EVALUATION OF PHOSPHORUS USE EFFICIENCY IN WINTER WHEAT VARIETIES AND USING OPTICAL SENSORS TO PREDICT THE MAIZE POPULATION (ZEA MAYS L.)

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POPULATION (ZEA MAYS L.)

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Abstract: This dissertation includes two topics; 1) evaluation of P use efficiency in winter wheat varieties. 2) using optical sensors to predict the maize population. The objective of the first topic was to (i) simulate a soil with insoluble forms of phosphorus and to evaluate seventeen winter wheat varieties for P uptake and utilization efficiency based on the total mass of P present in the soil; (ii) screen seven wheat varieties for P use efficiency in filed experiments (iii) predict the possibility of using the normalized-difference vegetative index (NDVI) for determining P limiting conditions. For P use efficiency, several studies were conducted in the growth chamber and filed experiments. The results of greenhouse studies suggested that Gallagher and Endurance were the most efficient varieties for both P uptake efficiency and P utilization efficiency. Also, The Ok11755W was the most P utilization efficient while OK10430-2 was the most P uptake efficient. Otherwise, the Ruby Lee was among the less efficient varieties either to uptake or utilize P under greenhouse conditions. Under experimental field conditions, Duster and Endurance were found to be more efficient in extracting or utilizing more P while Ruby Lee was found to be less efficient. NDVI was also possible to estimate the P deficiency due to a strong correlation between the NDVI and yield prediction under low P conditions; this observation may be used as an indicator for P recommendation. The objective of the second topic was to identify whether there is a correlation between normalized difference vegetation index (NDVI), the Coefficient of variation (CV), and the maize population. For using optical sensors, data was collected from 76 plots located at the Agronomy Research Station (EFAW) near Stillwater, OK, and the Lake Carl Blackwell Research Station (LCB) near Bray, OK. Finding and conclusion of this study suggested that since NDVI and CV were correlated to plant population, the growth stage V4 would be the appropriate stage to predict the plant population and biomass estimation, which could be useful for producers for making replant decisions and precise estimations of replanting rates.

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CHAPTER I

REVIEW OF LITERATURE

1) Introduction

Modern agricultural production depends on the phosphorus (P) derived from rock phosphate, which is a non-renewable resource and has been estimated to be depleted in the next 80 years at the current rate of extraction (Smil, 2000). In addition, it is broadly known that the quality of the remaining phosphorus reserves will decline, and the cost of P extraction will also increase (Driver, 1998; Runge-Metzger, 1995; Smil, 2000). Therefore, researchers should investigate means of P conservation, recycling, and increased efficiencies. Despite dwindling reserves of phosphorus, farmers apply P fertilizers more than plant require because of low P availability and low fertilizer use efficiency in agriculture soils. However, only 10-20% of the P applied with fertilizer is absorbed by plants in the first year of application and the remainder of the P will be fixed or precipitated into poorly soluble forms (Vu et al., 2008). The amount of P application depends on the crop P requirements as well as on the amount of extractable soil P and the soil capacity to fix P into unavaiable forms. Therefore, accurate estimating of P availability and precise predicting of P requirements are significantly important for sustainable agricultural production (Wang et al., 2001). The recovery of applied phosphorus by plants over a growing season is relatively very low in comparison to the amount held in the soil. Therefore, breeding of P-efficient plant varieties that can grow and yield well under low P conditions has become an important aspect of increasing P use efficiency

(Hoagland and Arnon, 1950; Wang et al., 2010). The effect of P deficiency on various wheat varieties has been widely studied to investigate the wheat's response to low P in the greenhouse and filed conditions (Fageria and Baligar, 1999; Gardiner and Christensen, 1990; Osborne and Rengel, 2002; Ozturk, et al., 2005; Penn et al., 2015). To keep up improved growth under P insufficient conditions, plant species and genotypes of given species can develop different adaptive responses by two major mechanisms, P acquisition and P utilization (Ozturk et al., 2005). A recent study revealed that wheat cultivars responded differently to low P conditions and varied in their behavior of uptake or ability to utilize P under this condition (Penn et al., 2015).

Wheat is one of the most important cereal crops for most of the world's population, and it is considered the essential source of human food in several countries. Together with rice and corn, wheat provides 60 percent of our energy intake (Tilman et al., 2002). Because of increasing worldwide demands for wheat, wheat grain production should be increased annually at a rate of 2% to meet estimated demands of 2050 (Gill et al., 2004). According to the Crop Production Summary report by the USDA's National Agricultural Statistics Service (NASS), Oklahoma was the sixth most productive state for wheat production in 2014 (NASS, 2015). However, soil acidity in central and western Oklahoma has been a significant factor in limiting crop yields (Zhang and Raun, 2006). Organic matter decomposition, nitrification, and acid rain are the primary factors of increasing the soil acidity (Power and Prasad, 1997; Sparks, 2003). In 1996, a survey was conducted and revealed that over 35% of the total wheat production in Oklahoma was planted in the soil with pH less than 5.5. (Zhang et al., 1998).

1.1 Phosphorus in the Plant

Although phosphorus is required in lower amounts than other macronutrients, it is an essential nutrient for plant growth and reproduction because it is a vital component of nucleic acids that carry genetic information to produce proteins and other essential compounds for plant structure. Phosphorus is also a major constituent of adenosine triphosphate (ATP), which is responsible for transferring the chemical energy for metabolism; therefore, plants cannot complete their life circle without a reliable supply of this element (Havlin et al., 2005). Also, P is involved in key enzyme reactions and the regulation of metabolic pathways (Schachtman et al., 1998). Regardless of the total P concentration in soil, soil solution P varies widely among soils between 0.003 ppm to 3 ppm. P deficiency symptoms are usually noticeable in young plants because phosphorus is relatively mobile in plants and can be transferred from older growth tissues to new. Phosphorus deficient leaves generally become dark green; however, as P deficiency increases, this color turns into a purplish to reddish color, especially in the older leaves (Havlin et al., 2005). However, it is difficult to diagnose the P deficiency through visual symptoms alone because there is a similarity between P deficiency and Al toxicity. As a result, soil and plant tissue analyses must be taken into consideration to confirm this deficiency correctly.

Since P is subjected to strong reactions with soil components and being immobile in soil, diffusion and interception are the only pathways of P movement to plants. For that reason, the roots must grow through the soil to get the necessary phosphorus. The young root tips discover phosphorus concentrations found in the bulk soil solution. Although phosphorus is quickly absorbed along the root surface, the phosphorus depletion zone of 0.2 to 1.0 mm develops around the root. Therefore, it is highly recommended to apply P fertilizers within the root zone.

Phosphorus is taken up by plants mostly as orthophosphate ions in either monovalent form ($H_2PO_4^-$) or divalent form (HPO_4^{-2}), and the distribution of orthophosphate species is depending on the soil pH. For example, below pH 6, the most dominant species is the monovalent form ($H_2PO_4^-$), whereas above that pH the soil solution P is present as the divalent form (HPO_4^{-2}) (Mikkelsen, 2013; Schachtman et al., 1998).

1.2 Phosphorus in the Soil

Although the total P concentration in soils may be higher, it is usually present in unavailable forms in acidic soils due to strong chemical reactions that occurred in the soil solution. These soils often have a low content of available P due to high P fixation by Al and Fe oxides, and therefore, the quantity of P fertilizer required depends not only on plant necessities but also includes the amount of P fixing capacity of the soil (Wang et al., 2001). Soil phosphorus occurs in different chemical forms including inorganic P and organic P. It is important to highlight that 35% to 70% of total P in the soil is found in inorganic form (Harrison, 1987; Shen et al., 2011). However, the available P is very low because when P is not absorbed by plant roots or immobilized by microorganisms, it will adsorb to clay minerals or precipitate as secondary P compounds. In acidic soils, for example, P precipitates as secondary Fe and Al minerals due to the high solubility of these ions in the solution (Weil and Brady, 2016). While in alkaline soils, P precipitates with Ca or adsorbs to clay minerals and calcium carbonate; however, the solubility of these minerals reduces as the soil pH increases (Tunesi et al., 1999).

Soil phosphorus occurs in the three broad pools: solution P, active P, and fixed P. The solution P pool mostly occurs as orthophosphate form and is 100% plant available; however, its amount is extremely low in comparison to the amount needed by plants. The solution pool is then buffered by the active P pool. The active P contains inorganic phosphate that is weakly held to clay minerals found as highly soluble precipitants with Ca, Mg, Fe, or Al. This P pool is relatively easily released in the soil solution and considered the main source of available P for plants as it buffers the soil solution. The fixed P pool contains insoluble inorganic forms that can release P into the active pool in a small quantity, also this pool can contain certain organic forms that are resistant to mineralization by microorganisms in the soil (Busman et al., 2009).

1.3 Organic P

In general, phosphorus in the soil can be separated into organic P, calcium phosphate compounds and iron or aluminum phosphate compounds (Bohn et al., 2001). However, it is crucial to highlight that nearly half of soil phosphorus is found in the organic forms, which are mainly found in the topsoil. However, these forms of phosphorus must be mineralized to inorganic phosphors before it can be absorbed and utilized by plants for their development (Busman et al., 2009; Schachtman et al., 1998). P organic fraction may be derived from plant residues and soil organisms, and a large proportion of it remains phospholipids, nucleic acids, and inositol phosphates. Phospholipids are responsible for both the development and the growth of plants, and they can be found naturally as waxes and fats. The common phospholipids are derivatives of glycerol and are insoluble in water, but they are easily decomposed by soil microbes. Thus, they represent a small proportion of the total organic P. The inositol phosphates represent a series of phosphate esters that can make up 10-60% of total organic P. Their abundance in soil is likely related to the stability of these compounds in both acidic and alkaline soils. Most inositol phosphates and nucleic acids in soils are products of microbial decomposition of plant residues; however, nucleic acids especially DNA and RNA are released into the soil in higher amounts than inositol phosphate, and they represent a small portion of the total soil organic P (Weil et al., 2016). Organic P becomes available to plants after being mineralized into inorganic P. The mineralization of organic P is likely related to the activity of soil microorganisms and free enzymes, and soil phosphatase, derived from the microbial population; therefore, organic P in the soil solution may be important for plant nutrition since plant roots can excrete phosphatase enzymes. Thus this fraction could be hydrolyzed by phosphatase enzymes. The accumulation of organic P is mostly correlated to microbial activity in the soil, and soil organic matter content can also serve as a significant factor to increase the amount of soil organic P (Dalai, 1977).

2) Factors Controlling the Availability of Phosphorus in Soils

2.1 Influence of pH and different cations

Phosphorus availability is controlled by different factors; however, the most critical factor is soil pH. Plants can only absorb P in the orthophosphate forms ($H_2PO_4^-$ or HPO_4^{2-}). The availability of orthophosphate is determined by soil solution pH. At a pH of 7.2, equal amounts of these two ionic forms ($H_2PO_4^-$ or HPO_4^{2-}) exist in solution. In general, the maximum availability of P is typically between a pH of 6-7; as a lower or higher pH, the orthophosphate will be subjected to several chemical reactions based on the activity of free ions in the soil solution, which make it less available for plant uptake. For example, in the highly weathered and acidic soils, the essential cations responsible for binding of P in soils are aluminum (Al^{3+}) and iron (Fe^{3+}). These cations readily react with phosphate ions, forming insoluble aluminum and iron phosphate; therefore, the most limiting factor that actively controls the P availability is the concentration of free ions of these cations in the soil solution. This concentration is mainly controlled by the hydrolysis reactions of Al^{+3} and Fe^{+3} (Bohn et al., 2001; Havlin et al., 2005; Weil et al., 2016). Also, it has been reported that as the exchangeable Al and Fe content in the soil increases, the amount of extractable P decreases. Therefore, the high concentration of these ions in the soil solution significantly impacts on the P availability in a wide range of agricultural soils; these soils tend to absorb large quantity of applied P to reach equilibrium P concentration. Even though acidic soils may have a high amount of P, it is difficult to grow plants in these soils because of large quantities of Al and Fe oxides, which can adsorb significant amounts of applied P. Therefore, it is recommended to apply high rates of P fertilizer to achieve the optimum yield production (Haynes and Mokolobate, 2001). On the other hand, in the alkaline soils, the abundance of calcium ions is also the most crucial factor for tying P and reducing its availability because calcium ion readily reacts with phosphorus, forming insoluble calcium phosphate. It has been documented that the solubility of calcium phosphate is controlled by soil pH and the Ca^{+2} activity. Therefore, the P solubility decrease as a result of increasing the activity of the calcium ion whereas the P solubility increases as the activity of hydrogen ions increased (Olsen, 1954; Weil et al., 2016). In calcareous soils, calcium carbonate also has a significant impact for P adsorption and precipitation reactions and reduces the P availability because when P fertilizers are added to the soil, P rapidly reacts with calcium carbonate, forming dicalcium phosphate and tricalcium phosphate. However, these forms of P become increasingly soluble when the soil pH decreases (Tunesi et al., 1999; Weil et al., 2016).

2.2 Influence of mineral types

The amount of clay minerals and P adsorption are mostly correlated to each other because clay surface is the major site of P sorption. Therefore, clay minerals that have greater anion exchange capacity can adsorb a large amount of solution P. For example, iron and aluminum oxides strongly attract and adsorb phosphate ions due to the greater surface area (Havlin et al., 2005). It has been reported that a significantly greater amount of P was absorbed by loamy soil than was absorbed by in sandy loam due to high clay content in the former (Rashmi et al., 2016). Phosphorus availability also tends to be high in the loam texture, and results have confirmed the significant correlation between clay content and available P (Pandey et al., 2000). Phosphorus fixation is not only affected by the amount of clay minerals but also on the type of clay minerals. For example, 1:1 clays have the greater capacity to bind phosphorus than 1:2 clays because of the higher amount of iron and aluminum oxides associated with 1:1 clays that are dominant in the highly weathered soils (Weil et al., 2016).

2.3 Influence of organic matter

Organic matter (OM) has a significant impact on soil fertility because it can retain plant nutrients and block them from leaching into the soil profile. Organic matter can increase the water holding capacity of soils by adhesion soil particles together. Also, it is important to buffer soil pH, leading to increasing the nutrient availability for plant growth. Microorganisms, which excrete organic acids, have an essential role in P mineralization and immobilization through the decomposition of OM (Bot and Benites, 2005). Moreover, the soil's OM has been observed to reduce the P-sorption of such soils (Uehara and Gillman, 1985), also it has been documented that application of OM has led to decreasing the P adsorption and enhancing the P availability (Antelo et al., 2007; Posso et al., 2017; Sibanda and Young, 1986). In general, OM increases the solubility of phosphate and reduces P fixation by several mechanisms. Firstly, OM can compete with phosphate ions for the binding site, leading to decrease P adsorption on the soil particles. Secondly, organic acids produced by plant residues and microbial decomposition can act as organic anions, which can chelate the cations such as Al^{3+}, Fe^{3+}, and Ca^{2+}, resulting in decreasing the phosphorus precipitation of these cations in the soil solution. A third factor is that OM can increase the quantity of organic P that can be mineralized into inorganic P (Hoagland and Arnon, 1950; Weil et al., 2016). A recent study evaluating five bacterial and fungal strains revealed that P availability was positively correlated with the organic production, and microorganisms have produced different organic acids and contributed to providing available P in the culture medium (Posso et al., 2017). Therefore, incorporated OM in the soil may increase the P solubility through the formation of organic acids. This has been technically demonstrated by research, using both low and high molecular organic acids, and leached OM (Guppy et al., 2005). These studies have confirmed the fact that OM increases the solubility and availability of P in calcareous soils.

2.4 Influence of soil temperature and moisture

The soil temperature is one of the most important factors for influencing the mineralization of soil organic P and chemical reactions of P. In incubation experiments, the available P concentration increased with increasing temperature from 27°C to 50°C, and results were confirmed that the increase of available P is mostly related to accelerated mineralization of organic P fraction due to heating conditions (Acquaye, 1963). Soil temperature is also positively correlated to the rate of biological reactions that are responsible for converting the organic P to an inorganic form due to the increase in the microbial activity, which increased as the temperature increased (Jiang et al., 2008). In general, the soil solution P is strongly influenced by the soil temperature as reported by (Hodges, 2010) the ability of the plant to remove P is decreased either in the low or high temperature.

Besides the soil temperature, soil moisture also has a significant influence on the nutrient availability. Water content strongly influences plants both directly through water requirements and indirectly through nutrient availability in the soil solution. The effect of wetting and drying conditions on the solubility of plant nutrients has been widely studied, and it has been reported that moisture content in soil helps to dissolve fertilizer granules, which increases the solubility of fertilizers (Haynes and Swift, 1985; Keith et al., 1997; Saleque et al., 1996). A recent study has been confirmed that low soil moisture can increase P fixation while high soil moisture can enhance dissolution and P removal in acidic soils (Fayiga and Obigbesan, 2017).

3) Mechanism of Phosphorus Efficiency in Plants

It has been defined that P efficiency is the ability of plant cultivars to provide higher yields when P is not readily available. Therefore, in P limiting conditions, plants have developed various strategies to overcome P deficiency such as improving the capability of plants to absorb more P in low P conditions, and the ability of plants to produce high biomass per unit of P uptake (Gourley et al., 1993; Graham and Gregorio, 2001; Penn et al., 2015). Hence, the mechanisms that can enhance plant P uptake efficiency may include the modification of root architecture (e.g. root hair, finer roots) (Balemi and Schenk, 2009), development of large and shallow root systems (Rubio et al., 2001), and increasing root exudates (e.g. organic anions, phosphatases) (Bhattacharyya et al., 2013). All of these mechanisms contribute to the increased P uptake efficiency of the plant. On the other hand, there are also other mechanisms related to enhancing P utilization efficiency; these mechanisms comprise the use of alternative P-independent enzymes and glycolytic pathways, efficient cytoplasmic P homeostasis and higher ability to translocate P from other plant parts (Czarnecki et al., 2013). Briefly, the ability of the crops to produce the highest biomass under P limiting condition may be explained by improved uptake efficiency, or increased utilization efficiency, which is the capability of producing higher dry matter per unit of phosphorus in tissue of the plant (Gahoonia and Nielsen, 1996; Penn et al., 2015).

3.1 Root morphology

As mentioned previously, plants can evolve and possess several morphological adaptations to enhance P use efficiency in low P conditions. One of the primary morphological mechanisms is to maximize the ability of the plant roots to uptake P from the soil by promoting the root growth, which can lead to the increase of root-soil contact to access any available P or utilize insoluble P (Schjørring and Nielsen, 1987). Hence, the root architecture alteration is a critical factor for the development of crop cultivars to acquire P under low P supply (Niu et al., 2012). The root architecture is defined as the spatial location of the root system in the soil, and it has been observed to be substantially important to influence the efficiency of P acquisition (Lynch, 1995). In general, plants can adjust the root architecture to low P condition through the reduced growth of primary roots, the promoted growth of lateral roots, and enhancing of root hair density and cluster root formation, which all enhance the plant's ability to utilize the insoluble P (Niu et al., 2012). Some studies have shown that some crops, including maize, rice, bean, tomato, and potato, were able to modify the length of primary roots and lateral roots, and increasing the root hair density and cluster root formation under low P condition (Fernandes et al., 2014; Foehse and Jungk, 1983; Gahoonia and Nielsen, 1998; Jungk, 2001; Schenk & Barber, 1979). The increasing of root/shoot ratio of the potato plant in response to P deficiency has been observed in studies conducted both in soil and in hydroponic nutrient solution

(Fernandes et al., 2014). However, consistent with Fernandes et al. (2014), this reaction might vary depending on the cultivar. In spite of the other research demonstrating that potato responds to P-deficiency by raising surface area by growing adventitious roots, root hairs, and by increasing the root length density. Fernandes et al. (2014) claimed that the root length and root surface area of some potato cultivars reduced as decreasing of the P supply. This conclusion was also observed in maize, peanut, and rapeseed (Bhadoria et al., 2004; Hu et al., 2010). Cluster root formation is also one of the major adaptive strategies to enhance nutrient acquisition for plants adapted in low-nutrient soils (Lambers et al., 2006; Skene, 1998). Also, cluster roots have been found to produce large amounts of organic acids under low P conditions and acidify the rhizosphere as reported by Dinkelaker et al., (1989), who observed that cluster roots of white lupin acidified the rhizosphere pH in the calcareous soil. In a study comparing the P efficiency of various Brazilian wheat cultivars, da Silva et al. (2016) found that root length varied significantly between cultivars where the most efficient varieties exhibited a shorter root length compared with cultivars that were less efficient in P uptake. Contrary to the published literature, Yuan et al. (2016) found that wheat roots developed a greater root-hair density and root hair length at relatively high P conditions, but the root-length density was greater under P limiting conditions. In fact, most published literature indicates that increasing of root hairs is produced with decreasing in P availability below the critical P concentration (Brown et al., 2013; Brown et al., 2012; Haling et al., 2014). Similar results were reported by Wang et al. (2016) who observed that root hair traits of six spring wheat varieties responded differently to low P condition where some varieties substantially increased their root hair density in response to P stress. Additionally, root hairs of some wheat varieties have been observed to be longer under P limiting conditions (Foehse and Jungk, 1983; Gahoonia et al., 1999). However, this conclusion has not been observed for some other varieties because some varieties have different morphological strategies to respond to P deficiency such as root exudation of organic acids and phosphate enzymes (Pypers, 2006; Yuan et al., 2016). Besides the important role of root hairs and cluster roots, reduced growth of primary roots and increased growth of lateral roots also show significant variation among plants such as Arabidopsis under P stress (Linkohr et al., 2002; Williamson et al., 2001). Williamson et al. (2001) studied the root system architecture of Arabidopsis, and they concluded in their study that primary roots of Arabidopsis decreased and the growth of lateral roots increased under low P conditions, indicating different morphological adaptations. Therefore, the root architecture may have a significant effect in maximizing P acquisition because root systems with the greater surface area can explore and exploit the water and nutrients effectively.

