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Comparative Analysis of The Effect of Salt and Sugar Stress on Acid Phosphatase Activity of *Triticum Aestivum* (Wheat), *Cicer Arientinum*(Chick Pea) and *Vigna Radiata* (Moong Bean)

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Abstract

Acid phosphatases are a group of enzymes with ubiquitous distribution throughout the plant kingdom. The main function of these enzymes is to obtain inorganic phosphate from organic sources making it usable to the plants. Some studies have revealed that under salt stress conditions, acid phosphatase activity increases. In lieu of this, the current study aims at studying three of the commercially most important plant species, wheat (*Tritcum aestivum*), moong bean (*Vigna radiate*) and chick pea (*Cicer arientinum*) and involves analysis of the acid phosphatase activity of their imbibed seeds. Different enzyme extracts were subjected to different concentrations of salts like $MgCl_2$ and NaCl and analysed for the effect on their acid phosphatase activity. Further, effect of glucose was also analysed on the activation of enzymes and a comprehensive analysis was conducted between all three species.

Key Words: Salt stress, acid phosphatises, activity, $MgCl_2$, NaCl

Introduction

A number of nutrients are required in energy transfer and metabolic regulation and phosphorous is one of the important mineral found in organic molecules like nucleic acids. It is found in many different forms and plays different functions and is absorbed in its soluble inorganic form of $H_2PO_4^-$ or HPO_4^{2-} ions (inorganic phosphate or Pi) in plants. It serves as cofactor in many biochemical transformations in the form of pyridoxal phosphate (PLP) [1].

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Although soil is rich in phosphorous compounds but the concentration of Pi ranges from 1 µM to 10 µM which is far below the optimum amount required by the plant for its growth and development [2]. For proper growth and development of plants, phosphorous needs to be completely assimilated, stored and metabolised. The assimilation of phosphorous requires a ubiquitous class of enzymes known as phosphatases that function to hydrolyse Pi from orthophosphate monoesters. On the basis of their catalysis under different pH conditions, phosphatases have been classified as acid and alkaline phosphatases. Acid phosphatases are those which work below the pH7 while alkaline phosphatases are the ones that perform their function above the pH 7 [3].

Studies have revealed that PAPs can be classified into two groups based on molecular weight and the mode of interaction; ~ 35 kDa monomeric forms, and ~ 55 kDa homodimeric forms [4]. The smaller type lacks catalytic function, which is present in larger PAPs [5].

Expression patterns of PAP genes are tissue-specific, for example in tomato protein levels of several selected PAPs are different in leaf, stem and root tissues (Bozzo *et al.,* 2006). In *Arabidopsis*, PAPs are expressed at moderate levels in roots, stems, leaves, flowers and siliques [6].

Phosphorus plays a major role in various processes like photosynthesis, respiration, energy storage and transfer, cell enlargement and cell division in plants. Phytase enzyme, a storage form of phosphorous plays a vital role in germinating seeds specially, during nitrogen fixation within nodules of legumes. It has been studied that the phytase enzyme shows different behavioural characteristics in different parts of plants from seeds to nodules and influences the use of phosphorous and nitrogen fixation required for seed germination [7].

Plants show different biochemical behaviour when allowed to survive under different conditions. A number of factors are involved in enhancing or declining the germination of seeds and growth of plants. Salt and sugar stress are one of those factors that can alter the activity of enzyme like acid phosphatase which in turn help in the assimilation of phosphorous required by plants for their cellular activities.

Different plants face different range of environmental stresses and most of the cultivated crops are sensitive to them like salt stress [8]. The rate of soil salinity has been increasing

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worldwide and it may lead to loss of 50% of cultivable land by 2050 [9]. Salt mainly affects two developmental processes: establishment and reproduction in crops. Salt can reduce branching, plant height, branch length, nymber of leaves, root/shoot weight, and even early development [8].

The effect of salt on plant establishment was analyzed in many different crops like *Zea mays Sorghum bicolor, Oryza sativa, Triticum aestivum* and *Glycine max* . It has been studied that the seedling growth of soybean is enhanced against 100 Mmol NaCl stress condition [10].

Another study revealed that salt stress affects different stages of development by altering the phenotypes of each stage. Salt stress applied at ratio NaCl:$CaCl_2$, 5.7:1 ratio showed down regulation of the growth of plants [11].

Phosphorous deficiency and salinity are both responsible for the restricted growth of plants in many species. Although when the plants are allowed to survive under saline conditions alone they respond in the same manner lowering the plant growth [12].

Under the conditions of salinity, lowest leaf water potential is observed. The speculated reasons include high accumulation of Na^+ and proline in the shoots of salt treated plants suggesting the involvement of these solutes in osmotic adjustment. Also, the shoot soluble sugars accumulation under phosphorous deficiency play an important role in the adaptation of *Aeluropus littoralis* plants, a halophyte to phosphorous shortage when treated with or without salt [12].

It has been observed that acid phosphatase activities increases by salt and drought stress in cultivars. Explants of *Medicago sativa* cultured under aseptic condition on MS medium containing supplements showed that acid phosphatase activity was increased by salt and osmotic stress [13].

Other factor that affects the plant growth by reducing or elevating the activity of acid phosphatase is sugar stress. Sugars are a good source of metabolic energy and functions as signaling molecules within the cell [14].

Phosphatases are capable of trans-phosphorylation in addition to hydrolysis. Phosphatases thus play an important role in the metabolism of carbohydrates, phospholipids and nucleotides. D-galactose is a reducing sugar that accelerates the process of aging by increasing oxidative stress.

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Protein kinases and plant phosphatases have critical functions in plant development and stress responses [15]. Members from different sub-families of phosphatases have been studied for their biological function.

In the current study, three different seeds of wheat (*Tritcum aestivum*), moong bean (*Vigna radiate*) and chick pea (*Cicer arientinum*) have been used for experimentation and the acid phosphatase activity has been determined under the effect of different salts like NaCl and KCl on the activity of APases. Further, effect of different sugars like glucose and sucrose have been studied on the activity of APases from *Tritcum aestivum*, *Cicer arientinum* and *Vigna radiate*.

