, either in moistened paper or the aeration system and the water content of the seeds stabilized at around 52 and 57%, after 48 hours, in the respective imbibition systems. The protrusion of the primary root was verified after 48 hours imbibition in distilled water, when the water content of the seeds was about 52% that characterized phase III of the imbibition process reported by Bewley and Black (1994). Therefore, in this case, imbibition phase II was short, because after 48 hours imbibition in water there was primary root emission. Water absorption by sweet pepper seeds was slow after 12 hours imbibition, indicating the start of phase II that lasted about 72 hours, when the primary root emission process started (Posse et al., 2001). Lopes et al. (1996) observed the start of primary root protrusion in onion seeds with water content around 44 to 46%, after 132 hours imbibition in distilled water and PEG 6000 and -0.75 PMa, indicating that the ideal phase for osmotic conditioning of these seeds was between two and four days. Usberti and Valio (1997) carried out a similar study and observed that *Panicum maximum* L. seed attained values close to 50% water after 48 hours imbibition in distilled water, while in PEG 6000 solution (288g/L) they attained 37.7 and 43.6% in the same phase, at 15 and 25°C, respectively. More recently, Caseiro et al. (2004) detected smaller water contents for onion seed osmatically conditioned in PEG solutions compared to those submitted to water conditioning techniques and "drum priming" (gradual hydration in a rotating drum). According to the authors this result was expected because the low water potential of the PEG 8000 solution (-0.5 and -1.0 MPa) reduced water absorption by the seeds.

In the seed imbibed in PEG solution, after 12 hours imbibition, stabilized water absorption was observed that was maintained up to 312 hours imbibition, and the primary root emission did not occur (Figures 2 to 5), clearly characterizing the continuity of phase II imbibition. In this phase, according to Bewley and Black (1994) water absorption stabilizes because the cells are turgid and are the wall potential is more active and it is a lag phase. Thus, starting at 24 hours imbibition in the PEG solutions, the water content of the seeds remained close to 40 and 45% in the imbibition in moistened paper and between 45 and 50% when imbibed in aerated solutions. The permanence of the seeds in phase II imbibition, without primary root protrusion, is desirable for osmotic conditioning, because it allows a suitable tissue hydration level to be maintained for a determined period of time, while the pre- germination metabolism is reactivated.

Regarding the imbibition methods, it was verified generally that the use of aerated solution favored water absorption by the seeds that was demonstrated by the higher water contents attained by the seeds in the aerated system compared to imbibition in moistened paper (Figures 2 to 5). Lopes et al. (2000) observed that in the system of immersion in aerated solutions these imbibition rates were four times greater than those attained by the moistened paper method, probably due to the greater contact surface of the carrot seeds with the PEG solutions

It was further observed in the aerated system that the water contents attained by the seeds imbibed in the most concentrated solution (-1.2 PMa) were relatively lower than those observed in solution at -1.0 PMa. However, both solutions were efficient in maintaining the seeds in phase II imbibition, reported by Bewley and Black (1994), even after a contact period of 312 hours, and did not permit the advance of the germination process and primary root emission, that is, that the seeds reached phase III. Carrot seeds conditioned in osmatic solutions of PEG 6000 at -0.6 PMa with and without aeration, emitted the seed primary root after six days treatment; therefore, this osmotic potential was not sufficient to prevent the seeds reaching phase III germination (Lopes et al., 2000). Bittencourt et al. (2004) worked with asparagus seeds imbibed in PEG 6000 solutions and -1.0 and -1.2 PMa and verified that there was no primary root emission during 28 days conditioning and the seeds remained in phase II imbibition. Machado Neto et al. (2006) worked with bean seeds and detected that substances such as manitol, calcium chloride and other salts can limit water entry in the seeds, simulating a water shortage that prevented them from carrying out the metabolic activities necessary for complete germination.

