

# **A minute P application contributes to a better establishment of pearl millet (*Pennisetum americanum*) seedling in P-deficient soils**

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**Short running title:** P microdosing in pearl millet

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## **Abstract**

Many soils of the semi-arid tropics are deficient in P, and under such adverse conditions, the establishment of pearl millet seedlings is a critical step to achieve satisfactory crop stands. Phosphorus fertilizer is expensive for small holder farmers and is only applied at low rates insufficient to give satisfactory crop stands. Methods are needed to enhance productivity at low rates of application. Here, we tested the hypothesis that a minute application of P at early seedling stage, equivalent to 125-500 g P ha<sup>-1</sup>, would enhance the plant establishment under P-limited conditions. We measured the minimum application of P needed to elicit a response of different genotypes. Pot experiments were conducted with pearl millet (*Pennisetum*

*americanum*) hybrids to measure the response to P placed close to the root system, 5 days after sowing (DAS), compared to a non-limiting P control (DAP). The placement treatments were 0, 0.25, 0.50, and 1.0 mg soluble P per seedling, applied as  $\text{KH}_2\text{PO}_4$  solution. The localized placement of P increased biomass in all 3 soils. Plant biomass at 1000  $\mu\text{L}$  (1 mg P) reached about 50 % of the control in one soil. If applied later than 19 DAS the placement had no effect on the plant biomass at 40 DAS. Hybrid ICMP 451-P8 was more responsive than 81B-P6. Placement to 20 inbred lines of pearl millet increased biomass by an average of 105 % compared to no placement with large genotypic variation. Although this work was not intended to be a way of applying P fertilizer to pearl millet under field conditions, it showed that applying minute amount of P to pearl millet seedling (equivalent to 125-500 g P  $\text{ha}^{-1}$ ) enhanced their establishment and led to improved growth for at least 5-6 weeks after sowing. Further work is in progress to develop a feasible technology for field crops based on the results of this study.

**Key words:** Biomass production, seedling establishment, pearl millet, phosphorus, seed reserves, microdosing

## Introduction

In areas where soils are deficient in P, like those in the semi-arid tropics of Africa, access and affordability of phosphorus fertilizer limits the quantities that farmer apply to their fields. This in turn leads to poor seedling emergence, which results in poor stand establishment and low yields in many semi-arid regions (Ragwa *et al.*, 2001). Phosphorus deficiency is one of the major constraints that limit crop yields in pearl millet (Rebafka *et al.*, 1993) and research is needed to optimize the scarce P fertilizer that is available. The essential physiological role of P and the issues at stake in improving crop performance under low P conditions have been reviewed (Sinclair and Vadez, 2002). In particular, early season deficiency of P lead to early unrecoverable restrictions in crop development that can drastically reduce crop populations.

The supply of nutrients in seed to the developing seedling lasts for only a short period after germination (Rebetzke *et al.*, 2004), and is crucial for ensuring seedling establishment until external nutrients become accessed by roots (Foster, 1986). Partial removal of seed tissue can reduce the size of the seedlings produced (Zhang and Maun, 1991) and the partial or total

removal of cotyledons can reduce biomass production and even cause death in young seedlings (Armstrong and Westoby, 1993). A strong correlation has been found between common bean seed size and P uptake in P deficient soils (Yan *et al.*, 1995; Allsopp and Stock, 1995), which can be interpreted as a consequence of better root establishment of the large seeded beans leading to greater P uptake. Small seeded crops with limited nutrient reserves such as pearl millet tend to establish poorly when sown on soils with inadequate available nutrients (Welch, 1986).

The seeds of pearl millet are very small in size ( $\sim 0.15 \text{ cm}^2$  surface area seed<sup>-1</sup>) and mass (7-20 mg seed<sup>-1</sup>) with small nutrient reserves, especially P ( $\sim 0.05 \text{ mg P seed}^{-1}$ ). These reserves support seedling establishment for a very limited period, and require a rapid external supply of nutrients to support further growth (Rebafka *et al.*, 1993). Poor establishment of pearl millet seedlings in soils deficient in P leads to smaller than optimum plant populations of irregularly spaced plants with restricted root systems which in turn restricts the capacity of young plants to exploit the small amounts of P in the soil. Our hypothesis is that minute amounts of P, equivalent to 125-500 g P ha<sup>-1</sup> made available to seedlings will enhance the establishment of pearl millet by increasing the capacity of young plants to survive and exploit the small amounts of P available in deficient soils, as previously suggested for rice (Wissuwa, 2003). To test this hypothesis we placed small amounts of P in solution close to the roots of seedlings. We also tested the effect of this placement on a range of pearl millet inbreds and hybrids to assess variation in genotypic response.

