INDIRECT SELECTION ON ROOT ARCHITECTURE IN THE MSU PINTO DRY BEAN BREEDING PROGRAM

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ABSTRACT

Historically, dry bean breeding programs have primarily focused on a shoot architecture ideotype to improve crop yield. An upright growth habit is thought to improve yield, adaptation, maturity, and mechanized harvesting. Growth habit is largely classified by three heritable characteristics: determinacy, length of main stem internodes, and climbing ability. Growth habits are not controlled by a singular genetic component but rather a plethora of genetic components combined. Indirect selection occurs when a trait is inadvertently selected upon, frequently by artificial selection, without it being the primary target. Shoot architecture is commonly targeted for breeding programs, whereas selection on root architecture is less common, often focusing on a related breeding target (i.e. pathogen resistance, nutrient availability, etc.). Roots and shoots have overlapping genetic and hormonal mechanisms for development. This work aims to identify whether breeding for an upright shoot architecture has indirectly selected upon root architecture. Using MSU pinto bean breeding program cultivars, a panel (composed of common pinto, UI-111, Sierra, Kodiak, and Eldorado) was assessed at MSU's Montcalm research farm in 2020 and in 2021 where shoots, and roots were collected at the R1 stage. Architecture data collected includes but is not limited to solidity, taproot width, basal root number, basal root width, and plant length. We aim to determine if or which root architectural changes have occurred over a historical timeline. In the long term, these results will help improve the quality of dry beans and support future root architecture research in breeding programs.

This thesis is dedicated to my mom, dad, family and friends who have nonstop supported me throughout the years and helped me keep going to achieve my dreams.

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LIST OF ABBREVIATIONS

AR GapT Adventitious root gap from top

AR GapB Adventitious root gap from bottom

ARN Adventitious root number

AVG Average

BR Angle Basal root angle

BRN Basal root number

BR Width Basal root width

DistFLR Distance from the first lateral root

LRN Lateral root number

RLD-1 Root length diameter 1

RLD-2 Root length diameter 2

RLD-3 Root length diameter 3

Xnods or % of Nodules Percentage of nodulation.

CHAPTER 1: HISTORICAL OVERVIEW OF DRY BEAN DOMESTICATION AND BREEDING ADVANCEMENTS LITERATURE REVIEW

ABSTRACT

Plant breeding is an improvement of a plant whose traits were directed and selected by humans. A cross between two parent plants that produce offspring with the desired traits. Domestication occurred over 12,000 years ago, and domestication events are important to note to give a background of the genetic diversity within a crop species. Breeding programs typically target and or focused on shoot architectural traits such as yield and disease resistance, however, root architecture is important to a crop's survival, anchorage, and nutrient and water assimilation. Root and shoots have overlapping mechanisms and biological processes in many species. Some overlapping roles in shoot and root development are not limited to phototropism, and or photomorphogenesis, gravitropism, growth regulating factors, plant growth regulators, and macro- and micronutrients. Recent studies over the past twenty years have shown that indirect selection has occurred on root architectural traits in maize, lentils, and dry beans. The indirect selection that was observed in dry beans occurred when breeding for shoot architectural traits. Shoot architecture in dry beans are categorized into four different types of growth habits each with its own various characteristics that attribute to its growth. Thus, making them key targets for breeding programs to improve upon yield, adaptation, plant architecture, stress tolerance and sustainability.

INTRODUCTION

Root Architecture

Root architecture refers to the configuration of a root system, which determines a plant's anchorage, nutrient and water uptake, and plant communication and competition (Sofi, et al., 2021). Root architecture, like shoot architecture, varies between species, environments, and several other factors (Lynch, 1995). Root architecture is evolutionary distinct. For example, monocots have a fibrous root system, lacking a single main root at maturity, but instead form seminal and nodal roots from the root crown. A dicot root system primarily consists of a taproot with lateral roots bracing out from the taproot (Grabowski, 2015). An ideotype is a combination of characteristics and or traits that optimize crop performance (Martre, et al., 2015). No standardized ideotype ideal for root architecture; it changes constantly depending on such as what you are breeding for, the soil content, and environment. For example, roots grow toward nutrient and water rich deposits in the soil. Root architecture with improved nitrogen efficiency should effectively be steep, deep, and cheap (Lynch, 2019; Lynch 2013). Major architectural traits involved in this adaptation include sparse lateral root branching, hardly any nodal roots, and steep root angles. On the contrary, if you are breeding for phosphorous efficiency, the root architecture system that you would want is one that resembles topsoil forging. A topsoil forging system typically has greater axial roots, small axial root angles, and increase root density and root hair length (Lynch, 2019).

Relationship between shoot and root architecture

Shoot and root development are controlled by conserved and organ specific mechanisms. Some conserved factors include phototropism, transcription factors that regulate photomorphogenesis, gravitropism, growth regulating factors, plant growth regulators, and macro- and micronutrients. Plants are sessile organisms, meaning they are immobile and must continuously adapt to their environment and surroundings to grow and develop. One of these adaptations is response to light. Plants use a vast array of photoreceptors to capture different kinds of lights; such as red sensing phytochromes, blue light cryptochromes, phototropins, and UV sensing UV resistance locus (Frankhauser, et al., 2015). These vast photoreceptors allow the plant to stretch the usage of sunlight in different intensities, directions, durations, and qualities (Kathare and Huq, 2021). Sunlight may penetrate the upper lays of the soil and thus root tissues may be exposed to it in the field (Mandoli et al., 1990). Phototropism refers to the ability of a plant to re-orient oneself to light. In *Arabidopsis* it has been noted that both root and shoot phototropism are regulated by blue phototropins (Kutschera and Briggs, 2012). *Arabidopsis* has two phototropins phot1 and phot2 (Christie et al., 2015). Phot1's is the photoreceptor for root phototropism, and shoot phototropism (Kutschera and Briggs, 2012; Christie et al., 2015). Phot2 is primarily for shoot phototropism at high light intensities (Sakai et al., 2001; Christie et al., 2015). Classic laboratory experimentations concluded that root response in phototropism is the opposite of the shoot (Darwin, 1882; Sachs, 1882; Pfeffer, 1904; Strasburger et al., 1911; Kutschera and Briggs, 2012). It is also interesting to note that not every root exhibits a negative phototropic response, about 50% of the plant species that were studied for phototropism only displayed the typical response, meaning that it is a species-dependent response (Schaefer, 1911; Hubert and Funke, 1937; Kutschera and Briggs, 2012). On the contrary, the typical response is shoots bend to light to optimize it for photosynthesis using blue and white light, and roots grow away (Kutschera and Briggs, 2012).

Photomorphogenesis is the developmental process of how organisms perceive light and optimize it for metabolism, growth and development (Montgomery, 2017). When *Arabidopsis* seedlings partake in photomorphogenesis, a series of photoreceptors are activated which in turn causes cotyledon expansion and regression of the hypocotyl and primary root (Stafen, et al., 2022). The light stabilization of elongated hypocotyl 5 (HY5) in the shoot advances to shoot-to-root transport and promote root growth (Chen, Xiangbin, et al., 2016; Guo, Zhixin, et al., 2021). While roots are not typically studied in photomorphogenesis, many positive transcription factors are expressed in the roots, suggesting that roots have a light sensing mechanism (Stafen, et al., 2022). Other phytochromes in the shoots have been noted to exhibit long distance effects on root growth and development (Costigan, et al., 2011; Salisbury, et al., 2007). Light also enhances auxin transport which encourages root growth as well (Bhalerao, et al., 2002; Fu and Harbed, 2003).

The ability to deal with mechanical stress is crucial to a plant's survival. Such mechanical stresses include touch, wind, rain, herbivory, other climatic, environmental, and weather conditions. Gravity is one of the mechanical stresses plants constantly undergo. Plants combat gravity using gravitropism; gravitropism is a plant's growth in response to gravity (Toyota and Gilroy, 2013). It has been shown in various studies and species that when a plant is laid horizontally the shoots will reorient their growth upwards and roots will reorient their growth downward (Blancaflor and Masson, 2003; Morita and Tasaka, 2004; Mortia, 2010). The overall consensus amongst scientists is that the hypothesis for this gravitropic response in plants is the starch-statolith hypothesis (Toyota and Gilroy, 2013). The hypothesis states that high-density amyloplast in specialized statocytes of the shoot endodermis and root cap reorient the cells and begin tropic response (Sack, 1991, 1997; Kiss, 2000). When amyloplasts are sedimented to the bottom it signals within the statocytes to regulate transport of auxin (Nakamura, Nishimura, and Morita, 2019). Auxin is known to be a main signaling hormone across multiple plants with shoot and root characteristics (Li, et al., 2022). During gravitropism, the plant hormone auxin is transported to the lower side of the plant organ, but cell growth in the shoots is promoted whereas in the roots it is inhibited (Li, et al., 2022; Rakusová, et al., 2016; Pařízková, et al., 2017). Low auxin concentrations tend to promote lateral root initiation influence apical dominance (Davies, 2010; Rademacher, 2015), shoot growth (George, Hall & De Klerk, 2008), root formation when wounded (Small & Degenhardt, 2018), and regulating root growth and development when there is a sustained shoot cell niche (Liu, et al., 2017).

Whilst hormones are naturally produced by plants, plant growth regulators are applied by humans onto plants. Plant growth regulators and hormones affect flowering, aging, root growth, stem elongation, leafing, color enhancement in fruits, and other various biological processes. Not all plant growth regulators are naturally occurring hormones and can be synthetic compounds such as Indoleacetic acid. Plant growth regulators is an encompassing term for plant growth regulating compounds. It consists of auxin, gibberellin, cytokinin, ethylene and abscisic acid. auxin is involved in several biological responses. Auxin is involved in phototropism, gravitropism, promotion of apical dominance, flower formation, fruit growth and set, and formation of adventitious roots. Gibberellins are typically known for germination as it can help germinate even the most stubborn of species. However, gibberellins are also known for cell division and elongation, and breaking seed dormancy. Cytokinins are found in both plants and animals. Often cytokinins are used for tissue culture, cellular division and plant growth (VanDerZanden, 2024). Cytokinin has been known to promote root growth, and shoot development from internodes (Carrow & Duncan, 2011; George, Hall & De Klerk, 2008). In shoot development, cytokinin induces shoot elongation, development, and regeneration (Amoanimaa-Dede, et al., 2022, Carrow & Duncan, 2011; George, Hall & De Klerk, 2008).

