كلية العلوم و التقنيات فاس
Faculté des Sciences et Techniques de Fès

جامعة سيدي محمد بن عبد الله
Université Sidi Mohamed Ben Abdellah

MOHAMMED VI POLYTECHNIC UNIVERSITY

GRADUATION PROJECT

PRESENTED WITH A VIEW TO OBTAINING THE DIPLOMA OF

MASTER OF SCIENCES AND TECHNIQUES

MANAGEMENT AND CONSERVATION OF BIODIVERSITY

Effect of Phosphorus deficiency on chlorophyll a fluorescence and visible near-infrared reflectance of wheat plants (*Triticum aestivum* L.) grown in hydroponic conditions

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Defended on: 13 july 2021

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Acknowledgements

First and foremost, praises and thanks to God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully and inspiring me with the right steps and the right reflexes.

I would like to express my deep and sincere gratitude to Mr. OUKARROUM Abdallah, my supervisor at Mohammed VI Polytechnic University, for giving me the opportunity to join his team and providing invaluable guidance throughout this research. His dynamism, vision, sincerity, and motivation have all left an indelible impression on me. It was a great privilege and honor to work under his guidance. I am extremely grateful for what he has offered me, especially his confidence on me. I will never thank you enough Sir.

I am extending my heartfelt thanks to Ms. EL MEJJAOUY Yousra who always knew how to answer me with cheerfulness and kindness, for her time, patience, continued support and encouragement. My completion of this project could not have been accomplished without her help and precious advices. I would also like to thank her for her friendship, empathy and great sense of humor.

I'm extending my thanks to Mr. RACHIQ Saad, my pedagogical supervisor at Faculty of Sciences and Techniques of Fez, for his advices, support, assistance and his sense of responsibility.

I'm extending my thanks to Mrs. BENJELLOUN Meryem and Mrs. MIKOU Karima, for the tremendous honor they have bestowed upon me by agreeing to judge this work.

I would like to thank Ms. NACIRI Rachida, LATIQUE Salma and all the team of plant stress physiology laboratory.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Dedications

This work is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual, emotional, and financial support. I'm extremely grateful to them for their love, prayers, caring and sacrifices for educating and preparing me for my future.

To my brothers Ilyas and Soufian, as well as my one and only sister Sara, for always being a constant source of support and encouragement during the challenges of all my college life.

To my forever bestie Hanae, for her unwavering support and love.

To my friend Ali, your words of encouragement have always inspired me during my difficult times. You'll never know how much your help meant to me. I will always remember your kindness and support. Thank you.

To all my friends, Nada, Meriem, Dominic, Otmane, Mstapha, Asmae and Fatima. I love you.

To my classmates, good luck to all of you, I wish you a bright future.

This work is only a beginning of my journey.

FIGURES LIST

TABLES LIST

Abbreviation list

P: Phosphorus

SOM: Soil Organic Matter

PUE: Phosphorus Use Efficiency

PP: Polyphosphate

ADP: Adenosine Diphosphate

ATP: Adenosine Triphosphate

RuBP: Ribulose-1.5-Biphsphate

LHC II: Light Harvesting Reaction Complex II

RC: Reaction Center

PSI: Photosystem I

PSII: Photosystem II

Q_A: Quinones

VNIRS: Visible Near-Infrared Spectroscopy

SWIR: Short-Wave Infrared

NDVI: Normalized Difference Vegetation Index

RVI: Ratio Vegetation Index

VIs: Vegetation Indices

ChlF: Chlorophyll Fluorescence

F_o: Initial fluorescence after the onset of actinic illumination

F_m: Fluorescence maxima under saturating illumination

PC: Plastocyanin

CCI: Chlorophyll Content Index

PRI: Photochemical Reflectance Index

Cyt b_6f: Cytochrome b_6f complex

F_M : Fluorescence maximal

F_I : Fluorescence minimal

Contents

Introduction

Wheat (*Triticum aestivum* L.), a member of the Poaceae family, is one of the second main grain crops in the world. It is the important staple food of the world which meets most of the protein requirement of the people (FAO, 2020). The nutrient plays a vital role in the production of wheat (Pandey et *al*., 2020).

Among essential plant nutrients, phosphorus (P) is the second most critical plant nutrient next to nitrogen (N), and second most widely limiting macronutrient in soil for plant growth (Balemi and Negisho, 2012; Mustonen et al., 2012)

Phosphorus is an essential element for plant growth because of its pivotal role in many metabolic processes (Vance et *al*. 2003). It is an essential macronutrient that greatly influences root development, plant growth and crop productivity (Shen et *al*., 2011). Moreover, P has a significant function in various metabolic processes in plants, such as protein formation, photosynthesis, cell division, respiration, energy storage and nutrient movement within the plant, and is an integral constituent of nucleic acids, phospholipids, and coenzymes activating amino acid production (Vance et *al*., 2003).

Phosphorus deficiency causes morphological, physiological, and biochemical alterations, such as a decrease in the shoot/root ratio, root architectural changes, anthocyanin buildup, and a delay in plant maturity(Yaryur et al., 2009). It also has a deleterious impact on NADPH regeneration, lowering quantum yield, carboxylic efficiency, and electron transport efficiency during photosynthesis(Kalaji et al., 2014).

For a variety of reasons, diagnosing P deficiency in plants is extremely challenging. One of them is that crop phosphorus response has been found to be unrelated to the total amount of phosphorus in the soil. Furthermore, obvious foliar symptoms of phosphorus deficiency plants are uncommon, and when they do occur, they frequently overlap with other nutrient-deficiency symptoms(Cetner et al., 2020). Thus, the current methods of plant nutrient diagnostics are destructive, time-consuming, resource dependent, and require specialized labor and equipment. Consequently, there is a demand for a quick, cheap and non-invasive method/tool that would provide immediate data/information regarding plant nutrient status, allowing for rapid intervention in crop production before the effects of nutrient stress become irreversible (van Maarschalkerweerd and S. Husted,2015).

In this context, reflectance and fluorescence spectroscopies may provide a rapid, non-destructive diagnostic method for detecting and quantifying environmental stresses and damage to the photosynthetic apparatus of leaves, thus allowing these analytical procedures to be tested on intact biological material (Yaryur et al., 2009).

Frydenvang et al. (2015) have suggested an analytical method based on Chl fluorescence measurements, and the analysis of the OJIP transients. Yaryura et al. (2009) have also studied the effect of phosphorus deficiency on reflectance and chlorophyll fluorescence of cotyledons of Oilseed Rape (Brassica napus L.).

In this study, a mix of methods and tools were used to analyze changes in the structure and function of the photosynthetic apparatus in order to gain a better understanding of photosynthesis impairment caused by P deficiency in wheat plants grown in hydroponic conditions.

The main purposes of our work were:

To elucidate the effects of P depletion on photosynthetic apparatus of wheat leaves, to assess fluorescence emission and reflectance as indicators of premature stress in wheat plants and to evaluate the effects of phosphorus deficiency on other physiological parameters such as stomatal conductance and chlorophyll content.

Literature review

I. Phosphorus

Phosphorus (P) is quantitatively the most important inorganic nutrient for plant growth after nitrogen, and often limits primary productivity in natural systems as well as cropping systems, unless supplied as fertilizer (Lambers et al., 2006). It is necessary for energy transfer and storage, and a key component of phospholipids and nucleic acids (Schneider et al., 2019). In agriculture, phosphorus applied in the form of fertilizer can also stimulate seed germination, the strength development of roots and stems; the formation of flowers and seeds; and increase the yield and quality of crops. Although the forecast estimates the existing phosphate rock reserves that can be used for fertilizer production in the next 300 to 400 years, it should be emphasized that phosphate is a non-renewable resource (Aracena Santos et al., 2021).

1. Phosphorus availability

Plants take up P as phosphate (Pi) from the soil solution. Since little Pi is available to plants in most soils, they have evolved mechanisms to acquire and use P efficiently and foster symbiotic relationships to help them acquire P sources beyond their immediate range. Whilst in agricultural systems P limitations are frequently overcome by the application of P-fertilizers.

1. 1 Phosphorus in soil

The large number of the available methods of estimating available phosphorus (P) in the soil indicates the complex nature of the crop nutrient, mostly due to its strong interaction with other elements and soil colloids present in the soil solution (Silva and Van Raij, 1999). When compared to other macronutrients in the soil solution, phosphorus concentration has much lower ranges of 0.001mg/l to 1 mg/l (Brady and Weil, 2002). Phosphorus is primarily found in the soil parent material as mineral soil P and especially as apatite (Tiessen *et al*., 1984). However, the behaviour of phosphorus in soils depends on the particular characteristics of each soil and, in addition to microbial activity, factors such as temperature, pH, and the degree of oxygenation, which influence its mobility. The figure 1 illustrates phosphorus up take by plants, and factors affecting its availability in soil.

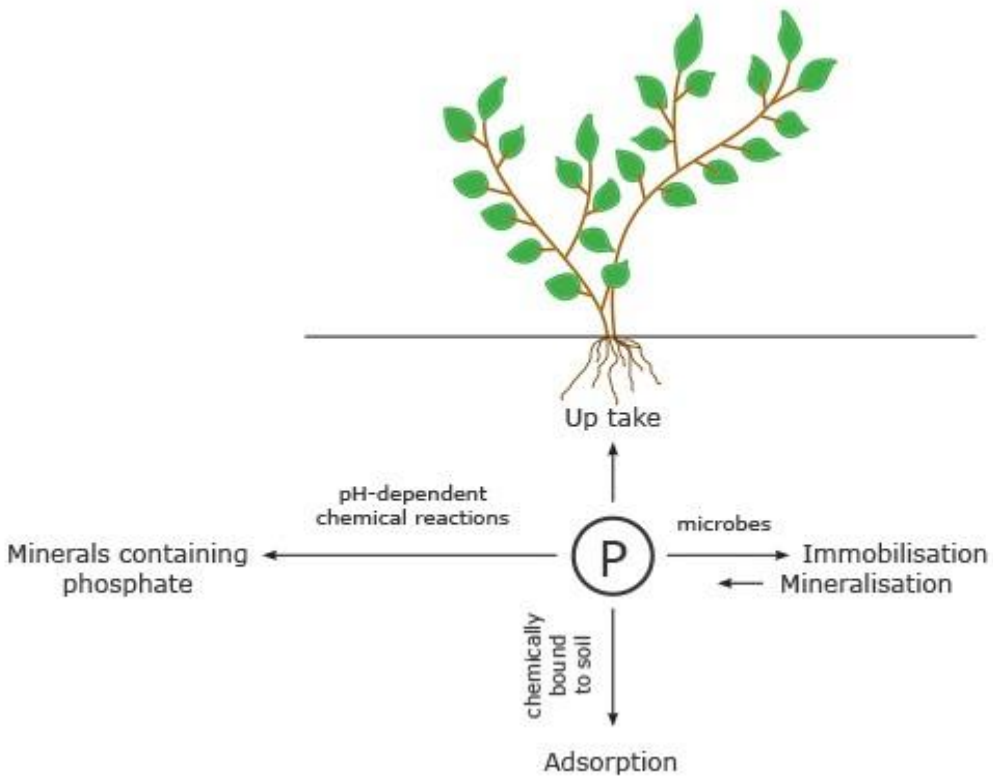

Figure 1: soil phosphorus availability and unavailability to plants. (University of Waikato, 2013)

pH

Soil pH is considered to be the "master variable" of soil chemistry due to its profound impact on countless chemical reactions involving essential plant nutrients. In the classic view, there are two main "valleys" of maximum P solubility occurring at around pH 4.5 and 6.5, coinciding with the lowest degree of P fixation by Calcium (Ca), Aluminium (Al), and iron (Fe) minerals. The dynamic between surface adsorption, anion exchange, and precipitation of Ca, Al, and Fe phosphates also explain why P solubility often increases with increasing pH among acid soils (Penn and Camberato, 2019)

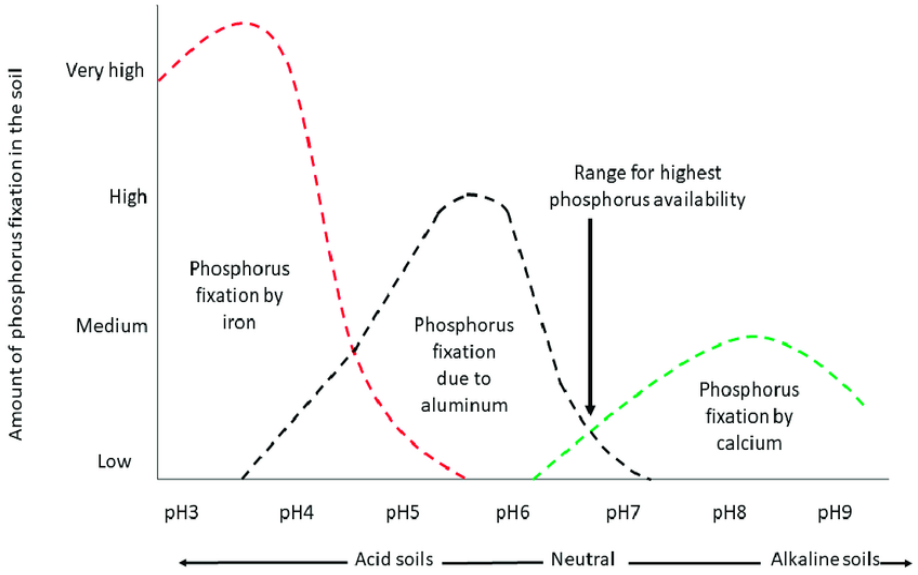

Figure 2: General qualitative representation of soil phosphorus availability as impacted by pH(Penn & Camberato, 2019)**.**

1.1. 1 Temperature

Temperature affects mechanisms of plant phosphorus (P) uptake and phosphorus-deficient plants have been associated sometimes with low soil temperatures, suggesting that P supply to the root is restricted with low soil temperature (Barber, 1986). Temperature can also increase the rate of reaction between soil and added P, resulting in a rapid decrease in soluble P (Bramley and Barrow, 1992). Phosphorus availability increased in manure-treated soils when temperature increased from 10 to 20°C (Whalen, Chang and Olson, 2001).

1.1. 2 Organic matter

In soil, P can be bound to soil organic matter (SOM), and the degree to which P is attracted to SOM will also be influenced by different forms of P present in soil (Zhang et *al.*, 2014). Some studies have shown that SOM can directly enrich the P pool in soil, and SOM has been found to be an important factor, through various mechanisms, affecting the adsorption and desorption of P in soil (Ye *et al.*, 2006; Wang and Liang, 2014). However previous research shows that high SOM content corresponds to a high concentration of organic P forms in the soil and low fixation of inorganic P forms, consequently high P availability (Costa *et al.*, 2016, Negassa and Leinweber, 2009, Rodrigues *et al.*, 2016).

