Evaluation of diagnostic tools for potassium management in soybean

by

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Abstract

Three studies were conducted to evaluate soil test methods and tissue analysis as diagnostic tools for potassium (K) in soybean (*Glycine max*). The first study assessed the relationship between K adsorption by cation exchange resins (CER) and K uptake by soybean in field conditions. The study was conducted at two locations with contrasting soil test K levels and two treatments, including a control (0 kg K_2O ha^{-1}) and a high K rate with 168 kg K_2O ha^{-1} applied pre-plant and incorporated. Cation exchange resins were buried in the field in multiple periods to cover the entire soybean reproductive growth stages. In addition, whole plant samples were collected at R2, R4, and R6 stages to measure plant K uptake. Soil volumetric water content and soil temperature were measured using a TEROS 11 sensor. This study found that CER tends to decrease in inverse proportion to plant K uptake, suggesting a measure of soil K surpluses because of root competition. The fertilized plots were able to maintain higher K supply rates during the peak plant demand. Depending on each location, soil temperature and soil moisture content were highly correlated with CER adsorption in control plots. The second study evaluated tissue nutrient concentration and nutrient ratios as predictors of soybean response to K fertilization. It was conducted at eight locations throughout eastern Kansas during 2019 and 2020. Four treatments were selected to evaluate soybean response to K fertilization. Treatments included a control with no K fertilization and rates with 56 kg K_2O ha^{-1} increments until reaching a maximum of 168 kg K_2O ha^{-1}. Aboveground plant samples were collected at V4, R2, R4, and R6 stages to measure plant K and Magnesium (Mg) concentration. K concentration and K/Mg ratio at V4 growth stage were well correlated to K uptake at R6 and grain yield. Considering grain yield, the critical concentration range for K and K/Mg ratio was 16.4 to 18.0 g kg^{-1} and 2.3 to 2.4, respectively. The nutrient ratio was slightly better in predicting K uptake. The third study compared different soil test K (STK) methods and evaluated the correlation to soybean yield and K uptake response in low testing soils. Additionally, the study assessed the effect of sampling moment on STK results for NH_4OAc and Mehlich-3 tests using dry and field moist samples. It was conducted at eight locations throughout eastern Kansas during 2019 and 2020. The treatments were a control with no K fertilization and rates with 56 kg K_2O ha^{-1} increments until reaching a maximum of 168 kg K_2O ha^{-1}. Aboveground plant samples were collected at R6 stage to measure plant K uptake. In general, moist tests were better correlated to K response than dry tests, especially with NH_4OAc. Among all evaluated methods, the $CaCl_2$ dry and moist, NH_4OAc moist, Resin K, and $NaBPh_4$ tests were the best when correlating to relative yield and K uptake. $CaCl_2$ dry is one the easiest and cheapest tests, also having a consistent correlation coefficient (around 0.70 for both variables). Furthermore, it might be an alternative to the NH_4OAc moist test because of the high correlation (r=0.91). Three out of eight locations had STK changes for dry samples regardless of K fertilization between fall and the subsequent spring sampling. However, when considering moist samples, almost all locations had little or no STK change. Overall, the NH_4OAc moist test was one of the best methods to estimate K availability in low testing soils; however, other non-conventional tests like $CaCl_2$ dry might perform similarly but without the typic disadvantages of moist samples.

Abbreviations: K, potassium; CER, cation exchange resins; Mg, Magnesium; STK, soil test potassium;

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Chapter 1 - General Introduction

Introduction

Soybean (*Glycine max*) was planted on 1.6 million hectares in 2020 in Kansas. This area is less than the amount of corn (2.4 million hectares), or wheat (2.6 million hectares) planted that year (USDA, 2020). The estimated soybean yield average for the 2020 season in Kansas was 2755 kilograms per hectare (USDA, 2020).

Potassium (K) is considered an essential nutrient for plant growth and is taken up in large quantities during the soybean life cycle. Most Kansas's soils can supply enough K for crop production, but there are still areas (e.g., eastern Kansas) where it is common to observe K deficiencies during the growing season. The purpose of this research is to evaluate diagnostic tools for K in soybean, including soil test, tissue analysis, and cation exchange resins.

Soil testing is one of the best tools to identify responsive soils to K fertilizer. However, more research is needed to find the K extraction method that better correlates to soybean grain yield across soils and regions. Some soil test methods used to estimate K availability (e.g., 1 M NH_4OAc) are not always good grain yield indicators. Also, tissue analysis has practical applications in assessing possible K deficiencies and predict future fertilizer needs. However, there are still limitations for this method (Stammer and Mallarino, 2018). In case of cation exchange resins, most of the studies focused on greenhouse conditions and more information is needed in order to make recommendations (Barber and Matthews, 1962; Qian et al., 1992, 1996). The use of ion-exchange resins to measure soil nutrient supply has potential applications for fertilizer management. In order to make recommendations, it is imperative to have a strong understanding of sampling moment, burial length, and critical levels.

Thesis Organization

This thesis is divided into five chapters. Following the introduction, there are three chapters, each of them consisting of one research project. Titles of chapter 2, 3, and 4 are: "Assessing in-season potassium supply to soybean using cation exchange resins", "Evaluation of soybean tissue analysis to assess response to potassium fertilizer," and "Evaluation of soil test methods and sampling moment to assess potassium response in soybean". The three chapters are preceded by a general introduction and proceeded by general conclusions (Chapters 1 and 5). Each chapter contains an abstract, introduction, materials and methods, results and discussion, conclusions, and references.

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Chapter 2 - Assessing In-season Potassium Supply to Soybean Using Cation Exchange Resins

Abstract

The use of ion-exchange resins to measure soil nutrient supply has potential applications for fertilizer management. The objective of this study was to evaluate the relationship between potassium (K) adsorption by cation exchange resins (CER) and K uptake by soybean (*Glycine max*) in field conditions. The study was conducted at two locations with contrasting soil test K levels and two treatments, including a control (0 kg K_2O ha^{-1}) and a high K rate with 168 kg K_2O ha^{-1} applied pre-plant and incorporated. The CER resin strip (CMI-7000) was used to assess in-season K supply to soybean. The number, length, and time between burial periods were defined to cover soybean reproductive growth stages. In addition, whole plant samples were collected at R2, R4, and R6 stages to measure plant K uptake. Soil volumetric water content and soil temperature were measured using a TEROS 11 sensor. Plant K uptake was significantly increased by K fertilization in the low soil test K (STK) location. Cation exchange resins were able to adsorb more K (measured as cumulative adsorption) when K fertilizer (150 lbs K_2O $acre^{-1}$) was applied in both locations. There was a relation between CER and plant K uptake, especially in the low STK site. Cation exchange resins values decreased as K uptake increased, suggesting a measure of soil K surpluses due to root competition. The fertilized plots were able to maintain higher K supply rates during the peak plant demand. This was reflected in higher plant K uptake compared to control. Depending on each location, soil temperature and soil moisture content were highly correlated with CER adsorption in control plots. Preliminary results from this study suggest that CER can be used as an indicator of K supply, particularly in soils with low soil test K levels.

Abbreviations: K, potassium; CER, cation exchange resins; STK, soil test K

Introduction

Some soil test methods used to estimate K availability (e.g., 1 mol l^{-1} NH_4OAc) are not always good indicators of K uptake by plants. Compared to soil test methods, ion exchange resins can be used to measure nutrient supply rates during specific adsorption periods. Therefore, soil processes such as nutrient release and transport can be considered. Membranes in cation exchange resins (CER) are negatively charged to adsorb positively-charged ions, like K^+. Since the 1950s, synthetic ion exchange resins have been used for assessing the bioavailable fraction of soil nutrients (Qian and Schoenau, 2002). The first time that ion-exchange resins were introduced in membrane form was in 1964 (Saunders, 1964). Several studies focused on assessing K supply using CER under controlled conditions (Barber and Matthews, 1962; Qian et al., 1992, 1996; Springob and Richter, 1998; Qian and Schoenau, 2002; Askegaard et al., 2005). Conversely, there are few studies conducted in field conditions (Woods et al., 2006; May et al., 2012) and are focused on species like *Agrostis stolonifera* and *Phalaris canariensis* L. Different burial lengths have been selected to evaluate K availability. Exchange membranes could assess the immediate nutrient supply rate by selecting short burial periods (1 hour) (Qian et al., 1996). Also, long periods are used to capture nutrients released from mineral and non-exchangeable forms (Cooperband and Logan, 1994).

Measuring plant K uptake at different growth stages and fertilizer rates allows us to contemplate plant K demand. Several authors have reported plant K uptake response to K fertilization in soybean. Oltmans and Mallarino (2015) observed an increase in K accumulation when fertilizing with K across multiple site years (including responsive and non-responsive sites). Heckman and Kamprath (1995) observed a K uptake response in 2 out of 3 years in low to medium STK soils (66 to 180 mg kg^{-1}). Qian et al. (1992) reported a correlation of 0.54 when canola K uptake was compared to CER values in a growth chamber experiment. This correlation was higher than with 0.5 mol l^{-1} $NaHCO_3$ K extraction method ($r^2 = 0.37$). Qian et al. (1996) also found that the CER method could be highly correlated to plant K uptake and be more precise in predicting plant availability in a wide range of soil types.

Better knowledge of when peak K uptake rate occurs could be useful to improve the assessment of soil K availability. As mentioned before, CER can be used to measure nutrient supply rates during specific adsorption periods. Therefore, it is possible to compare soil K supply rates between treatments at maximum plant K uptake rate. In a review of K accumulation studies, Gaspar et al. (2017) reported that soybean peak K uptake rates ranged from 1.5 to 2.8 kg ha^{-1} d^{-1}. These values occurred around R3 growth stage (Bender et al., 2015). However, Heckman and Kamprath (1995) reported K uptake rates as high as 5.11 kg K ha^{-1} d^{-1} obtained with 271 kg K_2O ha^{-1}. The same authors concluded that different soil moisture conditions between years conditioned K uptake by soybean plants. Qian and Schoenau (1997), working with CER, reported higher K supply rates when increasing soil moisture content. But soil water content is not the only factor affecting K diffusion in the soil. (Schoenau et al., 1993) reported lower nutrient adsorption values as soil temperature decreased. Also, they concluded that both factors impacted the amount of nutrients extracted from the resin strips, but the differences were not always significant.

This technology has potential applications in numerous areas, including agronomic research, because of its potential to represent the plant root activity in undisturbed conditions. However, there are still limitations such as unfamiliarity of units used to express results such as µg 10 cm^2 7 d^{-1} (Qian and Schoenau, 2002), and lack of calibration studies relating values to crop response. Commonly, K management is based on pre-plant soil sampling to assess soil K supply for the entire season. Finding an indicator that considers the kinetics of K release from the soil could be useful to improve future management. The objective of this study was to evaluate whether K adsorbed by CER could be used as an indicator of in-season K supply to soybean (*Glycine max*) in field conditions.

Materials and Methods

Treatments, Experimental Design, and Implementation

Field experiments were conducted at two locations throughout eastern Kansas during 2020. Sites were located near Ottawa, Franklin Co, and Scandia, Republic Co in Kansas. Both were under a conventional tillage crop system; however, the first location was under a rainfed system while the latter received supplemental irrigation. Franklin Co had deficient soil K levels (120 mg kg^{-1} NH_4OAc), based on Kansas state critical value for STK (130 mg kg^{-1}). In contrast, STK levels in Republic Co were considered very high (409 mg kg^{-1}) The experiments were a randomized complete block design, and two treatments and two replicates were selected to evaluate the CER. Treatments included a control with no K application and one with the application of 168 kg K_2O ha^{-1}. Both treatments had a blanket application of 90 kg P_2O_5 ha^{-1}. The fertilizer applications were a surface broadcast at pre-plant using triple superphosphate (TSP) (0-46-0) and potassium chloride (KCl) (0-0-60) as P and K sources. For this study, we used the CMI-7000 CER resin strip from Membranes International Inc. as an indicator of in-season K supply to soybean. This product consists of an exchange resin membrane that was inserted into the soil to measure *in situ* ion supply. Cation exchange resins were buried in the field, and the number, length, and time between burial periods were defined in order to cover soybean reproductive growth stages (R1 to R7). Both locations had a burial length of 7 days for each set of CER. The end of the previous period was the start of the next one; therefore, CERs were present in the soil during the entire measuring period. A total of 4 strips were distributed within the plot to obtain a composite sample. The CER was inserted vertically into the soil (facing a plant row), between 5-10 cm soil depth at 7.5 cm from the soybean row during the sampling season. The CER was buried 12 cm apart from the previous period (parallel to the row) for every new burial period to avoid sampling the same portion of the soil. A 12 cm zip tie was inserted on the top of the resin membrane in order to retrieve it easily from the soil.

Preparation and Extraction of Cation Exchange Resins

Cation exchange resins were cut into 5.5 cm by 1.7 cm strips and immersed in a 5% NaCl solution for 24-48 hours to allow membrane hydration and expansion. This process is needed only with new membranes. The next step was the washing process using a horizontal shaker at 120 rpm. The first step consisted of repeated shaking for 30 minutes with distilled water with sufficient volume to cover the vessel. This procedure was repeated three times. After each shaking period with distilled water, the vessel was rinsed three times before discarding the liquid. After that, membranes were shaken with a 1 mol l^{-1} NaOH solution for 10, 30, and 30 minutes. Every time a different solution was used, CER was washed with plenty of distilled water, and the liquid was discarded. Then, the entire first step was repeated using distilled water instead. Later, 1 mol l^{-1} HCl and 1 mol l^{-1} NH_4Cl solutions were used. Both steps consisted in repeated shaking for 10, 30, and 30 minutes for each solution. The washing process ends after shaking the membranes for 30 minutes with distilled water.

Once the washing process was completed, they were stored in distilled water until the saturation process. In this step, the CER was immersed into 1 mol l^{-1} $NaHCO_3$ solution (pH adjusted to 8.5) and shaken for 10, 30, and 120 minutes at 120 rpm to allow for membrane saturation with Na^+ ions. No rinsing was needed between the three saturation periods. This process was done 24 hours before each burial date. Cation exchange resin strips were reused during the experiment when needed. It was necessary to wash and saturate the CER that was buried in previous periods in order to use it again.

After recovering the CER strip from the field, the zip tie was removed, and the membrane was cleaned with distilled water using a soft brush. A container with lid was prepared with 40 ml of 0.8 mol l^{-1} NH_4Cl in 0.2 mol l^{-1} HCl. A membrane strip was left to stand for 90 minutes with the lid open. Later, the container was closed and shaken for 60 minutes on a horizontal shaker at 120 rpm, ensuring constant movement with the CER. After that, the membrane was removed using a clamp and K^+ in the solution was determined by inductively coupled plasma (ICP) spectrometry. The result was expressed in µg 10 cm^{-2} 7 d^{-1} as a K supply unit.

Sampling and Analysis

Composite soil samples were taken at pre-plant (one per replicate) at the 0-15 cm depth dividing each into two sub-samples. The first group was oven-dried at 40 °C, and ground to pass through a 2 mm screen. The second one was kept in a refrigerator at 3 °C at field moisture until further analysis. All dry samples were analyzed for soil pH (soil:deionized water; 1:1) (Watson and Brown, 1998), organic matter (OM) by loss on ignition (Combs and Nathan, 1998), extractable P and K with the Mehlich-3 extraction (Frank et al., 1998), exchangeable cations (1 M NH_4OAc pH 7.0, Flame Atomic Absorption) (Warncke and Brown, 1998), cation exchange capacity (CEC) with the displacement method (Chapman and Pratt, 1962), and soil texture (hydrometer method) (Gee and Bauder, 1986). In addition, exchangeable K (1 M NH_4OAc pH 7.0) was analyzed in field-moist samples.

Aboveground plant samples were collected at the R2, R4, and R6 growth stages to measure plant K uptake. Stages of soybean development are based on Fehr and Caviness (1977) publication. Plant samples were dried at 60°C, ground to pass through a 2 mm screen, weighed, and digested by nitric-perchloric acid digestion (Gieseking et al., 1935). The total K concentration of the extractant was determined by ICP spectrometry.

Soil volumetric water content and temperature were measured using a sensor from METER Group, Inc. Annual and historical weather data were collected from an automated weather station located < 1 km from the field sites (Kansas Mesonet, 2021). Rainfall and temperature data were used from May to November for both locations, covering the entire soybean growing season.

