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Rice Bean (*Vigna umbellata* Thunb.) Acid Phosphatase: Effect of Metal Ions and Metabolites

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Abstract: **Rice bean acid phosphatase dialyzed against ethylenediaminetetracetic acid (1 mM), yielded no loss in activity at 0.5 mM *p*-nitrophenylphosphate. It had a mild activating effect at 0.1 mM *p*-nitrophenylphosphate. Divalent cations belonging to group II of Periodic Table like Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} at 0.5 mM concentration *p*-nitrophenylphosphate did not affect the enzyme activity. At 0.1 mM *p*-nitrophenylphosphate, however, only Mg^{2+} ions showed little inhibition. The transition metal ions viz. Zn^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} showed inhibition at both *p*-nitrophenylphosphate concentrations. Na^{+}, K^{+}, and NH_4^{+} ions did not influence the enzyme activity, but Li^{+} inhibited the activity at 0.1 and 0.5 mM *p*-nitrophenylphosphate concentrations, respectively. Molybdate and phosphate anions proved to be strong inhibitors, but vanadate, a moderate inhibitor. Tartrate anion did not exhibit an effect on rice bean acid phosphatase. Sugars, plant hormones, medicines, vitamins, and amino acids at 1.0 mM concentration did not affect the enzyme activity. NADH exhibited the maximum activation, followed by citric acid, isocitrate, and oxaloacetate. Caffeine showed activation in the enzyme activity. Phosphate esters (1-Naphthylphosphate, phenylphosphate, PEP, and ADP) exhibited competitive inhibition when *p*-nitrophenylphosphate was used as a substrate. Non-ionic detergents Triton X-100 and Tween-20 brought activation to the enzyme. The ionic detergent SDS brought complete loss to the enzyme activity. Dithiothreitol brought inhibition to the enzyme activity. The presence of β-mercaptoethanol did not influence the enzyme activity.**

Index terms: **Acid phosphatase, Metabolites, Metal ions, Phosphate, Rice bean.**

I. Introduction

Acid phosphatase (EC 3.1.3.2) (APase) catalyzes the hydrolysis of phosphate esters to yield alcohol and inorganic phosphate at pH below 7.0 (Duff et al., 1994). APase got isolated and characterized from different plant sources (Roknabadi et al., 1999; Turner & Plaxton, 2001; Koffi et al., 2010; Anand & Srivastava, 2013; Tagad & Sabharwal, 2018; Zaman et al., 2020; Chafik et al., 2020; Nongpiur et al., 2021). Reports are there on the influence of ions (cations and anions) and metabolites (effectors) on the acid phosphatase activity (Guo & Roux, 1995; Turner & Plaxton, 2001; Coello, 2002; Greiner & Jany, 2003; Koffi et al., 2010; Nadir et al., 2012; Khan et al., 2016; Chafik, 2020).

EDTA demonstrated differential effects on APase from different plant tissues. Most APases did not exhibit an effect with EDTA, indicating that divalent cations were not essential for catalytic activity (De-Kundu & Banerjee, 1990; Miernyk, 1992; Kawarasaki et al., 1996; Nakazato et al., 1997; Granjeiro et al., 1999; Cirkovic et al., 2002; Greiner & Jany, 2003; Senna et al., 2006). In the presence of EDTA, inhibition in the enzyme activity was observed in some APases, suggesting that there is an obligate requirement of divalent cations (Gellatly et al., 1994; Asaduzzaman et al., 2011). EDTA brought a 2.5 fold stimulating effect on tyrosine phosphatase from pea nuclei (Guo & Roux, 1995). A similar activation effect was noticed in the coleoptiles and seeds of barley and *Lagenaria siceraria* APase (Pasqualini et al., 1992; Koffi et al., 2010). Among the metal ions, a study was on the use of Zn^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, and Ca^{2+} (Biswas & Cundiff, 1991; Kawarasaki et al., 1996; Garcia et al., 2004; Senna et al., 2006; Asaduzzaman et al., 2011; Anand & Srivastava, 2013; Khan et al., 2016).

The effect of anions such as phosphate, molybdate, and vanadate has been extensively studied (Biswas & Cundiff, 1991; Guo & Roux, 1995; Cirkovic et al., 2002; Garcia et al., 2004; Hoehamer et al., 2005; Nadir et al., 2012; Khan et al., 2016). Phosphate and vanadate ions are structurally similar. Phosphorus

