

## **ADAPTIVE MECHANISMS OF WHEAT GENOTYPES IN LOW P SUPPLY**

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### **ABSTRACT**

The objective of this study was to elucidate some possible adaptive mechanisms governing P-use efficiency of two wheat varieties. To achieve this objective, two pot experiments were conducted; in the first experiment two wheat varieties differing in P-use efficiency (Gemmaiza 10 representing the efficient and responsive varieties and Giza 168 representing the non-efficient and non-responsive varieties) were grown in P-deficient soil at three P-levels; low (without P application) moderate (50 mg P<sub>2</sub>O<sub>5</sub> / kg soil) and high (75 mg P<sub>2</sub>O<sub>5</sub> / kg soil) for 90 days after sowing. Plant samples were collected at 35, 55 and 90 days after sowing. The results of this experiment showed that both shoots and roots dry weight differed significantly between the two wheat varieties at each P-level and during all growth stages. The highest shoots and roots dry weight were recorded by Gemmaiza 10 at 75 mg P<sub>2</sub>O<sub>5</sub>/ kg soil during all growth stages. Gemmaiza 10 had higher root/shoot ratio at all P-levels and during all growth stages and the highest values of root/shoot ratio were recorded at low P level. During early growth stage (35 days old), Gemmaiza 10 had longer roots at low P-level and the root length decreased with increasing P-levels, while the reverse was true for Giza 168. However, during later growth stages (55 and 90 days old) the root length of the two wheat varieties increased by increasing P-levels where Gemmaiza 10 had much longer root at all P-levels compared with Giza 168. At each P-level, and during all growth stages, Gemmaiza 10 had larger root surface area and higher root fineness values than Giza 168.

On the other hand, at low P-level and during the early growth stage (35 days old), Gemmaiza 10 accumulated more amounts of P in both shoots and roots compared with Giza 168. Increasing P application induced an increase in P-content by roots and shoots of the two wheat varieties during all growth stages and this effect was more pronounced in Gemmaiza 10.

In the second experiment, the same two wheat varieties used in the first experiment were grown in the same P-deficient soil which used in the first experiment at two P-levels, low (without P application) and high (75 mg P<sub>2</sub>O<sub>5</sub>/ kg soil) for 35 days after sowing. Soil rhizosphere samples were collected at 21, 28 and 35 days after sowing to determine pH values and organic acids concentration in soil rhizosphere.

The results of this experiment showed that the lowest pH values were recorded in soil rhizosphere of Gemmaiza 10 at low P-level during all growth stages. The lower pH in soil rhizosphere of Gemmaiza 10 (efficient variety) could be one of the strategies for increasing P-uptake under condition of P-deficiency. Organic acids exudation were greatly stimulated in both wheat varieties by P-deficiency during early growth stage (21 days after sowing) and this effect diminished as the plant advanced in age. The increases of organic acids exudation in soil rhizosphere were greater for the efficient variety (Gemmaiza 10) than for the non-efficient variety (Giza 168).

## INTRODUCTION

Faced with the challenging task of increasing agricultural production in P-deficient soils and maintaining the environment, scientists have tried to select plant species and genotypes which can absorb P efficiently from soil and utilize applied P economically. In this respect, Ram *et al* (2000) postulated that plant species differ in the efficiency with which they acquire and utilize phosphorous. They clarify that phosphate efficiency relates to the different extents to which plants are able to mobilize phosphorous from poorly soluble sources or to take up the soluble phosphorous available in the soil solution.

There are several possible mechanisms for P-efficient varieties to overcome soil P-deficiency including root system enlargement (Lynch, 1995), increased organic acid exudation (Johnson *et al*, 1996), rhizosphere acidification (Moorby *et al.*, 1988) and enhanced phosphate uptake rate (Schachtman *et al.*, 1998).

Plants that are efficient at acquiring phosphorous often enhance P-uptake by modifying root structure. Great biomass allocation to the roots will increase the volume of soil that can be explored. Similarly, thinner roots or formation of more or longer root hairs increase the surface area for P-uptake (Horst *et al*, 1996).

The biosynthesis, accumulation, transport and root exudation of organic acids is dramatically increased in response to environmental stress. Root exudates may affect P solubility by changing the pH in the rhizosphere or organic components desorbed from the soil solid phase (Gerke *et al.*, 1994) or from soluble P complexes (Gardner *et al.*, 1983).

Under P deficiency, plants exude a wide range of organic and inorganic compounds. Acidification of rhizosphere by excretion of protons causes dissolution of plant-unavailable P forms, such as rock phosphate and Ca-P complexes in calcareous soils (Yan *et al.*, 1996). They also added that rhizosphere acidification is more prominent in P-efficient plant species and genotypes within the species than in their P-inefficient counterparts.

The present study was conducted to elucidate the mechanisms of P-efficiency by comparing the differences in shoot and root growth, P-content, rhizosphere acidity and root exudates between efficient and non-efficient wheat varieties.

