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PRIMING MAIZE (*Zea mays*) SEED WITH PHOSPHATE SOLUTIONS IMPROVES SEEDLING GROWTH AND YIELD

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ABSTRACT

In this study field experiments were conducted to assess the effect of different phosphorus priming sources on seedling growth and yield of maize cv. Azam at two locations i.e. The University of Agriculture and Agricultural Research Institute, Peshawar, Khyber Phukhtunkhwa, Pakistan during 2008 and 2009. Phosphorus concentration (1% P), using potassium dihydrogen phosphate (KH_2PO_4), single super phosphate (SSP) and di-ammonium phosphate (DAP) along with amended solutions of SSP (20 g l^{-1} KOH, 15 g l^{-1} NaOH and 12.5 g l^{-1} Na_2CO_3) were included in the experiment. Water primed and non primed seeds were also used as controls. Seeds were primed for 16 h and were then air-dried for 30 minutes. They were sown in mini plots and in the field. Seedlings were cut at 21 days old stage and remaining field plants were harvested after maturity. Three weeks old mini plots and field seedlings grown from seeds primed with water, P or P amended solutions showed higher vigor than unprimed seeds as reflected in increased fresh and dry shoot weights, fresh shoot height and shoot P content. Additionally the nutrient uptake of seedling was increased four times due to 1 % P solution priming with KH_2PO_4. Yield of maize was also increased in response to P priming showing significant results in cobs yield, grain and straw yields. Phosphorus content of grain was also enhanced as compared to control. Priming maize with SSP + 20 g l^{-1} KOH showed almost the same effect as that of KH_2PO_4. From the experiment it is concluded that priming maize seeds with SSP + 20 g l^{-1} KOH is more effective alternate than KH_2PO_4 as SSP is generally used fertilizer and KOH is also easily available in market. Thus KOH + SSP would be an easier and cheaper available source of P for the poor farmer.

Key words: maize; phosphorus priming sources; potassium hydroxide; single superphosphate; sodium carbonate; sodium hydroxide; yield.

INTRODUCTION

Phosphorus (P) is an important plant macronutrient, making up about 0.2% of a plant's dry weight. After nitrogen, P is the second most important nutrient that limit crop yield particularly in developing countries where high cost, lack of infrastructure and poorly operating markets limit their use. Resource-poor farmers in marginal areas are particularly unlikely to meet the P requirements of their crops. Even when farmers use fertilizers, the properties of many tropical soils are such that recovery rates, particularly for P, are very low. Maize production during 2006-07 showed a decrease of 0.68% as compared to 2005-06, which was due to low rates of the crop fertilizers received by growers, germination affected due to high temperature at the time of sowing, and average yield affected by lodging due to wind storms (ASP, 2006-07). In semi-arid tropic of Khyber Pukhtunkhwa, maize production is widely limited by poor stand establishment and nutrient deficiencies. Rapid and uniform field emergence is also an essential prerequisite to reach the yield potential, quality, and ultimately profit in crops. It is reported that P-deficiency had a detrimental effect on morphogenesis and physiological mechanism in maize, and P-deficiency symptoms and biomass have been known as indicative traits of maize in response to low-P stress (Liu, *et al.* 1993; Hajabbasi and Schumacher, 1994; Duan, *et al.* 2002).

The most obvious strategy to alleviate P deficiency is to add large quantities of phosphate fertilizers to P deficient soils, either as soluble P fertilizer or as rock phosphate (von Uexküll and Mutert, 1995). However, amelioration of P deficiency with cost intensive fertilizers is not a viable option for many resource-poor farmers. Treating or priming the seeds with small amounts of nutrients before sowing has been shown to partially overcome nutrient immobilization problem in soils and to increase nutrient use efficiency. Seed dressing of limiting nutrients has been advocated as a low-cost and highly effective approach since the 1970's (Roberts, 1973; Ros, *et al.* 2000). Seed priming with concentrating limiting plant nutrients around or within the seed may be an attractive solution to overcome poor establishment and P and Zn deficiencies (Asgedom and Becker, 2001). Priming with dilute P solutions has proved to be particularly effective in promoting rapid seedling growth and seedling performance is known to be related to seed P content (DeMarco, 1990; Derrick and Ryan, 1998).

