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SALT STRESS AND SALT–TEMPERATURE INTERACTION EFFECTS ON THE GERMINATION OF *PELTOPHORUM DUBIUM* SEEDS

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PEREZ, S. C. J. G. A., FANTI, S. C. & CASALI, C. A. 2001. Salt stress and salt–temperature interaction effects on the germination of *Peltophorum dubium* seeds. *Peltophorum dubium* is a tree species native to the Brazilian semideciduous forest and considered a threatened species. A study was undertaken to determine its maximum tolerance limit to different salts (KCl, NaCl, $CaCl_2$) and the salt–temperature interactions at 24, 27 and 30 °C. Four replicates of 50 seeds were used for each treatment. Germination was carried out in sterilised Petri dishes covered with autoclaved filter paper moistened with salt solution or Captan (0.2%). *Peltophorum dubium* is a salt tolerant species, but the average germination rate and germinability decreased with decreasing osmotic potential. At 24, 27, 30 °C and for all osmotic potentials used, $CaCl_2$ was the most toxic, followed by KCl and finally NaCl. An interaction between salt and temperature was registered. At 30 °C the maximum tolerance limit was reduced when KCl, NaCl and $CaCl_2$ solutions were added. When the incubation temperature was lowered (from 27 to 24 °C), the maximal tolerance limit was extended for NaCl and reduced for KCl and $CaCl_2$ salt solutions.

Key words: Canafístula - salt stress - temperature - osmotic potential - germination - tolerance

PEREZ, S. C. J. G. A., FANTI, S. C. & CASALI, C. A. 2001. Tegasan garam dan kesan saling tindakan suhu–garam terhadap percambahan biji benih *Peltophorum dubium*. *Peltophorum dubium* ialah spesies pokok asli di hutan separa daun luruh Brazil dan dianggap spesies yang terancam. Satu kajian dijalankan untuk menentukan had tolerans maksimumnya terhadap saling tindakan garam yang berbeza (KCl, NaCl, $CaCl_2$) dan suhu–garam pada 24, 27 dan 30 °C. Empat ulangan bagi 50 biji benih digunakan pada setiap rawatan. Percambahan dilakukan di dalam piring Petri disteril yang ditutup dengan kertas turas autoklaf yang dilembapkan dengan larutan garam atau Captan (0.2%). *Peltophorum dubium* ialah spesies yang toleran garam, tetapi kadar percambahan purata dan kebolehcambahan berkurangan dengan pengurangan potensi osmosis. Pada suhu 24, 27 dan 30 °C dan bagi semua kegunaan potensi osmosis, $CaCl_2$ adalah paling toksik, diikuti oleh KCl dan akhir sekali NaCl. Saling tindakan antara garam dan suhu direkodkan. Pada suhu 30 °C, had tolerans maksimum adalah berkurangan apabila ditambah larutan KCl, NaCl dan $CaCl_2$. Apabila suhu pengeraman direndahkan (dari 27 hingga 24 °C), had tolerans maksimum dipanjangkan bagi NaCl dan dikurangkan bagi larutan garam KCl dan $CaCl_2$.

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Introduction

Peltophorum dubium (Spreng.) Taub. (Leguminosae-Caesalpinoidae) or canafistula is native to the brasilian flora. It is an important ornamental tree, with good shade and attains 15–25 m height and 50–70 cm diameter. Yellow flowers are formed between December and February each year and the pods are 4–8 cm long, each containing only one seed. It is a useful tree for reforestation of degraded areas because of its fast growth (Lorenzi 1992).

Salinity is an increasing problem for irrigated agriculture, and increase in soil salinity causes deterioration of native vegetation due to adverse effects of salts on plant growth. Effects of salinity on germination and plant growth can adversely affect regeneration of woody species (Ghassemi *et al.* 1995). Irrigation with saline water and fertiliser applications are the major factors responsible for increasing soil salinity. In addition, the osmotic and toxic effects of different salts may be enhanced or attenuated according to external temperature, probably due to an interaction between salt and temperature. Osmotic potential and temperature are important environmental factors affecting seed germination. Individually, each factor has been studied rather extensively, but little is known about the effects of salt stress at different temperatures. A decreased salt tolerance limit is observed when the seeds are exposed to supra-optimum temperature, but sometimes sub-optimal temperature can extend this limit (Fitter & Hay 1990).

Seeds are generally in a more saline environment than the established plants, whose roots could use less of the saline portions of the soil profile. In many studies of salinity, especially those concerned with germination, a single salt solution of sodium chloride is used as osmotic substrate. In other investigations, salts such as calcium chloride are used (Kozlowski 1997).

Susceptibility to salt injuries varies according to the contamination source (Stevens *et al.* 1996) and the different salts present different effects on seed germination (Campos & Assunção 1990).

The aim of the present work was to study the effect of reduced osmotic potential, as a result of increasing salinity in the seed incubation media, and the temperature interaction with salinity on the germination of *P. dubium* seeds.

