Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.10 (1)pp. 21- 35 (2018)*

Egypt. Acad. J. Biolog. Sci., 10(1): 21-35 (2018)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Phosphorus Efficiency of Wheat (*Triticum aestivum*) Genotypes Inoculated with Mycorrhizal Fungi under Calcareous Soil Conditions

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ARTICLE INFO
Article History
Received:13/1/2018
Accepted:25/2/2018

Keywords:
wheat genotypes-
P levels-
mycorrhizal fungi-
calcareous soil

ABSTRACT

This research aimed to study P efficiency of six wheat genotypes *(Triticum aestivum)* inoculated with mycorrhizal fungi under three levels of P under calcareous soil conditions and to quantify the contribution of root growth and hyphae length in P uptake. Plants were grown in pots with three levels of P supply in soil P0 (without P fertilizer), P1 (50% of recommended P fertilizer, 75 mg P/kg soil), and P2 (100% of recommended P fertilizer, 150 mg P/kg soil). Half of the pots were treated with mixed mycorrhizal species (*Glomus intraradices* and *Glomus macrocarpium*) in with six replicates in randomized complete block design. The wheat genotypes were harvested 85 days after planting. The results showed that the response of shoot dry weight of wheat genotypes without mycorrhizae to increase of P supply was vigorous except genotype V6. In contrast, all the wheat genotypes inoculated with mycorrhizal fungi were less. All wheat genotypes with mycorrhizal inoculation except V4 with and without mycorrhizal and V6 without mycorrhizal attained more than 80% of its maximum shoot yield already at the lower level available. So all wheat genotypes with mycorrhizal inoculation were considered to be P efficient except V4 and all genotypes without mycorrhizal were regarded as P inefficient except V6. The wheat genotype V6 had extensive roots growth in limited P supply (P0) and the shorter roots observed in wheat genotype V4. The wheat genotype V6 had long hyphae length as compared to other wheat genotypes whereas the shorter hyphae length observed in wheat genotype V4. The adaptation of wheat genotypes to low level of soil P is closely related to better improved root system and the increase in the mycorrhizal hyphae length may be two of the main possible mechanisms of P efficiency in wheat. At low and high P level, the genotype V6 was given the highest values with and without mycorrhizal inoculation followed by V3 and V2, whereas genotype V4 was given the lowest values of the shoot P uptake followed by V5. In contrast, the results of this study suggested that root growth system and mycorrhizal hyphae length would be suitable parameters for selecting P efficient wheat genotypes especially under limited P supply.

INTRODUCTION

Wheat is one of the main cereal crops in Egypt and the world. In the world, wheat ranks the second crop passing only by rice. Phosphorus (P) is one of the main macro-essential elements necessary for plant growth and reproduction. Phosphorus is necessary to all forms of life because of its genetic function in nucleic acids and role in biological energy transfer via ATP. Ozanne (1980) reported that P is one of the main yield limiting factors in many arid and semi-arid regions. To solve this problem, the addition of commercial P fertilizers was the main common recommendation. However, there are different concerns associated with the application of commercial fertilizers in general and P fertilizers in special (Marschner, 2011).

In calcareous soils, $CaCO_3$ has a dominating influence due to its properties of relatively moderate solubility, higher buffer capacity, and alkalinity. Under such conditions, the predominant phosphates are the inorganic phosphates, which constitute as much as 90–95% of total soil phosphates. Organic P forms constitute the remaining 5–10% of total soil P. In calcareous soils, phosphorus may be immobilized by several mechanisms (FAO, 1984) which led to reduce the P availability. The solution according to Marschner, (1995) is the use of A-mycorrhizal fungi (AMF) that have the ability to obtain P and produce high yield under limited available P.

The treatment of host plants with arbuscular mycorrhizae (AM) fungi enhances the availability of nutrients as P to plants, especially when phosphorus is the limits growth (Smith and Read, 2010). Most studies approved the assistance of A-mycorrhizae fungi to P uptake efficiency (Sawers *et al.,* 2017). That could be due to the mycorrhizal hyphae which have the very small radius, about 1.5 µm (Tinker *et al.,* 1992), that means large surface area of the hyphae, leading to extension of the nutrients absorbing surface area especially P, the realization of organic acids and phosphatase enzyme which catalyzes the release of P from P organic components (Aono *et al.,* 2004). Plant species and genotypes vary in their ability to grow under low available phosphorus in soil, i.e. they differ in their P efficiency (Bhadoria *et al.,* 2002; Rose and Wissuwa, 2012). Phosphorus efficiency can be generally defined as the ability of the plants to achieve a specific percentage of their maximum yield (80% of maximum yield) at low level available P in soil (Fohse *et al.,* 1988).

This work aimed to select the efficient wheat genotypes inoculated with mycorrhizal fungi under calcareous soils conditions to improve the wheat crop yield.

MATERIALS AND METHODS

Soil:

The surface soil sample was collected from Abd El Baset village, Burg Al Arab, Alexandria, Egypt. The soil was air-dried, sieved through a 2 mm sieve, and roots were removed from the soil then mixed before using to homogenize. Main soil physical and chemical properties are presented in Table (1). The methods used for soil analyses were those described by Page *et al.* (1982).

