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Gaspé Flint corn as a seed-to-seed model to study the effect of phosphorus on maize growth and development

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ABSTRACT. Phosphorus (P) is a vital macronutrient for plant growth and development. Thus, P deficiency represents a bottleneck in the production of maize (*Zea mays*, L.). Therefore, there is a need for a prompt identification of new P-use efficient lineages and hybrids. The Canadian landrace Gaspé Flint (GF) race of maize was used to identify molecular and morphological traits due to its short life cycle and ease of growing in a hydroponic system under controlled conditions. First, GF was grown in a hydroponic system containing different Pi regimes for 15 d, and harvested tissues were assayed for various morphophysiological and molecular traits. Second, GF was grown in hydroponics under P+ (250 µM) and P- (10 µM) conditions until seed maturity. Pi deficiency led to a lack of synchrony between male and female reproductive organs, reducing fertilization, cob development, and productivity. Although typical Pi deficiency-mediated morphophysiological responses, such as increased root biomass relative to the shoot, accumulation of anthocyanins in the roots and leaves, and elevated acid phosphatase

activity in the shoot could be observed in any maize variety, the use of GF abbreviated the analysis of these traits from 120 days in commercial varieties to 40 days. Furthermore, Pi transporters ZmPT5 and ZmPT6 were induced in Pi-deprived roots and leaves and suppressed upon Pi replenishment, suggesting a transcriptional regulation. The study validated the efficacy of GF for accelerating studies on agronomic traits and plant response to stress, from seeds to seeds, in the grass family. The Gaspé Flint corn was confirmed as a plant model to study the effect of phosphorus on the growth and development of maize in a hydroponic system.

Key words: Maize (*Zea mays* L.); Gaspé Flint corn; Inbred line; Pi transporters; Agronomic traits

INTRODUCTION

Cereal crops contribute 42% of the human energy (Elert, 2014). Maize (popularly called corn) is among the most widely grown crops in the world after rice and wheat and has become a key source of global food security for the ever-increasing human population. In addition, maize is widely used as feed for livestock and bioethanol production as a substitute for nonrenewable fossil fuels (Zambrano et al., 2021). The USA, China, and Brazil are the top three countries, accounting for ~48% of the corn-producing area (Latifundist, 2020). In Brazil, corn is cultivated in ~22 Mha area with an average yield of 5.7 t/ha and a total production of ~127 Mt in 2023 (IBGE, 2023). Cerrado is a vast tropical savanna ecoregion of Brazil with highly acidic soils, and inorganic phosphate (Pi) is often present at low concentrations (Moreira et al., 2014; Vasconcelos et al., 2021). P is a crucial component of organic molecules, e.g., ATP, nucleic acids, and phospholipids, and plays a pivotal role in energy transfer, sensing and signal network, and several metabolic pathways (Chiou and Lin 2011; Sun and Zheng, 2022). P fertilizer is used mainly in soils poor in Pi. However, the cultivated crops acquire only ~ 20% to 30% of the applied Pi, and the rest is fixed in soil or lost in water bodies, causing eutrophication, which is a severe environmental concern (López-Arredondo et al., 2014). Moreover, Pi rock reserves are nonrenewable and limited and are anticipated to be exhausted in about ~100 years if they continue to be exploited globally at the current rate (Cordel et al., 2009; Li et al., 2020). Therefore, it is imperative to breed and engineer crops with better Pi acquisition efficiency (PAE) and Pi use efficiency (PUE) (Veneklaas et al., 2012; Paz-Ares et al., 2022). In this context, quickly deciphering the intricate Pi deficiency-mediated morphophysiological and molecular responses is pivotal for developing new maize crops with sustainable PAE and PUE.

