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The Timing of Phosphorus Availability to Corn: What Growth Stages Are Most Critical for Maximizing Yield?

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Abstract: Phosphorus (P) is critical for maximizing agricultural production and represents an appreciable input cost. Geologic sources of P that are most easily mined are a finite resource, while P transported from agricultural land to surface waters contributes to water quality degradation. Improved knowledge of P timing needs by corn (maize) can help inform management decisions that increase P use efficiency, which is beneficial to productivity, economics, and environmental quality. The objective of this study was to evaluate P application timing on the growth and yield components of corn. Corn was grown in a sand-culture hydroponics system that eliminated confounding plant–soil interactions and allowed for precise control of nutrient availability and timing. All nutrients were applied via drip irrigation and were therefore 100% bioavailable. Eight P timing treatments were tested using "low" (L) and "sufficient" (S) P concentrations. In each of the three growth phases, solution P application levels were changed or maintained, resulting in eight possible combinations, LLL, LLS, LSL, LSS, SLL, SSL, SLS, and SSS, where the first, second, and third letters indicate P solution application levels from planting to V6, V6 to R1, and R1 to R6, respectively. All other nutrients were applied at sufficient levels. Sacrificial samples were harvested at V6, R1, and R6 and evaluated for various yield parameters. Plants that received sufficient P between V6 and R1 produced a significantly higher grain yield than plants that received low P between V6 and R1 regardless of the level of P supply before V6 or after R1. The grain yield of plants that received sufficient P only between V6 and R1 did not differ significantly from plants that received only sufficient P (SSS), due to (1) a greater ear P concentration at R1; (2) an efficient remobilization of assimilates from the stem and leaf to grains between R1 and R6 (source–sink relationship); (3) a higher kernel/grain weight; and (4) less investment into root biomass.

Keywords: nutrient use efficiency; phosphorus uptake; corn fertility; nutrient timing; nutrient strategy; nutrient conservation

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1. Introduction

Phosphorus is one of the essential nutrients required by plants for growth activities. The increased demand for food and energy has taxed the world's most concentrated non-renewable phosphate mines for the purpose of fertilizer production [1]. Although conventional inorganic P fertilizers are highly soluble and immediately plant-available, P becomes "fixed" with decreasing solubility due to precipitation and adsorption reactions with soil Fe, Al, and Ca as it approaches equilibrium [2,3]. During this initial period of high solubility after application, the risk for non-point losses to surface waters is greatest [4], which can lead to eutrophication [5]. Therefore, proper management of P fertilizers is critical to both sustaining crop yields and preventing water quality degradation.

The robust development of crops is dependent on early establishment [6]. The P requirements of corn seedlings can be categorized into two main phases: (1) plants utilize stored seed P reserves during the initial stages of growth and after seed P reserves diminish, and (2) seedlings rely on external P supply for growth. Corn seeds contain predominantly organic P, which is stored in the form of phytate [7]. During early growth stages, the phytate is hydrolyzed into inorganic P (Pi) by phytase so that P can be utilized by plants for their metabolic and physiological functions [8,9]. The application of external P at planting does not prevent seed phytate from hydrolysis [10]. Although germinating seeds make P available to the growing roots and shoots, corn seedlings respond to external P supply despite high initial internal seed P contents [10].

Early establishment of crops has been associated with the application of starter fertilizers—concentrated bands of fertilizer placed near, or with, the seed at planting [11,12]. Gordon and Pierzynski [13] conducted a field experiment with four hybrids over three years to evaluate the effect of starter P fertilizer on corn biomass and P concentration at the V6 growth stage. They found that corn biomass and P concentration increased significantly in plants that received starter P fertilizer compared to those that received no starter P, and starter P fertilizer increased root surface area in two of the four hybrids tested. Kaiser et al. [14] also evaluated the effect of starter P fertilizer application in several fields across the United States on early corn growth. They found that corn dry weight increased significantly in plants that received starter P fertilizer, but P concentration did not differ between no starter and starter P treatments at the V6 growth stage. The increase in stem and leaf growth during early stages is due to P involvement in cell division and expansion [15]. At the V6 growth stage, all the leaf primordia formed [16] and therefore any change in growth conditions could not change the number of leaves but could impact, to some extent, stem and leaf growth. Although leaf number and surface can sometimes affect grain yield, greater ear initiation is an important component that can have a tremendous effect on yield compared to the former parameters [17]. In corn, ear primordia begin to form after V6 [16]; therefore, a change in growth conditions can have a great impact on ear formation in corn. An increase in P concentration in corn vegetative tissues after increasing the solution P level from low to sufficient at an early corn growth stage has been reported [18,19]. Barry and Miller [18] conducted an outdoor hydroponics experiment in which they changed the solution P level at the V6 or V10 growth stage from low to high and vice versa. They found that when the solution P level was changed from low to high at V6, yield did not differ from plants that received high P throughout. However, when solution P level was changed at V10 from low to high, yield was significantly less than the continuously sufficient treatment. These results suggest that the correction of P deficiency at the V10 growth stage was too late to maximize potential ear size, which adversely affected grain yield components. The study of Barry and Miller [18] considered the effects of varying P supply during vegetative growth stages on corn growth, but did not investigate the effects of P supply during the reproductive stage where P uptake and utilization remain high [20,21] on corn yield components and grain yield.

Although many have found that starter P increases early plant growth and P uptake [11,22–25], grain yield increases do not always occur. For example, Camberato and Nielsen [26] conducted several corn field trials in which they evaluated starter P, N, and K fertilizers on corn growth and yield. In the trials that included P as a starter fertilizer, P did not increase grain yield in most of the trials but rather shortened the corn growth cycle such as early silking, resulting in early maturity and drier grain. Kaiser et al. [14] also found no increase in corn grain yield when starter P fertilizer was applied in 7 of the 16 field trials tested. The absence of consistent yield response to starter P fertilizer prompts investigation into the critical growth stage at which corn P requirement must be met to achieve optimum yield. This research has become essential as it is estimated that up to 85% of metabolic P needs of corn is supplied by the stored P in the vacuoles after silking as a result of P recycling within plants [21,27,28].

Considering that vacuolar P can be recycled to maintain metabolic and physiological processes after the R1 growth stage, ear size primordia determination is critical between V6 and R1, and early P nutrition does not guarantee yield increase; supplying resources such as P between V6 and R1 (silking) is likely the most critical stage for optimum yield production. Therefore, we hypothesize that P supply between V6 and R1 is the most critical period for P uptake by corn. The objective of this study was to evaluate P application timing at different growth stages on the growth and yield components of corn.

2. Materials and Methods

2.1. Experimental Conditions and Phosphorus Treatments

Corn was grown under controlled conditions in a sand-culture hydroponics system described in detail by Wiethorn et al. [29]. Sand-culture hydroponics allowed for the precise control and manipulation of P availability at different corn growth stages, which would be impossible in soil due to its strong buffering of solution-phase P.

Dekalb hybrid DKC56-65RIB was selected for this experiment because of its popularity among corn growers in the Midwestern USA. Plants were grown to maturity (R6) in 28 L plastic pots containing 30 kg of silica-sand in a semi-automated grow room. All nutrients were supplied via fertigation by automated injectors (Dosatron D14MZ520; Dosatron International Inc., Clearwater, FL, USA) and drip tubing. The photoperiod was varied to reflect day length for Central Indiana from May to September. Light-emitting diode lamps were positioned ~80 cm directly above the plant row and adjusted to 65% capacity to maintain photosynthetic active radiation (PAR) between 700 and 900 µmoles m^{-2} s^{-1}. Average temperature and humidity were 26.2 °C and 55%, with diurnal fluctuation. The air in the room was constantly mixed using two oscillating pedestal fans.

The experimental design was a randomized complete block with four replicates. Phosphorus supply level and timing was randomized within each block. The solution P level was varied according to growth stage to achieve every combination of low and sufficient solution P concentrations over three growth periods: emergence to V6, V6 to R1, and R1 to R6. Low and sufficient P levels were 4 and 12 mg L^{-1}, respectively (L and S), as determined by previous experiments [29,30]. At V6, there were 2 treatments, L and S. At R1, treatments were LL, LS, SS, and SL, where the first letter indicates the P level from planting to V6 and the second letter is the P level from V6 to R1. At R6, treatments were LLL, LLS, LSL, LSS, SSS, SSL, SLS, and SLL, where the third letter indicates the P level applied between R1 and R6 (Table 1).

Table 1. Solution phosphorus (P) level applied during each corn growth period.