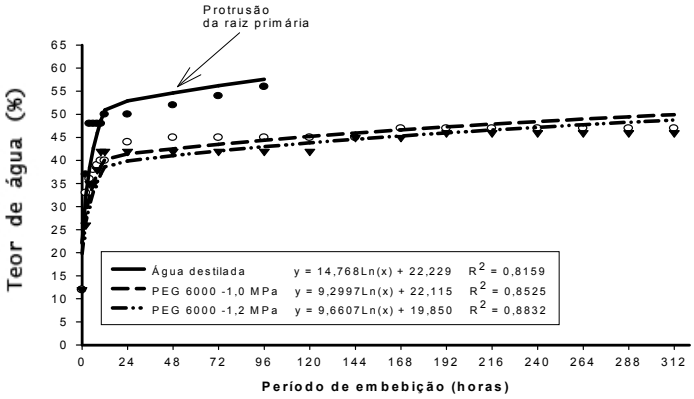

FIGURE 2. Water content of carrot seeds lot 1 after different imbibition periods in moistened paper with distilled water and PEG 6000 solutions at -1.0 and -1.2 PMa.

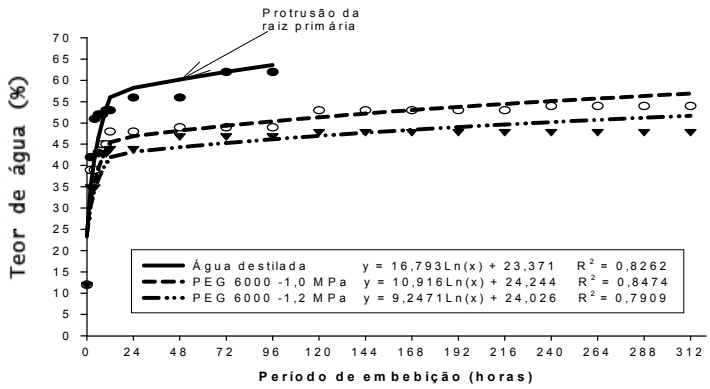

FIGURE 4. Water contents of carrot seeds lot 1 after different immersion periods in distilled water and PEG 6000 solutions at -1.0 and -1.2 PMa, with aeration system.

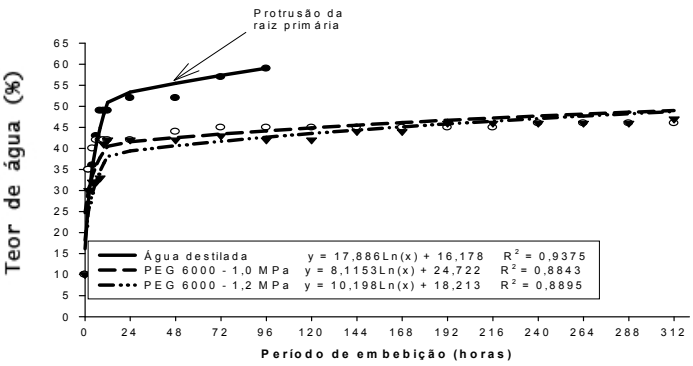

FIGURE 3. Water content of carrot seeds lot 2 after different imbibition periods in moistened paper with distilled water and PEG 6000 solutions at -1.0 and -1.2 PMa.

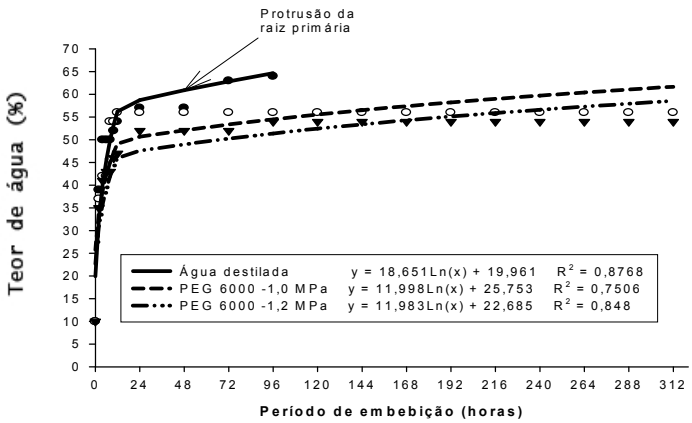

FIGURE 5. Water contents of carrot seeds lot 1 after different immersion periods in distilled water and PEG 6000 solutions at -1.0 and -1.2 PMa, with aeration system.

