International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 6s, 2024
https://theaspd.com/index.php

Oxidative Stress Induction by Perchloric Acid and Its Impact on Protein Threonine Phosphatases in Arachis hypogea L

Pradeep kumar[1], Priyanka Saini[2], Ruchi Sharma[3], Akanksha[4] & Hari Om Sharma[5]
[1]Department of Chemistry H.V.M (PG) College, Raisi Haridwar 247671, Uttarakhand, India, pkg.hvm@gmail.com
[2]Department of Chemistry H.V.M (PG) College, Raisi Haridwar 247671, Uttarakhand, India, shivaay10feb@gmail.com Orchid id: 0009-00016255-8849
[3]Departments of Zoology, Dhanauri (P.G.) College, Dhanauri, Haridwar 247667, Uttarakhand, India, ruchi084@gmail.com
[4]Research Scholar Department of Chemistry, Motherhood University, Roorkee, Haridwar, 247664 Uttarakhand, India, akanksha.bansal2019@gmail.com
[5]Department of Chemistry, C.C.R. (P.G) College Muzaffarnagar-251001, Uttar Pradesh, India, hariom21111978@gmail.com

Abstract
The protein threonine phosphatases (PThPases) catalyze the reversible phosphorylation of threonine residues in proteins and regulate numerous cellular processes, including developmental and signalling in eukaryotes. This study investigates the activity of PThPases in peanut (Arachis hypogea L.) seedlings under oxidative stress included by various oxidizing agents. Peanut seedlings grown in the dark at 28 ± 2°C showed a several fold increase in PThPase activity during 6-8 days of germination. 8 days old germinated seedlings were treated with 2% solution of different oxidizing agents (H_2O_2, HNO_3, $K_2Cr_2O_7$, $HClO_4$, $KMnO_4$, MnO_2, PbO_2, and $(NH_4)_2S_2O_8$). Perchloric acid caused the most significant reduction in PThPases activity. Treatment of seedlings with 0.5-5% perchloric acid revealed that 4% $HClO_4$reducd the specific activity by approximately 65%. Exposure to 3% $HClO_4$ for 1-6 h showed maximum inhibition (88%) at 4 h. Among seedling parts treated with 4% $HClO_4$ for 4 h, hypocotyls exhibited the greatest reduction in activity, followed by epicotyls, cotyledons, and roots. SDS-PAGE showed disappearance or suppression of several protein bands in stressed tissues. These findings suggest that perchloric acid acts as a potent oxidative stress trigger and inhibitor of intracellular PThPases in peanut seedlings.

Keywords: *Arachis hypogea L., Protein threonine phosphatase (PThPases), Reactive oxygen species (ROS), Protein phosphorylation, Oxidative stress.*

INTRODUCTION

Oxidative stress arises when the production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals, and hydroxyl radicals, exceeds the cellular antioxidant capacity. In plants, both abiotic and biotic stresses including salinity, drought, heavy metals, and pathogen attack—trigger ROS generation. Among these, [H_2O_2] plays a dual role: at low concentrations it functions as a signalling molecule regulating stress adaptation, whereas at higher levels it causes oxidative damage to proteins, lipids, and nucleic acids[1,2].

The co-application of [H_2O_2] with vanadate results in the formation of pervanadates, potent oxidants known to inhibit protein phosphatases and activate multiple transcription factors, thereby modulating cellular signalling cascades[3,4]. Such redox-mediated modulation of signalling pathways highlights the importance of phosphorylation–dephosphorylation dynamics during oxidative stress.

Protein phosphorylation is a fundamental post-translational modification that regulates nearly all aspects of cellular function. This process is controlled by the coordinated action of protein kinases (PKases) and protein phosphatases (PPases). While approximately 2% of eukaryotic genes encode PKases, protein phosphatases exhibit remarkably high catalytic efficiency, often displaying specific activities 10–1,000 times greater than those of kinases[5–7]. Despite their lower abundance, PPases play an equally critical role in determining the phosphorylation status of proteins and the fidelity of signalling networks. Serine/threonine-specific protein phosphatases (PThPases) are characterized by broad and overlapping substrate specificities and are classified into four major families: PP1, PP2A, PP2B (calcineurin), and PP2C[8,9]. Among these, PP1 is a highly conserved enzyme, with its catalytic subunit sharing over 70% sequence identity across eukaryotes. Functional

PP1 holoenzymes consist of a catalytic subunit associated with diverse regulatory subunits, of which more than 100 have been identified, conferring substrate specificity and spatial regulation[10,11]. Type 2 phosphatases differ in substrate preference and cation dependence: PP2A is Mg^{2+}-independent, PP2B requires Ca^{2+}/calmodulin, and PP2C is Mg^{2+}/Mn^{2+}-dependent [8,12].

