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# Hydroponic Screening of Arid Crop Genotypes for Phosphorus Mobilization Efficiency via Acid Phosphatase and Phytase Activity

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## ABSTRACT

Phosphorus deficiency is a major limitation to crop productivity in arid and semi-arid regions, where a large proportion of soil phosphorus remains unavailable to plants. The current study aimed to identify effective genotypes of arid crop species based on their capacity to mobilize phosphorus via enzyme-mediated mechanisms under regulated hydroculture conditions. Ten genotypes representing five important arid crops were evaluated under phosphorus-sufficient and phosphorus-deficient conditions at different growth stages. The activities of acid phosphatase and phytase were measured as key indicators of phosphorus mobilization.

The results showed considerable variation among crops and genotypes, with enzyme activity consistently increasing under phosphorus-deficient conditions and with plant age. Among the crops studied, pearl millet exhibited the highest enzyme activity, followed by moth bean and mung bean, indicating their superior capacity to mobilize phosphorus under stress conditions. In contrast, sorghum showed relatively lower phytase activity. Overall, the findings demonstrate that enzymatic responses play an essential role in phosphorus acquisition and highlight the effectiveness of hydroculture systems for screening nutrient-efficient genotypes. The superior genotypes identified in this study have potential for use in breeding programs aimed at improving phosphorus-use efficiency in arid environments.

***Keywords:*** Acid phosphatase, Arid crops, Genotypic variation, Hydroponic screening, Phosphorus deficiency, Phosphorus mobilization, Phytase activity

## 1. NTRODUCTION

Phosphorus (P) is a vital nutrient that is needed to facilitate number of critical physiological and biochemical functions in plants such as transfer of energy, development of roots and photosynthesis. Although phosphorus is essential, it is usually found in low quantities in arid and semi-arid soils because of fixation, low content of organic matter and low biological activity [1, 2]. Much of the phosphorus within the soil is in organic forms e.g. phytate which transformed into inorganic phosphate through enzymatic action as it cannot be directly used by plant [3]. This limited availability makes phosphorus one of the most critical constraints to crop productivity in nutrient-poor environments.

Plants have adopted various adaptive measures in order to survive phosphorus shortage. One of the most important mechanisms is the secretion of phosphatase enzymes, including acid phosphatase and phytase, which help in the breakdown of organic phosphorus compounds into plant-available forms [4, 5]. The release of these

enzymes is often enhanced under phosphorus-deficient conditions, reflecting a physiological response aimed at improving nutrient acquisition [6]. However, the efficiency of this response varies among plant species and even among genotypes within a species, indicating the presence of genetic variability that can be exploited for crop improvement [7].

Hydroculture systems provide a reliable and controlled platform for studying plant responses to nutrient stress. By eliminating soil-related variability, these systems allow precise evaluation of root processes, enzyme secretion, and nutrient uptake [8, 9]. This makes hydroculture particularly suitable for screening genotypes based on their biochemical and physiological efficiency under defined nutrient conditions.

In this context, this study was undertaken to determine the performance of various genotypes of arid crop species with respect to their ability to produce acid phosphatase and phytase under conditions of low phosphorus availability. The study also aimed to assess variation in enzyme activity at different growth stages and to identify superior genotypes with enhanced phosphorus mobilization capacity. Such genotypes can play an important role in developing sustainable crop production systems for phosphorus-limited environments.

## 2. MATERIALS & METHODS

To identify efficient genotypes of arid crop plants, a solution culture experiment was carried out. Seeds of ten widely cultivated genotypes belonging to five arid crop species grown in different regions of India were collected. Enzyme activities of these plants were evaluated under hydroponic conditions at different growth intervals. Based on enzyme performance, two superior genotypes were selected.

The sterilization of healthy and viable seeds done using a 1:1 (v/v) solution composed of hydrogen peroxide and absolute ethyl alcohol for 2 minutes, then 0.05 percentage mercuric chloride ($HgCl_2$) with a little amount of hydrochloric acid (HCl) for another 2 minutes. The chemicals were then drained after sterilization and seeds were then rinsed with sterile distilled water 10-12 times so as to remove any forms of residue. The sterilized seeds thereafter were put under sterilized beakers with vermiculite to germinate. All tests were performed in a controlled chamber in an aseptic environment of photoperiod (14 hr, 2500-3000 lux) with a relative humidity of 65 ± 5 and day temperature of $30\pm2^0$ C and night temperature of 20°C.