The applications of N and k fertilizers were corporate with each kg soil at a rate of 150 mg N as NH_4NO_3 and 150 mg K as K_2SO_4 per Kg soil. The N fertilizer was added at three equal doses at the rate of 50 mg/20 ml water for each pot. The K fertilizer was added before filling the pots with soil. Three levels of phosphorus fertilizer were added to obtain P0 (without P fertilizer), P1 (50% of recommended P fertilizer (75 Kg calcium superphosphate (15.5% P_2O_5)/fed.)), and P2 (100% of recommended P fertilizer (150 Kg calcium superphosphate/fed.)) were added before planting to obtain three levels of available P as determined by sodium bicarbonate method (Olsen *et al.,* 1954).

Table (1): Some initial soil physical and chemical properties.

Values	Soil properties
Mechanical analysis	
Clay %	22.88
Silt %	8
Sand %	69.12
Soil texture	Sand clay Loam
pH (1:1)	8.3
EC dSm^{-1} (1:1)	1.3
Total $CaCO_3$ (%)	21.05
Organic matter (%)	0.59
Soluble Cations and Anions (meq/ L)	
Calcium	2.3
Magnesium	2.32
Sodium	6.5
Potassium	0.9
Bicarbonate	1.88
Chloride	5
Sulphate	4.27
Available macronutrients, (mg/ kg soil)	
Nitrogen	113.3
Phosphorus	7.8
Potassium	230

Arbuscular Mycorrhizae:

The mixture of two mycorrhizal species (*Glomus intraradices* and *Glomus macrocarpium*) which is used in this experiment, was obtained from Germany and activated in the soil microbiology lab, Soil and Agriculture Chemistry Department, Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria, Egypt. The first specie of mycorrhizal fungi (*G. intraradiaces*) was obtained from Hanover University, Germany; and the second specie of mycorrhizal fungi (*G. macrocarpium*) was obtained from Gottingen University, Germany.

Hyphae Length (m/g dry soil):

The length of mycorrhizal hyphae in the soil was measured using the grid interception method after collecting the hyphae on nitrate cellulose filters with grid (Brundrett et a l., 1994).

Pot Experiment:

Plastic pots were filled with 1.0 kg of the calcareous soil leaving the upper 5 cm without soil. Six genotypes of wheat *(triticum aestivum)* which are used in this study were obtained from the Agricultural Research Center Nubaria, Ministry of Agriculture and Land Reclamation, Egypt and their detailed information is given in Table 2.

Table 2: List of wheat genotypes and experimental code numbers used in the experiment.

Genotypes	**Sakha 93**	**Gemmeiza 11**	**Egypt 2**	**Sides 13**	**Gemmeiza 7**	**Gemmeiza 12**
Code	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**

Three seeds of wheat genotypes were sown in each hill on 22 /12 /2015. The seedlings were thinned to one healthy and uniform plant per hill after 16 days from planting date. In mycorrhizal pots, the soil was mixed with 20 ml of mixed mycorrhizae (*Glomus intraradices* and *Glomus macrocarpium*) one week before planting as described by Malibari *et al.,* (1990). Also, 10 ml inoculums were added with the seedlings at transplanting time, (in total the rate of 500 spores per pot). The wheat genotypes were harvested 85 days after planting and the shoots were separated from roots. The shoots were washed by distillated water and then dried at 70^{o}C for 48 hours (Steyn, 1959) to constant weight and then weighed and milled for analysis. Samples of plant material were wet digested with H_2SO_4–H_2O_2 (Lowther, 1980) and phosphorus content was determined by vanadomolybdophosphoric method (Jackson, 1967).

Root length was measured using the line intersect method according to Tennant (1975) method.

Statistical Analysis:

The collected data were arranged in a randomized complete block design and replicated six times. Data were statistically analyzed for ANOVA and means comparison to fulfill the significance according to (Steel and Torrie, 1982). A significance level of α = 0.05 was used in all data.

RESULTS AND DISCUSSION

1- Plant Dry Matter and Shoot P Concentration:

Distinct differences in plant dry weight were observed among the genotypes at low and high P levels (Fig. 1). The response of genotypes without mycorrhizae to increase of P supply was vigorous except genotype V6 whereas the same genotypes inoculated with mycorrhizal fungi has less response to increase P level (Fig. 1). This is in line with Neumann *et al.,* (2001) who reported that the application of P fertilizer significantly improved plant growth. However, when the soil was inoculated with mycorrhizal fungi; the effect of fertilization on plant growth remained less pronounced. Treatment of plants with arbuscular mycorrhizae (AM) fungi enhances the availability of P to plants, especially when phosphorus supply of soils limits growth (Smith and Read, 2010).

At low and high P level, the data showed that wheat genotype V6 was given the highest shoot dry weight with and without mycorrhizal inoculation as compared to other wheat genotypes and followed by genotypes V3 and V2, whereas genotype V4 was given the lowest values of shoot dry weight followed by genotype V5. At low P level, the plant dry weight of wheat V6 treated with AMF had about 2.3 fold higher than V4 whereas, untreated wheat genotype V6 had about 3 fold higher than untreated wheat genotype V4.

Also, at low P level there was highly significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes. In contrast, at high P level there was no significant difference between plants treated and untreated with mycorrhizal fungi (Fig. 1). This is in line with the suggestion of Teste *et al.,* (2016) who reported that mycorrhizal root associations are stimulated by P deficiency in the soil, but are suppressed by high P supply. Ratnayake *et al.,* (1978) attributed the inhibition of mycorrhizal growth at high P level to increased phospholipids levels, which reduce membrane permeability and decrease exudation of organic acids, amino acids, and sugars, which are the main source of growth and development of mycorrhizal spores germination.