Statistical Analysis

Statistical analysis (ANOVA) was performed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, 2013). Blocks were included as a random effect in the model; alpha = 0.05 was set as the statistical significance level. The NLIN procedure in SAS 9.4 was used to fit the logistic growth model for plant K uptake, which is also known as the Verhulst growth model (Bacaër, 2011). The same SAS procedure was used for quadratic models as a predictor of potassium adsorption by cation exchange resin (CER-K). For correlation plots (Pearson's coefficient), the psych package was used in R version 3.6.1 (R Core Team (2020).

Results and Discussions

Climate and Soil Data

The average monthly rainfall and temperature from 1981 to 2010 and observed monthly rainfall and temperature during the experimental period are shown in Figure 2-1. For most of the crop season, there was less rainfall than normal in both locations, except in July, with near-normal or above-normal precipitation. Franklin Co was more dependent on precipitation than Republic Co due to its lack of irrigation. Overall, the air temperature was below normal in both locations, apart from June and November 2021.

There are two main differences regarding soil properties when comparing locations (Table 2-1 and 2-2). Firstly, Franklin Co has lower exchangeable K than Republic Co. The latter has 3.4 and 9.1 times higher STK values for dry and field-moist samples, respectively. Secondly, soil test P (STP) values are more than two times higher in Franklin than in Republic Co. Apart from the main differences mentioned before, Franklin Co has slightly higher CEC and more clay. However, soil properties like OM and pH are nearly the same in both locations.

The soil water content and temperature evolution during the soil measurement period in both locations are shown in Figure 2-2. Generally, soil water content and temperature have an important effect on K availability by impacting nutrient diffusion. The period from August to September experienced less precipitation than the 30-year average in Republic and Franklin (Figure 2-1). This caused the soil water content to decrease below 0.2 m^3 m^{-3} in Franklin Co. In contrast, Republic Co had various irrigation events in addition to precipitation, maintaining a higher soil water content throughout the season (Figure 2-2). Therefore, each burial period had a slightly different soil condition regarding soil water content and temperature.

Potassium Uptake and CER–K Cumulative Adsorption

In Franklin Co (STK = 120 mg kg^{-1}), at all reproductive stages (R2, R4, and R6), soybean grown with K fertilization resulted in greater plant K uptake than without fertilization. This location had significantly higher plant K uptake measured at R2 and R4 ($p < 0.05$), but not at R6 growth stage ($p < 0.15$) when 168 kg K_2O ha^{-1} was applied. In contrast, Republic Co (STK = 409 mg kg^{-1}) had higher plant K uptake at certain stages, but differences were not statistically significant (Figure 2-3). The inconsistent result in Republic was likely due to high soil K levels. Based on Kansas State University recommendations (Leikam et al., 2003), these locations had soil K levels that were above the critical level of 130 mg kg^{-1}, and no K fertilizer was needed (Table 2-1). The soybean K uptake response in both locations coincides with previously published results (Oltmans and Mallarino, 2015). In both cases, CER was able to adsorb more K (measured as cumulative adsorption) at a high K rate. However, the potassium adsorption rate by the CER was nearly the same between treatments from R2 to R6 in high STK sites. This suggests that a big part of the K adsorption difference occurred on the early stages (before R2). On the other hand, potassium adsorption by cation exchange resin (CER-K) at Franklin maintained higher in fertilized plots up to the R6 stage. At this stage, 86% more K uptake and 130% more cumulative CER-K was observed compared to the control in Franklin. The higher STK location (Republic Co) had -7% and 13% for K uptake and CER-K, respectively.

Potassium Uptake Rate and Cation Exchange Resin K

Potassium uptake, K uptake rate, and CER-K was compared for contrasting STK locations. As discussed earlier (Figure 2-3), there was a significant treatment effect in Franklin Co when looking at plant K uptake. The model shows how fertilized plots began to accumulate more K early in the season (Figure 2-4), as reported previously by (Heckman and Kamprath, 1995). Moreover, plots without K fertilization had around 60% relative K uptake at maturity compared to fertilization. In contrast, Republic Co showed no difference between treatments, likely due to the already high STK levels (409 mg kg^{-1}). Additionally, the difference in total uptake between both locations is clear, having Republic Co almost double the amount compared to Franklin Co (240 and 130 kg K ha^{-1}, respectively).

Comparing CER-K and K uptake rate, there was a trend, especially in the low STK site (Figure 2-5). First, the CER sensibility to capture a difference in K adsorption seems to be higher at the beginning of the measurements. Thus, the gap between fertilized and control was larger in the first burial periods. Second, the quadratic models resulting from regressing CER-K against days after planting ($R^2 = 0.94$ and $R^2 = 0.88$) suggest competition from plant roots, soil K fixation, or both. In this scenario, resin membranes are likely measuring the balance between soil K supply and plant K uptake, therefore, yielding a measure of K surpluses (Huang and Schoenau, 1997). The minimum supply rate for Franklin was 38 µg K $10cm^2$ $7d^{-1}$ in plots fertilized with 168 kg K_2O ha^{-1} compared to 18 µg K $10cm^2$ $7d^{-1}$ in the control. These lower values occurred between R2 to R4 growth stages, corresponding to the period of maximum plant K uptake rate in both treatments. Thus, not only did fertilized plots have a higher plant K uptake rate, but they were also able to maintain more CER-K throughout the experiment. As competition from plant roots reduces close to maturity (K uptake rate decreased), the gap in CER-K between treatments became larger, but not to the same extent compared to the first burial period.

Little difference was observed in the number of days after planting at which maximum K uptake rate occurred (3 days), based on the fitted model for Franklin Co. The same was observed in Republic Co when fertilized plants reached their maximum K uptake rate just 3 days before the control (50 and 53 days after planting, respectively). In this location, the quadratic model for CER-K under fertilization had a similar coefficient of determination compared to Franklin Co (R^2=0.88), having larger values at the beginning and the end of the measurements. In contrast, not a clear pattern was observed in the control treatment, resulting in scattered points and a low coefficient of determination ($R^2 = 0.13$). This high STK site had a similar cumulative K uptake curve in both treatments (Figure 2-4); therefore, the resulting K uptake rate curve had small differences. The fertilized plants seemed to have higher K uptake rates and an earlier maximum rate (3 days). However, considering that nutrient rates are still very high for the control, the differences in both curves are negligible.

Potassium adsorption by cation exchange resin was influenced by soil water content and temperature, or the combination of both depending on each location. At Franklin Co, soil temperature was the only factor that was highly and positively correlated to CER-K only in control plots ($r = 0.62$, $p < 0.08$) (Figure 2-6). Similar results were reported by Qian and Schoenau (1997). Neither soil water nor water by temperature interaction had significant relationships in both treatments ($p < 0.10$). Even though Republic Co had a high and positive correlation coefficient between soil temperature and CER-K in control plots ($r = 0.56$, $p < 0.09$), the water by temperature correlation coefficient was higher in this treatment ($r = 0.60$, $p < 0.06$). Potassium K diffusion is considered dependent on soil moisture and temperature (Schoenau et al., 1993). Since soil water content was at levels to sustain plant growth during most of the growing season, moisture might not be a significant limiting factor for K diffusion in this study. Soil temperature impacted CER-K regardless of K fertilization; however, it was only statistically significant in control plots ($p < 0.10$). Potassium adsorption by cation exchange resin in unfertilized plots was more dependent on soil conditions. When looking at fertilized plots, CER-K shows no statistical correlation with soil water content and temperature ($p < 0.10$).

Conclusions

In general, CER strips buried in field conditions provided detailed information about soil K supply. Since STK levels were high at Republic Co (409 mg kg^{-1}), relative CER values between fertilized and control treatments become negligible. Potassium supply rates observed in these conditions seem high enough to allow soybean growth without nutrient limitation. In contrast, CER becomes useful in low STK soil because they capture the difference in supply rate between treatments, and their values are highly related to K uptake rate by the plants. By allowing the CER to compete with plant roots, we could indirectly estimate K supply rates. Not all the available K in the soil solution will get adsorbed by the membrane since the plants are competing for the same nutrient. In this condition, CER is probably measuring soil K surpluses. This was clearly observed at Franklin Co, suggesting that it could be used as an indicator of K supply to soybean during the growing season, but further research is needed to confirm these findings.

Not only STK is important when looking at K supply rates. Results of this study show that control plots were significantly affected by soil conditions, especially soil temperature. Soil water content alone did not explain the difference in CER adsorption rates, but the interaction between water and temperature correlated better. Also, soil temperature had a significant impact on CER adsorption regardless of K fertilization.

This method needs to be calibrated to understand its performance under a wide range of soils. Looking at Franklin Co, it could be possible to extrapolate these results to other low STK soils, but validation is needed.

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Table 2-1. Selected soil properties for the 0-15 cm sampling depth.

Location	pH	OM	P_{M3}	$K_{AA\text{-}dry}$	$K_{AA\text{-}fm}$	Ca_{ex}	Mg_{ex}	Na_{ex}
		g kg^{-1}	------------------------		mg kg^{-1}		------------------------	
Franklin	6.5	32	11	120	50	2659	368	54
Republic	6.2	33	4	409	455	2031	333	27

M3: Mehlich-3 extraction, AA: Ammonium acetate extraction, fm: field-moist, ex: exchangeable

Table 2-2. Soil classification and clay characterization for the 0-15 cm sampling depth.

	Soil classification					
Location	Series	Subgroup	Sand	Silt	Clay	CEC
			---------------	%	-------------	cmol^{+} kg^{-1}
Franklin Co	Woodson	Abruptic Argiaquolls	10	64	26	23.0
Republic Co	Crete	Udertic Argiustolls	14	64	22	20.8

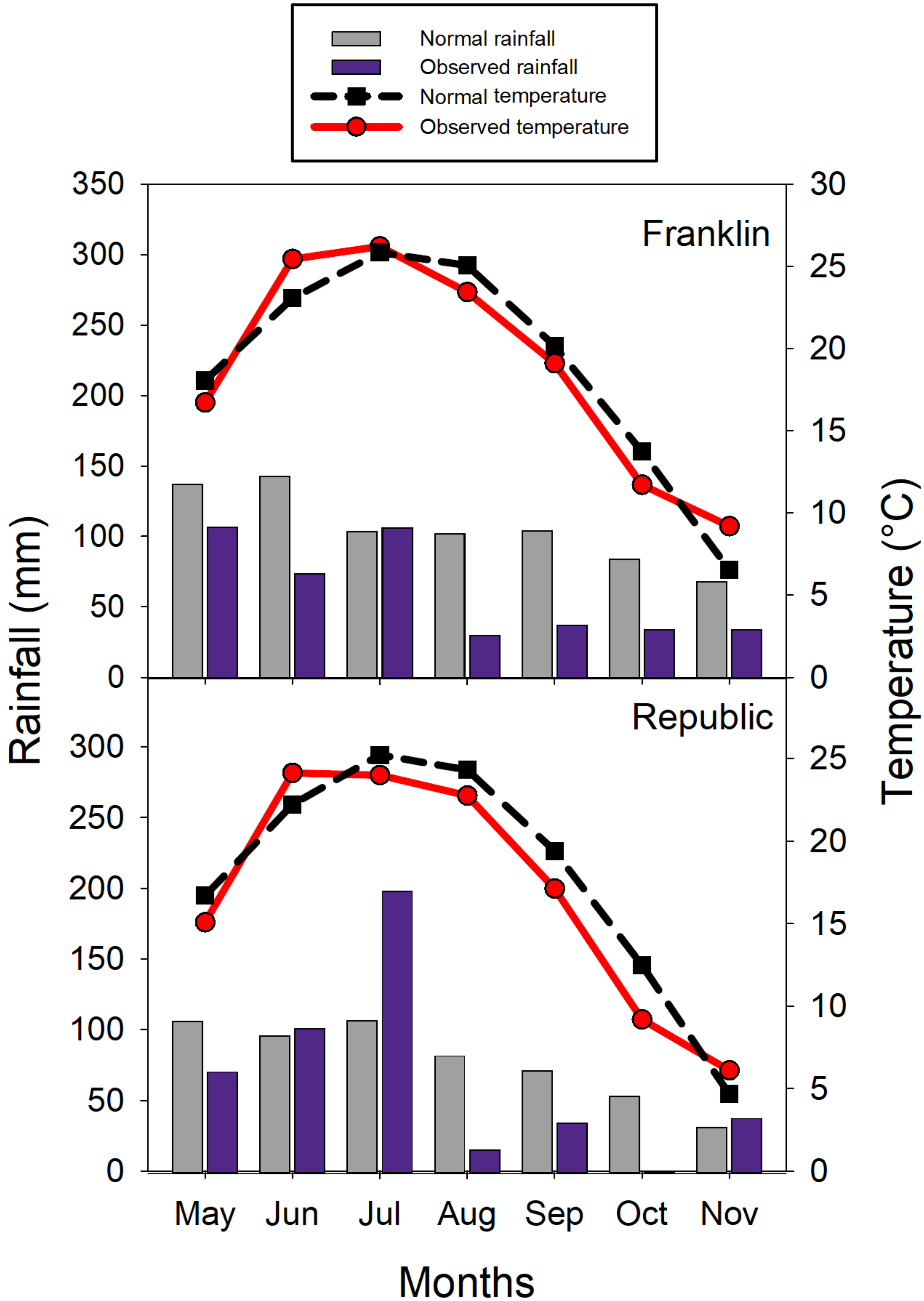

Figure 2-1. Average monthly rainfall and temperature (1981-2010) and observed monthly rainfall and temperature during the experimental period.

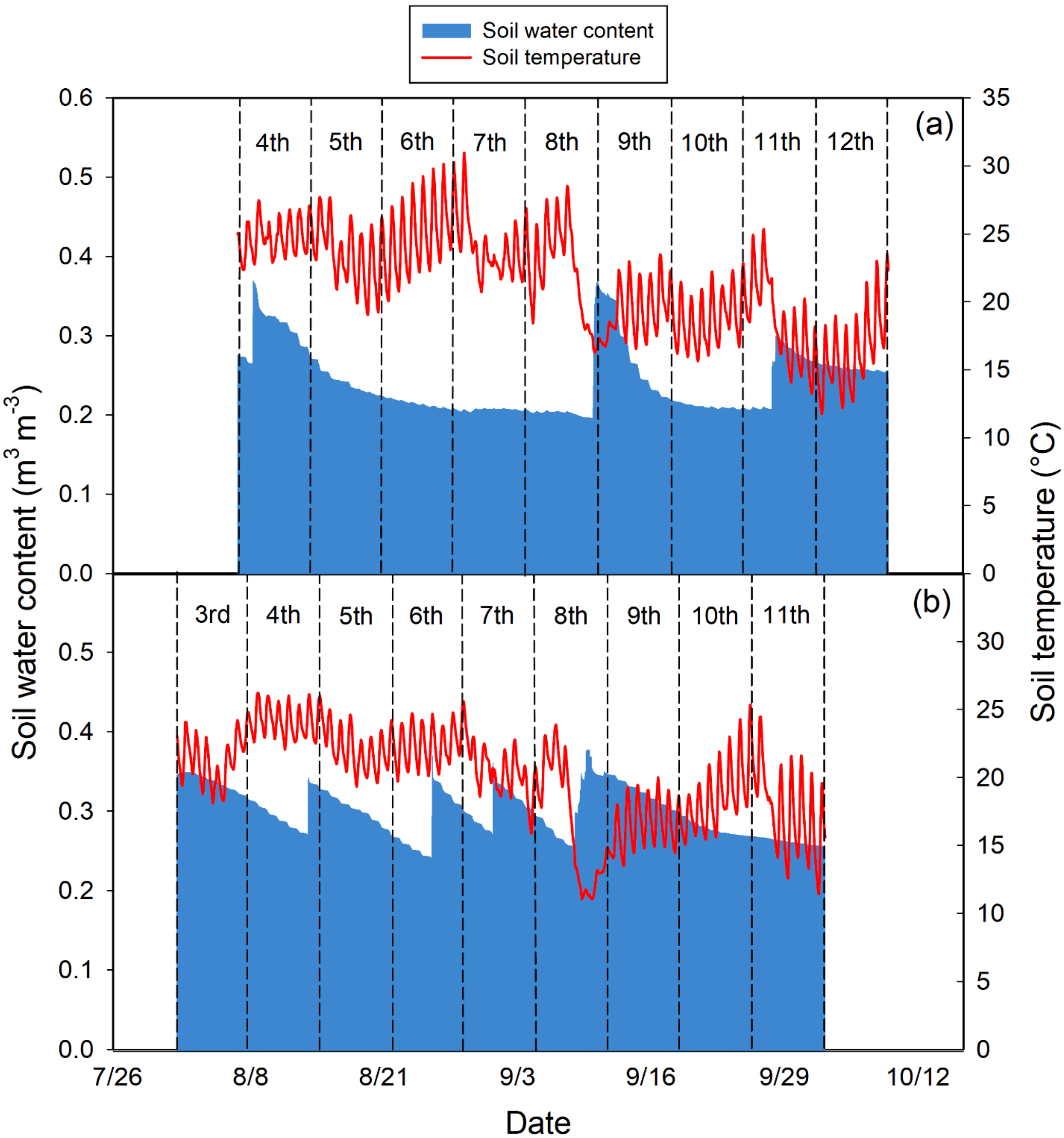

Figure 2-2. Soil water content and temperature evolution during the experimental period in Franklin (a) and Republic (b). Cation exchange resins (CER) burial periods are delimited by vertical dashed lines.