and vanadium are located centrally with four oxygen attached in a tetrahedral arrangement. Three oxygen are in the ionic form. Inorganic phosphate (Pi), a product of APase catalyzed hydrolysis, whatever the substrate may be, is a potent competitive inhibitor (Kaneko et al., 1990; Biswas & Cundiff, 1991; Miernyk, 1992; Granjeiro et al., 1999; Roknabadi et al., 1999; Turner & Plaxton, 2001; Cirkovic et al., 2002; Coello, 2002; Greiner & Jany, 2003; Andriotis & Ross, 2004; Hoehamer et al., 2005; Nadir et al., 2012). It is agreed upon that Pi could play a physiological role in sustaining a stable phosphate level in the cell through feedback regulation of enzyme activity. Vanadate is also one of the anions that show inhibition in most cases (Gellatly et al., 1994; Granjeiro et al., 1999; Roknabadi et al., 1999; Turner & Plaxton, 2001; Coello, 2002; Greiner & Jany, 2003; Andriotis & Ross, 2004; Senna et al., 2006; Nadir et al., 2012).

Molybdate anion has a central Mo with four oxygen attached, of which only two are in the ionic form. Molybdate is also a potent inhibitor to almost all APase tested so far (De-Kundu & Banerjee, 1990; Kaneko et al., 1990; Miernyk, 1992; Gellatly et al., 1994; Olczak et al., 1997; Tso & Chen, 1997; Granjeiro et al., 1999; Zhang & McManus, 2000; Turner & Plaxton, 2001; Cirkovic et al., 2002; Coello, 2002; Greiner & Jany, 2003; Senna et al., 2006; Nadir et al., 2012). Tartrate had no significant effect on APase activity of hazel seeds (Andriotis & Ross, 2004), potato tuber (Gellatly et al., 1994), lupin seeds (Olczak et al., 1997), buckwheat (Greiner & Jany, 2003), and *Vigna radiata* (Nadir et al., 2012) and this is a typical feature of the purple APases. Animal purple APases got reported to be resistant to tartrate inhibition, while most plant purple APases are either slightly inhibited or did not show an effect (De-Kundu & Banerjee, 1990; Roknabadi et al., 1999).

Literature is also available on the effect of metabolites on acid phosphatase activity. The presence of citrate, oxalate, DTT, SDS, Triton X-100, β-mercaptoethanol on the enzyme activity got tested (Sugiura et al., 1981; De Kundu & Banerjee, 1990; Roknabadi et al., 1999; Greiner & Jany, 2003; Nadir et al., 2012; Chafik, 2020).

Non-ionic detergent like Triton X-100 brought activation in most cases. This detergent has a strong affinity for hydrophobic side chains, and its interaction with a hydrophobic domain is responsible for activation. Further, its effect on different isozymes varies. It stimulated the activities of AP-I, AP-II, and AP-III but did not change AP-IV activity (Biswas & Cundiff, 1991). AP-1 was activated, while AP-2 showed inhibition in the presence of Triton X-100 (Koffi et al., 2010). SDS being an anionic detergent brings inhibition in activity only. Its effect on two isozymes AP-I and AP-II from *Vigna radiata* showed a marked difference. AP-I was severely affected, whereas much less inhibition got observed for AP-II.

Recently a report on the purification of acid phosphatase from rice bean (*Vigna umbellata* Thunb.) has come from our laboratory (Nongpiur et al., 2021). In the present paper, we put forward the results of the effect of cations, anions, and metabolites on the acid phosphatase activity and analyze the results in the light of available literature.

II. RESULTS AND DISCUSSION

A. Effect of cations

The enzyme dialyzed against EDTA showed no loss in the specific activity. The result suggests that none of the divalent cations are bound to the enzyme. The effect of divalent and monovalent cations on the APase activity were tested at 1.0 mM concentration at two different *p*-NPP concentrations (0.1 and 0.5 mM). The results got summarized in **Table I**.

Table I: Effect of cations (1mM) on (EDTA dialyzed) APase activity

[Cation] (1.0 mM)	**Activity (%) [*p*-NPP] (0.1mM)**	**Activity (%) [*p*-NPP] (0.5mM)**
Control	100	100
EDTA	112.3 ±1.69	107.2 ±4.72
$MgCl_2$	86.5 ± 2.6	97.5 ± 5.2
$MnCl_2$	72.7 ± 0.7	76.9 ± 2.2
$CaCl_2$	94.3 ± 2.0	101.4 ± 1.0
$SrCl_2$	93.2 ± 1.8	96.9 ± 3.5
$FeCl_3$	42.3 ± 2.3	59.6 ± 2.9
$BaCl_2$	94.3 ± 6.0	102.5 ± 1.7
$CoCl_2$	66.9 ± 0.2	86.6 ± 0.9
$ZnCl_2$	0.0	ND*
$CuSO_4$	0.0	ND*
NaCl	95.4 ± 3.2	97.8 ± 2.1
KCl	95 ± 4.0	99.7 ± 2.3
LiCl	45.8 ± 3.1	61.5 ± 2.2
NH_4Cl	95.7 ± 1.7	110.1 ± 1.1