The genome sequencing of the maize inbred line B73 (Schnable et al., 2009; Jiao et al., 2017) has greatly facilitated the functional genomics of an array of genes involved in nutrient homeostasis, responses to various abiotic and biotic stresses, growth, and development (Calderón-Vázquez et al., 2011; Zhang et al., 2022). However, the duration of its life cycle is comparable to the hybrids of commercial maize. Thus, using a maize genotype with a short cycle is desirable. The open-pollinated maize landrace Gaspé Flint corn (GF) from Quebec, Canada, is characterized by early seedling emergence, allelic variation related to early flowering, low stature, a few brace roots, multiple ears, abundant

tillers, meets these requirements (Eagles and Brooking, 1981; Swarts et al., 2017; Salvi et al., 2022). Therefore, in this study, we evaluated the efficacy of GF for elucidating the morphophysiological and molecular responses to Pi from seed to seed in a hydroponic system.

MATERIAL AND METHODS

Plant material and growth conditions

GF seeds were germinated in seedling trays containing Scott's ready earth plug mix (Scotts Co., Marysville, OH, USA) and grown in the greenhouse for a week. One-week-old seedlings were removed from the soil medium; the roots were washed and transferred to hydroponics containing one half-strength modified Hoagland's nutrient solution. After one week, seedlings were transferred to a nutrient solution containing different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) for 15 d, and the tissues were harvested for different morphophysiological and molecular assays. The solution was changed on alternate days during treatment to maintain the pH and nutrient concentrations. GF was also grown in hydroponics under P+ (250 µM) and P- (10 µM) conditions until maturity for seed collection.

Dry weight and root-to-shoot ratio

The roots and shoots of GF plants grown in hydroponics under different Pi regimes were collected separately, their fresh weight determined, dried at 50 ºC for 48 h in an oven, and then stored under desiccation until weighing for dry biomass and root-to-shoot ratio.

Quantification of soluble Pi

Harvested GF root and shoot tissues were rinsed thoroughly with deionized distilled water, blotted-dry, frozen in liquid nitrogen, and ground to a fine powder. Ground tissue (~50 mg) was homogenized with 250 ml of glacial acetic acid (1%, v/v), mixed thoroughly, and centrifuged at 10,000 rpm for 10 min. The supernatant was collected for assaying Pi content by phosphomolybdate colorimetric assay as described (Ames, 1966). A standard curve generated with KH_2PO_4 was used to determine the concentration of soluble Pi.

Quantification of anthocyanin

After 15 days of development in nutrition solution, freshly harvested roots and shoot tissues of GF were rinsed with deionized distilled water and ground to a fine powder in liquid nitrogen. Grounded powder (~100 mg) was used to quantify anthocyanin at A_{530} as described (Lange et al., 1971).

Quantification of acid phosphatase enzyme activity

After 15 days of growth in nutrition solution, freshly roots and GF shoot tissues were used to quantify acid phosphatase (APase) enzyme activity as described (Johnson et

al., 1973) with minor modifications. The enzyme extract was prepared by macerating 500 mg of tissues in 10 ml citrate buffer (0.1 M, pH 5.2) for ~2 min, and the crude enzyme solution was centrifuged at 10,000 rpm at 4 °C for 10 min. The clear supernatant was used for the quantification of APase enzyme activity. The reaction mixture (3 ml) contained 0.4 ml chilled citrate buffer (0.1 M, pH 5.2) and 0.5 ml para-nitrophenylphosphate (pNPP) (10 mM, pH 5.2) to which 0.1 ml of supernatant was added to initiate the enzymatic activity and kept at 24°C for 10 mins. The reaction was stopped by adding 2 ml of Na_2CO_3 (0.2 M). Enzyme activity was calculated by measuring para-nitrophenyl (pNP) at 405 nm, and the values were compared to a standard curve prepared with pNP.