## Materials and methods

A series of eight separate experiments were conducted during 2005 and 2006 with different objectives to elicit the effects of placement of phosphorus on seedling growth in P deficient soils. Two pearl millet (*Pennisetum americanum* L.) hybrids (inbred parents 81B-P6 and ICMP 451-P8 as pollinator cross with male sterile line ICMB 90111, referred to in the paper as 81B-P6 and ICMP 451-P8, 20 inbred lines, LGD 1-B-1-10, ICMP 85410 - P7, Tift 23D2B1 - P5, WSIL - P8, 81B-P6, ICMP 451-P8, ICMP 451 - P6, H 77/833-2-P5 (NT), H 77/833-2-Bulk, PRLT 2/89-33-Bulk, W 504-1-P1, P310-17-Bulk, PT 732B-P2, P1449-2-P1, 841B-P3, 863B-P2, IP 18293-P152, Tift 238D1-P158, Tift 186, and Tift 383, used in the marker-assisted breeding programme of ICRISAT, and 80 testcross hybrids resulting from the crossing of the 20 inbred

above to 4 male-sterile lines, were used in different experiments. Hybrids 81B-P6 and ICMP 451-P8 were used for all the experiments except experiment 3 and 4. (pH, Olsen P and exch. K, Mg and psd

Surface samples (0-15 cm) of three sandy-clay loam Alfisol soils with low P content (RL 24-A, E, and G) was collected from ICRISAT fields for pot experiments. The Olsen P of the soils were respectively 0.9, 3.78 and 2.62 ppm P. The soils were air-dried, sieved and mixed with sand (Olsen P of sand was 0.9 ppm) in 1:1 ratio to ease root extraction. The resulting Olsen P values of the mixtures were 0.8, 2.1, and 1.5 ppm for soils RL 24-A, E, and G. The same mixtures were used for all the experiments. Five kg of a mixture was put in 20 cm diameter pots and 1000 mL of water added to wet the soil. Seeds were sown and thinned to 3 plants per pot at 5 days after sowing (DAS). Prior to treatment application, 200 ml of 10 mM urea solution was applied to all pots just before sowing and at 3 weeks after sowing in all the experiments except experiment 8 in which response to N of P placement, was tested. All the experiments were arranged in fully randomized blocks with five replicates per treatment. Pots were irrigated to maintain soils close to 80 % of field capacity, but avoiding drainage. Depending on the experiment, plants were harvested between 35-45 DAS and separated into shoots and roots, dried at 70 °C for 72 h and dry weights recorded.

Very small quantities of P in solution (32.2 mM  $\text{KH}_2\text{PO}_4$ ) were placed as close as possible to the roots of young seedlings through small holes in the soil 2-3 cm deep using a micropipette 5-6 DAS, and compared with a non-limiting P control (300 mg  $\text{kg}^{-1}$  soil of diammonium phosphate, DAP). The localized application supplied the equivalent of 0.25, 0.50 or 1.0 mg P to each seedling, depending on treatment. After placement the holes were closed with soil and irrigation withheld for 2 days to avoid dispersing the placed P.

### *Experiment 1 and 2*

The objective of these two experiments was to test the response of pearl millet hybrids 81B-P6 and ICMP 451-P8 grown outdoors to placement of P in the three low P soils. Seeds were sown on 10 August 2005 (experiment 1) and 23 March 2006 (experiment 2) and seedlings thinned to 3 per pot at 6 and 5 DAS, respectively. Five rates of P were used 0, 250  $\mu\text{L}$  (0.25 mg P), 500  $\mu\text{L}$  (0.50 mg P) and 1000  $\mu\text{L}$  (1.0 mg P) of a  $\text{KH}_2\text{PO}_4$  solution (32.2 mM) at 6 and 5 DAS in experiment 1 and 2 respectively together with the non-limiting P treatment in both years. At 35

DAS (15 September 2005) and 37 DAS (28 April 2006), all plants were harvested, separated into shoot and root, and dry weights recorded.

#### *Experiment 3 and 4*

The objective of these two experiments was to compare the genotypic response to placement of P in 20 pearl millet inbred lines (Experiment 2) and in 80 hybrids (20 inbred lines crossed with male sterile testers, ICMB 89111B, ICMB 90111B, ICMB 92666B, and ICMB 95333B), in one low P soil (RL 24 E). We used RL 24-E soil because it gave the largest response to P placement in experiment 1. Experiment 3 was sown in a glasshouse on 8 December 2005, and experiment 4 was sown outdoors on 4 April 2006. Two concentrations of 0 and 1000  $\mu\text{L}$  of  $\text{KH}_2\text{PO}_4$  solution (32.2 mM) were placed at 6 DAS, equivalent to 0 and 1 mg P per seedling together with a non-limiting P control (300 mg DAP  $\text{kg}^{-1}$  soil). Phosphorus solution was applied as described earlier. At 45 and 40 DAS (21 January; 13 May 2006), all plants were harvested, separated into shoot and root and dry weights were recorded.

