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DATA NOTE **Open Access**

Seedling growth rate and root traits in the maize Nested Association Mapping (NAM) panel

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Abstract

This paper characterizes variation in shoot and root traits collected from the founders of the Maize Nested Association Mapping panel, which was designed to maximize genetic diversity while ensuring appropriate flowering in eastern North America. Here, we present a detailed account of greenhouse experiments conducted by four cohorts of undergraduate research interns at the University of Hawaiʻi at Mānoa. We summarize data collection, data cleaning procedures, and present data for 38 phenotypic variables for 24 genotypes with the number of plant replicates ranging from 3 to 20. The genotype B73 served as our experimental control to enable comparison over the four years. We also grew a subset of genotypes under different abiotic stress treatments to assess the phenotypic plasticity. These data can be used to predict the potential for different lines to function and capacity to adapt to different environments. Data are published on GitHub repositories, and have large reuse potential by the scientific community, as well as educators of undergraduate and graduate instruction.

Keywords Undergraduate research, Phenotypic diversity, Nested association mapping population parents, Tropical, Temperate, Phenotyping

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Objective

Maize (*Zea mays*) is a global staple produced on every continent. It is used in numerous products, serving as a vital food source and a major raw material with many uses. One way to better understand variation between maize genotypes is to explore diversity collections such as the Maize Nested Association Mapping (NAM) population, a structured genetic resource comprising 25 diverse parental maize lines crossed with the common reference line B73 [9]. The NAM founders represent temperate and tropical genotypes that were sourced from Asia, Africa, and the Americas [4, 5]. Exploring the extent of phenotypic variation associated with specific genotypes provides an expectation of the range of phenotypic plasticity these novel traits exhibit [10]. Variability in phenotypes via environmental conditions (i.e., climatic season, condition) informs potential responses to unknown environments [15]. Understanding variation and sources of variation may help deal with future food (in)security as crop production is expected to shift to different regions under future climate scenarios [11]. Since genetic diversity is the raw material for both natural and human-mediated selection [1], it will be imperative to understand plant traits, such as the unique growth rate, and root morphology to select genotypes that will withstand seedling stress and avoid disease [2, 6].

There has been much effort to explore the above ground characteristics [3], but there has been less effort with respect to the roots and growth rate. For instance, while it is well known that there are tremendous intraspecific differences in root traits, the variation between cultivars is not well quantified nor is it very comprehensive [12, 16]. Therefore, we need to explore the variation in shoot and root traits across diverse plant material. Here we characterize the seedling shoot and root traits of 24 NAM population parents and connect these traits to variation in aboveground seedling growth rate. We demonstrate that undergraduate researchers can be an integral part of generating datasets that provide baseline information on variability for important traits. Through this effort, we also developed methodology for standardizing data collection and analysis in multiple years by different cohorts of researchers.

Data description

Data were generated as part of an REEU cohort program [8]. Plants were grown in a completely random design with 3–20 replicates depending on survival. Within each treatment group, 24 of the 26 NAM founder parents were grown over the course of the program. The general experimental workflow can be seen in Fig. 1.

Leaf elongation rates (LER) were taken when leaf #4 emerged from the whorl and growth reached a steady state (three days with no change in growth rate). Every 24 h, leaf #4 was measured as the distance from the insertion point of leaf #1 at the base of the plant to the tip of leaf #4. Plants were dissected and leaf blade length, leaf blade width, and leaf sheath length were measured on leaf #1–4, scanned, segmented (conversion of image into different parts for analysis), and analyzed with the ImageJ software [13]. Roots were harvested from each pot and each part of the root system was scanned using an Epson v850 scanner. Images were analyzed with RhizoVision Explorer 2.0.3 software [14]. Trait distributions were visualized using ggplot2 [17] showing large variation in root and leaf traits within and across years (Figs. 2, 3 and 4). There were also significant differences in the amount of within genotype variation. The amount of variation was a result of both the differences between the genotypes and the differences between the environments. There are clear differences between genotypes as seen by the confidence intervals around the slope and asymptote of the linear plateaus. For each treatment and condition, distributions were created for leaf 2–4, showing a large within and across year variation in root traits (Fig. 3). There was also variation within genotypes (Fig. 4). To check the technical validity, we explored variation in the control inbred line B73 (Fig. 5).

