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Quantification of Nutrient Uptake and Allocation under Phosphate Stress in Rice using Ionomics Techniques

Priyanka Pandey

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Supervisors: Chungui Lu and Dov Stekel

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ABSTRACT

The rampant use of phosphate fertilizers, a non-renewable resource with limited supply, for increasing crop productivity is beset with problems linked to human health and environment. With the use of biotechnology, development of varieties with high phosphate (Pi) uptake efficiency and utilization capabilities is possible but it requires thorough understanding of physiological mechanisms regulating phosphate stress. Ionomic profile of the tissue undergoing phosphate stress can be of use to understand physiology of stress response.

In this experiment effects of hydroponic solutions with low or high concentration of Pi on plants were studied on *Oryza sativa* ssp *japonica* cultivar Nipponbare for 21 days. Rates of nutrient uptake by the whole plant from hydroponic solutions with different Pi treatments and their allocation to shoot and root tissues were calculated by using ionomic profiles of hydroponic solutions and shoot/root tissues over a period of 21 days. The plants were initially grown on hydroponic solutions with 320 µM Pi for 15 days before being subjected to different Pi treatments. Stunted shoots with reduced uptake as well as allocation of potassium and phosphorus to shoot and increased allocation of molybdenum and copper to roots were markers of Pi stress in Nipponbare. Ionomic profiles of macronutrients revealed opposite allocation patterns in shoot and root tissues during Pi stress. Study of marker elements for Pi stress in rice is done for the first time. Judicious use of phosphate fertilizers and addition of required nutrients to crop lands can be planned based on study of ionomic profile of crop plants to obtain their optimum production and mitigate problems related to overuse of phosphate fertilizers.

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LIST OF ABBREVIATIONS

ANCOVA: Analysis of covariance

AMT superfamily: Ammonium transporter superfamily

ATP: Adenosine triphosphate

B: Boron

Ca: Calcium

cHATS: Constitutive high-affinity system for transport of nitrate

Cl: Chlorine

cLATS: Constitutive low-affinity system for transport of nitrate

CO_2: Carbon dioxide

CRD: Completely randomized design

Cu: Copper

Fe: Iron

HKT: High-affinity Potassium Transporters

HPO_4^{2-} : Monovalent cation, Monohydrogen phosphate

$H_2PO_4^-$: Divalent cation, Dihydrogen phosphate

$H_3PO_4^{3-}$: Phosphoric acid

ICP-AES: Inductively Coupled Plasma- atomic emission spectroscopy

ICP-MS: Inductively Coupled Plasma- Mass Spectrometry

iHATS: Inducible high-affinity system for transport of nitrate

iLATS: Inducible low-affinity system for transport of nitrate

IPS1: Induction by Pi starvation

K: Potassium

MiR399: Micro RNA399

Mg: Magnesium

Mn: Manganese

Mo: Molybdenum

MoO_4^{2-}: Molybdate

N: Nitrogen

NH_4^+: Ammonium

Ni: Nickel

NO_2: Nitrogen dioxide

NO_3^-: Nitrate

OsPTF1: Pi starvation induced transcription factor in rice

P: Phosphorus

PHO family: Phosphate genes family

PHT family (1:4): Phosphate Transporter, Pi transporter genes family

PHR1: Phosphate starvation response 1

Pi: inorganic phosphate

PO_4^{2-} : Phosphate

RSA: Root system architecture

S: Sulphur

SO_2: Sulphur dioxide

SO_4^{2-} : Sulfate

SULTR: Sulfate transporter

SIZ1: SUMOE3 ligase

UBC24: a member of E2 ubiquitin conjugase family

UTP: Uridine triphosphate

Zn: Zinc

INTRODUCTION

PHOSPHATE STRESS IN PLANTS

Origin of problem with phosphate fertilizers

Inorganic phosphate (Pi) is essential for plant growth and productivity (Rouached et. al., 2010). Pi is immobile in soil and is also bound to organic molecules in soil particles, making it inaccessible to plants. Also, acidic soils and weathering impair its use by plants. To mitigate the effects of immobility, inaccessibility to plants, excessive application of phosphate fertilizers is prevalent to enhance crop productivity (Alexova and Millar, 2013). Phosphate fertilizers are derived from non-renewable rock deposits after mining of Pi. These resources are limited in amount and are predicted to become a major bottleneck in food production within next century (Nesset and Cordell, 2011). Another point of the concern with phosphate fertilizers is their contamination with heavy metals like Arsenic and Cadmium from mining of Pi, these metals are human health hazard on accumulation in crops due to heavy phosphate fertilization (Chen et. al., 2007; Cheraghi et. al., 2012; Jiao et. al., 2012). The demand for phosphate fertilizers is continuously increasing and hence, the risk of contamination of soil (Chien et. al., 2011). Agricultural runoff of soil with repeated use of phosphate fertilizers can cause surface water eutrophication (Levine and Schindler, 1989). Enrichment of surface waters with phosphorus limits its use for various purposes and contributes to environmental pollution, pointing to judicious use of these fertilizers (Sharpley et. al., 1995). Water run-off, soil erosion and leakage in soils undergoing repeated phosphate fertilization are believed to cause environmental problems like eutrophication of lakes and rivers (Poirier and Bucher, 2002).

To prevent the harmful effects of excessive use of phosphate fertilizers, crops with improved phosphate uptake and utilization are required which can be done through advanced biotechnological techniques (Alexova and Millar, 2013). For improving phosphate utilization, study of biochemical characteristics involving ionome, metabolome is needed (Alexova and Millar, 2013) especially in phosphate efficient cultivars and ecotypes of species like in barley, rice, maize, soybean and oilseeds. It also needs input from **study and understanding of mechanisms that plants use to overcome Pi limitation/ stress** (Alexova and Millar, 2013).

Uptake and transport of nutrients in plants

Nutrients

There are 14 essential mineral elements apart from water, oxygen and carbon dioxide which are required by plants for growth and reproduction. These mineral elements are classified into MAJOR ELEMENTS/ MACRONUTRIENTS: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Sulphur (S) and Magnesium (Mg), as they are needed in large amounts (>1000 mg/kg dry weight) and TRACE ELEMENTS/MICROELEMENTS: Chlorine (Cl), Iron (Fe), Boron (B), Nickel (Ni), Copper (Cu), Manganese (Mn), Zinc (Zn) and Molybdenum (Mo) as they are needed in smaller amounts (<100 mg/kg dry weight) (Mengel et. al., 2001).

Plants uptake sufficient quantities of essential nutrients from soil, which is determined by their availability and mobility in soil (Giovanni et. al., 2014). There is another challenge for plants from uptake of non-essential elements and accumulation of toxic levels of essential elements in tissues (Giovanni et. al.,

2014). To deal with these challenges, plants have developed strategies for uptake and allocation of nutrients to maintain homeostatic ion levels (Williams and Salt, 2009). For allocation to tissues, the metals are mobilized and absorbed from the soil by roots where they undergo further modifications (sequestration) and then loaded into xylem for transport to aerial parts of the plant (Giovanni et. al., 2014). Each step of transport of nutrients is governed by interactions of chelating compounds and metal transporters (Clemens, 2001). Plants also have another vascular tissue phloem, which together with xylem constitute their vascular system. The role of phloem is to translocate and redistribute the products of photosynthesis, phytohormones and nutrients between sources and sinks in the plant body (Giovanni et. al., 2014). Some ions undergo rapid xylem to phloem transfer in leaves and stems for re-translocation back to roots which is essential for nutrient redistribution like N, K, P and Mg (Hall and Baker, 1972).

Nutrient uptake by roots

Nutrients such as SO_2 (Sulphur dioxide, S), NH_3 (Ammonia) and NO_2 (Nitrogen dioxide, N) can be absorbed in the form of gases through stomata and undergo metabolism directly by leaves (Yang et. al., 2006; Vallano and Sparks, 2008). Most minerals are absorbed by roots and plants have developed a few strategies for efficient uptake or absorption which include acidification, secretion of organic metal binders and expression of high affinity metal transporters (generally in low availability of metal) (Rauser, 1999). Acidification plays a role in solubilisation of metals after which they can be easily adsorbed to the cell wall or undergo movement through apoplasm of roots (Giovanni et. al., 2014). During adsorption into root, there can be modification of metals like for

redox active metals and they are chemically reduced by membrane proteins to promote easy transport (Robinson et. al., 1999). From root apoplasm, the metals are then transported to symplasm which is hindered by endodermis with casparian strips and hence, involves active transport (Chen et. al., 2011). This involves many high affinity metal transporters, some with broad substrate affinity (Nishida et. al., 2011).

Allocation of nutrients (root to shoot transport)

After passage through the root endodermis, the ions are loaded in the xylem tissue of root vasculature forming a part of xylem sap which is brought to the shoot with the transpiration stream where they are allocated to aerial tissues (Clemens et. al., 2002). Xylem loading is thought to be regulated by metal specific transporters (Giovanni et. al., 2014). The unloading of the metals from the xylem is not well characterized, with possible role of several membrane proteins families but it also requires additional mechanisms for maintaining metal homoeostasis (Grotz and Guerinot, 2006).

During phloem translocation also, in shoot, the metal ions are complexed with a variety of compounds, including low and high molecular weight molecules (Giovanni et. al., 2014). There are two processes involved in phloem transport: apoplastic loading into companion cells and sieve elements and unloading at the target sink tissues (Curie et. al., 2001).

Generation of signals for uptake or acquisition of nutrients

Essential mineral nutrients are acquired from rhizosphere by plant roots, translocated to shoot through the vasculature for shoot and leaf development where photosynthesis produces sugars which is then delivered to the non-

photosynthetic sink tissues, young growing tissues and roots for growth (Lin et. al., 2014). Communication between root and shoot is dependent on movement of various non- cell autonomous signals that are synthesized on one site but function on another site (Lin et. al., 2014).

Signalling pathways

There are two types of signalling pathways: LOCAL and SYSTEMIC regulatory signalling pathways regulating plant responses (Turnbull and Lopez-Cobollo, 2013).

Local regulatory signals: these are produced in the cell and function in response to local stimuli (within shoot or root tissue), remain in the site of production or move to neighbouring cells through plasmodesmata (symplasm) or the intercellular spaces (apoplasm) (Turnbull and Lopez-Cobollo, 2013).

Systemic regulatory signals: these are long distance regulatory signals that are loaded into vascular bundles and then transported to distant target cells through symplasm or apoplasm to execute their functions in these cells (Turnbull and Lopez-Cobollo, 2013).

Integration of local and systemic signals brings about responses to internal and external stimuli like absence or presence of Pi in cell, soil or nutrient medium (Turnbull and Lopez-Cobollo, 2013).

Pi uptake and allocation in plants

Phosphorus is the second most limiting macronutrient for growth of plants after nitrogen (Schachtman et. al., 1998) making 0.2% of a plant's dry weight (Theodorou and Plaxton, 1993). According to Holford (1997), 80% of

phosphorus in soil is either immobile or unavailable for the uptake by plants, due to adsorption on soil particles, precipitation with other nutrients (like Iron at acidic pH) or because of conversion to organic forms which make up to 20% to 80% of phosphorus pool in soil (Richardson, 1994). The movement of phosphorus in soil occurs mainly by diffusion (Schachtman et. al., 1998) which in turn, along with other factors makes its least mobile in soil (Poirier et. al., 1991).

Pi, orthophosphate (Inorganic phosphate: HPO_4^{2-} and $H_2PO_4^-$) is the main source of phosphorus taken by the roots from soil (Marschner, 1995) through plasma membrane of epidermal and cortical cells and then transported to different cell organelles and cell membranes using different phosphate transporters (Poirier and Bucher, 2002).

Pi acquisition (uptake) and distribution within the plants (allocation) occurs in steps which include: uptake of Pi from soil by roots involving root/soil interface, Xylem loading of Pi in root, transport of Pi with xylem sap to shoot involving release from the xylem to aerial parts and redistribution of Pi from shoot to sink tissues (growing tissues, roots) involving phloem loading and unloading (Poirier and Bucher, 2002).

It is reported that Pi concentration in soil solution is approximately 10 µM (Bieleski, 1973). Uptake of Pi is not easy due to its low concentration in soil (in micromoles) and high requirement in plants (Schachtman et. al., 1998) of the order of 5-20 mM in plant tissues (Raghothama, 1999). As an adaptation to this problem, plants have specialized transporters on the root surface (especially root tip) for effective extraction of Pi from the soil solution (Schachtman et. al.,

1998). Two different Pi uptake systems exist, high affinity transporters (PHT, Phosphate Transporter family) with inducible activity depending on Pi availability and low affinity transporters with constitutive activity (Ullrich-Eberius et. al., 1984; Furihata et. al., 1992) for example, several genes of the PHT (Phosphate Transporter) family, *PHT1*, *PHT2*, *PHT3* and *PHT4*, encode proteins that are involved in uptake of Pi across the plasma membrane, chloroplast, mitochondria and golgi apparatus, respectively in *Arabidopsis* (Cubeco et. al., 2009; Guo et. al., 2008; Mudge et. al., 2002; Poirier and Bucher, 2002; Versaw and Harrison, 2002). Latest reports reveal that Pi is taken up by roots with the help of 9 high affinity-transporters (involves cotransport of H^+ with Pi, H^+ ATPase) in *Arabidopsis thaliana* and 13 high affinity-transporters in *Oryza sativa* (Nussaume et. al., 2011). Plants have also developed mechanisms of Pi transport in cells where its concentration is 1000 fold higher (in millimoles) than in the external solution (in micromoles) (Schachtman et. al., 1998). Pi can exist in 2 forms ($H_2PO_4^-$, divalent cation or HPO_4^{2-}, monovalent cation in equilibrium with H_3PO_4, phosphoric acid) depending on pH of soil solution, below pH 6.0, most of the Pi occurs as divalent cation (Schachtman et. al., 1998). Pi uptake is also pH dependent as rates of uptake are highest between pH 5.0 and 6.0, where $H_2PO_4^-$ dominates (Ullrich-Eberius et. al., 1984; Furihata et. al., 1992). The transport of Pi from soil solution to root requires energy because of difference in concentration of Pi in external and internal solutions and also because of existence of negative plasma membrane potential, which is a characteristic feature of plant cells (Schachtman et. al., 1998).

