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RELATIVE GROWTH RESPONSE OF HYDROPONICALLY GROWN WHEAT GENOTYPES TO DEFICIENT AND ADEQUATE PHOSPHORUS LEVELS

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ABSTRACT

Phosphorus (P) nutrition is indispensable for plant growth and development. Seven wheat varieties (Sarsabz, Kiran-95, Khirman, NIA-Amber, NIA-Sunhari, NIA-Sunder and NIA-Saarang) were evaluated for their growth response to P deficient (0.02 *mM*) and adequate (0.20 *mM*) levels in hydroponic culture. A factorial combination of treatments, repeated four times, was arranged in a completely randomized design. Wheat varieties differed significantly (P< 0.01) in shoot and root dry matter production, root: shoot ratio, shoot and root P concentration and uptake, and shoot P utilization efficiency. Shoot P uptake and utilization efficiency were strongly correlated (r = 0.89, r = 0.77) with shoot dry matter production. On the basis of shoot dry matter production and P utilization efficiency, cultivars were grouped into four categories. NIA-Sunder, Sarsabz and Kiran-95 were efficient as well as responsive (ER) genotypes. Khirman, NIA-Sunhari, NIA-Saarang and NIA-Amber were categorized as non-efficient and non-responsive (NENR) genotypes. Prevalence of such genetic variations among these wheat cultivars can be useful in developing P- efficient genotypes.

Keywords: hydroponics, phosphorus levels, phosphorus utilization efficiency, wheat genotypes

INTRODUCTION

Phosphorus (P) deficiency impedes plant growth and development in more than 30% of the world's cultivated soils (Runge-Metzger, 1995; Vance *et al*., 2003). Application of synthetic P fertilizers is generally recommended to overcome the P limitation problem. However, tendency of P fertilizers to rapidly form insoluble complexes with iron and aluminum ions in acidic soils and with calcium ions in alkaline soils leave a major part of P unavailable for plant growth (Lynch, 2007). Consequently, plant use efficiency of both native and applied P remains only up to 15-20% (Delgado *et al*., 2002). The finite, non-renewable nature of rock phosphate and increased prices of synthetic P fertilizers make the situation even more critical (Bouwman *et al*., 2009; Cordell *et al*., 2009; Van Kauwenbergh, 2010). The severity of the problem demands investigation and utilization of other

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sustainable options rather than relying on chemical P fertilizers alone (Vance *et al*., 2003). Plants have adapted diverse morphological, physiological and molecular strategies to grow on P deficient soils. Adaptive strategies include reduced growth rate, remobilization of internal P (Plaxton and Carswell, 1999), increased root surface area due to extended root systems and root hairs (Gahoonia and Nielsen, 1998; Lynch and Brown, 2001), rhizospheric exudation of organic acids (Gilbert *et al*., 1999; Watt and Evans, 2003) and enhanced expression of P transporters (Bucher *et al*., 2001).

Crop plants vary widely in these adaptive mechanisms (Akhtar *et al*., 2002; Gill *et al*., 2002; Aziz *et al*., 2006). Such genetic variations can be exploited to develop P efficient genotypes (Fageria and Baligar, 1997; Kosar *et al*., 2003; Akhtar *et al*., 2011), which will help to improve fertilizer use efficiency, reduce production costs and prevent nutrient losses (Aziz *et al*., 2006). Wheat is a staple food crop of Pakistan. It is being cultivated on more than 9.0 million ha area, and consumes almost 50% of P fertilizer used in the country (Economic Survey of Pakistan, 2014-15). Improving P use efficiency in wheat crop alone can have a substantial impact on fertilizer demand in future. Therefore, genetic differences for P utilization efficiency present among wheat genotypes should be investigated and exploited in future breeding programs. In this perspective, the current investigation was carriedout to study the relative response of wheat genotypes to deficient and adequate P levels in hydroponics.

