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Determining a critical phosphorus dilution curve for spring wheat

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Prashanta Raut

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Summary

Phosphorus (P) fertilizer is an important input for crop production. Phosphorus deficit limits crop productivity, while excess use creates environmental problems and depletes limited phosphate reserves. Therefore, the use of P fertilizer in agriculture must be optimized to sustain crop production at a desired level, without loading the environment. Values reported in the literature about recommended P concentration for optimal plant growth vary, and precise knowledge of the critical plant P concentration, which is the minimum concentration sufficient for maximum plant growth, is lacking. The critical concentration is expected to decrease with the biomass of the crop, due to dilution of the cytoplasm in the plant, as the share of fibers increases. In order to explore this dilution effect, and tentatively estimate a critical P dilution curve as function of standing biomass, a pot trial was conducted with spring wheat (Triticum aestivum L.). Eight different rates of P fertilizer (0, 1.67, 3.33, 5, 6.67, 10, 20 and 30 mg P/kg soil) were applied to soil with low P content (P-AL 1.6 mg/100g soil). Wheat plants were grown in a growth room at room temperature of 20 ^{0}C, at the rate of 9 plants per 3L pot, under otherwise well fertilized and irrigated condition. Plants were sampled five times from the three leaves stage to nearly maturity. P concentration and shoot biomass was measured at each sampling event. Green area, tillering and several other growth parameters were studied. Shoot biomass increased from lowest to the highest P application rate. Therefore, I was not able to identify the critical plant P concentration. However, my study indicated that P dilution occurs with growth, furthermore the lowest P concentration observed was 0.5 mg P/g DM, which could suggest this is a physiological minimum. The concentrations of P in the flag leaf and in the penultimate leaf were approximately constant, while the leaf area increased with P application. There was no effect of P application on the photosynthesis rate of the flag leaf. This indicates that, at least at the low P supply of this experiment, plant reduce leaf area to maintain a sufficient P concentration for photosynthesis.

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1 GENERAL PART

1.1 General Aim of the study

"It has been estimated that, crop yield on more than 30% of the world's arable land is limited by Phosphorus (P) availability. The acid-weathered soil of tropics and sub-tropics are particularly prone to P deficiency" (Vance, Uhde-stone, & Allan, 2003). While on the other hand, the practice of applying large amounts of P to agricultural land over several previous decades, particularly in regions with intensive livestock production, has resulted in P accumulation in soils with an increased risk of P losses into water bodies and thus of eutrophication (Lemercier et al., 2008). Insufficient use of P fertilizer has serious implication on the crop production and consequently on the livelihood of farmers in one part of the world, while on the other part, excess use of fertilizer is creating environmental problems. Although various characteristics of an agroecosystem affect the local phosphorus cycle, the problem of P imbalance particularly stems up from the inappropriate use of inorganic P fertilizer, either in deficit or at surplus. Since P is a finite resource and its reserve on earth is depleting, P needs to be used prudently in agriculture.

The general procedure for applying phosphorus (P) fertilizer to soil involves three main steps: (i) measurement of soil-P availability, (ii) classification of the soil-P fertility level and (iii) estimation of the recommended P dose (Jordan-meille et al., 2012). Discrepancies arising during any of the above steps can lead to error in P fertilizer application resulting in either under application or over application of P fertilizer.

This study aims to deal with the problem of under or over application of P fertilizer by investigating into efficient methods of estimating P nutrition status of wheat crop, so as to find out more accurate measures to correct P deficiency or excess.

1.2 Role of phosphorus in plants

Phosphorus is essential to all life forms because it is a key element in many important life processes. It is an important component of organic molecules, membranes and genetic components like DNA and RNA and is vital in energy metabolism within cells and tissues. In addition to this, plants require phosphorus for photosynthesis too. Phosphorus is therefore one of the three essential macronutrients in plants.

1.2.1 Role in energy transfer

Phosphorus is present in energy rich intermediates like ATP, ADP and AMP. The phosphate ions in these molecules are linked by pyrophosphate bond which allows energy transfer. Energy liberated during glycolysis, respiration, or photosynthesis is utilized for the synthesis of the energy-rich pyrophosphate bond and on hydrolysis of this bond, energy of nearly 30 kJ per mole ATP is released. This energy can also be transmitted with the phosphoryl group in a phosphorylation reaction to another compound, which results in the activation of this compound(Marschner & Rimmington, 1988). Almost every metabolic reaction of any significance involves phosphate derivatives (Havlin, Beaton, Tisdale, & Nelson, 2005). Involvement of ATP molecules in a glycolysis cycle (Figure 1) explains how indispensable P is in metabolic pathways.

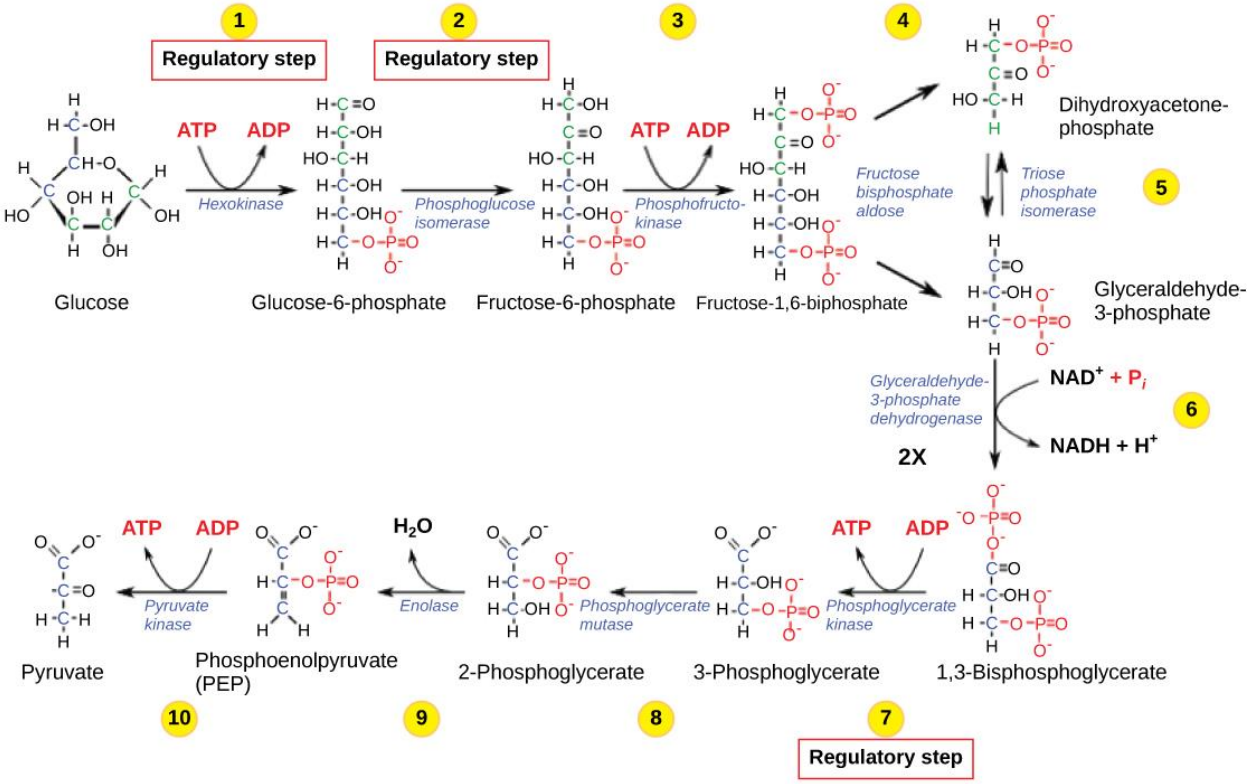

Figure 1 Glycolysis showing the steps involved in conversion of glucose to pyruvate. ATP is consumed in the early phase while in the later phase ATP is produced.

(Retrieved from https://biochemistry3rst.wordpress.com/category/glycolysis/ 14 March 2016)

1.2.2 Role as structural element

Phosphorus is an essential element in the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Figure 2) that contain the genetic code of the plant to produce proteins and other compounds essential for plant structure, seed yield and genetic transfer (Havlin et al., 2005).

In both DNA and RNA, phosphate forms a bridge between ribonucleooside units to form macromolecules. Phosphorus is also a constituent of another important structure of cells, the phospholipids which form the bio-membranes. In phospholipids, the phosphorus diester forms a bridge between a diglyceride and another molecule (amino acid, amine or alcohol) (Figure 3) (Marschner & Rimmington, 1988).

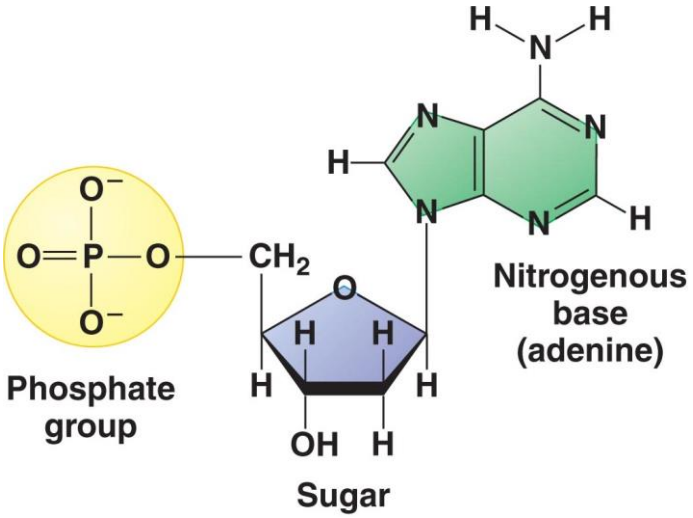

Figure 2 A DNA nucleotide

(Retrieved from http://pmgbiology.com/2014/10/21/dna-structure-and-function-igcse-a-understanding/ 9th March 2016)

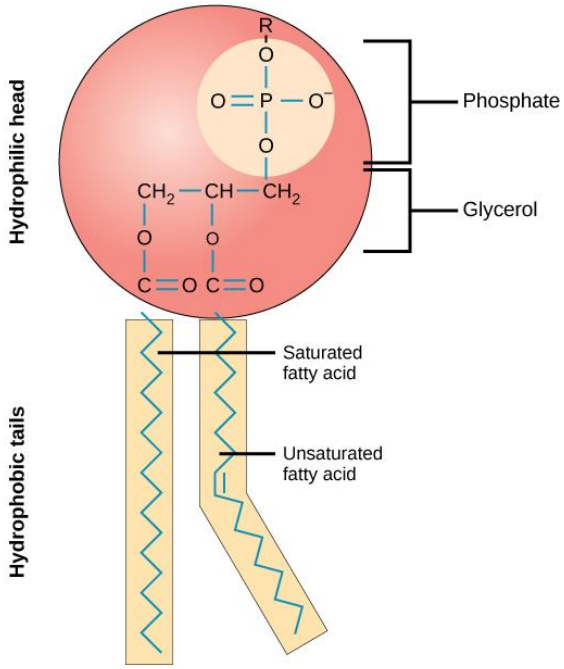

Figure 3 Structure of phospholipids

(Retrieved from https://www.boundless.com/biology/textbooks 9th March 2016)

1.2.3 Regulatory role of inorganic phosphate

Inorganic phosphate (Pi) controls some key enzyme reactions in cells. For instance, orthophosphate fluxes from vacuole to cytoplasm in fruits of tomato can stimulate the activity of phosphofructokinase (an enzyme responsible for regulating respiration) thus affecting fruit ripening (Woodrow & Rowan, 1979).

In leaves, photosynthesis and carbon partitioning in the light-dark cycle are strongly affected by the Pi concentrations in the stroma of chloroplasts and the compartmentation between chloroplasts and cytosol. The inhibition of starch synthesis by high concentrations of Pi is also caused by the inhibitive effect of Pi on ADP-glucose pyrophosphorylase (the key enzyme of starch synthesis in chloroplast) (Marschner & Rimmington, 1988).

1.2.4 Role of Phytate in seed germination

Phytate is the typical storage form of phosphorus in grains and seeds. Phytate phosphorus makes up 60-70% of total phosphorus in cereal grains and about 86% in wheat mill bran (Lolas, Palamidis, & Markakis, 1976). Phytate plays an important role in seed germination and seedling growth by providing the necessary P to the growing plant (Marschner & Rimmington, 1988).

1.3 Physiology of Phosphorus in plants:

1.3.1 Absorption

Plants acquire P predominantly as orthophosphate from the soil solution (Bieleski, 1973). Generally the phosphate content of root cells and xylem sap is about 100 to 1000 fold higher than that of the soil solution. This shows that phosphate is taken up by plant cells against a very steep concentration gradient. Phosphorus uptake is therefore an active metabolic process requiring energy (Mengel & Kirkby, 1987).When plant roots come in contact with the phosphate of soil solution, they absorb phosphate at high rate and the soil solution in the direct root vicinity is depleted of phosphate. This depletion creates a gradient regulating the rate of phosphate diffusion towards the plant root. Mass flow can also play a part in the transport of phosphate towards plant roots; however, its contribution is minimal since the phosphate concentration of soil solution is so low (Mengel & Kirkby, 1987).

Maximal uptake rates occur at pH range 5–6 (Ullrich Eberius, Novacky, Fischer, & Luttge, 1981). The ability of plants to uptake phosphate also differs between species and even cultivars and it is fixed genetically (Mengel & Kirkby, 1987).

The P supply to plant roots is greatly enhanced by a symbiotic relationship between plant roots and fungal microorganisms called mycorrhizae. Mycorrhizal fungi infect roots of most plants and function primarily by enhancing nutrient uptake. Ectomycorrizae predominantly infect tree species, while endomycorrhiza(Vesicular Arbuscular Mycorrhizae, VAM) infect most other plants. As new roots develop, mycorrhizal fungi infect the root and develop extensive structures extending into and beyond the rhizosphere. Under low soil nutrient availability, VAM-infected roots explore a substantially larger soil volume from which to absorb nutrients. In many cases excessive N and P fertilization and soil tillage can reduce the contribution of mycorrhiza related nutrient uptake (Havlin et al., 2005).

1.3.2 Translocation

Phosphate is readily mobile in the plant and can be translocated in an upward or downward direction (Mengel & Kirkby, 1987). Although Phosphorus is absorbed by plants in the form of orthophosphates, this phosphate rapidly becomes involved in metabolic processes. The regulation of Pi uptake and transport is mediated by phosphate transporters. These phosphate transporters are localized in the plasma-membrane and operate as H^+ co-transporters. P starvation or mycorrhizal infection increases the expression of phosphate transporters thus making P uptake more efficient. Pi uptake and transport however are complex processes involving roles of various phosphate transporters (Hawkesford, Kopriva, & De Kok, 2014).

Plants tend to transport P towards newer parts from older parts. In cereals P translocation towards flag leaves during the later growth stages and towards grains during maturity occurs thus making the grains ultimately the major sink of P. At maturity, wheat plants for instance may contain up to 90% of the total shoot P, with 20%-90% of this being re-translocated from other tissues (Peng & Li, 2005).

1.3.3 Role of P in growth of cereal crops

Biomass production in crops is a consequence of two major processes: i) the interception of incoming photosynthetically active radiation (PAR) by leaves ii) the ability of plants to transform the intercepted radiation to biomass (Monteith & Moss, 1977). Radiation Interception (RI) and Radiation Use Efficiency (RUE) both can have significant effect on shoot dry weight. When plants are growing under P deficiency, shoot dry matter is clearly reduced but less is known about whether the reduction in yield is due to reduced RI or reduced RUE. Results of some studies suggest that the yield reduction under P deficiency is primarily due to the interruption in canopy expansion that affects the interception of solar radiation (Fletcher, Moot, & Stone, 2008). However some others found that leaf

photosynthesis is reduced significantly in wheat plants due to P deficiency (D. Rodríguez, W. Keltjens, & J. Goudriaan, 1998b). Further, biomass production in cereals also depends on the tillering ability, which however has consequence on radiation interception.

Since it is possible for plants to increase its volume without gaining shoot dry matter, increase in volume is a poor measure of growth. Therefore, dry matter accumulation is often used as a parameter to measure growth.

The simplest measure of growth is Absolute Growth Rate (AGR), which is the absolute change in mass over a given time interval. The limitation of AGR as a measure to compare growth is that, it varies if individuals under comparison have different initial sizes. Therefore, Relative Growth Rate (RGR) is used widely to compare intrinsic growth physiology of different genotypes or species independent of difference in sizes (Hunt & Cornelissen, 1997). RGR is measured as the mass increase per aboveground biomass per day.

However, RGR is not size independent because most organisms including plants become increasingly inefficient as they get larger, through for example, self-shading, tissue aging and turnover and allocation to structural components. Despite these problems, RGR has a simple intuitive biological meaning in terms of growth efficiency and is a natural parameter to consider when analyzing growth (Rees et al., 2010).

In order to better understand how and why RGR varies among species, it is often factored into three components

RGR = NAR*SLA*LMR

Where,

NAR is the Net Assimilation Rate

SLA is Specific Leaf Area and

LMR is Leaf Mass Ratio

1.3.4 Phosphorus deficiency in plants

It is commonly reported that the phosphorus requirement for optimal growth is in the range of 0.3 – 0.5% of the plant dry matter during the vegetative stage of growth. The probability of phosphorus toxicity increases at contents higher that 1% in dry matter. In plants suffering from phosphorus deficiency, reduction in leaf expansion, leaf surface area, number of leaves

is most striking effects (Marschner & Rimmington, 1988). Plants suffering from P deficiency are retarded in growth and the shoot/root dry matter ratio is usually low. In cereals tillering is affected. Generally, the symptoms of P deficiency appear in the older leaves which are often of a darkish green color. The stems of many annual plant species suffering from P deficiency are characterized by a reddish coloration originating from an enhanced formation of anthocyanins (Mengel & Kirkby, 1987).