Materials and methods

Seeds of *P. dubium* from I.P.E.F. (Forestry Institute of Studies and Research), São Paulo State, Brazil, with 12% moisture content were used in this study. The seeds are orthodoxous and were stored in hermetic containers in a refrigerator (± 5 °C). Before the experiments, the seeds were selected according to the visual criteria of size, colour and intact coat. The best ones were scarified by sulphuric acid (98%) for 20 min (Perez *et al.* 1999) and then sterilised with sodium hypochlorite (10%) for 5 min. Following washing in distilled and deionised water, the seeds were placed in Petri dishes and sterilised for 3 h at 150 °C. The Petri dishes were covered with autoclaved filter paper moistened with 6 ml of saline solution or

Captan (0.2% - control group) (Clark & Scott 1982). The excess solution was drained after seed imbibition (24 h) and the filter paper changed when necessary. Four replicates with 50 seeds were used for each treatment. The seed incubation was conducted at constant temperature in a growth chamber (precision ±0.2 °C) where a fan kept the air thermically stable. The lights of the growth chamber were turned off, except during observations. The seeds were examined every 24 h and considered germinated when the radicle exceeded 2 mm longer, with positive geotrophic curvature (Anonymous 1992). The tests were continued until all the seeds were germinated or deteriorated. It was not necessary to do a viability test because all the non-germinated seeds were completely deteriorated.

Determination of maximum salt tolerance limit

In order to determine the salt tolerance of *P. dubium* seeds, a salt stress was induced using NaCl, $CaCl_2$ and KCl. The solutions were prepared at the following osmotic potentials: 0.0; -0.2; -0.4; -0.6; -0.8; -1.0; -1.2 and -1.4 MPa. The amounts of the different salts used to prepare the solutions were calculated using J. H. Van't Hoff's formula as given in Salisbury and Ross (1992).

Interaction between salt temperature

The seeds were incubated at optimum (27 °C), sub-optimum (24 °C) and supra-optimum (30 °C) temperatures (Perez *et al.* 1998), using salt solutions with different osmotic potentials, as described above.

Mathematical approach

The parameters of the germinative process were estimated using the formulas of germinability, average germination rate, mean time, relative frequency, and informational entropy according to Labouriau (1983).

Statistical analysis

The values of germinability were arc sine transformed before being submitted to variance analysis. The F-test or ANOVA procedure was used, with significance level fixed in $\alpha = 0.05$. H_0 (null hypothesis) was rejected when $p < 0.05$, in which case the Tukey test was employed (Sokal & Rohlf 1980).

Results and discussion

The salt effects are summarised in Tables 1–3. A decrease, in general, was observed in germinability, average germination rate and informational entropy, when NaCl, KCl and $CaCl_2$ were added to the germination media in increasing concentrations.

Near the maximum tolerance limit, the seed coat became dark, leaching a gelatinous substance on the germination media when the different salts were added. Under stress conditions, this substance may promote survival for a brief time. Perhaps this substance could reduce water uptake and gas exchange, producing secondary dormancy until the conditions become better (Fitter & Hay 1990). After a long time of exposure, the viability is lost as detected in these experiments. It would be interesting to investigate how and why this gelatinous substance can actually improve seed survival and when and if it presents deleterious effects.

Salt stress under optimal temperature (27 °C)

An osmotic, ionic or a combination of both effects produced, in general a decrease in the average germination rate, germinability and an increase in informational entropy. In this study, no attempt was made to isolate the osmotic and ionic effects.

A significant deleterious effect of NaCl solutions on the germinability occurred at -0.6 MPa but, in relation to the average germination rate, significant reductions occurred at osmotic potential beyond -0.4 MPa. Using KCl solutions, significant decreases on germinability and average germination rate values were observed after -0.8MP and -0.6MPa respectively. When $CaCl_2$ solutions were used, drastic reductions on the germinability (-0.6 MPa) and in the average germination rate (-0.2 MPa) were registered (Table 1).

Under salt stress, the average germination rate of *P. dubium* was more affected than the germinability. In relation to the average germination, Cavalcante and Perez (1995), Nassif and Perez (1997), Hebling (1997), Jeller (1997), and Jeller and Perez (1997) found the same behaviour for *Leucaena leucocephala, Pterogyne nitens, Enterolobium contortisiliquum, Cassia excelsa* and *Copaifera langsdorffii* respectively.

In order to evaluate the synchronisation of the individual seeds, the index E (informational entropy) was used (Labouriau & Valadares 1976). According to Labouriau (1983), the lowest values of informational entropy may be associated with a more organised system, presenting a lot of information. On the other hand, the highest values of entropy are related to a disorganised system, presenting a low degree of information.

In this study, the lowest mean values of informational entropy were found at -0.2 MPa, indicating that in this situation, the seed system was more organised. The lowest mean values of the parameters obtained at -1.2 MPa (NaCl solutions) and -1.1 MPa (KCl solutions) were due to a minority of seeds that were able to germinate, even under stress conditions. However, the average germination rate and germinability were very low (Table 1).

Some observations have been reported on the stimulation of germination in salt solutions at low concentrations (El-Sharkawi & Springuel 1979). For *P. dubium*, this was verified as the addition of NaCl (-0.2 MPa) produced an improvement on seed germination process.

Whether certain salts incorporated in germination media may have specific effects other than remedying minor nutrient deficiencies is still unclear (Heydecker & Coolbar 1997). It is well established that some salts are more toxic than others with respect to a given species. Mayer and Poljakoff-Mayber (1989) cited *Hypericum perfortum* as being extremely sensitive to calcium ions, like *P. dubium*. The maximum tolerance limits for *P. dubium* seeds under salt stress were -1.2, -0.8 and -0.6 MPa for NaCl, KCl and $CaCl_2$ respectively (Table 1).

Susceptibility to salt injury varies with species (Kozlowski 1997). Woody plants usually are relatively salt tolerant during seed germination, much more sensitive during the emergence and young seedling stages, and become progressively more tolerant with increasing age through the reproductive stage. There are many examples of variations in salt tolerance of species and genotypes of leguminous wood plants, for example, *Prosopis juliflora* (Perez & Moraes 1994), *Adenanthera pavonina* (Fanti 1996), *Pterogyne nitens* (Nassif & Perez 1997), and *Copaifera langsdorffi* (Jeller & Perez 1997).

