i-ACES Vol. 1 No. 1 (2014)
Inquiry in ACES: An Undergraduate Research Journal
Special Edition: Research Apprentice Program 2014
College of Agricultural, Consumer and Environmental Sciences
University of Illinois at Urbana-Champaign

i-ACES
{inquiry-ACES: Highlights of Undergraduate Research in ACES}

Evaluating the Complexity of the Embryogenic Root System

Nalysha Torres[1], Tony Grift[2], Martin Bohn[2,3]
[1]Research Apprentice Program Student Participant
[2]University of Illinois, Urbana-Champaign, IL, USA
[3] mbohn@illinois.edu, Department of Crop Sciences, University of Illinois, Urbana-Champaign, IL, USA

ARTICLE INFO

Article history:
Received 30 July 2014
Accepted 30 September 2014

Keywords:
Maize,
Root system;
Complexity;
Diversity

ABSTRACT

Roots are of key importance for plant development and productivity. Roots are responsible for the uptake of water and nutrients, as well as stability. Complex root systems are very important for the overall plant development. In this study, we evaluated the complexity of the embryogenic root system, i.e., the root system which develops from the seed during germination, by measuring the primary root length (PRL) and the number of seminal roots (NSR). To determine the complexity of the primary root system seed of a genetically highly diverse set of maize inbred lines (N=50) were germinated for six days. Subsequently, the embryogenic root systems were imaged, the image processed, and analyzed for their PRL and NSR. The maize inbred lines significantly ($P<0.05$) differed for PRL and NSR revealing a strong genetic basis for both traits. Interestingly, we showed that inbred line geographic origin and breeding program explained a significant part of the diversity for root complexity within the diverse set of maize inbreds.

INTRODUCTION

Corn is the first most consumed crop in the U.S. and many people and livestock depend on it as their main source of calories. Roots are the base of plants and are strongly related to plant development. Root systems of terrestrial plants serve many important tasks among which anchorage of the plant, and uptake of water plus nutrients are the most important ones (Aiken and Smucker, 1996). The amount of water and mineral nutrients available for the plant depends on how much soil is occupied by its root system. Complex root systems, in terms of architecture, morphology, and structure, are more likely to grow into a healthier plant that will have better yield results. Also, this trait helps the plant to survive under biotic (e.g., pests, microorganisms, or weeds) and abiotic (e.g., pesticide, environment or nutrient problems) conditions.

Root complexity is determined by the primary root and a variable amount of seminal roots. Conventional methods cannot provide accurate information about root expansion or complexity. The fractal dimension (FD) has been shown suitable to describe the complexity of natural as well as abstract objects (Mandelbrot, 1983). A fractal is an object that displays self-similarity on all scales. They are created by repeating a simple process over and over in an ongoing feedback loop. Nonetheless, root complexity and development depend on genetic and environmental factors and their interactions (O'Toole and Bland, 1987).

A limited number of genetic studies is available relating root architecture and development with yield, root lodging, and tolerance to stresses under field conditions (Bohn et al., 2006). To genetically improve root structure in the future, there is a need to identify the genes that govern root complexity (Zhong et al., 2009). Genome-wide association (GWA) mapping is useful in modern genetics to identify genes or narrow regions in the genome that contribute to genetically complex phenotypes. This present lack of knowledge greatly

affects the crop science field.

For that reason, the overall goal of this investigation was to provide important insights into the diversity of maize root architectural characteristics at an early developmental stage. The specific objectives of this study were to:

1. Evaluate the complexity of the embryogenic root system of a large and highly diverse set of maize inbred lines (N=50),

2. Determine the association between traditional and innovative root characteristics,

3. Estimate the genetic variability between maize inbred lines for their embryogenic root complexity.

Subsequently, this study will be the first step to locate genes involved in the inheritance of maize root architecture and will supply the breeders with the knowledge necessary to design selection strategies for optimizing root system efficiency. We hypothesized that maize inbred lines significantly differ for root architectural traits and that these differences are associated with the inbreds geographic origin and breeding program.

LITERATURE REVIEW

What you see above ground in your plants is really determined by what happens underground, where the plant roots live. The bigger and healthier the root system, the bigger and healthier the plant. In the present paper, it is intend to explore the diversity of maize root characteristics. In order to do that, different phenotypes of corn roots will be compared. It is hypothesized that since every corn plant is genetically different, so will be their root systems. Hence, plant roots, corn root architectural complexity, and past researches were investigated. The following references were analyzed to govern and sustain this paper with background information. Furthermore, to prove and support the hypothesis.