Despite severe inhibition of leaf expansion, protein content and chlorophyll per unit leaf area are not affected by P-deficiency. The chlorophyll concentration, in fact, is increased under P deficiency resulting in darker green leaf color as cell and leaf expansion are more retarded than chloroplast and chlorophyll formation [Rao and Terry, 1989; Hecht-Buchholz, 1967 as quoted in (Marschner & Rimmington, 1988)]. However photosynthetic efficiency per unit of chlorophyll is much lower in phosphorus deficient leaves [Lauer et al, 1989b as quoted in (Marschner & Rimmington, 1988)]. Phosphorus deficiency inhibits shoot growth more than it inhibits root growth. Therefore, P deficient plants have lower shoot-root dry weight ratio (Marschner & Rimmington, 1988). Phosphorus deficiency can even enhance elongation rate of individual root cells and of the roots (Anuradha & Narayanan, 1991).

Despite the adaptive responses in increasing P acquisition by roots, P limitation does not only retard shoot growth rate but also retards the formation of reproductive organs. Flower initiation is delayed (Rossiter, 1978), the number of flowers is decreased (Bould & Parfitt, 1973) and seed formation restricted in particular (Barry & Miller, 1989) due to P limitation. Premature senescence of leaves is another factor limiting yield in P deficient plants (Marschner & Rimmington, 1988).

1.4 Phosphorus in soil

The textbook, [Soil fertility and fertilizers (Havlin et al., 2005)] has been used as a reference for most of the contents of this section.

1.4.1 P composition of soil

The earth's crust contains about 1,200 mg P kg^{-1}, making it the 11th most abundant element. Common concentrations for total P in soils are between 200 and 800 mg kg^{-1},with older soils containing lower amounts of P and younger soils containing higher amounts of P (White & Hammond, 2008). Phosphorus in soil occurs almost exclusively in the form of orthophosphate. Quite a substantial amount of this P is associated with soil organic matter(Williams, 1959). For most mineral soils, apatites are believed to be the primary

phosphate containing minerals from which the other P containing soil fractions are derived (Mengel & Kirkby, 1987). In primary rocks and young soils, P is largely bound to calcium or magnesium, giving P a typical water solubility near 0.5 mg P L^{-1}.The weathering of minerals changes the solubility of P, as Ca is preferentially leached out, the relative abundance of Fe and Al increases and the solubility of P becomes controlled by Fe- or Al-phosphates, which have much lower solubilities than Ca- phosphates. As a result of the sequestration of P in low-solubility Fe and Al-phosphate compounds and the effect of leaching and erosion, many older and tropical soils are P deficient(White & Hammond, 2008).

1.4.2 Forms of soil P pools

In soil, P is present in various forms, which are interchangeable. These forms are: solution P, inorganic soil P and organic soil P.

1.4.2.1 Solution P

Phosphorus is present in the form of $H_2PO_4^-$ and HPO_4^{2-}in soil solution. P concentration in soil solution varies widely among soils from 0.003 – 3 ppm. Soil solution P required by plants depends on crop species and level of production. With relatively low soil solution P (~ 0.05 ppm) supplying the quantity of P needed by plants (~0.3% P) requires soil solution P to be frequently replenished. Young tissues near the root tips actively absorb P from the soil surface in contact thus depleting the solution P. As roots absorb P from soil solution, diffusion and mass flow transport additional P to the root surface.

1.4.2.2 Inorganic soil P

Primary and secondary P containing minerals present just a fraction of a total inorganic soil P found in agricultural soils. Phosphate fertilizers (rock phosphates, superphosphates, ammonium phosphates or polyphosphates) are commonly used to boost soil P levels. When inorganic P is added to the soil in the form of fertilizer, the excess inorganic P not adsorbed by roots or immobilized by microorganisms can be adsorbed to mineral surfaces or precipitated to secondary P compounds. Surface adsorption and precipitation reactions are collectively called P fixation. P adsorption in soils occurs by an initial rapid reaction followed by a much slower reaction. Generally, soils of temperate regions have higher soil solution P concentration due to slower reaction rates and lower Fe/Al oxide content. The extent of inorganic P fixation depends on many factors, most importantly soil pH. P availability in most soils is at a maximum near pH 6.5. At low pH, P fixation is largely from reaction with Fe/Al oxides and precipitation as $AlPO_4$ and $FePO_4$. Fe/Al oxides are abundant in acid soils and have the capacity to adsorb large amounts of solution P. In soils with significant Fe/Al oxide

content, P-fixation is also caused by the oxides greater surface area. Adsorption reactions involving exchange of P for anions on Fe/Al oxides are rapid, while reactions involving formation of covalent Fe-P or Al-P bonds on Fe/Al oxide surfaces and precipitation of P compounds are much slower.

1.4.2.3 Organic soil P

Organic P represents about 50% of total soil P and typically varies between 15 and 80%. Most organic P compounds are esters of orthophosphate including inositol phosphates, phospholipids and nucleic acids. Most inositol phosphates and nucleic acids in soils are products of microbial degradation of plant residues. The common phospholipids are derivatives of glycerol and are insoluble in water, but readily degraded by soil microbes. Organic compounds in soils increase P availability by 1) formation of organophosphate complexes that are more soluble, 2) organic anion replacement of $H_2PO_4^-$ on adsorption sites, 3) coating of Fe/Al oxides by humus to form a protective cover and reduce P adsorption, and 4) increasing the quantity of organic P mineralized to inorganic P. Mineralization of P from soil organic matter or crop residues also depends on soil biological activity which increases with both increasing temperature and soil water content.

1.4.3 Phosphorus cycle

The relationships and interactions between these various forms can be illustrated in a P cycle (Figure 4)

The decrease in soil solution P with absorption by plant roots is buffered by both inorganic and organic P fractions in soils. Primary and secondary P minerals dissolve to resupply $H_2PO_4^-$ and HPO_4^{2-} in solution. Inorganic P ($H_2PO_4^-$, HPO_4^{2-}) adsorbed on mineral and clay surfaces can also desorb to buffer solution P. P adsorption is greater in 1:1 clays like kaolinite than 2:1 clays because of the higher amount of Fe/Al oxides associated with kaolinitic clays that predominate in highly weathered soils. Soils containing large quantities of clay fix more P than soils with low clay content. P ions are absorbed better by clay mineral surfaces occupied by divalent cations than those occupied by monovalent cations. For example, clays saturated with Ca^{2+} retain greater amounts of P than those saturated with Na^+. Increased concentration of exchangeable Al^{3+} also increases precipitation and adsorption of P.

Soil microorganisms digest plant residues and other organic amendments (manures, bio-solids, etc.) producing organic P compounds that are mineralized through microbial activity to supply solution P. Water-soluble fertilizer or waste P applied to soil increases P in soil

solution. In addition to P uptake by roots, inorganic and organic P fractions buffer the increase in solution P through P adsorption on mineral surfaces, precipitation as secondary P minerals, and immobilization as microbial or organic P. Maintaining solution P concentration for adequate P nutrition depends on the ability of adsorbed, mineral and organic P to replace soil solution P taken up by the plant.

Both soil pH and soil water content have an influence on soil P dynamics. As pH increases, solution Fe and Al decreases which reduces P adsorption and precipitation and increases solution P concentration. Above pH 7, Ca^{2+} precipitates with P as Ca-P minerals and P availability decreases. Minimum P adsorption at pH 6.0-6.5 corresponds with the pH range of maximum P solubility. In neutral and calcareous soils, inorganic P precipitates as secondary minerals of Ca-P or is adsorbed to surfaces of clay minerals. In most soils, plant available P increases after flooding, largely due to conversion of Fe^{3+}-P minerals to more soluble Fe^{2+}-P minerals. Other mechanisms include increased mineralization of organic P in acid soils and increased solubility of Ca-P in calcareous soils.

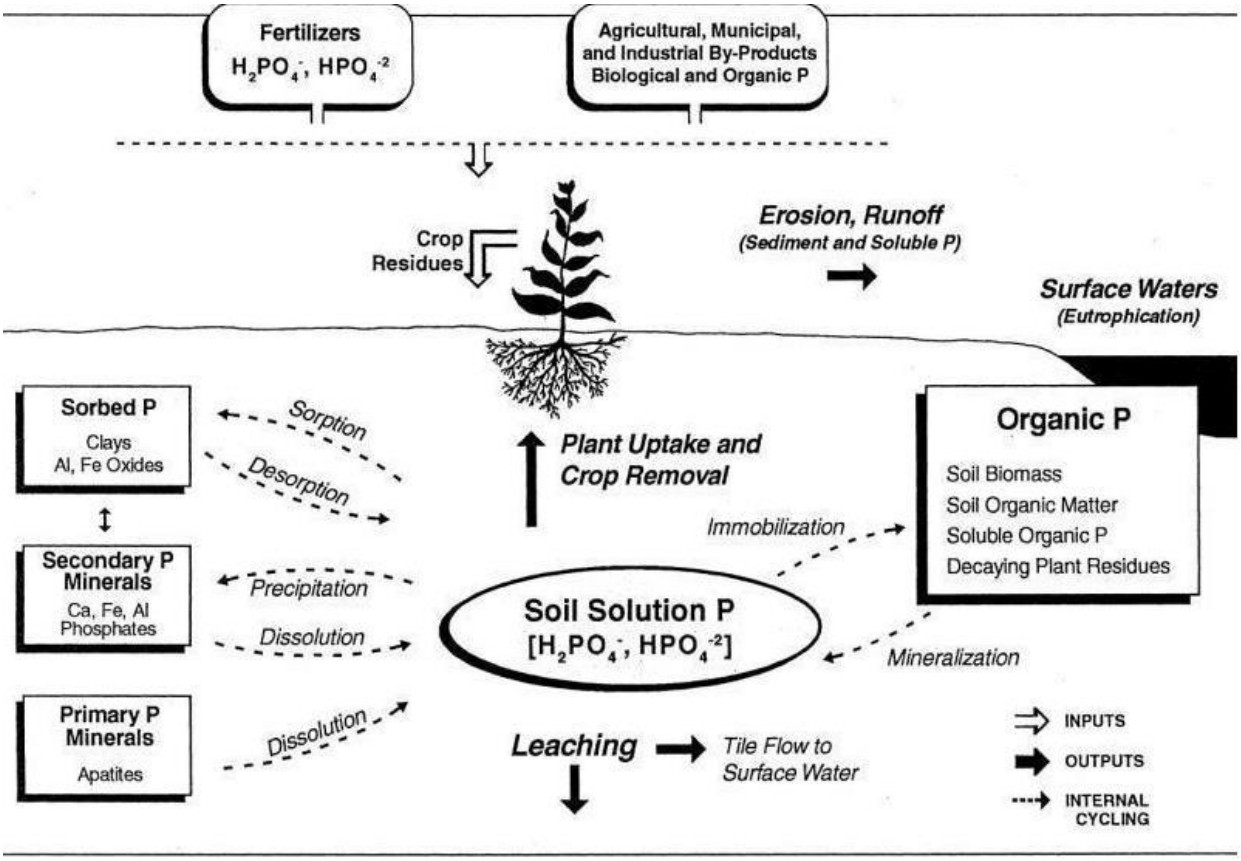

Figure 4 The phosphorus cycle in soil, showing the main mobilization processes in soil as well at the sources and sinks.

[Retrieved from (Pierzynski, McDowell, et al. 2005)].

1.4.4 P run off and eutrophication

While a large part of the world agricultural land is depleted of P, there is P run off occurring at other areas. Agriculture is a major contributor in the non-point pollution of fresh water with P at harmful level. It is mainly due to over application of P fertilizer and the consequent run off leading to eutrophication of fresh water bodies.

The excessive enrichment of waters with anthropogenic sources of nutrients especially nitrogen (N) and phosphorus (P) lead to the transformation of oligotrophic water bodies to mesotrophic, eutrophic, and finally hypertrophic. Mesotrophic and eutrophic phases exhibit intermediate and rich levels of nutrients and show increasing and serious water quality problems, respectively (Ansari & Gill, 2013).

Excess phosphorus inputs to water bodies usually come from two types of nutrient sources, point sources such as sewage, industrial discharges, and nonpoint sources such as runoff from agriculture, construction sites, and urban areas(Ansari & Gill, 2013).

P inputs increase the biological productivity of surface waters by accelerating eutrophication that is responsible for the impairment of surface water quality and restricts water use for fisheries, recreation, industry, and drinking because of increased growth of undesirable algae and aquatic weeds and the oxygen shortages caused by their death and decomposition (Ansari & Gill, 2013). In the Morsa catchment in south-eastern Norway 48% of all main sources of nutrients that led to harmful algae bloom could be contributed to agriculture (Figure 5) (Bioforsk Soil and Environment Fact sheet, November 2012). Furthermore, leaching of P from agriculture remains usually the determining factor of eutrophication of water bodies since point source emissions of sewage are mainly under control (Orderud & Vogt, 2013).

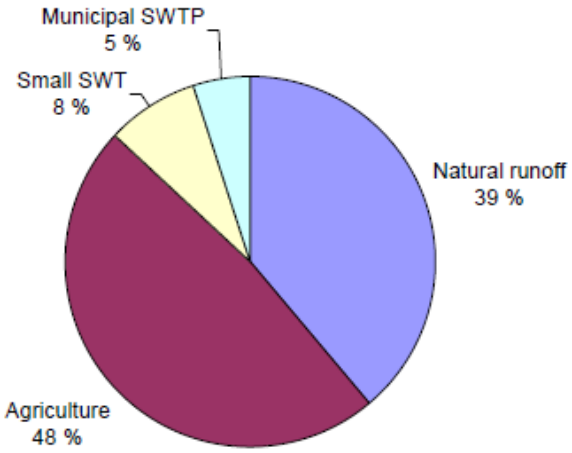

Figure 5 Pie chart showing the share of various sources of P loading in Morsa catchment, Norway

(Retrieved from: http://www.bioforsk.no/ikbViewer/Content/100538/Fact_sheet_Vansj%C3%B8_Morsa_english.pdf 14 March 2016)

1.4.5 Phosphorus in crop nutrition

Phosphorus is a basic element, essential for growth. Therefore, P deficit in soils can limit crop growth and reduce productivity of crops. In natural ecosystems P is recycled between soil and biota. However, in agroecosystems P removed by crop harvest must be replenished in the form of manure, fertilizer or other measures. Due to specific chemical properties of its various forms in the soil, P is readily rendered unavailable to plant roots despite being an abundant element in the soil. Therefore, in order to supply the P required by crops to maintain a good productivity level, we must supply the crops with a constant rate of organic or inorganic P fertilizer.

1.4.5.1 Inorganic Phosphate fertilizer

Historically, phosphorus has been supplied for crop production in manure, human excreta, bone meal and to some extent through guano, but since the discovery of phosphate rock in the 19th century, the rapidly increasing demand for mined phosphate rock has dominated global fertilizer production and has contributed to supplying food to billions of people (Neset & Cordell, 2012). Since the early days of applying mineral fertilizers to soils, phosphate fertilization has always been important. Vast areas of potentially good land are still agriculturally poor because of P deficiency (Mengel & Kirkby, 1987).

Rock phosphate (RP) is the primary raw material used in the manufacture of P fertilizer. The major RP materials are sedimentary deposits found in Morocco, China, Uniterd States and Russia representing nearly 72% of the total world production. RP minerals are apatites [Ca10(PO4)6(F,Cl,OH)2], Fluorapatites being the most common. Solubility of RP increases as soil pH decreases. Therefore, use of RP as a P fertilizer is restricted to very acidic soils in warm, moist climates characteristic of tropical region. RP are slow releasing and therefore result in residual availability. Calcium Phosphates like single super phosphate (SSP) and triple super phosphate (TSP) were widely used as P fertilizer. At present Ammonium phosphates like Monoammonium phosphate (MAP) and Diammonium phosphate (DAP) are widely used due to their high nutrient content. Potassium phosphates are commonly used in horticulture industry. Their high P and K content make them suitable for solanaceous crops such as potato, tomato and many leafy vegetables.

1.4.5.2 Organic sources

Organic wastes are excellent sources of plant available P, with manure accounting for 98% of organic P applied to cropland. The form and content of P in fresh organic materials vary widely depending on source and handling prior to application. With animal wastes, inorganic P ranges from 0.3 to 2% of the dry weight, while organic P ranges from 0.1 to 1%. It is doubtful whether organic P compounds are directly taken up by plant roots (Mengel & Kirkby, 1987). However, organic P compounds are mineralized by microbes to orthophosphate forms and then taken up by roots.

Animal excreta, including human is an excellent organic source of P and has been used in agriculture since long ago. Various industrial wastes like filter cake from sugarcane industries and poultry waste (Mohammad Mohsin, Syed, Sikander, & Syed Azam, 2005), bone meal and wood ash (Boen & Haraldsen, 2011), human urine and wood ash (Pradhan, Holopainen,

Weisell, & Heinonen-Tanski, 2010) are some other rich sources of P that can be utilized to fertilize several crops with P.

1.4.5.3 P fertilizer recommendation for cereal cultivation in Norway

Inorganic P fertilizer has been used in wheat cultivation for more than a century. The application rates differ from place to place and even between cultivars depending on the soil P status and the yield potential.