Salt tolerance of closely related species often varies widely, as among species of *Acacia* (Craig *et al.*1990), *Casuarina* (Clemens *et al.* 1983), *Melaleuca* (Van der Mozel & Bell 1987), *Sonneratia* (Ball & Pidsley 1995), *Pinus* (Townsend & Kwolek 1987) and *Eucalyptus* (Sharma *et al.* 1991, Fernando 1992, Sun & Dickinson 1993, Dunn *et al.* 1994). Variations in salt tolerance have also been demonstrated among provenances of *Pinus pinaster* (Saur *et al* 1993, 1995), *Eucalyptus microtheca* (Prat & Fathi-Ettai 1990, Morabito *et al.* 1994, Farrel *et al.* 1996), and *Taxodium distichum* (Allen *et al.* 1994).

A complementary view was obtained when the polygons of relative frequencies were analysed. The determination of the mean time allows classification of the germination isotherms in two groups, according to whether their frequency distributions are unimodal or not. This is an assessment of the homogeneity of these seeds and polymodal frequencies distributions clearly display subsets of seeds, with different germination times (Labouriau 1983).

Labouriau and Agudo (1987) affirmed that relative frequencies with polymodal distributions mean an adaptative performance with a possibility for the seedlings to find the best conditions in a changeable environment.

The polygons of relative frequencies and salt stress under optimal temperature are given in Figures 1–3. Using NaCl, KCl or $CaCl_2$ solutions, it was possible to observe that most of the polygons of relative frequencies are unimodal. There was an increase on the average germination time at -0.6 MPa. This behavior was confirmed by a displacement of the mean time to the right of the main mode (Figures 1–3).

Table 1. Mean values of germinability (G%), average germination rate (V days^{-1}) and informational entropy (E) for *Peltophorum dubium* seeds at 27 °C

	NaCl				KCl				CaCl$_2$			G%
π (MPa)	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E
0.0	94.5	76.5 AB	0.38 a	1.75 ab	94.5	76.5 A	0.38 a	1.75 ab	94.5	76.5 A	0.38 a	1.75 ab
- 0.2	100.0	90.0 A	0.36 a	1.11 c	98.5	85.1 A	0.36 a	1.12 c	96.5	79.2 A	0.29 a	1.39 b
- 0.4	98.0	83.0 AB	0.29 b	1.29 bc	98.0	81.8 A	0.30 ab	1.29 bc	84.5	66.4 A	0.20 c	2.17 a
- 0.6	84.5	67.3 BC	0.19 c	2.04 a	93.5	73.3 A	0.21 ab	1.75 ab	16.0	23.6 B	0.12 d	1.72 ab
- 0.8	69.5	56.7 C	0.14 d	1.97 a	52.0	41.0 B	0.18 ab	1.86 a	9.0	17.4 B	0.10 d	-
- 1.0	37.5	34.7 D	0.13 de	1.52 abc	5.5	13.3 C	0.12 b	1.09 c	4.0	11.5 B	0.10 d	-
- 1.2	28.5	32.2 D	0.12 de	1.93 a	8.5	15.2 C	0.14 b	0.79 c				
- 1.4	3.0	9.8 E	0.11 e	1.54 abc	-	-	-	-				
F		58.81	680.0	7.26		20.95	6.41	8.43		83.01	130.0	8.91
Fc		2.87	2.87	2.87		3.38	3.38	3.38		3.38	3.38	4.47
Δ		17.25	0.021	0.58		28.18	0.21	0.54		15.62	0.04	0.45

*Means followed by the same letter do not differ at 95% confidence.

Table 2. Mean values of germinability (G%), average germination rate (V $days^{-1}$) and informational entropy (E) for *P. dubium* seeds at 24 °C

	NaCl				KCl				CaCl2			
π (MPa)	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E
0.0	95.5	78.1 A	0.26 b	1.76 abcd	95.5	78.1 A	0.26 b	1.76 a	95.5	77.7 A	0.26 a	1.76 a
- 0.2	99.0	85.9 A	0.33 a	1.29 d	98.5	85.1 A	0.33 a	0.97 b	92.5	74.1 A	0.26 a	1.28 b
- 0.4	100	90.0 A	0.25 b	1.36 cd	95.5	78.3 A	0.26 b	1.56 ab	84	66.5 A	0.16 a	2.16 a
- 0.6	98.5	85.1 A	0.20 c	1.48 bcd	95.5	78.1 A	0.21 c	1.72 a	36.5	37.2 AB	0.13 a	2.01 a
- 0.8	96.0	80.3 A	0.16 d	1.59 abcd	57.5	49.4 B	0.16 d	1.75 a	3.5	10.8 B	0.11 a	-
- 1.0	82.0	64.9 B	0.13 d	1.89 ab	14.0	23.4 C	0.11 e	1.60 ab	-	-	-	-
- 1.2	21.5	26.9 C	0.08 e	2.07 a					-	-	-	-
F		69.26	129.04	6.65		68.96	109.9	3.98		5.50	3.75	11.8
Fc		3.09	3.09	3.09		3.38	3.38	3.38		3.38	3.80	4.47
Δ		12.13	0.03	0.49		13.01	0.03	0.68		51.67	-	0.47

*Means followed by the same letter do not differ at 95% confidence.

