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*Full Length Research Paper*

# Influence of flooding on bambara groundnut (*Vigna subterranea* L.) germination: Effect of temperature, duration and timing

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**To evaluate the response of bambara groundnut seeds to flooding stress a laboratory study (experiment 1) and a greenhouse study (experiment 2) were conducted. In the laboratory study seeds of a brown-coloured local bambara groundnut landrace were completely immersed in distilled water (pre-sowing soaking) for 2, 4, 6 and 8 d in an incubator at 20, 25, 30 and 35°C. In the greenhouse study flooding stress was imposed on seeds of the uniswa red landrace at 1, 3, 5 or 7 d after start of imbibition. In experiment 1 pre-sowing soaking enhanced germination rate, but final germination percentage decreased drastically as the duration of soaking increased beyond 2 days. There was a significant flooding duration x temperature interaction on final germination percentage. The germination ability of bambara groundnut seeds was reduced by 60 and 80% when seeds were soaked for 6 days at 20 and 30°C, respectively, and a complete loss in germination occurred when seeds were soaked for 6 days at 35°C, and for 8 days at all temperatures used in the study. However, seeds germinated well (68%) even after 6 days of soaking at 25°C. In experiment 2, flooding bambara groundnut seed for 1 or 12 h at 1, 3, 5 or 7 days after start of imbibition did not reduce germination percentage significantly. However, flooding at any time for 24 or 48 h significantly reduced germination percentage, compared with non-flooded seed. These results suggest that, at least for the two landraces used in this experiment, short-term flooding of fields during the germination phase of bambara groundnut is detrimental to germination and uniform emergence, but that the response is influenced by the duration of flooding, temperature, germination stage and the interaction between flooding duration and temperature.**

**Key words:** Bambara groundnut, *Vigna subterranea*, seed flooding tolerance, pre-sowing hydration, germination.

## INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), is a legume crop that is widely cultivated in much of sub-Sahara Africa (Stephens, 1994), especially in semi-arid regions where the seeds are considered a healthy component of the diet (Linnemann and Azam-Ali, 1993). It provides a significant source of protein and carbohydrates to rural populations and supplementary income to subsistence farmers (Sesay et al., 1999; Hampson et al., 2000). However, the crop is cultivated from landraces, and farm yields are low and unpredictable (Linnemann and Azam-Ali, 1993; Sesay et al., 1999). Germination or seedling emergence in Bambara groundnut is often erratic and variable. The problem of unpredictable yields has been attributed, at least in part, to variable or poor field establishment due to poor germination and/or seedling emergence (Linnemann and Azam-Ali, 1993).

Since bambara groundnut is grown throughout much of sub-Sahara Africa the crop is thus often exposed to a wide range of local conditions. In addition to seedbed conditions, water level is an important factor affecting the emergence and development of seedlings (Pollock, 1972). In the semi-arid regions soil dryness, associated with high temperatures, is probably the most important factor affecting seed germination and emergence. However, short-term flooding at sowing, caused either by poorly drained soils or heavy rainstorms, and lasting for a number of hours or days could be another major factor. Reports in the literature indicate that seeds of many crop species are sensitive to excessive water or flooding stress during germination (Wuebker et al., 2001; Sung, 1995; Cerwick et al., 1995; Hou and Thseng, 1991; Lagan et al., 1986; Fausey and McDonald, 1985; Duke and Kakefuda, 1981, Wenkert et al., 1981; Pollock, 1972). In soybean (*Glycine max* L.) pre-emergence flooding stress may result in 50 to 100% reductions in emergence (Sung, 1995). Hou and Thseng (1991) indicated that soybean cultivars experienced severe losses in germination when soaked for 4 days prior to germination. Langan et al. (1986) observed that flooding for 3 days starting 1 day after planting delayed emergence of corn (*Zea mays* L.), soybean, and wheat (*Triticum aestivum* L.).

