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Variation in the Response of Seed and Embryonic Axes to Incubation Temperature Gradients during Seed Treatments in Pearl Millet and Sorghum

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Abstract

Incubation temperature during the presowing soaking of seeds plays a significant role in determining the rate and characteristics of post-treatment germination. Three experiments were conducted on sorghum (*Sorghum bicolor* L Moench) and pearl millet (*Pennisetum glaucum* L. R. Br.) genotypes to determine the influence of constant, alternating, ascending and descending temperature regimes on germination characteristics of seeds after treatment. Incubation temperatures ranging from 10 to 35°C were applied as well as alternating the magnitude and range of day/night temperatures. A third experiment tested a 3-day temperature gradient and its impact on germination and seedling characteristics. All three incubation temperature regimes were combined with various hormonal and mineral seed soaking treatments to test for possible interactive effects. Temperature did not affect the final germination percentage of seeds but influenced the germination rate. Constant temperatures of 20 or 25°C induced higher germinative capacity than alternating or constant temperatures of higher or lower magnitude. Increasing the variance in day/night temperature reduced the rate of germination. Incubating seeds during soaking treatments at a constant 20°C for 3 days yielded better germination characteristics than a thermal gradient of 25/20/15°C. An 8g l^{-1}NaCl treatment induced greater plumule (shoot) growth than non-treated counterparts and treating seeds with GA_3 or salts improved germination characteristics and synchrony of treated seed lots.

Keywords: seed treatments, treatment temperature, germination, plumule, radicle

1 Introduction

Emergence and establishment of rainfed sorghum and pearl millet may not always be completely successful since, after imbibition, any water shortage delays emergence, exposing the seeds to stress (Al-Mudaris, 1998b; Kader, 2001; Kader and Jutzi, 2001). Therefore, there has recently been an upsurge of interest in the use of presowing seed treatments involving full or partial hydration of seeds, which may improve emergence and subsequent establishment (Gurushinghe *et al.*, 1999; Powell *et al.*, 2000; Gallardo *et al.*, 2001; Harris, 2001; Araus *et al.*, 2002; Kader and Jutzi, 2002).

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Such treatments include the soaking of seeds in high osmotic potential solutions for various periods of time (Heydecker, 1978; Heydecker and Gibbins, 1978; Brocklehurst and Dearman, 1984; Demir and van de Venter, 1999; Gosling *et al.*, 1999; Lin and Sung, 2001). Temperature, which is an important variable in such treatments, has both qualitative and quantitative effects on subsequent germination rates of treated seeds (Heydecker *et al.*, 1973; Argerich and Bradford, 1989; Hardegree, 1994; Hampton *et al.*, 2000). Reports on the optimum incubation temperature have been inconsistent and do not lend themselves to easy interpretation. Booth (1992) imbibed seeds of *Eurotica lanata* at temperatures from 0 to 20°C in 5°C increments and found that as imbibition temperature increased from 5 to 15°C the probability of successful germination after soaking decreased. Brocklehurst and Dearman (1984) primed carrot, celery, leek and onion seeds at 15°C, whereas Rennick and Tiernan (1978) used 18°C. Other treatment temperatures have been reported ranging from 20°C for carrot (Austin *et al.*, 1969) to 25°C for pepper seed (Georghiou *et al.*, 1987) spanning a wide array of temperature gradients (Welbaum *et al.*, 1998; Pritchard *et al.*, 1999; Kolasinska *et al.*, 2000; Steinmaus *et al.*, 2000; Iannucci *et al.*, 00; Wuebker *et al.*, 2001). The priming of sorghum and pearl millet has not been well documented in the literature, and investigation of the effects of both constant and alternate priming temperature gradients is important in stress acclimation treatments (Al-Mudaris, 1998b; Glenn and Brown, 1998). The objective of the experiments reported here was to study the influence of incubation temperature during priming with various agents on subsequent germination rate of sorghum (*Sorghum bicolor* L. Moench) and pearl millet (*Pennisetum glaucum* L. R. Br.) seeds. Both constant and alternate temperature regimes were tested in addition to a sequential regime involving gradual temperature increases or decreases throughout the treatment period, thus creating a temperature gradient.