The transition metal ions viz. Co^{2+}, Mn^{2+}, Fe^{3+}, and Cu^{2+} inhibited the enzyme at the two *p*-NPP concentrations (0.1 and 0.5 mM). Mn^{2+} ions inhibited some APases (Sugiura et al., 1981; Guo & Pesacreta, 1997; Yenigun & Guvenilir, 2003; Garcia et al., 2004; Anand & Srivastava, 2013). Fe^{3+} also inhibited APase isoforms from *Vigna* species (*V. sinensis* and *V. aconitifolia*) and *Opuntia* (Biswas & Cundiff, 1991; Biswas et al., 1996; Anand & Srivastava, 2013; Chafik, 2020). Co^{2+} inhibited some APases (Turner & Plaxton, 2001; Garcia et al., 2004). The effects of Zn^{2+} and Cu^{2+} got tested on APase activity at 0.1 mM *p*-NPP. A complete loss in the enzyme activity was observed. Inhibition of APase activity in the presence of Zn^{2+} is consistent with other APases (Sugiura et al., 1981; Kruzel & Morawiecka, 1982; Pan et al.. 1987; Gellatly et al., 1994; Olczak et al., 1997; Cashikar et al., 1997; Granjeiro et al., 1999;

Roknabadi et al., 1999; Turner & Plaxton, 2001; Koffi et al., 2010; Asaduzzaman et al., 2011; Anand & Srivastava, 2013; Khan et al., 2016). Inhibition in the presence of Cu^{2+} ions got observed in some APases (Sugiura et al., 1981; Waymack & Van Etten, 1991; Tso & Chen, 1997; Granjeiro et al., 1999; Yenigun & Guvenilir, 2003; Garcia et al., 2004; Singh & Luthra, 2011; Anand & Srivastava, 2013; Chafik, 2020).

Na^+, K^+, and NH_4^+ ions do not influence APase activity. Na^+ and K^+ ions did not affect other APases (Sugiura et al., 1981; Tagad & Sabharwal, 2018). Li^+ inhibited the rice bean APase in the present study. APase from *Phaseolus vulgaris* leaf got inhibited by Li^+. The latter ions did not influence APase from *P. vulgaris* nodule (Garcia et al., 2004). Other enzymes also did not show an effect in the presence of Li^+ (Guo & Pescareta, 1997; Khan et al., 2016).

B. Effect of anions

The results of effect of anions (1 mM) on APase activity is shown in **Fig. 1**.

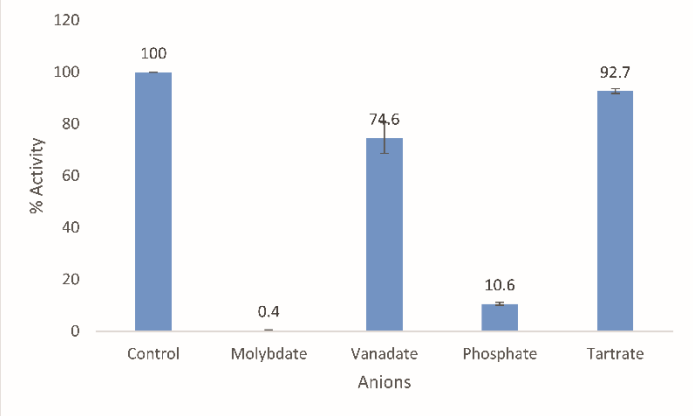

Fig. 1: Effect of anions (1 mM) on APase activity at [*p*-NPP] 0.1 mM.

Molybdate and phosphate anions proved stronger inhibitors to the vanadate anion. Inhibition in the enzyme activity got reported in the presence of phosphate in different APases (Sugiura et al., 1981; Ching et al., 1987; Gellatly et al., 1994; Olczak et al., 1997; Granjeiro et al., 1999; Turner & Plaxton, 2001; Cirkovic et al., 2002; Singh & Luthra, 2011). Phosphate is one of the products of the APase catalyzed reaction. There are reports of inhibition of APase activity in the presence of molybdate (Sugiura et al., 1981; Kruzel & Morawiecka, 1982; Ching et al., 1987; Miernyk, 1992; Pasqualini et al., 1992; Gellatly et al., 1994; Olczak et al., 1997; Tso & Chen, 1997; Cashikar et al., 1997; Granjeiro et al., 1999; Ferreira et al., 2000; Turner & Plaxton, 2001; Coello, 2002; Senna et al., 2006; Singh & Luthra, 2011; Khan et al., 2016). Phosphate and molybdate are most often inhibitors of APase did not affect the APase from tomato leaves (Tanaka et al., 1990).

