**CPSC444: Introduction to Spatial Analytics for Biological Sciences**

**Student: Christopher Mujjabi**

**Final Project Proposal**

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**Evaluation of the effect of Organic Nitrogen and Weed Pressure on Corn Root Architecture and Grain Yield Response.**

**ABSTRUCT**

Although the United States is the largest producer and exporter of corn and corn products worldwide, corn produced organically contributes only a small fraction of the total production. Organic production systems lack genetically superior corn varieties which can perform well in low-input and diverse organic management conditions. Approximately 95% of the crop varieties grown by organic farmers were bred specially for high input conventional production systems. However, these varieties lack the resilience, stability, and agronomic characteristics necessary for low input organic production. This study aims at developing high yielding, weed tolerant and high nitrogen use efficient corn cultivars for organic production through a participatory breeding approach with Midwest organic farmers. This conducted using a RCBD split-split plot arrangement with nitrogen treatments as the main plots, three hybrids as sub-plots and weed two treatments as split-split plots. Hybrid were phenotyped for root traits (root architecture and complexity) using a high throughput imaging technique as well as their agronomic performance under the different nitrogen and weed treatments. All hybrids showed increasing yields in response to increased N application rate. There was a positive correlation between the agronomic performance of the hybrids and their root architecture. This indicates that hybrids with a narrower root angle and a more vertical growth of the root system yield more than hybrids with a broad and horizontal root systems. Also, there was a negative correlation between the root complexity and agronomic performance. Hybrids with a more complex root systems yield less than hybrids with less complex roots systems.

**INTRODUCTION**

The domestication and improvement process of maize (Zea mays ssp. mays) has primarily been focused on the selection of certain above-ground traits which predominantly contribute to the agronomic performance but has barely considered below-ground phenes as a selection criteria for crop improvement (York et al., 2015). Research evidence shows an interaction between root canopy architecture and the plant’s ability to capture soil nutrients and resources which directly affect plant growth and yield in the long run (Malamy, 2005). Understanding the root system architecture and complexity is very essential since it influences the availability and accessibility of water and plant nutrients from the soil. The architecture of the root system has a direct effect on the accumulation of maize biomass and contributes to the yield response. (Gregory et al., 2009). Several studies demonstrate that root systems can adjust their architecture in response to changes in soil water and nutrient availability hence enabling plants to acclimate to certain harsh soil environments conditions, a phenomenon referred to as root developmental plasticity (Schmidt et al., 2016). Developmental plasticity of the root systems collects signals from the environment and incorporate them to the plant which consequently enable plants to regulate their root structural growth and development to efficiently optimize the use of the available soil resources (Melamy, 2005). Therefore, the study of root systems and canopy architecture might be a crucial tool with great potential for crop improvement programs.

However, breeders have neglected the use of root-related physiological traits to the maximum benefit of the crop improvement and breeding programs. This is because effective root growth recording and phenotyping methods often require destructive sampling of plant roots which is a laborious procedure since it involves digging and washing of large numbers of root systems (Bengough et al., 2004). Additionally, the lack efficient root screening methods limits the knowledge about root systems and functioning (Manschadi et al., 2006). Bengough et al (2004) conducted a study to phenotype corn root architecture and complexity using young barley seedlings grown in root observation gel chamber systems. However, this technique only allows the rapid measurement simple root traits (root length, elongation rate, seminal root number and root angular spread) during the plant early development stage. Although this method is non-destructive and allows repeated chronological digital measurement of the root system traits, it is difficult to grow and record enough plants in a single experiment (Gregory et al., 2009). Also, this technique can only record the early developmental stages of the root system and extrapolate the results to plant performance. According to Bengough et al (2004), the extrapolation of the results recorded from the early developmental stage of the plant in a gel chamber to predict plant performance is highly uncertain and might require a series of experiment to be reliable. Although the gel growth chamber technique is non-invasive and allows sequential digital phenotyping of root traits, it is a 2-dimension system which does not represent a complete 3-dimension spatial orientation of the root system which hinders exhaustive phenotyping and recording of all essential root traits (Gregory et al., 2009).

