

Ammonium fertilization increases pearl millet yield by promoting early root growth, higher tillering, and water use during grain filling in a low P Sahelian soil

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Abstract

Background: Adaptation of pearl millet [*Pennisetum glaucum* (L.) R. Br.] to low soil phosphorus (P) at early seedling stages and efficient P fertilizer application are crucial for its survival in the West African Sahel. While addition of NH_4^+ -N to P in the microdose technique has been reported to stimulate early growth of pearl millet, there is little information regarding root length (RL) at different timings.

Aims: Our study aimed at assessing the effects of added NH_4^+ -N and NO_3^- -N to P on (1) pearl millet agronomic traits such as root and shoot growth and (2) the water use dynamics during the cropping cycle.

Methods: Twenty-four “RhizoTubes” filled with P-deficient soil were used to grow pearl millet under three treatments: (T1) placed P addition alone at 0.4 g per seed hole, P_{placed} , (T2) P_{placed} + NH_4^+ -N, and (T3) P_{placed} + NO_3^- -N. At 2, 3, 4, and 5 weeks after sowing (WAS) we took non-destructive measurements of RL. Water use (transpiration) was measured from 3.5 to 9 WAS.

Results: At early growth, roots in the topsoil of T2 were longer than T3 and T1 roots (at 4 WAS: 129.3 cm for T2, 87.5 cm for T3, and 93.3 cm for T1, $p < 0.05$). Total RL at 2 WAS correlated positively with seedling height and final grain yield. Fertilization with NH_4^+ -N reduced the time to flowering and increased the number of tillers and grain yield, whereas addition of NO_3^- -N increased vegetative dry matter at harvest and water use efficiency.

Conclusions: Our findings suggest that fertilization with NH_4^+ -N plays a critical root stimulating role at early growth stages, seemingly by increasing lateral root initiation, which carries through to a larger water use during grain filling and higher grain yield.

Key words: ammonium versus nitrate fertilization / pearl millet / root length / RhizoTubes / West Africa

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1 Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the main staple crop grown on the predominantly acid sandy soils of the West African Sahel and it heavily contributes to food security of 50 million people. In this area, phosphorus (P) deficiency at the early growth stages critically affects plant survival, reproduction, and final yield (Grant et al., 2001; Karanam and Vadez, 2010; Beggi et al., 2015). Developing farming systems that require less P or use available P more effectively at the farm level is crucial to enhance small farmers' food sovereignty (Gilbert, 2009). Due to the limited availability and slow movement of P in the soil, one of the most effective plant strategies of enhancing P uptake is to increase the volume of soil explored by the root system, that is expansion of the root surface area by increasing root length and lateral root formation or by exploiting symbiosis with mycorrhizae (Bagayoko

et al., 2000; Williamson et al., 2001). A human option to foster millet growth is to improve the efficiency of fertilizer application. Ammonium and nitrate are the two main N sources for plants and root responses to their presence may have important growth effects (Omrane et al., 2009). At low concentration, ammonium (NH_4^+) has been reported to be the preferential N source for many plants (Gazzarrini et al., 1999; Gu et al., 2013) and its assimilation requires less energy than that of nitrate (Reisenauer, 1978). Though only a few species perform well when NH_4^+ is the only or predominant N source as compared with growth on nitrate (NO_3^-). At higher and exclusive supply, NH_4^+ -N may even inhibit primary root development (Britto and Kronzucker, 2002).

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Ammonium is taken up from the soil through specific ammonium transporters or through non-specific systems, including potassium channels, aquaporins and non-selective cation channels (Lima et al., 2010). Thereafter, the amino acids converted from NH_4^+ -N in the roots are loaded into the xylem via specific transporters (Schobert and Komor, 1992; von Wirén and Merrick, 2004; Winkler, 2006). In herbal species, most of the NO_3^- is transported from root-to-shoot via the xylem followed by NO_3^- -N reduction in the leaves. Ammonium-fed leaves of *Commelina communis* showed increased water use rates compared to nitrate-fed ones due to different pH effects in the xylem sap (Jia and Davies, 2007). The lower water use and growth of NO_3^- -N-fed plants confirms the role that NO_3^- -N plays in sensitization (closure) of stomata to abscisic acid (Wilkinson and Davies, 2002; Jia and Davies, 2007), thereby possibly enhancing water use efficiency under drought conditions.

Over the last few years, intensive work on *Arabidopsis thaliana* (L.) Heynh allowed to make significant progress in elucidating the molecular and cellular basis of root reactions to N signals in the rhizosphere (Ohkubo et al., 2017; Poitout et al., 2018; Jia et al., 2019). In crops, however, much work remains to be done on root and shoot response to the presence of soil-borne NH_4^+ -N versus NO_3^- -N.

Rebafka et al. (1993) observed that early growth of pearl millet in low P soils at ICRISAT Sahelian Centre (Niger) is improved when P is combined with NH_4^+ -N in seed coatings; they postulated that this was due to root stimulation by NH_4^+ -N. Subsequent pot trials conducted at the same location indicated that the addition of NH_4^+ -N to P in the seed hole at sowing may indeed stimulate early root growth (unpublished data), probably via enhanced lateral root initiation (Lima et al., 2010), but there is little information regarding root length at different timings in field-like conditions. The same putative effect of early NH_4^+ -N addition in combination with P on root and shoot growth of seedlings is also the basis for the “micro-dose technology” which, for the last two decades, has reportedly triggered major yield increases on millet, sorghum (*Sorghum bicolor* Moench.), and maize (*Zea mays* L.) in drylands throughout sub-Saharan Africa (Buerkert and Hiernaux, 1998; Buerkert et al., 2001; Ouedraogo et al., 2020). This study therefore addresses this knowledge gap on the interaction between early P and NH_4^+ -N using a “RhizoTube” system. This allowed to mimic field conditions and to take non-destructive measurements of root and shoot growth of millet from seedling stage to maturity. We hypothesized that addition of NH_4^+ -N to P in a low P soil increases early root length, particularly lateral root initiation, with a significant effect on final yield. We hereby assumed that roots appearing at the plexiglass surface of inclined RhizoTubes are representative of the entire root system (constant fraction). In view of this, our study aimed at assessing the effects of added NH_4^+ -N and NO_3^- -N to P on (1) pearl millet agronomic traits such as root and shoot growth and (2) the water use dynamics during the cropping cycle.