Data set 1 contains records of the blade dimensions (width, length, surface area) and sheath length of leaf #1–5 from 2021 to 2024 of 20 genotypes (Fig. 6), across growth treatments (control, growth, shade) from 11 geographic regions (Iowa, Mexico, North Carolina, Kwazulu Natal, Ohio, Indiana, Texas, Missouri, Thailand, Michigan, Kentucky) and five genetic origins (stiff stalk, subtropical, mixed, non-stiff stalk, and popcorn) grouped by climate zone (temperate or tropical). Data set 2 contains records of root types (crown, primary, seminal) and trait measurements of 21 genotypes from data collected between 2021 and 2024 across growth treatments from 11 geographic regions and five genetic origins grouped by climate zone. The Data Set 3 file contains the leaf measurements (mm) of leaf #4 with daily measurements until cessation from 2021 to 2023 of 20 maize genotypes across growth treatments from 11 geographic origins and five genetic origins. In 2024, leaf measurements (mm) of leaf #5 were measured daily until cessation. Data Set 4 contains the growth rate (mm) of 24 genotypes collected between 2021 and 2024 of leaf #4 (i.e., the plant's fourth leaf with visible leaf collars, beginning with the lowermost, short, rounded-tip true leaf and ending with the uppermost leaf with a visible leaf collar) or leaf #5 with duration of days until cessation, height in mm at start of leaf growth across season (fall, spring, summer) and treatment. All the treatments and growing seasons can be seen in Data Set 5, and definitions of phenotypes can be seen in Data Set 6.