The allocation of Pi to shoot (transfer from root to shoot) is based on xylem loading in root through PHO (Phosphate) transporters in *Arabidopsis* and is based on two members of the PHO1 (Phosphate) family: *PHO1* and *PHO1, H1* (Hamburger et. al., 2002; Stefanovic et. al., 2007).

Pi homeostasis, maintenance of stable Pi concentration in cells, is based on strict regulation of Pi concentrations and uptake in plants (Poirier and Bucher, 2002). It involves complex connections between regulatory molecules of Pi homeostasis and other nutritional elements and regulatory molecules (Rouached et. al., 2010).

According to Poirier and Bucher (2002), **understanding of molecular and biochemical mechanisms involved in Pi uptake at root/ soil interface and its subsequent allocation to plant tissues and organelles** is needed for revealing molecular basis of Pi homeostasis in plants.

Phosphate uptake and metabolism under Pi stress

Phosphate stress/ limitation

There is limited availability of Pi in the soil due to precipitation with oxides and hydroxides of aluminium and iron in acidic pH and precipitation with calcium at alkaline pH and also by conversion to organic forms (Raghothama, 1999) that results in phosphate limitation or stress, low availability of Pi in the environment. Pi stress is managed by methods of enhanced acquisition of external Pi acquisition (UPTAKE) and conservation and remobilization of internal Pi (ALLOCATION in shoots and roots) (Lin et al., 2013). The responses in shoot and root tissues are regulated by local or systemic signals (Lin et. al., 2013). Genes involved in regulation of root architecture are mostly

Pi acquisition or uptake genes related to external Pi and are regulated locally whereas genes involved in conservation and remobilization of internal Pi are regulated systemically (Thibaud et. al., 2010).

Enhanced Pi acquisition can involve some of these processes:

a. Induction of high affinity Pi transport systems.

b. Secretion of root exudates for releasing Pi from unavailable soil Phosphorus pool.

c. Modification of root morphology and architecture: reduction in primary root length, increased density of branch roots and root hairs and increase in mass of the root. It may involve mycorrhizal associations.

d. Anthocyanin accumulation in aerial tissues.

(Lin et. al., 2009)

The key regulators of Pi homeostasis in plants that alter cellular Pi concentrations play a crucial role in managing Pi stress (Lin et. al., 2009). Some of the key regulators involved in Pi Stress are:

1. *IPS1* (Induction by Pi starvation 1): transgenic with *IPS1* promoter expression in barley was detected under Pi sufficient conditions but it is highly induced by low Pi in *Arabidopsis* (Martín et. al., 2000).

2. *PHR1* (Phosphate starvation response 1): it was identified under low Pi conditions (Rubio et. al., 2001). It regulates many Pi starvation-induced genes, is also a key transcriptional activator in controlling Pi uptake and allocation, and has a role in anthocyanin accumulation and carbon metabolism in *Arabidopsis* (Lin et. al., 2009). *OsPHR1* and *OsPHR2* are two homologues of *PHR1* in rice,

regulating several Pi-starvation induced genes and *Psr1 (*Phosphate stress response 1) is a *PHR1* homologue in green algae (*Chlamydomonas reinhadtii*) (Lin et. al., 2001).

PHR1 protein is sumoylated by SIZ1, a SUMOE3 ligase and SIZ1 can act as either positive or negative regulator of Pi stress (Miura et. al., 2007). The expression of *SIZ1* gene is modulated positively or negatively by sumoylation, either directly or indirectly through *PHR1* or other regulators (Lin et. al., 2009). *PHF1* (phosphate traffic facilitator1) encodes a specific protein that functions as an ER (endoplasmic reticulum) located facilitator for movement of PHT1 family proteins in *Arabidopsis*, its expression is upregulated by Pi deficiency (Lin et. al., 2009)

3. *PHO1*: it is involved in transport of Pi (Hamburger et. al., 2002). It is upregulated by Pi deficiency but is not regulated by *PHR1* (Stefanovic et. al., 2007).

4. *PHO2* (*UBC24* gene, a member of E2 ubiquitin conjugase family): it causes Pi toxicity due to enhanced uptake and translocation from roots to shoot and retention of Pi in the old leaves in *Arabidopsis* (Lin et. al., 2009).

5. MiR399 (Micro RNA399): it is highly upregulated by low concentrations of Pi (Chiou, 2007). It controls Pi homeostasis by regulating the expression of *PHO2* gene in *Arabidopsis* (Lin et. al., 2009). The expression of miR399 is regulated by *PHR1* (Bari et. al., 2006).

6. *OsPTF1* (Pi starvation induced transcription factor): it encodes for transcription factor in rice which is similar to yeast *PHO4* genes. It is upregulated by Pi deficiency in roots but is constitutively expressed in shoot

and its overexpression enhances tolerance to Pi deficiency by increasing total root length and surface area and inducing greater Pi uptake activity (Yi et. al., 2005).

Forward and reverse genetics approaches are quite useful to understand gene functions as in the case of Pi stress. Recent development of the technique of leaf ionomics (Baxter et. al., 2008; Salt et al., 2008) for high throughput profiling of elemental composition as has been done in *Arabidopsis* (Baxter et. al., 2008) and can facilitate study of physiological changes that occur during Pi stress. A **set of marker elements identified through the technique of ionomics** can also be used with other datasets to investigate nutritional and physiological status of plants (Pii et. al., 2015)

The concept of ionome has been in existence for the last ten years and is known to have provided for high throughput profiling of elements in at least 10 different organisms (Baxter, 2015). The basic advantage of ionomics studies over experiments dealing with single elemental analysis, lies in dealing with **interactions of elements** which is crucial to understand cellular responses to genetic or environmental changes (Baxter, 2009). Further study on the interaction of the ionome with a systems integrative biology approach leads to understanding of plant's response to these changes.

IONOME AND IONOMICS

Ionome was first described by Lahner et. al. (2003) for all the metals, metalloids and non-metals present in an organism including nitrogen, phosphorus, sulphur, selenium, chlorine and iodine. Ionome can be thought as the inorganic subsets of the metabolome (quantitative complement of all the low molecular weight

molecules) present in the cells in a particular physiological or developmental state (Hollywood et. al. 2006). Ionomics is the quantitative and simultaneous measurement of the elemental composition of living organisms (Salt et. al., 2008).

The central importance in survival and evolution of sessile, multicellular and terrestrial organisms is uptake of ions from the environment and its movement to the appropriate tissue (Salt, 2004). **Ion transport involving ion transporters and multiple genes or gene families is the key focus of characterization of ionome in a tissue at particular physiological state.** Integration of ion homeostasis (represented by ionome) and gene expression is based on developing high throughput ion-profiling strategy (Salt, 2004). Use of inductively coupled plasma mass spectrometry (ICP-MS) technology for ion profiling has recently been explored to study biomarkers in *Arabidopsis* (Baxter et. al., 2008). Advantages of using ICP-MS for ion profiling are manifold such as:

1. Measurement of multiple elements in the sample

2. High sensitivity

3. Use of smaller sample size, leading to non-destructive sampling.

4. Dynamic range of measurement allowing for simultaneous measurement of macronutrients and micronutrients concentrations in the sample.

(Salt et. al., 2008).

Use of ionomics in assessing plant physiology

Physiological processes that control the ionome of a plant initiate in the rhizosphere and end with evapotranspiration and phloem loading and unloading (TRANSPORT and ALLOCATION of inorganic ions from soil solution to shoot and distribution in the whole plant). (Baxter 2009, 2010)

Alteration in the physiological processes changes the ionome of the plant (Conn and Gilliham, 2010). Thus, changing ionome can reflect changing physiological status of the plant (Conn and Gilliham, 2010). **Different ionomic profiles may reflect different plant physiological states.** These characteristic ionomic profiles can be used as **biomarkers for the particular physiological condition with which they are associated (**like phosphate stress in plants), IONOMIC BIOMARKERS (Salt et. al., 2008)

Little work has been done in plants to establish ionomic biomarkers, which is a potential concept for understanding plant physiology and deciphering genetics involved.

OBJECTIVES

With this background, the research work was undertaken to determine the rates of uptake and allocation of nutrients to tissues during Pi stress using techniques of ionomics. The advantage of working with ionomics lies in its multi element analysis and possibility of studying interactions of nutrients which can give snapshot of underlying physiological regulation of Pi stress. The analysis of nutritional status using single element analysis is time consuming, expensive and less informative. The study has been conducted with the following objectives:

1. Study the impact of Pi stress (absence, low: 10 µM Pi and high: 320 µM Pi concentrations of Pi in the hydroponic solutions) in plant using ionomic approaches.

2. Generation of characteristic ionomic profile for Pi stress in plant.

3. Identification of groups of macronutrients or micronutrients that show similar patterns of uptake and allocation with different Pi treatments.

4. Finding a relationship between phenotypic modifications and ionomic profile under Pi stress to suggest marker elements for this physiological state in the plant.

MATERIAL and METHODS

PLANT MATERIAL

A modern cultivated variety of rice, *Oryza sativa* ssp. *japonica* cultivar Nipponbare was used for research. Rice plants were grown in hydroponic solutions. Seeds were provided by Zhejiang University, China and after careful screening provided for experimentation by Prof Chungui Lu.

Basic procedure for plant growth

Seeds underwent a process of sterilization with sodium hypochlorite before germination under controlled conditions. The seeds were thoroughly washed with 100 mL of Mili-Q distilled water to remove any adhering surface dust particles or contaminants and then sterilized by exposure to 50 mL of 70% ethanol for 5s. Then the seeds were soaked in 50 mL of 2% sodium hypochlorite solution for 30 minutes. After, this treatment, the seeds were rinsed with Milli-Q distilled water thrice and left overnight in it. These seeds were then germinated on whatman filter paper lined petriplates at a temperature of 28˚C ± 2 for 5 days. The moisture content of the petriplates was routinely checked and replenished for successful germination. The seeds showed emergence of primary root and shoot after two days of germination in dark (Figure 1a) and then were transferred to light conditions (12 h photoperiod at 25˚C ± 5) for further growth. The transfer of seedlings to light conditions led to development of green coloured primary shoots with leaves and elongated primary adventitious roots with a few laterals (Figure 1a). At this stage, seedlings were transferred to hydroponic growing system composed of nutrients of Yoshida solution (Table 1) with pH maintained at 5.6 to 5.8 with 1 mM HCL (Yoshida

et. al., 1976) in hydroponic boxes, each accommodating 17 seedlings, for a period of 15 days (Figure 1c) in a controlled regime of 12 h photoperiod with day temperature of 28˚C and night temperature of 23 ˚C.

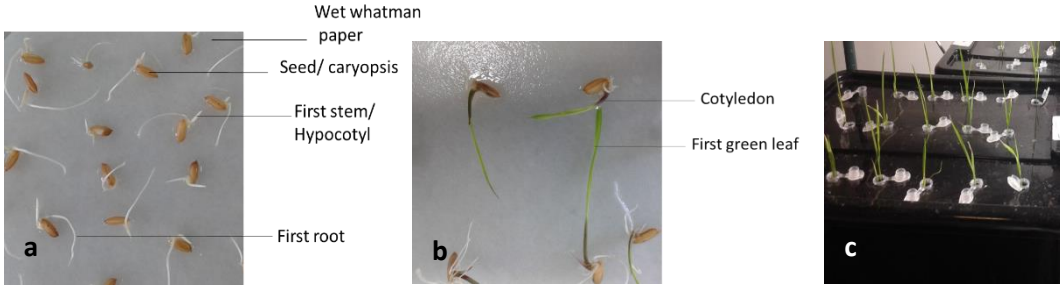


Figure 1 a: Germination of seeds on wet whatman filter paper, b: Greening of seedlings on wet whatman filter paper and c: Seedling growth in hydroponic system.

The hydroponic box/tub contained 9L of Yoshida's solution providing 0.5 L of solution per seedling. The solutions were changed every 3rd day and pH was maintained between 5.6 to 5.8 and constant growth regime of 28 ˚C/23 ˚C, 12 h photoperiod, was provided in the environmental controlled growth chamber. The position of the hydroponic boxes was changed randomly on 3rd day every time so as to avoid any positional variations and in this way completely randomized design (CRD) was followed for the work.

After 15 days of growth on full balanced hydroponic solutions (with 320 µM Pi), plants were subjected to Pi stress.

Table 1: Yoshida solution used in hydroponic system.

Type of nutrient	S.no.	Chemical (Formula)	Chemical (full form)	Molecular weight (g)	Nutrient	Concentration
Macronutrients	1	NH_4NO_3	Ammonium nitrate	80.04	N	1.42 mM
	2	$MgSO_4.7H_2O$	Magnesium sulfate	246.48	Mg, S	1.64 mM
	3	K_2SO_4	Potassium sulfate	174.25	K, S	0.51 mM
	4	$CaCl_2.2H_2O$	Calcium chloride	147.02	Ca	0.99 mM
	5	$NaH_2PO_4.2H_2O$	Sodium dihydrogen phosphate	156.01	P	0.32 mM
Micronutrients	6	FeEDTA.Na	Ethylene diamine tetra-acetic acid ferric sodium salt	367.05	Fe	0.12 mM
	7	$MnCl_2.4H_2O$	Manganese chloride	197.91	Mn	9 µM
	8	$(NH_4)_6Mo_7O_{24}.4H_2O$	Ammonium molybdate	1235.86	Mo	0.07 µM
	9	H_3BO_3	Boric acid	61.83	B	19 µM
	10	$CuSO_4.5H_2O$	Copper sulfate	249.68	Cu	0.15 µM
	11	$ZnSO_4.7H_2O$	Zinc sulfate	287.53	Zn	0.15 µM
Additional nutrient	12	$Na_2SiO_3.9H_2O$	Sodium silicate	284.20	Si	0.25 µM

Pi stress treatments

For providing Pi stress, three types of Pi treatments were applied to the experiment (Table 2)

Table 2: Modifications of Yoshida solution.