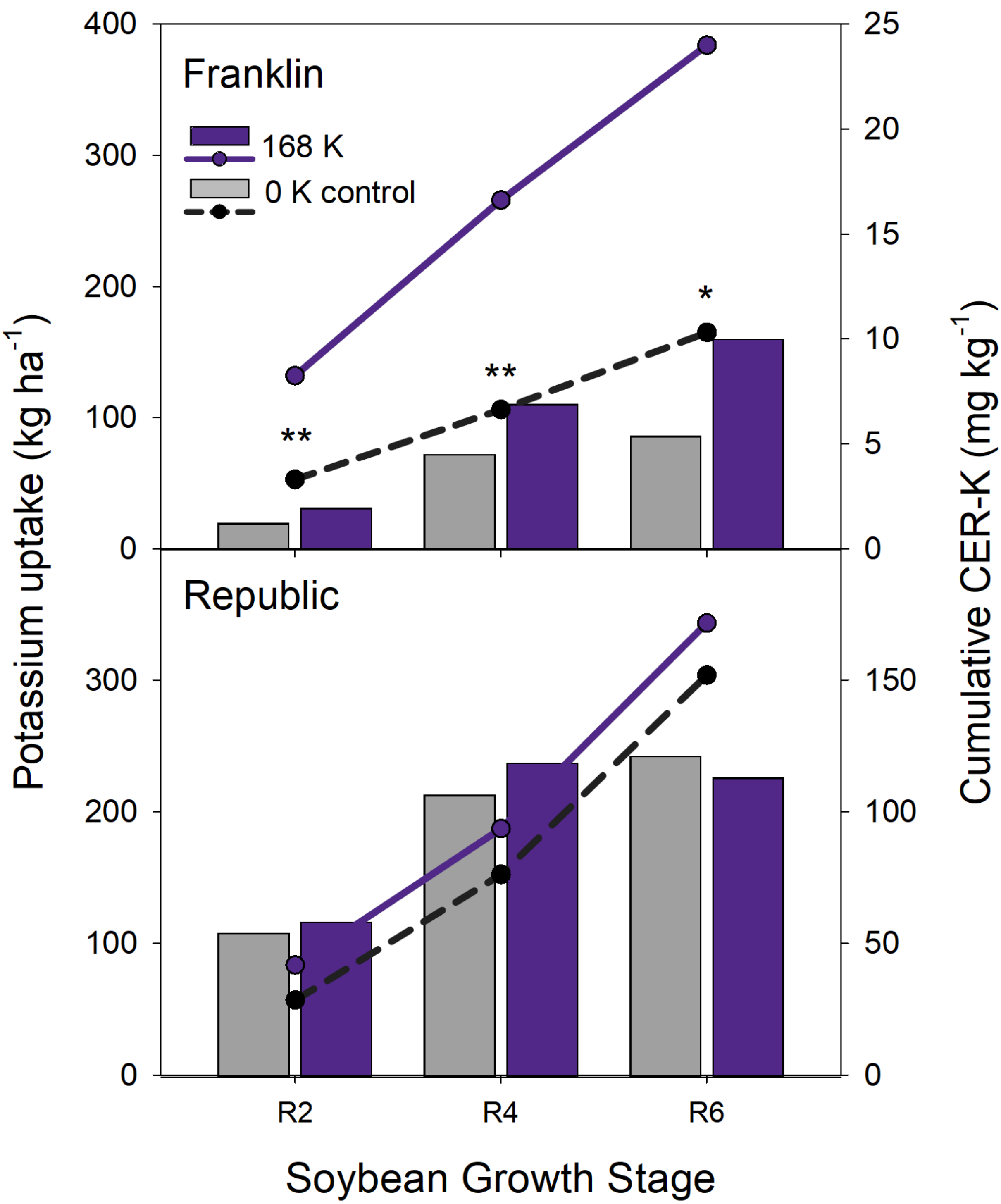

Figure 2-3. Potassium uptake (bars) and cumulative cation exchange resin potassium (CER-K) adsorption (lines) as affected by two levels of K application. Pairwise comparisons of K fertilizer application rate within each stage are indicated by "**" when both uptake and CER-K are statistically significant at $p<0.05$, and by "*" when only CER-K is statistically significant at $p<0.05$.

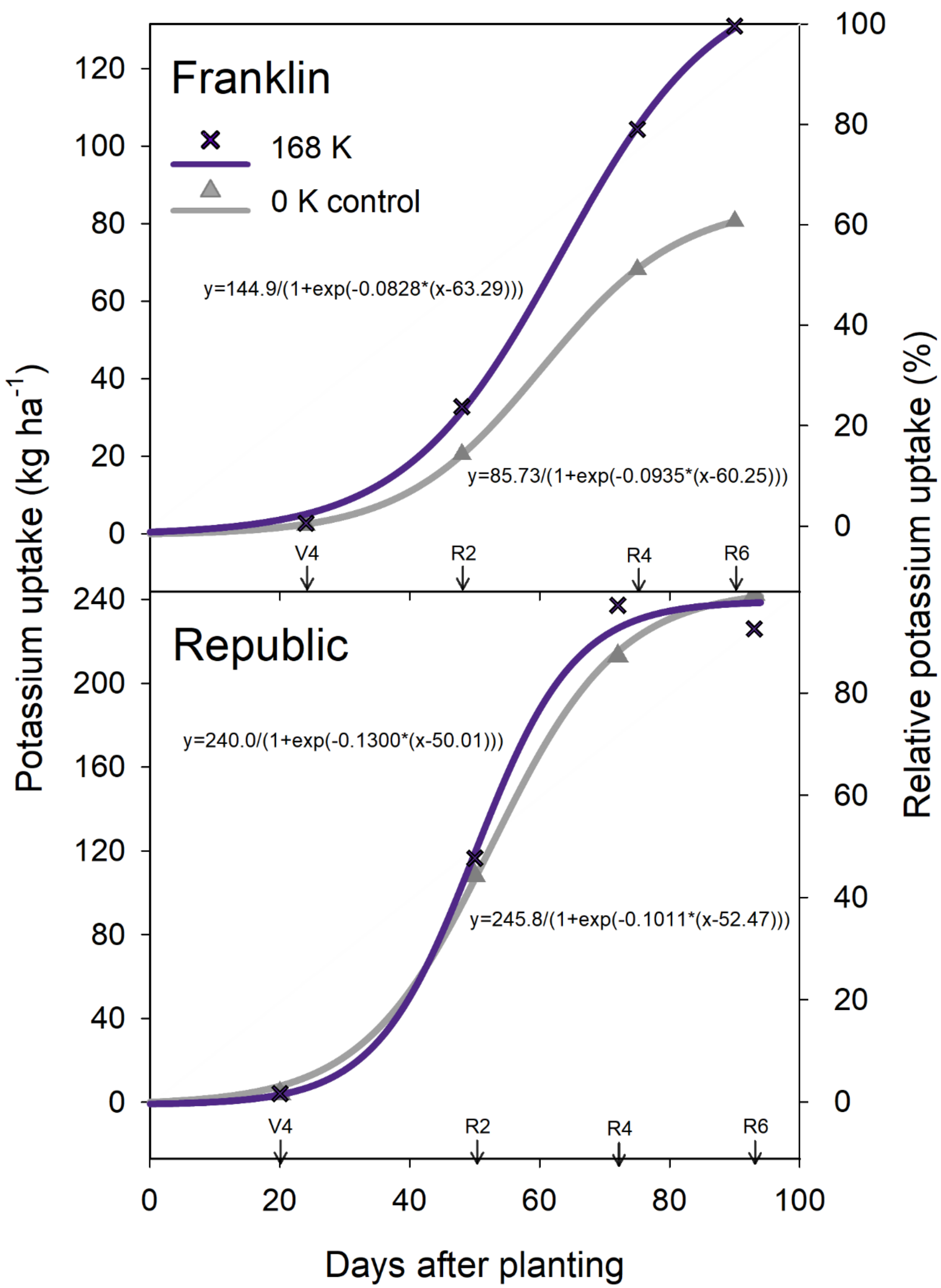

Figure 2-4. Potassium uptake at V4, R2, R4, and R6 growth stages (symbols) and regression curves (solid lines) as affected by two levels of K application. Plant K uptake in fertilized plots at maturity represents maximum relative uptake.

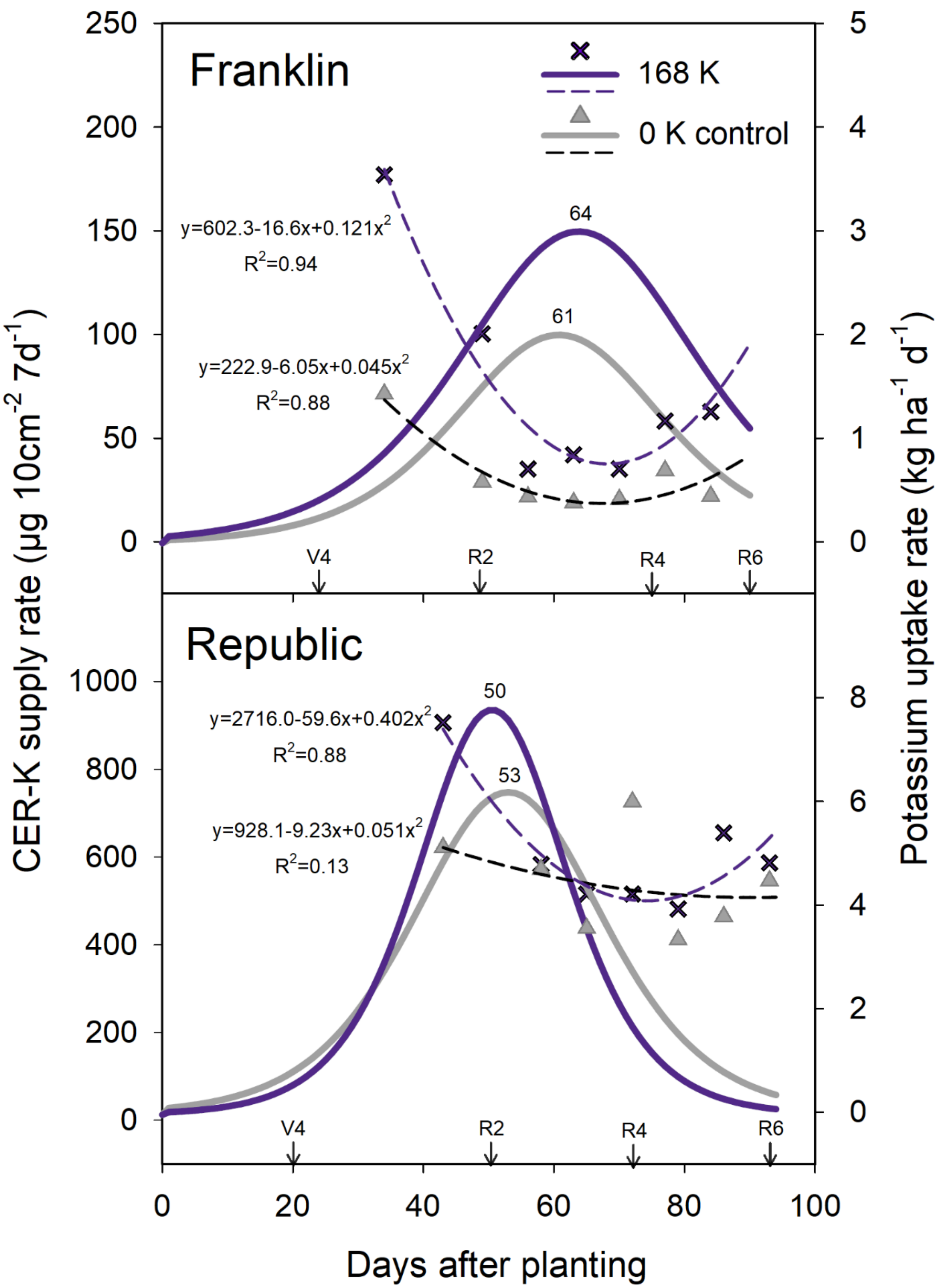

Figure 2-5. Cation exchange resin potassium supply rate (symbols) and potassium uptake rate (solid lines) as affected by two levels of K application. Dash lines represent regression curves with equations for K supply rate models. Values above uptake rate curve indicate the number of days after planting at which maximum K uptake rate was observed.

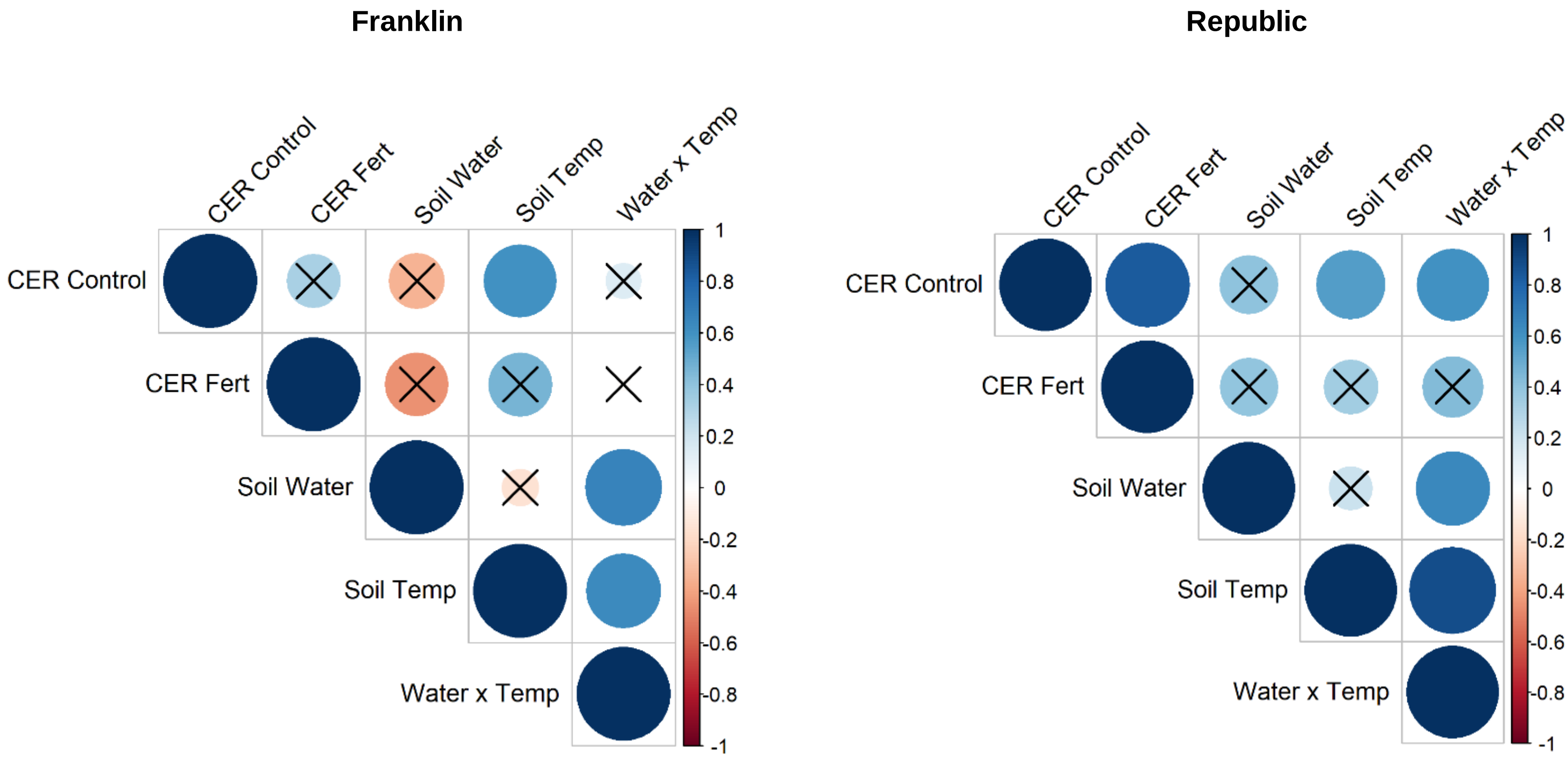

Figure 2-6. Correlation between potassium adsorption by cation exchange resin and soil variables (p<0.10). Darker colors indicate a higher (positive or negative) correlation coefficient; non-significant correlations are indicated by an “X”.