## MATERIALS AND METHODS

Tow pot experiments were conducted to elucidate the possible adaptive mechanisms of P-use efficiency of two wheat cultivars differing in P-use efficiency under P-deficient soil

### 1 First experiment:

A pot experiment was carried out to evaluate the role of root growth in P-uptake efficiency from P-deficient soil. To achieve this goal, two wheat cultivars differing in P-use efficiency were selected according to the results of a screening experiment (El-Kherbawy *et al*, 2007), the two wheat cultivars were; Gimmaiza 10 (efficient and responsive) and Giza 168 ( non-efficient and non-responsive). The two wheat cultivars were grown under greenhouse

conditions in plastic pots containing 5 kg soil. The soil used was deficient in plant available P and obtained from El-Nubaria, Behera Governorate. Concentration of  $\text{NaHCO}_3$ - extractable P in soil was 3 mg P/kg soil as measured by the method of Olsen *et al* (1954). The soil characteristics were pH 7.8 (1:1 soil water ratio), organic matter percent was 0.2 determined by oxidizing with chromic acid according to Walkley and Black(1934). Total calcium carbonate percent was 4.1 estimated using Collins Calcimeter according to Wright (1939) and the texture of tested soil was sandy.

Phosphorous added at three levels, low without P- application medium (50 mg  $\text{P}_2\text{O}_5$  /kg soil) and high (75 mg  $\text{P}_2\text{O}_5$  /kg soil) using single supper phosphate (15%  $\text{P}_2\text{O}_5$ ), together with basal dose of 75 mg N/kg soil as ammonium sulphate (20.5 % N) and 24 mg  $\text{K}_2\text{O}$ /kg soil as potassium sulphate (48%  $\text{K}_2\text{O}$ ). All fertilizers were mixed thoroughly with soil before planting. The treatments were performed in four replicates according to complete randomized design. About 20 seeds of both Gemmaiza 10 and Giza 168 wheat cultivars were sown separately in each pot, and after germination, seedlings were thinned to 13 plants per pot. Soil moisture content was kept near field capacity during the experimental period which extended to 90 days from sowing. Plant sample were collected at 35, 55 and 90 days from sowing. Evaluation of the tested cultivars and P-treatments was carried out through the following parameters: Dry weight of shoots and roots (recorded after oven drying at  $70\text{ }^\circ\text{C}$ ), root / shoot ratio, phosphorous content in shoots and roots (as described by Jackson, 1974), root length (fresh roots used for root length measurements by using the method described by Newman ,1966), root surface area (RSA) was calculated using the equation:  $\text{RSA} = 2 \pi \times \text{root length} \times \text{root fineness}$  which can be expressed as mean root diameter, but the root length/root volume ratio has the advantage of including the functionally important parameter, root length. Assuming a specific weight of the root similar to that of water ( $1\text{mg} = \text{mm}^{-3}$ ) Root volume in  $\text{mm}^3$  corresponds to root fresh weight in mg. (Ryser and Lambers, 1995). Thus root fineness = root length/root fresh weight

**b) Second experiment:**

A pot experiment was conducted to evaluate the role of rhizosphere pH and organic acids exudates in efficient P-uptake from P-deficient soil. The two wheat cultivars used in the previous experiment were grown in the same P-deficient soil which used in the first experiment. The levels of phosphorous were low (without P application) and high (75 mg  $\text{P}_2\text{O}_5$ / kg soil). A complete randomized design was used in a factorial arrangement, and treatments were replicated three times. All agricultural practices were carried out typically as in the first experiment.

Soil rhizosphere was collected at 21, 28 and 35 days from planting to determine pH value and organic acids exudation in the soil rhizosphere, the entire root system from all plants in each pot was treated as one unit, soil rhizosphere was separated from bulk soil by gently shaking the root system.. Soil adhering to the root system was defined as rhizosphere soil (Rovira and McDougall, 1967).The pH was determined in the rhizosphere. To determine organic acids concentration, the roots were transferred to a beaker with a known amount of 0.2 mM  $\text{CaCl}_2$ , and gently shaken to remove the soil

rhizosphere. A sub sample of the soil rhizosphere extract was taken and filtered into 1ml HPLC vial and analyzed by HPLC as described by (Madeleine et al, 2004).

Analysis of variance was performed with the SAS statistical package (SAS Institute, 1991).

## RESULTS AND DISCUSSION

### 1-First experiment:-

#### 1-1- Roots and shoots dry weight:

Effect of various P-levels on roots and shoots dry weight of the two cultivars, Gemmaiza 10 and Giza 168; during three growth stages (35, 55 and 90 days old) was illustrated in fig. (1).