2 Materials and Methods

2.1 Constant incubation temperatures

Four seed treatments including a dry control were applied to four sorghum and pearl millet genotypes. All four accessions were obtained from the Asia Centre of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India. These included sorghum varieties ICSV 745 and M35-1, the pearl millet variety CZ-IC 923 and the pearl millet hybrid HHB 67. All seeds were tested following International Seed Testing Association regulations (ISTA, 1993) and revealed germination percentages of 95.7 to 99.3%, moisture content of 13.3 to 14.9% and viability (tetrazolium) of 99.7 to 100%. One thousand (1000) seed weights were 30.3, 38.9, 13.3 and 13.5g for ICSV 745, M35-1, CZ-IC 923 and HHB 67, respectively.

Seed treatments included soaking seed in 150 mg l^{-1} gibberellic acid (GA_3) (150 ppm), 150 mg l^{-1} kinetin (150 ppm), 5g KNO_3 l^{-1} (5%) or 5g l^{-1} NaCl (5%) for 3 days (d). The control included dry, untreated seeds. All 4 seed treatments and the dry control were incubated during the 3-day (d) period at one of six temperatures. These were 10, 15, 20, 25, 30 or 35°C in incubation chambers in the dark (Conviron Industries,

Canada). After treatment, seeds were retrieved from solutions, washed in distilled water and sown in 1-litre polystyrene trays. Two hundred (200) seeds were sown per tray between creased filter paper and each treatment combination replicated 6 times. Trays were placed in a germination cabinet set at a constant 35°C temperature in the dark to allow germination. Germination counts were taken at 24 hour (h) intervals for 10 d and from them the final germination percentage (FGP), first day of germination (FDG), mean germination time (MGT) and germination rate index (GRI) calculated. MGT and GRI were calculated following ORCHARD (1977) and BENECH ARNOLD *et al.* (1991), respectively. Data were arcsine transformed (YANG *et al.*, 1999; HOULE *et al.*, 2001) and subjected to an analysis of variance with mean separation at the 5 % level of probability using the General Linear Model of the SAS® statistical package (SAS Institute, USA) (SAS, 1989; BARRILLEAUX and GRACE, 2000). Trays were arranged in a Randomised Complete Block Design (RCBD) inside incubators and data exposed to one-way and two-way ANOVA (WEBER and ANTONIO, 1999).

2.2 Alternating incubation temperatures

A dry, untreated and a wet, water-soaked (distilled water) control were included in this experiment in addition to two sodium chloride-based (NaCl) treatments. These were 4 and 8g l^{-1}NaCl solutions having an osmometer-measured (Wescor, Utah, USA) osmotic potential (Ψ_s) of -3.2 and -5.7 bar, respectively (circa -0.3 and -0.5 MPa, respectively).

Seeds of sorghum SPV 462, an ICRISAT variety, were either untreated (dry control), soaked in distilled water (wet control) or soaked in the NaCl solutions for 3 d. Incubation temperatures during treatment included a constant 25°C regime and 3 alternate regimes. These were 25/20°C (12 h/12 h day/night), 25/15°C and 25/10°C. Treatments were conducted in the dark. After treatment, seeds were washed in distilled water and dried back at 25°C for 48 h to their original weight in a constant air flow cabinet (Heraeus Voetsch, Germany). Batches of 200 seeds were then sown in 1-liter polystyrene trays between creased filter paper. The paper was moistened with 50 ml of a polyethylene glycol solution (PEG molecular weight 10,000 Sigma Chemical, St Louis, USA) producing a drought level of -10 bar (-1 MPa). As an osmotic agent, PEG is metabolically inert and is ideal for simulating drought (SALISBURY and ROSS, 1992; SWAGEL *et al.*, 1997).

Trays were covered with transparent lids, replicated 6 times and placed in an incubator at 42/18°C (12 h/12 h day/night). Germination was scored daily for a period of 10 d and from the data the FGP, MGT and germination index (GI) were calculated (ALMUDARIS, 1998a). GI assigns maximum weight to seeds germinating on the first day and less weight to seeds germinating thereafter (BENECH ARNOLD *et al.*, 1991). At the end of the test, 20 seedlings were randomly taken from the 20 middle creases in the filter paper and their plumules and radicles excised and weighed after drying at 80°C for 4 d in a reverse cycle oven (Conviron Industries, Canada). These produced the dry weight of plumule (DWP), dry weight of radicle (DWR) and the plumule to radicle ratio (PRR), which is the product of DWP divided by DWR. Statistical procedures were similar to the constant incubation temperature experiment.