Importance of Roots for Plant Development

As stated by Waisel et al. (2002) in “Plant Roots; The Hidden Half”, root systems of any plant are expected to fulfil two primary functions: the acquisition of soil-based resources (e.g., water and ions), and anchorage. Along with those functions, roots also have other secondary functions such as: storage, production of growth regulators, propagation - process of reproducing or breeding- and dispersal. Complex root systems of modern plants are now seen as achieving the more effective performance of these overall functions. The scarce variation in external features of roots is presumably related to the limited range of variation of the root environment. Unlike other parts of the plant, roots show few distinctive external features that would permit identification. Nevertheless, external features of the roots can be identified by its complexity. Root complexity is defined as the number of root branching points per soil volume.

Corn Root Imaging Box

This study was possible because of a high-throughput image analysis system composed of integrated hardware and software components to evaluate root complexity. In 2011, professors in the Departments of Agricultural and Biological Engineering and Crop Sciences at the University of Illinois developed the Corn Root Imaging Box (CRIB). This imaging box provides the necessary amount of light to prevent shadows when taking images. According to the researchers, this instrument can take up to 600 images of maize roots per day. The digital cameras of the CRIB are computer controlled. Once the picture is in the computer, the CRIB is able to evaluate root complexity by using MATLAB® and other software. MATLAB® is a high-level language and interactive environment for numerical computation, visualization, and programming. This program can be used for image and video processing, control systems, test and measurement, computational biology, and others. “More than a million engineers and scientists in industry and academia use MATLAB®, the language of technical computing.” (MATLAB® official page).

Study Related to the Present Research

In 2006, Bohn et al. investigated the inheritance of the primary root system complexity using 231 recombinant inbred lines derived from the IBM (B73×Mo17) population. Digital images were taken after four and eight days of germination and the fractal dimensions were calculated for each root system. Researchers found significant differences in the FD between the different days of imaging. As a result, a certain amount of QTLs were founded on all ten chromosomes explaining a large proportion of the

phenotypic variance. Differing with Bohn et al. (2006), the present research intends to evaluate root complexity variation using the primary root length and number of seminal roots.

METHODOLOGY

This experiment was conducted following procedures outlined by Bohn et al. (2006). Unlike these researchers, this investigation was run with a predetermined amount of 50 inbred lines (ILs) selected from a diverse set of inbreds (N=300). During the selection process all kinds of genotypes were selected including Tropical inbreds, inbreds from the U.S. Midwest, field corn, sweet corn, and popcorn varieties. These inbreds were developed in breeding programs from South Africa, Thailand, Mexico, and Canada.

Experimental Design

We applied an incomplete block design to efficiently control for variable growing conditions in the growing chamber (see below). The 50 ILs were subdivided into ten blocks. Each block containing five genetically different ILs. Two replications were used.

Sterilization and Germination Procedure

The 500 seeds (10×genotype) were divided into two repetitions (REP) using 5 seeds per genotype for each REP. Afterwards, all seeds were surface sterilized in 60 ml of commercial 6% Clorox® for 10 minutes. After this treatment, the seeds were washed three times with distilled water (DW) and placed (5 seeds of the same genotype) in the upper third of a nontoxic germination paper. These were placed with the embryo facing down and the space was maximized to prevent contact between different root systems. The germination paper was moisturized with a Captan® (BAYER) 2.5 g l^{-1} solution. Thereafter, the germination paper was rolled up vertically and placed into a 2 l plastic bucket with 1/3 l of Captan® solution (1500 mL DW + 0.99g Captan®). The idea of conducting this procedure is to minimize infection with fungus.

Subsequently, all the buckets were placed into a sterile growth chamber with 0% illumination at 25^{o} C and 100% relative humidity. The amount of Captan® solution was maintained at 250 ml during 6 days until the germination process was over.

Data Collection

After 6 days of germination the new root systems were evaluated. To make this process more flexible, images of each root system were taken. The images were obtained using the Corn Root Imaging Box (CRIB) (Grift et al., 2011). The color model of the CRIB was changed into a gray scale image to refine the details of the root system. Moreover, the background surface and the light inside the imaging box were taken into consideration to improve the quality of these pictures. Afterwards, a MATLAB software package was used to evaluate the digital images of the maize embryogenic root systems.

The pictures of each root system were interpreted. Using a Matlab® subroutine the length of each embryogenic root system (PRL) was measured. This data was registered with its corresponding genotype number and seed number for further analysis.

Since root complexity is described by the amount of branching points, the number of seminal roots (NSR) is an important trait when evaluating complexity. Therefore, this trait was also evaluated.