In Norway, the ammonium-acetate-lactate method (P-AL) by Egner et al. developed in 1960 has been used for estimating the content of plant available P in the soil since 1960.P-AL (mg per 100g soil) has been classified in the main classes as low (0-2),medium (3-6), high (7-15) and very high (>15).The fertilization practice in the last50 years has increased the amount of plant available P in soil (Krogstad, Øgaard, & Kristoffersen, 2008). Therefore, new P recommendations were introduced in grass and cereal farming in 2005 which recommended no need for a P surplus if the P-AL level is medium to high or above. Consequently, a balanced P fertilization strategy for P-AL 5-7 was introduced for meadow and pasture in 2007 and cereal farming in 2008. A balanced fertilization implies adding the same amount of P as removed by the yield. The term P norm is used for the recommended P fertilization to a standardized yield level at P-AL 5-7.The P norm for cereals was reduced from 20 kg P per ha to 14 kg P per ha for a yield of4000 kg/ha (15% water content). A linear correction by 3.5 kg P per 1000 kg deviation from 4000 kg grain per ha is used. If the straw is removed from the field it is recommended to increase the fertilization by 3 kg P per ha (Krogstad et al., 2008).

So, a wheat farmer in Norway, growing wheat in a soil with P-AL 5-7 is recommended to use 14 kg P per ha for standard yield and 17 kg P per ha if straw is removed.

1.5 P availability in the world

Yearly, about 22 million tons of phosphorus (P) from mined fossil phosphate resources is added to the world economy. The size of remaining fossil phosphate resources is uncertain but practically finite (Reijnders, 2014). Ensuring long-term availability and accessibility of phosphorus sources is critical to the future of humanity yet unlike water and energy scarcity, this topic has been largely ignored in research and policy debates on global food security and sustainable resource use until relatively recently (Cordell & White, 2011).

1.5.1 Phosphorus reserves of the world

It is estimated that the total P in Earth's crust amounts nearly to $4x10^{15}$ tons, however economically feasible phosphate rock reserves only amount to $2x10^{9}$ tons (Cordell & White,

2011). World's phosphate reserves are under control of a handful of countries like Morocco, China and US. Out of the estimated 67 billion tons of phosphate reserves in the world, Morocco has 50 billion tons of it. Production rates are increasing in Morocco, while it is decreasing in USand China. USGS (2015) (Table 1). Phosphate reserves are located in very few areas and countries around the world (Figure 6)

Table 1 Annual production of phosphate rocks and reserves around the world (Data is in thousand metric tons) USGS (2015).

	Mine production		Reserves[4]
	2013	2014[e]	
United States	31,200	27,100	1,100,000
Algeria	1,500	1,500	2,200,000
Australia	2,600	2,600	1,030,000
Brazil	6,000	6,750	270,000
Canada	400	--	76,000
China[5]	108,000	100,000	3,700,000
Egypt	6,500	6,000	715,000
India	1,270	2,100	35,000
Iraq	250	250	430,000
Israel	3,500	3,600	130,000
Jordan	5,400	6,000	1,300,000
Kazakhstan	1,600	1,600	260,000
Mexico	1,760	1,700	30,000
Morocco and Western Sahara	26,400	30,000	50,000,000
Peru	2,580	2,600	820,000
Russia	10,000	10,000	1,300,000
Saudi Arabia	3,000	3,000	211,000
Senegal	800	700	50,000
South Africa	2,300	2,200	1,500,000
Syria	500	1,000	1,800,000
Togo	1,110	1,200	30,000
Tunisia	3,500	5,000	100,000
Vietnam	2,370	2,400	30,000
Other countries	2,580	2,600	300,000
World total (rounded)	225,000	220,000	67,000,000

Retrieved from http://minerals.usgs.gov/minerals/pubs/commodity/phosphate_rock/mcs-2015-phosp.pdf

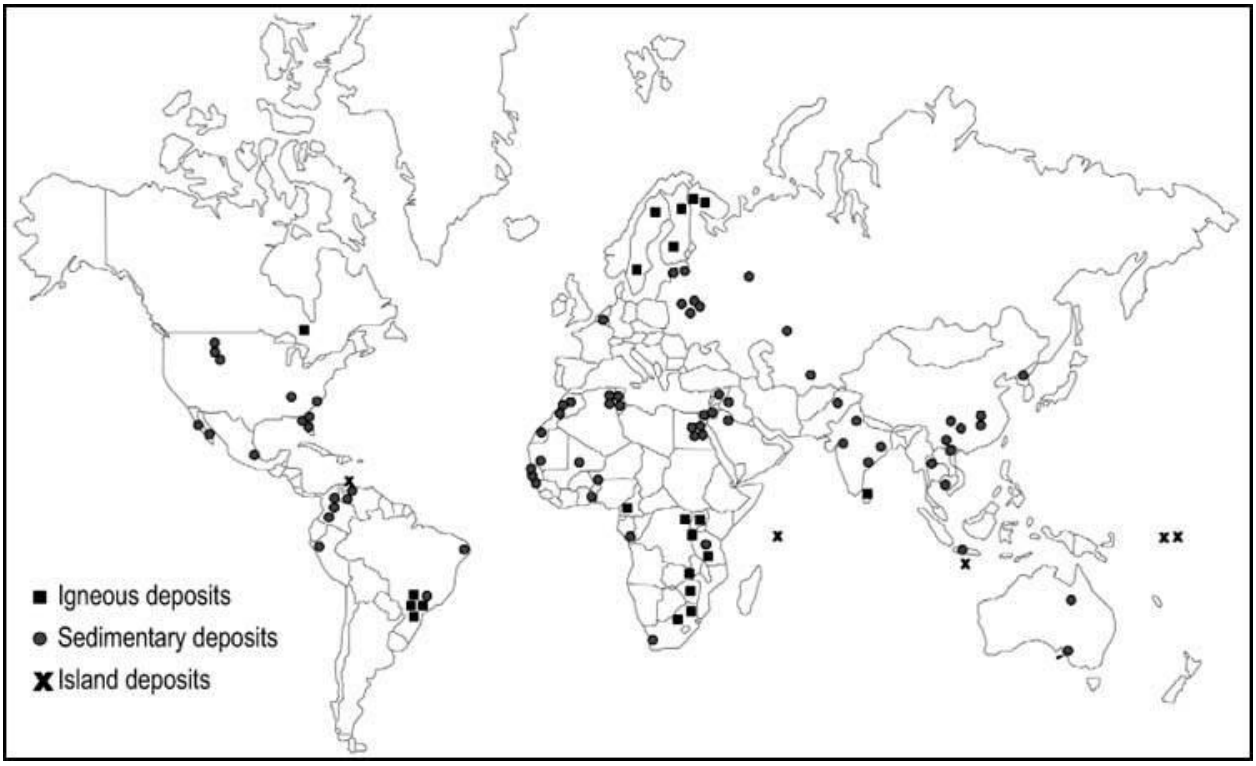

Figure 6 Economic and potentially economic phosphate deposits of the world FAO (2004). Retrieved from ftp://ftp.fao.org/agl/agll/docs/fpnb13.pdf

1.5.2 Peak Phosphorus

Phosphorus is a finite and depleting natural resource like oil. Recently, concept of oil peak has also been adapted to P availability. Various estimates with varying assumption predict peak phosphorus. Some of them predict that existing rock phosphate reserves could be exhausted in 50-100 years (Steen, 1998). However, some others estimate that there is enough reserve of rock phosphate to sustain for 300 to 400 years in future (Van Kauwenbergh, 2010). However, if we look at the historical phosphate rock consumption pattern (Figure 7), it has sky rocketed in the latter half of 20th century.

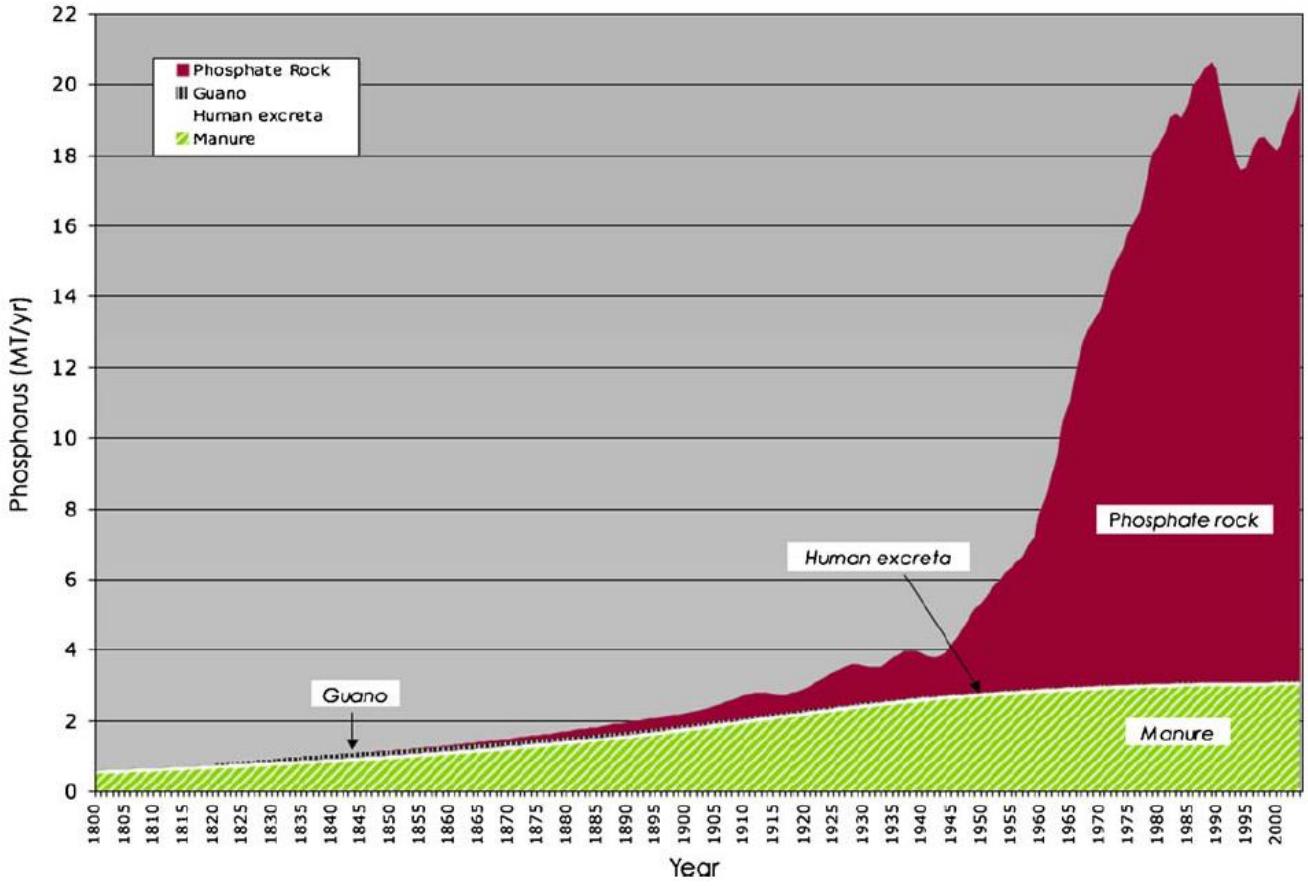

Figure 7 Historical sources of Phosphorus for use as fertilizer (Dana Cordell, Drangert, & White, 2009).

2 REVIEW OF LITERATURE

The concept of critical Nitrogen dilution curve was first developed by Lemaire and Salette (1984) for tall fescue. It was based on whole-plant N concentration represented by an allometric function: $N_c = aW^{-b}$,where W is the total shoot biomass expressed in mg dry matter (DM) ha^{-1}, N_c is the total N concentration in shoots expressed in g kg^{-1} DM, and 'a' and 'b' are estimated parameters. For winter wheat Justes et al (1994) proposed a unique critical nitrogen dilution curve described by the equation, $N_{ct} = 5{\cdot}35DM^{-0{\cdot}442}$,where N_{ct} (critical nitrogen concentration) was expressed in % DM (dry matter) and DM in ton ha^{-1} (Justes, Mary, Meynard, Machet, & Thelier-Huché, 1994). N dilution curve ($N_c = 38.5\ W^{-0.57}$) was determined for spring wheat and was different from those reported for winter wheat (Ziadi et al., 2010). The equation $N_c = 3.40(W)^{-0.37}$ was proposed for maize by (Daniel Plénet & Lemaire, 1999).

The concept of critical nutrient dilution was extended to Phosphorus by Salette and Huche in 1991 (M. Venkatesh, K. Hazra, & P. Ghosh, 2014). Diagnostic critical phosphorus, which is

the P concentration in tissue related to 90% of the maximum, P non-limiting yield, was studied by several researchers. Bolland and Brennan (2005) found out that diagnostic critical P decreased with increasing age in crops like oat, barley, triticale etc (Bolland & Brennan, 2005). However, critical P concentration as a function of shoot biomass was not studied in those experiments.

P uptake by plant is often closely related to N uptake and vice-versa. So, relationship between shoot P and N concentrations was studied by some researchers to determine critical P concentration required to diagnose P deficiency. Ziadi et al (2008) studied the relationship between P and N concentration in spring wheat and found out that, the relationship between shoot P and N concentrations under non-limiting N conditions is described by a linear function (P = 0.94 + 0.107N) in which concentrations were expressed in g kg^{-1} dry matter (DM). Under limiting N conditions, the relationship was different (P = 1.70 + 0.092N) with greater P concentrations for a given N concentration (Ziadi et al., 2008).

An attempt to study critical P concentration as a function of shoot biomass was made by Belanger et al (2015) through their study in wheat. They were not able to develop a critical phosphorus dilution curve, like the ones developed for N. However they proposed a correction of the previous linear model developed by Ziadi et al (2008) by introducing a polynomial model ($Pc = -0.677 + 0.221N - 0.00292N^2$) for critical Phosphorus concentration in relation to N concentration. (Bélanger, Ziadi, Pageau, Grant, et al., 2015)

3 EXPERIMENTAL

3.1 Introduction

Phosphorus deficiency limits the productivity of many crops, including wheat, in many parts of the world (Rashid, Awan, & Ryan, 2005), particularly in many tropical areas, while in temperate areas particularly Europe, excessive use of P fertilizer specially during 1960-1980 created environmental problems like eutrophication of fresh and sea water (Tóth, Guicharnaud, Tóth, & Hermann, 2014). The problem of P application in deficit or excess calls for judicious application of P in agriculture. Optimum P application is also important due to the fact that phosphate rock is a finite and scarce resource and it is depleting. The present challenge with P is therefore not about maximizing or minimizing P application, it is about optimizing P application, so as to be able use the available scarce and expensive P resources more wisely to sustain a desired yield without a load to the environment (Tóth et al., 2014).

Applying P fertilizer more accurately requires estimating P deficiency more accurately. It is necessary to estimate P requirement of crops to be able to apply P fertilizer in correct amount to avoid over or under application. Fertilizer recommendation to farmers is a common practice in agriculture extension globally but recommendation systems differ considerably among countries (Tóth et al., 2014). Soil testing for analysis of soil P concentration is commonly used for making fertilizer P recommendation. Nevertheless, availability of soil P to plants depends on multiple factors ranging from chemical/physical properties of soil, weather and climate to crop species, cultivar and yield potential, besides P status of soil. Therefore, soil P content is often regarded as a poor indicator of P nutrition status of crops. Plant analysis could be a more reliable for estimating plant available P because it measures nutrients that actually have been absorbed by plant (Rashid et al., 2005). Reliability of P recommendation for crops based on plant P concentration rests on the fact that adequate P concentration in the plant tissues ensures maximum crop growth and yield e.g. (Ziadi et al., 2008). Plant P concentration can be used to identify and correct P deficiency more accurately than soil P. However, a critical P concentration, over which there is no further yield increase, needs to be determined in order to diagnose P deficiencies in crops (Ziadi et al., 2008). Once critical P concentration is calculated, Phosphorus nutrition index (PNI) can be calculated in the same way as Nitrogen nutrition index (NNI) by calculating the ratio of plant P concentration to critical P concentration, which further can be used for P fertilizer recommendation.

Plant analysis can play an important role in determining the P nutritional status of crops but diagnostic indices to quantify deficiency are not so well defined for crops, not even for major ones like wheat(Rashid et al., 2005). "Plant-based methods for identifying and quantifying P deficiencies depend on the definition of optimal or critical concentrations, that is, the minimum concentration of a given nutrient required to achieve maximum shoot growth and yield" (Bélanger, Ziadi, Pageau, Grant, et al., 2015). A constant P concentration however cannot quantify P deficiency because many studies show that plants are subject to P dilution by growth. Estimations of nutrient concentration that do not account for this are thus biased and subject to erroneous interpretation (Génard, Baldazzi, & Gibon, 2013).Contrary to critical Nitrogen concentration, which has been well defined for many crops, studies of critical P concentration are very few. The lack of study in this particular area might be due to the fact that P does not directly contribute to emission of green house gases (GHG) like Nitrogen and Sulphur and thus global environmental consequences. A recent attempt to determine a critical P dilution curve for wheat crop was made by Belanger et al (2015). However, their attempt to

derive a model of critical P concentration as a function of shoot biomass could not be successful. In most of the8 experimental site x year combinations, wheat crop did not respond to applied P, because the soil already had enough P. They instead proposed a model which expressed critical P concentration as a polynomial function of shoot nitrogen concentration.

The determination of critical P dilution curve is therefore a novel area of study. Through this pot experiment, we aim to study the effect of P application on P concentration and shoot dry matter of wheat plants at various stages of growth. My working hypothesis was: "Critical P concentration can be expressed as a decreasing exponential function of shoot dry matter".