Chapter 3 - Evaluation of Soybean Tissue Analysis to Assess Response to Potassium Fertilizer

Abstract

Practical application of tissue analysis at early stages could help assess possible deficiencies and predict future fertilizer needs. The objectives of this study were to i) determine critical plant potassium (K) concentration for soybean at early stages and ii) identify critical levels using plant K uptake and grain yield. The study was conducted at eight locations throughout eastern Kansas during 2019 and 2020. Four treatments were selected to evaluate soybean response to K fertilization. Treatments included a control with no K fertilization and rates with 56 kg K_2O ha^{-1} increments until reaching a maximum of 168 kg K_2O ha^{-1}. Aboveground plant samples were collected at the V4, R2, R4, and R6 stages to measure plant K and Magnesium (Mg) concentration. Potassium concentration and K/Mg ratio at the V4 growth stage correlated well to K uptake at R6 and grain yield. Considering grain yield, the critical concentration range for K and K/Mg ratio was 16.4 to 18.0 g kg^{-1} and 2.3 to 2.4, respectively. The nutrient ratio was slightly better in predicting K uptake. Preliminary results from this study suggest that tissue analysis at early stages can be used as a diagnostic tool to assess the K status of the soybean crop. A new reference for K concentration was presented along with a K/Mg ratio that could be useful when sampling flexibility is needed.

Abbreviations: K, potassium; Mg, Magnesium.

Introduction

Plant tissue analysis has been used as a diagnostic tool in agriculture for a long time (Jones, 1967). However, there are still limitations such as insufficient research related to nutrient concentration and yield response, the moment of diagnosis when corrective management might be late, and worse or similar results compared to soil testing (Stammer and Mallarino, 2018). Sample contamination and deterioration could be additional limitations to tissue testing. On the other hand, sometimes soil levels are in the optimum range, but the plants are unable to absorb them for different reasons. Therefore, tissue testing might complement soil testing to improve nutrient management. Critical level or sufficiency level concepts have been used for decades to assess the nutritional status of plants. (Jones Jr. et al., 1990) define critical level as "that concentration below which yields decrease, or deficiency symptoms appear." For nutrients like K, yield typically decreases well before the appearance of deficiency symptoms. It is expected to observe a yield reduction below the critical level (deficient range), whereas the plateau represents the sufficiency range.

Previous studies reported critical levels for different nutrients in agriculture production, but there is scarce information regarding K on soybeans. Among other factors, a critical level will depend on the plant growth stage, and plant part sampled (Parvej et al., 2016). Most of the studies focused on K (Vitosh et al., 1995; Yin and Vyn, 2004; Fernández and Hoeft, 2009; Mills and Bryson, 2015; Parvej et al., 2016) considered soybean leaves and petioles in reproductive stages. Recent studies working with whole vegetative plants at early stages (V4-V5) had inconsistent results. (Clover and Mallarino, 2013) found a poor correlation between whole plant K concentration at early stages and grain yield. However, in a recent study, (Stammer and Mallarino, 2018) determined critical K concentration ranges by sampling whole plants at V4-V5. The obtained range was 18.9 to 22.6 g kg^{-1} of K with an R^2 of 0.35. Despite a low coefficient of determination, it is a good reference for this specific sampling time. There is no reference about critical levels considering the nutrient rations (such as K/Mg ratios) on soybean. As Ranade-Malvi (2011) reported, there is an interaction between K and Mg (K-Mg antagonism). When soil K availability is low, it is expected to have higher Mg absorption and, therefore, a lower K/Mg ratio. When analyzing both nutrients, this interaction could be considered and might be an advantage over the traditional approach using only the K concentration. Considering both nutrients could also be helpful when avoiding sampling error because of nutrient dilution effect at different growth stages. The objectives of this study were to i) determine critical plant K concentration for soybean at early growth stages, and ii) identify critical tissue K levels using plant K uptake and grain yield response.

Materials and Methods

Treatments, Experimental Design, and Implementation

Field experiments were conducted at eight locations throughout eastern Kansas during 2019 and 2020. Sites were located near Ottawa (Franklin Co), Parsons (Labette Co), Scandia (Republic Co), Ashland Bottoms (Riley Co), and Wetmore (Nemaha Co) in Kansas. All of them were under a conventional tillage crop system; however, the Scandia location was the only one under supplemental irrigation. Soybean was planted during the first weeks of June, with maturity groups ranging from 3.9 to 4.6. All experiments were planted with a row spacing of 76 cm, except for Nemaha Co (38 cm). Three sites had very high soil K levels (STK > 324 mg kg^{-1} NH_4OAc), while the remaining five locations were below the current critical value for Kansas (STK < 130 mg kg^{-1} NH_4OAc). The experiments were a randomized complete block design, and four treatments were selected to evaluate soybean response to K fertilization. Treatments included a control with no K fertilization and 56, 112, 168 kg K_2O ha^{-1}. Two additional treatments were considered in locations with low soil test phosphorus (STP) levels (e.g., Republic Co). They consisted of control with no K fertilization and 168 kg K_2O ha^{-1} but with a blanket application of 90 kg P_2O_5 ha^{-1}. The fertilizer applications were a surface broadcast at pre-plant using triple superphosphate (TSP) (0-46-0) and potassium chloride (KCl) (0-0-60) as P and K sources.

Sampling and Analysis

Composite soil samples were taken at pre-plant (one per replicate) at the 0-15 cm depth dividing each into two sub-samples. The first group was oven-dried at 40 °C, and ground to pass through a 2 mm screen. The second one was kept in a refrigerator at 3 °C at field moisture until further analysis. All dry samples were analyzed for soil pH (soil:deionized water; 1:1) (Watson and Brown, 1998), organic matter (OM) by loss on ignition (Combs and Nathan, 1998), extractable P and K with the Mehlich-3 extraction (Frank et al., 1998), exchangeable cations (1 M NH_4OAc pH 7.0, Flame Atomic Absorption) (Warncke and Brown, 1998), cation exchange capacity (CEC) with the displacement method (Chapman and Pratt, 1962), and soil texture (hydrometer method) (Gee and Bauder, 1986). In addition, exchangeable K (1 M NH_4OAc pH 7.0) was analyzed in field-moist samples.

Aboveground whole plant samples were collected at the V4, R2, R4, and R6 growth stages in order to measure plant K and Mg concentrations (6 plants per plot). Stages of soybean development are based on Fehr and Caviness (1977) publication. The samples were dried at 60°C, ground to pass through a 2 mm screen, weighed, and digested by nitric-perchloric acid digestion. Total K and Mg concentration was determined by inductively coupled plasma (ICP) spectrometry. Grain was harvested from the center rows (11.3-m length) with a plot combine. Yield was corrected at 13% moisture. A grain sub-sample was taken from each plot and analyzed for K concentration using the sulfuric acid/peroxide procedure (Matsunaga and Shiozaki, 1989).

Relative yield and relative K uptake were obtained, dividing each plot value by the maximum value for that location.

Statistical analysis

Statistical analysis (ANOVA) was performed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, 2013). Blocks were included as a random effect in the model, and alpha was set to 0.05 for the statistical significance level. Critical nutrient concentrations were obtained by using the NLIN procedure in SAS. Linear-plateau and quadratic-plateau response models were selected for this purpose. For correlation plots, the psych package was used in R version 3.6.1 (R Core Team, 2019).

Results and Discussion

Table 3-1 shows selected soil properties for the 0-15 cm sampling depth for each location. Almost all locations had similar contents of sand and clay, except for Riley Co, with more sand (30%) and less clay (18%). When looking at the eight locations, soil pH ranged from acidic to neutral (5.7 to 7.7), and OM varied from 24 to 36 g kg^{-1}. It is clear how Republic and Riley counties (locations 1 to 3) are very high in K regardless of soil test method. Conversely, Labette Co (locations 7 and 8) is very low in STK, especially when using the field-moist method. Phosphorus levels were very low at Republic and Labette counties (3 to 5 mg kg^{-1}), while the highest STP values were found at Riley Co (55 mg kg^{-1}). The highest CEC values were found at Republic and Franklin counties (> 20 $cmol^{+}$ kg^{-1}). In contrast, CEC in Riley, Nemaha, and Labette counties was around 15 $cmol^{+}$ kg^{-1}.

In this study, the mean grain yield varied from 2986 to 4463 kg ha^{-1}. In 4 out of 8 soybean locations, there was a statistically significant ($p < 0.10$) yield response (Table 3-2). One of these locations (#3) had a decrease in grain yield (180 kg ha^{-1}) in high K fertilization plots compared to the control. Recent studies have shown a small yield reduction when applying higher rates (more than 62 kg K_2O ha^{-1} as KCl) in high STK locations (Kaiser, 2021). The author mentioned potential chloride (Cl) issues on soybean when using KCl as a K source. The same study reported an adverse effect not only in poorly drained conditions but also in sandy soils. In our case, the only location that experienced yield reduction when applying high rates of KCl, had high STK levels and a sandier texture (30% sand) compared to the rest of the sites (Table 3-1). However, we do not have Cl tissue analysis to support these findings. The other 3 locations had an increase in grain yield ranging from 530 to 1815 kg ha^{-1} when compared to the control.

Variables such as grain yield, K removal, K uptake, and K/Mg ratio were used to classify locations as responsive and non-responsive. Soil test K values were also used to support this classification (Table 3-1). Locations 4 through 8 had a response to K fertilization for almost all variables. In contrast, locations 1 through 3 were not significant in most of these variables, classifying them as non-responsive (Table 3-2). Figure 1 shows the grain yield, plant K uptake, and K/Mg ratio at R6 as affected by the K rate using the aforementioned classification. In responsive locations, 56 kg K_2O ha^{-1} was enough to increase yield. Although higher rates resulted in slightly higher yields, they were not statistically significant ($p<0.05$). However, K uptake and K/Mg ratio were not statistically different at the first K rate level. Therefore, 112 kg K_2O ha^{-1} was necessary to obtain a significant response, and 168 kg K_2O ha^{-1} to obtain the maximum significant uptake and ratio.

Nutrient Dilution Curves

The relationship between nutrient concentrations (K and Mg) and aerial dry matter accumulation (DM) are shown in Figures 3-2a and 3-2b. The K/Mg ratio was also regressed against DM in figure 3-2c. In non-responsive locations plant K concentration was higher than responsive locations throughout the season regardless of K fertilization (Figure 3-2a). However, the opposite occurs with plant Mg concentration (Figure 3-2b). Ranade-Malvi (2011) stated that high soil K availability reduces the plant capacity to absorb Mg (K-Mg antagonism). In low STK sites, fertilized plots had significantly higher K and lower Mg concentrations at all growth stages ($p < 0.05$). The K concentration for whole plants did not differ considerably throughout the season. Plant K concentration in control plots varied from 10.7 to 9.1 g kg^{-1} between the V4 and R6 growth stages in low STK sites. However, Mg concentration decreased more relative to K, from 8.4 to 5.4 g kg^{-1} within the same period. Thus, the effect of variation in tissue age is minimized in the case of K, but not for Mg concentration when considering all growth stages.

In contrast, if we only consider the V4-R2 periods, both variables had relatively similar variations. Therefore, plant nutrient concentration in control plots varied from 10.7 to 9.6 and 8.4 to 7.5 g kg^{-1} for K and Mg, respectively. The low dilution effect in nutrient concentration may be important when using tissue sampling as a diagnostic tool. Overall, a significant difference was observed in DM, especially between high and low STK locations.

In general, when regressing K/Mg against DM (Figure 3-2c), the ratio increased in all cases as more biomass was accumulated, except for R6 in high STK sites. In these soils, no significant difference was observed between treatments. In contrast, fertilized plots had a significantly higher K/Mg ratio than the control in low STK locations, but consistently lower than high STK sites. In low STK sites, K/Mg ratio in control plots only varied from 1.30 to 1.32 between V4 and R2 growth stages. This variation only represents 1.5% compared to 10.3% and 10.7% for K and Mg concentrations, respectively. These results suggest that the K/Mg ratio might be less affected by sampling moment than K and Mg.

Critical Levels at V4 Growth Stage

Relative grain yield

The whole plant critical K concentration range at V4 determined by linear-plateau (LP) and quadratic-plateau (QP) response model were 16.4 to 18.0 g kg^{-1} (Figure 3-3a). The coefficient of determination (R^2) was 0.40 and 0.39 for LP and QP, respectively. The critical range is lower than a recent study that obtained 18.9 to 22.6 g kg^{-1} with an R^2 of 0.35 for V5-V6 whole plant tissue (Stammer and Mallarino, 2018). The relative yield was consistently higher than 80% when plant K concentration was above the new critical range. However, lower concentrations had relative yields as low as 42%, with a wider range of values. Although the R^2 obtained is relatively low, this finding could be helpful when using plant tissue information combined with soil analysis to better understand the soil K supply to soybean.

In the case of Mg concentration, the critical range at V4 was 6.0 for QP to 6.5 for LP with an R^2 of 0.35 for both models (Figure 3-3b). There is very limited work regarding Mg concentration in whole soybean plants at V4. Higher yields were obtained when the Mg concentration was lower than the critical range, indirectly suggesting a better K status in the plant. However, it is not clear how accurate this critical range could be in an Mg deficient environment.

Apart from plant K and Mg concentration at V4, the ratio between these nutrients was also considered to find a better response model (Figure 3-3c). The obtained critical range was 2.3 for LP to 2.4 for QP, with an R^2 of 0.40 for both models. As Mg, there is not too much bibliography regarding this specific ratio in whole soybean plants at early stages. Although there is not a big difference compared to the K model, the K/Mg ratio might be more precise because it does not depend too much on sampling moment (specifically at early stages).

Relative K uptake

The whole plant critical K concentration level at V4 determined by LP and QP response models was 18.5 g kg^{-1} (Figure 3-4a), higher than the 16.4 to 18.0 g kg^{-1} when regressed against grain yield. The coefficient of correlation (R^2) was 0.58. This critical value is closer to a recent study that obtained 18.9 to 22.6 g kg^{-1} (Stammer and Mallarino, 2018). Relative K uptake was consistently higher than 60% when plant K concentration was above the critical level. Conversely, lower concentrations had as low as 20% relative uptake, with a wider range of values. The R^2 obtained for these models is considerably higher than the previous ones (0.58 vs 0.40). Considering all K models tested in this study, the general critical range could be 16.4 to 18.5 g kg^{-1} at V4 growth stage.

Magnesium critical concentration range changed a little bit more when considering relative K uptake. The critical range at V4 was 5.1 for QP to 5.7 for LP with an R^2 of 0.54 and 0.55 for QP and LP, respectively (Figure 3-4b). Like was mentioned before, there is not too much bibliography regarding Mg concentration in whole soybean plants at V4. Considering all Mg models tested in this study, the general critical range could be 5.1 to 6.5 g kg^{-1} at V4 growth stage.

K/Mg ratio was the variable with the highest R^2 when regressed against relative K uptake. This coefficient was 0.61 for the LP and QP models. The range varied from 2.7 to 3.3 for LP and QP, respectively. This range is considerably higher than the previous models (2.3 to 2.4), suggesting a wider range of values.

Potassium Concentration and Soybean Growth Stage

Figures 3-5 and 3-6 show the correlation between K concentration at different growth stages. In general, K concentration was highly correlated between stages at responsive sites (Figure 3-5). All of them were significant ($p<0.001$). However, K concentration at V4 was not highly correlated, especially with late reproductive stages (0.48 and 0.54 for R4 and R6, respectively). The highest coefficient was obtained between R4 and R6 sampling (0.84).

On the other hand, K concentration was poorly correlated across non-responsive locations (Figure 3-6). Although some were significant ($p<0.01$), all coefficients were below 0.45 without a clear pattern. For example, the correlation coefficient between V4 and R6 was -0.06 and not significant ($p<0.05$) for these sites.