MATERIALS AND METHODS

A hydroponic study was carriedout in a rain protected net house at Nuclear Institute of Agriculture, Tandojam, during 2013. Seven wheat genotypes, i.e. Sarsabz, Kiran-95, Khirman, NIA-Amber, NIA-Sunhari, NIA-Sunder and NIA-Saarang were tested under P deficient (0.02 *mM*) and adequate (0.20 *mM*) levels (Gill *et al*., 2004). Seeds were germinated in plastic bowls containing washed gravels and irrigated with distilled water. Seven day-old, uniform and healthy seedlings were transferred to continuously aerated, half strength modified Johnson's nutrient solution (Johnson *et al*., 1957), in two polythene lined iron tubs (20- L capacity). After one week of transplanting, nutrient solution was exchanged with full strength solution. The full strength solution contained 16 mM N, 6 *mM* K, 4 *mM* Ca, 1 *mM* S, 1 *mM* Mg, 50 *µM* Cl, 25 *µM* B, 2 *µM* Mn, 2 *µM* Zn, 0.5 *µM* Cu, 0.5 *µM* Mo and 50 *µM* Fe. Phosphorus levels, i.e. deficient (0.02 *mM*) and adequate (0.20 *mM*) were maintained with ammonium dihydrogen phosphate salt ($NH_4H_2PO_4$). The solution was completely replaced on weekly basis. The pH of culture solution was adjusted at 5.5 ± 0.5 with ammonium hydroxide and glacial acetic acid on daily basis. Treatments, each replicated four times, were established according to completely randomized factorial design. After four weeks of transplanting, the plants were harvested, washed with distilled water and subsequently divided into root and shoot portions. The plant material was oven-dried at 70 ^{0}C for 72 hours till constant weight. Dried plant material was finely ground and one gram of each plant sample was digested with 10 ml of di-acid mixture of HNO_3 and $HClO_4$ (3:1) as reported by Miller (1998). Plant digests were analyzed for phosphorus concentration by vanadate-molybdate yellow color method using a spectrophotometer (Chapman and Pratt, 1961). Phosphorus uptake in shoot/root and relative reduction in shoot dry matter yield, also called as phosphorus stress factor (PSF) were calculated by the formulae recommended by Hunt (2003).

Shoot/root P uptake (mg P plant^{-1}) = Shoot/root P concentration (mg P g^{-1}) x Shoot/root dry matter yield (g plant^{-1})

$$\text{PSF (\%)} = \frac{\text{SDM (adequate P)} - \text{SDM (deficient P)}}{\text{SDM (adequate P)}} \times 100$$

Here, SDM represents shoot dry matter (g plant^{-1}) in the respective treatment.

The following formula of Siddiqi and Glass (1981) was used to calculate phosphorus utilization efficiency (PUE) of wheat genotypes:

$$PUE\ (g^2\ SDM\ mg^{-1}\ \text{P}) = \frac{Shoot\ dry\ matter\ (\text{g plant}^{-1})}{Shoot\ P\ concentration\ (\text{mg Pg}^{-1})}$$

The data collected for various growth parameters (shoot and root dry matter, root: shoot ratio, relative reduction in shoot dry matter) and P relations (shoot and root P concentration, shoot and root P uptake, P utilization efficiency) were subjected to ANOVA using computer software MSTAT-C (Gomez and Gomez, 1984) and treatment means were differentiated by least significant difference (LSD) method at $P< 0.01$. Further, the genotypes were differentiated into four categories on the basis of SDM production and PUE, as described by Fageria and Baligar (1997). The correlations among various parameters were derived by using Microsoft Excel (Redmond, WA, USA).

RESULTS

Biomass production

All the wheat genotypes exhibited remarkable differences in their biomass production at deficient and adequate P supplies in the growth medium. The P levels, the genotypes and their interaction had significant ($P< 0.01$) effects on shoot and root dry matter production, and root: shoot ratio (Table 1). Data revealed that SDM production by wheat genotypes was considerably reduced from 2.0 to 1.46 g plant^{-1} (27% reduction) due to P deficiency stress. NIA-Sunder produced higher SDM (1.68 and 2.30 g plant^{-1}) at deficient and adequate P levels, respectively. Minimum SDM accumulation (1.13 and 1.68 g plant^{-1}) was recorded in NIA-Saarang at deficient and adequate P levels, respectively. Performance of Sarsabz and Kiran-95 was statistically at par with that of NIA-Sunder at both P levels. Substantial genetic differences were observed in root dry matter (RDM) production by wheat varieties at both P levels. Plants grown in P deficient medium produced higher RDM (0.68 g plant^{-1}) than those (0.52 g plant^{-1}) supplied with adequate P level. At deficient P level, NIA-Amber produced maximum root dry matter (0.78 g plant^{-1}), while NIA-Saarang, statistically equivalent to Kiran-95, Khirman, NIA-Sunhari and NIA-Sunder produced the minimum (0.61 g plant^{-1}). At adequate P supply, RDM ranged between a minimum value of 0.46 g plant^{-1} (Khirman) and maximum value of 0.62 g plant^{-1} (Sarsabz). Root: shoot ratio (RSR) was also significantly ($P< 0.01$) affected by P levels and wheat genotypes as well as their interaction. Cultivars revealed almost twofold higher RSR (0.47) in P deficient medium than in P sufficient medium (0.26). Wheat variety 'NIA-Amber' produced maximum RSR (0.58 and 0.29) at deficient and sufficient P supplies, respectively. Minimum values of RSR (0.38 and 0.22) were attained by NIA-Sunder at deficient and adequate levels, respectively. Variations among wheat genotypes for RSR were more prominent at deficient P level.