Ethylene induces ripening, epinasty, abscission, and promotes senescence. Ethylene production is often in response to stress during the end of a plant's life. Abscisic acid is known to be a plant growth inhibitor; prevents germinating stomatal closure, abscission, and induces dormancy (VanDerZanden, 2024). Overall, in general plant growth regulators increase yield, improve plant metabolism, and regulate expression of endogenous hormones (Zeng et al., 2012). Root and shoot growth is promoted by plant growth regulators (Kim, et al., 2018; Qi, et al., 2013; Steffens, Wang & Sauter, 2006). In roots, the plant growth regulator ethylene regulates adventitious root formation (Amoanimaa-Dede, et al., 2022). In soybeans, in particular, gibberellins and ethylene increase plant height, adventitious root formation, and improve root growth (Kim, et al., 2018).

Growth regulating factors, not to be confused with plant growth regulators, also play an important role in shoot and root development. Growth regulating factors (GRF) are highly conserved plant transcription factors that play a role in various biological processes, including shoot and root development. GRF's are regulated by a microRNA, specifically *microRNA396*. GRF's have been found to be present in various species such as *Arabidopsis*, soybean, maize, and more. GRF's also control agronomic traits such as grain size, shape, and number, plant height, seed shattering, branch number and more. GRF's have conserved QLQ and WRC domains at their N-terminus (Liu, et al., 2023). In *Arabidopsis*, there are known to be nine GRF's that are exhibited in growing tissues (Horiguchi, et al., 2005; Kim et al., 2003; Kim and Kende, 2004). *AtGRF1*, are expressed in primary roots and help give rise to lateral roots (Kim, et al., 2003). *AtGRF2*, are expressed in root tips, and the meristematic zone (Bao, et al., 2014; Rodriguez, et al., 2010). *AtGRF3* is expressed in the elongation zone, lateral roots, and the meristematic zone (Ercoli, et al., 2018; Kim, et al., 2003; Rodriguez, et al., 2015). *AtGRF4* and *AtGRF5* in the meristematic zone (Bao, et al., 2014; Pajoro, et al., 2014), and *AtGRF7* and *8* in shoot tips with little expression in roots (Kim, et al., 2003). Certain GRF's are known to regulate leaf development and cotyledon growth such as *AtGRF1*, *AtGRF2*, *AtGRF3*, whereas other GRF's can promote and hinder root growth and development (Liu, et al., 2023). These are just a few examples of the knowledge on what is known about shoot and root mechanisms combined and how one affects the other.

Root Phenotyping

Roots can communicate, sense, and respond to abiotic and biotic stimuli. Another way to refer to root architecture is the spatial distribution of all roots in a growing environment (Paez-Garcia, et al., 2015). Roots are crucial to a plant's survival. Since they impact growth and development of the shoot architecture from root to shoot transport, and activation of signaling molecules in molecular processes (DoVale, et al., 2015). There are numerous amounts of root architectural traits that encompass a root system. On the other hand, the overall encompassing root traits are rooting depth, root hairs, and root branching. Rooting depth refers to how deep a root travels in the soil to acquire water and nutrients (Paez-Garcia, et al., 2015). Rooting depth is an important trait and is often the most evaluated trait since it deals with water acquisition, and nutrient uptake; specifically, nitrogen and soluble nutrients (Wasson, et al., 2012). Root hairs grow from root epidermal cells and are single celled (Paez-Garcia, et al., 2015). Root hairs contribute up to 50% of water uptake and are the largest trait in root total surface area (Paez-Garcia, et al., 2015). Root branching is an encompassing term that can be a variable amount of root traits but generally are lateral roots. Lateral roots are roots that branch from the primary root from differentiated cells (Malamy and Ryan, 2001). Lateral roots contributed to root biomass, surface, and length (Paez-Garcia, et al., 2015). Thus, lateral roots are hypothesized to also contribute to nutrient and water assimilation (Robbins and Dinneny, 2015; Sun, et al., 2015;

Tian, Smet, and Ding, 2014). To reiterate, root branching depends on soil conditions, such as water and nutrient availability, and environment (Paez-Garcia, et al., 2015).

Introduction to selection and breeding programs

According to Darwin, natural selection is when variations and favorable attributes are preserved and then there is destruction of traits not favored (Darwin, 1859/1998). Whereas artificial selection is a modification event to an organism by humans that brings about phenotypic variation, either intentionally or not (Yamasaki, et al., 2007). Plant breeders and plant geneticists use phenotypic variation to determine important agricultural traits to improve upon (Yamasaki, et al., 2007). There are three types of selection: positive, negative, and balancing. Artificial selection is known as a type of positive selection where it favors a one-sided shift in allelic frequency, Balancing selection refers to several constraints that are considered the optimal concession wherein allele frequencies for multiple alleles are shifted to an intermediate level (Loewe, 2008). Negative selection, also known as purifying selection, removes deleterious variations (Choudhuri, 2016). Selection of certain alleles may improve these genetic factors, but unknown alleles may affect other various agronomic traits (Yamasaki, et al., 2007). Indirect selection occurs when a selection inadvertently or unintentionally affects other genes that were not the primary target (Kirkpatrick and Barton, 1997).

Plant breeding is the improvement of a plant, crop, or variety, that was directly selected for by humans. The improved plant will have a combination of genes that are beneficial to various factors such as environment sustainability and adaptability, increased diseases and pest resistance, higher yield, nutritional content, and more (Kelly, 2010). Detecting and determining desirable traits based on visual aspects is known as phenotypic breeding and selection (Reynolds, et al., 2020). Traits are inherited two different ways: qualitatively through control by a single or few genes, or quantitatively through multigenic pathways. Breeding for qualitative traits is the fastest since they are easy to predict in genetic ratios. For example, many disease resistance traits are qualitative. When genes are quantitative and the environment plays a role in expression is where challenges arise. On the contrary, quantitatively inherited traits are the most important in crop plants. To determine how much a quantitative trait is under genetic control they express it as heritability. Combining a multitude of genes is not one easy task, as some genes are negatively correlated such as early maturity and high yields (Kelly, 2010).

Humans started to domesticate wild plants about 12,000 years ago (Lee, et al., 2015). Breeding targets focused on visual aspects and our senses, especially for early age breeding when it was farmers cultivated and breed these plants. Once Mendel's plant genetics was not only accepted by the scientific community, but also confirmed, that is when breeders started to breed and make new varieties based on genetic principles. Between the years 1965 and 1985, yields increased by 56%, and further increased by 28% between the years 1985 to 2005 (FAO, 2014). The increase in crop yield from 1965 to 1985 is due to the ‘Green Revolution’. The Green Revolution promoted the use of fertilizers and pesticides, improved irrigation, mechanized agriculture, and made new varieties via genetic breeding. During the Green Revolution, semi-dwarf varieties of wheat and rice were created that increased crop productivity and is notably highlighted as the most important milestone during the green revolution. Nowadays plant breeders are facing global warning, abnormal weather, water shortages, reduced arable land, and more. It is increasingly more difficult to have a stable food supply and make improved varieties due to these constraints (Lee, et al., 2015).

Arguably, the most well-documented domestication event can be found in maize (*Zea mays* ssp. *mays*). Maize was originally domesticated from its wild progenitor teosinte, *Z. mays* ssp. *parviglumis* between 6,000 and 9,000 years ago (Piperno and Flannery, 2001; Matsuoka, et al., 2002). Maize domestication resulted in many landraces which were then spread throughout the United States (Smith, 1998). A recent study that came out in December of 2023 showed that another wild teosinte contributed to make modern maize. It was noted that across 1,000 wild and domesticated lines, as well as 338 new varieties, there was only one line that lacked admixture with *Zea mays* ssp. *mexicana.* It is hypothesized that around 4,000 years after domestication maize hybridized with *Zea mays* ssp. *mexicana.* It was also found that 15-25% of the maize genome is contributed to *Zea mays* ssp. *mexicana,* as well as the admixture contributes to the genetic variation of many traits within maize. Such as, 50% disease phenotypes, and 25% variation in kernals per row (Yang, et al., 2023). Most maize breeding programs in the United States and Canada focus on two goals in their breeding programs; high yield and wide adapation in maturity zones (Troyer, 1996). Both of these traits protect the plant from abiotic and biotic stresses such as root or stalk lodging or dropped ears (Lee and Tracy, 2009).

Indirect selection on root architecture in dry bean, lentil, and maize

In 2009, a study was published linking shoot and root growth and development together on its tolerance to low soil phosphorus in dry beans. In the Andean bean population, it was found that root length density and root surface area had increased under phosphorus sufficiency under growth habits that had an indeterminate line (Cichy, et al., 2009). Determinate plants are categorized as having distinct reproductive and vegetative growth stages. Indeterminate plants continuously grow even if flowering is occurring elsewhere on the plant (Clark and Ma, 2023). Determinate beans have a compact, upright growth habit, but have lower and less stable yields compared to indeterminate counterparts (Kelly and Adams, 1987; Beaver, et al., 1985, 1996). According to Debouck, 1984, determinacy is a deciding factor in categorizing dry beans growth habit. Growth habit simply refers to the form or shape that a plant takes. In Soltani, et al., (2016), stem diameter and plant length were targeted traits for selecting an upright growth habit over time. Depending on the growth habit (bush or vining) and even market class of a bean, the plant's height varies. Some varieties can be as small as 20 centimeters small and others as large as 3 meters tall. The stem of a dry bean depends on the variety as well, it can be either trailing, climbing, or erect (Carton and Young, 2023).

Indirect selection on root architecture has occurred in an Australian lentil breeding program (Rao, et al., 2024). Over 36 historical genotypes were assessed for the relationship between shoot and root traits and their impact on yield selection on root traits. Results measured maximum root depth, length, surface area, diameter, and biomass. It was concurred that root architecture across the various genotypes and data years has significant variations. In 2020, it was concluded that there was great total root length above 20 centimeters, root dry weight, and root to shoot ratio. Whereas, in 2021, there was great total root length, surface area, and root dry weight below 20 centimeters. Roots in 2021 were shallow, short, and thick, and in 2020 roots were much deeper. It was found that when breeding for yield based selection that there was decreased root length and surface area over year of release. There were other root traits that negatively correlated with year of release as well. The major objective for the lentil breeding program in Australia was to increase yield and increase disease resistance; such parameters for selection were increase plant height, height to pod, and increase shoot biomass. Results showed that whilst selecting for yield based genotypes, simultaneously small root architecture systems were selected indirectly. This also coincides with selection of early maturity in lentils to have a smaller root architectural system (Rao, et al., 2024).