Conclusions

This study developed reference values for tissue testing to address K deficiency on soybean. The accuracy of the presented models was evaluated and discussed, resulting in recommendations that could complement current soil testing guidance. Looking at whole plants at V4 stage, K concentration and K/Mg ratio were similarly correlated to relative grain yield. Magnesium concentration gave us additional information but was less correlated than the variables mentioned before. The critical concentration range for K and K/Mg ratio was 16.4 to 18.0 g kg^{-1} and 2.3 to 2.4, respectively. When regressing against K uptake at R6, the best correlation was attained with the K/Mg ratio with an R^2 of 0.61. The critical range varied from 2.7 to 3.3, somewhat higher than relative grain yield. Potassium concentration had a lower R^2 than K/Mg but was still high, 0.58. The critical level for this nutrient alone was 18.5 g kg^{-1}. Also, Mg concentration correlated with a critical level ranging from 5.1 to 5.7 g kg^{-1} (R^2 = 0.54-0.55). Higher concentrations resulted in lower K uptake and grain yield due to K-Mg antagonism in this particular case.

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Table 3-1. Locations, soils, and selected soil properties for the 0-15 cm sampling depth.

Location	County	Soil		Sand	Clay	pH	OM	P_{M3}	K_{AA}	K_{AA-fm}	Ca_{ex}	Mg_{ex}	CEC
		Series	Subgroup										
				----- % -----			g kg^{-1}	--------------- mg kg^{-1} ---------------					$cmol^{+}$ kg^{-1}
1	Republic	Crete	Udertic Argiustolls	10	26	6.3	36	5	364	394	1916	260	17.2
2	Republic	Crete	Udertic Argiustolls	14	22	6.2	33	4	409	455	2031	333	20.8
3	Riley	Rossville	Cumulic Hapludolls	30	18	7.7	32	55	324	393	2749	117	15.6
4	Franklin	Woodson	Abruptic Argiaquolls	14	26	5.7	34	14	94	57	2399	322	20.9
5	Franklin	Woodson	Abruptic Argiaquolls	10	26	6.5	32	11	120	50	2659	368	23.0
6	Nemaha	Kennebec	Cumulic Hapludolls	20	20	6.1	24	8	60	46	1466	200	14.4
7	Labette	Parsons	Mollic Albaqualfs	10	20	6.6	26	3	34	16	1916	171	14.7
8	Labette	Parsons	Mollic Albaqualfs	10	20	6.4	26	4	66	25	1932	186	13.0

M3: Mehlich-3 extraction, AA: Ammonium acetate extraction, fm: field-moist, ex: exchangeable

Table 3-2. Statistical significance of potassium application rate on selected variables.

			K uptake				K/Mg ratio			
Location	Yield	Grain K removal	V4	R2	R4	R6	V4	R2	R4	R6
	---	---	---	---	--- p>F ---	---	---	---	---	---
1	0.662	0.549	0.620	0.644	0.006	0.067	0.641	0.827	0.391	0.974
2	0.655	0.488	0.179	0.499	0.991	0.491	0.250	0.817	0.329	0.138
3	0.067	0.190	0.306	0.468	0.079	0.842	0.799	0.775	0.321	0.168
4	0.361	0.031	0.050	0.032	0.009	0.002	<0.001	0.003	<0.001	<0.001
5	0.236	0.074	0.041	0.016	<0.001	0.066	0.011	0.001	<0.001	0.022
6	0.088	0.005	0.014	0.118	<0.001	0.018	0.004	0.028	<0.001	<0.001
7	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
8	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

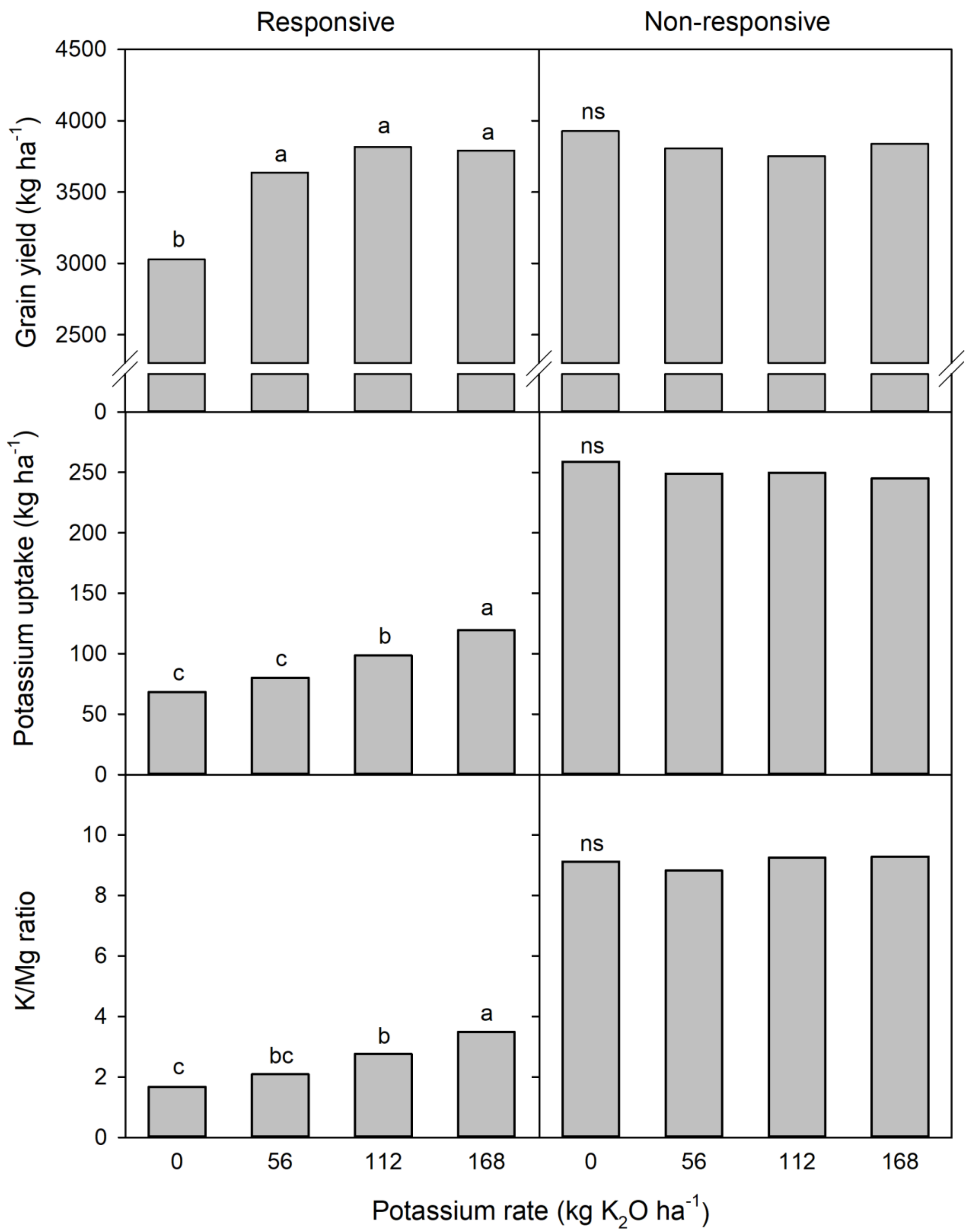

Figure 3-1. Grain yield, plant K uptake, and K/Mg ratio at R6 as affected by K rate in responsive and non-responsive locations. Mean values followed by the same letter do not differ significantly ($p<0.05$). ns, not significant.

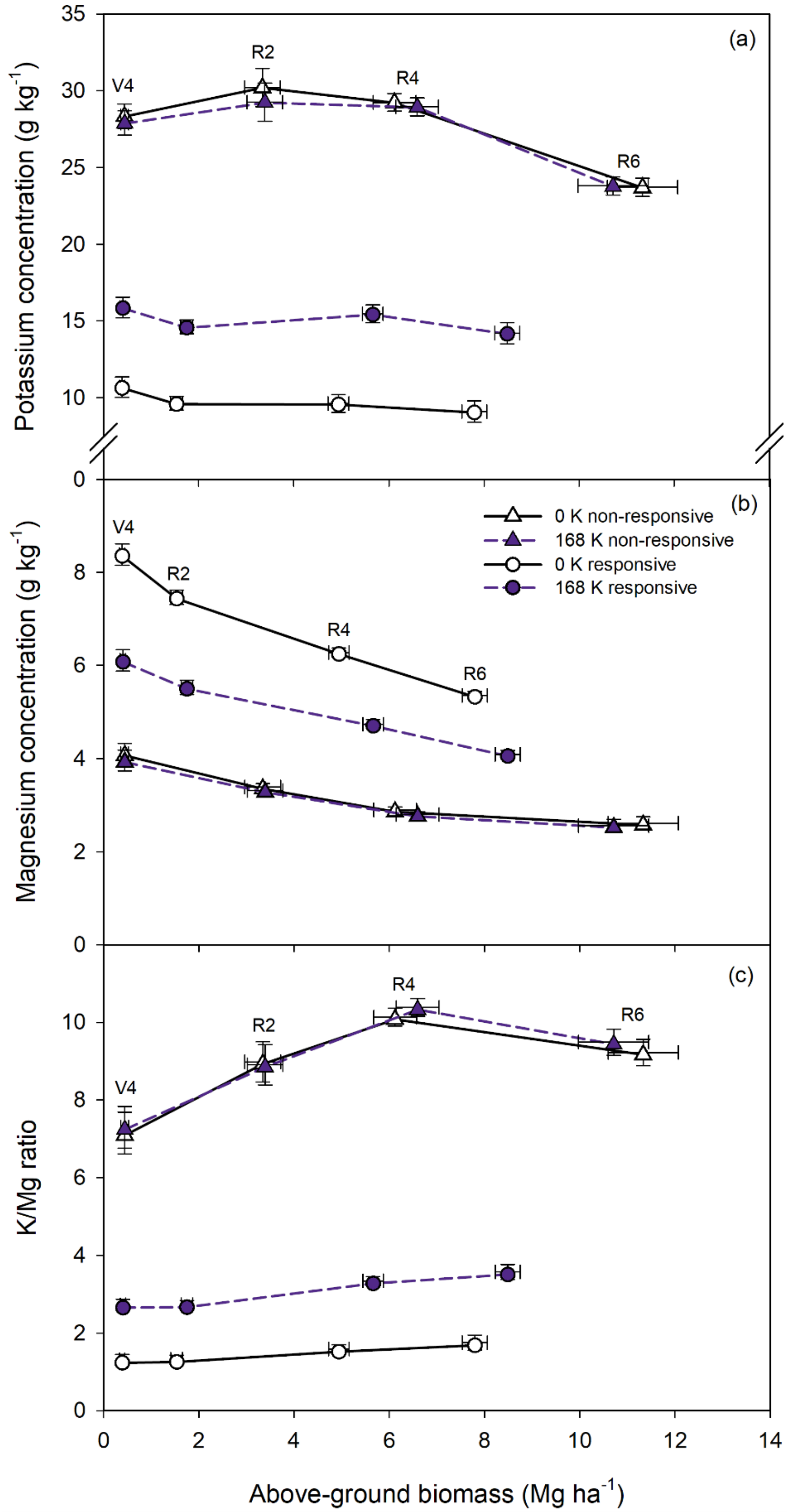

Figure 3-2. Relationship between aboveground biomass and K concentration (a), Mg concentration (b), and K/Mg ratio (c) from V4 to R6 growth stages. Horizontal and vertical bars show the standard error for estimated values.

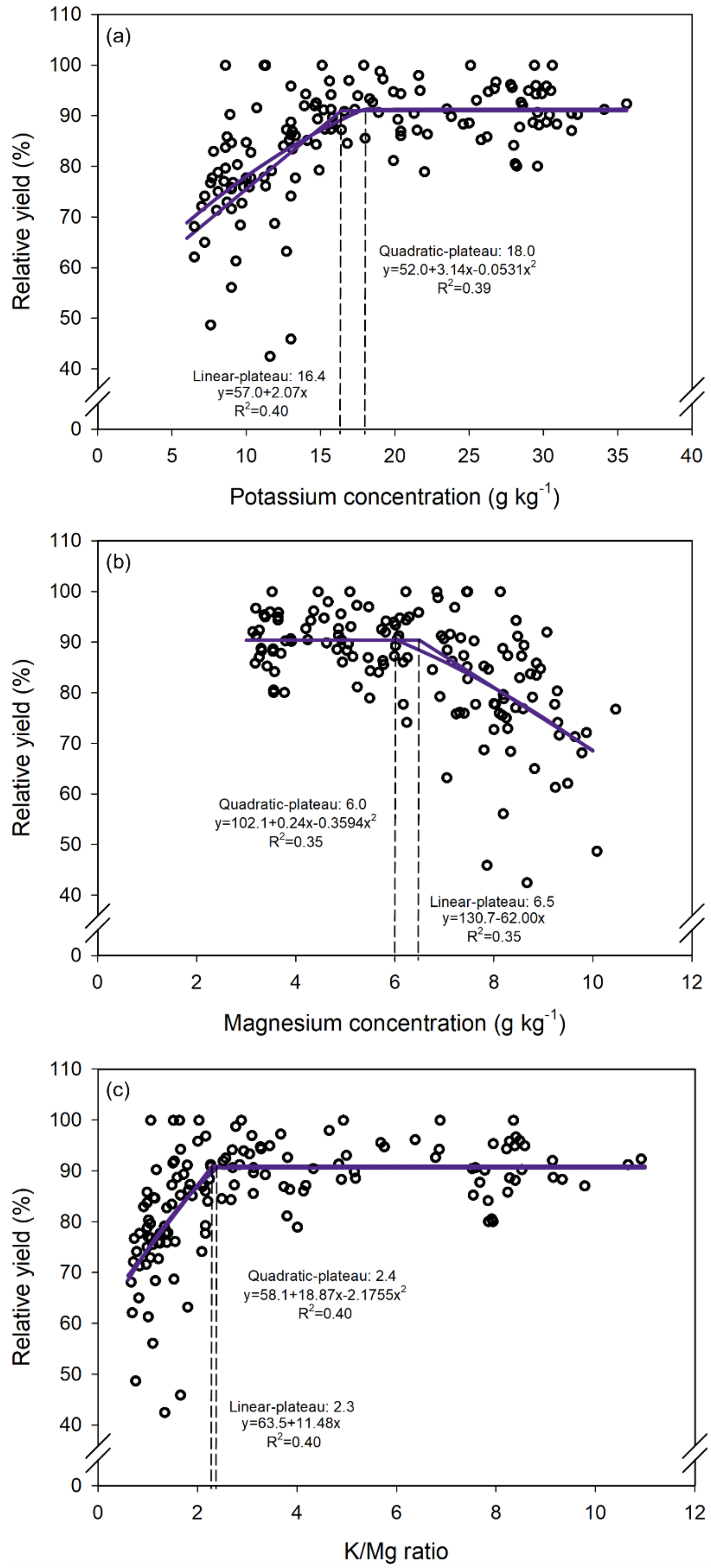

Figure 3-3. Relationship between soybean relative yield and K concentration (a), Mg concentration (b), and K/Mg ratio (c) of whole plants at the V4 growth stage.