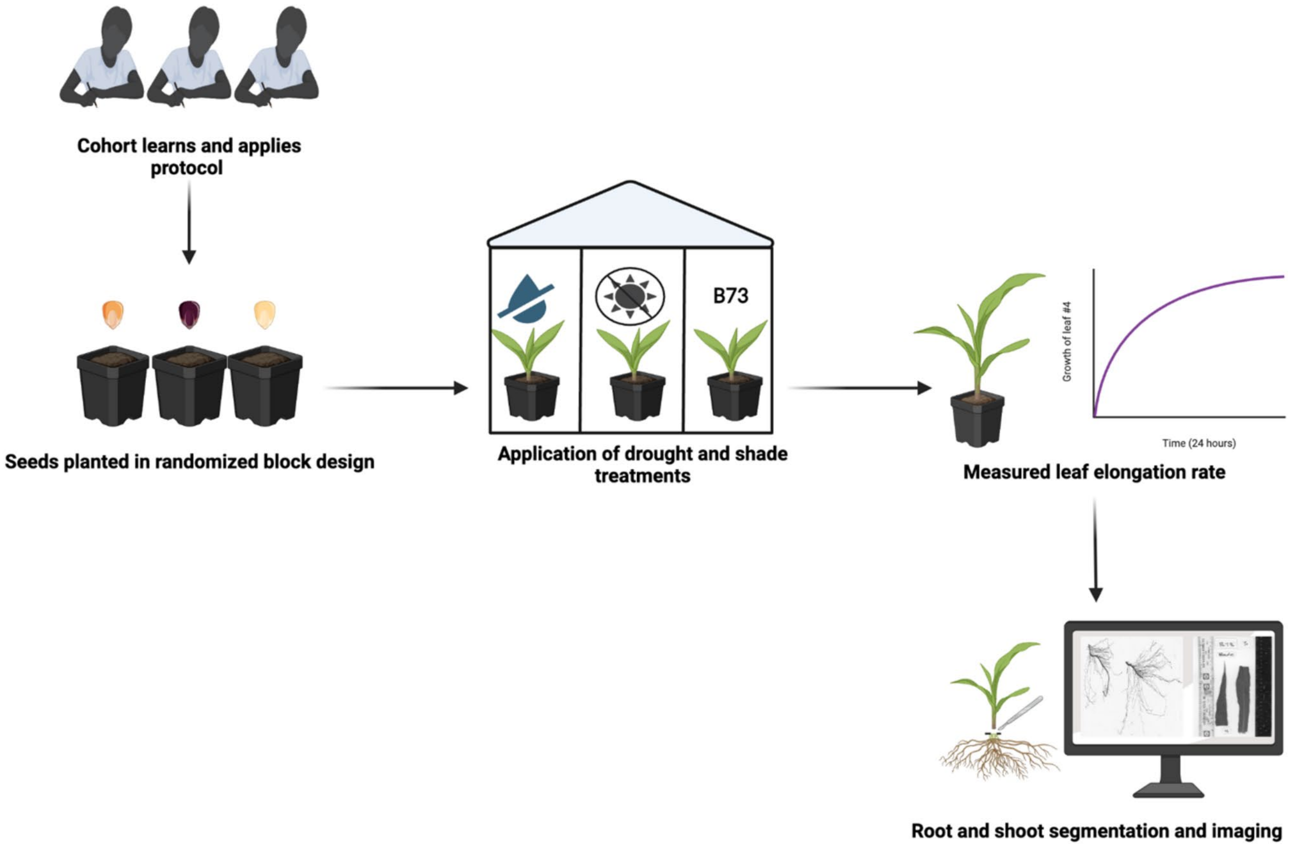

Fig. 1 This figure shows the project workflow to grow seedlings and phenotype leaf and root characteristics. The project started with cohort recruitment, followed by training, followed by planting the experiment. Approximately 15 days post germination leaf #4 of the plants are ready for measurement. When leaf four stops growing, the experiment is harvested, and roots are washed and measured. The experimental data is then analyzed by the cohort [8]. This structure can be used for any plant or soil course that wants to incorporate experiential student learning into the curriculum

This data has the potential to provide a baseline expectation for root growth and growth rate for important parental lines representing a large proportion of maize diversity. There is great utility in this data to understand genetic variation and growth rate plasticity. Additionally, we created various subsets of the data (see github - https://github.com/kant0063/REEU---Data-Science-in-Agriculture-data-and-code/tree/main/Data_Description_Manuscript and https://doi.org/10.6084/m9.figshare.27964536.v3*)*.

These subsets were as follows:

1. B73 under controlled conditions for all season/year.
2. Nested genotype under season/year under control conditions excluding genotypes B73, HP301, NC350, OH7b, Ki11.
3. Crossed genotype under control conditions by season/year.
4. Drought treatment by genotype.

The visual depictions of these structures can be seen in (Fig. 6). We have omitted one treatment (shade) because it was only conducted in one season/year combination and experimental notes suggest confounding environmental factors (e.g. temperature) which make that specific treatment unreliable. Leaf #5's growth rate data was also omitted because it was only recorded one year. *For data subset one (B73 under controlled conditions for all season/year)*, we observed that 68.72% of variation is explained by season/year and found that 31.28% is experimental error. *For data subset two (Nested genotype under season/year under control conditions excluding genotypes B73, HP301, NC350, OH7b, Ki1)*, we found that 44.30% is explained by season/year with 49.97% explained by genotype within season/year and 5.73% is experimental error. *For data subset three (Crossed genotype under control conditions by season/year)*, we determined that 15.0% of variation is explained by genotype and find that 85.0% is experimental error. *For data subset four (Drought treatment by genotype)*, we estimated that 19.11% of variation is explained by genotype, with 3.72% explained by treatment, and 77.17% is experimental error. *For normalized data subset one*, we found that 2.93% of variation is explained by season/year, we find that 97.07% is experimental error. *For normalized data subset two*, we determined that 7.94% is explained by season/year with 83.11%