Studies have strongly established the central role of duration of flooding and temperature and their interaction in determining the effect of flooding stress on germination and establishment of seedlings. Massawe et al. (1999) reported that pre-sowing hydration reduced final germination percentage in bambara groundnut landraces, but only after 72 h of hydration; while in one landrace increasing hydration time from 0 - 72 h resulted in a consistently greater germination percentage. Wuebker et al. (2001) indicated that the duration of flooding stress interacts significantly with germination temperature for germination percentage. They (Wuebker et al., 2001) indicated that flooding for 1 h at 15°C lowered germination percentage by over 20 percentage points compared with the non-flooded control, but it took approximately 36 more hours of flooding for seed at 25°C to experience the same loss in germination. However, earlier studies suggested that flooding at warmer temperatures caused greater losses in seedling emergence compared with flooding at lower temperatures. Hou and Thseng (1991) reported that soaking soybean seeds for up to 8 days at 10 and 15°C prior to germinating the seed caused no loss in germination, but that germination decreased as the duration of soaking increased at 25 and 30°C. Harfield and Egli (1974) found that at 10°C, soybean hypocotyl elongation was extremely slow and that the rate of hypocotyl elongation reached a maximum at 30°C.

There is no available information on the effect of flood-ing on bambara groundnut seed that have begun to im-bibe water or that have just started germinating. Such information may offer insights into critical times of seed susceptibility and be useful in predicting bambara groundnut germination and establishment. Wuebker et al. (2001) observed little differences in germination percentage of soybean seeds among flooding duration treatments when flooding began 1 d after the start of imbibition. When seeds were flooded 2 or 3 d after start of imbibition, however, a significant drop in germination percentage occurred in seed flooded for 48 h. Flooding seed 3 days, compared with 1 day, after the start of imbibition was more detrimental, regardless of temperature.

In Botswana the climate is semi-arid, with temperatures during the summer cropping season rising to 38°C and higher. Few studies have been conducted to study the response of bambara groundnut seed to flooding stress. Thus, the objective of this experiment was to characterize the response of bambara groundnut seed to flooding by investigating the effect of flooding duration, temperature and timing.

## MATERIALS AND METHODS

Two experiments were conducted; Experiment 1 in a laboratory environment and Experiment 2 in a greenhouse environment, at the Botswana College of Agriculture, Gaborone, in 2008. A brown seeded local landrace was used in the laboratory study, and the Uniswa red landrace (red seeded from Swaziland) was used in the greenhouse study. Prior to the start of the experiments seeds were selected visually for uniform size and the quality of the seeds was established by conducting a warm germination test according to "Rules for Testing Seeds" (AOSA, 1999). The initial germination percentage ranged from 90 to 100. Seeds were not surface sterilized, but fungal growth was minimal. The initial moisture content of the seeds was 7.7 (s.e. 0.56)% and 8.6 (s.e. 0.65)% for the brown and uniswa red landraces, respectively. The seed moisture content was measured by oven-drying at 105°C for 28 h and expressed as a percentage of the oven dry weight.

### Experiment 1: Effect of temperature and duration of pre-germination soaking

Seeds of a brown-coloured local bambara groundnut landrace were soaked by complete immersion in distilled water for 2, 4, 6, and 8 days in an incubator at temperatures of 20, 25, 30 and 35°C. After soaking, seeds were dried on filter paper overnight (18 to 20 h). Then 30 dried seeds were uniformly distributed on two layers of filter paper in 9 cm Petri dishes, moistened with 10 ml of water. The Petri dishes were kept at 25°C in an incubator for germination. The number of germinated seeds in each dish was recorded every 24 h, with the last determination at 168 h (7 days) after the initiation of the experiment. Seeds with radicle 2 - 3 mm long were regarded as germinated. The complete experiment was performed three times, to give three replications. The treatment combinations were completely randomized with respect to the placement of the Petri dishes in the incubator.

### Experiment 2: Effect of timing and duration of flooding on germination and seedling emergence

Seeds of the Uniswa red bambara groundnut landrace were planted in plastic tubs measuring 12 cm in length, 10 cm in width, and 10 cm in depth. Four holes were drilled in the bottom of each tub. A layer of cotton wool was placed in the bottom of each tub to prevent blocking of the holes by sand particles. The cotton wool was covered with a layer of sand. Thirty seeds were planted and pressed into the sand. The seeds were covered uniformly with a layer of sand to a depth of 3 cm. The tubs were left overnight on benches to allow the sand and seed to equilibrate to the greenhouse temperature (28 ± 2°C). Each tub, including the control treatments, was watered with 70 ml of tap water, and covered with aluminum foil. Flooding treatment was imposed on individual tubs at 1, 3, 5 or 7 d after start of imbibition. The seeds were flooded by placing the tubs in large plastic tubs to submerge the tubs to a depth of 5 cm with tap water. The tubs were totally submerged in water for periods of 1, 12, 24 and 48 h. The water level was main-

