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Pre-germination hydration affects seed performance in bambara groundnut

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In a study to determine the response of local bambara groundnut (*Vigna subterranea* (L.) Verdc.) germplasm to seed enhancement, seeds were subjected to hydration (12, 75 and 93% RH) to influence pre-germination water activity (a_w) and subsequent germination and seedling establishment. This study also reports on seedling characteristics 28 days after sowing under a controlled environment at 75% field capacity. There were highly significant ($P < 0.01$) differences between hydration treatments. The low RH resulted in a_w ranging from 0.06, 1 h after suspension to 0.39, 72 h after suspension, whereas the 93% RH resulted in a_w ranging from 0.2, 1 h after suspension to 0.68, 72 h later. Subjecting seeds to pre-germination hydration caused a significantly improved seed imbibition ($P < 0.05$) (up to 40%), germination (35%) ($P < 0.05$) and seedling establishment ($P < 0.05$) (as indicated by seedling size and utilization of seed storage tissue). It is concluded that the delay in seedling establishment due to lack of pre-germination hydration or low RH (12%) is linked to the observed collapse of seedling leaf stomata. Hence, the findings of this study have a bearing on drought tolerance.

Key words: Hydration, germination, seed, water activity.

INTRODUCTION

The world has a large number of underutilized crops that have a potential to contribute to food security (Mayes et al., 2011). The contribution of these crops to food security is undermined by lack of studies that explain their physiological and genetic characteristics. Recent efforts are biased towards breeding, when there is a need for multidisciplinary work to include seed physiology, crop protection and food science, among others. Bambara groundnut (*Vigna subterranea* L. Verdc) is an indigenous African leguminous crop grown primarily for its seeds. It is increasingly becoming popular as a food source in rural areas across the African continent (Abu and Buah, 2011; Nyongesa et al., 2013). The crop has potential to provide food security in dry areas of Africa (Smith, 2006) and has been identified as a drought tolerant crop that can produce reasonable yields where other crops, such as groundnut, fail (Harris and Azam-Ali, 1993). A global mapping system for Bambara groundnut was produced by Azam-Ali et al. (2001). In that study, it was revealed that Bambara groundnut has the advantage of being able to grow under harsh environmental conditions in the dry areas of Africa. Compared to other legumes, bambara groundnut has a high protein yield of 16 to 25% in seeds and is the third most preferred legume food crop in many areas where small-scale farmers already grow it (Linnemann, 1990; Brough and Azam-Ali, 1992), after peanuts (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Sellschop, 1962). It can also be used in various formulations and can play an important role in protein supply to rural populations, hence alleviating malnutrition (Nti and Plahar, 1995; Massawe et al., 2002). As such, bambara groundnut has a potential to provide

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food security in dry areas of Africa. With the potential risk of drought associated with climate change, drought tolerance is likely to become even more important in African agriculture. However, despite it being a drought tolerant crop, bambara groundnut remains an underutilized crop owing to limited research that has been done on the crop.

What is encouraging is that there have been simulating models to investigate the agronomic potential of agricultural crops in ecological contexts (Uehera and Tsuji, 1993; Penning de Vries and Teng, 1993; Bannayan and Crout, 1999). These models, however, are focused on dominant food crops, and less work has been done on underutilized food crops. One of the major reasons for lack of models on underutilized crops is lack of data on different physiological and agronomic aspects, including seed science and technology.

Seed treatments have been shown to improve germination capacity and crop stand establishment (Mabhaudhi and Modi, 2011). Manipulation of the moisture and temperature environment where seeds will germinate and grow has also been shown to affect seed performance (Zulu, 1989). The semi-arid regions where bambara groundnut is a dominant crop (Azam-Ali et al., 2001) require that seed treatments to improve germination and seedling establishment take place prior to planting. It is important that these treatments are environmentally friendly and link to water use during crop establishment and beyond. The objectives of this study were to determine the response of a bambara groundnut landrace targeted for drought tolerance to pre-germination treatment with saturated salts as established by the international rules for testing seeds. Further, the seedling establishment capacity of the seeds was determined.

MATERIALS AND METHODS

Study design

A completely randomized design was used in an experiment where the treatment structure consisted of three saturated salt-derived humid environments. Components of the Y-variate included changes in water activity, seed imbibition, seed germination, seedling establishment and seedling size. The block consisted of three replications for all measurements.