Phosphorus stress factor (PSF) indicates comparative decrease in SDM production of different cultivars at low P level. Cultivars varied significantly ($P<$ 0.01) in their relative tolerance to P deficiency stress (Figure 1). Phosphorus stress factor ranged between 18% (NIA-Sunhari) and 32.3% (NIA-Saarang), indicating the presence of genetic diversity in adaptability to P deficient conditions.

Table 1. Shoot dry matter, root dry matter and root: shoot ratio of different wheat genotypes grown with deficient (0.02 *mM*) and adequate (0.20 *mM*) phosphorus levels in culture solution

Genotypes	**Shoot dry matter (g plant^{-1})**		**Root dry matter (g plant^{-1})**		**Root: shoot ratio**	
	Deficient P	**Adequate P**	**Deficient P**	**Adequate P**	**Deficient P**	**Adequate P**
Sarsabz	1.56de	2.25a	0.76 a	0.62bc	0.49b	0.27ef
Kiran-95	1.54def	2.28a	0.67b	0.60c	0.43c	0.26efg
Khirman	1.47ef	1.95b	0.67b	0.46d	0.45bc	0.23fg
NIA-Amber	1.34fg	1.72cd	0.78a	0.49d	0.58a	0.29e
NIA-Sunhari	1.50def	1.84bc	0.64bc	0.47d	0.42c	0.26efg
NIA-Sunder	1.68cde	2.30a	0.63bc	0.51d	0.38d	0.22g
NIA-Saarang	1.13g	1.68cde	0.61c	0.48d	0.54a	0.29e
Mean	1.46B	2.00A	0.68 A	0.52B	0.47A	0.26B
$LSD_{0.01}$ (P,G,P×G)	0.08, 0.15, 0.22		0.02, 0.04, 0.06		0.02, 0.03, 0.05	

Means sharing same letters are statistically similar at 1% probability. P stands for phosphorus levels, G for genotype and P x G for P level x genotype interaction.

Table 2. Phosphorus concentration in shoot and root of different wheat genotypes grown with deficient (0.02 *mM*) and adequate (0.20 *mM*) phosphorus levels in culture solution

Genotypes	**Shoot P concentration (mg P g^{-1})**		**Root P concentration (mg P g^{-1})**	
	Deficient P	**Adequate P**	**Deficient P**	**Adequate P**
Sarsabz	1.80e	4.00c	1.85f	3.53d
Kiran-95	1.89e	4.37b	1.90ef	4.45c
Khirman	1.97e	4.33b	2.00ef	4.33c
NIA-Amber	1.97e	5.00a	2.00ef	5.28a
NIA-Sunhari	1.80e	4.42b	2.13e	4.90b
NIA-Sunder	1.75e	4.29b	2.12e	4.28c
NIA-Saarang	1.49f	3.62d	1.82f	3.70d
Mean	1.81B	4.29A	1.97B	4.35A
$LSD_{0.01}$ (P,G,P×G)	0.10, 0.18, 0.26		0.09, 0.17, 0.25	

Means sharing same letters are statistically similar at 1% probability. P stands for phosphorus levels, G for genotype and P x G for P level x genotype interaction.

