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Hydrothermal Variations and Physio-Osmotic Conditioning Effects on Five African Millet Varieties during Short Term Substrate Desiccation

M. A. Kader [*1] and **S. C. Jutzi** [2]

Abstract

Environmental factors differentially affect the germination of millet (*Pennisetum americanum* L.) and impact both the rate and extent of field emergence. The extent and uniformity of emergence depends on hydrothermal variations in both soil moisture and temperature levels. To determine the impact of these two factors and counteracting physiological and osmotic conditioning seed treatments, two growth chamber trials were conducted on African millet. Five genotypes responded to differences in temperature or osmotic seed conditioning. Seed conditioning with GA_3, Kinetin, $NaCl$ and KNO_3 was tested. Increasing incubation temperature decreased the final proportion of seeds germinating and slowed germination for each of the five genotypes tested when exceeding a 29°C threshold. GA_3 improved the performance of seed lots, while physio-osmotic conditioning and temperature interacted to affect the proportion of germinating millet seeds. These germination tests partially explain interspecific differences in the impact of timing of heat fluctuations in the field. Patterns of millet germination in response to temperature and rainfall fluctuations could be explained by its response to seed conditioning, temperature or moisture levels.

Keywords: hydrothermal variations, desiccation, GA_3, *Pennisetum americanum* (L.)

1 Introduction

Osmotic priming is widely used to improve seed quality. It causes an arrest in seed germination after phase II of the triphasic pattern of water uptake, when no changes in water content occur. Major metabolic events occur at this time to prepare the seed for radicle emergence (Dewar *et al.*, 1998) and the seed is restrained from entering phase III, which includes radicle elongation and completion of germination (Garcia-Maya *et al.*, 1990). Osmotic priming of seed generally causes faster germination (Kader and Jutzi, 2001) and faster field emergence (Kader, 2001) which may result in greater mean plant dry weights, leaf areas and ground cover percentages (Posmyk *et al.*, 2001; Kader and Jutzi, 2002).

* corresponding author

[1] Dr. M. A. Kader, Director, Consultica Worldwide, PO Box 3089 Tamarama NSW 2026 Australia

[2] Prof. Dr. S. C. Jutzi, Director, Animal Production and Health Division, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy

Both higher (FINCH-SAVAGE *et al.*, 1998) and unchanged (COLBACH *et al.*, 2002) final germination percentage at reduced osmotic potential have been reported. There is a linear relationship between germination rate and hydrothermal priming time, indicating that both the external osmotic potential during priming and the duration of the priming period contribute to the improved germination rate (ALVARADO and BRADFORD, 2002). The threshold temperature at which seeds can be treated dictates the extent of the beneficial effect of osmoconditioning in alleviating drought and/or heat stress within seed. Heat-stressed seeds, however, have been found to be more responsive to hormonal applications than osmotic induction (ZHU, 2002).

This experiment was designed to investigate the influence of physio-osmotic seed conditioning with both osmotic agents and hormones on millet (*Pennisetum americanum* L.) seed response to drought and heat stress. The range of temperatures and the concentrations of hormones were based on previous work (KADER, 2001, 2002) which were effective in osmoconditioning sorghum seed.

2 Materials and Methods

2.1 Constant Incubation Temperatures

Four seed treatments including a control were applied to four millet genotypes. These included the varieties Tupatupa (Malawi), ICMV88908 (Namibia), Shibe (Tanzania) and Tuso (Zambia). All seed lots were analysed following International Seed Testing Association regulations (ISTA, 1993) and revealed 1000 seed weights of 7.9 to 13g, moisture content of 12.7 to 14.3% and viability of 98.1 to 99.6%. Seed treatments included soaking seed in 150mg gibberellic acid (GA_3) per litre, 150mg kinetin per litre, 5g KNO_3 per litre or 5g $NaCl$ per litre for 3 days (d). The control included water-soaked seeds (distilled water). All 4 seed treatments and the wet control were incubated during the 3 d period at one of six temperatures. These were 9, 14, 19, 24, 29 or 34°C in incubation chambers in the dark. After treatment, seeds were retrieved from solutions, washed in distilled water and sown in 1000cm^3 trays between germination paper. One hundred seeds were sown per tray and each treatment combination replicated 5 times. Trays were placed in a germination cabinet set at a constant 42/29°C (11hr/13hr) temperature in the dark. Germination counts were taken at 24 hour (h) intervals for 9 d and from them the final germination percentage (FGP), first day of germination (FDG), mean germination time (MGT) and germination rate index (GRI) (ESECHIE, 1994) calculated. Data were arc sine transformed (BROWN and ROTHERY, 1993) and subjected to an analysis of variance (ANOVA) with mean separation at the 5 % level of probability through Duncan's Multiple Range Test (CHEW, 1980; DAY and QUINN, 1989).