Type of solution	Concentration of Pi (µM)
Absence of Pi	0
Presence of Pi (low)	10
Presence of Pi (high)	320

The plants were shifted to three different Pi treatment conditions, Yoshida solution with absence of Pi (P deficient) made by removal of source of P, sodium dihydrogen phosphate from the solution and replacing it with NaCl (sodium chloride) after 15 days of growth on 320 µM Pi . The other two concentrations of Pi were, hydroponic solutions with low concentration (10 µM) and high (320 µM, Pi sufficient, normal Yoshida solution). The levels of Pi were chosen based on the existing reports of Bieleski (1973) and Schachtman (1998) that the concentration of Pi rarely exceeds 10 µM in soil solutions probably being optimum for plant growth and 320 µM Pi was a component of balanced Yoshida solution used for growth of plants in hydroponic system in this study. In all these treatments pH was maintained at 5.6 to 5.8 using 1 mM HCl. In this way plants were kept for 21 days in controlled growth conditions of 28 ˚C/23 ˚C, 12 h photoperiod, changing the respective treatment solutions on every 3rd day and following completely randomized design (CRD) of experimentation.

SAMPLING

On 15th day (0 day of treatment), 9 plants with 3 replicates for each treatment were measured for their morphological characteristics; length of shoot and root, fresh weight of shoot and root. Plants were removed from the hydroponic boxes, placed in an oven (kept at 60 ˚C for 48 h) for dry weight analysis and further processing for ionomics experiments.

For each treatment, 25 plants were transferred to individual hydroponic bottles (2 L) maintained in the same controlled regime of temperature, light intensity and photoperiod as mentioned above.

From day 0 to 21 days, 3 plants each were collected after every 3rd day from each of the treatment and after measurement of morphological characters, were separated into shoot and root tissues. The tissues were subjected to drying in an oven at 60˚C for 48 h for dry weight measurement. These dried shoot and root samples were placed in individual paper bags for ionomic analysis.

Similarly, 50 mL of hydroponic solutions from individual bottles from which the plants were collected for analysis, were also removed after every 3rd day in a period of 3 to 21 days for nutrient analysis.

IONOMIC TECHNIQUES

The analysis of the elements can be carried out by two major groups of techniques:

1. Techniques based on electronic properties of an atom: Emission, Absorption or fluorescence spectroscopy (Salt et. al., 2008)

2. Techniques based on nuclear properties of an atom: radioactivity or atomic number (Salt et. al., 2008)

Inductively Coupled Plasma- Mass Spectrometry (ICP-MS):

This technique is widely used for elemental analysis and is based on the study of electronic properties of an atom. Inductively coupled plasma is a gas of ionized atoms which are detected either by optical atomic emission spectroscopy (ICP-AES) or mass spectrometry (ICP-MS) (Salt et. al., 2008). ICP-MS is based on measurement of mass/charge (m/z) ratios.

Total organic carbon analyser (TOC)/ Total nitrogen analyser (TN):

Though the technique is called TOC analysis, it actually quantifies total carbon (TC) by sum of the organic carbon (OC) and inorganic carbon(IC) present in a sample. The samples are acidified to convert IC into carbon dioxide (CO_2) which is then quantified. OC is oxidized into CO_2 by high temperature or UV irradiation. A non-dispersive (NDIR) detector correctly calibrated to samples can then quantify the TC present in the samples (Bernard, 2013).

720 °C catalytic thermal decomposition chemiluminescence methods are adopted for Total nitrogen (TN) measurement. This method is used widely, since total nitrogen in water can be measured only in several minutes (3 to 5 minutes). First, nitrogen (N) compound is oxidized at high temperature (600 to 900 °C) by the catalytic thermal decomposition method to generate nitrogen monoxide. Next, this nitrogen monoxide (NO) is reacted with Ozone (O*). Nitrogen dioxide (NO_2*) excited in metastable state having arisen then generates chemiluminescence, when it becomes stable nitrogen dioxide (NO_2). Intensity of this chemiluminescence is proportional to the nitrogen concentration. So, the chemiluminescence is detected to measure the nitrogen concentration in water (McGeehan and Naylor, 1988).

ICP-MS analysis

The analysis of macronutrients and micronutrients was carried out in hydroponic solutions and dried tissue samples of shoot and root using the technique of ICP-MS. Hydroponic solutions were used to study the rate of uptake of nutrients and dried root and shoot tissues from plants grown in these

hydroponic solutions were used for study of rate of allocation of nutrients in these plants over a span of 21 days.

Hydroponic solutions

Hydroponic bottles from which the water samples (50 mL) were removed, weighed prior to and after removal of water for water uptake analysis. 9.6 mL of the collected hydroponic solution was mixed with 0.4 mL of 50% HNO_3 (nitric acid) for acid digestion and stored at room temperature. 1 mL of these digested samples were mixed with 9 mL of MiliQ water in ICP tubes, covered and then directly analysed for minerals in ICP-MS instrument (Thermo Scientific, iCAPQ) in AES division of School of Biosciences.

Tissue samples

The roots and shoots separated in the collected dried plant samples grown in hydroponic solutions with different Pi, were finely pulverized using mortar and pestle. 0.2 g of each tissue was subjected to hot plate digestion. The tissue was transferred to digitubes, mixed with 8 mL of HNO_3 and covered with a glass lid. These samples were left in the heating blocks overnight in the fume hood, then to each of the digitube, 2 mL of H_2O_2 was added and after covering with glass lids, these digitubes were heated for 2h at 95˚C in the fume cupboard. After heating, these digitubes were left for cooling for 10 minutes and then mixed with 50 mL of MiliQ distilled water. The samples were then filtered using syringe filters into 7 mL vials. From the filtered samples, 1 mL of the sample was mixed with 9 mL of MiliQ water (dilution factor=10) in the ICP tubes which were covered and analysed using the ICP-MS machine (Thermo Scientific iCAPQ) for nutrients. Same procedure was adopted for two blank

solutions, composed of HNO_3 and H_2O_2, respectively and a control composed of pulverized tomato plant tissues.

TOC/TN analysis

The hydroponic solutions were also analysed for their C and N content using Total Organic Carbon/ Total Nitrogen analyser (Shimadzu TOC-VCPH total organic carbon analyser; TNM-1 total nitrogen measuring unit) in the AES division of School of Biosciences. 2 mL of hydroponic solution samples was mixed with 18 mL of MiliQ distilled water (Dilution factor=20) and analysed in the machine. The comparison of nutrients in the samples was done with the standard solutions prepared (Table 3).

Table 3: Standard solutions for quantification of Total carbon (TC), Inorganic carbon (IC) and Total nitrogen (TN) in hydroponic solution samples.

S.no	Chemical	Concentration (mg/L)
	For Total Carbon (TC)	
1	Blank	0
2	KHP (Potassium hydrogen phthalate)	10
3	KHP	20
4	KHP	30
5	KHP	40
6	KHP	50
	For Inorganic Carbon (IC)	
1	Blank	0
2	Na_2CO_3/$NaHCO_3$ (Sodium carbonate/ sodium bicarbonate)	10
3	Na_2CO_3/$NaHCO_3$	20
4	Na_2CO_3/$NaHCO_3$	30
5	Na_2CO_3/$NaHCO_3$	40
6	Na_2CO_3/$NaHCO_3$	50
	For Total Nitrogen (TN)	
1	Blank	0
2	KNO_3 (Potassium nitrate)	2
3	KNO_3	4
4	KNO_3	6
5	KNO_3	8
6	KNO_3	10

CALCULATIONS FOR NUTRIENT UPTAKE AND ALLOCATION

After the ICP-MS, TOC and TN analysis, ion profiles of the nutrients were obtained for hydroponic solutions and tissue samples. The concentration of nutrients was calculated in mg/L for hydroponic solutions and mg/kg for shoot and root tissue samples.

The concentration of nutrients in hydroponic solution samples was adjusted with the amount of water loss to account for differences in concentration of nutrients in the hydroponic solutions. This was done by measuring the weights of empty hydroponic bottles (E), hydroponic bottles filled with solutions of different Pi treatments (absence, 10 µM and 320 µM) initially (F1) and hydroponic bottles with the respective treatment solutions after 3 days of plant growth (F2) for a period of 21 days.

W1: Weight of water in hydroponic bottles on 0 day = F1-E

W2: Weight of water in hydroponic bottles on 3rd day of plant growth = F2-E.

W: Weight of water loss from the hydroponic bottles in three days = W1 –W2.

This formula for calculation of water loss was used for similar calculations for water loss in 21 days of plant growth.

The amount of nutrient measured in mg/L was multiplied with W for obtaining total nutrient present in hydroponic bottles after every 3rd day for a period of 21 days as:

A0: Amount of nutrient present on day of initiation of Pi stress to plants (0 day) = Amount of nutrient measured by ICP-MS (mg/L) on 0 Day **X** W

A3: Amount of nutrient present on 3rd day of Pi stress to plant (3rd day)

= Amount of nutrient measured by ICP-MS (mg/L) on 3rd day **X** W

In this way, the values of nutrient content present in hydroponic solutions were calculated for 21 days.

The nutrient content values so obtained from 3rd to 21st day were subtracted from the starting nutrient content values to obtain values for nutrient loss after every 3rd day over a period of 21 days during plant growth.

Amount on nutrient uptake on 3rd day: A0-A3.

The value of nutrient loss represented nutrient uptake by plants as the nutrient lost from the hydroponic solution was assumed to be equivalent to nutrient uptake by plants through roots. The loss due to evaporation of solutions from the hydroponic bottles was taken care of by covering the bottles and their mouths completely with layers of aluminium foils

The values for nutrient uptake by plants (in mg) were plotted in the graphs for 21 days representing **rate of uptake of nutrients** during plant growth in 21 days.

Similarly, the concentration of nutrients in tissue samples was adjusted with the dry weight of the tissue processed to account for differences in concentration of nutrients in the tissues. This data was used for calculating rate of allocation of nutrients in the shoot and root tissues.

The amount of nutrient measured in mg/kg was multiplied with dry weight of the shoot or root tissues for obtaining total nutrient present in shoot or root tissues after every 3rd day for a period of 21 days as:

A0: Amount of nutrient present on day of initiation of Pi stress to plants (0 day) = Amount of nutrient measured by ICP-MS (mg/L) on 0 Day **X** dry weight of tissue on day of initiation of Pi stress

A3: Amount of nutrient present on 3rd day of Pi stress to plant (3rd day) = Amount of nutrient measured by ICP-MS (mg/L) on 3rd day **X** dry weight of tissue on 3rd day of Pi stress

In this way, the values of nutrient content present in both shoot and root tissues were calculated for 21 days.

The nutrient content values so obtained from 3rd to 21st day were subtracted from the starting nutrient content values to obtain values for nutrient allocation after every 3rd day over a period of 21 days during plant growth.

Amount of nutrient allocated to shoot or root tissue on 3rd day: A0-A3.

The values for nutrient allocation (in mg) for shoot and root tissues were plotted in the graphs for 21 days representing **rate of allocation of nutrients to shoot or root tissues** during plant growth in 21 days.

STATISTICAL ANALYSIS

Statistical analyses were performed on data for morphological characters (length (measured as height in centimetres) of shoot and root, fresh weight of shoot and root (g) and dry weight of shoot and root (g), rate of uptake and allocation of macronutrients and micronutrients, pH and water absorption to assess their significance. ANCOVA (Analysis of Covariance) was performed for all the experimental data using three factors: Days (continuous variable), Concentration of Pi (discrete variable) and interaction between the two main

factors. The analyses were performed using Genstat software (17th edition, VSN International, 2013).

RESULTS

The objective of this research was to find out significant differences in **morphological characters**, **rates** (3 to 21 days of plant growth) **of uptake, rates of allocation of nutrients** i**n shoot and root** and **pH** with different Pi treatments. Macronutrients studied were Mg, P, K, Ca, P and S. Micronutrients studied were Fe, Mn, Mo, B, Cu and Zn. Nitrogen: N and Carbon: C were also analysed for their rates of uptake during Pi stress.

MORPHOLOGICAL CHANGES DURING PLANT GROWTH

Length and fresh weight under Pi treatments

Shoot length was affected significantly ($F_{(2,57)}$=43.08, p <0.01) by the Pi treatments (Table 4). Shoots were stunted in the absence of Pi but root length was not significantly affected by the absence or presence of Pi in the hydroponic solutions (Figures 2 and 3). Shoot length was more with 10 µM as well as 320 µM phosphorus (Pi) than in its absence (Figure 4a) in the hydroponic solutions after 21 days of plant growth. Similarly fresh weight of shoot ($F_{(2,57)}$=24.83, p <0.01, Table 4) as well as root ($F_{(2,57)}$= 3.83, p=0.028, Table 4) increased significantly from 3 to 21 days in the presence of phosphorus in the hydroponic solution. The fresh weight of shoot and root with10 µM Pi treatment showed intermediate level compared to other Pi treatments (absence and 320 µM) (Figure 5a, b). Root length and ratio of dry weight of shoot to root were not significantly affected by Pi treatments in 3 to 21 days (Table 4, Figure 4b; Figure 6).

Table 4: Significance of morphological changes calculated by using Genstat software. Coloured boxes are significant values. Significance level taken was $p<0.05$ (5%).