Plant material and seed treatment

Seeds of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) were collected from smallholder subsistence farms of Jozini, KwaZulu-Natal (27° 26' 0" South, 32° 4' 0" East), South Africa. Seeds of similar dry mass were surface sterilised and their moisture content was determined to be 11% ± 3. Saturated salt solutions of $LiCl.H_20$ (12% RH), NaCl (75% RH) and KNO_3 (93% RH), respectively, were prepared according to the Association of Official Seed Analysts (AOSA, 1992). Seeds were suspended over the different solutions for 72 h to influence their water potential, which was measured as water activity (a_w), and germination was determined, according to the International Seed Testing Association (ISTA, 2011).

Seedling establishment and electron microscopy

Subsequent to seed treatment to influence water potential, seeds were planted under controlled environment conditions (27/21°C day/night, 60% RH) to grow seedlings in 17-cm round plant pots (2 litres) containing fine sand (90.57 cm^2 g^{-1}). Water content in the pots was maintained at 75% field capacity (Zulu and Modi, 2010). Twenty eight days after sowing seedlings were harvested to determine establishment and dry mass. Seedling leaf stomatal appearance and mineral content were determined using X-ray diffraction (Modi et al., 2004).

Statistical analysis

Data were analysed using Genstat® version 14. Statistical differences were accepted at P ≤ 0.05. Standard errors of were used to indicated differences.

RESULTS

Seed hydration treatments resulted in significantly (P < 0.05) different water activity levels between salts (Figure 1). The key finding in this respect was that the lowest water activity was observed in seeds that were subjected to 12% RH, and the highest was found in seeds subjected to 93% RH (Figure 1). The low RH resulted in a_w ranging from 0.06, 1 h after suspension to 0.39, 72 h after suspension, whereas the 93% RH resulted in a_w ranging from 0.2, 1 h after suspension to 0.68, 72 h later. The 75% RH treatment resulted in a_w values that were between the low RH and the high RH treatments (Figure 1). This finding was associated with low RH causing a slow hydration, whereas higher RH treatments caused rapid imbibition (Figure 2). Consequently, seedling germination (Figure 3) and emergence (Figure 4) in response to 12% RH treatment were significantly (P < 0.05) delayed and less, compared with 75% RH and 93% RH treatments. Seedlings derived from 12% RH treatment were significantly (P < 0.01) smaller by ~8% and ~15%, respectively, compared with those derived from 75% RH and 93% RH seed treatments (Figure 5). The 12% RH caused pre-germination stress that led to possibly delayed utilisation of seed reserves for seed germination and seedling establishment as indicated by the large seed remnant due 12% RH treatment and no visible seed remnant due to 93% RH treatment (Figure 5).

This study showed that pre-hydration treatment of seeds before germination and planting influences seed performance in terms of seedling emergence, growth and possible response to water stress. Further investigations into the appearances of stomata on the leaves of seedlings derived from 12% RH and 93%RH treatments, respectively (Figure 6) indicated that the delay in hydration has positive effects of protecting seedlings from water stress damage during later stages of seedling