It has been proven that on-farm' seed priming with a 1% P solution using KH_2PO_4 improved fertilizer-use efficiency and increased yield and profit for different crops grown on P-deficient soils (Ali, *et al.* 2008). Although an attractive option for resource-poor farmers, seed priming with KH_2PO_4 can be difficult for them to adopt because of the scarcity of the chemical in rural areas and its high unit cost relative to more commonly available phosphatic fertilizers. In this paper we tested cheaper, more readily available alternatives to KH_2PO_4 as a source of water-soluble P for priming maize seeds.

MATERIALS AND METHODS

Mini plot and field experiments were conducted during 2008-2009 to compare the relative effectiveness of priming maize seeds with a 1% P solution derived from KH_2PO_4, single superphosphate (SSP), and di-ammonium phosphate (DAP).

Solution Preparation: To prepare solutions containing 1% P, the calculated amounts (Table 1) of KH_2PO_4, SSP or DAP were dissolved in one liter of distilled water. The suspensions formed were shaken occasionally during 24 h and the supernatant liquid was decanted and used to prime seeds. Using SSP as a source of P, the effects on pH, electrical conductance (EC) and osmotic potential of adding various amounts of commonly available alkalis to the P solution were tested. A 1% P solution was made using SSP and known amounts of NaOH, KOH or Na_2CO_3 were added to it (Table 2). Solutions of 1% P using SSP alone and KH_2PO_4 were included for comparison. The pH of the solutions was measured as noted in Table 2 and EC was measured using a Solubridge conductivity meter.

Osmotic potential (MPa) of solutions was calculated by multiplying EC (dSm^{-1}) by 0.0364 (US. Salinity Lab. Staff, 1954). Based on inspection of the pH values and colligative properties in Table 2, a range of solutions of amended SSP (1% P) was prepared. On the basis of solution properties and P uptake by seeds, a single concentration of each alkali was selected to amend an SSP solution. A miniplot experiment was then conducted to measure the effect of amended SSP solutions on seedling growth of maize.

Mini plot experiment: On the basis of the properties of SSP amended solutions, three alkalis (20 g l^{-1} KOH, 15 g l^{-1} NaOH and 12.5 g l^{-1} Na_2CO_3) were selected to study their effect on growth parameters of maize. Treatments of water, KH_2PO_4 (1 % P) and SSP alone (1% P) were also included in the experiment as controls. This experiment was conducted in The University of Agriculture, Peshawar in randomized complete block (RCB) design and replicated three times. The plot size was kept 14 m^2 and row to row distance was 30 cm apart. A basal dose of 120 kg N ha^{-1} as urea and 60 kg K_2O ha^{-1} as sulfate of potash (SOP) was applied before sowing. Twenty primed seeds per plot were sown in rows. The seedlings were harvested after three weeks, washed with distilled water, bundled and then weighed for fresh seedling biomass. These seedlings were then kept in oven at 80^oC in paper bags for 24 h and weighed after complete drying.

Field Experiment: Two field experiments were carried out at ARI, Tarnab during July 2008 and 2009 in RCB design. SSP solution amended with 20 g l^{-1} KOH was chosen from the mini plot experiment. The seeds were weighed in plastic bags at the recommended rate and primed with water and KH_2PO_4, SSP, SSP + 20 g KOH l^{-1}, and DAP for 16 h. Non-primed seed were used as control. The seeds were air dried for half an hour and were sown in prepared soil. A basal and starter dose of N at the rate of 60 kg ha^{-1} as urea and 60 kg ha^{-1} K as SOP was applied to the field at the time of sowing. Remaining half dose of urea was applied afterwards with irrigation.