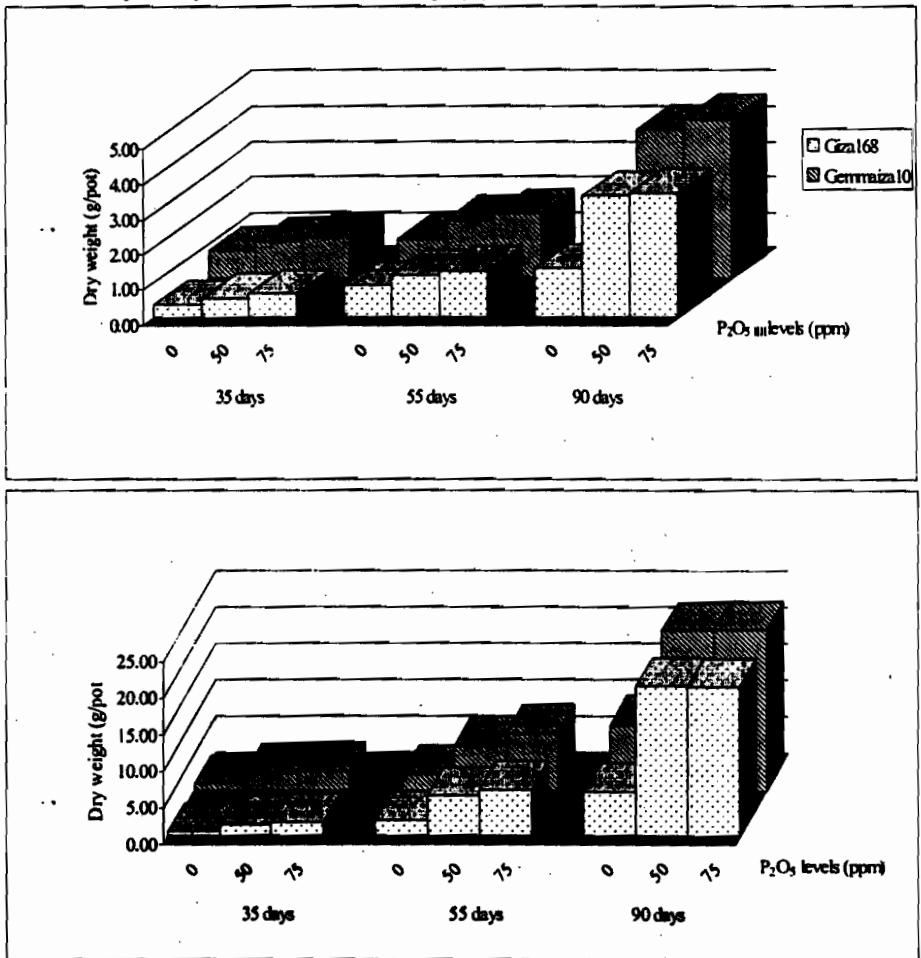
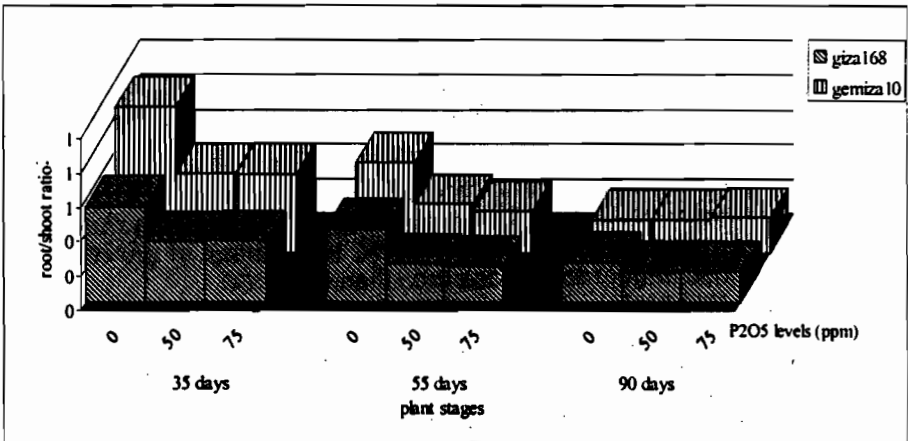


Fig. 1: Effect of different phosphorous levels on root /shoot ratio dry weight of the two wheat varieties (g/pot)

It could be noticed from the obtained data that, there were significant differences in roots and shoots dry weight between the two varieties at each P-level and during all studied growth stages. The highest root and shoot dry weight during all examined growth stages, were recorded by Gemmaiza 10 at 75 ppm  $P_2O_5$ . This confirm the results obtained by Chunqin *et al* (2002) as it indicated that wheat plant responded to increasing amounts of soil P, but the magnitude of the response varied markedly among varieties.