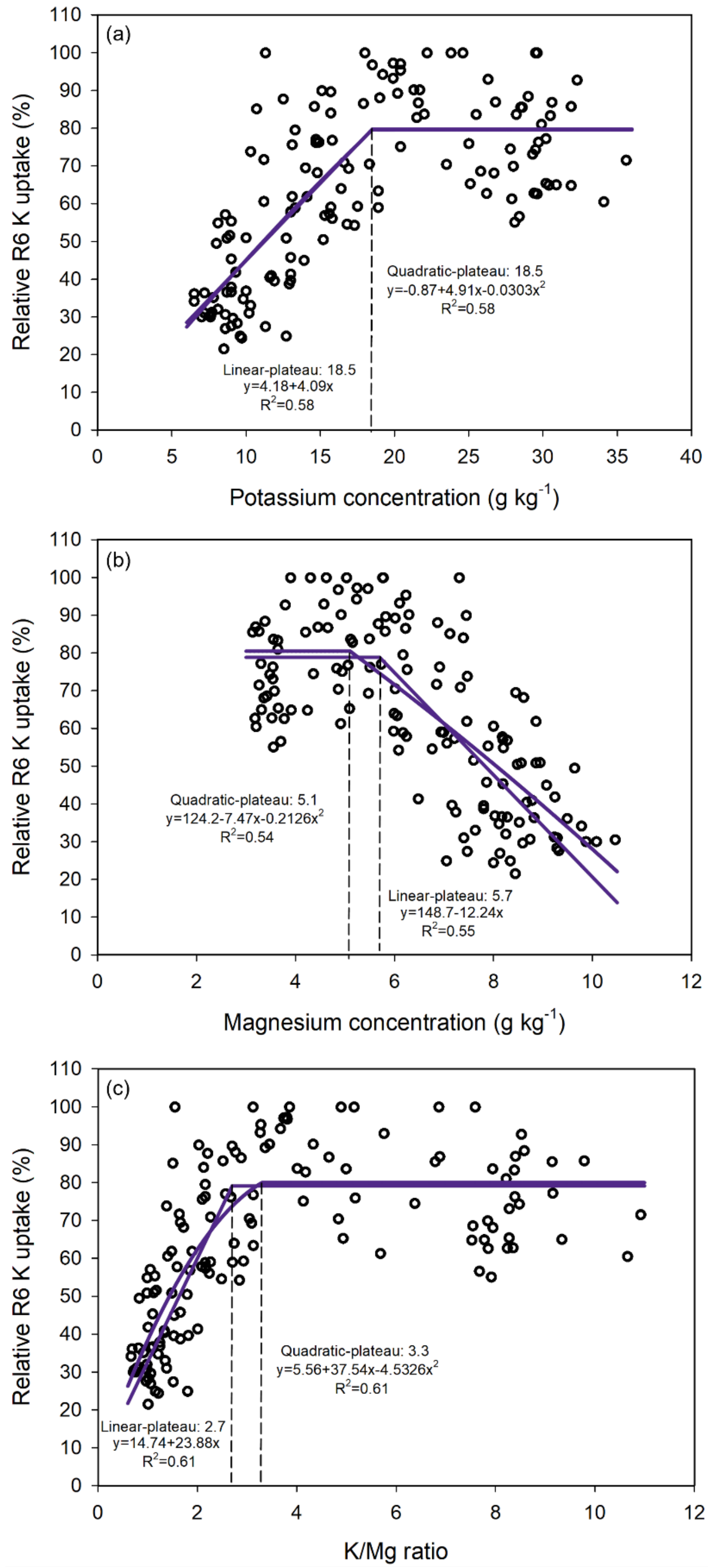

Figure 3-4. Relationship between soybean relative K uptake and K concentration (a), Mg concentration (b), and K/Mg ratio (c) of whole plants at the V4 growth stage.

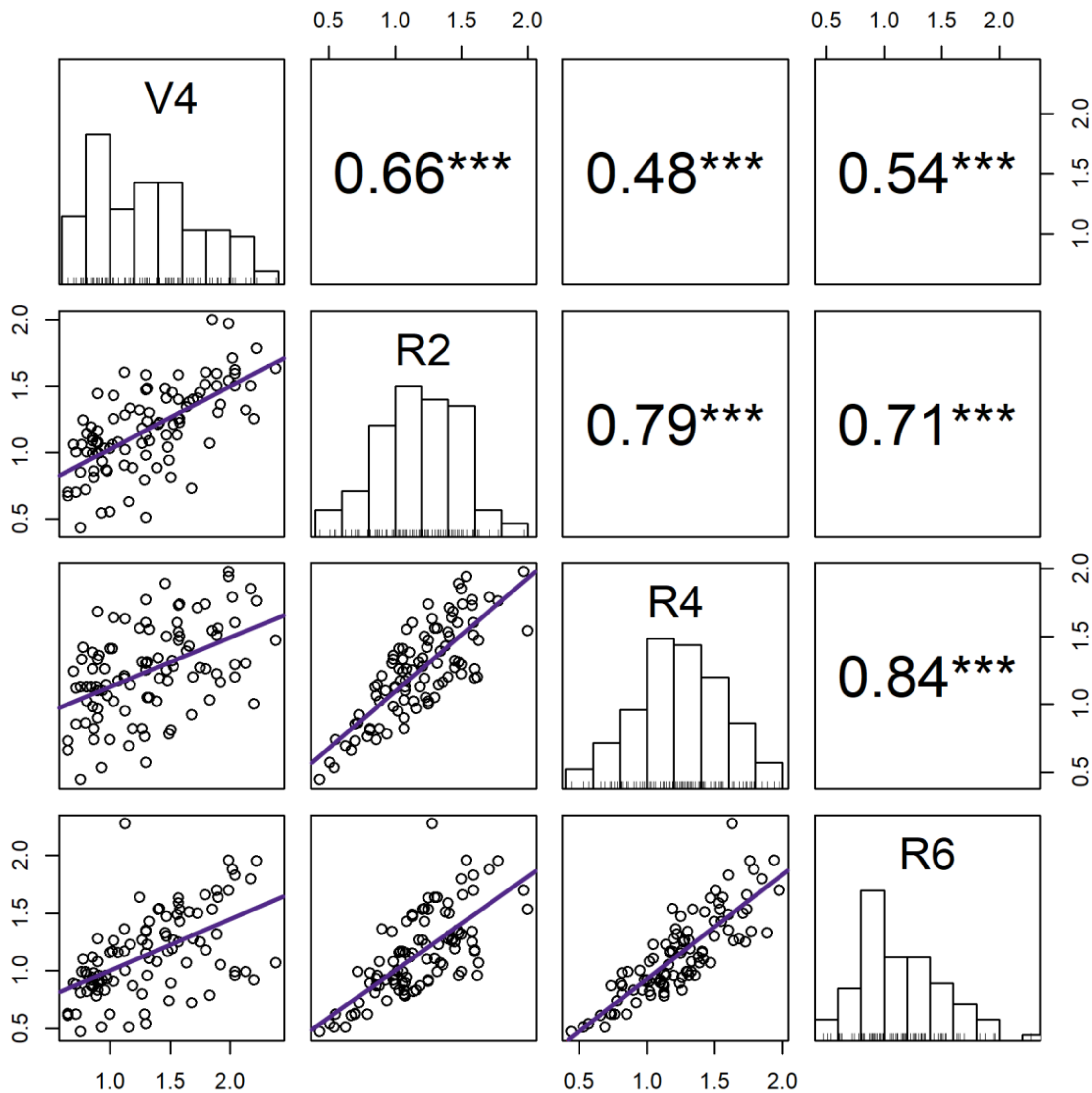

Figure 3-5. Pearson correlation coefficients for plant K concentration at different growth stages at responsive locations. ***, p<0.001

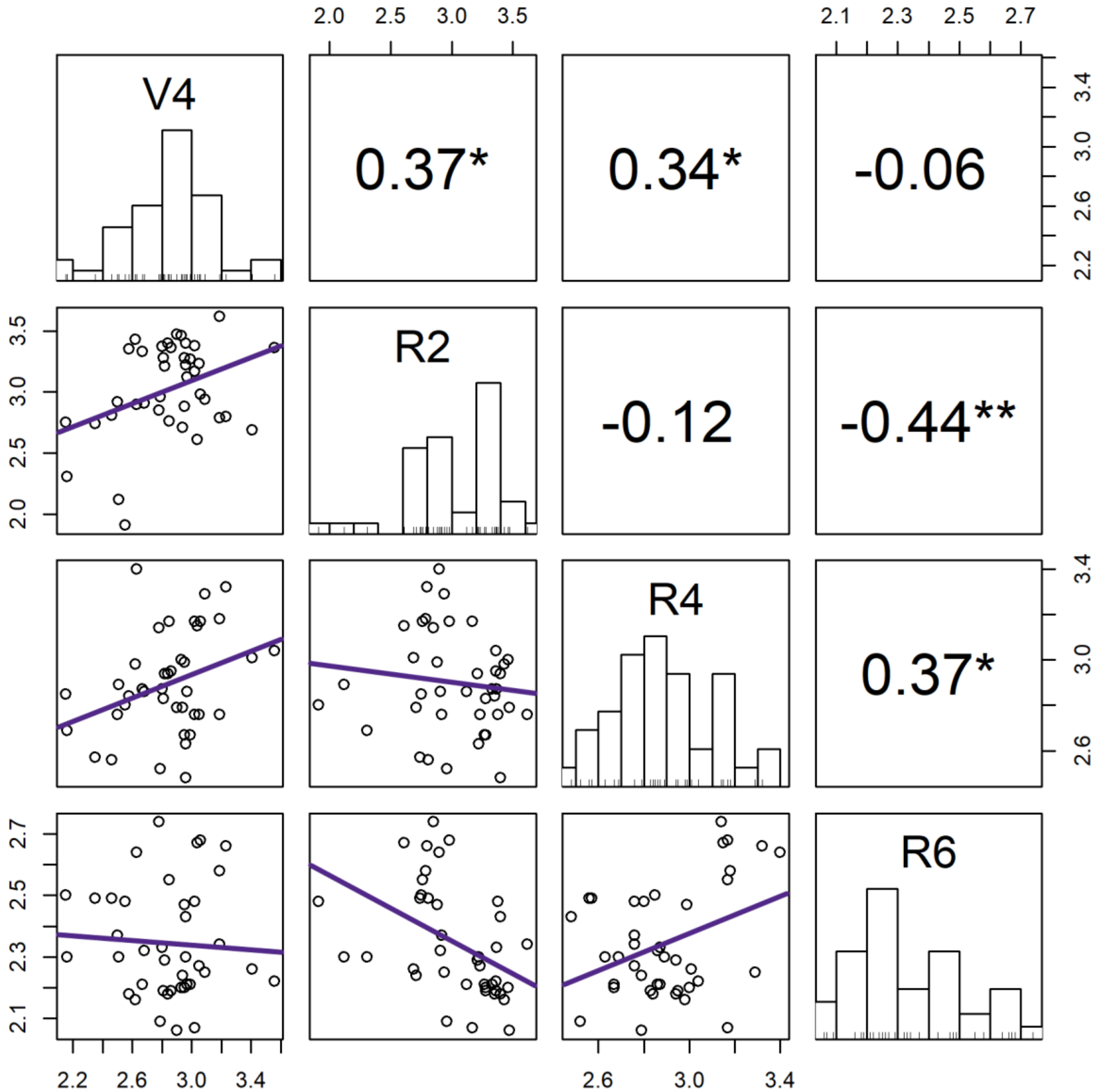

Figure 3-6. Pearson correlation coefficients for plant K concentration at different growth stages at non-responsive locations. *, p<0.05. **, p<0.01.

Chapter 4 - Evaluation of Soil Test Methods and Sampling Moment to Assess Potassium Response in Soybean

Abstract

Ammonium-acetate (NH_4OAc) soil potassium (K) extraction using field-moist samples has been one of the best tests predicting crop response to K fertilization. Still, it could be challenging to be implemented when high throughput is needed. This study compared different soil test K (STK) methods and evaluated the correlation to soybean yield and K uptake response in low testing soils. Additionally, the study assessed the effect of sampling moment on STK results for NH_4OAc and Mehlich-3 tests using dry and field moist samples. It was conducted at eight locations throughout eastern Kansas during 2019 and 2020. The treatments were from zero to 168 kg K_2O ha^{-1} in 56 kg K_2O ha^{-1} increments. Aboveground plant samples were collected at the R6 stage to measure plant K uptake. In general, tests using field moist were better correlated to K response than dry tests, especially with NH_4OAc. Among all evaluated methods, the $CaCl_2$ dry and moist, NH_4OAc moist, Resin K, and $NaBPh_4$ tests were the best when correlating to relative yield and K uptake. The $CaCl_2$ dry is one the easiest and lowest cost tests, also having a consistent correlation coefficient (around 0.70 for both response variables). Furthermore, it might be an alternative to the NH_4OAc moist test because of the high correlation (r=0.91). Three out of eight locations had STK changes regardless of K fertilization between fall post-harvest and the subsequent spring sampling (when using dry samples). However, almost all locations had little or no STK change when using moist samples. Overall, the NH_4OAc moist test was one of the best methods to estimate K availability in low testing soils; however, other non-conventional tests like $CaCl_2$ dry perform similarly but without the disadvantages of moist samples.

Abbreviations: NH_4OAc, Ammonium-acetate; K, potassium; STK, soil test potassium.

Introduction

Soil testing is one of the best tools to identify responsive soils to K fertilizer. However, more research is needed to find the K extraction method that better correlates to soybean grain yield across soils and regions. Some soil test methods used to estimate K availability (e.g., 1 M NH_4OAc) are not always good K uptake and grain yield indicators. Another extraction like Mehlich-3 has been similar to NH_4OAc, explaining yield response. Moreover, we know from previous research that these two extraction methods are strongly correlated, suggesting they could be used indistinctively (Rutter and Ruiz Diaz, 2019), and are likely measuring the same soil K pool.

A different procedure for the NH_4OAc and Mehlich-3 extraction methods has been evaluated in order to improve the K availability assessment in soils. It consists in using field-moist in contrast to the conventional oven-dried or air-dried soil samples. Previous greenhouse studies showed a better correlation between soil test K from field-moist samples and plant K uptake in various crops (Barber, 1961; Hanway, 1961, 1962). It is recognized that drying samples can increase or decrease the amount of K extracted with NH_4OAc, the former the most common case. Furthermore, studies have shown that K fixation could occur in high exchangeable K soils while K release is more common in low K exchangeable soils when drying samples (Cook and Hutcheson Jr., 1960; Hanway, 1962; Haby et al., 1988). The difference in exchangeable K between dry and moist soil samples was similar when using Mehlich-3 instead of the NH_4OAc extracting solution (Mallarino, 2012). The moist soil test for K was a better predictor of crop response to K fertilization than the dry test. The main downside of adopting the moist test in laboratories is the complex procedure when preparing soil samples, especially in fine-textured and very moist soils (Barbagelata and Mallarino, 2013).

Apart from the extractant solutions mentioned before, different extractions have been tested for K. For various reasons such as poor correlation or lack of calibration studies; they are not widely adopted. One example is the $CaCl_2$ method that has been evaluated since the 1950's as a single extraction for a wide range of nutrients, including K. The authors argue in favor of this method mainly because the solution ionic strength is similar to the average salt concentration found in many soils, the procedure is relatively cheap and easy to implement in soil laboratories, and its capacity as an alternative extractant due to the possibility to measure different nutrients in a single extract (Houba and Huybregts, 1986; Houba et al., 1990, 2000). The most recommended solution concentration is 0.01 M $CaCl_2$ with a 1:10 w/v soil:solution ratio (Houba and Huybregts, 1986; Salomon, 1998). Salomon (1998) concluded that there was a strong correlation between the dry and moist K extraction. However, most of the work has been conducted with dry soil samples.

Another soil test method for K has been used for phosphorus for many years. This method uses an exchange resin strip in contact with the soil to extract the available K, trying to mimic plant root absorption. Potassium extracted by this method is lower than NH_4OAc, but it might be more precise in predicting K response. Unfortunately, compared to the previous methods, there is a lack of bibliography related to this specific procedure for K.

The sodium tetraphenyl boron ($NaBPh_4$) K extraction method was developed by Scott et al. (1960), and since then, it has received attention as a potential method to estimate plant-available K. This method extract exchangeable K and part of the non-exchangeable forms of K. Furthermore, $NaBPh_4$ extracts less K from the mineral structures compared to the boiling 1 MHNO_3 method used for non-exchangeable K. The BPh_4 anion facilitates the release of non-exchangeable K by combining with K in solution and precipitating it as $KBPh_4$, while Na exchanges with interlayer K (Scott and Reed, 1962). Consequently, this method mimics the action of K uptake by depleting soil K solution and promoting the further release of exchangeable and non-exchangeable K. Previous experiments showed a good correlation between $NaBPh_4$ extraction and K uptake by plants (Cox et al., 1999). However, most studies have been conducted under greenhouse conditions; therefore, field calibration is needed.

The objectives of this study were to i) compare different soil test K methods (STK) and evaluate the correlation to soybean yield and plant K uptake response in low testing soils, and ii) assess the effect of sampling moment on STK results.