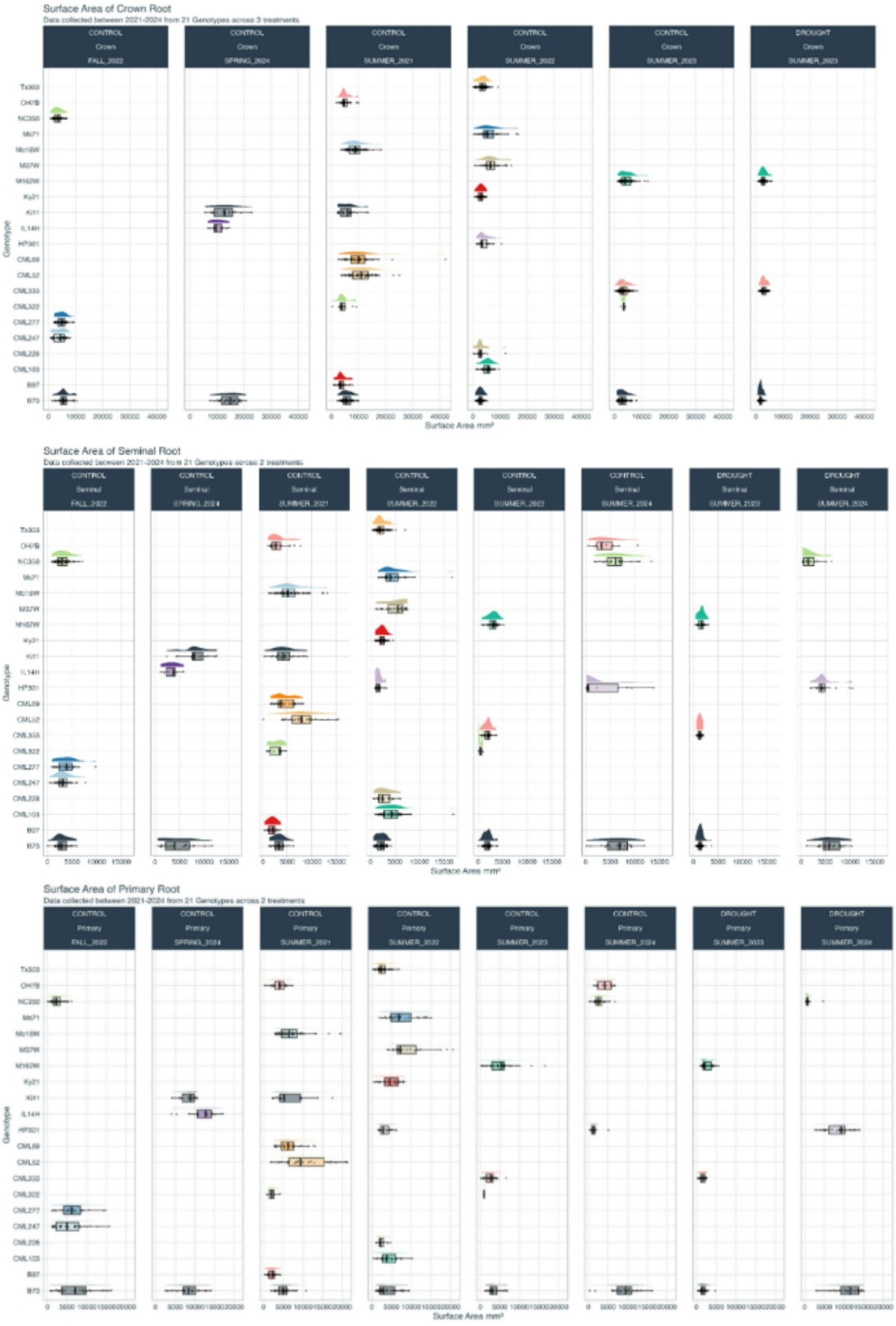

Fig. 2 Rain Cloud plot of 22 maize genotypes showing the surface area (mm^2) of the root system components (crown, seminal, and primary, top to bottom), each maize genotype is represented on the Y axis as a box plot paired with the distribution of root surface areas (x-axis)