**1-2 –Root/shoot ratio:**

Calculation of root/shoot ratio (fig.2) revealed significant differences in this ratio between various P- levels. It was noticed that increasing P levels diminished the differences between varieties in root/shoot ratio. Gemmiza10 had higher root/shoot ratio at all P levels and all growth stages. However it could be observed that the highest root/shoot rition were recorded at low p-level which indicated a large contribution by retarded shoot growth than root growth. This was in harmony with the finding of Sveinn and Paul (1988). Root /shoot ratio decreased by increasing P-levels and as the plant advanced in age, which revealed that shoot biomass increased more than root biomass with increasing P-levels. The same trend was obtained by Alain *et al* (2001). In this respect, Rufty *et al*, (1993) demonstrated that, when P supply is deficient, P-efficient genotype favor root growth, which leads to larger root/shoot ratios.



**Fig. 2: Effect of different phosphorous levels on root /shoot ratio for the two wheat varieties**

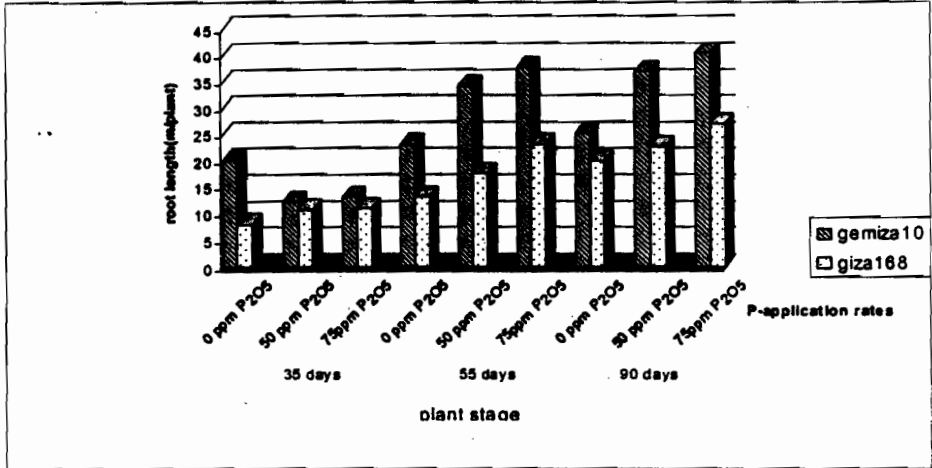
**1-3- Root morphology:**

Some root characters, including the size and structure of the root system, appear to affect P-acquisition efficiency (Bates and Lynch, 1996). Therefore, it was desirable to determine the morphological differences in the root system among selected varieties to detect its potential correlations with the observed differences in P-use efficiency.

**1-3-1 Root length:**

Root length of the two cultivars was found to be affected by P levels in the soil. The data presented in figure (3) illustrated that, during early growth

stage (35 days old) Gemmaiza 10 (which is efficient and responsive varieties) had longer roots at low P level and the root length decreased by increasing P- levels, while the reverse was true for Giza 168 ( which is non-efficient and non-responsive). This was in accordance with Linkohr *et al.* (2002) who identifier that high root length are decisive for efficient P acquisition from soil. However during later growth stages (55, and 90 days old), the root length of the two cultivars increased by increasing P levels, but in Gemmaiza 10 it was much longer at all P levels compared to Giza 168.



**Fig.3: Effect of different phosphorous levels on root length of two wheat varieties (m/plant)**

**3-1-3-6- Root surface area:**

The results obtained from the current study concerning root surface area (table 1) indicated that at each P-level, and during all growth stages, Gemmaiza 10 had larger root surface area than Giza 168.

**Table (1) : Effect of different phosphorous levels on root surface area (mm<sup>2</sup>/plant) of two wheat varieties**

Plant Age	Wheat varieties	Phosphorous levels (ppm P <sub>2</sub> O <sub>5</sub> )			mean
		0	50	75	
35 days	Gemmaiza10	11269.33	9604.96	10659.00	10511.09
	Giza 168	5604.37	7847.71	8600.852	7350.98
	mean	8436.85	8726.33	9629.926	
		P-level (L)	wheat V	LxV	
		ns	ns	ns	
55 days	Gemmaiza10	13406.79	21757.00	24387.95	19843.70a
	Giza 168	9174.62	12310.49	17750.02	13078.3b
	mean	11290.7b	17033.7a	21058.98a	
		P-level (L)	wheat V	LxV	
		2993.8	2444.42	ns	
90 days	Gemmaiza10	17000.32	25360.89	27771.76	23377.6 a
	Giza 168	13841.54	18163.22	21029.07	17677.7 b
	mean	15420.9c	21762.05b	24400.415a	
		P-level (L)	wheat V	LxV	
		775.66	633.32	895.31	

Root surface area=2 π r<sub>0</sub> L where r<sub>0</sub> is root radius(mm)and L is the root length (mm)

These differences were significant only at third growth stage (90 days). In this respect Ram *et al* (2000) postulated that the presence of long roots with large surface area in efficient genotype might well explain the superior phosphorous acquisition efficiencies exhibited by this genotype when grown in sparingly soluble phosphate source.