Materials and methods

Reagents used: Stock of Sodium acetate buffer (0.1M, pH 4.6), PNP (10mg/ml), 0.2M NaOH, Biuret reagent, Standard BSA protein (10mg/ml), 0.01M Sodium acetate buffer, 0.05M PNPP, 0.01M $MgCl_2$, 5M NaCl (stock), 5M Glucose (stock).

Seeds used:Wheat (*Triticum aestivu*, Moong bean (*Vigna radiate*), Chick pea (*Cicer arientinum*) were procured locally and same batch of seeds were used to perform all the experiments

Preparation of sodium acetate buffer (stock): 1M stock solution of sodium acetate (100ml) was prepared and pH was adjusted to 4.6 with glacial acetic acid.

Preparation of para nitrophenol standard cerve: PNP stock solution at final concentration of 10mg/ml was prepared. Six different concentrations of PNP were prepared from stock solution; 2mg/ml, 1.5mg/ml, 1mg/ml, 0.5mg/ml, 0.25mg/ml. Absorbance was measured at 405nm on UV spectrophotometer and standard curve was plotted

Preparation of enzyme extract: 5gm seeds of wheat, moong bean, chick pea were weighed and soaked overnight separately in distilled water. Seeds were then crushed and crude extract of total acid phosphatase was prepared using sodium acetate buffer. Following this, all the three extracts were centrifuged at 10,000 rpm for 10 minutes at 4°C. Supernatant containing the enzyme extract was collected at stored at 4°C until use

Preparation of biuret reagent: 1.5g of copper sulphate and 4.5g of sodium potassium tartrate were dissolved into 250ml 0.2N sodium hydroxide solution and then 2.5g of potassium iodide was added to make final volume of 500ml with 0.2N sodium hydroxide.

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Preparation of NaCl solutions: From 1M NaCl (stock) different concentrations as 0.05M, 0.1M, 0.25M, 0.5M were prepared.

Preparation of Glucose solutions: From 1M glucose (stock) different concentrations as 0.05M, 0.1M, 0.25M, 0.5M were prepared.

Estimation of protein: 10 mg/ml of BSA (Bovine serum albumin) stock solution was prepared. Standard curve was prepared by using different concentrations and absorbance was measured at 540nm. Protein concentrations of different samples from the standard curve were then estimated.

Detection of acid phosphatase activity: 0.1mg/ml of each enzyme extract was used for detection of enzyme activity. 100µl of 0.05M PNPP, 100µl of 0.01M $MgCl_2$ were added to the respective extracts and final volume was made to 1 ml with sodium acetate buffer. Reaction mixture was incubated for 15min after which 2ml sodium acetate was added and each tube was incubated at 37 °C. Finally, 200µl of NaOH was added and absorbance was measured at 405nm.

Detection of acid phosphatase activity in different $MgCl_2$ concentrations: Different $MgCl_2$ concentrations were used to detect the acid phosphatase activity of different samples of concentration 0.1mg/ml. Five test tubes as W_1, W_2, W_3, W_4 with varying concentrations of $MgCl_2$ as 0.01M, 0.001M, 0.025M, 0.05M respectively and blank. Enzyme (0.1 mg/ml) was added to all the tubes except for blank. Rest of the reaction was performed as described above and activity was measured. The same reaction was repeated for different concentrations of enzyme extract under different $MgCl_2$ concentrations

Estimation of acid phosphatase activity at different NaCl concentrations of different samples of seeds with concentration 0.5mg/ml: Varying concentrations of NaCl such as 0.05M, 0.1M, 0.25M, 0.5M respectively, without NaCl and blank. 0.5 mg/ml of protein extract was used for each sample and activity was analysed using the same reaction as described above.

Detection of acid phosphatase activity at different glucose concentrations: Varying concentrations of glucose such as 0.05M, 0.1M, 0.25M, 0.5M respectively, without glucose and blank. 0.5 mg/ml of protein extract was used for each sample and activity was analysed using the same reaction as described above.

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Results and Discussion

1. Preparation of standard curve using PNP

A standard curve of PNP was plotted using different concentrations and the absorbance was observed at 405nm and a linear curve was obtained and the absorbance of samples of wheat, moong bean and chickpea was also observed.

S.No	Volume of PNP	Conc. of PNP	Sodium acetate Buffer	Incubation for 10 min	NaOH	Absorbance (405nm)
Blank	-	-	**3 ml**	**37°C**	**200µl**	**0.00**
1	**1 ml**	**0.25 mg/ml**	**2 ml**	**37°C**	**200µl**	**0.048**
2	**1 ml**	**0.5 mg/ml**	**2 ml**	**37°C**	**200µl**	**0.139**
3	**1 ml**	**1 mg/ml**	**2 ml**	**37°C**	**200µl**	**0314**
4	**1 ml**	**1.5 mg/ml**	**2 ml**	**37°C**	**200µl**	**0.379**
5	**1 ml**	**2 mg/ml**	**2 ml**	**37°C**	**200µl**	**0.529**

Table 1. PNP dilution table

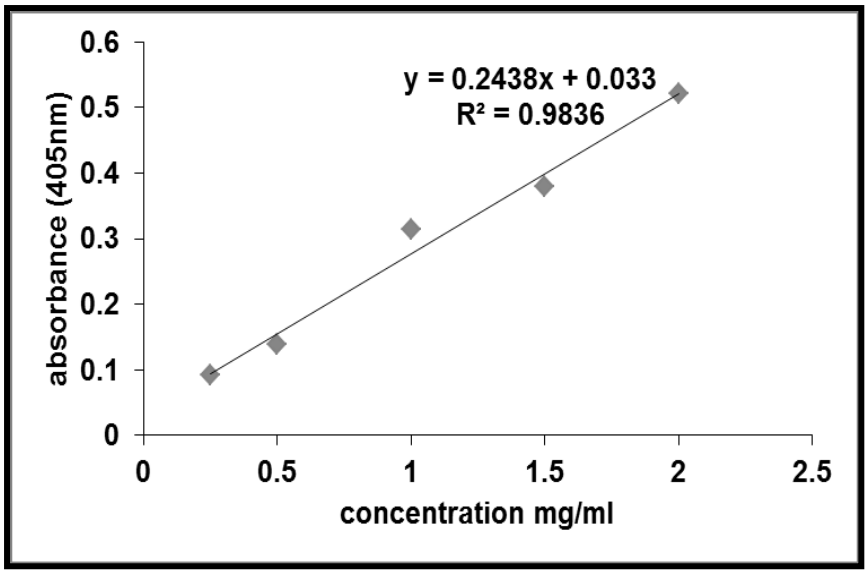

Figure 3. PNP Standard curve

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2. Preparation of standard curve of BSA for protein estimation

Using different concentrations of BSA (standard protein), the absorbance was observed at 540nm and a liner curve was obtained and used to determine the unknown concentrations of samples of wheat, moong bean and chickpea.