3.2 Methods and Material

3.2.1 Experimental Site and Plan

A trial was conducted in the growth room of the Department of Environmental Sciences (IMV) from 20th of July to 28th of September 2015. Altogether, 168 pots were sown with wheat (*Triticum aestivum* cv. Bjarne) and 8 different levels of phosphorus ranging from 0 to 135 mg P/pot, applied as a triple super phosphate (TSP) solution [$Ca(H_2PO_4)_2*H_2O$] were used as treatments (Table 2). The two upper doses were split into two applications: half at sowing and half 30 DAS (days after sowing). The first 3 samplings included treatments A, B, C, D, E, F and G. The 4th sampling included all treatments and 5th sampling included B, C, D, E, F, G and H treatments (Table 3). Varying number of replicates were used for various sampling events for the purpose of root sampling. Additional 3 pots were used to study plant phenology.

Table 2 Amount of phosphorus added as TSP solution to various treatments.

Treatments	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Phosphorus level (kg/daa)	0	0,5	1	1,5	2	3	6	9
Phosphorus level (mg/pot)	0	7,5	15	22,5	30	45	90	135
Phosphorus level (mg/kg soil)	0	1,67	3,33	5	6,67	10	20	30

Table 3 No. of pots used for each sampling and treatment.

Treatments	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Total**
Replicates for Sampling 1	3	3	3	3	3	3	3		21
Replicates for Sampling 2	4	4	4	4	4	4	4		28

Replicates for Sampling 3	4	4	4	4	4	4	4		28
Replicates for Sampling 4	4	4	4	4	4	4	4	4	32
Replicates for Sampling 5		9	9	9	9	9	9	5	59
Total	**15**	**24**	**24**	**24**	**24**	**24**	**24**	**9**	

3.2.2 Preparation of growth medium:

The soil medium used for the experiment was Norderåsskogen sand with 94% sand, 3% silt and 3% clay. Soil pH was measured to be 5.1 and soil bulk density 1.46 gcm^{-3}. Total carbon content of the soil was 2.8%, N content 0.1%, P-AL 1.6 mg/100g and K-AL 1.0 mg/100 g.

On 20th of July, pots were filled with air-dried soil at the rate of 3 liter per pot (4.4 kg approx.) and given amount of Phosphorus (Table 2) were added to the designated pots. The first half of the split P application was applied for G and H treatments. Water solutions of other plant nutrients were also added to the soil (Table 4). Soil acidity was adjusted to pH 6.5 with ($CaCO_3$) at a rate of 1 g per liter of soil. Pots were watered up to 80% of field water holding capacity and left overnight before sowing..

Half the amount of initial dose of all the nutrients mentioned in Table 4 were added on 14th of August 20 DAE because some undefined deficiency symptoms appeared in some of the pots. In addition to this, all the pots were fertilized with full amount of Nitrogen one more time at 40 DAE.

Table 4 Amount of nutrients added before sowing in the soil preparation.

Elements	mg element/l soil	Elements	mg element/g soil
N	100	**Cu**	6,77
K	100	**Mo**	0,23
Mg	10,27	**B**	0,24
Fe	8,95	**Zn**	2,84
Mn	5,13		

3.2.3 Growing Condition

On 21st of July wheat seeds were sown 2 cm deep at the rate of 12 seeds per pot. After germination, they were thinned to 9 plants per pot to maintain the plant density at approximately 380 plants m^{-2}(diameter of each pot 17.4 cm and the area 238 cm^2).

Plants were grown at a room temperature of 20^0C and a Photosynthetic Active Radiation (PAR) around 200 μmol m^{-2} s^{-1}. Day length was maintained at 16 hours. Pots were placed randomly in the room and randomized again twice a week (Figure 8). Soil water content was kept at about 80% of the water holding capacity when freely drained, throughout the experiment.

Figure 8 Experimental plants growing in pots inside growth room at IMV

3.2.4 Sampling

Whole pots were sampled 5 times during the experiment from the emergence of the 3rd leaf to maturity. Plants were harvested by cutting with scissors close to the soil surface. Fresh weight was taken right after harvesting. Leaf area were measured by using a leaf area meter (LI-3100C area meter), suitable for both large and small leaves. Total green area was measured in first 3 samplings. Area of flag leaf, second leaf (leaf right below the flag leaf) and remaining part were measured separately in 4th sampling, while area of yellow leaves were not measured.

Harvested plants were dried to constant weight at 60^0C and dry weight was measured. Dry weight of whole plant was measured on first 3 sampling events. Dry weight of flag leaf, second leaf, yellow leaves and the remaining part were measured separately on 4th sampling and dry weight of spike on main tiller, spikes on lateral tillers and remaining part were measured separately in 5th sampling.

Number of tillers per pot were counted on first 3 samplings. Number of lateral tillers with spikes and number of lateral tillers without spikes were counted separately on 5th sampling Number of spikes that reached flowering was counted at 4th sampling. Various parameters measured during sampling events 1 to 5 are listed in Table 5. Time of initiation of important phenological stages were observed throughout the experiment (Table 6)

Table 5 Sampling events and the parameters measured in each sampling

Sampling event	Date	DAS	Parameters measured									
			Fresh wt.	Dry wt.	Leaf Area	Tillers per pot	No. of leaves	No. of spikes that reached flowering	Relative chlorophyll content	No. of spikelets on main tiller	Phosphorus concentration	Concentration of other nutrients
1	10.aug	20	√	√	√	√	√				√	
2	17.aug	27	√	√	√	√	√				√	√
3	26.aug	36	√	√	√	√	√				√	
4	09.sep	50		√	√			√	√		√	√
5	28.sep	69		√		√				√	√	

Table 6 Time of initiation of some phenological stages

Growth Stage	Date	DAS
Sowing	21.jul	0
Emergence	25.jul	4
Booting	26.aug	36
Heading	01.sep	41
Flowering	04.sep	44
Milk development	15.sep	55
Maturity	28.sep	69

3.2.5 Calculation of relative growth rate (RGR)

RGR is measured as the mass increase per aboveground biomass per day.

RGR is calculated using the following equation,

$RGR = (\ln W2 - \ln W1)/(t2 - t1)$

Where, ln= natural logarithm

t1= time one (in days), t2= time two (in days)

W1= Dry weight of plant at time one (in grams),

W2= Dry weight of plant at time two (in grams)

3.2.6 Measurement of Photosynthesis

Net CO_2 assimilation rate of the leaf (**A** in μmol CO_2 m^{-2} s^{-1}), was measured using the infra-red gas exchange analyser CIRAS-1 (Amesbury, MA, USA) which is a portable system that features integral control of CO_2 (using mini CO_2cartridges) and H_2O.

Photosynthesis was measured once at 43-44 DAE. We selected two flag leaves from each sample pot (6 replicates from each treatment A, D, F and H) based on representativeness and placed in the cuvette. Temperature was set at 20°C and the CO_2 level nearly 400 ppm for all measurements. Leaf area inside the cuvette was measured and the settings were adjusted accordingly. Photosynthesis was measured at decreasing levels of irradiance (1000, 500, 350, 200, 100, 50 and 0 μmol photons m^{-2} s^{-1})and the parameters. Values of **A** in μmol CO_2 m^{-2} s^{-1}were plotted against the irradiance values to get an Assimilation curve

3.2.7 Measurement of chlorophyll content

Relative Chlorophyll content was measured with Hansatech CL-01 in pots used for light curves determination. The content was measured on nine flag leaves from each pot.

3.2.8 Determination of phosphorus

P concentration of all samples was determined by Gilford's instrument using molybdate spectrometric method. However, P concentration was also measured by Inductively coupled plasma atomic emission spectroscopy (ICP-AES) for some samples. P concentration of flag leaf, second leaf, yellow leaf and remaining part was determined separately only in 4th sampling. At 5th sampling spikes were separated from the remaining part before determining P concentration. The P concentration of spikes however were not measured due to time constraint.

3.2.9 Gilford's instrument using molybdate spectrometric method

The first step was to digest the sample using Nitric acid (HNO3) win an Argon autoclave at a temperature of 250 ˚C and a pressure of 50 bar for 2 hours. For this, the whole sample was finely ground, and 0.2 – 0.3 g of it (0.1 in for some samples) was weighed and placed in teflon tubes, to which 2 ml of de-ionized water and then 5ml of HNO3 was added. In the Argon autoclave, the water bath was filled with 370 ml of deionized water, 3 ml of sulphuric acid (H_2SO_4) and 25 ml of Hydrogen peroxide (H_2O_2). The teflon tubes were transferred to the sample racks and then placed above the water bath inside the autoclave. The digested samples were taken out and diluted to 50 ml by adding deionized water, shaken well and left to sediment.

The actual determination of P content was done using Gilford's instrument using molybdate spectrometric method. A volume of 5 - 10 ml of the digested samples were taken for the analysis (diluted 5, 10 or 12.5 times as required). Blank tests were carried out parallel with the determination, by the same procedure, using the same quantities of all reagents but using appropriate volume of deionised water instead of test portion. A standard orthophosphate solution of concentration 50 mg/L was taken and diluted to 4 different concentration (0.25, 0.5, 0.75 and 1 mg/L). These solutions were used as standards for calibration. Appropriately diluted samples were taken in glass tubes. 0.4 ml of ascorbic acid and molybdate were added to each sample and mixed well. The samples were left for 15 minutes for colour development. Absorbance of each sample was measured using Gilford's instrument at 700 nm. A graph was plotted with Absorbance at Y-axis against concentration in X-axis. Slope of the graph was determined. Standards were repeated for each analysis batch to get a new standard curve to verify the graph. Slope of standard curve was used for calculating the phosphorus concentration of the sample solution based on the absorbance values from spectrometric measurements.

3.2.10 Inductively coupled plasma atomic emission spectroscopy (ICPAES)

Amount of flag leaf and second leaf from single pot was very low to be analysed by spectrometric method. So, P concentration of flag leaves and second leaves from 4th sampling was determined by using the ICP-AES facility at the laboratory of Department of Environmental Science, NMBU. This method was also employed for samples from 2nd sampling so as to check the accuracy of Gilford's method.

3.2.11 Data Analysis:

The data program R (R i386 3.2.2) was used to perform linear regression analysis and analysis of variance (ANOVA). When ANOVA indicated a statistically significant effect, a Tukey's test at 95% confidence interval was run to compare treatments.

3.3 Results

3.3.1 Deficiency symptoms at early growth

As early as 20 DAE, deficiency symptoms started to appear in the lowest leaves. In treatments A, B, C and D, the lower leaves started dying starting from the lowest leaves. The symptom was different from the normal senescence in that leaves appeared dry but still green (bluish green) in the beginning and later turned yellow. In treatment E, F, G and H these symptoms were not prominent except in some pots. However, yellowing of tips in random leaves was observed in all these treatment.

Half the amount of all the nutrients (except for P) added at soil preparation was added to each pot as soon as deficiency was observed. This helped to correct the deficiency.

3.3.2 Effect of P application on shoot dry matter

3.3.2.1 Yield response to P application

Shoot dry matter showed a positive correlation with P application in all growth stages (Fig. 9). Plants that received higher P doses were bigger than the ones that received lower doses Fig 10). The linear relationship between shoot dry matter and P application in all samplings indicates that, our wheat plants were growing under limited P supply. Shoot dry matter did not level off even at doses as high as 135 mg P/pot. In the third sampling, there was no response to the last P application. A two sample t-test showed that there was no statistically significant difference between yields from treatment F and G. This can be explained by the fact, that the P application to treatment G and H was split into two doses and the second dose had been applied only a week before the third sampling.

Our data points did not show whether the shoot biomass observed for highest P application treatment was the maximum value or not. So, we are not sure if the corresponding P concentration was critical P concentration or not. The plants provided with second half of the split dose of P recovered well and showed a positive response to P application in the later sampling events. However, the response was relatively smaller compared to the treatments, in which P application was not split. So, a separate regression line was fitted more accurately for the last three treatments.

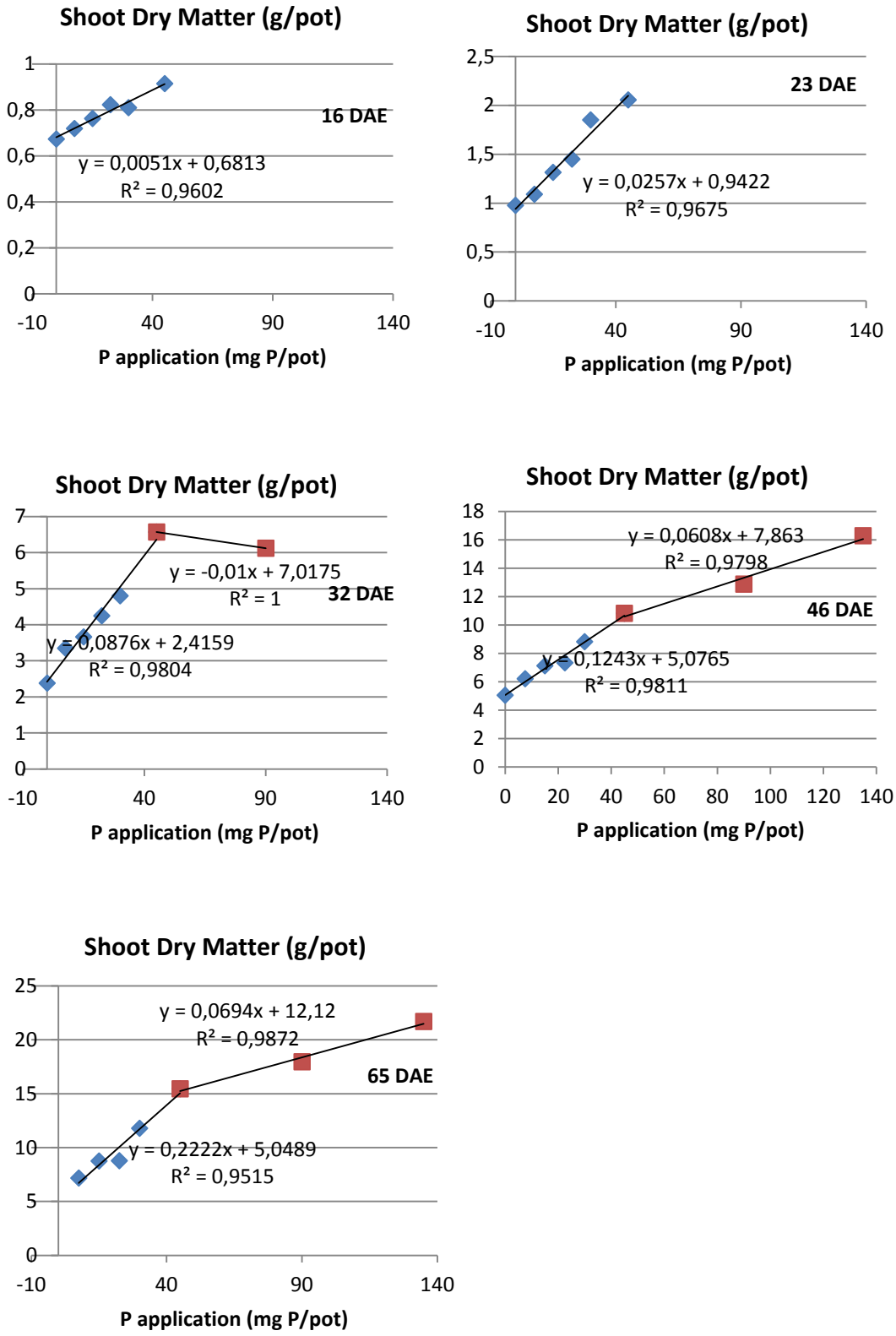

Figure 9 Shoot dry matter weight in g/pot by samplings, in response to P application in mg P/pot. The two upper P doses were split into two equal applications, one at sowing and the other at 26 DAE (Day after plant emergence). The treatments with the highest P application.

(H) was omitted in the first three samplings, while the treatment without P application (A) was omitted in the last sampling.

Figure 10 Difference between plants of treatment A and G observed during 1st sampling, 16 DAE (left) and plants of treatment A and H observed during 4th sampling, 46 DAE (right). Pictures are not to scale.

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3.3.2.2 Distribution of dry matter at the 4th sampling (46 DAE)

At this sampling event plants were divided into flag leaf, second leaf, yellow leaves and the remaining part and weighed separately. The flag leaf had lower weight than the second leaves and the yellow leaves (Figure 11). Although treatment A had higher dry matter of yellow leaf than treatment B, a two sample t-test showed that there was no significant difference between these two treatments.

