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## Root, Shoot and Nutrient Acquisition Responses of Mycorrhizal and Nonmycorrhizal Wheat to Phosphorus Application to Highly Calcareous Soils

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**Abstract:** Greenhouse pot experiment was conducted to evaluate the response of wheat grown in calcareous soil to arbuscular mycorrhizal fungi (AMF) and P application. The treatments consisted of two factors, five rates of P application (0, 50, 100, 200, 400 kg P ha<sup>-1</sup>) and two levels of AMF inoculation (inoculated and non inoculated). Wheat was grown for 7 weeks and at harvest wheat dry matter (DM), P and micronutrients uptake were determined. Roots were extracted and root length and surface area were measured. Roots also were stained in trypan blue and mycorrhizal colonization was determined. AMF inoculation increased mycorrhizal colonization which decreased at higher rates of P levels. Maintaining the maximum DM while the AMF colonization was depressed at higher P rate indicates that the increase in DM was solely due to P. AMF inoculation decreased root length and surface area at higher rates of P. Phosphorus uptake was higher for mycorrhizal plants at 50 and 100 kg ha<sup>-1</sup> P rates only. Linear and quadratic relationship between P uptake and the root surface area were observed for nonmycorrhizal and mycorrhizal wheat, respectively. Higher specific P uptake and higher physiological and agronomical use efficiencies at lower P rates by mycorrhizal wheat suggests that they are more efficient in P absorption and utilization of soil P that is unavailable to nonmycorrhizal wheat. AMF enhanced Fe, Zn and Cu uptake except at high P rates where both mycorrhizal and nonmycorrhizal wheat had similar values of copper uptake.

**Key words:** Mycorrhiza, calcareous soil, wheat root, nutrient uptake

### INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are normally colonizing field crops grown under various soil and environmental conditions<sup>[1,2]</sup>. AMF is considered the most important microbial symbiosis for the majority of crops especially under inadequate soil phosphorus levels<sup>[3]</sup>. However, the activity of the AMF is differently affected by these environmental and soil conditions and by the different agricultural practices and farming systems. Jacobson<sup>[4]</sup>, in a study of the AMF distribution in an arid environment, found that soil moisture availability significantly influenced AMF population and colonization levels. The soil organic matter (OM) and CaCO<sub>3</sub> percentages enhanced the activity of the AMF while the effect of soil P was negative<sup>[2]</sup>. Soils developed under arid and semiarid conditions are generally low in organic matter, alkaline in reaction and mostly calcareous<sup>[5]</sup>. These soil properties limit P availability due to the precipitation and sorption reactions of P that keeps P concentration in soil solution very low<sup>[6]</sup> and therefore most crops grown on these soils suffer from P stress and

low fertility conditions. To overcome this problem farmers annually apply high rates of P fertilizers to calcareous soil resulting in accumulation and build up of high levels of soil P. This usually leads to lower fertilizer P use efficiency. Therefore, optimizing P availability and exploring strategies for enhancing fertilizer P use efficiency and utilization of residual P in the soil are critical components of cereal production management in calcareous soils.

AMF through symbiotic relationships with the plant can enhance plant growth and nutrient acquisition under low soil fertility levels through several possible mechanisms: Mycorrhizal plants can i) explore greater volume of soil beyond the zone of P depletion<sup>[7]</sup>, ii) lower the threshold concentration for absorption from soil solution<sup>[8]</sup>, iii) produce exudates which enhance the availability of P<sup>[9-11]</sup>, iv) alter the rhizosphere pH as a results of anion and cation absorption by the mycorrhizal plants, which may affect the availability of P to plant<sup>[12]</sup> and v) solubilize the organic P by the production of phosphatase<sup>[13]</sup>.

Positive influences of AMF colonization on wheat yield in P-deficient soils have been reported<sup>[14]</sup> and the low level of available P in soil was compensated by AMF colonization. Mycorrhizal plants had commonly higher growth and tend to accumulate more P and other immobile nutrients than do nonmycorrhizal plants, especially when grown on soil with low P availability<sup>[15]</sup>. On the other hand, heavy P fertilization of agricultural fields decreases AMF colonization and reduces Zn accumulation in wheat tissue<sup>[16]</sup>.

