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Effect of urdbean (*Vigna mungo*) cultivars and phosphorus levels on dynamics of soil phosphorus fractions and enzyme activity

ASIK DUTTA[1]*, KRISHNASHIS DAS[2], M SENTHILKUMAR[1], NARENDRA KUMAR[1] and CHAITANYA P NATH[1]

ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208 024, India

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ABSTRACT

A field experiment was conducted during the rainy (*kharif*) seasons of 2019 and 2020 at ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh to study the impact of soil inorganic phosphorus fractions and enzyme activity in different urdbean [*Vigna mungo* (L.) Hepper] cultivars. Experiment was conducted in a randomized block design (RBD) with 4 replications with two urdbean cultivars, viz. IPU-2-43 and Uttara. Experimental results suggest, higher sol-P content (6 and 14% in 2019 and 2020, respectively) under Uttara than IPU 2-43 under low P-plots whereas, no prominent change of the same was recorded under normal P-plots. Similar to sol-P, the content of Ca_2-P has jumped significantly in Uttara than IPU 2-43. However, Uttara recorded higher acid and alkaline phosphatase activity as compared to IPU 2-43. In low-P plots the activity of acid phosphatase was higher by 12% (2019) and 10% (2020) in case of Uttara than IPU 2-43. Under low P soils the productivity of Uttara was significantly higher (~12% and ~9% in 2019 and 2020, respectively) than IPU 2-43. Therefore, based on the findings it can be said that with higher soluble P and productivity, the performance of Uttara was better over IPU 2-43 under low-P condition.

Keywords: Acid phosphatase, Cultivars, P- fractions, Urdbean, Yield

Protein (24%), minerals (3.2%) and array of different nutritional components have made urdbean [*Vigna mungo* (L.) Hepper] an integral component of Indian diets and covers nearly 4.63 Million hectare (Mha) with an average productivity of 600 kg/ha in India (Sen Gupta *et al.* 2020, Agricultural Statistics 2022). Short life span, high per day productivity and ability to adapt in different agro-ecological conditions are some of the excellent characteristics of urdbean which makes it a perfect choice for the farming community (Singh *et al.* 2016). Sustained efforts by the researchers over the time such as, development of biotic stress tolerant and photo-thermo insensitive short duration urdbean varieties augmented the productivity level by manifolds but meagre attention has been given on nutrient management in this crop (Singh *et al.* 2016). As urdbean can meetup the nitrogen (N) requirement through biological nitrogen fixation (BNF), phosphorus (P) is the cardinal nutrient for them associated in many biological functions like energy conversion, mediating root growth and translocating photosynthates in different plant parts (Dutta *et al.* 2022).

In soil, inorganic P (P_i) has been present in different fractions like (soluble-P, di-calcium P, occluded-P) based on *p*H, relative abundance of calcium (Ca), iron (Fe), and aluminium (Al) compounds however, in alkaline soil different compounds of calcium (Ca) and sometimes sesquioxides coating on calcium carbonate ($CaCO_3$) surface is the predominant factor hindering soil P availability *vis-a-vis* legume production especially in the tropics (Shen *et al.* 2004, McLaughlin *et al.* 2011). Undoubtedly, rhizospheric acidification (phosphatase and exuding carboxylates) enable legumes to utilise recalcitrant P-pools (Rose *et al.* 2010, Tian *et al.* 2020) but, the ability to forage and solubilise the native soil P varies along the crop species even within the cultivars due to difference in rooting behaviour, degree of rhizospheric modifications, intra plant P-translocation and mycorrhizal association (Nandwa *et al.* 2011). Therefore, there is a dire need to screen of efficient urdbean cultivars which can solubilise non-labile P-fractions and making it available peculiarly in the world where P-reserve is highly limited. Therefore, keeping the above facts in view, a biennial field experiment was conducted to assess the effect of urdbean cultivars and phosphorus levels on soil inorganic P fractions, enzyme activity and productivity.