**3-1-3-7- Root fineness:**

Root fineness can be expressed as mean root diameter, but the root length/ root volume ratio has the advantage of including the functionally important parameter, root length. The root length/root volume ratio reflects not only the mean root diameter, but also the pattern of diameter distribution (Ryser and Lamber, 1995). Root fineness was significantly increased by decreasing P-levels, during all growth stages, as recorded in table (2), which was in accordance with Krisztina *et al* (1997) who illustrated that, longest and finest root system might be an adaptation of plants to P stress. Moreover, at each P-level, Gemmaiza 10 had higher root fineness values than Giza 168. The differences between the two varieties were significant at all P-levels during whole growth period except during early growth stage at 50 and 75 ppm P<sub>2</sub>O<sub>5</sub> levels, and during late growth stage (90 days) at 0 P-levels, where the differences were not significant. Moreover, for both varieties, the root fineness values were decreased by increasing P-levels. In this respect, Marschner (1998) postulated that the roots become finer in P-deficient plants, which can be considered a strategy for enhancing phosphorous acquisition from soil.

**Table 2 : Effect of different phosphorous levels on root fineness (mm/mm<sup>3</sup>) of two wheat varieties**

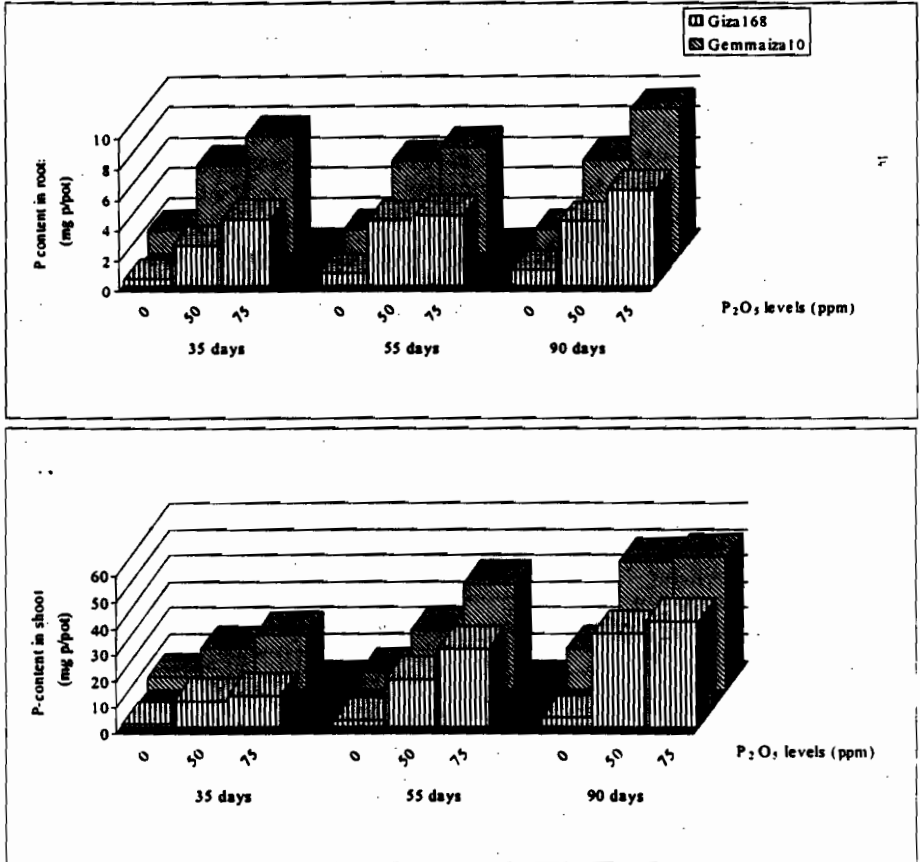
Plant Age	Wheat varieties	Phosphorous levels (ppm P <sub>2</sub> O <sub>5</sub> )			
		0	50	75	mean
35 days	Gemmaiza10	39.1	20.19	19.87	26.32 a
	Giza 168	26.26	23.79	20.44	23.50 b
	mean	32.68 a	21.99 b	20.06 b	
	(L)	V	LxV		
55 days	Gemmaiza10	37.47	31.08	30.29	32.94 a
	Giza 168	25.81	26.30	21.73	24.62 b
	mean	31.64 a	28.69 b	26.01 c	
	(L)	V	LxV		
90 days	Gemmaiza10	27.897	26.84	26.72	27.12 a
	Giza 168	26.01	19.53	20.73	22.089 b
	mean	26.95 a	23.18 b	23.68 b	
	(L)	V	LxV		
		1.962	1.602	2.360	