#### *Experiment 5 and 6*

The objective of these two experiments was to test the effect of timing of the placement on the response of plants. Two pearl millet hybrids 81B-P6 and ICMP 451-P8 were grown on soil RL 24-E. Seeds were sown on 22 December 2005 (experiment 5) and 23 March 2006 (experiment 6), and thinned to 3 plants per pot at 6 and 5 DAS, respectively. Experiment 5 was conducted in a glasshouse and experiment 6 outdoors. Two concentrations of  $\text{KH}_2\text{PO}_4$ , 0 P and 1000  $\mu\text{L}$  were placed on each of three occasions i.e. 6, 19, and 30 DAS in 2005, and 5, 12, and 19 DAS in 2006, together with a non-limiting P control (300 mg DAP  $\text{kg}^{-1}$  soil). Treatments were applied by the methods described earlier. At 40 DAS (31 January 2006) and 37 DAS (28 April 2006), all plants were harvested and dry weights of shoots and roots recorded.

#### *Experiment 7*

The objective of this experiment was to assess whether part of the placed P would be fixed by the soil before seedling uptake. For this, we used two soils (RL-24 E and RL-24 G) which differed in sorption capacity. Before filling the pots with soil, three small PVC tubes (15 cm length and 4 cm diameter) were placed vertically in half the pots and filled with sand. The sand

in each tube was wetted before removing them. Seeds of two pearl millet hybrids 81B-P6 and ICMP 451-P8 were sown in the middle of the sand cores on 22 December 2005 in a glasshouse and thinned to 3 plants per pot 6 DAS. Two concentrations of  $\text{KH}_2\text{PO}_4$  solution 0, 1000  $\mu\text{L}$  (1.0 mg P) were applied 6 DAS in pots with and without sand cores, together with a non-limiting P control (300 mg  $\text{kg}^{-1}$  DAP). At 41 DAS, all plants were harvested and dry weights recorded.

### *Experiment 8*

Preliminary observations had shown positive interaction between N source and genotype for the biomass achieved under low soil P conditions, with some genotypes being more responsive to urea than to ammonium nitrate under low soil P conditions. We considered these differences could be due the cation-N source inducing proton excretion into the rhizosphere thereby favouring P mobility and absorption. The objective of this experiment was to confirm these findings and test whether P placement would enhance this effect of N source.

A single experiment was conducted, with hybrids 81B-P6 and ICMP 451-P8 grown outside on the low P soil RL24-E. Before sowing, 200 mL of solutions of either urea (10 mM) or ammonium nitrate (10 mM) were supplied to pots. The seeds were sown on 23 March 2006 and thinned to 3 plants per pot on 5 DAS. Two concentrations of  $\text{KH}_2\text{PO}_4$  solution (0, 1000  $\mu\text{L}$ , equivalent of 0 and 1 mg P per seedling) and one non-limiting P control (300 mg  $\text{kg}^{-1}$  DAP) were placed. All plants were harvested at 37 DAS and shoot and root fractions were separated and dry weights recorded.

### *Statistical analysis*

Dry matter weights and P uptake were subjected to analysis of variance (ANOVA) using Genstat release 9.1. As our main objective was to compare the effect of different treatments in the low P soil, the results of the non-limiting P control were not included in the ANOVA..

## **Results**

### *Seedling establishment (experiments 1-4)*

In experiment 1 placement of P gave very large increases in dry weight of shoots and roots of both hybrids grown on all three soils. The maximum response was reached at 500  $\mu\text{L}$  with

hybrid 81B-P6 but with ICMP 451-P8 the response continued up to the 1000 uL level so the maximum response was not identified (Table 1A). However in experiment 6 the dry weight of the nil treatment was much larger and the response to placement although statistically significant was correspondingly less (Table 1B). The increased total biomass produced in response to P placement varied from as low as 31 % and 42 % in 81B-P6 and ICMP 451-P8 in 2006, to as high as 330 % and 285% % in 81B-P6 and ICMP 451-P8 in 2005 (Table 1A and 1B).

Although overall the total plant dry mass was significantly ( $P < 0.001$ ) increased by the placement there were large differences between the three soils particularly in 2005 when the response to placement was large (Figure 1). When P was non-limiting there was little difference in total dry weight (TDW was about 5.41, 5.17 and 5.10 g  $\text{pl}^{-1}$  in RL 24-A, E and G soils respectively, data not shown).

Overall total biomass of the twenty inbred parents of ten mapping populations was increased by P placement (Table 2). However, there was substantial variation in response between inbred lines (Figure 2) ( $P = < 0.001$ ) with the biomass achieved with the placement of P well and significantly correlated with the biomass of the nil treatment ( $R^2 = 0.44$ ) (Figure 3A).