**Table 1.** Effect of duration of soaking and temperature on germination of bambara groundnut seeds.

| Flooding duration | Temperatures (°C) | | | |
|---|---|---|---|---|
| | 20 | 25 | 30 | 35 |
| Days | Germination (%) | | | |
| 2 | 85.0b* | 97.8a | 86.7b | 63.3b |
| 4 | 35.0c | 80.0b | 75.0b | 58.3b |
| 6 | 36.0c | 67.8c | 16.7c | 0.0c |
| 8 | 6.7d | 0.0d | 0.0c | 0.0c |
| Non-soaked control | 96.7a | 96.7a | 96.7a | 96.7a |
| SE | 3.6 | 3.6 | 3.6 | 3.6 |

*Test of significance performed on arcsin √x +1 transformed data. Means followed by the same letter within a column are not significantly different at P = 0.05.

**Table 2.** Effect of timing and duration of flooding stress on mean germination percentage of bambara groundnut seeds.

| Duration of flooding h | Time after start of imbibition | | | |
|---|---|---|---|---|
| | 1 d | 3 d | 5 d | 7 d |
| | Germination % | | | |
| 1 | 96.7a† | 96.7a | 98.3a | 100.0a |
| 12 | 97.9a | 96.7a | 96.7a | 100.0a |
| 24 | 59.2b | 68.3b | 13.3b | 70.0ab |
| 48 | 34.6c | 30.0c | 3.3b | 51.7b |
| Non-flooded control | 92.8a | 93.8a | 95.8a | 94.2a |

†Test of significance performed on arcsin √x +1 transformed data.Means followed by the same letter within a column are not significantly different at P = 0.05.

tained by addition of tap water as required. Following the flooding treatment, the tubs were returned to the greenhouse benches. For the control treatment, the seeds were watered with 70 mL of water, but no flooding stress was imposed. Daily emergence counts were taken, with emergence defined as the number of seedlings with first true leaves appearing above the sand surface.

### Statistical analysis

To determine the effect of each treatment, Analysis of variance was performed on the data using STATISTIX 8 for windows (Analytical Software, Tallahassee, Florida. USA), Final germination data as percentage of number of seeds treated were arcsin-transformed prior to statistical analysis (Fowler et al., 1998). The least significant difference (LSD) test was used to compare treatment means. Statistical significance was determined at $P < 0.05$.

## RESULTS

### Experiment 1

The results from experiment 1 (Table 1, Figures 1 – 3) show a significant reduction in bambara groundnut seed germination in response to increasing duration of soaking, and a significant interaction between the duration of soaking and the temperature of soaking for germination percentage. Bambara groundnut seeds soaked for 2 and 4 days germinated over the range of temperatures used in this study (20, 25, 30 and 35°C), with optimum germination (97.8 and 80.0%) occurring at 25°C (Table 1). Soaking bambara groundnut seeds for 2 days at 25°C did not affect final germination percentage significantly, but significantly reduced germination at 20, 30 and 35°C. The germination ability of bambara groundnut seeds was reduced by 60 and 80 percentage points when seeds were soaked for 6 days at 20 and 30°C, respectively (Table 1, Figure 1), and a complete loss in germination occurred when seeds were soaked for 6 days at 35°C, and 8 days at all temperatures used in the study (Table 1, Figure 3). Pre-sowing hydration enhanced the rate of germination (Figures 1 - 3), with the fastest rate recorded following 2 days of pre-sowing soaking at all temperatures. Enhancement following 6 days of soaking occurred only at 25°C, with no enhancement following 8 days of soaking.