2 Material and methods

2.1 Description of RhizoTubes and soil preparation

RhizoTubes are cylindrical rhizotrons in which plants can be phenotyped non-invasively across their growth cycle. To produce these RhizoTubes, twenty-four PVC tubes [35 cm wide and 100 cm high as previously used in Beggi et al. (2015) for a lysimeter study; Fig. 1] were longitudinally cut and fitted with a plexiglass transparent surface to determine root penetration. All RhizoTubes were placed in a 1-m deep trench. To mimic a low P soil profile, we collected topsoil (0–20 cm) and subsoil (20–80 cm) from a severely P deficient Arenosol at ICRISAT Sahelian Centre (Buerkert et al., 1995). The topsoil had the following characteristics: 5.5 $\text{pH}_{\text{H}_2\text{O}}$ (1:2.5), 3.7 mg Bray1-P kg^{-1} soil, 0.3% C_{org} , and 247 mg total N kg^{-1} soil. Below 20 cm the soil was characterized as follows: 5.8 $\text{pH}_{\text{H}_2\text{O}}$ (1:2.5), 3.6 mg Bray1-P kg^{-1} soil, 0.1% C_{org} , and 81 mg total N kg^{-1} soil. Both soil types were kept separate, air-dried and homogenized thoroughly by hand prior to filling the tubes with 77 kg subsoil followed by 13.5 kg topsoil. Tubes were placed at a spacing of 1 plant m^{-2} . Two days after sowing, the RhizoTubes plexiglass surface was covered with a black PVC sheet and the tubes were inclined to 50°, whereby the plexiglass surface faced the ground. This allowed to avoid sun light interfering with root behavior. The RhizoTubes allow to obtain relevant agronomic and root data in a soil volume close to field conditions whereby soil physical and chemical conditions are much more homogeneous than under field conditions from where large heterogeneity occurs, severely hampering physiological studies (Buerkert et al., 1995; Buerkert and Stern, 1995). The RhizoTube system has recently been used to determine water use in different crops throughout the cropping cycle (Vadez et al., 2011a, 2011b; Beggi et al., 2015).



Figure 1: Root length measurement taken four times with a different marker color each time: at 2 WAS (blue), 3 WAS (green), 4 WAS (red), and 5 WAS (black) of pearl millet plants grown in twenty-four RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. Three treatments were applied: (T1) P_{placed} , (T2) $\text{P}_{\text{placed}} + \text{NH}_4^+$ -N, and (T3) $\text{P}_{\text{placed}} + \text{NO}_3^-$ -N. On the left, root marking on a transparent plastic sheet which is then placed on a white sheet (on the right) for scanning.

2.2 Experiment

The pearl millet variety PE05387 (hereafter referred to as “Geno26”), developed from a landrace of Mali, was previously identified as tolerant to low soil P (3.6 mg Bray-P kg⁻¹ soil) out of a collection of 102 pearl millet varieties from West Africa (Beggi et al., 2015, 2016). Under low P conditions, Geno26 accumulated almost three times more P in the shoot and presented higher early mycorrhization than varieties sensitive to low P (Beggi et al., 2016). Geno26 was sown in RhizoTubes on November 05, 2012, and grown until physiological maturity. We applied three treatments with 4 kg P ha⁻¹ (0.4 g P per RhizoTube) placed with the seed reflecting the microdose technique and referred to as P_{placed} (T1), P_{placed} + 5 kg NH₄⁺-N ha⁻¹ (T2), and P_{placed} + 5 kg NO₃⁻-N ha⁻¹ (T3) with 8 repetitions. Phosphorus was applied at 1.5 cm depth in a circle 2–3 cm around the central hole containing 5 seeds, while N was applied as 5 kg N ha⁻¹ (0.5 g N per RhizoTube) in three equal splits: at sowing, 1.5 WAS, and 3 WAS. A total of 24 RhizoTubes were used. Seedlings were thinned to one per tube at 1.5 WAS. Plants were harvested at physiological maturity. Subsequently, all biomass was sun-dried to constant weight in cotton bags. The parameters measured in this study were: (2) plant growth (height, number of tillers and leaves, time to booting and flowering, final root DM); (2) root length and root initiation; and (3) final yield (grain, panicle, stem, leaves DM, weight of 100 seeds at harvest). Vegetative DM was referred to as the sum of dry weight of leaves, stem and roots, whereas reproductive DM was considered as the panicle DM. During the cropping period, maximum and minimum air temperatures ranged from 32.1–41.1°C and 16.0–24.0°C, respectively, and relative air humidity at 1 pm averaged 12% (November 2012–January 2013), indicating high vapour-pressure deficit (VPD) conditions.

2.3 Measurements of root length

Visible roots were marked with a marker pen on transparent plastic removable sheets placed on the plexiglass at the time of measurement. The plastic sheet was split horizontally in two equal parts to facilitate subsequent scanning: Section 1 represented the upper soil compartment (0–40 cm) and Section 2 the lower one (deeper roots, 40–80 cm). Within Section 1, the topsoil section was measured separately, representing 0–20 cm soil depth. During the growing season root measurements were taken four times during the vegetative stage with a different marker color each time: at 2 WAS (blue), 3 WAS (green), 4 WAS (red), and 5 WAS (black; Fig. 1). Afterwards, all sheets were scanned at 800 dpi (Epson Perfection V700 Photo, Seiko Epson Corp., Suwa, Japan) and the images were analyzed using WinRHIZO Pro 2009c software (Regent Instruments Inc., Quebec, Canada) to determine root length for each color. To estimate root initiation, we manually counted the overall number of lateral roots at 0–40 cm.

2.4 Water use measurements and statistical analysis

At 3 WAS the soil surface of the RhizoTubes was covered with a round plastic sheet superposed with a 2 cm layer of low-density polyethylene beads to minimize soil evaporation.

Thereafter the RhizoTubes were placed in a trench across which a weighing support could be moved. Soil was kept at 90% field capacity by weighing the amount of water lost by transpiration (subsequently referred to as “water use”) and compensation by re-watering when necessary. The tops of the cylinders were equipped with metal collars and chains to allow the tubes to be lifted and weighed twice a week from 3.5 to 9 WAS eleven times: at 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 8.5, and 9 WAS. Water extraction related to plant water use was calculated from differences of tubes weights between consecutive weightings and additions of water. Transpiration data were assigned to the date of the latest weighing so that, e.g., transpiration at 4 WAS refers to the water transpired by the plant in the interval between 3.5 and 4 WAS. Water use efficiency (WUE) was calculated as the sum of reproductive and vegetative DM (g) produced per unit of water transpired (L).

