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Thermal Induction, Salt Treatment and the associated Plumule/Radicle Growth Response of Sorghum at 42/19°C

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Abstract: This investigation tested the influence of thermal induction of seeds at various stages of imbibition prior to exposure to heat shock on the germinative and growth response of those seeds and seedlings. Seeds of sorghum (*Sorghum bicolor* L. Moench) were treated in 2, 4 or 6 g NaCl/L solutions and exposed for 2 hours to 45°C during the first, second or third days of imbibition (thermal induction). Thereafter, seeds were dried and germinated at 42/19°C (day/night temperature). Salt treatments did not improve the final germination percentage but increased germination speed over untreated seeds. The higher the salt concentration used, the greater the dry weights of plumules (shoots) and radicles (roots). Thermal induction on the third day of imbibition yielded higher germination percentages than un-induced seeds, while induction on the second day gave faster germination. Both the second and third day induction treatments gave superior germination indices and higher plumule to radicle ratios. It is concluded that thermal induction may assist in acclimating seeds to heat stress.

Keywords: Thermal induction, stress, germination, growth

INTRODUCTION

Seed germination is usually the most critical factor determining the success or failure of stand establishment. Recent interest in presowing seed treatments for improving field emergence under stress has generated considerable advances. Priming with sodium chloride has been used in sorghum and shown to advance germination under drought but not under heat stress (Kader and Jutzi 2001, Kader 2002a). The principle of inducing seeds or whole plants to stress is also well documented (Amzallag et al. 1990) and has likewise been applied to NaCl treatments in sorghum (Kader 2001). Therefore, the possibility of inducing seeds to heat stress by pre-exposure to high temperatures seems to be feasible. In this connection, it would be interesting to investigate the effects of high temperatures during seed soaking on responses to post-treatment heat stress. The objective of this investigation was to test the influence of thermal induction by way of pre-stress acclimative heat exposure and its timing on germination of sorghum variety SPV 462 under heat stress.

MATERIALS AND METHODS

Salt-based seed priming treatments were applied to SPV 462 seeds stored prior to treatment at 5°C and 50% relative humidity for 2 months. Seed lots were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and had moisture content of

11%, viability (tetrazolium) of 97.3% and germinability of 98.1%. Seed treatments included soaking in 2, 4 or 6 g NaCl/L solutions for 3 days (d) in the dark. These solutions had osmotic potentials of -65, -135 and -180 mOsmol kg^{-1}, respectively, corresponding to -1.5, -3.2 and -4.3 bar, respectively. Electrical conductivity (EC) values for these solutions were 3.8, 7.3 and 9.3 mS cm^{-1}, respectively. A water-soaked control was also tested based on previous work (Kader and Jutzi 2001, Kader 2002a, Kader 2002b).

Thermal induction treatments were conducted on the 1^{st}, 2^{nd} or 3^{rd} day of imbibition by exposing seeds in solutions to a temperature of 45°C for 3 hours (h) and to 13°C for the remainder of the soaking period. After treatment, seeds were surface dried at 25°C for 3 h and plated for germination in polystyrene trays at a rate of 100 seeds/tray. A concomitant day/night temperature of 42/19°C (12 h/12 h) was used in germination cabinets under dark conditions and filter paper moistened with distilled water. Trays were replicated five times and observations of seed germination made on 24-h intervals for 10 d. After 10 d, seedling plumules (shoots) and radicles (roots) were excised for 10 seeds/tray from the 5 middle creases of the filter paper, dried in an air-forced cabinet at 80°C for 3 d and averaged. This yielded the dry weight of plumule (DWP) and dry weight of radicle (DWR). By dividing the DWP by the DWR the plumule: radicle ratio (PRR) was obtained. From daily germination scores, the final germination percentage (FGP), mean germination time (MGT) [$MGT = \sum fx/f$ where f is the number of seeds germinated on day x] (Orchard 1977) and germination index (GI) [$GI = (10 \times n1) + (9 \times n2) + \cdots + (1 \times n10)$ where n1, n2 ... n10 = no. of germinated seeds on the 1^{st}, 2^{nd} and subsequent days until the 10^{th} day; 10, 9 ... and 1 are weights given to the number of germinated seeds on the 1^{st}, 2^{nd} and subsequent days, respectively] (Benech Arnold et al. 1991) were calculated. Analysis of variance (ANOVA) was used to test for salt treatment and thermal induction effects as well as their interaction on arcsine transformed germination percentages. Duncan's Multiple Range Test was used for mean separation. Significance was evaluated at $p \leq 0.05$ using the Statistical Analysis System (SAS ®).