Seedling Growth parameters: Five plants along with the roots from each plot were harvested 21 days after sowing. Roots were cut and washed thoroughly with tap water. The plant shoots and roots were tap dried with tissue paper. The following growth parameters were recorded from the seedlings collected.

1. Fresh shoot and root weights (g $plant^{-1}$)
2. Dry shoot and root weights (g $plant^{-1}$)
3. Seedling height (cm)

The shoots and roots were then kept in an oven at 80^oC in paper bags till dried. Shoots were grinded and the seedlings were analyzed for their phosphorus content.

Yield Parameters: The remaining plants were harvested at maturity stage and agronomic parameters including Cob yield (kg $plot^{-1}$), Plant height (cm), Number of grains cob^{-1}, Grain weight cob^{-1} (g), 1000 grain weight (g), Grain yield (kg ha^{-1}), Straw yield (kg ha^{-1}), and Grain P content (%) were recorded.

Phosphorus analysis: Dried samples (shoot, root and grain) were ground using a Wiley mill to pass through a mesh sieve. Samples were digested in an acid mixture of 10 ml HNO_3 + 4 ml $HClO_4$ (Isaac and Kerber, 1971). Color was developed by using a single solution reagent containing ammonium molybdate, ascorbic acid and a small amount of antimony. Phosphorus was then measured spectrophotometrically at 880 nm (Ryan, *et al.* 2001).

Statistical analysis: The data was statistically analyzed by analysis of variance appropriate for Randomized Complete Block Design using MStatC. Means were compared using LSD test at 0.05 level of probability (Jan *et al.* 2009).

RESULTS AND DISCUSSION

Mini plot Experiment: Data regarding seedling growth parameters as affected by different SSP amended solutions (Fig 1) depicted higher shoot fresh biomass (67.2 g $plot^{-1}$) in seedlings primed with SSP (1% P) + 20 g l^{-1} KOH. Same pattern was observed in the dry biomass data showing more dry weight (9.06 g $plot^{-1}$) in seedlings primed with SSP (1% P) + 20 g l^{-1} KOH while less (7.17 g $plot^{-1}$) was observed in seedlings primed with SSP (1% P). This increase may be attributed to the fact that the availability of phosphorus (P) is one of the most significant determinants of plant growth. Recent reports have confirmed that deprivation of P has a detrimental effect on the rate of photosynthesis in maize (Khamis, *et al.,* 1990, Usuda and Shimogawara, 1992). These findings are in agreement with Ullah, *et al.* (2002a) who reported maximum values of shoot fresh and dry weights with $ZnSO_4$ treatment. However, shoot P concentration (%) of seedling was non-significantly affected by priming (Fig 2). Maximum P concentration (0.28 %) was observed in the seedlings primed with SSP (1% P) + 20 g l^{-1} KOH. SSP solution amended with 20 g l^{-1} KOH was chosen from this experiment as a source of water-soluble P for priming maize seeds.

Field experiments

Seedling growth parameters: Although equivalent solutions (1% P), SSP is more highly acidic than KH_2PO_4 and inhibited germination and damaged seedlings drastically. The DAP solution was alkaline and depressed emergence a little but reduced fresh shoot weight significantly relative to H_2O, KH_2PO_4 and, to some extent, SSP. Although SSP alone significantly reduced grain yield relative to using water-primed seeds (Table 4). However, using SSP as the source of P, in combination with KOH was nearly as effective as KH_2PO_4 in raising the P content of seeds. It can be noted from the seedling growth data (table 3) that more fresh shoot weight (7.286 g $plant^{-1}$) was observed in the seedlings primed with KH_2PO_4 1% P as compared to non primed seedlings (4.104 g $plant^{-1}$). Similarly, maximum dry shoot weight (1.267 g $plant^{-1}$) was observed from seedlings primed with KH_2PO_4 1% P which was higher from control plot seedlings (0.521 g $plant^{-1}$). These results were in line with Arif, *et al.* (2005) who recorded high fresh shoot weights primed with 1% P + 2% Zn as compared to controls. Increased shoot dry weights of seedlings treated with micronutrients were recorded by Pill, *et al.* (1997) and Adiloglu, *et al*. (2005). Increase in fresh weight (1.021 g $plant^{-1}$) was observed in roots primed with KH_2PO_4 1% P which was in line with root weight increase due to priming reported by Afzal *et al.* (2006). Jacob and Lawlor (2003) reported that P deficiency decreased the rate of regeneration of ribulose 1, 5-bisphosphate, as well as the activity and the content of ribulose 1, 5-bisphosphate carboxylase in maize leaves which eventually affects photosynthesis in leaves. Phosphorus priming in our study led to enzymatic changes in the seed and hence resulted in increased shoot biomass.