APase also got inhibited in the presence of vanadate (Waymack & Van Etten, 1991; Gellatly et al., 1994; Granjeiro et al., 1999; Roknabadi et al., 1999; Ferreira et al., 2000; Turner & Plaxton, 2001; Coello, 2002; Cirkovic et al., 2002; Senna et al., 2006). Tartrate anion did not affect the rice bean APase like other APases (Sugiura et al., 1981; Vincent & Averill, 1990; Olczak et al., 1997; Granjeiro et al., 1999; Turner & Plaxton, 2001; Coello, 2002; Andriotis & Ross, 2004).

C. Effect of metabolites

The result of sugars influence on the APase activity is in **Table II**.

The influence of effectors on the APase catalyzed reaction got tested; the *p*-NPP concentration kept 0.1 mM which is close to the K_m value (0.108 mM) (Nongpiur et al. 2021). In these conditions, the enzyme is 50% saturated, and therefore effectors are more likely to exert their effects (Ambasht et al., 1997). In some experiments, however, the *p*-NPP concentration was kept 0.5 mM.

Disaccharides do not significantly affect APase catalyzed reaction. Sucrose did not affect APase activity from ripened banana fruit (Turner & Plaxton, 2001). Glucose and fructose exhibited mild activating effects. However, the above sugars did not demonstrate any effect on APase from banana fruit (Turner & Plaxton, 2001).

Table II: Effect of biomolecules (sugars) on rice bean APase activity

[Biomolecule] 1mM; [*p*-NPP] 0.1 mM	**Activity (%)**
Sugars	
Control	100
Glucose	111.2 ± 2.5
Sucrose	106.4 ± 4.3
Galactose	108.3 ± 7.5
Lactose	106.9 ±8.8
Maltose	106.9 ±1.0
Fructose	111.7 ± 6.8

All 20 amino acids tested had no effect on APase activity (data not shown). Asp and Glu did not demonstrate any end-result on APase from ripened banana fruit (Turner & Plaxton, 2001).

The outcome of effects of some plant hormones like indole-3-acetic acid, gibberellic acid, abscisic acid, salicylic acid and some medicines like acetaminophen, aspirin, ibuprofen, etc. at 1.0 mM got tested on APase activity. None of them exert any effect on APase activity (data not shown).

The effects of some metabolites of at 1.0 mM got tested on APase activity ([*p*-NPP] 0.5 mM). Results are displayed in **Table III**.

Amongst the metabolites, NADH showed maximum activation. Intermediates of the TCA cycle like citrate, isocitrate, and oxaloacetate mildly activated the APase activity. Citrate activated different APases (Guo & Roux, 1995; Kawaraski et al., 1998). Citrate in some other APases did not show any effect (Cirkovic et al., 2002; Greiner & Jany, 2003; Nadir et al., 2012).

Table III: Effect of metabolites and phosphate esters (1mM) on APase activity

Metabolites and Phosphate esters	**Activity (%)**
Control [*p*-NPP] 0.5 mM	100
Metabolites (1 mM)	
Citrate	121.9 ± 1.2
Isocitrate	116 ± 3.1
Oxalic Acid	113 ± 3.8
Oxaloacetic acid	118.1 ± 2.2
Fumaric acid	112.7 ± 5.2
α Ketoglutaric acid	109.8 ± 5.2
Pyruvic acid	112.7 ± 2.2
Urea	107.9 ± 3.8
NADH	127.4 ± 2.0
Glycolic acid	109.2 ± 3.1
Gluconic acid	103.8 ± 1.3
Caffeine	116.3 ± 4.6
Phosphate esters (1mM)	
Glucose-6-phosphate	97.4 ± 3.4
Fructose-6-phosphate	83.6 ± 2.4
PEP	58.4 ± 0.3
ATP	59.6 ± 0.6
ADP	64.1 ± 1.3
AMP	101.5 ± 5.3
$NADP^+$	116.5 ± 0.9
1-Naphthyl phosphate	60.1 ± 1.3
Phenyl phosphate	50.5 ± 1.7
Phytic acid	89.2 ± 5.0

Other dicarboxylic acids like oxalic acid and fumaric acid-activated rice bean APase marginally. The presence of oxalate did not manifest any effect on the APase activity (Olczack et al., 1997; Greiner & Jany, 2003). Caffeine brought activation to the APase. The role of fumaric acid, isocitrate, and oxaloacetate on APase activity is still not established. It is interesting to note that PEP, ADP, 1-naphthyl phosphate, and phenyl phosphate in presence of *p*-NPP as substrate inhibited strongly APase activity. ATP also strongly inhibited the enzyme activity. Fructose-6-phosphate moderately inhibited APase, but glucose-6-phosphate had no effect. $NADP^+$ moderately activates APase activity. AMP has no effect on APase activity.