With regard to the plant dry weight, nutrient efficiency is the power of a plant to produce higher plant yield at low level of phosphorus in soil (Gourley *et al.,* 1994). Also, may be defined as the ability of a plant to achieve specific percentage of its top yield (80% of maximum yield) at low level of P (Föhse *et al.,* 1988). Based on this definition, all wheat genotypes treated with mycorrhizal fungi were considered to be P efficient except the wheat genotype V4 was regarded as P inefficient and all wheat genotypes without mycorrhizal inoculation were regarded as P inefficient except the wheat genotype V6 was considered to be P efficient (Fig. 2). In other words, the wheat genotype V6 treated and untreated with mycorrhizal fungi was P efficient at low P level. In contrast, the P deficiency genotype was V4 which has shoot yield less than 80% at low P level in both treated and untreated with mycorrhizal fungi compared to the same genotype at high P level (maximum shoot yield).

Relative yield was measured to compare the differences between shoot dry weight at low and high P levels. All wheat genotypes with mycorrhizal inoculation except V4 and V6 with and without mycorrhizal attained more than 80% of its maximum shoot dry weight (Fig. 2). Several authors (Fageria and Costa, 2000; Bhadoria *et al.,* 2002) reported that plant species or even genotypes of the same plant species varied in their power to grow on the low available soil. In the same line, Akter (2003) found that clear differences were observed among the potato genotypes grown in low available P in soil.

The shoot P concentration of all wheat genotypes inoculated and uninoculated with mycorrhizal fungi increased with increasing P levels (Fig. 3). The response of all wheat genotypes without mycorrhizae to increase P supply was strong whereas that of genotypes inoculated with mycorrhizal fungi was less (Fig. 3). Mycorrhizal hyphae extended considerable distances into the soil and because of their large surface areas can acquire P, which would be unavailable to the plant without this association (Hetrick, 1991).

At low P level, there was highly significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes. The A-mycorrhizal fungi spread a network of hyphae some centimeters out the surrounding soil, thereby developing the effective soil volume that plant can develop (Frank, 2002). On the other hand, at high P level there was no significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes (Fig. 3). That could be due to high amount of available P in soil which prevents both spore germination and the growth mycorrhizal hyphae (Miranda and Harris, 1994). Also, that could be impute to rise of phospholipids levels, which reduce membrane permeability and decrease exudation of organic acids, amino acids, and sugars, which are the main sources of food for developing of mycorrhizal growth (Ratnayake *et al.,* 1978). This is in line with (Abou El Seoud, 2008) who reported that the effect and growth of mycorrhizal fungi at high available P are limited.

At low P level, it can be noticed that genotype V6 was given the highest values with and without mycorrhizal inoculation and followed by genotypes V3 and V2, whereas genotype V4 with and without mycorrhizal fungi was given the lowest values of the shoot P concentration.

The P concentration of V6 inoculated with mycorrhizae at low P level was significantly higher by about 1.2 fold than the same genotype without mycorrhizae at the same P level. In contrast, the P concentration of V4 inoculated with mycorrhizae at low P level was highly significant than the same genotype without mycorrhizae at the same P level by about 1.9 fold. This means that the response of genotype V6 to inoculate with mycorrhizae was less than the genotype V4 at low P level.

