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DOI: <https://dx.doi.org/10.4314/acsj.v32i2.4>



SEEDBALL TECHNOLOGY OVERCOMES EFFECT OF SMALL SEED-SIZE AND LOW SOIL FERTILITY ON EARLY PEARL MILLET SEEDLING PERFORMANCE

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(Received 16 May 2024; accepted 28 June 2024)

ABSTRACT

In the African Sahel region, pearl millet (*Pennisetum glaucum* (L.) R. Brown) is mostly produced in low-nutrient soils. Available evidence shows that low soil fertility and small seed size significantly reduce seedling establishment and in turn, cause low grain yield. Seedball technology, which refers to a gravimetric mixture of loam soil, seeds, water and additives that improve plant performance, could overcome this effect in the field. The objective of this study was to optimise the influence of seedball on pearl millet (*Pennisetum glaucum* (L.) R. Brown) seedling establishment in the African Sahel region. Conventionally sown and seedball-derived pearl millet seedlings, of a local and improved varieties, were grown for 29 days, from small and large seed sizes, in low- and medium-nutrient soils, in a greenhouse. Results showed that under low-nutrient conditions, and with small seed sizes, *P. glaucum* produced generally inferior biomasses compared to normal nutrient conditions and large seed sizes. On the other hand, seedball technology significantly enhanced ($P < 0.05$) seedling vigour, leaf number, plant height, dry matter accumulation, root length and fine root development; and nutrient uptake, irrespective of soil nutrient level and seed size in the two varieties used in this study. These enhancement effects were more obvious in the local variety compared to its improved counterpart.

Key Words: *Pennisetum glaucum*, Sahel region, seedling vigour

RÉSUMÉ

Dans la région du Sahel Africain, le mil perlé (*Pennisetum glaucum* (L.) R. Brown) est principalement produit dans des sols pauvres en nutriments. Les données disponibles montrent qu'une faible fertilité du sol et une petite taille des graines réduisent considérablement l'établissement des semis et, à leur tour, entraînent un faible rendement en grains. La technologie des boules de graines, qui fait référence à un mélange gravimétrique de sol limoneux, de graines, d'eau et d'additifs qui améliorent les performances des plantes, pourrait surmonter cet effet sur le terrain. L'objectif de cette étude était d'optimiser l'influence des boules de graines sur l'établissement des plantules de mil perlé (*Pennisetum glaucum* (L.) R. Brown) dans la région du Sahel Africain. Des plantules de mil perlé semées de manière

conventionnelle et dérivées de boules de graines, de variétés locales et améliorées, ont été cultivées pendant 29 jours, à partir de petites et grandes tailles de graines, dans des sols pauvres et moyens en nutriments, dans une serre. Les résultats ont montré que dans des conditions de faible teneur en nutriments et avec de petites tailles de graines, *P. glaucum* produisait des biomasses généralement inférieures par rapport aux conditions nutritionnelles normales et aux grandes tailles de graines. D'autre part, la technologie des boules de semences a amélioré de manière significative ($P < 0,05$) la vigueur des plantules, le nombre de feuilles, la hauteur de la plante, l'accumulation de matière sèche, la longueur et le développement des racines fines, ainsi que l'absorption des nutriments, indépendamment du niveau de nutriments du sol et de la taille des graines dans les deux variétés utilisées dans cette étude. Ces effets d'amélioration étaient plus évidents dans la variété locale par rapport à ses homologues améliorées.

Mots Clés : *Pennisetum glaucum*, région du Sahel, vigueur des plantules

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L. R. Brown)) is a staple crop mainly in the arid and semi-arid regions of Africa and elsewhere in the world (Mason *et al.*, 2015). Seed size plays crucial roles in pearl millet seedlings establishment, particularly under stressful climatic conditions. Large seeds tend to produce more vigorous and high dry matter-endowed seedlings under drought stress (Manga and Yadav, 1995); while small seeds tend to produce poorly established seedlings under parallel stressful conditions (Gardner and Vanderlip, 1989). Pearl millet seed size range from 1000 grain mass (TGM) of 4 g (Nwankwo and Herrmann, 2023), to 44 g seed⁻¹ (Baryeh, 2002). Therefore, manipulation of seed sizes, together with soil nutrient levels to obtain optimal combinations, could serve as a possible intervention in this context.

The rationale behind the relatively better seedling performance from large seeds is the higher nutrient content of the endosperm, which nourishes the embryo at the earliest development stage (Kaufmann and Guitard, 1967). To mitigate the negative effects of small seed sizes on seedling performance, technologies that compensate for low nutrients amounts are needed. Moreover, with the conversion of agricultural lands into recreational and social activities (Moussa *et al.*, 2021). and the growing Sahelian human population exceeding food production gains

(Abdi *et al.*, 2014), options for increased pearl millet productivity are imperative.

The seedball technology is such an option that has a focus on crop establishment and early flush seedling growth. It is a simple seed-pelleting technique that combines loam, sand, seed and nutrient additives intended to increase pearl millet yield in low fertile soils (Nwankwo *et al.*, 2018a). The objective of this study was to optimise the influence of seedball on pearl millet (*Pennisetum glaucum* (L. R. Brown)) seedling establishment in the African Sahel region.

