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Effect of Temperature and Water Restriction On *Piper aduncum* L. Seed Germination

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Abstract: This study aimed to evaluate *Piper aduncum* L. seed germination restricted of water, simulated by solutions prepared with polyethylene glycol (PEG 6000) and thermal action influence, using germination chambers with temperatures controlled from 24 to 30°C. The experimental design was carried out in a 3×5 factorial scheme which counted three temperatures (24, 27 and 30°C), combined with five osmotic potential levels (0.0, -0.1, -0.2, -0.3 and -0.4 MPa). The analyzed variables were germination percentage and mean germination time. The results revealed that as the water restriction rose there was a decrease in the germination percentage and an increase in the mean germination time, demonstrating that seeds of this species are extremely sensitive to water shortage. The temperature increase from 24 to 30°C, accelerated germination.

Key words: *Piper aduncum* L., germination, temperature, water restriction

INTRODUCTION

The Amazon interests many researchers from different scientific areas since it is the largest tropical forest in the world, possessing flora with elevated economic potential. In this environment, many existenting native species are being commercially explored and others are being investigated for cultivation so their beneficiary processes can be completely explained to become an alternative of rational and biologically correct exploring. *Piper aduncum* is an aromatic plant from the Piperaceae family, native to the Amazon Region, with a high concentration of essential oil (2.5 to 4.0%). The oil extracted from the aerial part (leaves and branches) possesses dillapiol as the major active principal (Maia *et al*., 1998), a phenyllic ether that has been tested successfully as a fungicide, molluscicide, acaricide, bactericide and larvicide (Okunade *et al*., 1997; Morandim *et al*., 2005). This species adapts to the edafoclimatic conditions found in the Amazon Region and can represent a possibility of use in short term, as a biodegradable agent for industries in Brazil and throughout the world which currently use synthetic compounds as prime material for fabricating fungicides, molluscicides, acaricides, bactericides and larvicides.

The *P. aduncum* seed is extremely small, less than 1 mm in size, presenting water percentage from 10 to 30%, being vulnerable to dehydration, as well as being considered a recalcitrant (Lobato *et al*., 2005).

Germination is a biological process that requires, as pre-requisite, seed viability with structures and physiological pathways capable of activating its metabolism as well as specific abiotic conditions where water and temperature are some of the factors that have direct influence (Carvalho and Nakagawa, 2000).

Water availability is necessary for the seed imbibition process, since from absorption there will be reserved polysaccharide degradation, which is located in the seed endosperm in the majority of the plant species, producing ATP for the embryo to tear the tegument, develop and through cellular differentiation, form rootlets (Copeland and McDonald, 1995; Bray, 1995).

Temperature provokes variable effects that vary among the seeds of different species where the ideal average germination temperature is a genetic characteristic depending on seed morphology and physiology. This factor also acts directly on water absorption velocity and biochemical relations that occur during germination (Ferreira and Borghetti, 2004).

This study aimed to evaluate the germinative answers provoked by water restriction, simulated by mediums with prepared polyethylene glycol 6000 solutions and by temperature, imposed on the seeds in control chambers.

MATERIALS AND METHODS

The *P. aduncum* seeds were collected in the experimental station of Tauá, municipal of Santo Antônio

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do Tauá, Pará State, Brazil, during March, 2006. The experiment was carried out at the Laboratory of Plant Physiology at the Instituto de Ciências Agrárias of the Universidade Federal Rural da Amazônia, in the Municipal Belém, Pará State, Brazil.

Preparation consisted of seed obtainment, fermentation and washing, followed by immersion in a solution of tetramethyl tiuram disulphide ($C_6 H_{12} H_2 S_4$) 0.1% for 30 sec, then placed on filter paper for drying and finally conditioned in an aluminum flask containing silica until continuing with the experiment.

