Examining Phosphorus Use Efficiency Across Different Lettuce (Lactuca sativa L.) Genotypes

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Research Article

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Abstract

Most agricultural soils worldwide present limited availability of phosphorus (P) and crops require supplemental application of P fertilizers. Due to the economic and environmental concerns derived from the use of P fertilizers, identifying and breeding P-efficient lettuce (*Lactuca sativa* L.) cultivars is imperative for the reduction of production costs and implementation of more sustainable practices. Phosphorus use efficiency (PUE) remains unexplored in lettuce. In this research, 66 lettuce genotypes of six morphological types were evaluated between standard recommended P rate (202 kg·ha^{-1} of P_2O_5) and half-P rate (101 kg·ha^{-1} of P_2O_5). Lettuce genotypes were tested in two field experiments conducted during the 2017-2018 and 2019-2020 growing seasons in the organic soils (Histosols) within the Everglades Agricultural Area of South Florida. Head weight, marketability, tissue P concentration, soil total-P concentration, and soil extractable P were measured. Genetic variation was detected for PUE within romaine, crisphead, butterhead, Latin, and loose leaf. Eight genotypes were found to produce similar or higher head weight and good marketability when grown in the half-P rate compared to the standard P rate. No correlations were detected between head weight and tissue P concentration, indicating possible variation in P uptake and utilization on the tested lettuce genotypes. A significant, positive correlation was observed for soil total-P and soil extractable P, indicating that the increase in total P concentration of Histosols resulted in higher availability of P to plants. Lettuce genetic variation for PUE may allow further development of P-efficient cultivars for conventional and alternative production systems. More comprehensive investigations must be conducted to elucidate the genetic mechanisms controlling PUE in lettuce.

Introduction

Most agricultural soils worldwide present suboptimal levels of plant essential nutrients such as phosphorus (P) and/or are severely degraded due to intense crop cultivation and inappropriate soil management practices (Baligar and Fageria 2015). Low fertility and degradation of arable lands can negatively impact crop yield, and therefore, agricultural soils require additional fertilizer inputs to achieve adequate crop nutrition, resulting in higher production costs to farmers (Maqsood et al. 2013). Increased use of fertilizers has been associated with eutrophication of natural ecosystems (Fageria et al. 2008). These negative factors have led to a constant search for alternatives to minimize the utilization of fertilizers and their drawbacks (Kanter et al. 2015; Wu and Ma 2015).

Improving nutrient use efficiency (NUE) is considered one of the most cost-efficient methods to reduce fertilizer expenses and environmental degradation (Ali et al. 2018). Nutrient use efficiency can be defined as the plant's ability to absorb and utilize soil nutrients more efficiently, and consequently, produce adequate yield or biomass (Baligar and Fageria 2015). Mechanisms of NUE such as root morphology adaptation, induction of transporters, improved nutrient assimilation, translocation from roots to shoots, storage, recycling, and remobilization have been documented in the model plant Arabidopsis (*Arabidopsis thaliana*), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and coffee (*Coffea arabica* L.) (Horst et al. 1993; Walker et al. 1996; Jia et al. 2008; Kellermeier et al. 2013; Chietera and Chardon 2014; Yu et al. 2014; Moura et al. 2019). These studies, along with screening experiments to identify genotypes capable of producing good yield with less fertilizer inputs, allow for a better comprehension of the complex functioning of plant NUE and for the potential improvement of new nutrient use efficient cultivars (Reich et al. 2014).

A large fraction of NUE studies conducted hitherto have focused on P use efficiency (PUE). After nitrogen (N), P is the second most essential element to plants, though, one of the least mobile and available nutrients in soil (Gruen et al. 2014). While plant type, soil, and climatic factors can all influence the mobility and solubility of soil P, addressing soil pH in understanding the fate and transport of P in soils is also critical (Bhadha et al. 2010; Fageria et al. 2017). Phosphorus availability is reduced in soils with low pH (<5.5) due to P sorption by iron (Fe) and aluminum (Al) ions, whereas in high pH (>7.0) soils, P is fixed by calcium (Ca). Phosphorus' high fixation and low mobility in soils reflect in low recovery rates of applied P fertilizers by plants; often less than 25% of applied P fertilizer is recovered by crops in the year of application (Fageria et al. 2017).

Genetic variation for PUE has been reported in coffee (*C. arabica* and *C. canephora* Pierre) (Neto et al. 2016), faba bean (*Vicia faba* L.) (Daoui et al. 2011), soybean [*Glycine max* (L.) Merr.] (Yan et al. 2006), rice (Vandamme et al. 2016), wheat (*T. aestivum* L. and *T. durum* L.) (Ozturk et al. 2005; McDonald et al. 2010), white clover (*Trifolium repens* L.) (Pereira-Carvajal et al. 2016), barley (*H. vulgare* L.) (McDonald et al. 2010), and tea (*Camellia sinensis* L.) (Salehi and Hajiboland 2008). Phosphorus use efficiency has been gradually incorporated as a target trait by plant breeders worldwide, especially in rice and wheat breeding programs; P-efficient cultivars can contribute to the reduction of fertilizer use and production costs, and to more sustainable cultivation practices (Baligar et al. 2001; Ortiz-Monasterio et al. 2001; Ozturk et al. 2005; Wang et al. 2005; Ali et al. 2018). However, PUE remains unexploited in most vegetable crops, including lettuce (*Lactuca sativa* L.), that is one of the top-ten most consumed leafy vegetables in the United States.

In the U.S., California and Arizona are responsible for approximately 94% of the total national lettuce production, followed by Florida with approximately 3.5%. The crop is also produced in very minimal proportions throughout many states (USDA NASS 2019). In Florida, lettuce is primarily planted in the Everglades Agricultural Area (EAA). The EAA is an area in South Florida well-known for its high relevance to the state's agricultural industry and for its rich organic soils (Histosols), typically referred to as "muck". Histosols in the EAA contain nearly 65% of organic matter and have become shallower over time due soil subsidence, in which organic matter is lost by decomposition, oxidation, erosion, etc. Soil loss helps with the incorporation of calcium carbonate from the underlying limestone bedrock into upper parts of the soil profile increasing soil pH (Bhadha et al. 2020). In turn, the availability of some nutrients, especially P, is drastically reduced.

Hence, the identification of lettuce genotypes with higher PUE will allow the introgression of this trait into lettuce breeding programs for further improvement. In turn, these genotypes may help mitigate problems associated with low soil P availability in conventional cultivation areas such as the EAA and in protected production systems (i.e. hydroponics, vertical farming), where the waste of fertilizers generates economic and environmental concerns (Gruda and Tanny 2014). The objectives of this study were to: (1) test a large set of lettuce genotypes of six different morphological types in Histosols fertilized with the standard and half of recommended P rates; (2) identify lettuce genotypes that produce similar head weight in both P rates; (3) examine the correlations between soil and tissue P concentration; and (4) describe the differences in other horticultural traits of lettuce grown in half and standard P rate.

Materials And Methods

Plant material

Sixty-six lettuce cultivars, experimental lines, and plant introductions (PI) of six different morphological types were used in the study, including 17 iceberg, 18 romaine, 12 butterhead, 12 loose leaf, 4 Latin, and 1 oilseed (Table 1). These genotypes include lines/cultivars bred and adapted to Florida's conditions, obsolete commercial cultivars utilized in inland and southern production areas of California and Arizona, and heirloom cultivars and genotypes that were introduced to the United States Department of Agriculture (USDA) – National Plant Germplasm System / Germplasm Resources Information Network (NPGS-GRIN). Seeds of 20 breeding lines/cultivars were previously increased from the University of Florida's Institute of Food and Agriculture Sciences (UF/IFAS) Lettuce Breeding Program. The rest of germplasm was requested from the NPGS-GRIN collection and from the seed company 3 Star Lettuce (Gonzales, CA).

Field experiments

Two field experiments were conducted to screen lettuce genotypes for PUE. The experiments were planted at the UF/IFAS Everglades Research and Education Center (EREC), in Belle Glade, FL. The first experiment was conducted from November 2017 to March 2018, while the second experiment was conducted between November 2019 to January 2020. In both experiments, the soil was Dania muck (euic, hyperthermic Lithic Haplosaprists) with a record of minimum P fertilization in at least 5 years before these experiments were planted. Prior to the experiment, a soil-test analysis was conducted by collecting 10-15 samples across the fields and analyzed at the UF/IFAS Soil Laboratory at the EREC (Table 2).

Both experiments were direct seeded on 0.15 m raised double-row beds, and each row consisted of a unique genotype, with plots 7.62 m long. At four-leaf stage, seedlings were thinned to a 0.30 m in-row spacing. Pest and disease management were followed using standard procedures for commercial production of lettuce listed in the Vegetable Production Handbook of Florida (Kanissery et al. 2020). Over-head irrigation was provided throughout the crop cycle as needed. The herbicide Pursuit® was applied post-emergence once in each experiment at a rate of 0.14 $L \cdot ha^{-1}$ to control grasses and broadleaf weeds at 7 days after planting. Weeds were manually removed through hoeing in two opportunities.

The total area where the experiments were planted were divided in two zones, the first one was fertilized with the standard recommended rate (SR, 202 $kg \cdot ha^{-1}$ of P_2O_5), and the second zone with half recommended rate (HR, 101 $kg \cdot ha^{-1}$ of P_2O_5). Fertilizer rates were based on recommendations from the UF/IFAS Soil Testing Laboratory at the EREC (Table 2). The P fertilizer derived from ammonium polyphosphate 11-37-0 (Wedgworth's Inc., Clewiston, FL) and was banded during bedding at a depth of 5-10 cm below bed surface. Two post-planting split applications of 4.5 $kg \cdot ha^{-1}$ of multipurpose fertilizer 20-20-20 (Plant Foods Inc., Vero Beach, FL) were performed in each experiment to provide supplemental nutrients as needed.

Horticultural data collection

Genotypes were evaluated at their horticultural maturity, except for the genotype PI 251246 (oilseed type). Prior to harvest, the percentage (%) of marketable heads was estimated for each genotype by dividing the number of plants that would meet market requirements (shape and size) by the total number of plants per plot. At harvest, ten heads were randomly chosen from each plot to obtain the average head weight (HW), expressed in grams (g). The incidence (%) of tipburn, a lettuce disorder characterized by necrosis of newly developed leaf margins, was estimated for each plot by slicing the ten harvested heads in half.

