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Effects of Phosphorus on Different Genotypes of Wheat and Canola Differing in P-Efficiency in Acidic Soils of Western Australia

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Abstract. We hypothesized that phosphorus addition would result in plant morphological changes and changes in rhizosphere carboxylates among wheat and canola cultivars in different acidic soils. Concentration of carboxylates in the rhizosphere extracted with 0.2 mM CaCl2, expressed per unit root dry mass. Dry weight of root and shoot were measures after harvest; total root length, and average root diameter were determined using a scanner. Also, the concentration of phosphorus (Colwell P) in rhizosphere and bulk soil was measured using UV-VIS Spectrophotometer. Shoot and root dry mass of wheat and canola increased significantly with increasing P supply. There was significant difference in total root length and average root diameter between treatments and genotypes in both acidic soils. Citrate was the dominant carboxylate in the rhizosphere of wheat genotypes, and malate was the second one. In canola genotypes, concentration of carboxylates in the rhizosphere were at least 10 times higher than rhizosphere of wheat genotypes. Surprisingly, malonate which there was not in the rhizosphere of wheat genotypes, was the most important carboxylate in the rhizosphere of canola genotypes followed by malate and citrate. This study showed there were significant differences between plant P-efficient and non-efficient in acidic soils when we used different level of P.

Keywords: carboxylates, Colwell P, P-efficient, Rhizosphare, spectrophotometer

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1. Introduction

Phosphorus (P) is one of the most abundant elements in soil and is present in both inorganic and organic forms [1]. P is an essential plant nutrient, and its deficiency in soils severely restricts crop yields [2]. Only 0.1% of the total soil P is available to plants because of poor solubility and fixation with other metallic elements in soil (Ca-P and Mg-P in alkaline soils or Fe-P and Al-P in acidic soils) [1], [3]. Thus, farmers need to use relatively large amounts of phosphorus to achieve good plant growth, which may have detrimental effects on both the environment and economy;

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for example, many Australian inland rivers are eutrophicated due to excessive P applications in agriculture and horticulture [4]-[6]. It is therefore crucial to acknowledge the plant adaptations to the low accessibility of P in order to enhance the efficacy of plant P-acquisition, in fact to absorb the less usable soil P [7], [8]. Consequently, P-fertilizer application and environmental effects need to be reduced, and sustainable food supply should be guaranteed.

A widely agreed goal is the production of agricultural genotypes and cultivars which yield well when grown in soils with lower extractable P concentrations compared with those commonly required for high yields. Because of study on rhizosphere/non-rhizosphere in this research, it is worth mentioning that the structure of rhizosphere communities differs from that in the bulk soil [9], and compared to bulk soil, phosphatase activity in the rhizosphere is highest, with higher levels of microbial activity [9], [10]. This project will deal with wheat (*Triticum aestivum* L.) and canola (Brassica napus), as two important crops grown in acidic soils in Western Australia. Soil acidity represents a major growth-limiting factor in plant production [11]. In Western Australia, approximately 4.25 million hectares of land are currently acidic, or at risk of becoming acidic, and around 10% of agricultural soil are becoming acidic annually. The availability of certain nutrients such as phosphorus can reduce with low soil pH and consequently concentrations of toxic elements such as aluminum and iron in the soil solution can increase [12].

Organic acid synthesis is well known and generally recognized that bacteria use this strategy as the key mechanism for solubilization of P. Organic acids have the ability to enhance the amount of P present in the soil [13]. This event happens because of the organic acids chelating properties. In addition, the synthesis of organic acids contributes to the acidification of the bacterial environment; this will facilitate apatite solubilization through proton substitution of H+ and release of Ca2+. Low molecular weight carboxylates are prevalent compounds which influencing metabolism or organisms in the soil. Carboxylates can activate soil solid P depending on the chemical processes at the solid phase of the soil.

Root exudation is primarily a passive mechanism that occurs between the intact root cell cytoplasms and the external rhizosphere (substrate about the root area) that contributes to product releases in the rhizosphere. Banksia grandis an Australian Proteaceae species, when grown on sand comprising either poorly soluble Al-phosphate or Fe-phosphate as the only resource of P, adjusted its exudation template, and developed reasonably well on both source of P. The role of the P produced by carboxylates to plant growth is challenging to measure though [14] for Kennedia spp. documented a positive correlation between level of rhizosphere carboxylates and plant P volume.