For a better analysis of the data, the effect of such metabolites at 1.0 mM and 0.1 mM was tested keeping the *p*-NPP concentration 0.1 mM. The result is presented in **Table IV**. The results are first compared when the metabolite concentration was kept 1.0 mM and [*p*-NPP] reduced from 0.5 mM and 0.1 mM. The activation in the presence of tricarboxylic acids citrate and isocitrate was more pronounced. Oxaloacetate and caffeine which were moderately activating the enzyme, showed no effect.

Table IV: Effect of selected metabolites on APase activity

Metabolite	**Activity % [*p*-NPP] (0.1 mM) [Metabolite] (0.1mM)**	**Activity % [*p*-NPP] (0.1 mM) [Metabolite] (1.0 mM)**
Control	100	100
Citrate	122.5 ± 2.5	129.7 ± 3.9
Isocitrate	117.7 ± 4.3	121.4 ± 2.8
Oxaloacetic acid	97.9 ± 1.7	103.8 ± 0.7
Caffeine	97.2 ± 3.4	94.0 ± 5.5
NAD^+	84.28 ± 2.2	72.5 ± 3.3
PEP	51.6 ± 0.8	24.5 ± 1.0
ATP	53.3 ± 1.1	25.9 ± 0.7
ADP	44.4 ± 1.7	18.5 ± 0.7
1-Naphthyl phosphate	68.3 ± 2.7	25.37 ± 2.6
Phenyl phosphate	49.46 ± 0.9	16.84 ± 0.7

It is interesting to note that PEP, ATP, ADP, 1-naphthyl phosphate and phenyl phosphate exerted a stronger inhibition of APase activity. The results are now compared when metabolite concentration was also reduced from 1.0 mM to 0.1 mM and reducing *p*-NPP from 0.5 mM to 0.1 mM. Citrate and isocitrate show almost similar activation pattern. Oxaloacetate and caffeine which had shown moderate activation earlier have no effect under present situation. Phenyl phosphate has practically no effect. PEP and ATP showed little more inhibition. In the presence of ADP, however, the inhibition was more pronounced. The inhibition in the presence of 1-naphthyl phosphate was lesser in comparison to others.

Table V: Nature of inhibition and *K*i of inhibitors with respect to [*p*-NPP]

Inhibitor	**[Inhibitor] (mM)**	**Nature of Inhibition**	***K*i (mM)**
1-Naphthyl phosphate	0.2 mM	Competitive	0.228
ADP	0.2 mM	Competitive	0.109
PEP	0.1 mM	Competitive	0.1
Phenyl phosphate	0.2 mM	Competitive	0.076

In all the cases, competitive inhibition has been observed. The K_i values for PEP, ADP and phenyl phosphate are quite close to K_m value of *p*-NPP, suggesting that the above organic phosphates have a strong affinity for the active site. In case of 1-naphthyl phosphate, K_i value is almost twice the K_m

value of *p*-NPP, suggesting it to have a weaker affinity for active site.

The effect of some compounds and detergents got tested on APase activity. Result is shown in **Fig. 2**.

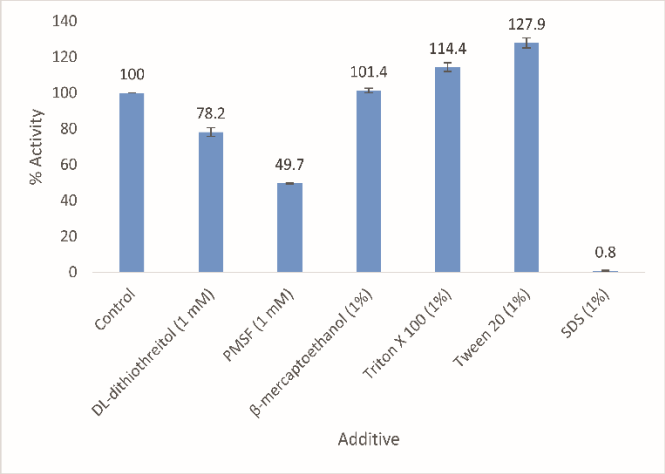

Fig.2: Effect of some compounds and detergents on APase activity

It is interesting to note that non-ionic detergents Triton X-100 and Tween-20 brought activation to the enzyme-like some others (De-Kundu & Banerjee, 1990; Biswas & Cundiff, 1991; Cirkovic et al., 2002; Nadir et al., 2012). APase isoforms of *Vigna sinensis* showed activation in the presence of non-ionic detergent Triton X-100 due to its strong affinity for hydrophobic side chains of enzymes. The interaction of the detergent with the hydrophobic domain of the enzyme may be the reason for the activation (Biswas & Cundiff, 1991). Peanut APase got stabilized in the presence of non-ionic detergent (Gonnety et al., 2006). Ionic detergent SDS brought complete loss in activity of APase in the present study like in APase from the castor bean, bringing about modification of tertiary structure with exposure of hydrophobic side chain (Granjeiro et al., 1999). APases from other sources also got inhibited in the presence of SDS (De-Kundu & Banerjee, 1990; Biswas & Cundiff, 1991; Chafik, 2020).