S.No	Distilled water	BSA	Conc. of BSA	Biuret reagent	Incubate for 15 min	Absorbance (540nm)
Blank	**1 ml**	**-**	**-**	**1.5 ml**	**37°C**	**0.00**
1	**-**	**1 ml**	**1 mg/ml**	**1.5 ml**	**37°C**	**0.332**
2	**250 µl**	**750 µl**	**0.75 mg/ml**	**1.5 ml**	**37°C**	**0.264**
3	**500 µl**	**500 µl**	**0.5 mg/ml**	**1.5 ml**	**37°C**	**0.160**
4	**750 µl**	**250 µl**	**0.25 mg/m**	**1.5 ml**	**37°C**	**0.068**
5	**900 µl**	**100 µl**	**0.1 mg/ml**	**1.5 ml**	**37°C**	**0.015**

Table 2. BSA dilution table

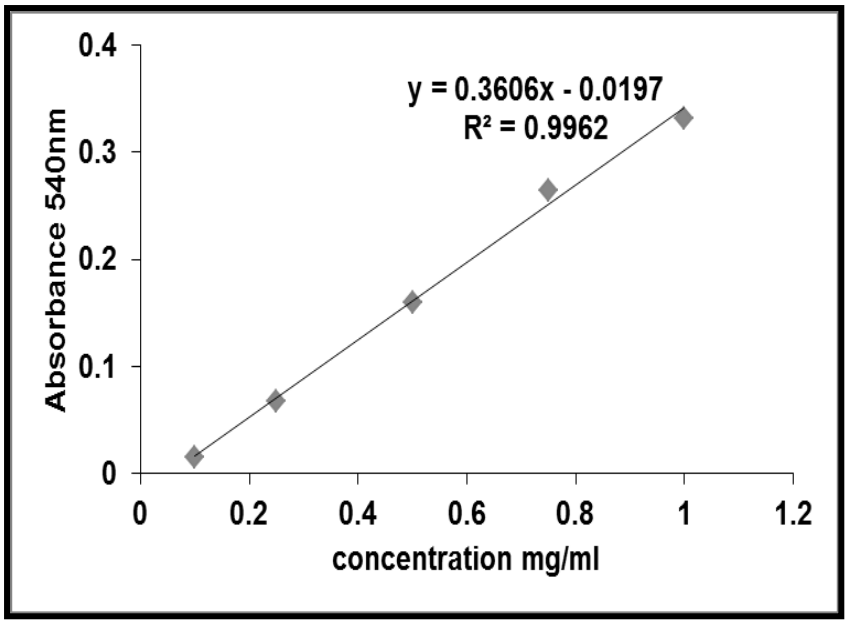

Figure 4. Standard curve of BSA

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Test samples	Volume of sample	Biuret reagent	Incubate for 15 min	Absorbance (540nm)	Conc. (mg/ml)
Wheat	1 ml	1.5 ml	37°C	0.138	1.749
Moong bean	1ml	1.5 ml	37°C	0.428	4.96
Chick pea	1ml	1.5 ml	37°C	1.068	12.06

Table 3. Estimation of protein concentration of enzyme extracts

3. Detection of acid phosphatase activity at different concentrations of $MgCl_2$ in wheat (0.1 mg/ml)

The acid phosphatase activity was determined using different concentrations of $MgCl_2$ as 0.1M, 0.001M, 0.025m, 0.05M in wheat at concentration 0.1 mg/ml and the maximum activity was shown at 0.025M at 405nm.

$MgCl_2$ conc.	Wheat (OD 405nm)
0.01 M	0.379
0.001 M	0.342
0.025 M	0.4
0.05 M	0.361

Table 4. Acid phosphatase activity in wheat (0.1 mg/ml)

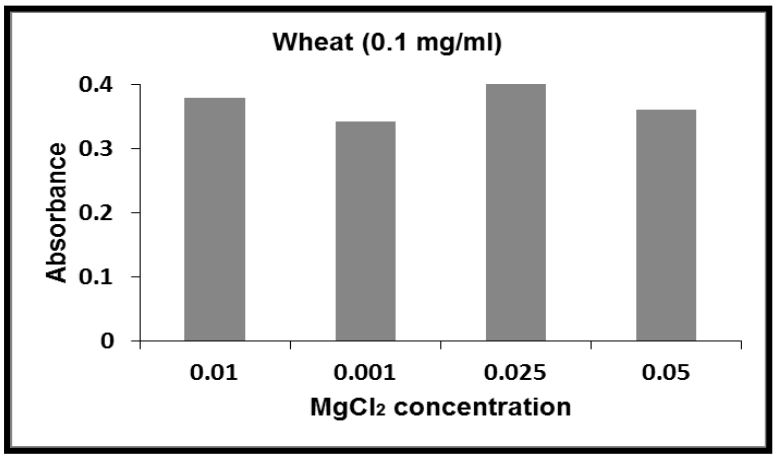

Figure 5. Acid phosphatase activity in wheat (0.1 mg/ml)

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4. Detection of acid phosphatase activity in Moong bean (0.1 mg/ml) at different concentrations of $MgCl_2$:

Using different concentrations of $MgCl_2$, the enzyme activity was observed in moong bean at 405nm and the maximum activity was shown at 0.001M concentration.