### Experiment 2

The results from experiment 2 are presented in Table 2 and Figures 4 - 6. The results show a significant interaction between timing and duration of flooding stress for final germination percentage. For seeds flooded at 1, 3, 5 or 7 days after start of imbibition no reduction in germination percentage was observed for the 1 and 12 h flooding duration treatments (Table 2). However, flooding at any time for 24 or 48 h significantly reduced germination percentage, compared with non-flooded seed. The average decrease in germination percentage for 24 and 48 h of flooding at 1 day after start of imbibition was 34 and 58 percentage points, respectively; and 83 and 92 percentage points, respectively, for flooding at 5 d after start of imbibition. Flooding at 7 days after start of imbibition, for 24 and 48 h, reduced germination percentage only by 34 and 43 percentage points, respectively.

An interesting result of the present study was that flooding at 1 - 7 days after start of imbibition for 1 or 12 h significantly enhanced the rate of germination, compared with the non-flooded seed (Figures 3 - 6).

## DISCUSSION

The successful performance of a crop in the field demands that the seeds germinate and establish sufficient plants. Germination occurs only after seed imbibition of sufficient water has occurred to reactivate growth in the seed. The aim of the study was to evaluate the response of bambara groundnut seeds to excessive water during germination, and to determine the influence of temperature, duration and timing of flooding. The results clearly indicate that increasing the duration of flooding stress significantly lowered germination percentage of bambara groundnut seed compared with the unsoaked or non-flooded seed. The results also indicate a significant inter-

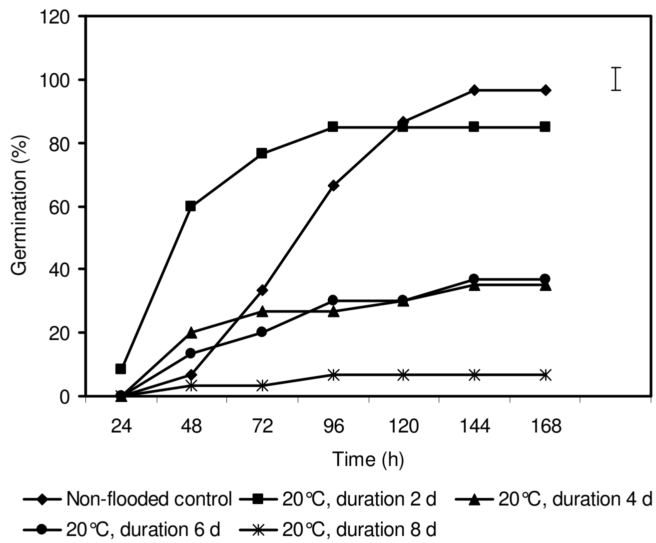


**Figure 1.** The effect of duration of soaking at 20°C on the time course of germination of bambara groundnut seeds. Error bar represents the SE of the difference between two means (n = 24).

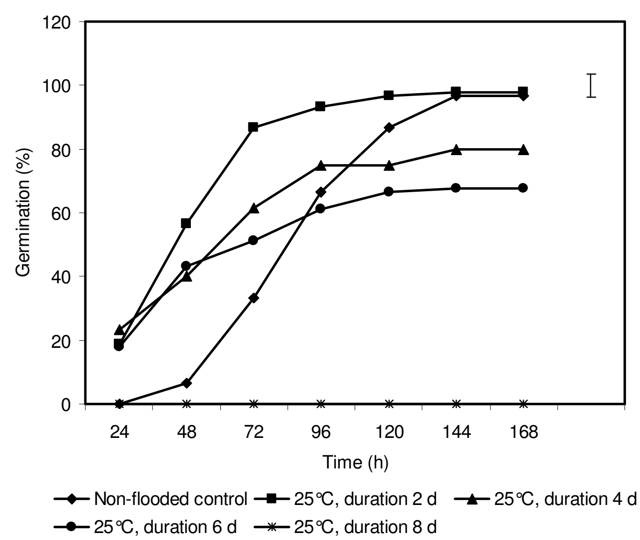


**Figure 2.** The effect of flooding duration at 25°C on the time course of germination of bambara groundnut seeds. Error bar represents the SE of the difference between two means (n = 24).