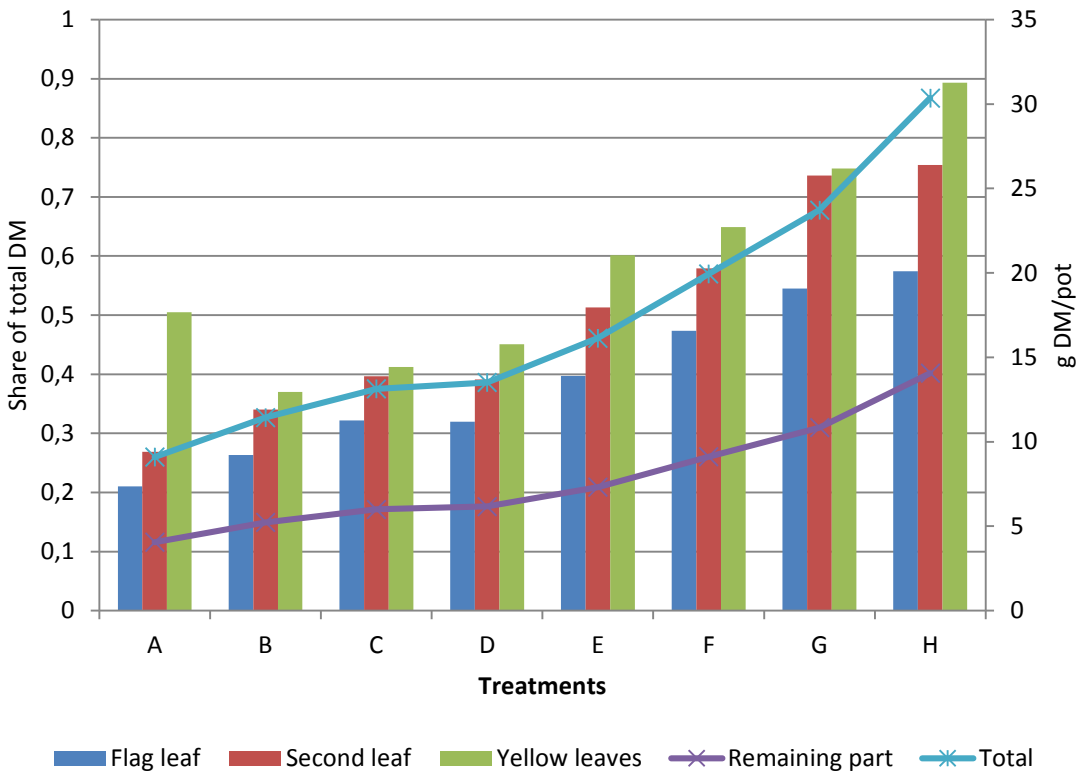


Figure 11 Dry matter (g/pot) of flag leaf, second leaf, yellow leaf, remaining part and total shoot dry matter of all 8 treatments during 4th sampling (46 DAE). Dry matter of flag leaf second leaf and yellow leaves are to the scale of Y-axis in the left and that of remaining part and total plant are to the scale of Y-axis in the right.

3.3.2.3 Dry matter distribution between shoot and root

The root biomass was assessed on treatment A, D, F and H only once at 4th sampling. It increased with higher P application in the same way as shoot dry matter (Figure 12) ANOVA conducted for root dry matter weight showed significant difference between treatments. Shoot to root ratio remained constant except for treatment H (Table 7), though this difference was statistically insignificant.

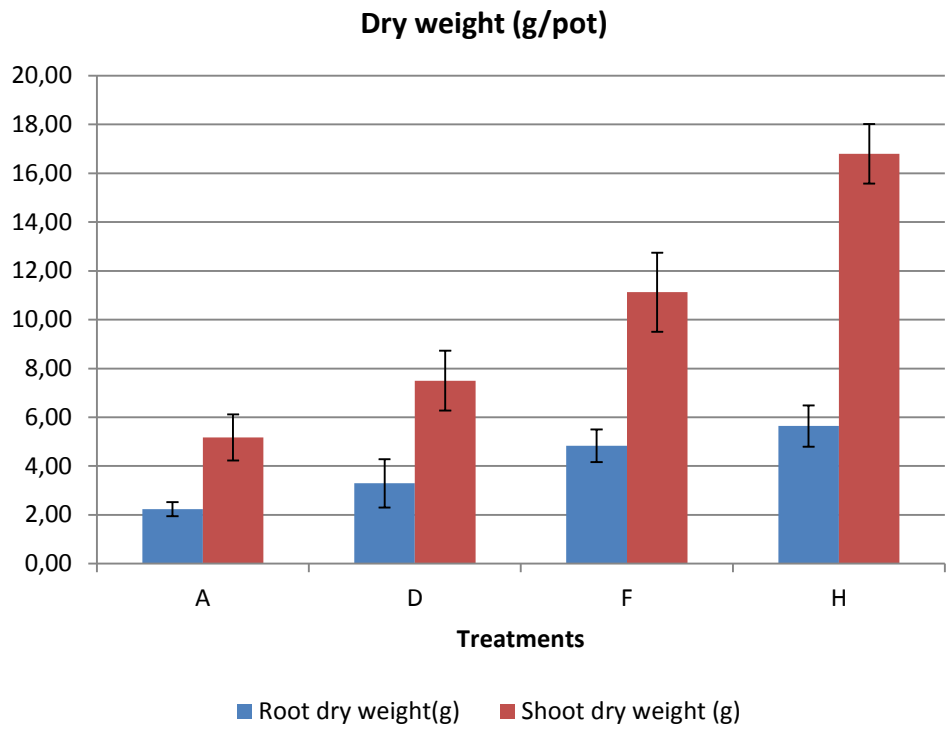


Figure 12 Root and shoot biomass (g/pot) at 4th sampling (46 DAE). Data are the average of 4 replicates from treatments A, D, F and H. Standard errors are also displayed.

Table 7 Shoot/root dry weight at 4th sampling (46 DAE). Data are the average of 4 replicates from treatments. Differences in shoot to root ration were not significant.

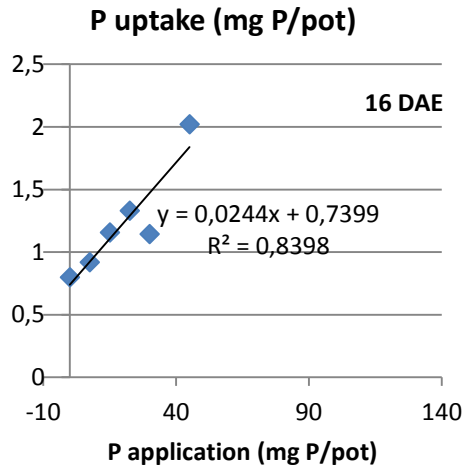
Treatment	Root dry weight(g)	Shoot dry weight (g)	Shoot/root dry weight
A	2,23	5,17	2,32
D	3,29	7,50	2,28
F	4,84	11,12	2,30
H	5,64	16,80	2,98

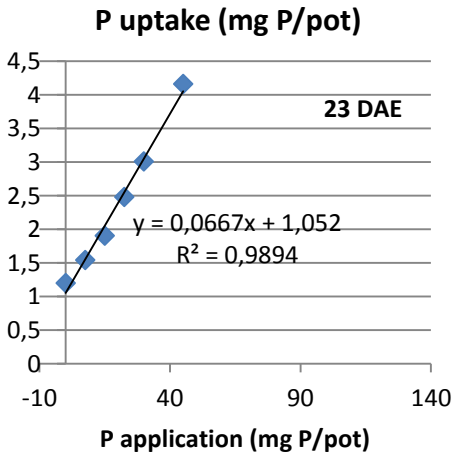
3.3.3 Effect of P application on P uptake

3.3.3.1 P uptake in response to P application

Dry weight and phosphorus concentration was not measured for the root. So, P uptake refers to P accumulated in the aerial part. Also, we did not measure the P concentration of the spike in the last sampling and therefore we do not have the data for total P concentration in the last sampling.

P accumulation in the shoot was proportional to the P application, except treatment E at sampling 1 (16 DAE). This treatment in is probably not representative of the real response, as it is quite lower than expected from response to adjacent applications levels (Figure 13)





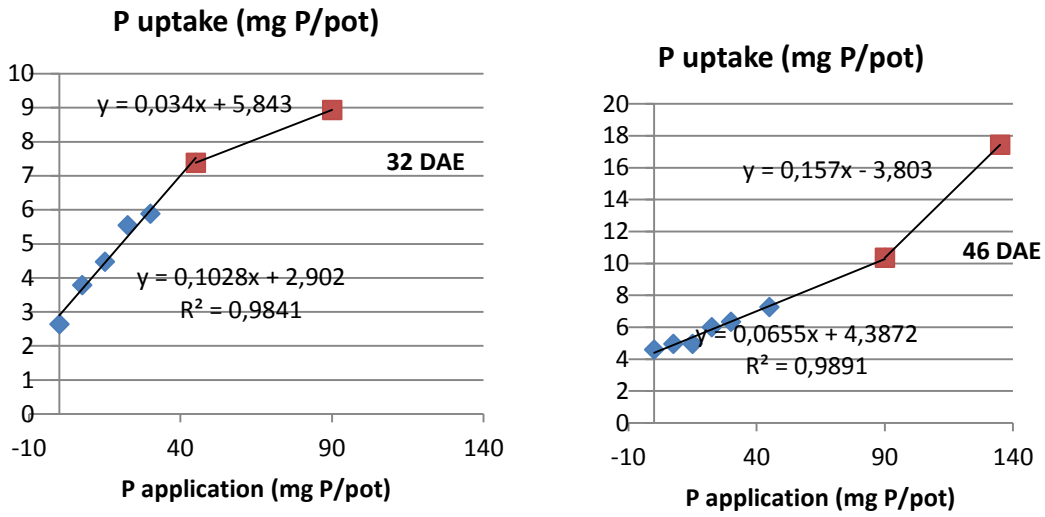


Figure 13 P accumulation by plants shoots in mg P/pot in the first four sampling events, in response to P application (mg P/pot). Data are averages of 3 or 4 replicates

3.3.3.2 Phosphorus use efficiency (PUE)

In literatures, PUE has been defined as the efficiency of plants to uptake P from soil and thus expressed as the ratio of P uptake to P applied. However, based on the basic definition of efficiency, PUE can also be defined as shoot dry matter output per unit P uptake by plants. I have used the following formula to calculate PUE in this study.

PUE during a growth period 't' days = gain in shoot biomass during time 't' in g /P uptake during time 't' in mg

In the 1st and 2nd sampling PUE gradually decreased with increased P application Table 8). In the 3rd sampling, the differences between treatments were not pronounced. However, in the 4th sampling PUE increased with increased P application, peaked to 8.93 g DM/mg P at treatment E and then again decreased to 0.72 g DM/mg P at treatment H. The peak PUE also corresponded to the lowest P concentration at treatment E in the 4th sampling. PUE for treatment F in the 4th sampling gave a negative value, which is not possible practically under our experimental condition. So, this value was not considered.

Table 8 PUE (in g DM/mg P) of treatment A - H calculated at four different sampling dates (16, 23, 32 and 46 DAE).

Treatment	PUE 16 DAE(g DM/mg P)	PUE 23 DAE(g DM/mg P)	PUE 23 DAE(g DM/mg P)	PUE 23 DAE(g DM/mg P)
A	0,84	0,75	0,98	1,36
B	0,78	0,59	1,00	2,44
C	0,66	0,74	0,91	7,33
D	0,62	0,54	0,91	7,01
E	0,71	0,56	1,03	8,93
F	0,44	0,56	1,49	-31,81
G	0,47	0,50	0,84	4,76
H				0,72

3.3.3.3 Allocation of P to different plant parts:

In the 4th sampling, the leaf area, dry matter and P concentration were measured separately for flag leaves, second leaves, yellow leaves and the remaining part. Phosphorus was not distributed evenly between these plant parts. Remaining part contained the highest share of total phosphorus in the shoot for all treatments. For the rest, with an exception to treatment A, the flag leaf contained the highest amount of P, followed by the second leaf and then yellow leaves (Figure 14).

The amount of P in the flag, second leaf and remaining green parts increased with P application. Treatment G and H differed significantly in P uptake from all other treatments and also between each other. the difference was significant for treatments above F from, There was little difference between treatments in the total amount of P remaining in yellow leaves, yet significant difference was observed between the pairs B-F, B-G and C-G.

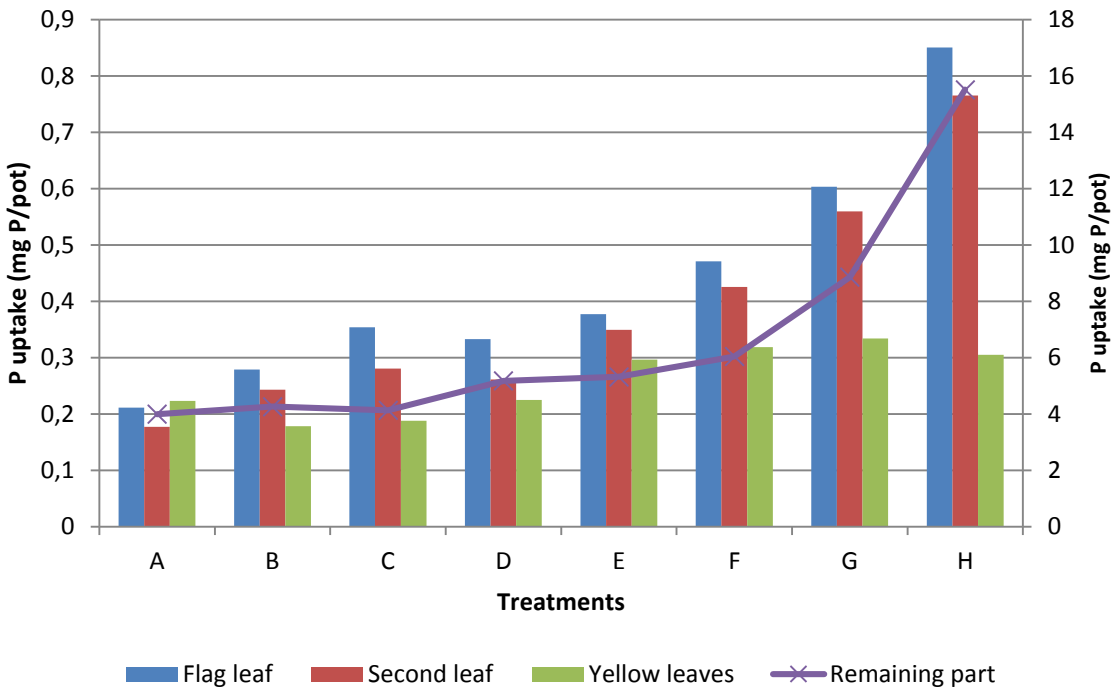


Figure 14 The allocation of phosphorus uptake (mg/pot) to flag leaf, second leaf, yellow leaf and remaining part in plants for 8 P applications. The data are from (46 DAE). P uptake of flag leaf, second leaf and yellow leaves are to the scale of left Y-axis while P uptake of remaining part is to the scale of right Y-axis.

3.3.4 Effect of P application on P concentration in the shoot:

3.3.4.1 P concentration in response to P application

P concentration in the total shoot DM increased with P application in the first two sampling from roughly 1,2 to about 2 mg P/g DM. In the third sampling the effect of P application on the P concentration was less pronounced (Figure 15). In the 4th sampling P concentration decreased from 0.88 mg/g DM at no P application (treatment A) to 0.65 mg/g DM at 45 mg P/pot (treatment F) and then again increased until it reached to 1.07 mg/g DM at highest P application (treatment H). The increase observed with the two highest P doses could be a consequence of split application.

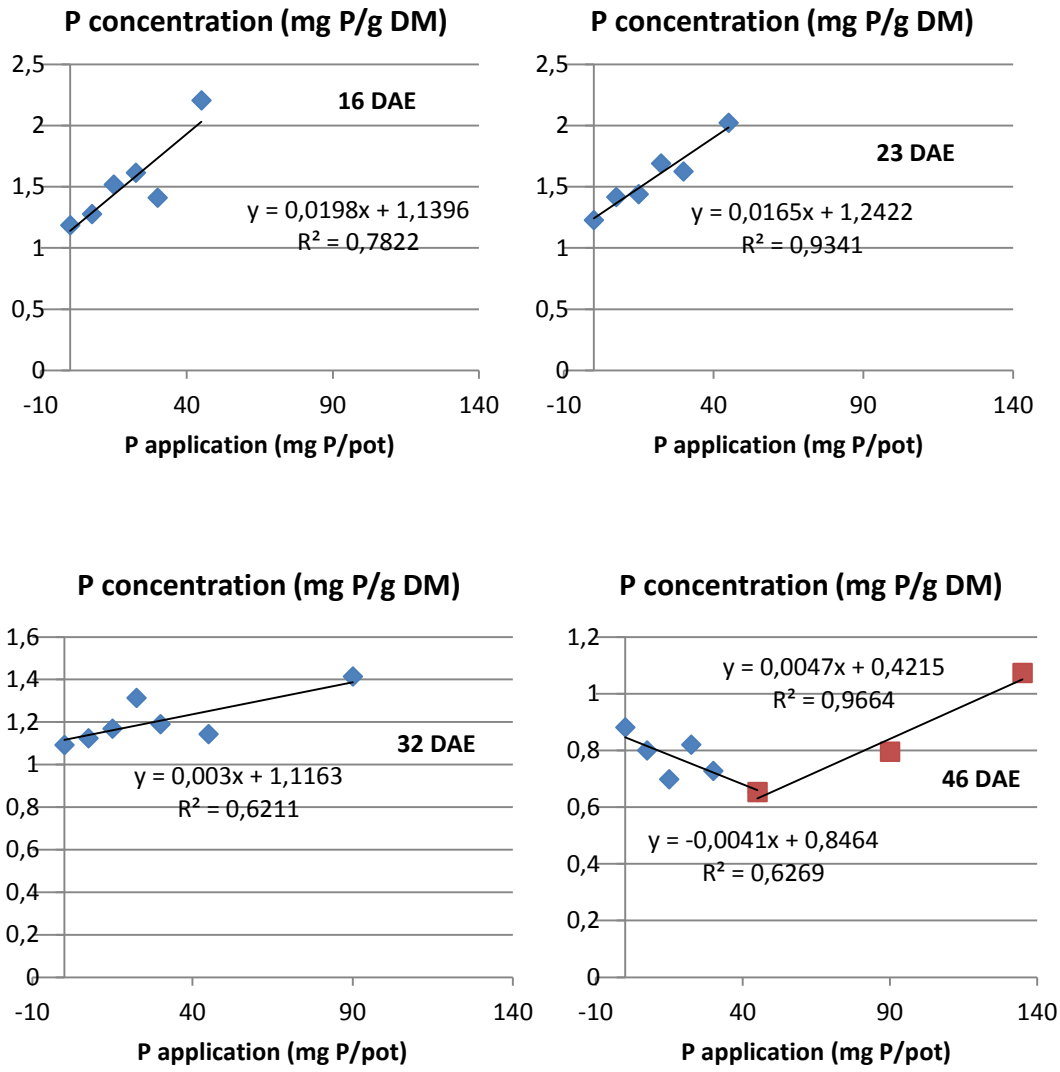


Figure 15 P concentration (mg P/g DM) in plants supplied with various amount of P during sowing, at 4 sampling events. Data are average values of 3 or 4 replicates. Data are averages of 3 or 4 replicates. Treatment E at the first sampling event was an outlier here too.