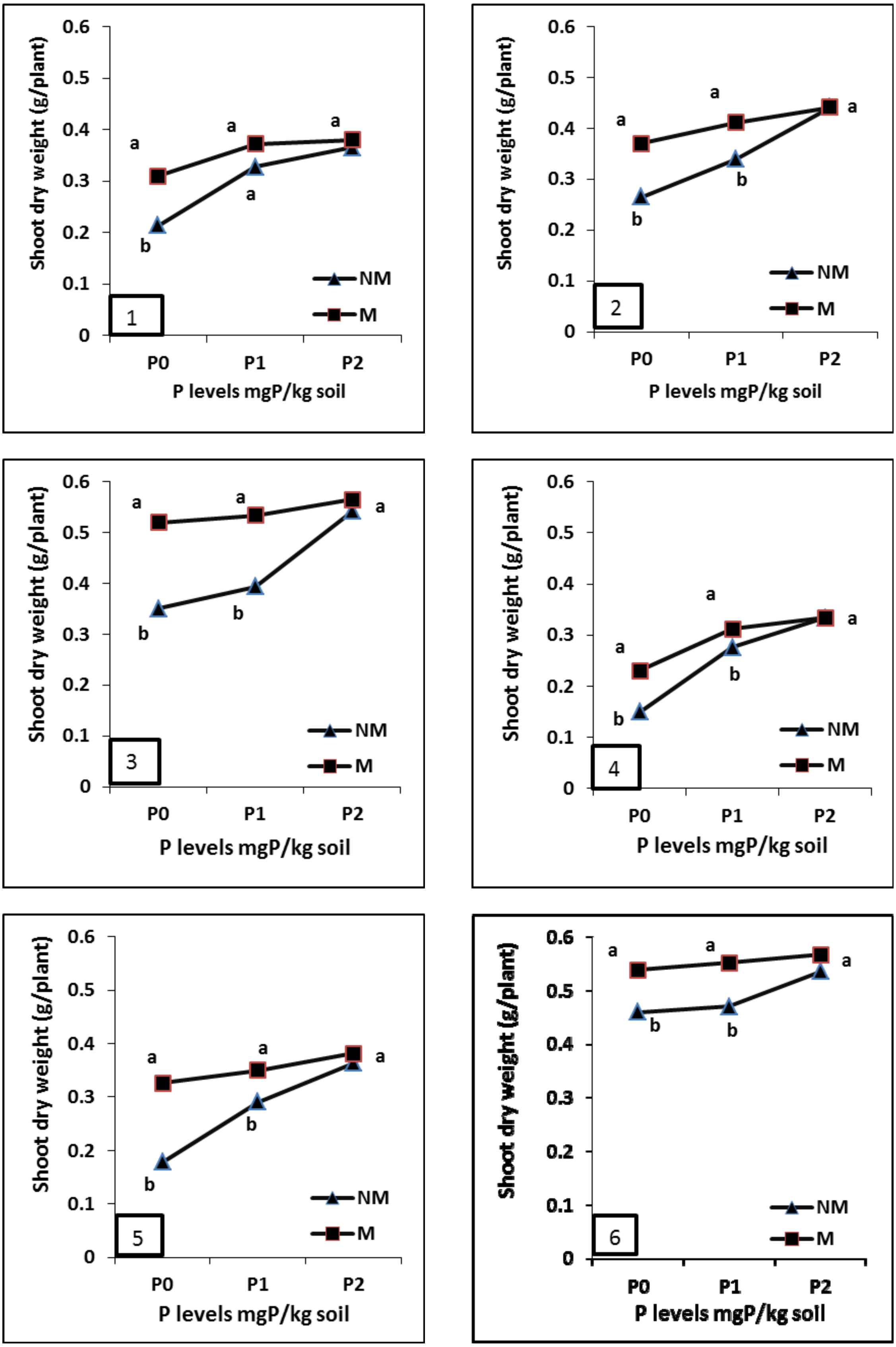

Fig. 1: Shoot dry weight (g/plant) of wheat genotypes as affected by mycorrhizal inoculation and P supply. (different letters indicate significant differences between NM and M; $P \leq 0.05$; NM = without mycorrhizal fungi, M = inoculated with mycorrhizal fungi).

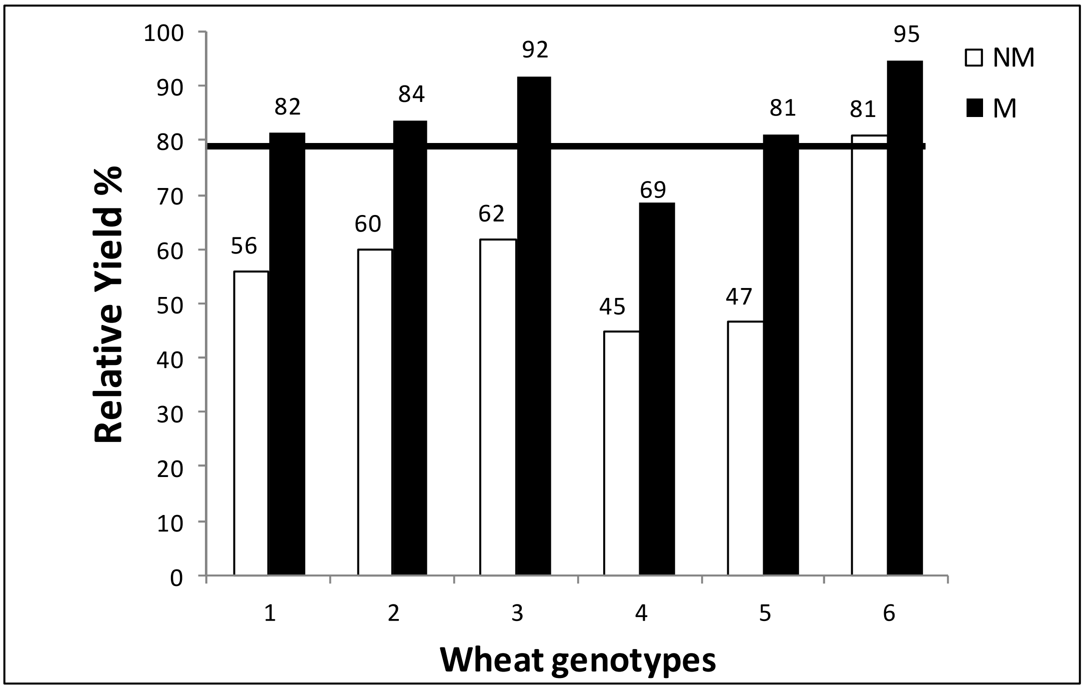

Fig. 2: Relative yield (%) of wheat genotypes as affected by P supply.

2-Wheat Root Growth and Mycorrhizal Hyphae Growth:

2.1 Wheat Root Growth

The root length of all wheat genotypes treated and untreated with mycorrhizal fungi increased with increasing P supply (Fig. 4). Similarly, Bloom *et al.,* (1993) reported that if the rhizosphere is needy in nutrients, root growth is short, with enhancing rhizosphere conditions the growth of root becomes more extensive. Bhadoria *et al.,* (2002) reported that increase in the growth of the root under low available of P might be one of the possible mechanisms of P efficiency in plants.

At low P level, there was highly significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes except V4, there was no significant difference. On the other hand, at high P level there was no significant difference between all plants with and without mycorrhizal inoculation in all wheat genotypes (Fig. 4). Similarly, Abou El Seoud, (2005) reported that the root length of plant treated with mycorrhizal fungi was significantly extensive than that of the untreated with mycorrhizal fungi at the low available P in soil. Also, Moradi *et al.,* (2013) reported that the root length of Chickpea plants was developed with inoculation plants with mycorrhizal fungi at low available P under calcareous soil conditions.