RESULTS AND DISCUSSION

The main effects, i.e. NaCl concentration and treatment timing, and their interactions were significant in their impact on germination and seedling characteristics of sorghum. The results of germination counts following treatment of seeds showed that a 2 g NaCl/L treatment gave the same FGP as the control, but higher percentages than the 4 or 6 g treatments (Table 1).

Enhanced germination rates, as reflected by lower MGT values, revealed a faster germination pattern in all three NaCl treatments when compared to the control. This was accompanied by high GI means in the case of a 2 g NaCl/L treatment as seen from Table 1. The highest DWP and DWR values were observed in 6 g NaCl/L-treated seeds, but untreated seeds appear to have allocated more growth activity to the plumule than to the radicle since they attained the highest PRR ratios (Table 1).

Germination and seedling characteristics as affected by thermal induction are presented in Table 2. Whereas the FGP was not influenced by thermal induction, MGT exhibited progressive decreases when seeds were induced on the 2nd or 3rd days of imbibition. When data in Table 2 are examined, it becomes apparent that the 2nd and 3rd day treatments advanced not only germination speed (lower MGT and higher GI values), but also gave the highest PRR ratios.

Agreement between pooled effects (Tables 1 and 2) and interactive effects of seed treatments and thermal induction, which are shown in Figures 1 and 2, was evident. The FGP was not affected by seed treatment or thermal induction, but germination speed was enhanced by NaCl soaks (Figs 1a–1c). Days 2 and 3 were the best periods to induce seeds during imbibition.

Seed Treatment (g NaCl/L)	FGP (%)	MGT (day)	GI	DWP (mg)	DWR (mg)	PRR
0 (Control)	88.6 ab	3.0 a	699.9 b	4.8 b	1.3 c	3.6 a
2	89.7 a	2.5 b	760.5 a	4.3 c	1.5 b	2.7 c
4	86.1 c	2.5 b	730.2 ab	4.5 bc	1.5 b	3.0 bc
6	86.9 bc	2.5 b	729.3 ab	5.9 a	1.9 a	3.1 b

Table 1. Effect of NaCl-based seed priming treatments on germination and seedling characteristics of sorghum SPV 462 seeds.
Means in columns followed by similar letters are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). 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.

Acclimation Time (Days after Imbibition)	FGP (%)	MGT (day)	GI	DWP (mg)	DWR (mg)	PRR
No Acclimation	86.7 a	2.9 a	689.2 b	4.5 b	1.6 b	3.0 b
Day 1	87.9 a	2.7 ab	720.7 b	5.0 a	1.8 a	2.8 b
Day 2	87.4 a	2.3 c	755.0 a	4.8 ab	1.4 b	3.3 a
Day 3	89.4 a	2.5 bc	755.0 a	5.2 a	1.5 b	3.4 a

Table 2. Effect of pre-stress heat acclimation treatments on germination and seedling characteristics of sorghum SPV 462 seeds on exposure to post-treatment heat stress.
Means in columns followed by similar letters are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). 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.

As seen from Figure 2a, the highest DWP values were obtained in the 6 g NaCl/L treatment with acclimation on the 1st day of imbibition being superior to other timings at this NaCl level. The highest DWR was observed in the 6 g NaCl/L treatment, when seeds were acclimated on the 1st day (Fig. 2b). Control seeds which were not acclimated yielded the highest PRR (Fig. 2c).

