# Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 5 (6): 948-955, 2006
ISSN 1682-3974


# Germination, Vigour and Dehydrogenase Activity of Naturally Aged Rice *(Oryza sativa* L.*)* Seeds Soaked in Potassium and Phosphorus Salts

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**Abstract:** The effectiveness of different concentrations of potassium and phosphorus salts and the duration of soaking on germination, vigour and dehydrogenase activity of rice seed, cvr IDSA 85, was studied in the laboratory. Treatments comprised untreated control, soaking in KCL at K concentrations of 0.5, 1.0%, 1.5 and 2.0% w/v and in $KH_2PO_4$ at P concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% w/v and water for 12 or 24 h. Soaking seeds in P for 24 h increased germination capacity ($p = 0.013$) from 79% in the control to 94% in 0.5% P for 24 h. Irrespective of the soaking medium, soaking duration did not increase germination capacity. Germination energy increased from 28% in the control to 77% in water, 83% in 0.5% K and 89% in 0.2% P. Soaking IDSA 85 seeds in K and P solutions or water significantly reduced the mean germination time and increased the daily rate of seedling emergence. Seeds soaked in P and K solutions produced significantly taller plants than the untreated control and water. Seeds soaked in KCL or $KH_2PO_4$ solutions had higher dehydrogenase activity compared to water suggesting higher viability and vigour of seeds soaked in K and P solutions.

**Key words:** *Oryza sativa*, germination energy, germination capacity, mean germination time, dehydrogenase activity

## INTRODUCTION

An improved upland rice (*Oryza sativa* L.) cultivar IDSA 85 introduced in the Hohoe district, Ghana has been widely adopted by farmers within the Region (DfID, 2004). This cultivar was selected because of its preferred long slender grain and high market price similar to that of high quality imported rice (Dogbe *et al*., 2002) and was being grown as a cash crop. However, one of the major constraints limiting its production in direct-seeded upland rainfed system is its poor stand establishment (DfID, 2004) due to low germination capacity.

It has been established that the rate of seedling emergence, uniformity of emergence and total emergence are critical determinants of crop establishment and yield. A successful crop establishment therefore requires planting high quality seed which germinates promptly under a wide range of field conditions (Phill, 1995; Yamauchi and Winn, 1996; Mcdonald, 1998). However, loss of seed viability and vigour prior to sowing is a major problem in tropical and subtropical regions (Ellis, 1988) due to poor storage conditions, resulting in low field emergence (Khan *et al*., 2003).

Poor storage conditions result in the impairment of cell membrane integrity to remain intact and hold up cell contents. Thus, upon rapid uptake of water deteriorated cells rapture to release their contents into the surrounding water. Among the cell contents are phosphorus (P), potassium (K) and other ions (Pandey, 1989; Marcos-Filho, 1998; Miguel and Marcos-Filho, 2002) which are essential constituents of metabolic enzymes that catalyse germination processes. Phosphorus and K are also important nutrient elements with large influence on early seedling growth.

Pre-sowing seed treatments such as osmopriming, hydropriming, hardening and osmohardening have been used to hasten germination, improve uniformity of germination and vigour of various field crops including rice (Basra *et al*., 2004, 2006). Osmotica that have shown good potential to enhance germination, emergence and seedling vigour include solutions of $KH_2PO_4$ (Das and Choudhury, 1996), KCL (Misra and Dwibedi, 1980; Basra *et al*., 2004) and polyethylene glycol (PEG) (Ruan *et al*., 2002; Basra *et al*., 2006). Water has also been used successfully as priming medium for many crops including rice by Harris *et al*. (1999). In rice, on farm seed priming with water (hydropriming) resulted in faster

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seedling emergence and vigorous growth (Harris *et al.*, 1999; Harris and Jones, 1997 ).

The objective of the study was to evaluate the effect of soaking rice seed in different concentrations of P and K salt solutions at different soaking durations on seed germinability and vigour.

## MATERIALS AND METHODS

This study was conducted in February 2004 at the Seed Science laboratory of the Crops Research Institute in Kumasi, Ghana.

**Seed material:** Rice cultivar, IDSA 85 that had been stored at the Crops Research Institutes cold storage room for 12 months was used for this study.