The wheat genotype V6 had extensive roots in limited P supply (P0) compared to the other wheat genotypes whereas the short roots observed in wheat genotypes V4. The adaptation of wheat genotype V6 to low available P in soil is closely related to better improved root growth which is able to take up high amount of P from the soil and obtain greater yields than those having short root system (Abou El Seoud and Wafaa, 2010). That may be one of the main possible mechanisms of P efficiency (Ozturk *et al.,* 2005). This result affirms the proposition that efficient plants growing in low available P in soil tend to have extensive roots (Manske *et al.,* 2000; Dechassa, 2001; Gaume *et al.,* 2001). This mechanism is an adaptation of plants to increase their uptake efficiency when P is a main limiting growth factor. Also, Alves *et al.,* (2001) showed that the acclimation of maize genotypes to low available P in soil is closely related to a better improved in root system.

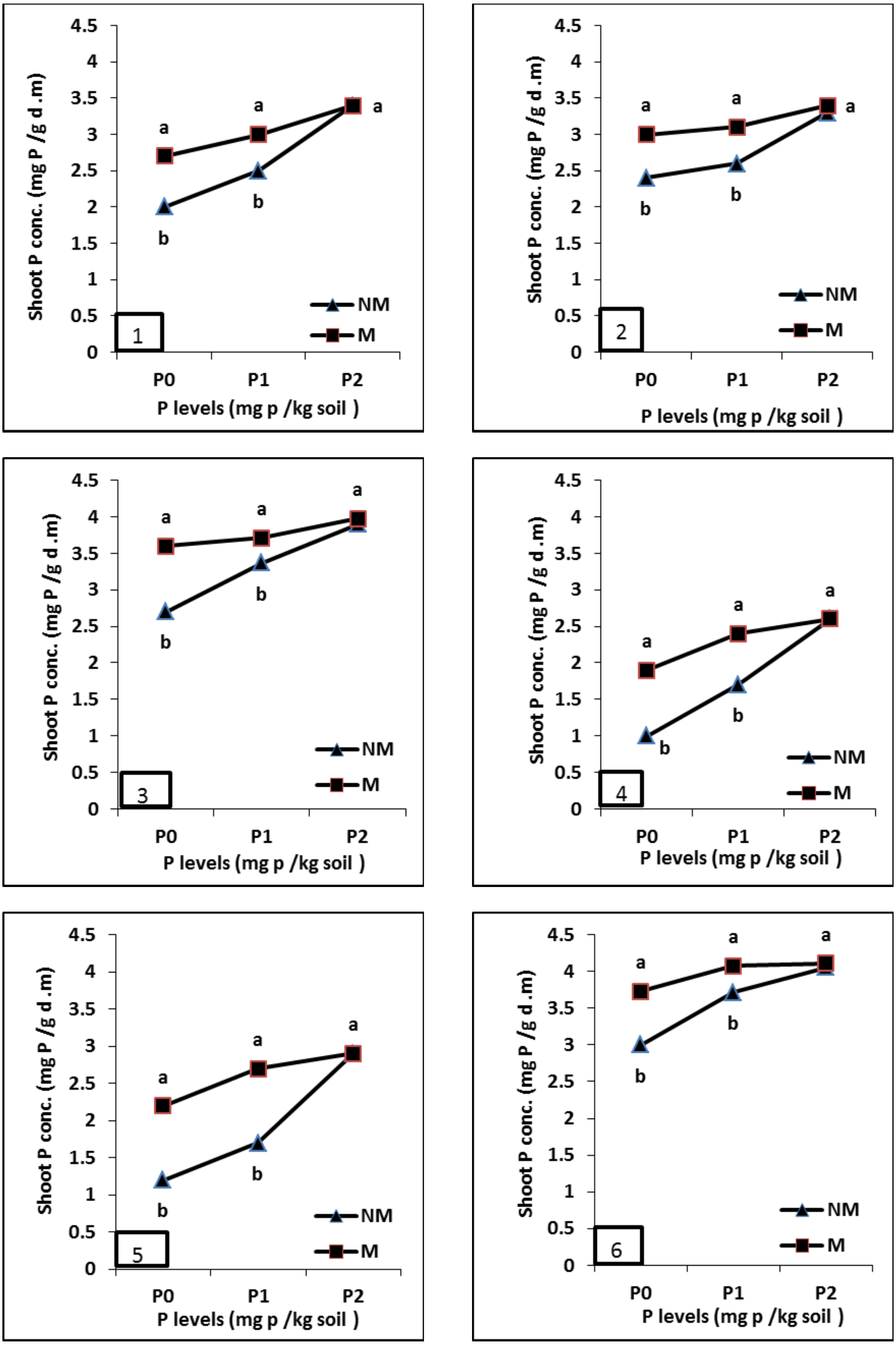

Fig. 3: Shoot P concentration (mg P/g d.m.) of wheat genotypes as affected by mycorrhizal inoculation and P supply. (different letters indicate significant differences between NM and M; P ≤ 0.05; NM = without mycorrhizal fungi, M = inoculated with mycorrhizal fungi).