Data were statistically analyzed by one-way ANOVA (with model residuals normally distributed) and graphs performed using R-Studio software [R Core Team (2019); <https://www.R-project.org/>]. Linear regression was used to calculate *r* (square root of *R*²). Significance levels were *p* < 0.05, *p* < 0.01, and *p* < 0.001, whereas results at *p* > 0.05 are shown as absolute numbers.

3 Results

3.1 Agronomic parameters

At 2 WAS, T2 plants were on average taller (35.7 cm) than T1 and T3 plants (29.4 cm and 30.7 cm respectively; Fig. 2).

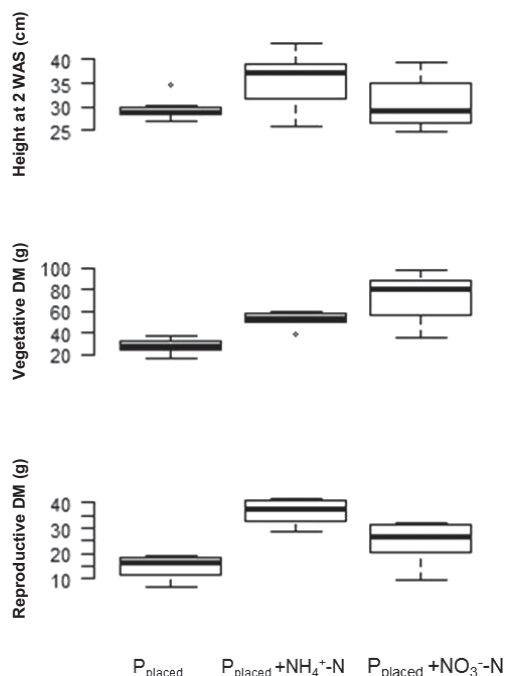


Figure 2: Boxplots of plant height at 2 weeks after sowing (WAS), total vegetative dry matter (DM; corresponding to sum of root, leaves, and stem DM) and reproductive dry matter (DM; corresponding to spike DM) at maturity of pearl millet plants grown in 24 RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. Three treatments were applied: (T1) P_{placed}; (T2) P_{placed} + NH₄⁺-N, and (T3) P_{placed} + NO₃⁻-N.

despite not significant. This early trend of a quicker development of NH_4 -fed plants was compensated by 3 WAS when plants from all treatments were of similar height which was maintained until maturity. T2 plants produced more tillers from 3.5 WAS onwards (Fig. 3), whereas at 4 WAS T3 plants had more leaves (7) than T2 plants (6), this difference was kept until maturity (data not shown). Thereafter, T3 plants produced the highest vegetative DM (73.2 g, $p < 0.001$; Fig. 2) as compared to T1 (28.1 g) and T2 plants (52.3 g). Root DM at harvest was also highest in T3 plants, 40.9 g compared to 13.7 g in T1 and 26.4 g in T2. In contrast, T2 plants flowered 4–5 days earlier (from 39 to 43 to 45.5 days than T3 and T1 plants) and they had highest reproductive DM (36.7 g, $p = 0.05$). Booting followed the same trend as flowering. At maturity, panicle DM and grain yield were higher in the T2 treatment than in the T3 treatment, itself higher than in the T1 treatment. In details, panicle DM was 14.7 g, 36.7 g and 24.8 g ($p < 0.05$; Fig. 2) and grain yield was 10.96 g, 22.86 g, and 17.44 g, for T1-T2-T3, respectively.

Plant height at 2 WAS was positively related to final grain yield ($p < 0.001$, $r = 0.69$) and to reproductive DM ($p < 0.001$, $r = 0.66$). Weight of 100 seeds at harvest didn't vary significantly among treatments. The difference in grain yield was rather due to number of seeds, which was higher in T2 (2400) followed by T3 (1900) and T1 (1500). Flowering time was inversely related to height at 2 WAS, to grain yield, and to panicle DM, indicating that taller seedlings flowered earlier ($p < 0.05$, $r = 0.53$) and yielded more ($p < 0.01$, $r = 0.59$ and $r = 0.63$, respectively). Root DM was positively related to shoot DM, given by the sum of leaves, stem and panicle DM ($p < 0.001$, $r = 0.83$).

3.2 Root length

Total root length at 5 WAS (last measurement, immediately before flowering) correlated linearly with total root DM at maturity ($p < 0.001$, $r = 0.7$). At 5 WAS, root length was larger in T3 than in T1 and T2 plants (Fig. 4). This also corre-

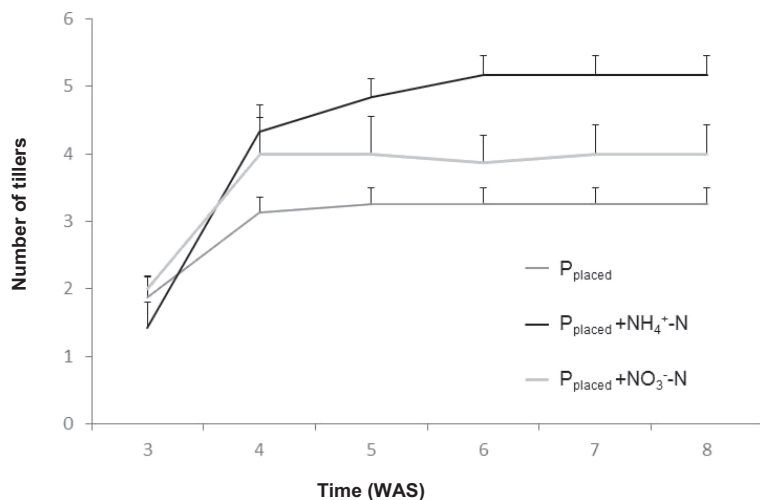


Figure 3: Number of tillers per plant across the growing period (weeks after sowing, WAS) of pearl millet in 24 RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. Three treatments were applied: (T1) P_{placed} , (T2) $P_{\text{placed}} + \text{NH}_4^+ - \text{N}$, and (T3) $P_{\text{placed}} + \text{NO}_3^- - \text{N}$. Error bars represent Standard Error.

sponded to the highest root DM in T3 plants. However, at 2 WAS, T2 roots in the topsoil (upper 20 cm) were longer than T3 and T1 roots, and at 4 WAS T2 roots were longer than T3 ones at $p = 0.08$). Root length in the topsoil at 2 WAS correlated with plant height at 2 WAS ($p < 0.001$, $r = 0.69$), with grain yield ($p < 0.001$, $r = 0.56$) and with reproductive DM ($p < 0.05$, $r = 0.5$), while it didn't correlate with any vegetative biomass component (stem, leaves, roots). The same trend was observed for root length in the topsoil at 4 WAS.