P Level †	Description
LLL	Low P supply throughout growth period
SSS	Sufficient P supply throughout growth period
LLS	Low P supply at planting-V6 and V6-R1; sufficient P at R1
LSL	Low P supply at planting-V6; sufficient P at V6-R1; low P at R1-R6
LSS	Low P supply at planting-V6; sufficient P at V6-R1 and R1-R6
SLL	Sufficient P at planting-V6; low P at V6-R1 and R1-R6
SSL	Sufficient P at planting-V6 and V6-R1; low P at R1-R6
SLS	Sufficient P at planting-V6; low P at V6-R1; sufficient P at R1-R6

† L: low P level; S: sufficient P level.

All nutrients other than P were supplied at sufficient levels from emergence to R6 [29]. Targeted concentrations of N, K, S, Ca, Mg, Fe, Zn, Mn, B, Cu, and Mo were 180, 120, 74, 80, 35, 2, 0.05, 0.25, 0.25, 0.02, and 0.01 mg L^{-1}, respectively. This solution was kept separate from the P solution until application to prevent P precipitation with Fe, Ca, and Mg [2].

2.2. Parameter Measurements, Harvesting, and Sample Preparation and Analysis

Note that the number of replications varies depending on sampling time because of the nature of this study in that nutrient concentrations were changed at V6 and R1. Therefore, the number of replications at any given sampling time for destructive analysis was always four or six. Four replicates of each treatment were sampled at V6 and R1 (prior to changing solution P) and six replicates of each treatment were harvested at R6. Plants in the V6 growth stage were separated into roots, leaves, and stems. Plants harvested at the R1 growth stage were separated into stem, leaves below ear (LBE), leaves above ear (LAE), ear, and roots. Plants harvested at R6 were separated into stem, LBE, LAE, CHI (cob, husk, and immature ear), grain, and roots. Samples were dried for 3–5 d at 50 °C before determining dry weight. Dried samples were ground to pass a 0.50 mm screen using a Thomas Wiley Mill model ED-5 (Arthur H. Thomas Co., Philadelphia, PA, USA). One gram of ground plant tissue was digested with 20 mL of concentrated nitric acid on a BD40^{HT} graphite heating block (Lachat Instruments, Milwaukee, WI, USA) by heating to 120 °C for about 60 min, followed by addition of 2 mL of hydrogen peroxide, before continuing heating to 160 °C for about 3 h. Phosphorus concentration was analyzed with inductively coupled plasma–optical emission spectroscopy (ICP-OES; iCAP PRO, Thermo Fisher Scientific Inc., Waltham, MA, USA). Phosphorus concentrations in all corn plant parts are presented on a dry weight basis except grain, which is presented at 15.5% moisture.

Photosynthetic rate, leaf stomata conductance, and transpiration rate were measured at V6 (on the 6th leaf), R1, and two weeks after R1 (on the 3rd leaf above the main ear) between 9 and 11 am using a LICOR 6800 portable photosynthetic system (Li-Cor Bioscience, Lincoln, NE, USA). Total leaf area was scanned at the R1 growth stage using LI-3100C (Li-Cor Bioscience, Lincoln, NE, USA). Total leaf area per plant was calculated by adding the area of individual leaves omitting senescent leaves. The number of kernel rows per ear and the number of kernels per row were determined by counting. Kernel dry weight was determined for 100 kernels after drying them at 50 °C for 5 days.

2.3. Statistical Analysis

All data were subjected to the Shapiro–Wilk test for normality before conducting Analysis of Variance (ANOVA) using the 'stats' package in R (R Core Team, 2022). Since the data were normally distributed, no transformations were applied. Analysis of Variance (general linear model) was performed and if a significant treatment effect was detected with ANOVA (α = 0.05), differences between means were determined by Tukey's Honest Significant Difference test (HSD; α = 0.05). Simple linear correlations were also conducted with the 'stats' package in R.

3. Results

3.1. Biomass Production

Plants that received sufficient P from planting to V6 produced 30 and 35% more stem and leaf dry weight at V6 compared to low-P-treated plants (Figure 1a,b). However, root dry weight and root/shoot ratio at V6 were unaffected by P supply (Figure 1c,d). Changing the solution P level at V6 from sufficient to low (SL) did not reduce the stem and leaf dry weight at R1 compared to plants that received sufficient P from planting to R1 (SS; Figure 2a–c). Plants that received sufficient P between V6 and R1 after receiving low P at planting (LS) did not respond to the increased P level after V6 and, as a result, the roots, stem, LBE, and LAE did not differ from plants that received low P until R1 (LL) (Figure 2a–c). Root dry weight at R1 was increased by the increased P supply between planting and V6, irrespective of whether P level was maintained or changed to the low level between V6 and R1 (SS, SL). This occurred despite no effect of P supply on root weight at V6. The root/shoot ratio at R1 was similar for all treatments due to proportional increases in root and shoot growth from V6 to R1 (Figure 2f).

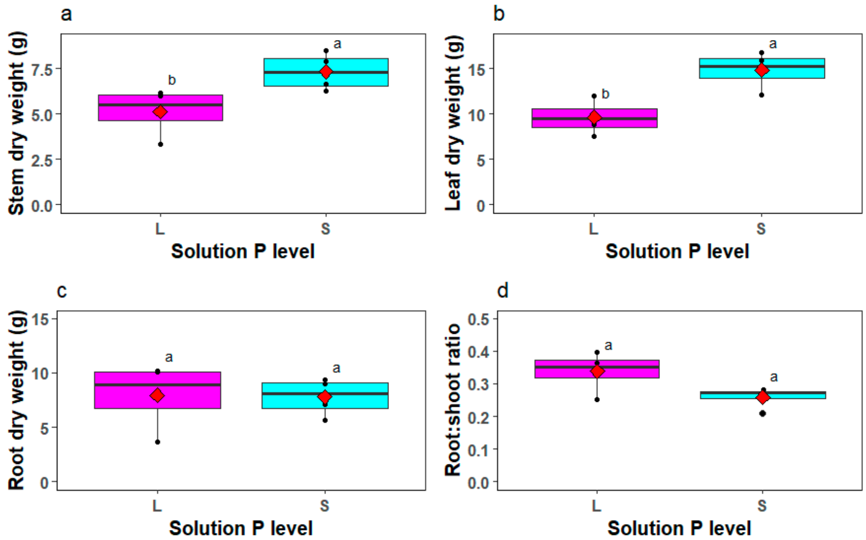

Figure 1. Dry weight at the V6 growth stage for corn plant parts in low (L) and sufficient (S) phosphorus (P) treatments applied from emergence to V6: stem dry weight (**a**); leaf dry weight (**b**); root dry weight (**c**); and root/shoot ratio (**d**). Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

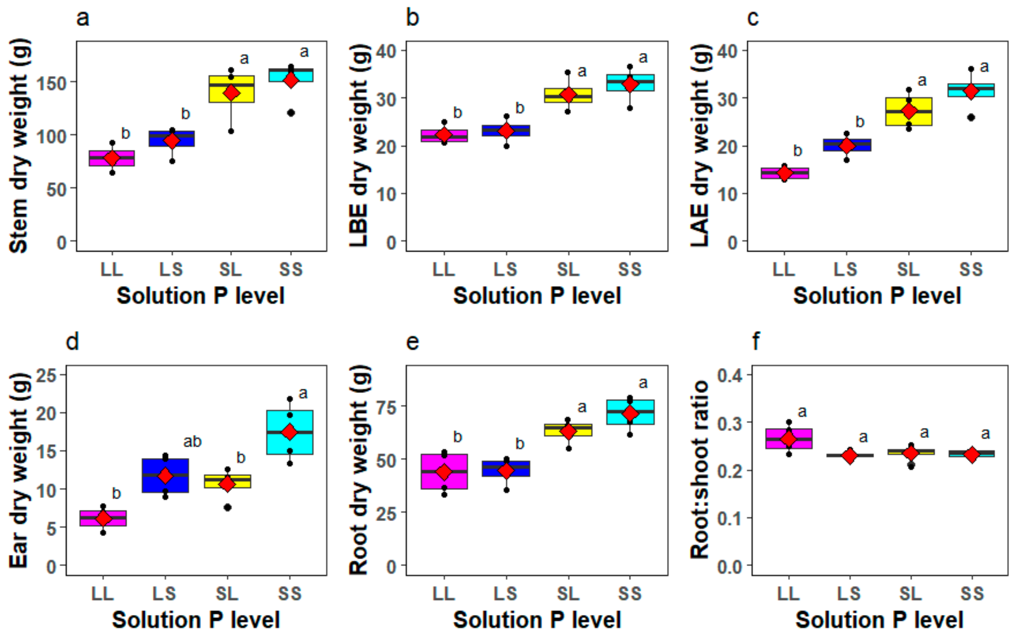

Figure 2. Dry weight for corn plant parts at R1 grown with low (L) and sufficient (S) solution phosphorus (P) levels: stem dry weight (**a**); leaf below ear (LBE) dry weight (**b**); leaf above ear (LAE) dry weight (**c**); ear dry weight (**d**); root dry weight (**e**); and root/shoot ratio (**f**). Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6 and the second letter indicates from V6 to R1. Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

The total dry weight of plants that received low P between V6 and R1 after receiving sufficient P at planting (SL) did not differ from plants that received sufficient P through R1 (SS), while total dry weight of plants that received sufficient P between V6 and R1 after receiving low P before V6 (LS) did not differ from plants that received low P from emergence to Rl (LL; Figure 3). From V6 to R1, plants that received low P between V6 and R1 after receiving sufficient P at planting (SL) added more dry matter (75 g) compared to plants that received low P at planting and sufficient P at V6 (LS; Table 2), indicating a minimal response of corn plants to a change in solution P level after V6. Even though dry matter production was lower in plants that received low solution P at planting and changed to sufficient P from V6 to R1 (LS) compared to plants that received sufficient P from planting to R1 (SS) or sufficient P from planting to V6 followed by low P from V6 to R1 (SL), ear dry weight at R1 did not differ between them (Figure 2d; Table 2).