Table 3. Phosphorus uptake in shoot and root of different wheat genotypes supplied with deficient (0.02 *mM*) and adequate (0.20 *mM*) phosphorus levels in culture solution

Genotypes	Shoot P uptake (mg P plant $^{-1}$)		Root P uptake (mg P plant $^{-1}$)	
	Deficient P	Adequate P	Deficient P	Adequate P
Sarsabz	2.80e	8.98bc	1.40fg	2.17bc
Kiran-95	2.90e	9.98a	1.27gh	2.67a
Khirman	2.90e	8.46c	1.33fgh	1.97cd
NIA-Amber	2.64e	8.57c	1.56ef	2.60a
NIA-Sunhari	2.71e	8.11c	1.35fg	2.30b
NIA-Sunder	2.95e	9.87ab	1.33fgh	2.18bc
NIA-Saarang	1.68f	6.06d	1.10h	1.78de
Mean	2.66B	8.58A	1.33B	2.24A
$LSD_{0.01}$ (P,G,P×G)	0.35, 0.65, 0.91		0.09, 0.16, 0.23	

Means sharing same letters are statistically similar at 1% probability. P stands for phosphorus levels, G for genotype and P x G for P level x genotype interaction.

Phosphorus concentration, uptake and utilization efficiency

Plants supplied with deficient P level had significantly (*P*< 0.01) lower shoot P concentrations than those grown with adequate P (Table 2). Significant (*P*< 0.01) variations among cultivars were present for shoot P concentration. However, differences were more pronounced at adequate P supplies. At 0.02 *mM* P supply, shoot P concentration in all genotypes was statistically at par with each other except NIA-Saarang (1.49 mg P g^{-1}). At deficient P level, shoot P concentration ranged between 1.49 mg P g^{-1} in NIA-Saarang and 1.97 mg P g^{-1} in NIA-Amber and Khirman. When plants were grown in P adequate medium, it ranged between 3.62 mg P g^{-1} in NIA-Saarang and 5.0 mg P g^{-1} in NIA-Amber.

Phosphorus levels and wheat genotypes showed significant (*P*< 0.01) main and interactive effects on root P concentration (Table 2). The P concentration in root varied from 1.82 to 2.13 mg P g^{-1} at deficient P level and from 3.53 to 5.28 mg P g^{-1} at adequate P level. On the whole, adequate P supply to the plants resulted in 120% increase in root P concentration over deficient P level. Disparity among wheat genotypes for root P concentration was more obvious at adequate P supply than at deficient P level.

Plants accumulated significantly (*P*< 0.01) more P (almost 3 folds) in their shoots, when P supply in rooting medium was increased from 0.02 to 0.20 *mM* (Table 3). Cultivars alone and together with P levels significantly (*P*< 0.01) affected shoot P uptake. When grown in P deficient medium, NIA-Sunder accumulated maximum P (2.95 mg P plant^{-1}) in shoot. However, shoot P uptake in NIA-Sunder was statistically equivalent to other cultivars except NIA-Saarang. Variations amongst wheat cultivars for shoot P uptake were more pronounced at adequate P level than at P deficient level in rooting medium. Kiran-95 and NIA-Sunder had higher shoot P (9.98 and 9.87 mg P plant^{-1}) at adequate P level.

On overall basis, there was 68% increase in root P uptake at adequate P supply over deficient P supply (Table 3). Wheat varieties also differed for their root P uptake, however, differences were more prominent at adequate P level. At deficient P supply, root P uptake ranged between 1.10 mg P plant^{-1} (NIA-Saarang) to 1.56 mg P plant^{-1} (NIA-Amber). When supplied with adequate P, Kiran-95 and NIA-Amber recorded the highest root P uptake (2.67 and 2.60 mg P plant^{-1}) while Khirman and NIA-Saarang showed least root P (1.97 and 1.78 mg P plant^{-1}).

Shoot P utilization efficiency was significantly (P< 0.01) affected both by P levels and genotypes as well as their interactions (Figure 2). Genotypes grown in P deficient medium were about 72% more efficient in their shoot P utilization than those grown with adequate P level. Among various genotypes grown in deficient P level, NIA-Sunder was ranked as the most efficient, followed by Sarsabz and NIA-Sunhari. NIA-Amber produced lower shoot P use efficiency at both P levels.