Plant growth responses to mycorrhizal inoculation can be affected by the rate and form of N and P fertilizers<sup>[17]</sup>. Most research on AMF has focused on evaluating shoot and yield response of different crops to AMF inoculation. Fewer studies have addressed root morphological parameters as related to nutrient acquisition and AMF colonization under different calcareous soil P levels. The objectives of this study were to evaluate shoot, root growth, nutrients uptake and pattern of colonization by mycorrhizal and non mycorrhizal wheat to application rates of fertilizer P on calcareous soil.

## MATERIALS AND METHODS

Surface soil (top 30 cm) that is fine-loamy, mixed, thermic, calcic Paleargid<sup>[18]</sup> was collected from the Research Center of Jordan University of Science and Technology. The soil was air dried and sieved through a 5 mm screen. Soil samples were analyzed for pH on 1:1 soil suspensions and for electrical conductivity in the 1:1 soil:water extracts<sup>[19]</sup>, for P by extraction with NaHCO<sub>3</sub><sup>[20]</sup> and organic matter by rapid oxidation<sup>[21]</sup>, soil texture analysis by the hydrometer method<sup>[22]</sup>. The soil is basic and alkaline, poor in organic matter, N and P. Available K is considered to be adequate for normal plant growth. Some of the major soil characteristics are shown in Table 1. The soil was fumigated with methyl bromide under air-tight plastic sheets and the fumigant allowed to dissipate for one week<sup>[23]</sup>.

Greenhouse experiment was conducted at 27±3°C under natural illumination. The treatments consisted of two factors: I) Five rates of P application (0, 50, 100, 200, 400 kg P ha<sup>-1</sup>) as triple super phosphate; and ii) Two levels of AMF inoculation (inoculated and non inoculated). For the inoculated treatments, the soil was inoculated with a mixture of AMF. The mycorrhizal inoculum mixture consisted of 100 CC of mixture of soil with the adhering spores and AMF-colonized chickpea root segments. The mixture contained 500±50 chlamydospores. The inoculum's mixture was thoroughly mixed with the middle third of the soil in the pot. The

Table 1: Selected properties of the soil

Soil characteristics	Surface soil
pH	7.93
EC (dS m <sup>-1</sup> )	0.72
N-NH <sub>4</sub> (mg kg <sup>-1</sup> )	6.34
N-NO <sub>3</sub> (mg kg <sup>-1</sup> )	14.70
P (mg kg <sup>-1</sup> )	7.34
K (mg kg <sup>-1</sup> )	564.00
CaCO <sub>3</sub> (%)	12.60
Organic matter (%)	0.81
Sand (%)	5.50
Silt (%)	61.00
Clay (%)	33.50

control treatments received the same quantity of non-inoculum soil to ensure the same microflora<sup>[23]</sup>.

Before planting, the soil in each pot (4 kg soil per pot) was watered with distilled water to field capacity and equilibrated. Wheat (*Triticum aestivum* cv. 'Horani') was then seeded at a rate of 10 seeds per pot where after germination they were thinned to five homogeneous plants per pot. All pots were amended with 125 mg N kg<sup>-1</sup> soil as ammonium sulfate. During the growing period each pot was periodically watered to maintain the soil moisture at approximate field capacity.

Aboveground portion of each plant was harvested at 7 weeks after planting, oven dried at 68°C and weighed. The plant tissue samples were analyzed for P in dry ash by calorimetric determination of P using a vanadate-molybdate-yellow method<sup>[24]</sup>. In addition, the trace elements in the digestate were measured with atomic absorption spectrophotometry. P uptake was measured by multiplying the DM by the P concentration, while the specific P uptake was calculated per unit of root surface area.

Two components of phosphorus use efficiency (PUE) were determined. Physiological PUE (PUE<sub>p</sub>) and agronomical PUE (PUE<sub>a</sub>) were calculated using the following formulas used by Pandey *et al.*<sup>[25]</sup> for component analysis of nitrogen use efficiency:

$$\begin{aligned} \text{PUE}_p &= (\text{DWF}-\text{DWC})/(\text{PUPf}-\text{PUPc}), \text{ kg kg}^{-1} \\ \text{PUE}_a &= (\text{DWF}-\text{DWC})/\text{PA}, \text{ kg kg}^{-1} \end{aligned}$$

Where:

DWF=dry weight of fertilized wheat; DWC=dry weight of nonfertilized (control) wheat; PUPf=P uptake by fertilized wheat; PUPc=P uptake by nonfertilized (control) wheat; PA=P applied

For determination of root morphology parameters, roots were extracted from each pot by washing with tap water, stained with methyl violet and the root length and root surface area were measured by edge discrimination analysis using flatbed desktop scanner<sup>[26]</sup>. Subsamples were taken from the roots and were fixed in a 90:5:5 (by volume) formaldehyde-acetic acid-ethanol solution. Using a modified version of Phillips and Hayman<sup>[27]</sup> method, root

samples were cleared in 2% KOH and stained in trypan blue and cut into 1 cm pieces. From each root sample, ten 1-cm pieces randomly selected and arranged parallel to each other on a microscope slide and the mycorrhizal root colonization was determined microscopically at 100X<sup>[28]</sup>. Five vision fields were examined in each 1-cm root section, therefore, 50 field visions were examined for each sample and the infection was recorded when hyphae, vesicles, or arbuscules were observed. The %age infection was calculated as a ratio the infected to the total section examined.

**Statistical analysis:** Data were statistically analyzed by analysis of variance using SYSTAT software program<sup>[29]</sup>. Probabilities of significance were used to test for significance among treatments and LSDs ( $P < 0.05$ ) were used for means separation.

## RESULTS AND DISCUSSION

AMF colonization %age was observed only when AMF was inoculated (Fig. 1). Sterilization of soil resulted on absence of AMF colonization in the non-inoculated soil. AMF colonization decreased at higher rates of P. Lower colonization at higher P rates has been reported by others<sup>[17]</sup> and was attributed mainly to the fact that under P stress the plant roots responded by exudation of organic compounds that believed to enhance spore germination and therefore, colonization. Such exudation under high soil P levels is absent. On the other hand, other researchers reported that application of up to 50 kg P ha<sup>-1</sup> increased AMF colonization activity on infertile soils low P soils<sup>[30]</sup>. The mycorrhizal dependency of wheat genotypes with relatively high P efficiencies was lower than that of the genotypes with lower P efficiencies<sup>[31]</sup>.

Addition of P increased wheat shoot DM of both M and NM plants, however, the patterns of their response were different (Fig. 1). Inoculation with AMF increased shoot DM at lower and moderate P application rates (50 and 100 kg P ha<sup>-1</sup>) while, at higher rates (200 and 400 kg P ha<sup>-1</sup>), the beneficial effect of AMF inoculation was not observed. When P was not added, both M and NM plants gave similar shoot DM. This indicates that AMF was not efficient under both very low and very high soil P levels. Although at these higher rates of P the maximum DM was maintained, the AMF colonization was suppressed and therefore, the increase in DM was solely due to P application. Growth depression has been reported for some mycorrhizal legume crops at high P fertilization levels<sup>[32]</sup>. However, the highest DM was achieved at lower P rates for M than NM plants. As

Table 2: Arbuscular mycorrhizal fungi (AMF) colonization, root length and root surface area of wheat as affected by P rates and AMF inoculation

AMF	P (kg P ha <sup>-1</sup> )	Root length (cm plant <sup>-1</sup> )	Root surface area (cm <sup>2</sup> plant <sup>-1</sup> )
M	0	674.3c*	95.3d
	50	938.7d	111.9d
	100	1274.1b	145.7bc
	200	1237.6bc	137.2bc
	400	1092.4cd	126.8cd
NM	0	646.8e	70.9e
	50	887.4d	93.6d
	100	1345.4b	158.2b
	200	1703.1a	203.5a
	400	1788.2a	210.8a

Table 3: Micronutrient uptake by wheat as affected by P rates and Arbuscular Mycorrhizal fungi (AMF) inoculation

AMF	P (kg P ha <sup>-1</sup> )	Fe uptake (ug plant <sup>-1</sup> )	Mn uptake (ug plant <sup>-1</sup> )	Cu uptake (ug plant <sup>-1</sup> )	Zn uptake (ug plant <sup>-1</sup> )
M	0	160.7f	133.2a	6.5d	44.7d
	50	278.8c	198.8a	13.0c	71.8b
	100	417.6a	323.5a	20.4a	89.3a
	200	332.3b	271.1a	16.6b	79.2b
	400	266.5c	257.0a	10.4cd	52.0d
NM	0	125.2e	135.0a	6.0d	31.7e
	50	227.8d	143.9a	7.4d	61.8c
	100	285.0c	180.1a	8.4cd	64.8bc
	200	315.8bc	236.0a	12.6c	78.9b
	400	265.5c	247.5a	10.3c	49.7d