MATERIALS AND METHODS

Nature of study and varieties used. This study was carried out in a greenhouse of the University of Hohenheim in Germany. Seeds of two pearl millet varieties, a local (Seré) and improved (Zatib), were selected by size from seed lots, and graded into small and large seeds, using a digital weighing scale. The local seed variety was selected from the 2020 harvest in the Maradi region, Niger; while the improved variety, was collected from the cross of the local varieties Zanwarfa (Za) and Tchinan (TI) Bijini (B) (Moussa, *Oral comm.*). The improved variety is tolerant to millet head miner, but sensitive to drought and Striga. It represents a regionally known improved variety in the Sahel (Maman *et al.*, 2000a). In terms of seed size, the local variety seeds had 1000 total grain mass (TGM) of 8.53 g for small

seeds and 14.14 g for large seeds; while the improved variety seeds had TGMs of 9.25 and 18.63 g, respectively.

Treatments. Three treatments were considered in this study, namely (i) seedball technology: no seedball (-) and with seedball (+); (ii) nutrient levels: (low and medium nutrient); and seed size (small and large); and (iii) varieties, local (Seré) and improved (Zatib). The seedball technology included conventional sowing, (i) control; and (ii) the seedball. The control comprised of seeds without the seedball coating, in the form often used by the local farmers. The seedball treatment represented about 2 cm diameter-sized balls, made from a mixture of 80 g sand, 50 g loam, and 1 g mineral fertiliser - NPK 15:15:15 (NPK).

The nutrient level factor was represented by low nutrients (no nutrient application), and medium level (mixture of low nutrient soil and quartz sand). Seed size, on the other hand, was represented by small size (8.53 g per TGM) and large seeds (14.14 g per TGM). The treatments were laid out in a completely randomised design, replicated six times. (Fig. 1).

Growth medium and environment. The growth medium comprised of soil material from dune sands, collected at Rastatt in Germany. It was sieved through a 2-mm mesh, to remove non-soil materials. The low-nutrient substrate represented a mixture of four parts of quartz sand and one part of Rastatt soil. The physical and chemical properties of these substrates were reported in detail in Nwankwo and Herrmann (2021).

Experimental management. Two seeds of each pearl millet variety were sown per germination pot, in six replications; in a completely randomised design. The seedlings were thinned to one per pot; each filled with substrate at a bulk density of 1.8 g cm^{-3} . The gravimetric water content of each pot was 16.5%; this was restored to the original weight at every 48 hr throughout the experimental 29 days. Temperatures in the greenhouse were $24 \text{ }^{\circ}\text{C}$ during night, and $32 \text{ }^{\circ}\text{C}$ during day. The relative humidity was 40 and 65% during day and night, respectively.

Data collection. Seedling vigour was determined on a scale of 1 to 10, in the order of weakest to strongest, at 12 days after

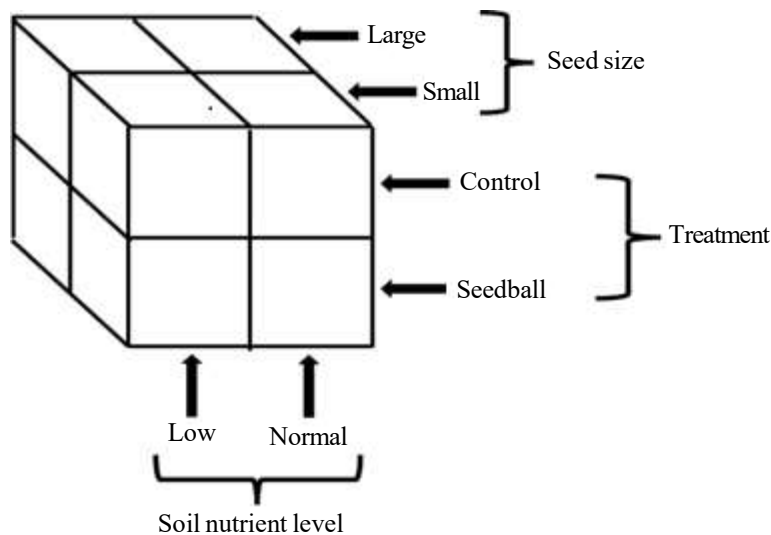


Figure 1. Sketch of the experimental design of the treatments and factors used in this study.

sowing (DAS). Plant height and stem basal diameter were also determined using a tape measure at the same growth stage. Leaf count was determined at 12, 21 and 29 DAS, number of tillers developed was also determined for each treatment at the 12, 21, and 29 DAS.

Shoot biomass was measured at harvest and separated into fresh shoots and roots, before drying of both portions at an oven temperature of 59 °C. Dry weight of the samples was taken using a digital balance. Roots were scanned and measured for total root length and diameter class, using an EPSON Perfection V700 PHOTO dual lens scanner; and WinRhizo[®] software V2009c (Regent Instruments, Nepean, Canada).

Nutrient contents in root (improved variety, only) and shoot (in both varieties) were analysed after harvest. Total phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) were analysed as major nutrients. Trace elements analysed for included zinc (Zn), iron (Fe) and copper (Cu).

Dry root and shoot samples were separately pooled by treatment and the materials ground using a mixer mill. Concentrated solutions of HNO₃ and H₂O₂ were used in a ratio 2:1, to digest the samples, for about 10 minutes in a ventilated microwave (MLS 1200 mega, Leutkirch, Germany) at 50 Watts. Microwave Plasma-Atomic Emission Spectrometer (MP-AES 4100, Agilent, Waldbronn, Germany) was used to measure K, Ca, Mg, Zn, Fe and Cu. Phosphorus was measured with a spectrometer using molybdenum blue method, at 710 nm wavelength (Varian, Cary 50).

Data analysis. The data were analysed using SAS version 9.4 and Sigma Plot version 13.0. All repeated and one-time data were subjected to medium distribution and variance homogeneity test based on the Shapiro-Wilk test. For unevenly distributed data, Welch's one-way analysis of variance using the procedure of general linear model (GLM) was applied, with line display option for statistical differences was used. All treatment means

were compared using standard deviations at 5% probability level.