DISCUSSION

Higher shoot dry matter production is generally considered the most reliable indicator of P use efficiency for screening genotypes under P deficient conditions (Ahmad *et al*., 2001; Alloush, 2003). Wheat genotypes demonstrated significant variations in SDM in response to P levels and these genetic differences can successfully be used for crop improvement in future research studies. Shoot dry matter production revealed a significant and positive correlation (r = 0.77) with shoot P utilization efficiency at deficient P supply (Figure 3A). Therefore, SDM can be an appropriate selection criterion for evaluating cultivar P use efficiency at early growth stages of wheat crop. The same criterion has effectively been used by Osborne and Rengel (2002) and Ozturk *et al*. (2005) to rank wheat genotypes for P utilization efficiency.

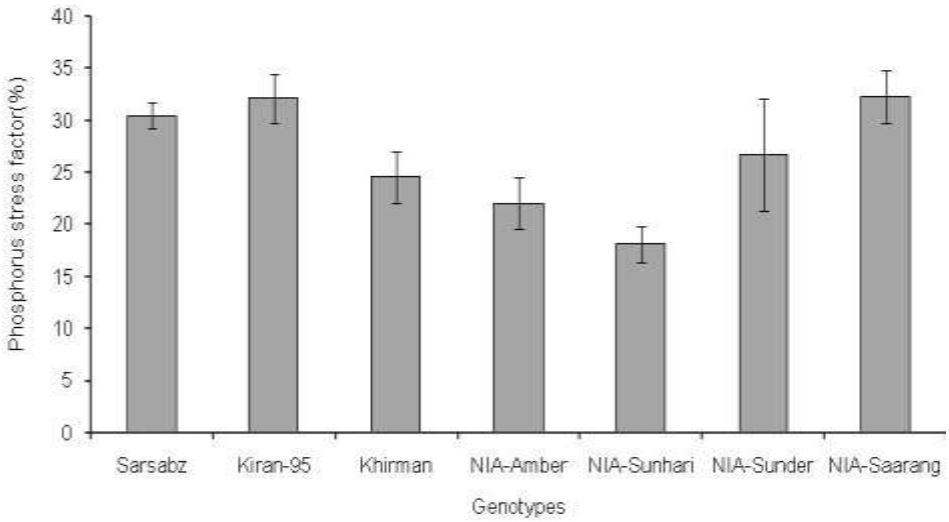


Figure 1. Relative reduction in shoot dry matter (phosphorus stress factor) of different wheat genotypes due to P deficiency stress

Root growth of plants was comparatively less inhibited than shoot growth under P deficient conditions. Increase in root: shoot ratio in wheat under low P stress has also been reported by Yaseen and Malhi (2009). The sustained root growth at the cost of shoot growth results from increased partitioning of photosynthates towards roots under low nutrient availability (Blair and Wilson, 1990; Chamk *et al*., 1994; Marschner, 1995), helping stressed plants to absorb more phosphorus from P deficient medium. Similar findings were observed by other scientists (Aziz *et al*., 2006; Lambers *et al*., 2006). The RDM positively correlated with shoot P concentration (r = 0.51) and shoot P uptake (r = 0.34) (Figure 3B, 3C). The present findings are in line with those of Machado and Furlani (2004). It can be inferred from the finding of this study that higher RDM production partly resulted in increased shoot P uptake.