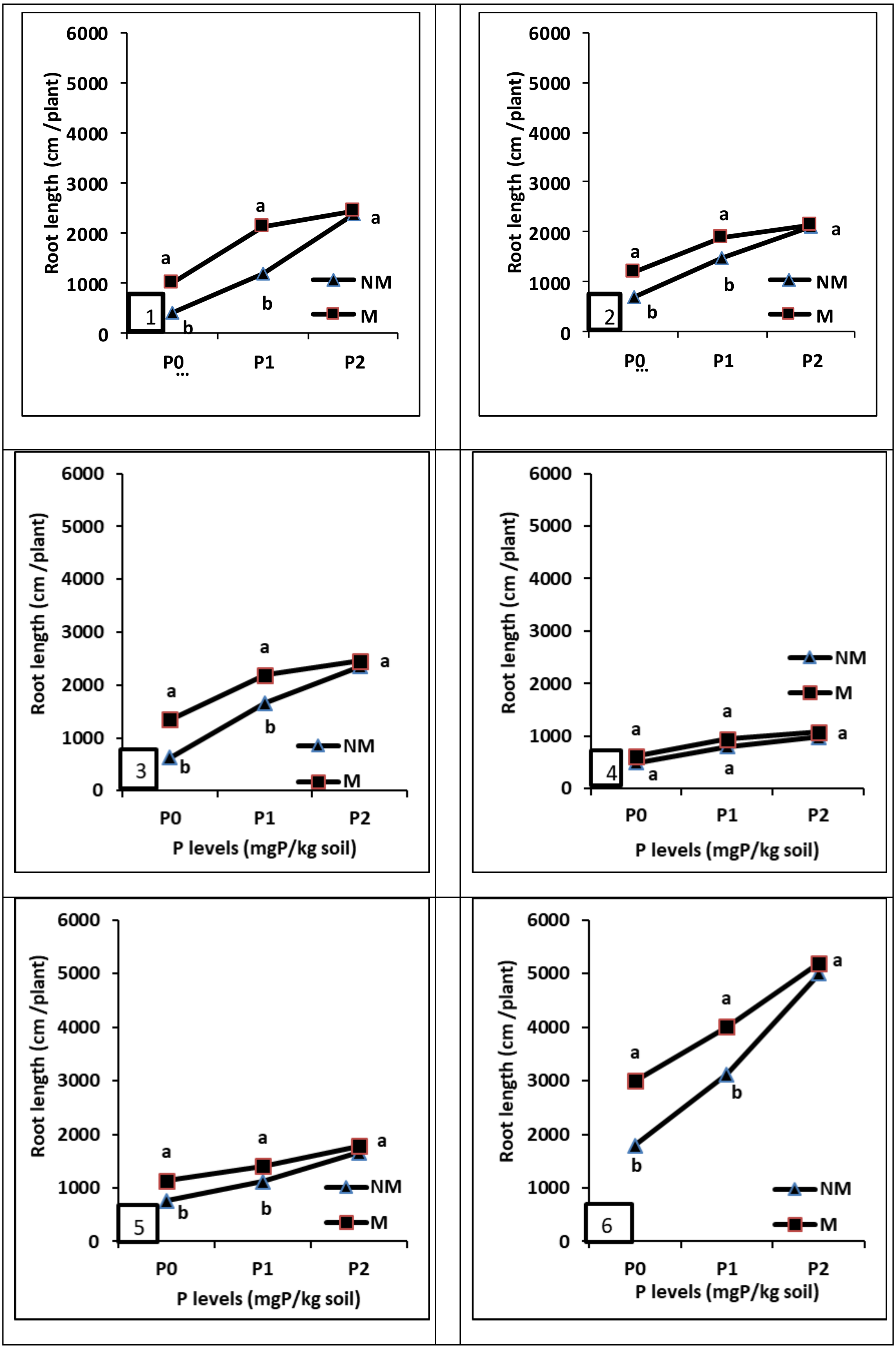

Fig.4: Root length (cm/plant) of wheat genotypes as affected by inoculation with mycorrhizae and P supply. (different letters indicate significant differences between NM and M; $P \leq 0.05$; NM = without mycorrhizal fungi, M = inoculated with mycorrhizal fungi).

2.2 Mycorrhizal Hyphae Length

At low P level of P, there was a highly significant difference between all wheat genotypes. In contrast, there was no significant difference between all wheat genotypes at high P level. On the other hand, the mycorrhizal hyphae length at high P level was shorter than the lower P level. In other words, the mycorrhizal hyphae length of all wheat genotypes decreased with increasing P levels in soil as show in (Fig. 5). Similarly, Deressa and Schenk, (2008) found that the amount of extra radical mycorrhizal hyphae produced in soil were greatest at the lowest available P level in soil. The highest level of P application decreased mycorrhizal hyphae length as compared to the lower P level. That could be due to the addition of phosphate fertilizer decreased root membrane permeability, and subsequently the amount of amino acids and sugars were reduced in root exudates. Then the mycorrhizae activities are reduced and the mycorrhizal hyphae length decreased. The external mycorrhizal hyphae extend considerable spaces around the soil and because of their high surface areas they can take up P from the soil, which would be unavailable to the plant root without this association (Hetrick, 1991).

The wheat genotype V6 had long hyphae length as compared to the other wheat genotypes whereas the shorter hyphae length was observed in wheat genotypes V4 (Fig. 5).