RESULTS

Plant height. Plant height was significantly influenced ($P < 0.05$) by treatments at all the observed development stages (Table 1). At 12 DAS, seedball technology increased plant height in local variety by 107 and 45% in low- and medium-nutrient soils, respectively. For the improved variety, seedball technology had a negative effect on the plant height in low-nutrient seedling; however, seedling vigour was relatively higher in all seedball treatments compared to the control (data not presented).

At 21 and 29 DAS, treatment effect was marginal in particular for the improved variety (Table 1). Plant height was consistently taller in seedball treatments than in the control, and medium-nutrient; compared to the low-nutrient seedling. Seedball demonstrated stronger effects on the height of local variety of medium-nutrient seedling, compared to the low-nutrient control seedling. Seedball treatments reduced the absolute plant height differences in the small-sized seeds between the local and improved varieties. Similar observations were made for leaf development (data and photos not shown).

Root and shoot variables. Seedling root density, root to shoot dry matter ratio and shoot dry matter were significantly influenced ($P < 0.05$) by the seedball treatment. With respect to the local variety compared to the control, the low- and medium-nutrient seedball treatment increased seedling root density by > 3.5 folds (Fig. 2a). The root to shoot dry matter ratio was reduced by > 30% in the seedball medium-nutrient treatment, compared to the low-nutrient condition. It appears that the plant invests relatively less in root biomass the more nutrients are supplied (Fig. 2b).

Seedballs increased seedling shoot dry matter by > 870 and 140% in low- and medium-nutrient treatments, respectively (Fig.

TABLE 1. Effect of seedball, seed size and nutrient levels on pearl millet plant height at 29 DAS

Variable	Time	Variety	Control		Seedball	
			Low nutrient	Medium nutrient	Low nutrient	Medium nutrient
Plant height (cm)	Day 12	Local	7.4 ^c (1.4)	11.2 ^b (3.0)	15.3 ^a (4.8)	16.2 ^a (3.3)
		Improved	22.7 ^a (3.2)	25.2 ^a (3.4)	17.1 ^b (5.2)	22.9 ^a (4.1)
	Day 21	Local	13.4 ^c (2.5)	29.2 ^b (4.3)	33.3 ^b (7.2)	43.5 ^a (6.4)
		Improved	32.3 ^b (5.6)	39.6 ^a (3.3)	31.3 ^b (8.3)	38.4 ^a (5.7)
	Day 29	Local	19.6 ^d (3.8)	54.7 ^b (5.9)	43.8 ^c (7.5)	64.8 ^a (5.8)
		Improved	50.1 ^b (7.8)	58.8 ^a (4.7)	52.7 ^b (7.3)	59.2 ^a (6.0)

Values show arithmetic means (n = 6), standard deviations are represented in brackets, letters show significance per treatment observed time at 5% level (Tukey test)

2c) for the local variety compared to the control. These figures were 21 and 33% for the improved variety (Fig. 2d). Treatment effects were more visible and consistent for the local variety than for the improved variety (Fig. 2a, c).

The seedball technology was invariably effective at increasing seedling total root as well as fine root length, irrespective of soil nutrient level (Figs. 3a and 3b). For instance, about 6- and 3-folds increase in total root length were observed in the low- and medium-nutrient seedball treatments, compared to the control (Fig. 3b).

The effect of soil nutrient level on root development was visible in all treatments (Table 2, Figs. 2, 3 and 4). In particular, fine root length was increased by > 200% in the control and by 62% in the seedball treatment (Fig. 3a). Again, the more nutrient deficient the growth medium was, the more effective seedballs appeared to be. Similar observations were made with respect to medium (0.2 - 0.8 mm diameter) and thick root lengths (> 0.8 mm) (Fig. 3a). In contrast, root diameter was not influenced ($P > 0.05$) by the treatments (data not presented).

The values above the bars in Figure 3 show the ratio of fine to total root length. Seedball treatments presented relatively finer roots than their control counterparts (Fig. 3). These root

variables were not measured in the improved variety, due to the destructive nature of WinRhizo^r analytical procedure applied; root dry matter measurement was considered of utmost important.

Compared to the control, total root length was increased by about 3.5 folds ($P < 0.05$) in seedball treatments, irrespective of seed size (Fig. 3b). Again, the nutrient supply nature of seedball was evident in the fine root length to total root length ratio. Fine root length to total root ratio was about 65% in seedball, compared to > 60% in the control, in small and large seed seedlings, respectively.

The effect of nutrient levels was more evident in the local variety, compared to its improved variety counterpart. For instance, seedball increased P uptake by about 2.8 and 13 folds in the local and improved varieties of medium- and low-nutrient seedlings, respectively; compared to control (Table 2). For K uptake in the local variety, it was about 2 and 11 folds higher in seedball medium-nutrient seedlings compared to control.