Another study was conducted in March of 2015, noting that indirect selection of root shoot architectural and anatomical phenes occurred in maize over the course of 100 years of breeding. Over the past 100 years, and particularly in the United States, maize breeding programs focused on yield and adapting to changing agronomic conditions (York, et al., 2015). Plant breeders started to develop inbred lines from these maize landraces to make hybrid maize (Walden, 1979). From 1930 through 1989 there was an increase in maize yields from 10 q ha^{-1} to 75 q ha^{-1} (Russell, 1991). This increase in yields can largely be attributed to increased fertilizers, weed control, higher plant density, improved management, but most importantly to the usage of hybrids (Yamasaki, et al., 2007). It has been hypothesized that maize selection has been changing intensive management systems. Thus, root systems have evolved to have an increase of resource acquisition. However, selection in maize has been typically yield driven above ground phenotypes rather than selection for root architecture thus having less understanding how it has evolved over time (York, et al., 2015). One open-pollinated variety and 15 hybrids were used in this study representing the entire Dupont Pioneer Era panel (Duvick, et al., 2004). They found that brace root angle decreased 12% from the oldest to the newest Era period. Crown root angle decreased 10% once again compared to the oldest to the newest Era period. The length of crown root lateral roots was significant by Era period, increasing 29% (York, et al., 2015). Indirect selection occurred on the root phenes in maize over the last century due to increased nitrogen stress (York, et al., 2015, Duvick, et al., 2004).

Dry bean domestication and breeding history at MSU

Dry bean, *Phaseolus vulgaris*, is a major legume consumed by Americans and people all over the world. Dry beans are an essential crop for food security and nutrition since they provide key nutrients such as zinc and iron. Michigan is the second largest producer of dry beans in the country (U.S. Dry Bean Council, 2017). DNA origin analysis indicates that wild bean *P. vulgaris* originated in Ecuador and Peru regions (Gepts, 1998; Kelly, 2010). Wild *P. vulgaris* had already diverged into two gene pools prior to domestication: the Middle American and the Andean. Genotypes from the Middle American gene pool originated from the region considered northern Mexico into Central America along with Colombia and Venezuela. Genotypes from the Andean gene pool originated from the regions that now encompass Peru, Bolivia, and Argentina (Gepts, 1998; Kelly, 2010). In addition to geographical location, the Middle American gene pool has greater genetic diversity and smaller sized seeds, whereas the Andean gene pool has less genetic diversity and larger sized seeds (Kelly, 2010).

The overarching goal in a plant breeding program is to improve specific characteristics of a variety without losing and retaining other desirable traits that the variety already possesses. These traits are chosen upon based on producer, and consumer needs; whilst also understanding what is achievable within biology (Kelly, 2010). Plant breeders face the challenge of identifying 'superior' lineages with desirable traits for the next generation. In dry bean, there have been a plethora of traits that have been selected upon over the years, however, which specific trait is usually associated with which cultivar has the best yield (Kelly, 2001). *P. vulgaris* is the most commonly cultivated species out of the genus due to its high plasticity under selection (Garc, et al., 1997). The genus *Phaseolus,* is vast and genetically diverse; dry bean breeding targets include tolerance to drought, heat, cold, alkalinity, viruses, bruchids, etc. (Bates, 1985; Nabhan, et al., 1986).

The MSU dry bean breeding program focused on breeding for yield, adaptation, plant architecture, stress tolerance and sustainability in many market classes including pinto (Kelly, 2010). Plant breeders often target shoot architecture to increase yield. Dry bean market classes differ in seed size and pod number making them harder to breed for yield (Kelly, et al., 1998). Over the years in the Michigan State dry bean breeding program, a focus on breeding for upright shoot architecture (growth habit) was important due to the Michigan environment. Type IV growth habit is the most similar to a wild growth habit, exhibiting a very large, long, vining, twining shoot architecture and is easier for hand threshing for countries that harvest in that capacity. In Michigan, however, a type II growth habit is more efficient for combine harvesting; the upright shoot architecture improves flooding and lodging resistance, and allows for pods to be higher in the canopy (Kelly, et al., 1987; Kelly, 2010). Pinto beans typically exhibit a type III growth habit and have higher yields (Eckert et al., 2011). However, it is considered to be less stable due to its issues with diseases spread, specifically white mold (Kelly and Adams, 1987). As well as a type III growth habit tends to lodge making it difficult for direct harvest (Eckert et al., 2011).

CHAPTER 2: INDENTIFICATION OF INDIRECT SELECTION ON ROOT ARCHITECTURE IN MSU DRY BEAN PLANT BREEDING PROGRAM

ABSTRACT

Dry beans in the United States typically growth in a bush type growth habit. Beans are quite susceptible to abiotic and biotic stresses. Beans are a self-pollinated crop unlike other crops, and pure line varieties are needed to be sold to farmers. In the United States over 60% of the dry bean acreage is pinto beans. However, pinto beans have major constraints within its market class such as susceptibility, mineral issues, weather and climatic conditions, and yield. The MSU dry bean breeding program focused on shoot architectural traits. To combat these constraints in pinto beans and to grow them in the state of Michigan, a Type II growth habit was produced in pinto beans. Type II growth habits are useful for combine harvesting, lodging and flooding resistance, and reducing disease spread.

INTRODUCTION

All commercially grown beans in the United States exhibited a bush type growth habit with some exceptions. Bean yields are limited not only by the short growing season but also due to susceptibility to diseases and pests, abiotic and biotic stresses, nutrient deficiencies, and investment in crop research. All beans in the United States are planted as a row crop, planting can be as early as in June and harvest can be up until the end of September. A combination of insecticides, fungicides, bactericides, and herbicides are applied to control pests (Kelly, 2010). Dry beans are harvested by direct harvest (Kelly, 2010; Thomas et al., 2016). Direct harvesting is a process in which dry beans are harvested by a combine through a single pass (Thomas et al., 2016). Beans are a self-pollinated crop, unlike other wind-pollinated or insect/animal related methods. Thus, unlike other crops, only pure line varieties are sold to farmers, which take through ten generations of breeding to ensure they are pure lines. Yield typically lies in the heritable range of about 10 to 15%, yield gain from selection will always be lower than other desirable traits as yield is a low steady constant gain. To counteract environmental effects on trait expression, breeders must continuously work in the field year after year to reduce environmental effects. These factors included but not limited to weeds, soil profiles, crop rotations, soil type, diseases and pests (Kelly, 2010).

Shoot architecture refers to the configuration of the body of the above ground organs of a plant. Dry beans can be grouped into four growth habits using shoot architecture characteristics (Table 1). Type I is a determinate growth habit that has limited node and leaf production after flowering. Type II-IV are indeterminate growth habits that vary between climbing ability, terminal buds, stem and branch strength, and twining (Singh, 1981). For example, type II typically lacks climbing ability, contrasting with the strong climbers of type IV (Kelly, 2001;

Debouck, 1984). In dry bean, improved yield in segregating populations often exhibits association with climbing bean growth habit, vine length, climbing ability, node number, and plant height (Kelly, et al., 1998). Whilst most breeding efforts for dry beans focus on disease resistance, maturity, growth habit, yield, and quality, major constraints diverge from each. For example, selecting for high yield but ignoring growth habit would likely indirectly select upon a type IV growth habit (Kelly, et al., 1998). According to the international center for tropical agriculture (CIAT), breeding for yield in dry beans must consider the effects of growth habit, seed size, maturity, and gene pool (Kornegay, et al., 1992).

In the United States over 60% of dry bean acreage is the pinto bean market class; with the highest production in North Dakota, Michigan, Nebraska, Minnesota, Colorado, and Idaho. The major constraints in the pinto market class are disease susceptibility, mineral deficiencies and toxicity, drought, frost or early spring weather causing the early flowering, and pod set that affect seed quality and yield (Terán, et al., 2009). There is high demand for an indeterminate upright type II growth habit in pinto cultivars to counteract these constraints (Singh, 1982). The first pinto bean cultivars were produced in 1925, by Dr. C. Hungerford, at the University of Idaho. These three cultivars were UI-72, UI-78, and UI-111. These three cultivars were specifically bred to counteract bean common mosaic virus and beet curly top virus (Terán, et al., 2009). The first dry bean breeding program was established here at MSU when it was known as Michigan State College in the 1900s, although it is not specified when exactly pinto beans were introduced into the MSU dry bean breeding program. Around the 1980s, there was a cry for diversification for dry beans, but particularly in pinto and great northern commercial classics (MSU, 2009). The major issues at the time are unclear, however, the MSU dry bean breeding program focused on breeding for yield, adaptation, plant architecture, stress tolerance and sustainability (Kelly, 2010). In pinto beans, breeding for a type II growth habit helps reduce disease spread and is best for direct harvest (Terán, et al., 2009; Kelly and Cichy, 2012). Thus, causing MSU to develop a pinto bean cultivar that exhibited a type II growth habit shoot architecture. One of the first successful pinto bean cultivars among the Durango race is Sierra, it exhibits a type II shoot architecture that was made by the MSU dry bean breeding program (Kelly, et al., 1990).

RATIONALE AND SIGNIFICANCE

Root architecture is often overlooked in the plant breeding community. Changes in root architecture affect yield, plant health, and nutrient capture, which then influence management practice decisions. In turn, root architecture is affected by the environment, climate, soil conditions, and management practices such as nutrient sprays. Breeding efforts towards certain root architecture can increase nutrient content within the crop. This in-depth examination of root architecture traits will determine whether specific traits were indirectly selected upon in various pinto bean cultivars when selecting upon shoot architecture.

OBJECTIVE AND HYPOTHESIS

Objective: To assess whether root traits over time have been affected by breeding for growth habit over time.

Hypothesis: Root architecture was indirectly selected upon through breeding efforts towards upright shoot architecture.