Statistical Analysis

The data for the statistical analysis consisted NRS and PRL measurements obtained from 436 images taken of healthy embryogenic root systems. An analysis of variance (ANOVA) was conducted using procedures of the SAS software package. The effect of inbred lines, their geographic origin, and breeding program on root characteristics were tested using appropriate statistical models. The relationship between PRL and NSR was evaluated conducting a correlation analysis. A multivariate analysis was performed to group the used set of maize inbreds into root complexity groups.

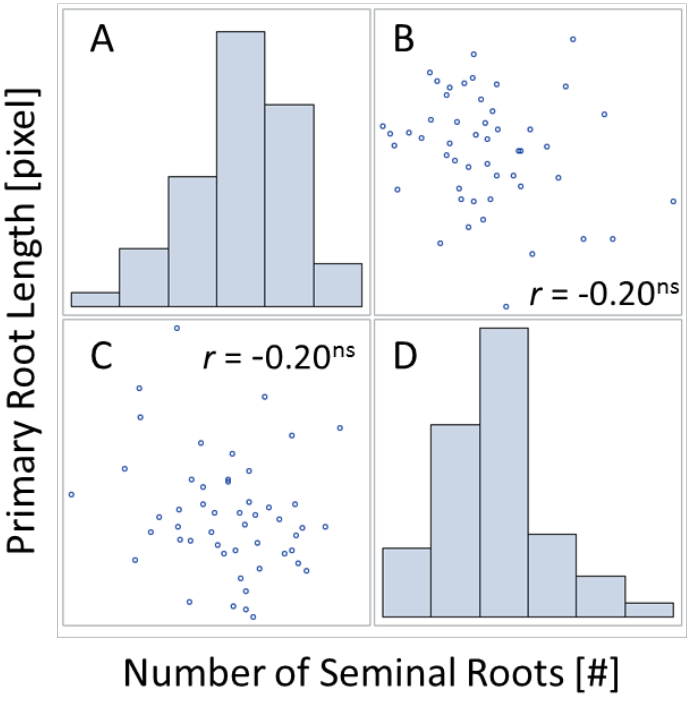
FINDINGS

Maize inbreds significantly differed ($P<0.001$) for PRL and NSR after six days of germination (Fig. 1, A, D). A combined analysis of NSR and PRL revealed three contrasting root morphology classes (see Table 1 Clusters 1 to 3).

Moreover, it was shown that the structure (geographic origin) of the inbred line has no impact on the length of the primary root. In addition, breeding

programs with extreme growing conditions showed relatively shortness in the primary root length. However, in Table 2 is shown that the geographic origin and breeding program of the tested inbred lines significantly ($P<0.001$) affected expression of NSR (Table 2). It was conclude that the Iowa program displays the smallest NSR as the South Africa program shows the largest number and the inbreds from adapted to tropical growing conditions express a high number of seminal roots of 4.4 and Sweet Corn displays a particularly small number of seminal roots with NSR=1.0.

Figure 1
Distribution (A, D) of maize inbred lines (N=50) for primary root length (PRL) and number of seminal roots (NSR) and scatter plots (B, C) showing the relationship between PRL and NSR.

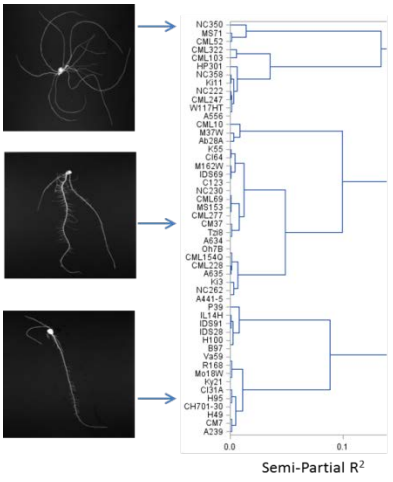


Root traits NSR and PRL were not significantly correlated (Fig.1, B, C). However, results suggest a negative relationship between both traits.

As observed in our set of maize inbred lines, roots tend to fall into three contrasting root complexity classes:

Class 1 Long but not high NSR
Class 2 High NSR but not long
Class 3 Medium length and medium NSR

Table 1
Dendrogram of 50 diverse maize inbreds revealed by UPGMA cluster analysis based on embryogenic root characteristics primary root length (PRL) and number of seminal roots (NSR). For each cluster PRL and NSR means are provided. Means with different letters within a column are significantly different at a 0.05 probability level.



	PRL [pixel]	NSR [#]
Cluster 1	906^{A}	4.1^{A}
Cluster 2	1108^{B}	4.0^{A}
Cluster 3	1188^{C}	2.2^{B}

Table 2
Mean of primary root length (PRL) and number of seminal roots (NSR) for various breeding programs and the complete set of 50 maize inbreds. Representative root images are provided to demonstrate root characteristics for each breeding program.