The hybrids (80) responded positively to the placement, with an overall total dry weight increase of 25 % (Figure 3B). The total dry weight of hybrids was about 7 times greater than inbreds with no placement. With placement, total dry weight of hybrids was about 3 times higher than inbreds. The biomass increase placement of P was well and significantly correlated with the biomass produced by the nil treatment ( $R^2 = 0.62$ ) (Figure 3B).

#### *Effect of timing of P placement on millet growth (Experiment 5-6)*

In experiment 5 regardless of genotype, the earlier the P placement the greater the increase in biomass (Figure 4 A). In experiment 6, the effect of an early application was less marked than in experiment 5. However, with an application at 5 DAS the total dry weight was still 29 % higher than the nil treatment, whereas application at 19 DAS, gave only an 8% increase (Figure 4 B).

#### *Type of soil and localized placement*

In Experiment 7, we tested whether using a sand core, to isolate the placement of soluble P from any sorption activity of the soil, could enhance the effect of the placement. It was found that the the biomass increase due to sand core placement exceeded the benefit of placement in soil ( $P < 0.001$ ). As expected, plants in RL 24-G soil grew very poorly in the 0 P treatment. However, the effect of the 1000  $\mu$ L P treatment applied in the sand core increased biomass relatively more in the case of RL 24-G than in RL 24-E. Total biomass increase due to the placement in RL 24-E was about 110% in both core or non-core treatment, with only a slight increase in the core treatment. By contrast, in RL 24-G, the increase in total biomass due to the placement in the non-core treatment was about 250%, whereas that in the core treatment was 400%.

#### *Effect of N source and placement*

Plant shoot dry weight in the nil P treatment was about 25% less with ammonium nitrate than with urea (Figure 5). With the placement of P, there was a further biomass enhancement in ICMP 451-P8, of about the same magnitude for both N sources but with 81B-P6 the increase (31 %) resulting from placement of P occurred only with the ammonium nitrate source.

#### *Effect of localized placement on the root/shoot (R/S) ratio*

Placement resulted in a trend for increased root/shoot ratio(R/S) of 0.46 meaned across experiments in all the inbreds and hybrids of pearl millet.. On average, placement of P showed higher R/S over non localized P (0.39) across all inbreds and hybrids (Figure 6). However, these differences were usually not large and in most cases not significant. The 80 hybrids appeared to have the largest increase in R/S ratio of about 20 %.

#### *Phosphorus acquisition of millet plants*



Although the Olsen P of soil RL24-A was less than in soil RL24-E and RL24-G, the plant P uptake in the nil and 0.25 mg treatments in this soil was greater than in plants grown in the same treatments in RL24-E and RL24-G. With the placement dose of 0.5 to 1.0 mg P, P uptake from RL24-E was similar to or greater than in RL24-A. P uptake from RL24-G was always smaller than uptake from RL24-A. Consequently the P balance at the end of the experiment with placement doses of 0.5 to 1.0 mg P, i.e. total P uptake minus Olsen P available in pots, was generally positive in RL24-A, and negative for both RL24-E and RL24-G.

## Discussion

Although the pearl millet was grown in pots rather than in field conditions, the positive response of young plants to minute applications of P, equivalent to 250-500 g P/ha, suggests that a practical field treatment based on this work would benefit this crop grown on soils very deficient in phosphorus.

The effect of the placement of P in our work is similar to that reported previously showing that adequate supplies of P in the early growing season promote early vigour, leading to more above ground biomass in cereals (Peltonen-Sainio *et al.*, 2006). These results also agree with Bolland and Baker (1988) who reported that wheat plants grown from seed with increasing P concentrations (1.4 to 3.7 g P kg<sup>-1</sup>) produced higher dry matter yields up to 35 days after sowing under greenhouse conditions. In our study, both hybrids and inbreds responded positively to the placement with no interaction indicating that the response to placement added to the intrinsic ability of some genotypes to perform better under low soil P conditions.

Therefore, an effect on the early establishment and development of young pearl millet seedling is likely to be the major reason for the success of the microdosing method described previously (Tabo *et al.*, 2005). Here we show that the microdosing method described by Tabo *et al.* could be improved and optimized, because improved growth was achieved using smaller quantities of P (equivalent to 250-500 g P/ha) than in the microdosing approach (equivalent to 12 kg P/ha). We are currently working on a seed coating and priming technique applying these small amounts of P.