3.2 Root exudation

In most agronomic soils, organic P ranges between 30% to 80% of the total P and this form of P must be hydrolyzed first by phosphatases to be available for plant uptake (Tarafdar and Claassen, 1988). Phosphatases can be divided into acid phosphatase and alkaline phosphatase; alkaline phosphatase is mainly found in alkaline soils, and acid phosphatase is mostly predominant in acid soils (Eivazi and Tabatabai, 1977). It has been noted that plant roots secrete more acid phosphatase under P deficiency (Tadano and Sakai, 1991; White and Hammond, 2008). In a study comparing acid phosphatase activity among plant species grown in P deficiency, Tadano and Sakai (1991) found that the acid phosphatase activity secreted by roots was remarkably higher in lupin and tomato compared to the P-sufficient controls. Similarly, it was found that the acid phosphatase activity of wheat cultivars increased when they were grown under P stress (Ciereszko et al., 2011). Therefore, root responses to secrete acid phosphates are an important consideration that acid phosphates are primarily contributed to the hydrolysis of soil organic P (Hayes et al., 1999).

Besides the activity of phosphatase, organic acid exudation is playing a significant role to enhance the mobilization of sparingly soluble P in the rhizosphere. It has reported that plant roots can secrete either low or high weight molecular compounds into the rhizosphere in response to biotic and abiotic stress (Bertin et al., 2003). The root exudates can include a compound mixture of organic acid anions such as citric, oxalic, malic, fumaric, succinic, acetic, butyric, valeric, glycolic, piscidic, formic, aconitic, lactic, pyruvic, glutaric, malonic, aldonic, erythronic, and tetronic acid (Fox and Comerford, 1990; Lipton et al., 1987). A recent study has been found that rice cultivars releasing tartaric, citric, and acetic acids could extract P from the strongly absorbed soil P fraction (NaOH-P), thereby increasing the P utilization efficiency. In acidic soils where P fertilization is required, a large percentage of applied P is fixed as strongly absorbed P in soils (Bhattacharyya et al., 2013). Bhattacharyya et al. (2013) found that rice cultivars releasing large amounts of organic acids in the rhizosphere would be able to utilize P more successfully (Bhattacharyya et al., 2013). Several studies have reported that some wheat cultivars exude substances that can apparently solubilize P from Al and Fe oxides (Osborne and Rengel, 2002; Pearse et al., 2007; L. Wang et al., 2010). A study to determine root exudation of Rape (Brassica napus L.), Hoffland et al., (1992) found the excretion of citric and malic acids from roots increased under P deficiency, resulting in a decrease of soil pH around the root zone and consequently, and enhanced P solubilization. A similar result reported by Dinkelaker et al. (1989) found the cluster roots of white lupin excreted large amounts of citric and malic acids, and the pH of the rhizosphere decreased from 7.5 to 4.8. Moreover, results of the other study measuring root excretions of wheat varieties grown in rhizo-cells have shown that P efficient varieties had the highest organic acid exudation especially oxalic and critic acids, which indicate significant roles for improving P uptake efficiency (Bell, 2013). Also, this observation is consistent with other studies suggested that organic acid exudation from plant roots can significantly contribute to P- uptake efficiency by increasing the solubility of fixed soil P, making it available for plant uptake (Hocking, 2001; Zhang et al., 1997). Interestingly, the role of root exudation is not only to promote the mobilization of sparingly soluble P in the soil but also it can involve in aluminum (Al) tolerance (Liang et al., 2013; Ward et al., 2011). Al toxicity and P deficiency are often associated together and heterogeneously distributed in acid soils; however, a few studies have been made to understand the underlying mechanism for plant's adaptation to this condition. In a hydroponic study mimicking acid soils to demonstrate these multiple factors, Liang et al. (2013) found that soybean released more malate exudation, which was critical for soybean adaptation to both Al toxicity and P deficiency. All the above findings indicate that the root exudation of organic acids is indeed one of the most critical mechanisms for maximizing the P use efficiency (PUE).

4) Strategies to improve PUE

Cereal crops require P fertilizers to improve yield and quality, and P fertilizers are the second most important nutrients to maintain high productivity (Fageria et al., 2017). However, PUE in the cereal crops is generally low, and it has been estimated to be around 16% (Dhillon et al., 2017). Also, it has been documented that P is limiting around 67% of agricultural soils (Batjes, 1997). The remainder of applied P is lost due to different chemical reactions, including adsorption on the soil particles and precipitation. Also, the excessive application can cause environmental contamination through runoff. Loss of P also contributes significantly to increase the production cost. Consequently, initiatives to increase PUE have become important to minimize both fertilizer losses and the production cost of crops. Over the last decade, many different strategies are evaluated to improve the P use efficiency to achieve greater agricultural profitability. These strategies generally include the following; 1) application of correct rates of P fertilizers based on the soil P testing to determine accurate P recommendations and reduce the potential impact of excessive P applications; 2) selecting the right source of P fertilizers to determine the effectiveness and plant availability. For example, Diammonium Phosphate (DAP) may have slightly more effectiveness compared to Monoammonium Phosphate (MAP) on acidic soils due to the different pH values when they are dissolved with water; 3) selecting the optimum method to apply P fertilizers to reduce P fixation. For example, band application of P in the root zone is more efficient than broadcast application because it can help to reduce the possible impact of runoff of soluble P. That is why this method is highly recommended for erosive soils; 4) selecting the right time of application to ensure adequate amount during peak uptake and critical growth stages (Johnston and Bruulsema, 2014). However, P supplementation is not a sustainable option due to high P fixation, especially in acidic soils. Hence, looking to other permanent options has become a top priority for agricultural scientists to reduce simultaneously the P fertilizer rates and avoid P depletion in the future. In this case, P use efficiency may be achieved by genetic improvements that are developed through plant breeding programs. In general, the possibilities of plant breeding for PUE include the following: 1) screening techniques of the efficient plants for P use; 2) identification of genes that are involved in P use efficiency; 3) using mycorrhiza by inoculation of plant roots through mycorrhizal fungi that can enhance P uptake through exchange carbon compounds with plants (Van de Wiel et al., 2016).

4.1 Screening of P efficient cultivars

The low P availability is considered to be the main limiting factors for crop production on acid soils because phosphate sorption to Fe and Al oxides reduce phosphorus availability to plants (Kochian et al., 2004; Shen et al., 2011). As a result, it is broadly believed that selecting P efficient cultivars can decrease the consumption of P resource and upgrade crop production; this may become an important strategy to contribute towards agricultural sustainability. In general, plants can be divided into four categories according to their response to nutrients: (1) efficient responders, plants having high productivity at low nutrient levels and showing high responses when adding the nutrients, (2) inefficient responders, plants with low productivity at low nutrient levels and showing high responses when adding the nutrients, (3) efficient non-responders, plants with high productivity at low nutrient levels but do not respond to the addition of nutrients, (4) inefficient non- responders, plants that have low productivity at low nutrient levels and show low responses with the addition of the nutrients (Gerloff, 1977). Many studies have reported that wheat cultivars show significant genetic variation in their response to P deficiency (Osborne and Rengel, 2002; Ozturk et al., 2005; Penn et al., 2015), which leads to further improvement by breeding programs.

As mentioned previously, the P use efficiency for cereal crops is very low; therefore, breeding of varieties that can adapt for P deficiency could be a part of solutions to address this issue. Different methods have been specially designed for screening plants for P efficiency. The easiest method is the use of nutrient solutions with varying levels of phosphorus under greenhouse pot experiments. Yan et al. 2004 used insoluble phosphate forms to simulate a similar situation to the soil, and they suggested in their study that bean genotypes releasing organic acids such as citrate, malate, and oxalate could extract and mobilize P from fixed sources. Consequently, P efficient varieties were significantly observed with high levels of acid exudation as opposed to inefficient varieties (Yan et al., 2004). Another example of these methods is using the rhizosphere study container technique (RSC) to separate roots from the soil to understand P depletion at different depths of roots and to study the rhizosphere processes as previously described (Zoysa et al., 1997). Hydroponics systems are also the other aspect of screening due to easy control of supplying nutrients directly to roots. As a result, many studies have used these systems with various levels of phosphorus to evaluate P use efficiency (Gong et al., 2011; Hayes et al., 2014). Using coating sand with aluminum may also be another efficient technique in studies of P nutrition because this method can easily eliminate the influence of organic matter and clay mineral that can affect the P availability, also roots are easily removed from the soil, allowing for accurate estimation of root mass. The main purpose of this project is to; 1) differentiate between P uptake efficiency and utilization efficiency under greenhouse conditions; 2) identify the variation in the P use efficiency across wheat varieties in the field experiment; 3) possibility of using NDVI for determining and predicting P limiting conditions and provide information for future research as well.

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CHAPTER II

EVALUATION OF PHOSPHORUS USE EFFICIENCY AMONG DIFFERENT WHEAT CULTIVARS UNDER ACIDIC SOIL CONDITION

ABSTRACT

Nationwide, most agriculture areas for wheat production are limited by the low phosphorus (P) availability (Batjes, 1997). Also, the P use efficiency (PUE) for cereal crops rarely exceeds 16%. Thus, farmers usually apply P fertilizers in excess rates of plant's requirements. Breeding cultivars to grow under low phosphorus condition may be beneficial to ensure global wheat production and increase PUE. The purposes of this study were to simulate a soil with insoluble forms of phosphorus and to evaluate seventeen winter wheat cultivars for P uptake and utilization efficiency based on the total mass of P present in the soil. Wheat cultivars were grown in coated sand with aluminum for 30 days in a growth chamber in two different sized pots. After 30 days of growth, shoots and roots were separately harvested for determination the biomass and P uptake for each cultivar. Results of this study proved the ability of some wheat varieties to uptake and utilize the unavailable P that is tightly bound to soil particles. Gallagher and Endurance were the most efficient varieties for both P uptake efficiency and P utilization efficiency. Also, the Ok11755W was the most P utilization efficient while OK10430-2 was consistently the most P uptake efficient. Other varieties evaluated in this study showed less efficient under the condition of this study. This observation proved the genetic variation among varieties, also this would suggest that P deficiency was associated with Al toxicity.

1- Introduction

Phosphorus is an imperative nutrient required for plant growth, and it cannot be substituted by any other element because it is a vital constituent of both DNA and RNA molecules, which are the major components to carry genetic information (Uchida, 2000). Phosphorus also plays a significant role in photosynthesis and respiration and the promotion of root development (Vance et al., 2003). However, P is limiting around 68% of most agricultural soils (Batjes, 1997). Also, the global P use efficiency (PUE) for cereal crops has been estimated to be around 16% (Dhillon et al., 2017). Due to low PUE, farmers usually apply P fertilizers more than plant's requirements; this may lead to increasing the potential risks of water contamination either by promoting algae growth or by infiltration into groundwater, which provides drinking water for millions of American people (Horrigan et al., 2002). Hence, plant breeding has become so important by selecting new varieties that can be resistant to P limiting conditions; this may be the permanent option for increasing PUE and also reducing environmental contamination. Plants can develop different mechanisms under low P environments to increase the efficiency at which plants uptake or utilize P, which ultimately contributes to yield stability. These mechanisms include the development of root system, increasing root exudation, and modification of root architecture (Balemi and Schenk, 2009; Bhattacharyya et al., 2013; Rubio et al., 2001).

In general, PUE can be classified into P utilization efficiency and P uptake efficiency. P utilization efficiency is defined by the plant's ability to produce a high yield with low amounts of P in the plant tissue (Hammond et al., 2004). P uptake efficiency is defined by the plants' ability to produce high yield under low P condition by extracting unavailable P

(Föhse et al., 1991). Plant roots can exudate organic acids, phosphatases and phytases to mobilize additional inorganic P (Jones, 1998; Li et al., 1997; Tadano and Sakai, 1991).

The main purpose of this study was to simulate a soil with highly insoluble P to evaluate the ability of wheat varieties to uptake and/or utilize P that is tightly bound to soil particles and to distinguish between P uptake efficiency and P utilization efficiency. The short-term impact of this study would decrease the P fertilizer rates, leading to an increase in the income of Oklahoma producers and decrease the agricultural cost production as well. For long-term, this study could be one of the solutions to reduce the potential risks of environmental contamination associated with excessive P fertilizer rates especially areas adjacent to rivers and lakes.

2- Materials and methods

2.1 Surface Modification of Sand

The inert sand (pool filter sand) was prepared by a coating method. The source of sand is a properly graded clean silica sand from the QUIKRETE ® Company, Atlanta, GA USA. The coating method was done in two stages. The first stage was to saturate the sand surface with negative charges using sodium hydroxide (NaOH). The following stage was to create a tightly aluminum bound in soil surface that can be linked with P using aluminum chloride $AlCl_3$ (Penn, 2016, personal communication). For the surface saturation with NaOH, 104 g of NaOH was dissolved with five litters of distilled water, and then 13 kg of sand was soaked in this solution for 24 hours. For the coating sand with $AlCl_3$, 250g of $AlCl_3$ was dissolved with five litters of distilled water, and then 13 kg of sand was soaked in this solution for 24 hours. After coating, P adsorption isotherm was determined according to the procedure developed by Nair (Nair et al., 1984). The purpose of P adsorption isotherm is to determine the capacity of treated sand to fix P and to identify the P concentration in the soil solution. The P adsorption isotherm performed as followed; a 2.5 g of air dried sample was equilibrated with 25 mL of different concentrations of P in 0.01 M CaCl2 solution in 50 mL centrifuge tubes. The concentrations of the solutions were 0, 0.01, 0.1, 1, 5, 10, 25, 50, and 100 mg P L^{-1}. The tubes were shaken for 24 hours at 25 C^{o}. After equilibration, samples were centrifuged at 2000 rpm (43 on the IEC centrifuge) for thirteen minutes and filtered through Whatman #42 filter paper. The amount of P adsorbed was calculated from the difference of P added and P remaining in solution after P equilibrium is established, and the adsorption data was fitted to the modified Freudlich equation (Reddy et al., 1980).

2.2 Soil Analyses

Before the experiment, the coated sand sample was dried at 65° C, and analyzed for pH, K, Ca, Mg, S, Mn, Zn, Fe, Cu, and B. Soil pH was analyzed with a 1:1 soil to water ratio using Mettler Toledo pH meter. Micronutrients and macronutrients were measured using Mehlich 3 extraction, which consists of a combination of different reagents (0.015M NH_4F, 0.2 M CH_3COOH, 0.25 M NH_4NO_4, 0.013 M HNO_3, and 0.001 M EDTA) (Mehlich, 1984). The Mehlich 3 extraction performed as followed; 2 g of air-dried sample was added to 20 mL of extracting solution and shaken for five minutes at room temperature, and then the extracting solution was filtered through Whatman #42 filter paper and analyzed by inductively coupled plasma emission spectroscopy (ICP-AES). Total P was analyzed by using acid digestion according to EPA 3050 method, and the filtrate was then submitted to analsis by ICP-AES (Edgell, 1989). KCl-extractable aluminum was determined by shaking 5 g of soil sample and 20 mL of 1.0 M potassium chloride (KCl) for 30 minutes and centrifuged at 2000 rmp (43 on the IEC centrifuge) and followed by ICP analysis of aluminum (Bertsch and Bloom, 1996). The analysis of the extractable aluminum was done to make sure Al levels were below critical threshold for Al toxcicity “25 ppm” (Abdulaha-Al Baquy, Jiu-Yu, Chen-Yang, Mehmood, & Ren-Kou, 2017).

2.3 P fractionation

P fractions were estimated to separate the different forms of P according to the Hedley method (Hedley et al., 1982). In short, sequential extraction was done by using different chemical reagents to separate each form. First of all, 0.5 g of air-dried sample was added to a plastic centrifuge tube and subjected to five sequential stages. First, the solution P was removed by adding 30 mL of distilled water and one anion exchange resin and shaken overnight. After that, the resin was rinsed with distilled water and placed in 40 mL of 1 M HCl and shaken for four hours and then was removed resin and the remaining solution was analyzed for inorganic phosphorus by spectrophotometer using the reagents of Murphy and Riley method (Murphy and Riley, 1962). The soil pellet was then saved for the next extraction stage. Second, the soluble and loosely bound P were extracted by adding 30 mL of 0.5 M $NaHCO_3$ to soil and shaken overnight and centrifuged at 4000 rpm (43 on the IEC centrifuge) for five minutes. The resulting extract was then analyzed for inorganic and organic P. Third, the P bound to Al and Fe hydroxide minerals was extracted by adding 30 mL of 0.1 M NaOH to soil and shaken overnight. The solution was centrifuged at 4000 rpm (43 on the IEC centrifuge) for five minutes, and then the resulting extract was analyzed for both inorganic and organic P. Fourth, the Ca-bound P was extracted with 30 mL of 1 M HCl and shaken overnight. The supernatant was then centrifuged at 4000 rpm (43 on the IEC centrifuge) for five minutes and analyzed for inorganic P. Finally; the residual P was digested by different concentrated acids according to the EPA 3050 method (Edgell, 1989).

2.4 Wheat Varieties

In experiments, the following winter wheat varieties were tested: Duster, Ruby Lee, Gallagher, Iba, Doublestop CL+, Endurance, Bentley, Spirit Rider, Stardust, Smith's Gold, OK10130, OK11755W, OK10430-2, OK11311F, OK11231, OK12621, and OK13625. All the varieties in this study were developed by Oklahoma State University breeding programs. Since P deficiency is often related with soil acidity and aluminum toxicity, it is important to include Al tolerant varieties in this study. Four varieties were chosen for a high tolerance of Al toxicity. These include Duster, Endurance, Doublestop CL+, and Gallagher (Edwards et al., 2012). Both Duster and Endurance contain the Al-tolerance gene

“AlMT1”, which is responsible for malate acid exudation from root rips as reported by previous studies (Carver et al., 2006; Edwards et al., 2012; Zhou et al., 2007). Two wheat varieties included were known to not be tolerant of aluminum toxicity, including Ruby Lee and Iba (Carver et al., 2012). Smith’s Gold is considered the next generation of the Gallagher, which is considered tolerant of the acid soil. Spirit Rider is also the following generation of the OK Bullet, which was released by OSU in 2005, and it is moderately resistant to acid soils (Edwards et al., 2010; McKindra, 2017). Bentley is also considered moderately tolerant of acid soils. However, all other varieties evaluated in this study have not been documented for aluminum toxicity. Prior experiment, the germination test of all varieties has been done according to the Quarberg’s procedure to predict the ability of seeds to germinate (Quarberg, 2012), and the results of the percent germination rate are presented in Table 2.1.