Phosphorus quantification in plant tissue and soil

At harvest, a sample of 10 inner leaves of the ten harvested heads from each plot were placed into individual plastic bags. Samples were then washed with deionized (DI) water to remove soil particles, placed into paper bags, and oven-dried at 65 ºC for 7 days. Once dried, each sample was ground using a Wiley® mill (Model 4, Thomas Scientific, Swedesboro, NJ), and stored in 20 mL polyethylene scintillation vials (Fisherbrand™, Fisher Scientific, Suwanee, GA).

Soil samples were collected from each field plot by scooping rhizosphere soil around 10 plants; all samples within a plot were evenly mixed to collect approximately 400 g of soil. One sample per plot was placed into individual plastic bags and dried at 65 ºC for 7 days. The dried soil was passed through a 1 mm sieve (16 mesh), collected into plastic scintillation vials, and stored for further analysis of P concentration.

Phosphorus tissue samples were extracted using a total-P (TP) protocol adapted from the UF/IFAS Extension Soil Testing Laboratory Analytical Procedures and Training Manual. Soil samples were extracted following TP and Mehlich-3 protocols (Mehlich 1984).

The TP extraction consisted in weighing 0.4 g of ground plant tissue (tissue TP) or dried and sieved soil (soil TP) into a 20 mL glass scintillation vial. Samples were then placed in a muffle furnace and burnt to ashes at 550º C for 5 h 30 min. Once samples reached room temperature, they were removed from the muffle furnace and moistened by adding 5 drops of deionized (DI) water. After, each sample received 2 mL of 6M hydrochloric acid (HCl) and was maintained at room temperature for 2 hours. The volume of each vial was then brought up to 20 mL, filtered with qualitative P5 filter paper (12.5 cm in diameter) and transferred to 15 mL polypropylene test tubes. Soil extractable P (M3P) was estimated following a Mehlich-3 extraction protocol (Mehlich

1984). All samples were analyzed for P concentration at the UF/IFAS Soil, Water, and Nutrient Management Laboratory using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Agilent Technologies 5110 ICP-OES, Santa Clara, CA, USA).

Experimental design and data analysis

The experiments were arranged following a randomized complete block design (RCBD) with three replications. Each block, or replicate, was further divided into two subblocks of 32 genotypes each. Combined analysis of variance (ANOVA) was performed for HW, marketability, tissue TP, soil TP, and soil M3P across genotypes, P rates, and experiments. Due to the absence of tipburn in most genotypes in both experiments, tipburn data were not analyzed. The factors genotype, P rate, experiment, and the interactions of genotype × P rate, genotype × experiment, P rate × experiment, and genotype × P rate × experiment were considered as fixed effects, while the factors subblock nested within block, and block nested within experiment were considered as random effects. Since the genotype × P rate × experiment interaction was not significant, a new ANOVA was performed by year.

The sums of squares were partitioned into each lettuce type. The oilseed type was represented by one single genotype (PI 251246), and therefore, it was not used in the models containing the genotype factor. Multiple comparisons were performed based on Fisher's least significant difference test (α=0.05). All analyses were conducted using the GLIMMIX procedure in SAS® software, Version 9.4 (SAS Institute Inc., Cary, NC, USA). Pearson correlation coefficients were calculated between HW, marketability, tissue TP, soil TP, and soil M3P values from each of the two P rates.

Results

Lettuce head weight under two P rates

Significant differences were identified for HW in the combined analysis for lettuce genotypes (*P<0.0001*) used in this experiment indicating genetic variation for HW under two P rates. Differences were also observed between the two P rates (*P<0.0001*) but no differences between the two experiments (*P=0.7331*) were detected. Only two interactions, genotype × experiment (G×E) and rate × experiment (R×E) were important in this study (*P>0.0001*), while other interactions had no effect on the overall analysis of HW (Table 3).

As morphological types are different in size and in morphology from one another, a separation of the square mean within type was conducted. Therefore, butterhead (*P=0.0251*), crisphead (*P<0.0001*), loose leaf (*P=0.0328*), and romaine (*P<0.0001*) yielded (HW) differently when fertilized with the standard and half P rates (Table 3). The opposite was observed in Latin (*P=0.0942*), and oilseed (*P=0.1734*) genotypes that showed non-significant differences (Table 3).

High similarity was identified in HW when lettuce was fertilized with the standard and half-P rates (*P>0.05*) (Table 3). These similarities were found in butterhead breeding line 60176 (Fig. 1), crisphead cultivars Honcho II, Cibola, and Sun Devil (Fig. 2), loose leaf cultivars North Star and RSX743, and PI 358001-1 and PI 278109 (Fig. 3), romaine breeding lines 50098 and 60183, and cultivar Okeechobee (Fig. 4), and Latin cultivar Little Gem (Fig. 5). However, a butterhead breeding line (B1196), two loose leaf cultivars (Cordoba and Revolution), and a Latin cultivar (Floribibb) had slightly higher HW when fertilized with half-P rate (Fig. 5). For this study, these genotypes were considered as P-efficient. The only oilseed accession tested, PI 251246, experience a HW reduction in the half-P rate, though, this difference was found to be non-significant (*P>0.05*) (Figs. 6 and 7).

Head weight performance across genotypes

Regardless of P rates, there were significant differences (*P>0.05*) for HW within each lettuce type among the tested genotypes (Table 3). For instance, the butterhead PI 342440 had a similar HW to Odyssey (a commercial butterhead cultivar) (Fig. 1S). In contrast, none of the crisphead cultivars and breeding lines were as productive as the commercial 'Chosen' but breeding line 60158 and cultivars Bubba and Coyote had similar HW as 'Flagler', another commercial cultivar currently used in Florida's field production (Table 2S). Similarly, three Latin lettuce genotypes were not as productive as the cultivar Floribibb; however, Latin lettuce is not currently planted in commercial fields in Florida (Fig. 3S). The cultivar RFX-0901 presented the highest HW of all loose leaf genotypes, followed by 'North Star', 'Tehama', 'Two Star', and 'RSX743' that performed statistically the same. Among these genotypes, only 'RSX743' has been cultivated in Florida (Fig. 4S). 'Valmaine' and 'C1145' had significantly higher HW than commercial romaine cultivars Hialeah or Manatee (Fig. 5S).

In addition, there were important experiment dependent differences as noted by the significant interactions in G×E and R×E (Table 3). For instance, the HW of specific genotypes was higher when lettuce was fertilized with half-P rate, depending on the experiment. Butterhead breeding lines 60176 and B1196 had higher HW in experiment 1 but breeding lines 18076, 50111, 70882 and B1196 had higher HW in experiment 2 when fertilized with half-P (Table 1S). In experiment 1, crisphead lettuce lines and cultivars 60167, 60172, Bubba, Chosen, H1078, and Sun Devil in experiment 1, and only Honcho II in experiment 2 had higher HW in half-P rate (Table 1S). In Latin lettuce, a higher HW was observed in the half-P rate for cultivars Little Gem in experiment 1 and Floribibb in experiments 1 and 2 (Table 1S). Two loose leaf genotypes, PI 358001-1 and RSX743 in experiment 1, and three cultivars Cordoba, North Star and Revolution in experiment 2, presented a higher HW under half-P rate (Table 1S). Ten romaine cultivars and breeding lines had a higher HW in the half-P rate in the first experiment but only PI 278108 had a higher HW under half-P rate in the second experiment (Table 1S).

Related traits

Lettuce fertilized with the two distinct P rates resulted in significant differences in the percentage of marketable heads for butterhead (*P=0.0223*), crisphead (*P<0.0001*), Latin (*P=0.0128*), and romaine (*P<0.0001*), but not for loose leaf lettuce (*P=0.1490*) (Table 3). The butterhead genotypes 70882 and PI 342440; crisphead cultivars Cibola, Flagler, and Honcho II; loose leaf PI 278109 and PI 358001-1 and cultivars North Star, Cordoba, Red Rage, and

Tehama; romaine cultivar Terrapin; and Latin breeding line 49530 presented equal or higher number of marketable heads in the half-P rate than in the standard P rate (Table 4).

In the first experiment, tipburn incidence was observed in the butterhead PI 342440 planted in both, standard and half-P rates (1.7 and 2.3%, respectively). In experiment 2, the butterhead breeding line B1196 had <1% tipburn when grown in the recommended P rate. All other genotypes did not present tipburn symptoms (data not shown).

Head weight and its relationship with P concentration

No significant correlations between HW and TP concentration in lettuce tissue were identified in the standard and half-P rates (Tables 5 and 6). Total P concentration in tissue differed significantly ($P<0.05$) among genotypes within all lettuce types tested, except for Latin type ($P=0.9325$) (Table 3). Tissue TP concentration on butterhead, crisphead, and Latin types was not significantly ($P>0.05$) affected by the two P rates (Table 3). The average tissue TP concentration of romaine and loose leaf genotypes was significantly ($P<0.05$) higher when lettuce was grown in the half-P rate, whereas the opposite was true for the only oilseed line tested (Table 4).

In addition, HW was not correlated with soil TP in any of the two P rates used (Tables 5 and 6). In this study, the soil TP concentration was estimated for all genotypes, but no differences were observed ($P>0.05$) for any of the lettuce types tested (Table 3). A significant effect of P rates was observed on soil TP concentration only in the overall analysis ($P=0.0001$) and within the loose leaf type ($P=0.0065$) (Table 3). Furthermore, positive significant correlations were observed between soil TP and soil extractable P (M3P), both in standard P rate ($r=0.85$; $P<0.0001$) (Table 5) as well as for the half-P rate ($r=0.66$; $P<0.0001$) (Table 6), indicating that the increase in soil TP of Histosols resulted in higher availability of P to plants.

A slightly significant correlation between HW and M3P was detected when lettuce was fertilized with both, half and standard P rates (Tables 5 and 6). However, significant differences for soil M3P were only detected within the butterhead type ($P=0.0484$) (Table 3). Variation was also observed for M3P among P rates in the overall analysis and within all lettuce types ($P<0.05$), except for oilseed lettuce ($P=0.1885$) (Table 3), indicating that the application of different P rates influenced the availability of extractable P in the soil.