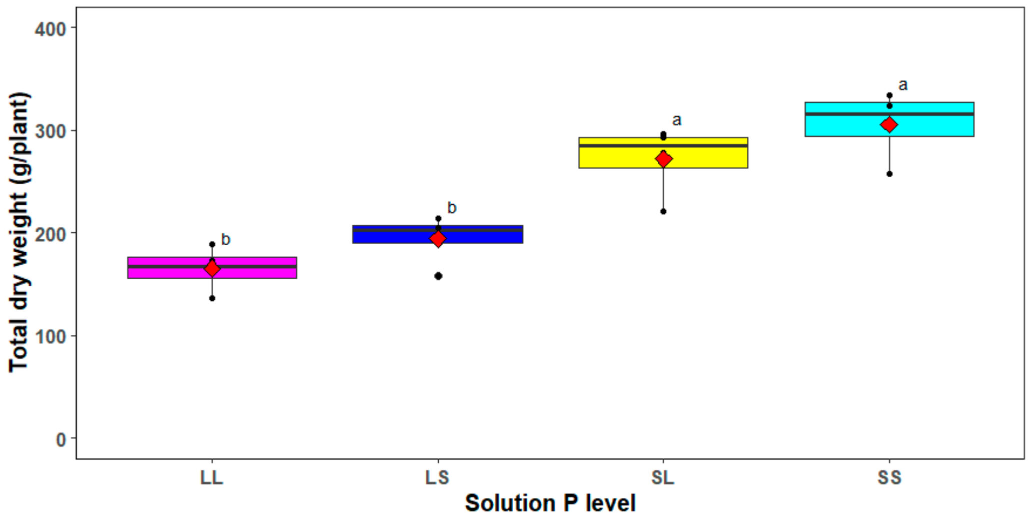

Figure 3. Total dry weight for corn plants at R1 grown with low (L) and sufficient (S) solution phosphorus (P) levels. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6 and the second letter indicates from V6 to R1. Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

Table 2. Change in corn plant dry weight (DW) from V6 to R1 after a change in solution phosphorus (P) level at V6. The same letter within row indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

Solution P Level Applied from Planting to V6 †	**L**		**S**	
Solution P Level Applied from V6 to R1	**L**	**S**	**L**	**S**
Plant Part	**Change in DW from V6 to R1, g/Plant**			
Leaf	27.0 b	33.5 b	43.2 a	49.6 a
Stem	70.8 b	87.3 b	134.9 a	146.9 a
Root	36.1 b	36.7 b	55.4 a	63.8 a
Ear	6.12 b	11.7 ab	10.7 b	17.5 a
Total	**140.0 b**	**169.2 b**	**244.2 a**	**277.8 a**

† L: low P level; S: sufficient P level.

At R6, treatments that provided sufficient P during one or two growth stages (LSL, LSS, SLL, SLS, and SSL, but not LLS) produced similar total plant dry weight, but less than that from plants that received sufficient P throughout (SSS; Figure 4). Despite the equivalence in total dry weight at R6 among several P treatments, dry weight partitioning differed among plant parts due to the timing and level of P supply (Figure 5a–f). Notwithstanding, plants that received sufficient P at emergence accumulated more biomass in their roots, stem LBE, and LAE by R6 compared to plants that received low P at emergence regardless of receiving low or sufficient P at V6 and/or R1 (Figure 5a–e). Plants that received sufficient P throughout (SSS) partitioned more biomass into their stem than all other treatments except SLS plants (Figure 5a). There was no clear difference between treatments' root/shoot ratio at R6 (Figure 5f).

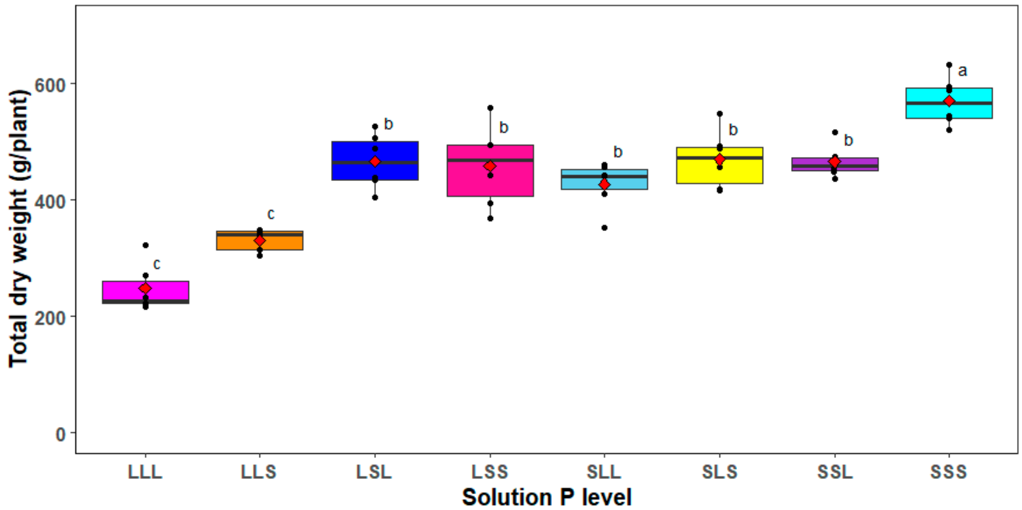

Figure 4. Total dry weight for corn plant grown with low (L) and sufficient (S) solution phosphorus (P) levels at R6. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Boxes indicate the interquartile spread (1st and 3rd quantiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

Even though plants that received low P from emergence to V6 followed by sufficient P to R1 (LS) had less total dry weight at R1, compared to plants that received sufficient P at emergence followed by low P between V6 and R1 (SL) or received sufficient P from emergence to R1 (SS; Figure 2; Table 2), the effect of sufficient P supply between V6 and R1 was later manifested after R1 (Table 3). As a result, the total dry matter accumulated between R1 and R6 was not different from plants that received sufficient P throughout (SSS), irrespective of supplying sufficient or low P from R1 to R6 (272.3 g for LSL; 264.3 g for LSS; 273.8 g for SSS). Plants that received low P after receiving sufficient P until R1 (SSL) or between planting and V6 (SLL) accumulated about 40% less dry matter compared to plants that received sufficient P throughout (SSS), or at least once at V6 or R1 (LSL or LSS) due to the great reduction in net accumulation of LBE, LAE, and stem and root dry weight (Table 3). The resumption of sufficient P after plants received sufficient P at planting and low P at V6 (SLS) resulted in about a 30% reduction in dry matter production between R1 and R6 compared to plants that received only sufficient P (SSS) or at least once at V6 or R1 (LSL or LSS).