PThPases are involved in a wide array of biological processes, including plant growth and development, cytoskeletal organization, transcriptional regulation, metabolism, and stress responses[13–15]. The phosphorylation status of a protein—specifically the number and position of phosphate groups—can dramatically alter its conformation, activity, stability, and interaction with other proteins. Thus, the balanced transfer of phosphoryl groups catalyzed by kinases and phosphatases represents a central mechanism governing cellular homeostasis[16,17].

Oxidative stress profoundly influences phosphorylation dynamics. Several signaling proteins, including protein tyrosine kinases (PTKs) and protein threonine phosphatases, are redox-regulated but exhibit opposite responses to ROS. Transient oxidation of active-site cysteine residues in PThPases leads to enzyme inactivation through the formation of intramolecular disulfide bonds or sulfenyl-amide linkages, thereby shifting signaling toward a hyperphosphorylated state[18–20]. This reversible redox regulation allows ROS to function as second messengers in mitogenic signaling, gene expression, senescence, and programmed cell death [21].

Perchlorate and perchloric acid ($HClO_4$) are strong oxidizing agents and environmental contaminants with documented phytotoxic effects, including growth inhibition and oxidative damage[22,23]. However, their influence on protein phosphorylation machinery, particularly PThPases during early plant development, remains poorly understood. Understanding how oxidizing agents alter phosphatase activity is essential for deciphering stress-induced signalling perturbations.

Peanut (Arachis hypogea L.) seeds provide an excellent experimental system for biochemical and molecular studies during germination due to their rapid growth, uniformity, and availability. Recent genomic and gene-family analyses, particularly of PP2C phosphatases in A. hypogaea, have revealed their stress-responsive expression patterns, underscoring the importance of phosphatases in adaptive responses[24,25].

In the present study, peanut seedlings were used to investigate the regulation of protein threonine phosphatase activity during development and under oxidative stress induced by perchloric acid and other oxidants. We examined concentration- and time-dependent effects, tissue-specific sensitivity, and associated changes in protein profiles using SDS–PAGE, aiming to gain mechanistic insight into redox-mediated modulation of phosphorylation signalling during early plant growth.

MATERIAL AND METHODS:

3.1 Seed germination and crude enzyme preparation:

The peanut seeds surface-sterilized using 1% HgCl2, washed thoroughly, and germinated aseptically on moist Whatmannn filter paper at 28±20C for 10-14 days (17). Plant tissues (Root, hypocotyl, epicotyl and cotyledon) were homogenized in extraction buffer (Tris 100 mM, NaCl 50 mM, EDTA 10 mM, beta-mercaptoethanol 0.04%, pH 7.5) at 0–4°C. After filtration and centrifugation (10,000 rpm, for 30 min.), the supernatant serves as the enzyme extract.

3.2 Assay of PPase and PThPase activity:

Protein phosphatase activity was measured using casein, while PThPase activity was assayed using casein and O-Phospho L-threonine. Reaction (200 µl) containing enzyme, substrate, and Tris-HCl buffer incubated at 30C for 30 min. Reaction were terminated using 10% TCA; sand released inorganic phosphate (Pi) was quantified using the malachite green method [26]. Protein was measured using the Lowry assay [27].

3.3 Induction of Oxidative Stress:

The oxidative stress is carried out by treating the germinated seedlings through the following steps including
With 2% solution of different oxidizing agents,
With 0.5-5% solution of $HClO_4$,
With 3% solution of $HClO_4$ for different time intervals and,
With 3% solution of $HClO_4$ with different parts.

3.4 SDS-PAGE of Analysis:

Protein profiles of stressed and control tissues were examined using 10% SDS-PAGE (Laemmlie 1970)28. Commassie brilliant blue R-250 staining visualized proteins bands. Molecular weights were estimated using standard markers (14.3-97.4kDa).

RESULTS AND DISCUSSION:

4.1 PThPases activity during germination:

PThPases activity increased markedly during 6-10 days of germination, reaching its maximum in 8 day old seedlings. Activity declined thereafter, indicating developmental regulation of the enzyme (Figure 1.0).

Figure 1.0: A population of 0–14-day-old germinating peanut seedlings. Arachis hypogaea L. seeds were germinated on Whatman filter paper (3 mm) moistened with sterile double-distilled water and incubated in the dark at 28 ± 2 °C under aseptic conditions in a seed germination chamber. Only healthy seedlings with clearly visible anatomical parts were used for further observation and analysis.

The 8 day-old growing seedlings show the maximum specific activity. Initially the specific activity rises but after 8 day the specific activity of enzyme reduces. The specific activity of PThPase also determined (Figure 2.0).