2.2 Alternating Incubation Temperatures

A dry and wet control were included in this experiment in addition to two sodium chloride-based treatments. These were 4 and 8g $NaCl$/l solutions with an osmometer-measured (Knauer, Germany) osmotic potential (Ψ_s) of -3.2 and -5.7 bar, respectively. Pearl millet PMV 3 seeds (Zimbabwe) 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 25/15°C (12 h/12 h), 25/10°C and 25/5°C. Treatments were conducted in the dark. After treatment seeds were washed in distilled water and dried back at 25°C for 4 h. Batches of 100 seeds were then sown in 1000 cm^3 trays between germination paper, and 150 ml of a PEG (polyethylene glycol, molecular weight 10.000) solution (Sigma Chemical, US) producing a drought level of -10 bar added to them. Boxes were covered with lids, replicated 5 times and placed in an incubator at 44/13°C (8 h/16 h). Germination was scored daily for a period of 10 d and from the data the FGP, MGT and germination index (GI) were calculated (Benech Arnold *et al.*, 1991). At the end of the test, 16 seedlings were randomly taken from the 8 middle creases in the filter paper and their plumules and radicles excised and weighed after drying at 80°C for 4 d. 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.

3 Results and Discussion

3.1 Constant Incubation Temperatures

Simple effect analysis showed that soaking treatments did not have a significant effect on the FGP or GRI of 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 yielded lower FDG values than all other treatments excluding kinetin.

Genotypes differed significantly in their germination characteristics (Table 1). The variety Tupatupa gave the highest overall FGP and GRI pooled over treatments and incubation temperatures followed by Shibe, Tuso and ICMV88908. The slowest initiation and rate of germination were observed in Tuso as illustrated in Table 1. Incubation temperature also had a significant effect on the FGP, FDG, MGT and GRI. The 34°C incubation temperature resulted in the lowest FGP followed by 29°C, whereas the 9°C regime caused germination to initiate later and take longer time to complete. The 24°C regime was optimal in terms of this initiation and ending of germination (Table 1)

Interactive analysis of genotype and temperature effects (data not shown) revealed the same trend where 29 and 34°C reduced the FGP. Germination speed was generally increased by an increase in incubation temperature and seed treatment $\times$ genotype analysis showed no preference of a genotype to one specific treatment (data not shown). The same applied to seed treatment $\times$ incubation temperature effects, where no single treatment preferred a particular temperature.

3.2 Alternating Incubation Temperatures

Unexpectedly, the FGP of the dry control 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 1: Effect of seed treatments, genotype and incubation temperature on germination characteristics of millet

	FGP (%)	*FDG (day)*	*MGT (day)*	*GRI (%/day)*
Seed Treatments				
Dry Control	65.9 a	3.6 a	3.8 a	15.3 a
GA_3	68.6 a	3.3 b	3.4 b	16.2 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				
Tupatupa	87.2 a	3.3 b	3.4 b	22.1 a
ICMV88908	45.1 d	3.4 b	3.5 b	10.3 d
Shibe	66.1 b	3.5 b	3.6 b	15.6 b
Tuso	61.0 c	3.7 a	3.8 a	13.3 c
Incubation Temp. (°C)				
9	71.5 a	3.9 a	4.0 a	14.8 ab
14	7.8 a	3.6 b	3.7 b	15.5 ab
19	70.3 a	3.4 b	3.5 b	16.0 a
24	68.2 a	3.1 c	3.2 c	17.2 a
29	58.5 b	3.4 b	3.5 c	15.2 ab
34	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 according to Duncan's Multiple Range Test ($p \leq 0.05$). 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.