The combination of organic anions of root exudation is incredibly changeable and relies on plant species. Malate and citrate seem to be the key ingredients generated during P deficiency by roots. Various organic anions demonstrate varying potential to mobilize P in soil; in most cases, citrate

is the most efficient [15]. Sampling procedures of organic anions have a significant impact on the defined data of rhizosphere organic anion concentrations [16]. The 'effective' organic anions in the rhizosphere are highly dependent on the level of root exudates that is indeed defined by stage of plant development, internal P concentrations [17], plant species and genotype [18], the volume of organic anions missed by soil sorption and soil microorganism breakdowns [19].

We hypothesized that P addition would result in plant morphological changes (like generating more root and shoot), changes in the rhizosphere carboxylates among wheat and canola cultivars in different acidic soils and the genotypes are P-efficient should show better result rather than inefficient genotypes.

2. Materials and Methods

Two different acidic soils were collected from two different regions from Western Australia named Kalannie and Dorper. The air-dried soils were sieved to 2 mm and thoroughly mixed. Kalannie soil has the following properties: pH (CaCl2) 3.9; Colwell P 9 mg kg−1; Colwell K 63 mg kg−1; NH4+ 1.65 mg kg−1; and NO3 15.8 mg kg−1. Dorper soil has the following properties: pH (CaCl2) 4.5; Colwell P 6 mg kg−1; Colwell K 136 mg kg−1; NH4+ 3 mg kg−1; and NO3 25 mg kg−1. Basal nutrients (mg kg−1 soil) were added to the soil at the following rates showed in the table 1. NH4NO3 95.2, K2SO4 139.9, KCl 50.0, MgSO4 40.0, CaCl2.2H2O 150.3, CuSO4·5H2O 2.0, MnSO4·H2O 10.0, CoSO4.7H2O 0.5, H3BO3 0.7, Na2MoO4·2H2O 0.2, and ZnSO4·7H2O 9.0. Phosphorus was added to the soil as FePO4 at a rate of 0, 20 mg P kg−1 soil to test the differences between two genotypes are differing in P-efficiency to know that can they use P in acidic soils.

The plant species studied were wheat (*Triticum aestivum*), and canola (*Brassica napus* L.). For each plant we had two different genotypes differing in P-efficiency. The preliminary test showed that wheat genotypes named Westonia and Janz and canola genotypes named Drum and Outback are different in P efficiency. Westonia and Drum were more P efficient. Seeds of similar weight per species were germinated on wet filter paper at 25°C in the dark for 24 h. After radicle emergence, eight uniform seeds were sown into a pot containing 3.5 kg air-dried soil equivalent with four replicates. Phosphorus was added as FePO4 at a rate of 20 mg P kg–1 soil. This rate was chosen to prevent severe P deficiency but still provide an inadequate P supply so the differences in releasing carboxylates between the genotypes that are different in P efficiency should be different. After germination, seedlings were thinned to four plants per pot. The pots were watered with deionized water to maintain 70% of field capacity. For each soil type, there were four unplanted control pots. The experiment was conducted under glasshouse conditions at The University of Western Australia (31°98’ S, 115°81′ E) from 10 May to 30 June 2019 with natural light at 22±2°C. All pots from the four replicates were randomly relocated within the area

occupied by the experiment weekly to minimize the influence of temperature and light gradients in the glasshouse. After 7 weeks of growth, plants were harvested in order of replicate.

Roots were further rinsed in deionized water before shoots were separated. Shoots and roots were oven-dried at 70 °C for 5 days. Once harvest was complete, total root length, and average root diameter (of representative subsamples) were determined using an Epson 1680 scanner and WinRHIZO 4.1 computer software (Regent Instruments Inc., Quebec, Canada). Also, the concentration of phosphorus (Colwell P) in rhizosphere and bulk soil was measured using UV-VIS Spectrophotometer based on Rayment and Lyons [20].