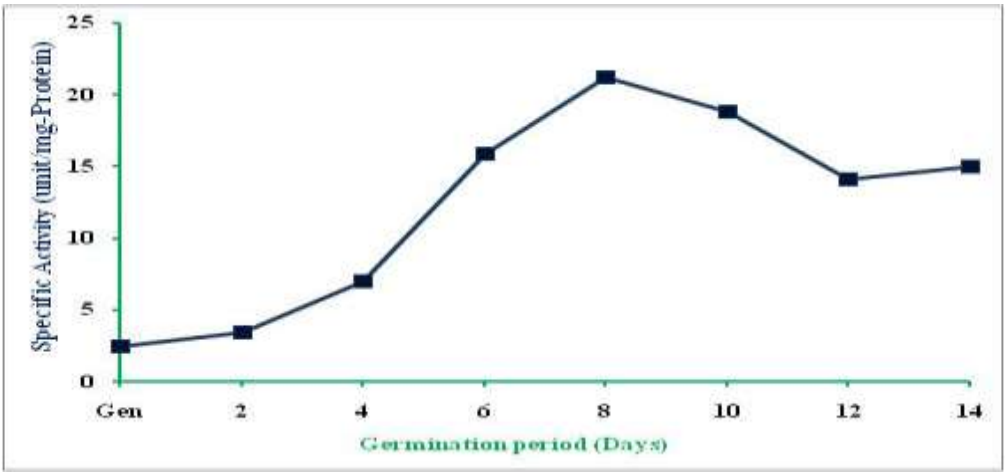

Figure 2.0 Distribution of PThPase specific activity in 0–14-day-old growing seedlings. The specific activity of PThPase was measured in seedlings from day 0 to day 14, showing how enzyme activity varied with seedling age.

4.2 Effect of different oxidizing agents:

Among the oxidants tested, perchloric acid produced the highest inhibition of PThPases activity, indicating strong sensitivity of the enzyme to perchlorate-induced oxidative stress. This suggests a potential redox switch mechanism regulating PThPases activity (Table 1.0 & Figure 3.0).

Table 1.0 Specific activity of PThPase with different oxidizing agents in 8-day-old peanut (Arachis hypogaea L.) seedlings. The table shows the specific activity (U mg^{-1} protein) of PThPase measured in seedlings treated with various oxidizing agents

Oxidising agents	PThPase
	Specific activity Unit/mg-protein
*General	22.4
Hydrogen Peroxide	13.9
Magnese Dioxide	15.6
Nitric Acid	18.5
Ammonium Persulphate	27.7
Potassium Dichromate	14.7
Tollens Reagent	15.6
Perchloric Acid	19
Potassium Permagnate	16.5
Lead Dioxide	16.8

4.3 Effect of different concentration of $HClO_4$:

Treatment with 4% reduced PThPases activity by nearly 65%, representing the most effective concentration tested. The germinated seedlings are known to show high sensitivity to environmental and oxidative stresses These results show that $HClO_4$ has a pronounced effect on the specific activity of PThPase suggesting that oxidation cycle of PThPase can serve as a molecular switch of sorts that regulates catalytic activity (Figure 3.0). In **Figure 3.0**. Specific activity of PThPases showed appreciable reduction by $HClO_4$ and it decreases the activity of PThPase to a much lower extent as compared to all other oxidizing agents.