1.1. 3 Phosphorus and nutrient interaction

Comprehension of the interaction of phosphorus with other nutrients is very important to maintain a balanced supply of nutrients in order to improve crop yields. Generally, phosphorus has positive significant interaction with nitrogen (N) and magnesium (Mg), since Mg is an activator of kinase enzymes and activates most reactions involving phosphate transfer (Fageria, 2006). Moreover, Fageria (2006) studied potassium (K) interaction with P in rice plants grown in

nutrient solution, and he noticed a significant quadratic decrease in the uptake of P with increasing K concentration in the solution culture.

1.1. 4 Phosphorus Fertilizer forms

The world's main source of phosphorus is phosphate rock (Schröder *et al*, 2010). Plants absorb phosphorus in the form of $H_2PO^-_4$ and HPO^{2-}_4 ions, the so-called orthophosphates (Schachtman and Shin, 2007). However, a popular form is several combined water-soluble phosphorus molecules identified as polyphosphates (PP) (Zhu *et al*, 2019). Crop response to P fertilization, in terms of productivity, phosphorus use efficiency (PUE), and residual fertility in soil, can depend on the form of P applied. Therefore adequate phosphorus fertilization plays a key role in precision agriculture (Rana *et al*, 2018).

In addition to its rarity, when P is added to soils, a large portion of it is rendered unavailable to plants due to fixation or sorption reactions with soil constituents, which results in only 10 to 30% of the P added to soils being recovered by plants(Dick, 1985).

a. Polyphosphates

The key to the availability of polyphosphate is the rate of hydrolysis which is mediated chemically and biochemically by a group of enzymes known as phosphatases, they must first undergo a hydrolysis reaction in order to release orthophosphate for absorption by plant roots. However, their degradation should not proceed too rapidly; otherwise, the favorable characteristics of polyphosphates would be quickly lost, thus diminishing their potential for increasing P efficiency (Dick, 1985).Polyphosphates are an anionic linear polymer of orthophosphate residues joined by hydrogen phosphate bonds similar to those found in ATP. They can affect the transcription and translation of specific genes, modulate several stress responses, provide an alternative source of energy and act as a metabolic regulator (Zhu *et al*, 2019; Laha *et al*, 2016). Their use in the nutrient solution has a significant effect on strong root system development, plant growth and earlier flowering (Torres-Dorante *et al*, 2006).

b. Orthophosphates

Orthophosphate (Pi) is a major regulator of carbon metabolism in plants, it is the preferred uptake form by plants(Carstensen et al., 2020). Moreover, orthophosphate (Pi) is an important regulator of the rate of photosynthesis, and of the partitioning of triose phosphates between starch and sucrose biosynthesis(Rao et al., 1987).

1. 2 Plant phosphorus uptake

Plant availability of P is a complex outcome of P fertilization history, but also of multiple and partly interacting soil properties. Phosphorus is one of the most important macro elements in nucleic acids, high-energy compounds cell membranes, which participates in the process of photosynthesis and respiration, influences gene expression and activates or causes inhibition of enzymes by phosphorylation (Ciereczko, 2006; Richardson, 2009). It is absorbed in the form of $H_2PO^-_4$ and HPO_4^{2-} ions, the so-called orthophosphates (Schachtman and Shin, 2007). Moreover, Phosphorus is an essential part of numerous physiological functions such as energy accumulation

and transmission, photosynthesis, respiration, cell differentiation, and cell expansion, which implies energy-rich phosphate compound synthesis as adenosine triphosphate (ATP), adenosine diphosphate (ADP). Phosphoproteins, nucleic acids, nucleotides, phospholipids, are also essential components (Anwar, 2016). Khan and colleagues (2007) found that when wheat was provided P at 90 kg ha-^{1}, the production of wheat was substantially enhanced from 2920 kg ha-^{1} up to 3560 kg ha-^{1}; the yield was increased by 22% and the number of lengths, tillers, spikes, and wheat plants was substantially higher.

2. Phosphorus deficiency

For many years and in most agricultural production systems, phosphorus (P) has been identified as the most frequently occurring essential element deficiency limiting crop yields. However, the deficiency of phosphorus available to plants causes inhibition of shoot and root growth, reduction of leaf blade area and reduction of plant weight (Rychter and Rao, 2005). Therefore phosphorus deficiency in plants disturbs the production of chlorophyll, causing leaf chlorosis (Choi and Lee, 2012; Viégas *et al*, 2018). It is well known that prolonged P deficiency may further result in the accumulation of anthocyanins, consequently leading to purple discoloration on the leaf surface (Osborne *et al*, 2002; Ticconi and Abel, 2004). In addition to the above many investigations indicated that P deficiency significantly depressed plant CO_2 assimilation capacity (Rao and Terry, 1989; Jacob and Lawlor, 1991).

2. 1 Morphological changes

a. Root growth

The growth and development of the root system is crucial for the early absorption of phosphorus (P) by plants, because phosphorus is relatively difficult to use and immobile in many soils. Authors generally believe that phosphorus deficiency will lead to a higher root: shoot ratio (Mollier & Pellerin, 1999). The root structure of plants may undergo many changes due to phosphorus deficiency. In beans, lateral root growth and increased branching of secondary roots were observed at the expense of primary root elongation (Wissuwa et al., 2005). Anuradha and Narayanan (1991) research on horsegram showed that plants grown on a nutrient solution lacking P have increased elongation of primary and secondary roots.

b. Shoot growth

Many studies have pointed out the effect of phosphorus deficiency on the process of photosynthesis. However, when the availability of phosphorus is limited, it usually reduces plant growth before the rate of photosynthesis per leaf surface (Plénet et al., 2000). On wheat, Rodriguez et al. (1998) showed that even if the phosphorus content in the plant exceeds the limit of photosynthesis, phosphorus deficiency will increase the interval between two adjacent leaves, reduce the number of tills, and reduce the leaf expansion rate. Further Cetner et *al*. (2020) noticed that phosphorus deficiency causes the purpling of petioles, and the appearance of pale or chlorotic spots on the older leaves and/or cotyledons began showing at 7th day after treatment.

2. 2 Physiological effects of P deficiency

a. On the photosynthetic characteristics

Photosynthesis is the most important photochemical well for the energy absorbed by the leaves, and therefore the photosynthetic apparatus is likely to be exposed to an excess of harmful light energy due to the strong inhibition of CO_2 assimilation in plants caused by P deficiency. Inhibition of photosynthesis by P limitation has often been explained by depressing the Calvin cycle activity, in particular, by depressing the amount and activity of Rubisco and the regeneration of ribulose-1,5-bisphosphate (RuBP). Although some investigations have demonstrated that P deficiency induces possible photo- inhibition and damage to PSII (Xu *et al.*, 2007). Thus, photosynthesis is sensitive to low P stress (Balemi and Negisho, 2012).

b. On leaf fluorescence

A study was carried out by Frydenvang et al. (2015) on barley, showed that phosphorus deficiencies induced a significant modification of OJIP Chl a fluorescence transient, which has been translated by the "disappearance" of stage I. Furthermore, Cetner et al. (2020) have observed that the JIP analysis chlorophyll test fluorescence under phosphorus deficiency stress showed LHCII detachment (increase in OF) and decrease in active RC PSII (gradually QA) by Chl antenna (RC / ABS), followed by a decrease quantum results of primary photochemistry PSII, electron transport and electron transport to PSI acceptors as well as the efficiency / conversation that the electron trapped in PSII RC is transferred beyond QA, are the main causes of the removal of ETC.

c. On leaf reflectance

Light utilization through leaf drives major photosynthetic and physiological traits. Thus leaf spectral traits based on the spectral reflectance properties are important components to understand the various physiological status of the plant (Gamon et al., 1997; Ray et al., 2005). Yaryura et al., 2009 have conducted a study on effect of phosphorus deficiency on reflectance, and have noticed that the most prominent effect of P on reflectance spectra in leaves was the decrease in reflectance values at 500–650 nm for P-depleted plants(Yaryur et al., 2009).

These paragraphs will be discussed in details in the next section (II) of this part.

II. Reflectance and fluorescence spectroscopy systems

As it is known, natural or artificial incident light presents different possibilities of evolution when it impacts the plant. As shown in Figure 3 for solar radiation, a significant part of the light is absorbed by the chlorophyll contained in the chloroplasts, another is transmitted and a third is reflected. Of the part absorbed, only a small fraction enters the photochemical processes (in relation to photosynthesis), a very large fraction dissipates in the form of heat and the remaining fraction dissipates via a so-called chlorophyll fluorescence emission. These proportions vary

greatly, on the one hand, according to the chemical and physical composition of the leaves and on the other hand, according to the regulatory dynamic which is a function of the light intensity absorbed (Ben Abdallah *et al.*, 2016).

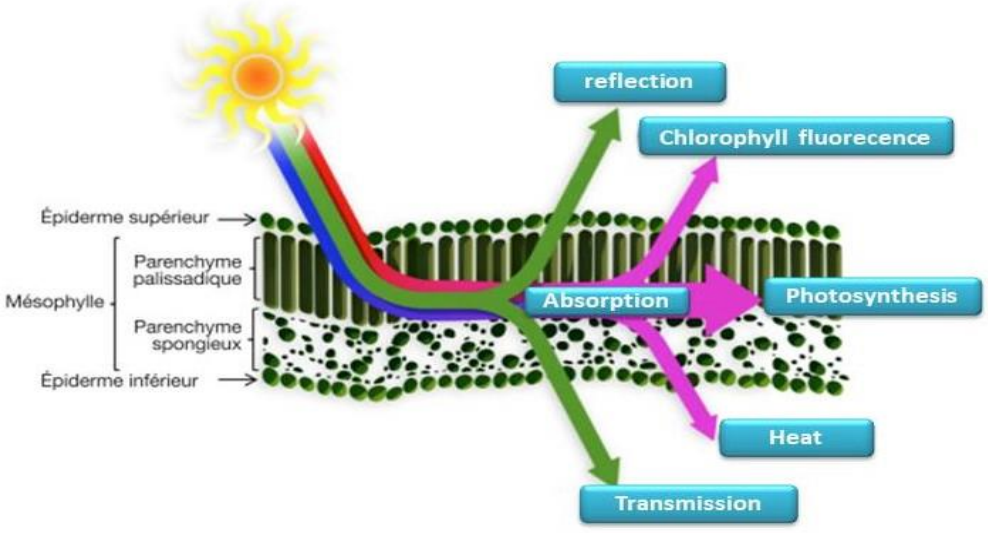

Figure 3: Schematic representation of the incident solar light behavior impacting a dicotyledonous leaf surface in the visible spectrum (Ben Abdallah *et al.*, 2016).

1. VIS- NIR reflectance spectroscopy

Near-infrared spectroscopy (NIRS) is an emerging technique for soil and plant nutrient analysis. This spectroscopy technique, which uses reflectance at a wavelength range of 700–2500nm, offers a rapid, non-destructive, cheap, less labor-intensive, and real-time plant nutrient analysis. Using NIRS at a leaf level allows a direct measurement of the crop's nutrient status, which bypasses soil nutrient analysis and allows growers to use this information for fertilizer management. Moreover, NIRS enables the detection of hidden deficiencies and prevents any excess application of fertilizers which would allow growers to be proactive in their nutrient management by maintaining the synchrony between nutrient demand from the crop and the supply from the soil, consequently reaching optimum yield and increasing profits, while reducing unnecessary input costs and environmental footprint of agriculture enterprises (Prananto *et al.*, 2020).

1. 1 History of VNIRS for plant nutrient analysis

Early studies of NIRS focused mostly on forage qualities such as digestibility, crude protein contents and acid detergent fiber (Abrams *et al*., 1987; Clark et al., 1987; Mc Lellan *et al*., 1991; Norris *et al*., 1976; Shenk and Westerhaus, 1985). Out of these early studies, Shenk *et al*. (1979) were one of the first studies that successfully estimated concentrations of nutrients such as N, P, Ca, K and B in forage grasses using NIRS. However, the earliest study on using NIRS on plants was performed by Knipling in 1970, who assessed the physical and physiological basis for the reflectance of visible and NIR radiation of vegetation.

1. 2 Fundamentals of VNIRS

The VNIR part of the electromagnetic spectrum includes both the visible (350–780 nm) and near-infrared (780–2500 nm) ranges, which overlaps with the optical radiation range (100–1000 nm) as shown in figure 4. Sometimes, the 350–1000 wavelength range is referred as VNIR (visible-near-infrared), and the 1000–2500 range is referred as the SWIR (short-wave infrared) in remote sensing literature (Clarck, 1999).

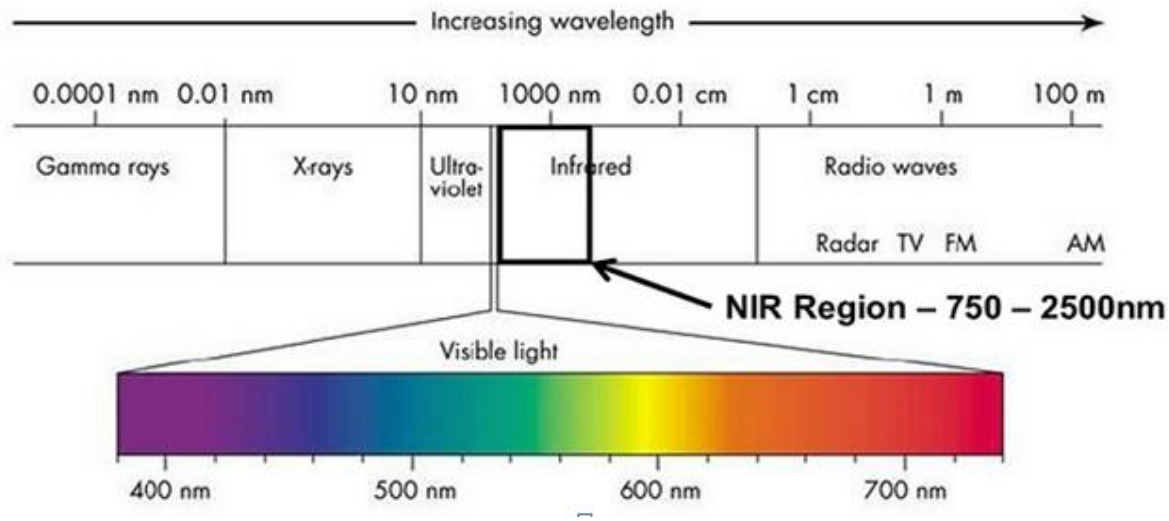

Figure 4 : The electromagnetic spectrum, showing the spectral range and location of visible and near infrared (VNIR) relative to other types of electromagnetic radiation.

1. 3 Plant leaf spectra

The reflectance or absorbance spectra of plant leaf tissues in the visible (400–700 nm) and NIR (700–2500 nm) spectral ranges are unique compared to other materials. Furthermore, each plant species has its unique reflectance spectra; however, there is a general trend that can be observed as shown in figure 5 (Richardson *et al.*, 2004).