The four plants in each pot were removed and placed into a tray where their roots and adhered soil were carefully teased apart from the bulk soil, taking care not to break roots. Once the root systems could be lifted from the tray without roots breaking, they were held by the base of the shoots above the tray and gently shaken; the soil remaining adhered to the roots was classified as the rhizosphere. The root systems from each pot were then placed into a 500 ml beaker and rinsed with 50 ml of 0.2 mM CaCl2 solution using a large syringe until all visible soil had been washed into the solution. One millilitre of the solution was then filtered through a 0.22 µm Acrodisc syringe filter into a 1 ml Waters HPLC vial containing 25 µl of orthophosphoric acid. Vials were immediately capped and placed on ice before being transferred to a −20 °C freezer at regular intervals. Rhizosphere carboxylates were analysed by reversephase liquid chromatography, as described by Cawthray [21] except for oxalate where the method detailed in [22] was followed. The amount of rhizosphere carboxylate was calculated relative to root DM.

2.1. Data Analyses

Rhizosphere carboxylates that were below the detection limit were given a value of zero. Acetate were present in a small number of pots and did not vary consistently with plant species; data are therefore not presented or included in calculations of total carboxylates. For each measured variable, data were examined for outliers and normality, and transformations undertaken if required. Data were then analysed in Genstat version 18.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK) using a two-way ANOVA to assess the effect of the 4 genotypes on each variable; the effect of replicate was included in the analysis. The means for each species/line were graphed with the standard error of the mean using Excel.

3. Results and Discussions

There was significant difference in plant growth among treatments, genotypes and soils. Shoot and root dry mass of wheat and canola increased significantly with increasing FePO4 supply (Fig. 1, 2). Dry mass of wheat genotypes was higher which for example in Westonia genotype shoot and root dry mass at 20 mg P /kg soil were increased by 32% and 34% in comparison with 0 P in Kalannie soil, respectively (Fig. 1a, 1b). There was a same result in Dorper soil regarding the

plant growth in wheat genotypes. Westonia showed a higher growth rate in Dorper soil with 49% in root dry mass and 30% in shoot dry mass rather than Janz genotype (Fig. 1a, 1b). It is worth mentioning that findings of Schulthess et al. [23] and Manske et al. [24] confirms that P use efficiency in wheat varied depending on genotypes and can increase the plant growth. The root and shoot dry mass of the P-inefficient wheat genotype, Janz, at 20 mg P /kg soil in comparison with 0 P had a better growth which indicate that adding 20 mg P /kg soil could help this genotype to grow better rather than the control (Fig. 1a, 1b). But the most important result is the same growth rate for Westonia 0P and Janz 20P, suggesting the P-efficient genotypes (Westonia) could show same result regarding root and shoot dry weight without receiving P in both soils.

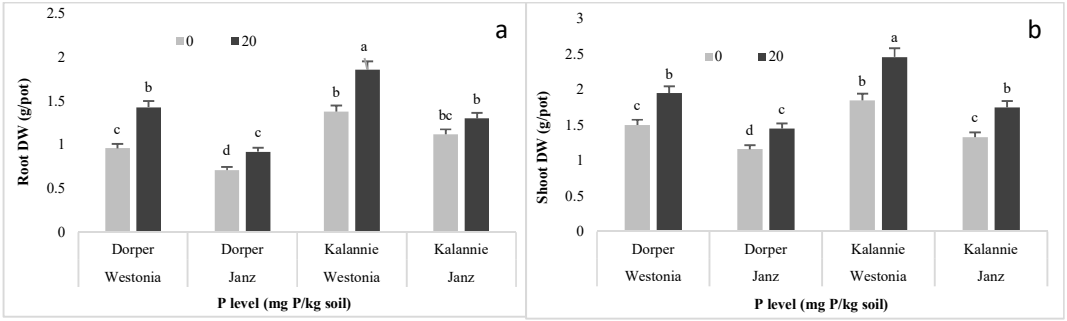

Figure 1. Results of comparing **a)** root and **b)** shoot dry weight related to wheat plants (Westonia & Janz) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P = 0.05)

The situation was the same in canola genotypes and the best reaction was seen at Drum genotype (P-efficient genotype) with 36% and 33% in shoot and root respectively in Kalannie soil (Fig. 2a, 2b). In addition, Drum in Dorper soil illustrates a better response to acidic soils and different rates of phosphorus with a better result in root and shoot dry mass (Fig. 2 a, b). Outback, another canola genotypes, showed a higher growth at 20 mg P /kg soil in comparison with 0 P regarding its root and shoot dry mass (Fig. 2a, 2b). Korkmaz and Altıntaş [22] tested ten canola genotypes at three P rates and indicated that the adaptation of canola genotypes to low levels of soil P is closely related to genotypes differ in P-use efficiency. P efficient plants are able to produce high yield at relatively low soil P supplies [25]. Like wheat genotypes, the same growth rate for Drum 0P and Outback 20P proved that the P-efficient genotypes (Drum) could show same result regarding root and shoot dry weight without receiving P in both soils.