2.3 Ascending and descending temperatures

The same batch of SPV 462 seeds was used in this test. Seeds were either untreated (dry control), soaked in 4g NaCl l-1 (4%) or soaked in 4g l^{-1} KCl (4%) for 3 d. Three temperature regimes were applied during soaking treatments as follows:

Regime 1 (R1): Seeds in soaking solutions exposed to 25°C on the first day of treatment, 20°C on the second day and 15°C on the third day.

Regime 2 (R2): Seeds in soaking solutions exposed to 15°C on the first day of treatment, 20°C on the second day and 25°C on the third day.

Regime 3 (R3): Seeds exposed to a continuos 20°C during the whole 3 d treatment period.

Seeds were retrieved from the solutions, dried as in the previous experiment and sown in batches of 200 in polystyrene trays in 6 replicates. Fifty (50) ml of the -10 bar PEG solution was applied to each tray and, thereafter, trays incubated at 39/15°C (12 h/12 h day/night) in the dark. Germination scores were taken daily for the first 10 d and the FGP, MGT and GI calculated. On the 11^{th} day, 20 seedlings were randomly taken as in the previous experiment and their DWP, DWR and PRR recorded.

3 Results and Discussion

3.1 Constant incubation temperatures

Single factor analysis showed that soaking treatments did not have a significant effect on the FGP or GRI of sorghum or pearl millet seed (Table 1). Germination speed as reflected by the FDG and MGT was, however, significantly increased by seed treatments in comparison to controls. GA_3 generally gave the fastest germination (Table 1).

Genotypes differed significantly in their germination characteristics (Table 1). The sorghum variety ICSV 745 gave the highest overall FGP and GRI pooled over treatments and incubation temperatures followed by the pearl millet variety CZ-IC 923, the hybrid HHB 67 and the sorghum variety M35-1. The slowest initiation and rate of germination were observed in HHB 67 as illustrated in Table 1. Incubation temperature also had a significant effect on the FGP, FDG, MGT and GRI. The 35°C incubation temperature resulted in the lowest FGP followed by 30°C, whereas the 10°C regime caused germination to initiate later and take longer time to complete. The 25°C regime was optimal in terms of this initiation and ending of germination as seen from FDG and MGT values (Table 1).

Interactive analysis of genotype×temperature effects (Table 2) revealed the same trend. Thirty and 35°C reduced the FGP and germination speed was generally increased by an increase in incubation temperature. Seed treatment × genotype analysis showed no general preference of a genotype to one specific treatment (data not shown). The same applied to seed treatment×incubation temperature effects, where no single treatment generally preferred a particular temperature but rather an overall effect of temperature in reducing the FGP as it rose to 35°C was detected (data not shown).

Table 1: Effect of seed treatments, genotype and incubation temperature on germination characteristics of sorghum and pearl millet.

	FGP (%)	*FDG (day)*	*MGT (day)*	*GRI (%/day)*
Seed Treatment				
Dry Control	65.8 ^{a}	3.6 ^{a}	3.8 ^{a}	15.4 ^{a}
GA_3	67.6 ^{a}	3.3 ^{b}	3.4 ^{b}	16.3 ^{a}
Kinetin				
64.2 ^{a}	3.5 ^{ab}	3.6 ^{b}	15.1 ^{a}	
KNO_3	64.1 ^{a}	3.5 ^{a}	3:6 ^{ab}	15.4 ^{a}
$NaCl$	63.6 ^{a}	3.5 ^{a}	3.6 ^{ab}	14.4 ^{a}
Genotype				
ICSV 745	87.0 ^{a}	3.3 ^{b}	3.4 ^{b}	22.1 ^{a}
M35-1	45.2 ^{d}	3.4 ^{b}	3.5 ^{b}	10.3 ^{d}
CZ-IC 923	66.1 ^{b}	3.5 ^{b}	3.6 ^{b}	15.6 ^{b}
HHB 67	62.0 ^{c}	3.7 ^{a}	3.8 ^{a}	13.3 ^{c}
Incubation Temp. (°C)				
10	71.3 ^{a}	3.9 ^{a}	4.0 ^{a}	14.8 ^{ab}
15	71.8 ^{a}	3.6 ^{b}	3.7 ^{b}	15.5 ^{ab}
20	70.3 ^{a}	3.4 ^{b}	3.5 ^{b}	16.0 ^{a}
25	68.2 ^{a}	3.1 ^{c}	3.2 ^{c}	17.2 ^{a}
30	58.5 ^{b}	3.4 ^{b}	3.5 ^{c}	15.2 ^{ab}
35	50.3 ^{c}	3.5 ^{b}	3.6 ^{b}	13.2 ^{b}

Means of treatment effects within columns followed by a similar letter are not significantly different at 5%. The same applies to means of genotype and incubation temperature effects. FGP: Final Germination Percentage, FDG: First Day of Germination, MGT: Mean Germination Time and GRI: Germination Rate Index.