RNA isolation and Northern blot analysis

The total RNA was extracted from different tissues (10 g) of GF by the lithium chloride (LiCl) and hot phenol extraction method (Huap et al., 2011). Total RNA (10 µg) was electrophoretically separated on 1.2% (w/v) denaturing formaldehyde agarose gel and blotted onto a Magna pure nylon membrane (0.45 µm). After blotting, the RNA was immobilized on the membrane by UV cross-linking (120 mJ/cm^2) in a UV Stratalinker. Pre-hybridization was carried out for ~3 h in a hybridization solution containing denatured salmon sperm DNA (150 µg mL^{-1}), Denhardt's solution (5X), formamide (50%, v/v), 6X saline sodium phosphate EDTA (SSPE) buffer, sodium dodecyl sulfate (SDS) (0.5%, w/v) at 42°C. Subsequently, nylon filters were hybridized overnight with ^{32}P-labelled DNA probes (10^6 cpm mL^{-1}) of Pi transporters *ZmPT*5 and *ZmPT*6 in a hybridization solution at 42°C and then washed thrice with a solution containing 2X saline sodium citrate (SSE) and SDS (0.2%, w/v) for 15 min each at 55°C before autoradiography.

Statistical analysis

The data were computed from two to three independent experiments, and the statistical significance of differences between mean values was determined using the student's t-test. Different letters on the histograms indicate statistically different means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth and development of GF during Pi deficiency

Pi availability in the rhizosphere promotes plant growth and enhances productivity, and its deficiency generates serious morphophysiological alterations that affect growth and yield (López-Arredondo et al., 2014; Sun and Zheng, 2022). To determine the effects of different Pi regimes on the growth and development of GF, one-week-old seedlings grown in hydroponics containing one half-strength modified Hoagland's nutrient solution were transferred to a complete nutrient solution with different Pi regimes (250 µM [P+], 100 µM, 50 µM, 25 µM, 10 µM [P-], and 0 µM Pi) in a greenhouse until maturity. With the decrease in Pi concentration, there was a noticeable delay in the growth and development of tillers in both the vegetative (shoot and leaves) and reproductive traits (tassel and cob) in GF (Figure 1). An antagonistic correlation between root/shoot ratio and Pi concentration indicated a

shift in the allocation of photosynthates to the roots to promotes its growth for exploring the scarcely available Pi in the medium (Figure 2A). He et al. (2022) found that the low phosphate response of the root showed stronger genotypic variations than the low phosphate response observed in in shoots. In addition, Poli et al. (2021) found that a rice mutant NH787 exhibited higher root and vegetative biomass, number of tillers, and grain yield under Pi deprivation. The reduced concentration of Pi in the medium also resulted in a significant delay in the emergence of the cobs with a fully receptive stigma. Although different Pi regimes did not exert any significant influence on the growth and development of tassel and produced viable pollen (data not shown), a lack of synchrony with the female reproductive organ affected pollination efficacy, which resulted in an adverse effect on the development and number of cobs per plant (Figure 2B). Overall, there were commensurate declines in the dry weight (Figure 2C) and Pi content in the root and shoot (Figure 3) of GF with a reduction of Pi concentration in the medium.

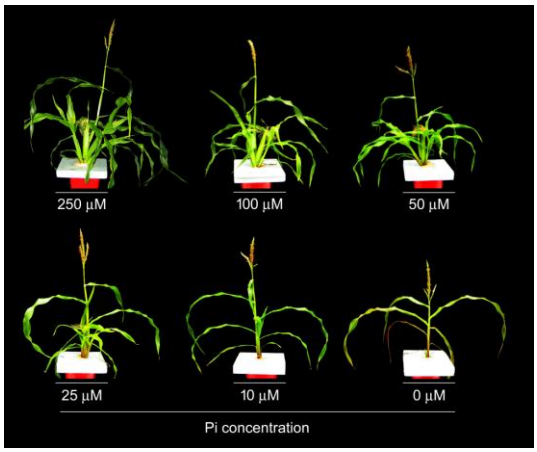

Figure 1. Different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) influence the growth and development of Gaspé Flint corn. Seedlings (one week old) were initially grown in hydroponics containing one half-strength modified Hoagland's nutrient solution, transferred to a nutrient solution with different Pi regimes, and grown until maturity under greenhouse conditions for documenting the growth performance.