In general, we observed that T3 roots developed more vertically from 4 WAS onwards, having more than 100 cm root length in the lower section (40–80 cm) of the RhizoTubes at that time (Fig. 5), possibly due to higher mobility of NO_3^- . T2 plants in contrast had a higher percentage of their total root length within the topsoil or the upper section layer at 4 and 5 WAS.

Finally, lateral root initiation was highest for T2 plants (94.5 ± 26) followed by T1 and T3 plants (72.5 ± 12.9 and 56 ± 9.18 , respectively), despite differences were not statistically significant. In particular, lateral root initiation positively correlated with root length at 4 WAS ($r = 0.86$, $p < 0.001$).

3.3 Water use

Overall, T1 plants used significantly less water (14.8 L) than plants in the other two treatments (26.3 and 24.2 L, respectively; $p < 0.05$). T2 and T3 plants had similar water use curves until 6 WAS (Fig. 6), with T3 plants using more water until 5 WAS ($p < 0.05$; Fig. 6). At 5.5 WAS and from 6.5 WAS, during the grain filling period, T2 plants presented higher water use than T3 plants (Fig. 6).

WUE was higher for T3 plants than for T1 and T2 ones, whereas T2 and T3 plants used a similar amount of water (26.3 and 24.2 L respectively; Fig. 7) but with a different pattern. During pre-anthesis plants in T3 used the highest amount of water (13.6 L) compared to T2 (8.13 L) and T1 (9.6 L; $p < 0.01$). During post-anthesis, at the stage of grain filling, plants in T2 used more water (18.2 L) than in T1 (5.2 L) and T3 (10.6 L; $p = 0.09$). These results are similar to those of Beggi et al. (2015) indicating that pearl millet varieties tolerant to low soil P, i.e., those that yield more under low soil P, as the T2 plants do in this experiment, had higher water use during post-anthesis, while sensitive varieties transpired more water during the vegetative stage.

4 Discussion

In this study, Geno26 seedlings fed with P and $\text{NH}_4^+ - \text{N}$ produced longer roots in the topsoil and overall more lateral roots at an early stage, which translated into taller seedlings, more tillers, earlier flowering, more seeds, and higher grain yield. These traits are typical attributes of high resistance to low P. This is in line with the $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ seed coating results of Rebařka et al. (1993) who

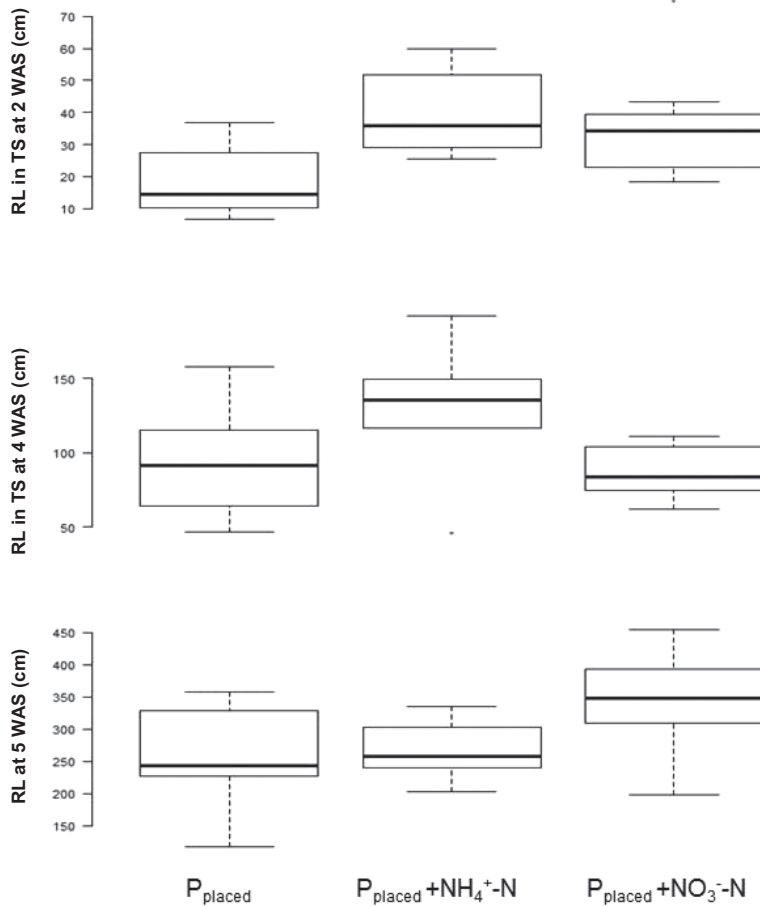


Figure 4: Total root length (RL; 0–80 cm) per plant at 5 weeks after sowing (WAS) and root length in topsoil (TS; 0–20 cm) at 2 and 4 WAS of pearl millet grown in 24 RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. Three treatments were applied: (T1) P_{placed} , (T2) $P_{\text{placed}} + \text{NH}_4^+\text{-N}$, and (T3) $P_{\text{placed}} + \text{NO}_3^-\text{-N}$.

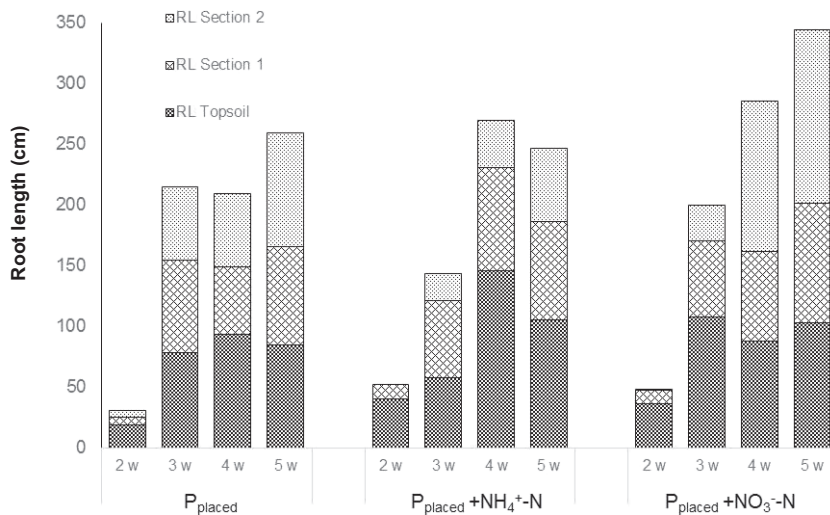


Figure 5: Root length of pearl millet plants grown in 24 RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger measured at 2, 3, 4, and 5 WAS. Three treatments were applied: (T1) P_{placed} , (T2) $P_{\text{placed}} + \text{NH}_4^+\text{-N}$, and (T3) $P_{\text{placed}} + \text{NO}_3^-\text{-N}$. Root length was measured in “Section 1” (upper 40 cm soil) of the RhizoTubes, whose upper 20 cm refer to the “Topsoil” and also measured in “Section 2” (lower 40 cm soil).