3.2 Alternating incubation temperatures

The FGP of dry controls was significantly higher than that of either the wet control or the two $NaCl$ treatments. However, the MGT of the dry control was also higher meaning that it germinated slower than those seeds that were soaked (Table 3). Due to the higher FGP of the dry control it attained a higher GI value at the end of the test. The dry weight of plumules of seeds treated with the 8g l^{-1} $NaCl$ solution was significantly greater than those of all other treatments (Table 3), which did not differ from each other in this respect. The DWR and PRR did not differ amongst treatments.

Table 2: Interactive effects of genotype and incubation temperature on germination characteristics of sorghum and pearl millet.

Genotype	*Incubation Temp. (°C)*	*FGP (%)*	*FDG (day)*	*MGT (day)*	*GRI (%/day)*
ICSV 745	10	87.5 ^{ab}	4.1 ^{ab}	4.1 ^{ab}	18.7 $^{b-d}$
	15	92.5 ^{a}	3.8 $^{b-d}$	3.8 $^{b-d}$	20.9 $^{a-c}$
	20	86.0 ^{b}	3.4 $^{d-f}$	3.6 $^{d-f}$	20.9 $^{a-c}$
	25	88.0 ^{ab}	3.2 $^{e-g}$	3.3 $^{e-g}$	23.3 ^{ab}
	30	85.0 ^{b}	2.9 ^{gh}	2.9 ^{g}	24.0 ^{a}
	35	83.0 ^{bc}	2.7 ^{h}	2.8 ^{g}	24.9 ^{a}
M35-1	10	42.0 ^{gh}	4.0 $^{a-c}$	4.1 $^{a-c}$	8.1 ^{i}
	15	47.5 ^{gh}	3.6 ^{de}	3.6 ^{de}	9.2 ^{hi}
	20	51.5 ^{g}	3.5 $^{d-f}$	3.6 $^{d-f}$	11.8 $^{e-i}$
	25	48.5 $^{f-h}$	3.2 $^{e-g}$	3.2 $^{e-g}$	10.4 $^{g-i}$
	30	45.0 ^{gh}	3.5 $^{d-f}$	3.6 $^{d-f}$	11.7 $^{e-i}$
	35	37.0 ^{h}	2.9 ^{gh}	2.9 ^{g}	10.6 $^{g-i}$
CZ-IC 923	10	81.5 ^{bc}	3.8 $^{b-d}$	3.8 $^{b-d}$	17.1 ^{cd}
	15	81.5 ^{bc}	3.5 $^{d-f}$	3.5 $^{d-f}$	18.4 $^{b-d}$
	20	68.5 ^{de}	3.4 $^{e-f}$	3.5 $^{d-f}$	15.7 $^{d-f}$
	25	67.0 ^{de}	3.1 $^{e-g}$	3.2 ^{fg}	17.0 ^{cd}
	30	60.5 ^{ef}	3.2 $^{e-g}$	3.2 $^{e-g}$	16.0 $^{c-e}$
	35	38.0 ^{h}	4.1 ^{ab}	4.2 ^{ab}	9.4 ^{hi}
HHB 67	10	74.3 ^{cd}	3.8 $^{a-d}$	3.9 $^{b-d}$	15.1 $^{e-g}$
	15	66.0 ^{de}	3.7 ^{cd}	3.7 $^{c-e}$	13.7 $^{e-h}$
	20	75.5 ^{cd}	3.4 $^{d-f}$	3.5 $^{e-f}$	15.5 $^{d-f}$
	25	69.5 ^{de}	3.1 $^{e-h}$	3.1 ^{fg}	18.1 ^{cd}
	30	43.5 ^{gh}	4.3 ^{a}	4.4 ^{a}	9.1 ^{hi}
	35	43.5 ^{gh}	4.2 ^{a}	4.4 ^{a}	8.0 ^{i}

Means in columns followed by similar letters are not significantly different at 5%.
FGP: Final Germination Percentage, FDG: First Day of Germination, MGT: Mean Germination Time and GRI: Germination Rate Index.