Materials and Methods

Treatments, Experimental Design, and Implementation

Field experiments were conducted at eight locations throughout eastern Kansas during 2019 and 2020. Sites were located near Ottawa (Franklin Co), Parsons (Labette Co), Scandia (Republic Co), Ashland Bottoms (Riley Co), and Wetmore (Nemaha Co) in Kansas. All of them were under a conventional tillage crop system; however, the Scandia location was the only one under supplemental irrigation. Soybean was planted during the first weeks of June, with maturity groups ranging from 3.9 to 4.6. All experiments were planted with a row spacing of 76 cm, except for Nemaha Co (38 cm). Three sites had very high soil K levels (STK > 324 mg kg^{-1} NH_4OAc), while the remaining five locations were below the current critical value for Kansas (STK < 130 mg kg^{-1} NH_4OAc). The experiments were a randomized complete block design, and four treatments were selected to evaluate soybean response to K fertilization. Treatments included a control with no K fertilization and 56, 112, 168 kg K_2O ha^{-1}. Two additional treatments were considered in locations with low soil test phosphorus (STP) levels (e.g. Republic Co). They consisted of a control with no K fertilization and 168 kg K_2O ha^{-1} but with a blanket application of 90 kg P_2O_5 ha^{-1}. The fertilizer applications were a surface broadcast at pre-plant using triple superphosphate (TSP) (0-46-0) and potassium chloride (KCl) (0-0-60) as P and K sources. Control plots (0 kg K_2O ha^{-1}) were selected when correlating soil test methods with relative yield.

Sampling and Analysis

Composite soil samples were taken at pre-plant (one per replicate) at the 0-15 cm depth dividing each into two sub-samples. The first group was oven-dried at 40 °C, and ground to pass through a 2 mm screen. The second one was kept in a refrigerator at 3 °C at field moisture until further analysis. All dry samples were analyzed for soil pH (soil:water; 1:1) (Watson and Brown, 1998), organic matter (OM) by loss on ignition (Combs and Nathan, 1998), extractable P with the Mehlich-3 extraction (Frank et al., 1998), exchangeable cations (1 M NH_4OAc pH 7.0, flame atomic absorption) (Warncke and Brown, 1998), cation exchange capacity (CEC) with the displacement method (Chapman and Pratt, 1962), and soil texture (hydrometer method) (Gee and Bauder, 1986). Besides the ammonium acetate (AA) extraction mentioned before, additional K methods were evaluated, including Mehlich-3 (M3), $CaCl_2$, and cation exchange resin. The same K extractions were done to field-moisture samples for comparison. The $CaCl_2$ method used was based on Houba et al. (1986).

Aboveground plant samples were collected at the R6 growth stage to measure plant biomass and K concentration. Stages of soybean development are based on Fehr and Caviness (1977) publication. The samples were dried at 60°C, ground to pass through a 2 mm screen, weighed, and digested by nitric-perchloric acid digestion (Gieseking et al., 1935). Total K concentration was determined by inductively coupled plasma (ICP) spectrometry.

For the sampling moment study, the same treatments were sampled at harvest and the following spring before planting, keeping one sub-sample at field moisture, and the other was air-dried as explained before. By doing this, it was possible to study the evolution of K levels in different K extraction methods.

Grain was harvested from the center rows (11.3-m length) with a plot combine. The yield was corrected at 13% moisture. Relative yield (%) was obtained by dividing each plot value by the maximum value for that location.

Cation Exchange Resin Method

Cation exchange resin (CER) was cut into 2.0 cm by 1.0 cm strips and immersed in a 5% NaCl solution for 24-48 hours to allow membrane hydration and expansion. This process is needed only with new membranes. The next step was the washing process using a horizontal shaker at 120 rpm. The first step consisted of repeated shaking for 30 minutes with distilled H_2O with sufficient volume to cover the vessel. This procedure was repeated three times. After each shaking period with distilled H_2O, the vessel was washed three times before discarding the liquid. After that, membranes were contacted with a 1 mol l^{-1} NaOH solution for 10, 30, and 30 minutes. Every time a different solution was used, CERs were washed with plenty of distilled H_2O, and the liquid was discarded. Then, the entire first step was repeated using distilled H_2O. Later, 1 mol l^{-1} HCl and 1 mol l^{-1} NH_4Cl solutions were used. Both steps consisted in repeated shaking for 10, 30, and 30 minutes for each solution. The washing process ends after shaking the membranes for 30 minutes with distilled H_2O.

Once the washing process was completed, they were stored in distilled H_2O until the saturation process. In this step, CERs were immersed into 1 mol l^{-1} $NaHCO_3$ solution (pH adjusted to 8.5) and shaken for 10, 30, and 120 minutes at 120 rpm to allow for membrane saturation with Na^+ ions. This process was done 24 hours before soil analysis. CER strips were reused during the experiment when needed. It was necessary to wash and saturate CERs that were buried in previous periods to use them again.

The analysis procedure consisted in weighing 0.5 g of soil to centrifuge tubes with a crew cap. After that, add 10 ml of distilled water and the CER prepared in the previous steps. Shake for 16 hours in an "end-over-end" shaker (rotation of 33 rpm). After 16 hours of shaking, remove the CER and wash the excess soil. Next, prepare a container with a lid with 10 ml of 0.8 mol l^{-1} NH_4Cl in 0.2 mol l^{-1} HCl, place the CER strip, and leave to stand in the solution for 90 min (with the lid open). Cap and stir for 30 minutes on a horizontal stirrer (150 rpm, ensuring constant movement with strip). After that, the membrane was removed using a clamp and K^+ in solution determined by inductively coupled plasma (ICP) spectrometry.

Statistical Analysis

Statistical analysis (ANOVA) was performed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, 2013). Blocks were included as a random effect in the model. Alpha = 0.05 was set as the statistical significance level. For correlation plots (Pearson's coefficient), the psych package was used in R version 3.6.1 (R Core Team, 2019).

Results and Discussion

Table 4-1 shows selected soil properties for the 0-15 cm sampling depth for each location. Almost all locations had similar contents of sand and clay, except for Riley Co with more sand (30%) and less clay (18%). When looking at the eight locations, soil pH ranged from acidic to neutral (5.7 to 7.7) and OM varied from 24 to 36 g kg-1. Phosphorus levels were very low at locations 1, 2, 7, and 8 (3 to 5 mg kg^{-1}), while the highest STP values were found at location 3 (55 mg kg^{-1}). The highest CEC values were found at locations 1, 2, 4, and 5 (> 20 meq 100 g^{-1}). In contrast, CEC in locations 3, 6, 7, and 8 was around 15 meq 100 g^{-1}. Table 4-2 is focused on K soil test results for the same locations mentioned before. Locations 1 to 3 (locations 1 to 3) were very high in K regardless of the soil test method. Conversely, locations 7 and 8 were very low in STK, especially when using field-moist samples.

Amount of K Extracted by Soil Tests

The $CaCl_2$ moist extracted the lowest amount of K among the different methods (Table 4-2). The $CaCl_2$ dry extracted more than the moist version of the same test, but less than Resin K. In high STK locations, conventional K methods (1 M NH_4OAc and Mehlich-3) extracted more than the Resin K regardless of sample processing method (dry vs. moist). However, moist samples extracted with NH_4OAc and Mehlich-3 performed similarly to Resin K in low STK soils. In these locations, the moist analysis always extracted lower amounts of K than the dry soil. However, the opposite occurred in high testing soils, especially with the NH_4OAc extraction. These findings are in concordance with previous studies (Cook and Hutcheson Jr., 1960; Hanway, 1962; Haby et al., 1988). Although the Mehlich-3 test extracted slightly more K on average (17%) than the 1 M NH_4OAc test, the amount was generally similar as Rutter and Ruiz Diaz (2019) found. The biggest difference was observed in locations 1, 6, and 7 with 32%, 48%, and 35%. Finally, the highest amount of K was extracted with the $NaBPh_4$ test. As mentioned before, this method extracts exchangeable K and part of the non-exchangeable forms of K; thus, the higher amount compared to NH_4OAc and Mehlich-3 tests that mainly extract exchangeable forms of K.

Relative Grain Yield, K Uptake, and Soil Test Methods

Figures 4-1 shows correlation coefficients between relative grain yield, K uptake, and STK methods for low testing sites (locations 4-8, STK < 120 mg kg^{-1} NH_4OAc dry). All tests had a positive correlation with grain yield and K uptake, being the NH_4OAc dry test the only one that was not statistically significant for both variables ($p<0.05$). The strongest correlations were found with $CaCl_2$ dry and moist, NH_4OAc moist, Resin K, and $NaBPh_4$.

Figure 4-2 shows correlation coefficients and scatter plots for the highly correlated soil test methods mentioned before. Several authors have shown a better correlation between STK in moist samples and K uptake by plants (Barber, 1961; Hanway, 1961, 1962). Across conventional methods, the best result was obtained with the moist test. In contrast to (Mallarino, 2012), NH_4OAc moist was superior to Mehlich-3 moist. It is important to consider different soils and conditions in both studies. Correlation coefficients were r=0.76 and r=0.71 in NH_4OAc moist for yield and K uptake, respectively, while Mehlich-3 had r=0.62 and r=0.58 for the same variables. The NH_4OAc moist test could be considered a good reference for soil test method comparison due to its better prediction of crop response to K fertilization than the dry test, as stated by (Barbagelata and Mallarino, 2013). Both $CaCl_2$ tests performed similarly to the NH_4OAc moist test, having the moist version even slightly higher correlation for yield and K uptake. Resin K was positively correlated with a lower but significant correlation coefficient ($p<0.01$). Finally, $NaBPh_4$ test performed similarly to the NH_4OAc moist test when considering grain yield; however, the correlation coefficient was lower for K uptake.

As mentioned before, the NH_4OAc moist test could be considered a reference when looking at an accurate test to assess K availability in the soil. However, adopting the moist test in laboratories has some downside due to its difficulty when preparing soil samples, mainly in fine-textured and very moist soils (Barbagelata and Mallarino, 2013). Possible alternatives to moist samples are $CaCl_2$, Resin K, and $NaBPh_4$ (all of them in their dry versions). $CaCl_2$ dry and Resin K are highly correlated to the NH_4OAc moist test (r=0.91 and r=0.89, respectively). This might be useful because there is a possibility to adopt these two tests, avoiding moist sample preparation and, at the same time, obtaining as good results as the NH_4OAc moist test. $NaBPh_4$ test is highly (r=0.83) but less correlated, and it is one of the most complicated and time demanding procedures among all soil test methods evaluated in this study. On the contrary, $CaCl_2$ dry is the easiest and cheapest test, also having a consistent correlation coefficient around r=0.70 for both variables (yield and K uptake). Also, Resin K has similar correlation coefficients to $CaCl_2$, but the procedure is longer and demands more reagents.

Soil Test K and Sampling Moment

Figure 4-3 shows relative NH_4OAc STK evolution between fall and spring sampling for dry and moist samples. All locations were included in this analysis (low and high STK soils). This figure makes it possible to observe the difference in STK for the four K fertilizer treatments. Considering a 95% confidence level, the dry test did not change in high STK sites (locations 1-3) regardless of previous K fertilization rate, except for location 3 in low K rate and control plots (slightly increase). In contrast, the moist test decreased in location 1, while locations 2 and 3 were not different from zero. Drastic STK increments with the dry test were observed in locations 4 and 6, with around 80% and 40% of the variation, respectively. It did not happen with the moist test in these sites (confidence levels include or are very close to zero). Although the dry test in locations 5, 7, and 8 did not vary within the two sampling moments, the moist test had substantial changes, especially in locations 7 (increase) and 8 (decrease).

Figure 4-4 presents similar information but with Mehlich-3 results. Conversely to NH_4OAc, Mehlich-3 had similar values when looking at dry and moist tests. Although the moist test had values not different from zero in almost all locations, the trend was similar.

Conclusions

The NH_4OAc moist test is one of the best methods to assess potassium response in soybean. These results are similar to previously conducted studies. However, adoption by commercial laboratories could be affected due to its complex soil preparation process. Methods that use dry samples like $CaCl_2$, Resin K, and $NaBPh_4$ might be an alternative. The $CaCl_2$ dry seems to be a good candidate because of its simplicity and good correlation with K uptake and grain yield. There was not a clear trend regarding STK changes between fall post-harvest and the subsequent spring sampling. Only few locations had changes in STK with the dry test. However, almost all locations had little or no STK change when using moist samples. Potassium recycling from soybean leaves might influence the post-harvest sampling, affecting the comparison with the subsequent spring sampling. Another reason could be that exchangeable forms of K might become part of non-exchangeable fractions. Either NH_4OAc or Mehlich-3 extract mainly exchangeable K from the soil.

Overall, the NH_4OAc moist test was one of the best methods to estimate K availability in low testing soils; however, other non-conventional tests like $CaCl_2$ dry perform similarly but without the disadvantages of moist samples. More research is needed to find the best soil test method that captures soil potassium changes over time.

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Table 4-1. Locations, soils, and selected soil properties for the 0-15 cm sampling depth.

Location	County	Series	Subgroup	Sand	Clay	pH	OM	P_{M3}	Ca_{ex}	Mg_{ex}	Na_{ex}	CEC
				----- % -----			g kg^{-1}	----------- mg kg^{-1} -----------				meq $100g^{-1}$
1	Republic	Crete	Udertic Argiustolls	10	26	6.3	36	5	1916	260	41	17.2
2	Republic	Crete	Udertic Argiustolls	14	22	6.2	33	4	2031	333	27	20.8
3	Riley	Rossville	Cumulic Hapludolls	30	18	7.7	32	55	2749	117	11	15.6
4	Franklin	Woodson	Abruptic Argiaquolls	14	26	5.7	34	14	2399	322	29	20.9
5	Franklin	Woodson	Abruptic Argiaquolls	10	26	6.5	32	11	2659	368	54	23.0
6	Nemaha	Kennebec	Cumulic Hapludolls	20	20	6.1	24	8	1466	200	11	14.4
7	Labette	Parsons	Mollic Albaqualfs	10	20	6.6	26	3	1916	171	35	14.7
8	Labette	Parsons	Mollic Albaqualfs	10	20	6.4	26	4	1932	186	46	13.0

M3: Mehlich-3 extraction, AA: Ammonium acetate extraction, fm: field-moist, ex: exchangeable

Table 4-2. Potassium soil test results for the 0-15 cm sampling depth.

Location	NH_4OAc dry	NH_4OAc moist	Mehlich-3 dry	Mehlich-3 moist	$CaCl_2$ dry	$CaCl_2$ moist	Resin K	$NaBPh_4$
	----------	----------	----------	----------	mg kg^{-1}	----------	----------	----------
1	364	394	479	428	227	166	324	626
2	409	455	458	436	207	155	254	629
3	324	393	350	413	201	165	230	457
4	94	57	102	39	40	16	62	157
5	120	50	134	49	34	7	49	146
6	60	46	89	33	34	17	51	93
7	34	16	46	24	12	3	23	59
8	66	25	66	23	22	3	40	47

Table 4-3. Amount of potassium present in soybean stover as affected by K rate.

	Rate (kg K_2O ha^{-1})			
	0	56	112	168
Location	-----------------	kg ha^{-1}	-----------------	
1	65	60	59	67
2	69	66	57	78
3	117 b	111 b	122 ab	129 a
4	29 c	37 b	40 ab	44 a
5	15 c	20 b	17 bc	26 a
6	19	18	14	23
7	6 d	11 c	24 b	30 a
8	5 c	7 c	18 b	27 a

Means followed by the same letter are not significantly different at $p<0.05$ within each variable.