After initial growth, ten seedlings of each selected genotype were transferred into sterilized flasks containing 100 mL of sterile nutrient solution as described by Tarafdar and Claassen (1988). Phosphorus treatments included a sufficient level of 250 mg P $L^{-1}$ supplied as $KH_2PO_4$, while the phosphorus-deficient treatment received only 5 µg of inorganic P $L^{-1}$. A treatment without phosphorus was not included due to severe growth inhibition. There were three experiments of each treatment, and the plants were kept under sterile conditions during the experiment.

Sampling was done after 7, 14 and 21 days of transfer. A streak on the yeast extract mannitol agar was performed on an aliquot of each flask prior to harvesting in order to ensure the absence of microbial contamination and only samples that did not harbor the microbe contamination were to be subjected to further analysis. Immediately after harvest, the nutrient solution was analyzed for phosphatase and phytase enzyme activities.

### 2.1 Assessment of acid phosphatase activity

Assay of acid phosphatase was performed by following the recommended method of Tabatabai and Bremner [10]. The methodology that was given to determine the phosphomonoesterase activities is founded on what is called the colorimetric estimation of p-nitrophenol released upon the activity of phosphatase when an aliquot is mixed with buffer (pH 5.4) and 0.25%

sodium p-nitrophenyl phosphate solution. One millilitre of extractant was transferred into 15 mL screw-cap test tube. Subsequently, 0.2 mL toluene and p-nitrophenyl phosphate solution prepared in acetate buffer (pH 5.4) were added, and the mixture was incubated at 35°C. After 1 hour of incubation, 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH were added to the reaction mixture. The contents were shaken thoroughly for several minutes and then filtered using Whatman No. 42 filter paper. The yellow-coloured intensity developed in the filtrate was measured using a spectrophotometer at 420 nm. The concentration of p-nitrophenol in the filtrate was determined by plotting against a standard curve prepared by using 0, 10, 20, 30, 40, and 50 mg p-nitrophenol.

### 2.2 Assessment of phytase activity

An aliquot of 2 mL was transferred into 15 mL screw-cap test tubes. It was mixed with 4 mL of sodium acetate (100 mM) buffer (pH 4.5) and 1 mL sodium phytate solution (1 mM), and the mixture was incubated at 37°C for 1 h. The reaction was terminated after incubation by adding 0.5 mL of 10% trichloroacetic acid (TCA; $CCl_3COOH$). The precipitated proteins were removed by centrifugation at 10,000 rpm for 10 minutes, and the supernatant was used to determine the liberated inorganic phosphorus (Pi) using the chlorostannous-reduced molybdophosphoric acid method, as described by Jackson [11]. One unit of phytase activity was defined as the amount of enzyme required to release one micromole of inorganic phosphorus per second.

## 3. RESULT

A hydroculture-based experiment was conducted to identify efficient plant genotypes capable of producing higher levels of acid phosphatase and phytase under phosphorus (P) stress. Ten genotypes representing five arid crop species were evaluated at 7, 14, and 21 days after transfer (DAT) under both phosphorus-sufficient (+P) and phosphorus-deficient (–P) conditions. Two best genotypes of each crop were chosen based on enzyme activity, which would be further analyzed. It is worth noting that alkaline phosphatase activity was not observed in any of the genotypes that were tested. Table 1 shows the selected crops and genotypes.

In general, the acid phosphatase and phytase activities were significantly different across the crops, genotypes and the growth stages. Plant age effects were noted to increase enzyme activity, which was always greater in the phosphorus-deficient conditions than in the phosphorus-sufficient conditions, and there was a great biochemical response to phosphorus limitation.

### 3.1 Enzyme activity in mung bean

In mung bean, both genotypes showed a gradual rise in the acid phosphatase activity with the age of the plants with significantly higher activity in the phosphorus-deficient condition. SML-668 genotype exhibited moderate increases at the early stage which were enhanced by 21 DAT. Conversely, Pusa Vishal showed an increased enzyme activity at all the stages of growth and so it seems to be more adaptive to the phosphorus deficiency.

The same was also found on the activity of phytase that grew progressively with plant age on both genotypes. Nevertheless, the increase of Pusa Vishal was significantly higher in the later stages, which means that this fungus has higher ability to hydrolyze organic phosphorus sources than SML-668.