The results obtained in the present study showed that when distilled water was used there was rapid seed imbibition and primary root protrusion after 48 hours imbibition, when the seeds reached approximately 57% water, that is desirable when it is intended to use the physiological conditioning technique, because the seeds become intolerant to drying after primary root protrusion. Cauliflower seeds emitted primary roots when they presented between 39% and 43% water, after 36 hours imbibition (Kikuti, 2006). On the other hand, with the use of PEG 6000 and -1.0 and -1.2 PMa it is possible to adopt conditioning periods of up to 312 hours (13 days) maintaining the seed with water contents of approximately 48% (imbibition in moistened paper) and 54% (aerated solution) without primary root protrusion of the carrot seeds. Thus both the PEG concentrations were efficient in maintaining the seeds, both in lot 1 and lot 2, in the phase II imbibition, reported by Bewley and Black (1994) for a long period of time, permitting the activation of preparatory metabolism events essential for primary root emergence.

Consequently, determining the imbibition curve of the seeds in a lot to be conditioned is fundamental for success with the technique, because the imbibition rate varies with the initial seed quality, among other factors. Vigorous seeds tend to be able to absorb water quickly and re-start the germination metabolism for germination, emitting the primary root in a shorter period of time than lower quality seeds. For these, the imbibition speed is generally slower, indicating that the conditioning period should be longer for the same osmotic potential and temperature. According to Caseiro (2003), the imbibition period necessary for primary root emission varies with the physiological potential of the lot, and is longer for less vigorous lots. In the present study, the seeds of both the lots, whose initial quality was similar (70% and 76% germination) reached, in phase II, similar water contents, that is between 40% and 45% (imbibition in moistened paper) and between 45% and 50% (aerated solution). Thus the definition of water content is fundamental to mark the end of the seed conditioning period.

CONCLUSION

For osmotic conditioning, carrot seed imbibition is recommended in the PEG 6000 solutions at -1.0 and -1.2 PMa until water contents are obtained between 40 and 45% in moistened paper and 45% to 50% in aerated solution.

REFERENCES

BALBINOT, E.; LOPES, H.M. Efeitos do condicionamento fisiológico e da secagem na germinação e no vigor de sementes de cenoura. **Revista Brasileira de Sementes**, Pelotas, v.28, n.1, p.01-08, 2006.

BEWLEY, J.D.; BLACK, M. **Seeds:** physiology of development and germination**.** 2.ed. New York: Plenum Press, 1994. 445p.

BITTENCOURT, M.L.C.; DIAS, D.C.F.S.; ARAÚJO, E.F.; DIAS L.A.S. Controle da hidratação para o condicionamento osmótico de sementes de aspargo. **Revista Brasileira de Sementes**, Pelotas, v.26, n.2, p.99-104, 2004.

BRADFORD, K.J. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. **HortScience**, Alexandria, v.21, n.5, p.1105-1112, 1986.

BRASIL. Ministério da Agricultura e Reforma Agrária. **Regras para análise de sementes**. Brasília: SNDA/DNDV/CLAV, 1992. 365p.

BROCKLEHURST, P.A.; DEARMAN, J. A comparison of different chemicals for osmotic treatment of vegetable seed. **Annals of Applied Biology**, Warwick, v.105, p.391-398, 1984.

CANTLIFFE, D.J.; ELBALLA, M. Improved germination of carrot at stressful high temperature by seed priming. **Proceedings of the Florida State Horticultural Society**, Lake Alfred, v.107, p.121-128, 1994.

CASEIRO, R.F. **Métodos para condicionamento fisiológico de sementes de cebola e influência da secagem e armazenamento**. 2003. 109f. Tese (Doutorado em Fitotecnia) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2003.

CASEIRO, R.F.; BENNETT, M.A.; MARCOS-FILHO, J. Comparison of three priming techniques for onion seed lots differing in initial seed quality. **Seed Science & Technology**, Zurich, v.32, p.365-375, 2004.

COPELAND, L.O.; McDONALD, M.B. **Principles of seed science and technology**. 3.ed. New York: Chapman & Hall, 1995. 409p.

FRETT, J.J.; PILL, W.G.; MORNEAU, D.C. A comparison of priming agents for tomato and asparagus seeds. **HortScience**, Alexandria, v.26, n.9, p.1158- 1159, 1991.

HEYDECKER, W.; HIGGIS, J.; TURNER, Y.J. Invigoration of seeds. **Seed Science & Technology**, Zürich, v.3, p.881-888, 1975.