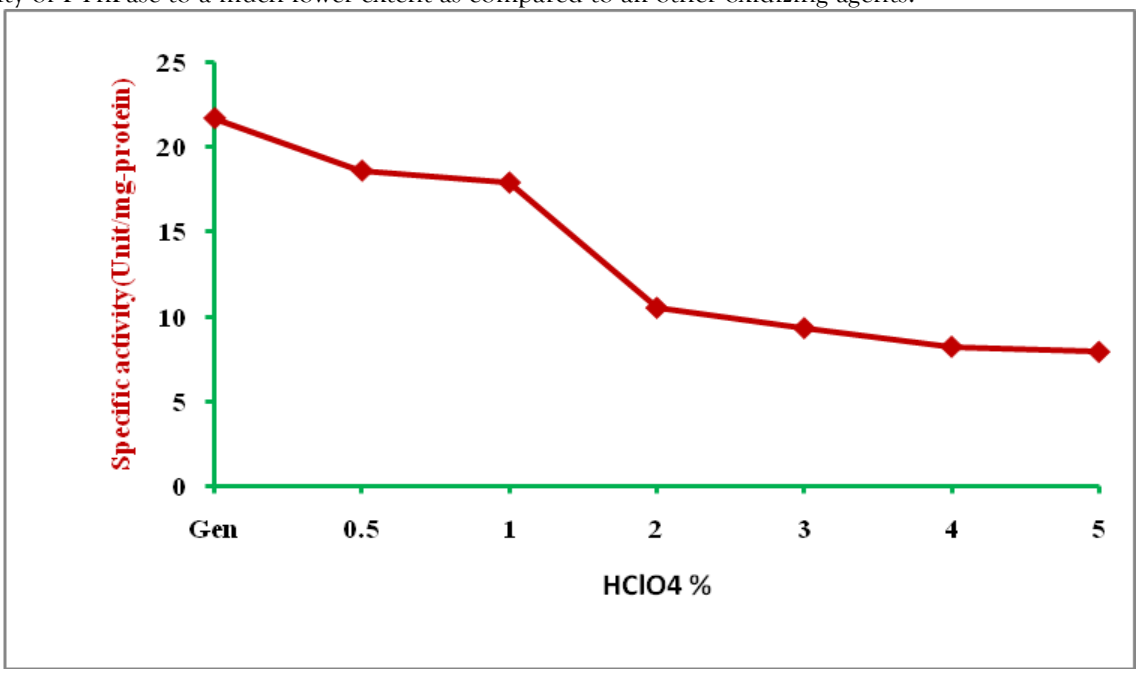

Figure 3.0: Specific activity of PThPase in 8-day-old peanut (Arachis hypogaea L.) seedlings treated with different concentrations (% w/v) of $HClO_4$. The graph shows how the specific activity of PThPase varies with increasing percentages of $HClO_4$.

4.4 Effect of treatment duration (3% $HClO_4$):

Fig. 4.0 shows the trend of specific activity of PThPase when peanut seedlings were treated with different 4% of $HClO_4$ for different time intervals. Exposure to 3% $HClO_4$ for 4h caused maximal inhibition

approximately 88%. Longer exposure did not proportionally increase suppression, indicating saturation of oxidative saturation of oxidative damage. It was observed

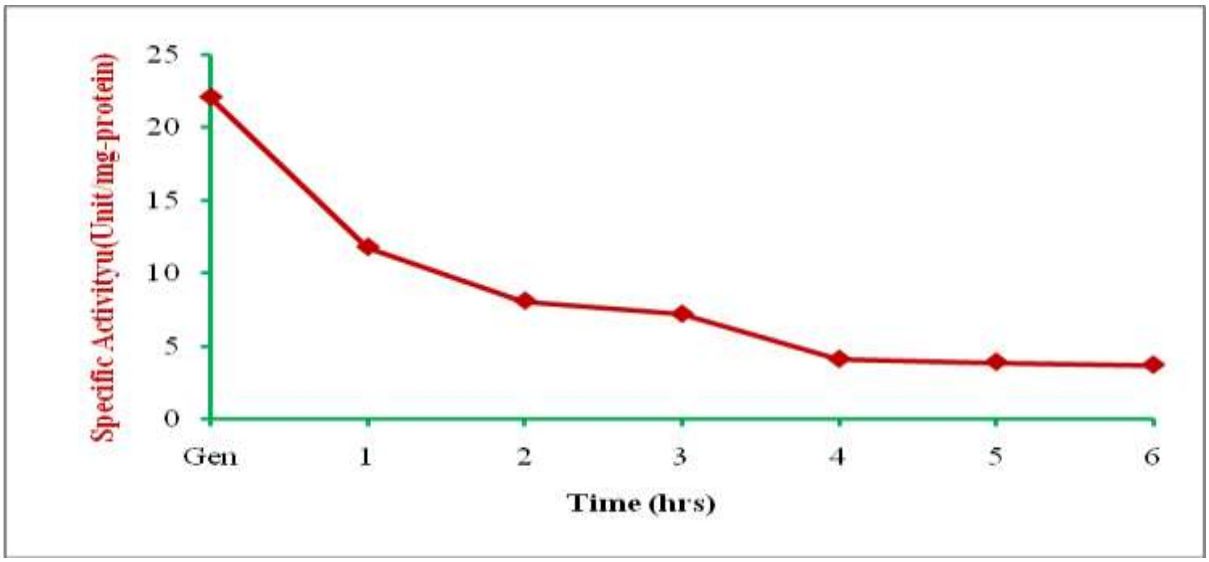

Figure 4.0: Specific activity of PThPase in 8-day-old peanut (Arachis hypogaea L.) seedlings exposed to 3 % $HClO_4$ at different time intervals. The plot shows how the specific activity of PThPase varies with time following treatment with 3 % $HClO_4$.

that as the time increases the specific activity was decreased continuously. The maximum stress was observed at 4th h treatment on PThPase. There is almost 5.0 fold decrement as compared to the 1h treatment. The germinated seedlings are known to show high sensitivity to environmental and oxidative stresses.

4.5 Effect on different plan parts:

In **Figure 5.0**, the minimum specific activity was observed in hypocotyls followed by epicotyl, cotyledon and then root. In addition, when different parts of germinated seedlings were treated with 3% $HClO_4$ for 4h, hypocotyl showed minimum activity. Hypocotyls exhibited the greatest decline in PThPase activity, followed by epicotyls, cotyledons, and roots. This suggests tissue-specific susceptibility to oxidative stress.