\* Means with different letters within each column are significantly different at the 0.05 probability level; M=mycorrhizal wheat; NM=non mycorrhizal wheat

shown in Fig. 1, the addition of 100 kg P ha<sup>-1</sup> with AMF was adequate to give DM as high as that obtained when 200 kg P ha<sup>-1</sup> was applied without AMF. Therefore, AMF maintained the high DM while reducing the P inputs<sup>[6]</sup>. This may illustrate the agronomic, economic and environmental advantages of the AMF inoculation through enhancing plant growth, decreasing cost of fertilizers P and minimizing possible adverse effect of the continuous overfertilization with P. The slope of the DM response to P rates when AMF was inoculated is positively steeper within the linear range of the polynomial curve and reached the maximum value faster and at lower P rates. On the other hand, when AMF was not added, the slope was less steeper and remained linear over a wider range of P rates.

Root length increased with increasing P rates up to 100 kg P ha<sup>-1</sup> for the M plants and up to 200 kg P ha<sup>-1</sup> for the NM plant then stayed the same after the addition of the highest two rates (Table 2). AMF inoculation had no effect on root length at the zero, 50 and 100 kg P ha<sup>-1</sup> rates, while at the highest two P rates (200 and 400 kg P ha<sup>-1</sup>) root length decreased with AMF inoculation. Root surface area increased with increasing P rates for the NM plants. On the other hand, root surface area of M plants increased only at the highest P rates. Root surface area for the M plants was higher when no P

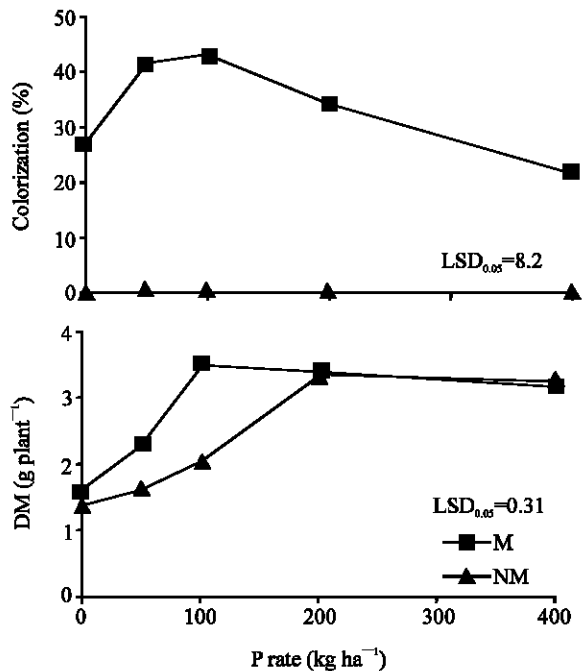


Fig. 1: Colonization % and dry matter (DM) of wheat as affected by P rates and mycorrhizal inoculation (M=mycorrhizal; NM=nonmycorrhizal plants)

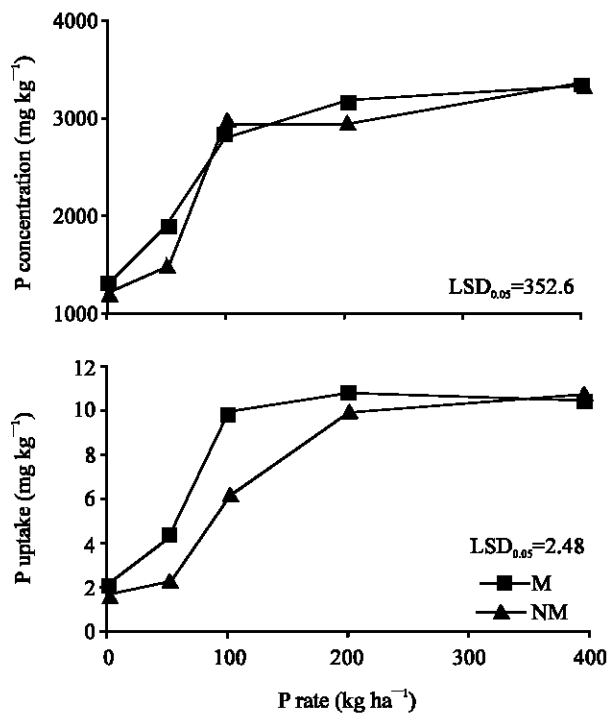


Fig. 2: Concentration and uptake of P by wheat as affected by P rates and mycorrhizal inoculation (M=mycorrhizal; NM=nonmycorrhizal plants)