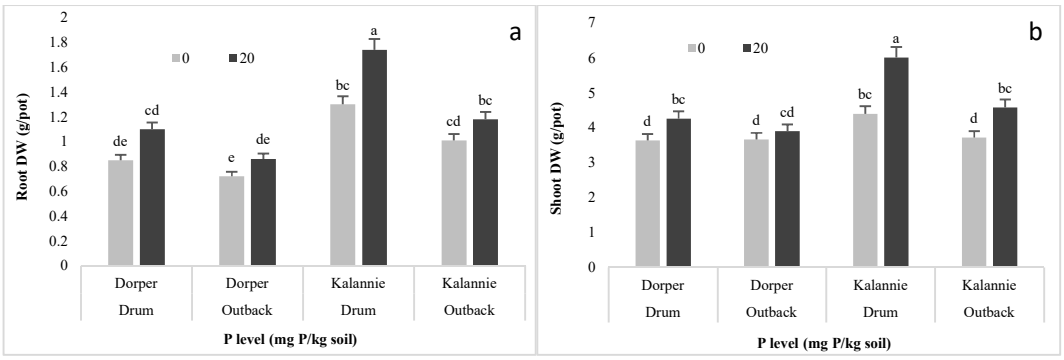

Figure 2. Results of comparing **a)** root and **b)** shoot dry weight related to canola plants (Drum & Outback) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P = 0.05)

There was significant difference in total root length and average root diameter between treatments and genotypes in both soils (Fig. 3, 4). In wheat genotypes, Westonia indicated a longer and thicker root in average in Kalannie soil in comparison to other treatments (soil and P level), and Janz in Dorper soil was the weakest (Fig. 3a, 3b). In canola genotypes, Drum showed a significant difference in total root length and average root diameter in comparison to Outback in both soils (Fig. 4a, 4b). Same like Janz, Outback depicted the lowest numbers, especially in Dorper soil they had only 74% of Drum's total root length in Kalannie soil (Fig. 4a), 20 mg P /kg soil in comparison to 0 P illustrated a significant difference in all treatments which describing even adding 20 mg P /kg soil could increase the plant growth in P-efficient genotypes. Root length density are some factors that may related to the genotypic differences in P uptake efficiency [2]. As mentioned earlier, root characteristics is the main factor that influence P uptake efficiency. Mean root diameter varied in most cases by less than 47.73% across soils, with mean values of 0.304 mm for wheat, and 0.272 mm for wheat roots. But the most important result is the same growth rate of Westonia and Drum with 0P against Janz and Outback 20P, suggesting the P-efficient genotypes (Westonia and Drum) could indicate same result regarding total root length and average root diameter without receiving P in both soils. Total root length in Westonia (P-efficient genotype) showed a little bit better result in comparison to Janz in Dorper soil.

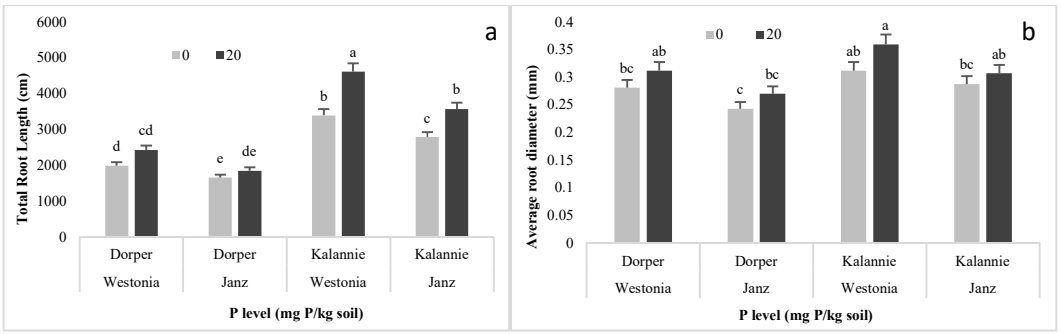

Figure 3. Results of comparing **a)** total root length and **b)** average root diameter related to wheat plants (Westonia & Janz) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P= 0.05)