HEYDECKER, W.; COOLBEAR, P. Seed treatments for improved performancesurvey and attempted prognosis. **Seed Science and Technology**, Zürich, v.5, n.2, p.353-425, 1977.

KHAN, A.A. Preplant physiological seed conditioning. **Horticultural Reviews**, New York, v.13, p.131-181, 1992.

KIKUTI, A.L.P. **Avaliação do potencial fisiológico, métodos de condicionamento e desempenho de sementes de couve-flor (Brassica oleracea L. var. botrytis) durante o armazenamento e em campo**. 2006. 155f. Tese (Doutorado em Fitotecnia) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2006.

KOSTER, K.L.; LEOPOLD, A.C. Sugars and desiccation tolerance in seeds. **Plant Physiology,** Lancaster, v.88, p.829-832, 1988.

LEPRINCE, O.; HARREN, F.J.M.; BUITINK, J.; ALBERDA, M.; HOEKSTRA; F.A. Metabolic dysfunction and unabated respiration precede the loss of membrane integrity during dehydration of germinating radicles. **Plant Physiology**, Lancaster, v.122, p.597-608, 2000.

LOPES, H.M.; MARIA, J.; SILVA, R.F. & MALAVASI, M.M. Influência do potencial osmótico e da temperatura na embebição e no crescimento da radícula de sementes de cebola (*Allium cepa* L.). **Revista Brasileira de Sementes**, Brasília, v.18, n.2, p.167-172, 1996.

LOPES, H.M.; ROSSETTO, C.A.V.; CARNEIRO, V. Embebição de sementes de cenoura (*Daucus carota* L.) em diferentes potenciais osmóticos por dois métodos. **Revista Brasileira de Sementes**, Londrina, v.22, n.1, p.81-87, 2000.

MACHADO NETO, N.B.; CUSTÓDIO, C.C.; COSTA, P.R.; DONÁ, F.L. Deficiência hídrica induzida por diferentes agentes osmóticos na germinação e vigor de sementes de feijão. **Revista Brasileira de Sementes**, Pelotas, v.28, n.1, p.142-148, 2006.

MARCOS FILHO, J. **Fisiologia de sementes de plantas cultivadas**. FEALQ: Piracicaba, 2005. 495p.

NASCIMENTO, W.M. **Condicionamento osmótico de sementes de hortaliças.** Brasília: EMBRAPA. Circular Técnica, n. 33, 2004. 12p.

PARERA, C.A.; CANTLIFFE, D.J. Presowing seed priming. **Horticultural Reviews**, New York, v.16, n.1, p.109-141, 1994.

PILL, W.G.; FRETT, J.J.; MORNEAU, D.C. Germination and seedling emergence of primed tomato and asparagus seeds under adverse conditions. **HortScience**, Alexandria, v.26, n.9, p.1160-1162, 1991.

PILL, W. G. Low Water potential and prossowing germination treatments to improve see quality. In: BARSRA, A. S. (ed). **Seed quality-basic mechanisms and agricultural implications.** Food Products Press, New York, 1995, p.319-359,

POSSE, S. C. P.; SILVA, R. F.; VIEIRA, H. D.; CATUNDA, P. H. A. Efeitos do condicionamento osmótico e da hidratação na germinação de sementes de pimentão (*Capsicum annum* L.) submetidas a baixa temperaturas. **Revista Brasileira de Sementes**, Londrina, v.23, n.1, p.123-127, 2001.

SENARATNA, T.; McKERSIE, B.D. Dehydration injury in germinating soybean (*Glycine max* L. Merr.) seeds. **Plant Physiology**, Lancaster, v.72, p.620-624, 1983.

TAYLOR, A.C. Seed storage, germination and quality. In: WIEN, H.C. (Ed). **The physiological of vegetable crops**. New York, 1997, p.1-36.

USBERTI, R.; VALIO, F.M. Osmoconditioning effects on germination of guinea gran (*Panicum maximum*) seeds. **Seed Science and Technology**, Zürich, v.25, n.2, p.303-310, 1997.

VILLELA, F.A., DONI FILHO, L.; SEQUEIRA, E.L. Tabela de potencial osmótico em função da concentração de polietileno glicol 6000 e da temperatura. **Pesquisa Agropecuária Brasileira**, Brasília, v.26, n.11/12, p.1957- 1968, 1991.

★☆★☆