Magnesium uptake in both local and the improved varieties was influenced in same order of magnitude; medium-nutrient seedball > medium-nutrient control > low-nutrient seedball > low-nutrient control (Table 2). Calcium uptake was significantly increased ($P < 0.05$) in the seedball treatment compared

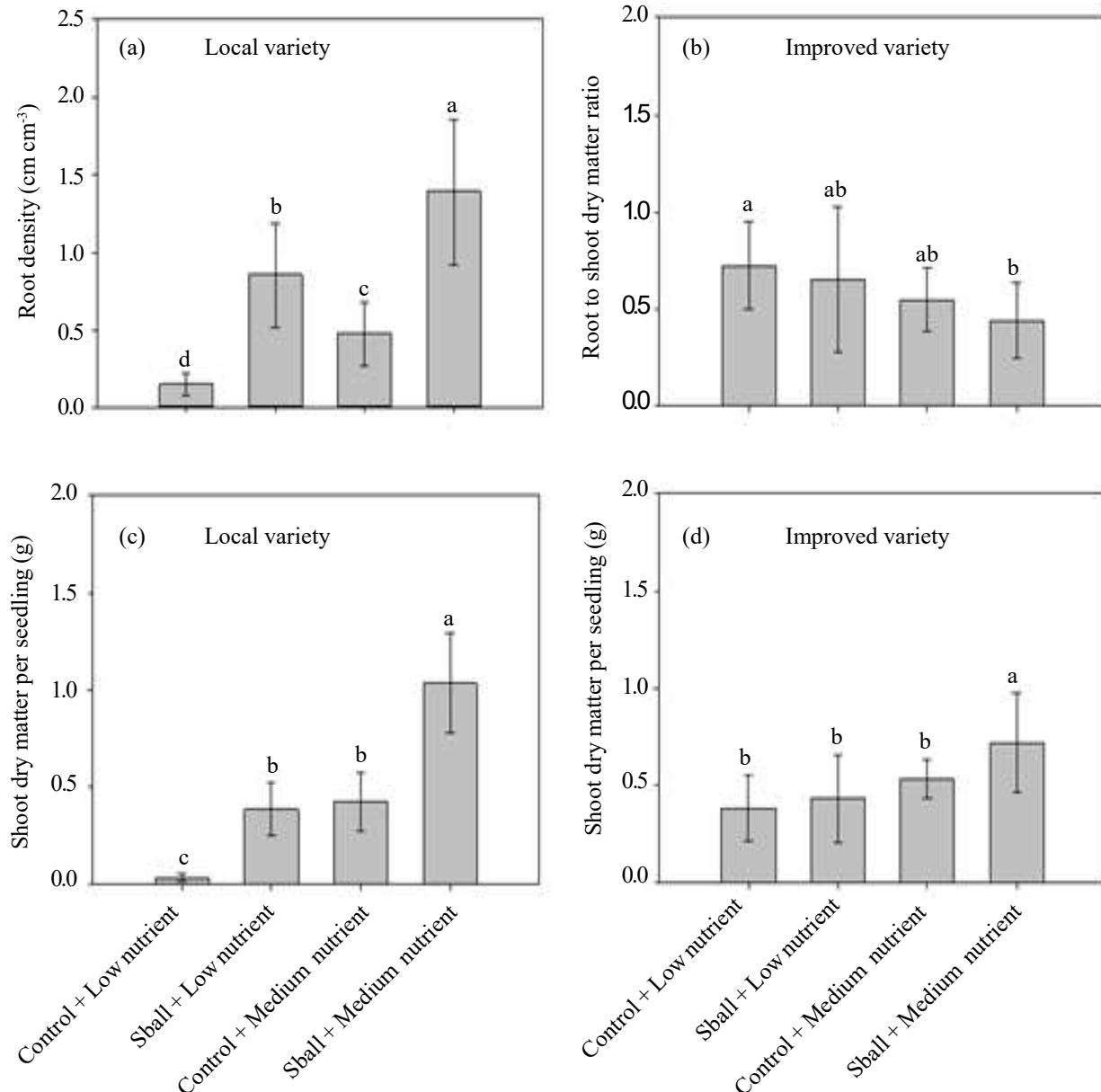


Figure 2. Root density (a), root to shoot dry matter ratio (b), and shoot dry matter (c, d) of local (a, c) as well as improved (b, d) pearl millet varieties 29 DAS for different treatments. Letters indicate significant differences ($P < 0.05$) of arithmetic means ($n = 6$) and error bars indicate standard deviations.

to control in the local variety, but it was lower in low-nutrient seedling.

Treatments did not influence Fe uptake in the improved variety (Table 2). The uptake of Zn and Fe was extremely low under low-nutrient condition in both local and improved

varieties (Table 2). The local variety repeated this already known pattern for Cu uptake; however, improved variety Cu uptake was marginal under low-nutrient control treatment (Table 2).