Average shoot P concentration data revealed significant increase in P (0.107 %) in KH_2PO_4 1% P primed seedlings while less P content (0.039 %) was recorded in seedlings that were not primed. The results were supported by the findings of Adiloglu, *et al*. (2005). Zhang *et al*. (1998) also recommended soaking seeds in P solution to improve their P content. Mean P uptake was increased by the seedling was treated with KH_2PO_4 1% P (0.135 g $plant^{-1}$) while less uptake (0.021 g $plant^{-1}$) was shown by non-primed seedlings. Findings reported by Slaton *et al*. (2001) indicated a significant increase in Zn uptake by seedling due to nutrient priming, which was in similar pattern with the present study. Ros, *et al*. (2000) also reported similar results of higher P uptake due to P seed soaking.

Yield parameters: Two years average yield data are presented in Table 4. Data pertaining cobs yield indicated that priming maize seeds had significantly affected the cob weight as higher weight (7.67 kg $plot^{-1}$) was observed in the plants treated with KH_2PO_4 while less cob weight (3.38 kg $plot^{-1}$) observed in non-primed plants. These findings agreed with positive responses to seed treatment and maximum cob yield reported for maize by Harris, *et al.* (1999). Similarly, taller plants (231.7 cm) were observed in plots primed with SSP + KOH whereas shorter plants were observed in control plots having average height of 119.3 cm. Average data of two years regarding the number of grains cob^{-1} revealed more grains (421 per cob) in plots primed with KH_2PO_4 1 % P while lesser number of grains per cob (325) were noted in plants primed with SSP 1% P. Number of grains cob^{-1} was increased up to 26 % by the P priming as compared to control. Ali *et al.* (2008) had reported similar increase in number of grains $spike^{-1}$ of wheat primed with 0.3 % P. Highest grain weight cob^{-1} (133.09 g) was noted in plants primed with KH_2PO_4 1% P while lowest grain weight cob^{-1} (99.95 g) was recorded in control plots. These results were in line with the findings of Rashid, *et al.* (2004) who observed increased grain weight pod^{-1} of mung bean due to priming. The priming-improved seed performance might be attributable in part to metabolic repair processes, a buildup of germination metabolites or osmotic adjustment during treatment (Haghpanah, *et al.* 2009).

Average grain yield was significantly increased by KH_2PO_4 priming (3509.0 kg ha^{-1}) which was followed by grain yield of plants treated with SSP + KOH (2743.2 kg ha^{-1}). Lowest yield (1365.2 kg ha^{-1}) was observed in control plots. Grain yield recorded from our work showed 157% increase over control due to KH_2PO_4 1 % P. Harris, *et al.* (2004) reported similar results as reflected in 48% grain yield increase due to Zn seed priming. These results were also supported by Harris *et al.* (2001; 2007 a, b). The increase grain yield may be due to the fact that priming advances the metabolism of the seed and the seed protein is synthesized which has a direct affect in increasing seed performance and hence yield (Varier, *et al*. 2010).