2.5 Experimental Section

The study was carried out in a growth chamber at the Controlled Environmental Research Lab (CERL) located on the OSU main campus during the summer season of 2016 and the winter season of 2017. The temperature was programmed at 25°C for 16 hours during the daytime and at 20°C for 8 hours during the nighttime with a relative humidity of 60% to 80%. Light intensity at the top of plants was 200 μmoles m^2 per second. The experimental design was a randomized complete block design with five replications, and data was analyzed using the SAS software and procedure Tuckey to analyze the least significant difference among wheat varieties at alpha value of 0.05 (SAS Institute, 2013). Each cultivar was grown in two different pot sizes for both experiments “2016 and 2017”. Each set is placed in the Hoagland’s solution, containing micronutrients and macronutrients with omitting phosphorus (Hoagland and Arnon, 1950). After 30 days of growth, shoots were harvested and dried in the oven for 28 hours at 65°C to determine the dry weight. Due to low biomass production, all replications for each variety were combined and digested to measure the P concentration in the plant tissue according to a method described by Jones Jr and Case (1990). P uptake was calculated using the following equation as described by Penn et al. (2015).

$$\text{P uptake (mg)} = \frac{\text{Plant P concentration} \times \text{total plant biomass}}{\text{number of plants in composite}} \qquad (1)$$

3- Results and discussion

3.1 Soil Properties

The general properties of the coated sand used in this study are listed in Table 2.2. The pure sand was chosen to create a tightly Al bound in soil surface and to eliminate the influence of clay minerals and organic matter that can affect the P availability. As a result of the coating method, the available P ranged from 1.9 mg/kg to 2.6 mg/kg for the experiment conducted in 2016 and 2017 respectively. One exception to this study was that a portion of P was converted to Al-bound P as opposed to Ca-bound P (Table 2.3). This was likely due to the high original concentration of calcium ion in sand, which is responsible for determining the ion speciation in the soil solution as described by Bohn et al. (2001). To support this conclusion, the additional extraction by Mehlich 3 was applied to make sure that Ca-bound P is not loosely soluble (Mehlich, 1984). Therefore, the P concentrations extracted with Mehlich 3 are 5.4 mg/kg, and 6 mg/kg for the experiment conducted 2016 and 2017 respectively, which is considered very low in the available P as reported by Mallarino et al.(2013).

3.2 Shoot dry weight and P concentration

Table 2.4 showed the average shoot dry weight (mg) and shoot P concentration for varieties grown in the large and small pots for experiments conducted in 2016. The shoot dry weight of 17 wheat varieties grown in the big pots ranged from 89.7mg for OK10430-2 to 15.4 mg for Iba. In the case of small pots, the shoots' dry weight ranged from 56.6 mg for Gallagher to 5.3 mg for Iba. With regard to the experiment conducted in 2017, the shoot dry weight of varieties grown in the large pots ranged from 127.7 mg for OK10430-2 to 52 mg for Bentley. While the shoot dry weight of varieties grown in the small pots ranged from 96.8 mg for OK11755W to 27.9 mg for Stardust (Table 2.5). In general, dry matter production was lower in the experiment conducted in 2016 as opposed to the experiment conducted in 2017. The lower dry matter production is probably due to loss of electricity that caused the shutdown of the temperature and light for 24 hours; however, wheat varieties in both experiments showed significant differences for dry matter production either in small pots or large pots. In the experiment conducted in 2016, the shoot P concentrations of wheat varieties grown in the large pots ranged from 38.8 mg P kg for OK10430-2 to 8.9 mg P kg for Smith's Gold. While the shoot P concentration in the small pots ranged from 53.4 mg P kg for Gallagher to 0.7 mg P kg for OK11231 (Table 2.4). In the experiment conducted in 2017, the shoot P concentrations in the large pots ranged from 55.2 mg P kg for OK10430-2 to 23.3 mg P kg for Bentley. While in small opts, the shoot P concertation ranged from 65.9 mg P kg for Doublestop CL+ to 27.3 mg P kg for Stardust (Table 2.5). The significant variation of dry matter production indicated that wheat varieties responded differently to low P condition. Also, the P concentrations in the plant tissue differed among wheat varieties; therefore, the variation of the dry matter production and P concentration can be summarized by substantial genetic variation to extract or utilize the native soil P. The different responses to low P condition take into consideration the description by Gerloff (1977). He reported that the cultivars could only be divided into efficient or inefficient categories according to their responses to nutrients when they are growing under nutrient stress (Gerloff, 1977). Thus, many greenhouse studies have been established followed by field experiments to classify a large number of wheat varieties according to their tolerance to low P conditions (McDonald et al., 2015; Ozturk et al., 2005; L. Wang et al., 2010). The results indicate that there is a genetic variation among wheat varieties with low P conditions, and wheat varieties responded differently in their growth and P content in the plant tissue.

3.3 P uptake efficiency versus P utilization

The difference of the total mass of P within two pot sizes can be used to distinguish between P uptake efficiency and P utilization efficiency as diffusion is the major pathway of P movement to roots as reported by Penn et al., 2015. Therefore, we can identify between two mechanisms based on the total mass of P present in the soil. Small pots represent the P utilization efficiency since there is less P, which contained 4 mg P and 4.6 mg P for studies conducted in 2016 and 2017 respectively compared to large pots, which contained 16 mg P and 18.6 mg P for studies conducted in 2016 and 2017 respectively. Therefore, cultivars in small pots will utilize the P to achieve the highest yield with little P due to less volume of soil for roots to extract more P and vice versa for P uptake efficiency in large pots due to the greater amount of soil, which means there is more P to be exploited by roots. P uptake efficiency and P utilization efficiency were calculated using the following equations as previously described by Penn et al., (2015) to maintain consistent results with their study.

$$\text{Relative P uptake} = \left(\frac{\text{P uptake of given cultivar}}{\text{P uptake of cultivar with highest P uptake}}\right) \qquad (2)$$

$$\text{Percent difference in biomass} = \left(\frac{\text{Biomass for large pots} - \text{Biomass for small pots}}{\text{Biomass for small pots}}\right) \times 100 \qquad (3)$$

$$\text{Relative biomass for small pots} = \left(\frac{\text{Biomass of given cultivar}}{\text{Biomass of cultivar with the highest biomass}}\right) \times 100 \qquad (4)$$

These equations were evaluated to differentiate between P uptake efficiency and P utilization efficiency where equation (1) was used to determine the average relative P uptake among large and small pots for each variety, and 75th percentile value for average relative P uptake was determined as the threshold to identify the most P efficient cultivars. Equations (2) and (3) were also used to determine the P utilization efficiency, and the 25th percentile value for relative dry matter production was also determined to identify the most P utilization efficient cultivars (Penn et al., 2015). Figures 2.1 and 2.3 illustrate the P uptake efficiency for separate experiments conducted in 2016 and 2017 respectively. The dashed line on the Y-axis shows the 75th percentile value of the average relative P uptake. Gallagher, Endurance, and Ok10430-2 are the most P uptake efficient due to their ability to extract and uptake more P to produce the highest biomass. These varieties showed consistent results in experiments conducted in both 2016 and 2017 compared to other cultivars, which fall below this threshold. Otherwise, all varieties located below threshold showed low biomass production and less intensity to uptake more P compared to Gallagher, Endurance, and Ok10430-2. Figures 2.2 and 2.4 shows the P utilization efficiency for both experiments conducted in 2016 and 2017 respectively. The dashed line on the X-axis represents the 25th percentile value of percent difference in biomass between small pots and large pots, which indicates varieties that produce the highest biomass in small pots in comparison to those who are grown in the large pots are located on the left side of the vertical line. Gallagher, Endurance, and Ok11755W were the most P utilization efficient in both experiments, which indicated their ability to produce the highest biomass with little soil P due to lower soil volume for roots to extract more P. On the other hand, all varieties located on the right side of the vertical line showed low biomass production and less intensity to utilize P compared to Gallagher, Endurance, and Ok11755W. The observation of this study concluded that Ok11755W was the most P utilization efficient, and Ok10430-

2 was the most P uptake efficient. While Gallagher and Endurance were the highest efficient varieties in both P uptake efficiency and P utilization efficiency, indicating their ability to extract the non-liable P and/or utilize the native soil P to achieve the highest biomass production. Varieties “Gallagher and Endurance” are considered to be tolerant to acid soils based on field experiments (Edwards et al., 2012)., and several studies have been reported that Al-resistant varieties excrete organic acids that might contribute to the detoxification of Al and increase P availability in the rhizosphere (Dakora and Phillips, 2002; Hinsinger, 2001; Jones and Darrah, 1994; Ström et al., 2002). Therefore, these varieties were able to extract non-labile phosphorus due to their root exudations since they are tolerant to acid soils. This observation is also consistent with similar results obtained by (Tang et al., 2007); who suggested that Al-resistant soybean species were the most efficient in utilizing AlPO4 or FePO4 under the greenhouse study. To support this conclusion, seven varieties have been selected to evaluate their responses on field experiments to reveal the cultivar P efficiency.

3.4 Data limitations

In this experiment, it was found that some wheat varieties have a low germination rate, leading to frequent germination failure throughout this study. This limitation may have impacted for obtaining the accurate estimation of biomass due to limited replications obtained within each variety. These varieties include Duster, OK11231, and OK13625. Ok11231 was completely eliminated in the experiment conducted in 2017 due to the seed germination failure.

4- Conclusion

This chapter presented the results of the study evaluating the P use efficiency among 17 wheat varieties and differentiated between P uptake efficiency and P utilization efficiency based on the different amount of the total of P present as a function of the volume of the soil. Wheat varieties responded differently to P deficiency; this variation indicates different responses of varieties for low P condition. Ok11755W was the most efficient variety to utilize the native P soil to produce high dry matter production compared to other varieties (Table 2.6). OK10430-2 was also the most efficient variety to uptake and extract more P to produce the highest biomass compared to other varieties in the table 2.7, which fall below the threshold "75th percentile value for average P uptake relative to the variety with the highest P uptake. Additionally, Gallagher and Endurance were the most efficient varieties either to extract and uptake more P and/or to utilize the native P soil to produce the highest biomass. Also, it is important to highlight that Gallagher and Endurance are tolerant to Al toxicity and Endurance contains Al-tolerance gene AIMT1, which is responsible for secreting malate acid that can effectively reduce the rhizosphere pH and solubilize P (Carver et al., 2006; Edwards et al., 2012; Zhou et al., 2007).This observation proved that there was genetic variation among wheat varieties to uptake and utilize unavailable P and the P deficiency was also associated with Al toxicity. Therefore, varieties have been selected to be evaluated in field experiments to support this observation.

Table 2.1 Results of the average germination percentage for winter wheat varieties evaluated in the greenhouse study.

Varieties	Temperature (°C)	Germination Test Period (Days)	Mean %
Doublestop CL+	21	7	96
Duster	21	7	70
Endurance	21	7	96
Gallagher	21	7	92
Iba	21	7	99
Bentley	21	7	92
Spirit Rider	21	7	92
OK10130	21	7	96
OK10430-2	21	7	92
Stardust	21	7	92
OK11231	21	7	72
OK11311F	21	7	80
OK11755W	21	7	100
Smith’s Gold	21	7	100
OK12621	21	7	80
OK13625	21	7	76
Ruby Lee	21	7	88

Table 2.2 Chemical characteristics of coated sand from composite samples collected before conducting of each experiment.

Coated sand	pH	Extractable P ¥	Total P ±	Exchangeable Al §	K	Ca	Mg	S	Fe	Zn	B
		--------- mg P g soil^{-1}---------		------------------------ mg kg soil^{-1}--------------------------------							
Experiment of 2016	4.3	0.0054	0.0799	103	7.4	481	13.0	9.9	15.7	0.7	0.8
Experiment of 2017	4.4	0.0060	0.0931	101	6.1	962	14.0	12.9	13.4	0.7	0.7

¥ Extractable P extracted with Mehlich 3

± Total P extracted with EPA method

§ Exchangeable Al extracted with KCl

Table 2.3 Soil phosphorus (P) forms for each experiment conducted in the greenhouse study.

	Inorganic Phosphorus				Organic Phosphorus		Total P
	Resin (Bio Avail)	NaHCO3 (Labile)	NaoH (FeP, AlP)	HCl (Apatite CaP)	NaHCO3	NaOH	EPA 3050 Method
				mg P kg soil^{-1}			
Experiment of 2016	1.9	1.0	4.5	38.6	3.9	2.0	79.9
Experiment of 2017	2.6	1.1	4.8	48.1	3.5	2.2	93.1
Pure sand	41.1	1.7	2.7	30.7	3.6	1.7	91.8

Table 2.4 Shoot dry weight (mg) and shoot P concentration for different wheat cultivars grown in low P in the large pots and small pots for the experiment conducted in 2016.

Cultivars	Large Pot			Small Pot		
	Shoot dry weight (mg)	Shoot P conc. (mg P g plant^{-1})	P uptake (mg)	Shoot dry weight (mg)	Shoot P conc. (mg P g plant^{-1})	P uptake (mg)
Doublestop CL+	53.6 abcde	35.5	0.007	7.1 efg	10.8	0.003
Duster	19.8 defg	9.9	0.002	6.3 fg	6.1	0.002
Endurance	30.1 bcdefg	24.8	0.005	28.3 bcdefg	25.0	0.005
Gallagher	48.2 abcdefg	21.7	0.004	56.6 abcde	53.4	0.013
Iba	15.4 defg	7.7	0.002	5.8 fg	4.2	0.001
Bentley	25.2 bcdefg	11.3	0.003	11.6 efg	11.5	0.003
Spirit Rider	34.6 bcdefg	14.5	0.003	8.2 efg	6.1	0.002
OK10130	21.5 cdefg	10.2	0.002	21.5 cdefg	16.5	0.004
OK10430-2	89.7 a	38.8	0.008	26.8 bcdefg	18.5	0.004
Stardust	28.3 bcdefg	11.4	0.006	5.3 g	5.2	0.001
OK11231	52.6 abcdef	20.2	0.004	7.7 efg	0.7	0.0001
OK11311F	52 abcdefg	23.2	0.006	13.2 defg	9.3	0.002
OK11755W	71.1 ab	19.8	0.004	60.2 abcd	27.9	0.006
Smith's Gold	20.0 defg	8.9	0.002	15 defg	12.2	0.002
OK12621	43.5 bcdefg	26.0	0.005	25.7 bcdefg	13.5	0.003
OK13625	68.4 abc	26.5	0.007	30.4 bcdefg	0.9	0.0002
Ruby Lee	17.7 defg	13.2	0.003	14.1 defg	12.9	0.003

* Means with the same letter are not significantly different at 5% level of probability

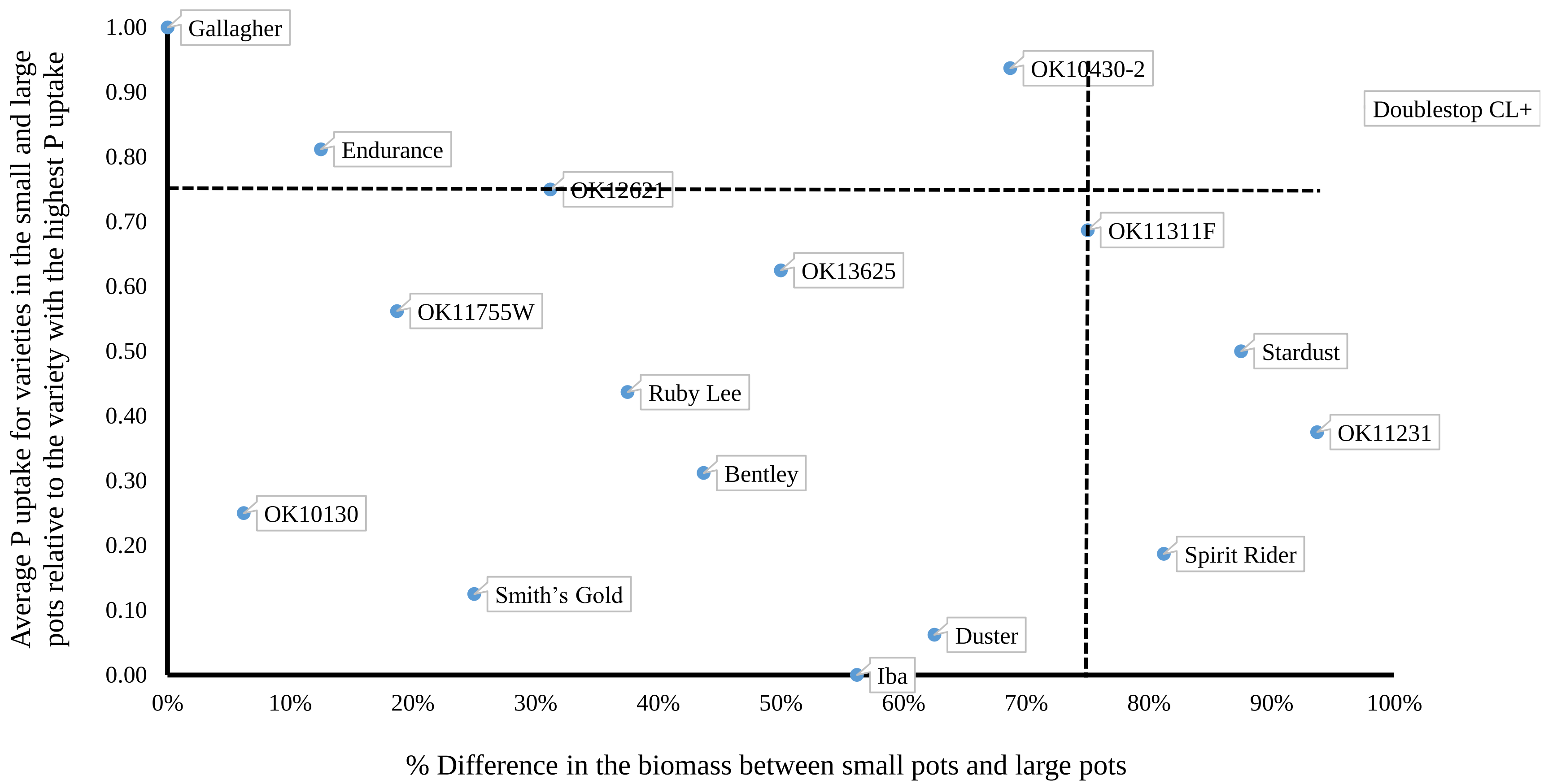

Figure 2.1 P uptake efficiency for the experiment conducted in 2016. The dotted line in Y-axis shows the 75th percentile value of the average P uptake for varieties in the small and large pots relative to the variety with the highest P uptake (equation 2). Also, the dotted line in X-axis shows the 75th percentile value of percent difference in the biomass between small pots and the large pots (equation 3). Therefore, all varieties above the dotted line in Y-axis are the most efficient varieties due to their ability to uptake more P under the experimental condition of this study.

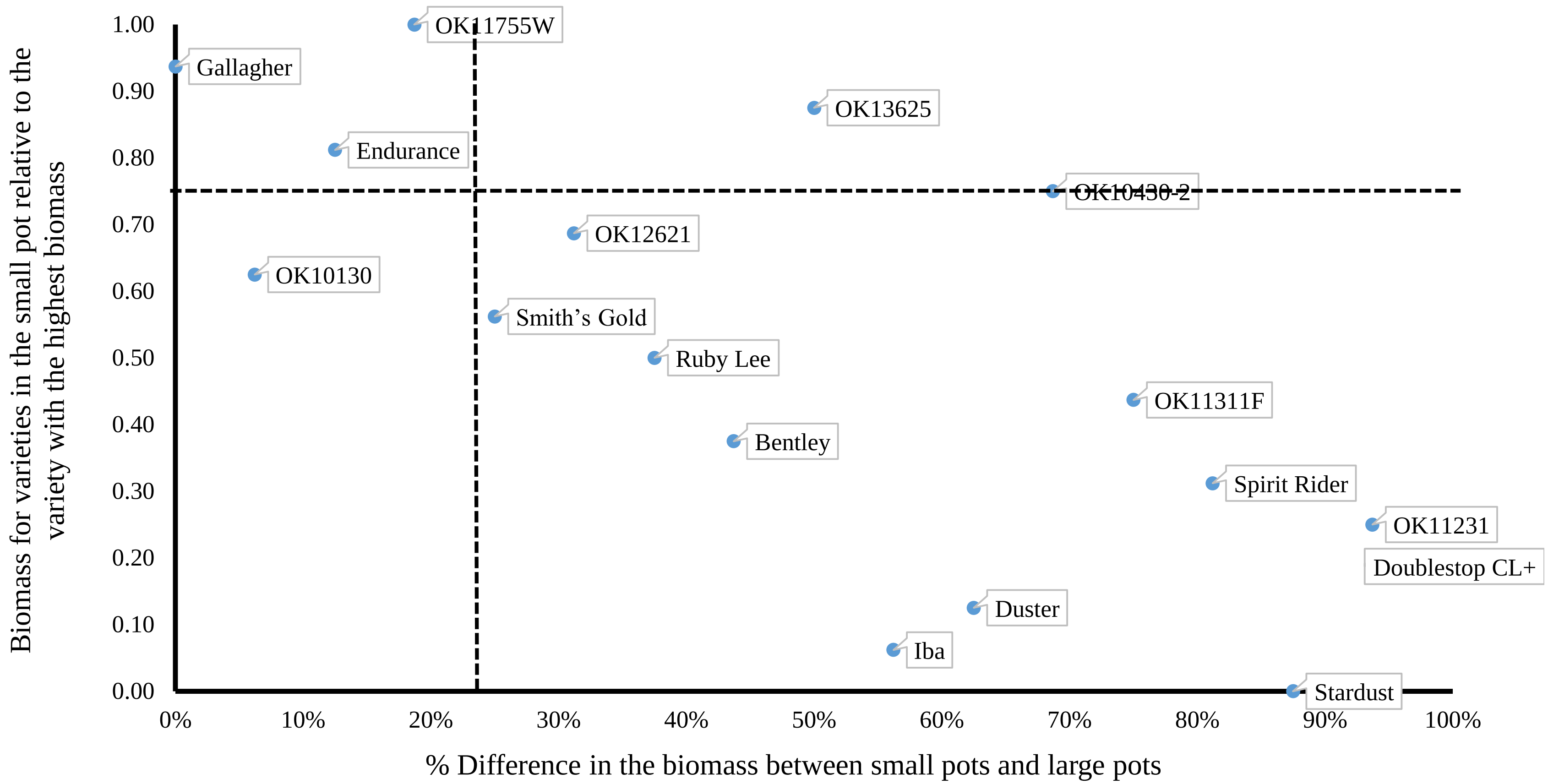

Figure 2.2 P utilization efficiency for the experiment conducted in 2016. The dotted line in Y-axis shows the 75th percentile value of the biomass for varieties in the small pot relative to the variety with the highest biomass (equation 4). Also, the dotted line in X-axis shows the 25th percentile value of percent difference in the biomass between small pots and large pots (equation 3), which indicates varieties produced the highest biomass in small pots relative to large pots are located on the left side of the line. Therefore, varieties above the horizontal line are the most efficient varieties due to their ability to utilize P under the experimental condition of this study.