Root fineness = root length mm/root volume mm<sup>3</sup>

**3-1-4-- Phosphorous content:**

Concerning the effect of various P levels on P-content by the two cultivars, the data presented in figure (4) revealed significant increase in P-uptake by root and shoot of Gemmaiza 10 reached 3.5 and 5.0 fold respectively compared to Giza 168 at low P level during early growth stage

(35 days from sowing). It could be noticed also that increasing P levels induced an increase in P uptake by root and shoot of the two cultivars during all growth stages, but this effect was more pronounced in Gemmaiza 10. These was in accordance with Chunqin *et al.*(2002) who demonstrated that the P-efficient genotype maintained relatively high P-concentrations in roots and shoots even under conditions of P deficiency. They added that, having relatively high P concentration in tissue could be one reason why P-efficient genotype can grow well in low available P-soil.



**Fig.4: Effect of different phosphorous levels on phosphorous content in roots and shoots of two wheat varieties (mg p/ pot)**

**2- Second experiment:**

**2-1 Rhizosphere pH and organic acid exudates:**

**2-1-1- Rhizosphere pH:**

Regarding rhizosphere pH, it could be observed from the data presented in figure (5) that there were slight differences in pH values between the two wheat varieties, the lowest pH value was recorded in soil rhizosphere of Gemmaiza 10 at unfertilized soil during all growth stages. In this respect, Yan *et al* (2004) postulated that the efficient and responsive wheat and maize



genotype, when grown with P-deficient, markedly reduced the pH of rhizosphere soil.

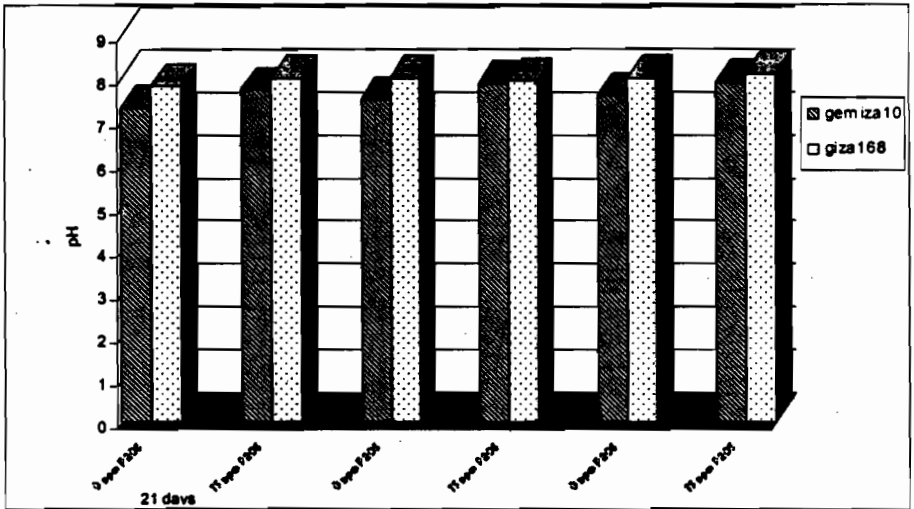


Fig 5: Effect of different phosphorous application levels on pH in the rhizosphere soil for two wheat varieties

**3-2-2- Organic acids exudate:**

Release of organic acids from wheat plant roots was generally higher under P-starvation condition than with 75 ppm P<sub>2</sub>O<sub>5</sub> treatment (fig.6.) Furthermore, the release of oxalic and malic acids (both were efficient in P mobilization from soil as demonstrated by Jones and Darrah,(1994) was significantly higher under P-deficiency particularly during early growth stage. In this respect Patricia *et al* (1997) and Rengel (1999) postulated that under P deficiency, plants exude a wide range of organic and inorganic compounds to increase mobilization of P from sparingly soluble sources.

The interaction effect of P-levels and wheat varieties revealed that organic acid exudation was greatly stimulated in both varieties by P-deficiency during early growth stage (21 days from sowing). These effect diminished as the plant advanced in age. P-starvation caused three fold increases in the total amount of organic acid excaudate by Gemmiza10 compared to 75 ppm P<sub>2</sub>O<sub>5</sub> fertilization level, during early growth stage (21 days). This was in accordance with Pratap *et al.* (2003) who demonstrated that organic acids were found to be more in rhizosphere at initial stages of crop and Pratap and Datta (2004) who reported that maximum root influx rate decreased with plant age.