reported that dry matter production of pearl millet in Niger increased by 280% and grain yield by 45% at 3 WAS. $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ produced the largest effect on plant growth and P content and this was attributed to the enhancement effect of NH_4^+ on P uptake (Rebafka et al., 1993).

Root phenotype at the seedling stage is fundamental for seed yield (Strock et al., 2019). It is genetically determined and can be modulated by plant’s nutritional status and the nutrient availability in the soil. Ammonium is the preferential N source for most crop plants (Gu et al., 2013) when supplied in similar concentrations as nitrate (Sasakawa and Yamamoto, 1978; Gazzarrini et al., 1999) which holds true as long as $\text{NH}_4^+\text{-N}$ supply is low, as in this experiment. At least in initial plant stages $\text{NH}_4^+\text{-N}$ uptake is also energetically less costly than $\text{NO}_3^-\text{-N}$ (Zerihun et al., 1998). On the other side, the diffusion coefficient of $\text{NO}_3^-\text{-N}$ is estimated to be 10 to 100-fold higher than that of $\text{NH}_4^+\text{-N}$ in soil water, which is easily adsorbed by soil particles (Giehl and von Wirén, 2014). This reflects the quicker colonization of the bottom section of the RhizoTubes by T3 roots. Ammonium has been shown to increase root length, branching and density in nutrient solution (Bloom et al., 2002), inhibit primary root elongation and favor lateral roots (Kojima, 2018). This would be consistent with our finding of a higher T2 root length in the topsoil, and less in deeper layers. Lateral roots account for a large part of the total root length. Employing split-root systems Lima et al. (2010) have shown that local $\text{NH}_4^+\text{-N}$ supply to *Arabidopsis thaliana* plants increases lateral root initiation and higher-order lateral root branching, whereas the elongation of lateral roots is stimulated mainly

by $\text{NO}_3^-\text{-N}$. These authors suggested that this response may reflect a specific adaptation. Finely branched lateral roots are efficient at adsorbing $\text{NH}_4^+\text{-N}$ fixed at the soil particles, whereas longer/deeper lateral roots can explore highly mobile nitrate sources. These responses are dependent on different transporters (for $\text{NO}_3^-\text{-N}$ and for $\text{NH}_4^+\text{-N}$) which can change root morphology independent of N uptake. These transporters are interestingly acting as sensors of external N availability (Hachiya and Sakakibara, 2017). De Souza et al. (2019) also demonstrated that maize plants fed with $\text{NH}_4^+\text{-N}$ produced greater root surface and higher density of root hairs than $\text{NO}_3^-\text{-N}$. Root proliferation is more important for ions with low soil mobility, such as $\text{NH}_4^+\text{-N}$ and phosphate. Therefore, we postulate that the response of the root system to $\text{NH}_4^+\text{-N}$, favoring early root growth development, occurred preferentially in the topsoil which would have fostered the adaptation to the typical Sahelian soils low in plant-available

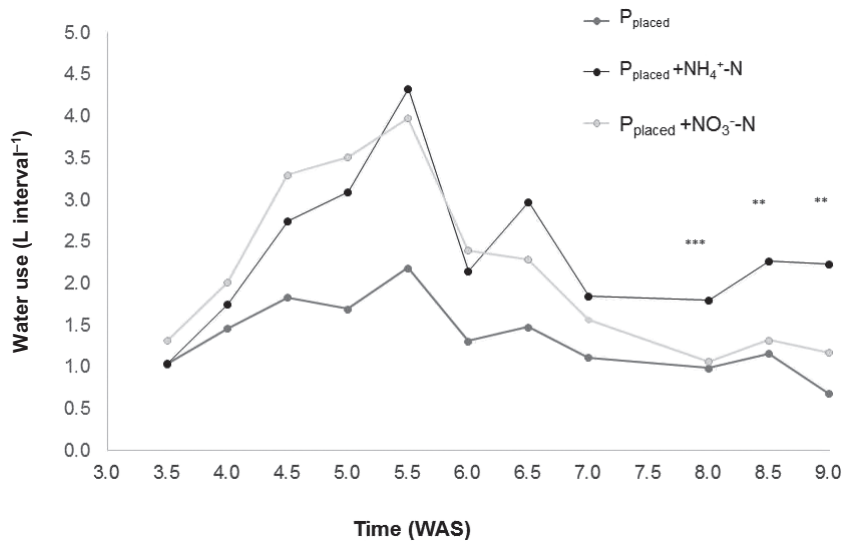


Figure 6: Water used (transpired) per time interval by pearl millet plants grown in 24 RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. Measurements were conducted eleven times from 3.5 and 9 weeks after sowing (WAS). Three treatments were applied: (T1) P_{placed}, (T2) P_{placed} + NH₄⁺-N, and (T3) P_{placed} + NO₃⁻-N. Asterisks indicate significant differences at $p < 0.01$ (**) and at $p < 0.001$ (***).