Dithiothreitol that usually stabilizes SH groups in a protein brought inhibition to APase in the present study. The presence of β-mercaptoethanol did not affect the APase activity. β-Mercaptoethanol also did not demonstrate any effect on other reported APases (Kruzel & Morawiecka, 1982; Anand & Srivastava, 2013).

III. EXPERIMENTAL

Rice bean APase used in the present study was as reported earlier (Nongpiur et al., 2021). The chemicals employed in the experiments were from Sigma Aldrich USA and Merck Germany. The other chemicals were AR Grade from Sisco Research Laboratory, HiMedia, Qualigens Fine Chemicals, and Sd. Fine Chemicals, India. De-ionized water was from the Milli-Q system (Millipore, USA). The enzyme activity was assayed using *p*-nitrophenylphosphate (*p*-NPP) as the substrate described as described earlier (Nongpiur et al., 2021).

The APase (80 U/mg) was dialyzed against in 50 mM Tris-HCl buffer, pH 7.5, containing 1.0 mM EDTA. The EDTA-dialyzed enzyme was used for the study for the effects of the divalent and the monovalent cations. The outcome of different divalent (calcium chloride, magnesium chloride, manganese chloride, cobalt chloride, strontium chloride, ferric chloride, barium chloride, zinc chloride, copper sulfate) and monovalent (potassium chloride, ammonium chloride, sodium chloride, and lithium chloride) cations got studied at 1.0 mM concentration on rice bean APase catalyzed reaction at two different concentrations of *p*-NPP (0.1 mM and 0.5 mM). Solutions of metal ion salt were prepared in the assay buffer.

Several metabolites of the TCA cycle, nucleotides, sugars, amino acids, plant hormones, and organic phosphates got tested for their effects on APase catalyzed reaction at 1.0 mM concentration. A 10 mM stock solution of the metabolite got prepared in the assay buffer. The 2.0 mL test solution contained 1.0 mL *p*-NPP, 0.75 mL assay buffer, and 0.2 mL of metabolite. The reaction got started by adding an enzyme aliquot (0.05 mL). The concentration of *p*-NPP in the assay mixture was 0.1 mM. In one set of experiments, a few selected metabolites were tested for their effects on APase activity at 1.0 mM and 0.1 mM while keeping the concentration of *p*-NPP in the assay mixture at 0.1 mM. In another experiment, a few additives were tested for their effects at 1.0 mM or 1%, keeping the *p*-NPP concentration 0.1 mM.

CONCLUSION

Rice bean acid phosphatase activity in the presence of divalent, monovalent, and trivalent cations were investigated. APase activity got inhibited in the presence of Zn^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, and Li^{+}, while Mg^{2+}, Ca^{2+} did not impart any effect. Among anions phosphate, molybdate, and vanadate were inhibitors. Intermediates of TCA cycle like citrate, isocitrate, and oxaloacetate brought activation. Non-ionic detergents like Triton X-100 and Tween-20 exhibited activation. This is the first report on activation of the acid phosphatase in the presence of NADH and this behavior needs an investigation in future.

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REFERENCES

Ambasht, P.K., Malhotra, O.P., & Kayastha, A.M. (1997). Regulatory properties and active site groups of cytosolic mung bean pyruvate kinase. *Indian Journal of Biochemistry and Biophysics* 34, 365-372.

Anand, A., & Srivastava, P.K. (2013). Isolation and enzymatic properties of a non-specific acid phosphatase from *Vigna aconitifolia* seeds. *Biotechnology and Applied Biochemistry* DOI: 10.1002/b.ab.1131.

Andriotis, V.M.E., & Ross, J.D. (2004). Isolation and partial characterization of acid phosphatase isozymes from dormant oilseed of *Corylus avellana* L. *Planta* 219, 346-35.

Asaduzzaman, A.K.M., Rahman, M.H., & Yeasmin, T. (2011). Purification and characterization of acid phosphatase from a germinating black gram (*Vigna mungo* L.) seedling. *Archives of Biological Science Belgrade* 63, 747-756.

Biwas, T.K., & Cundiff, C. (1991). Multiple forms of acid phosphatases in germinating seeds of *Vigna sinensis. Phytochemistry* 30, 2119-2125.

Cashikar, A.G., Kumaresan, R., & Rao, M. (1997). Biochemical characterization and subcellular localization of the red kidney bean purple acid phosphatase. *Plant Physiology* 114, 907-915.