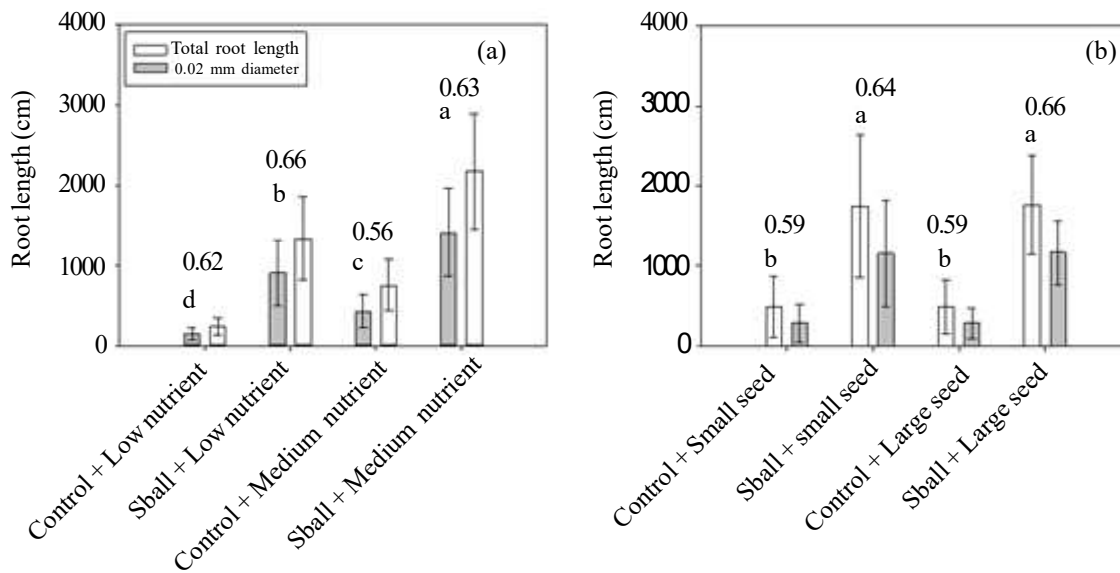


Figure 3. Root length of the local pearl millet variety 29 DAS for different treatments as influenced by soil nutrient level (a) and seed size (b). Values above the bars represent the ratio of fine root (0–0.2 mm diameter) to total root (0–> 1.2 mm diameter) length. Letters indicate significant differences ($P<0.05$) of arithmetic means ($n = 6$) for total root length and error bars indicate standard deviations.

TABLE 2. Treatment effect on total seedling nutrient uptake (dry matter mg per germination pot) of the improved pearl millet seedlings variety 29 days after sowing

Nutrient	Control		Seedball	
	Low nutrient	Medium nutrient	Low nutrient	Medium nutrient
Phosphorus	1.2 ^C (0.5)	2.2 ^B (0.7)	2.0 ^B (1.0)	3.3 ^A (1.0)
Potassium	21.6 ^B (9.0)	22.7 ^B (6.4)	26.6 ^{AB} (13.8)	35.3 ^A (11.7)
Magnesium	2.6 ^B (1.2)	3.7 ^B (0.8)	2.9 ^B (1.5)	5.3 ^A (2.3)
Calcium	5.5 ^A (2.5)	5.8 ^B (1.4)	6.0 ^A (3.1)	7.3 ^A (2.8)
Zinc	0.04 ^B (0.02)	0.06 ^A (0.02)	0.03 ^B (0.02)	0.07 ^A (0.02)
Iron	1.3 ^A (0.7)	0.96 ^A (0.3)	1.1 ^A (0.6)	0.97 ^A (0.5)

Values represent arithmetic means (\pm standard deviations; $n = 6$) and letters indicate significance at 5% level (Tukey test)

Plant leaf and height development vs seed size. In all treatments, the number of leaves and plant height were significantly increased ($P<0.5$) between 12 and 29 DAS. However, treatment effects were stronger for the local than for the improved variety (Table 3). For

instance, compared to control at 12 DAS, seedball increased the leaf number by 27 and 43% in small and large seeds, respectively; in the local variety (Table 3). The same was not significant ($P>0.05$) for improved variety. Observations for the improved variety at 21