MATERIALS AND METHODS

A field experiment was conducted during the rainy (*kharif*) seasons of 2019 and 2020 at ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh. The experimental

[1]ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh; [2]ICAR-Indian Agricultural Research Institute, New Delhi. *Corresponding author email: asikdutta975@gmail.com

plots were properly drained with sandy-loam soil texture with *p*H 8.27 and electrical conductivity 0.30 dS/m (non-saline). The experimental field was low in available N (215 kg/ha) content while, medium in available potassium (K) (163 kg/ha). The available P content in normal-P and low-P strip was medium (16.4 kg/ha) and low (9 kg/ha), respectively. The recommended dose of 20:40:40 kg/ha ($N:P_2O_5:K_2O$) was applied in normal P-strips. However, low P strips does not receive any phosphatic fertilizer since last 13 years and the others two nutrients (20 kg N: 40 kg K_2O /ha) were applied uniformly.

Two urdbean cultivars, viz. IPU-2-43 and Uttara were sown in both the low and high P-plots with a spacing of 30 cm × 10 cm. In both the strips, the genotypes were sown in 3 m × 2 m plots in a randomized block design (RBD) with 4 replications. *Rhizobium* culture (>10^7 bacteria/g inert material) at 20 g/kg seeds was used to treat the seeds prior to sowing. Crop was irrigated at pod formation and no plant protection measures were taken in the crop season. Soil samples from the rhizosphere of individual genotypes carefully collected and after collecting any kind of visible dirt or foreign particle was removed. Necessary steps like air-drying, grounding and passing through 2 mm sieve was carried out to process the collected samples and prepare the final one. The final sample was kept in two halves and one of that was placed inside refrigerator at 4˚C for determination of soil microbial parameters whereas, the other subsection was housed in ambient condition (~24˚C) for analysing soil inorganic P-fractions.

Kuo (1996) outlined the steps to extract the Pi-fractions and in this study the same has been adapted with definite changes to extricate the P-fractions conjoined with different Ca-compounds (Smillie and Syers 1972). Using different extracting reagent in a sequential manner a total seven Pi-fractions was determined. At first, in a 50 ml centrifuge tube, 0.5 g soil sample was taken and precaution must be taken to avoid unnecessary soil loss in every step. In the first step, soil samples were shaken with 1 M ammonium chloride (NH_4Cl) (25 ml) for half an hour to extract the soluble and loosely bound fraction (fraction 1). The left-over of the previous extractant was discarded and samples were treated with 0.25 M sodium bicarbonate ($NaHCO_3$) (*p*H 7.5) and ammonium acetate ($C_2H_7NO_2$) (*p*H 1.2) for extracting di-Ca bound P (Ca_2-P) (fraction 2) and octa-Ca bound P (Ca_8-P) (fraction 3), respectively. Solubility in both water and citrate *vis-a-vis* surface adsorption characteristics make Ca_2-P make crucial for plant P-availability. In the third step, washing of samples for 15 min in 95% ethanol was a must. For, extracting fraction 4 and 5, viz. Fe bound P (Fe-P) and aluminium bound P (Al-P) soil samples were shaken with 0.5 M ammonium fluoride (NH_4F) (*p*H 8.2) and 0.1 M sodium hydroxide (NaOH) for 1 h and 17 h, respectively. After collecting the extractant, samples were washed with saturated sodium chloride (NaCl) solution and both of them were combined. Combination of sodium citrate ($Na_3C_6H_5O_7.2H_2O$)-sodium dithionate ($Na_2S_2O_4$)-sodium bicarbonate or commonly known as CDB-treatment was carried out to bring out occluded P (fraction 6). Soil samples were initially shaken with 25 ml (20 ml of 0.3 M $Na_3C_6H_5O_7.2H_2O$ and 5 ml of 1 M $NaHCO_3$) of extracting solution followed by heating in a water bath for 15 min at 85˚C. Afterwards, samples were mixed rapidly after adding 0.5 g $Na_2S_2O_4$ and lastly washed with 25 ml NaCl solution before placing them over-night in a well-ventilated room. In the end, deca-Ca phosphate (Ca_{10}-P) sharing similar chemical structure of hydroxyl apatite [$Ca_{10}(PO_4)_6.(OH)_2$], was extracted with 0.25 M sulphuric acid (H_2SO_4). At the completion of every step, supernatant was collected in a 50 ml volumetric flask and final volume was adjusted by using double distilled water. Determination of individual soil P-fractions was carried out by phospho-molybdate method (Murphy and Riley 1972). Prescribed methodology by Tabatabai and Bremner (1969) was followed for assessing acid and alkaline phosphatase activity (µg p-nitrophenol/g dry soil/h) in the soil samples. The dehydrogenase activity in the soil was determined by Klein *et al.* (1971) using 2,3,5- triphenyl tetrazolium chloride (TTC) method and results are expressed as µM triphenyl formazan (TPF)/g/h of soil. Crop yield was expressed in g/plot basis. Student t-test ($P \leq 0.05$) was used to assess the significant difference among the genotypes.