METHODS

With guidance from Dr. James Kelly, the history of the MSU pinto bean breeding program was reviewed and evaluated. The first pinto bean cultivar used by the MSU breeding program was the vining cultivar UI-111, from the University of Idaho released in 1945, generally believed to be derived from the landrace, Common Pinto. We then chose three cultivars directly descended from UI-111 and one another that represented key achievements in growth habit or upright shoot architecture (Table 2). Sierra was one of the first pinto cultivars released by MSU and was directly derived from UI-111. Subsequently Sierra (1990) was used to breed Kodiak (1999), which in turn gave rise to Eldorado (2012). Each of the cultivars represents a key improvement in shoot architecture and direct descendants from one another. For example, starting with Common pinto, has been noted and recorded to exhibit a type IVa shoot architecture. From there, UI-111 was recorded and exhibited a type IVa shoot architecture (Table 2). UI-111 worked well in Michigan’s climate and therefore was used in making Sierra, which was one of the first to exhibit a type II shoot architecture (Kelly, et al., 1990). Subsequently, Kodiak and Eldorado are noted to have a type II shoot architecture as well; however, Eldorado in this study has been recorded to exhibit a type III shoot architecture despite its documentation.

Table 1. CIAT table of growth habit types in *P. vulgaris* (Debouck, 1984).

Growth Habit	**Growth**	**Stem and Branch Strength**	**Terminal Guide**	**Climbing Ability**
Type I	Determinate	Strong and upright	Absent or small	Absent or weak climber

Table 1. (cont'd)

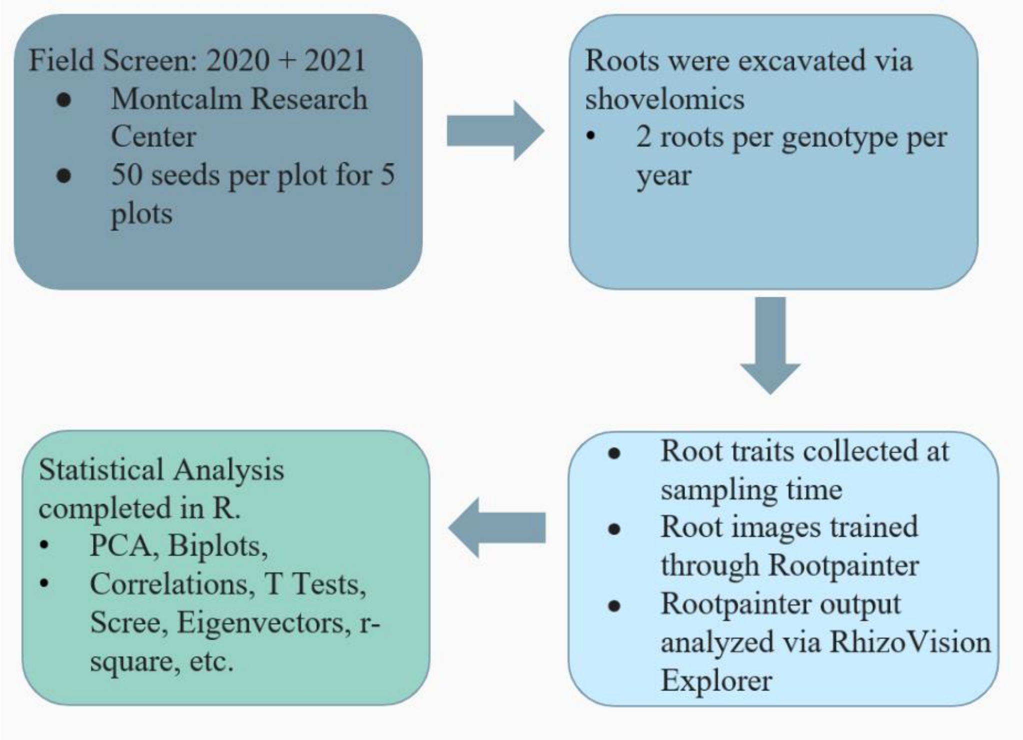
Type II	Indeterminate	Strong and upright	Absent or small twining	Absent or weak climber
Type III	Indeterminate	Weak, open or prostrate	Small or medium, twining	Weak or facultative climber
Type IV	Indeterminate	Very weak, twining	Very large, twining	Strong climber

Table 2. Pinto panel with growth habits recorded from field crops grown at MSU and referenced from a literature review (USDA-GRIN; Kolar and LeBaron, 1977; Terán et al., 2007).

Cultivar	**Year of Release**	**Growth Habit Recorded**	**Growth Habit Sourced**
Common Pinto (Landrace)	NA	IV	IVa
UI-111	1945	IV	IVa
Sierra	1990	II	IIb or IIIa
Kodiak	1999	II	II
Eldorado	2012	III	IIb

Each genotype was planted to replicate (50 seeds: per plot for 5 plots) at MSU's Montcalm Research farm in 2020 and 2021 as a completely randomized block design. At the end of data collection, over 50 pinto roots were collected and analyzed, 25 per year two per genotype. Two roots per plot were excavated via “shovelomics” at the R1 growth stage and imaged as previously described (Burridge, et al., 2016; and Haus, et al., 2020) (Figure 1).

Figure 1. Experimental design and workflow.



Roots were analyzed using RootPainter (Smith, et al., 2022), and RhizoVision Explorer (Seethepalli and York, 2020) (Figure 1). RootPainter is used for image segmentation by training a model to detect and annotate roots in images. RhizoVision was used to extract root architecture traits of the annotated images (Figure 1). Specific root traits (Table 3) that have been measured in both ImageJ and RhizoVision are provided. To provide context for root architecture, soil was randomly sampled in a Z pattern (Lawrence, et al., 2020), across the field and soil samples were grouped by block.

All statistical analyses were done in R (R 4.3.1). R packages that were used include FactoMineR, ggplot2, factoextra, dplyr, ggfortify, DataExplorer, ggpubr, sjmisc, corrplot. A t-test was used to determine significance between all varieties and cultivars when comparing plant length and stem diameter. The R function stat_compare_means was used with the method being t.test and the cutoff points 0, 0.0001, 0.001, 0.01, 0.05, Inf. Correlations tests were performed using the function cor and a pairwise correlation to uncover potential traits of interest; cor.mtest was used which uses p-values and confidence intervals for each pair of input variables, and the function cor to get r squared values. Cutoff points were $p<0.05$ and ($R^2 <0.5$). Confidence intervals were set to 0.95. A Pearson correlation was used given the normality of the data as the variables root traits move in a specific direction at a constant rate. Data analysis was performed with both years combined, and then separated into each individual year to understand how each field season impacted root architecture. As well as understanding what other underlying root traits are also being selected upon but not are not a significant association when both years are combined.

Table 3. Root traits measured in ImageJ and RhizoVision Explorer (Seethepalli, et al., 2020; Seethepalli, et al., 2021).

Root Traits	**Description**
Plant length	Length from soil line to tip of leader
Stem diameter	Diameter of stem above the adventitious roots
Taproot diameter	Diameter of taproot just below the basal roots
Basal root number	Number of basal roots at root crown
Basal root diameter	Average diameter near the root crown of two basal roots
Basal root angle	Angle of basal roots from soil to tips

Table 3. (cont'd)

Adventitious root gap from top (AR GapT)	Distance between the top two adventitious roots (nearest the soil line)
Adventitious root gap from bottom (AR GapB)	Distance between the bottom two adventitious roots (nearest the basal roots)
Adventitious root number (ARN)	Number of living adventitious roots near root crown
Length between basal roots and lateral roots (DistFLR)	Length (mm) between the lowest basal root and uppermost lateral root
Lateral root number (LRN)	Number of individual lateral roots within a 1cm length starting from the uppermost lateral root
Percentage of nodules (% of Nodules)	Visually estimated scale (0-100%) of fresh root system biomass that was made of nodules
Number of root tips	Total count of the number of tip pixels in a segmented skeletal image
Depth	Maximum depth of a root in a segmented image
Maximum width	Maximum width of a root in a segmented image
Network area	The total number of pixels in a segmented image.
Convex area	The convex hull is the minimal-sized convex polygon that may contain the root. The convex area is determined from the convex hull

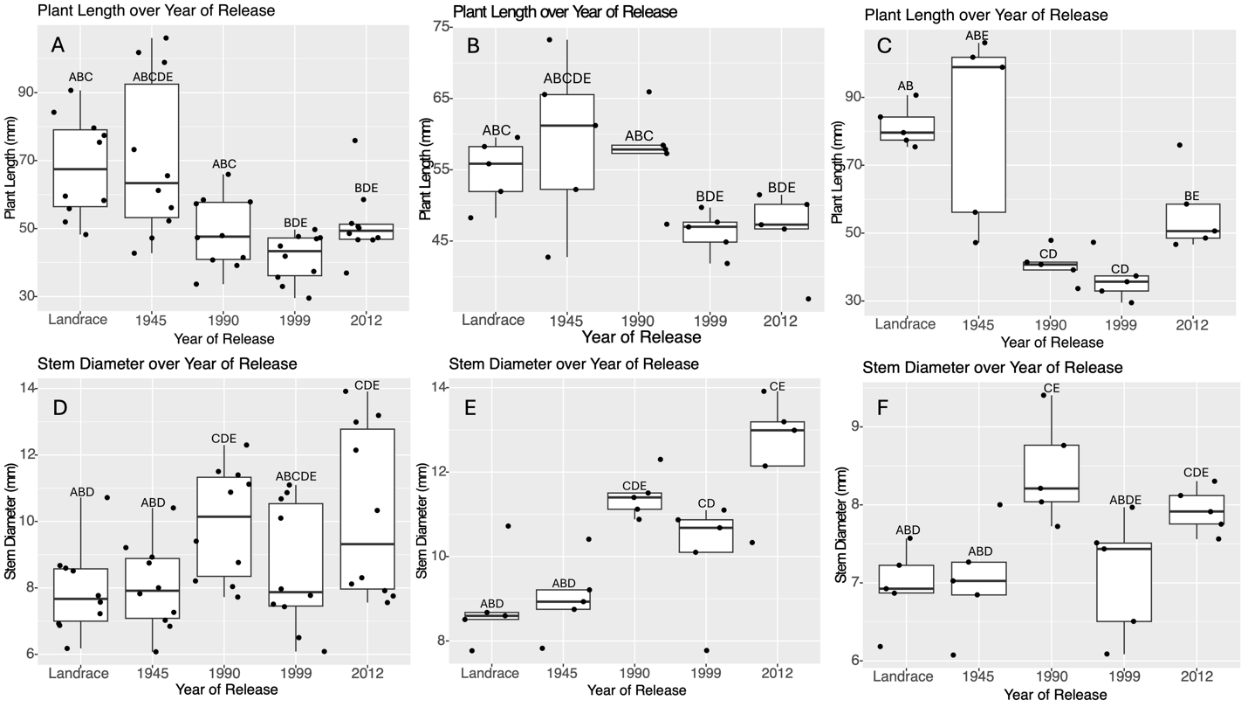
Table 3. (cont'd)