P. This is supported by the fact that at low pH the microbial conversion of NH₄⁺ to NO₃⁻ (nitrification) is generally suppressed, thus decreasing NO₃⁻ availability in the soil. Under the typically very low pH conditions of Sahelian sandy soil where root measurements were always conducted < 2 weeks after N application, we expected < 1/3 of the N being converted into nitrate (De Bruin et al., 1989). With equal mean NH₄⁺-N and NO₃⁻-N concentrations, a N-limited plant supplied solely with NH₄⁺-N would need a more extensive root/root hair/mycorrhiza hyphal system to attain the same N uptake rate on a per plant basis as would a NO₃⁻-N-supplied plant, with negative consequences for resource allocation by, and growth rate of, the NH₄⁺-N-grown plant (Raven et al., 1992). The understanding of the functional relevance of the three different types of early lateral roots of pearl millet suggested by

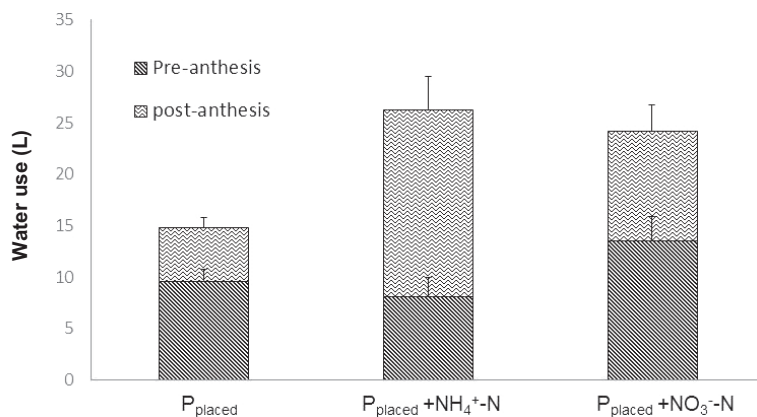


Figure 7: Water used (transpired) per plant during the pre-anthesis and the post-anthesis stage by pearl millet grown in twenty-four RhizoTubes at ICRISAT Sahelian Centre, Sadoré, Niger. The sum of the pre-anthesis and the post-anthesis water use corresponds to the total water used by plants between 3.5 and 9 weeks after sowing (WAS). Three treatments were applied: (T1) + P, (T2) + P + NH₄⁺, and (T3) + P + NO₃⁻. Error bars represent +/- one standard error of the mean.

Passot et al. (2016) may help shedding light on the synergistic mechanism of P and NH₄⁺-N.

In our study, NH₄⁺-N-fed plants initially had less tillers than those of the other treatments until at 3.5 WAS when this trend was inverted. This was reflected in the higher water use starting from 5.5 WAS which coincided with the average onset of flowering of NH₄⁺-N-fed plants. The NH₄⁺-N or NO₃⁻-N treatments show two different transpiration patterns: NH₄⁺-N-fed plants used less water during pre-anthesis and more water during post-anthesis, following a pattern as already observed in chickpea (*Cicer arietinum* L.; Zaman-Allah et al., 2011) and in pearl millet (Beggi et al., 2015). The latter authors reported that varieties tolerant to low P (including the one studied here) had higher post-anthesis water use, while sensitive ones transpired more water during vegetative stage, leaving less water available for grain filling. Differences between water extraction at pre- and post-

anthesis were independent of water availability. Since there was no experimental water stress imposed, this suggests that constitutive mechanism favoring stress avoidance may play a role, such as previously found for pearl millet in India (Kholová et al., 2010a, 2010b). For instance, the higher vegetative biomass production of T3 plants was mirrored by more leaves and less tillering at an early stage, causing the higher pre-anthesis water use, whereas in the T2 plants, more tillers would call for more photosynthate needs for grain filling and then higher water use post-anthesis.

In the current work, NH₄⁺-N-fed plants flowered earlier probably as a consequence of better P uptake as it has been demonstrated for pearl millet varieties tolerant to low P (Beggi et al., 2015). This higher P uptake may have come from the early root vigour in the top soil, yielding an effect similar to the seed coating of Rebařka et al. (1993). Hence, we hypothesized that earlier and better P supply to NH₄⁺-N-fed plants would have favoured panicle development and possibly reduced the period until the end of the juvenile phase, which could explain earlier flowering. Evidence for the relationship between early availability of P and grain yield of cereals dates back 30 years when Römer and Schilling (1986) reported that early P application caused a high number of fertile wheat (*Triticum aestivum* L.) ears per area, a high number of grains per ear, and a high P pool in the vegetative parts which could be partly mobilized during grain filling. Therefore, for high grain yield the soil has to meet P requirement at early stages of plant growth. This is also consistent with the findings of Karanam and Vadez (2010), where seed coating allowed plants to establish in a low P soil and eventually reached a similar vegetative biomass as in a non-limiting P treatment,

whereas the seed yield of the coating treatment remained lower. Their interpretation was that low soil P conditions had deleterious effect on early panicle development (panicle meristems and floret primordia setting) that could not be compensated for later. Our results suggest a likely early effect of enhanced P on final seed number, which led to the higher yield of T2 plants. In Sahelian pearl millet earlier flowering may decrease plants' risk of suffering from water deficient conditions during grain filling at the end of the rainy period.

In different experiments on fruit trees *Grasmanis* and *Leeper* (1967) showed that NH_4^+ -N led to earlier flowering and to a much higher proportion of flowers among total buds. But when NH_4^+ -N was supplied throughout the season alone, vegetative growth was less than with NO_3^- -N alone. This suggests that the choice of the N added to P can be critical to the partitioning of vegetative *versus* reproductive biomass. *Fageria* et al. (2011) found a 22% higher wheat grain yield when NH_4^+ -N was applied instead of urea, but no difference was found between NH_4^+ -N and NO_3^- -N for the same by *Wang* et al. (2015).

In our study, the water transpired by NH_4^+ -N - and NO_3^- -N -fed plants was similar, but in the former case the water was used for reproductive DM production, whereas in the later it enhanced vegetative DM. Overall, WUE was higher in NO_3^- -N -fed plants, in part explained by their higher vegetative biomass than in the other treatments. NO_3^- -N -fed plants are known to have stomata more sensitive to soil drying as an effect of NO_3^- -N addition on xylem sap pH and closure of stomata *via* an abscisic acid-based mechanism (*Davies* et al., 2002; *Jia* and *Davies*, 2007). Although there was no apparent water stress under our conditions, the high VPD conditions prevailing in the experiment could have elicited a transpiration restriction in NO_3^- -N -fed plants that would have saved water and increased WUE (*Vadez* et al., 2014), and in consequence would have allowed the observed higher biomass of NO_3^- -N -fed plants.

The RhizoTubes methodology employed proved to be a reliable, innovative setup for studying root and plant growth under the West African Sahelian conditions. Despite the RhizoTubes forcing plant roots to partly grow along the glass surface, roots could explore a soil volume which is closer to field-like conditions than in pots. After all no artificial systems can perfectly simulate natural conditions, but the RhizoTubes allow analyses of key phenotypic traits for root architecture and growth, comparable to those obtained either in controlled conditions or in the field (*Jeudy* et al., 2016).