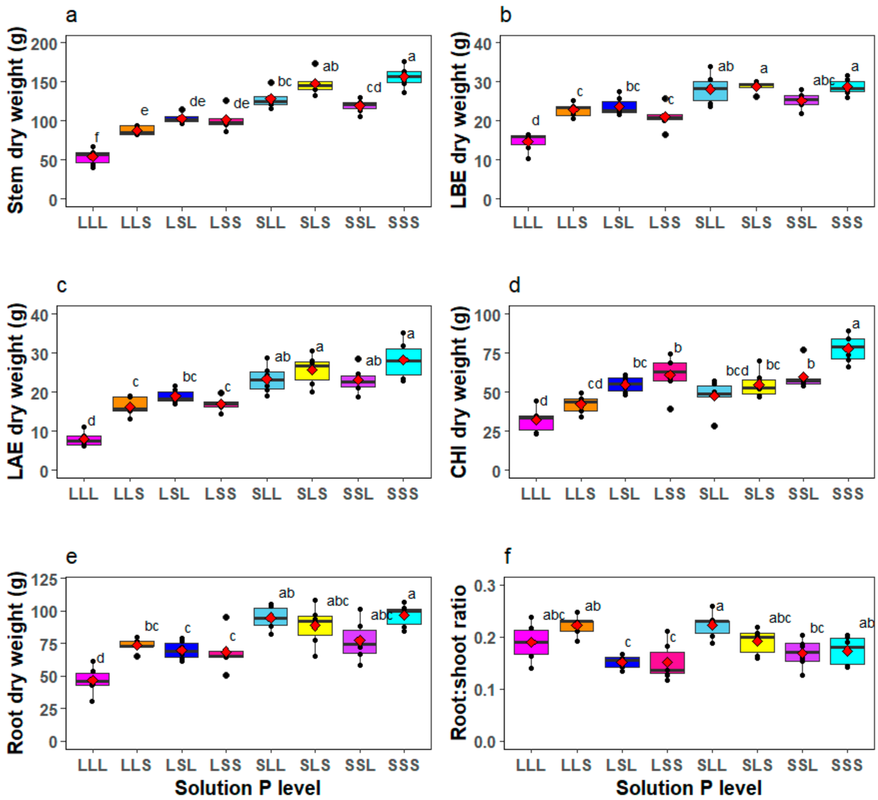

Figure 5. Dry weight for corn plant parts at R6 grown with low (L) and sufficient (S) solution phosphorus (P) levels: stem dry weight (**a**); leaf below ear (LBE) dry weight (**b**); leaf above ear (LAE) dry weight (**c**); cobb, husk, and immature ear (CHI) dry weight (**d**); root dry weight (**e**); and root/shoot ratio (**f**). Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

Table 3. Change in corn plant dry weight (DW) from R1 to R6 after a change in solution phosphorus (P) level at R1. The same letter within row indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

Solution P Levels Applied from Planting to V6 and V6 to R1	**LL**		**LS**		**SL**		**SS**	
Solution P Level Applied from R1 to R6 †	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**
Plant Part ‡	**Change in DW from R1 to R6, g/Plant**							
LBE	−7.6 b	0.6 a	0.6 a	−2.1 ab	−2.5 ab	−1.9 ab	−7.7 b	−4.1 ab
LAE	−6.4 bc	1.9 a	−1.1 ab	−3.1 abc	−4.1 bc	−1.6 abc	−8.4 c	−3.3 abc
Stem	−24.1 ab	9.7 a	8.4 a	6.4 a	−12 ab	−8.0 a	−33.0 b	4.0 a
Root	2.6 c	29.4 a	25.3 ab	23.9 ab	31.5 a	25.4 ab	5.6 bc	24.9 ab
Grain and CHI	118.6 d	123.7 d	239.1 ab	239.2 ab	142.7 cd	171.1 bcd	203.3 abc	252.3 a
Total	**83.1 d**	**165.3 bc**	**272.3 a**	**264.3 a**	**155.6 cd**	**185.0 bc**	**159.8 cd**	**273.8 a**

† L: low P level; S: sufficient P level; ‡ LBE: leaf below ear; LAE: leaf above ear; CHI: cob, husk and immature ears.

The ear (grain and CHI) dry weight produced between R1 and R6 in plants that received sufficient P at V6 and/or R1 (LSL, LSS, SSL) was not different from that of plants that received sufficient P throughout (SSS; 239 g for LSL; 239 g for LSS; 203 for SSL; 252 g for SSS; Table 3). Ear dry weight produced between R1 and R6 in plants that received sufficient P between planting and V6 followed by low P until R6 or sufficient P at planting followed by low P between V6 and R1 followed by sufficient P until R6 (SLL, SLS) was lower than plants that received sufficient P throughout (SSS). An increase in P after R1 did not allow ear dry weight to recover among plants that received low P between planting and R1 (LLS), thereby producing a similar ear dry weight between R1 and R6 as plants that received low P throughout (LLL; 123 g vs. 119 g; Table 3).

3.2. Grain Yield

The grain yield did not follow the same trend as vegetative dry matter production (Figure 6). Instead, plants that received low P from planting to V6, sufficient P between V6 and R1, and low P between R1 and R6 (LSL) or plants that received low P at planting followed by sufficient P between V6 and R6 (LSS) produced the highest grain yield and did not differ from plants that received sufficient P throughout (SSS) or sufficient P between planting and R1 followed by low P between R1 and R6 (SSL). In essence, any treatment that received sufficient P between V6 and R1 produced maximum yield, suggesting that maintaining P uptake during the period between V6 and R1 is critical for maximizing grain production. Plants that received sufficient P between planting and V6, followed by low P until R6 (SLL) or low P between V6 and R1 followed by sufficient P until R6 (SLS) produced a grain yield that did not differ from plants that received low P throughout (LLL), or low P until R1 followed by sufficient P until R6 (LLS), indicating that P uptake prior to V6 does not guarantee optimum grain yield. Plants that received low P between V6 and R1 produced lower yields regardless of receiving sufficient P before or after that period (SLL, SLS). These results suggest that corn plants can recover from early P deficiency without any significant decrease in yield when a sufficient P supply is resumed at V6, whereas yield will be reduced if the plants receive sufficient P prior to V6 but deficient P between V6 and R1. At R1, applying sufficient P to corn plants after receiving low P through R1 (LLS) was too late to recover grain yield.

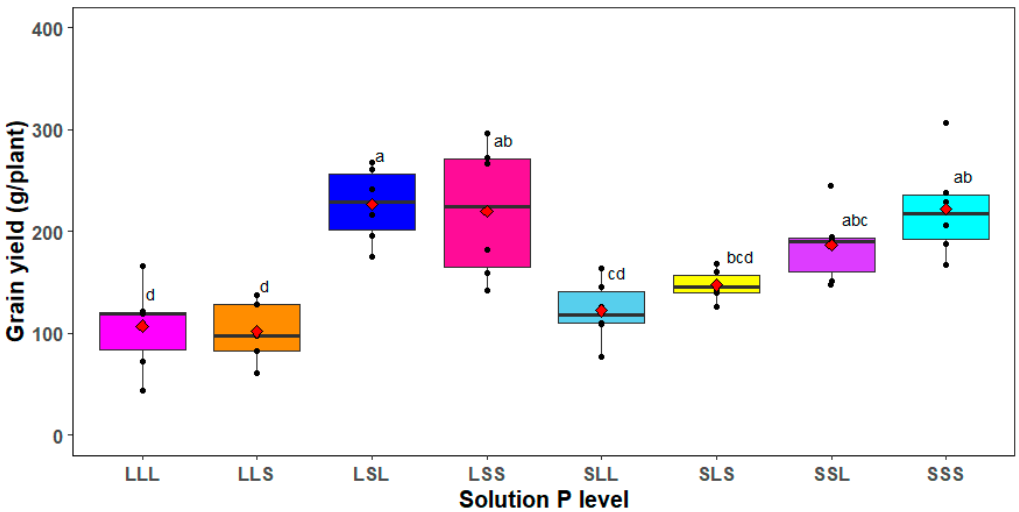

Figure 6. Grain yield at 15.5% moisture for corn plants at R6 grown with low (L) and sufficient (S) solution phosphorus (P) levels. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The

lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

3.3. Photosynthesis, Transpiration, and Stomatal Conductance

Net photosynthesis (Pn) increased from V6 to two weeks after R1 (Table 4). Net photosynthesis, transpiration rate (E), and Pn/E did not differ between treatments at V6. On the other hand, stomatal conductance (g_s) was higher in plants grown with sufficient P. Transpiration rate and g_s were low at V6 but increased at R1 and at 2 wk after R1. At R1, Pn and Pn/E were higher in SS than LL plants but E and g_s were higher in LL than SS plants. As a consequence, water use efficiency (Pn/E) was higher in plants that received only sufficient P until R1 (SS) than plants that received only low P (LL). Two weeks after R1, there were no treatment effects on Pn, E, g_s, and Pn/E. Overall, there was no clear effect of changes in solution P level on Pn.