Solidity	The ratio of the network area and the convex area
Lower root area	The total number of pixels that are located below the medial axis (maximum radius) in a segmented image
Average diameter	The distance from the nearest non-root pixel is used as a radius to fit a circle. The diameter of the circle is calculated to be the diameter for the entire root crown
Holes	The number of disconnects of the background to the root branching and root complexity by inverting the segmented image
Average hole size	The number of disconnects of the background to the root branching and root complexity by inverting the segmented image the average hole size is calculated based on this.
Average root orientation	For every medial axis pixels, the orientation is computed by determining the mean of the medial axis; it is the average of all of these orientations.
Root length diameter range 1, 2, 3 (RLD-1,2,3)	Fine, Medium, and Coarse root diameters. From the segmented skeletal image, the medial axis pixels are separated into fine, medium or coarse roots based off the diameter
Average basal root width	The average of two basal root widths, the width of a basal root

RESULTS

Plant length and stem diameter were key breeding targets for growth habit, (Soltani, et al., 2016). To confirm this trend in the pinto panel, measurements of plant length and stem diameter were collected and plotted according to year of release to assess a general trend of these two shoot architectural traits. Data across both years show that plant length has decreased over time and stem width has increased, supporting the previous study. Plant length of the Common Pinto and Idaho cultivar UI-111 are significantly longer from the three Michigan cultivars in both years combined; however, when breaking it down by year released, plant length had an increase in 1945 but then decreased until 1999 with a subtle increase in 2012 (Figure 2). In 2020, Kodiak and Eldorado are the only two significantly different from the landrace Common Pinto, and in 2021 all three Michigan cultivars are significantly different from the landrace Common Pinto (Figure 2). Looking at stem diameter there was a general increase over time especially in the Michigan cultivars, however, only the 1990 and 2012 were significantly different from the Common Pinto in both data collection years combined and in 2021 (Figure 2). The only exception being in 2020, where every Michigan cultivar is significantly different from the landrace, Common Pinto (Figure 2).

Figure 2. Plant Length and Stem Diameter over data collection years with significant differences ($p<0.05$). A. Plant Length in both 2020 and 2021 combined. B. Plant Length in 2020. C. Plant Length in 2021. D. Stem Diameter in both 2020 and 2021 combined. E. Stem Diameter in 2020. F. Stem Diameter in 2021.



Each root trait was assessed on how it correlates across stem diameter, plant length, the year of release and growth habit. When 2020 and 2021 are combined, 16 out of 24 root traits are significantly different with stem diameter and 6 out of 24 root traits with plant length ($p<0.05$) (Figure 3). There were 11 strong correlations with stem diameter, being taproot diameter, basal root angle, adventitious root gap from the bottom, percentage of nodules, number of root tips, network area, solidity, average diameter, root length diameter 1 and 3, and basal root width ($R^2 <0.5$/ $R^2 \sim 0.5$) (Table 4; Appendix). Whereas only 3 out of 24 and 1 out of 24 traits were significantly different in year of release and growth habit ($p<0.05$) (Figure 3). In year of release, taproot diameter, percentage of nodules, and basal root width were significantly different (Figure

3). When looking at growth habit only basal root width was significantly different (Figure 3). There only strong correlation with both year of release and growth habit was basal root width (R^2 <0.5/ R^2 ~0.5) (Table 4; Appendix). Notably, the year of release and growth habit have an opposite association of correlation.

In 2020, 6 out of 24 root traits are significantly different with stem diameter and 0 out of 24 root traits are significantly different with plant length ($p<0.05$) (Figure 3). There were six strong correlations with stem diameter being taproot diameter, adventitious root number, percentage of nodules, maximum width, convex area, and basal root width (R^2 <0.5/ R^2 ~0.5) (Table 5; Appendix). In 2020, 4 out of 24 root traits are significantly different with year of release and 1 out of 24 root traits are significantly different with growth habit ($p<0.05$) (Figure 3). In year of release, taproot diameter, percentage of nodules, solidity, and basal root width were significantly different (Figure 3). When looking at growth habit only basal root width was significantly different (Figure 3). There were two strong correlations with year of release and growth, being taproot diameter and basal root width for both respectively (R^2 <0.5/ R^2 ~0.5) (Table 5; Appendix).

In 2021, 5 out of 24 root traits are significantly different with stem diameter and 1 out of 24 root traits are significantly different with plant length ($p<0.05$) (Figure 3). There were three strong correlations with stem diameter being taproot diameter, lateral root number, percentage of nodules (R^2 <0.5/ R^2 ~0.5) (Table 6; Appendix). 3 out of 24 root traits are significantly different with year of release and 0 out of 24 root traits are significantly different with growth habit ($p<0.05$) (Figure 3). In year of release, the significantly different traits are length between basal roots and lateral roots (DistFLR), percentage of nodules, and basal root width (Figure 3). There were two strong correlations with year of release being length between basal roots and lateral roots (DistFLR) and basal root width ($R^2 < 0.5$/ $R^2 \sim 0.5$) (Table 6; Appendix). Full R^2 value table can be found in the appendix; Tables 4, 5, and 6.

Figure 3. Correlation plots with Stem Diameter, Plant Length, Year Released, and Growth Habit with significant associations across all data collection years (* indicates $p<0.05$).

In this study, the purpose of a PCA helps to start establishing similarities of groups in a given dataset. Both 2020 and 2021 combined PCA, each cultivar overlaps one another, with no major separation (Figure 4). When breaking the PCAs down by year, some distinctions occur. In 2020, there is a clear separation between Common Pinto, UI-111, and the most recently released cultivar, Eldorado (Figure 4). However, in 2021, Common Pinto overlapped with Eldorado, but Sierra had a clear separation from Common Pinto and Eldorado (Figure 4). No general distinctions can be made with biplots due to the overlapping of each growth habit in both years combined and separating them independently by year (Figure 5).

Figure 4. PCA plots measuring root traits grouping by year of release across data collection years.

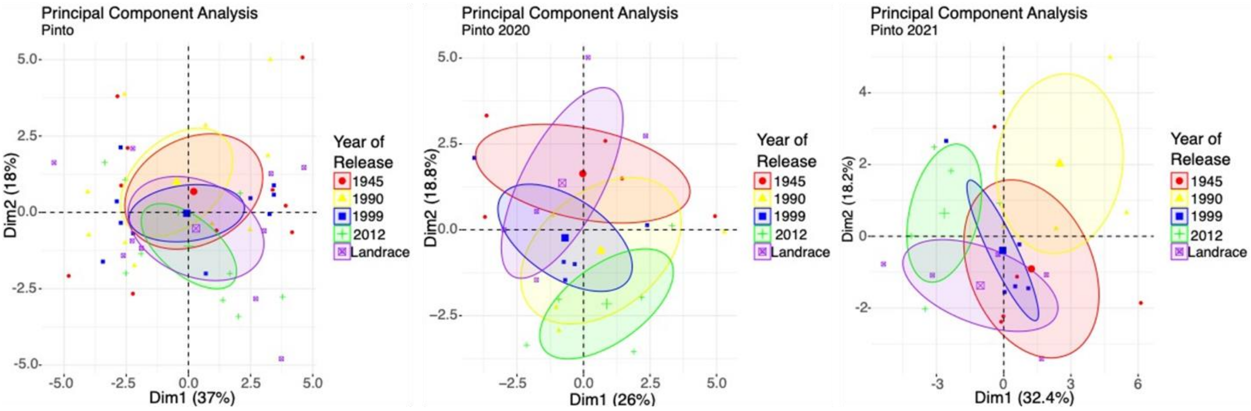
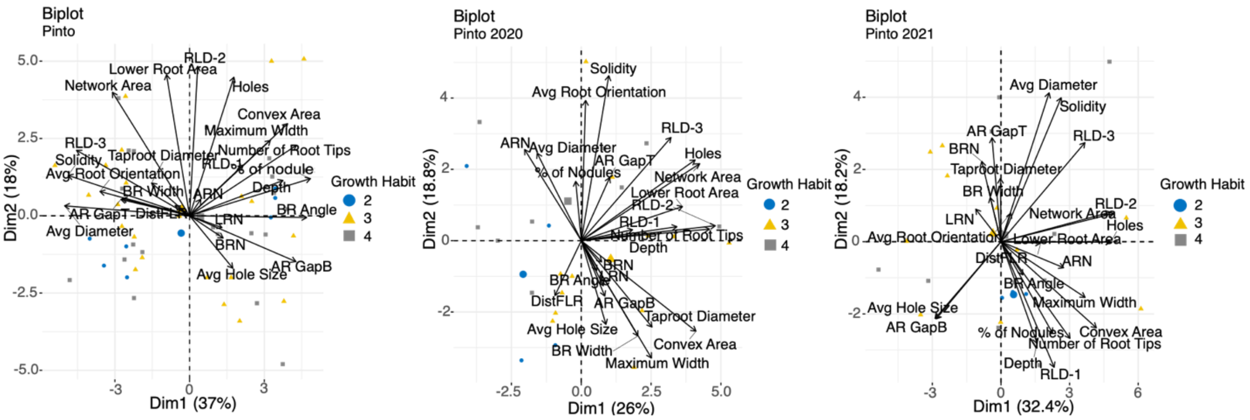
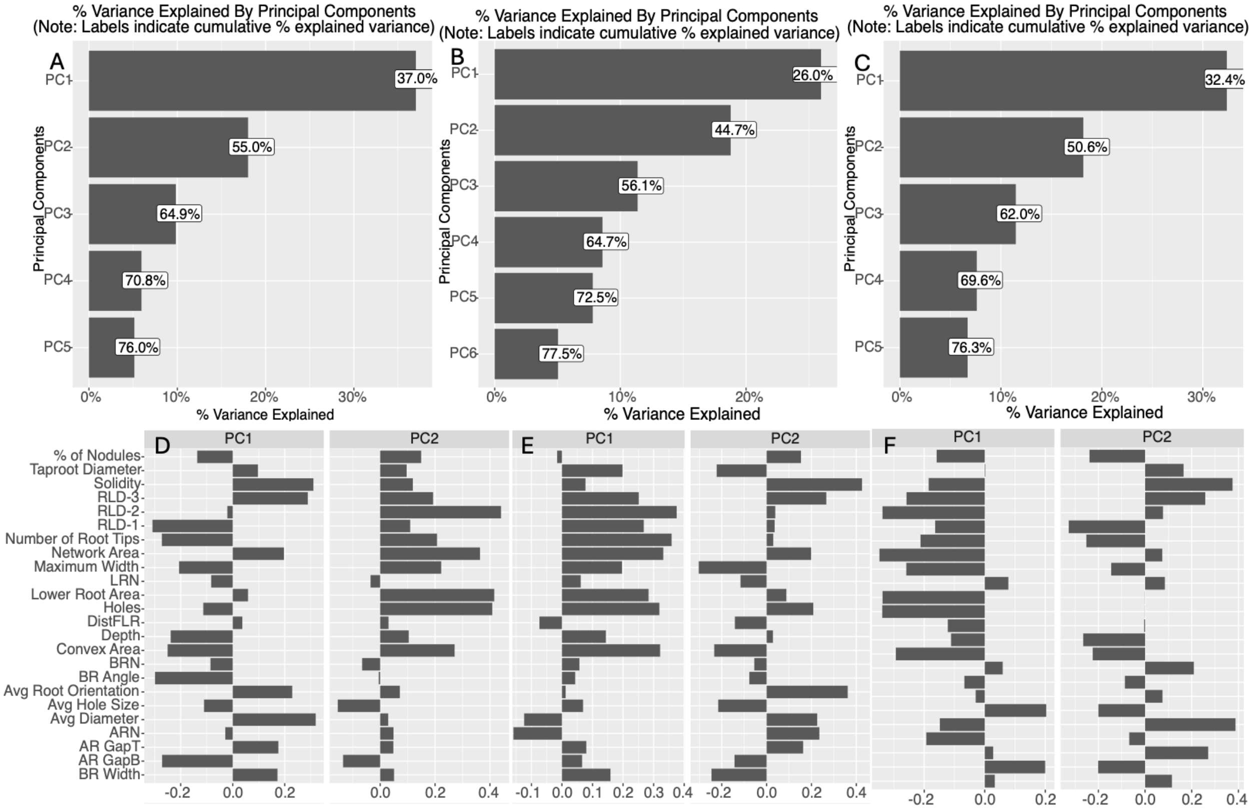