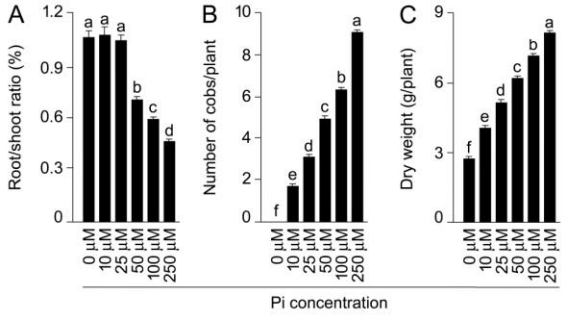

Figure 2. Effect of different Pi regimes on the agronomic traits. Gaspé Flint corn was grown in different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) under greenhouse conditions until maturity, as described in in the legend of Figure 1. Data are presented for (A) Percent root /shoot ratio, (B) Number of cobs/plant, and (C) Dry weight. Values are means ± SE (n = 6), and different letters on the histograms indicate that the values differ significantly ($P < 0.05$).

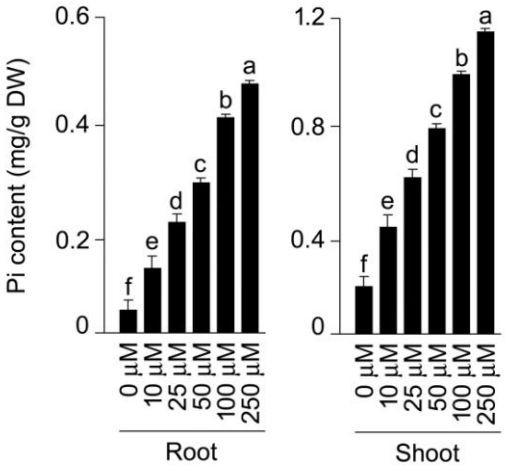

Figure 3. The effect of different Pi regimes on Pi content in different tissues. Gaspé Flint corn was grown in different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) under greenhouse conditions until maturity, as described in Figure Legend 1. Data are presented for Pi content in the root and shoot. Values are means ± SE (n = 6), and different letters on the histograms indicate that the values differ significantly ($P < 0.05$).

APase activity in GF during Pi deficiency

APases perform their activities over a broad range of pH (4.0-7.6) and maintain 80% activity over a broad temperature range (22°C-48°C) (Dick et al., 2011). During Pi deficiency, the roots secrete APase in the rhizosphere to hydrolyze different substrates, including organic P and inorganic Pi, to increase the Pi acquisition by plants (Ozawa et al., 1995). In addition, intracellular APase has been implicated in the rapid recycling and remobilization from internal P reservoirs during Pi deficiency (Żebrowska et al., 2017). Pi deficiency resulted in increased APase activity in both roots and shoots with much higher level observed in the latter (Figure 4). By contrast, there was a significant reduction in the APase activity in these tissues with increased Pi concentration in the medium. These results highlight the antagonistic correlation between APase and Pi. In addition, after Pi supplementation, the suppressed APase activity was more noticeable in shoots than in roots. This differential Pi deficiency-mediated tissue-specific response of APase activity could be due to the growth of GF in hydroponics, which, differently from the soil, lacks an organic source of P. Thus, internal mobilization of organic P resources could have significantly released much-needed P to GF. These results are consistent with earlier studies reporting a Pi deficiency-mediated increase in APase activity in maize (Kummerová, 1986; Yu et al., 2019; Ma et al., 2021). An intricate network of local and systemic signals and crosstalk with ethylene and sucrose signaling pathways related with the induction and secretion of APase (Wang and Liu, 2018). Also an interaction of MAPK--kinase with sucrose synthase and the root elongation factor 1 during Pi deficiency (He et al., 2022). Overall, the reversible induction of APase activity helps plants to modulate their responses according to the availability of Pi in the rhizosphere or internally in the tissues. Also, Poli et al. (2022) studying a rice mutant line NH787 found an augmented activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) under low Pi concentration.