3.3.4.2 Relationship between P concentration and shoot biomass

When P concentration was plotted against DM/pot (Figure 16), it increased rapidly with plant biomass, in the1st and 2nd sampling events. In the 3rd sampling, P concentration increased with plant size at a diminishing rate until it reached a plateau Finally, in the 4th sampling P concentration at first decreased with increased plant biomass P and then increased again.

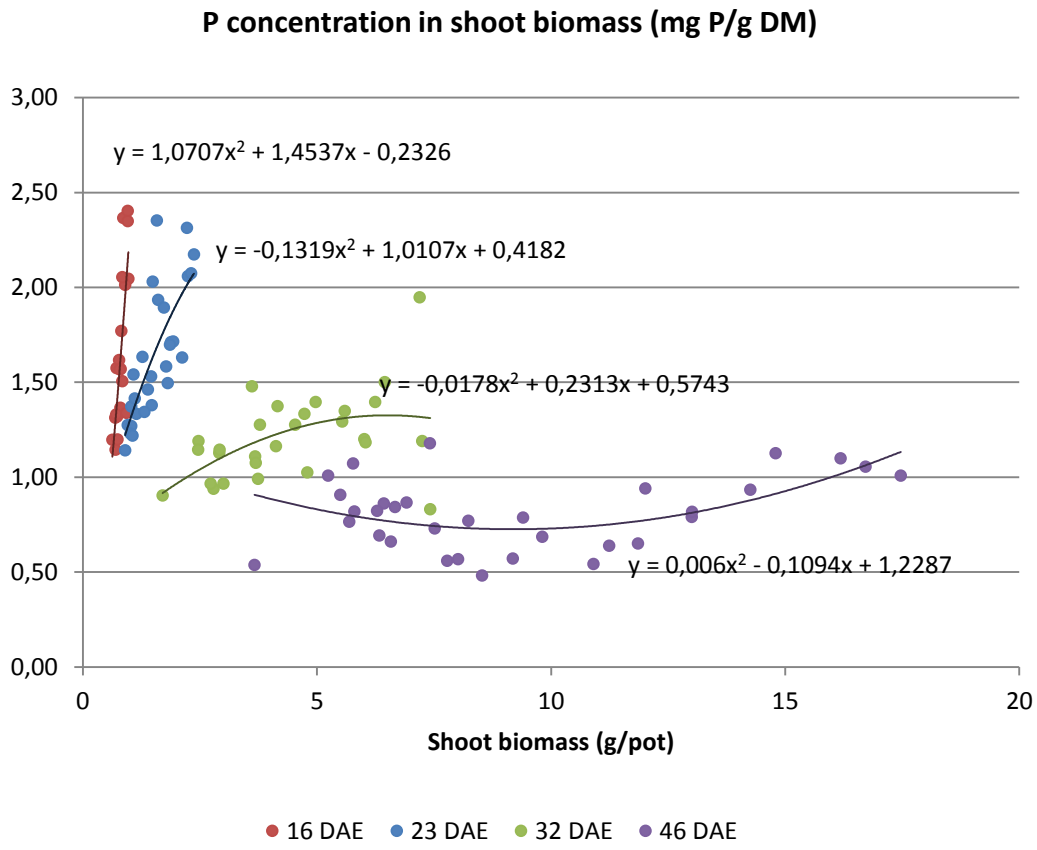


Figure 16 P concentration (mg P/g DM) of all pots from all treatments from sampling 1 to 4 plotted against their respective shoot dry matter (g/pot) and grouped by sampling event.

3.3.4.3 Phosphorus dilution by growth

When P concentration was plotted against dry matter (Fig. 17), it decreased with plant biomass at all P application levels. P dilution was observed in all treatments. P concentration leveled off close to 1 mg P/g DM in treatment A but for other higher P application treatments like E and F, P concentration leveled off closer to 0.5 mg P/g DM.

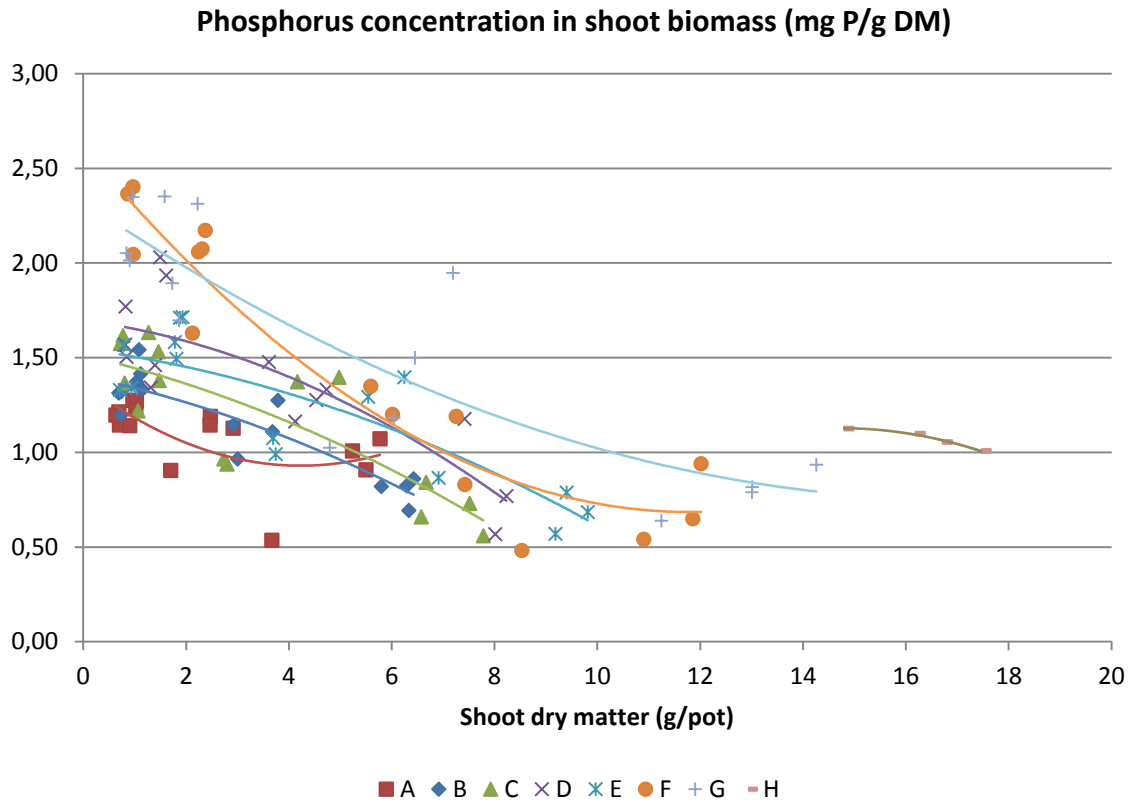


Figure 17 P concentration (mg P/g DM) of all pots from all treatments from sampling 1 to 4 plotted against their respective shoot dry matter (g/pot) and grouped by treatment.

3.3.4.4 P concentration and relative growth rate:

RGR increased remarkably with P application from 1st to 2nd sampling. However, it didn't differ much between treatments at other growth period (between sampling 2 and 3, 3 and 4) (Table 9).

Table 9 P concentration (mg P/g DM) of treatments A - G and their corresponding relative growth rate (g/g DM/day) at three different sampling events (2nd, 3rd and 4th)

Treatment	23 DAE		32 DAE		46 DAE	
	P concentration (mg P/g DM)	RGR (g/g DM/day)	P concentration (mg P/g DM)	RGR (g/g DM/day)	P concentration (mg P/g DM)	RGR (g/g DM/day)
A	1,23	0,05	1,09	0,10	0,88	0,05
B	1,42	0,06	1,12	0,12	0,80	0,04
C	1,44	0,08	1,17	0,11	0,70	0,05
D	1,69	0,08	1,31	0,12	0,82	0,04
E	1,63	0,12	1,19	0,11	0,73	0,04
F	1,98	0,13	1,14	0,12	0,70	0,04
G	2,06	0,10	1,41	0,13	0,80	0,05

3.3.4.5 Differential P concentration of various plant parts

The flag leaves had significantly higher P concentration than the second leaves, yellow leaves and remaining part (Figure 18). In general, the P concentration was remarkably constant irrespective of treatment, about 1 mg/g in flag leaves, 0.7 mg/g in second leaves, 0.7-1.1 mg/g in remaining parts and 0.35 – 0.5 mg/g in yellow leaves. Only treatment H had statistically significant higher P concentration than other treatments in the flag leaf, second leaf and remaining part (1.5, 1 and 1.1 mg P/g DM respectively). Yellow leaves showed a tendency towards decreased P concentration at higher P doses but the difference was not statistically significant.

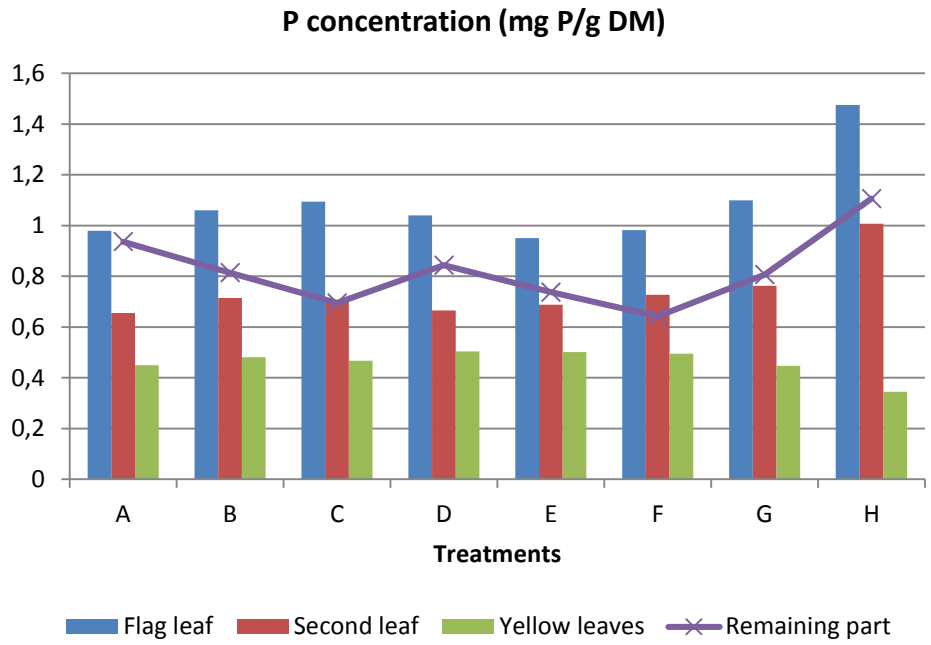


Figure 18 P concentration (mg P/g DM) in flag leaf, second leaf, yellow leaves and remaining part of plants from all 8 treatments. The data are from the 4th sampling (46 DAE)

3.3.5 Effect of P application on leaf expansion

3.3.5.1 Leaf area in response to P application

The green area of the whole shoot was measured at 4 sampling events. The green area was proportional to shoot dry matter at all sampling events, although the proportionality varied with date (Figure 19).

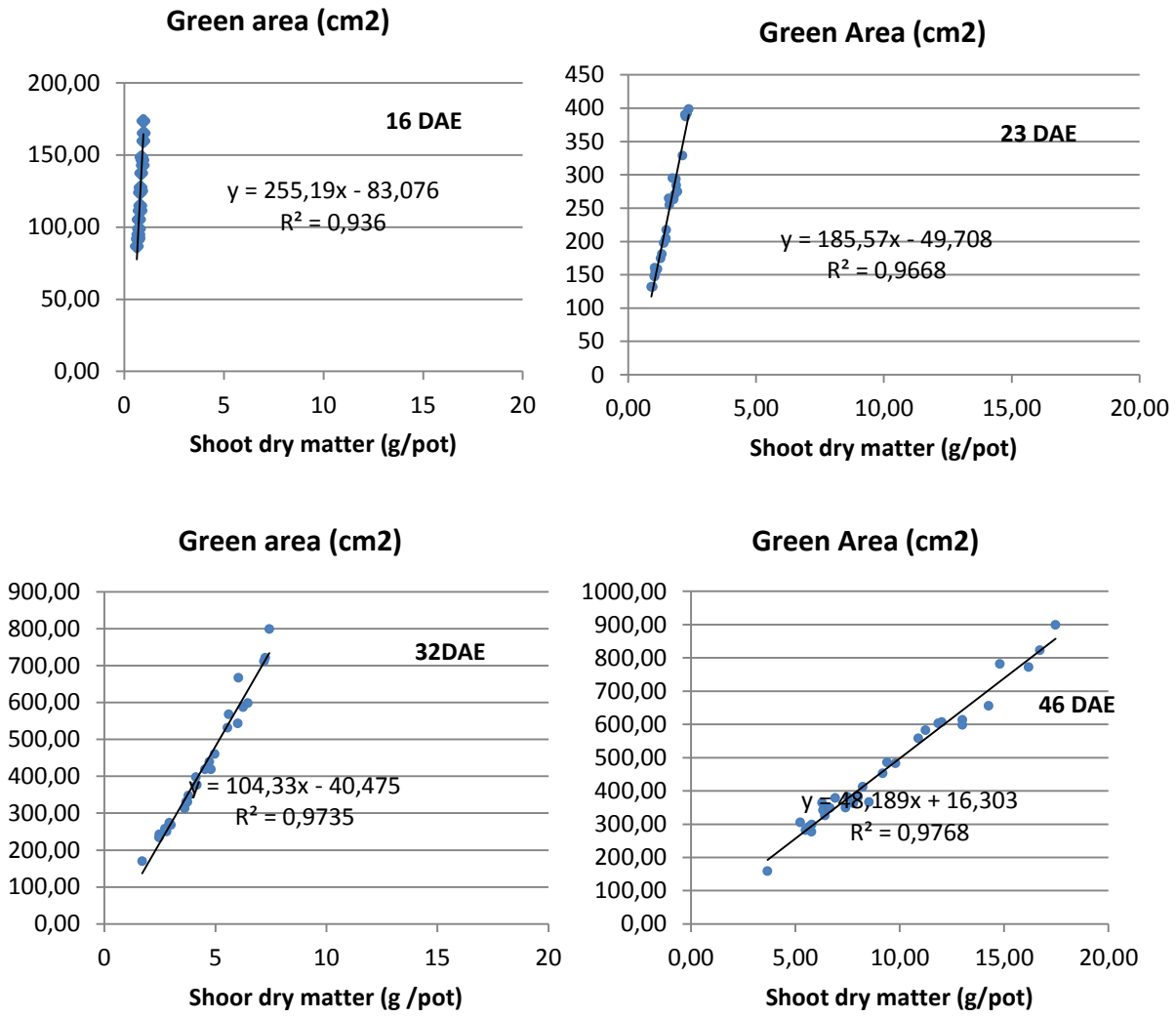


Figure 19 Green area of whole shoot of nine plants (cm^2/pot) plotted against total shoot dry matter (including yellow leaves) in g/pot at 4 sampling events. Points show values of 3 or 4 replicates.

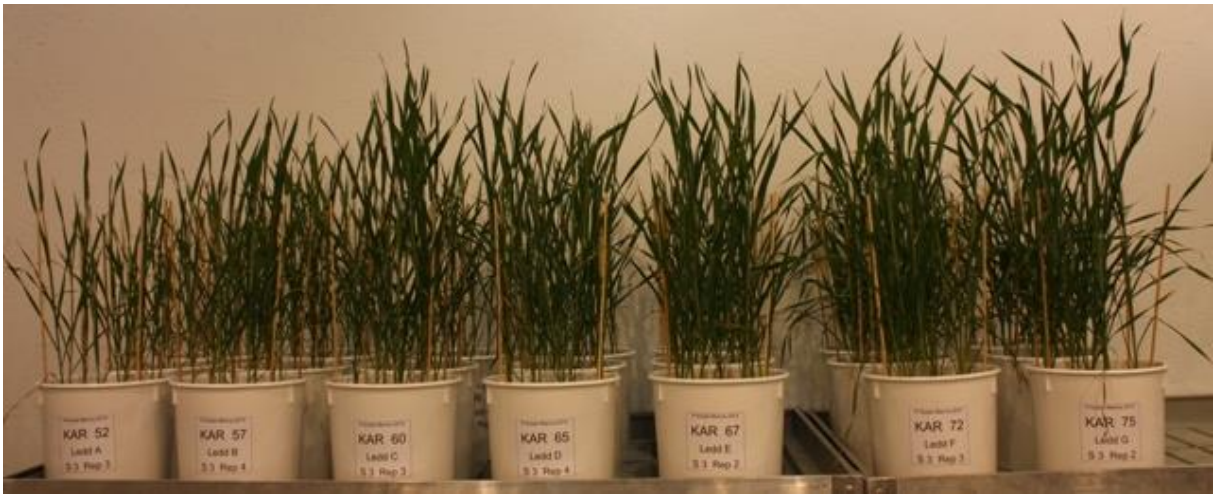


Figure 20 Difference in leaf area of treatments A to H (from left to right) observed during 3rd sampling (32 DAE)

3.3.5.2 Relationship between leaf area and P uptake:

Green area was also proportional to the total amount of P in the shoot (Figure 21) particularly in sampling 1 and 2 but the relationship was not as good as for DM yield.