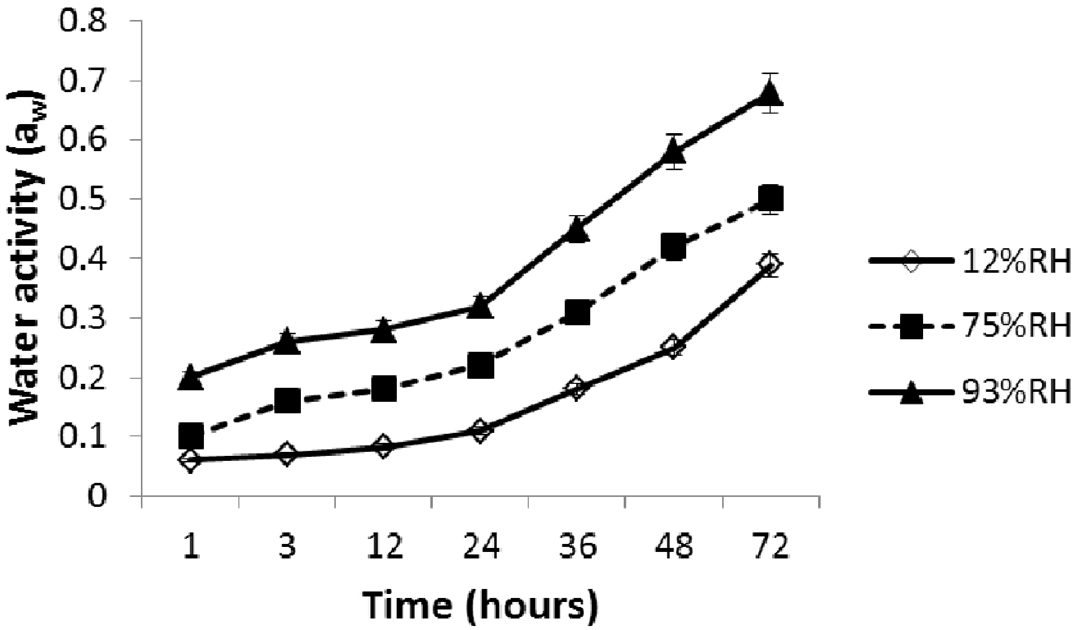

Figure 1. Changes in water activity of bambara groundnut seeds during pre-germination hydration in containers with different air relative humidity using saturated salt solutions (12%RH = $LiCl.H_2O$; 75% RH = NaCl and 93% RH = KNO_3) over 72 h.

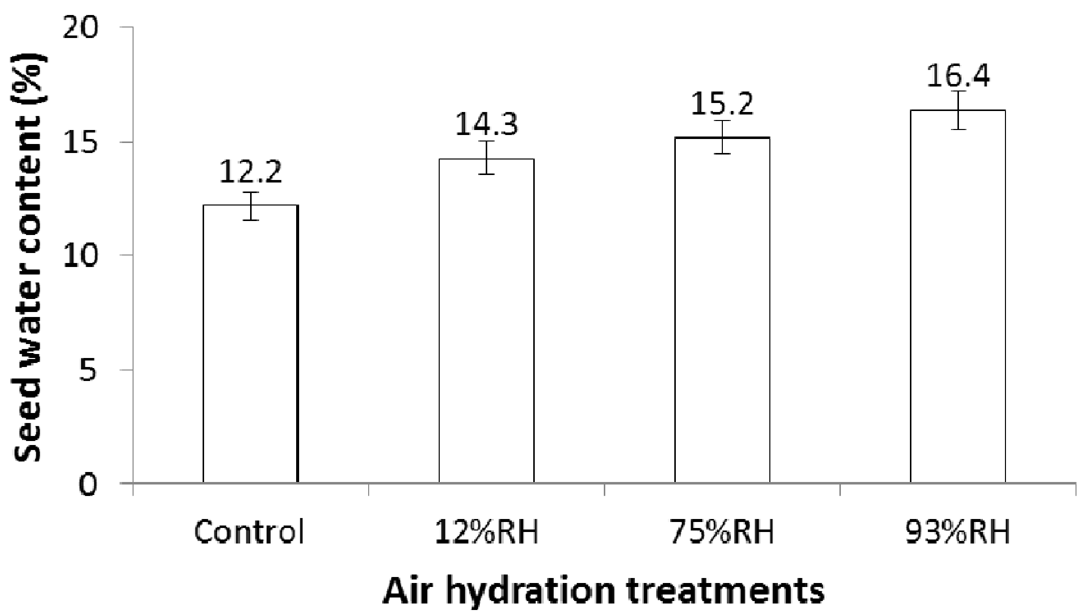

Figure 2. Bambara seed water content following pre-hydration by suspension over different relative humidity environments caused by $LiCl.H_2O$ (12%); NaCl (75% RH) and KNO_3 (93% RH). Control seeds were suspended over dry air (50% RH).

growth. The future direction for this study will also be targeting stomata for determination of mineral elements known to dominate osmolytes during water stress (for example, Ca, K, Cl, Mg and Al), chlorophyll content and stomatal activity in response to seed treatments. Hoai et al. (2003) and Liu and van Staden (2000) suggested that the biochemical response of plant cells to osmotic tress is the accumulation of organic and mineral osmolytes,