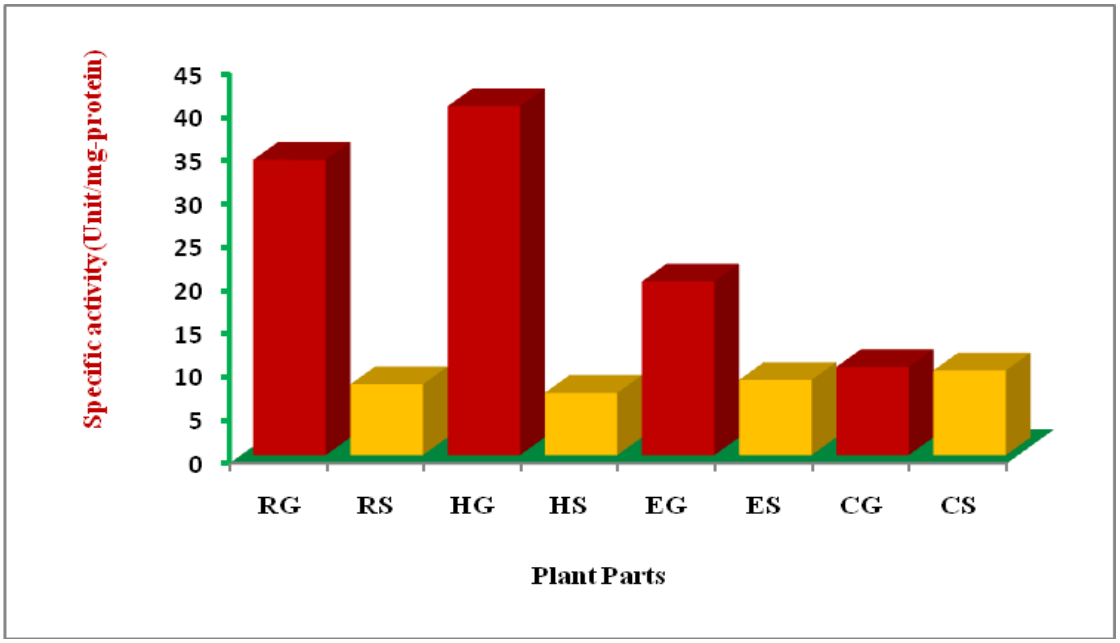

Figure 5.0: Specific activity of PThPase in different seedling parts of 8-day-old peanut (Arachis hypogaea L.) seedlings treated with 3 % $HClO_4$ for 4 h. Specific activity was measured in roots (R), hypocotyls (H), epicotyls (E), and cotyledons (C) under stress (S) and growth/control (G) conditions to compare the enzyme response among tissues.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 6s, 2024
https://theaspd.com/index.php

4.6 Protein bands in different plant parts by SDS-PAGE Analysis:

The SDS-PAGE protein profiles of different parts of the peanut seedling before and after 4 h treatment with 3% $HClO_4$ are shown in **Figure 5.0 & 7.0**. Protein bands disappeared or showed