$MgCl_2$ conc.	**Moong bean (OD 405nm)**
0.01 M	0.252
0.001 M	0.257
0.025 M	0.254
0.05 M	0.205

Table 5. Acid phosphatase activity of moong bean (0.1 mg/ml)

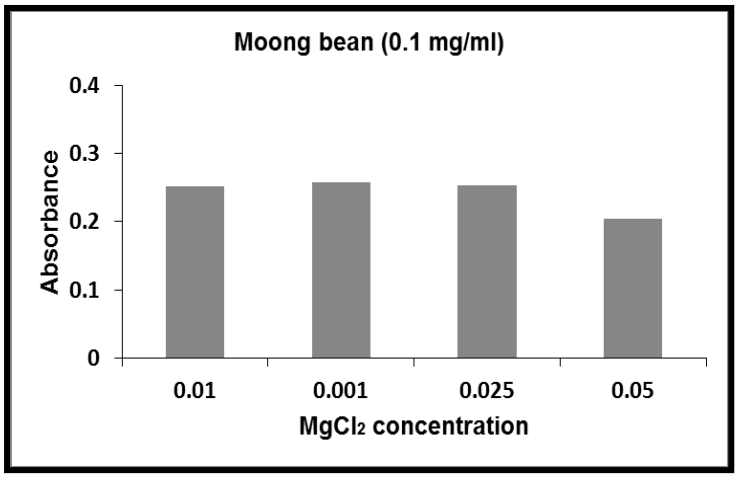

Figure 6. Acid phosphatase activity in moong bean (0.1 mg/ml)

5. Detection of acid phosphatase activity in chickpea (0.1 mg/ml) at different concentrations of $MgCl_2$:

The acid phosphatase activity of chickpea was determined at different concentrations of $MgCl_2$ that showed the maximum enzymatic activity at 0.25M at 405 nm wavelength

$MgCl_2$ conc.	**Chickpea (OD 405nm)**
0.01 M	0.235
0.001 M	0.203
0.025 M	0.240
0.05 M	0.193

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Table 6. Acid phosphatase activity of chickpea (0.1 mg/ml)

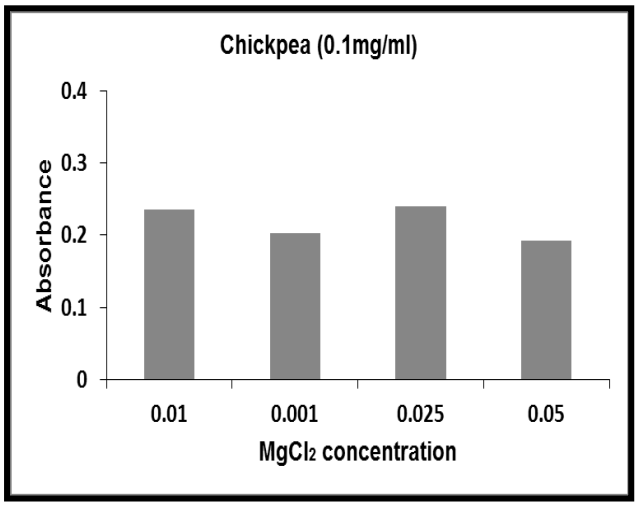

Figure 7. Acid phosphatase activity in chickpea (0.1 mg/ml)

6. Comparative acid phosphatase activities of all the extracts of samples at different concentrations of $MgCl_2$

When all the extracts of wheat, moong bean and chickpea were compared together, wheat showed the highest enzymatic activity at 0.025M .

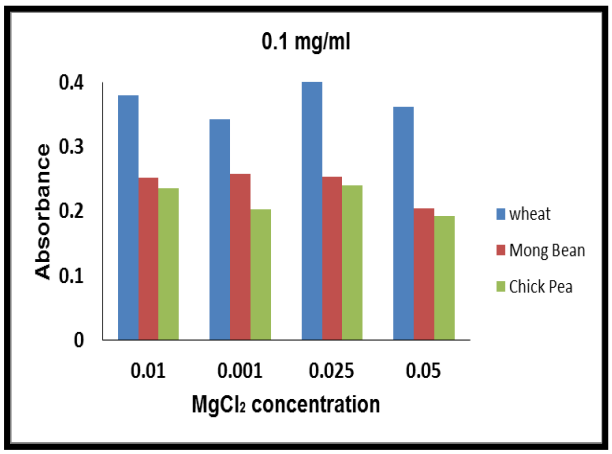

Figure 8. Comparison of APase activities of extracts (0.1mg/ml)

7. Detection of acid phosphatase activity in wheat (0.2 mg/ml) at different concentrations of $MgCl_2$:

The wheat extract (0.2mg/ml) was treated with different concentrations of $MgCl_2$ and the maximum activity was shown at 0.025M concentration.

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$MgCl_2$ conc.	Wheat (OD 405nm)
0.01 M	0.096
0.001 M	0.085
0.025 M	0.098
0.05 M	0.107

Table 7. Acid phosphatase activity of wheat (0.2 mg/ml)

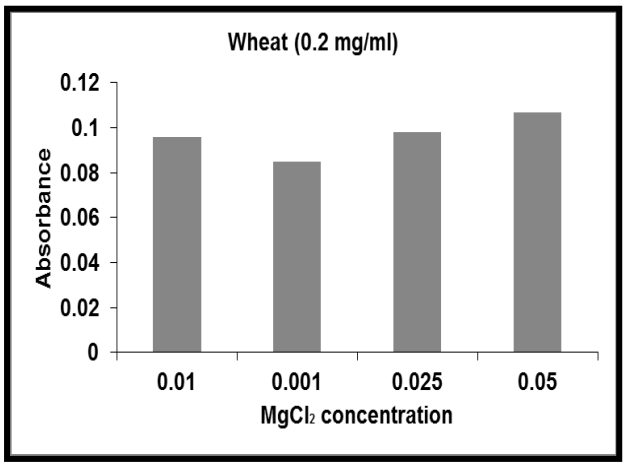

Figure 9. Acid phosphatase activity in wheat (0.2 mg/ml)

8. Detection of acid phosphatase activity in moong bean (0.2 mg/ml) at different concentrations of $MgCl_2$:

The moong bean extract (0.2mg/ml) was treated with different concentrations of $MgCl_2$ and the maximum activity was shown at 0.05M concentration.