5 Conclusions

The RhizoTubes setup allowed the detailed, non-destructive study of root and plant growth under Sahelian conditions where high short-distance soil variability often precludes detailed field measurements of plant physiological traits throughout the growing cycle. Our study indicates how the N source added to P in nutrient applications at sowing can determine the partitioning of vegetative *versus* reproductive biomass and suggests that NH_4^+ -N and NO_3^- -N trigger two dif-

ferent transpiration mechanisms with regard to pre- *versus* post-anthesis water use. At the farmers' level NH_4^+ -N in NP fertilizers now used in widely farmer-recommended "micro-dose approach" triggers early seedling growth, thus fostering millet survival under the harsh Sahelian early growing conditions, while the NO_3^- -N dominating soil N later in the season helps sustain root elongation and subsequent shoot growth.

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Data Availability Statement

The data that support the findings of this study are in the article itself.

References

- Bagayoko, M., George, E., Römheld, V., Buerkert, A.* (2000): Effects of mycorrhizae and phosphorus on growth and nutrient uptake of pearl millet, cowpea and sorghum on a West African soil. *J. Agr. Sci.* 135, 399–407.
- Beggi, F., Falalou, H., Buerkert, A., Vadez, V.* (2015): Tolerant pearl millet (*Pennisetum glaucum* (L.) R. Br.) varieties to low soil P have higher transpiration efficiency and lower flowering delay than sensitive ones. *Plant Soil* 389, 89–108.
- Beggi, F., Hamidou, F., Hash, C. T., Buerkert, A.* (2016): Effects of early mycorrhization and colonized root length on low-soil-phosphorus resistance of West African pearl millet. *J. Plant Nutr. Soil Sci.* 179, 466–471.
- Bloom, A. J., Meyerhoff, P. A., Taylor, A. R.* (2002): Root development and absorption of ammonium and nitrate from the rhizosphere. *J. Plant Growth Regul.* 21, 416–431.
- Britto, D. T., Kronzucker, H. J.* (2002): NH_4^+ toxicity in higher plants: a critical review. *J. Plant Physiol.* 159, 567–584.
- Buerkert, A., Stern, R. D.* (1995): Crop residue and P application affect the spatial variability of non-destructively measured millet growth in the Sahel. *Exp. Agr.* 31, 429–449.
- Buerkert, A., Stern, R. D., Marschner, H.* (1995): Post stratification clarifies treatment effects on millet growth in the Sahel. *Agron J.* 87, 752–761.