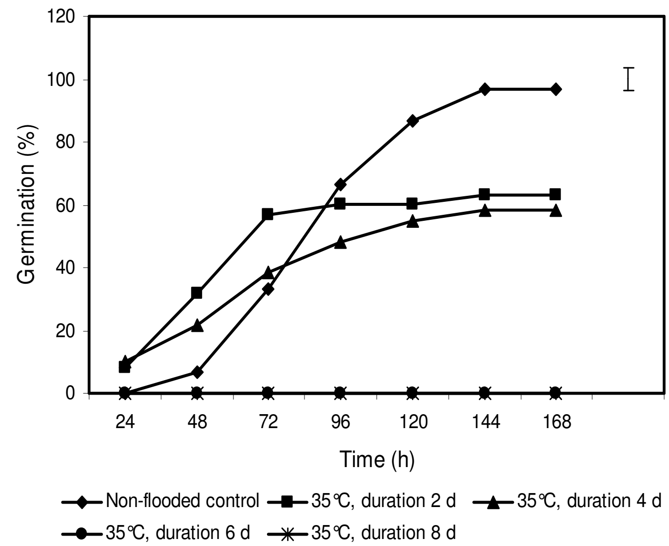


**Figure 3.** The effect of flooding duration at 35°C on the time course of germination of bambara groundnut seeds. Error bar represents the SE of the difference between two means (n = 12).

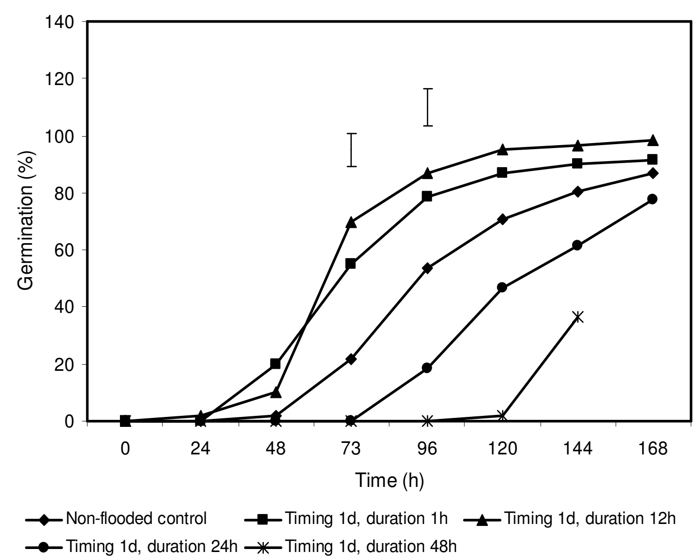


**Figure 4.** The effect of flooding at 1 day after start of imbibition on the time course of germination of bambara groundnut seeds. Vertical bars indicate standard error of the mean.

action between the duration of soaking and the temperature of soaking for germination percentage. Soaking bambara groundnut seeds for only 2 days has the potential to cause significant losses in germinability, depending on the temperature of soaking. The germination ability of bambara groundnut seeds was drastically reduced when soaked for 6 days at 30°C, and completely damaged when they were soaked for 6 days at 35°C and 8 days at 20, 25 and 30°C. These findings, however, were not consistent with those of Massawe et al. (1999). They reported that pre-sowing hydration of bambara groundnut seeds at 27°C reduced final germination percentage only after 72 h of hydration; and in one landrace, increasing hydration time from 0 to 72 h resulted in a consistently greater germination percentage. Possibly air-drying soaked seeds on filter paper overnight (18 to 20 h) caused the difference in the pattern of germination response in this study compared with the results of Massawe et al. (1999). Emmerich and

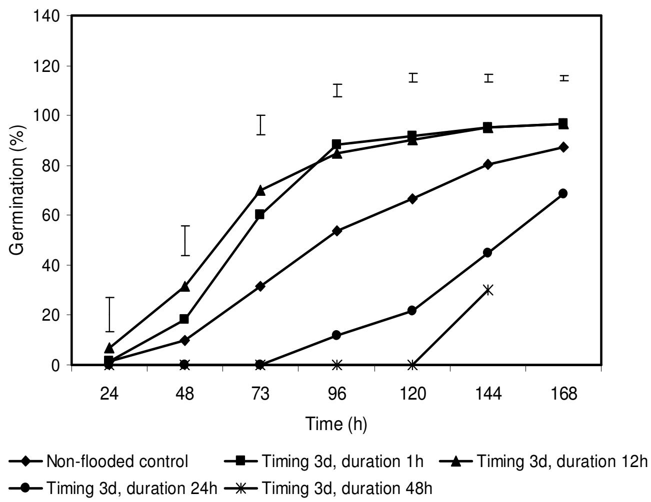


**Figure 5.** The effect of flooding at 3 days after start of imbibition on the time course of germination of bambara groundnut seeds. Vertical bars indicate standard error of the mean.