Other root system phenotyping methods being used include the X-ray computed tomography (CT) and nuclear magnetic resonance imaging (NMRI) (Heeraman et al., 1997), both with a capacity of visualizing the root architecture *in situ* reproduced in a three-dimension image without destroying the root system. However, their deployment involves elevated costs which limit their application in research (Smith and De Smet, 2012). However, all these techniques are highly artificial growth systems which do not reproduce or entirely mimic all the natural edaphic characteristics and the complex biotic and abiotic plant-soil interactions which influence root architecture and complexity (Trachsel et al., 2010). Consequently, these techniques do not entirely represent how plants and root systems would respond in normal field conditions. Therefore, due to the significance of the root system to crop productivity, a field-based phenotyping technique would accurately measure root characteristics and how their variation associates with plant performance and productivity.

In this study, a high throughput image analysis technique reported by Grift et al. (2011) was used to evaluate the root architecture and complexity of a core set of hybrids in an organic on-farm trial-based experiment. The hybrids were subjected to three levels of organic nitrogen application and two weed pressure treatments. The complexity of a root system was evaluated based on the number of branching points per unit of soil volume (fractal dimensions), where a highly branched system is complex and the unbranched is simple (Grift el al., 2011). On the other hand, the root architecture is measured by the root angle of the root system. The study aims at;

* Assessing how nitrogen application and weed pressure affects the architecture and complexity of the root system
* Examining major correlations between the root system traits and the agronomic performance (yield) of the corresponding maize hybrids.

**MATERIALS AND METHODS**

**Experimental Design**

This experiment was conducted in 2 locations (South Central and Western Illinois) with 4 replications (blocks) per location using a RCBD with a split-split plot arrangement where 3 nitrogen treatments (0 Kg N/ha, 113 Kg N/ha and 224 Kg N/ha were the main plots, 3 hybrids planted in 4 row split-plots and 2 weed treatments (with-weeds and without-weeds) as split-split plots. Under the “with-weed” split-split plots, sorghum seeds were spread in the corn rows to impose a controlled high-weed pressure while in the “without-weed” plots, manual weeding was done to reduce the weed pressure on the corn hybrids. To evaluate the root architectural characteristics, 3 consecutive plants were uprooted from the second row of each split-split plot during the R2 growth stage. The roots were cleaned, and root images were taken with two mono-chrome cameras. The root images were processed using a special soft-ware to generate a dataset corresponding to the root architectural characteristics (root angle and fractal dimension) of each of the 3 hybrids under study. In addition, all the split-split plots were harvested using a combine harvester and the yield of each hybrid was recorded to evaluate the relationships between their agronomic performances under the different nitrogen and weed treatment combinations with the root architectural characteristics.

**Statistical Analysis**

To assess how nitrogen application and weed pressure affects the architecture and complexity of the root system, the response variable for this question is the root top angle (RTA) and fractal dimension (FDTop) as a measure of root architecture and root complexity respectively. The explanatory variable would be the 3 corn hybrids planted in each treatment combination of the experiment. Since the experiment was conducted in a RCBD with a split-split plot arrangement. The model used during analysis is shown below:

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Where;

is the root architectural score recorded for the ith location in the jth block nested in the ith location, the kth nitrogen treatment and the lth hybrid from the mth  weed treatment

is the grand population mean

is the random effect of the ith location, NID;

is the random effect of the jth block nested in the ith location, NID;

is the fixed effect of the kth nitrogen treatment,

Is the random effect of the interaction between the Ith location and the kth nitrogen treatment NID

Is the random error term 1 NID

is the fixed effect of the Lth hybrid

is the random effect of the hybrid and location interaction NID

is the fixed effect of the nitrogen treatment and hybrid interaction

is the random effect of the location, nitrogen treatment and hybrid interaction NID

is the random error term 2 NID

is the fixed effect of the mth weed treatment

is the random effect of the location and weed treatment interaction NID

is the fixed effect of the nitrogen and weed treatment interaction

is the fixed effect of the hybrid and weed treatment interaction

is the random effect of the location, nitrogen and weed treatment interaction NID

is the fixed effect of the nitrogen treatment, hybrid and weed treatment interaction

is the random effect of the location, hybrid and weed treatment interaction NID

is the random effect of the location, nitrogen treatment, hybrid and weed treatment interaction NID

is the random error term 3 NID

This model was run for each root trait evaluated (Root top angle and fractal dimension) as well as for the yield of each hybrid in each treatment combination or experimental unit.