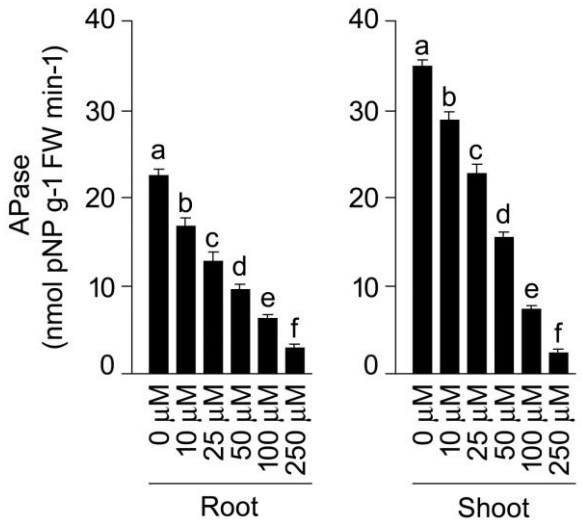

Figure 4. The effect of different Pi regimes on APase activity in different tissues. Gaspé Flint corn was grown in different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) under greenhouse conditions until maturity, as described in Figure Legend 1. Data are presented for APase activity in the root and shoot. Values are means ± SE (n = 6), and different letters on the histograms indicate that the values differ significantly ($P < 0.05$).

Accumulation of anthocyanin in different tissues of GF during Pi deficiency

Anthocyanins, a group of water-soluble glycosylated derivatives of polyphenolic anthocyanidins, are ubiquitously present in various plant species and responsible for imparting pH-dependent hues (blue, purple, red, and violet) to various tissues, e.g., roots, leaves, stems, flowers, and fruits (Belwal et al. 2019; Tan et al., 2022). The anthocyanin accumulation in cell vacuoles is an adaptive response to Pi deficiency that protects the leaves from the photoinhibition and photooxidative stresses by absorbing the excess light energy as heat and attenuating its interception by chlorophyll (Gould, 2004; López-Arredondo et al., 2014; Paz-Ares et al., 2022). Therefore, we investigated the effect of P+ (250 µM Pi) and P- (10 µM Pi) conditions on the anthocyanin accumulation in different tissues (stem, root, leaf, and panicle) of GF grown hydroponically until maturity under greenhouse conditions (Figure 5A-E). Although under P+ condition, there was no visible anthocyanin accumulation in the stem, root, leaf, and panicle, these tissues develop purple or pink hues during Pi deficiency (Figure 5A-D). The anthocyanin accumulation was relatively higher in older leaves under P- compared with P- younger leaves (Figure 5A). The results were consistent with earlier studies reporting Pi deficiency-induced accumulation of anthocyanins in maize leaves (Sun and Zheng, 2022). Further, a significant reduction in anthocyanin content in roots with an increase in the concentration of Pi revealed an inverse correlation between Pi concentration and accumulation of anthocyanin in tissues (Figure 5E). He et al. (2022) found a MAPK-kinase MKK6 orthologue with a putative regulatory role in roots. In Arabidopsis, a MKK6 kinase is a negative regulator of anthocyanin induction (Wersch et al., 2018). Moreover, this kinase is required for lateral root formation in Arabidopsis (Zeng et al., 2011).