### 3.2 Enzyme activity in moth bean

Genotypes of moth beans exhibited a strong response in the activity of enzymes, especially in the early growth phases. RMO-225 and RMO-257 showed a high rate of acid phosphatase activity at 7 DAT in phosphorus-deficient conditions, which was followed by a comparatively lower rate at subsequent stages. Such early response suggests the ability to mobilize phosphorus at an early stage of growth at a high rate.

**Table -1. List of selected arid crop species and their respective genotypes evaluated under hydroculture conditions.**

| Crop (Common Name) | Botanical Name with Author Citation | Genotypes /Varity |
|---|---|---|
| Mung bean (Green gram) | *Vigna radiata* (L.) R. Wilczek | Pusa Vishal, Pusa Baisakhi, Pusa Ratna, Pusa 9531, SML 668, SML 832, IPM 02-3, IPM 02-14, Pant Moong 5, Samrat |
| Moth bean | *Vigna aconitifolia* (Jacq.) Marechal | RMO-40, RMO-225, RMO-257, RMO-435 (Maru Bahar), CZM-2, CZM-3, Jadia, Baleshwar-12, CAZRI Moth-2 (CZM-45), CAZRI Moth-3 (CZM-99) |
| Cluster bean (Guar) | *Cyamopsis tetragonoloba* (L.) Taub. | RGC-936, RGC-986, RGC-1002, RGC-1038, RGC-1066, RGC-936-1, HGS-563, HGS-986, HGS-365, HGS-884, Pusa Navbahar |
| Pearl millet (Bajra) | *Pennisetum glaucum* (L) R.Br. | RHB 67, RHB 173, RHB 177, RHB 121, MPMH 17, GHB 558, ICTP 8203, ICMV 155, NBH 5767, Pusa Composite 443 |
| Sorghum (Jowar) | *Sorghum bicolor* (L) Moench | CSH 14, CSH 16, CSV 15, CSV 20, CSV 23, CSH 25, CSV 10, CSV 13, SPV 13, M-35-1 (Maldandi) |

**Table 2. Release of acid phosphatase at P-deficient and P-sufficient condition by selected genotypes of arid plants under hydroculture condition (± indicate standard errors of mean)**

| Crop (genotypes) | Acid phosphatase ((EU × 10 $^{-6}$) activity* | | | | | |
|---|---|---|---|---|---|---|
| | 7 DAT | | 14 DAT | | 21 DAT | |
| | +P | -P | +P | -P | +P | -P |
| Mung bean (SML-668) | 2.62 ± 0.05 | 2.90 ± 0.12 | 3.12 ± 0.31 | 3.50 ± 0.62 | 4.31 ± .08 | 6.33 ± 0.23 |
| Mung bean (Pusa Vishal) | 2.50 ± 0.02 | 3.21 ± 0.01 | 4.15 ± 0.07 | 4.83 ± 0.07 | 5.53 ± 0.09 | 6.42 ± 0.13 |
| Moth bean (RMO-257) | 1.29 ± 0.002 | 2.30 ± 0.002 | 3.00 ± 0.02 | 3.32 ± 0.02 | 5.36 ± 0.04 | 6.36 ± 0.05 |
| Moth bean (RMO-225) | 1.30 ± 0.003 | 2.32 ± 0.004 | 2.89 ± 0.02 | 3.18 ± 0.02 | 5.51 ± 0.04 | 6.21 ± 0.05 |
| Cluster bean (RGC-936) | 1.6 ± 0.05 | 1.20 ± 0.08 | 2.74 ± 0.02 | 3.51 ± 0.18 | 4.70 ± 0.16 | 4.98 ± 0.21 |
| Cluster bean (HG-365) | 1.9 ± 0.06 | 2.91 ± 0.42 | 3.51 ± 0.13 | 3.72 ± 0.32 | 4.15 ± 0.21 | 4.97 ± 0.24 |
| Pearl millet (HHB- 67) | 1.32 ± 0.01 | 2.35 ± 0.016 | 2.21 ± 0.014 | 3.88 ± 0.023 | 2.67 ± 0.021 | 4.43 ± 0.03 |
| Pearl millet (RHB-173) | 1.39 ± 0.01 | 2.28 ± 0.018 | 2.40 ± 0.019 | 3.78 ± 0.025 | 2.76 ± 0.027 | 4.33 ± 0.03 |
| Sorghum (CSH-16) | 1.28 ± 0.09 | 1.92 ± 0.026 | 2.95 ± 0.028 | 3.76 ± 0.034 | 3.90 ± 0.03 | 4.37 ± 0.03 |
| Sorghum (CSV-15) | 1.26 ± 0.01 | 2.04 ± 0.019 | 3.01 ± 0.028 | 3.82 ± 0.031 | 3.79 ± 0.03 | 4.42 ± 0.03 |