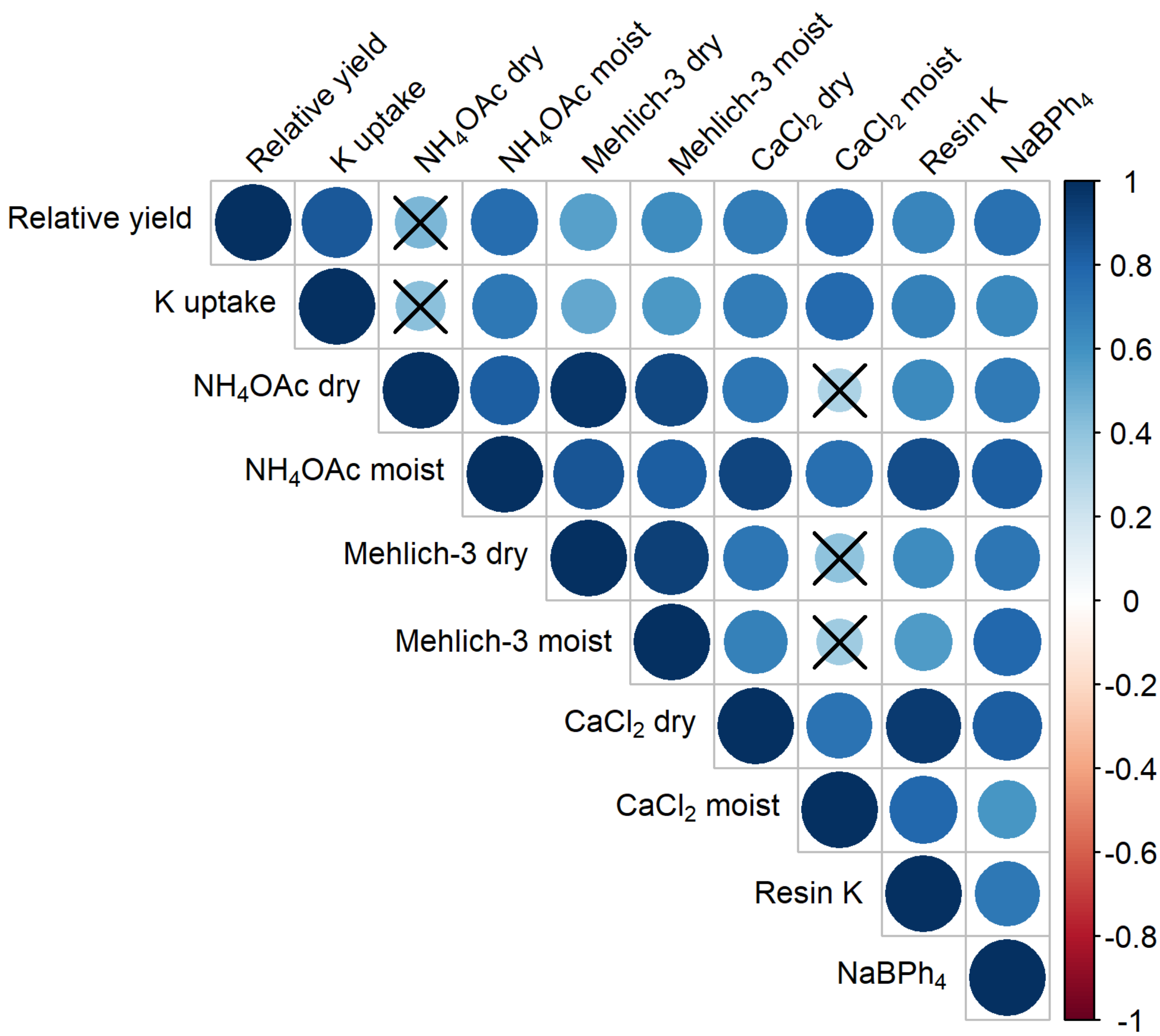

Figure 4-1. Correlation between relative grain yield, K uptake at R6, and soil test methods for K in locations 4-8 (STK < 120 mg kg^{-1} NH_4OAc dry). Crossed when not statistically significant at $p<0.05$.

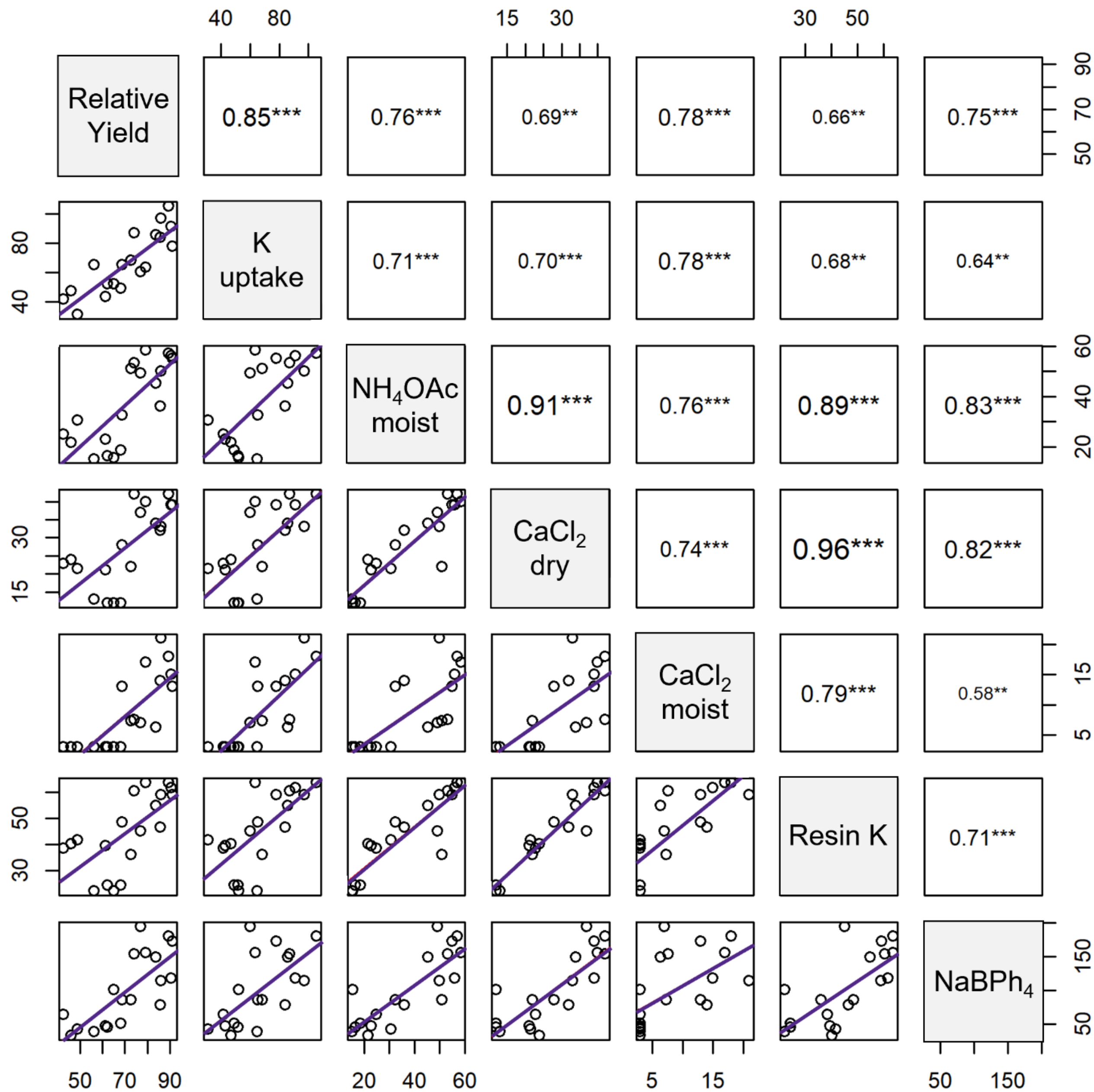

Figure 4-2. Correlation between Relative yield, K uptake at R6, NH_4OAc moist, $CaCl_2$ dry, $CaCl_2$ moist, Resin K, and $NaBPh_4$ in locations 4-8 (STK < 120 mg kg^{-1} NH_4OAc dry).

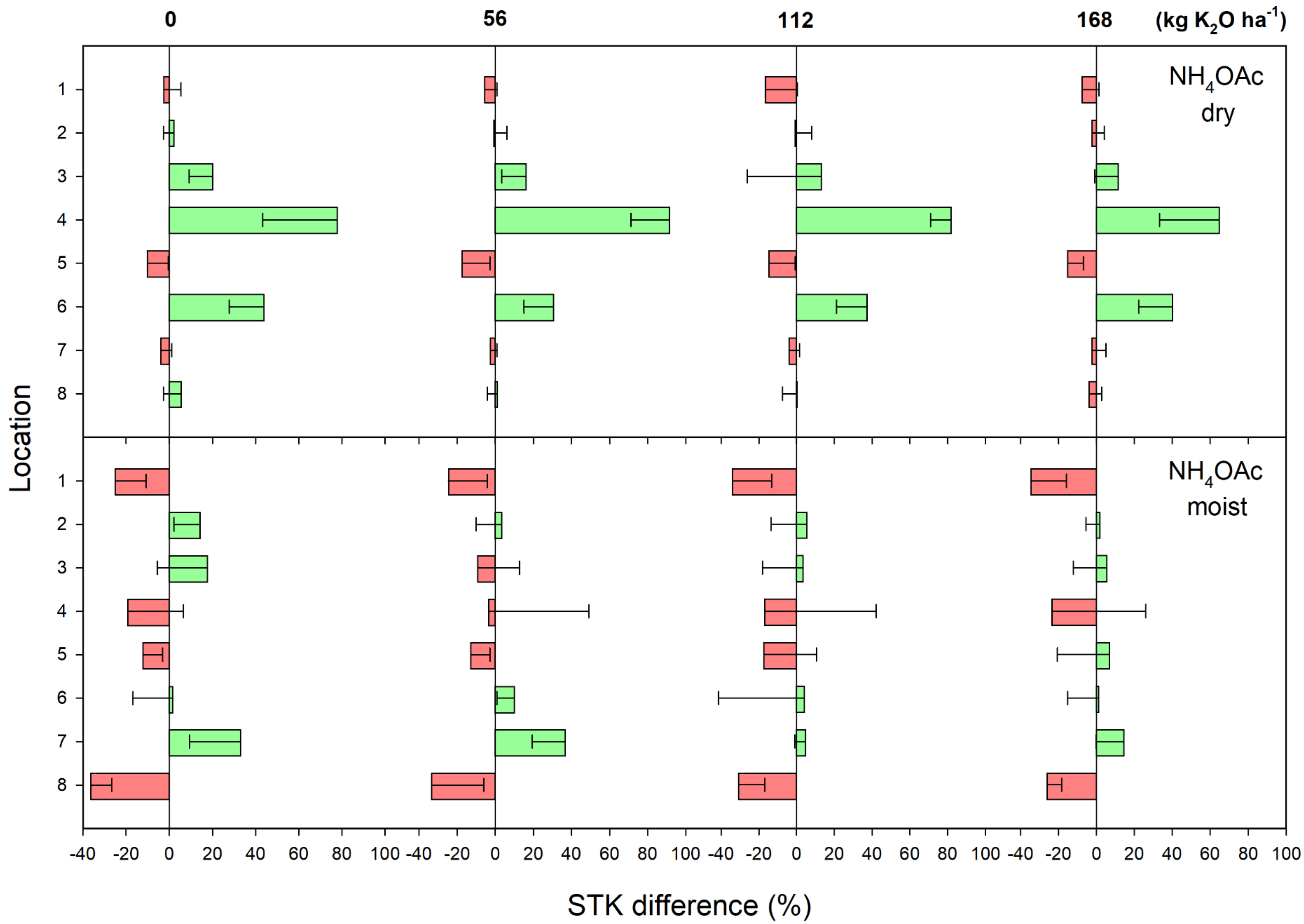

Figure 4-3. STK difference between fall and following spring sampling as affected by K rate for NH_4OAc dry and moist extractions in all locations (STK = 34-409 mg kg^{-1} NH_4OAc dry). Error bars represent 95% confidence level (direction towards zero).

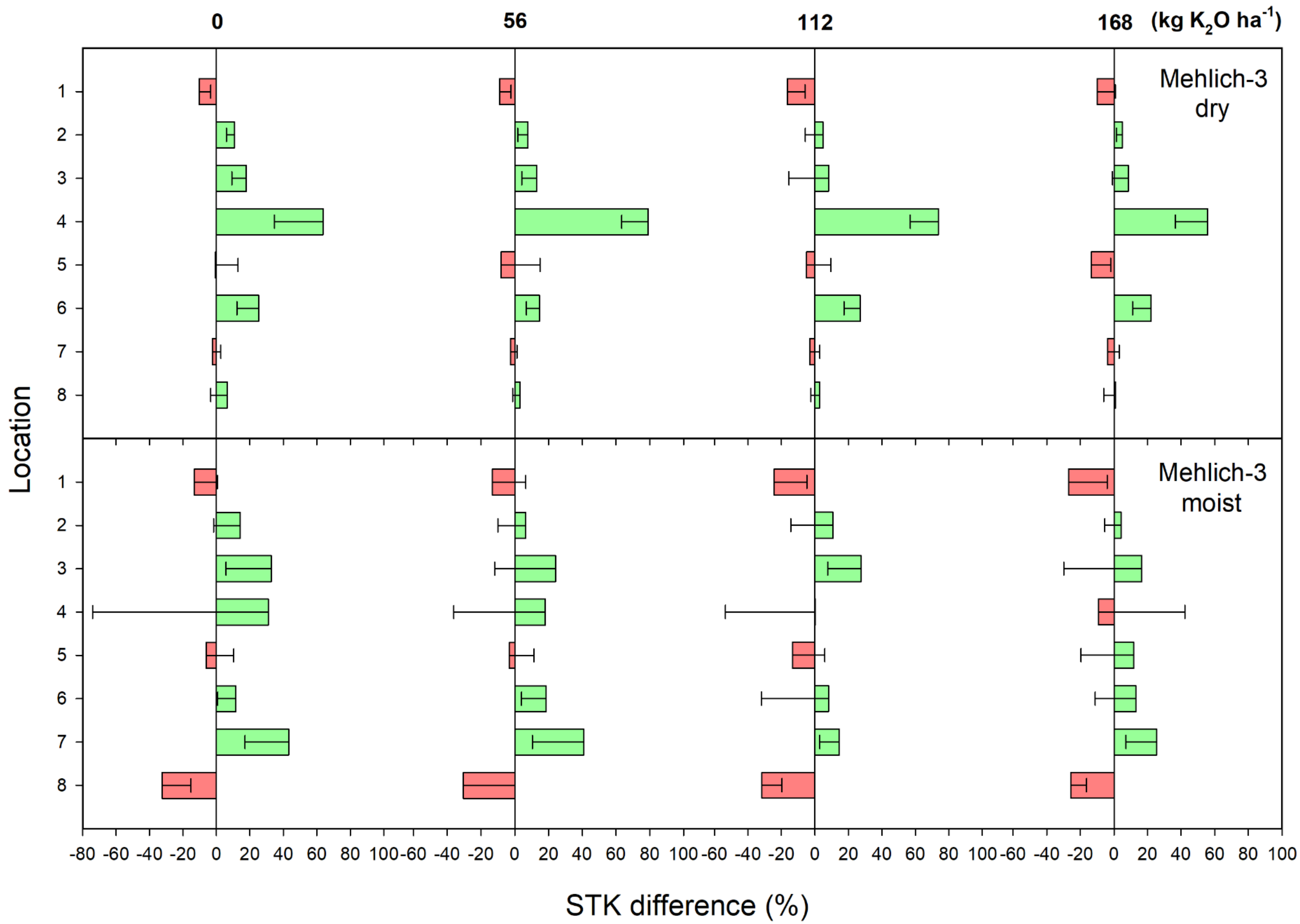

Figure 4-4. STK difference between fall and following spring sampling as affected by K rate for Mehlich-3 dry and moist extractions in all locations (STK = 46-479 mg kg^{-1} Mehlich-3 dry). Error bars represent 95% confidence level (direction towards zero).

Chapter 5 - General Conclusions

Understanding potassium availability to soybean plants can help predict future fertilizer needs to sustain higher yields. Results from our study show that there are several potential diagnostic tools for potassium management in soybean, but more research is needed in order to confirm our findings.

In general, CER strips buried in field conditions provided detailed information about soil K supply. This study found that CER tends to decrease in inverse proportion to plant K uptake, suggesting a measure of soil K surpluses because of root competition. Depending on each location, soil temperature and soil moisture content were highly correlated with CER adsorption in control plots. Preliminary results from this study suggest that CER can be used as an indicator of K supply, particularly in soils with low soil test K levels.

Regarding tissue analysis, our study developed reference values for tissue testing to address K deficiency on soybean. Looking at whole plants at V4 stage, K concentration and K/Mg ratio were similarly correlated to relative grain yield. Magnesium concentration gave us additional information but was less correlated than the variables mentioned before. The critical concentration range for K and K/Mg ratio was 16.4 to 18.0 g kg^{-1} and 2.3 to 2.4, respectively. Preliminary results from this study suggest that tissue analysis at early stages can be used as a diagnostic tool to assess the K status of the soybean crop. A new reference for K concentration was presented along with a K/Mg ratio that could be useful when sampling flexibility is needed. Soil testing is one of the best tools to identify responsive soils to K fertilizer. Overall, the NH_4OAc moist test was one of the best methods to estimate K availability in low testing soils; however, other non-conventional tests like $CaCl_2$ dry perform similarly but without the disadvantages of moist samples. Additionally, there was not a clear trend regarding STK changes between fall post-harvest and the subsequent spring sampling. More research is needed to find the best soil test method that captures soil potassium changes over time.