Chafik, A., Essamadi, A., Celik, S.Y., & Mavi, A. (2020). A novel acid phosphatase from cactus (*Opuntia megacantha* Salm-Dyck) cladodes: Purification and biochemical characterization of the enzyme. *International Journal of Biological Macromolecules* 160, 991-999.

Ching, T.M., Lin, T.P., & Metzger, R.J. (1987). Purification and properties of acid phosphatase from plump and shriveled seeds of triticale. *Plant Physiology* 84, 789-795.

Cirkovic, T.D., Gavrovic-Jankulovic, M.D.J., Bukilica, M.N., Mandic, L., Petrovic, S.Z., & Jankov, R.M. (2002). Isolation and partial characterization of an acid phosphatase from *Artemisia vulgaris* pollen extract. *Journal of Serbian Chemical Society* 67, 567-572.

Coello, P. (2002). Purification and characterization of secreted acid phosphatase in phosphorus-deficient *Arabidopsis thaliana*. *Physiologia Plantarum* 116, 293-298.

De-Kundu, P., & Banerjee, A.C. (1990). Multiple forms of acid phosphatase from seedling axes of *Vigna radiata*. *Phytochemistry* 29, 2825-2828.

Duff, S.M.G., Sarath, G., & Plaxton, W.C. (1994). The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum* 90, 791-800.

Ferreira, C.V., Taga, E.M., & Aoyama, H. (2000). Inhibition of acid phosphatase isoforms purified from mature soybean (*Glycine max*) seeds. *Journal of Enzyme Inhibition* 15, 403-410.

Garcia, N.A.T., Olivera, M., Iribarne, C., & Lluch, C. (2004). Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*. *Plant Physiology and Biochemistry* 42, 585-591.

Gellatly, K.S., Moorehead, G.B.G., Duff, S.M.G., Lefebvre, D.D., & Plaxton, W.C. (1994). Purification and characterization of potato tuber acid phosphatase having significant phosphotyrosine phosphatase activity. *Plant Physiology* 106, 223-232.

Gonnety, J.T., Niamke, S., Faulet, M.B., Kouadio, N.E.J.P., & Kouame, L.P. (2006). Purification and characterization of two acid phosphatases from germinating peanut (*Arachis hypogaea*) seed. *Iranian Journal of Biochemistry* 4, 26-35.

Granjeiro, P.A., Ferreira, C.V., Granjeiro, J.M., Taga, E.M., & Aoyama, H. (1999). Purification and kinetic properties of a castor bean seed acid phosphatase containing sulfhydryl groups. *Plant Physiology* 107, 151-158.

Greiner, R., & Jany, K.D. (2003). Purification and characterization of homogeneous acid phosphatase from non-germinated buckwheat (*Fagopyrum esculentum*) seeds. *Journal of Food Biochemistry* 27, 197-220.

Guo, J., & Pesacreta, T.C. (1997). Purification and characterization of an acid phosphatase from the bulb of *Allium cepa* L. var. Sweet Spanish. *Journal of Plant Physiology* 151, 520-527.

Guo, Y.L., & Roux, S.J. (1995). Partial purification and characterization of an enzyme from pea nuclei with protein tyrosine phosphatase activity. *Plant Physiology* 107, 167-175.

Hoehamer, C.F., Mazur, C.S., & Wolfe, N.L. (2005). Purification and partial characterization of an acid phosphatase from *Spirodela oligorrhiza* and its affinity for selected organophosphate pesticides. *Journal of Agricultural Food Chemistry* 53, 90-97.

Kaneko, T.S., Kuwabara, C., Tomioka, S., & Suzuki, K. (1998). 60 kD polypeptide of cell wall acid phosphatase from tobacco cells. *Phytochemistry* 48, 1125-1130.

Kawarasaki, Y., Nakano, H., & Yamane, T. (1996). Purification and some properties of wheat germ acid phosphatases. *Plant Science* 119, 67-77.

Khan, S., Khan, S., Batool, S., & Ahmed, M. (2016). Purification and biochemical characterization of acid phosphatase I from seeds of *Nelumbo nucifera*. *Natural Product Research* 30, 570-573.

Koffi, D.M., Gonnety, J.T., Faulet, B.M., Bedikou, M.E., Kouame, L.P., Zoro-Bi, I.A., & Niamke, S.L. (2010). Biochemical characterization of two non-specific acid phosphatases from Cucurbitaceae (*Lagenaria siceraria*) edible seeds exhibiting phytasic activity. *Journal of Animal and Plant Sciences* 7, 860-875.

Kruzel, M., & Morawiecka, B. (1982). Acid phosphatase of potato tubers (*Solanum tuberosum* L.). Purification, properties, sugar and amino acid composition. *Acta Biochimica Polonica* 29, 321-331.