Table 2.5 Shoot dry weight (mg) and shoot P concentration for different wheat cultivars grown in low P in the large pots and small pots for the experiment conducted in 2017.

Cultivars	Large Pot			Small Pot		
	Shoot dry weight (mg)	Shoot P conc. (mg P g $plant^{-1}$)	P uptake (mg)	Shoot dry weight (mg)	Shoot P conc. (mg P g $plant^{-1}$)	P uptake (mg)
Doublestop CL+	115.5 ab	53.2	0.011	43.4 cdef	65.9	0.033
Duster	71 abcdef	35.7	0.007	37.5 ef	35.9	0.009
Endurance	85.4 abcde	38.4	0.008	70.2 abcdef	56.4	0.028
Gallagher	87.6 abcde	39.4	0.008	67.4 abcdef	63.6	0.064
Iba	74.9 abcdef	37.4	0.007	49.9 cdef	36.3	0.007
Bentley	52 cdef	23.3	0.005	29.5 ef	29.1	0.015
Spirit Rider	70.6 abcdef	29.7	0.006	40.2 def	29.7	0.010
OK10130	71.7 abcdef	34.2	0.007	53.9 cdef	41.3	0.010
OK10430-2	127.7 a	55.2	0.011	70.9 abcdf	48.8	0.016
Stardust	82.7 abcde	43.8	0.009	27.9 f	27.3	0.007
OK11231	89.4 abcde	34.3	0.007	NONE	NONE	NONE
OK11311F	94.6 abcd	42.3	0.008	47.8 cdef	33.7	0.011
OK11755W	99.2 abc	40.9	0.008	96.8 abcd	56.0	0.019
Smith's Gold	91.3 abcde	36.8	0.007	52.1 bcdef	49.2	0.010
OK12621	78.7 abcde	41.2	0.008	55.7 bcdef	46.1	0.023
OK13625	91.3 abcde	40.9	0.008	40.9 def	60.5	0.020
Ruby Lee	89.2 abcde	42.8	0.009	52.2 cdef	47.9	0.016

* Means with the same letter are not significantly different at 5% level of probability

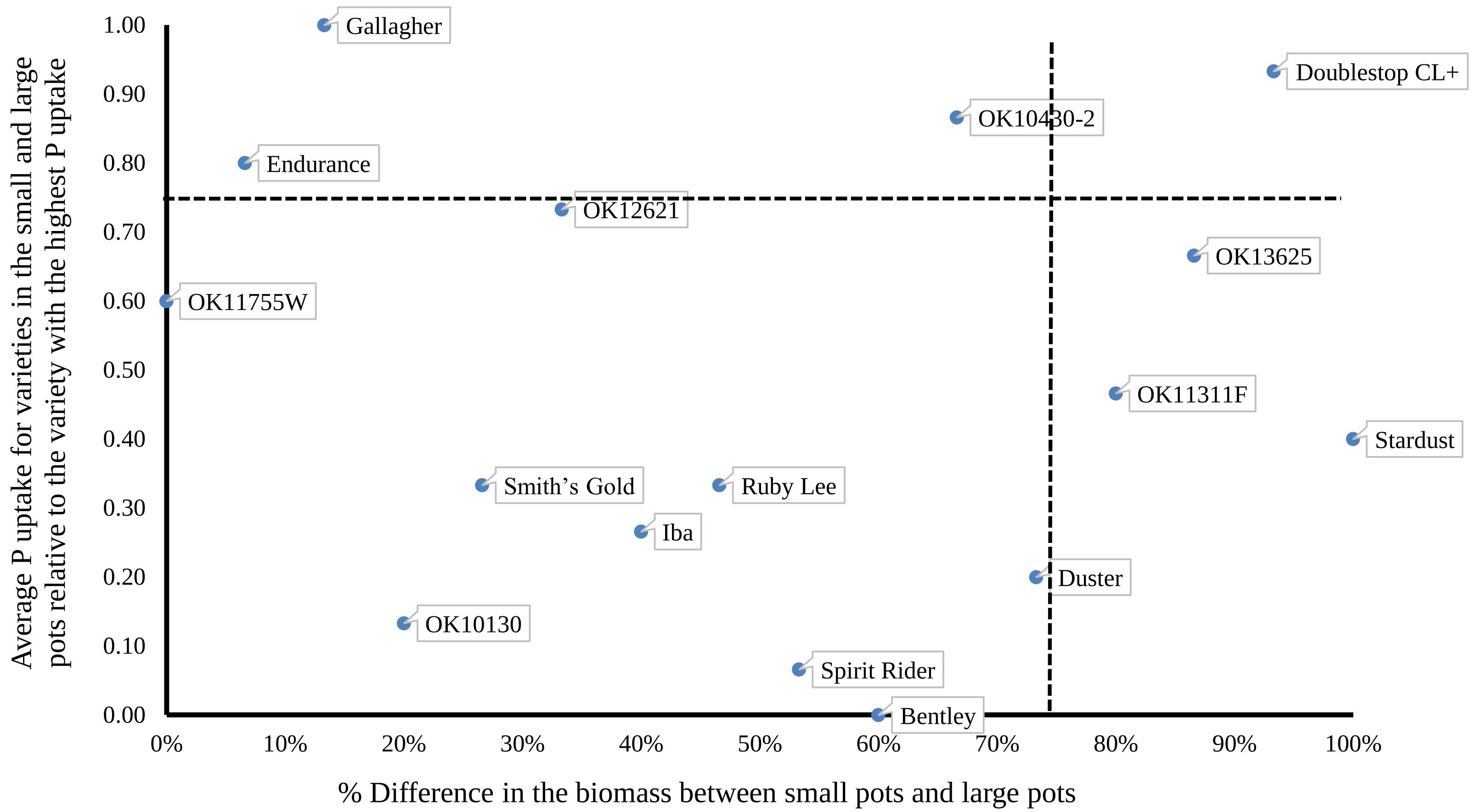

Figure 2.3 P uptake efficiency for the experiment conducted in 2017. The dotted line in Y-axis shows the 75th percentile value of the average P uptake for varieties in the small and large pots relative to the variety with the highest P uptake (equation 2). Also, the dotted line in X-axis shows the 75th percentile value of percent difference in the biomass between small pots and large pots (equation 3). Therefore, all varieties above the dotted line in Y-axis are the most efficient varieties due to their ability to uptake more P under the experimental condition of this study.

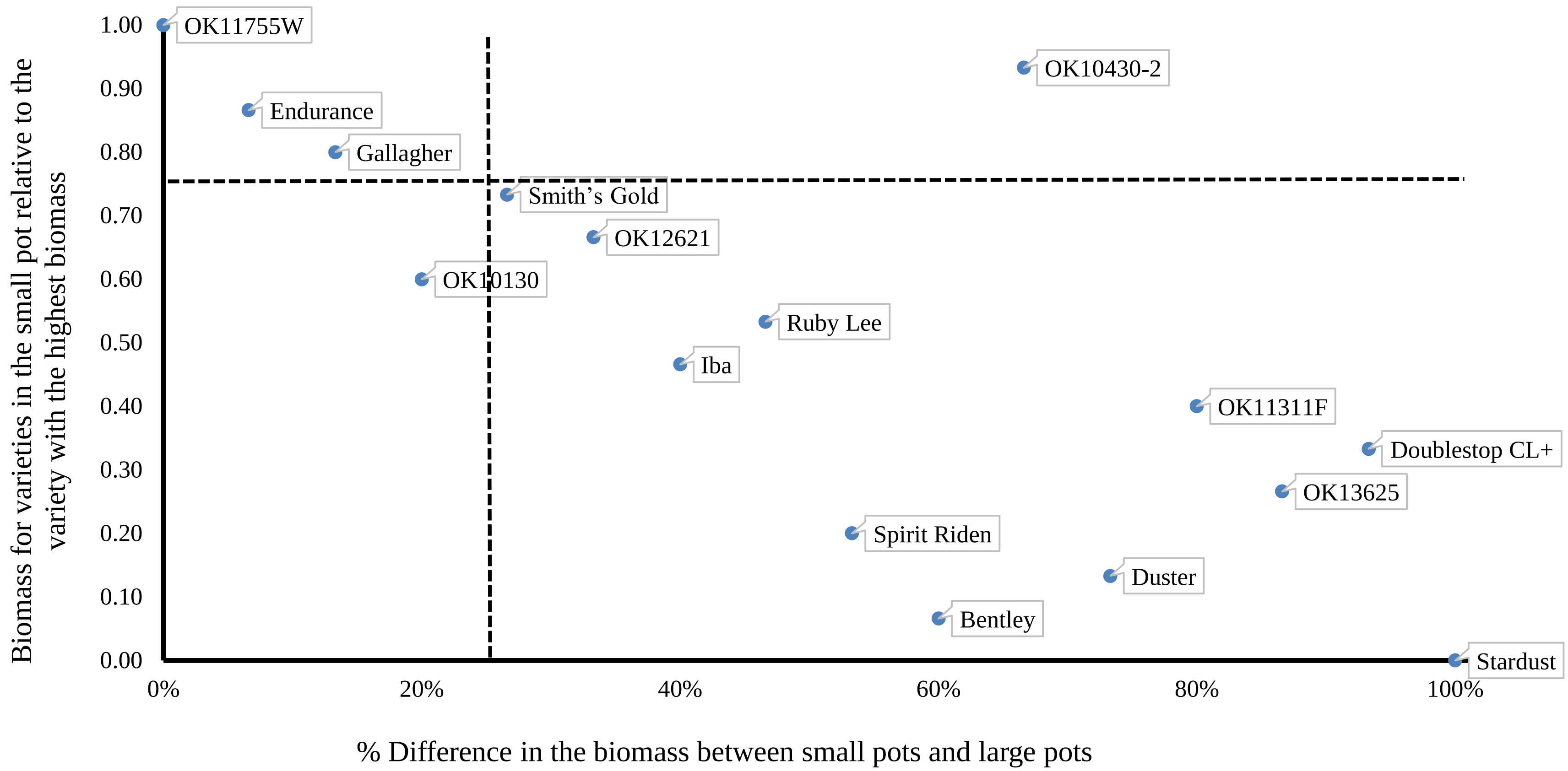

Figure 2.4 P utilization efficiency for the experiment conducted in 2017. The dotted line in Y-axis shows the 75th percentile value of the biomass for varieties in the small pot relative to the variety with the highest biomass (equation 4). Also, the dotted line in X-axis shows the 25th percentile value of percent difference in the biomass between small pots and large pots (equation 3), which indicates varieties produced the highest biomass in small pots relative to large pots are located on the left side of the line. Therefore, varieties above the horizontal line are the most efficient varieties due to their ability to utilize P under the experimental condition of this study.

Table 2.6 Distribution of winter wheat varieties for P utilization efficiency for both greenhouse experiments conducted in 2016 and 2017.

High efficient	Moderate efficient	Less efficient	Inefficient
OK11755W	OK10130	OK13625	OK12621
Endurance		OK10430-2	Smith's Gold
Gallagher			Ruby Lee
			Bentley
			Iba
			Duster
			Spirit Rider
			OK11311F
			Doublestop CL+
			Stardust
			OK11311F

*High efficient varieties mean greater than 75th percentile value of relative biomass in small pots and less than 25th percentile value of percent difference in the biomass.
* Moderate efficient varieties mean less than 75th percentile value of relative biomass in small pots and less than 25th percentile value of percent difference in the biomass.
* Less efficient varieties mean greater than 75th percentile value of relative biomass in small pots and greater than 25th percentile value of percent difference in the biomass.
* Inefficient varieties means less than 75th percentile value of relative biomass in small pots and greater than 25th percentile value of percent difference in the biomass.

Table 2.7 Distribution of winter wheat varieties for P uptake efficiency for both greenhouse experiments conducted in 2016 and 2017.

High efficient	Moderate efficient	Less efficient	Inefficient
Endurance	Doublestop CL+	OK13625	Stardust
Gallagher		Ruby Lee	Spirit Rider
OK10430-2		Smith's Gold	OK11231
		Iba	OK11311F
		Bentley	
		OK11755W	
		OK10130	
		Duster	
		OK12621	

*High efficient varieties mean greater than 75^{th} percentile value of average relative P uptake and less than 75^{th} percentile value of percent difference in the biomass.
* Moderate efficient varieties mean greater than 75^{th} percentile value of average relative P uptake and greater than 75^{th} percentile value of percent difference in the biomass.
* Less efficient varieties mean less than 75^{th} percentile value of average relative P uptake and less than 75^{th} percentile value of percent difference in the biomass.
* Inefficient varieties means less than 75^{th} percentile value of average relative P uptake and greater than 75^{th} percentile value of percent difference in the biomass.

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CHAPTER III

EVALUATION OF PHOSPHORUS USE EFFICIENCY AMONG DIFFERENT WHEAT CULTIVARS UNDER OKLAHOMA FIELD CONDITIONS

ABSTRACT

Since the global estimation of P use efficiency (PUE) for cereal crops is only 16%, it is very important to investigate other mechanisms that can maximize the PUE efficiency to ensure sufficient phosphorus to feed humanity in the future. The main purpose of this study was to evaluate seven winter wheat cultivars with low and adequate P levels in the field experiments and to predict the possibility of using the normalized-difference vegetative index (NDVI) for determining P limiting conditions. Wheat varieties varied in their ability to respond to P deficiency, which indicates the genetic variation among wheat varieties evaluated in this study. Duster and Endurance were found to be more efficient in extracting or utilizing more P. These varieties contain Al-tolerance gene ALMT1, which is responsible for excreting malate acid that effectively reduces the rhizosphere pH and solubilizes P bound in the soil. On the other hand, while Ruby Lee was found to be less efficient, and it is not tolerant to Al toxicity. This observation suggests that Al-tolerant varieties would be more efficient to utilize the P compared to other varieties that are not tolerant to Al toxicity. Also, NDVI was possible to estimate the P deficiency due to a strong correlation between the NDVI and yield prediction under low P conditions; this observation may be used as an indicator for P recommendation. These results may be the target of further studies for wheat specialists who are interested in increasing the PUE.

1- Introduction

Phosphorus is one of the primary nutrients for plant growth, and it cannot be substituted by any other element because it is considered the part of the DNA and RNA structures, which are responsible to transfer the genetic information within cells (Uchida, 2000). However, the increasing world population and limiting P resources currently represent a substantial challenge for future agricultural production, and the rock phosphate, which is the main resource of phosphorus, is a nonrenewable resource and has been estimated to be depleted in the next 80 years if extraction continues at the current rate (Smil, 2000). On the top of this situation, rock phosphate is mainly located in the United State, China, and Morocco. These countries would not be able to supply the demand of phosphorus to meet the growing population (Cordell et al., 2009). In addition, the global P use efficiency (PUE) for cereal crops was estimated to be around 16% (Dhillon et al., 2017). Therefore, it is important to increase PUE and to ensure we will have sufficient phosphorus to feed humanity in the future. In general, P deficiency is usually corrected by the application of P fertilizers; however, this is not a sustainable option because most of P applied will be converted into unavailable forms due to fixation and precipitation processes (Vu et al., 2008). The other way of overcoming P deficiency is to use plant varieties that are more efficient to low P; this might be the most productive permanent option and part of the solution for improving P efficiency. Several studies have recently been established to investigate the possibilities of breeding plant varieties that involve either enhancing P uptake or improving the P utilization efficiency (Penn et al., 2015; Vance et al., 2003; L. Wang et al., 2010; Yan et al., 2004).

Wheat is one of the most important cereal crops and staple foods for the majority of world population and has become imperative for human nutrition. The United States is one of major wheat producing counties, and the most productive states are Kansas, North Dakota, Montana, Washington and Oklahoma (USDA, 2017). Wheat consumption has increased during the last decade due to the world's growing population, which is expected to reach 9.15 billion by 2050 (Alexandratos and Bruinsma, 2012). Therefore, breeding of wheat varieties that can adapt and yield better under low P condition could be the most effective way to secure global wheat production and to increase the PUE. The main purpose of this study was to evaluate the PUE among seven winter wheat cultivars selected from the previous studies and to predict the possibility of using the normalized-difference vegetative index (NDVI) for determining the P stress status. The final results of study might recommend Oklahoma producers to use some of these varieties instead of others; also it might decrease the P fertilizer rates, which can help to decrease the production cost and increase income of local producers.

2- Materials and methods

2.1 Experimental Site

The study was established in the fall of 2016 at two locations with low available P at Cimarron Valley Research Station near Perkins, OK and N40 Experimental Filed near Stillwater, OK. Each plot size is 20 m long by 10 m wide with alley 20 m between replications. Two P rates (0, 39 kg ha^{-1}) were evaluated beside seven different wheat cultivars for a total of fourteen treatments. Both experiments were established in a randomized complete block design with three replications, and the treatment structure was the same for both experiments (see Table 3.1). Seven winter wheat cultivars (OK13625, O104320-2, Endurance, Spirit Rider, Duster, Ruby Lee, and Gallagher) were chosen from the previous greenhouse experiment because of their efficiency to uptake P and/or utilize P. Planting occurred in early October 2016 at a rate of 84 Kg ha^{-1} in rows 7.5 cm apart. Prior to establishment of field experiments, composite soil samples were collected from each site at depth of 0-15cm, dried at 65°C for 24 h, and ground to pass a 2 mm sieve. A routine soil analysis was made to determine the chemical properties for both experiments, soil texture was also determined according to a method previously descried by Bouyoucos (1962). All results are listed in Table 3.2.

2.2 Soil Sampling and Analysis

Soil samples were collected in 2016 and 2017 during the growth period and after harvesting for both locations. During the growth period, three stratified soil samples from each plot with zero P rate were taken at a different depth: 0-5, 5-10, and 10-15 cm, and analyzed for pH, exchangeable Al, and available P extracted by Mehlich 3. Soil pH was measured in a 1:1 soil: water ratio using a combination pH electrode (Thomas, 1996). Exchangeable Al was also analyzed based on a method previously developed (Bertsch and Bloom, 1996). In short, 5.0 g of air-dried sample was mixed with 25 mL of 1.0M KCl and shaken for thirteen minutes and then centrifuged at 2000 rmp for ten minutes. The extract was then filtered through Whatman #42 filter paper and followed by ICP analysis of aluminum. The available P was measured using the Mehlich 3 extracting solution where 2.0 g of soil sample was stirred with 20 ml of Mehlich 3, containing 0.015M NH_4F, 0.2 M CH_3COOH, 0.25 M NH_4NO_4, 0.013 M HNO_3, and 0.001 M EDTA (Mehlich, 1984). After harvesting, two stratified samples were taken from all plots at two different depths: 0-7 and 7-15 cm for analyzing the soil pH, exchangeable Al, and available P.