S. No.	Parameter	Days	Concentration	Days * Concentration
1	Shoot length	<0.001	<0.001	<0.001
2	Root length	<0.001	0.428	0.791
3	Shoot fresh weight	<0.001	<0.001	<0.001
4	Root fresh weight	<0.001	0.290	0.028
5	Ratio of dry weight of shoot to root	0.165	0.071	0.799

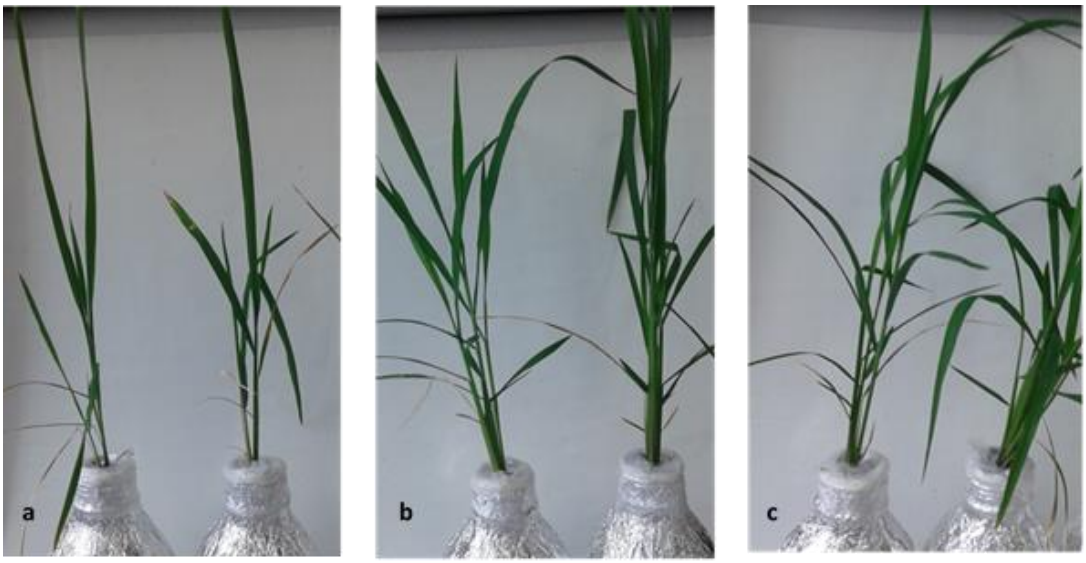


Figure 2: Shoot growth in absence and presence of Pi: a. Absence of Pi; b. 10 µM Pi and c. 320 µM Pi.



Figure 3: Root growth in absence and presence of Pi: a. Absence of Pi; b. 10 µM Pi and c. 320 µM Pi.

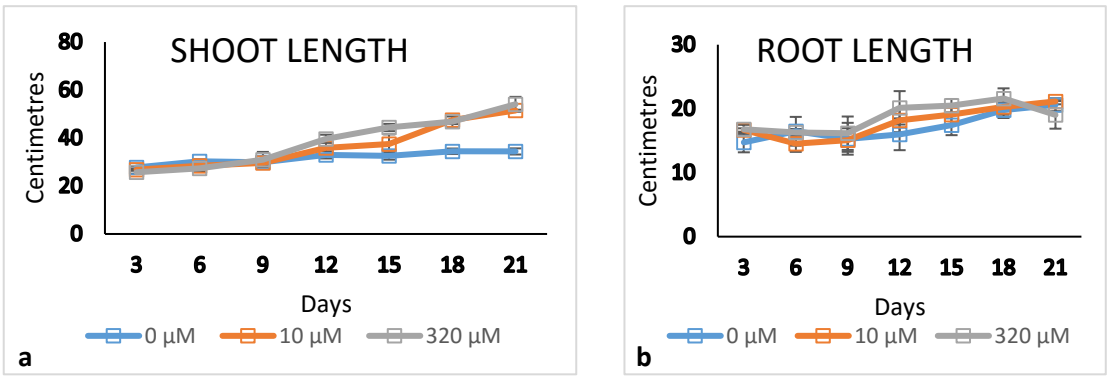


Figure 4 a, b: Shoot length and Root length. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

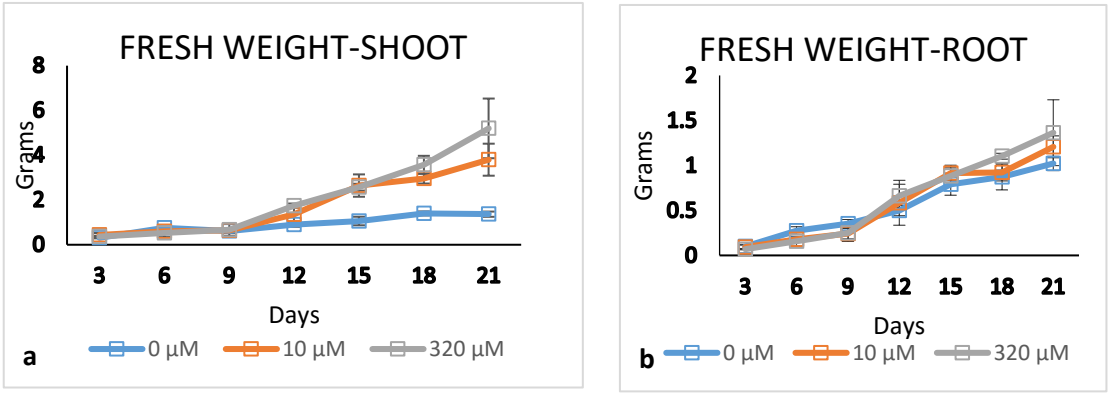


Figure 5 a, b: Fresh weight of Shoot and Root. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

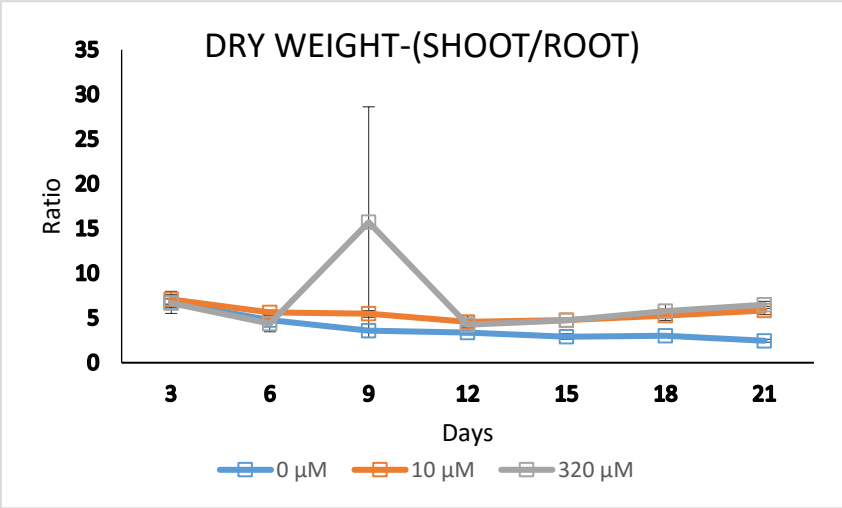


Figure 6: Ratio of Dry Weight of Shoot and Root. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

NUTRIENT UPTAKE

N, K, P, Mn, B, Zn and water uptake by plants under Pi conditions

The effect of Pi treatments on uptake of macronutrients, micronutrients and water from hydroponic solutions is depicted in Figures 7, 8 and 9. The significant effect of Pi on these nutrients is presented in Table 5.

The rates of loss of nutrients from the hydroponic solutions were assumed to be equivalent to rates of uptake of these nutrients by plants. The negative uptake of other macronutrients (Figure 7) is presumably because of existence of these nutrients in ample amounts required for growth of the plant, excess probably being released in the hydroponic solutions. These plants were transferred to different Pi treatments (absence, 10 and 320 µM) after their growth on hydroponic solutions supplemented with 320 µM Pi in hydroponic boxes for 15 days after seed germination.

Amongst the macronutrients, K and P, revealed marked differences in their rates of uptake due to presence of phosphorus in the hydroponic solutions from 3 to 21 days (Figure 7, Table 5). The amount of potassium taken up by the plants increased for all the treatments during plant growth but it was substantially high for presence of 320 µM Pi ($F_{(2,57)}$=68.03, p <0.01) (Figure 7b, Table 5).

Table 5: Significance of uptake of nutrients calculated by using Genstat software. Coloured boxes are significant values. Significance level taken was p<0.05 (5%).

S. No.	Nutrient	Days	Concentration	Days * Concentration
1	Magnesium	<0.001	0.577	0.282
2	Potassium	<0.001	<0.001	<0.001
3	Calcium	<0.001	0.137	0.553
4	Phosphorus	<0.001	<0.001	<0.001
5	Sulphur	<0.001	0.476	0.232
6	Total Nitrogen	<0.001	<0.001	<0.001
7	Inorganic Carbon	0.039	0.908	0.950
8	Total Organic Carbon	0.001	0.409	0.263
9	Total Carbon	0.017	0.310	0.314
10	Iron	<0.001	0.071	0.283
11	Manganese	<0.001	0.043	<0.001
12	Molybdenum	<0.001	0.796	0.338
13	Boron	<0.001	0.929	<0.001
14	Copper	<0.001	0.108	0.141
15	Zinc	0.081	<0.001	0.003
16	Water	<0.001	<0.001	<0.001

The rate of uptake of Pi from the hydroponic solution was also positively affected by presence of Pi in the solution ($F_{(2,57)}$=97.16, p <0.01) (Table 5). In the absence of Pi there was hardly any uptake from 3 to 21 days as expected (Figure 7d). From 12th to 21st days the amount of uptake of Pi was significantly higher for hydroponic solution with 320 µM Pi than 10 µM Pi (Figure 7d).

The rate of water uptake also increased significantly ($F_{(2,57)}$=26.76, p <0.01) in the presence of phosphorus in the hydroponic solution (Figure 7f).

Similarly, the rate of uptake of total N was significantly ($F_{(2,57)}$=23.12, p <0.01) affected by Pi in the hydroponic solution (Table 5). Figure 8 showed that on 6th day, the amount of uptake of total nitrogen was the highest for 10 µM Pi. From 12th to 21st days total N taken up by the plant increased under 320 µM Pi condition (Figure 8a). C uptake was not significantly affected by Pi treatments (Figures 8 b, c, d)

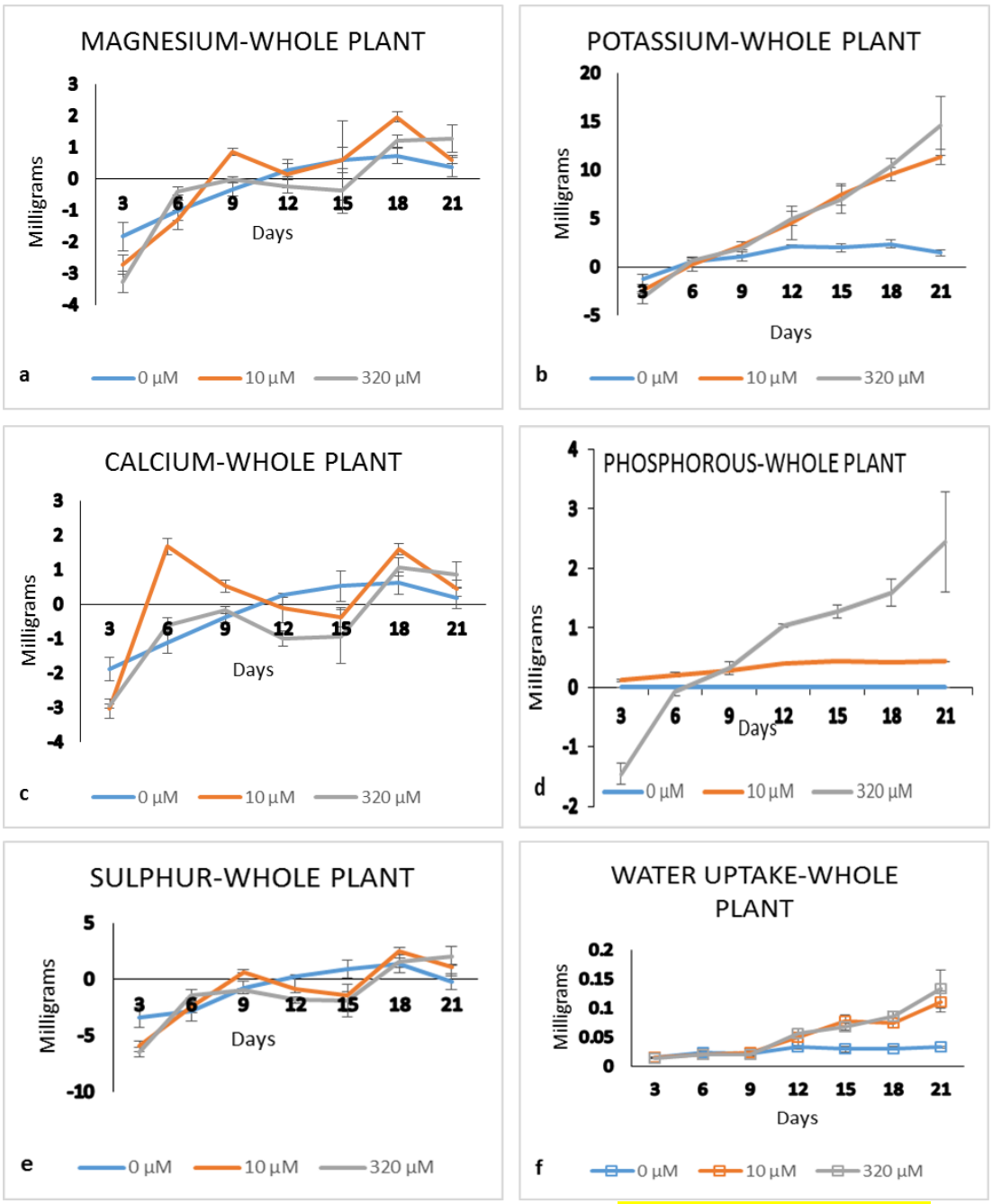


Figure 7 a-f: Uptake of macronutrients and water from hydroponic solutions during plant growth. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