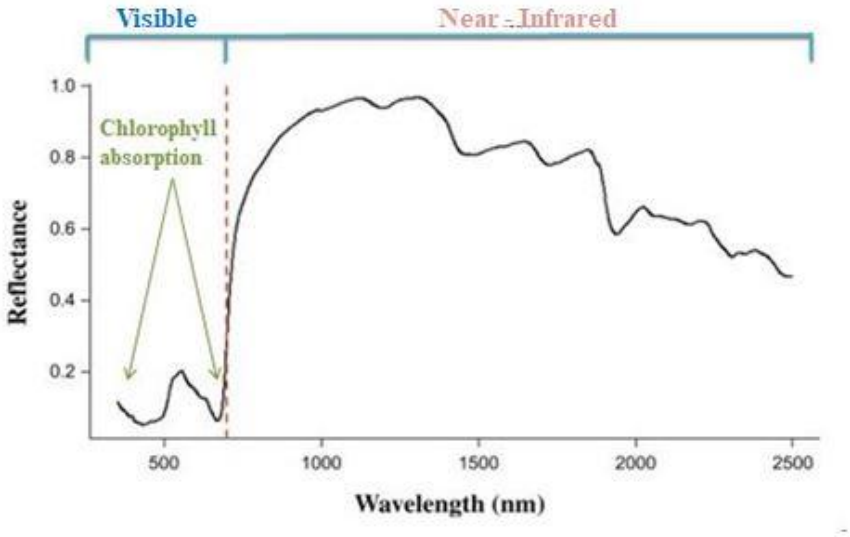

Figure 5: Reflectance spectra of a cotton leaf in the visible part of the spectra (400–700nm) and the NIR part of the spectra (700–2500nm) (Prananto et al., 2020)**.**

Leaf reflectance tissue differs from one region to another, it is relatively low in the visible spectral range (Vis), and relatively high in the NIR spectra (vice versa for the absorbance) and flattens at a wavelength of 800–1300nm called the NIR plateau. This unique spectrum is a result of the complex scattering and absorption pattern by various bio-chemical and structural components present in the tissue (Grant, 1987; Jie *et al*., 2014).

Absorbance by pigment structures (e.g., chlorophyll and carotenoids) is the major contributor to the reflectance in the visible spectral range (Grant, 1987; Richardson *et al.*, 2004). Visible light is absorbed by pigments as a primary process of photosynthesis (Sims and Gamon, 2002), however, internal structure and organic compounds such as carbohydrates and proteins have a higher contribution to the absorbance in the NIR range through different chemical bonds (Richardson *et al.*, 2004).

Several vegetation indices can be calculated using these absorption bands. Simple VIs that combine visible and NIR bands have significantly improved the sensitivity of green vegetation detection (Xue & Su, 2017).

1. 4 Basic vegetation indices

With the utilization of high-resolution spectral instrumentation, the amount of bands obtained by remote sensing is increasing, and therefore the bandwidth is getting narrower (Honkavaara *et al*., 2013).

- **NDVI**

There are several vegetation indices, one of the foremost used and implemented indices calculated from multispectral information as a normalized ratio between the red and near infrared

bands is the Normalized Difference Vegetation Index NDVI. It was proposed by Rouse Jr. *et al*., in 1974, which can be expressed as:

$$\text{NDVI} = \frac{(\rho\text{NIR} - \rho\text{R})}{\rho\text{NIR}} + \rho R$$

Since the index is calculated through a normalization procedure, the range of NDVI values is between 0 and 1, having a sensitive response to green vegetation even for low vegetation-covered areas(Xue & Su, 2017).

- **RVI**

Jordan proposed in 1969 one among the primary VIs named Ratio Vegetation Index (RVI), which is predicated on the principle that leaves absorb relatively more red than infrared light; RVI are often expressed mathematically as:

$$RVI = \frac{R}{NIR}$$

Where NIR is that the near-infrared band reflectance and R is band reflectance. The RVI is widely used for green biomass estimations and monitoring, specifically, at high density vegetation coverage, since this index is very sensitive to vegetation and has a good correlation with plant biomass. However, when the vegetation cover is sparse (less than 50% cover), RVI is sensitive to atmospheric effects, and their representation of biomass is weak(Xue & Su, 2017).

2. Chlorophyll Fluorescence

Photosynthesis is one among the foremost important metabolic processes in plants; therefore, measuring its activity also provides information about the plant's general "health status". In photosynthesis research, chl a fluorescence is one among the foremost widespread methods, both in basic and ecophysiological studies (Papageorgiou and Govindjee 2004).

Chlorophyll within a leaf exists as pigment–protein complexes in PSII, PSI, and within the light harvesting reaction complexes (LHCs) associated with each of these centers. Light energy absorbed by chlorophyll molecules has three possibilities; it can drive photosynthesis (photochemistry), be re-emitted as heat, or be re-emitted as light (fluorescence).thus chlorophyll fluorescence is a measure of re-emitted light (in the red wavebands) from PSII(Murchie & Lawson, 2013). Analysis of chlorophyll fluorescence (ChlF) induction curves allows the evaluation of the physiological condition of photosystem II (PSII) and photosynthetic electron transport chain components (Kalaji et al., 2016)

2.1 Principles of chlorophyll fluorescence analysis

The method is based on high-frequency record of ChlF emitted by dark adapted leaf during short (usually one second lasting) pulse of strong actinic light by fluorimeter. The ChlF rise during the first second of illumination shows a sequence of phases (labeled as O, K, J, I, P) from the initial (Fo) to the maximal (Fm) fluorescence value. The mathematical model of the polyphasic

transient was developed and named as JIP-test. The method enables calculation of specific biophysical parameters, quantum yields and probabilities characterizing structure and function of the photosynthetic electron transport system as well as some integrative parameters related to plant photosynthetic performance (Živčák et al., 2014).

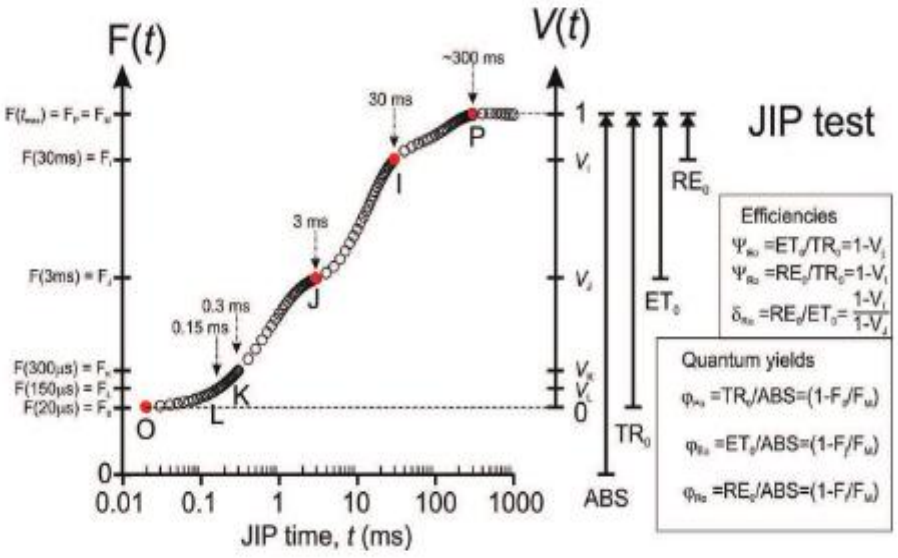

Figure 6: Typical OJIP-transient of chlorophyll fluorescence (Kautsky curve) exhibited on illumination of a dark-adapted leaf sample (4 mm2) by saturating red light (3000 µmol photons m22 s21)(Kalaji et al., 2014)**.**

2.2 Experimental fluorescence induction

The fluorescence measured experimentally at ambient temperature comes essentially from the PSII. The fluorescence signal of PSI, is much lower, and almost independent of the redox state of its cofactors (Briantais et al. 1986, Byrdin et al. 2000). When the primary acceptors of PSII, the Quinones Q_A, are oxidized or reduced, we respectively talk about open or closed reaction centers (Duysens et Sweers 1963). When the Q_A molecules are mostly oxidized (open reaction centers), only about 2% of the light absorbed is re-emitted through the fluorescence (Trissl et al. 1993). On the other hand, when the Q_A molecules are mainly reduced (closed reaction centers), approximately 10% of the light absorbed is re-emitted in the form of fluorescence. In order to ensure that the reaction centers opening prior fluorescence measurement, it is necessary to adapt the photosynthetic samples to the dark for a few minutes (Georgina, n.d.).

2.3 Complementary techniques of chl a fluorescence

In spite of the wide use of chl a fluorescence and the development in instrumentation during the last 10-20 years, there are several unanswered questions and mutually exclusive hypotheses concerning the interpretation of chl a fluorescence, especially the OJIP transient (Toth 2006). This may partially be due to the lack of complementary techniques.However, there have been

attempts to measure direct electron flow through PSI at 820 nm transmission using the PEA Senior instrument. This enabled studying the influence of electron transfer processes occurring in PSI on chl a fluorescence.

3. Absorbance at 820nm

The leaf absorbance at 820nm is a technique that allows the monitoring of the oxidation state of P-700 in vivo, and which does so using equipment that is as robust and simple as that used to measure chlorophyll fluorescence(HARBINSON & WOODWARD, 1987). Kinetic changes at 820 nm reflect changes in the redox states of the primary electron donors of photosystem I (P700) and plastocyanin (PC) with a small contribution of ferredoxin The oxidation of P-700 also results in an absorbance increase around 820 nm (Ke, 1972). Photo oxidation of P700 by illumination can be used as an indicator of PSI electron transport capacity (Klughammer and Schreiber, 1994).

III. Hydroponic culture

1. Hydroponic culture

Hydroponics is the science of growing plants without soil by providing them with chemical solutions with artificial forms of nutrients, which they usually draw from the soil. Interest in hydroponic culture has continued for several reasons. Firstly, no soil is needed and a large plant population can be grown in a very small area. Secondly, when fed effectively, optimum production can be attained (Deutschmann, 1998; Saffel, 1993). Thirdly, nutrients, water and aeration can be controlled to the highest degree. Today, hydroponics is an established branch of agronomical science (Steinberg *et al*., 2000).

2. Nutrient solution

A nutrient solution for hydroponic systems is an aqueous solution containing mainly inorganic ions form soluble salts of essential elements for higher plants(Trejo-téllez & Gómez-merino, 2012). For plant nutrition application, nutrient is understood as being one of the thirteen plant essential mineral elements that have been divided into two categories: the six major mineral elements—N, P, K, Ca, Mg, and S—found at percent concentrations in the plant dry matter, and the seven micronutrients—B, Cl, Cu, Fe, Mn, Mo, and Zn—found in the dry matter of the plant at less than 100% levels(Jr, 2008).

Materials & Methods

I. Plant growth conditions

1. Seed germination

The study was conducted at UM6P University in plant stress physiology laboratory in Bengurir, Morocco. To achieve this study, the wheat seeds (Karim variety) are sown in peat. three seeds per hole were sown. These are irrigated everyday with distilled water, for six days, under controlled conditions of temperature and light. (T = 25°C and 104 µmol.m^{-2}.s^{-1} of light intensity). The plumule is observed to break the surface after two days of sown.

Figure 7: seed germination assay

2. Hydroponic experiment wheat

Fourteen big plastic containers covered by wooden plates within each plate, nine planting positions existed where the germinated seeds are transferred (three plants per hole), the transplantation of the seedlings is carried out at two unrolled leaves stage, by transferring the seedlings into a modified Hoagland solution for wheat, which was continuously aerated, replaced twice a week and contained the following macro- and micronutrients: CL, Ca, N, K, S, Mg, P, Fe, B, Zn, Mn, Cu, and Mo. Water loss due to evaporation or plant transpiration was compensated by adding nutrient solution every three days.

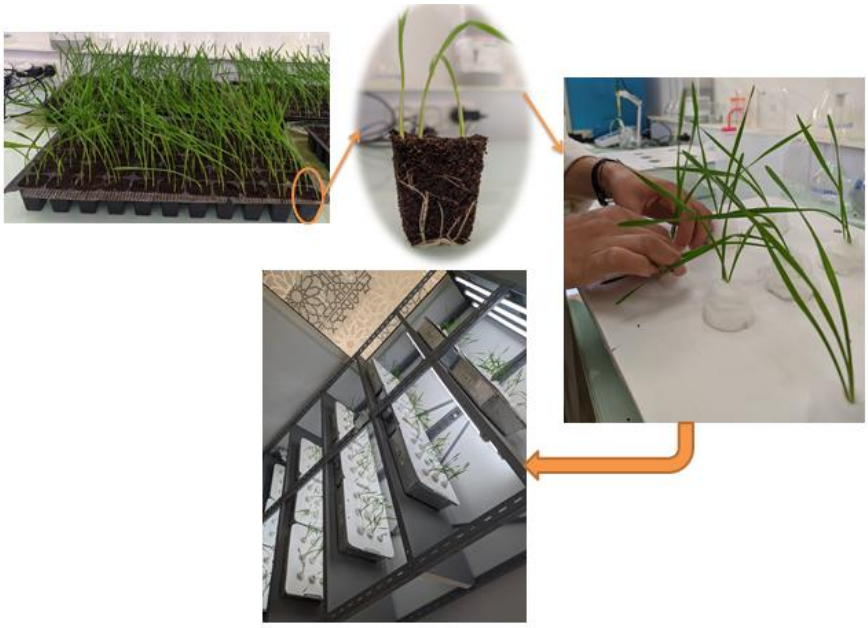

Figure 8: The steps of transplantation

3. Nutrient solution

Table 1: Wheat nutrients requirement in mg/l

Elements	Mass (mg/l)
Macronutrients	
N	105.05
P	15.49
K	117.29
Ca	100.20
Mg	24.31
Cl	17.72
S	32.07
Micronutrients	
Fe	3.3507
Cu	0.02986662
Zn	0.0601496
B	0.205409
Mn	0.19997432
Mo	0.0009595

4. Applied treatments

To respond to our objective, 2 doses of phosphorus combined with three fertilizer forms plus a control where no phosphorus were applied, were carried out. Wheat plants were hydroponically cultivated using three different doses: The –P dose, in which plants were treated with all nutrients except phosphorus, the P1/2 dose, in which plants were treated with 50% of their need of

phosphorus and the +P dose, in which plant were treated with sufficient phosphorus. The table below shows the applied fertilizer forms and P doses.