Table 3. Mean values of germinability (G%), average germination rate (V $days^{-1}$) and informational entropy (E) for *P. dubium* seeds at 30 °C

	NaCl				KCl				CaCl2			
π (MPa)	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E	G%	Arc sin √%	V	E
0.0	99.0	87.1 A	0.38 a	1.0 d	99.0	84.3 A	0.37 a	1.0 b	99.0	84.3 A	0.37 a	-
- 0.2	98.5	83.9 A	0.25 b	2.2 bc	97.0	80.1 A	0.33 ab	0.1 b	80.3	63.9 A	0.25 a	-
- 0.4	86.5	69.1 A	0.16 cd	2.8 a	99.0	85.9 A	0.24 bc	1.8 a	10.5	18.9 B	0.26 a	-
- 0.6	56.0	48.5 A	0.12 d	2.5 ab	82.5	65.4 A	0.18 c	1.8 a	-	-	-	-
- 0.8	12.5	20.5 A	0.10 d	1.7 c	29.5	32.8 B	0.17 c	1.5 ab	-	-	-	-
- 1.0	5.3	12.0 B	0.08 d	1.1 d	2.0	8.13 B	0.17 c	-	-	-	-	-
F		188.51	44.61	36.0		28.94	45.87	7.87		20.69	4.61	
Fc		3.38	3.38	3.38		3.38	3.38	3.80		5.71	5.71	
Δ		10.49	0.081	0.55		26.73	0.09	0.62		29.02	-	

*Means followed by the same letter do not differ at 95% confidence.

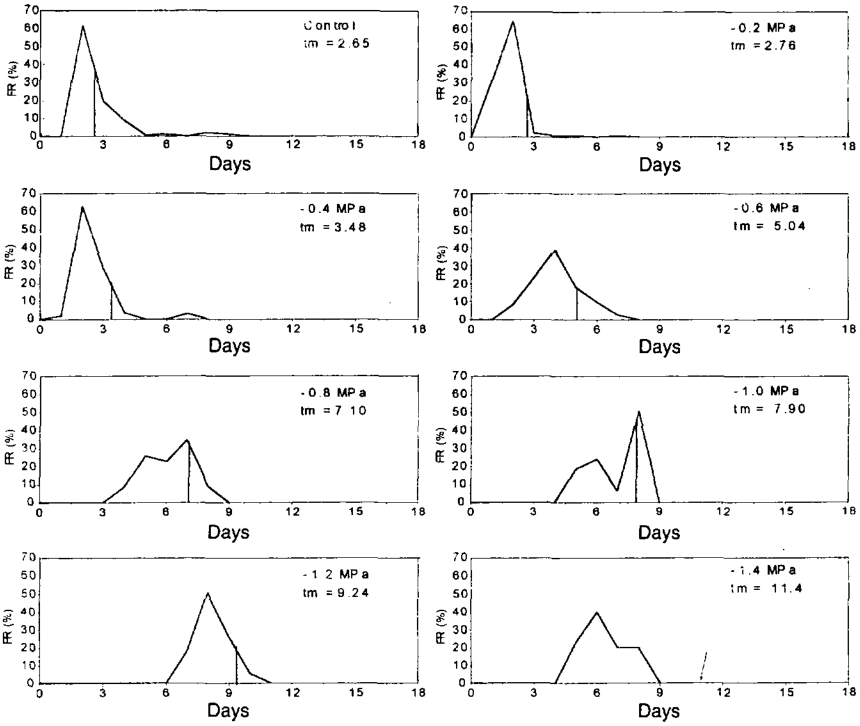

Figure 1. Relative frequencies of *Peltophorum dubium* seeds treated with NaCl solutions at 27 °C. Tm = average germination time.

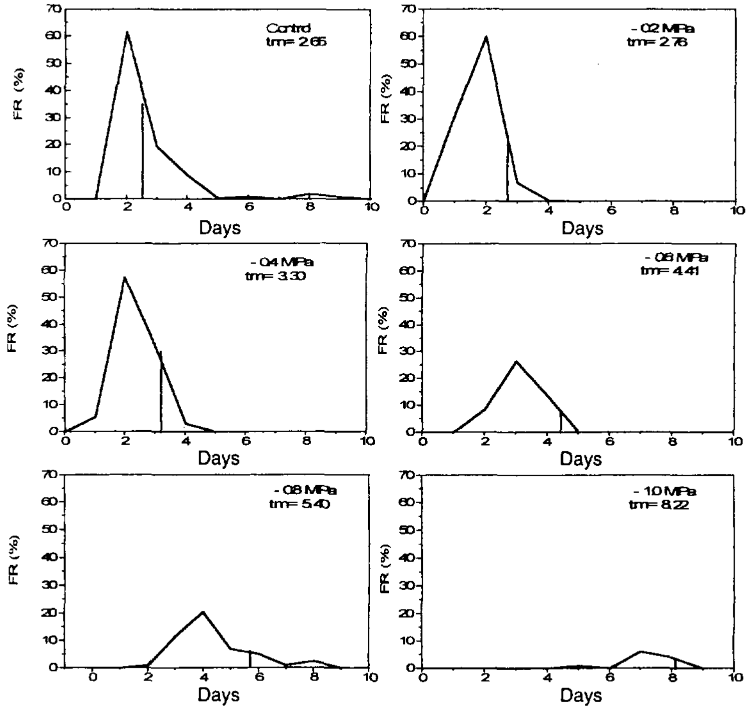

Figure 2. Relative frequencies of *P. dubium* seeds treated with KCl solutions at 27 °C. Tm = average germination time.