This experiment showed that wheat and canola genotypes were greatly affected by soil types and they had a better growth in Kalannie soil compared to Dorper soil. Low productivity in acid soil has been identified as a significant form of land degradation in Australia. Surface and subsoil acidity is common in Australia; around 50 million ha of surface soil and 23 million ha of subsoils in Australia are affected by soil acidity [26]. So, the acidity of the soils is one of the most important factors for these results. The genetic structures of variety and root lengths might cause differences in grain yield and nutrient use efficiency among the cultivars. Soil type, genotypic variability and phosphorus rates are the factors affecting P uptake by plants. Among mentioned factors, genotypic differences have been indicated to be more related to P use efficiency [27]. It completely shows why P-efficient genotypes (Westonia and Drum) were significantly different compared to P-inefficient genotypes.

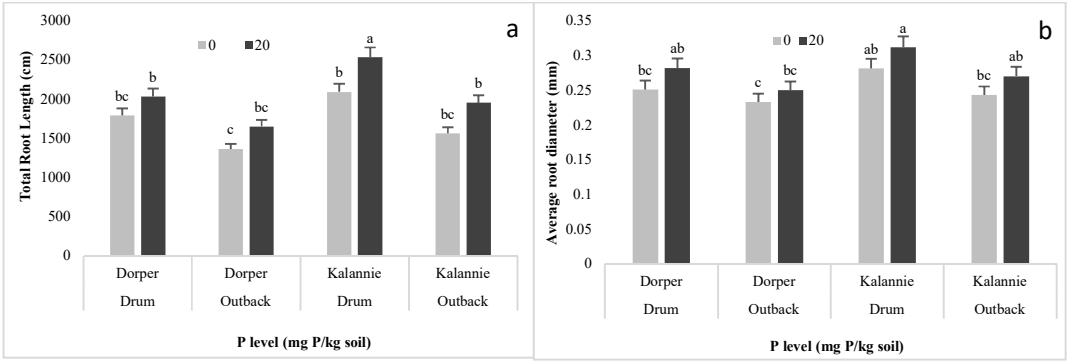

Figure 4. Results of comparing **a)** total root length and **b)** average root diameter related to canola plants (Drum & Outback) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P= 0.05)

Citrate was the dominant carboxylate in the rhizosphere of wheat genotypes (Fig. 5 a), and malate was the second one. Westonia had a significantly different citrate in Kalannie soil rather than Dorper soil; The highest was Westonia 20 mg P /kg soil in Kalannie soil with 675 nmol/g root DM and the lowest was Janz 0 P in Dorper soil with 154 nmol/g root DM. The amount of malate in the wheat genotypes was less than citrate, but the amount of citrate and malate in Westonia 20

mg P /kg soil in Dorper soil was less than Janz 0 P in Kalannie soil which describing that Janz in Kalannie soil which is a better soil rather than Dorper, could release more malate to liberate more P for the plant. The released amount of citrate and malate of Westonia with 0P against Janz 20P was almost same, suggesting the P-efficient genotype (Westonia) could release same amount of carboxylates while we did not add any P to the soils.

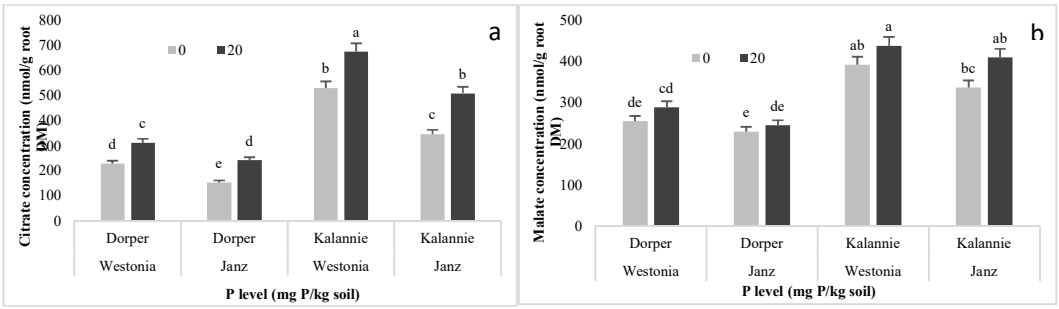

Figure 5. Concentration of carboxylates in the rhizosphere extracted with 0.2 mM CaCl2, expressed per unit root dry mass. Results are comparing **a)** citrate and **b)** malate related to wheat plants (Westonia & Janz) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P= 0.05).