Priming had a significant effect on average straw yield as highest straw yield (6322.9 kg ha^{-1}) was observed from the plants primed with KH_2PO_4 whereas lowest straw yield (3260.4 kg ha^{-1}) was noted in control plots. Priming of maize increased the straw yield up to 94 % over control that was in accordance with results reported by Iqbal and Chauhan (2003). Khalil *et al.* (2010) observed that wheat DM yield increased with each increment of priming and maximum DM yield (6051 kg ha^{-1}) was obtained from seeds primed in 0.2% P_2O_5 solution. Average data of grain P content showed that the amount of P (1.170 %) was increased significantly in the grains which were primed with KH_2PO_4 while less increase (0.813 %) was observed in control plot grains. Data regarding the seed P content showed 44 % increase due to priming. These observations fairly correspond to those of Liu, *et al.* (1993) who reported significant increased Zn content in seeds of maize due to priming. Also Alam, *et al.* (1995) observed that application of Zn increased its concentration in all plant parts of maize including grains. Similar increase in P concentration and uptake were obtained when maize was given 0, 20, 40 or 80 mg P kg^{-1} of seed by Erdal, *et al.* (2000).

Table 1. The amount of fertilizer needed to make an aqueous 1% P solution and the pH of the resulting solution.

Source of P	Quantity of fertilizer (g l^{-1})	pH
KH_2PO_4	43.9	5.6
SSP	114.5	2.8
DAP	49.8	8.1

Table 2. Some properties of SSP solution (1% P) amended with different alkalis

Solution	Alkali	Alkali added g l^{-1}	pH	EC dSm-1	Osmotic potential MPa
SSP	--	--	2.80	16.22	-0.59
SSP	KOH	11.5	5.20	21.08	-0.77
SSP	KOH	17.2	7.14	28.40	-1.03
SSP	KOH	22.9	8.32	37.70	-1.39
SSP	NaOH	11.5	6.30	22.2	-0.81
SSP	NaOH	17.2	8.0	37.90	-1.38
SSP	NaOH	22.9	11.12	48.4	-1.76
SSP	Na_2CO_3	10.0	3.89	11.49	-0.42
SSP	Na_2CO_3	12.5	5.09	12.11	-0.44
SSP	Na_2CO_3	15.0	5.57	12.96	-0.47
KH_2PO_4	--	--	5.60	31.7	-1.16

Table 3. Two years average growth data of maize seedlings from field experiment primed with water or dilute P solutions. Columns denoted by different letters differ significantly at P < 0.05.

Parameters	Treatments						
	Non primed Control	H_2O primed	KH_2PO_4 (1% P)	SSP (1% P)	SSP (1% P + 20 g l^{-1} KOH)	DAP (1 % P)	LSD $\leq$ 0.05
Fresh shoot mass (g pl^{-1})	4.104 ^{c}	5.314 ^{bc}	7.286 ^{a}	4.397 ^{c}	6.343 ^{ab}	4.587 ^{bc}	1.506 *
Fresh root mass (g pl^{-1})	0.701 ^{cd}	0.838 ^{bc}	1.021 ^{a}	0.583 ^{d}	0.999 ^{ab}	0.955 ^{ab}	0.1627 *
Dry shoot mass (g pl^{-1})	0.521 ^{c}	0.709 ^{bc}	1.267 ^{a}	0.541 ^{a}	0.807 ^{c}	0.744 ^{bc}	0.2636 *
Dry root mass (g pl^{-1})	0.180	0.195	0.248	0.138	0.205	0.175	NS
Shoot height (cm)	33.82	37.66	40.89	29.12	40.70	36.46	NS
Shoot P Content (%)	0.039 ^{b}	0.057 ^{b}	0.107 ^{a}	0.084 ^{a}	0.092 ^{a}	0.054 ^{b}	0.02573 *
Shoot P uptake (g $plant^{-1}$)	0.021 ^{d}	0.037 ^{cd}	0.135 ^{a}	0.043 ^{c}	0.074 ^{b}	0.037 ^{cd}	0.0181 *

Table 4. Two years average yield data of maize seedlings from field experiment primed with water or dilute P solutions. Columns denoted by different letters differ significantly at P < 0.05.