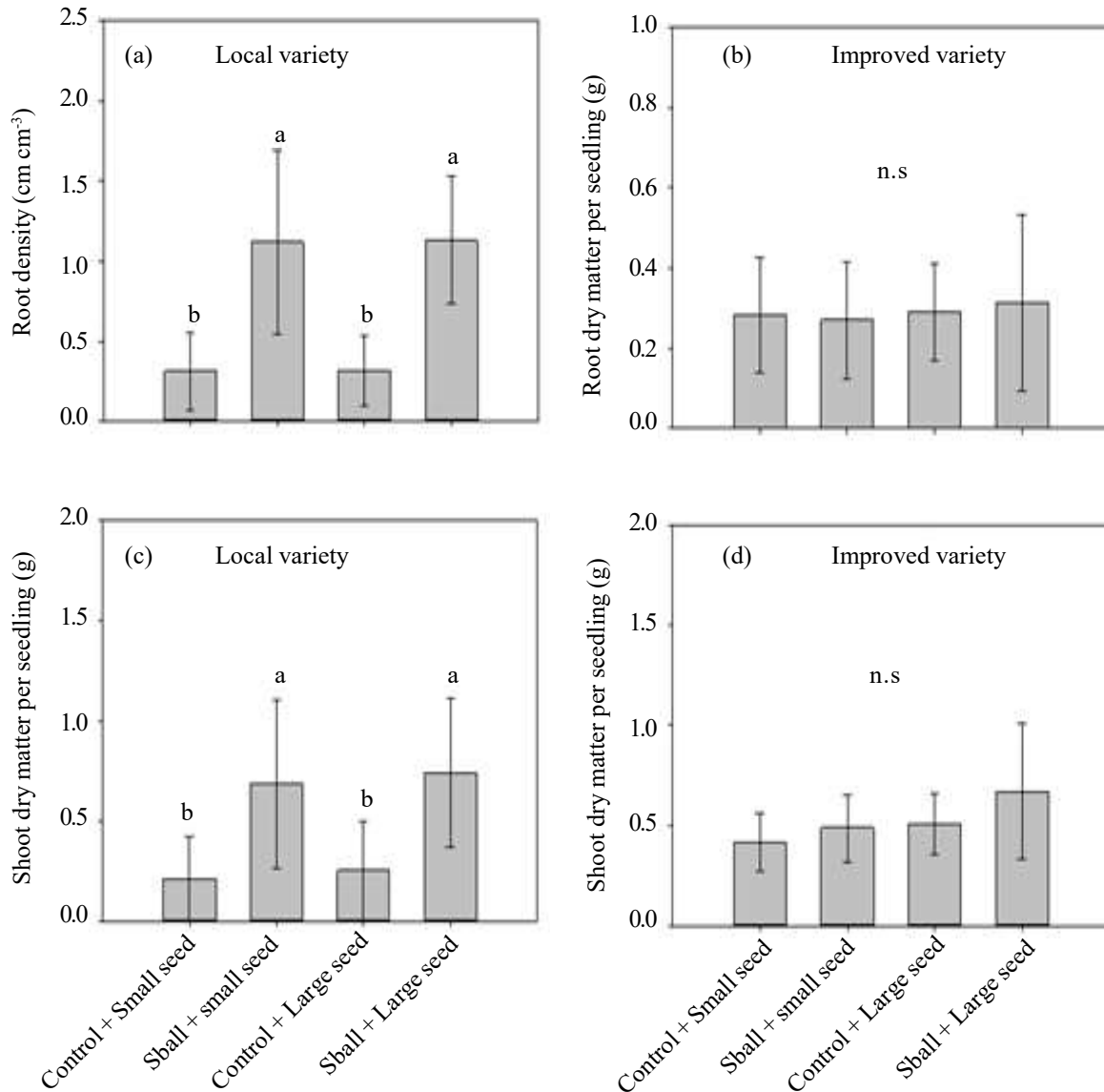


Figure 4. Root density (a), root dry matter (b), and shoot dry matter (c, d) of local (a, c) as well as improved (b, d) pearl millet seedling varieties 29 DAS for different treatments. Letters indicate significant differences of arithmetic means ($p < 0.05$), n. s. indicates non-significance ($p > 0.05$), and error bars indicate standard deviations.

and 29 DAS showed a seed size effect, with small seeds resulting into slower leaf height, the differences decreased over time. There were no significant significant seedball treatment effects ($P > 0.5$) on plant height at 21 DAP. This indicates that the seedball treatment outbalances the varietal effect.

The effect of seedballs increased plant height by 86 and 56%, at 12 DAS; and by 86 and 75% at 21 DAS, respectively. Although the improved variety outcompeted the local

variety in all control treatments effects on plant height, the differences decreased over time. There were no significant significant seedball treatment effects ($P > 0.5$) on plant height at 21 DAP. This indicates that the seedball treatment outbalances the varietal effect.

TABLE 3. Treatment effect on leaf and height development of pearl millet 29 DAS

Variable	Time	Variety	Control		Seedball	
			Small seed	Large seed	Small seed	Large seed
Plant leaf number (#)	Day 12	Local	2.2 ^b (0.3)	2.1 ^b (0.4)	2.8 ^a (0.6)	3.0 ^a (0.4)
		Improved	4.1 ^a (0.3)	4.3 ^a (0.7)	4.0 ^a (0.6)	4.5 ^a (0.8)
	Day 21	Local	3.9 ^b (1.1)	3.9 ^b (1.1)	5.7 ^a (1.0)	6.0 ^a (0.9)
		Improved	6.0 ^b (0.6)	6.7 ^{ab} (0.7)	6.5 ^b (0.8)	7.5 ^a (2.0)
	Day 29	Local	5.4 ^b (1.6)	5.3 ^b (1.3)	7.4 ^a (1.7)	7.1 ^a (1.1)
		Improved	7.6 ^b (0.7)	7.8 ^b (0.6)	8.0 ^b (1.2)	9.1 ^a (2.1)
Plant height (cm)	Day 12	Local	8.0 ^b (2.1)	10.5 ^b (3.4)	14.9 ^a (4.6)	16.6 ^a (3.4)
		Improved	23.3 ^{ab} (2.9)	24.7 ^a (3.9)	19.6 ^b (4.7)	20.7 ^{ab} (6.3)
	Day 21	Local	21.2 ^b (9.0)	21.3 ^b (9.0)	37.2 ^a (8.9)	39.6 ^a (8.3)
		Improved	35.5 ^a (5.4)	36.6 ^a (6.3)	34.7 ^a (7.0)	35.4 ^a (8.9)
	Day 29	Local	37.8 ^b (19.3)	36.5 ^b (18.6)	54.4 ^a (12.2)	54.1 ^a (13.5)
		Improved	56.0 ^a (7.6)	53.8 ^a (7.5)	56.3 ^a (6.6)	55.9 ^a (8.3)

Values show arithmetic means (n = 6), standard deviations are represented in brackets, letters show significance per treatment observed time at 5 % level (Tukey test)

Seed size on root and shoot variables at harvest. Seedballs showed no significant impact on biomass variables of the improved variety (Fig. 4b and d). In contrast, root density of the local variety was significantly increased ($P < 0.05$) by about 2.7 folds by seedball (Fig. 4a), independent of seed size. Likewise, shoot dry matter increased by about 3 folds (Fig. 4c). Again, seedballs could outbalance the negative effect of low grain size. Small grain sizes of the improved variety do not seem to result in nutrient limitations that would hamper early biomass production.

Soil nutrient levels vs nutrient uptake. Nutrient uptake was significantly higher ($P < 0.05$) in seedball seedlings (Table 4). For instance, large seeded seedball increased P and Mg uptake by 1.9 and 1.5 folds higher than in the control (Table 4). Potassium uptake was only slightly influenced in the following order

of magnitude: seedball > control, independent of seed size ($P > 0.05$). A difference in calcium, however, was only evident in the large seed of seedball treatment, compared to the absolute control i.e., small seed control (Table 4).

DISCUSSION

Plant height. The significant increase in seedling plant height at 12 DAS, in the local variety, by 107 and 45% in low- and medium-nutrient soils, respectively (Table 3), confirms the capability of seedball technology to favour pearl millet seedling growth at this early stage, and may in turn contribute to the overall grain yields of the crop. It is presumed that seedballs release nutrients at microsite spots, in favour of enhancing soil fertility around plant roots, without exposing the nutrients to the bulk of the soil; an eventuality that could lead to nutrient fixation and other losses.