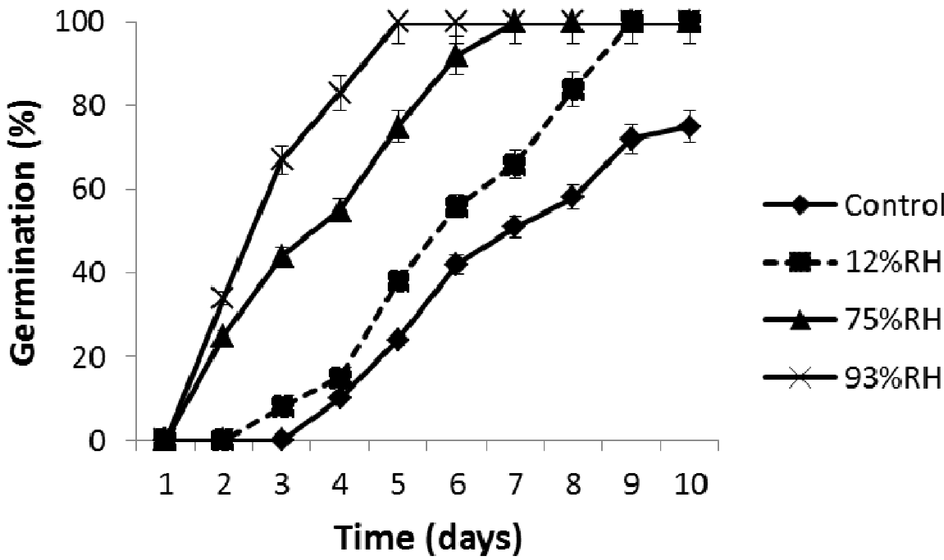

Figure 3. Bambara seed germination following pre-hydration by suspension over different relative humidity environments caused by $LiCl.H_2O$ (12%); NaCl (75% RH) and KNO_3 (93% RH). Control seeds were suspended over dry air (50% RH).

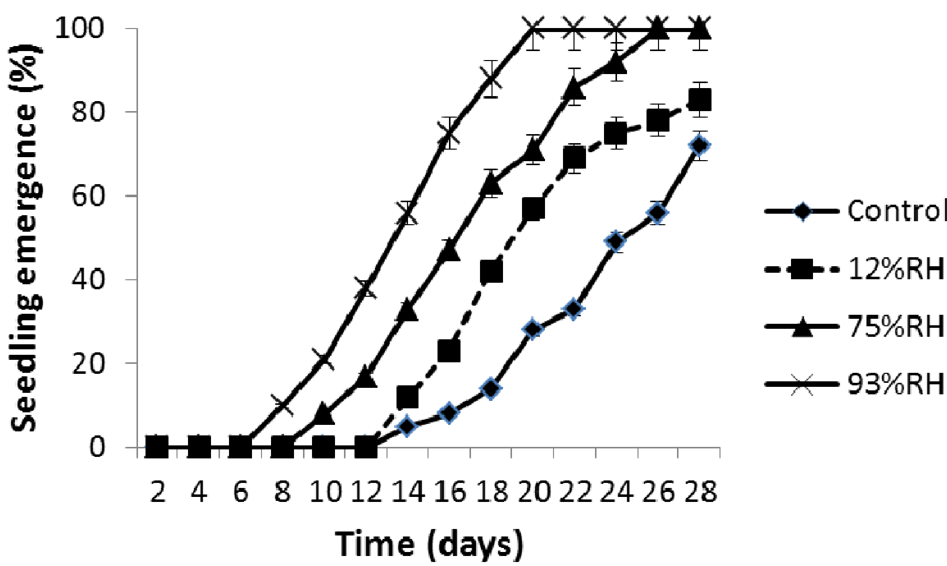

Figure 4. Bambara seedling establishment following pre-hydration by suspension over different relative humidity environments caused by $LiCl.H_2O$ (12%); NaCl (75% RH) and KNO_3 (93% RH). Control seeds were suspended over dry air (50% RH).

which act as osmoprotectants to prevent cell damage. However, this response has not been demonstrated to be associated with seed treatment.

DISCUSSION

Seed hydration has been known as an important vseed treatment to improve seed performance for a long time (Hegarty, 2006). Subjecting seeds to various levels of water stress has been suggested to affect the physiological processes linked to viability and dormancy (Ching, 1972; Hegarty, 2006). It has also been indicated that these processes have a direct linkage to seedling growth and development (Hegarty, 1977, 2006).

In this study there was a clear pattern of change in

12% RH Control 75%RH 93%RH

Figure 5. Effect of pre-hydration over saturated salts (control = dry air; 12%RH = $LiCl.H_20$; 75% RH = NaCl and 93% RH = KNO_3) on bambara groundnut seedling size 28 days after emergence.