The experimental design was in a 3x5 factorial scheme, with temperature and osmotic potential being the analyzed factors, counting on 3 temperature levels (24, 27 and 30°C), combined with 5 osmotic potential levels that simulate water restriction (0.0, -0.1, -0.2, -0.3 and -0.4 MPa). The solutions were obtained with Polyethylene glycol 6000 (Sigma Chemicals) according to the method by Michel and Kaufmann (1973) and described by Villela *et al.* (1991). For the 0.0 potential (control), only distilled water autoclaved at 120°C, 1 atm, for 20 min was added. The experiment was composed of 15 treatments and 5 repetitions with each parcel was composed of 100 seeds.

The seeds were placed in clear plastic boxes with the following dimensions (C x W x L; 11 x 11 x 4 cm), which were previously lined with sterile sheets of filter paper, dampened with the solutions to be evaluated until reaching 2.5 times the dry paper weight. The recipients hermetically sealed and containing the seeds were put into germination chambers with controlled temperatures, relative humidity at 70% and photoperiod of 12 h by white fluorescent light at 25 μ mol m^{-2} sec^{-1} irradiance.

The readings were carried out on the 13th and 23rd day after the implementation of the experiment taking away those seeds with rootlets with a length equal or longer than 1.0 mm. The variables observed were the germination percentage and the mean germination time, according to Edmond and Drapala (1957) and described by Silva and Nakagawa (1995). The results were submitted to variance analysis and the averages of the treatments were compared following Tukey at the 5% significance level using SAS (SAS Institute, 1996) and based on statistical theories by Gomes (2000).

RESULTS AND DISCUSSION

Germination: The results submitted to variance analysis reveal that germination was affected by two factors; the osmotic potential and temperature, where a significant interaction between them was observed (Table 1). The experiment demonstrated that under normal water conditions (control), the seeds presented high germination percentages. In the control treatment (0.0 MPa), it was observed that under 24, 27 and 30°C, the germination percentages 81, 99 and 97% were achieved respectively, without a statistical difference between the treatments under 27 and 30°C, besides proving with this *P. aduncum* seed assay that these temperatures can be considered as ideal for this species (Bewley and Black, 1994). At this temperature the hydrolytic enzymes involved in the germinative process and degradation of stored seed polysaccharides were possibly influenced by the temperature, since it was seen that in extreme, sub-ideal and supra-ideal temperatures these enzymes are deactivated or denaturized being essential for germination (Taiz and Zeiger, 1998). The water restrictions imposed on the experiments reveal that independent to temperatures, the studied osmotic potentials were capable of significantly reducing seed germination. Among the evaluated temperatures, 27°C was the only one that made germination under -0.2 MPa potential possible, besides this, at this temperature and under the potentials of 0.0, -0.1, -0.2, -0.3 and -0.4 MPa, germination percentages of 99, 47, 3, 0 and 0%, respectively were observed, showing an accented fall in germination percentage showing that seeds of this species are extremely sensitive to water restriction.

According to Mayer and Poljakoff-Mayber (1989) as the water availability decreases, a reduction of the hydraulic conductance, intensity and in some cases, water flow direction between the environment and seed occurs, where consequently the imbibition process is reduced

Table 1: *Piper aduncum* L. seed germination under different temperatures and osmotic potentials

Osmotic potentials (MPa)	Germination (%) 24°C	27°C	30°C
0.0	81.4aB[1]	99.2aA	97.4aA
-0.1	9.6bB	47.6bA	43.4bA
-0.2	0cB	3.5cA	0cB
-0.3	0c*	0d*	0d*
-0.4	0c*	0d*	0d*
CV (%)	7.19		
DMS (Tukey)	1.23		

[1]Mean followed by the same lowercase letter in the column and uppercase letter in the line, do not differ within themselves by the Tukey test at 5% probability, *Absence of germination and consequently, absence of the test indication for the line

Table 2: Mean *Piper aduncum* L. seed germination time under different temperatures and osmotic potentials

Osmotic potentials (MPa)	Mean time (days) 24°C	27°C	30°C
0.0	18.7aB[1]	17.8aB	15.5aA
-0.1	19.5bB	20.2bC	18.4bA
-0.2	0cB	21.0bA	0cB
-0.3	0c*	0c*	0c*
-0.4	0c*	0c*	0c*
CV (%)	7.99		
DMS (Tukey)	1.27		