Parameters	Treatments						
	Control	H_2O primed	KH_2PO_4 (1% P)	SSP (1% P)	SSP (1% P + 20 g l^{-1} KOH)	DAP (1 % P)	LSD ≤ 0.05
Cob yield (kg $plot^{-1}$)	3.38 d	5.97 b	7.67 a	3.08 d	7.60 a	4.97 c	0.9267
Plant height (cm)	119.3 b	196.2 a	222.8 a	131.2 b	231.7 a	189.8 a	46.66
Number of grains cob^{-1}	335 cd	377 b	421 a	325 d	369 bc	372 b	35.78
Grain weight cob^{-1} (g)	99.95 b	123.76 a	133.09 a	100.38 b	106.36 b	105.66 b	13.12
Thousand grain wt (g)	247.13	266.27	295.82	233.88	252.13	254.47	NS
Grain yield (kg ha^{-1})	1365.2 c	2498.8 b	3509.0 a	1475.3 c	2743.2 b	2228.1 b	721.3
Straw yield (kg ha^{-1})	3260.4 c	5395.8 ab	6322.9 a	3531.2 c	50560.4 b	3302.1 c	863.5
Grain P (%)	0.813 c	0.876 bc	1.170 a	0.956 b	0.986 b	0.913 bc	0.1409

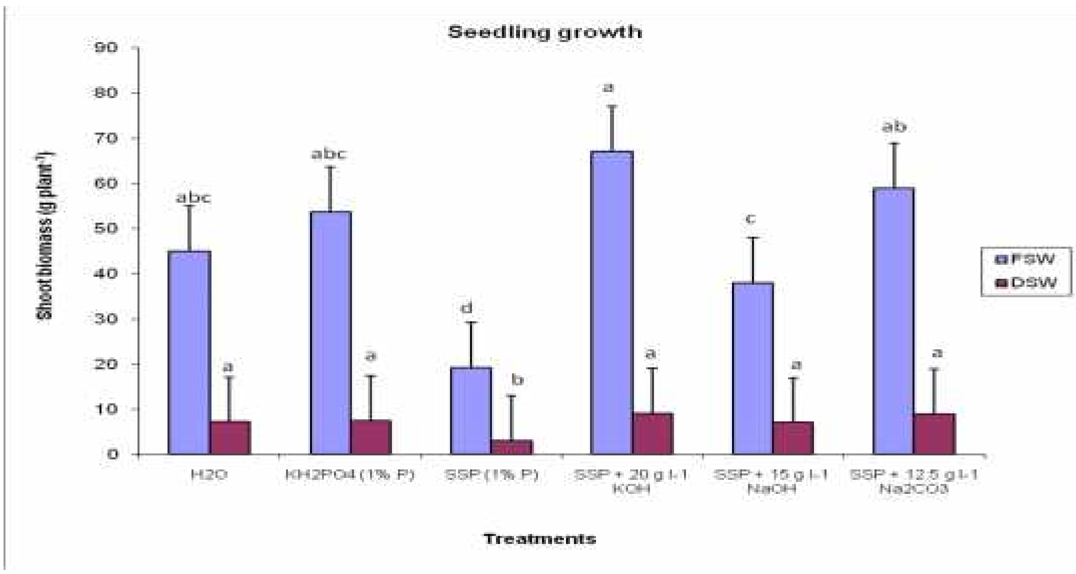

Fig.1. Seedling growth parameters from mini plot experiment as affected by different SSP amended priming solutions. Columns denoted by different letters differ significantly at P < 0.05.