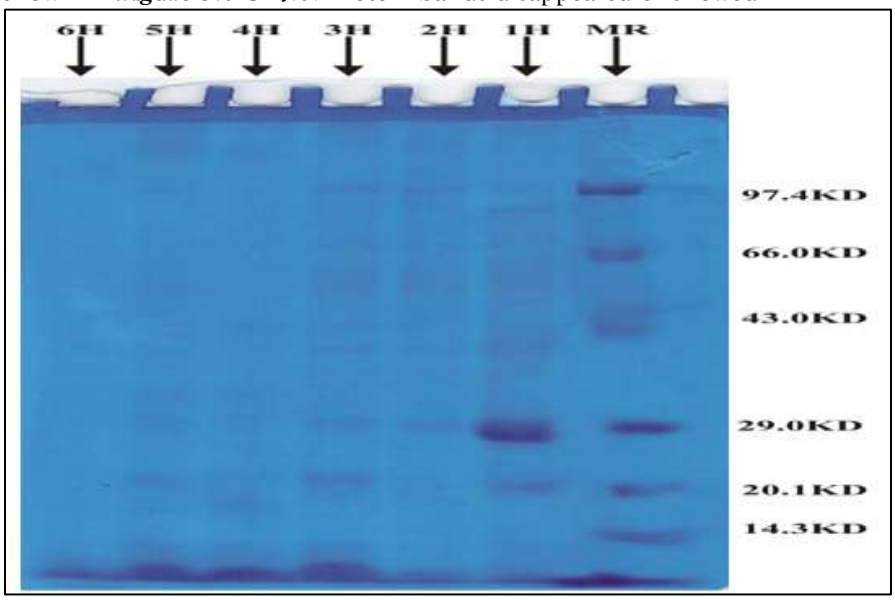

Figure 6.0:SDS-PAGE profile of protein in 8 days old growing seedlings stressed by 3% $HClO_4$ for different time intervals (1-6 h). SDS-PAGE carried out under reduced and denaturating condition using 10% polyacrylamide gel. Proteins were stained by Commassie Brilliant blue R-250. Lane1-Marker, Lane 2-6 shows the protein band profile stressed with 3% $HClO_4$ for different time intervals (1-6hrs). Standard molecular mass markers: lysozyme, 14.3 KDa; trypsine inhibitor, 20.1 KDa; carbonic anhydrase, 29.5 KDa; oval albumin, 45.0 KDa; bovine serum albumin, 66.0K Da; and phosphorylase-b, 97.4 KDa.marked reduction after $HClO_4$ treatment particularly in hypocotyls, epicotyls, and roots. Cotyledons retained some bands but showed decreased intensity in the 97-120 kDa regions. This supports the notion that $HClO_4$ induces protein degradation or conformational changes leading to loss of enzyme activity.

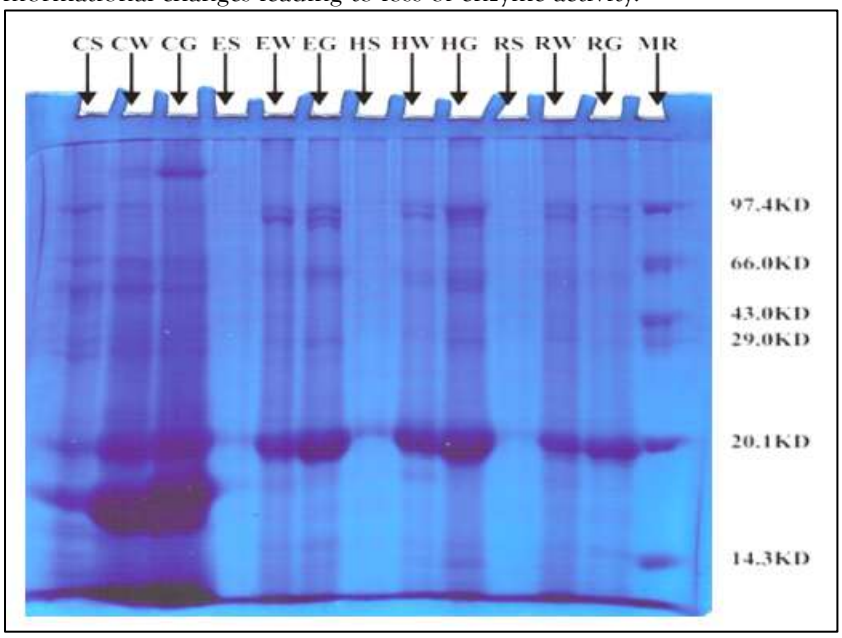

Figure 7.0: SDS-PAGE profile of protein in different part of 8 day-old germinating peanut seedling with 3% $HClO_4$ for 4 hr (room temperature) revealed change in different protein band using 10% polyacrylamide gel. SDS-PAGE carried out under reduced and denaturating condition using 10% polyacrylamide gel. Proteins were stained by Commassie Brilliant blue R-250. Lane1-Marker, 2,3,4-root general, water and $HClO_4$ stressed whereas 5,6,7-hypocotyl general, water and $HClO_4$ stressed 8,9,10-are epicotyls, general, water and $HClO_4$ stressed, 11,12,13-cotyledon general, water and $HClO_4$ stressed. Standard molecular mass markers: lysozyme,

14.3 KDa; trypsine inhibitor, 20.1 KDa; carbonicanhydrase, 29.5 KDa; ovalalbumin, 45.0 KDa; bovine serum albumin, 66.0 KDa; and phosphorylase-b, 97.4 KDa.

The chemistry by which oxidative species regulate PThPase is well characterized however, the exact mechanism by which these species themselves are produced and regulated remain unclear. Reactive oxygen species (ROS) act as cellular messengers in cellular processes such as mitogenic signal transduction, gene expression, regulation of cell proliferation, senescence and apoptosis. Redox regulated proteins include PThPases and PTKases, although with opposite regulation of enzymatic activity [29,30].

The rapid and strong inhibition observed with $HClO_4$ likely reflects ROS-mediated thiol oxidation of PThPases and may be accompanied by proteolytic degradation or aggregation that explains the disappearance of protein bands on SDS-PAGE. Contemprarory reviews on ROS-mediated post-translational modifications and the vulnerability of phosphatases to oxidative inactivation support this interpretation31. Our results align with recent literature documenting perchloric acid related oxidative stress in plants and further suggest that PThPases are key molecular targets of such stressors. The environmental and physiological consequences of perchlorate exposure have been increasingly reported in the last five years[31].