In canola genotypes, concentration of carboxylates in the rhizosphere were at least 10 times higher than rhizosphere of wheat genotypes (Fig. 6a, 6b, 6c). Surprisingly, malonate which there was not in the rhizosphere of wheat genotypes, was the most important carboxylate in the rhizosphere of canola genotypes with 11.3 umol/g root DM in Drum 20 mg P/kg soil in Kalannie soil (the highest) and 4.8 umol/g root DM in Outback 0 P in Dorper soil (the lowest) (Fig. 6c). After malonate, malate was the second more common carboxylate around the rhizosphere of canola genotypes followecd by citrate. Between the treatments, 20 mg P /kg soil; between the genotypes, Drum; and between the soils, Kalannie soil, indicated a significant difference in comparison to 0 P, Outback and Dorper respectively (Fig. 6a, 6b, 6c). The released amount of citrate, malonate and malate of Drum with 0P against Outback 20P was almost same, suggesting the P-efficient genotype (Drum) could release same amount of carboxylates while we did not add any P to the soils. In several species, the root-released organic anions in the rhizosphere rise during P deficiency. Also there are variations between cultivars or genotypes for same species [28]. In general, the concentrations of organic anion shown in the literature may differ from the real values; this is because of several factors, for instance, analytical techniques, soil microbial activity, soil sorption, the sampling procedure, and the trap solution used [29].

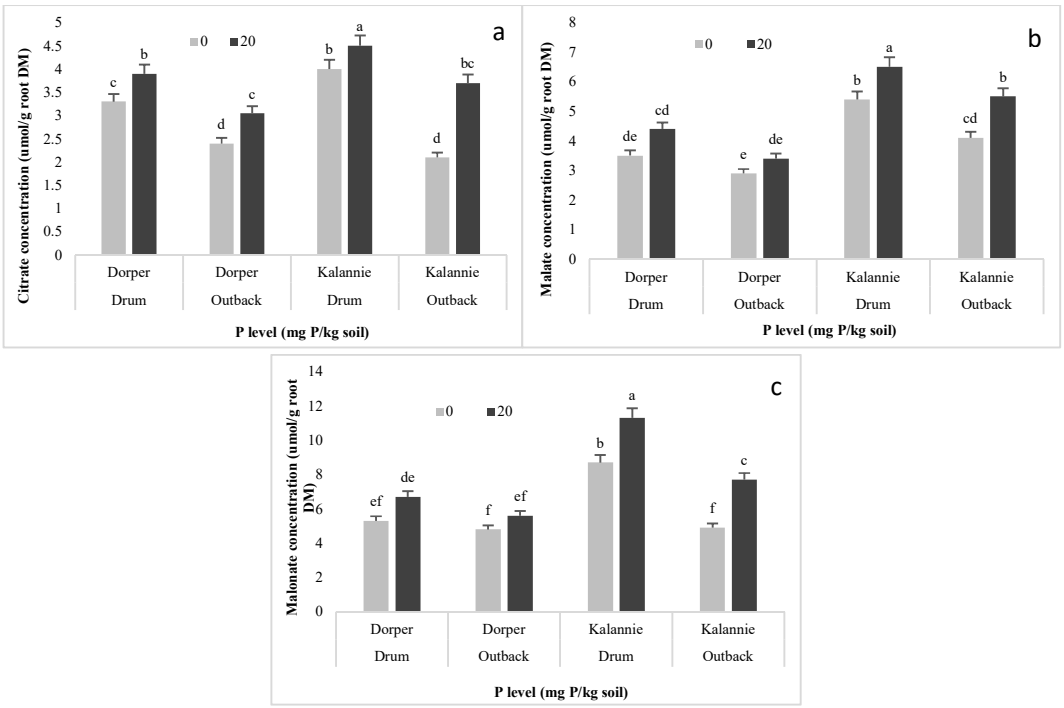

Figure 6. Concentration of carboxylates in the rhizosphere extracted with 0.2 mM CaCl2, expressed per unit root dry mass. Results are comparing **a)** citrate **b)** malate and **c)** malonate related to canola plants (Drum & Outback) growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different (P= 0.05)