The placement only had a beneficial effect on biomass when it was applied at early growth stages (Figure 5). We speculate that the early seedling development under P deficiency may limit the development of a plant structure that will eventually determine the growth potential

(for instance a reduction in the size and number of xylem vessels). The high P demand at early growth stages may also be due to the development of yield-determining structures (Ascher et al., 1987), because panicle initiation, formation and development of floral parts in pearl millet usually takes place within 30 days after planting (Bidinger and Hash, 2003). We interpret that the localized P treatment applied at an *early* stage, although minute in quantity, promoted a feed-forward effect, with an early “boost” to rooting leading subsequently to more P uptake from the soil. Work is in progress to test whether the improved early establishment of pearl millet seedling eventually leads to yield differences at maturity.

Significant differences between soils in how plants responded to the placement were observed in our study (Figure 1), with soil RL 24 E allowing the largest response and RL 24G a smaller response. We hypothesize that these soil-related differences were due to differences in their sorption capacity as described by Scott et al., (1991). Although we did not measure their sorption capacity, the greater response in soil RL 24 G than in soil RL 24 E when the placement was applied into a sand core, which protected the soluble P from the sorption effect (Figure 6), supports this hypothesis.

There were smaller responses to placement in experiments 4 and 6 than in experiment 3. All three experiments were conducted outdoors but 4 and 6 were during the dry season, whereas experiment 3 was during the rainy season. Our interpretation is that the maintenance of a wetter soil surface layer during the days after treatment, favoured by growing plants under more humid conditions would have kept the applied P in a soluble form for longer and promoted its absorption. Quick drying of the surface soil during the dry season might have limited the availability of soluble P, hence restricting the response to the treatment.

In our study, increased relative biomass allocation to roots was observed ( $>R/S$ ) in both inbreds and hybrids, although these differences were only statistically significant in the case of the 80 hybrids. These results indicate presumably that the enhanced phosphorus acquisition from the placement may have increased rooting at least initially. The enhanced P uptake resulting from the greater root development would in turn favour shoot development and limit the increase in  $R/S$  (Figure 8). This probable effect of the P treatment on the root development is particularly relevant to pearl millet growing areas of West Africa where P deficiency and water stress often occur together.

Our results showed that P placement combined with urea as the N source increased plant response to placement more than ammonium nitrate (Figure 7). This difference is supported by a recent study (Zhang et al., 2004), who found that initial P uptake in plants was greater with  $\text{NH}_4^+$ -N fertilizer than with the  $\text{NO}_3^-$ -N nutrition. Enhanced effects of N on P uptake have also been shown for maize (Engelstad and Allen, 1971) and wheat (Leikam *et al.*, 1983), particularly when N was applied as ammonium (Leonce and Millet, 1966), and might be explained by pH differences induced by the source of N used. Indeed, Gillespie and Pope (1991) found up to a five-fold increase in phosphorus diffusion rate with an increased acidity of one pH unit. More work would be needed to assess the interaction between localized pH and N source.

## **Conclusion**

Our work has shown that pearl millet seedlings grown in P deficient soil respond dramatically to minute amounts of placed P, leading to improved establishment. Practical applications of this approach would lead to better pearl millet stands on farms, so we are developing methods such as seed coating and priming as means of applying the small amounts of P required to give the response, i.e. only 10-20 times the amount available in pearl millet seeds which is equivalent to an application of 250-500 g P/ha,. The development of these practical applications, will need to be tested on a range of soils varying in their P deficiency,. It will be important to develop solutions that can be easily implemented by farmers using as far as possible local on farm resources and readily accessible sources of P fertilizer. This work is currently in progress.

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**Table 1A** Mean of shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), and root shoot ratio(R/S) of two pearl millet hybrids across 3 P deficient soils after P placement with 0, 250, 500 1000  $\mu$ L of a 32.2  $\mu$ M  $\text{KH}_2\text{PO}_4$  solution in Experiment 1 (2005). Data are means of 5 replicate pots

Genotype/Treatment	SDW	RDW	TDW	R/S				
<i>Experiment 1 (2005)</i>								
81B-P6								
0 μL	0.16	0.07	0.23	0.55				
250 μL	0.25	0.12	0.37	0.59				
500 μL	0.62	0.36	0.99	0.60				
1000 μL	0.40	0.21	0.61	0.61				
ICMP 451-P8								
0 μL	0.29	0.11	0.40	0.50				
250 μL	0.40	0.23	0.62	0.62				
500 μL	0.88	0.43	1.32	0.52				
1000 μL	1.03	0.50	1.54	0.59				
<i>Factors</i>	SDW		RDW		TDW		RS ratio	
	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>
Treatment (T)	<0.001	0.11	<0.001	0.06	<0.001	0.16	NS	0.06
Genotype (G)	<0.001	0.07	<0.001	0.04	<0.001	0.11	NS	0.04
Soil (S)	<0.001	0.09	<0.001	0.05	<0.001	0.14	<0.001	0.05
TxG	<0.001	0.15	<0.001	0.08	<0.001	0.23	NS	0.09
TxS	<0.001	0.19	<0.001	0.10	<0.001	0.28	<0.001	0.11
GxS	<0.001	0.13	<0.001	0.07	<0.001	0.20	NS	0.08

<sup>§</sup>At 5% level of significance; NS, Non-significant.