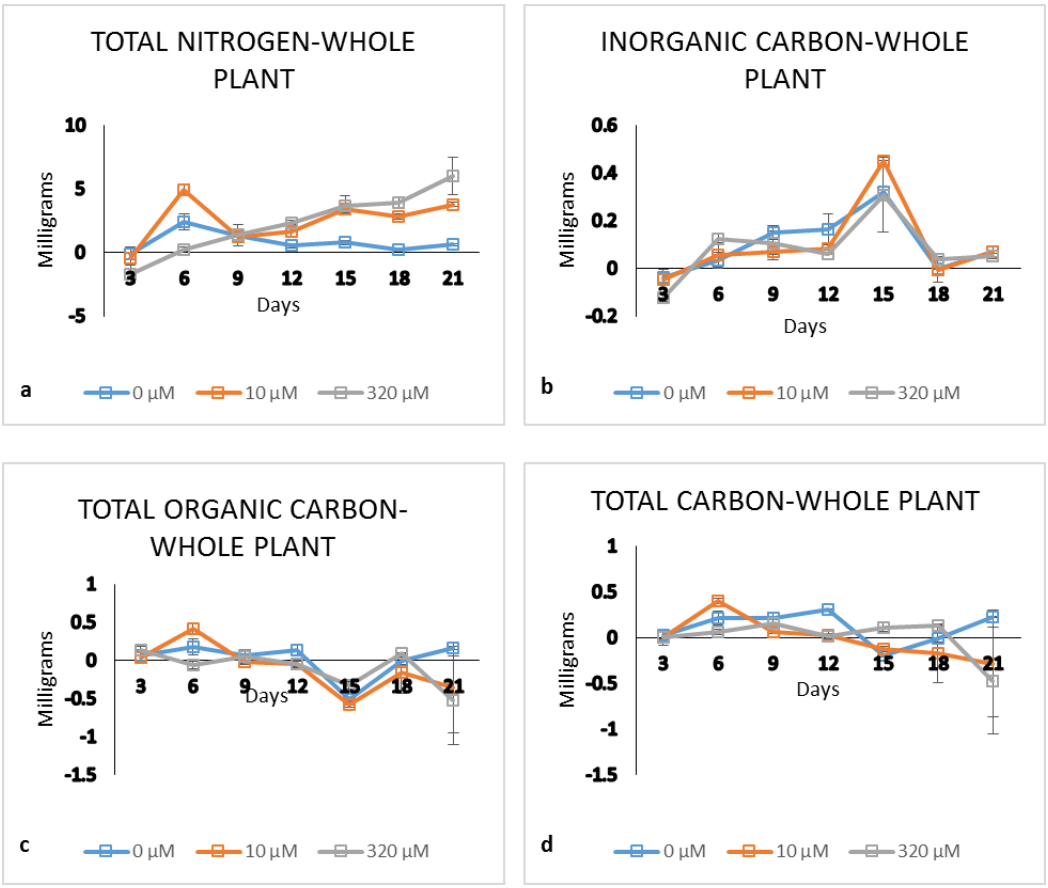


Figure 8 a-d: Uptake of Nitrogen and Carbon from hydroponic solutions during plant growth. The calculations for Carbon were from changes in dissolved inorganic and organic carbon in hydroponic solutions. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

In the case of micronutrients, Mn, B and Zn showed significantly different rates of uptake with different Pi treatments (Table 5). Both Mn ($F_{(2,57)}$=13.56, p <0.01) and B ($F_{(2,57)}$=24.37, p <0.01) demonstrated increased rates of uptake in the presence of phosphorus (Table 5). 10 µM Pi appeared to more effective than 320 µM Pi for uptake of Mn (Figure 9b) though for B, 320 µM Pi substantially promoted its uptake (Figure 9d).

The data for Zn as seen in Figure 9f appeared to be skewed (on 3rd day with 320 µM Pi, there was highest uptake of Zn not seen with other treatments won any

of the days) and hence non reliable, not fulfilling the assumptions for significance of linear data. There was anomalous significant behaviour of Zn for effect of presence of Pi (320 µM Pi) on its rate of uptake from the hydroponic solution.

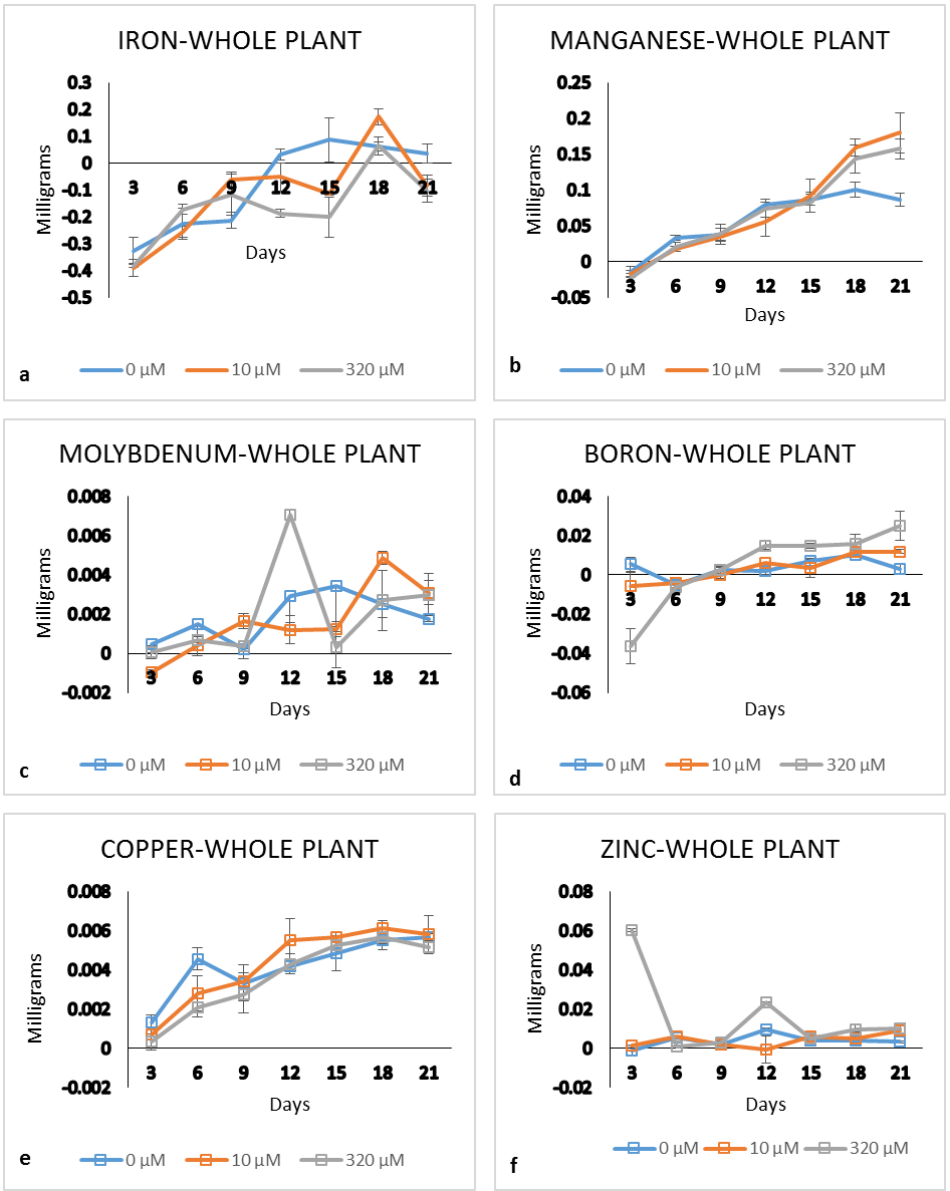


Figure 9 a-f: Uptake of micronutrients from hydroponic solutions during plant growth. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

NUTRIENT ALLOCATION

Nutrient allocation pattern in shoot

Significant rates of allocation of Mg, K, Ca and P under different Pi treatments

All the macronutrients except sulphur demonstrated differences in the rates of their allocation to shoot when plants were grown in presence of Pi in the hydroponic solutions (Table 6).

Table 6: Significance of allocation of nutrients calculated by using Genstat software. Coloured boxes are significant values. Significance level taken was $p<0.05$ (5%). (S: shoot, R: root)

S. No.	Nutrient		Days	Concentration	Days * Concentration
1	Magnesium	S	0.002	0.008	0.024
		R	0.005	0.907	0.904
2	Potassium	S	0.009	0.046	0.090
		R	0.002	0.879	0.792
3	Calcium	S	0.003	0.024	0.034
		R	0.018	0.887	0.932
4	Phosphorus	S	0.052	0.005	0.022
		R	0.030	0.117	0.174
5	Sulphur	S	0.040	0.083	0.257
		R	0.002	0.951	0.901
6	Manganese	S	0.054	0.869	0.600
		R	0.001	0.475	0.246
7	Iron	S	0.025	0.272	0.290
		R	0.266	0.309	0.228
8	Molybdenum	S	0.008	0.102	0.165
		R	0.003	0.030	0.233
9	Boron	S	0.017	0.186	0.054
		R	0.745	0.144	0.246
10	Copper	S	0.014	0.290	0.383
		R	<0.001	0.060	0.020
11	Zinc	S	0.111	0.0302	0.591
		R	0.003	0.924	0.699

The allocation of Mg to shoot in plants grown in hydroponic solution with 320 µM Pi increased conspicuously from 3rd to 21st day and especially from 18th to 21st day (Figure 10a). K level was also similar to Mg with increase in allocation with days (Figure 10b). Ca and P revealed similar patterns of increased rates of allocation to shoot especially when plants were grown in the presence of 320 µM Pi in the hydroponic solution (Figure 10c and d). S had non-significant difference in rate of its allocation to shoot either when plants were grown in absence or presence of phosphorus in the hydroponic solutions (Table 6) as also was seen in Figures 10e.

Uniquely, none of the microelements showed significant differences (Table 6) in their rates of allocation to shoot when plants were grown in the presence of Pi in the hydroponic solutions (Figure 11).

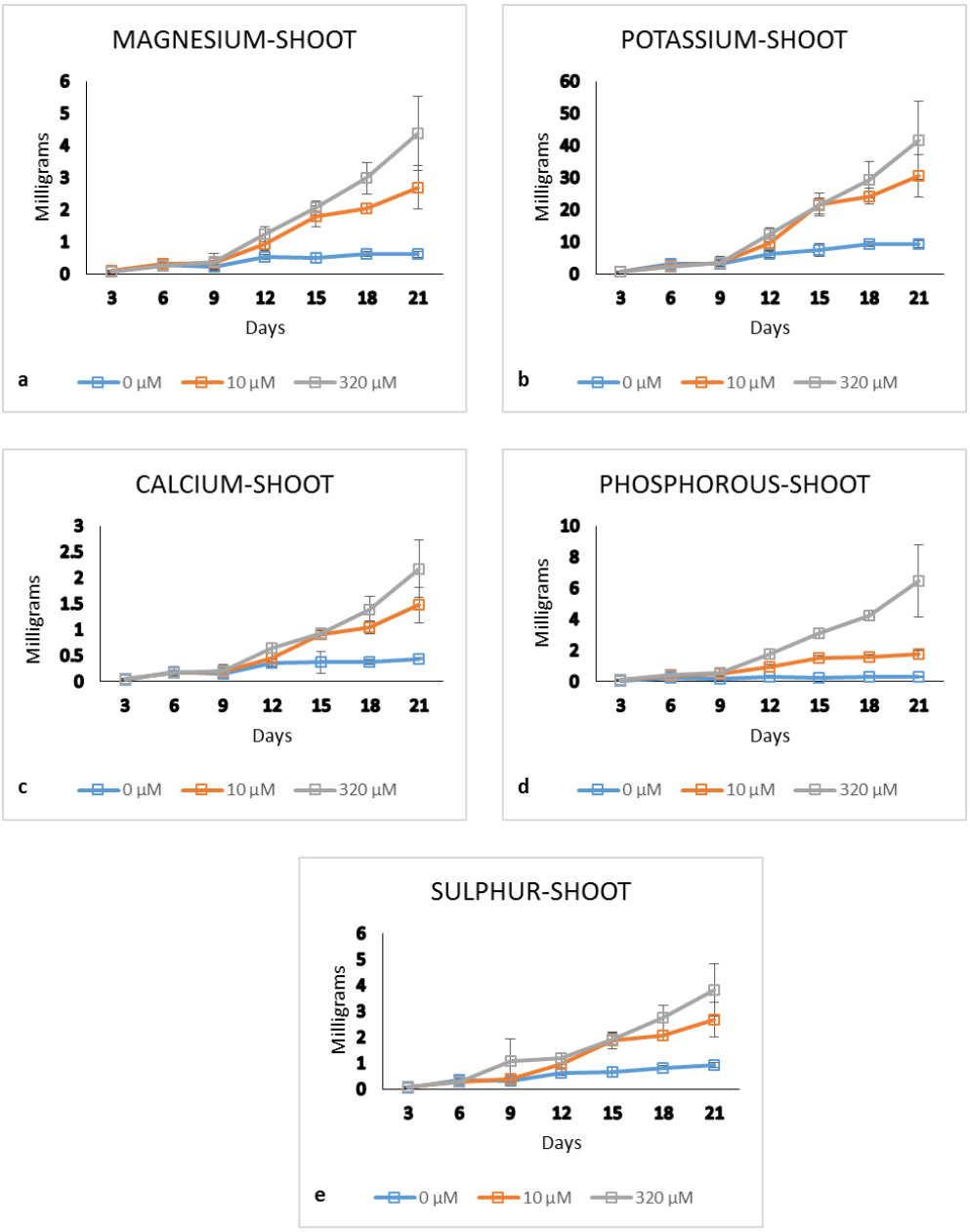


Figure 10 a-e: Allocation of macronutrients to shoots from plants grown in hydroponic solutions with different Pi treatments. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