Observation of seed and seedling characteristics confirmed that germination speed was improved and that a 6 g NaCl/L treatment increased both the DWP and DWR whereas control seeds had a higher PRR. Such a PRR value would indicate a tendency, on the seed's part, to shift growth activity to the shoot rather than the root. Speculations on the larger axis (heavier plumules and radicles) in 6 g NaCl-treated seeds can be deduced from reports of earlier differentiation in secondary xylem in seedlings exposed to NaCl conditions for both root and shoot (Valenti et al. 1992). Additionally, osmotic adjustment of plumules and radicles in NaCl-treated seeds may have contributed to this increase in weight due to the accumulation of Na^+ and Cl^- ions (Nabil and Coudret 1995). This, however, is in disagreement with the data of Roundy et al. (1985) who reported that sodium, chloride, sulphate and calcium ions interfered with germination and growth processes resulting in lower radicle growth. We tend to favour the assumption that the accumulation of ions in the seed during the 3 d soaking treatment led to the subsequent translocation of Na^+ and Cl^- to the newly growing parts in the plumule and radicle leading to osmotic adjustment (Kader and Jutzi 2002, Kader et al. unpublished data). This adjustment would render the axis capable of taking up more water from the surrounding medium, thus providing both plumules and radicles of treated seeds with an advantage over untreated counterparts.

While advancement in the speed of germination was obtained by thermally inducing seeds on the 2nd and 3rd days of imbibition, the FGP is postulated to be less "improvable" by acclimation than the MGT. However, the positive effects of thermal induction may not be ruled out since 2nd and 3rd day-treated seeds germinated faster and gave a higher PRR. We take this as evidence that the ratio between shoot and root is governed by the environmental conditions surrounding the seed. We speculate that stress tends to lead to larger radicles resulting in a lower PRR. This stress may take the form of limited moisture, high temperature or osmotic pressure, but in all three cases the reaction on part of the plant would be to enhance root growth in the search for moisture at lower soil profiles. This is reinforced by the fact that untreated seeds had a higher PRR than 6 g NaCl-treated seeds. This may suggest that untreated seeds, because not exposed to "stress" caused by NaCl treatment, "sensed" that no stress existed and thus focused on plumule elongation to emerge and make use of this "non-stress opportunity" (Kader and Jutzi 1998a). Seeds treated with salt, on the other hand, responded by increasing radicle elongation leading, in turn, to a lower PRR.

If the same were true for heat, it would mean that seeds acclimated to heat stress by pre-exposure to 45°C on the 2nd or 3rd day of imbibition did not "sense" stress when they were transferred to a 12-hours-a-day regime of 42°C. They would have been acclimated to such a temperature range through hormonal signals (Kader 2001) and thus the need for producing larger radicles would diminish, leading to a higher PRR. Such gradual acclimation of seeds to high temperatures has been reported in cowpea by El-kholy et al. (1997). Gong et al. (1997), working on maize, also presented evidence that plants have the capacity to acquire thermotolerance when they are prehardened at an elevated but nonlethal temperature. It can be argued that the function of such a treatment would be to activate so-called "Heat-Shock Proteins (HSPs)" (Kader and Jutzi 1997, Kader and Jutzi 1998b, Kader 2001). In studies with soybean, Jinn et al. (1997) found that in seedlings treated with 40°C for 2 h, low molecular weight HSPs were found in aggregated granular structures distributed in the cytoplasm and nucleus. These may assist in the resolubilization of proteins denaturated or aggregated by heat and may also participate in the restoration of organular functions after heat shock.

Abernethy et al. (1989) found that during the initial 9–12 h of imbibition, imbibing wheat cv. Lancer and Guard seed exhibited substantial tolerance to high temperature. This initial tolerance gradually declined with increasing time of seed imbibition. This timing of tolerance is shifted well into the later phases of germination in pearl millet (Carberry and Campbell 1989). The most responsive time to acclimate seeds was observed on the 2nd and 3rd days after soaking. We assume that the seed's inner mechanisms are responsive to external heat impulses during this period or else acclimation at this time would not have advanced subsequent germination (Kader 2001).

During the 1st day of soaking a seed is being forced out of its former dry, quiescent state into a metabolically and physiologically active one (Gong et al. 1997). During this imbibition phase it would not be reasonable to expect response from the seed because it is still in the process of fulfilling the threshold level of moisture needed to initiate this active form (Kader and Jutzi 2002). By the 2nd and 3rd days of soaking the seed is active enough to respond to external factors. From a practical standpoint, despite reports of air temperatures reaching 44.9°C in sorghum growing areas (Maiti 1996), soil temperatures within the vicinity of the seedbed would not stay at these levels for long, and such extreme cases are the exception rather than the rule (Kader 2002c). Finally, reflections from controlled-environment studies such as this are a potentially useful tool in screening for variation in response to temperature (Carauford et al. 1996), but field validation of results obtained is required to advance the application of recommendations.