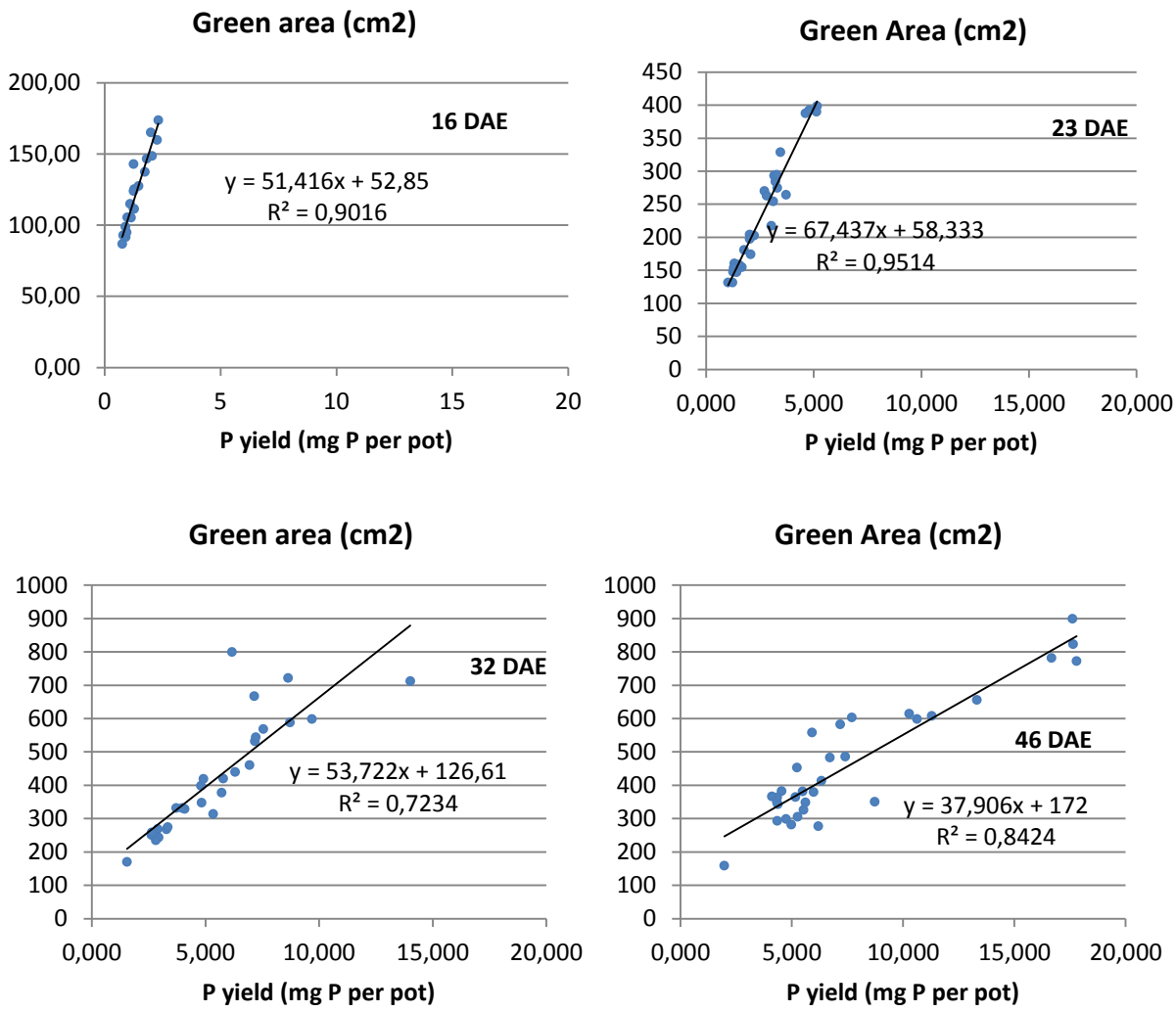


Figure 21 Green area of the whole shoot of 9 plants in cm^2 plotted against P yield in mg P/pot at different sampling events.

3.3.5.3 Green area of various plant parts

In the 4th sampling (46 DAE), the green area of the flag leaf, of the second leaf and of the remaining parts were measured separately while yellow leaves were excluded from green area at all times. The area of flag leaf, second leaf, remaining leaf and whole plant responded positively to increased P application (Figure 22). An ANOVA test conducted for testing the difference in green area of the whole shoot, flag leaf, second leaf and remaining part (excluding yellow leaves) between treatments confirmed significant difference between treatments.

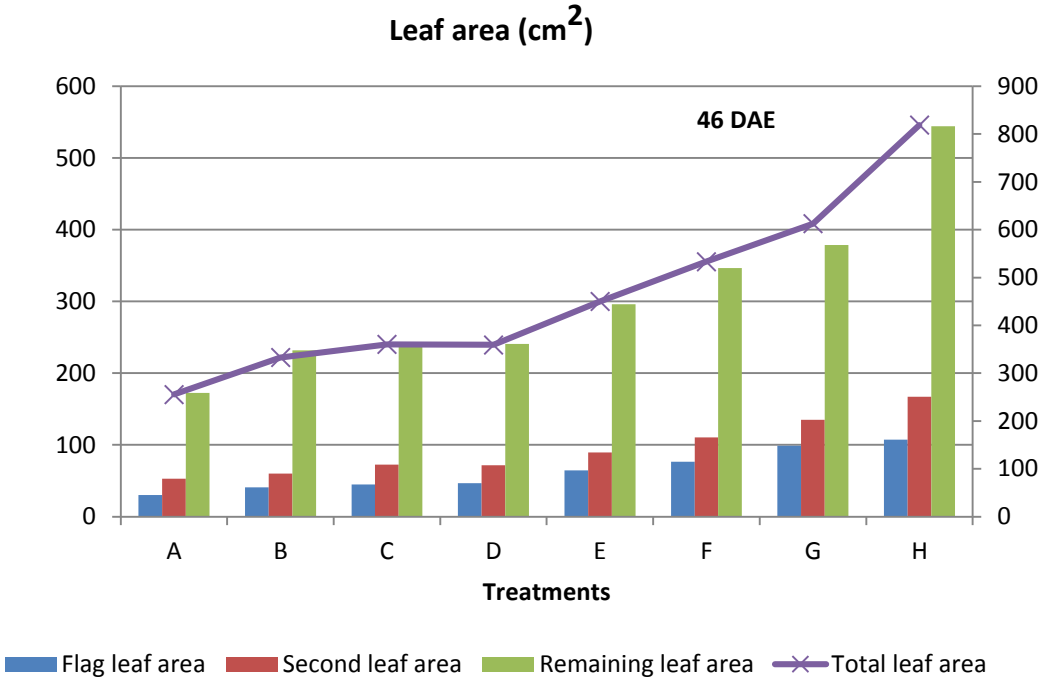


Figure 22 Green area (cm^2)of flag leaf, second leaf and remaining part, sorted by P application, measured at the4th sampling (46 DAE). Total green area is to the scale of right Y-axis.

3.3.6 Effect of P application on tillering and fertile spikelets

P application had a significant positive effect on tillering (Figure 23). Lateral tillers were observed in all treatments except treatment B and A. However, not all the lateral tillers did bear spikes at maturity. Both tillers with spikes and without spikes increased with increased P application. There was statistically significant difference in total number of tillers between treatments. A Tukey test was conducted to analyse the difference in number of tillers with spikes between treatments. Treatments with differences, which are statistically significant, are shown with letters in the plot area.

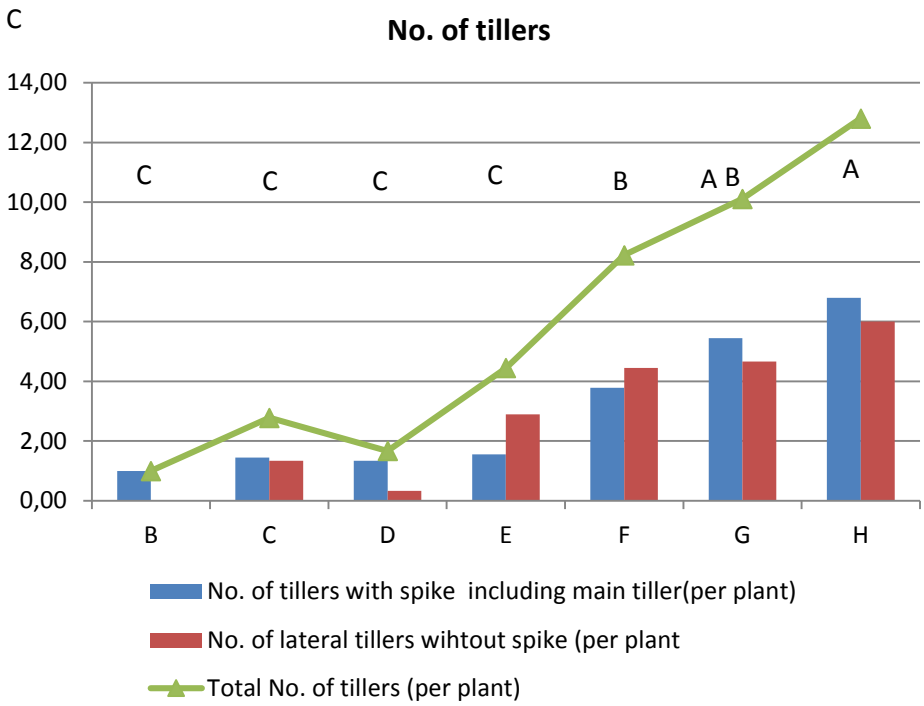


Figure 23 Number of unfertile, fertile and total tillers per plant at maturity (65 DAE), as affected by P application. The letters refer to Tukey's post-hoc pair wise comparison of total number of tillers. Treatments sharing at least one letter are not statistically significant.

The total number of spikelet on the main spike was moderately affected and increased from 15 at no P application to 18 at highest P application (Figure 24)

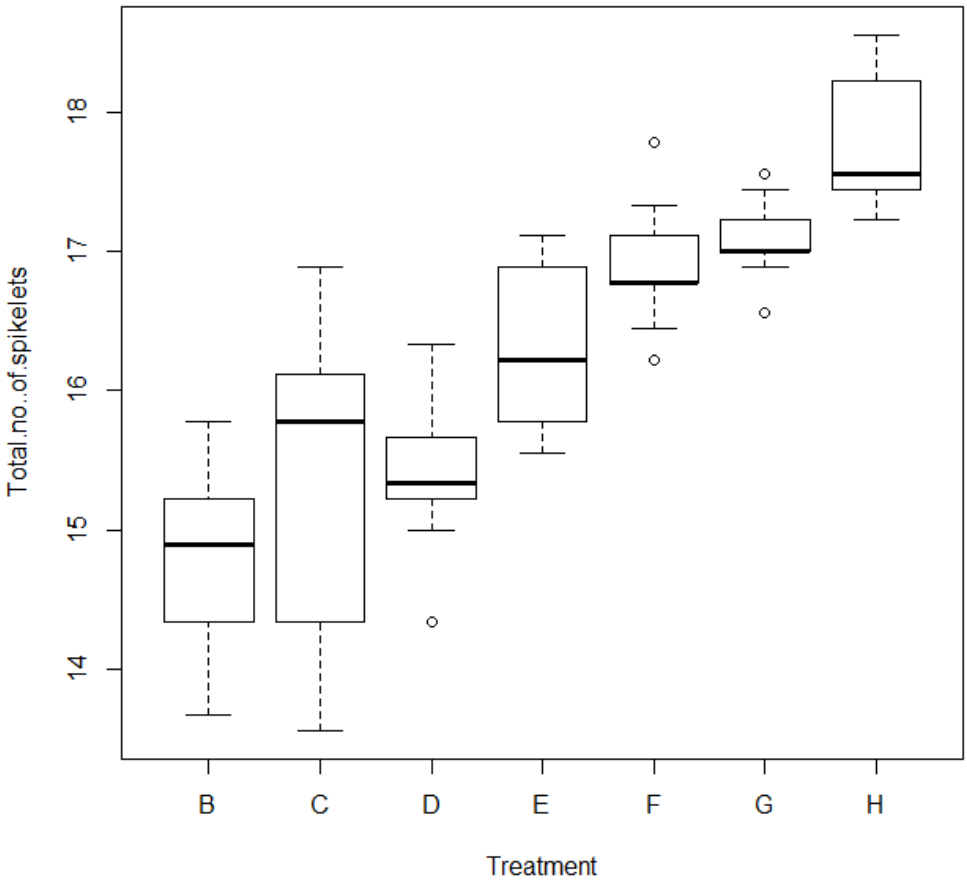


Figure 24 Variation in the total number of spikelets in the main tiller between 7 treatments at maturity (65 DAE). Treatment C showed a large variation in the total number of spikelets in the main tiller thus indicating insignificant difference.

However, the number of fertile spikelet on the main spike increased most , from 8 at no P application to 16 at highest P application. Thus the number of non fertile spikelet decreased with P application (Figure 25). Effect of P application on both the number of fertile and the number of non fertile spikelet was statistically significant. However, there was no difference between the three highest P applications

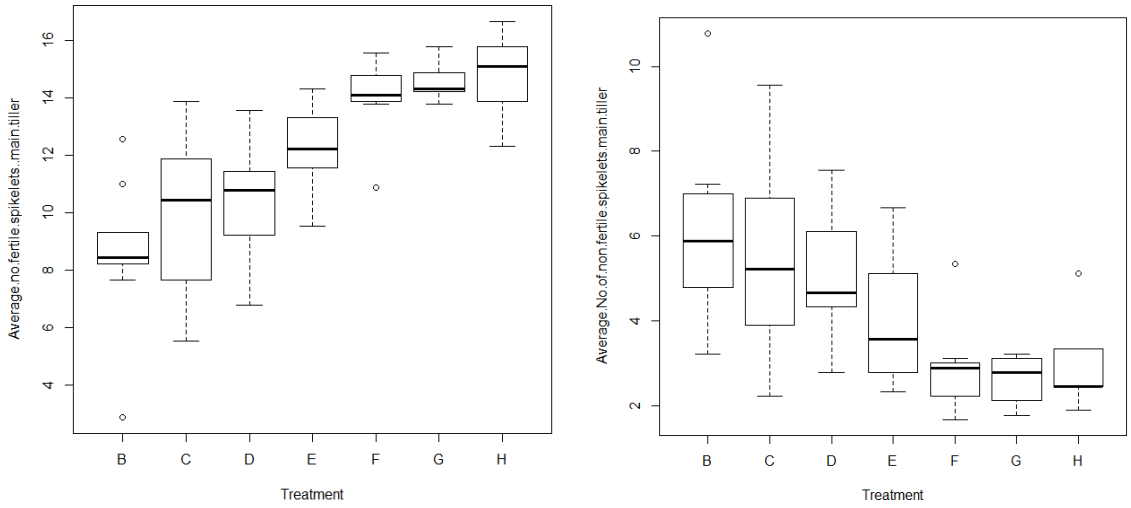


Figure 25 Variation in the number of fertile spikelet (left) and unfertile spikelet in the main tiller among 7 different treatments at the 5th sampling (65 DAE).

3.3.7 Effect of P application on the concentration of other nutrients at sampling 2 (23 DAE)

The concentration of other nutrients was measured only at the second sampling. The ICPAES method was used to control the quality of the Gilford's P analysis method, and at the same time the concentration of other minerals was checked because of symptoms on the leaves that could suggest deficiency of other nutrients, in particular K deficiency. The deficiency or other disorders symptoms appeared first and were most marked in the treatments with no or lowest P application. Potassium showed a large positive response to P application (Figure 26).

Sulphur showed very small but statistically significant, positive response to P application. A post hoc Tukey's test at 95% confidence interval (CI) confirmed that treatment F significantly differed from treatment A, B, C and D. Zinc shows a small, statistically significant, negative response to P application. Tukey's test at 95% CI shows that treatment A significantly differs from treatment B, C, D, E and F. Calcium shows statistically significant, positive response to P application. Treatment F differs significantly from treatment A, B, C and D. Manganese shows a statistically significant negative response to P application. Treatment A significantly differs from treatment B, D and E. P application does not have significant effect on Magnesium concentration.