$MgCl_2$ conc.	Moong bean (OD 405nm)
0.01 M	0.236
0.001 M	0.229
0.025 M	0.236
0.05 M	0.247

Table 8. Acid phosphatase activity of moong bean (0.2 mg/ml)

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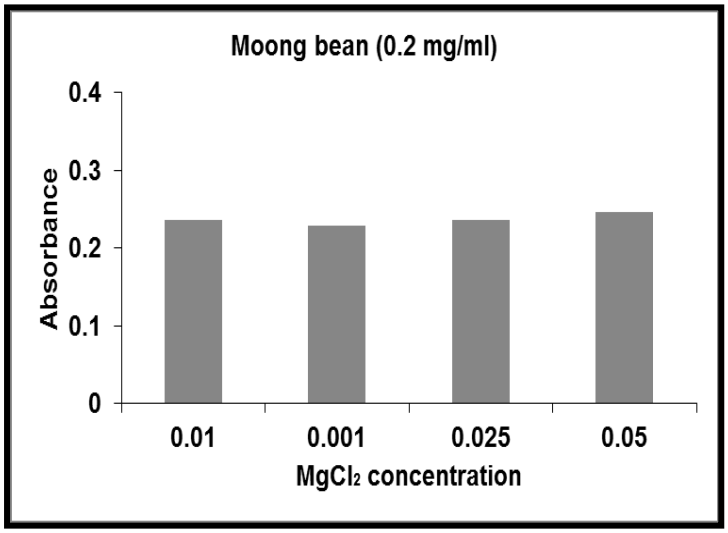

Figure 10. Acid phosphatase activity in moong bean at different $MgCl_2$ concentrations

9. Detection of acid phosphatase activity in chickpea (0.2 mg/ml) at different concentrations of $MgCl_2$:

When the chickpea extract (0.2mg/ml) was treated with different concentrations of $MgCl_2$, the maximum activity was shown at 0.025M concentration.

$MgCl_2$ conc.	**Chickpea (OD 405nm)**
0.01 M	0.251
0.001 M	0.276
0.025 M	0.238
0.05 M	0.259

Table 9. Acid phosphatase activity of chickpea (0.2 mg/ml)

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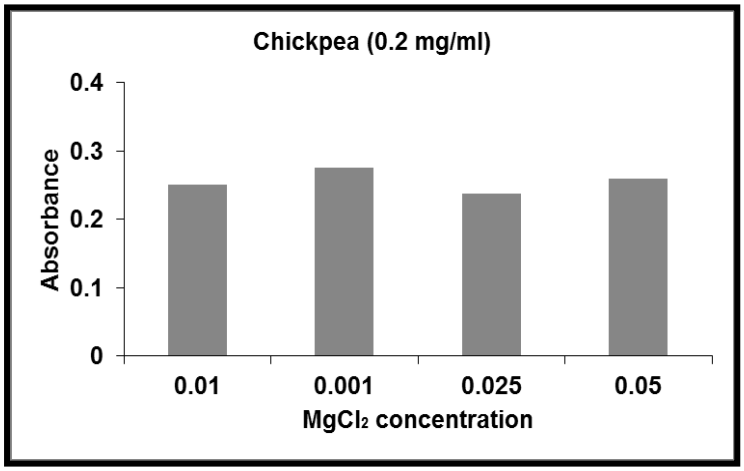

Figure 11. Acid phosphatase activity in chickpea at different $MgCl_2$ concentrations.

10. Comparative acid phosphatase activities of all the extracts of samples at different concentrations of $MgCl_2$:

When the enzymatic activities at different concentrations of $MgCl_2$ of all the extracts were compared together, the maximum activity was shown by chickpea at 0.001M concentration.

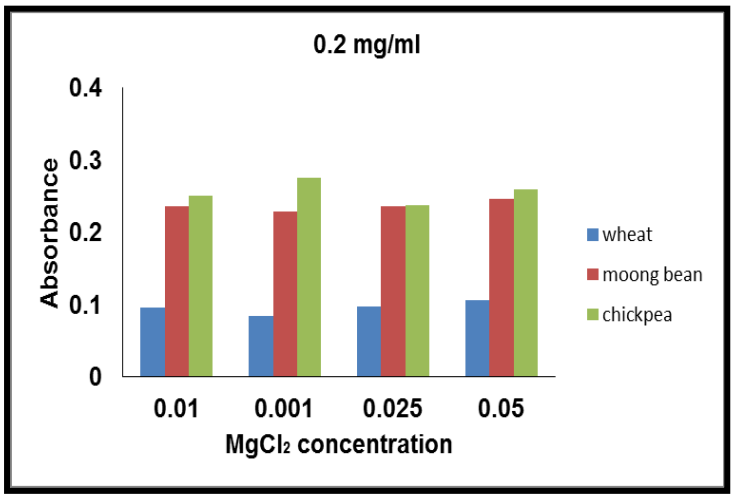

Figure 12. Comparison of APase activities of all extracts at different $MgCl_2$ concentrations.

11. Detection of acid phosphatase activity in wheat (0.5 mg/ml) at different concentrations of $MgCl_2$:

When the wheat extract (0.5mg/ml) was treated with different concentrations of $MgCl_2$, the maximum activity was shown at 0.025M concentration.