Table 2: P levels and fertilizer forms

Fertilizer forms	P doses
Ortho-A: 1st orthophosphate form	-P: 0P, no P was applied
Ortho-B: 2nd orthophosphate form	1/2P: 50% of P plant requirements is covered
Poly-B: polyphosphate form applied	+P: 100% of P plant requirements is covered

II. Measurements

1. Non-destructive measurements

1. 1 Chlorophyll Content Index (CCI)

Chlorophyll Content Index was measured using CHLOROPHYLL METER SPAD-502 Plus, which is a lightweight handheld meter for measuring the chlorophyll content of leaves without causing damage to plants. It allows determining the relative quantity of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions, as it is known that chlorophyll has two peaks of absorbance one in the blue (400-500 nm) and the other in red (600-700 nm) regions, with no absorbance in the near-infrared region. The SPAD-502 Plus measures the absorbance of the leaf in the red and near-infrared regions.

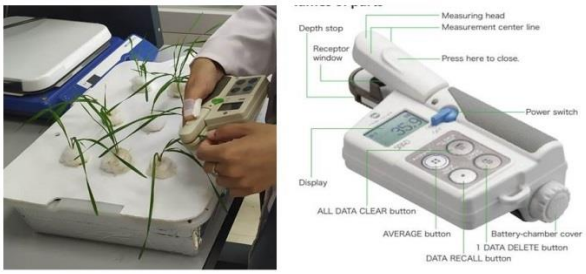

Figure 9: Measure of CCI with the SPAD-502 Plus

1. 2 Chlorophyll fluorescence and absorbance

Chlorophyll fluorescence and absorbance measurements were performed using the M-PEA (Multi-Function Plant Efficiency Analyzer). The leaf clips were placed on the leaves 20 minutes prior to the measurements to provide dark adaptation. Leaf measurements were taken every three days in the central part of the leaf, a total of six measurements was conducted the four first measurements were done always on the fully developed leaf, the third one from the top (leaf 3), however the fifth and the 6th measurement were done in the second leaf.

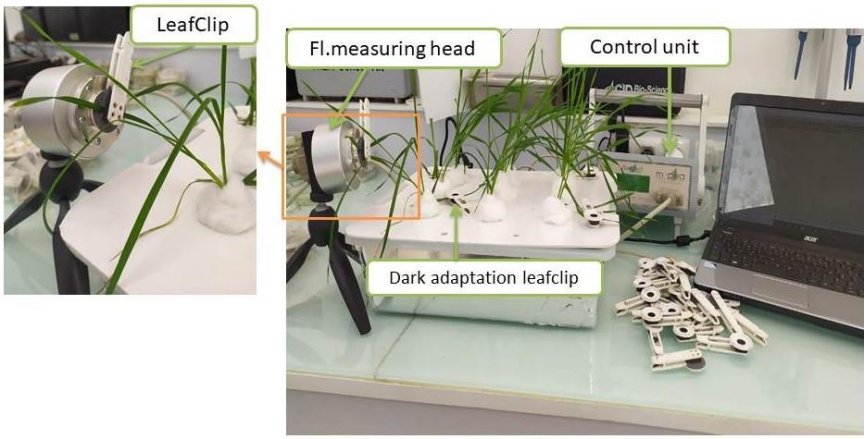

Figure 10: The M-PEA senior instrument

1. 3 Reflectance

Leaf spectral reflectance was measured using a spectrometer (CI-710/720, CID- Bioscience, USA). Six measurements of spectral reflectance were conducted from 450 nm to 1000 nm wavelength. Leaf reflectance is calculated as a ratio between the reflected energy of the leaf and the incident energy of the light source. The measurements were performed by placing the opening of the integrating sphere on the leaves. Subsequently, the reflectance spectra were smoothed 20 using the originlabPro. From every set of 12 smoothed spectra, the average value of leaf reflectance was obtained. Different spectral indices namely Normalized Differential Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI), were obtained from the leaf reflectance data exhibited by the Spectrometer-inbuilt software system.

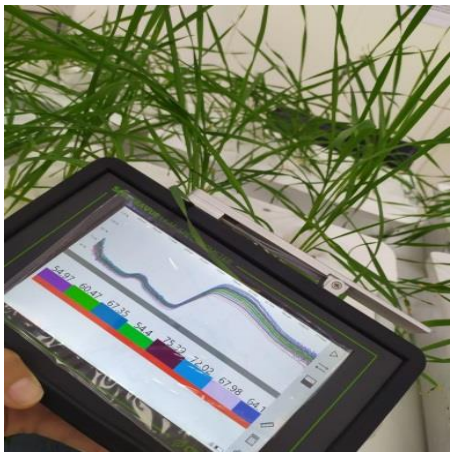

Figure 11: The CI-710/720 spectrometer

1. 4 Stomatal conductance

Stomatal control of leaf conductance is an important means by which plants limit water loss. Two measures of stomatal conductance (mmol m^{-2} s^{-1}) were taken using leaf porometer (SC-1, Decagon Devices, WA, USA). Instrument calibration was done prior each set of measurements. The leaf porometer measures stomatal conductance which is a measure of the degree of stomatal

opening and can be used as an indicator of plant water status, by putting the conductance of a leaf in series with two known conductance elements, and comparing the humidity measurements between them. Leaf measurements were taken by placing the leaf on the sensor head under the inner leaf pad.

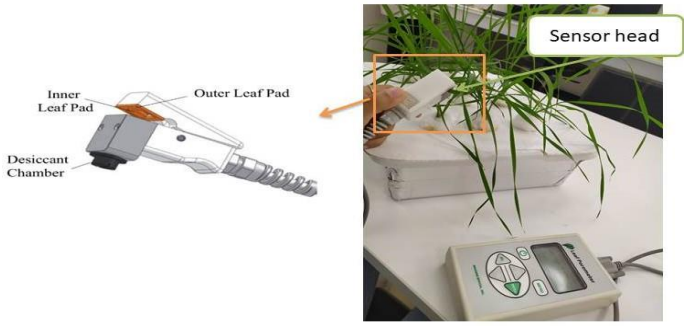

Figure 12: Measure of stomatal conductance using leaf porometer

2. Destructive measurements

Plants are harvested after 20 days by cutting off the shoot from the roots. The plants were separated into two components the leaves and the stalks were dried at 60°C for 2 days until they reached a constant weight for dry weight determination. The roots were a subject of root morphology analysis before drying.

2. 1 Leaf area

After harvesting, leaf area was measured using the AM350 Portable leaf area meter. Leaves were cut, arranged, and placed upon the leaf meter. The meter cover was slid over the leaf and scanning of the leaf surface area takes place.

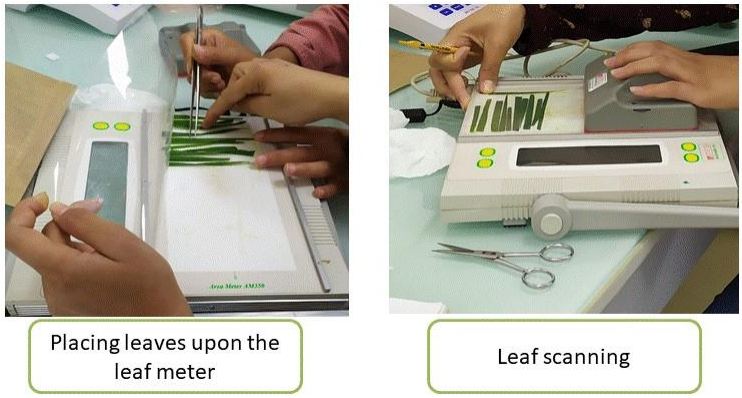

Figure 13: Leaf area measurements with the AM350 Portable leaf area meter

2. 2 Root analysis

Before the measurements, roots were carefully removed, and soaked in distilled water to eliminate all impurities. The root different parameters are calculated through a scanner WinRhizo. The WinRhizo system can detect and make corrections for areas of root overlap.

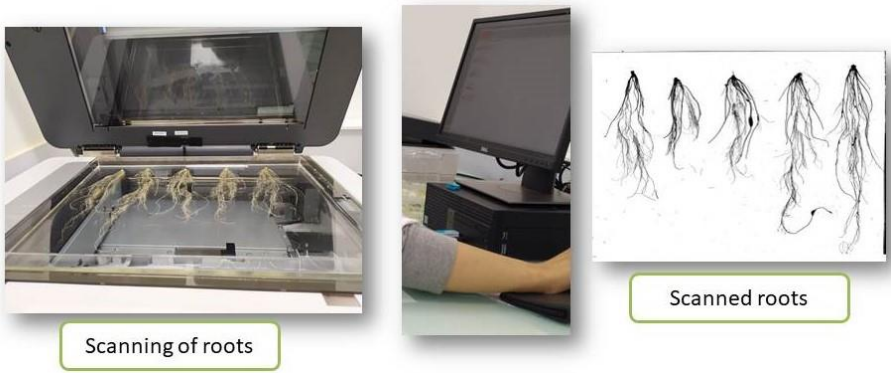

Figure 14: Root scanning using Winrhizo

2. 3 Phosphorus laboratory analysis

The dried plant samples collected (root and shoot), were grinded and digested using nitric acid after which they were analysed for phosphorus. Kjeldahl method was used to analyse total P content of root and shoot.

2. 4 Statistical analysis

In order to better understand the response of wheat leaves to different P fertilizer forms and doses, the results obtained were analyzed with analysis of variance (ANOVA) using IBM SPSS Statistics 20. The Phosphorus concentrations and fertilizer forms were intercompared and ranked using a post-hoc test (SNK).

Results

I. Morphological parameters

1. Root morphology

The morphological traits of roots wheat were examined after 20 days of growth under different levels of P, -P (0mM), 1/2P (0.25mM) and +P (0.5mM) by applying phosphorus fertilizer forms, which were two orthophosphates and one polyphosphate. We noticed a huge difference in size between the roots of plants grown in a deficient (0 mM) P concentration and those of plants supplied with optimal (0.25 and 0.5 mM) P.

One of the most conspicuous changes in root architecture that results from phosphorus deficiency is the induction of some form root hairs. For instance, under conditions of low P availability especially (-P), root hairs became longer and denser.

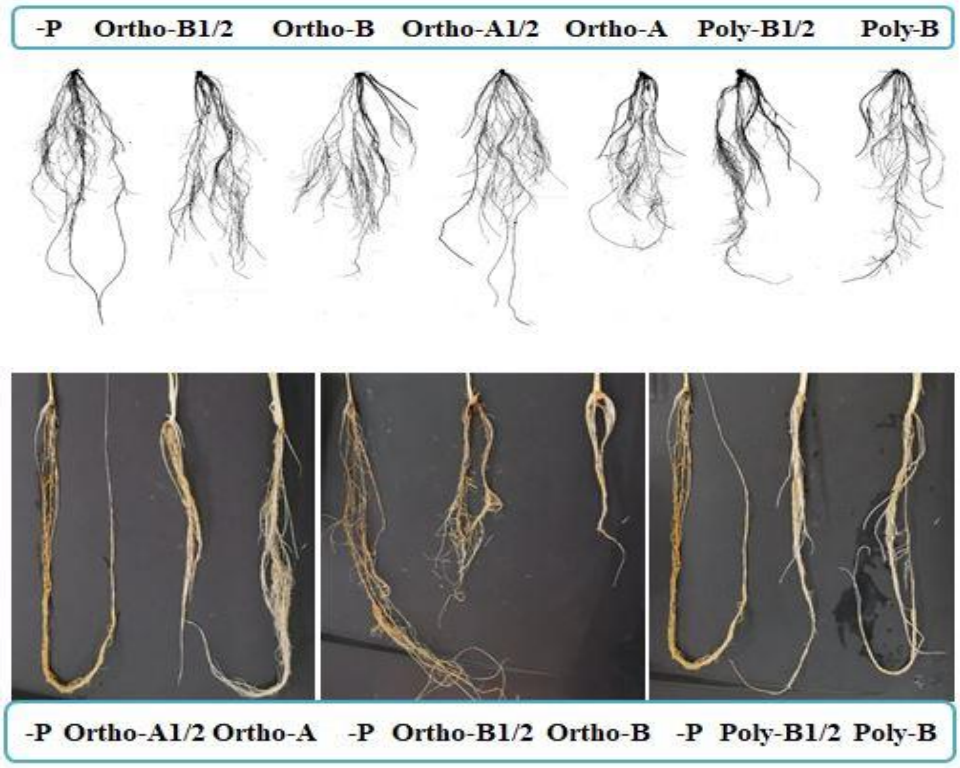

Figure 15: Responses of wheat root systems to different phosphorus levels and fertilizers forms.

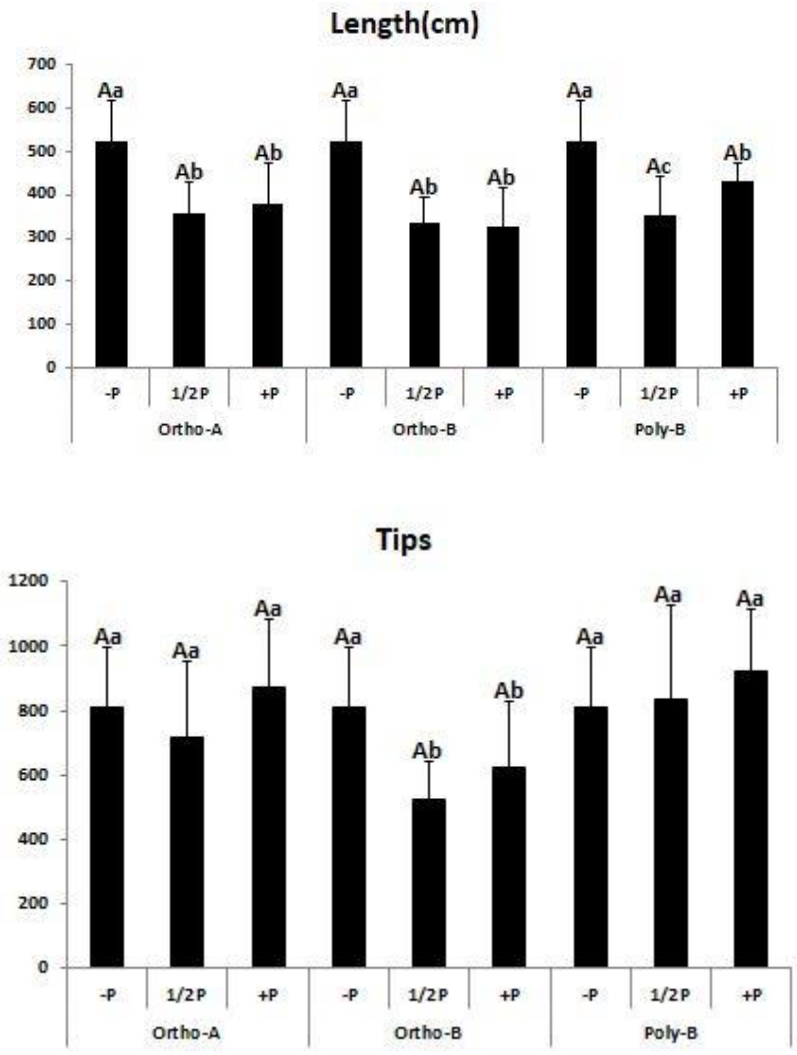

Figure 16: Effect of P doses and fertilizer forms on wheat roots morphological parameters (length and tips). (A; effect of fertilizer form) and (a, b and c; effect of P level).