Figure 5. Biplots measuring root traits grouping by year of release across data collection years.



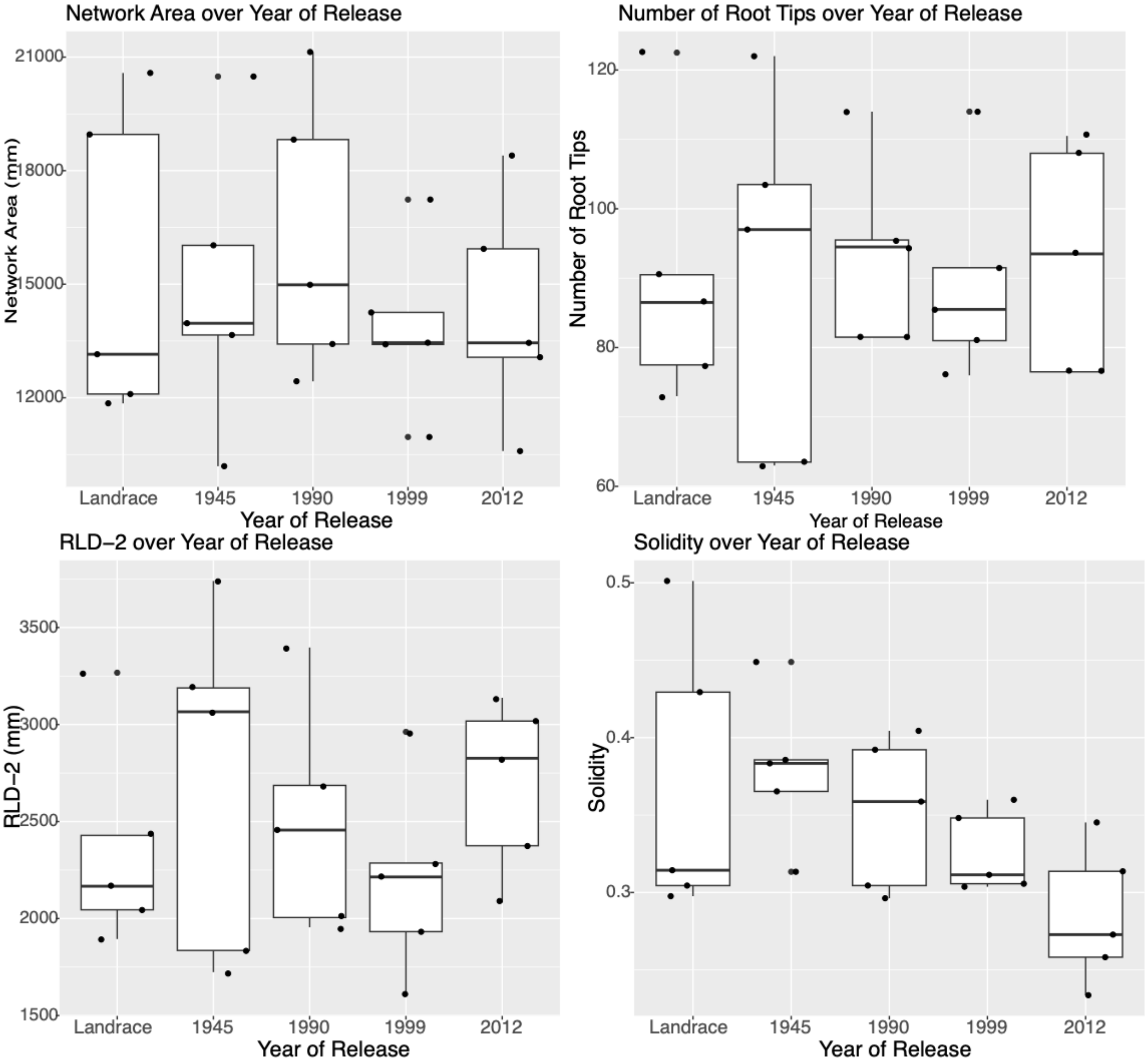
The PCA's were then broken down into Scree plots and Eigenvectors to look at the variance explained by each principal component and which root traits were pulling the principal component in a certain direction. With both years combined there is an 18% variance explained by PC1 and 9.9% variance explained by PC2 (Figure 6). In 2020, 18.7% of the variance was explained by PC1 and 11.4% of the variance was explained by PC2 (Figure 6). In 2021, 18.2% of the variance was explained by PC1, and 11.4% of the variance explained by PC2 (Figure 6).

Figure 6. Scree plots and Eigenvectors showing variance and root traits expressed by PC1 and PC2 across data collection years. A. Both 2020 and 2021 Scree plot. B. 2020 Scree plot. C. 2021 Scree plot. D. Both 2020 and 2021 Eigenvector graph. E. 2020 Eigenvector graph. F. 2021 Eigenvector graph.



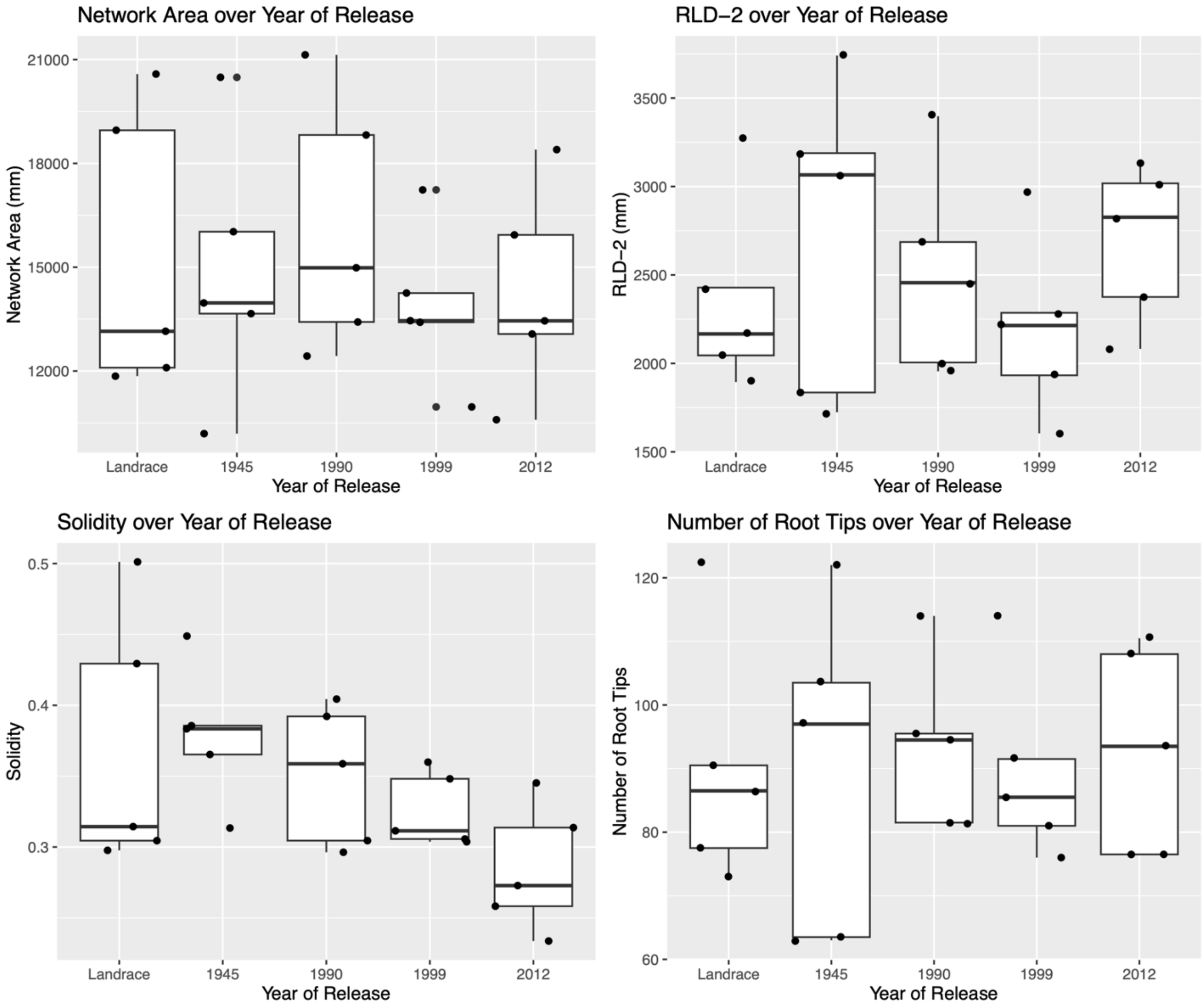
Diving deeper into the PCA plots and eigenvector graphs. Key traits were found to be significant from the PCA plots and were graphed to look at overall trends over time. In both years combined, 2020 and 2021, the top three root traits contributing to the first principal component was Average Diameter, Solidity, and Root Length Diameter 3, and in PC2 the top one being Root Length Diameter 2 (Figure 6). When plotting the general trend of Average Diameter, Solidity, Root Length Diameter 3, and Root Length Diameter 2 starting with Common Pinto, these traits increased until the 1990 cultivar Sierra, then after decreased to the latest cultivar Eldorado, even lower than Common Pinto (Figure 7).