**Table 1B** Mean of shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), and root shoot ratio(R/S) of two pearl millet hybrids across 3 P deficient soils after P placement of 0, 250, 500 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution in Experiment 6 (2006). Data are means of 5 replicate pots

Genotype/Treatment	SDW	RDW	TDW	R/S ratio				
<i>Experiment 6 (2006)</i>								
81B-P6								
0 μL	0.48	0.26	0.74	0.55				
250 μL	0.59	0.32	0.92	0.55				
500 μL	0.66	0.31	0.97	0.49				
1000 μL	0.63	0.31	0.94	0.52				
ICMP 451-P8								
0 μL	0.58	0.22	0.80	0.42				
250 μL	0.60	0.26	0.86	0.44				
500 μL	0.75	0.29	1.04	0.40				
1000 μL	0.81	0.33	1.14	0.42				
<i>Factors</i>	SDW		RDW		TDW		R/S ratio	
	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>	<i>p</i>	LSD <sup>§</sup>
Treatment (T)	<0.001	0.06	NS	0.04	<0.001	0.09	NS	0.06
Genotype (G)	<0.001	0.04	NS	0.03	NS	0.06	<0.001	0.04
Soil (S)	<0.001	0.05	<0.001	0.03	<0.001	0.08	<0.001	0.05
TxG	NS	0.09	NS	0.06	NS	1.13	NS	0.09
TxS	NS	0.11	NS	0.07	NS	1.16	NS	0.11
GxS	NS	0.08	NS	0.05	NS	1.11	NS	0.08

<sup>§</sup>At 5% level of significance; NS, Non-significant.

**Table 2** Analysis of variance of effects of P placement (1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution) on shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW), in  $\text{g plant}^{-1}$  of twenty mapping population pearl millet genotypes in Experiment 2

Factors	SDW		RDW		TDW	
	P	LSD <sup>§</sup>	P	LSD <sup>§</sup>	P	LSD <sup>§</sup>
Genotype (G)	< 0.001	0.28	< 0.001	0.15	< 0.001	0.41
Treatment (T)	< 0.001	0.11	< 0.001	0.06	< 0.001	0.16
G x T	NS	NS	NS	NS	NS	NS

<sup>§</sup>At 5% level of significance; NS, Non-significant.



## **Legends to Figures**

**Figure 1** Total dry weight ( $\text{g pl}^{-1}$ ) of two pearl millet hybrids on three types of soils following P placement treatments (0, 250, 500, 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution) in Experiment 1 (2005 A, B) and experiment 2 (2006 C, D). Data are means of 5 pots, and bars indicate SE.

**Figure 2** Total biomass production ( $\text{g P pl}^{-1}$ ) of twenty mapping population pearl millet parents under 0  $\mu\text{L}$  or 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution treatment (Experiment 3). Data are means of 5 pots, and bars indicate SE.

**Figure 3** Linear relationship between total plant biomass with P placement (1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution) and total biomass without placement in 20 inbred parents (Experiment 3) (A) and 80 hybrids (Experiment 4) (B).

**Figure 4** Influence of placement of 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution applied at 6, 19 and 30 DAS in Experiment 5 (A), or at 5, 12, 19 DAS in Experiment 6 (B) on total dry weight ( $\text{g/plant}$ ) of two pearl millet hybrids; means of 5 pots, bars indicate SE.

**Figure 5** Response of total dry weight ( $\text{g pl}^{-1}$ ) of two pearl millet hybrids to P placement at 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution using two sources of available nitrogen (urea and ammonium nitrate (AN)) (Experiment 8). Data are means of 5 replicated pots, and bars indicate SE.

**Figure 6** Effect of P placement (0  $\mu\text{L}$  and 1000  $\mu\text{L}$  of a 32.2  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  solution) on the root to shoot ratio (Root/Shoot) across 20 inbred lines, across 2 and 80 hybrids of pearl millet, Values in parenthesis indicate the number of genotypes used in each pair of bars. (data are means of 5 plants, bars indicate SE).