Table 3: Effect of seed treatments and incubation temperatures on germination and seedling characteristics of sorghum SPV 462 seeds.

	FGP (%)	*MGT (day)*	*GI*	*DWP (mg)*	*DWR (mg)*	*PRR*
Seed Treatment						
Dry Ctrl.	82.8 ^{a}	4.0 ^{a}	535.4 ^{a}	1.0 ^{b}	1.5 ^{a}	0.83 ^{a}
Wet Ctrl.	61.2 ^{b}	3.5 ^{b}	432.2 ^{bc}	1.3 ^{b}	1.7 ^{a}	0.88 ^{a}
4g/l NaCl	58.0 ^{b}	3.3 ^{bc}	401.7 ^{c}	1.1 ^{b}	1.6 ^{a}	0.75 ^{a}
8g/l NaCl	53.0 ^{b}	2.9 ^{c}	486.6 ^{ab}	2.0 ^{a}	2.1 ^{a}	0.97 ^{a}
Incubation Temp. (°C)						
25	68.5 ^{a}	3.1 ^{b}	521.4 ^{a}	1.4 ^{a}	2.3 ^{a}	0.64 ^{a}
25/20	64.9 ^{a}	3.3 ^{b}	476.1 ^{ab}	1.2 ^{a}	1.5 ^{b}	0.89 ^{a}
25/15	63.0 ^{a}	3.9 ^{a}	426.0 ^{b}	1.3 ^{a}	1.5 ^{b}	0.93 ^{a}
25/10	58.5 ^{a}	3.5 ^{ab}	432.5 ^{b}	1.5 ^{a}	1.6 ^{ab}	0.97 ^{a}

Means of treatment effects within columns followed by a similar letter are not significantly different at 5%. The same applies to means of incubation temperature effects.
FGP: Final Germination Percentage , MGT: Mean Germination Time, GI: Germination Index, DWP: Dry Weight of Plumule, DWR: Dry Weight of Radicle, PRR: Plumule/Radicle Ratio, Dry Control: untreated seeds, and Wet Control: Water-soaked seeds.

Incubation temperature also did not seem to have an effect on the FGP of sorghum seeds (Table 3), but affected germination speed as seen from the MGT values. The 25°C constant temperature regime gave faster germination than the 25/15°C regime. The GI was also higher for the 25°C regime than the 25/15 or 25/10°C regimes (Table 3). Neither DWP nor PRR were affected by incubation temperature even though the DWR was higher at 25°C than at 25/20 or 25/15°C.

Interactive analysis between seed treatments and temperature regimes revealed no preference of treatments for a certain temperature but a tendency of water-soaked seeds to perform better under the 25°C regime than under others (data not shown). Otherwise, the same results as those of single factor effects were observed.

3.3 Ascending and descending temperatures

As seen from Table 4, the 4g l^{-1} KCl treatment, pooled over all three temperature regimes, yielded a significantly lower FGP than the dry control and the 4g l^{-1} NaCl treatment. Again, the effect of soaking treatments was that of increasing germination speed as seen by lower MGT values in the salt soaks.

The 4g l^{-1} NaCl treatment gave the best FGP$\times$MGT relationship as it yielded the highest GI value. Seedling characteristics, represented by DWP, DWR and PRR were

Table 4: Effect of seed treatments and incubation temperature sequences on germination and seedling characteristics of sorghum SPV 462.

	FGP (%)	*MGT (day)*	*GI*	*DWP (mg)*	*DWR (mg)*	*PRR*
Seed Treatment [1]						
Dry Ctrl.	84.0 ^{a}	3.9 ^{a}	593.3 ^{b}	2.4 ^{a}	2.1 ^{a}	1.1 ^{a}
4g/l NaCl	84.7 ^{a}	2.9 ^{b}	678.8 ^{a}	2.6 ^{a}	2.6 ^{a}	1.0 ^{a}
4g/l KCl	78.2 ^{b}	3.3 ^{b}	593.7 ^{b}	2.8 ^{a}	2.6 ^{a}	1.0 ^{a}
Incubation Temp. (°C) [2]						
25/20/15	81.6 ^{a}	3.8 ^{a}	584.4 ^{a}	2.8 ^{a}	2.9 ^{a}	0.9 ^{b}
15/20/25	82.8 ^{a}	3.3 ^{ab}	631.4 ^{a}	2.7 ^{ab}	2.2 ^{b}	1.2 ^{a}
20	82.4 ^{a}	3.0 ^{b}	651.1 ^{a}	2.3 ^{b}	2.1 ^{b}	1.0 ^{ab}

[1]: Means of treatment effects within columns followed by similar letters are not significantly different at 5%. The same applies to means of temperature effects.