**Seed soaking:** Stock solution of phosphorus (P) was prepared from dihydrogen potassium orthophosphate ($KH_2PO_4$) and diluted to six concentration levels of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% P (w/v). A second stock solution of potassium (K) was also prepared from potassium chloride (KCL) and diluted to 4 concentration levels namely 0.5, 1, 1.5 and 2% K (w/v). All soaking media were prepared in distilled water. Seeds of IDSA 85 were then soaked in each nutrient solution treatment (P and K) and distilled water for either 12 or 24 h at room temperature. After soaking, the seeds were rolled on filter paper to remove excess water. Untreated dry seeds were used as control. Seed samples were taken from each treatment and the control for germination assay. Seed samples were also taken from P and K solutions and water to determine dehydrogenase activity after soaking for 24 h.

**Germination and vigour tests:** Plastic seed trays were filled with moistened sterilized river sand and used for germination assay. Thee replicates of 50 seeds from each treatment were sown in the trays and each tray covered with transparent polyethylene bag to reduce loss of moisture from the germination medium. The treatments were evaluated in two separate experiments. Experiment 1 evaluated the effect of P on germinability and vigour of IDSA 85. The treatments consisted of: 0.05, 0.1, 0.2, 0.3, 0.4, 0.5% P and water at 12 and 24h and non soaked control. Experiment 2 evaluated the effect of K on germinability and vigour of IDSA 85 and comprised the following treatments; 0.5, 1, 1.5, 2% K and water at 12 and 24 h and non-soaked control. All the treatments were arranged in a completely randomised design and were run concurrently.

Emergence counts were made on days 3, 4, 5 and 7 after sowing. Germination Energy (GE) which is the percentage of seeds germinating 3 days after sowing was determined 3 days after seeding while Germination Capacity (GC) which is the percentage of seeds germinating was determined 7 days after sowing. Seedling Length (SL) was measured in centimetres on day 7 and was taken from the surface of the soil to the tip of the most recently developed leaf. Mean Germination Time (MGT) in days was calculated according to the formula of Scott *et al.* (1984):

$$MGT\ (days) = \Sigma TiNi/S$$

Where:

$T_i$ = Number of days after beginning of experiment
$N_i$ = Number of seeds germinated and emerged on day i
S = Total number of seeds germinated and emerged

Mean germination time is the time to get 50% emergence. Mean rate of emergence ($R_m$, $day^{-1}$) was determined as the inverse of MGT.

**Determination of total dehydrogenase activity:** Samples of IDSA 85 seeds soaked in different concentrations of P and K and water for 24 h were used for the determination. Six replicates each of seed samples from each treatment were dehusked and 1 g of whole dehusked samples weighed into test tubes. To each test tube, 5 mL of 0.7% w/v of 2, 3, 5 triphenyl tetrazolium chloride was added and incubated at 40°C. After 16 h of incubation, seed samples were milled in methanol and the supernatant centrifuged at 3000 rpm for 10 min. The absorbance of the supernatants was measured spectrophotometrically at 485 nm.

**Statistical analysis:** Data collected were analysed using analysis of variance (ANOVA). Before ANOVA, germination energy (%) and germination capacity (%) were transformed using angular transformation;

$$Angles = \arcsin \sqrt{\{g/100\}}$$

where g is the percentage of seeds germinating.

## RESULTS

**Germination energy:** Soaking seeds in K or water for 24 h increased germination energy ($p<0.001$) over the untreated control. Similarly, germination energy increased ($p<0.01$) in the 12 h seed treatments over the control. Germination energy increased from 28% in the control treatment to 83% at 0.5% K and 77% for water in the 24 h