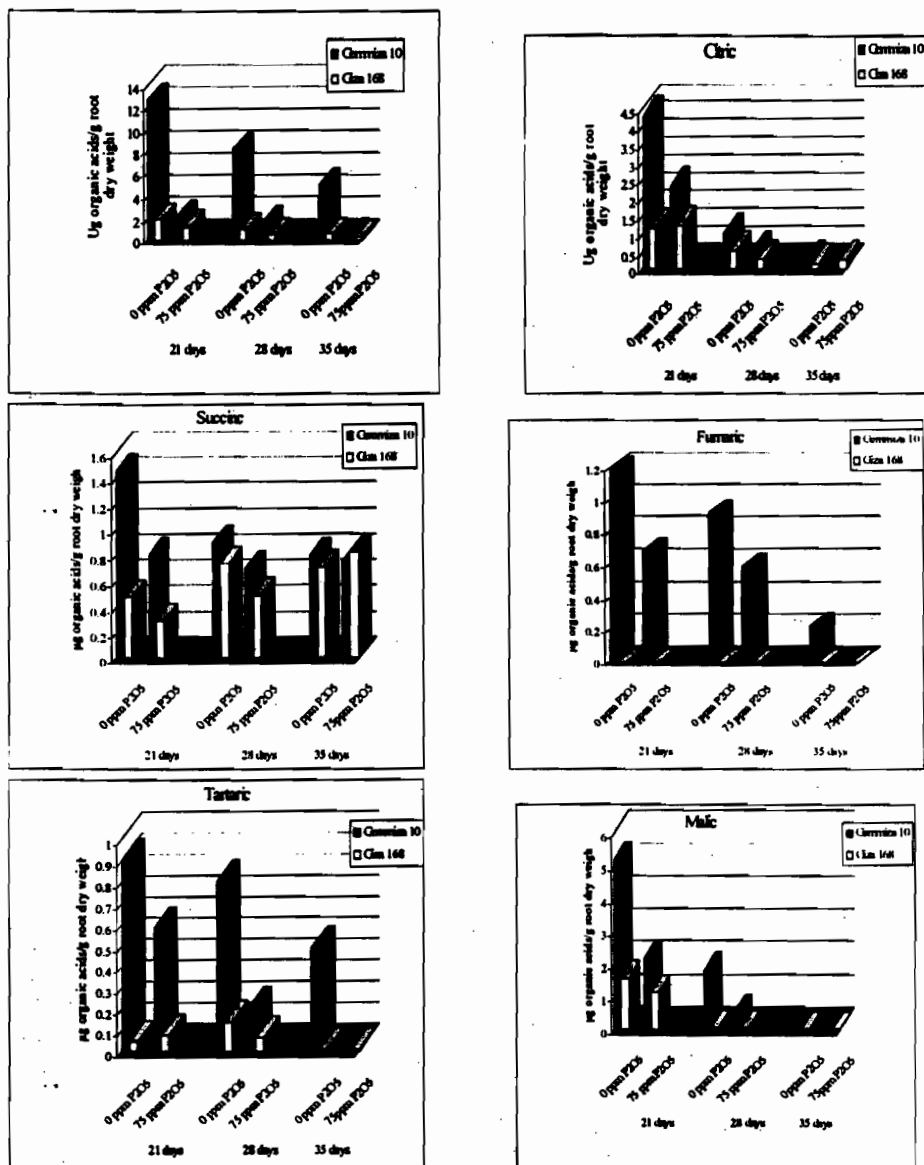


Fig.6 : Effect of different phosphorous levels on root exudates of two wheat varieties ( $\mu$  organic acids/ g root dry weight)

Moreover, Oxalic acid was the predominant organic acid in the root exudates of the two wheat varieties during the three growth stages (21, 28 and 35 days old). Roots of both varieties exudates large amount of malic acid under P-stress compared to plants received P-fertilizer. It was noticed that, during early growth stage (21 days), malic acid exudates by roots of Gemmaiza 10 variety grown under P-deficiency reached about 2.5 times that

exudates by plants grown at 75 ppm P<sub>2</sub>O<sub>5</sub> fertilization. However, at later growth stage (35 days) this acid was not detected even under P-deficiency condition. The organic acids exudates by roots of Gemmaiza 10 could be ranked (according to their concentration in rhizosphere) in the following order: Oxalic > malic > citric > succinic > fumaric > tartaric. However in Giza 168 fumaric acid disappear and the other five organic acids were detected in root exudates. This finding is in harmony with those obtained by Rengel *et al* (2002) who postulated that Citric, malic, malonic and succinic were identified in root exudates and increased by P- starvation in alfalfa, rape and pigeonpea. Hayes *et al.* (2004) demonstrated that root exudates such as organic acids may be important for increasing the availability of bound form of inorganic P in soil.