TABLE 4. Treatment effect on nutrient uptake (mg per germination pot) of the local (shoot, only) and improved (root + shoot) pearl millet seedlings 29 days after sowing

Nutrient	Control		Seedball	
	Small seed	Large seed	Small seed	Large seed
Phosphorus	2.0 ^{AB} (0.9)	1.5 ^B (0.5)	2.5 ^A (0.8)	2.8 ^A (1.5)
Potassium	23.0 ^B (7.4)	21.5 ^B (8.0)	28.2 ^{AB} (10.2)	33.8 ^A (15.7)
Magnesium	2.9 ^B (1.2)	3.4 ^B (1.0)	3.3 ^B (1.1)	5.0 ^A (2.8)
Calcium	4.9 ^B (1.6)	6.3 ^{AB} (2.0)	6.1 ^{AB} (2.4)	7.2 ^A (3.4)

Values represent arithmetic means (\pm standard deviations; n = 6) and letters indicate significance at 5% level (Tukey test)

Seedling biomass production. The significant interaction effect of seed size, soil nutrient level and seedball technology on early development of pearl millet seedling biomass (Figs. 2, 3 and 4; Table 1), is a compelling observation, implying that an optimum point exists at which the influence of the three factors should be harmonised to enhance the performance of the crop; and perhaps to bolster the overall performance of the crop.

From the stand point of the present study (Table 1 and Fig. 2), TGM differed between the two varieties, i.e., 8.53 and 14.14 g for local, and 9.25 and 18.63 g for improved variety. Considering that heigher TGM necessarily leads to higher nutrient mass per unit (Farahani *et al.*, 2011; Ambika *et al.*, 2014), the improved variety already had superior starting conditions. However, these negative effects were mitigated in seedball-derived seedlings compared to the conventionally sown (the control).

The effect of seed size on pearl millet (Gardner and Vanderlip, 1989; Manga and Yadav, 1995) and other small-seeded species (Marshall, 1986; Guberac *et al.*, 1998; Farahani *et al.*, 2011; Ambika *et al.*, 2014) is well documented. Small seeds generally produce relatively less vigorous seedlings due to the low availability of mobilisable embryonic food reserves needed for biomass production at this stage (Kaufmann and Guitard, 1967; Ambika *et al.*, 2014). In contrast, large seeds

of pearl millet and wheat often produce seedlings that are higher in dry matter weight, vigour and plant height (Manga and Yadav, 1995; Farahani *et al.*, 2011). Therefore, seedball technology significantly mitigates the negative impacts of small seed sizes and low-nutrient soils, and subsequently enhance seedling vigour, leaf number, plant height, dry matter accumulation, root length, fine root development, and nutrient uptake.

Our findings indicate that seedball technology effectively improves seedling performance across different seed sizes and nutrient conditions, particularly benefiting the local variety of pearl millet. The enhancement in seedling growth parameters suggests that seedball technology is a viable intervention to overcome challenges associated with small seed sizes and poor soil fertility, thereby potentially increasing pearl millet productivity in the Sahel region.

Root to shoot dry matter ratio. Root to shoot dry matter ratio was not measured for the local variety due to the destructive technique applied in assessing the root characteristics. However, previous studies (Nwankwo, 2019; Nwankwo and Herrmann, 2021) reveal seedball-derived seedlings invest relatively less resources in root development due to nutrient supply advantage over conventional seedlings, i.e., the control. Remarkably, the root variables are often more

enhanced in low-nutrient soils compared to medium nutrient soils (see Figs. 2a, 2b; 3a, 3b, 4a, 4b), a fact associated with plant adaptation under stressful circumstances (Baryeh, 2002). These results emphasize the potential of seedball technology to balance the disadvantages of small seed sizes by providing localised nutrient support, which increases better seedling establishment and growth under low-nutrient conditions typical of the Sahel region.

It is worth noting that the effect of seedball often outcompeted the effect of variety and seed size (Table 1); particularly leading to enhanced dry matter production for seedlings derived from small seeds; (Figs. 2b, c, d; 4b, c, d.), vigour (data not presented), height (Tables 1 and 3) as well as leaf (Table 3) development, irrespective of the low and normal applied soil nutrient level (Fig. 1). The general biomass effect could be explained by the early nutrient supply through seedballs that was shown to be effective for pearl millet as well as sorghum (Nwankwo *et al.*, 2018a). This is particularly true when NPK serves as the nutrient additive (Nwankwo *et al.*, 2018b; Nwankwo and Herrmann, 2021).

In the Sahel region, where the soil fertility level is regarded as low for pearl millet production (Adams *et al.*, 2020), application of seedball technology will likely mitigate the potential negative effects of small-sized pearl millet seeds. An additional advantage of the seedball technology is that sown seed count (factored in during seedball production) is uniform per planting hole. This in turn economises seed costs, decreases labour time for thinning, and concurrence between seedlings.

For the local variety, seedball application increased root density (Figs. 2a, 4a) and total root; as well as fine root length (Figs. 3a, 3b). Furthermore, it relatively increased the ratio of fine root to total root length. These findings are in line with previous studies (Nwankwo *et al.*, 2018a; 2018b), and are supported by Guberac *et al.* (1998), who observed higher

root length in spring oat derived from larger seeds. This is due to relatively high nutrient supply in the large seeds arising from the endosperm, compared to the smaller seeds that have smaller endosperm.