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$MgCl_2$ conc.	Wheat (OD 405nm)
0.01 M	0.379
0.001 M	0.342
0.025 M	0.400
0.05 M	0.361

Table 10. Acid phosphatase activity of wheat (0.5 mg/ml)

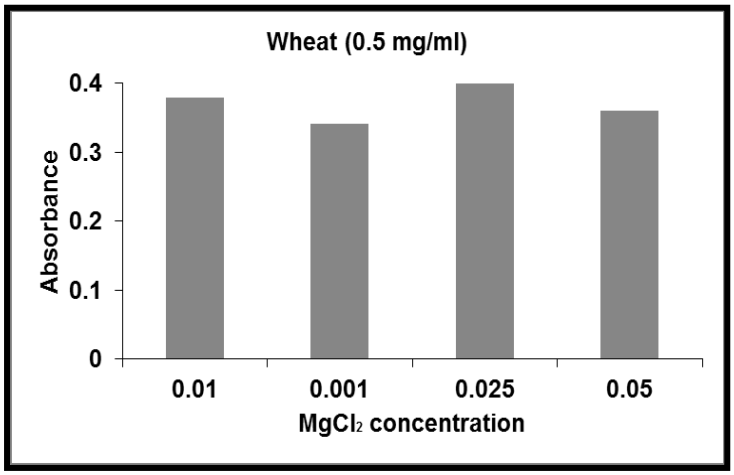

Figure 13. Acid phosphatase activity in wheat(0.5 mg/ml) at different $MgCl_2$ concentrations.

12. Detection of acid phosphatase activity in moong bean (0.5 mg/ml) at different concentrations of $MgCl_2$:

When the moong bean (0.5mg/ml) was treated with different concentrations of $MgCl_2$, the maximum activity was shown at 0.025M concentration.

$MgCl_2$ conc.	Moong bean (OD 405nm)
0.01 M	0.252
0.001 M	0.257
0.025 M	0.254
0.05 M	0.205

Table 11. Acid phosphatase activity of moong bean (0.5 mg/ml)

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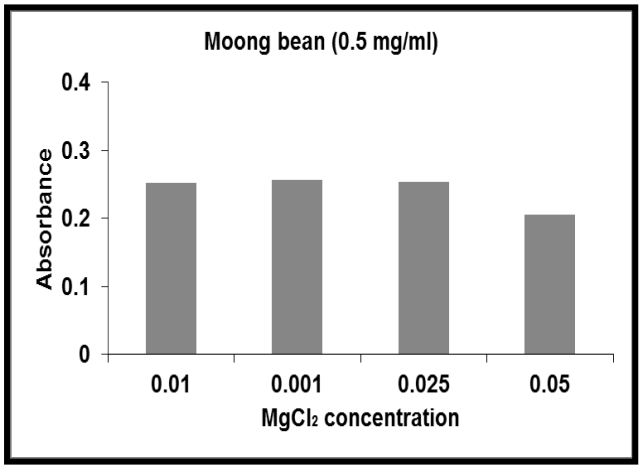

Figure 14. Acid phosphatase activity in moong bean (0.5mg/ml)at different $MgCl_2$ concentrations.

13. Detection of acid phosphatase activity in chickpea (0.5 mg/ml) at different concentrations of $MgCl_2$

When the chickpea extract (0.5mg/ml) was treated with different concentrations of $MgCl_2$, the maximum activity was shown at 0.025M concentration.

$MgCl_2$ conc.	**Chickpea (OD 405nm)**
0.01 M	0.235
0.001 M	0.203
0.025 M	0.240
0.05 M	0.193

Table 12. Acid phosphatase activity of chickpea (0.5 mg/ml)

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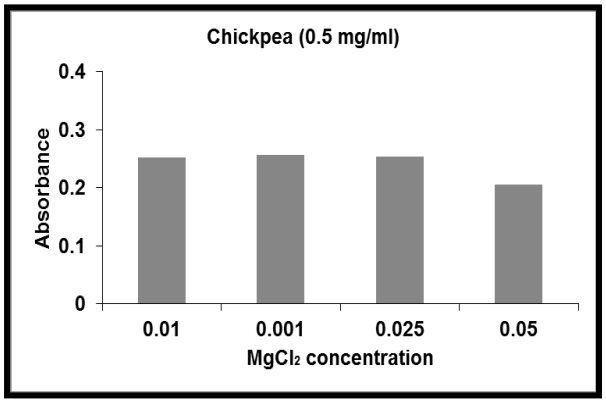

Figure 15. APase activity in chickpea (0.5 mg/ml) at different $MgCl_2$ concentrations.

14. Comparative acid phosphatase activities of all the extracts of samples at different concentrations of $MgCl_2$:

When the acid phosphatase activities of three samples of wheat, moong bean and chickpea at different concentrations of MgCl2 were compared, it was found that the maximum activity was shown byr wheat at 0.025M concentration.

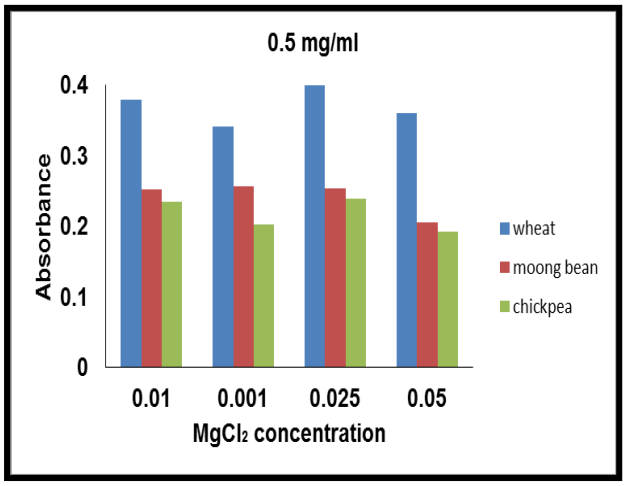

Figure 16. Comparison of APase activities at of extracts (0.5 mg/ml) different $MgCl_2$ concentrations.

14. Detection of acid phosphatase activity in wheat (0.5 mg/ml) at different concentrations of NaCl :

Using the different concentrations of NaCl as 0.05M, 0.1M, 0.25M, 0.5M, the acid phosphatase activity was observed. Wheat showed the maximum activity at 0.5M concentration of NaCl.