2.3 Grain harvest and NDVI readings

Grain was harvested in early June 2017 with a plot combine from an area of 1.5 x 4.5 meters of each plot. Sub-samples were collected from each treatment and dried at 70 C for 72 to determine moisture and protein content. Protein content was determined by using NIR Spectroscopy for each treatment. GreenSeeker^{TM} 505 handheld Sensor (Trimble Industries, Inc.) was used to calculate NDVI from all plots at Feekes growth stages; 3, 6 and 9.

2.4 Statistical Analysis

Data was analyzed using the SAS Version 9.4 (SAS Institute, 2013), and PROC Tukey was used to obtain the least significant differences between treatments at 5% level of probability. Regression analyses were also conducted using MS Excel software to determine the variation of NDVI for wheat varieties under low and adequate P conditions.

3- Results and discussion

3.1 Soil properties

Field experiments were conducted in two plots with and without P application at Cimarron Valley Research Station near Perkins, Ok and N40 Experimental Filed near Stillwater, Ok. The general properties of soils used in this study are listed in Table 3.2. For the experiment conducted in Perkins, the average soil P concentration extracted by Mehlich 3 ranged from 6.1 ppm to 5.4 ppm and 4.1 ppm to 1.9 ppm, and 2.9 ppm at depths 0-5, 5-10 and 10-15 cm, respectively (Table 3.3). For the experiment conducted in N40, the average soil P concentration extracted by Mehlich 3 ranged from 2 ppm to 1.2 ppm and 1.0 ppm to 0.6 and 1.3 ppm to 0.6 ppm at depths 0-5, 5-10, and 10-15 cm respectively (Table 3.4). It can be seen that the P concentrations were below 20 ppm, which is considered very low in the available P as described by Mallarino and others (Mallarino et al., 2013). Therefore, treatments without applied P was used as a standard, and the difference between P levels is what we are concentrating on this study.

4- Perkins Location

4.1 Biomass production

Measurements of NDVI were used to estimate the biomass production and to investigate the vegetative response to P conditions. Table 3.5 shows the effect of varying P levels and wheat varieties on NDVI score at different growth stags. In general, there was significant variation among varieties along all growth stages at low P condition, which indicates a strong correlation between NDVI and yield production (R^2=0.92) (Figure 3.1). For example, NDVI showed different variation among wheat varieties, which was also correlated with wheat responses to low P condition. Maximum values of NDVI were recorded by Duster and Endurance at Feekes growth stage 3 and 6; however, the Duster recorded the highest value in the Feekes 9. On the other hand, the Ruby

Lee was measured the lowest value in Feekes 6 and 9 (Figure 3.2). By contrast, varieties grown at adequate P condition (0, 39 kg ha^{-1}) did not show significant difference in Feekes of 3 and 6; however, there was a slightly significant variation among some varieties in Feekes 9. This variation might be due to the late season freeze, which occurred during Feekes 6 growth stage (Figure 3.3). The differences in NDVI among varieties may reflect the ability of a variety to utilize the native soil P because NDVI was strongly correlated to yield production as observed in Figure 3.1. This observation could also be supported by the fact that Duster and Endurance had the highest NDVI value and achieved the highest grain yield under low P condition compared to other varieties, which indicates that these varieties were able to utilize the native P. On the other hand, the Ruby Lee had the lowest NDVI value and produced the lowest grain yield compared to other varieties. This conclusion can be supported by the greenhouse study where these varieties had similar results excluding the Duster, which has low germination rate as reported in the previous chapter. These results are convincing to an extent that the estimated biomass production measured by NDVI responded differently to low P conditions, and the variation of NDVI value is likely related to the P deficiency. Thus, it can be concluded from these results that the probability of using NDVI could be possible as the effective tool to predict and estimate the P deficiency in the soil.

4.2 Grain yield and Protein %

Data in Table 3.6 shows the average grain yield (kg ha^{-1}) and protein percentage among wheat varieties grown under low and adequate P conditions. Overall, there was no significant variation among varieties for the protein content under both conditions, yet the grain yield showed significant differences among varieties. However, the interaction among varieties and P rates was found to be not significant. Grain yield of wheat varieties at low P ranged from 2813 kg ha^{-1} for Endurance to 1154 kg ha^{-1} for Ruby Lee. Duster and Endurance had significantly higher yield

(2816 and 2813 kg ha^{-1}, respectively) as opposed to other varieties, whereas the lowest grain yield was recorded in Ruby Lee and OK13625, producing 1154 and 1918 kg ha^{-1} respectively. Duster and Endurance produced almost the same in both low P and adequate P; indicating the ability of these varieties to extract more P or utilize the native P more efficiently compared to other varieties. These varieties have also been found to be tolerant for aluminum toxicity, and they contain the ALMT1 gene, which is responsible for malate acid exudation. Duster and Endurance were likely able to extract non-labile phosphorus since the exudation of organic acids has been reported to be one of potential mechanisms for P mobilization in the rhizosphere (Dakora and Phillips, 2002; Hinsinger, 2001; Jones and Darrah, 1994; Ström et al., 2002). This conclusion is also consistent with similar results reported by Tang et al. (2007), who suggested that soybean varieties that are resistant to acid soils were the most efficient to utilize the unavailable P of $AlPO_4$ or $FePO_4$. On the other hand, the grain production was the lowest in the Ruby Lee. Moreover, this variety in the experiment pot did not respond efficiently to uptake or utilize P, which was completely unavailable. Therefore, since the Ruby Lee is not tolerant for aluminum toxicity, this finding also supports the fact that organic acids from root exudation can solubilize unavailable P. Thus, the Ruby Lee is mostly considered the least efficient variety in this study. With regard to adequate P application, the grain yield varied from 2992 kg ha^{-1} for OK13625 to 1816 kg ha^{-1} for Ruby Lee; however, significant differences were not observed among varieties for the grain yield at 5% level of probability (Table 3.7; Figure 3.4). The difference in grain yields indicates the great ability between cultivars to access non-labile P and their tolerance to low soil P. Hence, P efficient cultivars are the most desirable for P-limiting environments because of their efficiency to extract more P from sparingly soluble P sources.

The results of this study concluded that Endurance and Duster are the most efficient varieties, and the Ruby Lee is the less efficient variety. This observation is consistent with our results from the greenhouse study, which suggested that the Ruby Lee was among the less efficient varieties, and the Endurance was among the highest efficient varieties that could uptake or utilize non-labile phosphorus, which was tightly bound to soil particles. However, in the greenhouse study, the Duster had a low germination rate, which was only 70 %. This results in a lack of accurate estimation of biomass due to few replications obtained, which was reported as data limitations on that study. Overall, this conclusion corresponds with other published studies that proved wheat varieties responded differently to low P environments, and some cultivars were able to extract more P and produce high biomass while others cannot extract more P under low P soils (Gunes et al., 2006; Manske et al., 2000; Ozturk et al., 2005; Penn et al., 2015).

5- N40 location

5.1 Biomass production

Table 3.8 showed the results of the effect of varying P level and wheat varieties on NDVI at three different s for the experiment conducted in the N40 site. The range of NDVI for all growth stages was 0.26 to 0.61 at low P condition (0 kg ha^{-1}), and 0.26 to 0.69 at adequate P condition (39 kg ha^{-1}) (Table 3.9). At low P condition, there was significant variation among varieties in the Feekes growth stage 3 where the highest value of NDVI was measured in Ruby Lee and Duster whereas the lowest value of NDVI was recorded in the OK104320-2. However, measurements of NDVI remarkably decreased at the Feekes 6, and there were no significant differences among varieties. This is probably due to the impact of freezing temperatures occurred in the month of January, which was approximately -28°C for one week (Simerel, 2017). During Feekes 6 growth stage, NDVI was increased without significant differences among wheat varieties (Figure 3.5). At adequate P condition, NDVI values varied among wheat varieties where the maximum values were observed in Ruby Lee and Gallagher in the Feekes growth stage 3 following by Duster and Endurance, and the minimum values were observed in Spirit Rider and OK104320-2. However, NDVI values decreased at the Feekes 6 due to freezing condition mentioned above, also there was a slightly significant differences among varieties in the growth stages 6 and 9 (Figure 3.6). Results from N40 study suggest that the variation of NDVI values is not directly related to low P conditions because germination failure has been observed in some plots; therefore, the variation of NDVI value is likely due to germination failure rather than the differences among varieties.

5.2 Grain yield and Protein %

Grain yield of wheat varieties at low P ranged from 2327 kg ha^{-1} for Endurance to 1842 kg ha^{-1} for OK13625. Also, grain yield ranged from 3100 kg ha^{-1} for Gallagher to 1889 kg ha^{-1} for OK13625 under sufficient P condition (Table 3.10). No statistical significances were noted in the grain yield among wheat varieties. Also, the protein content was found not to be significant between wheat varieties (Table 3.9). However, a trend of increasing yield was observed across all varieties either at low P or adequate P, and the general direction of yield changes could be possible to indicate the genetic variation among varieties. Endurance and Spirit Rider at low P produced the highest grain yield 2327 kg ha^{-1}, also Spirit Rider produced the 2^{nd} high yield at 39 kg ha^{-1} with Gallagher (Figure 3.7). Endurance also performed well at Perkins location and greenhouse study where the variety resulted in the highest grain yield at low P, and has been reported that Endurance is tolerance to Al toxicity due to root execution that can regulate the rhizosphere soil pH around the root system (Edwards et al., 2010; McKindra, 2017). Also, Gallagher produced the highest yield under sufficient P condition. Meanwhile, this variety performed relatively less in low P condition compared to Endurance and Spirit Rider (Figure 3.7). It is important to highlight that

Gallagher was also the most efficient variety to uptake and/or utilize P in the greenhouse study, and it produced the 2nd high grain yield at 0 kg ha-1 in this study. Therefore, the observation of N40 study would suggest that Endurance, Gallagher, and Spirit Rider performed better in low P condition compared to other varieties evaluated in this study. Even though the direction of trends is almost significant, there is no statically significant difference among these varieties because of several factors impacted this study, including germination failure and weed damage in some plots. These limitations might be the significant factor in detecting significant differences.

6- Conclusion

Two experiments were conducted in the Perkins experimental station and N40 station during the growing season of 2016-2017. Seven wheat varieties were chosen from previous studies conducted in the growth chamber. Overall, field experiments showed significant variation for grain yield and NDVI, which indicates that wheat varieties responded differently to low P conditions. In the experiment conducted in the Perkins station, Duster and Endurance had the highest NDVI values in all growth stages at low P condition. Also, these varieties showed the highest grain yield compared to other varieties, which indicated that NDVI was correlated with grain yield. Endurance produced better under greenhouse and field conditions and was classified as the most efficient variety for P uptake efficiency and P utilization in the greenhouse study. On the other hand, Ruby Lee had the lowest NDVI value along all Feekes growth stages, and it produced the lowest grain yield as compression to other varieties evaluated in this study. In relation to the experiment conducted at N40 station, no significant differences were noted for grain yield and protein content among varieties; this observation is likely due to the impact of several factors such as germination failure and weed damage in some plots. However, a trend of increasing yield production was observed across all varieties either at low P or adequate P, which indicates the genetic variation among varieties. Overall, the results of the N40 study would suggest that Endurance, Gallagher, and Spirit Rider performed better in low P condition compared to other varieties evaluated in that study. To summarize, Duster and Endurance are the most efficient varieties in extracting more P or utilizing the native P while the Ruby Lee is the most inefficient variety, showing less effective growth in a low P condition. Also, NDVI was possible to estimate the P deficiency due to a strong correlation between the NDVI and yield production under low P conditions; this observation may be used as an indicator for P recommendation, if a phosphorus unlimited plot or strip was available.

Table 3.1 Treatment structure implemented for field experiments in a randomized completely block design.

Treatment	Varieties*	P- Fertilizer Rate kg P ha^{-1}
1	OK13625	0
2	OK 13625	39
3	OK 10430-2	0
4	OK 10430-2	39
5	Endurance	0
6	Endurance	39
7	Spirit Rider	0
8	Spirit Rider	39
9	Duster	0
10	Duster	39
11	Ruby Lee	0
12	Ruby Lee	39
13	Gallagher	0
14	Gallagher	39

* Varieties were selected from the previous greenhouse experiment due to their efficiency to uptake more P and/or utilize P.

Table 3.2 General properties of soils and 0-15 cm composite soil sample results of the locations utilized for P use efficiency study.

Location	pH	P	K	N	S	Ca	Mg	Sand	Silt	Clay	Soil texture	Soil series classification
	Extracted by Mehlich 3 (ppm)								%			
Perkins	4.2	8	126	3	12	652	128	70	20	10	Sandy loam	Teller fine sandy loam
N40	6.6	18	119	4	11	1700	705	30	44	26	Loam	Kirkland silt loam

Table 3.3 Initial P test results extracted by Mehlich 3 from stratified samples at different depths for Perkins site during the growth period.

Treatment	Varieties	P rate ($kg\ ha^{-1}$)	pH	Depth 0-5 cm P (ppm)	Depth 5-10 cm P (ppm)	Depth 10-15 cm P (ppm)
1	OK13625	0	4.6	6.1	3.5	2.5
3	O10430-2	0	4.6	5.9	3.8	2.9
5	Endurance	0	4.7	5.4	3.7	2.7
7	Spirit Rider	0	4.6	5.8	3.4	2.7
9	Duster	0	4.8	5.7	1.9	2.6
11	Ruby Lee	0	4.6	6.0	3.4	2.7
13	Gallagher	0	4.6	5.4	41	28

Table 3.4 Initial P test results extracted by Mehlich 3 from stratified samples at different depths for N40 site during the growth period.

Treatment	Varieties	P rate ($kg\ ha^{-1}$)	pH	Depth 0-5 cm P (ppm)	Depth 5-10 cm P (ppm)	Depth 10-15 cm P (ppm)
1	OK13625	0	6.1	1.2	0.6	1.1
3	O104320-2	0	6.0	1.2	0.8	0.8
5	Endurance	0	6.2	2.0	0.6	0.6
7	Spirit Rider	0	6.2	1.3	0.5	0.6
9	Duster	0	6.0	1.4	0.1	0.8
11	Ruby Lee	0	5.8	1.2	0.9	1.3
13	Gallagher	0	6.2	1.3	0.6	0.5

Table 3.5 Effect of varying P levels and wheat cultivars on normalized difference vegetative index (NDVI) values collected at different Feekes growth stages for Perkins site. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

	Stage 3	Stage 6	Stage 9	Stage 3	Stage 6	Stage 9
Varieties	P rate (0 Kg ha^{-1})			P rate (39 Kg ha^{-1})		
OK13625	0.37 [b]	0.40 [bc]	0.49 [abc]	0.53 [a]	0.51 [a]	0.58 [a]
OK10430-2	0.37 [b]	0.43 [abc]	0.58 [ab]	0.55 [a]	0.54 [a]	0.66 [a]
Endurance	0.63 [a]	0.53 [a]	0.58 [ab]	0.71 [a]	0.54 [a]	0.57 [a]
Spirit Rider	0.44 [ab]	0.47 [abc]	0.57 [ab]	0.53 [a]	0.53 [a]	0.56 [ab]
Duster	0.58 [a]	0.51 [a]	0.61 [a]	0.60 [a]	0.53 [a]	0.62 [a]
Ruby Lee	0.38 [b]	0.34 [c]	0.37 [c]	0.56 [a]	0.43 [a]	0.42 [b]
Gallagher	0.35 [b]	0.38 [c]	0.44 [c]	0.54 [a]	0.48 [a]	0.54 [ab]

Values within each column followed by same letters are not significantly different at 5% level of probability

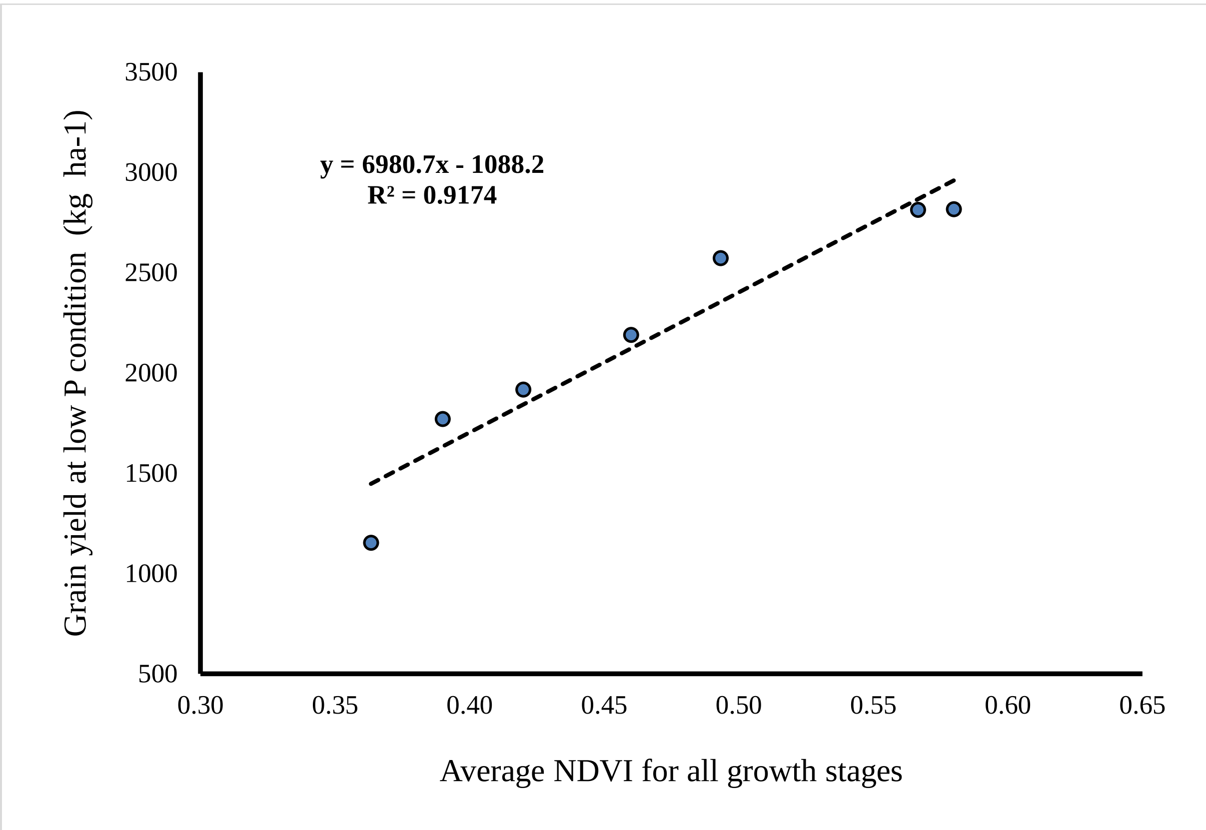

Figure 3.1 Correlation between average normalized difference vegetative index (NDVI) for all three growth stages evaluated (Feekes 3, 6, 9) and grain yield of winter wheat grown under low soil test P conditions in the Cimarron Valley Research Station located near Perkins, Ok.