Total root length of plants grown in (-P) increased till 510 cm and then decreased under subsequent concentrations with a maximum decrease at 300 cm which represents the roots of plants treated with Ortho-B level +P treatment. The same results are obtained for the tips parameter; the highest value (800) was noticed in roots of -P. The root growth showed a correlation with roots dry weight and which was highest at -P and thereafter dropped to a maximum decrease at Ortho-B level +P treatment (figure 3).

Phosphorus Fertilizer forms have no effect on roots growth and their morphological parameters. However, P level has almost affected all root parameters, especially the 0 level (-P) that has promoted a maximum growth.

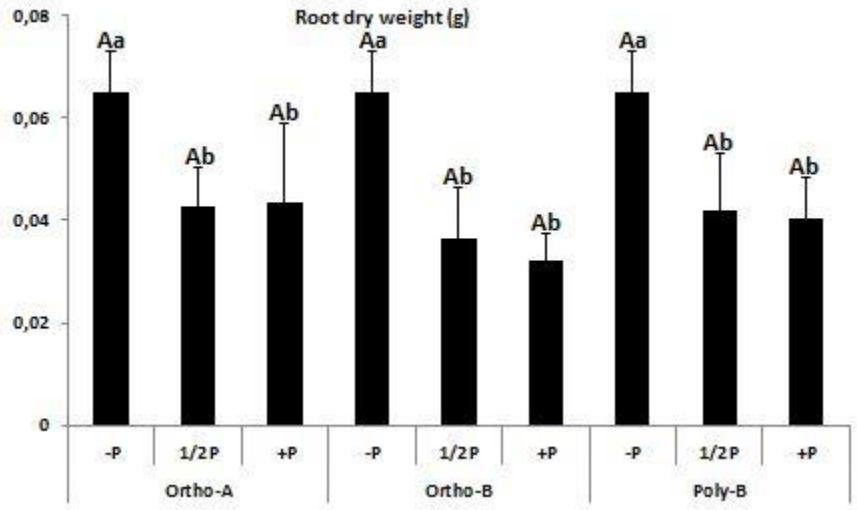

Figure 17: Effect of P doses and fertilizer forms on wheat roots dry weight.

2. Shoot morphology

Visible morphologic symptoms of P deficiency in wheat plants were observed. The plants (shoot) under phosphorus deficiency stress were slightly smaller than those grown in solution rich with phosphorus. Moreover, P deficiency was manifested by the occurrence of small purple spots on stems and leaves (figure 18).

Figure 18: Morphological effects of P deficiency (-P), P levels and fertilizer forms on shoot.

The symptoms of P deficiency included reduction in leaf expansion and number of leaves. In addition to the increased root/shoot ratio. The figure below shows the Root/Shoot ratio, the control plants (-P) revealed the highest ratio value of 0.39 which means that shoots of control (-P) are the lightest (0.18g) and less developed than shoot of plants treated with P. the fertilizer form had no effect on shoot dry weight and root/shoot ratio.

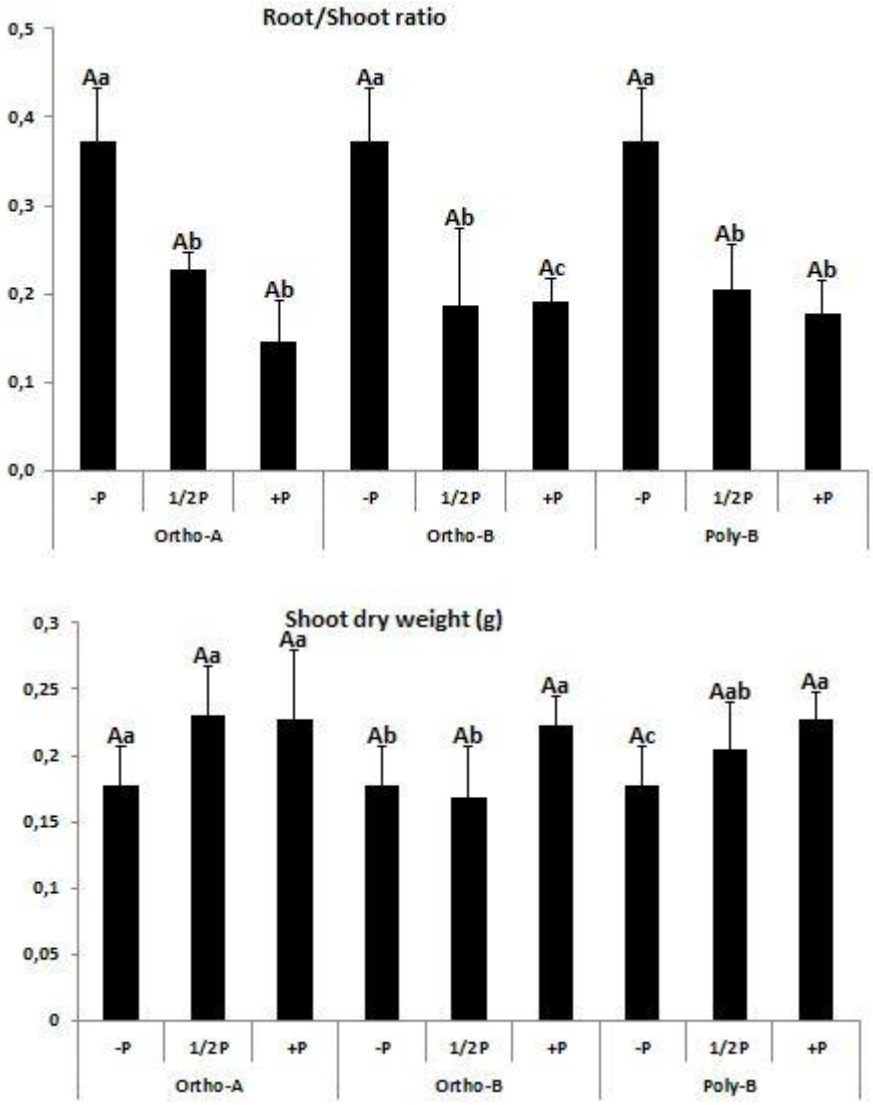

Figure 19: Shoot dry weight and Root/ Shoot ratio of wheat plants under P doses.

3. Leaf area

The results obtained by measuring leaf area shows that no P treatment has decreased the leaf area to 35 cm^2. This value begins to increase normally to reach a maximal value of 65 cm^2 in leaves treated of plants treated with Ortho-A level +P as shown on figure 8. Fertilizer forms have not influenced leaf area.

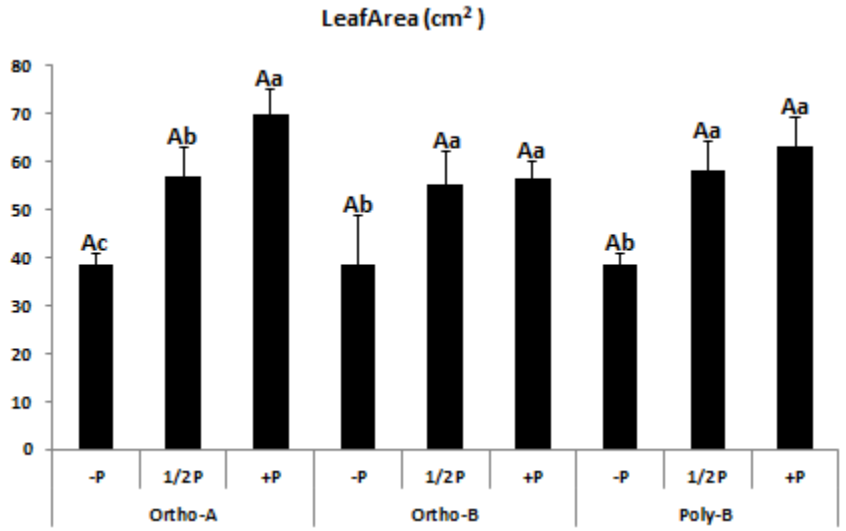

Figure 20: Effect of P levels and fertilizer forms on the leaf area of wheat leaves grown in hydroponic conditions.

II. Physiological parameters

1. Stomatal conductance

The Stomatal conductance using a leaf porometer over time of the treatment is presented in Figure 21. Stomatal conductance is a measure of the degree of stomatal opening. We noticed that the no P treatment (-P) in the first measure has significantly decreased the stomatal conductance to 90 mmol m^{-2} s^{-1}. Then this value begins to increase to reach a maximum value of 150 mmol m^{-2} s^{-1} in the leaves of plants treated with Ortho-B and Ortho-A +P level.

During the second measure stomatal conductance showed a slight decrease to120mmol m^{-2} s^{-1} in P-deficient leaves (-P), and returned to normal values to reach after a maximum value of 200 mmol m-2 s-1 in leaves of plants grown in solution containing Poly-B level $P_{1/2}$. No interaction effect has been noticed between P level and fertilizer forms during both, first and last measure.

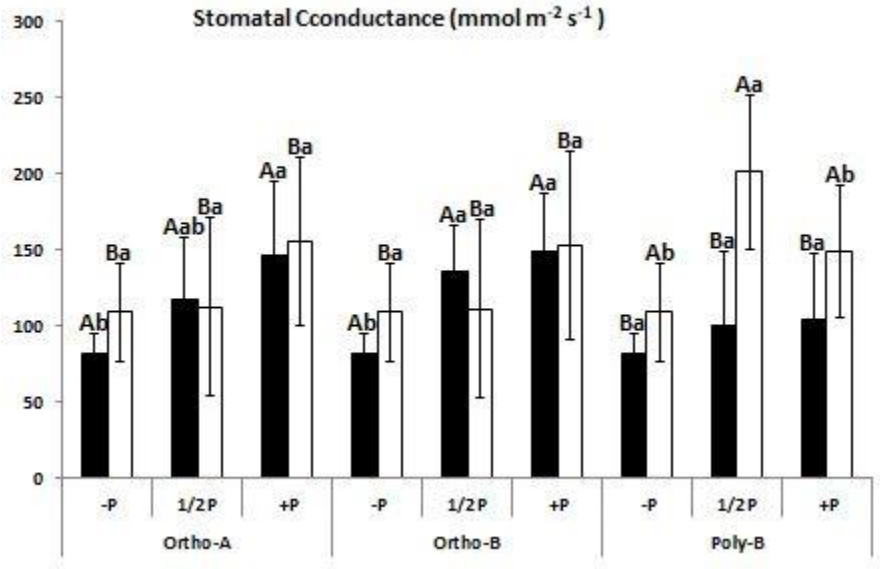

Figure 21: Responses of wheat stomatal leaves to different phosphorus levels and fertilizer forms.

2. Chlorophyll Content Index

The data for chlorophyll content in wheat plants are shown on Figure 22. No significant difference has been noticed between plants leaves under P efficient conditions and those grown in sufficient P concentration. This means that P-deficiency did not affect leaves chlorophyll content index. Also, there was no interaction effect between P level and fertilizer forms.

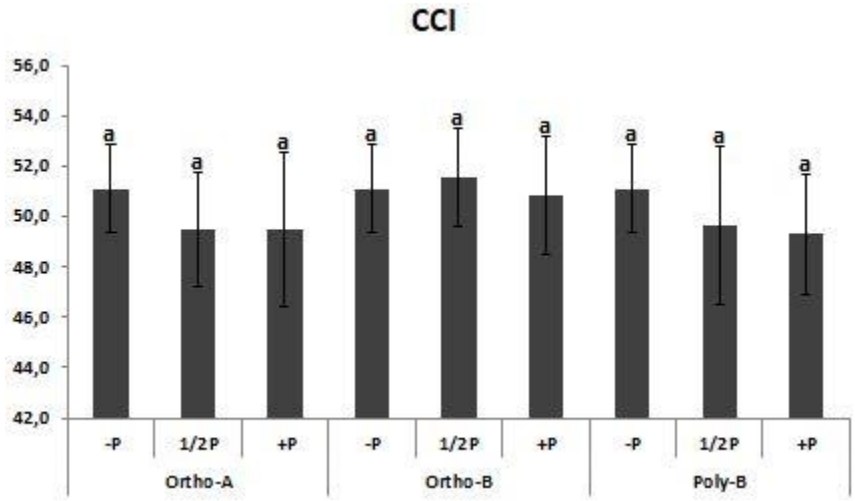

Figure 22: Effect of P doses and fertilizer forms on wheat leaves chlorophyll content index.

3. Leaf reflectance

To estimate plant nutrient status with spectral reflections, plants should be exposed to varying levels of the relevant nutrient (Mani and Shanmugam, 2019). Figure 7 shows the average reflectance curves for the three different Phosphorus doses and fertilizer forms of the first and the last measure.

As can be observed in the two figures bellow, the reflectance of the different treatments exhibited the typical features of fresh plant leaves. It is relatively lower in the visible region specifically between 450 nm and 700 nm; and relatively higher in the NIR range especially between 450 nm and 1000nm.

In the visible range, the maximal value of reflectance was 25% at 550nm and the minimum value is 14% at 670nm.this value start to increase in the debut of the NIR range to reach a maximal value of 62% throughout the wavelengths between 750nm and 1000nm, for all the treatments with the exception of control (-P) that started to decrease to 56% at 940nm only in the first measure. Unlike the last measurement where the control (-P) recorded the highest value of 64% at 800 nm.