- Buerkert, A., Hiernaux, P. (1998): Nutrients in the West African Sudano-Sahelian zone: Losses, transfers and role of external inputs. *J. Plant Nutr. Soil Sci.* 161, 365–383.
- Buerkert, A., Bationo, A., Piepho, H. P. (2001): Efficient phosphorus application strategies for increased crop production in sub-Saharan West Africa. *Field Crop. Res.* 72, 1–15.
- Davies, W. J., Wilkinson, S., Loveys, B. (2002): Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytol.* 153, 449–460.
- De Bruin, B., Penning De Vries, F. W. T., Van Broekhoven, L. W., Van de Geijn (1989): Net nitrogen mineralization, nitrification and CO₂ production in alternating moisture conditions in an unfertilized low-humus sandy soil from the Sahel. *Plant Soil* 113, 69–78.
- De Souza, E. A., Ferreira-Eloy, N. R., Grassmann, Cd. S., Rosolem, C. A., White, P. J. (2019): Ammonium improves corn phosphorus acquisition through changes in the rhizosphere processes and root morphology. *Pedosphere* 29, 534–539.
- Fageria, N. K., Dos Santos, A. B., Coelho, A. M. (2011): Growth, yield and yield components of lowland rice as influenced by ammonium sulfate and urea fertilization. *J. Plant Nutr.* 34, 371–386.
- Gazzarrini, S., Lejay, L., Gojon, A., Ninnemann, O., Frommer, W. B., von Wirén, N. (1999): Three functional transporters for constitutive, diurnally regulated, and starvation-induced uptake of ammonium into *Arabidopsis* roots. *Plant Cell* 11, 937–947.
- Giehl, R. F. H., von Wirén, N. (2014): Root nutrient foraging. *Plant Physiol.* 166, 509–517.
- Gilbert, N. (2009): The disappearing nutrient. *Nature* 461, 716–718.
- Grant, C. A., Flaten, D. N., Tomasiewicz, D. J., Sheppard, S. C. (2001): The importance of early season phosphorus nutrition. *Can. J. Plant Sci.* 81, 211–224.
- Grasmanis, V. O., Leeper, G. W. (1967): Ammonium nutrition and flowering of apple trees. *Aust. J. Biol. Sci.* 20, 761–768.
- Gu, R., Duan, F., An, X., Zhang, F., von Wirén, N., Yuan, L. (2013): Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (*Zea mays* L.). *Plant Cell Physiol.* 54, 1515–1524.
- Hachiya, T., Sakakibara, H. (2017): Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *J. Exp. Bot.* 68, 2501–2512.
- Jeady, C., Adrian, M., Baussard, C., Bernard, C., Bernaud, E., Bourion, V., Busset, H., Cabrera-Bosquet, L., Cointault, F., Han, S., Lamboeuf, M., Moreau, D., Pivato, B., Prudent, M., Trouvelot, S., Truong, H. N., Vernoud, V., Voison, A.-S., Wipf, D., Salon, C. (2016): RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation. *Plant Method.* 12. DOI: <https://doi.org/10.1186/s13007-016-0131-9>.
- Jia, W., Davies, W. J. (2007): Modification of leaf apoplastic pH in relation to stomatal sensitivity to root-sourced abscisic acid signals. *Plant Physiol.* 143, 68–77.
- Jia, Z., Liu, Y., Gruber, B. D., Neumann, K., Kilian, B., Graner, A., von Wirén, N. (2019): Genetic dissection of root system architectural traits in spring barley. *Front Plant Sci.* 10. DOI: <https://doi.org/10.3389/fpls.2019.00400>.
- Karanam, P. V., Vadez, V. (2010): Phosphorus coating on pearl millet seed in low P Alfisol improves plant establishment and increases stover more than seed yield. *Exp. Agric.* 46, 457–469.
- Kholová, J., Hash, C. T., Kakkera, A., Kočová, M., Vadez, V. (2010a): Constitutive water conserving mechanisms are correlated with the terminal drought tolerance of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *J. Exp. Bot.* 61, 369–377.
- Kholová, J., Hash, C. T., Kumar, P. L., Yadav, R. S., Kočová, M., Vadez, V. (2010b): Terminal drought-tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br.] have high leaf ABA and limit transpiration at high vapour pressure deficit. *J. Exp. Bot.* 61, 1431–1440.
- Kojima, S. (2018): The possible interaction of ammonium and auxin polar transport on root system architectures in the two ecotypes of *Arabidopsis thaliana*. *Soil Sci. Plant Nutr.* 64, 616–622.
- Lima, J. E., Kojima, S., Takahashi, H., von Wirén, N. (2010): Ammonium triggers lateral root branching in *Arabidopsis* in an AMMONIUM TRANSPORTER1;3-dependent manner. *Plant Cell* 22, 3621–3633.
- Ohkubo, Y., Tanaka, M., Tabata, R., Ogawa-Ohnishi, M., Matsubayashi, Y. (2017): Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. *Nature Plant.* 3. DOI: <https://doi.org/10.1038/nplants.2017.29>.
- Omrane, S., Ferrarini, A., D'Apuzzo, E., Rogato, A., Delledonne, M., Chiurazzi, M. (2009): Symbiotic competence in *Lotus japonicus* is affected by plant nitrogen status: transcriptomic identification of genes affected by a new signalling pathway. *New Phytol.* 183, 380–394.
- Ouedraogo, Y., Sibiri Taonda, J.-B., Sermé, I., Tychon, B., Bielders, C. L. (2020): Factors driving cereal response to fertilizer microdosing in sub-Saharan Africa: A meta-analysis. *Agron. J.* 2020. DOI: <https://doi.org/10.1002/agj2.20229>.
- Passot, S., Gnacko, F., Moukouanga, D., Lucas, M., Guyomarc'h, S., Moreno Ortega, B., Atkinson, J. A., Belko, M. N., Bennett, M. J., Gantet, P., Wells, D. M., Guédon, Y., Vigouroux, Y., Verdeil, J.-L., Muller, B., Laplaze, L. (2016): Characterization of pearl millet root architecture and anatomy reveals three types of lateral roots. *Front Plant Sci.* 7. DOI: <https://doi.org/10.3389/fpls.2016.00829>.
- Poitout, A., Crabos, A., Petřík, I., Novák, O., Krouk, G., Lacombe, B., Ruffel, S. (2018): Responses to systemic nitrogen signaling in *Arabidopsis* roots involve trans-zeatin in shoots. *Plant Cell* 30, 1243–1257.
- Raven, J. A., Wollenweber, B., Handley, L. L. (1992): A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs. *New Phytol.* 121, 19–32.
- Rebafka, F., Bationo, A., Marschner, H. (1993): Phosphorus seed coating increases phosphorus uptake, early growth and yield of pearl millet (*Pennisetum glaucum* (L.) R. Br.) grown on an acid sandy soil in Niger, West Africa. *Fert. Res.* 35, 151–160.
- Reisenauer, H. M. (1978): Absorption and Utilization of Ammonium by Plants, in Nielsen, D. R., MacDonald, J. G. (eds.): Soil–Plant–Nitrogen Relationships. Academic Press, New York, NY, USA, pp. 157–189.
- Römer, W., Schilling, G. (1986): Phosphorus requirements of the wheat plant in various stages of its life cycle. *Plant Soil* 91, 221–229.
- Sasakawa, H., Yamamoto, Y. (1978): Comparison of the uptake of nitrate and ammonium by rice seedlings: influences of light, temperature, oxygen concentration, exogenous sucrose, and metabolic inhibitors. *Plant Physiol.* 62, 665–669.
- Schober, C., Komor, E. (1992): Transport of nitrate and ammonium into the phloem and the xylem of *Ricinus communis* seedlings. *J. Plant Physiol.* 140, 306–309.
- Strock, C. F., Burrige, J., Massas, A. S. F., Beaver, J., Beebe, S., Camilo, S. A., Fourie, D., Jochua, C., Miguel, M., Miklas, P. N., Mndolwa, E., Nchimbi-Msolla, S., Polania, J., Porch, T. G., Rosas, J. C., Trapp, J. J., Lynch, J. P. (2019): Seedling root architecture and its relationship with seed yield across diverse environments in *Phaseolus vulgaris*. *Field Crop. Res.* 237, 53–64.
- Vadez, V., Deshpande, S. P., Kholová, J., Hammer, G. L., Borrel, A. K., Talwar, H. S., Hash, C. T. (2011a): Stay-green quantitative trait

- loci's effects on water extraction, transpiration efficiency and seed yield depend on recipient parent background. *Funct. Plant Biol.* 38, 553–566.
- Vadez, V., Krishnamurthy, L., Hash, C. T., Upadhyaya, H. D., Borrel, A. K. (2011b): Yield, transpiration efficiency, and water use variations and their relationships in the sorghum reference collection. *Crop Pasture Sci.* 62, 645–655.
- Vadez, V., Kholova, J., Medina, S., Kakkera, A., Anderberg, H. (2014): Transpiration efficiency: new insights into an old story. *J Exp Bot.* 64, 6141–6153.
- von Wirén, N., Merrick, M. (2004): Molecular mechanisms controlling transmembrane transport. *Top. Curr. Genet.* 9, 1–26.
- Wang, Z.-H., Miao, Y.-F., Li, S.-X. (2015): Effect of ammonium and nitrate nitrogen fertilizers on wheat yield in relation to accumulated nitrate at different depths of soil in drylands of China. *Field Crop. Res.* 183, 211–224.
- Wilkinson, S., Davies, W. J. (2002): ABA-based chemical signalling: the co-ordination of responses to stress in plants *Plant Cell Environ.* 25, 195–210.
- Williamson, L. C., Ribrioux, S. P., Fitter, A. H., Leyser, H. M. (2001): Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiol.* 126, 875–882.
- Winkler, F. K. (2006): Amt/MEP/Rh proteins conduct ammonia. *Pflügers Arch. Eur. J. Physiol.* 451, 701–707.
- Zaman-Allah, M., Jenkinson, D. M., Vadez, V. (2011): A conservative pattern of water use, rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea. *J. Exp. Bot.* 62, 4239–4252.
- Zerihun, A., McKenzie, B. A., Morton, J. D. (1998): Photosynthate costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot–root biomass partitioning. *New Phytol.* 138, 1–11.