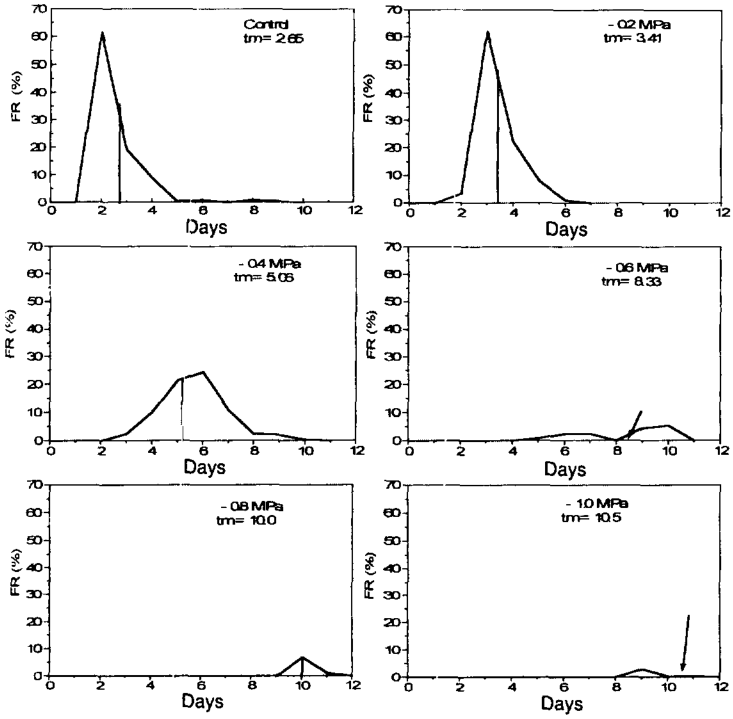

Figure 3. Relative frequencies of *P. dubium* seeds treated with $CaCl_2$ solutions at 27 °C. Tm = average germination time.

Under salt stress, both halophytes (Ungar 1978, Haradine 1982) and glycophytes (Rijven & Parkash 1970, Varshney & Baijal 1977, Ungar 1991) present the same behavior, that is a significant reduction on the average germination rate and germinability. Differences are found in the maximum tolerance limit.

Halophytes with high tolerance are able to germinate on media with up to 8% NaCl (Ungar 1978), but the germination of halophytes with low tolerance is inhibited on media with 1 and 2% NaCl. Several glycophytes do not germinate at salt concentration above 1.5% of NaCl, but are able to do so below this level. In this case are included several species of *Eucalyptus* and *Malaleuca* (Van der Mozel & Bell 1987). In spite of this, glycophytes such as *Prosopis farcta* (Bazzaz 1973), *Lactuca sativa, Helianthus annum, Capsicum annum* (Guerrier 1983, *apud* Perez & Moraes 1994) are very tolerant to salt stress.

Increased salt tolerance is perhaps related to increased proline concentration in the cytoplasm counteracting the salt excess in the vacuole; this is also an osmotic adjustment. In several species, the osmotic adjustment is induced by synthesis of organic solutes in the cytoplasm. These solutes in high concentrations can reduce the toxic salt effect, while the enzymes and bio-membranes are not damaged (Gucci *et al.* 1997).

Guerrier (1983, *apud* Perez & Moraes 1994) verified that very salt tolerant plants contain high levels of K^+ or Ca^{++} as mineral reserves, and the low tolerant ones have low levels of these elements. Thus, several complex mechanism as well as genetic and metabolic factors would be involved.

The effect of salinity on the germination of seeds is generally rather complex. Discrepancies in results reported in the literature are quite evident. Limits between the predominant osmotic effect and toxic effect of one and the same salt are not defined, and this may be the main cause of the discrepancies. Experiments in which single salts are used do not determine such limits, since the criteria taken for germination depend on two variables: (1) the effect of reduced water potential due to increased salinity (Ψs) on seed water relations, and hence metabolism; (2) the effect of ion toxicity on metabolism. The problem becomes more intricate when a third factor such as temperature is considered.

Salt stress under sub-optimum temperature (24 °C)

At 24 °C the maximum tolerance limits for NaCl, KCl and $CaCl_2$ were -1.3, -1.1, and -0.8 MPa respectively, and for $CaCl_2$ solutions, the maximum tolerance limit was lower than that registered at 27 °C (Table 2).

At 24 °C an improvement of the average germination rate at -0.2 MPa using NaCl and KCl solutions was observed. There was an attenuation of toxic and osmotic effects, expressed as an increase on the germinability, in relation to salt stress at 27 °C, but this behaviour was not observed for the average germination rate. The maximum tolerance limit was not extent. Using NaCl solutions a significant decrease in the germinability rate was only registered at -1.0 and -0.6 MPa respectively. KCl solutions reduced the germinability and average germination rate at -0.8 MPa and -0.6 MPa respectively. A decrease in the germinability and average germination rate was observed at -0.6 MPa with $CaCl_2$ solutions.

The lowest values of the informational entropy were found at -0.2 MPa for all salt solutions, indicating the highest synchronisation of the germinative process (Table 2).

Figure 4 shows the polygons of relative frequencies with NaCl solutions. Most of the polygons are unimodal. Exceptions were observed at -1.1 and -1.2 MPa, and a shift of the mean time to the right of the main mode was observed at -0.6 MPa. The use of KCl solutions produced unimodal polygons (except at -0.8 MPa) and a reduction of the synchronisation of the germinative process after -0.4 MPa (Figure 5). $CaCl_2$ solutions produced an early delay on the germinative process after -0.4 MPa (Figure 6).

Salt stress at supra-optimum temperature (30 °C)

The maximum tolerance limit at 30 °C was -1.0, -1.0 and -0.4 MPa for NaCl, KCl and $CaCl_2$ respectively.