RESULTS AND DISCUSSION

P-fractions: Non-significant results were obtained with respect to different P-fractions except soluble (sol)-P and di-calcium (Ca_2)-P fractions. In low-P plots both in 2019 and 2020, sol-P content was ~6% and ~14% higher in Uttara over IPU 2-43 (Table 1). Similar to sol-P, there was no change in Ca_2-P fractions under normal-P plots among the genotypes but, the same was ~7% and ~11% higher in Uttara over IPU 2-43 under low-P plots (Table 1). Different attributes of root like root hairs, length and intensity of root induced processes are the primary factors responsible for transformation of non-labile P into labile P fractions (Krasilnikoff *et al.* 2003). The other reason might be due to upregulation of phosphatase activity especially under Uttara which could be seen in this experiment also (Shen *et al.* 2011). Dutta *et al.* (2023) reported the difference in soil P-fractions in two lentil cultivars (IPL 316 and IPL 406) and the primary reason was the divergent root mediated activity. The surge in labile P with resultant decrease in Ca_2-P was a result of two counterbalance processes i.e. increase in rhizospheric acidification mediated P- desorption and diminution due to plant uptake and adsorption (Li *et al.* 2015). Nonetheless, the better efficacy of Uttara in mobilising soil P-pools may be due to higher effective absorbing surface are, amount of root mediated secretions, effective foraging ability and translocation of photosynthates in the roots (Ganguly *et al.* 2021). Also, specific strategies in legume cultivars like deposition of P and sugar in nodules than the above ground parts, efficacious N-fixation per unit of nodular mass and directly absorbing P inside nodules and bacteroids aid better P-nutrition in the deficient conditions (Araújo *et al.* 2008). Better photosynthetic allocation in the roots along with

Table 1 Dynamics of soil inorganic P-fractions under two urdbean genotypes in different P-levels