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NaCl conc.	Wheat (OD 405nm)
0.05 M	0.357
0.1 M	0.335
0.25 M	0.363
0.5 M	0.366
No NaCl	0.378

Table 13. Acid phosphatase activity of Wheat (0.5 mg/ml)

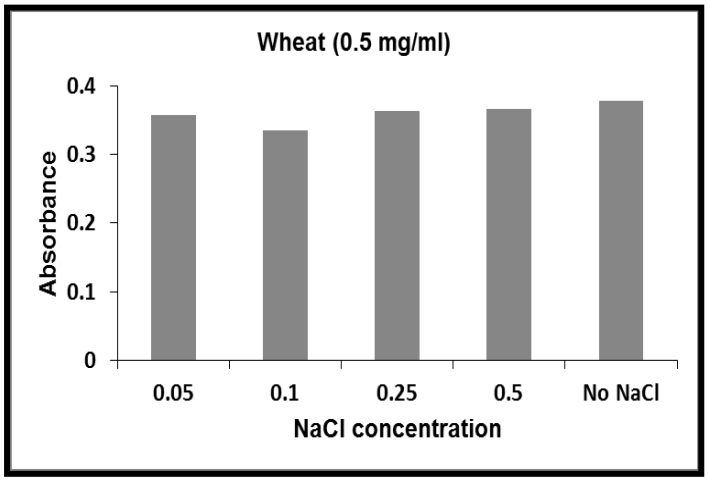

Figure 17. Acid phosphatase activity in wheat (0.5 mg/ml) at different NaCl conditions

15. Detection of acid phosphatase activity in moong bean (0.5 mg/ml) at different concentrations of NaCl :

Using the different concentrations of NaCl as 0.05M, 0.1M, 0.25M, 0.5M, the acid phosphatase activity was observed. Moong bean showed the maximum activity at 0.05M concentration of NaCl.

NaCl conc.	Moong bean (OD 405nm)
0.05 M	0.499
0.1 M	0.466
0.25 M	0.479
0.5 M	0.429
No NaCl	0.489

Table 14. Acid phosphatase activity of Moong bean (0.5 mg/ml)

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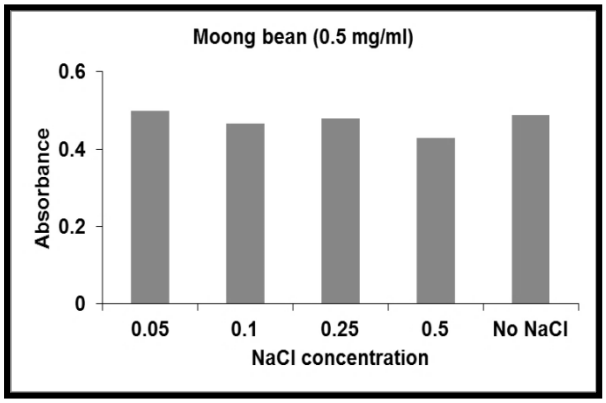

Figure 18. Acid phosphatase activity in moong bean (0.5 mg/ml) at different NaCl conditions

16. Detection of acid phosphatase activity in chickpea (0.5 mg/ml) at different concentrations of NaCl :

Using the different concentrations of NaCl as 0.05M, 0.1M, 0.25M, 0.5M, the acid phosphatase activity was observed. Moong bean showed the maximum activity at 0.25M concentration of NaCl.

NaCl conc.	Chickpea (OD 405nm)
0.05 M	0.264
0.1 M	0.244
0.25 M	0.266
0.5 M	0.234
No NaCl	0.250

Table 15. Acid phosphatase activity of chickpea (0.5 mg/ml)

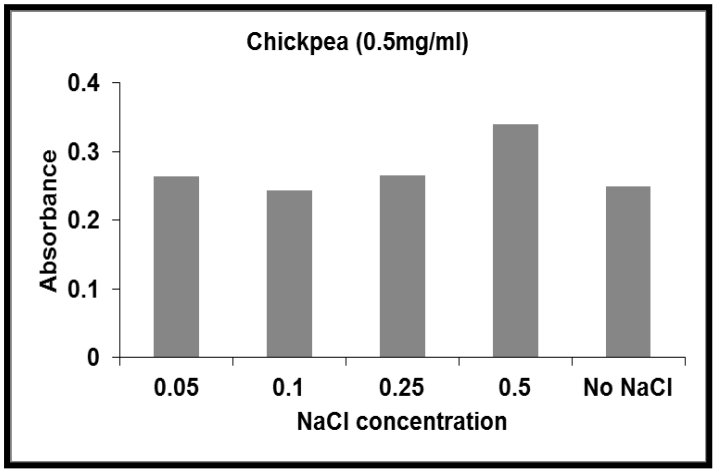

Figure 19. Acid phosphatase activity in chickpea (0.5 mg/ml) at different NaCl conditions

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17. Comparative acid phosphatase activities of all the extracts of samples at different concentrations of NaCl :

The comparative analysis of all the extracts of samples when subjected under salt stress of different concentrations suggests that the maximum enzymatic activity was shown by moong bean at 0.05M concentration.

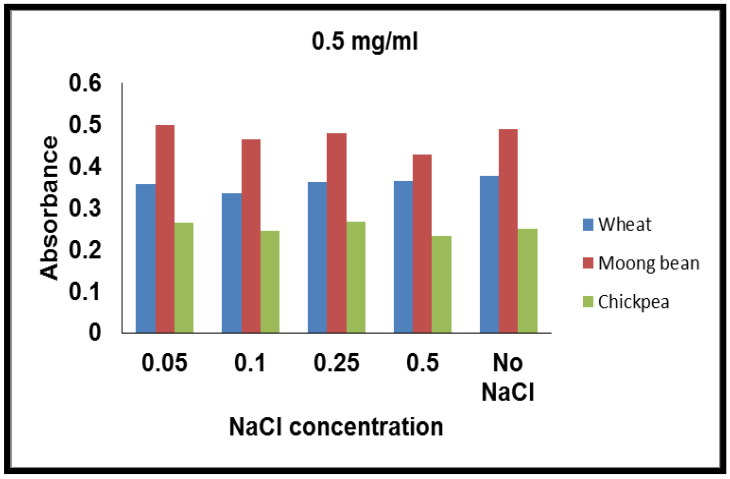

Figure 20. Comparison of APase activities of extracts at different NaCl concentrations.

18. Detection of acid phosphatase activity in wheat (0.5 mg/ml) at different concentrations of Glucose :

The acid phosphatase activity was analysed by subjecting the extracts of wheat under the sugar stress of glucose at different concentrations. Wheat showed the maximum activity at 0.25M concentration.