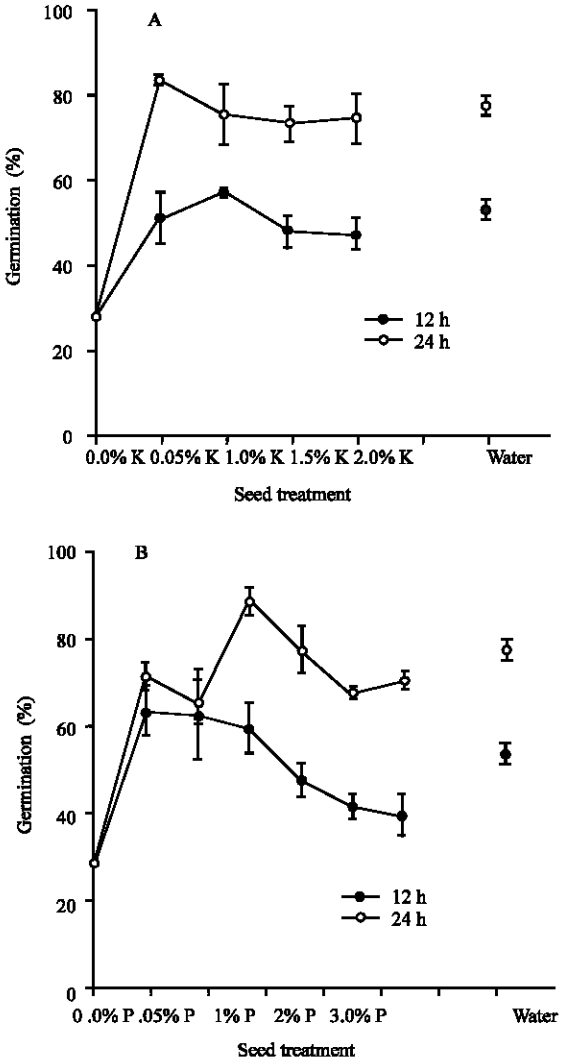


Fig. 1: Effect of soaking IDSA 85 seed in (A) potassium and water, (B) phosphorus and water and soaking duration on germination energy (number of normal seedlings emerging 3 days after sowing) of IDSA 85. Vertical bars represent mean±SE. Some of the SE bars are smaller than the symbols

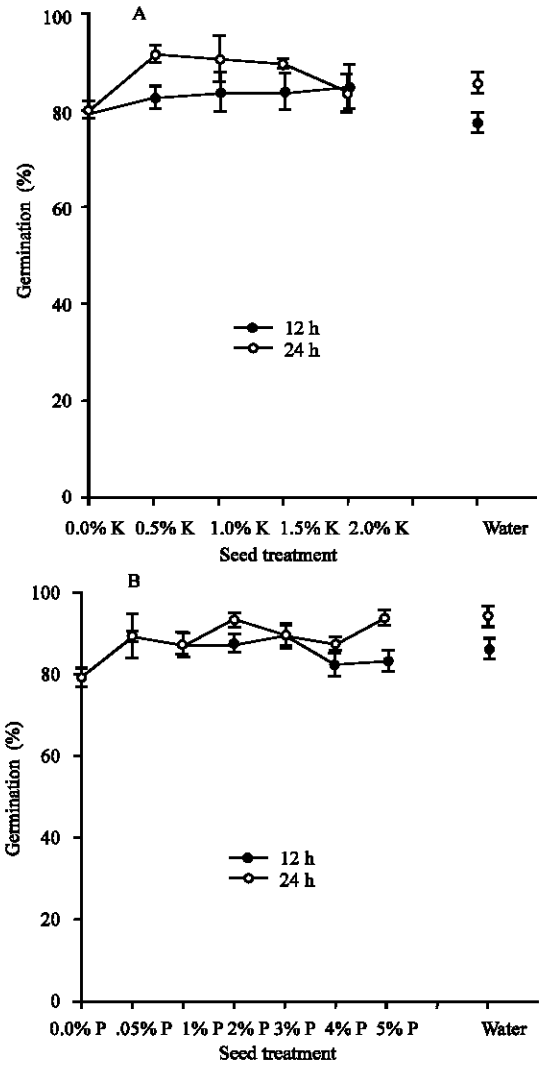


Fig. 2: Effect of soaking IDSA 85 seed in (A) potassium and water, (B) phosphorus and water and soaking duration on germination capacity (%) of IDSA 85. Vertical bars represent mean±SE

treatment. Delayed germinations were observed at higher concentrations of K (Fig. 1A).

Seeds soaked in P or water for 24 h increased the germination energy ($p<0.001$). The greatest germination energy (89%) was observed when seeds were soaked in 0.2% P compared with water (77%). Percentage germination at 0.2% P 3DAS represented about 96% of the total number of seeds which germinated 7DAS, 90.6% for water and 35% for the control. Germination was delayed at higher concentrations of P (Fig. 1B). Seeds soaked with P, K or water for 24 h produced greater ($p<0.001$) germination energy than those soaked for 12 h.