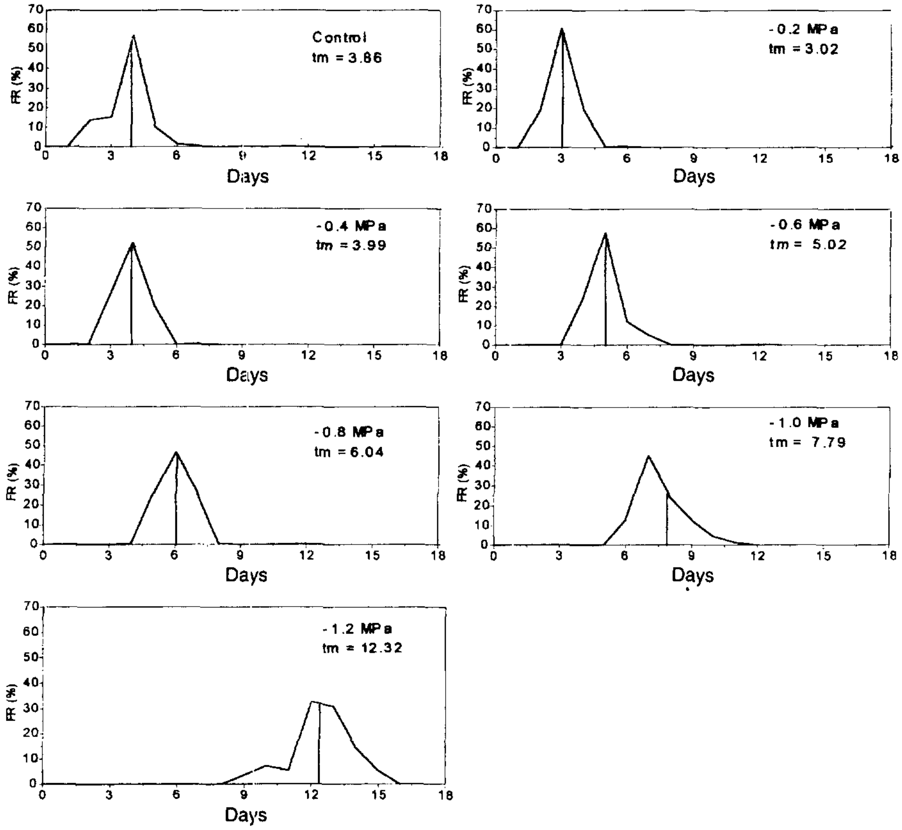

Figure 4. Relative frequencies of *P. dubium* seeds treated with NaCl solutions at 24 °C. Tm = average germination time.

With NaCl, significant reductions on the average germination rate and germinability were observed after -0.2 and -0.4 MPa respectively. At 30 °C an amplification of the toxic and/or osmotic effect was verified, and the maximum tolerance limit reduced. KCl solutions produced significant reductions in the average germination rate and germinability at -0.6 and -0.8 MPa respectively. $CaCl_2$ solutions did not produce a stimulatory effect at -0.2 MPa, and reductions on the average germination rate and germinability were registered at -0.2 and -0.2 MPa respectively (Table 3).

The smallest values of the informational entropy were found in the control group, and the highest at the more negative osmotic potentials, indicating that the germinative process is unsynchronised (Table 3).

At the same temperature, in general, the germinability was inversely proportional to salt concentration. In addition, at the same temperature, $CaCl_2$ solutions were more toxic than the other salt solutions, NaCl and KCl.

The smallest effect on average germination rate, germinability and informational entropy was found when NaCl was added to the germination media, at 27 and 24 °C. At 30 °C KCl was less toxic than the another salts (Tables 4 and 5).

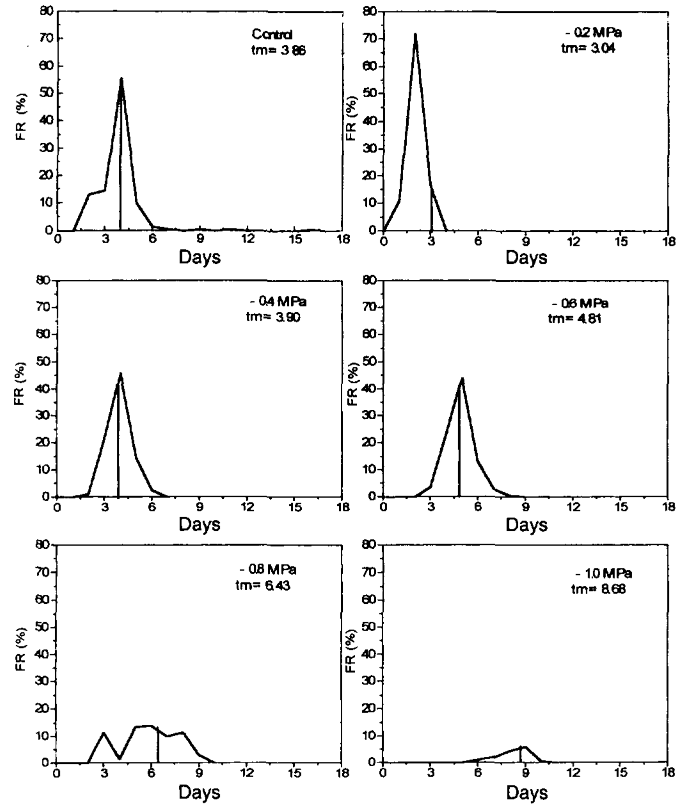

Figure 5. Relative frequencies of *P. dubium* seeds treated with KCl solutions at 24 °C. Tm = average germination time.