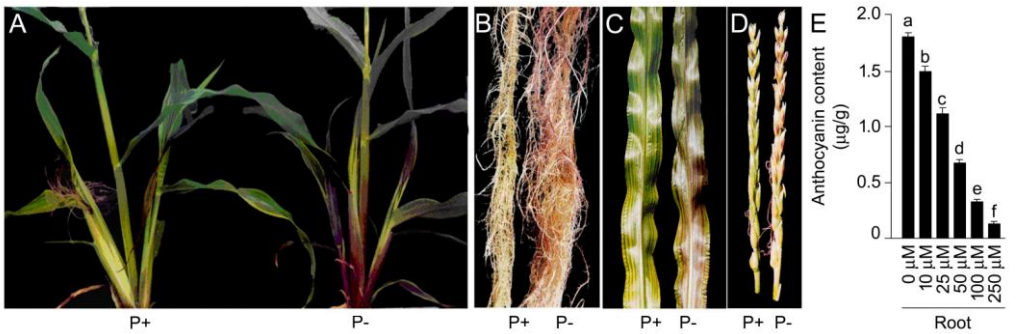

Figure 5. Pi deficiency induces accumulation of anthocyanin in different tissues. Gaspé Flint corn was grown in different Pi regimes (250, 100, 50, 25, 10, and 0 µM Pi) under greenhouse conditions until maturity, as described in Figure Legend 1. (A-D) Photographs show the effects of P+ (250 µM) and P- (10 µM) conditions on the accumulation of anthocyanin in (A) Stem, (B), Root, (C), Leaf, and (D) Panicle. (E) Data are presented for anthocyanin content in the root under different Pi regimes. Values are means ± SE (n = 6), and different letters on the histograms indicate that the values differ significantly ($P < 0.05$).

Pi deficiency triggers differential spatiotemporal expression of Pi transporters *ZmPT5* and *ZmPT6* and is transcriptionally regulated by Pi in GF

Plant Pi acquisition from rhizospheres is mediated by genes of the *PHOSPHATE TRANSPORTER1* (*Pht1*) family that encodes closely related plasma membrane-localized high-affinity Pi transporters, which are transcriptionally upregulated during Pi deficiency. The expression of *Pht1* genes was predominantly observed in the roots and often in aerial parts of taxonomically diverse plant species (Wang et al., 2017). The expression of the *Pht1* family members exhibits overlapping patterns, resulting in functional redundancy (Ayadi et al., 2015). In maize, five genes (*ZmPTs*) encoding *Pht1* Pi transporters were identified whose transcripts were significantly induced during Pi deficiency in the roots, young and old leaves, anthers, and pollen, suggesting their pivotal roles in the acquisition and allocation of Pi (Nagy et al., 2006; Vasconcelos et al., 2018; Vasconcelos et al., 2022). Therefore, we investigated the spatiotemporal expression of *ZmPT5* and *ZmPT6* and their likely transcriptional regulation by Pi in GF (Figure 6A-D). GF was grown hydroponically under different Pi regimes as described above for different time intervals (1d, 3d, 5d, 6d, 7d, 8d, 12d, and 15d), and roots, shoots, young and old leaves, tassels, and cobs were harvested for the expression analysis of *ZmPT5* and *ZmPT6*. Further, GF grew under P- condition for 15d were replenished with P+ at different time intervals (1d, 2d, 3d, 4d, and 5d) to determine whether Pi transcriptionally regulates *ZmPT5* and *ZmPT6* in GF. We have employed both Northern blotting (Karthikeyan et al., 2002; Nagy et al., 2006; Vasconcelos et al., 2021) and qRT-PCR (Jain et al., 2007, 2009; Ramaiah et al., 2022) for assaying the expression of functionally diverse genes involved in the maintenance of Pi homeostasis. Since the northern blot was used in our earlier studies to determine the effect of Pi deprivation on the expression of Pi transporters in maize and sorghum (*Sorghum bicolor* [L.] Moench) (Nagy et al., 2006; Vasconcelos et al., 2021), we decided for this technique for determining the effect of Pi deprivation on the transcript abundance of *ZmPT5* and *ZmPT6* of GF for comparative analysis.