Nutrient reserves are often exhausted within a few days in small-seeded species (Williams, 1955). e.g., P reserve of oat was depleted as fast as in 18 days (Williams, 1948). The seedball technology intends to compensate for nutrient deficiency. However, nutrient release from seedballs becomes less effective between 2-3 weeks of after sowing (Nwankwo *et al.*, 2018a). To ensure continuous smooth seedling growth, additional fertilisation with the most limiting nutrients, is recommended. In this context, a recent study by Moussa *et al.* (2021) demonstrated that farmers can apply sanitised human urine (OGA), in sole or combination with composted manure, as a strategy for promoting the already established strong seedlings vigour and development.

With respect to the improved variety, root and shoot dry matter variables were often not affected by seedball treatment (4b, d). However, the root to shoot dry matter ratio (Fig. 2b) indicates an effect, i.e., the plant needs to invest less in root biomass per unit shoot biomass. Nitrogen or P-deficient soil conditions often trigger root growth; with less response of shoot (Ericsson, 1995). In small seeded species, biomass allocation to roots is often associated with nutrient uptake (Mašková and Herben, 2018). This indicates the higher the availability of nutrient in the substrate for pearl millet seedlings uptake, the higher the allocation of roots as observed in this study.

Regardless of soil nutrient level and seed size, the seedball technology increased pearl millet shoot dry matter (Figs. 2c, d; 4c), in particular of the local variety. The effect of small seed size and low-nutrient conditions on pearl millet seedlings are visible in the control. The low-nutrient soil used in this study was more than 4 times poorer (Nwankwo and

Herrmann, 2021); this is coupled with the small size of pearl millet seeds (Peske and Novembre, 2011; Nwankwo, 2019), usually weighing between 4-8 mg, often associated with low nutrient reserve. In the chemically infertile soils of the African Sahel region, the application of seedball technology might improve pearl millet seedlings establishment.

Nutrient uptake response to soil nutrient level and seed size. Tables 2 and 4 indicate treatment influenced nutrient uptake in both varieties, with a more visible effect on the local variety in particular. This is particularly seen in P, K, Mg and Ca uptake between the seedlings of seedball medium-nutrient and absolute control i.e., the control low-nutrient seedlings. The soil nutrient level did not influence P, Ca, Zn and Fe uptake of the improved variety. However, even where no statistical significance could be reached, seedling nutrient uptake obviously depended on the interaction of treatment (seedball) and soil nutrient level as well as seed size. Fe and Zn uptake in the improved variety was not influenced by treatment (data not shown).

Ca and Mg uptake of stress-free 42 days old millet was relatively higher than what was observed in our study; however, K uptake is in same range, whereas P was relatively lower (Ashraf and Hafeez, 2004).

Iron, P, Zn and Cu uptake of our study was lower than pearl 24 DAS pearl millet seedlings grown in Hoagland solution of varying P levels as reported by Ajakaiye (1979). However, similar to our observations, the concentrations of Fe Zn and Cu were higher in the root.

Pearl millet N and P uptake is related to N and P availability in soil (Payne *et al.*, 1995; Ribeiro *et al.*, 2018) since these nutrients are moderately deficient in terrestrial environments. Pearl millet crops show positive responses to nutrient supplementation, but often without specific patterns (Payne *et al.*, 1995; Voortman, 2010). Nutrient uptake did not follow a specific pattern in both pearl millet varieties used in our study, regardless of seed

size and soil nutrient level. This is expected since the NPK-amended seedball used in our study has relatively high N, P and K contents. This observation is in line with the observed and reported antagonistic effects of nutrient uptake as well as content in pearl millet (Kumar *et al.*, 1986; Voortman, 2010). Therefore, the provision of one nutrient in pearl millet seedlings can sometime limit the availability of another nutrient.

There is no confirmed reasons for the marginal influence of treatment on improved variety, e.g. height (Table 1) and leaf (data not presented) development at 21 and 29 DAS, root as well as shoot dry matter and uptake of Ca, Fe and Cu (Tables 2, 4). *Zatib*, the improved variety used in this study is a well-adapted tall variety, with improved biomass and panicle yield in Niger (Maman *et al.*, 2000b). In addition, little is known about the physiological and nutritional performance of *Zatib* seedlings under greenhouse conditions.

Seedball has shown its potential in mitigating the negative effects of small seed size as well as low-nutrient soil on local pearl millet seedlings in particular. In the Sahel region, where increased pearl millet establishment is often a prerequisite for higher grain yield, seedball application may be crucial. Early supply of nutrients by seedball was recently proven to increase panicle yield (Nwankwo *et al.*, 2021) in over 4,000 Sahelian sites. One of the early works of Williams (1955) established that the 25% of the overall dry matter production in cereals is dependent on the > 90% of N and P uptake by the seedling during the development stage, indicating that early nutrient supply to cereals is essential.

CONCLUSION

The seedball technology enhances pearl millet seedling performance derived from small-sized seeds in low fertility soils in the Sahelian region. In this study, NPK-amended seedballs demonstrated consistent positive effects on root (length, fine root, and dry matter) and

shoot (height as well as leaf, and dry matter) development, as well as macro- and micro-nutrient uptake regardless of seed size and soil nutrient level. The major determining factor for the observed pearl millet enhancement is the nutrient release through seedballs to the seedling root zone as early as emergence. Nutritionally enhanced pearl millet seedlings have the potential to better tolerate stressful conditions (drought, nutrient deficiency) and subsequently increase panicle yield.

From a management perspective, the seedball technology is particularly recommended for small-sized seeds applied to chemically infertile pearl millet production sites. In order to verify the greenhouse results, on-farm field trials at Sahelian sites differentiating seed sizes and analysing soil nutrient status are recommended. In particular micro-nutrients are of interest to better tailor the technology with respect to soil conditions.

ACKNOWLEDGMENT

This study was made possible through the generous support by the American People to the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet through the United States Agency for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. Program activities are funded by the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047.

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