Program	#	PRL [pixel]	NSR [#]	Representative Root Samples
Iowa	1	1126	1.4	
Michigan	2	994	4.8	
Mexico	9	1029	4.5	
South Africa	2	1098	5.4	
Mean		1086	3.4	
Min-Max		587-1442	0.4-9.5	
STD		175	1.7	

The experimental set up was efficient to evaluate differences within a large number of embryogenic root systems (N=50 ILs). In general, fungi infections of plants raised in growth chambers are a common obstacle. However, our protocols for sterilizing the seed, germination paper, and growth chamber shown to have immensely minimized the levels of fungus and other bacteria. At the moment of the analysis, 435 out of 500 images (87%) were registered as useful. This number (N=435) prove that the sterilization process was efficient.

The used set of maize inbred lines was highly diverse. It comprised field corn, sweet corn, and popcorn varieties, as well as materials from South Africa, Thailand, Mexico, Canada, and the USA. Given this diversity we were not surprised that root characteristics reflected this diversity. Our analysis showed that geographic origin and the specific breeding program significantly influenced expression of root traits. It is interesting to note that roots from inbreds adapted to the growing conditions in the U.S. Midwest are on average long and show low seminal root numbers, whereas inbreds adapted to more extreme environments, like northern growing regions in the U.S. or tropical environments, display root systems with significantly larger number of seminal roots. Whether this observation is also reflected by the three classes of root shapes ("Root complexity classes") revealed by a multivariate analysis of PRL and NSR (Table 1) needs to be further investigated. Based on our findings in this study, we can formulate a hypothesis that embryogenic roots of maize cultivars adapted to harsher growing conditions (e.g., cold temperatures or dry conditions during germination) show short primary roots but an increased number of seminal roots. We suggest evaluating the embryogenic root systems of additional maize inbred lines to accommodate the prerequisites of a genome-wide association mapping study with the goal to identify genes involved in the inheritance of root traits.

CONCLUSIONS

Accordingly to the results' interpretation we can conclude that PRL is governed by the genetic composition of the corn plant. Furthermore, it seems that NSR still need deeper investigation. Moreover, we can hypothesize that NSR increase in extremes conditions to explore a larger soil space. Since the plant is growing in stressed conditions it could be possible that this number increases as a response in order to survive this climate. Since PRL and NSR showed a negative correlation, it would be great to explore the relations shown in Table 1 (Cluster 1 to 3) in the further research.

ACKNOWLEDGEMENTS

I would like to acknowledge my mentor Dr. Martin Bohn, as well as, Nicole Yana for their guidance during the past seven weeks. Also, thanks to Dr. Tony Grift for supplying my research with the needed programs and software. Moreover, Ana Martin-Ryals deserve to be congratulated for being with me and my peers through this great experience helping us achieve our goals and complete our requirements. And thanks to the RAP program and all its staff for this research opportunity that will help us exponentially in the future.

REFERENCES

Aiken, R.M., and A.J.M. Smucker. 1996. Root system regulation of whole plant growth. Annu. Rev. Phytopath. 34:325-347

Bohn, M., J. Novais, R. Fonseca, R. Tuberosa, and T. Grift. 2006. Genetic evaluation of root complexity in maize. Acta Agronomica Hungarica 54:291-303.

Grift, T., J. Novais, and M. Bohn. 2011. High-throughput phenotyping technology for maize roots. Biosystems Engineering 110:40-48.

Mandelbrot, B.B. 1983. The fractal geometry of nature. Freeman, San Francisco.

MATLAB.. - The Language of Technical Computing. Retrieved July 30, 2014, from http://www.mathworks.com/products/matlab.

O'Toole J.C. , and W.L.Bland WL.1987. Genotypic variation in crop plant root systems. Advances in Agronomy 41: 91–145.

Waisel, Y., Eshel, A., & Kafkafi, U. (2002). Plant roots the hidden half (3rd ed.). New York: M. Dekker.

Zhong, D., J. Novais, T.E. Grift, M. Bohn, and J. Han. 2009. Maize root complexity analysis using a support vector machine method. Comput. Electron. Agric. 69:46-50.

This special edition of *iACES* is composed of articles written by high school students enrolled in the College of ACES Research Apprentice Program (RAP), University of Illinois. We gratefully acknowledge the many faculty, staff, and graduate student mentors who account for the success of RAP, its Director, Dr. Jesse Thompson, as well as its generous sponsors. Further appreciation extends to Emily Mayhew for her editorial assistance.