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## آليات تأقلم اصناف القمح للإمداد المنخفض من الفوسفور

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تهدف الدراسة الحالية الى توضيح بعض الآليات المحتملة والتي تفسر الاختلاف في كفاءة استخدام الفوسفور بواسطة صنفين من اصناف القمح، ولتحقيق هذا الهدف نفذت تجربتي أصص، في التجربة الأولى تم انماء صنفين من اصناف القمح يختلفان في كفاءة استخدامهما للفوسفور (صنف جيميزة 10 ويمثل الاصناف الكفزه والمستجيبة للفوسفور وصنف جيزة 168 ويمثل الاصناف غير الكفزه وغير المستجيبة للفوسفور (في تربة بها نقص في عنصر الفوسفور وذلك تحت ثلاث مستويات من الفوسفور، المستوى المنخفض) بدون إضافة فوسفور، (المستوى المتوسط) إضافة 50مجم فورا لكل كجم تربة (، المستوى العالي) إضافة 75مجم فورا لكل كجم تربة (ولذلك لمدة 90يوما من الزراعة، وأخذت العينات النباتية عند ثلاث مراحل وهي 35، 55، 90يوما من الزراعة ولقد دلت نتائج هذه التجربة على ان الوزن الجاف لكل من الأجزاء الخضرية والجنور اختلف معنويا بين صنفى القمح عند كل مستوى من مستويات الفوسفور وخلال مراحل النمو الثلاث، وقد سجل صنف جيميزة 10أعلى وزن جاف لكل من الأجزاء الخضرية والجنور عند المستوى العالي من الفوسفور وخلال مراحل النمو الثلاث. وكذلك أوضحت النتائج ان صنف القمح جيميزة 10سجل أعلى نسبة بين الوزن الجاف للجنور الى الوزن الجاف للأجزاء الخضرية عند كل مستويات الفوسفور وخلال كل مراحل النمو. وان أعلى قيم لهذه النسبة كانت عند المستوى المنخفض من الفوسفور.

وبشكل عام وجد ان طول الجنور لنباتات القمح قد تأثر بكل من مستويات الفوسفور والاصناف وانه خلال مرحلة النمو المبكرة (35يوما من الزراعة (سجل صنف القمح جيميزة 10أعلى طول للجنور عند المستوى المنخفض من الفوسفور وان طول الجذر قل بزيادة معدلات الفوسفور، بينما حدث العكس خلال هذه المرحلة بالنسبة لصنف القمح جيزة 168، علاوة على ذلك فاقه خلال مراحل النمو المتأخرة (55، 90يوما من الزراعة (لوحظ زيادة طول الجذر لكل من صنفى القمح بزيادة معدلات الفوسفور وان صنف جيميزة 10كان له أطول جنور عند كل مستويات الفوسفور وخلال كل مراحل النمو وذلك بالمقارنة بالصنف جيزة 168.وكذلك أوضحت النتائج ان الصنف الكفو (جيميزة 10)كان له اكبر مساحة سطح للجنور وكذلك أعلى درجة نعومة للجنور وذلك عند كل مستويات الفوسفور وخلال كل مراحل النمو بالمقارنة بالصنف غير الكفو (جيزة 168).ومن ناحية أخرى، فقد سجل صنف جيميزة 10أعلى محتوى من الفوسفور في كل من الأجزاء الخضرية والجنور بالمقارنة بالصنف جيزة 168.أولئك عند المستوى المنخفض من الفوسفور، خلال مرحلة النمو الأولى (35يوما من الزراعة. وانه بزيادة مستويات الفوسفور حدث زيادة في محتوى كل من الأجزاء الخضرية والجنور من الفوسفور لكل من الصنفين وذلك خلال كل مراحل النمو وكان هذا التأثير أكثر وضوحا في حالة الصنف الكفو جيميزة 10.

في التجربة الثانية تم انماء نفس صنفى القمح المستخدمين في التجربة الأولى في نفس التربة المستخدمة في التجربة الأولى وذلك عند مستويين من الفوسفور وهما المستوى المنخفض بدون إضافة فوسفور (والمستوى العالي) إضافة 75مجم فورا لكل كجم تربة (ولذلك لمدة 35يوما من الزراعة. وتم جمع عينات من التربة من منطقة نمو الجنور عند ثلاث مراحل وهي 21، 28، 35يوما من الزراعة وذلك لتقدير رقم ال pH وافرازات الأحماض العضوية في منطقة نمو الجنور. وقد دلت نتائج هذه التجربة على ان اقل قيم لرقم ال pH في منطقة الجنور قد سجلت في حالة صنف القمح جيميزة 10عند المستوى المنخفض من الفوسفور وخلال مراحل الدراسة الثلاث وان انخفاض رقم ال pH في منطقة نمو جنود صنف القمح جيميزة 10من الممكن أن يكون احد الاستراتيجيات المسنولة عن زيادة امتصاص الفوسفور تحت ظروف نقصه. كما دلت النتائج أيضا على أن نقص الفوسفور شجع على زيادة إفراز الأحماض العضوية في منطقة نمو الجنور لكلا من الصنفين خلال مرحلة النمو الأولى (21يوما من الزراعة (وان هذا التأثير قل بزيادة عمر النباتات وان الزيادة في إفراز الأحماض العضوية في منطقة نمو الجنور كانت كبيرة في حالة الصنف الكفو (جيميزة 10)عن الصنف غير الكفو (جيزة 168).