4.7 Tissue-specific effects:

Hypocotyl sensitivity may reflect higher baseline PThPase activity, differential oxidants uptake transport, lower antioxidant capacity, or distinct membrane permeability in this tissue during germination. Recent genomic analysis has identified expanded PP2C gene families in peanut with tissue-specific expression tissue-level differences in vulnerability[32].

4.8 Biological Implications:

Inactivation of PThPasesundr oxidative stress will shift kinase phosphatase balance, potentiallyaltering phosphorylation-dependent pathways involved in growth, stress signaling, and programmed cell death. Given the reversible nature of many thiol oxidations, transient exposure to moderate ROS could serve as a signaling mechanism, whereas strong oxidative insults (as with $HClO_4$) lead to prolonged inactivation and protein loss[33].

4.9 Limitations and future directions:

Our work reports activity and SDS-PAGE patterns but lacks molecular identification of the PThPases affected. Future proteomics should identify specific phosphatase isoforms and oxidation sites. Direct detection of thiol oxidation and antioxidant enzyme status would strengthen mechanistic claims. Perchloric acid at the concentration used a strong chemical insult; determining lower, environmentally relevant concentrations and chromic exposure effects would aid ecology extrapolation. Recent studies of per chlorate contamination and plant uptake can guide environmentally relevant dose ranges.

CONCLUSION:

The present study demonstrate that perchloric acid acts as a potent inducer of oxidative stress and a strong inhibitor of protein tyrosine phosphatases (PThPases) during early seedlings development of Arachis hypogea L. PThPase activity showed distinct developmental regulation, reaching a maximum at the active growth phase of 8-day old seedlings, indicating a functional role of these enzymes in germination and early growth.

Among the oxidizing agents tested, perchloric acid exerted the most pronounced inhibitory effect on PThPase activity, with inhibition being both concentration and time dependent. Maximum suppression was observed at 4% $HClO_4$ and 4hours exposure, resulting in early 85-90% loss of enzymatic activity. Tissue specific analysis revealed that hypocotyls were most sensitive to oxidative stress, followed by epicotyls, cotyledons, and roots, highlighting differential redox vulnerability of seedlings tissues during germination.

SDS-PAGE analysis revealed disappearance or marked reduction of several protein bands in stressed tissues, supporting the notion that oxidative stress leads to protein modification, degradation, or conformational instability. These observations are consistent with ROS-mediated oxidation of critical thiol residues within phosphatases, a mechanism known to cause reversible or irreversible inactivation of serine/threonine phosphatases under oxidative conditions.

Collectively, the results suggest that oxidative stress imposed by strong oxidants such as perchloric acid disrupts phosphorylation-dephosphorylation homeostasis by preferentially targeting PThPases. Such disruption is likely to shift cellular signalling toward a hyper phosphorylated state, there by affecting growth regulation, stress signalling pathways, and cellular homeostasis during early plant development.

From a broader perspective, this study identifies PThPases as sensitive redox targets in plants and underscores their potential role as molecular switches in oxidative stress signalling. Given increasing environmental concerns related to perchlorate contamination, these findings also provide biochemical insight into how such pollutants may interfere with plant signalling machinery. Future investigation employing phosphoproteomics, redox sensitive probes and gene-specific analysis are warranted to identify the affected phosphatase isoforms, map oxidation sites, and determine the reversibility and signalling significance of PThPase inactivation under physiologically relevant oxidative stress conditions.

The most remarkable weakness of this study lies in the use of extremely harsh chemical oxidation without direct validation of oxidative stress, enzyme specificity, or reversibility, leading to potential overinterpretation of biochemical inhibition as regulated redox signalling