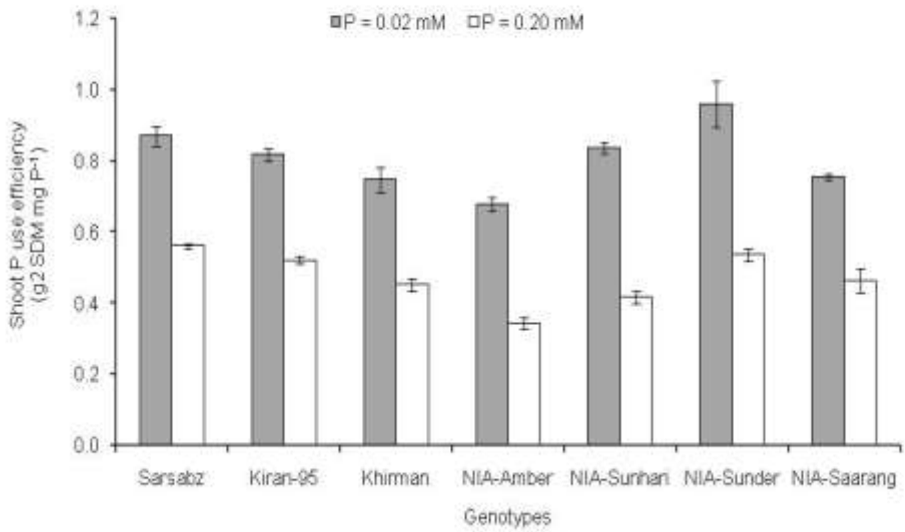
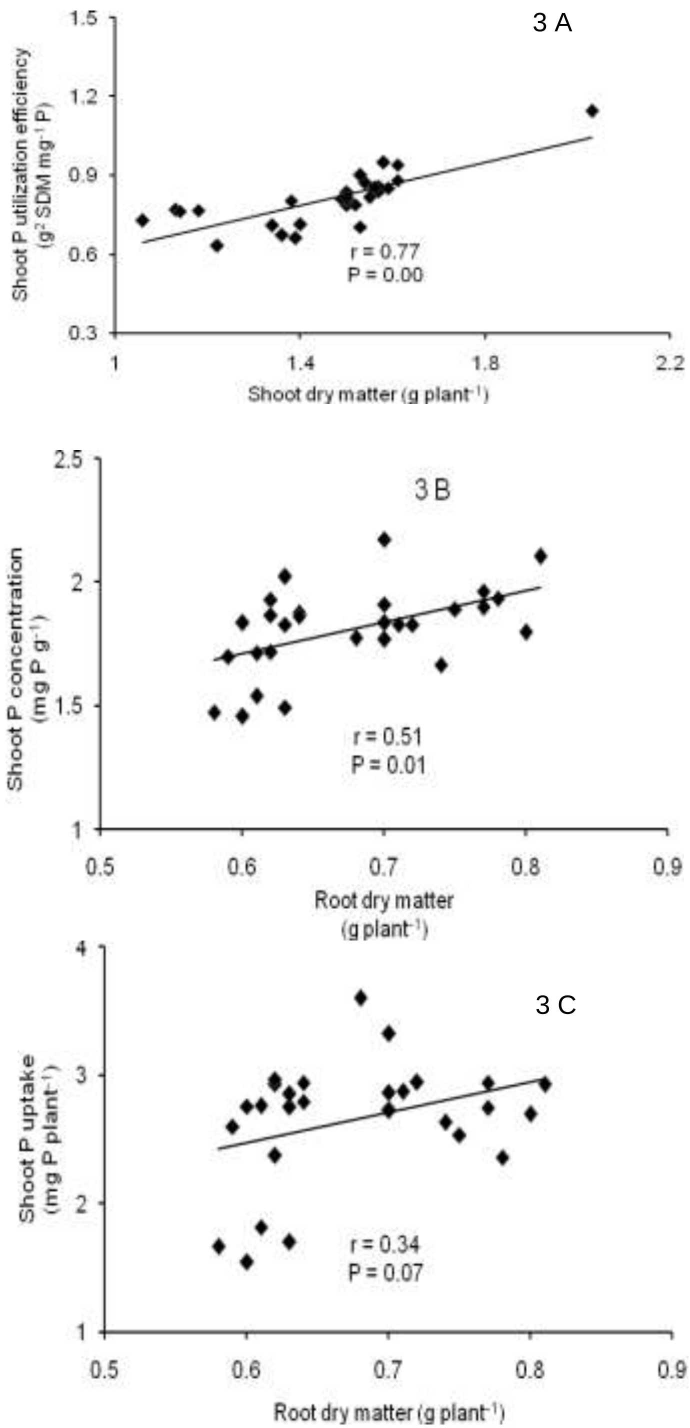


Figure 2. Phosphorus utilization efficiency of different wheat genotypes supplied with deficient (0.02 *mM*) and adequate (0.20 *mM*) phosphorus levels in culture solution

Phosphorus stress factor (PSF) is a useful indicator of relative tolerance of wheat cultivars to P deficiency stress (Ahmad *et al*., 2001; Gill *et al*., 2002). Remarkable differences in PSF values of the cultivars indicated the presence of genetic variability for adaptation to P deficient environments. Genotypes with relatively low PSF values can be suitable candidates for P deficient soils. However, genotypes cannot be selected on basis of PSF criteria only, since it only shows the extent of relative reduction in SDM under P deficient conditions (Shahbaz *et al*., 2006).