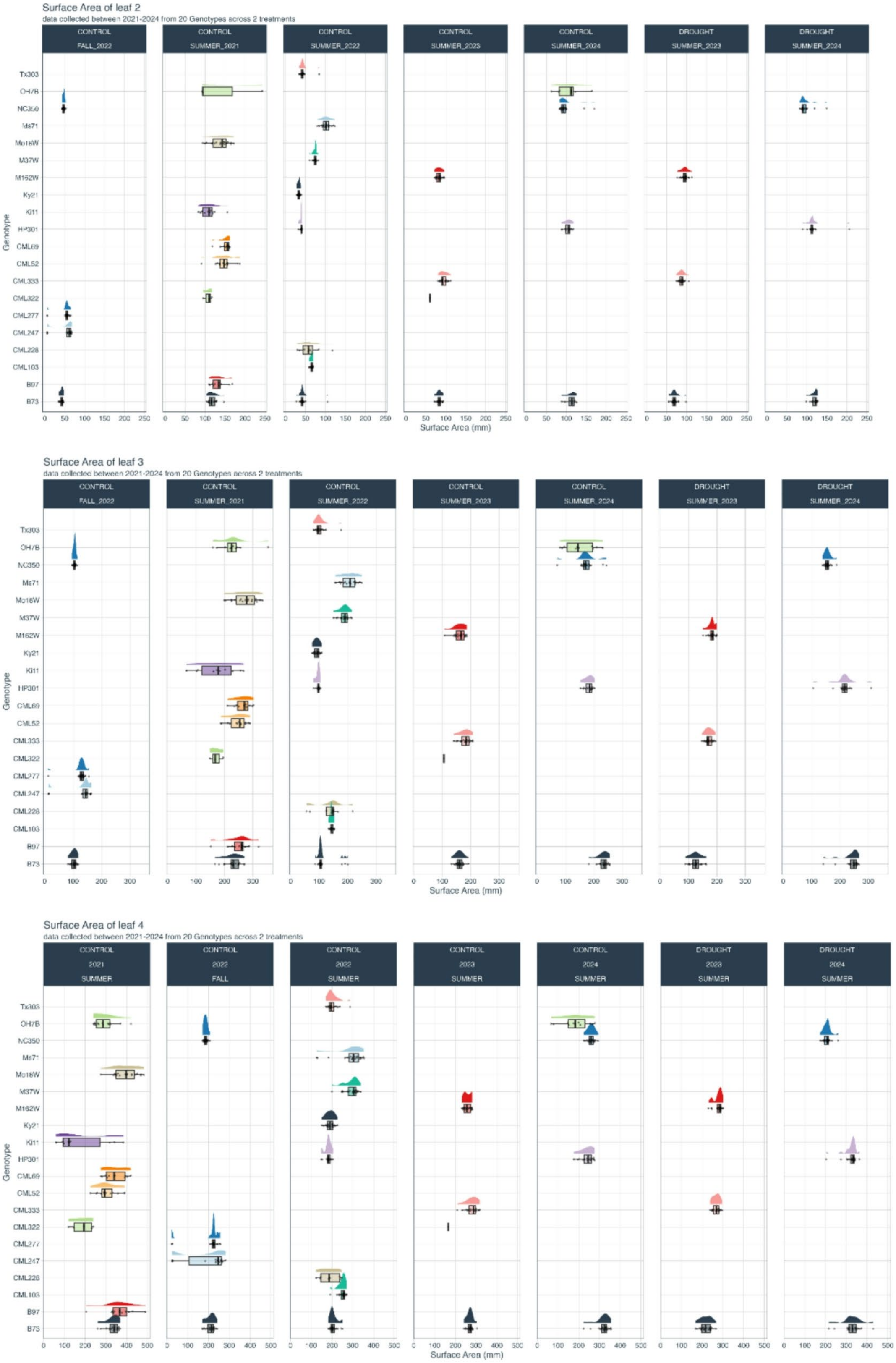

Fig. 3 Rain Cloud plot of 20 maize genotypes showing the surface area (mm^2) of measured leaf 2, 3 and 4, each maize genotype is represented on the y-axis as a box plot paired with the distribution of leaf surface areas on the x-axis. The plots are faceted to show the area per year (2021–2024) and treatment (control and drought)

Fig. 4 Rain Cloud plot of 24 maize genotypes showing seedling growth rate (mm^2). Each maize genotype is represented on the y-axis as a box plot paired with the distribution of leaf surface areas on the x-axis. The plots are faceted to show the area per year (2021–2024), leaf (four) and treatment (control and drought)