Soil P content	Soil P-fractions (ppm)													
	Sol-P		Ca_2-P		Ca_8-P		Al-P		Fe-P		Occ-P		Ca_{10}-P	
	N	L	N	L	N	L	N	L	N	L	N	L	N	L
2019														
IPU 2-43	30.38 ± 1.4^{A}*	26.25 ± 0.3^{A}	22 ± 1.2^{A}	19.65 ± 0.16^{B}	104.50 ± 1.8^{A}	100.82 ± 0.16^{A}	45.42 ± 3.4^{A}	49.96 ± 3^{A}	32.83 ± 3.2^{A}	47.44 ± 3.8^{A}	18 ± 2.4^{A}	17.42 ± 1.8^{A}	133.1 ± 0.35^{A}	127.88 ± 3.4^{A}
Uttara	26.38 ± 0.8^{A}	27.87 ± 0.1^{B}	21.25 ± 0.7^{A}	21.12 ± 0.13^{A}	102.25 ± 1.3^{A}	90.68 ± 2.4^{A}	49.16 ± 3.6^{A}	48.96 ± 2.3^{A}	27.20 ± 1.8^{A}	52.25 ± 1.7^{A}	18.95 ± 0.9^{A}	21.27 ± 2.3^{A}	132.73 ± 0.23^{A}	143.70 ± 2.5^{A}
2020														
IPU 2-43	33.31 ± 0.7^{A}	23.13 ± 0.1^{B}	26.61 ± 0.9^{A}	23.62 ± 0.4^{B}	118.45 ± 2.5^{A}	84.42 ± 1.2^{A}	49.02 ± 3^{A}	59.46 ± 0.6^{A}	34.65 ± 3.6^{A}	43.3 ± 1.3^{A}	21.97 ± 1.6^{A}	15.75 ± 1.3^{A}	133.85 ± 3.2^{A}	130.26 ± 11.1^{A}
Uttara	35.14 ± 0.5^{A}	26.79 ± 0.2^{A}	26.76 ± 1.3^{A}	26.48 ± 0.5^{A}	105.35 ± 1.8^{A}	86.74 ± 2.8^{A}	40.07 ± 0.4^{A}	40.7 ± 3^{A}	27.08 ± 3^{A}	51.8 ± 3.7^{A}	17.02 ± 1.9^{A}	20.49 ± 1.5^{A}	128.03 ± 3.4^{A}	127.87 ± 2.1^{A}

*Values followed by different upper case letters (A–B) are significantly different between treatments at $P \leq 0.05$. N, Normal P-dose; L, Low P-dose.

mycorrhizal colonisation in the roots is an important criterion for assessing genotypic potential for P-solubilisation and acquisition but, exceptions do exist (Cong *et al.* 2020). The quantity of sol-P increased in normal P-strips (*e.g.* Δ+9% in IPU 2-43) whereas, the sol-P declined in low P-strips (e.g. Δ-12% in IPU 2-43) (Table 1). Irrespective of urdbean cultivar higher alteration into Fe-P in the low P-plots due to higher acidification facilitating sorption of native soil P with concomitant reduction of soluble P (Tian *et al.* 2020). As mentioned in the earlier section any of the P-fractions namely Ca_8-P, Al-P, Fe-P, Occ-P and Ca_{10}-P showed no significant changes over the years in any of the genotypes. Non-significant change in Ca_8-P and Ca_{10}-P was due to high *p*H of the experimental fields (Mat Hassan *et al.* 2012). Over the years the content of Ca_8-P content reduced (16 and 4% in IPU 2-43 and Uttara) indicating the fact that both the cultivars were capable of utilising legacy P via root mediated acidifications (Gao *et al.* 2019). Irrespective of year, none of the cultivars are effective in utilising non-labile P (Ca_{10}-P) due to limited biochemical activities under poor fertility conditions of the soil.

Enzyme: IPU 2-43 recorded significantly higher dehydrogenase activity over Uttara. In normal P-plots dehydrogenase content was 46% higher in IPU 2-43 over Uttara whereas, in low-P plots the respective increase in dehydrogenase content was ~29% and ~20% under IPU 2-43 as compared to Uttara in 2019 and 2020, respectively (Fig. 1). Higher dehydrogenase activity in the rhizosphere of IPU 2-43 implies higher microbial activity specifically in the P-fertilised plots. Except in 2020, under normal P-plots, the content of acid and alkaline phosphatase content irrespective of year and P-content was significantly higher in Uttara as compared to IPU 2-43. In 2019, activity of acid phosphatase under normal P-plots was ~10% higher in Uttara (130.28 µg p–nitrophenol/g soil/h) than IPU 2-43 (118.3 µg p–nitrophenol/g soil/h). Whereas, in low-P plots the activity of acid phosphatase was higher by 12% (2019) and 10% (2020) in case of Uttara than IPU 2-43 (Fig. 2a). Since, secretion of acid phosphatase was an adaptive trait for legumes under low-P conditions which could be discerned from this study (Dutta *et al.* 2022). Ganguly *et al.* (2021) pointed out apart from root mediated secretions and acid