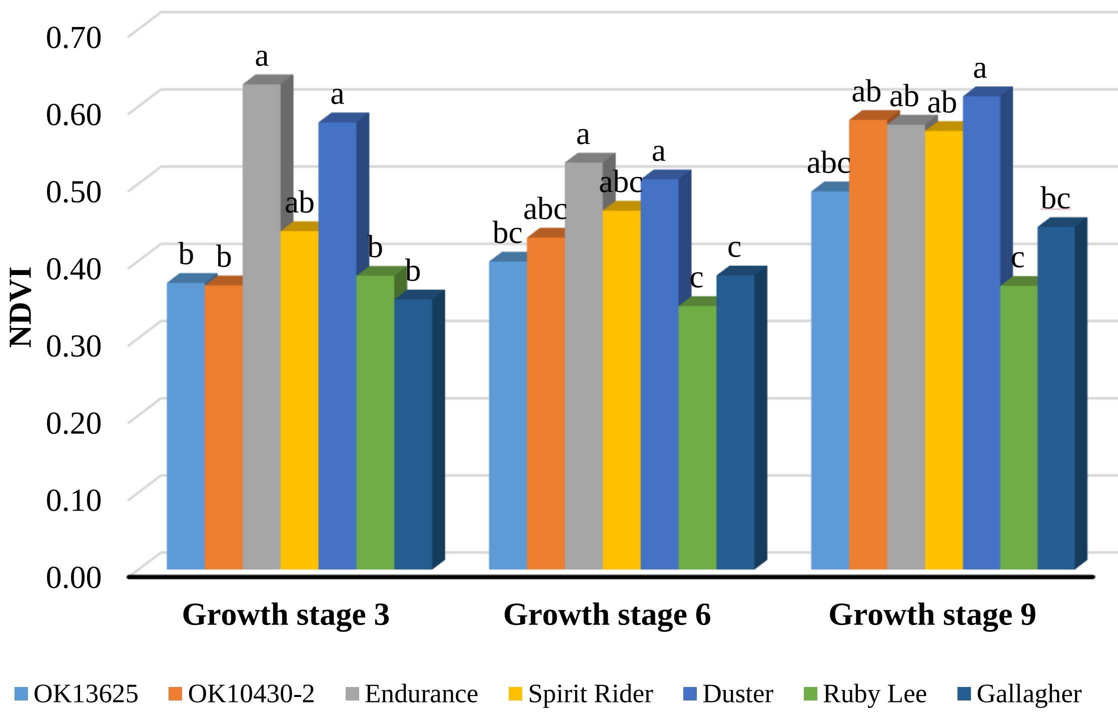

Figure 3.2 Variation of normalized difference vegetative index (NDVI) for winter wheat varieties received 0 Kg P ha^{-1} in the Cimarron Valley Research Station located near Perkins, Ok. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

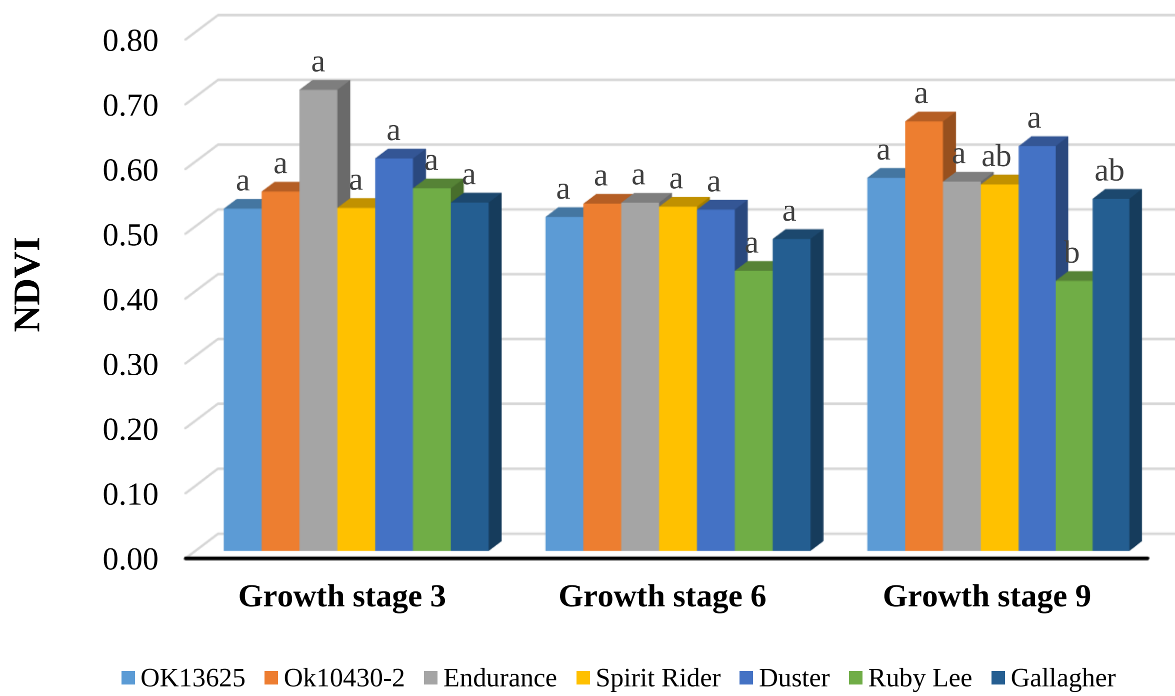

Figure 3.3 Variation of normalized difference vegetative index (NDVI) for winter wheat varieties received 39 kg P ha^{-1} in the Cimarron Valley Research Station located near Perkins, Ok. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

Table 3.6 The average grain yields and percent protein of winter wheat varieties grown under two P rates in the Cimarron Valley Research Station located near Perkins, Ok.

Treatment	Varieties	P rate (kg ha^{-1})	Yield (kg ha^{-1})	Protein %
1	OK13625	0	1918 [bcd]	12.6 [a]
2	OK13625	39	2443 [abc]	11.9 [a]
3	OK10430-2	0	2190 [abc]	12.8 [a]
4	OK10430-2	39	2992 [a]	12.7 [a]
5	Endurance	0	2816 [ab]	11.6 [a]
6	Endurance	39	2694 [abc]	11.5 [a]
7	Spirit Rider	0	2572 [abc]	13.2 [a]
8	Spirit Rider	39	2841 [ab]	10.8 [a]
9	Duster	0	2813 [ab]	10.8 [a]
10	Duster	39	2954 [a]	10.5 [a]
11	Ruby Lee	0	1154 [d]	13.3 [a]
12	Ruby Lee	39	1816 [cd]	13.5 [a]
13	Gallagher	0	1770 [cd]	12.3 [a]
14	Gallagher	39	2323 [abc]	12.3 [a]

* Means followed by the same letter are not significantly different at 5% level of probability

Table 3.7 The average grain yields of winter wheat varieties grown under two P rates in the Cimarron Valley Research Station located near Perkins, Ok.

Varieties	Yield (kg ha^{-1}) P rate (0 kg ha^{-1})	Yield (kg ha^{-1}) P rate (39 kg ha^{-1})
OK13625	1918 [c]	2443 [a]
OK10430-2	2190 [bc]	2992 [a]
Endurance	2816 [a]	2694 [a]
Spirit Rider	2572 [ab]	2841 [a]
Duster	2813 [a]	2954 [a]
Ruby Lee	1154 [d]	1816 [a]
Gallagher	1770 [c]	2323 [a]

* Means within each column followed by the same letter are not significantly different at 5% level of probability

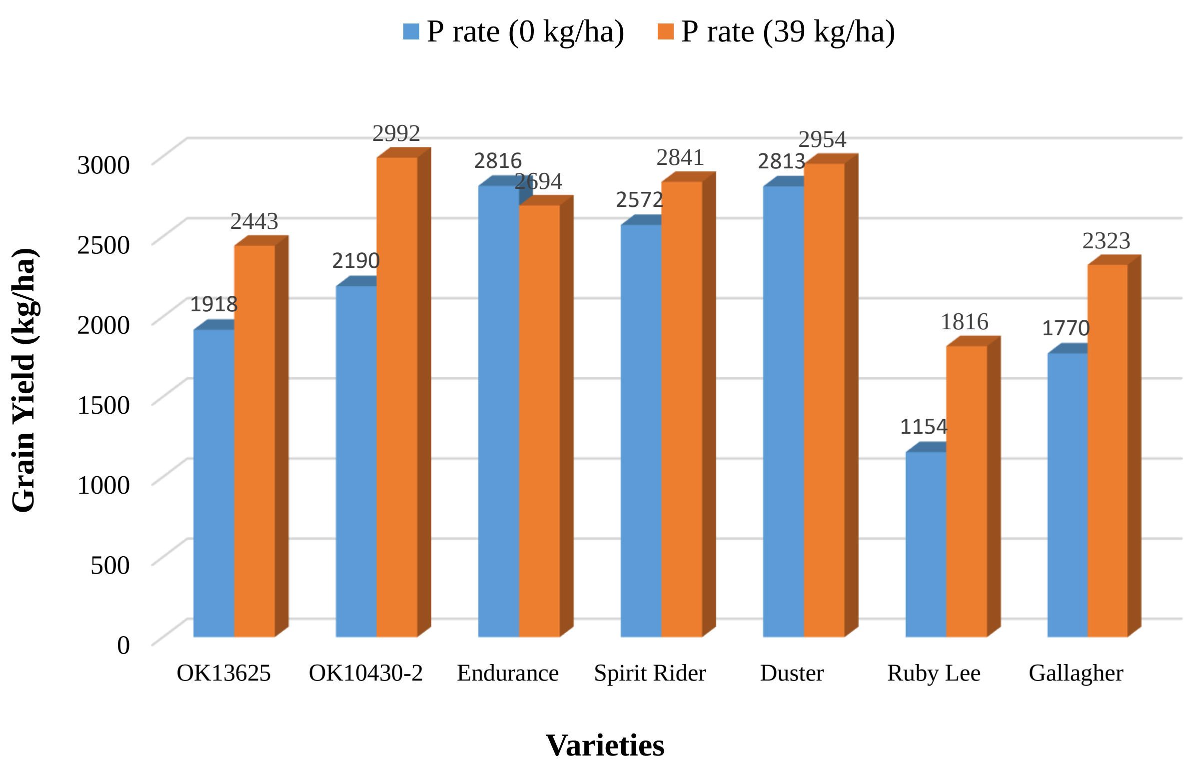

Figure 3.4 Variation of grain yield among winter wheat varieties grown at two P rates in the Cimarron Valley Research Station located near Perkins, Ok.

N40 site

Table 3.8 Effect of varying P levels and wheat cultivars on normalized difference vegetative index (NDVI) values collected at different Feekes growth stages in the N40 Experimental Filed near Stillwater, Ok. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

	Stage 3	Stage 6	Stage 9	Stage 3	Stage 6	Stage 9
Varieties	P rate (0 kg ha^{-1})			P rate (39 kg ha^{-1})		
OK13625	0.48 ^{abc}	0.27 ^{a}	0.47 ^{a}	0.60 ^{ab}	0.28 ^{abc}	0.46 ^{ab}
OK10430-2	0.41 ^{c}	0.26 ^{a}	0.53 ^{a}	0.44 ^{b}	0.27 ^{bc}	0.53 ^{a}
Endurance	0.52 ^{abc}	0.29 ^{a}	0.50 ^{a}	0.65 ^{ab}	0.32 ^{a}	0.50 ^{ab}
Spirit Rider	0.43 ^{bc}	0.26 ^{a}	0.45 ^{a}	0.46 ^{ab}	0.26 ^{c}	0.44 ^{b}
Duster	0.61 ^{a}	0.29 ^{a}	0.50 ^{a}	0.65 ^{ab}	0.31 ^{ab}	0.43 ^{b}
Ruby Lee	0.61 ^{a}	0.28 ^{a}	0.46 ^{a}	0.68 ^{a}	0.31 ^{ab}	0.45 ^{b}
Gallagher	0.56 ^{ab}	0.29 ^{a}	0.47 ^{a}	0.69 ^{a}	0.31 ^{ab}	0.46 ^{ab}

Values within each column followed by the same letters are not significantly different at 5% level of probability.

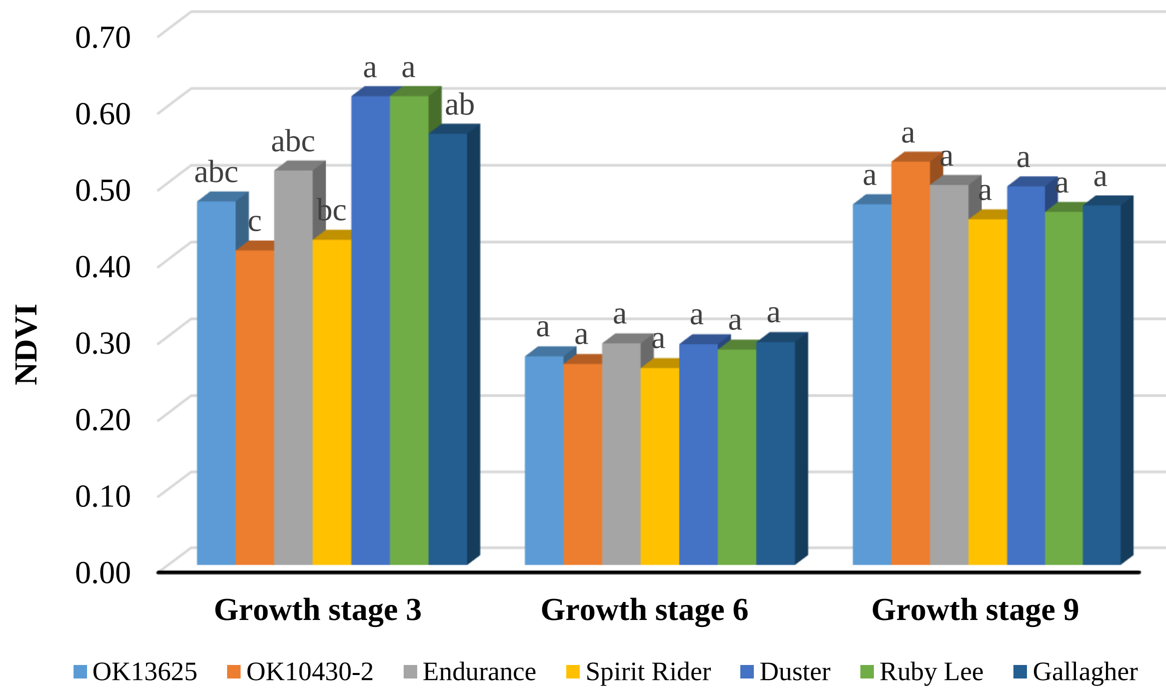

Figure 3.5 Variation of normalized difference vegetative index (NDVI) values for winter wheat varieties received 0 kg P ha^{-1} in the N40 Experimental Field near Stillwater, Ok. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

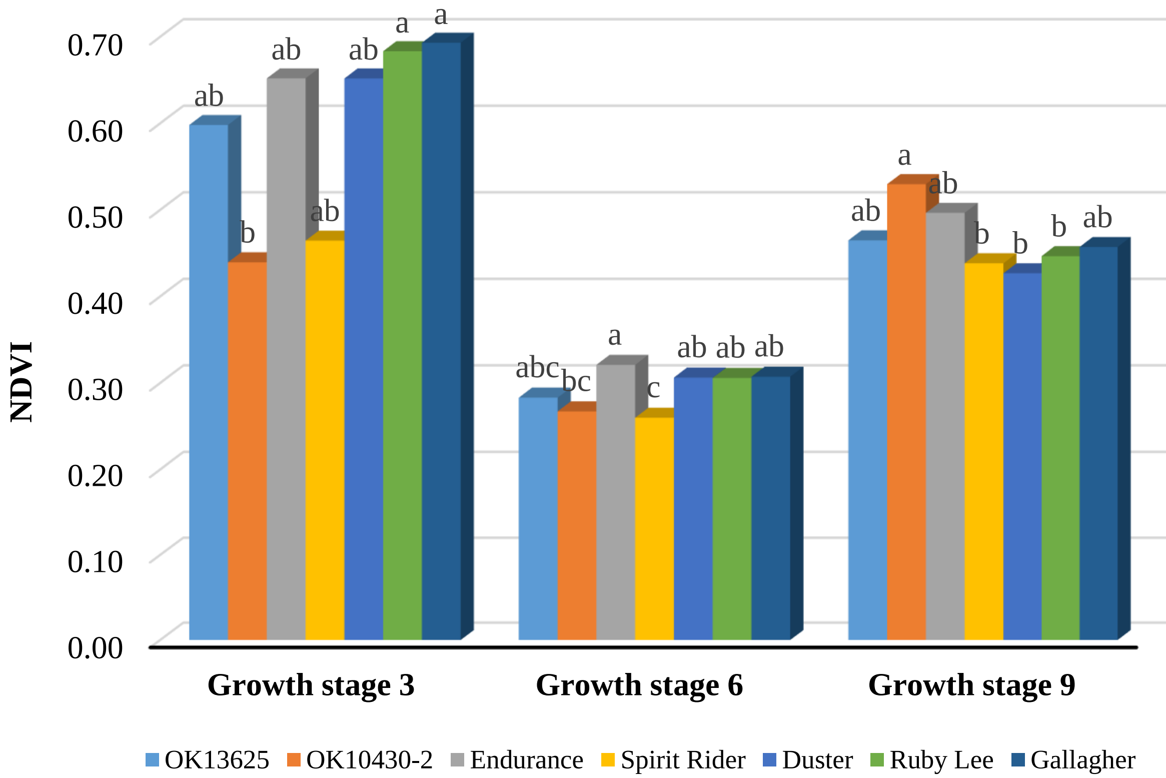

Figure 3.6 Variation of normalized difference vegetative index (NDVI) values for winter wheat varieties received 39 kg P ha^{-1} in the N40 Experimental Field near Stillwater, Ok. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

Table 3.9 The average grain yields and percent protein of winter wheat varieties grown at two P rates in the N40 Experimental Field near Stillwater, Ok.

Treatment	Varieties	P rate (kg ha^{-1})	Yield (kg ha^{-1})	Protein %
1	OK13625	0	1842 [a]	14.0 [a]
2	OK13625	39	1889 [a]	13.7 [a]
3	OK10430-2	0	2115 [a]	14.7 [a]
4	OK10430-2	39	2548 [a]	14.9 [a]
5	Endurance	0	2327 [a]	14.0 [a]
6	Endurance	39	2681 [a]	14.2 [a]
7	Spirit Rider	0	2327 [a]	14.8 [a]
8	Spirit Rider	39	3000 [a]	13.8 [a]
9	Duster	0	2049 [a]	14.7 [a]
10	Duster	39	2711 [a]	13.8 [a]
11	Ruby Lee	0	2021 [a]	14.9 [a]
12	Ruby Lee	39	2630 [a]	14.3 [a]
13	Gallagher	0	2181 [a]	14.0 [a]
14	Gallagher	39	3100 [a]	13.6 [a]

* Means with the same letter are not significantly different at 5% level of probability.

Table 3.10 The average grain yield of winter wheat varieties grown at two P rates in the N40 Experimental Field near Stillwater, Ok.

	Yield (kg ha^{-1})	Yield (kg ha^{-1})
Varieties	P rate (0 kg ha^{-1})	P rate (39 kg ha^{-1})
OK13625	1842 [a]	1889 [a]
OK10430-2	2115 [a]	2548 [a]
Endurance	2327 [a]	2681 [a]
Spirit Rider	2327 [a]	3000 [a]
Duster	2049 [a]	2711 [a]
Ruby Lee	2021 [a]	2630 [a]
Gallagher	2181 [a]	3100 [a]

* Means followed by the same letter are not significantly different at 5% level of probability

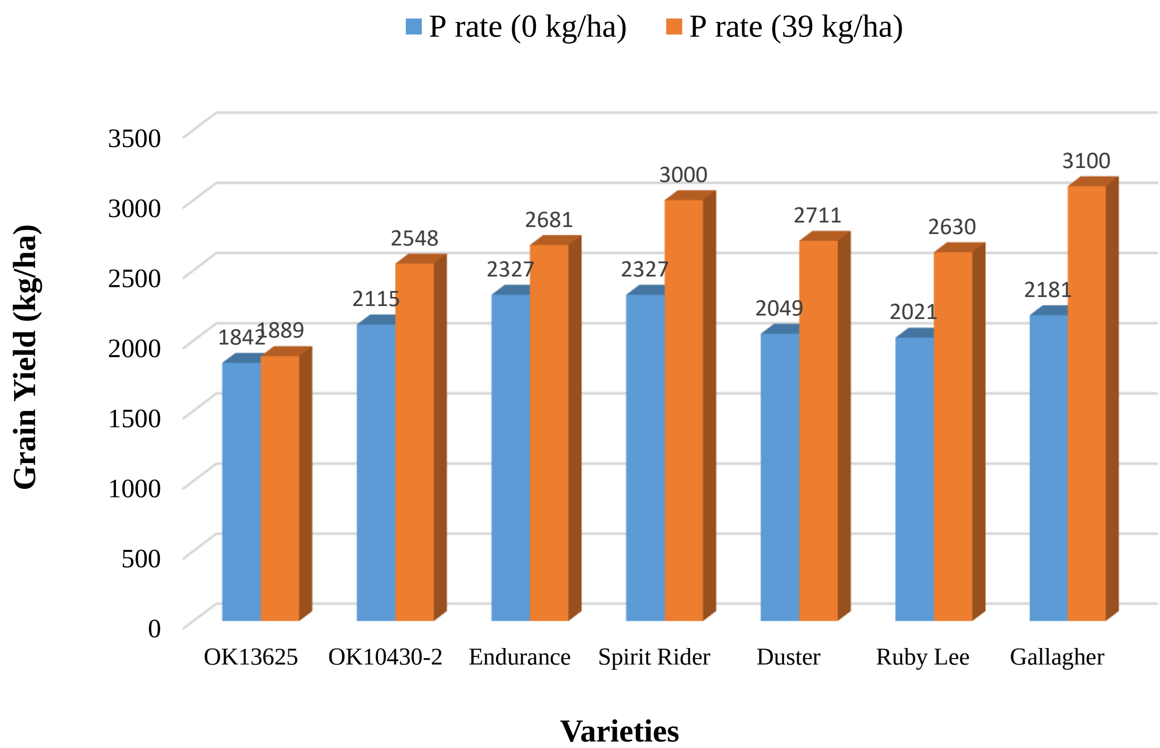

Figure 3.7 Variation of grain yield among winter wheat varieties grown at two P rates in the N40 Experimental Field near Stillwater, Ok.