**DAT: Days after transfer: * Release by ten plants**

**Table 3. Release of phytase at P-deficient and P-sufficient condition by selected genotypes of arid plants under hydroculture experiment (± indicate standard errors of mean)**

| Crop genotypes | Phytase activity (EU × 10 $^{-6}$) activity* | | | | | |
|---|---|---|---|---|---|---|
| | 7 DAT | | 14 DAT | | 21 DAT | |
| | +P | P | +P | -P | +P | -P |
| Mung bean (SML-668) | 3.02±0.025 | 3.75 ± 0.028 | 3.18± 0.028 | 3.79 ± 0.031 | 3.31 ± 0.022 | 3.91 ± 0.040 |
| Mung bean (Pusa Vishal) | 2.60± 0.021 | 3.30 ± 0.040 | 2.72 ± 0.021 | 3.75 ± 0.035 | 2.80 ± 0.018 | 4.01 ± 0.036 |
| Moth bean (RMO-257) | 2.05± 0.035 | 2.52 ± 0.033 | 3.68 ± 0.053 | 4.33 ± 0.069 | 3.90 ± 0.081 | 4.71 ± 0.093 |
| Moth bean (RMO-225) | 2.80± 0.030 | 3.12 ± 0.038 | 3.21 ± 0.039 | 3.60± 0.042 | 4.20 ± 0.062 | 4.31 ± 0.075 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cluster bean (RGC-936) | 1.90± 0.016 | 2.10 ± 0.018 | 2.75 ± 0.022 | 2.95 ± 0.025 | 2.81 ± 0.026 | 3.10 ± 0.028 |
| Cluster bean (HG-365) | 2.09± 0.018 | 2.35 ± 0.021 | 2.40 ± 0.022 | 2.79 ± 0.025 | 3.32 ± 0.027 | 3.63 ± 0.029 |
| Pearl millet (HHB- 67) | 1.35 ± 0.009 | 1.95 ± 0.050 | 1.70 ± 0.034 | 2.15 ± 0.019 | 2.05 ± 0.053 | 2.8 ± 0.024 |
| Pearl millet (RHB-173) | 1.42 ± 0.021 | 1.76 ± 0.095 | 1.84 ± 0.031 | 2.10 ± 0.058 | 1.77 ± 0.021 | 2.4 ± 0.012 |
| Sorghum (CSH-16) | 2.12 ± 0.021 | 2.20 ± 0.031 | 2.30 ± 0.032 | 2.41 ± 0.021 | 2.42 ± 0.032 | 2.52 ± 0.020 |
| Sorghum (CSV-15) | 2.30 ± 0.031 | 2.44 ± 0.030 | 2.33 ± 0.022 | 2.58 ± 0.041 | 2.38 ± 0.031 | 2.61 ± 0.025 |

**DAT: Days after transfer: * Release by ten plants**

Phytase activity in moth bean also became elevated under phosphorus stress though the increase was relatively moderate. The activity of RMO-257 was significantly greater than that of RMO-225, which indicates that there is genotypic variation in the capacity of using organic phosphorus.

### 3.3 Enzyme activity in cluster bean

There was a fluctuating response in the enzyme activity of cluster bean genotypes. The HG-365 genotype had a robust rise of acid phosphatase activity initially, and thereafter fluctuations occurred in the subsequent stages. Conversely, RGC-936 exhibited a late response and the activity was relatively low initially but as the growth progressed, the enzyme production was intensified by phosphorus-deficient conditions Phytase activity in cluster beans was slightly enhanced in the presence of phosphorus-deficient conditions. The gradual growth stages in each genotype were observed, but the magnitude of the increases was less than in other crops, which showed a comparatively moderate response to enzyme.

### 3.4 Enzyme activity in pearl millet

The increase in acid phosphatase activity was highest and most constant in pearl millet as compared to the other crops tested. Genotypes, HHB-67 and RHB-173, demonstrated significant growth at all stages of growth under phosphorus-deficient conditions, and therefore, they have a high adaptive trait to phosphorus stress.

The pearl millet phytase activity also showed a tremendous rise with HHB-67 having a higher activity compared to RHB-173. The high and uniform production of enzymes in pearl millet shows that it is more efficient in mobilizing the inorganic and organic sources of phosphorus.