[1]Mean followed by the same lowercase letter in the column and uppercase letter in the line, do not differ within themselves by the Tukey test at 5% probability, *Absence of germination and consequently, absence of the test indication for the line

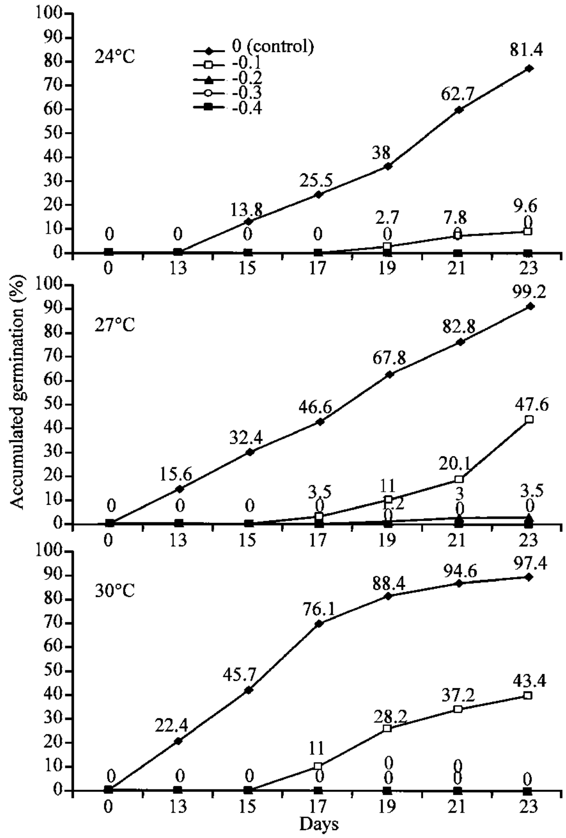

Fig. 1: Accumulated germination (%) of *P. aduncum* seeds at 24, 27 and 30°C, at the different osmotic potentials

and/or decelerated, which is necessary for protein, carbohydrate and the degradation of other metabolites used in the embryo development. From these results it is possible to demonstrate that *P. aduncum* seeds are more susceptible to water restriction than the seeds of other studied species (Rosa *et al.*, 2005; Fonseca and Perez, 2003; Bertagnolli *et al.*, 2003).

Mean time: The mean germination time was significantly affected (Table 2), according to variance analysis, both at the factor level related to the temperature and for the osmotic potentials used in the experiment. The treatments on the control (0.0 MPa) under 24, 27 and 30°C showed the following germination means 18, 17 and 15 days, respectively, demonstrating that with an increase in temperature a decrease in viscosity and an elevation in water kinetic energy occur making the seed imbibition easier (Bradford, 1990).

This process is responsible for intensifying the respiratory velocity due to inducing enzyme and hormone synthesis and activity, as well as the reserve translocation and assimilation that results in primary root protrusion in the majority of the plant species (Marcos Filho, 2005). Within these, the seeds of *P. aduncum* behave in a similar manner where a rise in the temperature probably increased its physiological and biochemical activity, consequently accelerating germination, decreasing the average time of this process (Fig. 1). These results confirmed that the temperature accelerated the germination process thus the behavior of this species is similar to the results obtained by Stefanello (2006) with *Pimpinella anisum* L. The influence of the osmotic potentials of 0.0, -0.1, -0.2, -0.3 and -0.4 MPa under 27°C, resulted in mean germination times of 17, 20 and 21 days, revealing that there is a deceleration in the germinative process making it longer because of enzymes involved that are not activated probably caused by a difficulty in reserve hydrolysis and mobilization (Bewley and Black, 1994). From these results, strategies studying on how to accelerate the germination process. Besides this, proving that the mean germination time of *P. aduncum* seed is affected negatively under water restriction and the necessary time to undergo germination is maximized was demonstrated.

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REFERENCES

Bertagnolli, C.M., N.L. Menezes, L. Storck, O.S. Santos and L.L. Pasqualli, 2003. Performance of bare and pelleted lettuce (*Lactuca sativa* L.) seeds exposed to hydric and thermal stresses. Revista Brasileira de sementes, 25: 7-13.