Figure 7. Boxplots showing general trends of top PC traits in 2020 and 2021 combined.



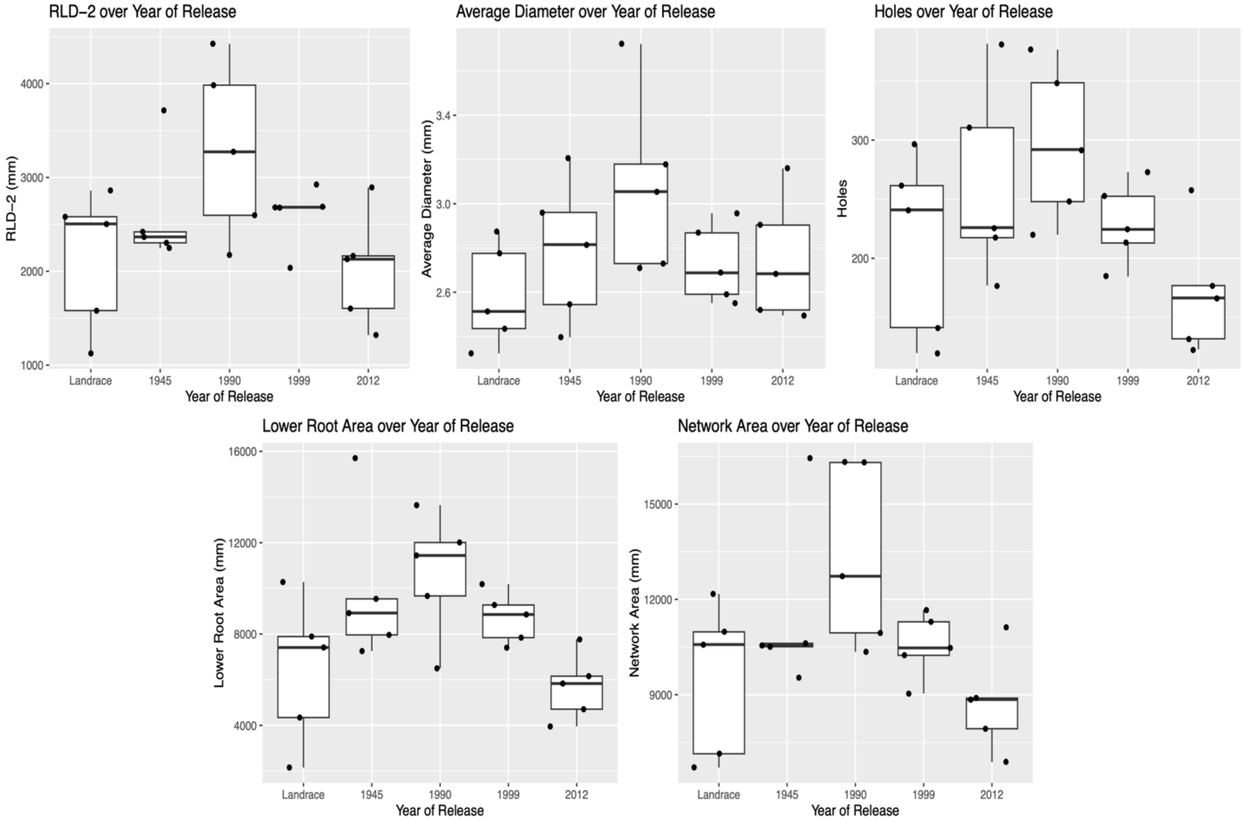
In 2020, the top three root traits pulling PC1 being Root Length Diameter 2, Number of Root Tips, and Network Area; and the top one in PC2 being Solidity (Figure 6). When plotting these root traits, the general trends are vastly different than both years combined. Network Area's general trend is in consensus with an increase until the 1990 cultivar Sierra, and then a decrease to the 2012 cultivar Eldorado (Figure 8). The Number of Root Tips when plotted had a similar trend with an increase in root tips until 1945 then had a steady decrease until 1999 then there was an increase in 2012 (Figure 8). Root Length Diameter 2 also exhibited this trend (Figure 8). However, when looking at Solidity, it followed the trend of increasing until 1945, but then had a steady decrease including the 2012 cultivar (Figure 8).

Figure 8. Boxplots showing general trends of top PC traits in 2020.



In 2021, the top three root traits pulling PC1 being Network Area, Holes, Lower Root Area, and Root Length Diameter 2; and the top one in PC2 being Average Diameter (Figure 6). Plotting these root traits exhibited the same general trend as both years combined, having an increase until the 1990 cultivar and then a steady decrease until 2012 (Figure 9). More data points, and experimentation are needed to conclude why these trends are appearing. Further investigating overall trends of these traits that have these significant associations would reveal what root traits were indirectly selected upon when breeding for shoot architecture.

Figure 9. Boxplots showing general trends of top PC traits in 2021.



DISCUSSION

Indirect selection is when a selection has occurred that was not intentional that brings along other genes that were not the target and causes a phenotypic change (Kirkpatrick and Barton, 1997). We hypothesized that since the MSU dry bean breeding program primarily focused on changes in shoot architecture to adapt to Michigan environments, it inadvertently or indirectly selected upon root architectural traits. Root and shoot architecture have overlapping mechanisms and mimic each other in maize as a result of domestication and artificial selection (Duvick, et al., 2004). Root and shoots also have overlapping mechanisms in dry beans when looking at phosphorus availability (Cichy, et al., 2009). In this study we investigated the possibility of indirect selection occurring on root traits when selecting breeding for shoot architecture in dry beans. This research topic has not been explored extensively in the scientific community.

Data across both years show that plant length has decreased over time and stem width has increased. Breaking it down by each data collection year there were slight variations in decreases and significant differences, but the general trend persisted. We found that stem diameter had an increase over the years especially when switching from the Idaho cultivar UI-111 to the Michigan cultivars. It was also found that plant length decreased over the years especially in the Michigan cultivars. Two out of three of the Michigan cultivars were significantly different from the Landrace, Common Pinto in all three data collections. The significance changes between Sierra, the 1990 cultivar, and Kodiak, the 1999 cultivar. The most recently released cultivar, Eldorado, was consistently significantly different from the landrace, Common Pinto. These two findings concur with the knowledge of breeding for type II shoot architecture for Michigan environments. On the contrary, not every Michigan cultivar when looking at both years combined and separately, were significantly different from the foundational Idaho cultivar.

Correlation plots indicate significant differences between root traits and plant length, stem diameter, year of release and growth habit. Across both years combined and separately in stem diameter; taproot diameter, percentage of nodules, and basal root width were significantly different. Across both years combined and separately in year of release; percentage of nodules and basal root width were significantly different. There were multiple correlations with stem diameter, plant length, year of release, and growth habit. However, the top reoccurring roots traits were taproot diameter, percentage of nodules, and basal root width. Nevertheless, the significant differences found from the p-values are not to be overlooked entirely and can provide additional information for future studies to examine closer at.

When looking at the PCA with both years, there is not a clear separation across cultivars that supports the hypothesis that indirect selection has occurred. In 2020 there is a clear separation between the Common Pinto and our most recent cultivar Eldorado, giving inclination that indirect selection could have occurred. On the contrary, there are no other separations of Common Pinto with any other cultivar besides Eldorado. In 2021, the only separation occurring with Common Pinto was the 1990 cultivar, Sierra; Which is another inclination that indirect selection could have occurred. Nevertheless, there are numerous root traits that are pulling the PCA in a certain direction. Therefore, indirect selection could have occurred within only a few traits but might not have been shown within our PCAs. A further in-depth analysis is needed to unravel which specific traits have been indirectly selected upon, and this work builds the foundation for further research.

Taking a closer look at our PCAs we plotted our eigenvectors to understand what specific root traits were pulling the PCA in a certain direction. It is interesting to note that not all of the Top PC’s did not fully align with the correlation plots significant associations. Instead, gave different root traits that could be candidates for future research in determining whether or not these specific root traits were indirectly selected upon. In both years combined the top three root traits pulling PC1 being Average Diameter, Solidity, and Root Length Diameter 3, and in PC2 the top one being Root Length Diameter 2. In 2020, the top three root traits pulling PC1 being Root Length Diameter 2, Number of Root Tips, and Network Area; and the top one in PC2 being Solidity. In 2021, the top three root traits pulling PC1 being Network Area, Holes, Lower Root Area, and Root Length Diameter 2; and the top one in PC2 being Average Diameter. The overall general trend across these individual root traits is that there was an increase until the 1990 first Michigan cultivar Sierra and then has a steady decline until the most recent cultivar 2012 Eldorado. The only exceptions to that were only found in 2020, where Number of Root Tips and Root Length Diameter 2 had that general trend but instead had an increase in the 2012 cultivar Eldorado. Solidity in 2020 had an increase in the 1945 cultivar, UI-111, but then had a steady decrease all the way until the most recent cultivar. The only overlapping root trait out of all three data collections was Root Length Diameter 2, being another high candidate to study further upon for indirect selection mechanistically wise.