Discussion

Phosphorus-efficient lettuce genotypes were identified in this research across different types. Genetic variation for PUE has been previously identified in lettuce (Buso and Bliss 1988; Bertossi et al. 2013), and in related species including sunflower and safflower in the Asteraceae (Compositae) family (Abbadi and Gerendás 2015). Many of the previous studies on PUE were conducted in greenhouses and in other cropping systems different to the one in this research. In *L. sativa*, genetic variability exists for nitrogen (N) use efficiency (Macias-González et al. 2021) and this species reacts differently to the deprivation of P, N, or potassium (K) (Hoque et al. 2010; Simko, 2020). Findings from the present study indicate the possibility to improve lettuce cultivars for PUE.

This study was performed in organic soils of the EAA; these soils have a significant amount of non-available P to plants, mainly due to their high pH, which in turn, is associated with a phenomenon known as soil subsidence (Bhadha et al. 2020). Lettuce grown under half of recommended P rate generally produced less HW as expected, but in occasions, HW was slightly higher when planted with less P, indicating that lettuce was capable of utilizing P more efficiently and/or acquiring the naturally present P in Histosols in the EAA. The mechanism of utilizing P more efficiently in lettuce cannot be confirmed with data generated from this research but perhaps specific lettuce lines have a higher P utilization efficiency and greater yield per unit of absorbed P due to the reallocation of P from shoot to root tissues as was found in cultivars of *Brassica* spp. (Akhtar et al. 2007). P-efficient lettuce genotypes detected in this study might present higher soil exploration capacity that contributes to an improved P acquisition by the plants. Soil exploration capacity is directly related to root architecture and distribution, which in turn, have been found to be critical for lettuce to uptake N and water when these two resources were limited (Johnson et al. 2000; Macias-González et al. 2021). Perhaps, some lettuce genotypes can access some of the P present in the insoluble complexes formed with Ca and Mg (Gruen et al. 2014), including calcium phosphate and magnesium phosphate, through the root exudation of organic compounds such as acid phosphatase enzymes, as observed in barley and rice genotypes (Gao et al. 2020; Nirubana et al. 2020). Furthermore, P solubilization and uptake by plants might be facilitated via symbiotic associations with soil microorganisms such as mycorrhizal fungi and plant-growth promoting bacteria, which were found to improve soil P availability in the rhizosphere of maize (Oliveira et al. 2009). Such symbiotic associations remain yet to be identified in lettuce.

Although correlations in HW between standard and half-P rates were high and positive, as expected, significant genotype × experiment and rate × experiment interactions identified in this research warrant a deeper analysis of the environmental factors influencing P uptake. In rice, environmental interactions were believed to be an important component of the phenotypic variation in NUE (van de Wiel et al. 2016, Vandamme et al. 2016). Therefore, PUE in *L. sativa* may not be different since N use efficiency of lettuce was found to be influenced by environmental factors including soil temperature (Macias-González et al. 2021). Phosphorus uptake could be influenced by the P application method, the soil pH, temperature and light, water availability, and biotic stressors as weeds (Gruen et al. 2014; Reich et al. 2014; Fageria et al. 2017). In this research, P was applied in bands as recommended to optimize P uptake by plants and presumably did not impact P availability to plants. Soil pH was 7.6 in the first experimental site, compared to 7.1 in the second experimental site. Although the optimum soil pH for lettuce is 6.5, the overall HW of most lettuce types was slightly higher in the first experiment, indicating that a higher soil pH did not directly impact the lettuce productivity in this study. The average soil temperature (at -10 cm) and above-ground temperature (at 60 cm) were similar in the two experiments (Table 2), whereas the average solar radiation was higher during the first experiment than in the second experiment (Table 2). The solar radiation might have contributed to the significant genotype × experiment and rate × experiment interactions observed in this study, since higher solar radiation was found to be associated with higher yields and P uptake in soybean (Zhou et al. 2019). Despite the higher precipitation observed in the second experiment, both experiments were overhead irrigated during periods of rainfall absence to provide adequate water availability to the plants. Thus, water availability was unlikely a limiting factor for P uptake in these experiments. Weeds in the Histosols of the EAA are a nuisance to vegetable production, due to the limited number of approved herbicides and the interaction of herbicides with the high organic matter concentration in muck soils that reduces their efficacy (Odero and Wright 2013); the higher presence of weeds in these trials likely resulted in competition for nutrients and may have diminished P uptake in lettuce, causing these interactions to be significant.

Applying less P-based fertilizers in lettuce led to a decrease in plant size, and consequently, less marketable heads. Lettuce plants more sensitive to P deprivation were found to produce a smaller number of leaves, leading to the absence of head formation on crisphead, romaine and butterhead types. Other unmeasured P-deficiency symptoms, such as foliage chlorosis and necrotic spotting on outer leaves were observed during the execution of these experiments, primarily in the half-P rate. However, neither tipburn (a physiological disorder) nor other biotic stresses were noticeable during the execution of these experiments. In contrast, some genotypes produced satisfactory yield when grown in half of the recommended P rate. Thus, P rates lower than the current recommendation could provide a similar or better crop while having less impact on crop production and the environment. The adequate reduction for P-efficient lettuce needs to be investigated without sacrificing HW and other important characteristics to the industry.

In the present study, only weak correlations between HW and tissue P concentration were observed. Two main mechanisms can contribute to a higher PUE in plants: (1) higher P uptake efficiency and (2) higher internal P utilization (Vandamme et al. 2016). In this research, we hypothesize that most lettuce genotypes had either a high P uptake and poor P utilization (not able to convert absorbed P into biomass), or poor P uptake and high P utilization. Both hypotheses could possibly explain the weak correlations observed between HW and tissue P. Therefore, tissue TP might not be an appropriate trait for discriminating P-efficient lettuce genotypes; rather, evaluating lettuce for PUE based on HW would be more reliable. Similar observations have been reported in a screening of wheat genotypes for PUE, in which no correlation between shoot dry weight and tissue P was detected (Ozturk et al. 2005).

The variation in tissue P concentration observed in this study is probably due to the complex dynamics involved in P uptake and P internal utilization of different lettuce genotypes under P stress. For instance, P uptake and P utilization have been found to be independent, weakly correlated mechanisms in maize planted in low and high P (Parentoni and Souza Júnior 2008) and in *C. arabica* and *C. canephora* cultivars (Neto et al. 2016), indicating independent mechanisms of action for each trait. In lettuce, more comprehensive investigations are required to determine and quantify the mechanisms involved in PUE, especially when it comes to absorption and utilization.

Conclusions

In conclusion, P-efficient lettuce was identified within butterhead, crisphead, loose leaf, and Latin types in this research. In general, applying half of the recommended P rate decreased HW and marketability of lettuce, however, genotypes 60176, Honcho II, Cibola, Sun Devil, North Star, RSX743, PI 358001-1, and PI 278109 were capable of producing an acceptable HW with less impact on marketability. The weak correlations detected between HW and tissue TP concentration may denote the complex mechanisms, such as P uptake and P utilization, controlling PUE in lettuce. The evaluation of lettuce genotypes for PUE based on HW, rather than tissue TP, was found to be a more appropriate approach. Improving P-efficient lettuce is a possibility but the genetics of yield and related traits under P depravation must be further investigated. Furthermore, the assessment of plant-microbe interactions in P-deprived conditions might help elucidate additional aid mechanisms involved in uptake and utilization of P in lettuce.

Declarations

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Conflicts of interest (include appropriate disclosures)

The authors declare no conflict of interest.

Availability of data and material (data transparency)

Not applicable

Code availability (software application or custom code)

Not applicable

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References

Abbadi J, Gerendás J (2015) Phosphorus use efficiency of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.). J Plant Nutr 38(7):1121-1142. https://doi.org/10.1080/01904167.2014.983115

Akhtar MS, Oki Y, Adachi T, Murata Y, Khan MHR (2007) Relative phosphorus utilization efficiency, growth response, and phosphorus uptake kinetics of brassica cultivars under a phosphorus stress environment. Commun in Soil Sci Plant Anal 38:1061-1085. https://doi.org/10.1080/00103620701280266

Ali J, Jewel ZA, Mahender A, Anandan A, Hernandez J, Li Z (2018) Molecular genetics and breeding for nutrient use efficiency in rice. Int J Mol Sci 19:1762. https://dx.doi.org/10.3390%2Fijms19061762

Baligar VC, Fageria NK (2015) Nutrient use efficiency in plants: an overview. In: Rakshit A, Singh HB, Sen A (eds), Nutrient use efficiency: from basics to advances. New Delhi, Springer, pp 1-14. https://doi.org/10.1007/978-81-322-2169-2

Baligar VC, Fageria NK, He ZL (2001) Nutrient use efficiency in plants. Commun Soil Sci Plant Anal 32(7-8):921-950. https://doi.org/10.1081/CSS-100104098

Bertossi APA, Thomazini A, Fonseca AS, Amaral JFT (2013) Nutritional efficiency of phosphorus in lettuce. J Agric Sci 5(8):125-131. http://dx.doi.org/10.5539/jas.v5n8p125

Bhadha JH, Harris WG, Jawitz JW (2010) Soil phosphorus release and storage capacity from an impacted subtropical wetland. Soil Sci Soc Am J 74:1816-1825. https://doi.org/10.2136/sssaj2010.0063

Bhadha JH, Wright AL, Snyder GH (2020) Everglades Agricultural Area soil subsidence and sustainability. University of Florida IFAS EDIS Publication. SL311. https://edis.ifas.ufl.edu/ss523

Buso GSC, Bliss FA (1988) Variability among lettuce cultivars grown at two levels of available phosphorus. Plant Soil 111:67-73.