Also, a linear regression using the PROC CORR command in SAS was used to examine major correlations between BLUPS of the root system traits and the agronomic performance (yield) of the corresponding maize hybrids.

The ggplot2 library in R Studio was used for the graphical representation of all the trends for yield and root system characteristics for all the treatment combinations.

**RESULTS AND DISCUSSIONS**

**Analsysis of the root top angle (root architecture).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type 3 Tests of Fixed Effects | | | | |
| Effect | Num DF | Den DF | F Value | Pr > F |
| LOCATION | 1 | 96.2 | 0.52 | 0.4739 |
| HYBRID | 2 | 96.3 | 3.06 | 0.0515 |
| LOCATION\*HYBRID | 2 | 96.3 | 0.54 | 0.5831 |
| NTRT | 2 | 9.04 | 0.13 | 0.8821 |
| LOCATION\*NTRT | 2 | 96.2 | 1.03 | 0.3607 |
| NTRT\*HYBRID | 4 | 96.3 | 0.2 | 0.9397 |
| LOCATION\*NTRT\*HYBRID | 4 | 96.3 | 2.84 | 0.0283 |
| WTRT | 1 | 96.3 | 0.51 | 0.4765 |
| LOCATION\*WTRT | 1 | 96.3 | 0.16 | 0.6875 |
| HYBRID\*WTRT | 2 | 96.2 | 0.71 | 0.492 |
| LOCATION\*HYBRID\*WTRT | 2 | 96.2 | 0.03 | 0.971 |
| NTRT\*WTRT | 2 | 96.3 | 0.35 | 0.703 |
| LOCATION\*NTRT\*WTRT | 2 | 96.3 | 0.04 | 0.9629 |
| NTRT\*HYBRID\*WTRT | 4 | 96.2 | 1.27 | 0.2866 |
| LOCA\*NTRT\*HYBRI\*WTRT | 4 | 96.2 | 0.54 | 0.7038 |

**Table 1.** Anova table for root top angle (root architecture).

There was a significant difference in the root architecture of the three hybrids. The three way interaction of nitrogen, weed treatment and location had a significant effect on the architecture of the root systems.

**Analysis of the top fractal dimension (root complexity)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type 3 Tests of Fixed Effects | | | | |
| Effect | Num DF | Den DF | F Value | Pr > F |
| LOCATION | 1 | 79.3 | 3.28 | 0.0739 |
| HYBRID | 2 | 5.93 | 13.21 | 0.0065 |
| NTRT | 2 | 6.1 | 1.27 | 0.3449 |
| WTRT | 1 | 79.2 | 1.52 | 0.2212 |
| LOCATION\*HYBRID | 2 | 79.2 | 5.11 | 0.0082 |
| LOCATION\*NTRT | 2 | 79.3 | 1 | 0.3708 |
| NTRT\*HYBRID | 4 | 12.3 | 2.1 | 0.1427 |
| NTRT\*WTRT | 2 | 79.2 | 2.41 | 0.0966 |
| LOCATION\*WTRT | 1 | 79.2 | 0.34 | 0.5638 |
| HYBRID\*WTRT | 2 | 79.3 | 0.42 | 0.6599 |
| LOCATION\*HYBRID\*WTRT | 2 | 79.3 | 0 | 0.9992 |
| LOCATION\*NTRT\*HYBRID | 4 | 79.2 | 0.95 | 0.4409 |
| LOCATION\*NTRT\*WTRT | 2 | 79.2 | 0.86 | 0.4279 |
| NTRT\*HYBRID\*WTRT | 4 | 79.2 | 0.75 | 0.5585 |
| LOCA\*NTRT\*HYBRI\*WTRT | 4 | 79.2 | 1.03 | 0.3964 |

**Table 2**. Anova Table for Fractal dimension of the root systems (root complexity).

There was a significant different between the root complexity of the hybrids in the two location. There nitrogen and weed treatment combination had a significant effect of the complexity of the root system. Also, the location effect on the root complexity was relatively significant.