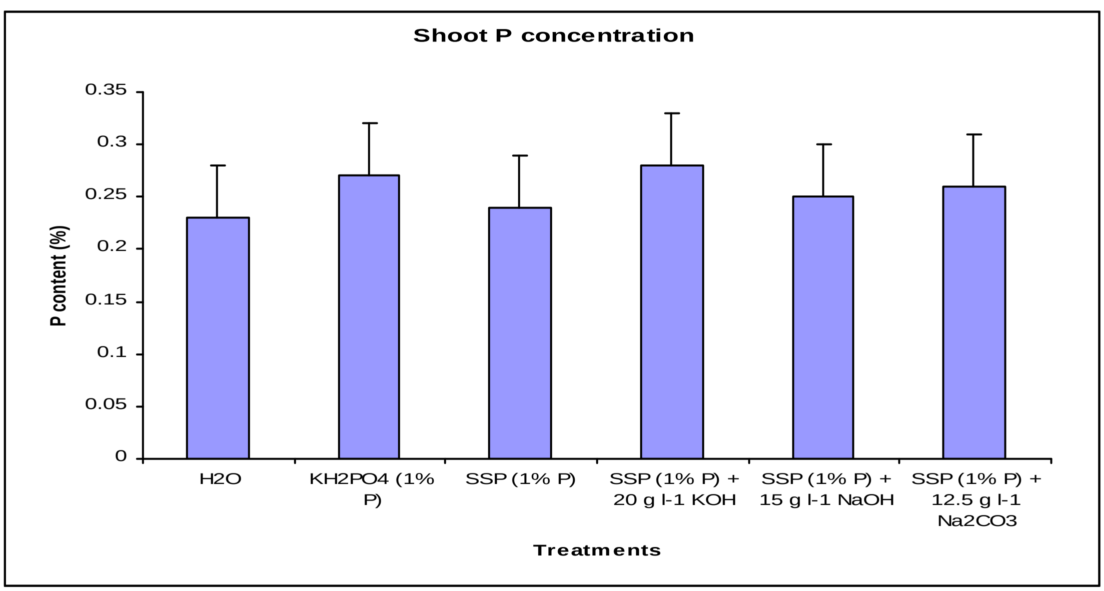

Fig. 2. Shoot P content (%) from mini plot experiment as affected by different SSP amended priming solutions

Conclusions and Recommendations: In rural areas of less developed countries where access to specialized agricultural inputs is difficult, it would be farmers' advantage to be able to increase the P-content of their seeds with cheaper, more readily available sources of P. In our study KH_2PO_4 was chosen because solutions at the appropriate concentrations are not too acidic (Table 2) and Tisdale *et al.* (1996) have noted its relatively non-toxic effects in proximity to seeds. However, its unit cost is high. Aqueous solutions of the common, commercially available P fertilizers (SSP and DAP) tested here as alternatives to KH_2PO_4 for priming maize seeds contain the orthophosphate (H_2PO_4) form of P and are highly water soluble (95-100%). SSP contains a common P compound, Ca $(H_2PO_4)_2$, whereas DAP has (NH_4) H_2PO_4 (Follett *et al.* 1981). Manufacturing chemistry, reactions and behavior of phosphate fertilizers are well documented in many text books (e.g. Follett *et al.* 1981; Tisdale *et al.* 1996; Brady and Weil, 2002). We have shown that equivalent solution, i.e. 1% P, of SSP is more highly acidic than KH_2PO_4 and inhibited germination and damaged seedlings drastically so is not recommended further. A DAP solution was alkaline and depressed emergence a little but reduced fresh shoot weight significantly relative to H_2O, KH_2PO_4 and, to some extent, SSP. The adverse effect of DAP on young seedlings might have been due to evolution of NH_3 gas, a situation associated with coating seeds or banding DAP near seed according to Tisdale *et al*. (1996). Consequently DAP was not considered further as an alternative source of P for priming. SSP alone significantly reduced grain yield relative to using water-primed seeds (Table 4). However, using SSP as the source of P, some combinations with NaOH, KOH or Na_2CO_3 were nearly as effective as KH_2PO_4 in raising the P content of seeds (Fig 2) and as effective at increasing the biomass and shoot P content (Fig 1) of seedlings.

It has been concluded from this study that priming maize seeds with neutralized SSP solutions, in particular using 20 g l^{-1} KOH, was as effective as using KH_2PO_4 in increasing biomass and grain yield. It would be available as cheaper and more readily available source of phosphorus priming for the resource poor farmers.

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