Shoot P concentration was negatively correlated (r = -0.29) with shoot P utilization efficiency (Figure 3D). The cultivars with higher P utilization efficiency had lower shoot P concentration than low efficient ones, reflecting that cultivars with high shoot P concentration were inefficient in P utilization. Similar findings were revealed by Jones *et al*. (1989) and Fageria and Baligar (1999). Plant physiologists linked lower shoot P concentration with more efficient utilization of P in metabolism (Gerloff, 1987; Glass, 1989).


3 A
Shoot P utilization efficiency (g² SDM mg⁻¹ P)
r = 0.77
P = 0.00
Shoot dry matter (g plant⁻¹)
3 B
Shoot P concentration (mg P g⁻¹)
r = 0.51
P = 0.01
Root dry matter (g plant⁻¹)
3 C
Shoot P uptake (mg P plant⁻¹)
r = 0.34
P = 0.07
Root dry matter (g plant⁻¹)

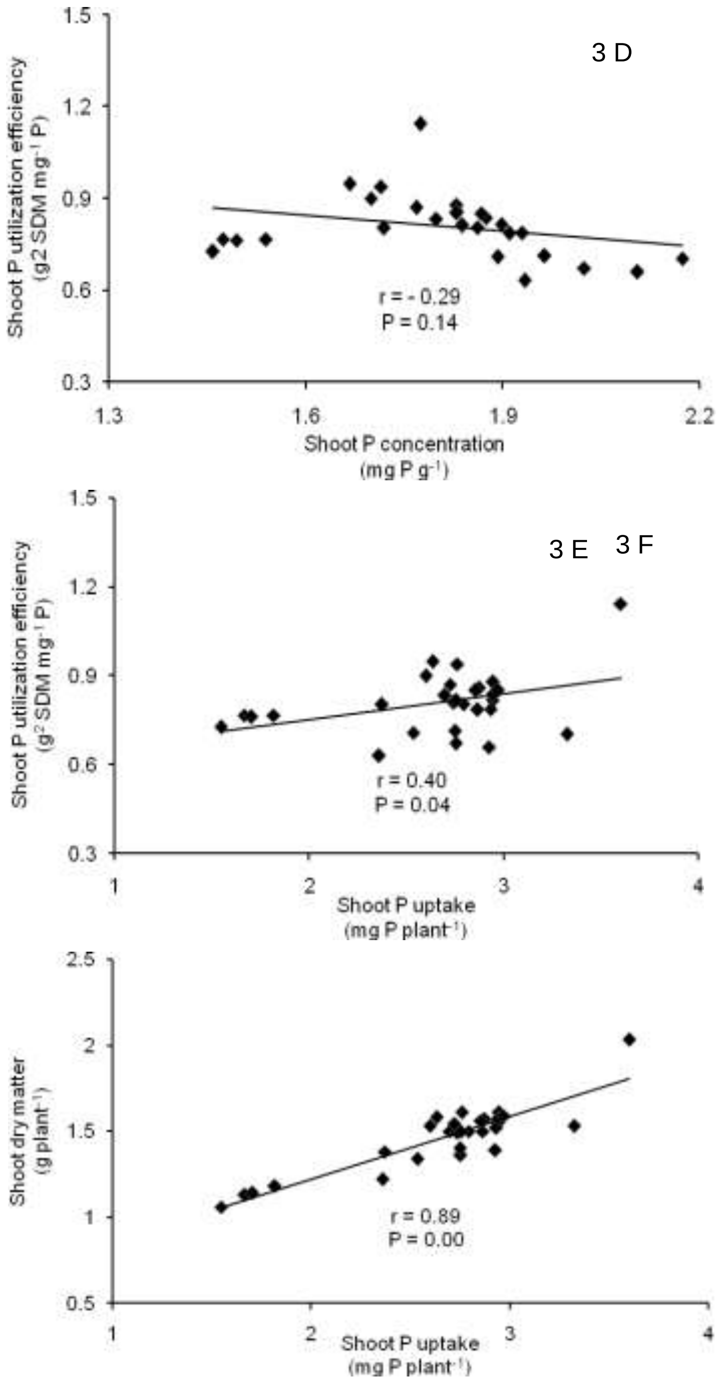


Figure 3. Relationship between various growth and growth related parameters of wheat genotypes at deficient P supply in culture solution