Table 4. Net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (g_s), and water use efficiency (Pn/E) as affected by low (L) and sufficient (S) phosphorus (P) treatment. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Values shown are the mean of four replications followed by standard error. The same lower-case letter within a growth stage indicates no difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

P Level	Pn (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	Pn/E × 10^{-3}
		V6 growth stage		
L	14.1 ± 0.2 a	0.9 ± 0.1 a	35.5 ± 4.89 b	15.7 ± 2.8 a
S	13.8 ± 0.2 a	1.3 ± 0.1 a	54.2 ± 6.11 a	10.6 ± 2.3 a
		R1 growth stage		
LL	17.1 ± 0.3 b	5.4 ± 0.2 a	161.6 ± 9.4 a	3.2 ± 0.1 b
LS	19.3 ± 0.4 ab	3.6 ± 0.8 ab	117.9 ± 24.5 ab	5.4 ± 1.3 ab
SL	19.2 ± 0.5 ab	3.3 ± 1.1 ab	123.4 ± 13.6 ab	5.8 ± 2.4 ab
SS	20.1 ± 0.8 a	2.6 ± 0.9 b	85.9 ± 15.9 b	7.7 ± 4.9 a
		2 weeks after R1		
LLL	30.6 ± 2.4 a	5.6 ± 0.6 a	151.2 ± 15.5 a	5.5 ± 0.2 a
LLS	28.9 ± 2.7 a	5.2 ± 0.5 a	131.3 ± 13.2 a	5.6 ± 0.1 a
LSL	21.5 ± 0.9 a	4.1 ± 0.3 a	99.7 ± 7.7 a	5.3 ± 0.3 a
LSS	30.4 ± 3.1 a	5.4 ± 0.6 a	142.5 ± 19.0 a	5.7 ± 0.2 a
SLL	24.8 ± 3.4 a	5.3 ± 0.5 a	139.2 ± 15.2 a	4.6 ± 0.3 a
SLS	28.0 ± 2.3 a	5.1 ± 0.3 a	135.0 ± 8.5 a	5.6 ± 0.5 a
SSL	24.2 ± 2.0 a	4.3 ± 0.4 a	104.7 ± 9.0 a	5.7 ± 0.2 a
SSS	25.0 ± 1.5 a	4.4 ± 0.2 a	108.9 ± 5.1 a	5.7 ± 0.1 a

3.4. Corn Plant Growth Parameters

The stem diameter at R1 among plants that received sufficient P followed by low P at V6 (SL) did not differ from plants that received sufficient P (SS; Figure 7a). A change in solution P level from low to sufficient at V6 (LS) did not cause a significant increase in stem diameter compared to plants that received either low P until R1 (LL) or sufficient P at planting and low P at V6 (SL). Plant height at R1 did not significantly differ among treatments that received sufficient P at planting or V6 (LS, SL, SS), but was lower in plants that received low P (LL; Figure 7b). The number of leaves at R1 was greater for plants that received sufficient P (SS) than plants that received low P (LL) or low P at planting followed by sufficient P at V6 (LS), but did not differ from plants that received sufficient P at planting followed by low P at V6 (SL; Figure 7c). The total leaf area at R1 was higher in plants that received sufficient P at planting (SS, SL) than plants that received low P at planting (LL, LS) regardless of an increase in P level at V6, suggesting that sufficient P supply at planting is very important for leaf area development (Figure 7d).

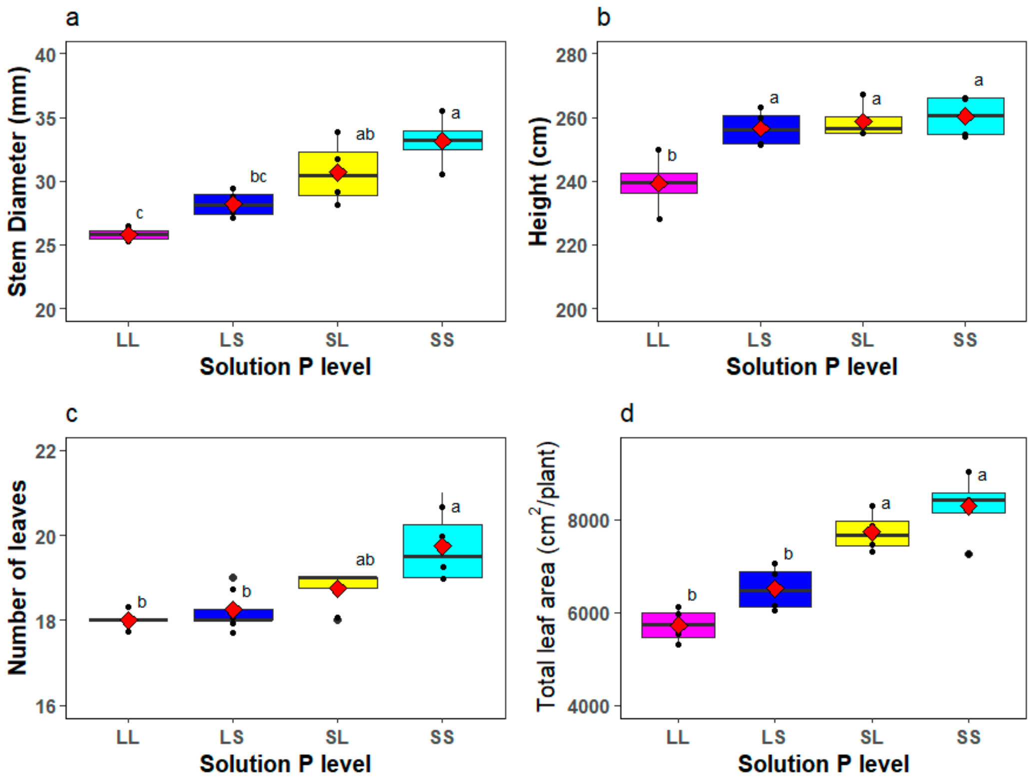

Figure 7. Corn plant growth parameters at R1 grown with low (L) and sufficient (S) solution phosphorus (P) levels varying by growth stage: stem diameter (**a**); height (**b**); number of leaves (**c**); total leaf area (**d**). Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6 and the second letter indicates from V6 to R1. Boxes indicate the interquartile spread (1st and 3rd quantiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

3.5. Yield Components

Plants that received sufficient P from planting to maturity (SSS) produced a greater kernel number compared to other treatments (Figure 8a). Plants that received sufficient P at planting followed by low P at V6 or R1 (SLL, SLS, SSL) produced kernel numbers that did not differ from plants that received low P at planting followed by sufficient P from V6 to R1 or V6 to R6 (LSL, LSS; Figure 8a). The application of sufficient P between R1 and R6 after plants received low P between planting and R1 (LLS) did not recover kernel number and therefore produced lower kernel numbers than LSL, LSS, SLL, SLS, SSL, and SSS plants but did not differ from plants that received low P throughout (LLL; Figure 8a). However, the individual kernel weight in plants that received low P at planting was generally more than that of plants that received sufficient P at planting regardless of a change in solution P level at V6 and R1 (Figure 8c). Kernel row number was not different between treatments except plants that received low P throughout (LLL; Figure 8b).

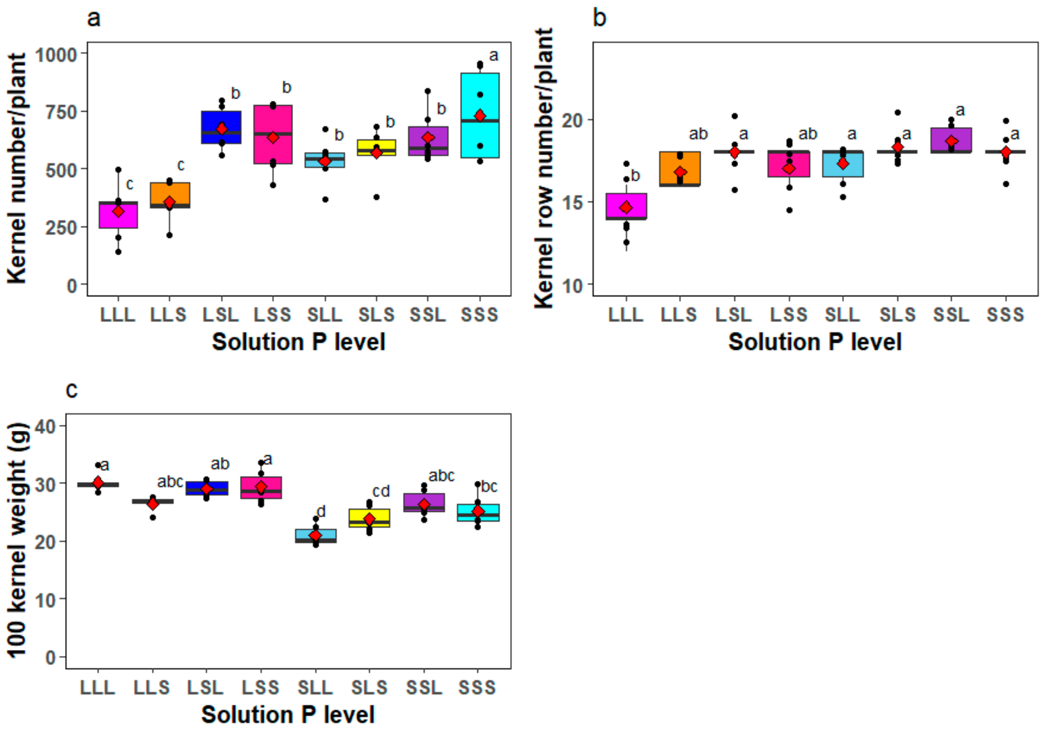

Figure 8. Yield components at R6 for corn plant grown with low (L) and sufficient (S) solution phosphorus (P) levels: grain number (**a**); kernel row number (**b**); and 100-grain weight (**c**). Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Boxes indicate the interquartile spread (1st and 3rd quartiles), while the horizontal line in the boxplot indicates median values of each P treatment. The lower and upper vertical lines of the box plot indicate the 1st and 3rd quartile, respectively. The rhombus in the boxplots indicates the mean value of four replicates. The same letter on boxplots indicates no significant difference between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

3.6. Phosphorus Concentration in Corn Plant Parts

Plants that received sufficient P from planting to V6 had a higher P concentration in their leaves, stems, and roots at V6 than plants that received low P from planting to V6 (Table 5).