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CHAPTER IV

PREDICTION OF MAIZE POPULATION (ZEA MAYS L.) BASED ON NORMALIZED-DIFFERENCE VEGETATIVE INDEX (NDVI) AND COEFFICIENT OF VARIATION (CV)

ABSTRACT

The process of improving crop management inputs by use of remote sensing devices is a new technology. This study presents the use of the normalized-difference vegetative index (NDVI) combined with the coefficient of variation (CV) to estimate the plant population of maize (Zea Mays L.) at different growth stages. Data was collected from 76 plots located at the Agronomy Research Station (EFAW) near Stillwater, OK, and the Lake Carl Blackwell Research Station (LCB) near Bray, OK. Each plot was 20 meters long by 10 meters wide. The results of this study showed that we were not able to reliably predict plant population because the canopy covered the soil with overlapping leaves at these growth stages. The linear relationship at the V4 growth stage was slightly higher than that of vegetative growth stages V6 and V8 (R^2 = 0.22, 0.01 and 0.001 respectively). This relationship decreased significantly as canopy closure occurred. The results also suggest that as the corn plant emerged from the soil at early growth stages, the biomass per unit area was small and the application of sensor technology for prediction of plant population was possible. Further studies should be designed based on this observation to investigate the possibility of using this technique at early stages such as V2 and V3, which may be useful for producers for making replant decisions and precise estimations of replant rates.

1- Introduction

With the increasing cost of production and environmental concerns, researchers have focused on investigating different methods of increasing yields while reducing fertilizer use (Arnall and Godsey, 2013). A recent study suggested that the crop populations are tied up to the end yields (Nielsen et al., 2016), also it has been reported that the plant population is correlated to seeding rate and row populations (Bushong et al., 2016). For example, if the plant population is high then crops compete with each other for nutrients, leading to decrease the grain production. On the contrary, if the population is low then the farmer is under-exploiting the farm and reducing the chances of optimum harvesting yield. A recent study revealed that the seeding rate, row spacing, germination rate, and seed placement determine the plant population (Arnall and Godsey, 2013). Seed placement, the depth, soil tillage, and type also determine how many plants survive after planting. When seeds are placed uniformly, the emergence is also uniform, thus, ensuring healthy plant-to-plant competition (Arnall and Godsey, 2013). Therefore, the farmer should estimate the percentage of pure grains and other characteristics such as germination percentage to determine the desired plant population. Most of the seed companies test the seeds before they sell their seeds, and thus, report on the potential germination rate under ideal moisture, and temperatures (Freeman et al., 2007). Nowadays, it is essential to achieve maximum yield production at minimum cost; therefore the remote sensors have been widely used to estimate the potential plant populations correlated to the agricultural environment through evaluating crop health, water, nutrients and crop yield production before harvested (Martin et al., 2007). The first remote sensor developed was known as normalized difference vegetation index (NDVI), which calculates the number of plant per acre. In recent studies, NDVI has been used successfully to estimate the nitrogen use and the potential yield production, which are responsible for making accurate agricultural decisions to minimize the production cost (Miller et al., 2017; Tagarakis and Ketterings, 2017). Also, it has been documented that NDVI is one of the effective tools to estimate yield (Hayes and Decker, 1996; Mika et al., 2002; Mkhabela and Mashinini, 2005; Prasad et al., 2006). Also, Turvey and Mclaurin (2012) proposed that NDVI could be a possible alternative index for insurance purposes. Therefore, the potential of using NDVI to predict the plant population is possible; this might be useful for agricultural insurance companies under circumstances of data scarcity.

2- Literature review

Plant placing determines the plant population. Narrow spacing with less equidistant spacing increases plant population. A recent study revealed that decreased soybeans spacing from 0.76cm to 0.38m doubled the realized crop population, and different spatial selections realized in varying row spacing affect the plant density since it influences crops resource competition (Nielsen et al., 2016). Consequently, the population and distribution of these plants in the field result in the final yield. According to Chim et al. (2014), seed spacing shows the spatial distribution of the crops, the canopy structures, light, efficiency distribution, interestingly, the biomass, and the grain yield. Thus, the plant population per square meter determines the nutrients undertaken from the soil. Uneven crop distribution gives less maize yield unlike when uniformly distributed. The corn yield is detrimental if the plants are extremely distributed. Proper plant spacing increases the yield because it enables the crops to absorb nutrients adequately. Plants should be given the spacing of about 0.05 to 0.07 m (Chim et al., 2014). The equidistant spacing gives the yield a more advantage than the undistributed spacing. The spacing gives the plants an increased resource utilization and absorption. The quantity harvested increases with increase in the plant density although the population might at times be uneconomical. Plant population is sensitive because uneven distribution of plants can decrease the expected yield compared to the uniform distribution (Wade et al., 1988). On the other hand, less plant population gives a low quantity due to lack of exploitation of the resources. The only problem with improper plant community is there is plant competition for the nutrients. However, the extent of influence depends on with the environment and hybrid conditions (Martin et al., 2012). Each mixture of maize has the minimum nutrients and conditions they can thrive. Plant population within a row determines the light access and thus photosynthesis and the end yield. The farmers can also manipulate the plant spacing to increase access to light for each plant. In the normal conditions, the growth of crop yields is related to how much light they intercept. Maximizing the light interception is significant in corn production to acquire optimal return. The light necessity for corn is required during early reproductive periods and vegetation (Martin et al., 2012). High plant densities and narrow spacing give the plants the chance to access adequate light. Nitrogen uptake is closely related to plant spacing just like all the other nutrients; therefore, narrowing the maize crops can increase the N uptake which otherwise could be lost (Chim et al., 2014). During the last decade, remote sensing has been used extensively in agricultural experiments because it enables farmers to predict accurately the yield that is likely to be harvested and the knowledge of the return expected to be collected from a particular field allows the farmer to be able to maximize the management practices on the field. It is essential to note that nitrogen is the usual limiting factor in most of the studies in the different production systems and accounting for the production costs. Therefore, it is easier to relate to the data collected on the yield and yield relationships potential estimates that were calculated on the wheat production (Teal et al., 2006). The growth strategies are crucial while determining the yield. Each crop has the appropriate developmental stages that are used while investigating the yield potential. The recent advancement in technologies including the sensors and modifications in weather and crops has enabled adjustments of nitrogen in-season, which allow the farmers to achieve potential yield (Martin et al., 2012). The three past decades show that the methodologies that incorporate sensors are responsible for the many agronomic decisions made within any farm. According to Bushong et al., (2016) the remote sensors are suitable for reflecting and monitoring the amount of nitrogen in a crop and the amount required to be added. However, other scientists used the Green-Seeker hand sensor to determine the individual plant NDVI difference (Teal et al., 2006). According to Chung et al., (2008) the capacity to test these variations was seen as the V8 psychological stage that disappeared at V10 development stage. Al-Abbas et al., (1974) had applied the NDVI method to evaluate nutrient deficiencies such as such as nitrogen, phosphorus, potassium, and sulfur in corn leaves. In a study to comparing the ability of 10 sensing indexes for accurate estimation of percent groundcover and the biomass of winter cover crops, Prabhakara et al., (2015) found that NDVI was strongly correlated with percent groundcover for all vegetation (R^2=0.93), and with green vegetation only (R^2=0.94). Also, Kapp Junior et al., (2016) evaluated the efficiency of GreenSeeker™ 505 handheld Sensor and Crop Circle ACS-470 to predict the biomass and wheat response to N fertilization, and they concluded the efficiency of both sensors are similar in determining biomass production and wheat responses to N rates applied. Additionally, NDVI values abstained by GreenSeeker ™ 505 handheld Sensor have proven to be more accurate in the early season to predict the yield potential of wheat. For example, Raun et al., (2001) reported that NDVI measurements had a strong correlation with the estimated yield of wheat at Feekes Growth Stage 4 and 6 (R^2=0.50). Similar studies have shown that NDVI measurements of winter wheat at Feekes Growth Stage 4 and 5 could provide a reliable estimation of both N uptake and biomass (Stone et al., 1996). Also, in a study to evaluate the relationship between NDVI and corn grain yield, the NDVI values obtained by the GreenSeeker has been found to be strongly correlated (R^2=77) with harvested grain yield of corn at V8 stage (Teal et al., 2006). A similar study to evaluate the NDVI values obtain by GeenSeeker for predicting the biomass and plant N content in four varieties of wheat found strong correlations between NDVI measurements, biomass and N content; results of that study have been confirmed the direct relationship between total biomass and NDVI readings (Cabrera-Bosquet et al., 2011). Recently, Ji et al., (2017) evaluated the GreenSeeker hand-held optical sensor to predict the yield production of cabbage varieties, and they concluded in their study that NDVI values were significantly correlated to harvested yields of cabbage. Moreover, NDVI has been proved to be a good indicator of biomass and leaf area in several crops such as wheat, and corn (Chewab and Murdock, 2002; Prabhakara et al., 2015). Ahmadi and Mollazade (2009) also concluded in their study that there was an acceptable correlation between NDVI and soybean population where high NDVI indicated the high plant population and vice versa with low NDVI.

Maize is one of the most major cereal crops, and currently, it is considered the second most significant product cultivated globally. Therefore it is so important to involve any technology that may increase the yield production or decrease the cost of production that is why sensor production is being adopted (Edmonds et al., 2013). The sensor technology enables farmers to be able to quantify yields and their variability in the field. The sensors give spatial resolution images that show the minimum distance between two different objects (Chung et al., 2008). According to Martin et al., (2007) coefficient of variation (CV) is used to assess the variability of the NDVI data collected, and it is calculated statistically by dividing the standard deviation by the mean and multiply by 100. CV has been used in various calculations involving wheat production, and it can provide a measure of the variability of the area of interest. The CV assesses the effects of added nutrients during any season of production. Meanwhile, the researchers discovered that the NDVI was related to the yield biomass and the corn population. The NDVI measurements recorded in various projects show that the corn grain in the fields varies from one plant to the next. In general, NDVI is highly useful to determine the vegetative areas and their conditions. Raun et al., (2005b) reported that 10 NDVI readings collected from $0.4m^2$ are considered enough to obtain a composite sample; however, few studies have been done to evaluate the possibility of using NDVI and CV to predict the plant population. The major goal of this research was to identify whether there is any relationship between NDVI, CV, and the plant population of maize.

3- Materials and methods

This study was employed to examine the relationship between the normalized difference vegetation index (NDVI) sensor reading and plant population. The study was conducted in two locations in summer 2016. The first site was located at the Agronomy Research Station (EFAW) near Stillwater, OK, and the second was located at the Lake Carl Blackwell Research Station (LCB) near Bray, Ok. In total, seventy-six plots were randomly selected from both locations; each plot size is 20 m long by 10 m wide with alley 10 m. A GreenSeeker^{TM} 505 handheld Sensor (Trimble Industries, Inc.) was used to collect normalized difference vegetation index (NDVI) measurements. This device is used to calculate NDVI by emitting red and NIR light where red radiation is absorbed by plant chlorophyll; therefore, a healthy plant will absorb more red radiation and reflect more massive amounts of NIR radiation compared to unhealthy plants. In short, sensing was done once a week until maturity by holding the sensor 40 cm above the top of crop canopies to obtain proper function when measuring NDVI in each row. Two sensor readings were vertically taken to the seed rows, and then the mean NDVI and CV of NDVI were calculated as the following; 1) NDVI was calculated based on the following equation as described by Raun et al., (2005a):

$$\mathrm{NDVI} = \frac{F\mathrm{NIR} - F\mathrm{Red}}{F\mathrm{NIR} + F\mathrm{Red}}$$

Where: F_{RIN} is the Fraction of emitted NIR radiation returned from the sensed area, and F_{Red} was the fraction of emitted red radiation returned from the sensed area. 2) the Coefficient of variation (CV), which can be defined as the ratio of the standard deviation divided by the mean, was also calculated by computing the standard deviation of NDVI values and the average of NDVI values based on the following equation as cited in Raun et al. (2005a).

$$\mathrm{C}V = \frac{\text{Standard deviation}}{Mean} \times 100$$

At the end of the study, the plant population was computed for each plot by counting all plants within two rows, and then the linear regression analyses were performed using MS Excel software to determine the correlation between the plant population, NDVI, and the CV.

4- Results and Discussion

4.1 NDVI at different growth stages

As Lukina et al. (2000) reported, NDVI can be an effective tool for estimation vegetation cover; therefore, this research was carried out to assess the possibility of using NDVI and CV to predict the plant population. Figures 4.1, 4.2, and 4.3 demonstrate the correlation between the plant population and NDVI at vegetative growth stages V4, V6, and V8. As expected, there was a significant correlation between plant population and NDVI values taken at the early growth stage (V4) where plant population increased with increasing NDVI with a correlation coefficient r^2=0.22 (Figure 4.1). This correlation was slightly higher compared to the growth stage 6 and the growth stage 8 where the relationship significantly decreased as canopy closure occurred. This observation can suggest that as the corn plant emerged from the soil in the early growth stages, the biomass per unit land area was very small and therefore the sensor technology application for prediction of plant population could be possible. This observation is also consistent with similar results reported by Ahmadi and Mollazade (2009); who suggested that plant population can be correlated with NDVI where they found that plant population increased with an increase NDVI with a correlation coefficient of 0.92. Therefore, using NDVI to predict the population could be possible at early growth stages. Another study also was observed a strong relationship with a correlation coefficient of 0.95 between NDVI and canopy cover, which is the percentage of the area covered by plant leaves (Trout et al., 2008). Therefore, results of both studies mentioned above are agreed that higher NDVI values indicate increasing of plant biomass. On the other hand, the linear relationship between plant population and NDVI was found poor at vegetative growth stages V6 and V8. The change of correlation in the growth stage V6 was relatively higher compared to the growth stage V8 where a correlation coefficient r^2 was 0.01 and 0.001 at the growth stage V6 and the growth stage V8 respectively (Figures 4.2 & 4.3). Overall, the results of this research suggested that we were not able to reliably predict the plant population because the canopy covered the soil with overlapping leaves at these growth stages; therefore, the plant population can only predict at early growth stages before plant canopies closed up and covered the soil surface completely.

4.2 CV at different growth stages

Figures 4.4, 4.5 and 4.6 showed the relationship between the plant population and the Coefficient of variation (CV), which is measured as the standard deviation divided by the mean, at different vegetative growth stages (V4), (V6) and (V8). Plant population was negatively correlated to the CV values in the growth stage V4, and the highest relationship was recorded on that stage where a correlation coefficient r^2 was 0.21 (Figure 4.4). However, there was the weak correlation between plant population and CV at growth stages, V6 and (V8), and the change in CV values was stable at that stages (Figure 4.5 & 4.6). Results of this study also suggest that when CV increased the plant population decreased, also the observation of growth stage V4 indicates that the prediction of plant population at this stage may provide the accurate estimation of plant population because it has been observed when CV was greater than 25 the plant population decreased. This conclusion is consistent with similar results reported by Lukina et al., (2000), who observed that the CV of NDVI values decreased with increasing the vegetation coverage. Also, results of this study correspond with another study conducted by Arnall et al., (2006), who observed that the plant density of the winter wheat was low when CVs reached around 20 at early growth stages. Therefore, the use of CV obtained from NDVI measurements could be used to estimate the plant population only in the early growth stages.

5- Conclusion

This study was conducted in the summer of 2016 to evaluate the possibility of using NDVI and CV to predict the plant population. Two sites were designed to collect the data near Stillwater, Ok. The results of this study suggest that the use of NDVI could be possible to predict the plant population, especially in the early growth stages before plant canopies cover the soil with overlapping leaves, which could be one of the obstacles that restrict the accurate prediction of plant population. Results of this research are consistent with similar results reported by Ahmadi and Mollazade (2009) and Trout et al., (2008). However, there was no significant relationship between NDVI and plant population in vegetative growth stages V6 and V8; this can be refuted by the fact that plant canopies covered the entire area during the late growth stages, resulting in the inaccurate prediction of plant population. On the other hand, the use of CV to predict the plant population could also be possible at early growth stages where CV was negatively correlated with plant population, and the CV decreased with increasing the plant population. This observation is also consistent with the other study reported by Lukina et al., (2000). Therefore, since NDVI and CV were correlated to plant population, the growth stage V4 would be the appropriate stage to predict the plant population and biomass estimation. Also, this observation should lead forward for further study to investigate the likelihood of using this technique at early stages such as V2 and V3, which could be beneficial for producers to estimate corn replant rates.

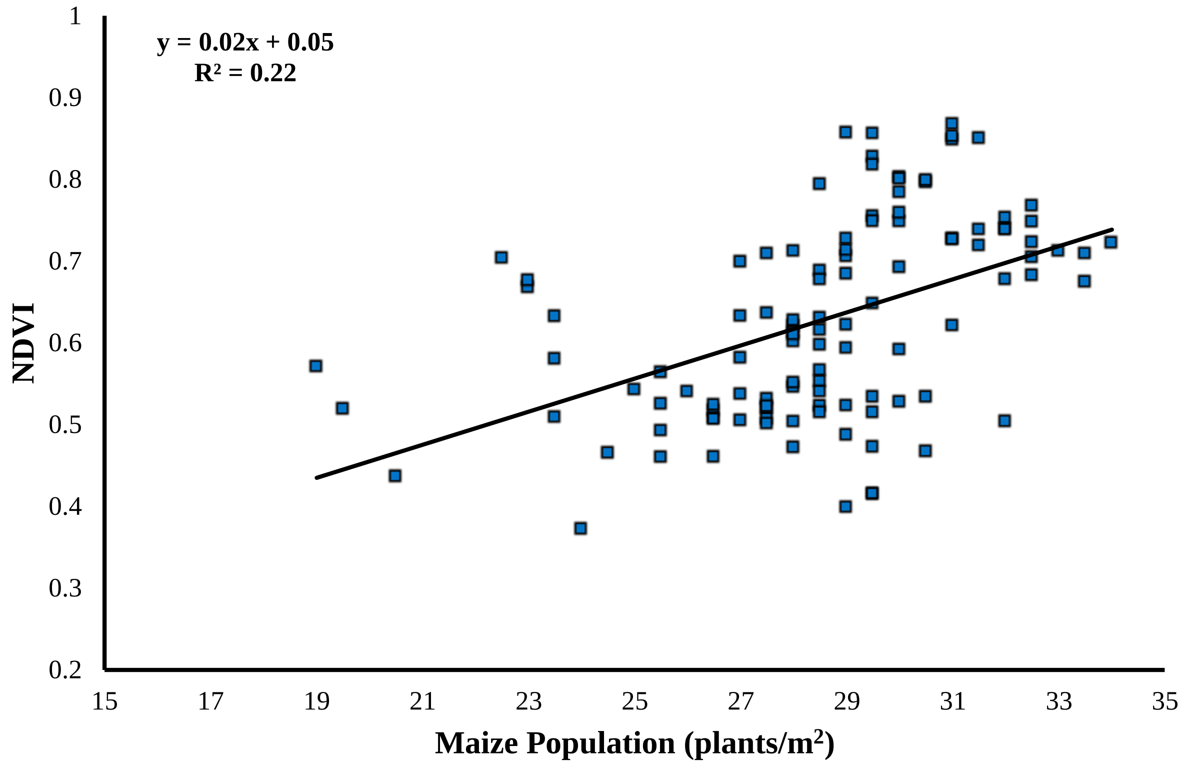

Figure 4.1 The relationship between the plant population of maize and normalized difference vegetative index (NDVI) values at growth stage V4 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