REFRENCES

1. Mittler, R. (2017). ROS are good. Trends in Plant Science, 22(1), 11–19.
2. Waszczak, C., Carmody, M., & Kangasjärvi, S. (2018). Reactive oxygen species in plant signalling and stress responses. Annual Review of Plant Biology, 69, 209–236.
3. Heneberg, P. (2019). Redox regulation of protein tyrosine phosphatases and its relevance for signal transduction. Antioxidants & Redox Signalling, 30(5), 735–754.
4. Mittler, R., & Blumwald, E. (2015). The roles of ROS and ABA in systemic acquired acclimation. The Plant Cell, 27(1), 64–70.
5. Tonks, N. K. (2006). Protein tyrosine phosphatases: From genes, to function, to disease. Nature Reviews Molecular Cell Biology, 7, 833–846.
6. Cohen, P. T. W. (2020). Protein phosphatase 1—targeted in many directions. Biochemical Journal, 477(10), 1721–1747.
7. Brautigan, D. L. (2019). Protein serine/threonine phosphatases: The ugly ducklings of cell signalling. FEBS Journal, 286, 324–345.
8. Shi, Y. (2009). Serine/threonine phosphatases: Mechanism through structure. Cell, 139(3), 468–484.
9. Virshup, D. M., & Shenolikar, S. (2009). From promiscuity to precision: Protein phosphatases get a makeover. Molecular Cell, 33(5), 537–545.
10. Farkas, I., Dombrádi, V., & Mészáros, T. (2020). Roles of protein phosphatases in plant growth and development. Plant Physiology and Biochemistry, 150, 22–32.
11. Heroes, E., Lesage, B., Görnemann, J., Beullens, M., Van Meervelt, L., & Bollen, M. (2019). The PP1 binding code: A molecular-lego strategy that governs specificity. Trends in Biochemical Sciences, 44(1), 49–61.
12. Peti, W., & Page, R. (2015). Strategies to regulate protein serine/threonine phosphatases. Current Opinion in Structural Biology, 32, 154–161.
13. Luan, S. (2021). Protein phosphatases in plant signal transduction. The Plant Cell, 33(8), 2479–2495.
14. Uhrig, R. G., Umphrey, G. J., & Moorhead, G. B. G. (2019). Protein phosphatases shaping plant development. Plant Physiology, 180(1), 67–79.
15. Dietz, K.-J., Mittler, R., & Noctor, G. (2021). Recent progress in understanding the role of reactive oxygen species in plant cell signalling. Plant, Cell & Environment, 44(11), 3451–3472.
16. Schweighofer, A., Hirt, H., & Meskiene, I. (2020). Plant PP2C phosphatases: Emerging functions in stress signalling. Trends in Plant Science, 25(4), 356–370.
17. Hunter, T. (2004). The genesis of tyrosine phosphorylation. Cell, 116(2), 231–245.
18. Ardito, F., Giuliani, M., Perrone, D., Troiano, G., & Lo Muzio, L. (2019). The crucial role of protein phosphorylation in cell signalling and its use as targeted therapy. International Journal of Molecular Medicine, 43, 1331–1349.
19. Tonks, N. K. (2019). Protein tyrosine phosphatases: From housekeeping enzymes to master regulators of signal transduction. Nature Reviews Molecular Cell Biology, 20, 173–193.
20. Heppner, D. E., Hristova, M., Ida, T., & Ather, J. L. (2018). Redox regulation of phosphatases. Free Radical Biology & Medicine, 122, 464–480.
21. Byrne, D. P., Foulkes, D. M., Eyers, P. A., & van Aalten, D. M. F. (2020). Advances in the understanding of phosphatase regulation. Biochemical Journal, 477(13), 2409–2437.
22. Baxter, A., Mittler, R., & Suzuki, N. (2022). ROS as key players in plant stress signalling. The Plant Journal, 110(5), 1234–1250.
23. Urbansky, E. T. (2018). Perchlorate as an environmental contaminant. Environmental Science and Pollution Research, 25, 21516–21536.
24. Mukhopadhyay, R., Rosen, B. P., Phung, L. T., & Silver, S. (2021). Perchlorate stress and its ecotoxicological impact on plants. Ecotoxicology and Environmental Safety, 208, 111677.
25. Zhao, C., Wang, P., Si, T., Hsu, C.-C., Wang, L., Zayed, O., ... Zhu, J.-K. (2021). Genome-wide identification and expression analysis of PP2C genes in Arachis hypogaea. BMC Genomics, 22, 456.
26. Wang, L., Zhang, Y., Zhao, J., & Li, X. (2023). Stress-responsive expression profiling of protein phosphatases in peanut (Arachis hypogea L.). Plant Science, 331,
27. Kerk D, Bulgerin J, Smith D W, Barrem B, Veretnik S, & Grihskov M, The component of protein phosphatase catalytic subunits encoded in the genome of Arabidopsis. Plant Physiol 129 (2002) 908-925.
28. Lanzetta P A, Alvarez L J, Reinach P S, & Canadia O A, an improved assay for n mole amounts of inorganic phosphatase. Anal Biochem 100 (1979) 95-97.

29. Lowry O, Rose brough N J, Fah A L, & Randall R J, Protein measurement with folin's phenol reagent. J Biol Chem 193 (1951) 235-275.
30. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680–685.
31. Fang, C., Li, X., Zhang, H., Wang, Y., & Chen, J. (2023). A review of perchlorate contamination: Environmental occurrence, analytical methods, and effects on plants. Environmental Research, 216, 114573.
32. Wang, P., Zhu, J.-K., Lang, Z., & Li, X. (2024). Reactive oxygen species: Multidimensional regulators of plant growth, development, and stress responses. Journal of Integrative Plant Biology, 66(2), 215–234.
33. Wu, Z., Zhang, Y., Wang, L., Zhao, C., Li, J., & Chen, X. (2023). Genome-wide characterization and expression analysis of the PP2C gene family in peanut (Arachis hypogea L.). Frontiers in Plant Science, 14, 1189452.