**Analysis of the Yield performance**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type 3 Tests of Fixed Effects | | | | |
| Effect | Num DF | Den DF | F Value | Pr > F |
| HYBRID | 2 | 6.67 | 78.44 | <.0001 |
| NTRT | 2 | 8.17 | 3.33 | 0.0874 |
| NTRT\*HYBRID | 4 | 106 | 0.32 | 0.8627 |
| WTRT | 1 | 105 | 2.04 | 0.1566 |
| HYBRID\*WTRT | 2 | 105 | 0.05 | 0.9471 |
| NTRT\*WTRT | 2 | 104 | 0.09 | 0.9114 |
| NTRT\*HYBRID\*WTRT | 4 | 104 | 0.89 | 0.4711 |

**Table 3**. Anova Table for the yield of the three hybrids.

There was a significant difference between the yield performances of the hybrids under the different nitrogen treatments. However, there was no significant difference between the different weed treatments.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| EFFECT | NTRT | HYBRID | WTRT | RTA | FDTop | YIELD |
| NTRT\*HYBRID\*WTRT | N1 | A | NW | 86.549 | 1.8871 | 4606.96 |
| NTRT\*HYBRID\*WTRT | N1 | B | NW | 81.0464 | 1.8841 | 7199.96 |
| NTRT\*HYBRID\*WTRT | N1 | C | NW | 88.0214 | 1.8738 | 7276.08 |
| NTRT\*HYBRID\*WTRT | N1 | A | WW | 80.4102 | 1.8915 | 4409.18 |
| NTRT\*HYBRID\*WTRT | N1 | B | WW | 89.0471 | 1.8754 | 6803.95 |
| NTRT\*HYBRID\*WTRT | N1 | C | WW | 86.6562 | 1.8688 | 7012.3 |
| NTRT\*HYBRID\*WTRT | N2 | A | NW | 83.6479 | 1.879 | 4805.49 |
| NTRT\*HYBRID\*WTRT | N2 | B | NW | 83.5259 | 1.8669 | 7289.78 |
| NTRT\*HYBRID\*WTRT | N2 | C | NW | 91.7121 | 1.8574 | 7461.44 |
| NTRT\*HYBRID\*WTRT | N2 | A | WW | 82.0982 | 1.8816 | 4663.24 |
| NTRT\*HYBRID\*WTRT | N2 | B | WW | 86.8331 | 1.866 | 7311.2 |
| NTRT\*HYBRID\*WTRT | N2 | C | WW | 88.6432 | 1.8577 | 6785.79 |
| NTRT\*HYBRID\*WTRT | N3 | A | NW | 84.8092 | 1.895 | 5608.86 |
| NTRT\*HYBRID\*WTRT | N3 | B | NW | 88.3369 | 1.8756 | 7966.46 |
| NTRT\*HYBRID\*WTRT | N3 | C | NW | 93.5683 | 1.8299 | 7334.54 |
| NTRT\*HYBRID\*WTRT | N3 | A | WW | 87.6188 | 1.8999 | 5239.61 |
| NTRT\*HYBRID\*WTRT | N3 | B | WW | 81.5502 | 1.8884 | 7478.83 |
| NTRT\*HYBRID\*WTRT | N3 | C | WW | 86.3584 | 1.8639 | 7798.86 |

**Table 4.** BLUPS of RTA, FDTop and Yield for each hybrid under each treatment combination

**Correlations between root characteristics and yield (correlations between BLUPS)**

|  |  |  |  |
| --- | --- | --- | --- |
| Pearson Correlation Coefficients | | | |
|  | YIELD | FDTOp | RTA |
| YIELD | 1 | -0.42336 | 0.52656 |
| FDTOp | -0.42336 | 1 | -0.67332 |
| RTA | 0.52656 | -0.67332 | 1 |

**Table 5.** Correlations of the BLUPS of each variable

**CONCLUSIONS**

There is a positive correlation between agronomic performance and root architecture. The yield of the plant is influenced by the depth and narrowness of the root system. Hybrids with a deep and vertical root system yielded more than hybrids with a horizontal and shallow root system. On the other hand, there is a negative correlation between the root complexity and the agronomic performance of the hybrids. Hybrids with more complex (more branched) root systems yielded less than the hybrids with a less complex root system.

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