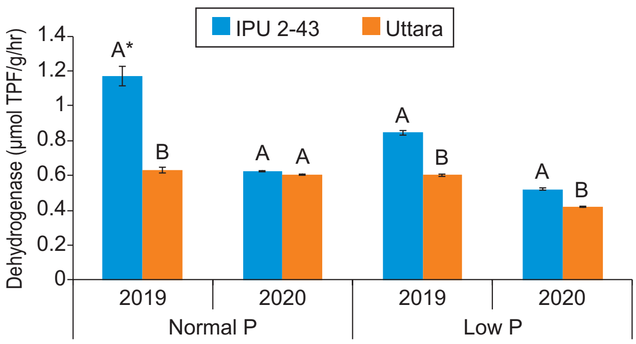

Fig. 1 Activity of soil dehydrogenase (µmol TPF/g/hr) under two urdbean genotypes in different P-levels.
*Values followed by different upper case letters (A-B) are significantly different between treatments at $P \leq 0.05$.

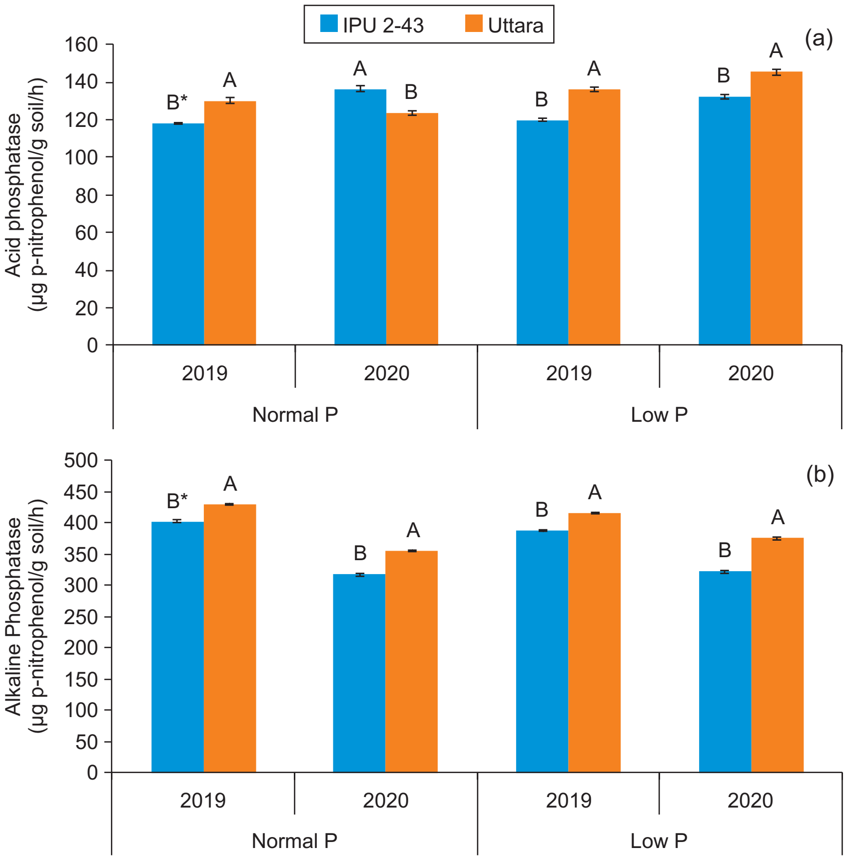

Fig. 2 Activity of soil (a) acid and (b) alkaline phosphatase under two urdbean genotypes in different P-levels.
*Values followed by different upper case letters (A–B) are significantly different between treatments at $P \leq 0.05$.