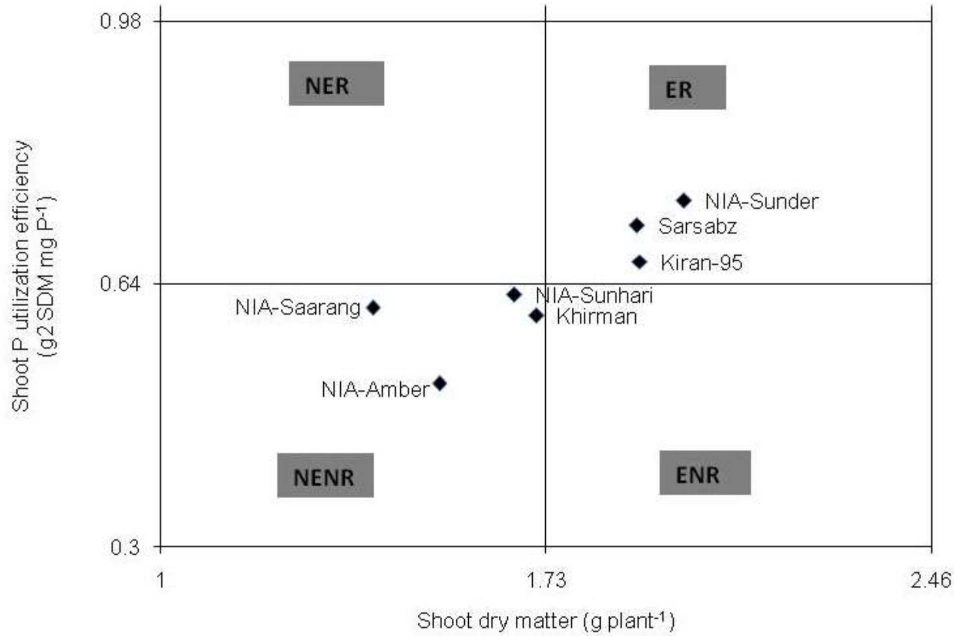


Figure 4. Classification of wheat genotypes on the basis of shoot dry matter and P utilization efficiency. The demarcating lines represent the average values of shoot dry matter and P utilization efficiency for all genotypes

NER: Non-efficient but responsive
ER: Efficient and responsive
NENR: Non-efficient and non-responsive
ENR: Efficient but non-responsive

A positive relationship (r= 0.40) was revealed between shoot P uptake and P utilization efficiency of genotypes (Figure 3E). Shoot P uptake also strongly correlated (r= 0.89) with shoot dry matter at deficient P supply (Figure 3F), implying that improved P uptake resulted in high P efficiency at low P level. Higher SDM yield of P efficient genotypes results from efficient utilization of accumulated P under P deficiency stress (Ozturk *et al*., 2005).

Present results depicted remarkable differences in wheat genotypes for P utilization efficiency. The PUE significantly correlated (r= 0.77) with shoot dry matter (Figure 3A). Based on SDM production and PUE, cultivars were grouped into four categories as described by Fageria and Baligar (1997) (Figure 4). Efficient and responsive genotypes (NIA-Sunder, Kiran-95 and Sarsabz) were the most desirable ones that produced SDM and PUE higher than their respective average values for all genotypes. These varieties are suitable to grow in P deficient soils, as they can produce well at low P level apart from responding well to fertilizer P. Non-efficient but responsive genotypes produced less than average SDM but PUE higher than average for all tested genotypes. None of the genotypes fell into this category. Khirman, NIA-Saarang, NIA-Amber and NIA-Sunhari can be classified as “Non-efficient and non responsive” because they produced SDM and PUE both lower than their respective averages. However, before final recommendations, these results should be confirmed under field conditions.

CONCLUSION

The findings of this study reveal that NIA-Sunder, Sarsabz and Kiran-95 are efficient and responsive genotypes, which have potential for better growth in P limited environments. However, these results should be confirmed under field conditions. The useful genetic differences that exist among wheat genotypes can be better exploited for breeding of high yielding and P efficient genotypes for P deficient soils in future.

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