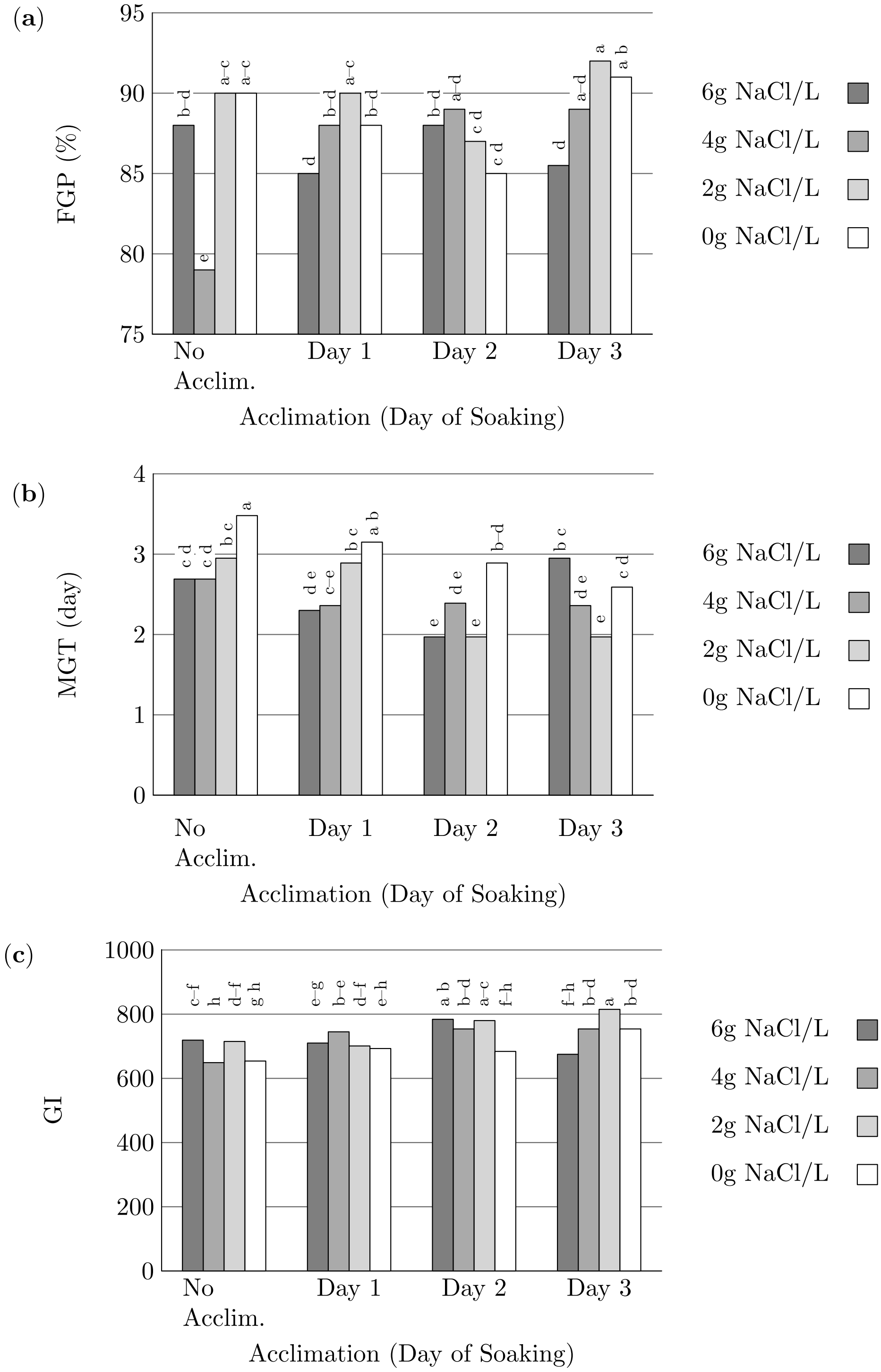

Figure 1. Interactive effects of NaCl-based seed priming treatment and heat induction on (a) the final germination percentage (FGP), (b) mean germination time (MGT) and (c) germination index (GI) of sorghum SPV 462 seeds. Bars having similar letters represent means that are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