was added (control), similar when 50 or 100 kg P ha<sup>-1</sup> were added or lower when 200 or 400 P kg P ha<sup>-1</sup> were added than the for the NM plants. Higher root length of wheat with AMF inoculation under low soil P condition has been reported in earlier study<sup>[14]</sup>.

Addition of up to 100 P kg ha<sup>-1</sup> increased shoot P concentration of both M and NM plants (Fig. 2). Shoot P concentration was higher in the M plant only at the 50 kg P ha<sup>-1</sup> rates. Phosphorus uptake was higher for M plants at lower P rates (50 and 100 kg ha<sup>-1</sup>) but remained the same for both M and NM plants at the highest two rates and at the control zero P treatments (Fig. 2). Increased P absorption is usually attributed to increased surface area and increased soil exploration by the root-AMF association<sup>[6]</sup>. Yao *et al.*<sup>[11]</sup> reported that under low soil P, mycorrhizal wheat translocated more carbohydrate to the roots promoting higher hyphal length and density which consequently enhanced P uptake. However, increased absorption rate by M plants was due to increased absorption site affinity and not from surface area since affinity is independent of surface area<sup>[6,33]</sup>. When surface area is higher by AMF then the mechanism will be exploration of larger soil volume.

For NM plants, there was a strong linear relationship between P uptake and the root surface area (R<sup>2</sup>=0.98) (Fig. 3). On the other hand, the relationship in the M plant

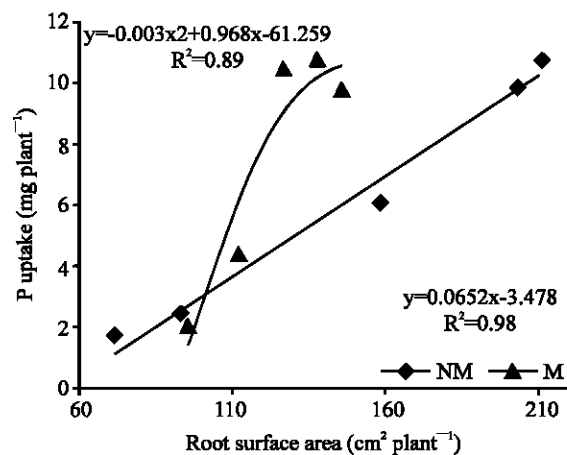


Fig. 3: P uptake response of mycorrhizal and non-mycorrhizal wheat to root surface area

followed the second degree of the quadratic polynomial relationship. Having such relationship, where the response tend to levels off (saturation zone) at low values of surface area (130 cm<sup>2</sup>/plant), may indicate that this low surface area was supported by the mycorrhizal hyphae that increases the actual absorption surface area for the plant roots which was not detected by the techniques used for measuring root parameters. In another word, the measured surface area of 130 for the M plant was

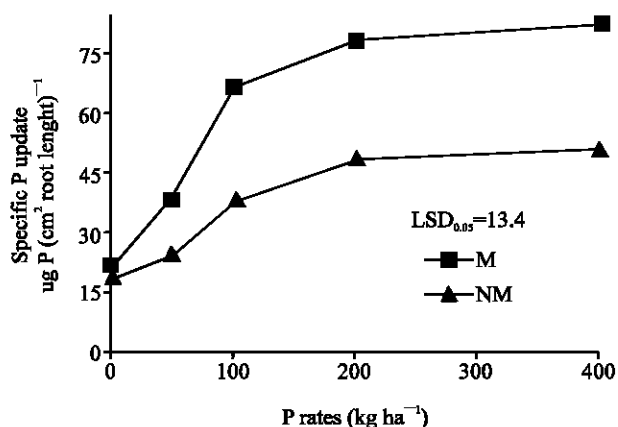


Fig. 4: Relationship between root surface area and specific P uptake for mycorrhizal and non-mycorrhizal roots

equivalent to the 180 measured for the NM plants. Such explanation is supported by the higher specific P uptake obtained for the M plants (Fig. 4). At all rates of P application, the values of specific P uptake were higher for the M than for the NM wheat. Therefore, the uptake or absorption efficiency for the M roots was higher. Higher specific P uptake by mycorrhizal barley roots grown in calcareous soils has been reported by other researchers who concluded that AMF played a large role in P uptake<sup>[34]</sup>.