Chietera G, Chardon F (2014) Natural variation as a tool to investigate nutrient use efficiency in plants. In: Hawkesford MJ, Kopriva S, De Kok LJ (eds) Nutrient use efficiency in plants, Plant Ecophysiology, vol 10. Springer, Cham., pp 29-50. http://doi-org-443.webvpn.fjmu.edu.cn/10.1007/978-3-319-10635-9_2

Daoui K, Karrou M, Mrabet R, Fatemi Z, Draye X, Ledent JF (2011) Genotypic variation of phosphorus use efficiency among Moroccan faba bean varieties (*Vicia faba* major) under rainfed conditions. J Plant Nutr 35:34-48. https://doi.org/10.1080/01904167.2012.631665

Fageria NK, Baligar VC, Li YC (2008) The role of nutrient efficient plants in improving crop yields in the twenty first century. J Plant Nutr 31(6):1121-1157. https://doi.org/10.1080/01904160802116068

Fageria NK, He Z, Baligar VC (2017) Phosphorus management in crop production. CRC Press, Boca Raton. https://doi.org/10.1201/9781315162096

Gao S, Xia J, Yuan S, Shen Y, Zhong X, Zhang S, Li Y, Hu D, Zeng J, Lan T, Liu Y, Chen G (2020) Novel QTL conferring phosphorus acquisition and utilization efficiencies in barley. Front Genet 11:580452. https://doi.org/10.3389/fgene.2020.580452

Gruda N, Tanny J (2014) Protected Crops. In: Dixon GR, Aldous DE (eds) Horticulture: plants for people and places, Production horticulture, vol 1. Springer Netherlands, Dordrecht, pp 327-405. https://doi.org/10.1007/978-94-017-8578-5_10

Gruen A, Broadley MR, Buchner P, Hawkesford MJ (2014) Efficient mineral nutrition: genetic improvement of phosphate uptake and use efficiency in crops. In: Hawkesford MJ, Kopriva S, De Kok LJ (eds) Nutrient use efficiency in plants, Plant Ecophysiology, vol 10. Springer, Cham., pp 93-132. https://doi.org/10.1007/978-3-319-10635-9_4

Horst WJ, Abdou M, Wiesler F (1993) Genotypic differences in phosphorus efficiency in wheat. Plant Soil 155/156:293-296. https://doi.org/10.1007/BF00025040

Hoque MM, Ajwa H, Othman M, Smith R, Cahn M (2010) Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers. HortScience 45(10):1539-1544. https://doi.org/10.21273/HORTSCI.45.10.1539

Jia Y, Yang X, Feng Y, Jilani G (2008) Differential response of root morphology to potassium deficient stress among rice genotypes varying in potassium efficiency. J Zhejiang Univ Sci B 9(5):427-434. https://dx.doi.org/10.1631%2Fjzus.B0710636

Johnson WC, Jackson LE, Ochoa O, van Wijk R, Peleman J, St Clair DA, Michelmore RW (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploration. Theor Appl Genet 101:1066-1073. https://doi.org/10.1007/s001220051581

Kanissery R, Raid, RN, Desaeger J, Noling JW, Beuzelin J, Miller CF (2020) Leafy vegetable production. In: Dittmar P, Freeman J, Paret M, Smith H (eds) Vegetable production handbook of Florida, 2020-2021. University of Florida/IFAS Extension, Gainesville, FL.

Kanter DR, Zhang X, Mauzerall DL (2015) Reducing nitrogen pollution while decreasing farmers' costs and increasing fertilizer industry profits. J Environ Qual 44:325-335. https://doi.org/10.2134/jeq2014.04.0173

Kellermeier F, Chardon F, Amtmann A (2013) Natural variation of Arabidopsis root architecture reveals complementing adaptive strategies to potassium starvation. Plant Physiol 161:1421-1432. https://doi.org/10.1104/pp.112.211144

Macias-González M, Truco MJ, Smith R, Cahn M, Simko I, Hayes R, Michelmore RW (2021) Genetics of robustness under nitrogen- and water-deficient conditions in field-grown lettuce. Crop Sci 1-38. https://doi.org/10.1002/csc2.20380

Maqsood MA, Hussain S, Aziz T, Ashraf M (2013) Sustainable agriculture through integrated soil fertility management on degraded lands. In: Shahid SA, Abdelfattah MA, Taha FK (eds) Developments in soil salinity assessment and reclamation: Innovative thinking and use of marginal soil and water resources in irrigated agriculture. Springer Science & Business Media, Dordrecht, pp 759-768. https://doi.org/10.1007/978-94-007-5684-7

McDonald G, Wheeler R, Porker K, Bovill B, McKay A, Smith A (2010) Phosphorus use efficiency in wheat and barley. The Regional Institute (online publishing). In: H. Dove, and R. Culvenor (eds.), Food security from sustainable agriculture. Proc. 15th Australian Agron Conf, New Zealand.

Mehlich A (1984) Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Comm. Soil Sci. Plant Analysis 15(12):1409-1416. https://doi.org/10.1080/00103628409367568

Moura WM, Cardoso PMR, Gravina GA, Cavalcante VS, Pedrosa AW, Ferreira TC (2019) Variability in the production traits and nutritional efficiency of coffee cultivars when submitted to low soil nitrogen. Genet Mol Res 18(2):gmr18175. http://dx.doi.org/10.4238/gmr18175

Neto AP, Favarin JL, Hammond JP, Tezotto T, Couto HTZ (2016) Analysis of phosphorus use efficiency traits in *Coffea* genotypes reveals *Coffea arabica* and *Coffea canephora* have contrasting phosphorus uptake and utilization efficiencies. Front Plant Sci 7:408. https://doi.org/10.3389/fpls.2016.00408

Nirubana V, Vanniarajan C, Aananthi N, Ramalingam J (2020) Screening tolerance to phosphorus starvation and haplotype analysis using phosphorus uptake (*Pup1*) QTL linked markers in rice genotypes. Physiol Mol Biol Plants 26:2355-2369. https://doi.org/10.1007/s12298-020-00903-1

Odero DC, Wright AL (2013a) Phosphorus application influences the critical period of weed control in lettuce. Weed Sci 61:410-414. https://doi.org/10.1614/WS-D-12-00107.1

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimarães CT, Schaffert RE, Sá NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem 41:1782-1787. https://doi.org/10.1016/j.soilbio.2008.01.012

Ortiz-Monasterio JI, Manske GGB, van Ginkel M (2001) Nitrogen and phosphorus use efficiency. In: Reynolds MP, Ortiz-Monasterio JI, McNab A (eds), Application of physiology in wheat breeding. Mexico, DF, CIMMYT, pp 200-207.

Ozturk L, Eker S, Torun B, Cakmak I (2005) Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. Plant Soil 269:69-80. https://doi.org/10.1007/s11104-004-0469-z

Parentoni SN, Souza Júnior CL (2008) Phosphorus acquisition and internal utilization efficiency in tropical maize genotypes. Pesq Agropec Bras 43(7):893-901. https://doi.org/10.1590/S0100-204X2008000700014

Pereira-Carvajal M, Valle S, Carrasco J, Balocchi O, Pinochet D (2016) Response of naturalized genotypes of white clover to low phosphorus condition in Andisols. Agro Sur 44(3):77-87. https://doi.org/10.4206/agrosur.2016.v44n3-08

Reich M, Aghajanzadeh T, De Kok LJ (2014) Physiological basis of plant nutrient use efficiency – concepts, opportunities and challenges for its improvement. In: Hawkesford MJ, Kopriva S, De Kok LJ (eds) Nutrient use efficiency in plants, Plant Ecophysiology, vol 10. Springer, Cham., pp 1-27. https://doi.org/10.1007/978-3-319-10635-9_1

Salehi SY, Hajiboland R (2008) A high internal phosphorus use efficiency in tea (*Camellia sinensis* L.) plants. Asian J Plant Sci 7(1):30-36. https://dx.doi.org/10.3923/ajps.2008.30.36

Simko I (2020) Genetic variation in response to N, P, or K deprivation in baby leaf lettuce. Horticulturae 6(1):15. https://doi.org/10.3390/horticulturae6010015

USDA NASS (2019) Quick Stats Dep Agric Natl Agric Stat Serv Available at https://quickstats.nass.usda.gov/. Accessed 13 Oct 2020

Vandamme E, Rose T, Saito K, Jeong K, Wissuwa M (2016) Integration of P acquisition efficiency, P utilization efficiency and low grain P concentrations into P-efficient rice genotypes for specific target environments. Nutr Cycl Agroecosyst 104:413-427. https://doi.org/10.1007/s10705-015-9716-3

van de Wiel CCM, van der Linden CG, Scholten OE (2016) Improving phosphorus use efficiency in agriculture: opportunities for breeding. Euphytica 207:1-22. https://doi.org/10.1007/s10681-015-1572-3

Walker DJ, Leigh RA, Miller AJ (1996) Potassium homeostasis in vacuolate plant cells. Proc Natl Acad Sci 98:10510-10514. https://dx.doi.org/10.1073%2Fpnas.93.19.10510

Wang QR, Li JY, Li ZS, Christie P (2005) Screening Chinese wheat germplasm for phosphorus efficiency in calcareous soils. J Plant Nutr 28:489-505. https://doi.org/10.1081/PLN-200049186

Wu W, Ma B (2015) Integrated nutrient management (INM) for sustaining crop productivity and reducing environmental impact: a review. Sci Total Environ 512-513:415-427. https://doi.org/10.1016/j.scitotenv.2014.12.101

Yan X, Wu P, Ling H, Xu G, Xu F, Zhang Q (2006) Plant nutriomics in China: an overview. Ann Bot 98:473-482. https://dx.doi.org/10.1093%2Faob%2Fmcl116

Yu P, White PJ, Hochholdinger F, Li C (2014) Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. Planta 240:667-678. https://doi.org/10.1007/s00425-014-2150-y

Zhou T, Wang L, Yang H, Gao Y, Liu W, Yang W (2019) Ameliorated light conditions increase the P uptake capability of soybean in a relay-strip intercropping system by altering root morphology and physiology in the areas with low solar radiation. Sci Total Environ 688:1069-1080. https://doi.org/10.1016/j.scitotenv.2019.06.344

Tables

Table 1 Lettuce accessions utilized in the two screening experiments for phosphorus use efficiency, morphological types, and plant introduction numbers