Table 5. The effect of applied phosphorus (P) levels on P concentration in corn plant parts at V6 growth stage. The treatment letters "S" and "L" indicate the solution P level applied from planting to V6. The mean values are averages of four replications across each treatment. Standard error calculated within each treatment follow the mean. Different letters in the same column indicate significant differences between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

P Level	P Concentration (g kg^{-1})		
	Leaf	Stem	Root
L	1.44 ± 0.16 b	1.74 ± 0.11 b	1.29 ± 0.11 b
S	2.79 ± 0.08 a	3.27 ± 0.06 a	1.88 ± 0.07 a

At R1, the P concentration decreased in all plant parts compared to V6 irrespective of low or sufficient P treatment between V6 and R1 (LL, SL, SS) except plants that received sufficient P at V6 after receiving low P at planting (LS) that increased P concentration in the LBE and LAE (Table 6). Stem P concentration decreased about 2–4-fold at R1, relative to V6, regardless of P treatment. Plants that received low solution P at planting responded positively to a change in P level from low to sufficient at V6 (LS) so at R1, the P concentration in the LBE, LAE, and stem did not differ from plants that received sufficient P until R1 (SS; Table 6). On the other hand, changing the solution P level from sufficient to low at V6 (SL) decreased the P concentration in the LBE, LAE, and stem to the degree that was similar to plants that received low P until R1 (LL; Table 6). The ear P concentration at R1 in plants that received low P at planting followed by sufficient P after V6 (LS) did not differ from plants that received sufficient P until R1 (SS). Ear P concentration in plants that received low P until R1 (LL) did not differ from plants that received sufficient P at planting until V6 followed by low P (SL) or sufficient P through to R1 (SS). At R1, plants that received sufficient P between V6 and R1 generally had the highest P tissue concentrations (SS and LS > LL and SL), with the exception of roots. Root P concentration at R1 did not differ among treatments.

Table 6. The effect of phosphorus (P) treatment on P concentration in maize plant parts at R1 growth stage. The mean values are averages of four replications across each treatment. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6 and the second letter indicates from V6 to R1. Standard errors calculated within treatments follow the mean. Different letters in the same column indicate significant differences between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

P Level †	**P Concentration (g kg^{-1})**				
	LBE *	**LAE** ‡	**Stem**	**Ear**	**Root**
LL	0.67 ± 0.10 b	1.32 ± 0.12 b	0.48 ± 0.04 b	1.50 ± 0.22 b	1.08 ± 0.26 a
LS	1.68 ± 0.09 a	1.92 ± 0.09 a	0.86 ± 0.06 a	2.50 ± 0.05 a	1.07 ± 0.05 a
SL	0.85 ± 0.08 b	1.28 ± 0.20 b	0.55 ± 0.03 b	1.76 ± 0.14 b	0.86 ± 0.03 a
SS	1.66 ± 0.05 a	1.88 ± 0.14 a	0.93 ± 0.05 a	2.07 ± 0.12 ab	1.11 ± 0.08 a

† L: low P level; S: sufficient P level; * LBE: leaf below ear; ‡ LAE: leaf above ear.

At R6, the P concentration decreased compared to R1 in the vegetative plant parts (LBE, LAE, stem, and roots; Table 7). The P concentration in the vegetative parts of plants that received sufficient P between R1 and R6 (LLS, LSS, SLS, SSS) were higher than in plants that received low P between R1 and R6 (LLL, LSL, SLL, SSL) regardless of the solution P level applied prior to R1 (0.9–1.8 vs. 0.4–1 g kg^{-1}, respectively; Table 7). Plants that received low P at planting (LLL, LLS, LSS) had a lower P concentration in the LBE than LAE except plants that received sufficient P exclusively between V6 and R1 (LSL) which had similar P concentrations in both the LBE and LAE. Plants that received sufficient P at planting (SSS, SSL, SLS, SLL) regardless of changes after V6 also had LBE concentrations that were similar to LAE. Plants that received sufficient P at V6 or R1, but low P between planting and V6 (LLS or LSS), increased P concentration in their LAE at R6 relative to R1. Plants that received sufficient P exclusively between V6 and R1 (LSL) had a decrease in LBE, LAE, stem, and CHI P concentration from R1 to R6, although these values were not different from those of plants that received sufficient P until R1 followed by low P (SSL) or sufficient P at planting until V6 followed by low P (SLL). The grain P concentration in plants that received sufficient P exclusively between V6 and R1 (LSL) was lower than almost all other treatments (1.70 vs. 2.14–2.54 g kg^{-1}).

Table 7. The effect of applied phosphorus (P) levels on P concentration in maize plant parts at R6 growth stage. The mean values are averages of six replications across each treatment. Treatments are indicated by letters "L" and "S", where the first letter indicates the solution P level applied from planting to V6, the second letter indicates from V6 to R1, and the third letter indicates from R1 to R6. Standard error calculated within each treatment follow the mean. Different letters in the same column indicate significant differences between treatments as assessed by Tukey's Honest Significant Difference ($p \leq 0.05$).

P Level †	P Concentration (g kg^{-1})					
	LBE *	LAE ‡	Stem	CHI #	Grain	Root
LLL	0.68 ± 0.10 ab	1.04 ± 0.23 ab	0.60 ± 0.06 bcd	0.59 ± 0.11 ab	2.14 ± 0.20 abc	0.46 ± 0.03 b
LLS	1.10 ± 0.29 a	1.80 ± 0.43 a	0.86 ± 0.12 abc	0.80 ± 0.08 ab	2.52 ± 0.16 ab	0.67 ± 0.09 ab
LSL	0.52 ± 0.05 b	0.49 ± 0.05 b	0.38 ± 0.03 d	0.39 ± 0.03 b	1.70 ± 0.14 c	0.58 ± 0.08 b
LSS	1.04 ± 0.22 a	1.61 ± 0.19 a	0.90 ± 0.13 ab	0.64 ± 0.10 ab	2.37 ± 0.20 ab	0.73 ± 0.10 ab
SLL	0.41 ± 0.05 b	0.43 ± 0.07 b	0.56 ± 0.07 bcd	0.54 ± 0.05 ab	2.52 ± 0.08 ab	0.54 ± 0.04 b
SLS	0.96 ± 0.10 ab	1.10 ± 0.23 ab	0.82 ± 0.04 abc	0.74 ± 0.11 ab	2.54 ± 0.12 a	0.75 ± 0.04 ab
SSL	0.53 ± 0.04 b	0.49 ± 0.03 b	0.49 ± 0.05 cd	0.56 ± 0.06 ab	2.23 ± 0.13 abc	0.65 ± 0.05 ab
SSS	0.93 ± 0.11 ab	0.92 ± 0.13 ab	1.00 ± 0.11 a	0.85 ± 0.17 a	2.50 ± 0.12 ab	0.96 ± 0.10 a

† L: low P level; S: sufficient P level; * LBE: leaf below ear; ‡ LAE: leaf above ear; # CHI: cob, husk, and immature ears.

4. Discussion

Phosphorus application timing in corn was assessed in sand-culture hydroponics to evaluate the effect of a change in solution P level on corn. This work aimed to quantify the effect of applying low and sufficient P at either planting, V6, and R1 on corn growth parameters and overall yield.

4.1. The Effect of Phosphorus on Corn Growth and Biomass Production

It is clear that starter P fertilizer application at least increases early shoot growth of corn [13,25]. In this study, stem and leaf dry weight, and P concentration in the stems, leaves, and roots at V6, were greater in plants that received sufficient P from planting to V6 compared to plants that received low P over the same period (Table 4; Figure 1). Interestingly, root dry weight was the same for both P treatments at V6. The greater shoot (stem and leaf) dry weight and P concentration in plants that received sufficient P from planting to V6 agree with the findings of Gordon and Pierzynski [13] and Kaiser et al. [14] that P starter fertilizer enhances early shoot growth. A typical response of P deficiency is an increase in root-to-shoot ratio. However, root/shoot ratio did not differ between treatments in our study. At the V6 growth stage, the nodal root system, which depends solely on the external P source, was not fully developed and was not inhibited by low-P conditions [16]. This might explain why there was no difference in root dry weight between sufficient and low P treatments at V6.