[2]: Alternating temperatures indicate temperatures on days 1, 2 and 3, respectively and 20°C represents a continuos temperature for the whole 3 d period.

FGP: Final Germination Percentage , MGT: Mean Germination Time, GI: Germination Index, DWP: Dry Weight of Plumule, DWR: Dry Weight of Radicle and PRR: Plumule/Radicle Ratio.

not affected by soaking treatments. The sequence of incubation temperature did not play a role in the FGP of seeds, but rather in the MGT (Table 4). Seeds incubated under the 20°C constant temperature regime germinated faster than those incubated under the 25/20/15°C sequence (R1). There was no significant difference between R1, R2 and R3 in GI terms. The growth of plumules and radicles in addition to their ratio was affected by temperature regime, as 25/20/15°C gave significantly higher DWP than both 15/20/25°C and 20°C. The difference in weight between plumules and radicles in favour of the former was more pronounced at 4g l^{-1} NaCl in R2 than in R1, thus yielding higher PRR values in the former (Table 5).

The general picture which emerges from the data is that the seed soaking treatments reported seem to be more efficient in increasing germination speed than its final percentage. This effect appears not to be altered by post treatment drying of the seed since the general line of effects observed in the first experiment where seeds were sown fresh was also observed in the dried-back seeds of the second and third experiments. Moreover, the three experiments included different temperature and moisture conditions. The constant temperature experiment was conducted at 35°C without inducing drought, whereas the alternating temperature experiment had a 42/18°C day/night temperature averaging 30°C on a 24 h basis. It also received a PEG-induced drought treatment of -10 bar as did the third experiment. This would tend to point to flexibility in the

Table 5: Interactive effects of seed treatments and incubation temperature sequences on germination and seedling characteristics of sorghum SPV 462 seeds.

Seed Treatment	*Incubation Temp. (°C)*[1]	*FGP (%)*	*MGT (day)*	*GI*	*DWP (mg)*	*DWR (mg)*	*PPR*
Dry Ctrl.	25/20/15	85.3 ^{b}	4.0 ^{a}	588.3 ^{d}	2.8 ^{a}	2.6 ^{bc}	1.0 $^{a-c}$
	15/20/25	84.6 ^{b}	3.9 ^{a}	598.6 ^{cd}	2.5 ^{ab}	2.0 ^{cd}	1.2 ^{ab}
	20	82.0 ^{bc}	3.7 ^{ab}	593.0 ^{d}	2.0 ^{b}	1.8 ^{d}	1.0 $^{a-c}$
4g NaCl/l	25/20/15	82.3 ^{bc}	3.6 ^{ab}	603.0 ^{cd}	2.8 ^{a}	3.3 ^{a}	0.8 ^{c}
	15/20/25	91.0 ^{a}	2.8 ^{cd}	742.3 ^{a}	3.1 ^{a}	2.3 $^{b-d}$	1.3 ^{a}
	20	81.0 ^{bc}	2.4 ^{d}	691.3 ^{ab}	2.1 ^{b}	2.1 ^{cd}	0.9 ^{bc}
4g KCl/l	25/20/15	77.3 ^{cd}	3.7 ^{ab}	559.0 ^{d}	2.8 ^{a}	2.9 ^{ab}	1.0 $^{a-c}$
	15/20/25	73.0 ^{d}	3.3 a-c	553.3 ^{d}	2.5 ^{ab}	2.5 $^{b-d}$	1.0 $^{a-c}$
	20	84.3 ^{b}	3.0 $^{b-d}$	669.0 ^{bc}	3.0 ^{a}	2.5 ^{bc}	1.1 $^{a-c}$

Means within columns followed by similar letters are not significantly different at 5%.
[1]: Alternating temperatures indicate temperatures on days 1, 2 and 3, respectively and 20°C represents a continuos temperature for the whole 3 d period.
FGP: Final Germination Percentage , MGT: Mean Germination Time, GI: Germination Index, DWP: Dry Weight of Plumule, DWR: Dry Weight of Radicle and PRR: Plumule/Radicle Ratio.

response of sorghum and pearl millet seeds to soaking treatments within the range between 27 and 35°C during germination, confirming earlier reports (Ziska and Bunce, 1993; Forcella *et al.*, 2000; Tiryaki and Andrews, 2001; Harris, 2001).