**Figure 1**

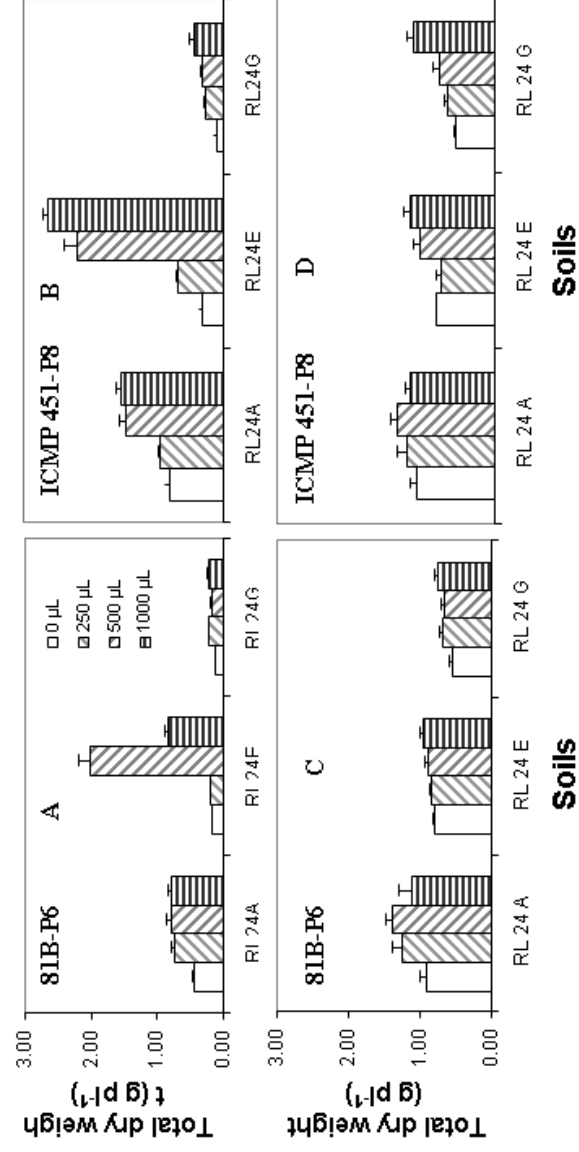


Figure 2

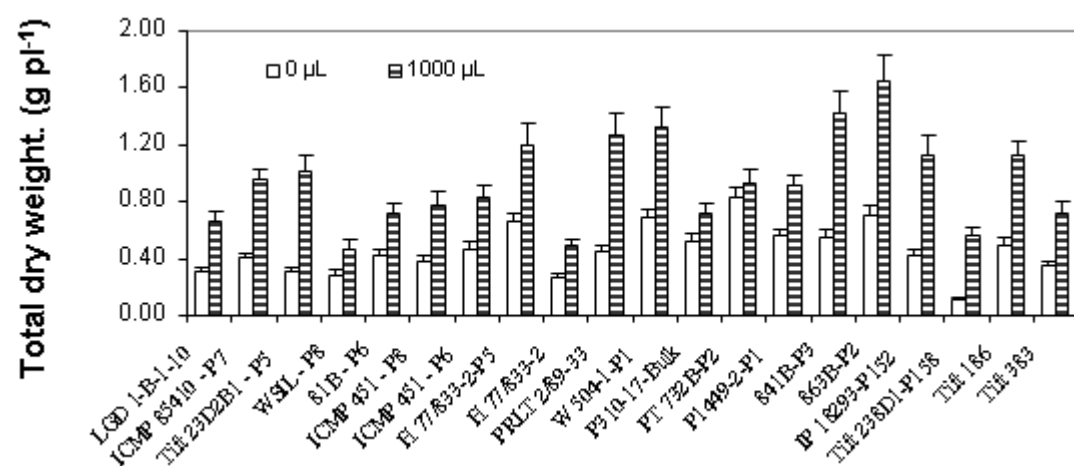


Figure 3

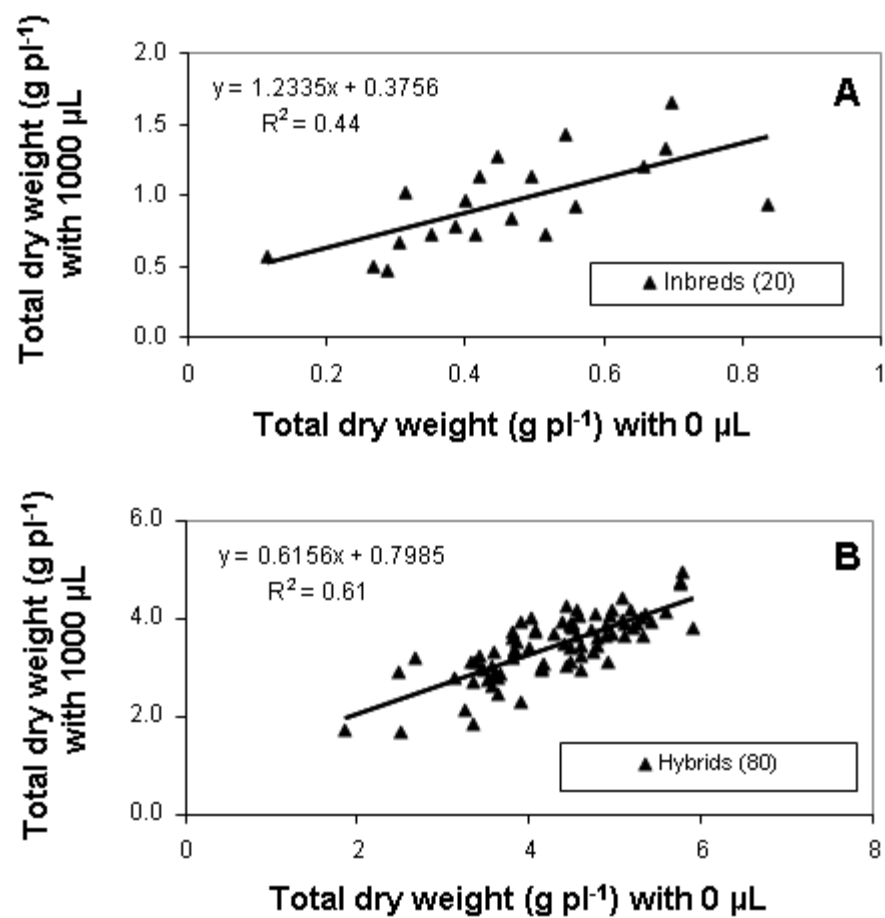