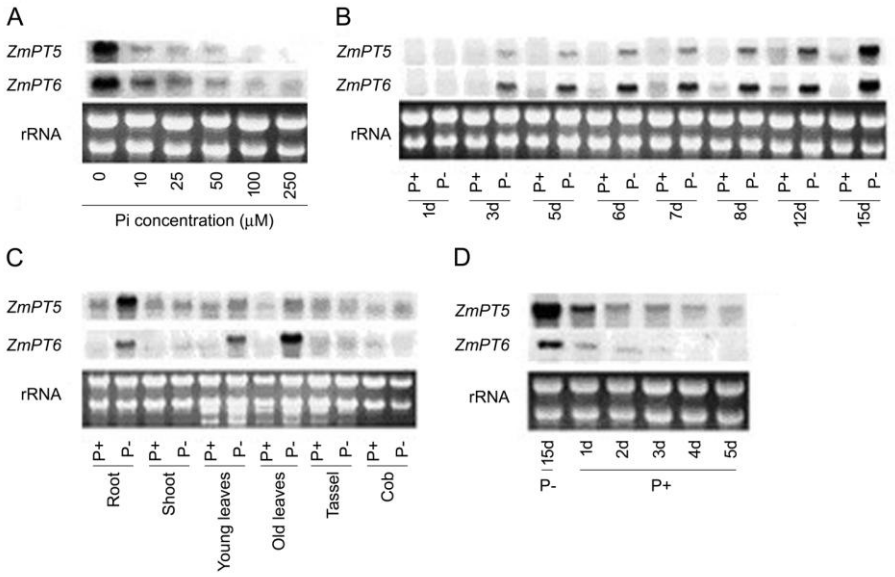

Figure 6. Expression of Pi transporters during Pi deficiency and upon replenishment with Pi. Gaspé Flint corn plants were grown hydroponically under different Pi regimes (250 µM and 10 µM), including P+ conditions and P- for different time intervals, and tissues were harvested for Northern blot analysis by probing with ZmPTs labeled with 32P. (A) Expression of ZmPTs in the roots under different Pi regimes for 15 d, (B) Temporal expression of ZmPTs in the roots under P+ and P- conditions, (C) P- induced tissue-specific expression of ZmPTs after treatment for 15 d, and (D) P- plants replenished with P+ for different time intervals suppresses the expression of ZmPTs in the roots. The equivalence of RNA loading in all the lanes is shown by ethidium bromide-stained rRNA (bottom panel).

The effect of treatment with different Pi concentrations for 15d on the expression of *ZmPT5* and *ZmPT6* in the roots is shown in Figure 6A. The Northern analysis revealed strong expression of both *ZmPT5* and *ZmPT6* at 0 µM Pi. The expression of *ZmPT5* declined rapidly with an increase in the concentration of Pi. The lower expression was noted at 10 µM Pi, 25 µM Pi, and 50 µM Pi, and barely expression at 100 µM Pi and 250 µM Pi. The expression of ZmPT6 was relatively higher at 10 µM Pi, 25 µM Pi, and 50 µM Pi, compared with ZmPT5, which was lower at 100 µM Pi and 250 µM Pi. An antagonistic correlation between the concentration of Pi and the transcript abundance of *ZmPT5 and ZmPT6* was evident, with the effect more pronounced on the former than the latter. The results suggested potentially differential roles of ZmPT5 and ZmPT6 in acquiring and mobilizing Pi in GF. The results were consistent with earlier studies employing Northern analysis for demonstrating a Pi deficiency-mediated increase in the transcript abundance of Pi transporters in Arabidopsis (Karthikeyan et al., 2002), maize (Nagy et al., 2006; Vasconcelos et al., 2022), and sorghum (Vasconcelos et al., 2021). We further investigated the Pi deficiency-mediated temporal expression of *ZmPT5* and *ZmPT6* in the roots by growing Gaspé Flint corn plants hydroponically under P+ (250 µM) and P- (10 µM) conditions in different time intervals (1d, 3d, 5d, 6d, 7 d, 8d, 12d, 15d) (Figure 6B). Although transcripts of both *ZmPT5* and *ZmPT6* were barely detectable after 1d treatment of Pi deficiency, their abundance could be detected when Pi deficiency was extended for 3d and increased with prolonged Pi deficiency (5d – 15d). The results were coherent with an