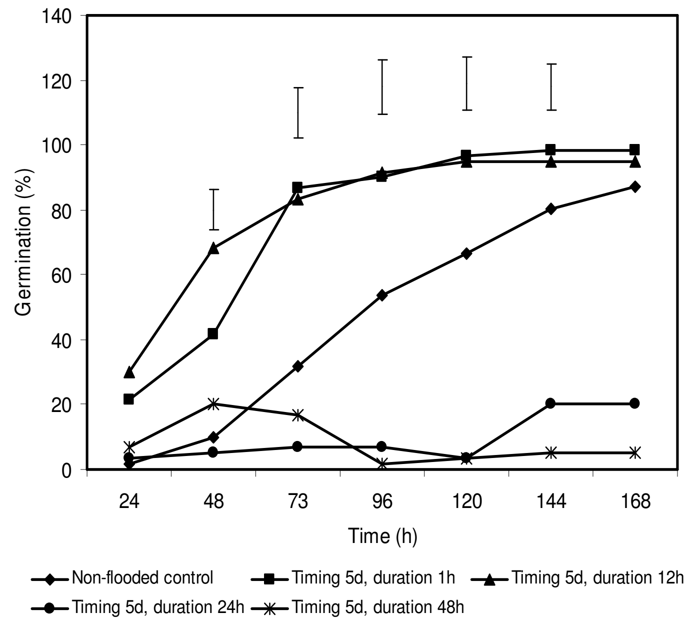


**Figure 6.** The effect of flooding at 5 days after start of imbibition on the time course of germination of bambara groundnut seeds. Vertical bars indicate standard error of the mean.

Hardegree (1996) reported a trend of reduced germination with increasing length of air-dry dehydration in warm-season grass seeds. However, the results generated in this study are in agreement with earlier findings in other crop species (Hou and Thseng, 1991; Fausey and McDonald, 1985). Hou and Thseng (1991) indicated that germination of soybean seeds decreased as the duration of soaking increased after 2 d of soaking at 25 and 30°C.

A notable result of the present study is the relative tolerance to flooding observed when the seeds were soaked at 25°C. Seeds germinated well (68%) even after 6 days of soaking at 25°C. The reason for this response was not readily clear, but the response is consistent with results reported in barley (Takeda and Fukuyama, 1987) and soybeans (Hou and Thseng, 1991). The implication is that a precipitation event that produces 1 or 2 days of flooding or excessive soil moisture at 25°C would not affect crop stand, at least for the landrace used in this study.

Widely varying results and conclusions have been reported in the literature concerning the physical and physiological causes of flooding injury. The potential physiological mechanisms include ethanol toxicity, oxygen deprivation, and carbon dioxide accumulation (Wuebker et al. 2001). Nakayama et al. (2005) indicated that flooding injury could be avoided by reducing the rate of water absorption during the early stage of seed hydration, and concluded that flooding injury was mainly caused by physical damage of seeds resulting from a rapid inrush of water into seeds, and not by oxygen deficiency. The accumulation of ethanol, acetaldehyde, and $CO_2$ during soaking of maize seed has been reported (Crawford, 1977; Holm, 1972), the suggestion has been made and that ethanol and acetaldehyde were toxic to seeds and resulted in cellular death (Crawford, 1977). However, Martin et al. (1991) concluded that neither ethanol nor acetaldehyde accumulated to sufficiently high levels in maize seed during flooding to result in reduced germination. Cerwick et al. (1995) demonstrated accumulation of $CO_2$ during soaking, inhibition of maize seed germination and seedling growth by $CO_2$, and suggested that one mechanism of inhibition by $CO_2$ was direct interference with oxidative metabolism of mitochondria. Whatever the actual mechanism or combination of mechanisms involved, it is now well established, that excessive water during germination causes the deterioration of seeds leading to decreased field emergence.