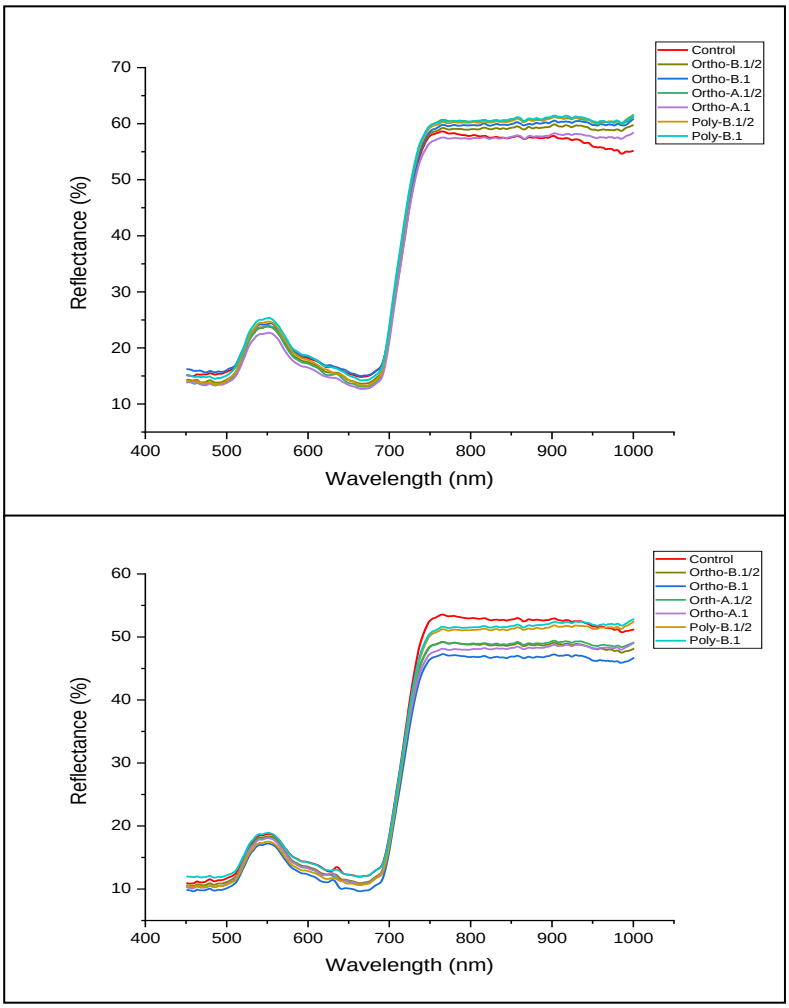

Figure 23: First and last measure of leaf reflectance

- **Vegetation Indices**

Spectral indices for phosphorus nutrient were calculated with all possible combinations of leaf reflectance at wavelengths between 450 and 1000 nm. The equations used for calculating the NDVI and PRI were as in the following.

NDVI = (R800 - R680) / (R800 + R680)
PRI = (R531 - R570) / (R531 + R570)

(Where R refers to reflectance at respective wavelength)

The obtained results are shown in the below figure (figure 10).fertilizer forms and P levels have no effect on NDVI indice, however as it can be noticed in the figure of PRI, they have influenced it. PRI values are very low; the highest value was recorded on Ortho-B P1 level while the lowest one was noticed on P deficient leaves (-P).

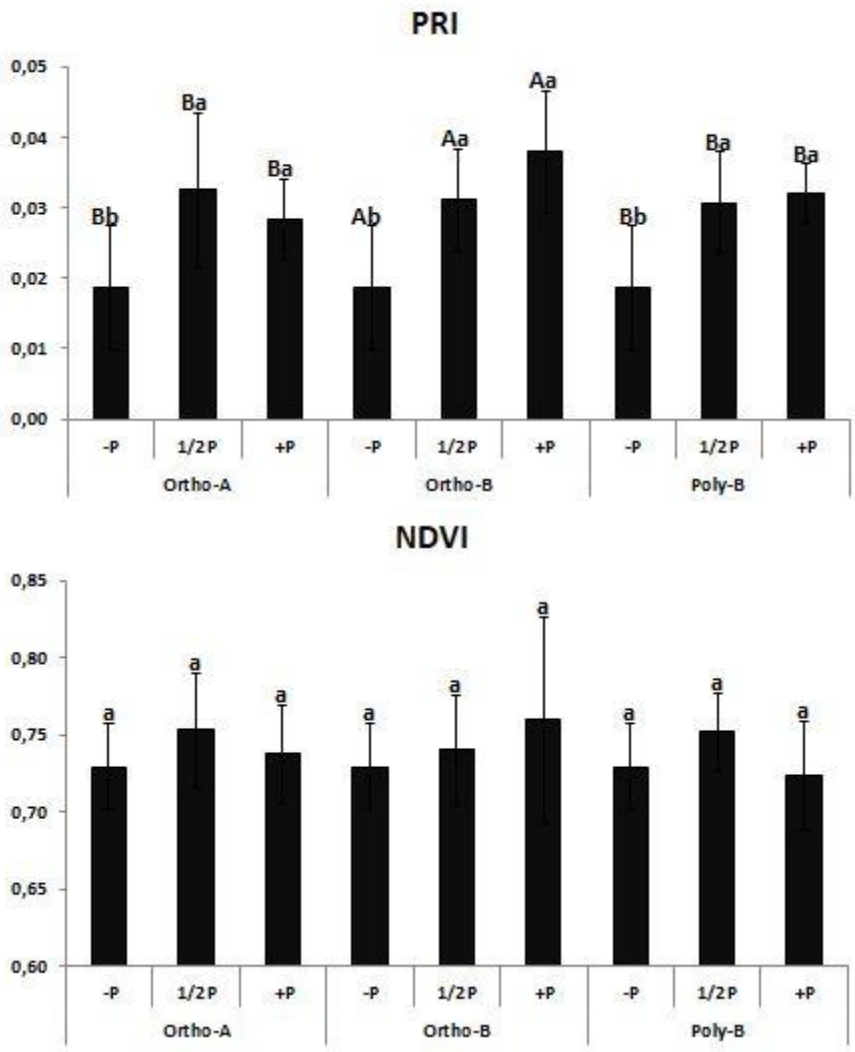

Figure 24: Responses of leaf spectral traits of wheat grown under varying P levels and fertilizer forms. (Normalized differential vegetation index (NDVI), Photochemical reflectance index (PRI)).

4. Leaf chlorophyll a fluorescence

- **OJIP curves**

The OJIP curves of Chl a fluorescence in wheat leaves are shown in the figure below. The figures showed no difference among the three treatments in the first two phases of the fluorescence curves, while starting from F_I a difference is noticed, the highest fluorescence intensity value (20000 mv) is recorded in phosphorus-deficient leaves (-P) at 100 ms followed by leaves of plants treated with 1/2P (50% of P) with a value of 18000 mv for the Ortho-B treatment and a value of 19000 mv for the Ortho-A and Poly-B treatments, and finally those grown in a solution containing the sufficient amount of phosphorus (+ P).

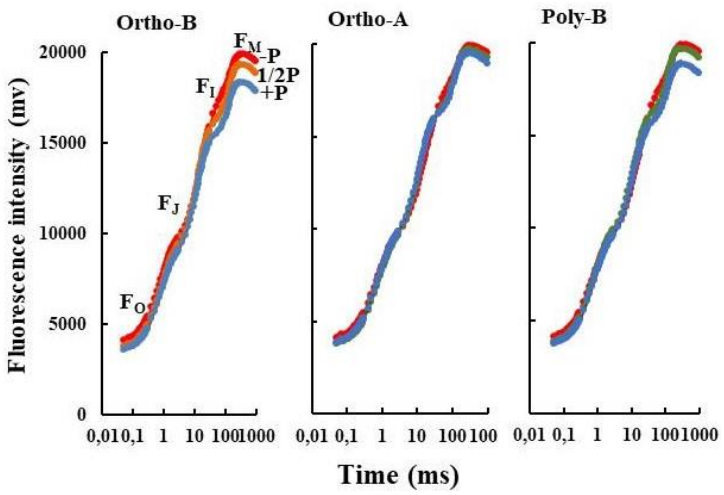

Figure 25: Transient chlorophyll a fluorescence induction curves of wheat leaves grown under low (-P), medium (1/2P) and high (+P) phosphorus stresses.

The Chl fluorescence measured in all plants showed the typical transient OJIP. In plant grown in nutritive solution with low P concentration showed a difference in fluorescence yield during I-P phase. We should note here that O-J phase is called the photochemical phase and it is light-dependent ((Delosme 1967, Neubauer and Schreiber 1987; Laza ´r 2006; Schansker et al. 2006). this photochemical phase provides information on antenna size and photosystem II (PSII) reaction centres connectivity (Strasser et al. 2004). The J-P phase is named the thermal phase (Delosme 1967). The J-to-I rise was proposed to be associated with the reduction of the PQ-pool (Toth et al. 2007a) and the I-to-P rise was associated with electron flow through PSI (Schreiber et al. 1989; Schansker et al. 2003; 2005). Under low P, the O-J, J-I phases seems to be unaffected. However, an increased I level was observed in plant growing without P.

- **Fluorescence parameters**
  - **ΔVt**

To observe the distinct changes among the fluorescence curves of different treatment, the ΔVt OJIP curves from O phase to P phase of the first (A) and the last (B) measure are presented in the figure below (figure 26).

In the first measure (A), compared with the P curve, the characteristic of the ΔVt curve of the plants treated by (-P) included a significantly decrease and recorded negative values from 0.1 to 100ms with a maximum decrease of -0.15 at 1ms and -0.19 at 100ms in Ortho-A and Ortho-B treatments, respectively. Then begins to increase progressively to reach the 0, for the three fertilizer forms. The same results are noticed in plants treated with 1/2P, with an exception of Poly-B treatment, values of ΔVt were positives close to zero before they increased to reach a maximum value of 0.08 at 10ms then decreased to -0.02 at 100ms.

In the last measure (B), compared with the P curve, P deficiency (-P) has introduced a big decrease in the ΔVt to -0.3 at 10 ms for all treatments, and then increased to 0.1 at 100ms.P optimal concentration has also increased the ΔVt to -0.1 between 1 and 10 ms.

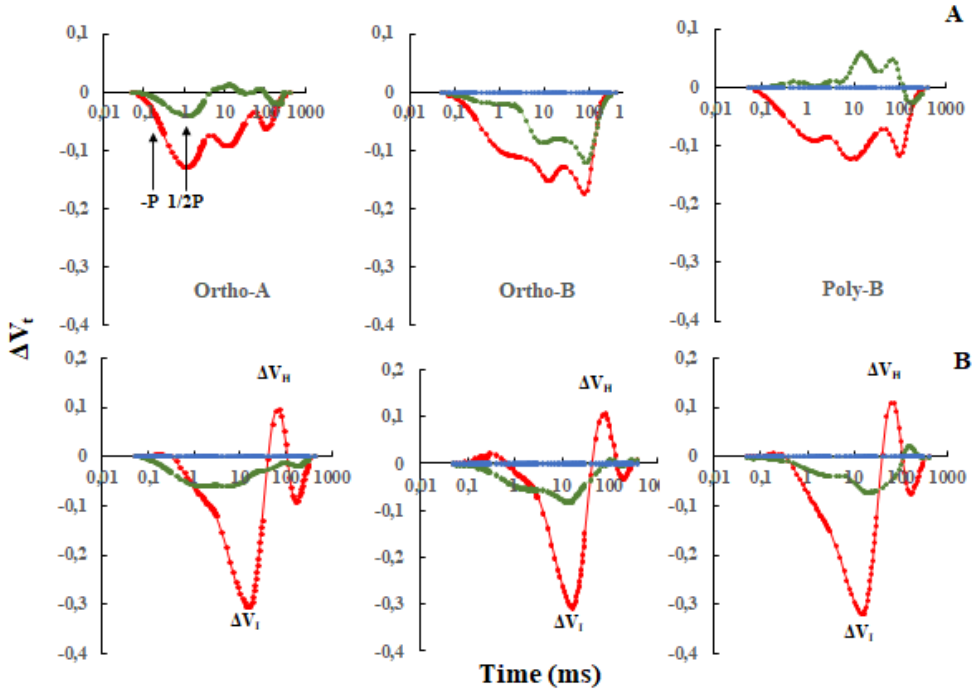

Figure 26: Effect of P deficiency and P fertilizer forms on OJIP fluorescence kinetics. (A: first measure and B: last measure).

- **F_t/F_I and $(F_t - F_I)/(F_M - F_I)$ ratio**

To further analyze the effect of P deficiency on the OJIP properties, the fluorescence curves were normalized to FI shown as FIP and double normalized between I and P shown as VIP which allows evaluating the behavior of the electron flux at the acceptors side of photosystem I (PSI). F_{IP} amplitude was higher in plants growing without P and its shape was distinguishable from plants growing at low or sufficient P concentration. VIP showed also the highest slope in plant growing in low or sufficient P compared to plants growing without P. highest slopes on phase I-P. The IP phase of the OJIP curve measured in plants growing in sufficient or low concentration of P exhibited a sigmoidal curve while this sigmoidality is lost in plants growing in nutritive solution without P.

All graphical illustrations of the transients were double-normalized between F_I and F_M to give the relative variable fluorescence at time t as follows:

V (t) = $(F_t-F_I)/(F_M-F_I)$. And F_t/F_I ratio

The below figures represent the I and P zoomed steps of the OJIP curves. The difference between plants under P deficiency and plants under P sufficient conditions is very clear, which confirms the differences noticed in the OJIP curve.

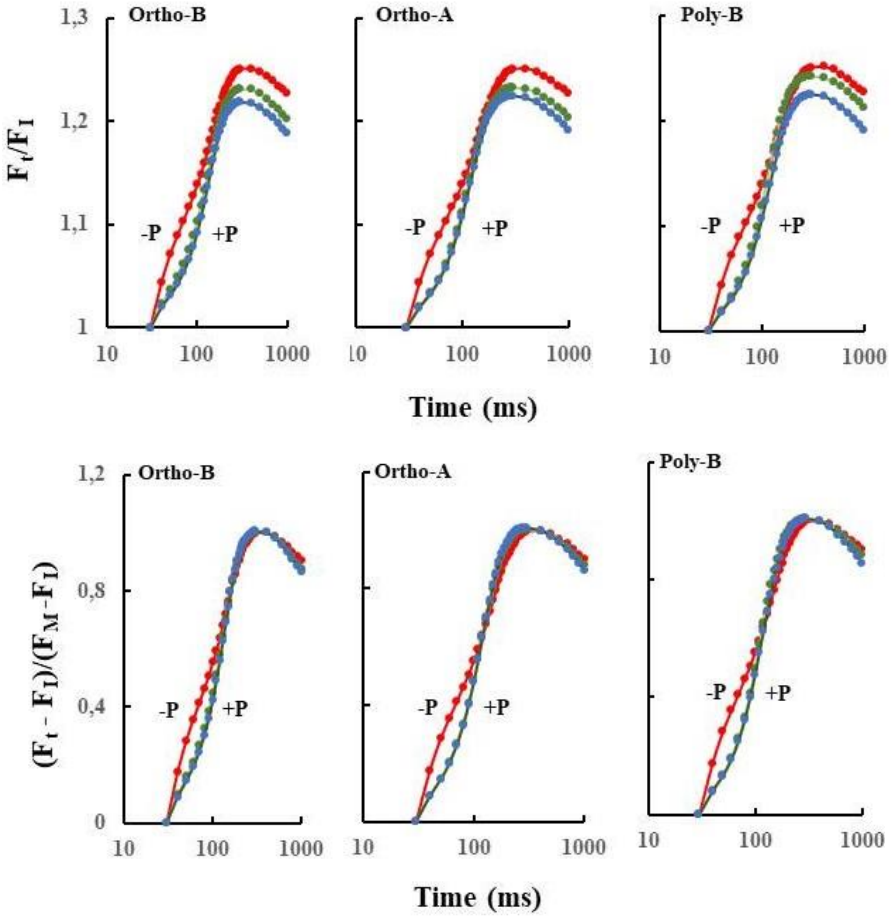

Figure 27: the O-J-I-P fluorescence transients zoomed in at the so-called I-step and P-step and double normalized between FI and FM.