(Table 2). 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 $NaCl$/l solution was significantly greater than those of all other treatments (Table 2) which did not differ from each other in this respect. The DWR and PRR were not significantly different between treatments.

Incubation temperature did not have an effect on the FGP of sorghum seeds (Table 2), 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 at 25°C than at 25/15 or 25/10°C (Table 2). 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.

Table 2: Effect of seed treatments and incubation temperatures on germination and seedling characteristics of PMV3 seeds

	FGP(%)	*MGT(day)*	*GI*	*DWP(mg)*	*DWR(mg)*	*PRR*
Seed Treatment [1]						
Dry Control	82.8 a	4.0 a	535.4 a	1.0 b	1.5 a	0.83 a
Wet Control	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

[1] Means of treatment effects within columns followed by a similar letter are not significantly different according to Duncan's Multiple Range Test (p(0.05). 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.

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).

A constant temperature during seed soaking appears to be more favourable for post-treatment germination than an alternating one. Generally, 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. This means that if a threshold temperature is reached, certain changes may occur within the seed that are dependent on future temperatures. 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), which has been linked to preferred temperature ranges during soaking (Kader and Jutzi, 2002). Hegarty (1978) concluded that reduced seed response after soaking at 10 or 30°C compared to 20°C is associated with greater losses of solutes from the seeds. Seeds, in this experiment, 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; Winiewski and Zagdaska, 2002). This may be one of the reasons why dry controls gave higher FGP values. Imbibition at the higher temperature range also increases sensitivity to ethylene (Zarnstorff *et al.*, 1994) which may explain reduced

FGP values. Also, 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. This may have caused the increased MGT values observed under lower incubation temperatures.

That an increase in soaking temperature reduced the MGT is in agreement with the results of Hardegree (1994) and Argerich and Bradford (1989) who showed increases in germination rate with rises in temperature up to 25°C. Khan *et al.* (1978) found that osmoconditioning celery seeds at 15°C was not as effective as at 20°C in shortening germination period. Pollock and Toole (1966), reported an immediate imbibitional uptake of water at 25°C for about 2 h, a lag period for the 2^{nd} to the 6^{th} h, and, finally, a rapid, linear uptake to about the 25^{th} h. Both the duration of the lag phase and the second rapid uptake phase were dependent on imbibition temperature. Imbibition at lower temperatures (5 or 15°C vs. 25°C) lengthened the lag phase. Additionally, higher temperatures may have reduced water viscosity surrounding the seed and, thus, increased its diffusion (Woodstock, 1988). Vertucci and Leopold (1983) suggested two components of early imbibition: An initial wetting reaction which is influenced by the surface tension of the water and a subsequent flow of water through seed tissue which is influenced by water viscosity.

The effect of soaking treatments on the germination and early axis growth of seedlings may not be attributed to the osmotic potential (Ψ_s) of solutions which would decrease water uptake as it drops (Gurmu and Naylor, 1991; Leubner-Metzger *et al.*, 1996), but rather to possible physiological or ionic effects. The Ψ_s of 4g $NaCl$/l and 8g $NaCl$/l solutions were -3.2 and -5.7 bar. Hence, 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; Dewar *et al.*, 1998). Additionally, cell extension of plant tissue is generally held to be regulated by hormones, especially auxins and gibberellins (Agu *et al.*, 1993; Rood, 1995). Since germination culminates in radicle emergence, which in most cases comprises only cell enlargement and not necessarily cell division (Bewley and Black, 1978b), the promotive role of GA_3 in increasing germination speed is not surprising. Exogenous application of GA_3 has been reported to stimulate growth (Kozlowski, 1972; Bewley, 1995; Steinbach *et al.*, 1997) 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; Kader, 2001).

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