phosphatase activity, other physiological and morphological changes of the roots plays critical role and helping out the plant to use soil P. Nannipieri *et al.* (2011) elucidated that excess phosphate fertilisers could supress phosphates activity due to down-regulation of PHO genes. However, the acidifying impact of legumes are strongly correlated with BNF and biomass and not low but a congruous P-dose is the key to attain optimum enzyme activity (Tian *et al.* 2020). Difference in rooting behaviour, N-utilisation efficiency, microbial association, root phenes and lateral branching density which clearly take one cultivar ahead of another in availing soil-P explicitly in deficit conditions (Camilo *et al.* 2021). Alkaline phosphatase content in normal P-plots was significantly higher in Uttara over IPU 2-43 by ~6.5% and ~11% in 2019 and 2020, respectively. Similar positive gain in alkaline phosphatase activity can be recorded in low P plots under Uttara than IPU 2-43 (Fig. 2b). Additionally, application of N in the plots which were deficient in N and P resulted in higher phosphatase activity as compared to normal P-plots (Janes-Bassett *et al.* 2022) but, at the same time N-addition in long run negatively impact phosphates activity due to upsurge in number of P-mineralisers (Chen *et al.* 2019). However, Venterink (2011) emphasizes the intrinsic physiological traits in regulating phosphates activity rather than the microbial activity. Also, it can be outlined that over the years there was a constant drop in alkaline phosphatase activity.

Yield: In both the year under normal P-plots, IPU-2-43 recorded significantly higher grain yield but, in contrary in the low P-plots a completely opposite scenario can be seen where, the productivity of Uttara was significantly higher than IPU 2-43. Grain yield of Uttara was ~12% and ~9% higher under low-P plots as compared to IPU 2-43 in 2019 and 2020, respectively (Fig. 3). Higher P-allocation *vis-a-vis* uptake regulated by acid and alkaline phosphates along with better source-sink relation revamped the yield of urdbean in the low-P plots as compared to the normal P-plots (Ganguly *et al.* 2021). Grain legumes like urdbean are highly efficient in sustaining production even in low nutrient supply as because they can be used both to produce a seed crop themselves and to generate high N-content plant residue (Sinclair and Vadez 2002). Irfan *et al.* (2017) by a screening study reported mungbean genotype: AEM-20/3/87 with highest grain yield and P-accumulation capability out of 10 mungbean genotypes under different P-levels. Discrepancy in different genetically mediated yield attributing characteristics like number of pods/plant and seeds/pod do exists among legume genotypes causing difference in yield (Irfan *et al.* 2017). Under P-limited conditions, cultivars are competent enough to translocate phosphorus in different plant parts and subsequently in the economic parts led to significantly higher grain yield (Irfan *et al.* 2017). On the basis of 2-years study, it was concluded that can be concluded that under low P plots, Uttara performed better with significantly higher soluble P (10%), acid phosphatase (11%) and productivity (9%) than IPU 2-43. But, long term trails with more in-depth information about the soil inorganic and organic P-fractions

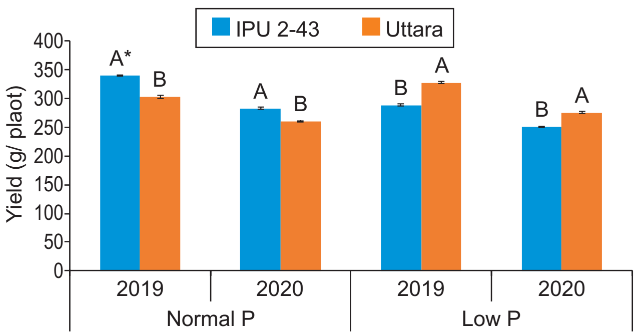

Fig. 3 Changes in yield (g/plot) under two urdbean genotypes in different P-levels.
*Values followed by different upper case letters (A–B) are significantly different between treatments at $P \leq 0.05$.

in association with plant morphological traits are need of the hour to draw a clear conclusion.

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