Genotype	Type	PI number[1]	Breeder
60158	Crisphead	N/A	UF/IFAS
60162	Crisphead	N/A	UF/IFAS
60167	Crisphead	N/A	UF/IFAS
60172	Crisphead	N/A	UF/IFAS
Beacon	Crisphead	PI 604232	Nunhems B.V.
Bubba	Crisphead	PI 601978	Seminis Vegetable Seeds, Inc.
Chosen	Crisphead	N/A	3 Star Lettuce, LLC
Cibola	Crisphead	N/A	Paragon Seed, Inc.
Cooper[3]	Crisphead	PI 661094	3 Star Lettuce, LLC
Coyote	Crisphead	PI 631465	Seminis Vegetable Seeds, Inc.
Eblin[2]	Crisphead	N/A	Unknown
Flagler[3]	Crisphead	N/A	3 Star Lettuce, LLC
Green Lightning	Crisphead	PI 599597	Progeny Advanced Genetics, Inc.
H1078	Crisphead	N/A	UF/IFAS
Honcho II	Crisphead	PI 601591	Seminis Vegetable Seeds, Inc.
Javelina	Crisphead	PI 631464	Seminis Vegetable Seeds, Inc.
Lantana	Crisphead	PI 658143	3 Star Lettuce, LLC
Reine des Glaces	Crisphead	PI 634668	Vilmorin, S.A.
Sun Devil	Crisphead	PI 603974	Progeny Advanced Genetics, Inc.
50098	Romaine	N/A	UF/IFAS
50100	Romaine	N/A	UF/IFAS
60182	Romaine	N/A	UF/IFAS
60183	Romaine	N/A	UF/IFAS
60184	Romaine	N/A	UF/IFAS
70096	Romaine	N/A	UF/IFAS
C1145	Romaine	N/A	UF/IFAS
Floricos 83	Romaine	N/A	UF/IFAS
Green Towers	Romaine	PI 601336	Harris Moran Seed Company
Hialeah	Romaine	N/A	3 Star Lettuce, LLC
King Henry	Romaine	PI 595620	Progeny Advanced Genetics, Inc.
Manatee	Romaine	PI 641790	3 Star Lettuce, LLC
Okeechobee	Romaine	PI 658142	3 Star Lettuce, LLC
46	Romaine	PI 278108	N/A
PIC	Romaine	N/A	Unknown
Tall Guzmaine	Romaine	PI 665208	UF/IFAS
Terrapin	Romaine	PI 614861	UF/IFAS
Valmaine	Romaine	PI 543959	UF/IFAS
18076	Butterhead	N/A	UF/IFAS
50111	Butterhead	N/A	UF/IFAS
60173	Butterhead	N/A	UF/IFAS
60174	Butterhead	N/A	UF/IFAS

60176	Butterhead	N/A	UF/IFAS
60179[2]	Butterhead	N/A	UF/IFAS
70202	Butterhead	N/A	UF/IFAS
70882	Butterhead	N/A	UF/IFAS
B1190	Butterhead	N/A	UF/IFAS
B1196	Butterhead	N/A	UF/IFAS
Odyssey	Butterhead	N/A	Unknown
66043	Butterhead	PI 342440	N/A
Bambino	Loose leaf	N/A	Unknown
Cordoba	Loose leaf	PI 595839	Seminis Vegetable Seeds, Inc.
Galactic	Loose leaf	N/A	Johnny's Selected Seeds
North Star	Loose leaf	PI 612155	Nunhems B.V.
47	Loose leaf	PI 278109	N/A
Strumicka	Loose leaf	PI 358001-1	N/A
Red Rage	Loose leaf	PI 603972	Pybas, Inc. and Douglas Peters
Revolution	Loose leaf	W6 38949	Unknown
RFX-0901	Loose leaf	N/A	Unknown
RSX743	Loose leaf	N/A	3 Star Lettuce, LLC
Tehama	Loose leaf	PI 632457	Nunhems B.V.
Two Star	Loose leaf	PI 562631	Orsetti Seed Company, Inc.
49530	Latin	N/A	UF/IFAS
Floribibb	Latin	N/A	UF/IFAS
Little Gem	Latin	PI 617959	Vilmorin, S.A.
Pavane	Latin	PI 667705	Unknown
N/A	Oilseed	PI 251246	N/A

[1] Plant introduction number obtained from U.S. Department of Agriculture, National Plant Germplasm System (USDA-NPGS at https://npgsweb.ars-grin.gov/gringlobal/search).

[2] Lettuce accessions Eblin and 60179 were utilized only in the first experiment

[3] Lettuce accessions Flagler and Cooper were utilized only in the second experiment.

Table 2 Total precipitation, average soil temperature (at -10 cm), average above-ground temperature (at 60 cm), average solar radiation soil pH, and nutrient recommendations prior to planting for each of the two experimental sites used to screen lettuce genotypes for phosphorus use efficiency

Year	Total precipitation (mm)[1]	Average soil temperature (ºC) [1]	Average above-ground temperature (ºC) [1]	Average solar radiation (W/m^2) [1]	Soil pH	Nutrient recommendation prior to planting ($kg{\cdot}ha^{-1}$)[2]			
						N	P_2O_5	K_2O	Mg
2017-18	69.08	20.5	18.6	150.59	7.6	0	215	112	0
2019-20	102.36	20.9	19.3	129.08	7.1	0	187	67	0

[1] Total precipitation (mm) during the execution of the two experiments in the 2017-18 and 2019-20 seasons. Data collected from the Florida Automated Weather Network station, located in the Everglades Research and Education Center, in Belle Glade, FL.

[2] Recommendations from the UF/IFAS Soil Testing Laboratory at the Everglades Research and Education Center, in Belle Glade, FL.

Table 3 Analysis of variance of head weight (HW) for the 66 lettuce genotypes planted under two phosphorus rates in the 2017-2018 and 2019-2020 seasons

Source of variation	Head weight		Marketability		Tissue Total-P (TP)		Soil Total-P (TP)		Soil Mehlich-3 (M3P)	
	DF	*P*-value	DF	*P*-value	DF	*P*-value	DF	*P*-value	DF	*P*-value
Genotype (G)	65	*<0.0001*	64	*<0.0001*	65	*<0.0001*	65	*0.8828*	65	*0.1758*
Butterhead (BH)	11	*0.0003*	11	*0.0002*	11	*0.0011*	11	*0.6107*	11	*0.0484*
Crisphead (CH)	18	*0.0075*	18	*<0.0001*	18	*<0.0001*	18	*0.7878*	18	*0.7298*
Latin (LA)	3	*0.1573*	3	*0.0025*	3	*0.9325*	3	*0.3520*	3	*0.5533*
Loose leaf (LF)	11	*<0.0001*	11	*<0.0001*	11	*0.0015*	11	*0.4928*	11	*0.2723*
Romaine (RO)	17	*0.0004*	17	*<0.0001*	17	*0.0004*	17	*0.9250*	17	*0.8900*
Rate (R)	1	*<0.0001*	1	*<0.0001*	1	*<0.0001*	1	*0.0001*	1	*<0.0001*
BH	1	*0.0251*	1	*0.0223*	1	*0.0596*	1	*0.0971*	1	*<0.0001*
CH	1	*<0.0001*	1	*<0.0001*	1	*0.3402*	1	*0.0781*	1	*<0.0001*
LA	1	*0.0942*	1	*0.0128*	1	*0.4835*	1	*0.5947*	1	*0.0403*
LF	1	*0.0328*	1	*0.1490*	1	*<0.0001*	1	*0.0065*	1	*<0.0001*
Oilseed (OS)	1	*0.1734*	-	-	1	*0.0117*	1	*0.6289*	1	*0.1885*
RO	1	*<0.0001*	1	*<0.0001*	1	*<0.0001*	1	*0.0724*	1	*<0.0001*
G × R	65	*0.7004*	64	*0.0009*	65	*0.0006*	65	*0.6022*	65	*0.3886*
BH × R	11	*0.9268*	11	*0.6014*	11	*0.4687*	11	*0.6018*	11	*0.0489*
CH × R	18	*0.6679*	18	*0.0124*	17	*0.3092*	18	*0.4053*	18	*0.6817*
LA × R	3	*0.0118*	3	*0.1230*	3	*0.5390*	3	*0.0077*	3	*0.0252*
LF × R	11	*0.8191*	11	*0.5418*	11	*0.0044*	11	*0.9030*	11	*0.8469*
RO × R	17	*0.7006*	17	*0.0365*	17	*0.1215*	17	*0.8781*	17	*0.5639*
Experiment (E)	1	*0.7331*	1	*0.0945*	1	*0.0989*	1	*0.2405*	1	*0.0107*
BH	1	*0.2287*	1	*0.4558*	1	*0.8817*	1	*0.6590*	1	*0.2529*
CH	1	*0.1608*	1	*0.1386*	1	*0.0175*	1	*0.1029*	1	*0.0352*
LA	1	*0.2680*	1	*0.1343*	1	*0.3973*	1	*0.3419*	1	*0.1380*
LF	1	*0.3434*	1	*0.0240*	1	*0.5201*	1	*0.4698*	1	*0.0308*
OS	1	*0.9428*	-	-	1	*0.0659*	1	*0.3622*	1	*0.1661*
RO	1	*0.2946*	1	*0.1399*	1	*0.0682*	1	*0.1667*	1	*0.0295*
G × E	61	*<0.0001*	60	*<0.0001*	61	*<0.0001*	61	*0.7779*	61	*0.4485*
BH × E	10	*0.1626*	10	*0.1254*	10	*0.2458*	10	*0.9656*	10	*0.8531*
CH × E	15	*0.5886*	15	*0.0333*	15	*0.0788*	15	*0.7932*	15	*0.8935*
LA × E	3	*0.1320*	3	*0.1143*	3	*0.6868*	3	*0.4457*	3	*0.1814*
LF × E	11	*<0.0001*	11	*<0.0001*	11	*0.0005*	11	*0.6195*	11	*0.0945*
RO × E	17	*0.0999*	17	*<0.0001*	17	*0.0011*	17	*0.7833*	17	*0.3574*
R × E	1	*<0.0001*	1	*0.0064*	1	*0.0156*	1	*<0.0001*	1	*<0.0001*

BH	1	*0.6107*	1	*0.7163*	1	*0.1715*	1	*0.0021*	1	*0.0715*
CH	1	*0.0016*	1	*0.0217*	1	*0.0023*	1	*<0.0001*	1	*<0.0001*
LA	1	*0.0548*	1	*0.1276*	1	*0.2898*	1	*0.0457*	1	*0.5042*
LF	1	*0.4223*	1	*0.2888*	1	*<0.0001*	1	*<0.0001*	1	*<0.0001*
OS	1	*0.3048*	-	-	1	*0.0658*	1	*0.0809*	1	*0.0360*
RO	1	*0.0004*	1	*0.0037*	1	*0.0346*	1	*<0.0001*	1	*0.0137*
G × R × E	61	*0.3015*	57	*0.0211*	58	*<0.0001*	61	*0.5224*	61	*0.6422*
BH × R × E	10	*0.9758*	9	*0.3460*	10	*0.3389*	10	*0.8039*	10	*0.7469*
CH × R × E	15	*0.0377*	15	*0.7345*	13	*0.1423*	15	*0.8409*	15	*0.8685*
LA × R × E	3	*0.4216*	2	*0.5538*	3	*0.8684*	3	*0.2844*	3	*0.1756*
LF × R × E	11	*0.7224*	11	*0.9624*	11	*0.0011*	11	*0.6788*	11	*0.7744*
RO × R × E	17	*0.7051*	16	*<0.0001*	16	*0.0082*	17	*0.1051*	17	*0.1647*
Covariance Parameters	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error
Subblock (Block)	528	672	19	15	0	-	2712	2462	3	50
Block (Year)	472	760	0	-	96612	75593	0	-	0	-
Residual	6035	397	361	24	793233	56241	117092	7675	7054	463