### 3.5 Enzyme activity in sorghum

The genotypes of sorghum showed an increase in the acid phosphatase activity under a phosphorus deficient state; the increase was moderately high. Both CSH-16 and CSV-15 were more active at the initial stages followed by a slowing of the rate of increase in the advanced stages.

The activity of phytases in sorghum was low in comparison with other crops. Even though a small increase was also seen under phosphorus deficiency, the response was minimal indicating that less dependence is made on phytate hydrolysis to acquire phosphorus.

### 3.6 Comparative performance of crops

In all crops, the levels of enzyme activity were always higher under phosphorus-deficient conditions and with the age of the plant. Pearl millet had the highest enzyme activity, then there was moth bean, then mung bean, cluster bean was moderate, and sorghum had the lowest phytase activity. These findings show clearly a high interspecific and genotypic difference in the mobilization of phosphorus.

## 4. DISCUSSION

The deficiency of phosphorus greatly promoted the activity of acid phosphatase and phytase in various crops and among different genotypes, and therefore, phosphorus mobilizing biochemical adaptation was high. This reaction is consistent with previous results that plants enhance the synthesis of phosphatase and phytase in low phosphorus status to gain access to inorganic and organic sources of phosphorus [2, 6]. The fact that the acid phosphatase activity increases with age of crops is an indication that there is a strong association between enzyme production, the growth of a plant, and nutrient demand especially in the conditions of phosphorus stress [1,12].

Of the crops that were studied, pearl millet exhibited the highest acid phosphatase activity; this was followed by moth bean and sorghum, which indicated their high ability to mobilize phosphorus in nutrient-limiting conditions. This could be explained by the fact that they have adapted to harsh climatic conditions and effective systems of nutrient acquisition [13]. Moth bean recorded a high level of early-stage enzyme activity, therefore enzyme acquisition through root-mediated phosphorus uptake was observed to be important in the initial growth of moth bean, but the ability of mung bean and cluster bean was moderate and, therefore, genotype-specific [14,15].

The phytase activity was also observed to be increasing significantly in case of phosphorus deficiency which validated the activity of phytase in hydrolyzing organic phosphorus, especially phytate [16]. Pearl millet and mung bean exhibited higher phytase activity implying better utilization of organic phosphorus sources whereas sorghum exhibited a relatively lower activity implying that there are differences in the enzyme dependency of crops [5].

Generally, the findings indicate that phosphorus deficiency triggers enzyme-mediated phosphorus mobilization, which is evidently different across crops and genotypes. Pearl millet, which was then overtaken by moth bean and mung bean, proved to be more effective in phosphorus uptake, and it is important to consider the genotypes of high enzymatic potential in enhancing phosphorus-use efficiency in nutrient-deficient conditions [2, 13].

## CONCLUSION

There was a high positive correlation between the phosphorus mobilization and the enzyme activity of the various crop genotypes. The positive relationship between increased phytase and acid phosphatase activity in phosphorus-deficient conditions and the increased release of inorganic phosphorus implied the proper mobilization of organic phosphorus sources. The current paper has clearly shown that phosphorus deficiency highly activates acid phosphatase and phytase activities in arid crop species, which is a significant biochemical adaptation to phosphorus uptake. There was a great difference between crops and genotypes, which points out the importance of genetic factors in deciding phosphorus-use efficiency. Pearl millet was found to have the highest enzymatic activity, then moth bean and mung bean, which showed that they have the highest potential to mobilize inorganic and organic phosphorus with nutrient-limited conditions. In comparison, sorghum recorded relatively reduced phytase activity indicating a low level of dependence on organic phosphorus hydrolysis.

The fact that progressively the enzyme activity has risen as plant age increases is also a sign that phosphorus requirement and mobilizing processes are also highly intertwined with the stage of plant development. The effectiveness of screening of specific genotypes using enzyme-based screening in the identification of nutrient-efficient plant materials is confirmed by the superior performance of the specific genotypes in the hydroculture environment.

Generally, this research has solid evidence that the acid phosphatase and phytase activity can be a good biological indicator to use in the selection of phosphorus-efficient

genotypes. The superior genotypes have great potential to be utilized in breeding programs to enhance crop productivity in arid soils with phosphorus deficiency. Future studies must be directed to field verification and combining these characteristics with molecular and microbial methods in order to come up with the strategy of sustainable management of nutrients.

## *Declaration by Authors*

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