Glucose conc.	Wheat (OD 405nm)
0.05 M	0.894
0.1 M	0.941
0.25 M	0.866
0.5 M	0.928
No Glucose	0.926

Table 16. Acid phosphatase activity of wheat (0.5 mg/ml) under sugar stress

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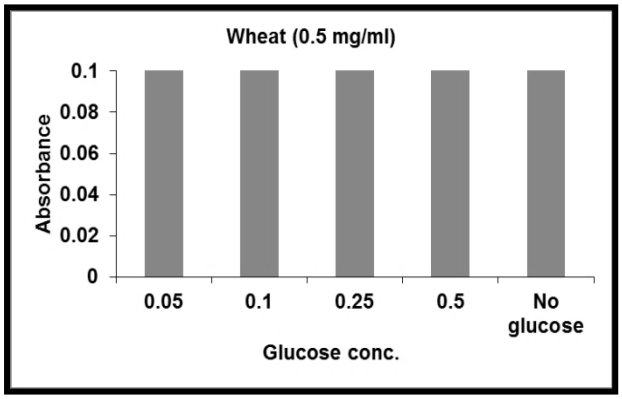

Figure 21. Acid phosphatase activity in wheat at different Glucose concentrations

19. Detection of acid phosphatase activity in moong bean (0.5 mg/ml) at different concentrations of Glucose :

The acid phosphatase activity was analysed by subjecting the extracts of moong bean under the sugar stress of glucose at different concentrations. Wheat showed the maximum activity at 0.25M concentration.

Glucose conc.	Moong bean (OD 405nm)
0.05 M	0.521
0.1 M	0.556
0.25 M	0.535
0.5 M	0.531
No Glucose	0.524

Table 17. Acid phosphatase activity of moong bean (0.5 mg/ml) under sugar stress

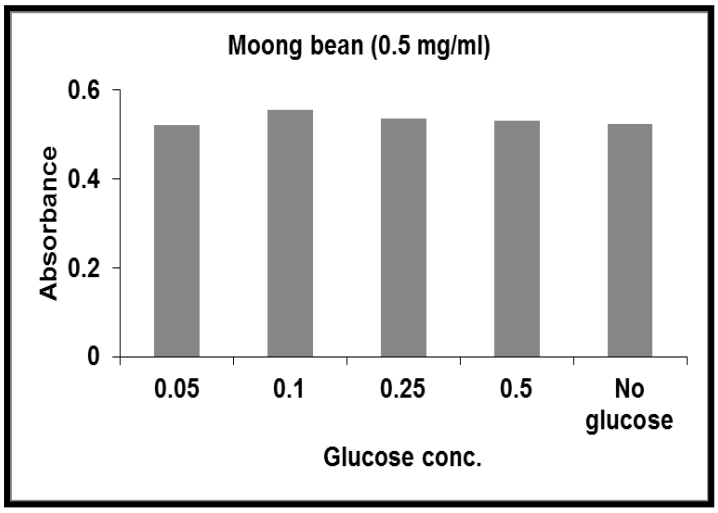

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Figure 22. Acid phosphatase activity at different Glucose conditions

20. Detection of acid phosphatase activity in chickpea (0.5 mg/ml) at different concentrations of Glucose :

The acid phosphatase activity was analysed by subjecting the extracts of chickpea under the sugar stress of glucose at different concentrations. Wheat showed the maximum activity at 0.25M concentration.

Glucose conc.	Chickpea (OD 405nm)
0.05 M	0.433
0.1 M	0.421
0.25 M	0.435
0.5 M	0.447
No Glucose	0.428

Table 18. Acid phosphatase activity of chickpea (0.5 mg/ml) under sugar stress

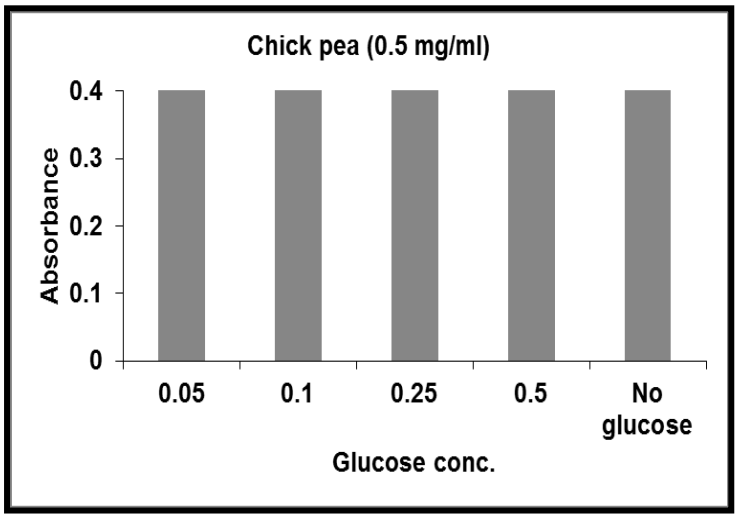

Figure 23. Acid phosphatase activity in chickpea at different Glucose conc.

21. Comparative acid phosphatase activities of all the extracts of samples at different concentrations of NaCl :

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The comparative analysis of acid phosphatase activities of all the extracts of wheat, moong bean and chickpea under the sugar stress showed the maximum activity at 0.25M but there was no significant effect found in all the samples.

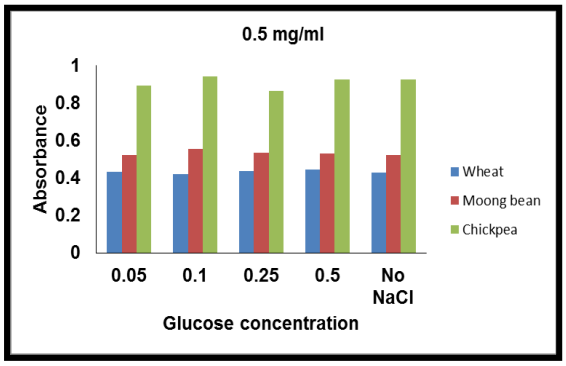

Figure 24. Acid phosphatase activity of all samples at different Glucose conditions

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