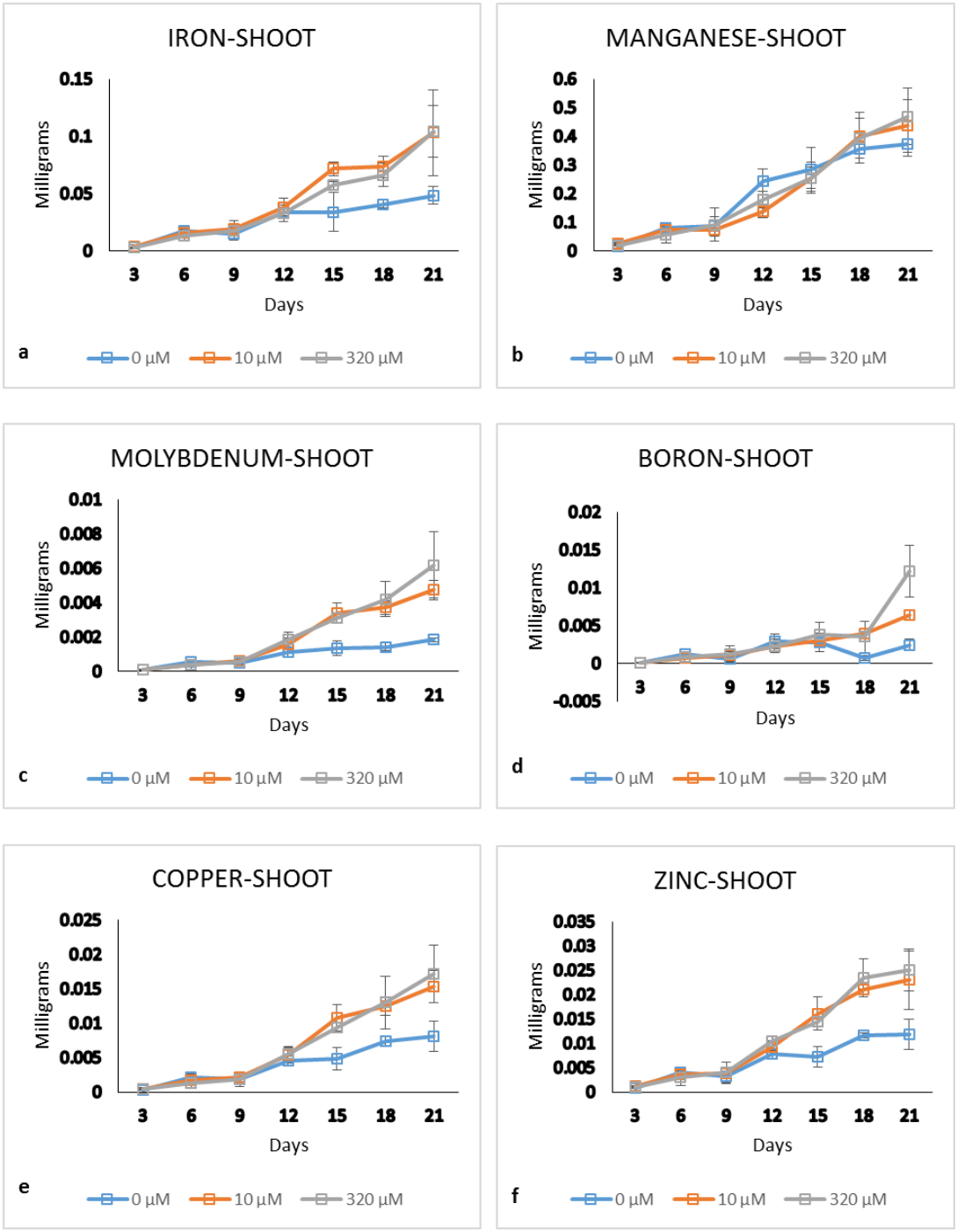


Figure 11 a-f: Allocation of micronutrients to shoots from plants grown in hydroponic solutions with different Pi treatments. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

Nutrient allocation pattern in root

Rates of allocation to root for none of the macronutrients were affected by Pi treatments

In the case of root, allocation pattern for macronutrients was totally opposite to shoot and as seen in table 6, none of the macronutrients showed significant differences in their rate of allocation to roots in plants grown in hydroponic solutions with different Pi treatments. It can be said that presence or absence of phosphorus in the hydroponic solutions was not the deciding factor in plants for allocation of macronutrients to roots (Figure 12).

Rates of allocation to root for Mo and Cu (micronutrients) were negatively affected under Pi treatments:

Out of all the micronutrients studied and statistically analysed, only two demonstrated significant differences in their rates of allocation to root, Mo and Cu (Table 6) in plants grown in hydroponic solutions with different Pi treatments.

Another interesting fact about allocation of Mo and Cu to root was that both these elements were allocated to root in substantially lesser amounts in plants grown in hydroponic solutions with Pi (Figures 13c and e) than in its absence in the hydroponic solution from 3 to 21 days (Figures 13c and e).

For the other micronutrients, there were non-significant differences in their rates of allocation to roots from plants grown either in the presence or absence of Pi in the hydroponic solutions (Table 6, Figure 13).

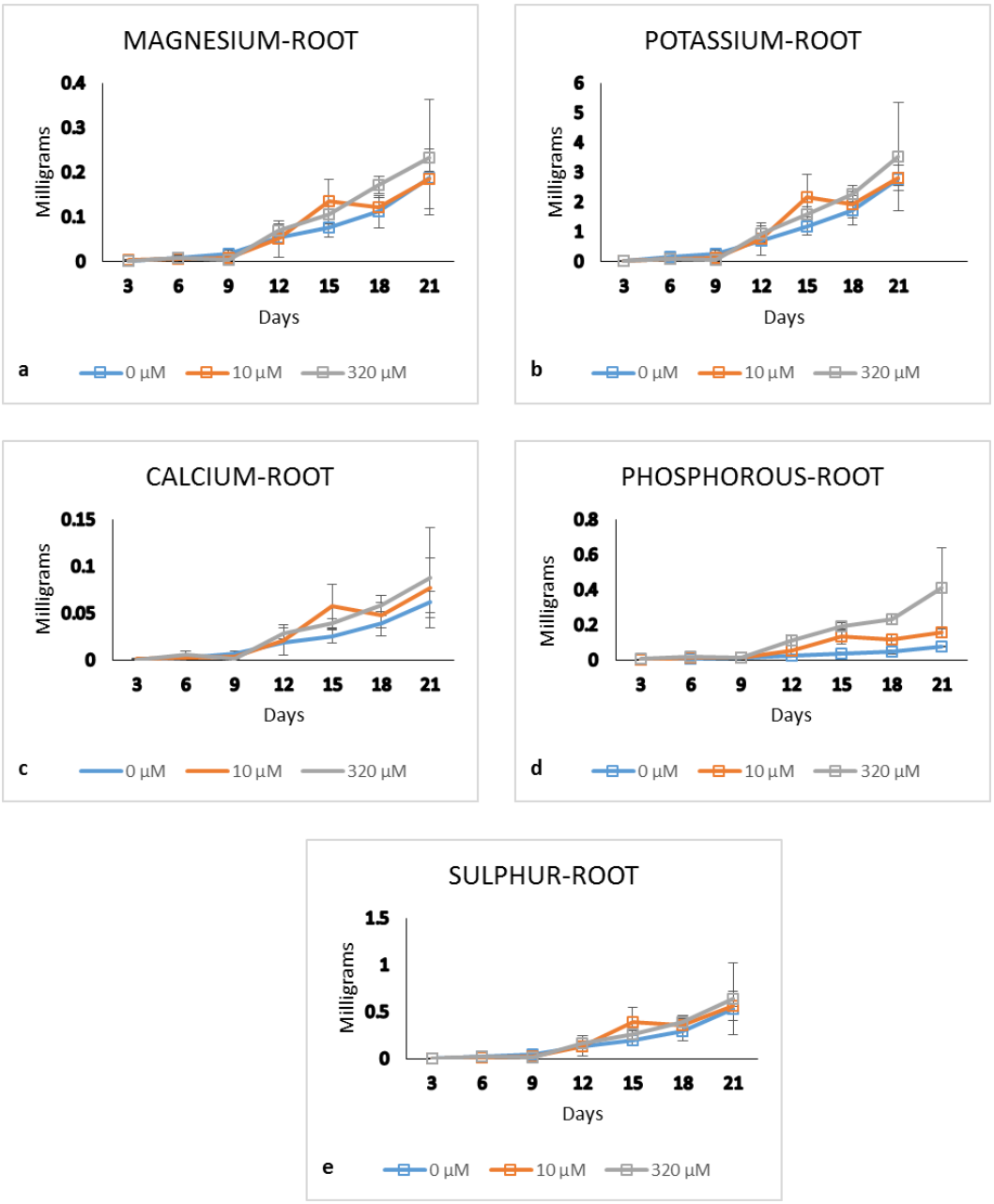


Figure 12 a-e: Allocation of macronutrients to roots from plants grown in hydroponic solutions with different Pi. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

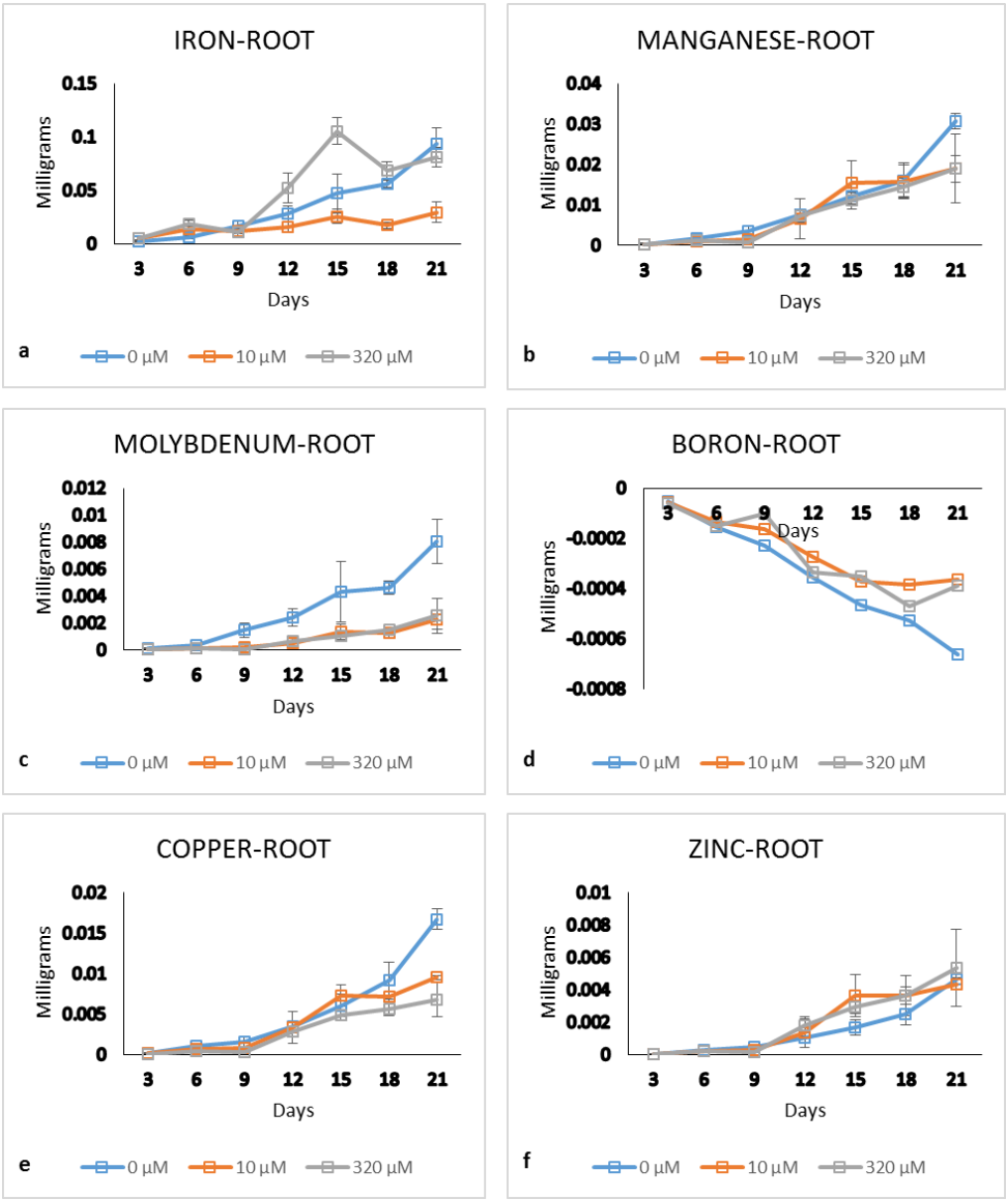


Figure 13 a-f: Allocation of micronutrients to roots from plants grown in hydroponic solutions with different Pi. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

pH

pH changes under Pi treatments

Decrease in pH of the hydroponic solutions collected after plant growth, was noted in the presence of Pi in the hydroponic solutions (Figure 14). The pH of hydroponic solutions with Pi became acidic in the range of 3.0 to 4.0 from 3 to 21 days of plant growth (Figure 14) though these hydroponic solutions were made with pH at 5.3 to 5.8 prior to plant growth.

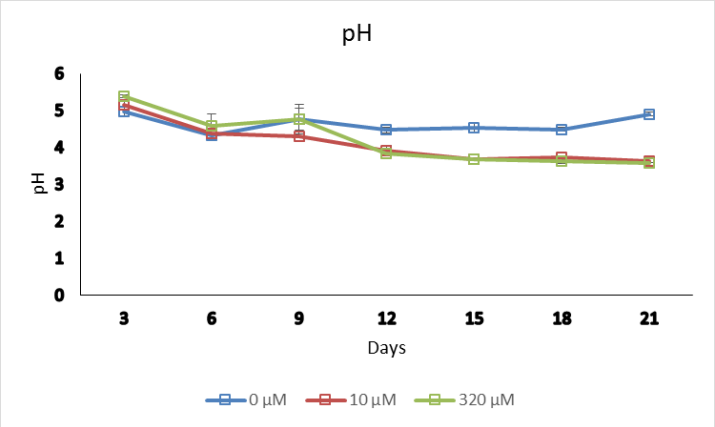


Figure 14: pH of hydroponic solution with plant growth from 3 to 21 days. Error bars represent ± standard error calculated for three replicates from individual hydroponic bottles.