5. Leaf absorbance at 820nm

Absorbance kinetic changes at 820 nm in wheat leaves induced by red actinic light that reflect states of PSI is presented in figure 28. In the first measure (A), the absorbance at 820nm has decreased to 33860 until about 20ms in P deficient plants followed by plants treated with 1/2P and then those of +P, especially on Ortho-B and Poly-B treatments, then increased to 34150 between 100 and 120ms. Contrary to the last measure where the maximum decrease on the absorbance at 820nm was noticed on plants treated with +P and 1/2P, then those deficient of phosphorus.

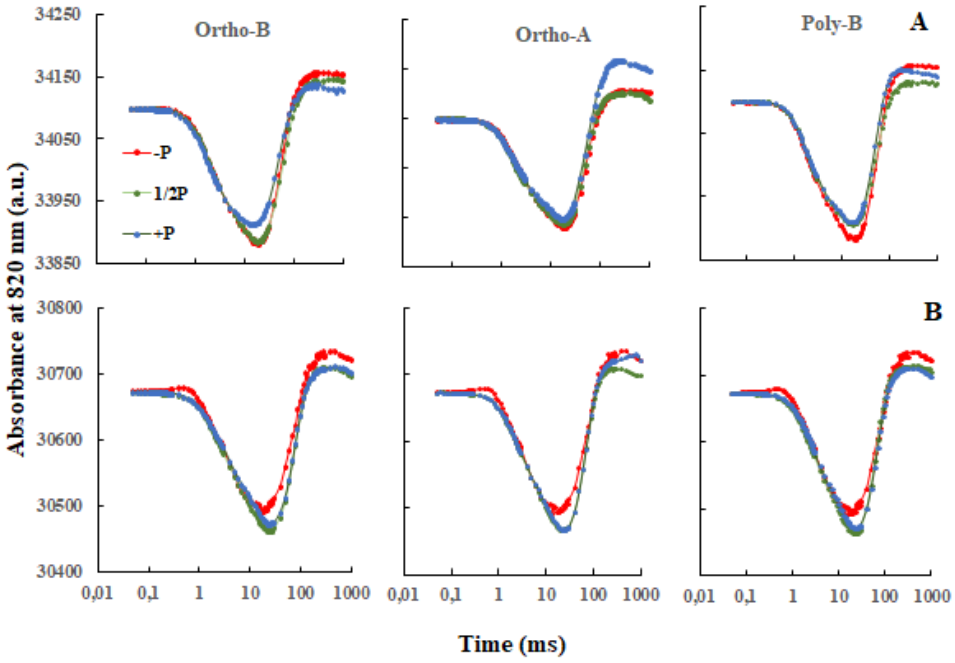

Figure 28: kinetics of absorbance at 820 nm of wheat leaves under different level of P and fertilizer forms in the first and last measure.

Figure 28 showed the Kinetic changes at 820 nm (MR) induced by red actinic light of 5000 mmol photons m2 s1 in leaves. These changes in MR reflect the redox states of P700 and PC. The kinetics of the normalized MR indicates initial oxidation followed by a re-reduction of P700 and PC. At the beginning of treatment, kinetic changes at 820 nm showed similar re-reduction of P700 and PC and this occurred after 20 ms except in plants growing in nutritive solution with Ortho-B and sufficient P which re-reduction of P700 and PC was faster and occurred after 16 ms. after 4 weeks of treatment, we observed that re-reduction of P700 and PC was faster and occurred after 14 ms in all plants growing without P.

Discussion

I. Morphological parameters

This study was conducted to determine whether P deficiency affected plant morphological and physiological parameters. The increase in root growth parameters (**figures 13, 14 and 15)** of wheat plants under P-deficient conditions is considered as a kind adaptation of plants to ensure a sufficient uptake of P. Plants establish their root systems in order to search for phosphorus which is immobile in the soil. This can be explained by Mollier and Pellerin's (1999) notion that P deficiency impacts root growth by affecting carbon partitioning between shoots and roots. The observed results are consistent with those of Lynch and Brown, (2001) who have noticed that the root architecture of plants can undergo several changes in response to P deficiency. These changes were reflected by an increase in lateral root growth and secondary root branching at the expense of primary root elongation in beans. P deficiency also led to an increase in the root/shoot dry weight ratio, this may be explained by the increase in the allocation of carbohydrates to root according to An-cheng et al, (2006). Since root/shoot ratios typically increase in P deficit, roots are generally regarded as stronger sinks than leaves (Mollier and Pellerin, 1999;Vance et al., 2003).

The reduction in the shoot and whole plant dry weight production observed in wheat plants in response to P limitation is related to a reduction in leaf area (**figures 16 and 17**). The measured LA was significantly lower in the -P treatment, which agrees with the results of Plénet et al. (2000) in a study conducted in the field on maize that showed the LA was severely reduced in the P0 treatment (up to 60%), especially during the first phase of the crop cycle. This reduction in leaf area of wheat plants grown under phosphorus deficiency indicates that P starvation affects the processes related to cell division and growth of the wheat plant. Wheat plants grown under P-sufficient conditions produced more shoot than plants grown under P-deficient conditions, which demonstrate the importance of an adequate P supply for the development of the wheat shoot.

II. Physiological parameters

The results obtained for the chlorophyll content index in wheat leaves under P-deficient were similar to those obtained in plants grown in sufficient P conditions (**figure 19**). Hence no differences had been noticed, which means that P deficiency did not affect the chlorophyll

content index in wheat leaves. These results are similar to those of Kalaji and colleagues ( ), in a study where they compared between different chlorophyll content meters under nutrients deficiency conditions, they found that deficiencies of all elements except P and S caused a significant decrease in total chlorophyll content. However, P deficiency has influenced the stomatal conductance. Plants under P deficient conditions had recorded the lowest value of stomatal conductance and which was higher in plants treated with sufficient P (**figure 18**). This means that P starvation has a negative effect on leaf stomatal conductance. Our results are in accordance with those found by An-cheng et al (2006), where they noticed that low P treatment decreased stomatal conductance for both genotypes of rice. Decrease in stomatal conductance under deficient P induced a decrease in gas exchanges. It is well known that the regulation of stomatal opening and closure has a direct influence in reducing transpiration and solute transport through the plant. We assume that plants under deficient P modulate their stomatal conductance to optimise the rate of CO_2 and water loss to preserve the energy used in photochemical phase in photosynthesis.

ChlF techniques are frequently employed in physiological analyses of phosphorus deficiency in plants. In the present work, effects of P doses and fertilizer forms on chlorophyll a fluorescence were studied. The obtained results demonstrated that P deficiency (-P) has modified the I-P phase on the OJIP curves, plant under P starvation (-P) revealed the highest fluorescence intensity value compared to those grown under P sufficient conditions (+P). This is a strong indication that P deficiency has affected the photosynthesis activity. As it is known that the I-P phase represents the reduction of photosystem I, thus phosphorus deprivation has a direct effect on PSI. Therefore, changes in the I-P step appear to be highly valuable for diagnosing P deficiency. Similar results are found by Carstensen et al.,(2020). Moreover, Studies have previously shown that the curvature of the I-step of OJIP transient can be used as a proxy to determine the bioactive P concentration in plants (Frydenvang *et al.* 2015).

Clear differences at the I-step and the P-step were observed between the treatments. To avoid a pure visual classification of changes in the two-steps curvature we applied mathematical formulas, as shown in **figure 25**. The differences in the two steps seemed clearer. This is similar to that presented in Carstensen *et al*. (2019). To analyze differences between the induction curves form in response to P-deficiency, curves of differential values (ΔVt), was performed (**figure 24**). As a result, three negative peaks were observed at J and P steps under –P level in the three forms, and three positive peaks were noticed at I-step. Under P optimal concentrations (1/2P) the peaks of all steps were negative.

Considering the fact that when fluorescence intensity is higher the photosynthetic activity is lower, it could be logical to deduce that phosphorus deficiency affects the photosynthetic apparatus and limits its activity. Moreover, phosphorus plays an important role in photosynthesis, particularly during the synthesis of ATP (ADP + Pi = ATP). A disorder in P nutrition can alter this process, which causes some disturbances in light-dependent photosynthesis processes (electron transfer from PSII to PSI), according to (Chtouki et al., 2021). Also, P deficiency

reduces ATP synthase activity, which causes a reduction in proton transport from the thylakoid lumen to the chloroplast stroma, resulting in lumen acidification and restriction of plastoquinones oxidation, as demonstrated by Carstensen et al. (2018). Same explanation was suggested by (Carstensen et al., 2020).

Regardless of the form of the polyphosphates, the effect of P deficiency on wheat depended on the concentration of P in the nutrient solution but also on the duration of treatment. At the beginning of the deficiency, it seems the deficiency in P affects the whole chain of electron transport shown by the appearance of two bands at J and I steps. However, in our experience and after 4 weeks of treatment, the IP phase is strongly affected. The appearance of ΔJ-band in OJIP transient is associated with the accumulation of Q_A^- which might be due to an inhibition of the Q_A^- reoxidation. The appearance of ΔI-band could be associated with the inactivation of ferredoxin-$NADP^+$ oxidoreductase (Schansker et al., 2003). The I-P phase suggests being linked to the subsequent electron flow through cytochrome b6f to the PSI acceptor side (Stirbet and Govindjee, 2011). We suppose that the decreases of ΔVt on I-P phase reflected a decrease in the efficiency in which an electron is transferred to the PSI acceptor side.

The observed decrease of absorbance at 820 nm, between 10 and 100 ms, reflects the photo induced oxidation of P700 and PC, followed by an increase at 500 ms which reflects the reduction of photosystem I and PC (**figure 28**).

At wavelengths between 450-500 and 600-700 nm, the reflectance spectra of both P-deficient and non-deficient plants showed no significant differences (**figure 21**). Indeed, chlorophyll-a and chlorophyll-b have so strong absorption in this region that the reflectance signal is probably saturated, and changes in pigment concentrations are not reflected in the spectra. This agrees with the results found by Yaryura *et al*., (2009). But the reflectance increase noticed between 500 and 600 nm maight be explained by the fact that anthocyanins typically absorb at around 530nm.

However, important differences were observed between plants under P deficiency and plants grown under P-sufficient conditions in the 750-1000 nm region especially in the last measure. Therefore we can conclude that the phosphorus deficiency might affect indirectly on the pigments absorption.

Conclusion

In this study conducted on wheat plants grown under hydroponic conditions, we focused on the response of the photosynthetic apparatus and other morphological parameters to phosphorus deficiency-induced stress. Our results showed that P deficiency affected several features, including plant morphology, photosynthesis activity, pigment light absorption on the vis-NIR reflectance and the stomata opening percentage. Visible symptoms of P deficiency were also observed, which were reflected by the decrease in shoot dry weight and leaf area. And an increase on root growth has been noticed. We found that P deficiency (-P) has modified the I and P steps, which might be due to disturbances in light-dependent photosynthesis processes (electron transfer from PSII to PSI). This induced stress has also indirectly affected leaf reflectance. We can conclude that these reflectance and fluorescence spectroscopies may provide a rapid, non-destructive diagnostic method for detecting and quantifying phosphorus deficiency and damage to the photosynthetic apparatus of leaves, thus allowing for early intervention in crop production before the effects of phosphorus stress become irreversible. We recommend using the two techniques of fluorescence and reflectance on a large scale as a rapid, non-destructive diagnostic method to detect and quantify nutritional deficiencies and damage to the photosynthetic apparatus, allowing rapid and early intervention in agricultural production.

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Résumé

Le phosphore (P) est un élément nutritif essentiel de la nutrition minérale des plantes au même titre que l'azote et le potassium. Il est le deuxième macronutriment le plus largement limitant dans le sol pour la croissance des plantes, après l'azote. Il est essentiel pour la croissance des plantes en raison de son rôle crucial dans de nombreux processus métaboliques. Parmi ces processus, les fonctions physiologiques telles que l'accumulation et la transmission d'énergie, la photosynthèse, la respiration, la différenciation cellulaire et l'expansion cellulaire, il est ainsi impliqué dans la synthèse de composés phosphatés riches en énergie comme l'adénosine triphosphate (ATP) et l'adénosine di-phosphate (ADP). Les plantes absorbent le P sous forme d'ortho-phosphates ; $H_2PO^-_4$ et HPO_4^{2-} . Cet élément existe immobile dans le sol.

Depuis de nombreuses années et dans la plupart des pratiques agricoles, le phosphore a été identifié comme l'élément essentiel limitant la croissance des plantes le plus courant. Sa carence provoque des altérations morphologiques, physiologiques et biochimiques, telles que la diminution du rapport pousses/racines, les changements architecturaux racinaires, l'accumulation d'anthocyanes et le retard dans la maturité de la plante.

Pour diverses raisons, il est extrêmement difficile de diagnostiquer une carence en P chez les plantes. L'un d'eux est que la réponse du phosphore des cultures s'est avérée sans rapport avec la quantité totale du phosphore dans le sol. De plus, les symptômes foliaires évidents des plantes carencées en phosphore sont rares, et lorsqu'ils surviennent, ils se produisent souvent en conjonction avec d'autres symptômes de carence en éléments nutritifs. Ainsi, les méthodes actuelles de diagnostic des éléments nutritifs des plantes sont destructives, prennent du temps, dépendent des ressources, nécessitant une main-d'œuvre et des équipements spécialisés. Par conséquent, il existe un besoin pour une méthode rapide, moins couteuse et non invasive qui pourra fournir des informations sur l'état nutritionnel des plantes, permettant ainsi une intervention précoce et rapide dans la production agricole, avant que les stress nutritionnel deviennent irréversibles.

Dans ce contexte, les spectroscopies de réflectance et de fluorescence peuvent fournir une méthode rapide et un diagnostic non destructif pour détecter et quantifier les stress environnementaux et leurs effets sur l'appareil photosynthétique des feuilles.

Dans cette étude, un mélange de méthodes et d'outils a été utilisé pour analyser les changements dans la structure et la fonction de la machinerie photosynthétique afin de mieux comprendre l'altération la photosynthèse induite par une carence en P dans les plantes du blé cultivées dans des conditions hydroponiques.

Les principaux objectifs de ce travail étaient :

D’élucider les effets de l'appauvrissement en P sur l'appareil photosynthétique des feuilles de blé, d’évaluer l'émission de fluorescence et de réflectance en tant qu'indicateurs de stress prématuré chez les plants de blé et d’évaluer les effets d'une carence en phosphore sur d'autres paramètres physiologiques, tels que la conductance stomatique et la teneur en chlorophylle, ainsi que morphologiques.