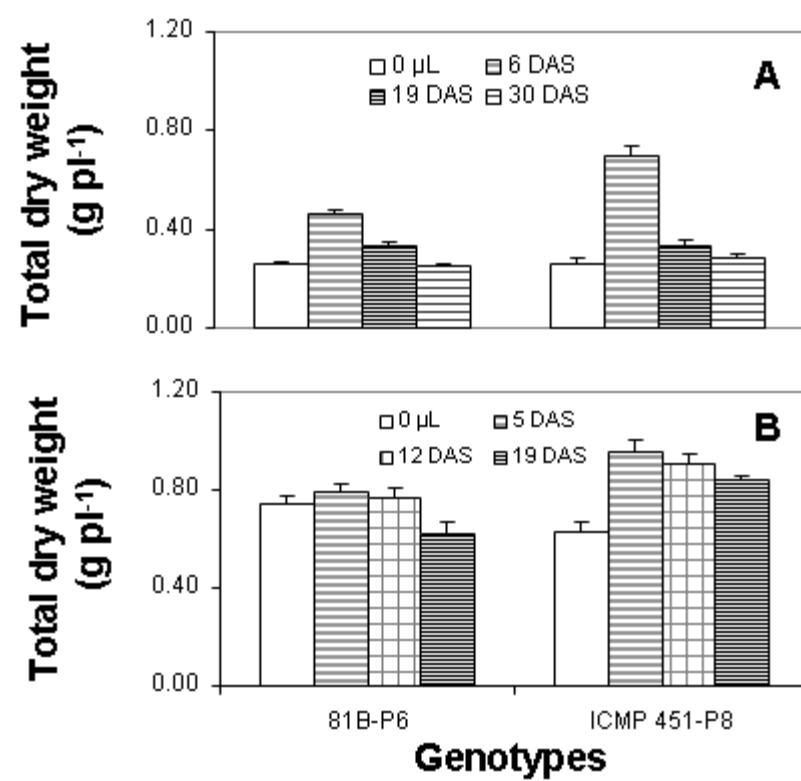
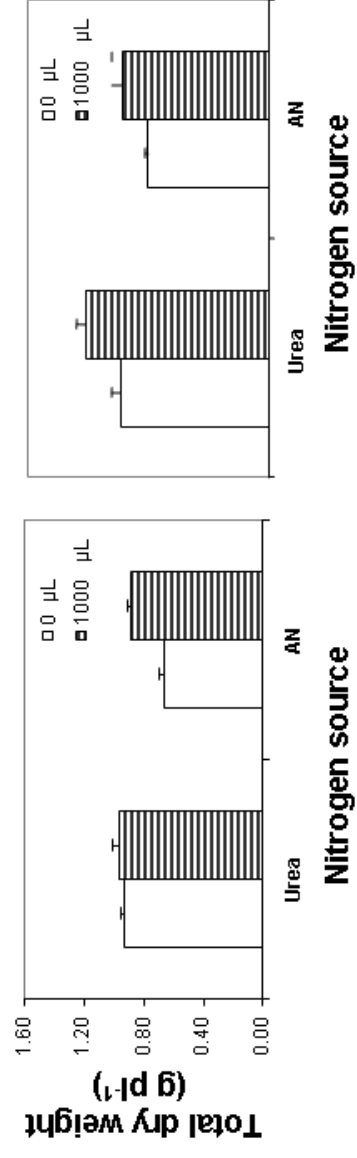


Figure 4



**Figure 5**



**Figure 6**