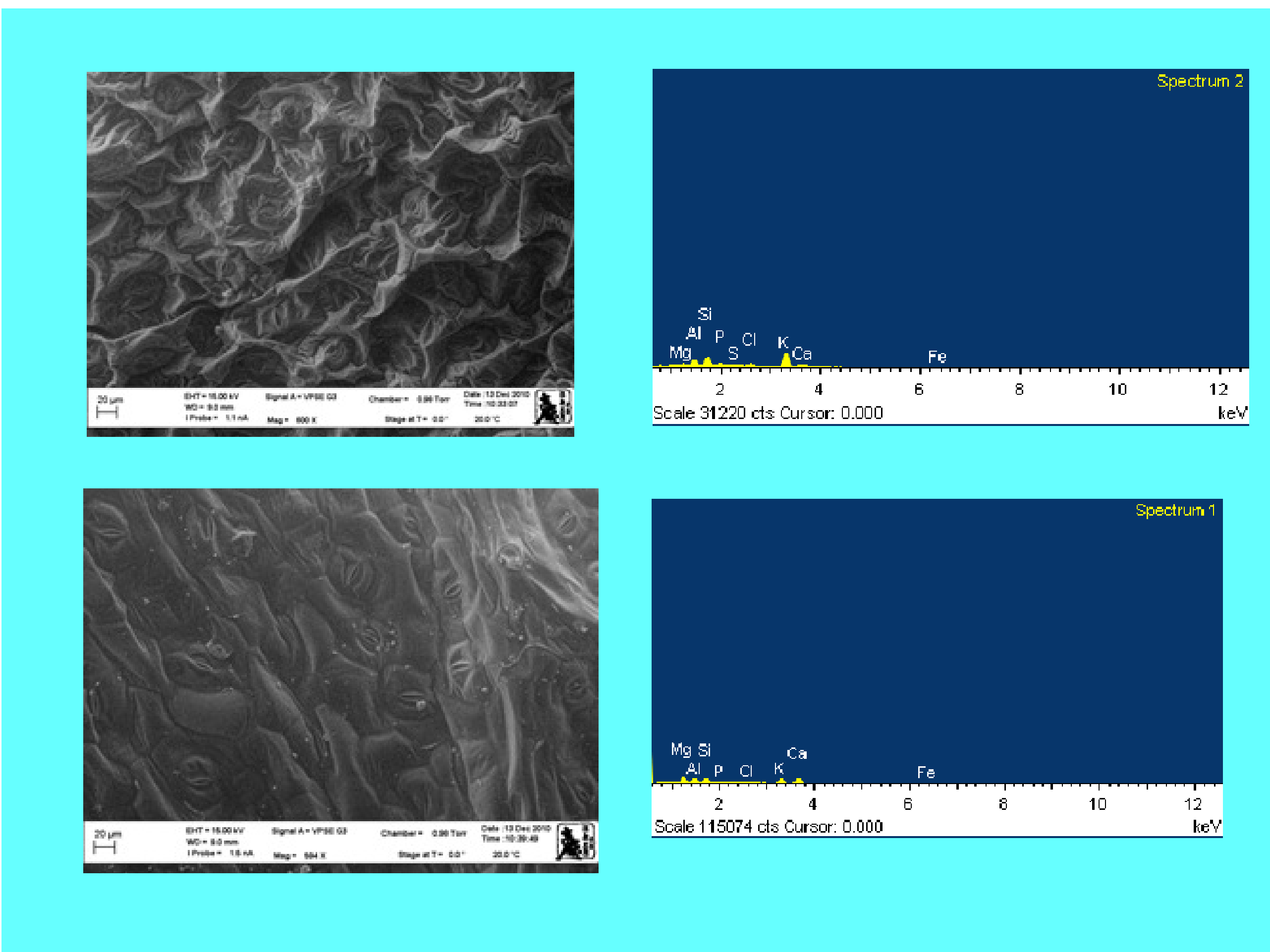

Figure 6. Effects of pre-hydration with 12% RH (top) and 90% RH (bottom) on stomatal appearances of upper leaf surfaces of bambara groundnut seedlings 28 days after planting.

water activity during imbibition (Figure 1). Nevertheless, the pattern of change was similar across water availability treatments. This finding, when linked with the results shown in Figures 2 and 3 was consistent with the accepted three-phase germination pattern in all seeds as explained by Ching (1972). Seed germination pattern is characterized by (i) the imbibition phase when macromolecules and organelles inactively absorb water, (ii) steady water uptake when respiration and biogenesis occur and (iii) cell division and growth with continuous respiration and increase in fresh weight happening simultaneously.

Bambara groundnut seeds responded quickly to pre-hydration and showed that 93%RH as a seed treatment can improve seedling emergence by 20% within 12 days. Under conditions of water stress, which may be accompanied by sodicity (12%RH), this positive effect can be doubled (Figure 4). In a study to determine the agronomic and physiological performance of bambara groundnut in southern Nigeria, Mudukwe et al. (2011) concluded that the crop is sensitive to water stress. Therefore, any treatments that can alleviate the negative effects of water stress on seedling establishment and subsequent plant growth should have a significant impact (Ferreras and Galetto, 2010).

It is concluded that seed pre-hydration enhances imbibition and germination capacity in bambara groundnut. Consequently, seedling establishment that is linked to rapid utilization of storage reserves occurs early during crop establishment (Figure 5). The findings of this study have a bearing on drought tolerance (Figure 6), a subject that requires further investigation.

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