Bewley, J.D. and M. Black, 1994. Seeds: Physiology of Development and Germination. Plenum, New York.

Bradford, K.J.A., 1990. Water relations analysis of seed germination rates. Plant Physiol., 94: 840-849.

Bray, C.F., 1995. Biochemical Processes During the Osmopriming of Seeds. Seed Development and Germination. In: Kigel, J. and G. Galili (Eds.), Marcel Dekker, New York, pp: 767-789.

Carvalho, N.M. and J. Nakagawa, 2000. Seeds-science, Technology and Production. Funep, Campinas.

Copeland, L.O. and M.B. McDonald, 1995. Principles of Seed Science and Technology. Macmillan, New York.

Edmond, J.B. and W.J. Drapala, 1957. The effect of temperature, sand and soil and acetone on germination of okra seed. Proceeding of the American Society for Horticultural Science, 71: 728-734.

Ferreira, A.G. and F. Borghetti, 2004. Germination: From Basic to Applied. Artmed, Porto Alegre.

Fonseca, S.C.L. and S.C.J.G.A. Perez, 2003. Germination of *Adenanthera pavonina* L. seeds: PEG and polyamines effects under different temperatures. Revista Brasileira de sementes, 25: 1-6.

Gomes, F.P., 2000. Experimental statistical course. USP, Piracicaba.

Lobato, A.K.S., D.G.C. Santos and M.H.L. Silva, 2005. Seed Biometry of *Piper aduncum* L. and *Piper hispidinervium* C. DC., Plants Producers of Essential Oils. In: Proceedings of the 56th Congresso Nacional de Botânica, 9-14 October, Curitiba, Brasil. Sociedade Brasileira de Botânica, São Paulo.

Maia, J.G.S., M.G.B. Zoghbi, E.H.A. Andrade, A.S. Santos, M.H.L. Silva, A.I.R. Luz and C.N. Bastos, 1998. Constituents of the essential oil of *Piper aduncum* L. growing wild in the Amazon region. Flavour Fragrance J., 13: 269-272.

Marcos Filho, J., 2005. Physiology of Seeds of Cultivated Plants. Fealq, Piracicaba.

Mayer, A.M. and A. Poljakoff-Mayber, 1989. The Germination of Seed. Pergamon Press, Oxford.

Michel, B.E. and M.R. Kaufmann, 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51: 914-916.

Morandim, A.A., D.C.B. Bergamo, M.J. Kato, A.J. Cavalheiro, V.S. Bolzani and M. Furlan, 2005. Circadian rhythm of anti-fungal prenylated chromene in leaves of *Piper aduncum*. Phytochem. Anal., 16: 282-286.

Okunade A.L., C.D. Hufford, A.M. Clark and D. Lentz, 1997. Antimicrobial properties of the constituents of *Piper aduncum*. Phytother. Res., 11:142-144.

Rosa, L.S., M. Fellipi, A.C. Nogueira and F. Grossi, 2005. Germination assessment on different osmotic potentials and seed and seedling morphologic characterization of the *Ateleia glazioviana* Baill. Revista Cerne, 11: 306-314.

SAS Institute, 1996. SAS/STAT User's Guid, Version 6. 12 SAS Institute, Cary, NC.

Silva, J.B.C. and J. Nakagawa, 1995. Study of formulas for germination velocity calculation. Informativ Abrates, 5: 62-73.

Stefanello, R., D.C. Garcia, N.L. Menezes and C.F. Wrasse, 2006. Influence of light, temperature and hydric stress in the germination and vigor of seeds of anise. Revista Brasileira Agrociência, 12: 45-50.

Taiz, L. and E. Zeiger, 1998. Plant Physiology. Sinauer Associates, Massachusetts.

Villela, F.A., L. Doni Filho and E.L. Sequeira, 1991. Table of osmotic potential as a function of polyethilene glycol 6000 concentration and temperature. Pesquisa Agropecuária Brasileira, 26: 1957-1968.