As the solution P level concentration was changed from low to sufficient (LS) at V6, plants responded strongly to sufficient P application with shoot (stem, LBE, and LAE) P concentration at R1 that did not differ from SS plants (Table 5). Similarly, changing from sufficient to low P at V6 (SL) produced shoot P concentrations that did not differ from plants that received low P from planting to R1 (LL; Table 5). Despite reduced shoot P concentrations in SL plants, the root, stem, LBE, and LAE dry weight did not differ from SS plants, whereas the increase in shoot P concentration in LS plants did result in those parameters being different from LL plants (Figure 2a–c,e). Although shoot P concentrations in LS plants were similar to SS plants at R1, total leaf number and area did not "catch up" with SS plants after the increase in P addition at V6 (Figure 7c,d). This could be explained by the fact that at the V6 growth stage, final leaf primordia have been formed and any change in limiting factors will have little impact on final leaf number [16]. Another reason for fewer leaves in plants that received low P from planting until V6 or R1 (LL and LS) might arise from the involvement of low P in increasing plastochron in plants [31]. To date, all studies that evaluated the effect of P on the total leaf area of corn have been conducted by applying exclusively low or sufficient P until maturity; those studies showed that total leaf area was significantly higher for sufficient than low-P plants [20,32]. In this study, R1 total leaf area did not recover after increasing from low to sufficient P application at V6 despite the recovery (relative to SS treatment) of leaf P concentrations. Similar results were reported by Henningsen et al. [19] who found that corn plant dry weight did not recover (i.e., "catch up" to sufficient plants) despite supplying sufficient P to 30-day-old corn plants that had received low P earlier. Chiera et al. [33] also found that when sunflower was exposed to limited P for 32 days, the number of cell divisions and cell size decreased, resulting in a smaller leaf area. A possible reason why the leaf area in LS plants at R1 did not recover despite the increase in P application at V6 is the limited number of cell division and cell size in the leaf primordia of the plants. The effect of early P nutrition on cell division and the expansion of plants is well documented [15].

Plants invest P mostly in organic forms rather than inorganic P, especially lipid-P during the active growth phase, to maintain photosynthesis under low-P conditions [27]. Wang and Ning [20] found that a concentration of 1.5 mg P kg^{-1} DW in actively growing leaves (i.e., leaves above the ear) of corn plants that received low P was enough to maintain maximum photosynthesis at R1, which was similar to plants that received sufficient P in the same period. In our study, the LAE P concentration in all treatments at R1 was in the range of 1.3–1.9 mg kg^{-1} (Table 5), which was similar to the photosynthesis threshold value reported by Wang and Ning [20]. As a consequence, Pn did not differ much between treatments at R1 (Table 4). While Pn (per unit leaf area) was the same for LS, SL, and SS treatments at R1, total photosynthesis was greatest in SS and SL plants due to a greater number of leaves and leaf surface area (Figure 7c,d). Although shoot P concentrations in SL plants were significantly less than those of SS plants at R1, the decrease in P application from sufficient to low at V6 produced similar dry matter as fully sufficient (SS) plants (Figure 3) due to similar biomass growth between V6 and R1 (Table 2). At the whole plant level, plants have the ability to use stored phosphate in the vacuoles to buffer the high P requirement for photosynthetic carbon metabolism under low P [15,32]. This means that the high P concentration in the shoots of SL plants at V6 might have buffered the cytosolic P that needs to be maintained to maintain metabolic activities. The similar dry matter increase between V6 and R1 for SL compared to SS plants (Table 2), despite the low shoot P concentration in SL plants, might have been due to the large leaf surface area, number of leaves, and cytoplasmic P buffering from the vacuoles.

4.2. Grain Yield and Yield Components

Phosphorus plays an important role in grain yield of corn by regulating photosynthetic carbon metabolism [32]. Sufficient phosphate in the cytosol increases sucrose synthesis by exchanging triose-P in the chloroplast, thereby decreasing starch accumulation in the chloroplast. Sucrose synthesis also liberates phosphate in the process, thereby increasing the recycling of phosphate between the chloroplast and the cytosol. A higher sucrose/starch ratio stimulates remobilization of assimilates in the form of sucrose to the sink [20]. However, sucrose transport to the sink is regulated by the sink size [34]. A small sink size sends a feedback regulatory mechanism to sucrose transporters to limit sucrose movement to the grain [35]. In corn, the sink size is determined by the ear size, which is basically composed of kernel row per ear and kernel number per row [16]. However, kernel row number is strongly influenced by the genetics of corn rather than environmental factors [16]. In the present study, kernel row per ear did not differ significantly between treatments (Figure 8b), supporting the assertion that environmental factors have little influence on kernel row number. On the other hand, kernel number and weight are influenced by environmental factors, which explains significant differences in grain yield between P treatments in this study (Figure 8a,c).

Our hypothesis was that supplying sufficient P to corn plants between V6 and R1 is critical for higher grain yield production at maturity. Plants that received sufficient P between V6 and R1 (LSL, LSS, SSL, SSS) produced significantly higher grain yield than plants that received low P between V6 and R1 (LLL, LLS, SLL, SLS) regardless of the P supply level before V6 or after R1 (Figure 6). These results support our hypothesis that sufficient P is critical between V6 and R1 in ensuring higher corn grain yield. The only study that varied solution P levels akin to our study via hydroponics was Barry and Miller [18]. In their study, sufficient, intermediate, or low P was applied to corn at planting to either the 6- or 10-leaf stage followed by either sufficient, intermediate, or low P until maturity. The results of their study showed that the yield increase was more sensitive to sufficient P applied after the 6- or 10-leaf stage rather than low or sufficient P before the 6- or 10-leaf stage, which agree with the results of our study. Barry and Miller [18] also found that plants that received sufficient P throughout produced the highest kernel number compared to plants in which solution P level was changed from low to sufficient and vice versa during growth. In our study, kernel number was significantly higher in plants that received sufficient P throughout (SSS). Excluding the fully sufficient treatment (SSS), there was no difference in kernel number among plants that received sufficient P at least once between planting and R1 (LSL, LSS, SLL, SLS, SSL; Figure 8a), but they were significantly greater than those that never received sufficient P, or not until R1 (LLL, LLS). On the other hand, kernel weight was sensitive to solution P level applied between planting and V6. Kernel weight was greater for plants that received low P between planting and V6 compared to plants that received sufficient P between planting and V6 (Figure 8c). The lower kernel number in LSL and LSS treatments compared to SSS was compensated for by the increased kernel weight. This explains why the grain yield of LSL and LSS plants did not differ from SSS plants (Figure 6). Phosphorus has not been directly implicated in assimilate transport in corn [34]. However, since application of low P between planting and V6 followed by sufficient P between V6 to R1 or R6 (LSL, LSS) ensured the efficient movement of assimilates to the sink (grain) and produced more kernel/grain weight than plants that received sufficient P between planting and R1 (SSL, SSS), it can be hypothesized that P application timing rather than P concentration in the vegetative tissues of corn per se stimulates source strength and sink activity.