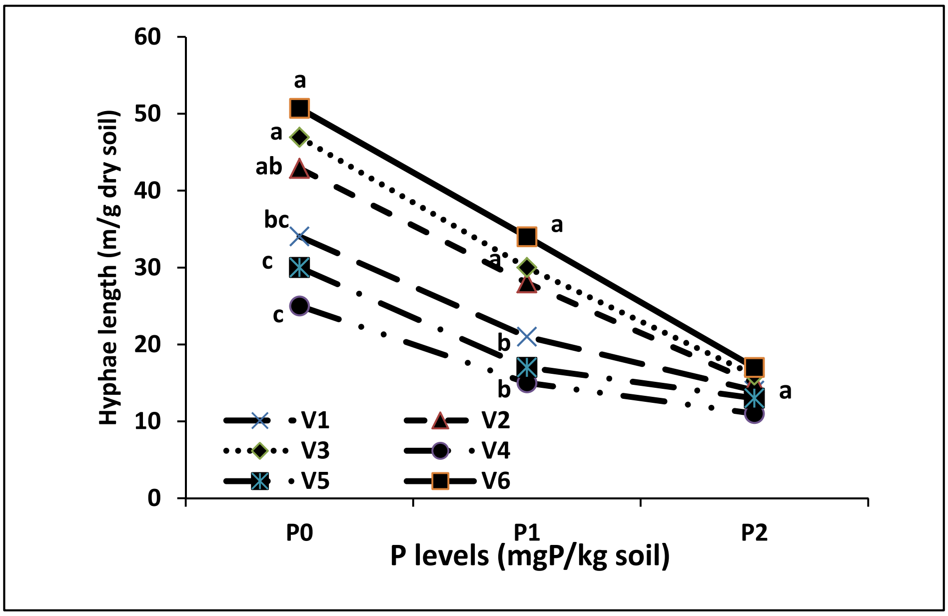

Fig. 5: Hyphae length (m/g dry soil) of wheat genotypes as affected by P supply (different letters indicate significant differences between different wheat genotypes $P \leq 0.05$; M= inoculation with mycorrhizal fungi).

3- Phosphorus Uptake:

At low P level, there was highly significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes. On the other hand, at high P level there was no significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes (Fig. 6). Mycorrhiza may improve nutrient uptake by decreasing the distance between nutrients must diffuse and plant roots (Dania *et al.*, 2013; Liu *et al.*, 2014). Also, the distribution of mycorrhizal hyphae in soil where the roots are absent (the micro-sized cross-section of the mycorrhizal hyphae facilitates the permeation of much smaller soil pore where plant roots cannot penetrate), as well as the high contact between the hyphae and the soil contributes largely to improve nutrient and water uptake (Joubert and Archer, 2000).

Phosphorus uptake by all wheat genotypes with and without mycorrhizae was increased with increasing P supply. The response of untreated plants to increase P levels was higher than the other plants treated with AMF (Fig. 6). Similarly, Alam *et al.,* (2003) observed development in the value of P take up by wheat plants when increasing the P addition to the soil.

At low and high P level, the data showed that genotype V6 was given the highest amount of shoot P uptake treated and untreated with mycorrhizal fungi followed by V3 and V2, whereas genotype V4 was given the lowest values of the shoot P uptake followed by V5. That could be due to the increase root growth and mycorrhizal hyphae length of efficient wheat genotypes as V6 which leads to develop P depletion zone, compared to the other inefficient genotypes as V4 which had few root growth and short mycorrhizal hyphae length. In the same line, Eticha (2000) reported that the efficient cabbage genotype plants which take up more P from the soil under low P addition.

In conclusion, the results of this study suggested that root growth system and mycorrhizal hyphae length would be main suitable parameters for selecting the P efficient wheat genotypes especially under limited P supply and under the same conditions of this experiment.