Table 4 Least Square Means (LSM) of marketability (%), total phosphorus (P) concentration in tissue (mg g^{-1}), total P concentration in soil (mg g^{-1}), and extractable P concentration in soil (Mehlich-3; mg g^{-1}) of the 66 lettuce genotypes planted under two phosphorus rates in the 2017-2018 and 2019-2020 seasons

Genotype	Marketability (%)		Tissue Total-P (mg g^{-1})		Soil Total-P (mg g^{-1})		Soil Mehlich-3 (mg g^{-1})	
	Half P	Standard P	Half P	Standard P	Half P	Standard P	Half P	Standard P
Butterhead								
18076	5	28	6.2	5.8	1.7	2.1	0.10	0.19
50111	56	62	6.5	6.3	1.9	2.0	0.11	0.18
60173	33	46	7.1	6.8	2.0	2.0	0.18	0.20
60174	35	43	6.3	6.8	1.8	1.8	0.13	0.16
60176	45	60	7.2	6.8	2.0	1.9	0.12	0.15
60179[1]	20	24	7.1	6.9	1.8	1.9	0.10	0.19
70202	33	58	8.3	6.8	2.0	1.9	0.16	0.18
70882	52	44	6.3	6.0	1.9	2.5	0.13	0.39
B1190	43	55	6.6	7.1	1.9	2.1	0.15	0.21
B1196	37	61	5.5	5.7	2.0	2.0	0.15	0.15
Odyssey	60	76	6.8	6.1	1.9	2.0	0.12	0.16
PI 342440	49	37	9.1	7.1	1.9	1.9	0.13	0.17
Average	39	49	6.9	6.5	1.9	2.0	0.13	0.19
Crisphead								
60158	38	49	6.1	6.8	1.9	2.2	0.14	0.26
60162	14	44	5.4	5.3	2.0	1.9	0.14	0.23
60167	28	29	5.2	5.5	2.1	2.2	0.17	0.27
60172	28	30	5.9	5.9	2.0	2.1	0.15	0.19
Beacon	38	63	6.8	6.1	2.0	2.2	0.18	0.25
Bubba	25	41	6.9	6.7	2.0	2.1	0.18	0.23
Chosen	67	69	6.0	5.8	2.0	2.1	0.14	0.20
Cibola	23	21	6.6	6.6	2.0	2.4	0.17	0.29
Cooper[1]	6	43	-	7.2	2.3	2.2	0.34	0.27
Coyote	21	28	6.5	6.6	1.9	2.0	0.15	0.23
Eblin[1]	0	1	5.5	5.8	2.3	1.6	0.16	0.11
Flagler[1]	57	54	6.3	6.6	1.7	3.0	0.13	0.43
Green Lightning	16	38	6.5	7.0	1.9	1.9	0.14	0.24
H1078	39	44	5.8	7.2	1.9	1.9	0.17	0.20
Honcho II	33	11	6.7	5.8	1.9	2.1	0.16	0.16
Javelina	29	68	6.6	6.5	1.7	2.1	0.12	0.21
Lantana	34	60	6.1	6.5	2.1	2.1	0.16	0.25
Reine des Glaces	21	50	6.6	6.6	2.1	1.9	0.16	0.20
Sun Devil	29	36	5.0	5.7	2.0	1.9	0.15	0.20
Average	29	41	6.1	6.3	2.0	2.1	0.16	0.23
Latin								
49530	52	49	6.6	7.1	1.9	2.5	0.13	0.29
Floribibb	55	68	6.4	6.8	2.1	2.0	0.17	0.19

Little Gem	34	52	6.5	7.1	2.3	2.0	0.19	0.18
Pavane	7	37	7.1	6.6	1.9	2.0	0.13	0.20
Average	37	52	6.7	6.9	2.1	2.1	0.16	0.22
Loose leaf								
Bambino	48	70	7.5	5.3	1.9	2.2	0.15	0.20
Cordoba	56	56	7.0	4.8	2.0	2.1	0.13	0.20
Galactic	42	61	6.6	4.9	2.0	2.3	0.13	0.27
North Star	80	77	7.3	5.9	1.9	2.2	0.14	0.26
PI 278109	69	61	7.6	5.8	1.9	2.2	0.11	0.20
PI 358001-1	23	16	6.3	6.3	1.9	1.9	0.12	0.21
Red Rage	63	54	5.4	6.0	1.8	2.0	0.09	0.17
Revolution	49	62	6.8	5.7	1.9	1.9	0.12	0.14
RFX-0901	60	78	7.1	6.9	2.1	2.1	0.15	0.23
RSX743	57	68	6.0	6.1	2.0	2.1	0.14	0.20
Tehama	70	67	5.8	5.2	1.9	1.9	0.12	0.17
Two Star	63	71	6.8	7.5	2.0	2.0	0.12	0.16
Average	57	62	6.7	5.9	1.9	2.1	0.13	0.20
Oilseed								
PI 251246[2]	-	-	5.4	6.0	2.1	2.0	0.13	0.14
Romaine								
50098	22	57	6.1	6.3	1.9	2.0	0.14	0.18
50100	35	63	6.6	5.8	2.1	2.2	0.18	0.25
60182	21	36	7.4	6.4	2.0	2.4	0.15	0.32
60183	27	51	6.9	6.2	2.0	1.9	0.20	0.20
60184	17	58	6.6	6.6	2.1	2.0	0.21	0.21
70096	25	72	8.0	6.1	1.9	2.1	0.13	0.20
C1145	37	53	7.6	6.8	2.0	2.1	0.16	0.20
Floricos 83	25	59	7.7	7.0	2.0	1.9	0.17	0.15
Green Towers	27	41	6.7	7.3	1.8	2.2	0.17	0.24
Hialeah	21	50	7.5	6.4	2.0	2.1	0.14	0.23
King Henry	0	5	7.0	6.5	2.0	2.0	0.17	0.20
Manatee	49	71	8.3	7.4	2.0	2.0	0.15	0.22
Okeechobee	17	59	7.3	7.6	2.0	2.3	0.15	0.24
PI 278108	13	15	7.2	7.1	1.9	2.2	0.13	0.20
PIC	13	34	7.2	7.1	2.0	2.1	0.19	0.17
Tall Guzmaine	24	36	7.4	6.7	2.2	2.2	0.17	0.22
Terrapin	52	46	7.0	6.8	2.1	2.1	0.17	0.21
Valmaine[3]	53	83	5.9	5.4	2.2	2.2	0.15	0.12
Average	27	49	7.1	6.6	2.0	2.1	0.16	0.21

[1] Lettuce genotypes Eblin and 60179 were utilized in the 2017-2018 experiment only. Lettuce genotypes Flagler and Cooper were utilized in the 2019-2020 experiment only.

[2] PI 251246 is a primitive lettuce accession that does not produce marketable heads.

[3] Plots of cultivar Valmaine were discarded due to seed contamination in 2019-2020.

Table 5 Correlations and P-values, in parentheses, between head weight, marketability, tissue total-P, soil total-P, and soil Mehlich-3 of the 66 lettuce genotypes grown in the standard P rate

Trait	Head weight	Marketability	Tissue Total-P	Soil Total-P	Soil Mehlich-3
Head weight	1	-0.02	0.19	0.09	0.31
	(-)	(0.8691)	(0.1307)	(0.4467)	(0.0111)
Marketability		1	-0.07	0.11	0.01
		(-)	(0.5858)	(0.3831)	(0.9423)
Tissue Total-P			1	-0.01	0.07
			(-)	(0.9838)	(0.5967)
Soil Total-P				1	0.85
				(-)	(<0.0001)
Soil Mehlich-3					1
					(-)

Table 6 Correlations and P-values, in parentheses, between head weight, marketability, tissue total-P, soil total-P, and soil Mehlich-3 of the 66 lettuce genotypes grown in the half-P rate

Trait	Head weight	Marketability	Tissue Total-P	Soil Total-P	Soil Mehlich-3
Head weight	1	-0.04	-0.07	0.13	0.28
	(-)	(0.7357)	(0.5902)	(0.3013)	(0.0248)
Marketability		1	0.04	-0.24	-0.38
		(-)	(0.7804)	(0.0506)	(0.0020)
Tissue Total-P			1	-0.04	0.05
			(-)	(0.7267)	(0.6796)
Soil Total-P				1	0.66
				(-)	(<0.0001)
Soil Mehlich-3					1
					(-)