Table 4. Variance analysis for average germination rate and germinability of *P. dubium* seeds treated with solutions with different osmotic potentials without considering the kind of salt

	24 °C		27 °C		30 °C	
π (MPa)	Arc sin √%	V (days^{-1})	Arc sin √%	V (days^{-1})	Arc sin √%	V (days^{-1})
0	78.1 A	0.26 b	76.5 B	0.38 a	87.1 A	0.38 a
- 0.2	81.7 A	0.28 a	84.9 A	0.33 b	76.1 B	0.27 a
- 0.4	78.2 A	0.24 b	78.3 B	0.26 c	56.9 C	0.21 a
- 0.6	66.7 B	0.20 c	55.0 C	0.14 d	-	-
- 0.8	48.4 C	0.16 d	38.5 D	0.15 d	-	-
- 1.0	-	-	19.7 E	0.12 d	-	-
F	77.53	48.38	51.98	4.55	111.85	4.14
Fc	3.13	3.13	2.79	2.79	4.24	4.24
Δ	5.20	0.016	4.12	0.04	5.14	-

*Means followed by the same letter do not differ at 95% confidence.

Table 5. Variance analysis for average germination rate and germinability of seeds of *P. dubium* germinated in different salts, without considering the osmotic potential

	24 °C		27 °C		30 °C	
Salt	Arc sin $\sqrt{\%}$	V (days^{-1})	Arc sin $\sqrt{\%}$	V (days^{-1})	Arc sin $\sqrt{\%}$	V (days^{-1})
$CaCl_2$	53.3 C	0.19 b	45.5 B	0.19 b	55.60 B	0.04 b
KCl	73.8 B	0.26 a	62.9 A	0.23 ab	84.54 A	0.31 a
NaCl	84.8 A	0.24 a	68.0 A	0.26 a	80.02 A	0.27 a
F	135.34	189.67	778.40	172.83	107.52	50.7
Fc	4.05	4.05	3.93	3.93	4.24	4.24
Δ	5.70	0.025	4.74	0.04	5.14	0.04

*Means followed by the same letter do not differ at 95% confidence.

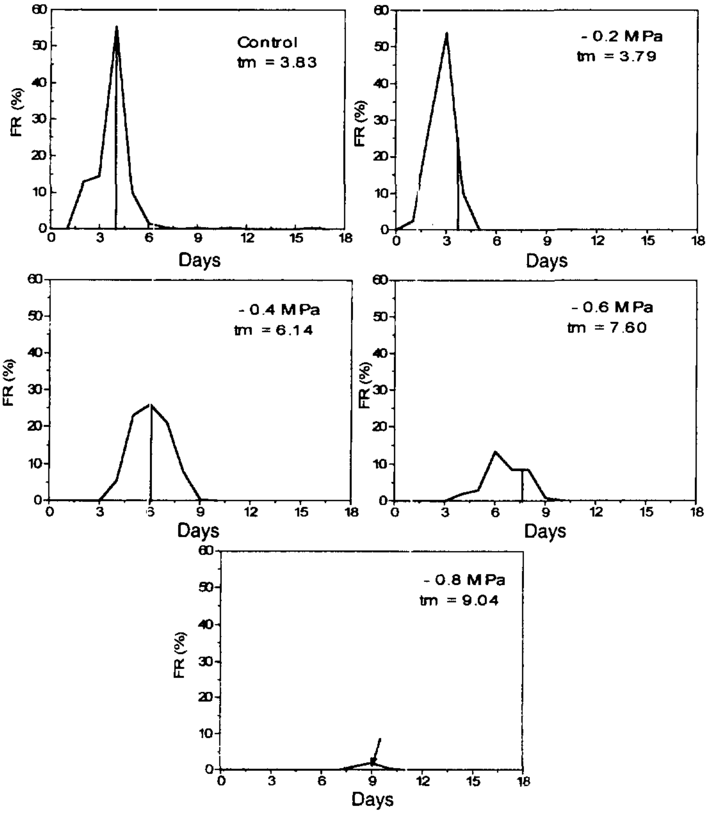

Figure 6. Relative frequencies of *P. dubium* seeds treated with $CaCl_2$ solutions at 24 °C. Tm = average germination time.

In the distribution of relative frequencies (Figure 7), it was observed that for NaCl solution at 30 °C most of the polygons are polymodal. The use of KCl solution produced unimodal polygons (except for the control group) and a reduction of the synchronisation of the germinative process after -0.4 MPa

(Figure 8). $CaCl_2$ solution produced an early delay on the germinative process after -0.4 MPa, confirming its toxic effects (Figure 9).