**Germination capacity:** IDSA 85 seeds soaked with K solution or water for 24 h increased germination capacity from 79% in the untreated control to 91% when soaked in 0.5% K and decreased to 83 for 2.0% K and 85% for water. However, germination capacity was not significantly affected by soaking treatment. Soaking in K or water for 12 h resulted in only slight increases in germination capacity above the untreated control (Fig. 2A). Germination capacity was not influenced ($p>0.05$) by soaking duration (i.e., soaking in K solution or water for 12 or 24 h).

Soaking seeds in 0.5% P for 24 h increased germination capacity from 79% in the untreated control to

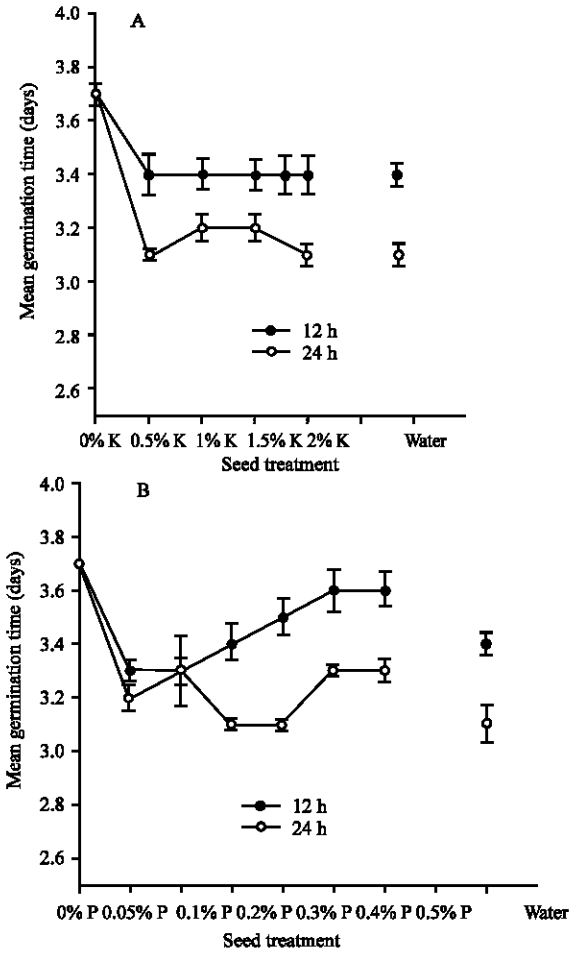


Fig. 3: Effect of soaking IDSA 85 seed in (A) potassium and water, (B) phosphorus and water and soaking duration on mean germination time (days). Vertical bars represent mean±SE

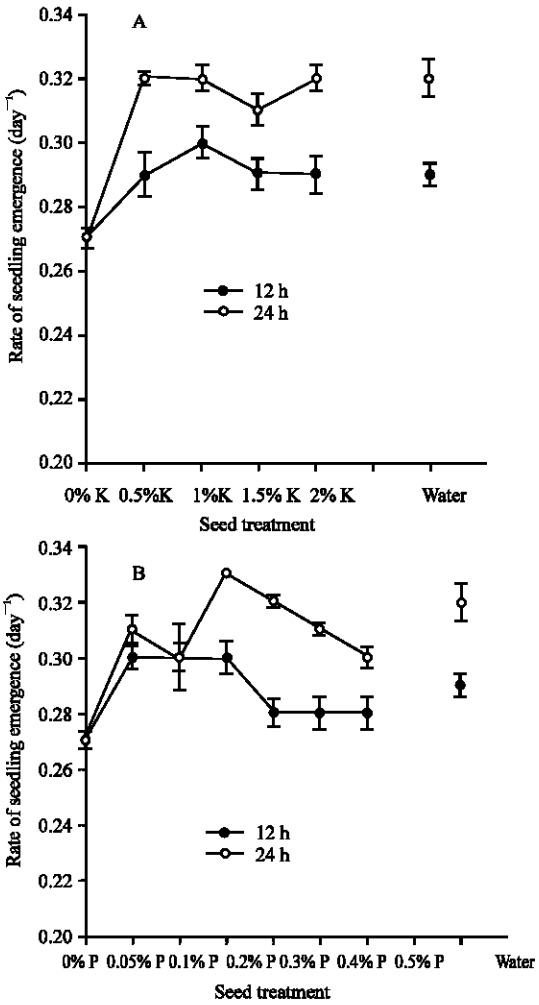


Fig. 4: Effect of soaking IDSA 85 seed in (A) potassium and water, (B) phosphorus and water and soaking duration on rate of seedling emergence ($day^{-1}$). Vertical bars represent mean±SE