In their model prediction and observed data, Borrás and Westgate [36] found that the sufficient accumulation of dry matter before grain filling is critical to ensuring a high grain filling potential of corn. It has been revealed in our study that a higher total biomass production before silking (R1) is not necessarily a guarantee to higher grain yield (R^2 = 0.10). Instead, a greater R1 ear P concentration (R^2 = 0.90) and, to some extent, R1 ear size were well related to grain yield (R^2 = 0.56; Figure A1). Essentially, ear P concentration > 2 g kg^{-1} at R1 growth stage in plants that received sufficient P between V6 and R1 (SS and LS) (Table 5) was sufficient to sustain the highest grain yield at R6 regardless of applying low or sufficient P after R1. This is an indication that at or before R1, P redistribution rather than dry matter partitioning into the ears is an important determinant of grain yield. Plants that received low P between planting and V6 followed by sufficient P between V6 and R1 (LS) produced lower shoot (stem and leaf) biomass at R1 compared to plants that received sufficient P at planting or V6 (SL or SS; Figure 2). But, after R1, when solution P was changed to low or sufficient (LSL or LSS), the final grain yield was similar between the LSL, LSS, and SSS, despite the biomass differences at R1 (Figures 3 and 6). In the same vein, when the solution P level was changed to low at R1 for initially sufficient plants (SSL), grain yield was similar between SSL and SSS plants (Figure 6). Based on these results, it can be extrapolated that low P availability before V6 or after R1 does not pose any threat to grain yield if the corn plant has access to sufficient available P between V6 and R1. One of the main mechanisms of P utilization efficiency is the internal remobilization of P from source tissues (mature leaves and stems) to the sink organ (grain) during the reproductive phase [28]. The P concentration at maturity in the plants that received low P after R1 (LSL, SSL) was significantly less in the LBE, LAE, stem, and CHI compared to plants that received sufficient P after R1 (LLS, LSS), implying greater remobilization of P to the grain (Table 6). Dry matter partitioning into the root, LBE, LAE, and stem was generally higher in plants that received sufficient P at planting (SLL, SSL, SLS, SSS) compared to plants that received low P at planting (LLL, LLS, LSL, LSS) (Figure 5). It appears that efficient dry matter partitioning between R1 and R6, from source (leaves and stem) into the sink (grain) of LSL and LSS plants, was another important mechanism responsible for grain yield recovery from earlier P-deficient conditions. For example, the grain P concentration in LSL plants was lowest (1.7 g kg^{-1}), yet the grain yield was not significantly different from that of plants fully supplied with sufficient P (SSS). The reported P concentration in corn grain at maturity was >2 g/kg [30,37]. The low grain P concentration (1.7 g/kg) might be explained by a dilution effect since this particular treatment (LSL) produced one of the largest grain biomass values. Overall, four main mechanisms might explain how low P supply at planting followed by sufficient P supply at V6 (LSL, LSS) did not reduce grain yield: (1) adequate ear P concentration at R1; (2) efficient remobilization of assimilates from stem and leaf to grains between R1 and R6 (source–sink relationship); (3) higher kernel weight; and (4) less investment into root biomass.

5. Implications and Conclusions

Sufficient P application at planting increased root and shoot P concentration and enhanced early crop establishment by increasing shoot growth, but not root growth at V6. A change in solution P level from sufficient to low between V6 and R1 (SL) produced a lower P concentration, but not lower root and shoot biomass compared to fully P sufficient plants. Conversely, root and shoot (leaf and stem) dry weight did not recover with an increase in solution P level from low to sufficient between V6 and R1 (LS), relative to fully deficient plants (LL). An ear P concentration >2 g kg^{-1} at R1 in plants that received sufficient P after V6 (LS, SS) was adequate to sustain the highest grain filling between R1 and R6. Plants that received sufficient P between V6 and R1 (LSL, LSS, SSL, SSS) produced a significantly higher grain yield than plants that received low P between V6 and R1 (LLL, LLS, SLL, SLS) regardless of the level of P supply before V6 or after R1. The grain yield of plants that received sufficient P at least once after V6 (LSL and LSS) did not differ from plants that received only sufficient P (SSS), due to (1) greater ear P concentration at R1; (2) efficient remobilization of assimilates from the stem and leaf to grains between R1 and R6 (source–sink relationship); (3) higher kernel weight; and (4) less investment into root biomass. Overall, several corn parameters could be recovered, where "recover" is defined as when a treatment that received a deficient P application level during at least one of the three stages was still able to produce the same mass, size, or concentration as the fully sufficient control (SSS). Those parameters are grain yield, height, ear size, 100-grain weight, ear P concentration, and all photosynthetic parameters.

It can be extrapolated from this study that it is unnecessary to have sufficient P levels between planting and V6 or from R1 to R6, but rather, it is critical for corn to have access to sufficient P from V6 to R1 to achieve optimum yield. Although plants that received sufficient P only between V6 and R1 (LSL) produced a high grain yield comparable to plants that received sufficient P throughout (SSS), grain P concentration was low (1.7 k kg^{-1}). This decreased grain P concentration represents a tradeoff if one were to minimize P application and yet not reduce grain yield. In some situations, lower grain P concentrations could be advantageous. Grain P is mostly in the form of phytate, which is somewhat unavailable to monogastric livestock that do not produce phytase enzyme (i.e., poultry and swine) [38]. As a result, inorganic P is added to their diets, which results in high P concentrations in manure and may present challenges to nutrient management [39]. One alternative is to apply phytase enzyme to animal feed in order to increase P absorption and eliminate the need for inorganic P supplements, or feed with grain from low-phytate hybrids that are more digestible [40], thereby reducing manure P concentrations [39,41]. However, low-phytate corn hybrids tend to produce low yields [42]. The results of this study present an additional alternative through the production of corn grain with lower P concentrations, yet not compromising in yield. To date, no known industrial use of corn grain requires a high P concentration or content. Therefore, producing corn grain with low P content will not affect its industrial use. Also, if one is fertilizing to replace P removed by grain harvest (i.e., maintain adequate soil test P), then a lower grain P concentration is an advantage.

On the other hand, if the purpose is to produce corn for silage in which total biomass is most important rather than grain yield alone, then the results of this study suggest that sufficient P availability throughout the corn life cycle is necessary. For example, the fully sufficient control (SSS) produced the highest biomass with all other treatments producing significantly less total biomass.

It must be noted that this study was conducted in a sand-culture hydroponics system in which soil P-solution dynamics were purposely eliminated as a confounding factor since the growing media was inert. In other words, there is no buffering of the solution P by soil, which allowed for nutrient availability to be controlled through fertigation concentrations and timing. This was necessary in order to clearly test the hypothesis; otherwise, soil sorption and desorption degree and kinetics would prevent the interpretation of data in the context of plant growth activity and uptake timing. The most obvious example is that while it is easy to add P to soil to increase solution P concentrations in moving from a deficient to sufficient soil condition, it is not possible to quickly change in the other direction from a sufficient to deficient soil condition. With clear plant response results without confounding soil interactions, it now becomes necessary to determine if soil P sorption and desorption kinetics will permit the benefits of P application at V6 to be realized. Fertilizer P will immediately increase solution P concentrations for plants, but later decrease to a fraction of the initial level, at variable rates, as a function of soil properties [2,3,43]. Therefore, roots can take advantage of this "window" of high solution P concentration if applied at V6. In addition, rhizosphere activity is elevated between V6 and R1 due to maximum root exudates, which may reduce strong soil P sorption [44,45]. If highly soluble P fertilizer is applied as a band or side dress around the root zone between V6 and R1, the possibility of P bioavailability within the vicinity of roots will increase, thereby increasing P uptake. This study clearly supports the potential for achieving optimum grain yield with lesser P application, through side dressing of P at V6; field studies are to be conducted.

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Data Availability Statement: Please contact authors regarding access to data.

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Appendix A

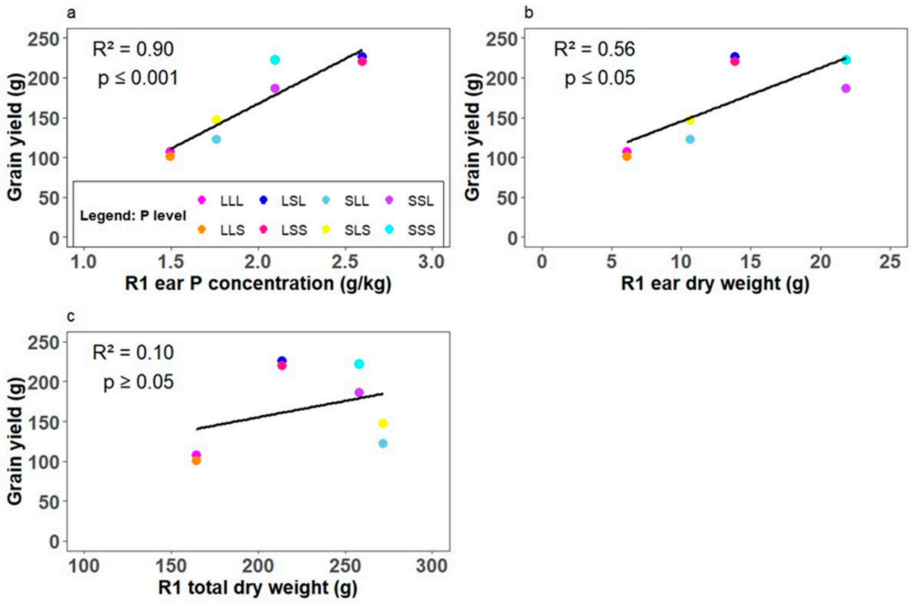

Figure A1. Grain yield at R6 shown as a function of R1 ear P concentration (**a**), ear dry weight (**b**), and total dry weight (**c**). Values for LL, LS, SL, and SS at R1 were plotted against R6 LLL and LLS, LSL and LSS, SLS and SLL, and SSL and SSS, respectively. The data points at R1 for ear P concentration, ear dry weight, and total dry weight are averages of four replications, and grain yields at R6 are averages of six replications.

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