The present study clearly indicates that bambara groundnut seeds that had begun to imbibe water were sensitive to flooding stress only after 12 h of flooding. Flooding for 24 or 48 h at 1, 3, 5 or 7 days after start of imbibition, was detrimental to emergence. Also, flooding at 1, 3 or 5 days after start of imbibition was more detrimental than flooding at 7 days after start of imbibition. This is consistent with the observation that at 7 days after start of imbibition germination was essentially completed for many seeds, and seedlings had already fully emerged. The implication is that from 1 to 7 days after sowing or start of imbibition a precipitation event that produces 1 to 12 h of flooding or excessive soil moisture may not affect crop stand in bambara groundnut. How-ever, for soybeans, Wuebker et al. (2001) reported that flooding at 1 to 3 days after start of imbibition, and for 1 to 48 h significantly lowered germination percentage.

Langan et al. (1986) observed that flooding for 3 d starting 1d after planting delayed emergence of corn (*Z. mays* L.), soybean, and wheat (*T. aestivum* L.).

The results of this study further indicate that pre-sowing flooding and flooding after the start of imbibition significantly enhanced the rate of germination and the rate of emergence compared with the non-flooded seed in bambara groundnut. Similar results had been reported for bambara groundnut (Massawe et al., 1999) and a range of other crop species, including tea (*Camellia sinensis* L.) (Habib et al, 1990), sunflower (*Helianthus annus* L.) (Singh, Singh and Vig, 1981), Maize (*Z. mays* L.), rice (*Oryza sativa* L.) and chickpea (*Cicer arietinum* L.) (Harris et al, 1999). Soaking in water (hydro-priming) has been proposed as a viable technology to reduce the time between seed sowing and seedling emergence and the synchronization of emergence in several crop species (Harris et al., 1999; Subedi and Ma, 2005).

The pattern of response of bambara groundnut seed to flooding as observed in this study may not be typical of all bambara groundnut landraces. Massawe et al. (1999) reported differences among bambara groundnut landraces in rate of water uptake. Studies involving pre-sowing soaking or hydration of soybean have indicated a relationship between flooding tolerance and seed colour, and between seed coat colour and rate of water uptake (McDonald et al., 1988; Hou and Thseng, 1991). Field observations have suggested that differences in rate of imbibition in bambara groundnut seeds are related to seed colour, with cream-coloured seeds imbibing faster than dark coloured seeds. It is therefore likely that cream-coloured bambara groundnut seeds would be more sensitive to excessive water than dark-coloured seeds due to rapid absorption and disruption of cell membranes. It is also likely that the tolerance exhibited by bambara groundnut seeds that had begun to imbibe water is attributable to a slow rate of imbibition, since a landrace with a red-pigmented seed coat was used in the study on the effect of timing and duration of flooding. Also, since final germination percentage decreased drastically as the duration of soaking increased beyond 2 days, and since bambara groundnut seeds that had begun to imbibe water were sensitive to flooding stress only after 12 h of flooding, the pattern of response of bambara groundnut seed to flooding as observed in this experiment may not likely occur, to the extent observed in the study, under conditions in Botswana.

Although heavy rainfall may occur in the summer cropping season the rains are often followed by strong sunshine so that a good deal of the rainfall does not penetrate the soil but is lost to evapo-ration, thus flooding caused by heavy rainfall may not frequently last beyond two days in the field. However, tem-perature may influence the response pattern, and field studies would be necessary to ascertain the relevance of the results to the field situation.

## Conclusion

The results presented are interpreted to indicate that flooding or water-logging at sowing is a potential contributing factor to the erratic and variable field establishment in bambara groundnut. Final germination percentage decreased significantly as the duration of soaking increased from 2 to 8 days, and a complete loss in germination occurred when seeds were soaked for 6 days at 35°C, and 8 days at all temperatures used in the study. Flooding bambara groundnut seed for 1 or 12 h at 1, 3, 5 or 7 days after start of imbibition did not reduce germination percentage significantly. The results generated in this study are generally in agreement with earlier findings in other crop species.

Field studies would be necessary to ascertain the relevance of the laboratory and greenhouse results to the field situation. In a field study, the influence of several uncontrollable environmental factors may be critical in determining the pattern of response of bambara groundnut seed to flooding. The ability of seeds of a crop to withstand temporary periods of excessive water or flooding stress during germination may determine its potential for successful regeneration under present and future climatic regimes.

## ACKNOWLEDGEMENT

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