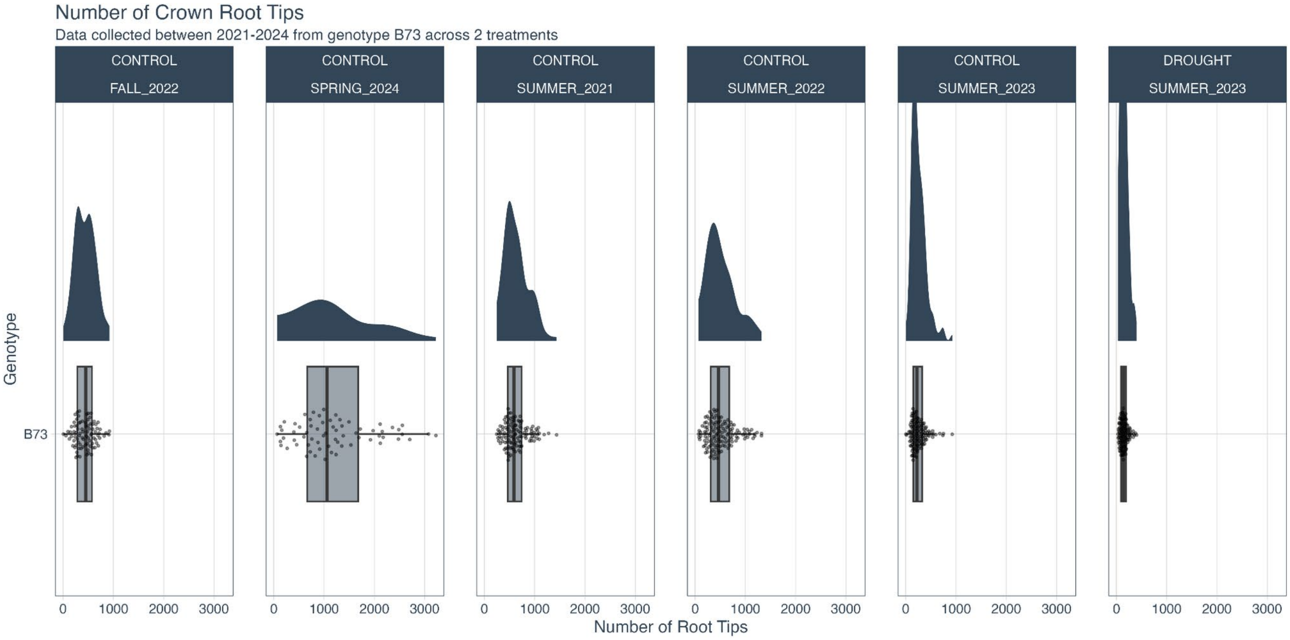

Fig. 5 Rain Cloud plot of maize genotype B73 showing the number of crown root tips. The plot is faceted to show the number of root tips per year (2021–2024) per treatment (control and drought)

explained by genotype within season/year and 8.95% is experimental error. *For the normalized data subset three*, we observed that 84.81% of variation is explained by genotype and find that 15.19% is experimental error. *For normalized data subset four*, we measured that 19.11% of variation is explained by genotype with 3.72% explained by treatment, and 77.17% is experimental error.

Consistent and comprehensive characterization of the shoot and root system has immense implications because differences in shoot and root architecture can greatly impact how the plant responds to stress. Understanding the total amount of variation and where that variation resides creates an opportunity to both understand what genotypes fit in different environments and what genetics