94%. Soaking seeds in P solution or water for 12 h also increased germination capacity by 10 and 3% when seeds were soaked in 0.3% P solution and water respectively (Fig. 2B). However, differences ($p>0.05$) between P soaking treatments at 12 h and the control were not significant.

**Mean Germination Time (MGT):** Soaking seeds for 24 h decreased ($p<0.05$) the mean time for 50% emergence at room temperature compared to 12 h. Soaking in either K solution or water for 24 h reduced ($p<0.001$) MGT from 3.7 days in the control to 3.1 days at 0.5% K and water. The mean germination time was also reduced by the 12 h soaking treatments from 3.7 in the control to 3.4 in all the soaking treatments (Fig. 3A).

Soaking in P solution and water reduced MGT for both 24 h ($p<0.001$) and 12 h ($p<0.05$) treated seeds. Mean germination time was reduced from 3.7 days in the control to 3.1 days for seeds soaked for 24 h in 0.2% P, 0.3% P solutions and water. Soaking seeds for 12 h in P solution and water reduced MGT from 3.7 days in the control to 3.3 days for 0.05% P and 0.1% P solutions but increased with increasing concentration of P (Fig. 3B).

**Rate of seedling emergence ($R_m$):** Relations between $R_m$ and seed treatments with K and water for 12 and 24 h are shown in Fig. 4A. The untreated control had the least rate (0.27 $day^{-1}$) of emergence. Soaking IDSA 85 seeds in different concentrations of K and water for 24 h resulted in faster seedling emergence than 12 h. For the 24 h soakings, $R_m$ was 0.32 $day^{-1}$ for the different

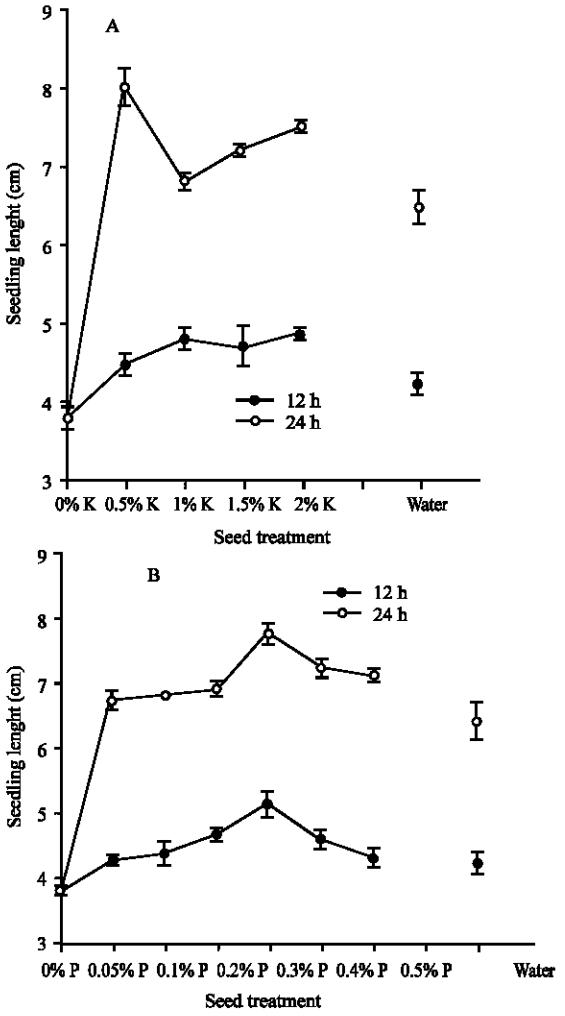


Fig. 5: Effect of soaking IDSA 85 seed in (A) potassium and water, (B) phosphorus and water and soaking duration on seedling length (cm). Vertical bars represent mean±SE. Some of the SE bars are smaller than the symbols