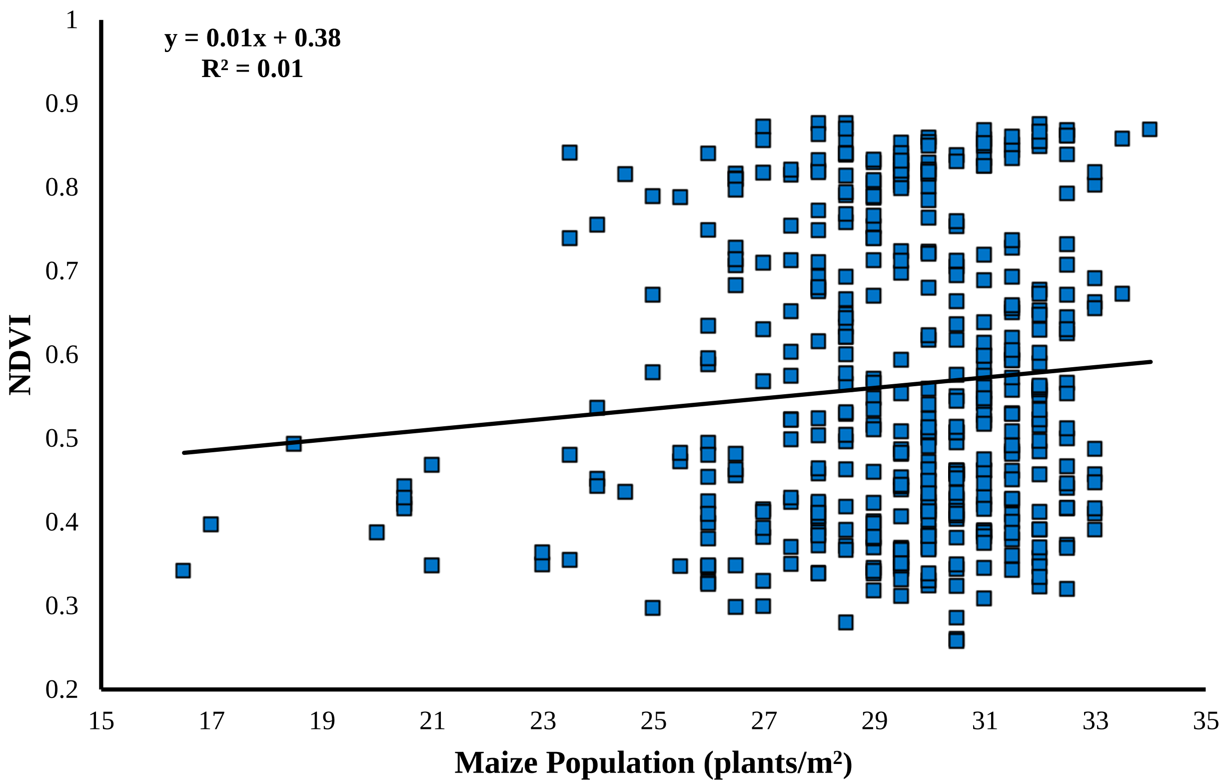

Figure 4.2 The relationship between the plant population of maize and normalized difference vegetative index (NDVI) values at growth stage V6 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

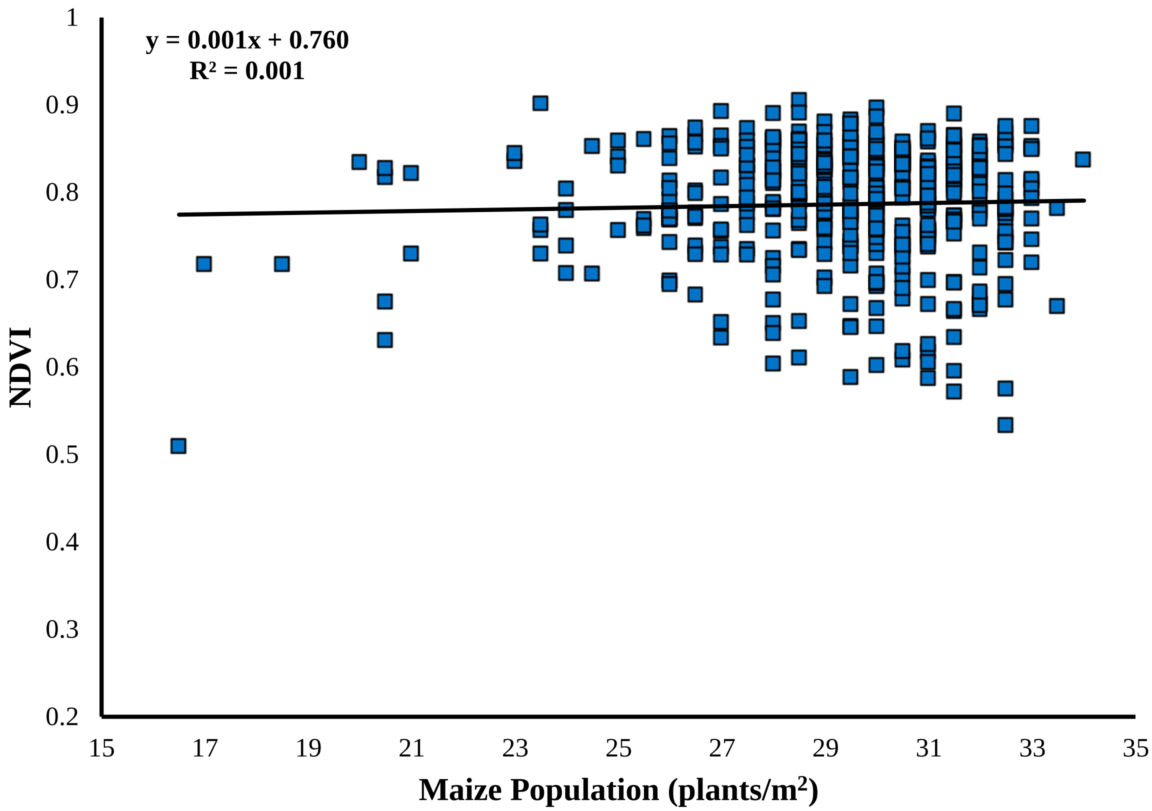

Figure 4.3 The relationship between the plant population of maize and normalized difference vegetative index (NDVI) values at growth stage V8 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

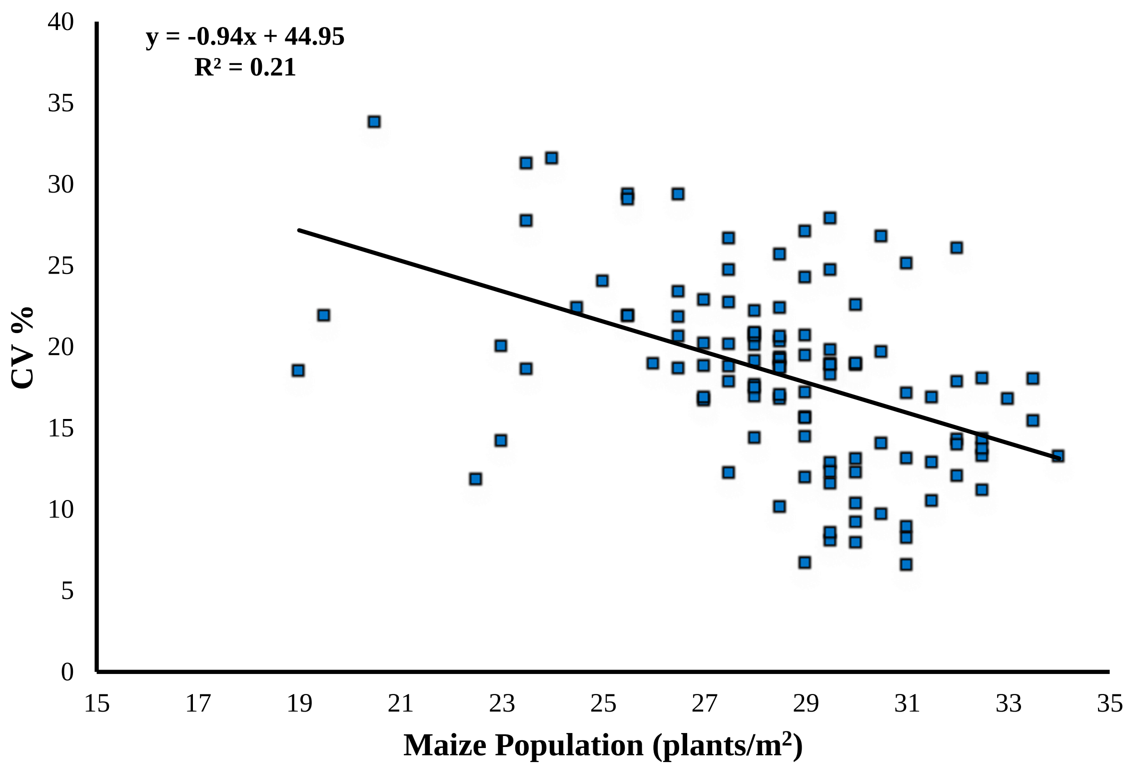

Figure 4.4 The relationship between the plant population of maize and the coefficient of variation (CV) collected from normalized difference vegetative index (NDVI) values at growth stage V4 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

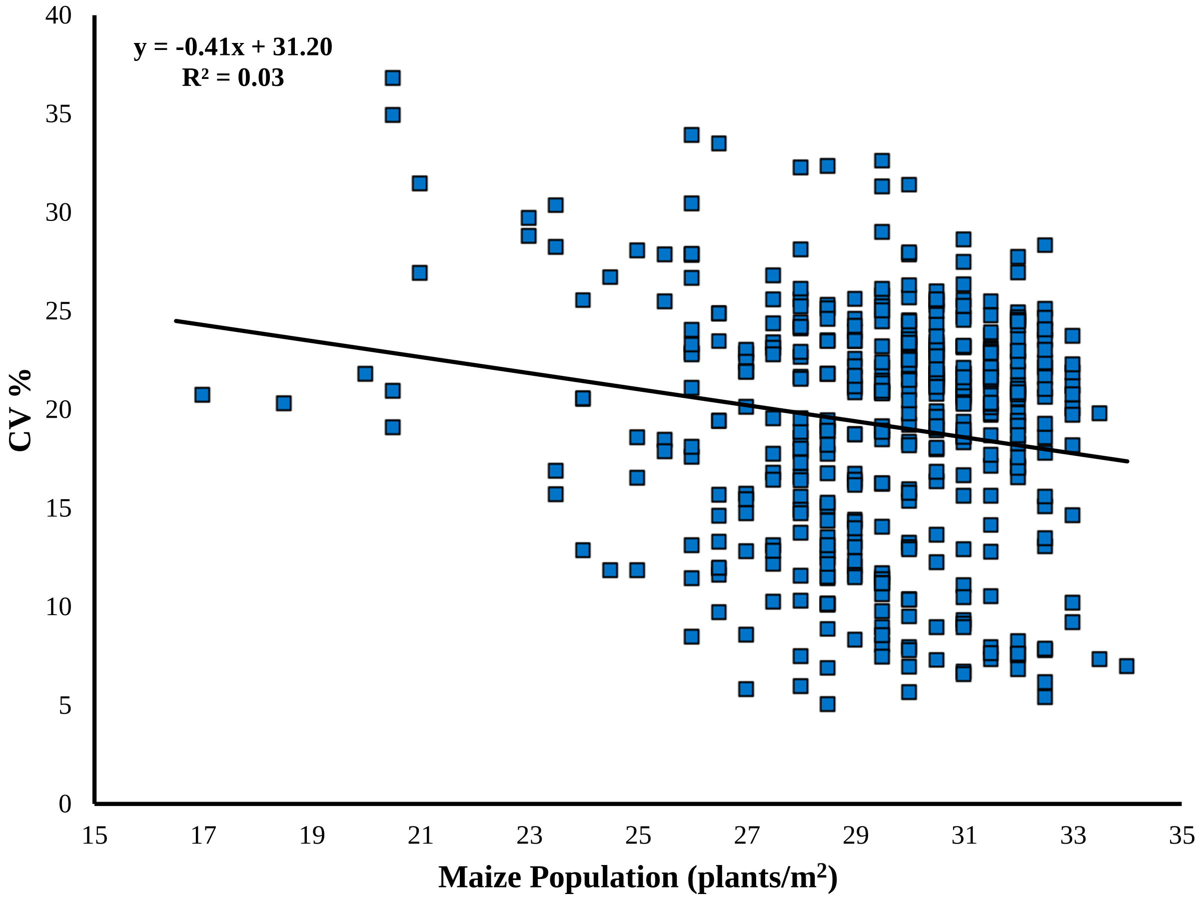

Figure 4.5 The relationship between the plant population of maize and the coefficient of variation (CV) collected from normalized difference vegetative index (NDVI) values at growth stage V6 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

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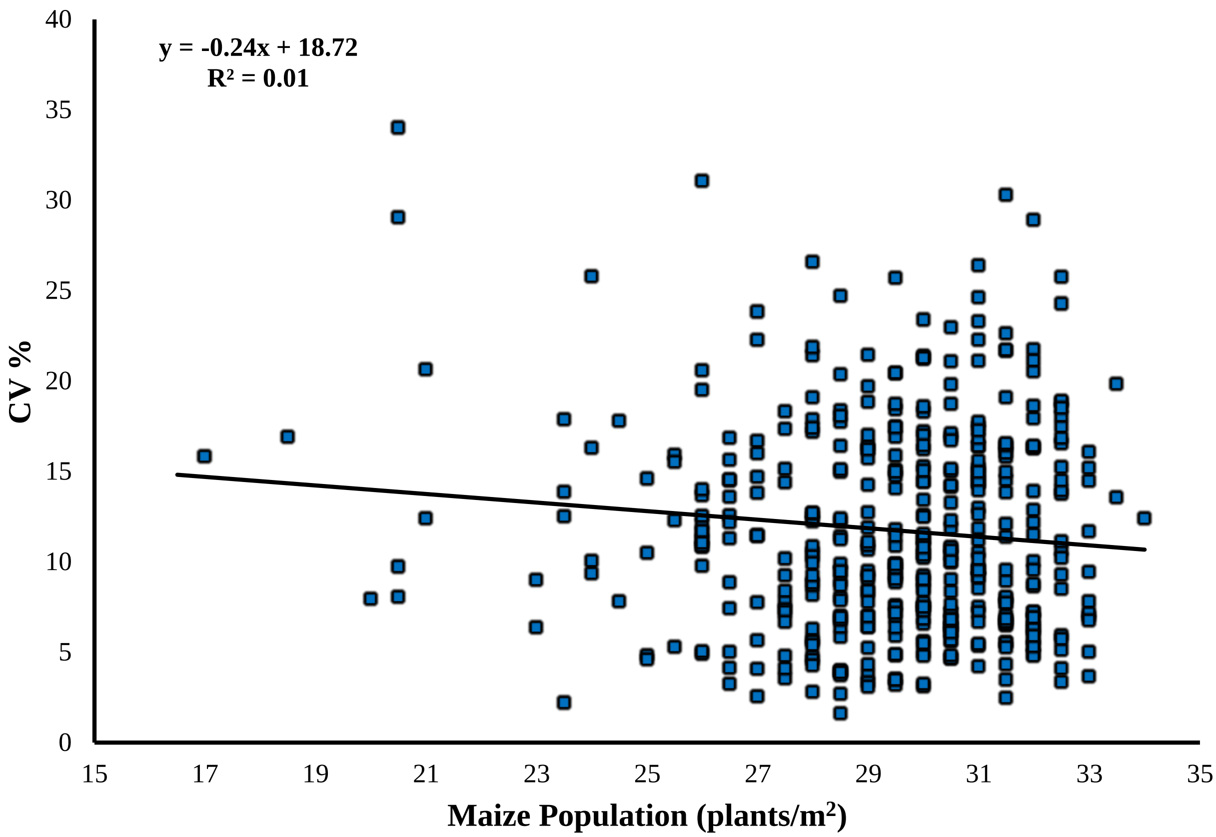

Figure 4.6 The relationship between the plant population of maize and the coefficient of variation (CV) collected from normalized difference vegetative index (NDVI) values at growth stage V8 from trials conducted in the Agronomy Research Station and Lake Carl Blackwell Research Station. NDVI was collected with a GreenSeeker™ 505 handheld sensor, Trimble Inc.

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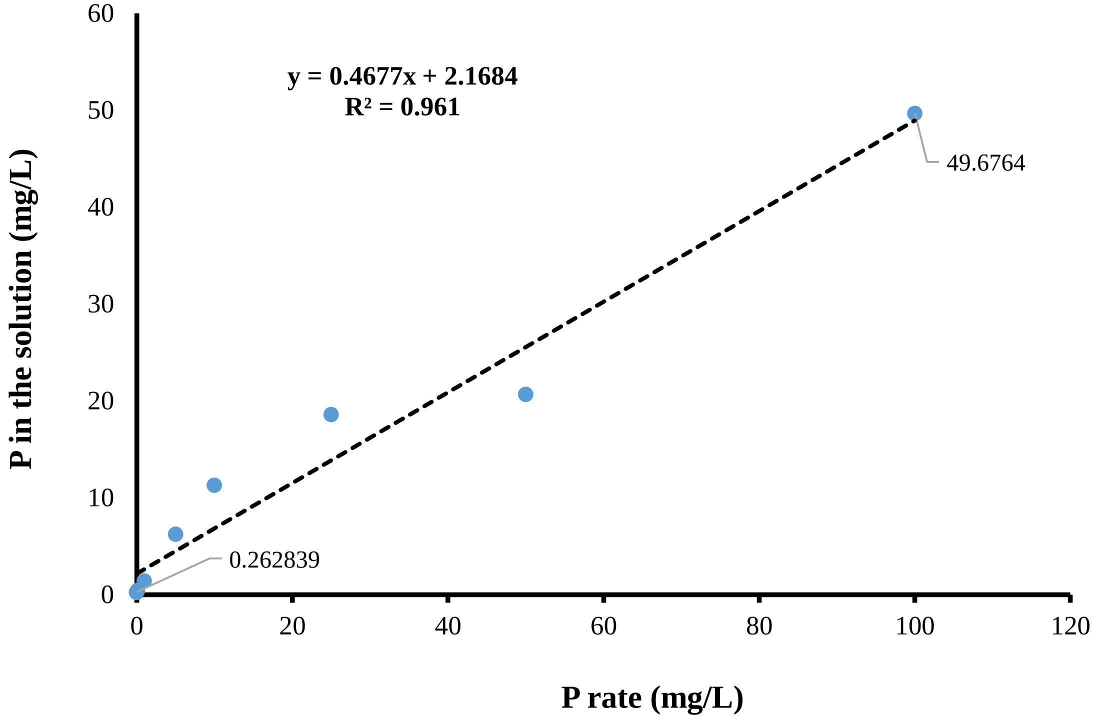
APPENDICES



Appendix 1. Phosphorus adsorption isotherm for coated sand with aluminum chloride ($AlCl_3$) in the greenhouse study.

Treatment	Varieties	P rate (kg ha^{-1})	Depth 0-7 cm P (ppm)	Depth 7-15 cm P (ppm)	Mean P (ppm)
1	OK13625	0	4.6	2.8	3.7
2	OK13625	39	4.7	3.8	4.3
3	O10430-2	0	5.4	3.1	4.2
4	O10430-2	39	6.3	3.5	4.9
5	Endurance	0	4.7	3.2	4.0
6	Endurance	39	6.0	3.1	4.6
7	Spirit Rider	0	6.0	3.8	4.9
8	Spirit Rider	39	6.7	3.4	5.1
9	Duster	0	4.4	3.1	3.7
10	Duster	39	5.5	3.3	4.4
11	Ruby Lee	0	5.4	3.4	4.4
12	Ruby Lee	39	6.2	3.1	4.6
13	Gallagher	0	4.9	2.9	3.9
14	Gallagher	39	5.7	4.4	5

Appendix 2. Postharvest P test results extracted by Mehlich III from stratified samples at two depths in the Cimarron Valley Research Station located near Perkins, Ok.

Treatments	Varieties	P rate (kg ha^{-1})	Depth 0-7 cm P (ppm)	Depth 7-15 cm P (ppm)	Mean P (ppm)
1	OK13625	0	1.8	0.9	1.3
2	OK13625	39	1.9	1	1.4
3	OK10430-2	0	1.3	1.1	1.2
4	OK10430-2	39	1.8	0.9	1.3
5	Endurance	0	1.5	0.9	1.2
6	Endurance	39	1.4	1.1	1.3
7	Spirit Rider	0	1.1	0.7	0.9
8	Spirit Rider	39	4.5	0.9	2.7
9	Duster	0	1.5	0.9	1.2
10	Duster	39	1.9	0.8	1.4
11	Ruby Lee	0	1.6	1	1.3
12	Ruby Lee	39	1.7	1.3	1.5
13	Gallagher	0	2	0.5	1.3
14	Gallagher	39	1.4	0.9	1.2

Appendix 3. Postharvest P test results extracted by Mehlich III from stratified samples at two depths in the N40 Experimental Filed near Stillwater, Ok.

VITA

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Doctor of Philosophy

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