There was a significant difference between treatments in Colwell P in the rhizosphere (Fig. 7 a, b) but in bulk soil there was not any difference. Treatments with 20 mg P /kg soil in wheat and canola in both soils were highly significant in comparison to 0 P treatments. As we expected, Colwell P was higher in Westonia and Drum 0P (P-efficient genotypes) compared to Janz and Outback 0P, respectively. The pH of the soil was measured before and after experiment and it showed that the rhizosphere pH has decrease in all pots (samples). Previously it was assumed that organic anion exudation may contribute to acidification of the rhizosphere, but it has already been properly recognized that exudation of organic anion and acidification of the rhizosphere are both spatially coordinated, biochemically independent procedures. Wheat had the smallest average root diameter, and therefore compared to canola many long roots, which explored a large soil volume. This probably enabled it to access reasonable amounts of readily available P; but in canola genotypes the amount of carboxylates covered this problem for Drum and Outback plants [30].

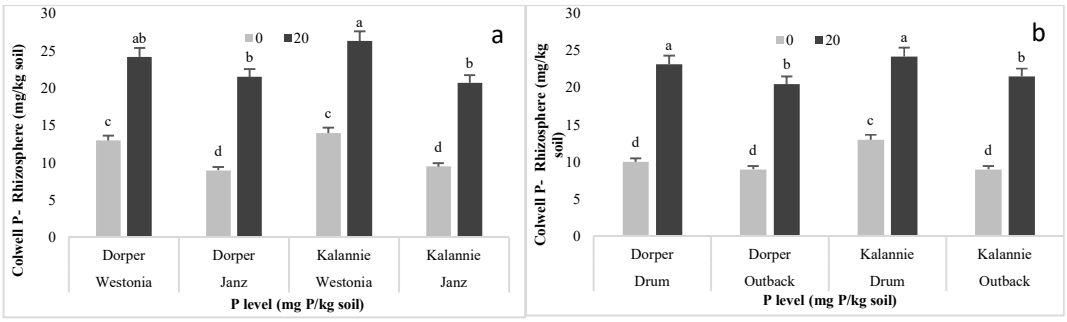

Figure 7. Results of comparing Colwell P around the rhizosphere of **a)** wheat and **b)** canola genotypes growing with different rates of P in different acidic soils. Values are means + standard error (SE) of four replicates. Means with the same letter are not significantly different

Comparison with other studies is problematic as rhizosphere carboxylate amount will differ with soil type [31], plant age [32] and different genotypes and may decline with rising the available P [33]. Also, the process has been used for measuring the carboxylates is different; for example, in this study the amount of carboxylates calculated based on root dry mass. In addition, some organic anions may originate from degraded roots; not all soil in the rhizosphere can be collected for organic anion extraction; various soil attributes provide different sorption capabilities, and soil microbes can dissolve organic anions or liberate organic anions to soil [34]. Therefore, the findings can be affected by 'inaccurate' organic anion information. Moreover, plants prefer to use root architecture or mycorrhizal colonization approaches to reach soil P where there is desirable volume of P accessible in the rhizosphere. On the contrary, root exudates will be more efficient where there is an exceptionally low volume of accessible P to maximize the concentration of P throughout the rhizosphere and thereby have sufficient P for the acquisition of the plant. Some researchers such as Wissuwa [35] and Korkmaz et al. [36] reported that genotypic differences might be an essential strategy for improved P acquisition during low P availability in soil. Also, rhizosphere microorganisms, especially indigenous phosphate solubilizing bacteria (PSB), are able to enhance or reduce the availability of P to plants [9], [37], [38] so, finding the relationship among PSBs, plants and the amount of released carboxylates is essential to investigate, which it would be the next experiment.

4. Conclusions

In conclusion, this study showed there are significant differences between P-efficient genotypes and P-inefficient in acidic soils and all plant growth factors shown different reactions to this situation and so the amount of carboxylates around the rhizosphere will differ.

Acknowledgments

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