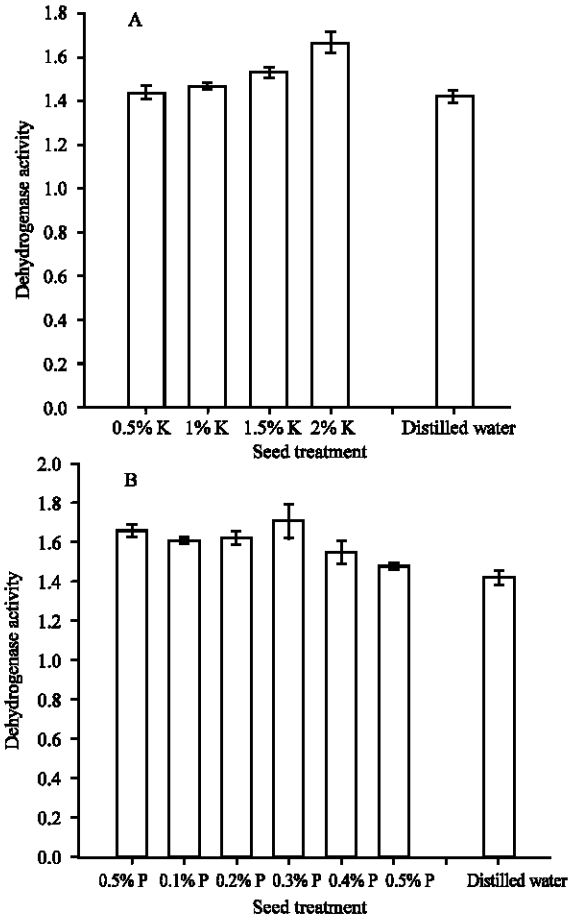


Fig. 6: Effect of soaking IDSA 85 seed in (A) phosphorus and water, (B) phosphorus and water on dehydrogenase activity. Vertical bars represent mean±SE

concentrations of K and water except 1.5% K. The maximum $R_m$ at 12 h was achieved at 1% K.

The maximum rate of emergence was achieved at 0.2% P when seeds were soaked in different concentrations of P and water for 24 h (Fig. 4). Higher concentrations of P (p>0.2%, w/v) at 24 h suppressed $R_m$. Soaking in P and water for 12 h also showed a maximum $R_m$ between 0.05% P and 0.2% P. Again, the rates of emergence were significantly higher in all the 24 h treatments than the 12 h treatments.

**Seedling length:** At 7DAS, seedlings were shorter in control plants compared to those treated with K and P solutions and water (Fig. 5A, 5B). Also, seeds soaked in K and P solutions and water for 24 h produced more vigorous (taller) ($p<0.001$) plants than 12 h soaked seeds.

Seeds soaked in 0.5% K produced the tallest seedlings (8.0 cm) among the 24 h K treated seeds and water. Seedling length was decreased with higher concentrations of K (K>0.5%) but seedlings in these treatments were all taller than those from water soaked seeds (Fig. 5A). Seedling length increased with increasing concentration of K when seeds were treated for 12 h.

For both soaking periods, seedling length was maximal at 0.3% P (7.4 cm for 24 h vrs 4.9 cm for 12 h) and decreased at p>0.3% w/v. Seedlings produced from seeds soaked for 24 h were also taller than the seedlings from seeds soaked in water (Fig. 5B). Similarly, except for 0.5% P w/v, all seeds soaked for 12 h produced taller seedlings than seedlings produced from the seeds soaked in water.

**Total Dehydrogenase Activity (TDA):** Total dehydrogenase activity increased with increased concentration of K and was maximal (1.66) in seeds soaked in 2% K (Fig. 6A). Total dehydrogenase activity increased from 1.65 when seeds were soaked in 0.05% P to 1.70 when seeds were soaked in 0.3% P but decreased to 1.47 at 0.5% P (Fig. 6B). Seeds soaked in distilled water recorded the least (1.42) TDA compared to both K and P treated seeds (Fig. 6A, B).