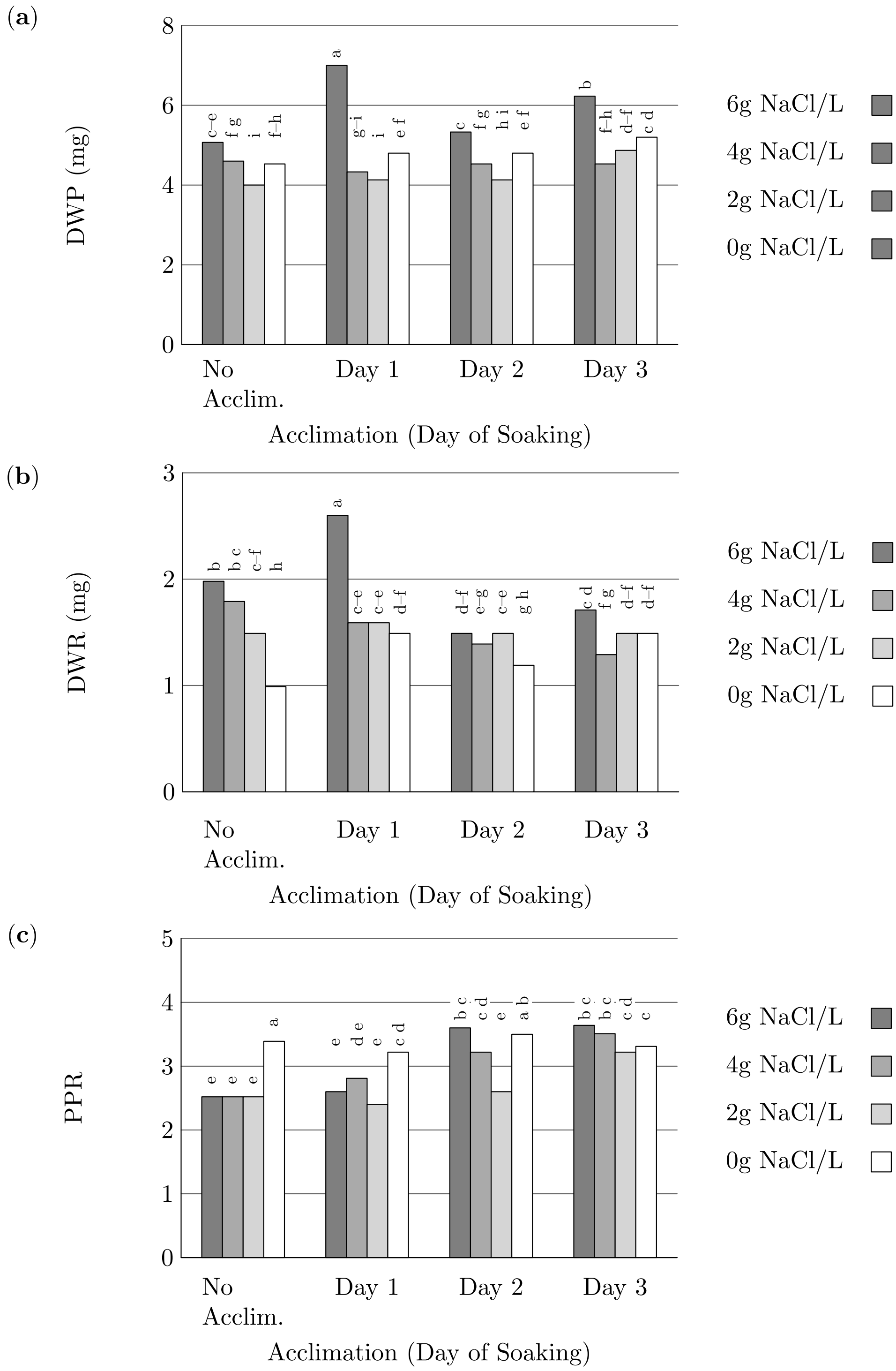

Figure 2. Interactive effects of NaCl-based seed priming treatment and heat induction on (a) the dry weight of plumule (DWP), (b) dry weight of radicle (DWR) and (c) plumule:radicle ratio (PRR). Bars having similar letters represent means that are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

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