Miernyk, J.A. (1992). Purification and characterization of the major acid phosphatase isozyme secreted by maize endosperm cultures. *Phytochemistry* 31, 2613-2616.

Nadir, S., Saeed, A., Naz, R., Siddiqua, A., Sherazi, M., Wazir, S.M., Saeed, A. (2012). Isolation, purification and characterization of acid phosphatase from germinating *Vigna radiata* seeds. *Journal of the Chemical Society of Pakistan* 34, 717-727.

Nakazato, H., Okamoto, T., Nishikoori, M., Washio, K., Morita, N., Haraguchi, K., Thompson, Jr. G.A., & Okuyama, H. (1998). The glycosylphosphatidylinositol-anchored phosphatase from *Spirodela oligorrhiza* is a purple acid phosphatase. *Plant Physiology* 118, 1015-1020.

Nongpiur, S.R., Kalita, T., Belho, K., & Ambasht, P.K. (2021). Purification and partial characterization of acid phosphatase from rice bean (*Vigna umbellata* Thunb.). *Journal of Proteins and Proteomics* 12, 325-335.

Olczak, M., Watorek, W., & Morawieka, B. (1997). Purification and characterization of acid phosphatase from yellow Lupin (*Lupinus luteus*) seeds. *Biochimica et Biophysica Acta* 1341, 14-25.

Pan, S.M., Huang, Y.F., & Chen, Y.R. (1987). Characterization and localization of acid phosphatase in the suspension cultured cells of *Nicotiana otophora*. *Taiwania* 32, 1-10.

Pasqualini, S., Panara, F., Bracardi, P., & Antonielli, M. (1992). High molecular weight acid phosphatase from barley coleoptiles. *Phytochemistry* 31, 3703-3706.

Roknabadi, S.M., Bose, S.K., & Taneja, V. (1999). A histidine thiol 150 kDa, tetrameric acid phosphatase from lentil, *Lens esculenta*, seeds with the characteristics of protein tyrosine phosphatases. *Biochimica et Biophysica Acta* 1433, 272-280.

Senna, R., Simonin, V., Silva-Neto, M.A.C., & Fialho, E. (2006). Induction of acid phosphatase activity during germination of maize (*Zea mays*) seeds. *Plant Physiology and Biochemistry* 44, 467-473.

Singh, S., & Luthra, P.M. (2011). Identification, characterization and partial purification of acid phosphatase from cotyledons of *Psoralea corylifolia* L. *American Journal of Plant Physiology* 6, 228-241.

Sugiura, Y., Kawabe, H., Tanaka, H., Fujimoto, S., & Ohara, A. (1981). Purification, enzymatic properties, and active site environment of a novel manganese (III)-containing acid phosphatase. *Journal of Biological Chemistry* 256, 10664-10670.

Tagad, C.K., & Sabharwal, S.G. (2018). Purification and characterization of acid phosphatase from *Macrotyloma uniflorum* seeds. *Journal of Food Science and Technology* 55, 2941-2949.

Tanaka, H., Hoshi, J., Nakata, K., Takagi, M., & Yano, K. (1990). Isolation and some properties of acid phosphatase-1 from tomato leaves. *Agricultural and Biological Chemistry* 54, 1947-1952.

Tso, S.C., & Chen, Y.R. (1997). Isolation and characterization of a group III isoenzyme of acid phosphatase from rice plants. *Botanical Bulletin of Academia Sinica* 38, 245-250.

Turner, W.L., & Plaxton, W.C. (2001). Purification and characterization of banana fruit acid phosphatase. *Planta* 214: 243-249.

Vincent, J.B., & Averill, B.A. (1990). An enzyme with double identity: purple acid phosphatase and tartrate-resistant acid phosphatase. *The FASEB Journal* 4, 3009-3014.

Waymack, P.P., & Van Etten, R.L. (1991). Isolation and characterization of a homogenous isoenzyme of wheat germ acid phosphatase. *Archives of Biochemistry and Biophysics* 288, 621-633.

Yenigun, B., & Guvenilir, Y. (2003). Partial purification and kinetic characterization of acid phosphatase from garlic seedling. *Applied Biochemistry and Biotechnology* 105-108, 677-687.

Zaman, U., Naz, R., Khattak, N.S., Rehman, K.U., Iqbal, A., Ahmad, S., & Shah, L.A. (2020). Investigating the thermodynamic and kinetic properties of acid phosphatase extracted and purified from seedlings of *Chenopodium murale*. *International Journal of Biological Macromolecules* 165: 1475-1481.

Zhang, C., McManus, M.T. (2000). Identification and characterization of two distinct acid phosphatases in cell walls of roots of white clover. *Plant Physiology and Biochemistry* 38, 259-270.

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