Figures

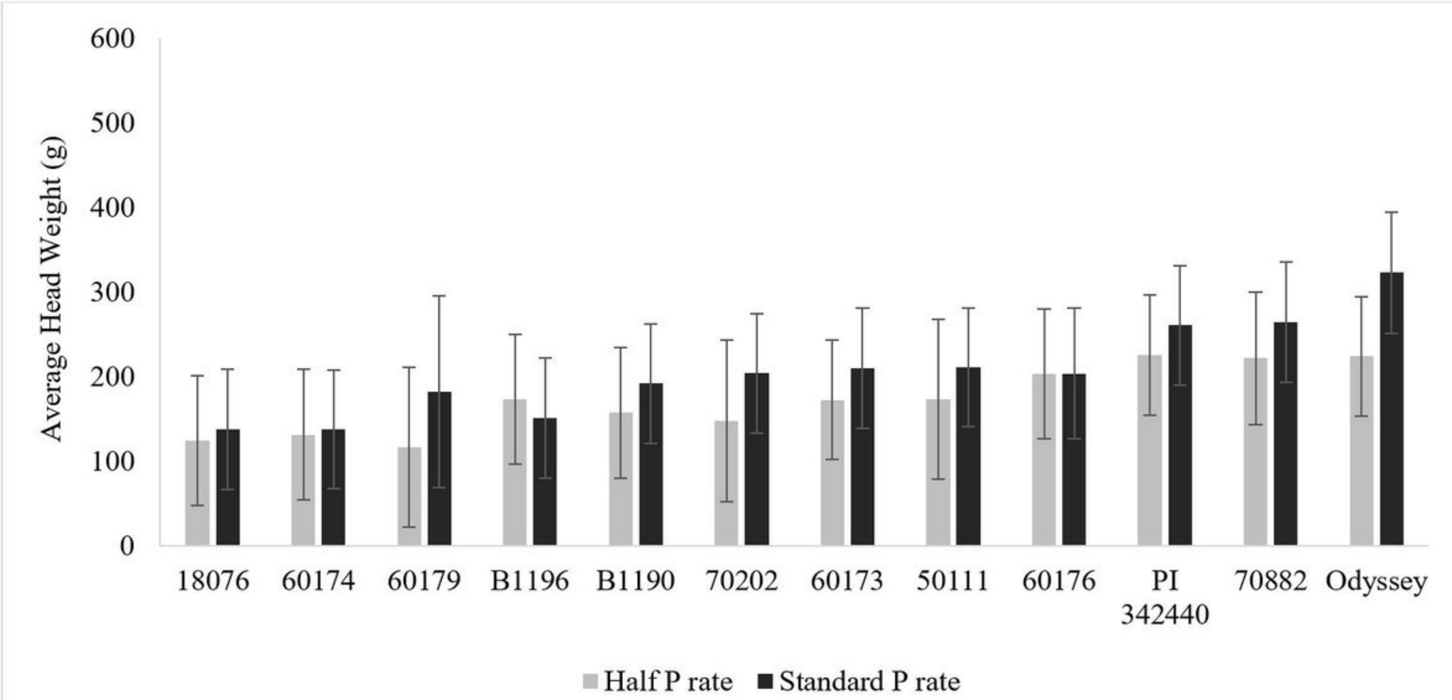

Figure 1

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 12 butterhead genotypes in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

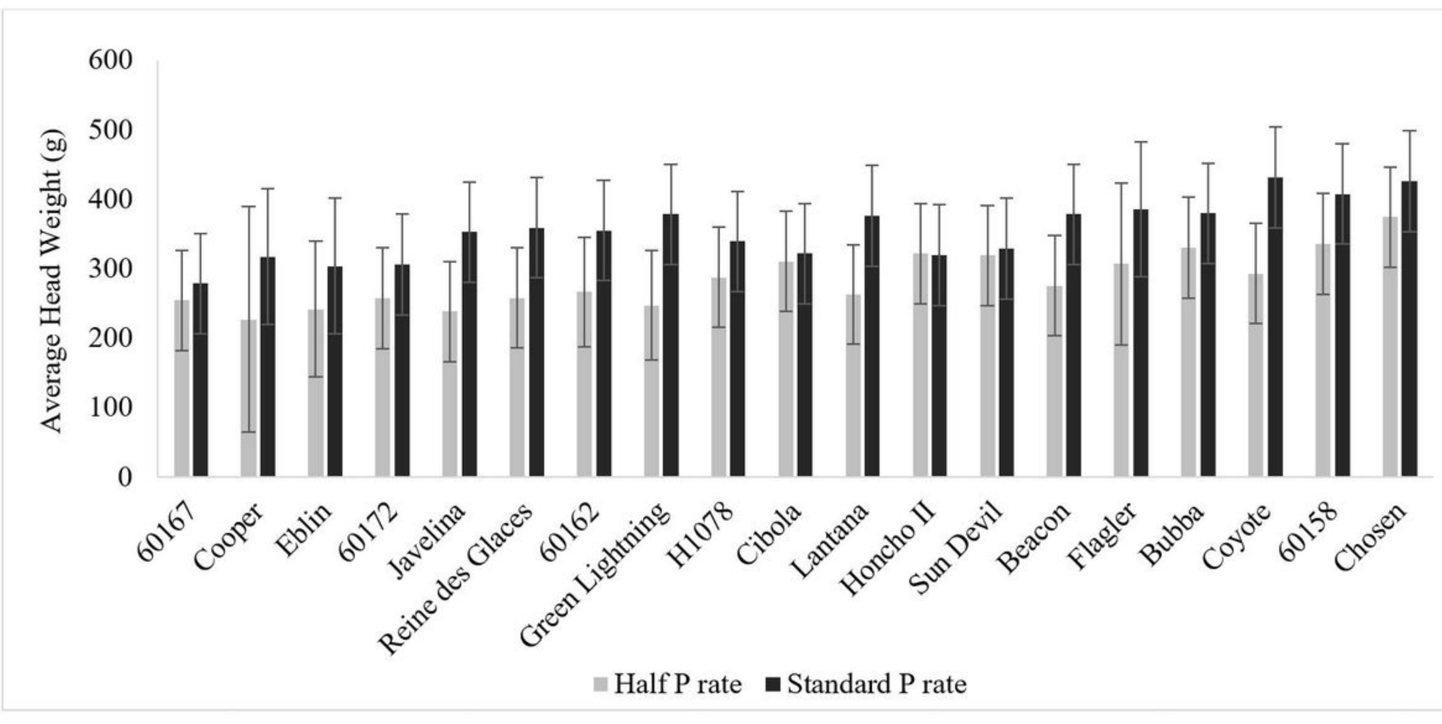

Figure 2

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 19 crisphead genotypes in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

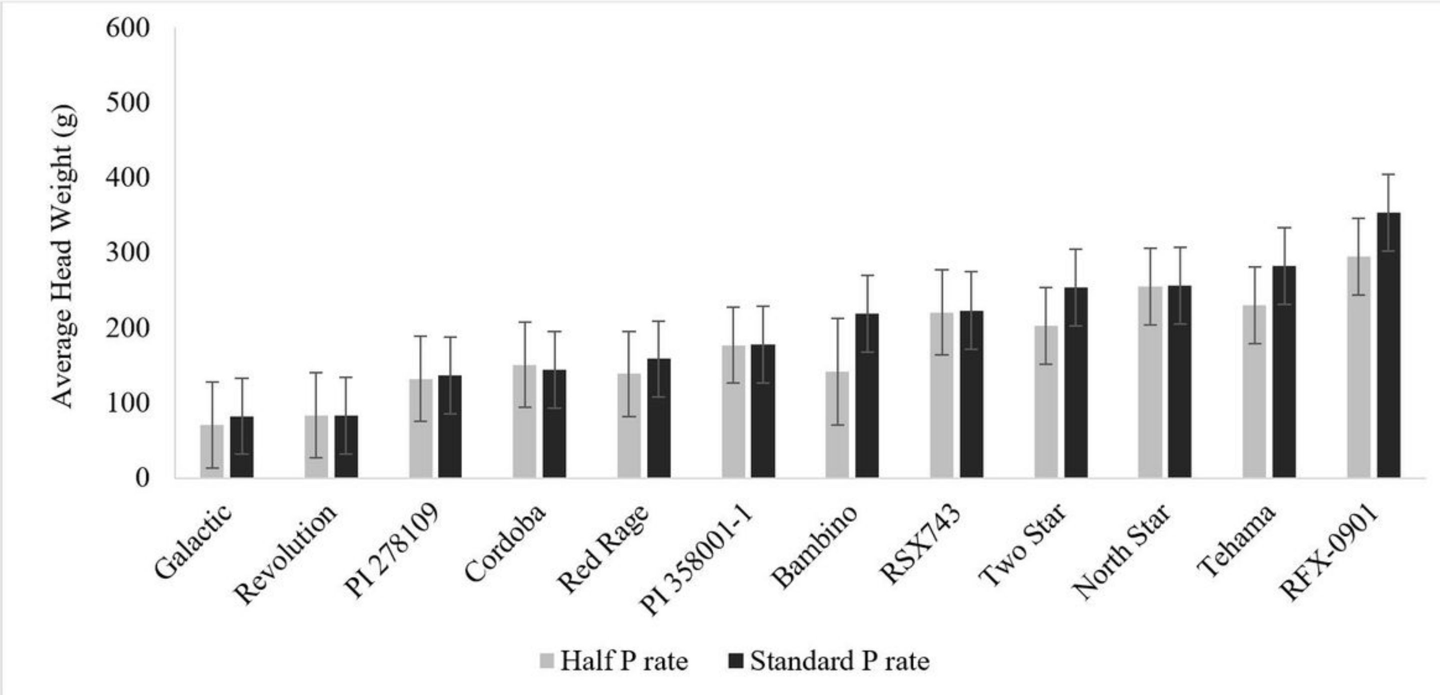

Figure 3

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 12 loose leaf genotypes in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

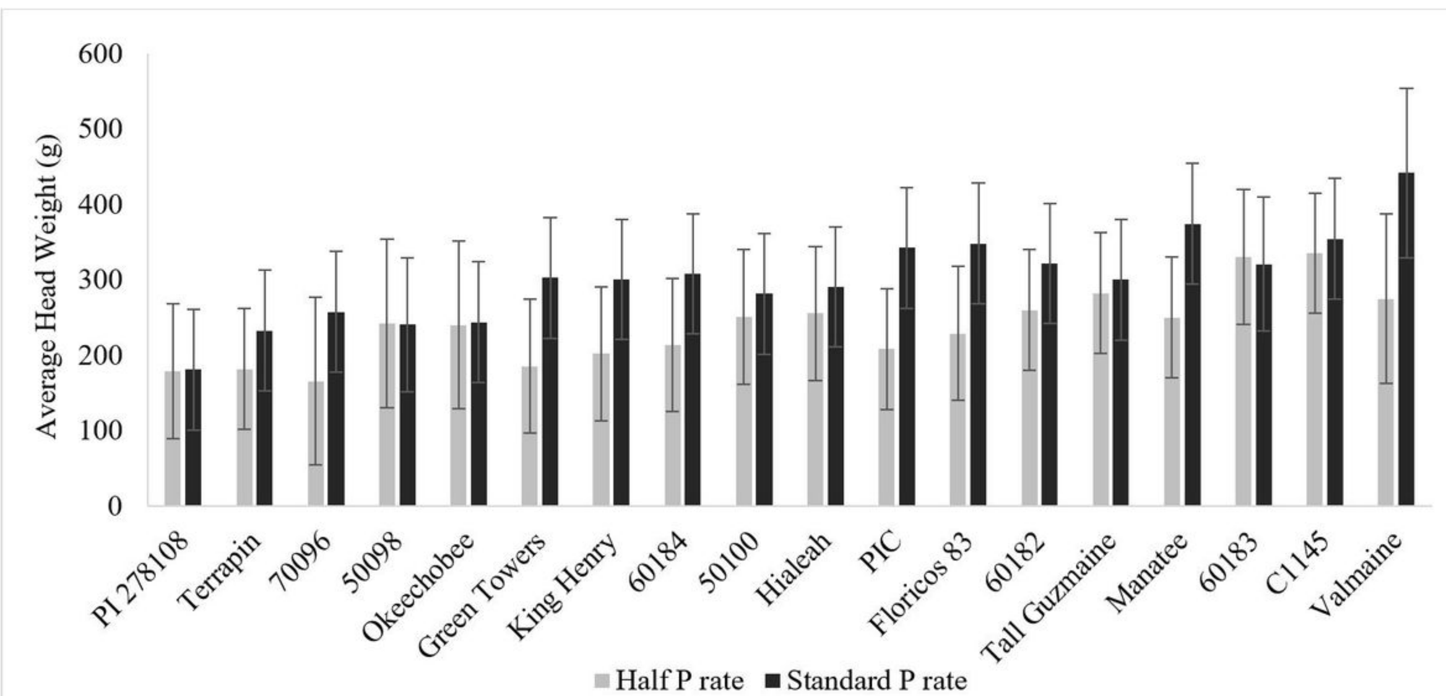

Figure 4

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 18 romaine genotypes in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