## DISCUSSION

The relative effectiveness of a priming (soaking) treatment in restoring the vigour of stored seeds is expressed by increased germination (Dell'Aquila *et al*., 1984). Increased germination capacity due to soaking in some nutrient solutions of P for 24 h over untreated control and water suggests that these nutrient solutions are more effective in restoring vigour of IDSA 85 seeds. Results obtained in this study are at variance with the results of Ros *et al*. (2000) who reported between 20 and 50% reduction in germination and seedling emergence compared to untreated control when rice seeds were soaked in 5, 10 and 20% P (w/v). The high concentration of the nutrient solutions may have damaged membranes and changed enzyme relations leading to reduced germination and emergence of seedlings. The P and K applied in this study were based on the amounts leached into seed steep water and may explain why no negative effect of these nutrient solutions was observed on germination capacity. Absence of differences in germination capacity in soaking duration and concentration of K between soaking treatments agrees with the findings of Giri and Schillinger (2003) who also reported no effect of priming duration (12 and 24 h) and concentration of KCL on final germination of wheat. Also, the absence of difference in germination capacity between non-soaked rice seed and rice seed soaked in water for 12 or 24 h agrees with the findings of Harris *et al*. (1999).

Germination energy is a direct measure of seed vigour. Differences in GE between seed treatments and untreated control suggest that the treated seeds had higher vigour levels than the untreated ones. The significant decline in GE at P>0.2% w/v may be due to phytotoxic effect or limited synthesis of protein in the germinating embryos. Sivritepe and Durado (1995) reported that fast germination is accompanied by high protein synthesis in germinating embryos. The significantly greater GE at 0.2% P may be due to faster repair of cell membrane damage during treatment. Sivritepe and Durado (1995) have also reported decreased frequency of visible cytological aberrations whilst percentage germination increased. The above reasons might also explain the decline in $R_m$ at P>0.2% (w/v).

Mean germination time is a good measure of vigour of viable seeds (Shen and Oden, 2000). Prolonged germination time is therefore an indication of deterioration of seed quality (Dell'Aquila, 1987). Prolonged germination time may expose the seed to harsh germination conditions and decreases germination capacity (ISTA, 1995). The negative relationship observed in this study between MGT and GC agrees with the findings of Dell'Aquila (1987) who reported that MGT and GC should be negatively related. The shortening of MGT in all the 24 h soaking treatments compared with the 12 h soaking treatments suggests that seeds soaked for 24 h were more invigorated and therefore germinated at a faster rate. Reduction in MGT due to soaking treatments agrees well with the findings of Lee and Kim (1999), Harris *et al*. (2001) and Khan *et al*. (2003). It is worth noting that although soaking treatments decreased MGT, differences in MGT between the concentrations of K and P for either 12 or 24 h soaking periods were not significant.

Rapid seedling growth is essential for early crop establishment Increased seedling length (seedling vigour) as a result of soaking seeds in K or P or water shows that seed soaking influences early seedling growth or vigour. Lee and Kim (1999) also reported faster radicle growth of primed rice seeds compared to non-primed seeds. The faster growth observed by the 24 h P and K soaking treatments over the control and water soaked treatments might be due to the influence of P and K on early seedling growth. Apart from the salts acting as low water potential osmoticum to control amount of water imbibed by the seed (McDonald, 1999), the salt ions may also enter into embryonic cells of the seed to affect pregerminative metabolic activities (Ruan *et al*., 2002).

Soaking IDSA 85 seeds in KCL or $KH_2PO_4$ resulted in higher dehydrogenase activity compared to water suggesting higher viability and vigour of seeds soaked in K and P than in water. Higher dehydrogenase activity has been reported by various workers to be associated with higher viability and vigour (Nautiyal and Joshi, 1991; Yanping *et al*., 2000). Saha *et al*. (1990) also reported that priming caused increased dehydrogenase activity in aged soybean seeds compared to non-primed seeds.

In conclusion, the results of the study have shown that soaking rice in $KH_2PO_4$ solution is a better treatment to increase germination capacity. Present data also suggest that KCL or $KH_2PO_4$ solutions increased seedling vigour and dehydrogenase activity compared to seeds soaked in water. In addition, soaking seeds in water or K or P solutions for 24 h increased germination energy and

rate of seedling emergence, seedling length and reduced mean germination time over soaking for 12 h.

**ACKNOWLEDGMENTS**

The authors express gratitude to Mr. Anthony Abutiate of the Soil Research Institute, Ghana and Rev. Eric Avah and all the staff of Seed Science Laboratory of the Crops Research Institute of Ghana for their assistance in conducting this experiment.

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