INFERENCE

The prominent results from this work can be presented as:

Table 7: Combination of uptake and allocation of nutrients under different Pi treatments.

Elements	Impact of Pi on uptake	Impact of Pi on allocation to shoot	Impact of Pi on allocation to root
Mg and Ca	No	+	
Mo and Cu	No		-
S, Fe, and Zn	No	No	No
K and P	+	+	
Mn and B	+	No	No
N and Water	+		
C	No		

Analysis of 14 elements has been presented in this work on rice variety Nipponbare subjected to three treatments of Pi: absence of Pi, Low and high Pi regime. Statistical analyses using ANCOVA revealed differences in ionomic profile of these three different treatment regimens. The differences in the ionomic profiles of the treatments can be understood as:

1. 4 elements: **no** impact of Pi on uptake but **positive** impact on allocation to shoot (**Mg and Ca**) or **negative** impact of Pi on allocation to root (**Mo and Cu**).

2. 3 elements: no impact of Pi on uptake or allocation to shoot or root (**Fe, S and Zn**).

3. 2 elements: positive impact of Pi on uptake and allocation to shoot (**K and P**).

4. 2 elements: positive impact on uptake but no impact on allocation (**Mn and B**).

5. For **N and Water** only positive impact of Pi on uptake has been studied.

6. For dissolved **C**: no impact of Pi on uptake.

There are a few elements that behaved in a similar manner for their uptake and allocation under different Pi treatments which can be used to draw a basic idea about the ionomic profile of Pi stress in this variety of rice. The possible grouping of these unique macronutrients and micronutrients as observed in this work is presented below:

Macronutrients: Mg and Ca; K and P

Micronutrients: Mo and Cu, Mn and B

Further work is needed to understand the exact nature of this behaviour of nutrients in this variety under conditions of Pi stress.

DISCUSSION

MORPHOLOGICAL ANALYSIS

Phosphate deprivation induces various morphological modifications which can be understood as adaptive responses in shoots ad roots of the plant.

Length and fresh weight in shoot

In the absence of Pi, both shoot length and fresh weight of shoot were reduced. General adaptive response in shoot that are known to occur with absence of Pi include retardation of shoot growth and branching (López-Bucio et. al., 2002) for reducing photosynthetic efficiency to conserve Pi pool (Lin et. al., 2014). Plants require more inflow of phosphorus to shoots than roots (Cogliatti and Santa Maria, 1990; Burleigh and Harrison, 1999; Raghothama, 1999) which is related to their involvement in photosynthesis that requires intensive phosphorylation (Rao and Terry, 1989; Louw Gaume et. al., 2010). In the absence of Pi, stunted shoots are typically seen (Kennelly et. al., 2012) as also observed in this work which could be because of reduction in photosynthesis, lack of phosphorus is known to reduce photosynthesis (Foyer and Spencer, 1986) which is needed for normal growth and development of a plant. Though alteration in expression of shoot specific genes has been observed due to lack of phosphorus in plants (Hammond et. al., 2003) but the molecular mechanism for Pi stress and signalling in shoot are still in infancy.

Fresh weight in root

Root system architecture (RSA) is known to be affected by deficiency of phosphorus (Lambers et. al., 2011; Niu et. al., 2012; Péret et. al., 2011). Roots

also respond to absence of Pi by extension of surface area, becoming more branched, modification of root architecture or by symbiosis with mycorrhizal fungi (Péret et. al., 2011; Smith et. al. 2011). In this work, there was no difference in root length between absence and presence of Pi (Figure 17) but fresh weight of root was affected by presence of Pi in the hydroponic solution.

The fresh weight of root was higher in the presence of both low and high Pi treatments, than in the absence of phosphorus probably pointing to retention of more water in root cells, the exact cause of this suggestion needs further experimentation. Root length hair length rather than density (fresh weight) or radius of roots, has been considered as the best correlated parameter to Pi uptake (Itoh and Barber, 1983; Jungk, 2001). The impact of Pi stress on root morphology needs further refined assessment in this work.

NUTRIENT UPTAKE AND ALLOCATION

The mechanism involved in elemental response to phosphate deficiency are far from clear and it can be done by identification of genes controlling the accumulation of individual elements or groups of highly correlated elements using mutant screens but this process is laborious and with limited application to a particular genetic background (Baxter et. al., 2012). Another approach is ionomics, measurement of nutrient and trace elements composition of plants to understand genetic and environmental (Baxter, 2009; 2010; Salt et. al. 2008) regulation and its effect on growth (Prinzenberg et. al. 2010).

The interactions between Pi and other nutrients which influenced their rates of for uptake and allocation can be discussed based on:

1. Immobilization of nutrients in the hydroponic solution limiting their availability for uptake,

2. Physiological nature of interactions between Pi and nutrients involving various transport proteins etc. within the root surface (uptake) and in the long distance transport (root to shoot through xylem sap) or phloem translocation (Allocation).

Both these influences can be due to either synergistic (positive) or antagonistic (negative) interactions between nutrients and Pi and would be discussed keeping one major factor determining these interactions, pH of the hydroponic solution, which has been found to decrease with Pi addition to it.

Synergism and antagonism between nutrients has been shown to define plant behaviour with respect to uptake of the elements and their allocation to different plant tissues in barley, cucumber and tomato using hydroponic solution and soil with different nutritional regime (Pii et. al., 2015).

Interactions between Pi and macronutrients

The key point to be considered here while discussing the interactions between Pi and macro nutrients is that Pi has either no or positive impact on uptake and allocation of macro nutrients and there were no negative interactions.

No impact of Pi on uptake but positive impact on allocation of Mg and Ca to shoot

Mg, Ca and K ions are quite similar in size and charge and compete with each other for uptake sites. Addition of any one of them reduces the uptake rate of

the other two (Ranade-Malvi, 2011). Uptake of Mg is strongly influenced by the availability of other cations like NH_4, Ca and K (Jacoby, 1961; Diem and Goldbold, 1993; Fageria, 2001; Römheld and Kirkby, 2007). There is existence of phenomenon of competition of cations for their uptake (Jacoby, 1961; Diem and Goldbold, 1993; Fageria, 2001, Marschner, 2012; Chen and Ma, 2012). In experiments with nutrient solutions, it is shown that high availability of cations like Ca, K and Mn can lead to strong decreases in Mg uptake (Marschner, 2012). Fageria (1973) has reported in rice plants that high concentration of Ca in nutrient solution causes antagonistic cation competition for uptake leading to reduction in uptake of Mg (and K). Evidence for K-Mg/Ca antagonism has been given by Shabala and Hariadi (2005) in leaf mesophyll cells of broad bean. There is a non-selective ion channel system capable of both K and Ca transport, suggesting reduction in uptake of Mg can occur due to K and Ca. Horie et. al. (2011) has provided an experimental proof to K- Mg/ Ca antagonism. Two members of class II of HKT transporters in rice are though principally transporters of K, also capable of transporting Mg and Ca. Under K supply, the uptake of both Mg and Ca is hampered. The availability of Ca has also been known to decrease with application of high phosphorus due to precipitation of insoluble phosphates.

In this study, both uptake of Mg and Ca were not affected by the presence of phosphorus suggesting possible K- Mg/ Ca antagonism rather than Mg- Ca antagonism, the role of phosphorus in this antagonism needs further experimentation.

Another possible cause of no impact of Pi on uptake of Mg and Ca could be related to decrease in pH that occurred in nutrient medium in the present study

though there are reports for increase in uptake of both the ions with application of phosphorus (Hassan et. al., 2013; Majumdar et. al. 2007 Suresh, 2008). The availability of cations, Ca and Mg decreases at acidic pH due to disruption of electrochemical gradient across the plasma membrane of root cells (Schubert et. al., 1990). Competition for uptake of ions is a consequence of lack of specificity of the individual uptake systems for each cation (Gardner, 2003; Deng et. al., 2006; Marschner, 2012). It has been observed that K- Mg antagonism affects translocation from root to shoot (allocation to shoot) being impaired by high K concentrations (Gransee and Führs, 2013). In the present study, Mg and Ca were positively allocated to shoot in the presence of phosphorus, also again suggesting possible role of phosphorus in Mg/ Ca -K antagonism operating at the level of shoot allocation. Fernandes and Soratto (2012) have reported increase in shoot allocation of Ca and decrease in shoot allocation of Mg with Pi addition in potato (*Solanum tuberosum*).

Another factor that has to be considered for positive effect of Pi on allocation of Mg and Ca to shoot despite of it casting no impact on their uptake is the growth of these plants for 15 days in a balanced full nutrient medium (Yoshida et. al., 1976) which could have allowed for accumulation of sufficient Mg and Ca for the development during this period as also reported for phosphorus in potato (Fernandes and Soratto, 2012) and micronutrients in castor bean (Lange et. al., 2005).

No impact of Pi on uptake or allocation of S

S plays important roles in many processes. The major form of S available to plants is anionic sulfate (SO_4^{2-}) taken up into root cells by sulfate transporters (SULTR) (Davidian and Stansislav, 2010). Uptake of sulfate is determined by

S status of the plant and is influenced by deficiency of S (Lappartient and Touraine, 1996). It is related to assimilation of N and C, Limitation of N reduces S uptake (Smith, 1980; Clarkson et. al. 1989).

Sulfate is actively transported into roots and distributed throughout the plant in an unmetabolized form (Leustek and Saito, 1999). S and P interact as anions, SO_4^{2-} and PO_4^{2-}, respectively. According to Aulakh and Pasricha (1977), sulfate and phosphate compete for the sites in the same transport pathway within the roots and stems, thus affecting their allocation also.

There are reports of synergistic as well as antagonistic relationships between S and P depending on their concentration and crop species (Marok and Dev, 1980; Sinha et. al. 1995) like in legumes (chickpea, pigeon pea, lentil, pea, urd bean and ground nut) synergistic interactions are known to occur (Shrinivasarao et al., 2004; Raina and Tanawade, 2005). These interactions may affect the critical levels of available P and S (Yadav, 2011).

There was no impact of P on uptake or allocation of S in the present study, which could be because of sufficient levels of S available to the plant which probably obscured the competition between sulfate and phosphate for transport and allocation and also uptake of S is demand driven (Lepertient and Touraine, 1996). The proposal for existence of sufficient levels of S in plants is derived from the fact these plants were grown on fully balanced nutrient medium (Yoshida et. al., 1976) prior to being subjected to various treatments, this period probably added ample amount of S for further growth of plants.

Positive impact of Pi on uptake and allocation of K and P

Significantly higher uptake of K with application of P has been reported by Hasan et. al. (2013) in aubergine and in rice by Kadu et. al. (2005).

The possible reason for the increased uptake of K in presence of Pi could be because of increased availability of K in this nutrient medium but mechanism regulating the interaction between P and K causing increased availability of K is far from clear and needs more experimentation. The uptake of K involves active and passive processes and is influenced by its concentration in the nutrient solution (Williams, 1961). Concentration driven uptake is known to involve mass flow of minerals with high concentration in soil solution like N, K, S, Ca, Mg and Cl (chlorine) (Gardner, 2003; Shaul, 2002; Deng et. al., 2006; Marschner, 2012) or Cu, Ni (Nickel), B and Mo (White et. al. 2013).

Contribution of pH to increased uptake of K in this study is not very evident as it is known that reduced pH that occurred in the nutrient medium with Pi, actually reduces the availability of K (White et. al, 2014).

Competition between K and other cations for exchangeable sites for adsorption on root transporters can also be one of the reasons for the increased uptake of K as it is a dominant cation in nutrient solution. Another factor contributing to the increased uptake of K is N availability which is known to influence K uptake (Zhang et. al., 2010). The role of Pi in cation competition or increasing availability of N which are thought to be inducing significant uptake of K here needs more work and analysis.

Fernandes and Soratto (2011) reported in aubergine, higher shoot K allocation with high levels of P as also is observed in the present study. Pi influenced K translocation has been suggested cause of this observation on the basis of work of Malavolta (2004, 2005). After Pi absorption by roots, it has been reported

that the ATP (Adenosine tiphosphate) and UTP (Uridine triphosphate) are rapidly formed. Energy provided by ATP helps in K transport to shoot by allowing movement of K through K^+-H^+ antiport system in xylem.

Diffusion of mineral elements which is a concentration gradient driven movement from soil solution to root surface (White and Greenwood, 2013) is operational over short distances and plays an important role in movement of P and K (Hissinger et. al., 2011). Combination of mass flow and diffusion of mineral elements are means of their movement in soil and uptake by roots (Lynch, 2007; Richardson et. al., 2011; Simpson et. al., 2011; White et. al., 2013). Mineral acquisition/ uptake can also be enhanced by combined effect of improving root characteristics, modification of rhizosphere's pH, high capacity uptake systems for mineral nutrients (White et. al. 2014).

Taken together, the increased uptake of Pi with different treatments of Pi in this work could have resulted from increased availability of Pi in hydroponic solution, thus, facilitating its movement towards the root surface, interestingly there was an increase in root fresh weight in the presence of Pi probably as a factor for improving Pi availability to plant. The availability of Pi could have also been improved by decreased pH of the hydroponic solution as seen in this work.

Besirat et. al. (2011) observed that in tomato plants well fed with P, there was higher total P content in leaves (shoot) as is also observed in this work. In plants, shoots are sources of photosynthesis which requires intensive phosphorylation process (Rao and Terry, 1989; Louw-Gaume et. al., 2010). Pi transport in plants requires low affinity transporter system that is often expressed and a high affinity transporter system that is induced only when plants face severe P

deficiency (Kim et. al., 1998; Daram et. al., 1998; Mukatira et. al., 1996; Liu et. al., 1998; Raghothama, 1999). In the present work, presence of Pi positively affected uptake of P and also its allocation to shoot probably involving low affinity transport system which is expressed in sufficient availability of Pi in the system.