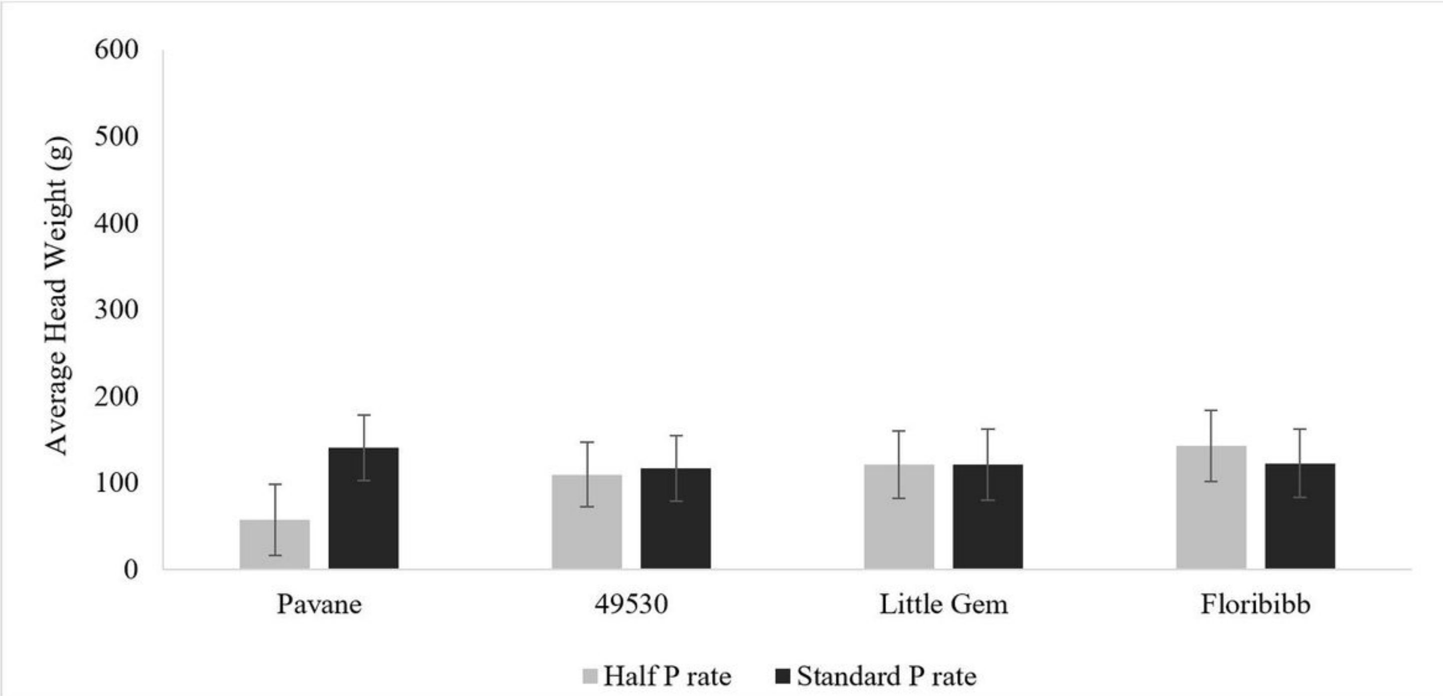

Figure 5

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 4 Latin genotypes in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

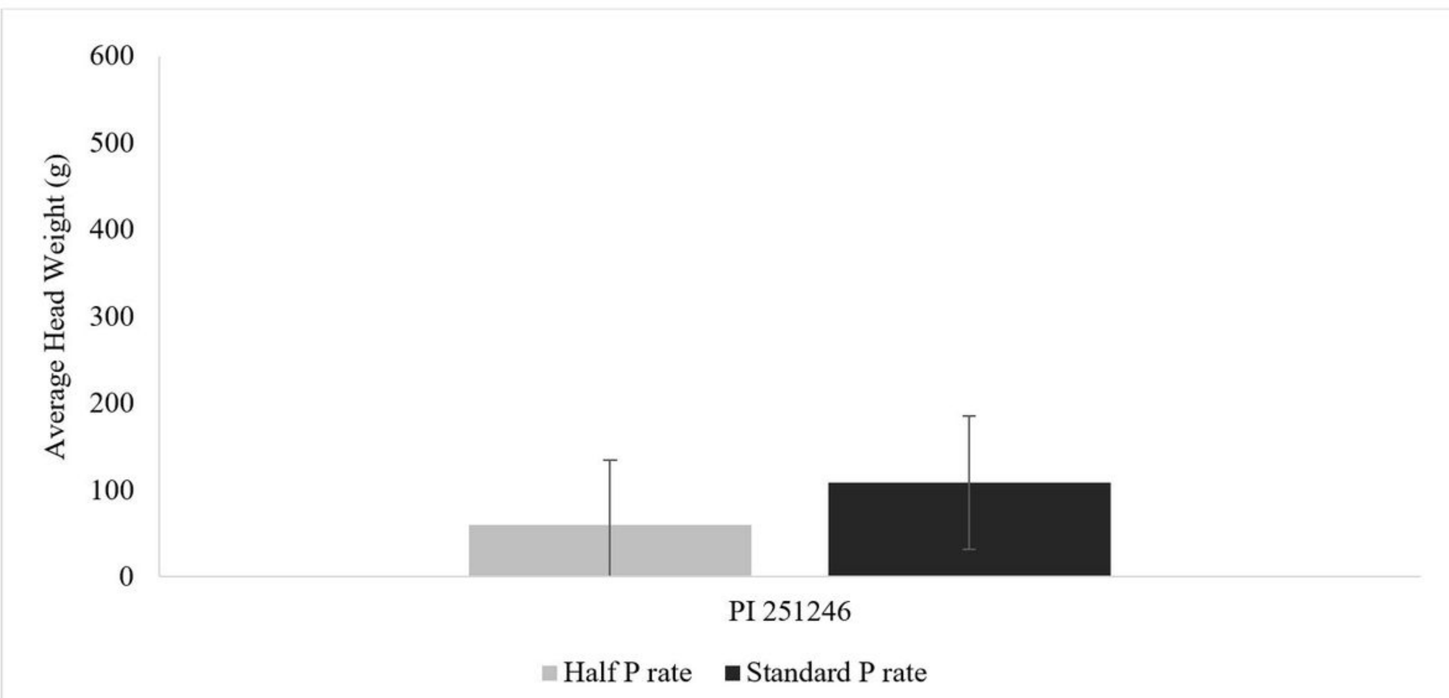

Figure 6

Least Square Means (LSM) of head weight (g) with 95% confidence intervals of 1 oilseed genotype in experiments conducted in the 2017-2018 and 2019-2020 seasons under half phosphorus (P) rate and standard P rate

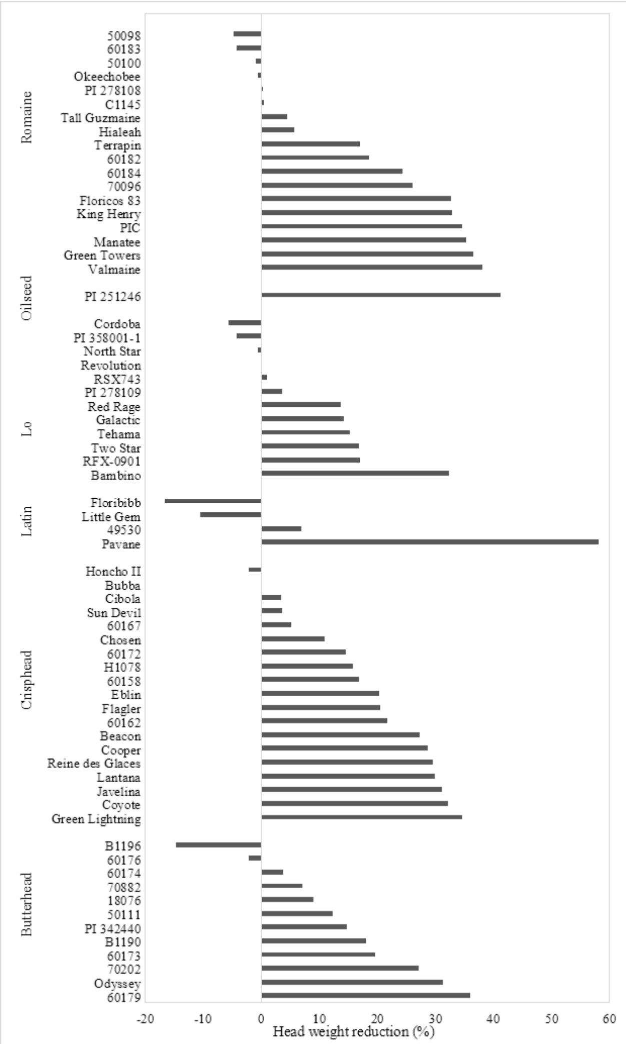

Figure 7

Head weight (HW) reduction (%) between standard and half-P rate of romaine, oilseed, loose leaf, Latin, crisphead, and butterhead lettuce. Negatives values indicate that HW was higher in the half-P rate. High positive values indicate high HW reduction when lettuce was grown in the half-P rate versus standard P rate