Incubation temperature during treatment, on the other hand, seems to act in another way. A constant temperature during seed soaking appears to be more favourable for post-treatment germination than an alternating regime. In the first experiment, seeds were exposed to constant temperatures ranging from 10 to 35°C. In the second experiment the 25/20, 25/15 °C and 25/10°C regimes gave a 24 h average of 22.5, 20.0 and 17.5°C, respectively. Nevertheless, the average temperature during a day in soaking seems not to be the critical point. More significant appears to be the change in temperature, be it increasing or decreasing, during treatment. This could be confirmed by the data of the third experiment. The 25/20/15 and 20°C regimes all averaged 20°C over the 3 d soaking period. This 20°C given in one constant bulk of heat units (R3), however, yielded better post-treatment results than an increasing (R1) or decreasing (R2) regime. The upper limit of temperature with which one may treat seeds of the genotypes tested is 30°C. Temperatures over 30°C (i.e 35°C in this investigation) yielded poor results. Also, some seeds were observed to germinate during treatment as early as 24 h after initial soaking at 30 and 35°C. This was most severe in pearl millet HHB 67 and less in the M35-1 sorghum variety, and confirms earlier tests (Al-Mudaris and Jutzi, 1998b,c,a, 1999a,b).

Generally, but not always significantly, a rise in incubation temperature during treatment increased post-treatment germination speed, which agrees with the data of Khan *et al.* (1980) who obtained higher germination rates at 20°C in comparison to 10 or 15°C. However, no effect on the FGP was detected. The fact that 12 h a day of temperatures 20°C or lower (i.e. 20, 15 and 10°C in the second experiment) during a 24 h cycle,

or for 24 h during a 72 h cycle (R1 and R2 treatments of the third experiment) were not as effective as the constant 20°C may point to an absolute temperature preference by soaked seeds. This means that if a threshold "low" is reached, certain changes may occur within the seed that are dependent on future temperatures in a way that may be similar to certain qualitative light responses in flowering plants. Lima bean (*Phaseolus lunatus* L.) seeds imbibed at 15°C and then allowed to germinate and grow at 25°C have been shown to produce smaller seedlings (Pollock and Toole, 1966). Thus, sensitivity to chilling injury during the first 10 minutes of imbibition has been proposed (Pollock and Toole, 1966; Kester *et al.*, 1997; Al-Mudaris, 1998b; Kolasinska *et al.*, 2000; Massardo *et al.*, 2000; Gallardo *et al.*, 2001; Kader and Jutzi, 2002). Hegarty (1978) concluded that increased injury during soaking in some species at 10 or 30°C compared to 20°C is associated with greater losses of solutes from the seeds. Simon and Wiebe (1975), on the other hand, reported that the extent of leakage depends on initial water content of the seeds, being very low if embryos already have a water content of 30% or more (Ψ_s of -80 bars) before soaking. This would not apply to the seed batches used in these experiments since moisture contents of seeds were within the normal limits of circa 13-15.0 %.

Seeds in experiments 2 and 3 were dried back after treatment and it has been reported that embryos imbibed for 60 minutes, dried and returned to water again show a rapid leakage of solutes (Bewley and Black, 1978a,b). This may be one of the reasons why dry controls gave higher FGP values in experiment 2. Imbibition at a high temperature of 35°C also increases sensitivity to ethylene (Zarnstorff *et al.*, 1994) whilst at 30°C cytokinin passage from the cotyledon to the embryonic axis is affected (Eloisa Revilla *et al.*, 1988). Hassan *et al.* (1985) observed decreased auxin concentrations with time in seeds of *Anemone coronaria* and *Ranunculus asiaticus* at 8°C compared with 24°C during soaking. Chen *et al.* (1983) reported reduced germination of chickpea seeds down to 30% when soaked at 2°C compared to 95% at 20°C. This tends to point to the presence of a threshold minimum and/or maximum below or above which seeds respond through a number of physiological events.