FUTURE WORK

Overall, this study set up the baseline for future research. Furthering this research would need to include another data collection year or two, determining whether or not indirect selection has occurred within the pinto market class, and finally determining a genetic relationship between shoot architectural traits and root architectural traits. It was not determined whether or not indirect selection has occurred within the pinto bean market class in both data years combined. When separating the two by data collection year there were trends and inclinations of indirect selection occurring. Furthermore, having at least two or three more field seasons to maximize replication and determine consistent root architectural traits that were selected upon as well as viewing which cultivars, if any; that indirect selection has occurred.

A GWAS could be performed to identify genetic markers and traits of interest across the landrace, Common Pinto, and across each cultivar. A GWAS or also known as genome-wide association study is a type of research approach to identify genetic variants that are statically significant with a particular gene (Hutter, 2024). When SNPS are identified that are associated with either shoot architecture or root architecture a GWAS can help pinpoint their potential regions of interest that influence that specific trait (Hutter, 2024). A GWAS in this case might prove to be useful due the fact that it can look at a large genetically diverse population. In our case, our beans are directly related to one another but phenotypically look different. At least between the landrace, and the 1945 cultivar, UI-111 in comparison to the Michigan cultivars. GWAS findings can also help identify what specific genes were targeted when breeding for shoot architectural traits over the years as a point of reference. Otherwise, to establish any indirect selection occurring, one might use any genetic correlation test. For example, a GWAS or a pedigree analysis.

To determine, mechanistically what shoot and root architectural traits overlap one another one might use QTL. QTL or quantitative trait locus analysis/mapping is a statical test that links phenotypic data and genotypic data to explain the genetic makeup and basis of complex traits (Falconer & Mackay, 1996; Kearsey, 1998; Lynch & Walsh, 1998). A QTL would be useful to find regions that are association with our shoot and root architectural traits. Once candidate genes are known from a QTL one can look at possible orthologs to determine mechanistically what the gene does. As if an ortholog or homolog exists, the code for proteins might have similar function within our common bean. Otherwise, if it is unknown, one might perform a gene knock-out to prevent expression of said gene to examine its impact upon the plant. Or one could otherwise make recombinant DNA with the gene of interest and make transgenic lines to understand the gene of interests' expression profile and function.

CONCLUSION

It is important to note that there were potential limitations and errors when conducting this study. Shovelomics is a technique that does not evaluate the entire root but rather limits the analyses to the root crown. Therefore, when excavating roots from the field soil, potential breakage could have occurred, and loss of other root trait features since it doesn't encompass the whole root system. Another potential limiting factor during this study was that the data collection year 2021 suffered from poor weed control and poor stand counts which can skew both years. Other potential limitations arise from hand measurements and RhizoVision measurements. When taking hand measurements each individual person has a different point of reference and opinion on where a measurement should begin and end. On the contrary, when using software such as RhizoVision, it has a fixed set of measurements that doesn't encompass every single individual root trait there is. Each software program has its own algorithm that is fixed and trained only to identify certain aspects of the root system, while other software options may provide alternative or improved measurements. Therefore, in our study not every single root trait was measured, and not every root trait that was measured was accounted for in this study due to redundancy.

Overall, this study suggests that possibly occurred within the pinto market class in the MSU dry bean breeding program but remains unclear. Future work is needed to determine the actuality of indirect selection occurring. As we found multiple root traits to be contributing to indirect selection. Basal root width, taproot diameter, and percentage of nodules had significance across multiple data collection years and across contributing shoot architectural variables. Future work will need to include a defining genetic mechanism governing this relationship between shoot and root architecture in dry beans. This research will be a foundation for expanding and analyzing how root architecture affected yield, plant health, and nutrient content in dry beans.

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APPENDIX

Table 4. R square values from correlation plot both years combined.

2020 & 2021	**Stem Diameter**	**Plant Length**	**Year of Release**	**Growth Habit**
Taproot Diameter	0.5417278	-0.0164884	0.3656774	-0.184807
BRN	-0.063045	-0.1000661	0.2274457	0.00585236
BR Angle	-0.7050549	0.1695348	-0.075006	0.1760018
AR GapT	0.3733831	-0.0224055	-0.0485773	0.0120346
AR GapB	-0.6365008	0.3409388	-0.0739481	0.07804121
ARN	-0.4169011	-0.1271598	-0.1959728	0.05859903
DistFLR	0.1226257	0.07555603	-0.2638048	-0.009183
LRN	0.01007564	0.07641911	0.2364746	-0.0266199
% of Nodules	-0.5241314	0.2937588	-0.3898187	0.1019473
Number of root tips	-0.49694	0.3382334	-0.0626668	0.1027469
Depth	-0.4703775	0.3082022	-0.0035116	0.08640718
Maximum width	-0.1821831	0.07468302	0.162985	-0.1676446

Table 4. (cont'd)

Network area	0.5242096	-0.0420338	-0.0280687	0.01688442
Convex area	-0.3145379	0.3109452	0.03300408	-0.0189369
Solidity	0.5642204	-0.1866979	-0.0981758	0.01678577
Lower root area	0.1947978	-0.0139214	0.01240894	0.06249928
Average diameter	0.6545334	-0.212156	-0.0341551	-0.1051498
Holes	-0.2311326	0.186277	-0.0975626	0.2264682
Average hole size	-0.1157713	0.05344709	0.1036759	-0.0917639
Average root orientation	0.4157499	-0.2530238	0.1290117	-0.1213079
Root length diameter 1	-0.6419318	0.3641505	-0.0855453	0.1480431
Root length diameter 2	0.113297	-0.0369432	0.1051826	0.04791877
Root length diameter 3	0.6420195	-0.1168692	-0.0579663	-0.0046327

Table 4. (cont'd)

Basal root width	0.8057925	-0.1580609	0.5738694	-0.5016107

Table 5. R square values from correlation plot in 2020.

2020	**Stem Diameter**	**Plant Length**	**Year of Release**	**Growth Habit**
Taproot Diameter	0.5072163	0.0010537	0.5654914	-0.3939987
BRN	0.2217538	0.276943	0.1449807	-0.196366
BR Angle	-0.0755264	0.02468419	-0.1018272	0.2778312
AR GapT	0.2308741	0.150641	-0.0303901	-0.0218633
AR GapB	0.2635769	0.08718612	0.3101491	-0.0060343
ARN	-0.6482924	-0.1361926	-0.2392897	0.04979951
DistFLR	0.1128789	-0.2526388	-0.0356182	-0.2738004
LRN	0.1674427	0.169468	0.289972	-0.1519841
% of Nodules	-0.5307696	-0.0950604	-0.4058545	0.1309469
Number of root tips	0.2039866	0.1651414	0.05532598	0.1276231
Depth	-0.0054194	0.2487251	0.0035521	0.3239759

Table 5. (cont'd)

Maximum width	0.5259007	-0.1773901	0.3950397	-0.3170032
Network area	0.1365799	0.3753941	-0.1183822	0.1757909
Convex area	0.5702519	0.1243126	0.3470716	-0.077812
Solidity	-0.2906963	0.3274555	-0.4340282	0.2532434
Lower root area	-0.0555847	0.1339498	-0.0546339	0.2520829
Average diameter	-0.2162139	0.209524	-0.2862047	-0.0398862
Holes	-0.0275707	0.1899263	-0.1881587	0.3690198
Average hole size	0.1874869	-0.0390493	0.2429346	-0.0590932
Average root orientation	-0.2784662	0.1324529	-0.1115373	0.1387973
Root length diameter 1	0.08803378	0.0179672	0.0369751	0.2890969
Root length diameter 2	0.252877	0.2588629	0.04376656	0.2201982
Root length diameter 3	0.02176622	0.3682326	-0.2012477	0.1348804

Table 5. (cont'd)

Basal root width	0.8156236	-0.0718861	0.7742969	-0.6486237

Table 6. R square values from correlation plot in 2021.

2021	**Stem Diameter**	**Plant Length**	**Year of Release**	**Growth Habit**
Taproot Diameter	0.580385	0.06472237	0.1697664	0.1167173
BRN	0.4799473	-0.3330758	0.3104399	0.1301495
BR Angle	-0.3250964	-0.0237156	-0.2717326	0.00392708
AR GapT	0.1270968	-0.0496879	0.09756395	0.1741779
AR GapB	-0.26913	0.3900928	-0.3373286	-0.0572857
ARN	-0.023442	-0.23118	-0.1425903	0.04807401
DistFLR	-0.217979	0.2865009	-0.5504717	0.3541595
LRN	0.5377814	-0.0271398	0.1881237	0.1759892
% of Nodules	-0.5519592	0.3455318	-0.4252571	0.03160788
Number of root tips	0.102536	0.32611	-0.1707653	-0.018581
Depth	0.09213605	0.2708219	-0.0114646	-0.2436954

Table 6. (cont'd)

Maximum width	0.00094571	0.02520974	-0.0150712	-0.2131087
Network area	0.2130455	-0.0155768	0.06931359	0.02273855
Convex area	-0.0006136	0.2912037	-0.1546419	-0.1484564
Solidity	0.2918149	-0.3666589	0.2881953	0.1913283
Lower root area	0.1665982	0.00358614	0.09002064	-0.0842276
Average diameter	0.263132	-0.3874735	0.2741343	0.09439545
Holes	0.1288453	0.1296145	-0.0288056	0.05082799
Average hole size	-0.3277566	0.04597312	-0.052336	-0.184018
Average root orientation	0.2066553	-0.2727192	0.3789125	-0.2213668
Root length diameter 1	-0.0583868	0.4914801	-0.2945887	0.01450889
Root length diameter 2	0.1948334	-0.1292425	0.153977	-0.0910528

Table 6. (cont'd)

Root length diameter 3	0.2619412	-0.0812753	0.06330953	0.2525873
Basal root width	0.399954	-0.113315	0.5695181	-0.3454453