The uptake of iron and Zn were higher in the M plants at the control (zero P) and lowest P rates (50 and 100 kg P ha<sup>-1</sup>) (Table 3). At higher P rates (200 and 400 kg P ha<sup>-1</sup>), both M and NM plants had similar iron and zinc uptake. Copper uptake was higher for the M plant when 50, 100 and 200 kg P ha<sup>-1</sup> were added. However, when no P or when the highest rate (400 kg P ha<sup>-1</sup>) was added both M and NM plants had similar values of copper uptake. Manganese uptake was not affected significantly neither by AMF inoculation nor by the P rates. Reports on acquisition of micronutrients are not consistent. Greater acquisition of Fe, Zn and Cu but lower Mn has been reported in mycorrhizal plants<sup>[15-16]</sup>. The mycorrhizal plants had similar concentration but higher content of shoot P and Mn compared to the nonmycorrhizal plants<sup>[35]</sup>. They also reported that mycorrhizal plants had higher concentration and contents of Fe, Zn and Cu than the nonmycorrhizal plants. Although increasing absorption surface area of roots has been reported as one of the mechanisms by which M plants are more efficient in nutrient absorption. However, such increase in surface area is due to mainly

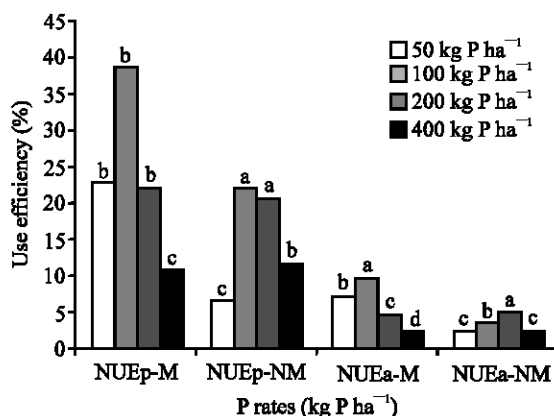


Fig. 5: Phosphorus use efficiency of mycorrhizal and non-mycorrhizal wheat as affected by the P rates (PUE=P use efficiency; M=mycorrhizal; NM=non-mycorrhizal; p=physiological; a=agronomical)

hyphae that can not be detected by the techniques used for measuring root parameters. It has been reported that since the P concentration is usually very low in the soil solution of calcareous soils and the diffusion rate of P in calcareous soil is very low, the depleted zone around the growing root can not be adequately replenished. Therefore, the root-external AMF hyphae of M wheat grown far beyond the depleted zone can explore more soil volume and absorb P that is position ally unavailable to NM wheat<sup>[30]</sup>.

Physiological use efficiency (PUEp) was higher for mycorrhizal wheat at the lowest two P rates (50 and 100) (Fig. 5). At higher P rates (200 and 400), PUEp was similar for both M and NM wheat. On the other hand M wheat has the highest PUEp at P rate of 100 kg P ha<sup>-1</sup> then decreased at higher P rates (200 and 400) and at the lowest rate (150). PUEp for the NM wheat remained the highest at 100 and 200 P rates then decreased at both higher and lower rates than these rates (Fig. 5). A general trend for both M and NM wheat can be observed that the PUEp increased by increasing the P rates from 50 to 100, then decreased with increasing P rates above the 100. Similar trends were observed for the agronomical P use efficiency (PUEa). However, the values of the PUEa were lower than those of PUEp. Lower values of PUE has been reported for crops grown in calcareous soils<sup>[36]</sup> and were attributed to the higher capacity of calcareous soils to fix P in an unavailable forms through adsorption and precipitation reaction. Therefore, obtaining higher PUE for M wheat may indicate that they are more efficient in P absorption and utilization of soil P that are unavailable to NM wheat.

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