Pour répondre à nos objectifs, des plantules du blé, 6 jours après semé, ont été transplanté dans une solution d’Hoagland modifiée pour le blé qui était continuellement aérée, changée deux fois par semaine (chaque trois jours) et contenait les macros et micronutriments suivants : CL, Ca, N, K, S, Mg, P, Fe, B, Zn, Mn, Cu et Mo. Les plantes du blé ont été cultivées en hydroponie en utilisant trois différentes doses de P : la dose –P, dans laquelle les plantes ont été traitées avec tous les nutriments sauf le phosphore, la dose P1/2, dans laquelle les plantes ont été traitées avec 50 % de leur besoin en phosphore et la dose +P, dans laquelle les plantes ont été traitées avec la quantité suffisante en phosphore. Trois formes de fertilisants sont utilisées pour apporter le P, deux ortho-phosphates et un poly-phosphate.

Pour évaluer l’effet de la carence en P sur la culture, six mesures ont été effectuées pour chaque paramètre physiologique sauf la conductance stomatique (deux mesures uniquement).

L'indice de teneur en chlorophylle a été mesuré à l'aide du CHLOROPHYLL METER SPAD-502 Plus. C’est un appareil qui permet de déterminer la quantité relative de chlorophylle en mesurant l'absorbance foliaire dans deux régions de longueur d'onde, car la chlorophylle a deux pics d’absorption, un dans le bleu et l'autre dans le rouge. Deux mesures de la conductance stomatique sont effectuées pour à l’aide de leaf porometer (SC-1, Decagon Devices, WA, USA), pour nous donner une idée sur le pourcentage de stomates ouvertes.

La photosynthèse est l'un des processus métaboliques les plus importants chez les plantes; par conséquent, la mesure de son activité fournit également des informations sur l'« état de santé » de la plante. Dans le domaine de la recherche sur la photosynthèse, la fluorescence de la chlorophylle a est l'une des méthodes les plus répandues, à la fois dans les études fondamentales et éco-physiologiques. L'énergie lumineuse absorbée par les molécules de chlorophylle a trois possibilités ; elle peut entraîner la photosynthèse, être réémis sous forme de chaleur ou être réémis sous forme de lumière. Par conséquent, la fluorescence chlorophyllienne est une mesure de la lumière du PSII. Dans ce travail les mesures de fluorescence et d'absorbance de la chlorophylle ont été effectuées en utilisant l’appareil M-PEA (Multi-Function Plant Efficiency Analyzer).

Cette méthode est basée sur l'enregistrement, à haute fréquence, de ChlF émis par une feuille adaptée a l’obscurité pendant une courte impulsion (généralement une seconde) de forte lumière actinique. L'augmentation de ChlF pendant la première seconde d'éclairage montre une séquence de phases (marquées O, K, J, I, P) de la valeur de fluorescence initiale (Fo) à la valeur de

fluorescence maximale (Fm). La méthode permet de calculer des paramètres biophysiques spécifiques, des rendements quantiques et des probabilités caractérisant la structure et la fonction du système de transport d'électrons photosynthétique ainsi que certains paramètres d'intégration liés aux performances photosynthétiques des plantes. La courbe OJIP contient trois phases distinctes : OJ, JI, et IP, qui ont rapport à trois processus différents. OJ est lié à la réduction du côté accepteur du PSII ; JI représente la réduction du pool de PQ ; enfin, IP représente la réduction du coté accepteur d'électrons du PSI. Par leur sensibilité à l'état redox des composants de la chaîne de transport d'électrons, les mesures de fluorescence permettent donc de suivre de manière indirecte et d'interpréter de nombreux processus ayant lieu durant le transport d'électrons.

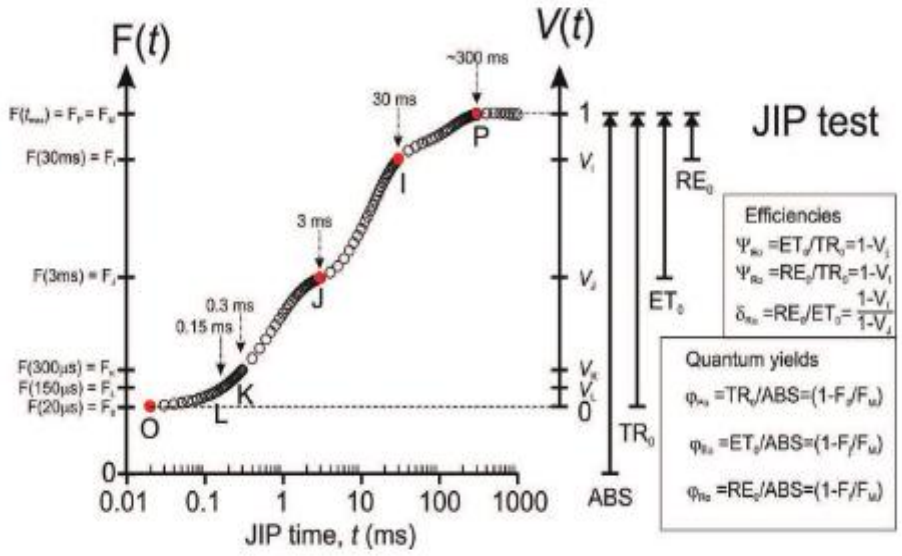

Figure 1: Typical OJIP-transient of chlorophyll fluorescence (Kautsky curve) exhibited on illumination of a dark-adapted leaf sample (4 mm2) by saturating red light (3000 µmol photons m22 s21).

Nous avons utilisé une méthode complémentaire, à savoir les mesures simultanées d'absorbance à 820 nm (en parallèle avec la mesure de la fluorescence chlorophyllienne). Cette technique permet d'obtenir des informations sur l'état redox de P700 et PC qui sont les composants de photosystème I. De cette façon, on peut obtenir des informations de l'autre côté de la chaîne de transfert d'électrons. Le principe de cette technique est le suivant : si le P700 et PC sont oxydés par les séparations des charges qui se font dans le photosystème I, l'absorbance à 820 nm diminuera. Quand les électrons arrivent de photosystème II, ils réduisent le P700+ et PC+, et l'absorbance augmente.

La réflectance spectrale des feuilles a été mesurée à l'aide d'un spectromètre (CI-710/720, CID-Bioscience, USA). Six mesures de réflectance spectrale ont été effectuées de 450 nm à 1000 nm de longueur d'onde. La réflectance de la feuille est calculée comme un rapport entre l'énergie réfléchie de la feuille et l'énergie incidente de la source lumineuse.

Cette technique est utilisée pour l'analyse des éléments nutritifs des sols et des plantes en mesurant la réflectance dans une plage de longueurs d'onde de 400 à 2500 nm, le visible et le proche-infrarouge, respectivement. Elle permet une mesure directe du statut nutritionnel de la plante, ce qui contourne l'analyse des nutriments du sol et permet aux producteurs d'utiliser ces informations pour la gestion des engrais. De plus, le VNIRS permet de détecter les carences cachées et empêche toute application excessive d'engrais qui permettrait aux producteurs d'être proactifs dans leur gestion des nutriments. La réflectance des feuilles diffère d'une région à l'autre, il est relativement faible dans le domaine spectral visible (Vis) et relativement élevé dans le spectre NIR. L'absorbance par les structures pigmentaires (par exemple, la chlorophylle et les caroténoïdes) est le principal contributeur à la réflectance dans la gamme spectrale visible.

Plusieurs indices de végétation peuvent être calculés à l'aide de ces bandes d'absorption, dans cette étude nous avons obtenu deux indices de végétation à savoir l'indice de végétation différentiel normalisé (NDVI) et l'indice de réflectance photochimique (PRI).

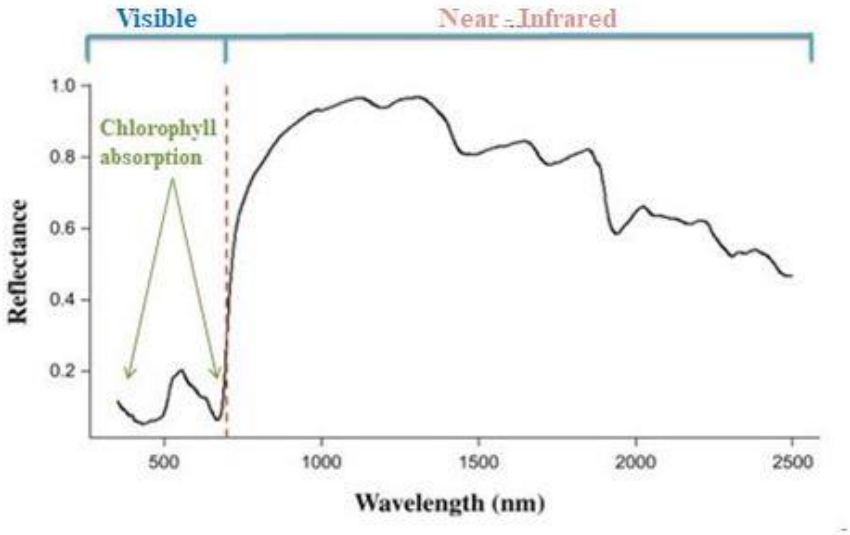

Figure 2: Reflectance spectra of a cotton leaf in the visible part of the spectra (400–700nm) and the NIR part of the spectra (700–2500nm).

Après 20 jours, les plantes ont été récoltées en séparant les deux parties de la plante (la partie aérienne et la partie racinaire). Les feuilles ont été mesurées, pour déterminer la surface foliaire, à l'aide de AM350 Portable leaf area meter. Les racines ont été soigneusement retirées, et trempées dans de l'eau distillée pour éliminer toutes les impuretés. Les différents paramètres racinaires sont calculés grâce à un scanner WinRhizo.

Dans la présente étude, nous avons pu montrer que la carence en P a influencé plusieurs paramètres physiologiques ainsi que morphologiques. Les plantes cultivées dans un milieu déficient en P avaient les racines les plus développées. Ceci est considéré comme une sorte

d'adaptation des plantes pour assurer une absorption suffisante de P. En outre, la déficience en P est traduite par une réduction de la surface foliaire et une diminution du poids sec de la partie aérienne au détriment de celui de la partie souterraine.

La diminution de la conductance stomatique sous un déficit en P induit une diminution des échanges gazeux. Il est bien connu que la régulation de l'ouverture et de la fermeture des stomates a une influence directe sur la réduction de la transpiration et du transport de soluté à travers la plante. Nous supposons donc que les plantes sous carence en P modulent leur conductance stomatique pour optimiser le taux de CO2 et la perte d'eau afin de préserver l'énergie utilisée dans la phase photochimique de la photosynthèse.

Les résultats obtenus à partir des mesures de la fluorescence chlorophyllienne ont montré que la carence en P a modifié la phase I-P sur la courbe OJIP. Considérant le fait que, lorsque l'intensité de fluorescence est plus élevée, l'activité photosynthétique est plus faible, il pourrait être logique d'en déduire qu'une carence en phosphore affecte l'appareil photosynthétique et limite son activité. De plus, le phosphore joue un rôle important dans la photosynthèse, notamment lors de la synthèse d'ATP (ADP + Pi = ATP). Un trouble de la nutrition P peut altérer ce processus, ce qui provoque quelques perturbations dans les processus de photosynthèse dépendant de la lumière (transfert d'électrons du PSII vers le PSI). En outre, la carence en P réduit l'activité de l'ATP synthase, ce qui entraîne une réduction du transport des protons de lumen des thylakoïdes au stroma chloroplastique, entraînant une acidification de lumen et une restriction de l'oxydation des plasto-quinones.

La diminution observée de l'absorbance à 820 nm, entre 10 et 100 ms, reflète l'oxydation photo-induite de P700 et PC, suivie d'une augmentation à 500 ms qui reflète la réduction du photosystème I et PC.

Aux longueurs d'onde comprises entre 450-500 et 600-700 nm, les spectres de réflectance des plantes déficientes et non déficientes en P n'ont montré aucune différence significative. En effet, la chlorophylle-a et la chlorophylle-b ont une absorption si forte dans cette région que le signal de réflectance est probablement saturé, et les changements de concentrations de pigments ne sont pas reflétés dans les spectres. Mais l'augmentation de la réflectance observée entre 500 et 600 nm pourrait s'expliquer par le fait que les anthocyanes absorbent généralement vers 530 nm. De ce fait, nous pouvons conclure que la carence en phosphore pourrait affecter indirectement l'absorption des pigments.

Nos résultats ont pu montrer que la carence en P affectait plusieurs caractéristiques, notamment la morphologie de la plante, l'activité de photosynthèse, l'absorption des pigments dans le domaine vis-NIR et le pourcentage d'ouverture des stomates. Des symptômes visibles de carence en P ont également été observés, reflétés par la diminution du poids sec des pousses et la réduction de la surface foliaire. Nous avons constaté que le déficit en P a modifié les étapes I et P, ce qui pourrait être dû à des perturbations dans les processus de photosynthèse dépendant de la lumière (transfert

d'électrons du PSII au PSI). Ce stress induit a également affecté indirectement la réflectance des feuilles.

Nous pouvons donc conclure que ces techniques de réflectance et de fluorescence peuvent fournir une méthode de diagnostic rapide et non destructive pour détecter et quantifier la carence en phosphore et les dommages causés à l'appareil photosynthétique des feuilles, permettant ainsi une intervention précoce dans la production agricole avant que les effets du stress phosphore ne deviendra irréversibles.

Master Sciences et Techniques

Gestion et Conservation de la Biodiversité

Abstract

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Année Universitaire: 2020-2021
Titre: Effect of Phosphorus deficiency and fertilizer forms on chlorophyll a fluorescence and visible near-infrared reflectance of wheat plants grown under hydroponic conditions

Wheat (*Triticum sativus* L.) seedlings of 6 days were transplanted and used to study effects of doses and fertilizer forms of phosphorus in the chlorophyll a fluorescence, leaf reflectance and other physiological and morphological parameters. Plants were raised in hydroponic culture.

As it is known that phosphorus is an essential macronutrient for plant growth, and its deficiency limits plant productivity. Recent research has shown that chlorophyll a fluorescence and leaf reflectance analysis can be used as a sensitive indicator of latent P deficiency in a variety of plant species. In the present study we showed that, chlorophyll a fluorescence OJIP transients and plant leaf spectra in the vis-NIR regions are powerful tools for detecting P deficiency in wheat directly in the plant.

The results showed a difference on the I and P steps on the OJIP transient in phosphorus plants deficient (-P) compared to the plants grown under P sufficient conditions. Besides the indirect effect of P starvation on pigment absorption in the near-infrared region, it was found that P deficiency also affected the stomatal conductance. However no effect was noticed on chlorophyll content index.In addition to physiological parameters, deficit on phosphorus has also affected some morphological ones. In fact, the induction of some forms of root hairs and the increase in root growth parameters are the most noticeable changes in root architecture caused by phosphorus deficiency. This was to the detriment of shoot growth, which was smaller.

Keywords: Phosphorus deficiency, Chlorophyll a fluorescence, leaf reflectance, root architecture, shoot growth, wheat.