Positive impact of Pi on uptake of N and no impact on uptake of C

N, P and K are the essential nutrients with significant effects on plant growth and development. Inorganic N (NO_3^-; nitrate and NH_4^+; ammonium) is absorbed by plants. Plant roots uptake nitrate by transporters and then reduce it to ammonium for further assimilation (Forde, 2000). Ammonium and nitrate are transported to root through different sets of transporters localized in the plasma membrane of root cells (Kiba and Krapp, 2016). The uptake of nitrate is based on active transport system even if its high concentration is available (Glass et. al., 1992; walker et. al., 1995). These transporters are classified into four categories: constitutive high-affinity system (cHATS), inducible high-affinity system (iHATS), constitutive low-affinity system (cLATS) and inducible low-affinity system (iLATS) (Forde, 2000). Ammonium transport is mediated by AMT superfamily of transporters (Ludewig et. al., 2007).

There are reports of uptake of N being promoted by high P concentrations in brinjal (Hasan et. al., 2013) and chilli (Dass and Mishra, 2002) as is also seen in the present work but it needs further work for explanation in terms of availability and form of nitrogen that was absorbed by roots, influence of pH on the uptake of available form and importantly effect of P on these forms of N taken up by the rice plants grown hydroponically.

Similarly though there is an observation of no impact of P on uptake of C but it is inconclusive as it is available to plants in the gaseous form also and any interaction between Pi and C in the plant needs further elucidation of availability of inorganic and organic C apart from total C.

Interactions between Pi and micronutreints

Pi showed negative or no impact on uptake and allocation of micronutrients except for rates of uptake of Mn and B which were positively affected.

No impact of Pi on uptake of Mo and Cu but negative impact on allocation to root

Mo, a transition essential element micronutrient, is required in lowest amount by the plants (Zimmer and Mendel, 1999).

Phosphate has high affinity to Fe oxihydroxides, leading to release of adsorbed Mo and thus, increasing its water soluble concentration which is readily available to plants (Ballistrieri and Chao, 1990). In this work no impact of Pi on Mo uptake could be because of acidity of nutrient medium induced by Pi.

There is still a lacuna in knowledge about Mo transporters in plants and it has been proposed to be transported unspecifically by anion transporter (Zimmer and Mendel, 1999). Pi deficiency has been experimentally shown to enhance uptake of radiolabelled Mo in tomato plants, led to this assumption that Mo uptake uses phosphate binding or transporting sites at the plasma membrane of root cells (Heuwinkel et. al., 1992).

Molybdate anion (MoO_4^{2-}) behaves more like sulfate than phosphate in soil and the uptake of molybdate decreases in presence of large amounts of sulfate (Sims et. al. 1979) possibly because both anions use the same transport system (Marschner, 1995) which is still to be validated as existence of separate transport systems for sulfate and molybdate has also been proposed in plants (Purakayastha and Nad, 1996). Since S uptake has not been affected by P in this work, its involvement with Mo uptake needs further assessment.

All these suggestions point to decrease in pH as the probable factor contributing to no impact of Pi on uptake of Mo in this work.

After uptake, soluble molybdate is transported in xylem as well as phloem depending on the pH (Marschner, 1995). There must have been limited availability of Mo in the root sap after its uptake as it showed no difference in uptake in the presence or absence of P and hence decreased allocation to root was seen in the present work but needs further experimental proof.

There was also no impact of Pi on the uptake of Cu in this work, as also reported by Racz and Haluschak (1974) in wheat. One of the mechanisms for efficient absorption of nutrients in plants involves the release of protons into the rhizosphere by root, which increases the process of solubilisation of cations like Fe, Cu and Zn (Palmer and Guerinot, 2009). H^+ (Protons) are released by ATP dependent proton pumps (H^+- ATPases) in the plasma membrane of the root cells (Giovanni et. al. 2014). After solubilization, the metal is absorbed by the root cells (Giovanni et. al. 2014). General agronomic practices also indicate that P exerts a negative influence on micronutrients. One of the factors that might have contributed to no impact of Pi on the rate of uptake of Cu in this work

could be precipitation of Cu into Cu phosphate in the nutrient medium as also suggested by Islam et. al. (2009) in chickpea. Precipitation makes the metal unavailable for absorption by root, along with the solubilisation process that is required for uptake of Cu must have led to no impact of P on its uptake. On the other hand, like Mo, there is negative influence of P on allocation of Cu to root as also is reported in potato by Fernandes and Soratto (2012). Since it is speculated that there was decrease in availability of Cu in the hydroponic solution with P, which needs experiments for proof, its uptake remained unaffected and the amount of Cu that was measured probably indicated that amount which got allocated in the plants exposed to 15 days of growth on full balanced hydroponic solution before being given different treatments. Another cause could be same as that for uptake of Cu, precipitation of the Cu that was taken up by roots which prevented its allocation to root as also suggested by Islam et. al. (2009) in chickpea.

No impact of Pi on uptake or allocation of Fe and Zn

P can have negative influence on utilization of micronutrients like Fe and Zn by plants (Rocers and Wu, 1948; Burlesson et. al., 1961, Brown and Tiffin, 1962). This effect has been attributed to: either immobilization of these micronutrients by phosphorus precipitation (Biddulph and woodbridge, 1952; Spencer, 1960) or physiological nature of Fe/ Zn and P interactions due to reduced root surface absorption phenomenon or reduced translocation within the plant (Langin et. al., 1962; Loneragan, 1951; Warnock, 1970).

Since no antagonism between Fe/ Zn and P is noticed in this study, pH of the nutrient medium with P could have been one of the factors responsible for no

impact of P on uptake or allocation of these two micronutrients in the present study. In case of Fe, it has been reported that there can be either increase or decrease in the Fe content of the plant due to reduced pH induced by addition of Pi (Racz and Haluschak, 1974).

There was no impact of Pi on allocation of Fe/ Zn in this study. It can be argued that for both these micronutrients, there was uptake of available amounts from the hydroponic solution but were not distributed to the shoot for allocation probably because of known antagonism between Fe/ Zn and P. These plants were grown on full balanced nutrient medium for 15 days before being treated with different concentrations of Pi, that exposure must have led to allocation of sufficient Fe/ Zn for growth and it was probably this amount which was measured and for the amount of these nutrients used for allocation after treatment with Pi, some more work is required. Fageria (2011) has documented antagonistic interactions between Fe and P through precipitation and formation of iron phosphate. Similarly for Zn, Barben et. al. (2010) reported reduced Zn activity with over accumulation of P because of involvement of Mn. Application of these proposals to discuss the interactions between these micronutrients and Pi in the present study needs further refined experimentation.

Positive impact of Pi on uptake of Mn and B but no impact on allocation

Barben et. al. (2010) has suggested role of P in Mn uptake as well as transport. Also there is increase in availability of Mn with reduced pH (Racz and Haluschak, 1974) which could be one of the causes of increased uptake of Mn in presence of Pi as seen in this work. On the other side, P addition is also known to decrease Mn content of the plant which is also attributable to pH of the

nutrient medium (Racz and Haluschak, 1974). In this work, there was no impact of Pi on allocation of Mn which might have occurred because of metal chelation induced by Pi. Chelation probably restricted movement of Mn from root to shoot despite positive impact of Pi on its uptake. Chelation occurs to facilitate translocation of highly reactive ions through the vasculature (Pich et. al., 2001; Von Wirén et. al., 1999).

B is the only element that is not taken up as an ion but as an uncharged molecule by the plant (Miwa and Fujiwara, 2010). Earlier B transport was thought to be a passive diffusion (not catalysed by proteins) (Raven, 1980) but it is known now that there is a transport protein involved in B homeostasis (Miwa and Fujiwara, 2010). Since the hydroponic solution was supplied with sufficient amount of B and uptake of B is also concentration driven passive transport in such a condition, presence of Pi facilitated it, probably by reducing the pH. Robertson and Loughman (1973) also reported synergistic interaction between P and B. P influences B uptake (May and Pritts, 1993). Excessive P addition are known to reduce B uptake in citrus (Bingham and Garber, 1960; Bingham et. al., 1958). Complex adsorption and precipitation reactions exist between B and P and it has been proposed that B and P interactions can be either synergistic or antagonistic depending on the environmental conditions or presence of other nutrients (May and Pritts, 1993). There was no impact of Pi on allocation of B despite positively affecting its uptake in the present study because of prior exposure of plants to 15 days growth on full balanced nutrient medium which probably allocated sufficient B for growth. High levels of B are known to be toxic to cells (Miwa and Fujiwara, 2010) and high uptake was balanced by

restricted movement of B to shoot in the presence of Pi and mechanism for this observation needs further experimentation.

pH

Decrease in pH with Pi treatments

Roots adopt many techniques to increase the uptake of nutrients, one of which is acidification of rhizosphere (Rauser, 1999). Acidification involves the release of proton into the rhizosphere for increasing the solubilisation of cations like Fe, Cu and Zn (Palmer and Guerinot, 2009). This function is carried on by ATP-dependent proton pumps (H^+-ATPases) in the plasma membrane of root cells (Palmer and Guerinot, 2009).

Pi is transported across the root plasma membrane into the root cell with 2-4 protons (Sakano, 1999; Ai et. al., 2009). Raghothama (1999) has reported acidification of the rhizosphere during P deficiency. In plants like *Brassica napus* and whie lupin, it has been reported that acidification of rhizosphere increases during Pi deficiency (Yan et. al., 2002). All these reports suggested that during P deficiency, activity of proton ATPase was likely to increase (Chang et. al., 2008). Upregulation of root plasma membrane H^+-ATPase gene was shown during P starvation in soybean (Shen et. al., 2006) but Chang et. al. (2008) found down regulation of *OsA8* gene that encodes for ATPase in rice during P starvation. This gene has a major role in nutrient uptake (Chang et. al., 2008). Sondergaaard et. al. (2001) proposed that some proton pumps have redundant function and this could be one of the reasons for less acidification during absence of P in rice by *OsA8* gene (Chang et. al., 2008). High acidification of rhizosphere in the presence of P in this work also needs further

molecular work as it is showing similarity to *OsA8* gene in its response but conclusively one can say that it is because of H^+-ATPases/ proton pumps in the plasma membrane of the root cells.

Increased mobilization (solubilisation) of phosphorus in soil leading to increase in its influx (acquisition) in sugarbeet has been attributed to chemical modification of rhizosphere by root exudates (Khorassani et. al., 2011). Root exudates are composed of various molecules including low molecular weight compounds like organic acids, sugars and others. Organic acids like citrate, malate and oxalate are known to affect phosphorus mobilization (Khorassani et. al. 2011).

It is speculated that for mobilization and uptake of available Pi in plants grown in hydroponic solution without Pi, there was exudation of organic acids which caused lowering of pH as also observed in sugarbeet (Khorassani et. al., 2011). In the hydroponic solution with phosphorus, there was possibily exudation of organic acids or other molecules which in the presence of Pi, reduced the pH of the solution during plant growth. The involvement of root exudates in decreasing the pH of hydroponic solutions in which they were grown need further experimentation and analysis. Proteoid roots are known to exude high amounts of organic acids which acidify soil causing chelation of metal ions around roots which impacts the mobilization of phosphorus along with other micronutrients (Marschner, 1995). Increase in fresh weight of roots and also uptake of Pi in plants grown in the presence of phosphorus in hydroponic solutions also points to involvement of root exudates in dropping of pH with increased supply of Pi in this research work.

CONCLUSIONS

It is impossible to state an ionomic biomarker on the basis of limited work done on ionomic profile in this work but certainly one can predict Pi stress in this variety on the basis of correlation of morphological characters and ionomic profile of Pi stress which include:

Table 8: Markers for Pi stress in Nipponbare:

Character	Parameter	Absence of Pi	Presence of Pi
Morphological	Shoot growth	Reduced	Luxuriant
Uptake	K and P	Reduced	Increased
	Mn and B	Reduced	Increased
Allocation to shoot	K and P	Reduced	Increased
	Mg and Ca	Reduced	Increased
Allocation to root	Mo and Cu	Increased	Reduced
Reduction in pH		Non-significant	Significant

Clearly the crux of the work is "**reduction in uptake and allocation of K and P to shoot and increased allocation of Mo and Cu to root**" defines Pi stress in Nipponbare.

Individual variety biomarkers like an effort for Nipponbare cultivar of rice in this work, combined with other such datasets can be used to predict physiological markers for Pi stress (ionomic biomarkers) for the entire species (Pii et. al., 2015). This has tremendous scope for application in:

a. Enhancing crop productivity especially food crops, oil producing crops.

b. Biofortification of food crops making them nutritionally rich.

c. Reducing cost of crop production by decreasing cost of fertilizer input.

d. Preventing crop failures due to pathogens.

e. Use of non-arable land for supporting agriculture and feeding the increasing population especially in developing nations.

f. Sustainable agriculture, forestry, horticulture etc.

Also ionomic profiles of mapping populations can be one of the sources to understand the genome of an organism and correlate the same to functions of genes deducing gene networks or they can be one of the most productive, highly applicable, less time consuming and most importantly low cost technique for deducing gene expression with high reliability.

The study on uptake of nutrients done through time series dataset can be used to develop mathematical models for uptake of nutrients during growth of plants. Phosphate stress shows its effect on root system architecture and it can be incorporated in further work by use of softwares like RootNav which would provide for quantification of important root characteristics like laterals. One of the important area of research based on this work would be correlation of Pi stress genes and Ionomic profile of Pi stress. This work can be based on assessment of expression of Pi stress genes in shoot and root tissues undergoing Pi stress. As observed in this work, there was increased allocation of Mo and Cu to root in the absence of phosphorus. Study related to transporters of these two nutrients and effect of presence of Pi on their regulation can be assessed.

Similarly K and P showed increased uptake and allocation to shoot with Pi stress, study related to the regulation of their transporters in uptake, transport and allocation can be conducted involving techniques like qRT-PCR. The datasets so collected can be extended to other cultivars of rice which are Pi stress tolerant and a holistic view of Pi stress in rice can be obtained which would include morphology, physiology and genetic makeup of this model monocot plant.

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