earlier study in maize demonstrating a steady increase in the transcript levels of Pht1 transporters during Pi deprivation from 1d to 15d (Nagy et al., 2006). Relatively, the accumulation of Pht1 Pi transporter transcripts has been observed as early as 12h to 24h of Pi deprivation in Arabidopsis (Karthikeyan et al., 2002; Misson et al., 2004) and tomato (*Solanum lycopersicon* L.) (Muchhal and Raghothama, 1999). Therefore, the species specificity and the growth condition could influence the Pi deficiency-induced differential temporal expression pattern of the Pht1 family members. For example, hydroponics and potting soil (Nagy et al., 2006), agar plate (Misson et al., 2004), liquid culture (Karthikeyan et al., 2002; Misson et al., 2005), and aeroponics (Muchhal and Raghothama, 1999). Noticeably, Pi deficiency-mediated accumulation of *ZmPT6* in GF roots was more abundant compared with *ZmPT5* deprived of Pi for 3d-15d, suggesting their differential roles in Pi homeostasis during Pi deficiency (Figure 6B). Further, we investigated the Pi deficiency-mediated spatial expression pattern of *ZmPT5* and *ZmPT6* in different tissues (root, shoot, young and old leaves, tassel, and cobs) by growing Gaspé Flint corn plants hydroponically under P+ (250 µM) and P- (10 µM) conditions for 15d (Figure 6C). Pi deficiency significantly induced the expression of *ZmPT5* in the roots, while in other tissues (young and old leaves, tassel, and cobs) its expression remained at the basal level under P+ and P- conditions. The result is consistent with earlier studies reporting strong expression of most of the *Pht1* family members predominantly in the roots of both monocotyledonous and dicotyledonous species strengthening the evidence of their pivotal role in Pi acquisition from rhizosphere roots (Wang et al., 2017). Although the transcript abundance of *ZmPT6* in Pi-deprived roots was relatively lower compared with *ZmPT5*, there was a significant accumulation of the transcripts in P- young leaves, which augmented further in old leaves (Figure 6C). The results suggest differential roles of *ZmPT5* and *ZmPT6* not only in the acquisition of Pi by roots but also its remobilization from old to young leaves. Our results are coherent with several earlier studies in diverse plant species, demonstrating the expression of various *Pht1* family members in the aerial parts of different vegetative and reproductive tissues (Karthikeyan et al., 2002; Nagy et al., 2006; Vasconcelos et al., 2022). Finally, we investigated whether GF plants grown under P- condition for 15d and subsequent replenishment with P+ for different time intervals (1d, 2d, 3d, 4d, and 5d). This treatment exerted any attenuating influence on the transcript abundance of *ZmPT5* and *ZmPT6* in the root (Figure 6D). There were significant and commensurate reductions in the transcripts of *ZmPT5* and *ZmPT6* with an increase in the duration of replenishment with P+. The effect was more pronounced in the latter than in the former. The results provided evidence for the transcriptional regulation of *ZmPT5* and *ZmPT6*, consistent with earlier studies on Pi transporters SbPT1 and SbPT2 in the contrasting genotypes for P-use efficiency in *Sorghum bicolor* (Vasconcelos et al., 2021) and AtPT1 and AtPT2 in Arabidopsis (Karthikeyan et al., 2002).

CONCLUSIONS

The present study revealed the efficacy of GF as a potent and easy-to-grow maize in a hydroponic system under different Pi regimes. GF exhibited an array of Pi-deficiency mediated morphophysiological and molecular adaptive responses, highlighting its efficacy for studying other agronomic traits of maize.

CONTRIBUTIONS

K.G.R. conceived the project, designed it, and assisted in writing the manuscript. M.J.V.V., J.E.F.F., M.F.O., and R.S.T conducted the experiments and helped prepare a draft manuscript. A.J. and P.Y. finalized the figures and manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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