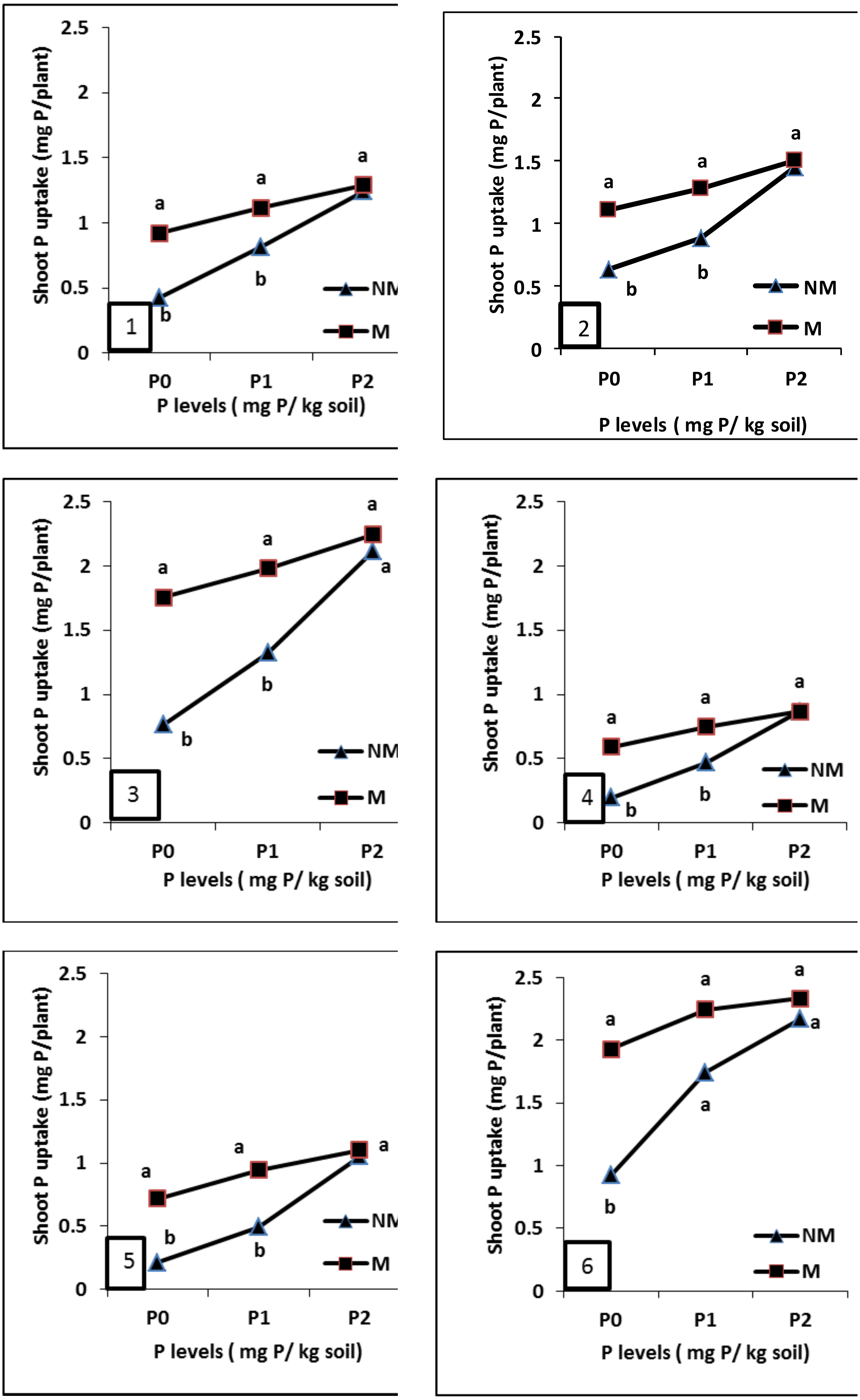

Fig. 6: P uptake of all plant (mg P/ plant) of wheat genotypes as affected by P supply (different letters indicate significant differences between NM and M; P $\leq$ 0.05; NM = without mycorrhizal fungi, M = inoculated with mycorrhizal fungi).

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ARABIC SUMMARY

كفاءة الفوسفور لأصناف القمح الملقحة بفطر الميكوريزا تحت ظروف الأراضى الجيرية

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يهدف هذا البحث الى دراسة كفاءة الفوسفور لستة أصناف من القمح الملقحه بفطر الميكوريزا عند ثلاث مستويات من الفوسفور المتاح فى أرض جيرية وذلك لتقييم أثر مساهمة نمو الجذر وطول هيفات فطر الميكوريزا فى إمتصاص الفوسفوربنباتات القمح. تم إنماء النباتات بأصص معبأه بالتربه المسمده بثلاث مستويات من الفوسفور: المستوى الاول p_0 (بدون اضافة) المستوى الثانى p_1 (50% من الجرعة الموصى بها) المستوى الثالث p_2 (100% من الجرعة الموصى بها) . و قد تم تلقيح نصف عدد الأصص بخليط من أنواع فطر الميكوريزا (*Glomus intraradices* and *Glomus macrocarpium*) وترك النصف الآخر بدون تلقيح. تم تنفيذ التجربة بتصميم قطاعات عشوائية كاملة مع ست مكررات. تم حصاد نباتات القمح بعد 85 يوم من الزراعة. ومن أهم النتائج المتحصل عليها أستجابة الوزن الجاف للمجموع الخضري لجميع اصناف القمح الغيرملقحة بفطر الميكوريزا استجابة عالية لزيادة مستويات الفوسفورباستثناء الصنف 6. في حين أن جميع الاصناف الملقحه بفطر الميكوريزا اعطت استجابة أقل. جميع أصناف القمح الملقحة بفطر الميكوريزا أعطت أكثر من 80% من المحصول الاعظم عند المستوي المنخفض من الفوسفورباستثناء الصنف 4 وعلي العكس جميع أصناف القمح غير الملقحة بفطر الميكوريزا أعطت أقل من 80% من المحصول الاعظم عند المستوي المنخفض من الفوسفورباستثناء الصنف 6. لذلك جميع أصناف القمح الملقحة بفطر الميكوريزا تعتبر ذات كفاءة للفوسفور باستثناء الصنف 4 وجميع أصناف القمح بدون فطر الميكوريزا تعتبر ذات كفاءة أقل للفوسفور باستثناء الصنف 6. الصنف6 أعطي أطول مجموع جذري عند امداد فوسفوري محدود بينما الصنف 4 أعطي أقصر مجموع جذري. صنف القمح 6 أعطي أكبر طول لهيفات فطر الميكوريزا مقارنة بالاصناف الاخري بينما صنف القمح 4 كان أقصر الاصناف لطول هبفات فطر الميكوريزا.ومن النتائج يتضح ان هناك تأقلم أصناف القمح مع المستويات المنخفضة من الفوسفور بالتربة قد يرجع لتطور أفضل في النظام الجذري وزيادة طول الجذر وزيادة طول هيفات فطر الميكوريزا تحت الاجهاد الفوسفوري قد تكون اثنين من الاليات الرئيسية الممكنة لكفاءة الفوسفور بنبات القمح.عند مستوي الفوسفور المنخفض والمرتفع كان الصنف 6 في وجود وعدم وجود الميكوريزا هو الأعلي في امتصاص الفوسفور ثم الصنف 3و2 وعلي العكس كان الصنف 4 في وجود وعدم وجود الميكوريزا هو الأقل في امتصاص الفوسفور ويسبقه الصنف 5. في النهاية فان نتائج هذه الدراسة تشير الي أن النظام الجذري وطول هيفات فطر الميكوريزا هما عاملان مناسبان لأختيار صنف القمح الأكفأ خاصة تحت ظروف الامدات المنخفضه من الفوسفور.