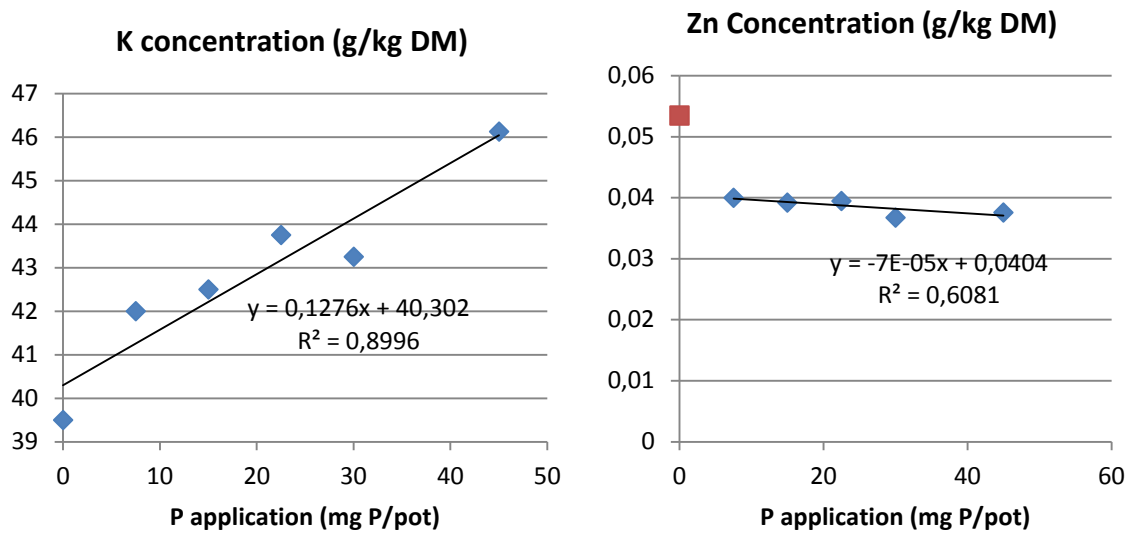


Figure 26 Concentration of Potassium (left) and Zinc (right) in g/kg DM of plants at the second sampling (23 DAE) in response to P application (mg P/pot). Average of 4 **replicates.**

3.3.8 Effect of P application on Photosynthesis

Light response curves were obtained for treatment A, D, F and H by plotting CO_2assimilation against irradiance at 46 DAE just after flowering stage. Assimilation increased at a diminishing rate with increased irradiance, thus giving a response curve with decreasing positive slope (Figure 27). There was a large variation between treatments, and not statistically significant difference was detected by ANOVA at irradiance 500, 200, 100 and 50 µmol photon/m^2s. At 1000 µmol photon/m^2s irradiance, a significant difference was observed between treatments H and F.

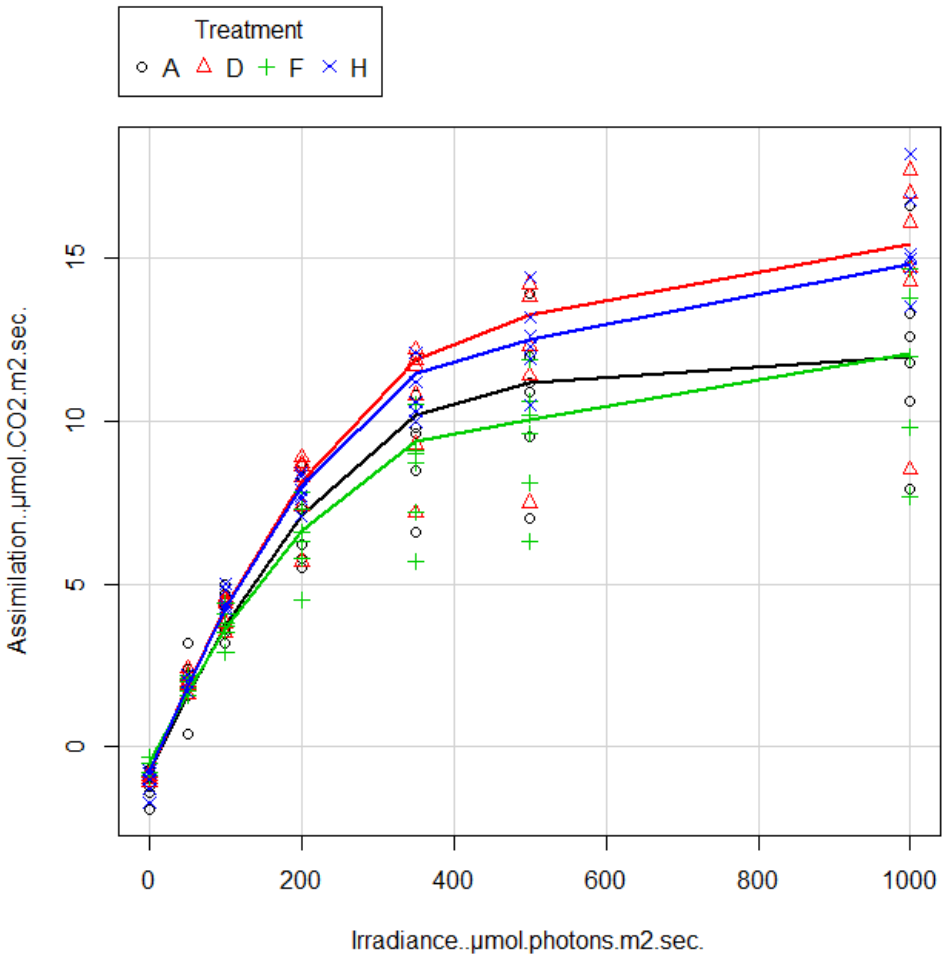


Figure 27 Light response curve showing average assimilation of 4 replicates from treatments A, D, F and H at increasing irradiance level. The data is from 4th sampling (46 DAE). Lines join average values of different treatments.

3.4 Discussion

3.4.1 Yield response and P availability

Shoot dry matter increased from the lowest to the highest P application at all sampling events. Thus, there is no information to say for sure that the critical P concentration in the plant was reached. The highest P concentration observed in the experimental wheat plants was only 0.24% of total dry weight for the highest treatment level at first sampling. Marschner & Rimmington (1988) suggest that the optimal growth for most plants occurs in the P concentration range of 0.3 – 0.5% of the plant dry matter during the vegetative stage of growth. Some other studies report even higher P concentration, for instance, tissue P concentration in well fertilized plants[1] were approximately in the range 0.4–1.5% of the dry

[1] Plants grown under nutrient film technique (NFT) hydroponic system in a nutrient solution containing 0.25 mM KH2PO4 in a glass house

matter. The mean P concentration of well fertilized wheat plants was found to be 0.93% of total dry weight (Broadley et al., 2004). This clearly indicates that our experimental plants were growing under P deficiency and could not reach the non limiting P levels at any stage of growth.

Shoot dry matter respond to P application depending on the P status of the soil or growth medium in which they grow. For instance, Belanger et al (2015), in their field experiment on wheat did not observe any response even at P application rates as low as 10 kg/ha since non-limiting P conditions was achieved with no applied P (Bélanger, Ziadi, Pageau, Lafond, et al., 2015). Contrary to this, in our experiment shoot dry matter did not level off even at P application rates as high as 135 mg P/pot (equivalent to about 90 kg/ha).

The initial P status of the soil is responsible for this kind of difference in dry matter response. Our soil was very poor in P with a P-AL value 1.6 mg/100g soil. A soil with very poor P concentration was chosen to ensure P limiting condition. As expected, at lower P application rates, most of the P applied was adsorbed by the P deficient soil it was not anticipated that the soil would adsorb nearly at all the P applied even at the highest P application. At 1st sampling, the highest P application level was 45 mg P/pot (10 mg P/kg soil). The adsorption curve for this soil determined by PhD student Iva Zivanovic (Figure 28) shows that nearly all the P applied to the soil was adsorped by the soil.

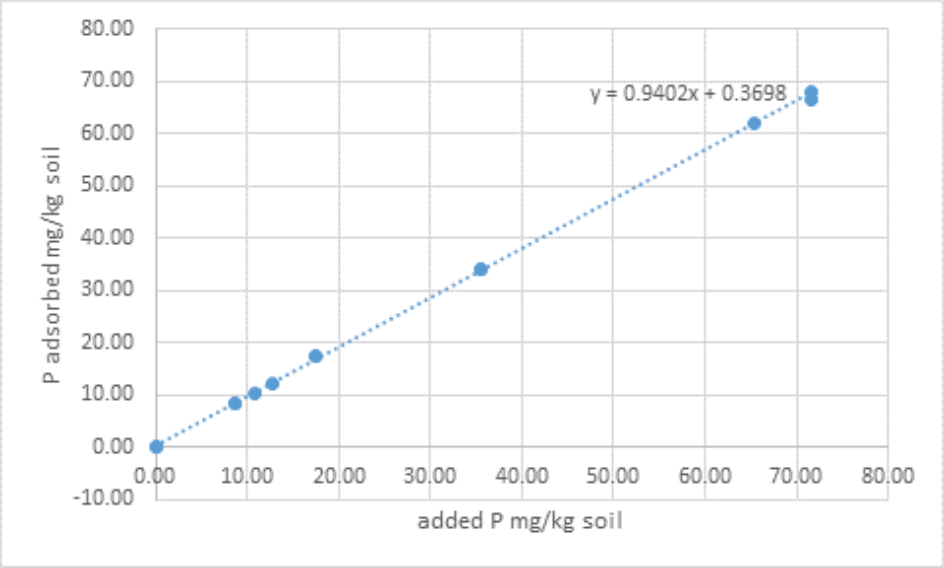


Figure 28 P adsorption curve for the experimental soil deterimed by PhD student Iva Zivanovic. Data points are values of P adsorped by soil against added P.

3.4.2 Dry matter distribution between root and shoot

We observed a significant increase in the root dry weight with increased P application. This results are in agreement with the results of root development in ryegrass where maximum root development was obtained at medium P application rates while shoot dry weight kept increasing until higher application rates (Mazza et al., 2012). Also, in wheat field experiments, dry weight of roots sampled at flowering stage increased with increased P fertilizer, peaked at higher P dose and then decreased (Teng et al., 2013).

However, shoot to root ratio in our pot experiment was constant for three application rates from no P to 45 mg P per pot, and the increase observed at the last treatment 135 mg P per pot was not statistically significant, which is contrary to several studies that report that under P deficiency plants tend to invest on roots more than on shoot.

3.4.3 P uptake and translocation

The P uptake by shoots was proportional to the P application in the soil. The highest P removal observed was 17 mg P/pot (12 % of applied P) at P application 135 mg P/pot during 4^{th} sampling. Our results are similar to that of many other field and pot experiments. In a pot experiment involving P analysis of several Brazilian wheat cultivars grown in pots, supplied with sufficient P and sown at the rate of 2 plants per pot, the P uptake increased with P application (Silva et al., 2016). In wheat field experiment carried out in sub humid region of China, shoot P at flowering and grain P at maturity increased with increase in P supply. (Teng et al., 2013). My results clearly showed that the flag leaves act as a sink for P translocation. Lower P concentration of yellow leaves on the other hand confirms that they act as source of P for re-translocation.

3.4.4 Minimum P concentration and dilution by growth

Unlike shoot dry matter and P uptake, P concentration responded to increased P application in different ways at different sampling events. At first sampling, P concentration increased sharply with increase in P application. At second sampling, P concentration increased with P application at a lower rate. However, in third sampling, P concentration increased with P application at first and then leveled off at higher P application. At the 4th sampling, P concentration decreased with increased P application at first and then increased at higher P application.

In the fourth sampling (43 DAE), the decline in P concentration from a mean value of 0.9 to 0.65 mg P/g DM from treatment with no P application (treatment A) to treatment with P

application at the rate of 45 mg P per pot (treatment F) indicates that lower P concentration is not always a sign of a more severe P deficiency. If we considered treatment A to have better P nutrition than treatment F at this sampling, it would be an error. Therefore, information of shoot dry matter must come together with P concentration in order to be able to quantify deficiency.

The P concentration of whole plants at any growth stage was never below a minimum value of 0.5 mg P/g DM. In the first three samplings, P concentration correlated fairly well with shoot dry matter but in 4th sampling, we observed higher P concentration at lowest P application treatments. Plants maintained a minimum P concentration of 0.5 mg P/g DM in my experiment. P concentration of even the yellow leaves was close to 0.5 mg P/g DM. This indicates that 0.5 mg P/g DM is the absolute minimum concentration below which growth is not possible. Higher RGR of plants supplied with higher P in early growth stage and similar RGR at later growth stage shows that P deficiency affects the growth of plants early in the life cycle). So, at later growth stages, plants of lower P application treatments had a comparative advantage of comparable relative growth.

Irrespective of the level of P application, P concentration decreased with growth in all experimental plants. Dilution however occurred the least in lowest P application treatment resulting in a relatively higher P concentration compared to other treatments at 4th sampling. P was most diluted in treatment F resulting in some of the P concentration values as low as 0.5 mg P/g DM at 4th sampling. Decreasing tissue P concentration with the advancement in growth was observed by various researchers in various crops like maize (Daniel Plénet & Lemaire, 1999), Stylo(*Stylosantheshumilis*L.)(Moody & Edwards, 1978), mung bean and urdbean(M. S. Venkatesh, K. K. Hazra, & P. K. Ghosh, 2014) observed it in mung bean and urdbean.

3.4.5 Green area and leaf expansion

Green area of the shoot increased significantly with increase in P application. The response however was highest at 32 DAE with a slope of 9.06, implying that for every 1 g increase in P application per pot, green area increased by 9 cm^2. The higher green area of plants grown under higher P application levels might have resulted in the higher shoot biomass due to increased radiation interception. Similar results have been observed, for examples in maize: under P deficiency yield reduction was primarily due to the interruption in canopy expansion that affects the interception of solar radiation (Fletcher et al., 2008). Colomb et al (2000) also

concluded from their experiment in maize due to limitation in interception of photosynthetically active radiation (PAR) rather than reducing efficiency of conversion of PAR into dry matter (Colomb, Kiniry, & Debaeke, 2000). By experimental and simulation techniques, (D. Rodríguez, W. G. Keltjens, & J. Goudriaan, 1998a) showed that P deficiency directly affected individual leaf area expansion. The area of individual leaf was reduced by low P inputs in field experiments with sweet corn (Fletcher et al., 2008).

3.4.6 Tillering and fertile spikelets

Tillering is an important determinant of shoot biomass and grain yield. Number of tillers increased with P application in our experiment. Similar results are obtained by other researchers in different crops. However (Mazza et al., 2012) reports that, P application at rates above the moderate P level has no significant effect on the number of tillers but in their biomass. We saw number of tillers increased continuously towards the highest treatment in our experiment. Tillering is found out to be most sensitive to P deficiency (Rodríguez et al., 1998a). However, a study of wheat cultivars of Japan from 1996 suggests that a wheat cultivar adapted to lower available P showed higher tillering at low available P. This also leads to the discussion that some low available P adapted varieties may respond to lower P by higher tillering(Sato, Oyanagi, & Wada, 1996).

Another study from Argentina suggests that phosphorus deficiency directly altered the normal pattern of tiller emergence by slowing the emergence of leaves on the main stem and by reducing the maximum rate of emergence for each tiller.(Rodríguez, Andrade, & Goudriaan, 1999)

Increased P application not only increased the number spikelet per spike but also decreased the number of unfertile spikelet per spike. We did not measure the length of spike but there are reports suggesting increase in spike length with increased P application (Rahim, Ranjha, Rahamtullah, & Waraich, 2010).

3.4.7 Uptake and concentration of other nutrients

Potassium (K) concentration showed a positive response to P application and P concentration. Positive P-K interaction in plants has been demonstrated by several studies. Experiment on greenhouse condition carried out on perennial ryegrass showed that P and K accumulation of plants were closely related to DM accumulation. Phosphorus deficiency can thus influence negatively K-uptake and K concentration in plants, in agreement with reports by(Sárdi, Balázsy, & Salamon, 2012).

Zn concentration of our experimental plants measured in the shoot 23 DAE were close to 0.4 g/kg DM. This concentration is nowhere close to deficiency region. So, increased P application had negative effect on Zn concentration but not so severe to observe any deficiency. Zn-P interaction studied in 2 wheat cultivars differing in P uptake efficiency showed that Zn supply had little effect on tissue P concentration and P uptake but an increase in P availability caused significant reduction in Zn uptake and tissue Zn concentration (Zhu, Smith, & Smith, 2001). Negative response of Zn concentration to P application has been confirmed by several studies. P induced Zn deficiency are also reported. The mechanism, however is explained in terms of plant dilution and reduced translocation from roots by some studies (J. P. Singh, Karamanos, & Stewart, 1988). Some others suggest that the reduction cannot be entirely explained by dilution effect and conclude that high P uptake efficiency may depress plant uptake of Zn.

3.4.8 Effect of P application on Photosynthesis

Although there was increase in total photosynthesis due to increased green area in higher treatment levels, we did not observe increase in photosynthesis per unit leaf area with increased P supply. This result is in agreement with the results of (D. Plénet, Mollier, & Pellerin, 2000) who conclude from their field experiment on maize that P deficiency does not affect RUE, even during the period when above-ground biomass accumulation is most severely reduced. Our results however are contrary to the results by (Rodríguez et al., 1998a) who found out that phosphorus limitation reduced light saturated photosynthesis per unit leaf area in pot experiment conducted on wheat. In their experiment, despite the impaired metabolism, the effect on leaf area expansion was minor. Also this result contrasts with the results of (Chapin, Groves, & Evans, 1989) in barley varieties, where they observed 42 – 60 % reduction in maximum photosynthetic rate with reduced P supply. Similarly, a study carried out on cotton (S. K. Singh & Reddy, 2014) showed that P deficiency reduced electron transport rate (ETR), the quantum yield of PSII, CO2 assimilation, and overall photochemical quenching and reduced the efficiency of energy transfer to the PSII reaction center.

Rate of photosynthesis was measured in the fully developed flag leaf of wheat plants. Since P concentration in the flag leaf was found out to be constant at various P application rates, it is not surprising that we observed no significant difference between photosynthetic rates at various treatments.

3.5 Conclusion:

This study confirms that plants are subjected to P dilution by growth. Although the critical P concentration could not be determined, my findings indicate that 0.5 mg P/g DM is an absolute minimum P concentration in wheat plants, which could suggest it is a physiological minimum.

There was no significant effect of P application on the rate of photosynthesis per unit area of flag leaf. P concentration in the flag leaf and penultimate leaf remained constant across lower P application treatments but leaf area increased significantly with P application.

Therefore, I conclude that under severe P deficiency, wheat plants reduce their leaf area to maintain sufficient P concentration for photosynthesis.

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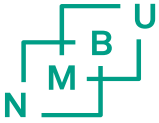

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