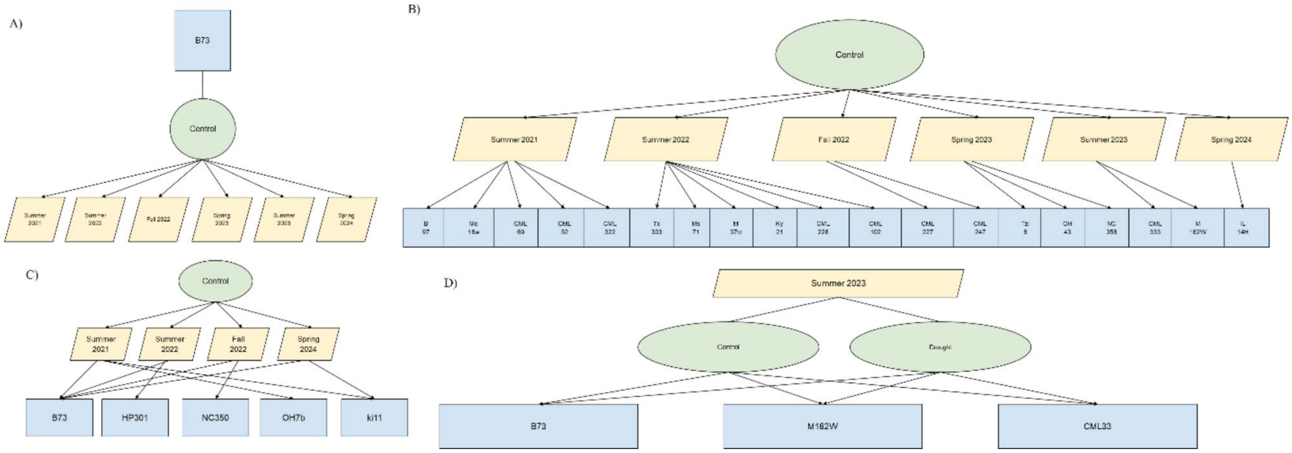

Fig. 6 Data structure for each of the datasets examined (**A**) B73 under controlled conditions for all season/year; (**B**) Nested genotype under control conditions by season/year; (**C**) Drought treatment by genotype within season/year (partially crossed); (**D**) Drought and shade treatment by genotype (fully crossed)

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	*all_years_growth_rate_L4_final*	*.csv*	*Figshare* -[7]
Data set 2	*all_years_leafs_final_fixed*	*.csv*	*Figshare* -[7]
Data set 3	*all_years_roots_final-2*	*.csv*	*Figshare* -[7]
Data set 4	*allyears_growthratedata_2021_2025*	*.csv*	*Figshare* -[7]
Data set 5	*Table Traits and Trait Descriptions*	*.xlsx*	*Figshare* -[7]
Data set 6	*Table_Experiment_Descriptions*	*.docx*	*Figshare* -[7]
Code	reeu_project	.Rmd	*Figshare* -[7]

can be a source of parental material for future breeding. These data not only have utility to further research efforts, they also have use for plant physiology/biology/plant breeding courses by allowing instructors to conduct a similar experiment and combine their data with the data presented to create project-based learning within the context of required and elective courses (Fig. 1 and Table 1 [7]).

Limitations

Each cohort of students measured variables in slightly different ways and students-imposed treatments in slightly different ways which contributed to variation in the datasets across the years. Individual students did not always follow the protocol, this led to differences in recorded data (e.g. sometimes different numbers of significant figures were used). For cohort-based data generation with undergraduates or teams of researchers, clear guidance on rounding and significant figures should be included as standards in protocols.

Abbreviations

REEU	Undergraduate Research and Extension Experiences

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Author contributions

M.M.P - data processing, validation, writing, T.M. - data processing, validation, writing, MM- project conception, writing, Y.M. project conception, writing, R.P. -site management, data acquisition, I.D. - site management, data acquisition, K.K. -site management, data acquisition, M.B. K - project conception, writing, A.H.A - data processing, data acquisition, validation, A. N - data processing, data acquisition, validation, A. T - data processing, data acquisition, validation, A.G. - data processing, data acquisition, validation, A.K.N. - data processing, data acquisition, validation, B.T. - data processing, data acquisition, validation, D.J.Y.S - data processing, data acquisition, validation, E.S. - data processing, data acquisition, validation, E.S.M. - data processing, data acquisition, validation, G.K. - data processing, data acquisition, validation, G.L.J - data processing, data acquisition, validation, G.L. - data processing, data acquisition, validation, I.K.C. - data processing, data acquisition, validation, J.M.K - data processing, data acquisition, validation, J.A. - data processing, data acquisition, validation, J.C.M.A. - data processing, data acquisition, validation, K. S. - data processing, data acquisition, validation, K.M.A.T. - data processing, data acquisition, validation, K.B. - data processing, data acquisition, validation, K.C.W - data processing, data acquisition, validation, L.R. - data processing, data acquisition, validation, M.L. - data processing, data acquisition, validation, N.C. - data processing, data acquisition, validation, N.B. - data processing, data acquisition, validation, O.J. - data processing, data acquisition, validation, R. A. B. M - data processing, data acquisition, validation, R. N. - data processing, data acquisition, validation, S. N. - data processing, data acquisition, validation, S.A.S. - data processing, data acquisition, validation, T. P. - data processing, data acquisition, validation, U. H. - data processing, data acquisition, validation, Z.G. P. M. - data processing, data acquisition, validation, Z. G. - data processing, data acquisition, validation, N. N. - project conception, writing.

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Data availability
The experimental data are available in Figshare with the identifier https://doi.org/10.6084/m9.figshare.27964536.v3

Declarations

Ethics approval and consent to participate
N/A.

Consent for publication
N/A.

Competing interests
The authors declare no competing interests.

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