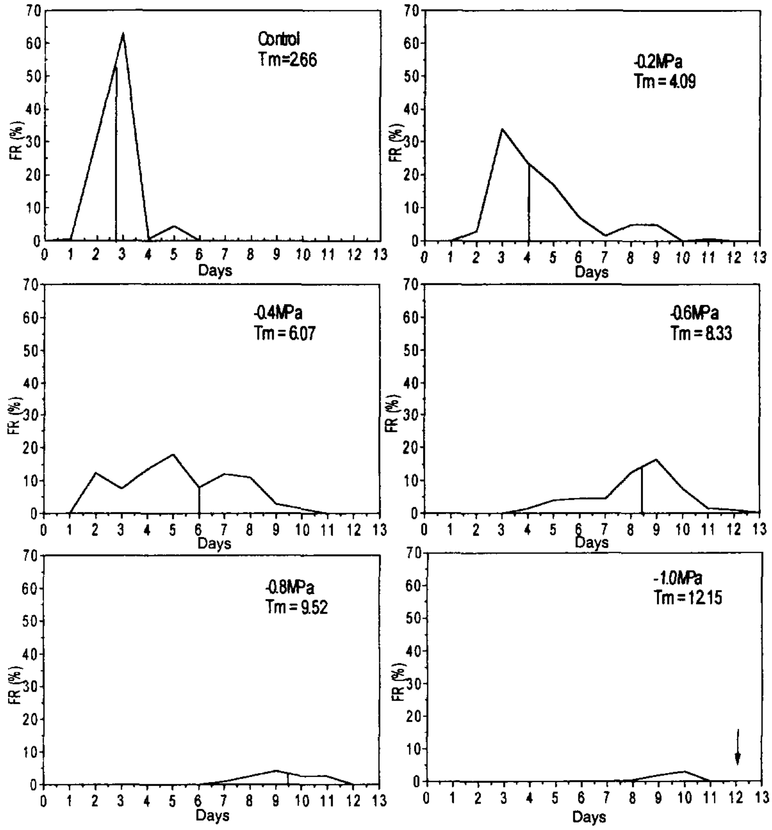

Figure 7. Relative frequencies of *P. dubium* seeds treated with NaCl solutions at 30 °C. Tm = average germination time.

Salinity injures cell membranes and increases solute leakage (Hautala *et al.* 1992). The effects of NaCl on membrane leakage are counteracted by Ca^{+2} (Cromer *et al.* 1985). Salt stress induced a variety of metabolic dysfunctions in nonhalophytes in enzymatic activity, protein and nucleic metabolism and respiration (Kozlowski 1997).

From the results obtained with *P. dubium* seeds under salt stress at different temperatures, it can be concluded that:

- salt tolerance of *P. dubium* seeds is generally high,
- salt toxicity increases with temperature,
- the average germination rate and germinability decrease when salt solution concentration increases,
- $CaCl_2$ solution promotes the highest toxic and osmotic effects, and NaCl the lowest. At 30 °C, KCl is the least toxic.

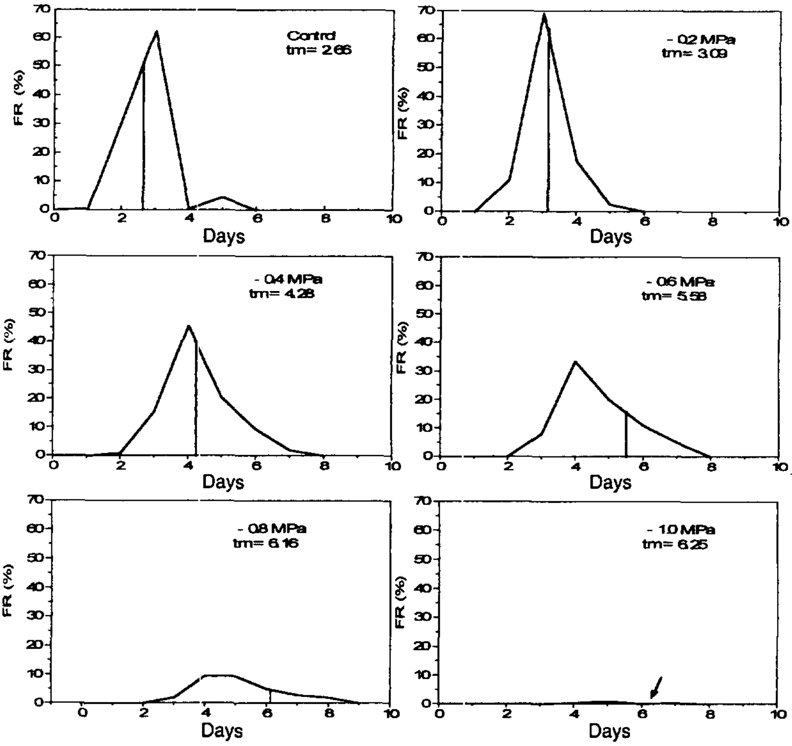

Figure 8. Relative frequencies of *P. dubium* seeds treated with KCl solutions at 30 °C. Tm = average germination time.

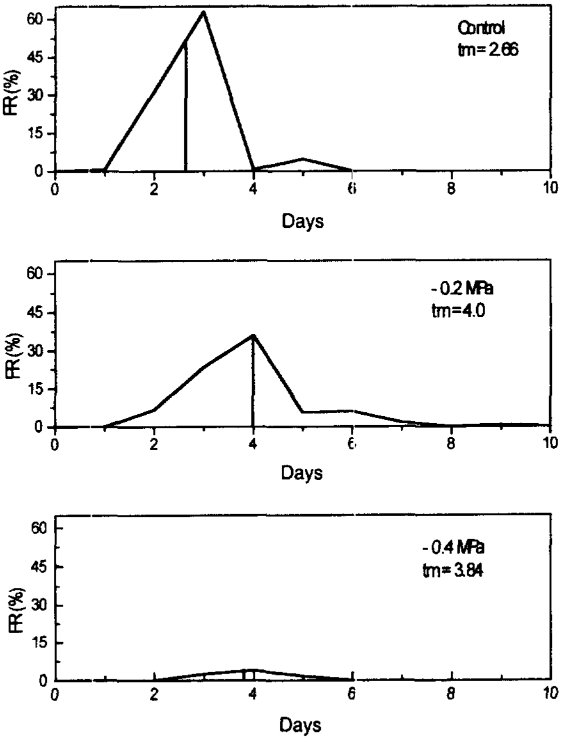

Figure 9. Relative frequencies of *P. dubium* seeds treated with $CaCl_2$ solutions at 30 °C. Tm = average germination time.

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