An increase in soaking temperature affected germination speed. This is in agreement with the results of Argerich and Bradford (1989) and Hardegree (1994), who showed increases in germination rate with rises in temperature up to 25°C. Heydecker *et al.* (1973) arrived at similar conclusions, and Khan *et al.* (1978) found that osmo-conditioning celery seeds at 15°C was not as effective as at 20°C in shortening the germination period. Cotton seed germination has been found to be affected by presowing imbibition temperature. McCarty (1992), studying cyclic temperature schemes, indicated that imbibing seeds at 10°C resulted in more adverse effects than imbibing at 25°C. Keeping seeds at 10°C for periods greater than 24 h reduced seedling emergence compared with keeping seeds at 10°C for 24 h then increasing substrate temperature. Increasing substrate temperature after 48 h of exposure to 10°C was found not to reverse the damaging effects of low temperatures. This tends to confirm the conclusion that 20 to 25°C is the optimal treatment temperature.

The effect of soaking treatments on the germination and early axis growth of seedlings may not be attributed to the Ψ_s of solutions, which would decrease water uptake as it drops (Gurmu and Naylor, 1991), but rather to possible physiological or ionic effects (Al-Mudaris, 1998b; Dewar *et al.*, 1998; Ren and Kermode, 1999; Richards *et al.*, 2001; Tiryaki and Andrews, 2001). In experiment 1 the 5g l^{-1} KNO_3 solution measured -2.4 bar on the osmometer vs. -3.9 bar for 5g l^{-1} NaCl. The Ψ_s of 4g l^{-1} NaCl and 8g l^{-1} NaCl solutions in the second experiment were -3.2 and -5.7 bar, respectively and that of 4g l^{-1} KCl in experiment 3 was -2.4 bar. It follows that differences were not large in the Ψ_s between treatments and it is, thus, difficult to trace back results to this factor, which would typically arise from notable differences in Ψ_s (Hadas and Russo, 1974).

The greatest increase in germination speed in the constant temperature experiment was in the GA_3 treatment. The production of gibberellin is speculated to be a prerequisite for radicle emergence (Bewley and Black, 1978a; Wang *et al.*, 1998; Lin *et al.*, 1998; Pedersen and Toy, 2001; Ljung *et al.*, 2001). Additionally, cell extension of plant tissue is generally held to be regulated by hormones, especially auxins and gibberellins and, since germination culminates in radicle emergence, which in most cases comprises only cell enlargement and not necessarily cell division (Bewley and Black, 1978a; Dominguez and Cejudo, 1999; Nascimento and West, 2000; Lahuta *et al.*, 2000), the promotive role of GA_3 in increasing germination speed is not surprising. Additionally, on the premise that germination may involve the synthesis of specific proteins/enzymes, the possibility that GA_3 may have an effect on protein and/or RNA synthesis (Bewley and Black, 1978b) still remains open. Exogenous application of GA_3 has been reported to stimulate growth (Kozlowski, 1972) and germination percentages and rates in sorghum seeds soaked for 4-6 days in 500 or 750 ppm GA_3 at 15 and 20°C (Santipracha, 1986).

KCl was not as effective as NaCl since it yielded lower FGPs in the third experiment. The observed difference may lie within the K^+ and Na^+ ions since Cl^- is common between the two compounds. Potassium is characterized by high mobility in plants at cellular, tissue or long distance transport levels (Marschner, 1995) and seems essential for the synthesis of metabolites (Kozlowski, 1972). Sodium is less essential than K^+ as a mineral nutrient (Marschner, 1995). The 4g l^{-1} KCl and 4g l^{-1} NaCl solutions had almost the same pH values of 5.84 and 5.87, respectively, and electrical conductivity values of 7.35 and 6.92 mS cm^{-1}, respectively. Thus, other internal effects may have played a role since a relationship between potassium, magnesium and phosphate ions, and gibberellic acid is known to exist (Bewley and Black, 1978a). Influx of Na^+, Cl^- and K^+ ions into the seed may have altered the response to temperature as these have an impact on physiological triggers (Keiffer and Ungar, 1997; Glenn and Brown, 1998; Howard and Mendelssohn, 1999; Gaxiola *et al.*, 2001).

In conclusion it is recommended that ambient room temperatures of 20 to 25°C be used for the soaking treatments reported since gains through alternating temperatures were not observed. It would also be interesting to validate the effect of GA_3 on sorghum and

millet seeds under other conditions and to further investigate the effects of Na^+ and K^+ in seed priming treatments.

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