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BREEDING AND GENETICS OF PERENNIAL MAIZE: PROGRESS, OPPORTUNITIES AND CHALLENGES

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ABSTRACT

Perennial crops have many hypothesized benefits (i.e. intercepted sunlight and nutrients over longer periods, increased yields, decreased soil erosion, improve stability of grain production, etc.) making them attractive for sustainable production. Annual maize (*Zea mays* L.), domesticated ~9 000 years ago, is one of the most important world crops and billions of dollars have been spent towards its improvement. However, there has been minimal effort to breed perennial maize and benefits hypothesized remain untested. Through crosses between annual maize and its two interfertile perennial maize relatives: tetraploid *Z. perennis* and diploid *Z. diploperennis*, maize germplasm and genomics knowledge can be leveraged to develop perennial maize. A few crosses and populations have been made by other investigators, notably Brewbaker, Carlson, Doebley, Holland, and Shaver but no adequate-yielding perennial maize has been developed. Compared with breeding perennial sorghum (a close maize relative) maize appears to have additional recessive quantitative traits that must be pyramided (senescence, tillering, perennial rhizomes/crowns, regrowth, heat and cold tolerance). I have now integrated the aforementioned perennial derived germplasm for nine breeding



seasons with additional crosses to *Z. diploperennis* but very few to annual maize. My methodology has involved a cycle of 1) selfing crosses to purge deleterious recessive alleles; 2) selecting at the end of each season for non-senescence, regrowth/tillering and large ear size and 3) crossing between germplasm derived from different sources to pyramid complementary advantageous traits. Dramatic progress in adaptation, ear size, non-senescence and stability has been obtained, but this is confounded with evaluation environments. As of yet, we have not found a derived plant that oversummers, overwinters and produces rhizomes in Texas as the wild species do. A number of novel phenotypes have been observed including “ear forest”, clumping grass types versus large tillered stalks, and prolificacy. A “one-best” ideotype for perennial maize still remains elusive.

Keywords: perennial, *Zea*, maize, sorghum, breeding, *Z. diploperennis*

INTRODUCTION

Nearly all domesticated grain crops are annuals that senesce and die after reproduction within the growing season in which they were planted, or they are managed as annuals, as in the case of sorghum, which is weakly perennial in warm climates. Perennials unlike our annual crop plants have an ability to overwinter and generally survive three or more successive years. Despite great successes in production, annual grain systems suffer from negative externalities including high input costs, soil erosion, and a limited growing season. Specific perceived economic and environmental benefits of perennial crops include increased vegetative cover over longer growing periods leading to increased photosynthate assimilation, decreased planting costs and reduction of energy-intensive inputs. Expanded root architecture of perennial crops could increase soil sequestration of carbon, further decrease off-season soil erosion, increase temporal access to water and nutrients among many other benefits. Perennial crops are one likely approach to improving sustainable food, feed, and fuel production with decreased land availability and access to inputs under a changing climate (Pimentel *et al.* 2012). Pastures and grasslands, which are generally managed as perennial systems, demonstrate improvements in critical ecosystem services; however, they do not produce grain needed for food, feed, and fuel uses. Despite these benefits the successful development of perennial grain crops has been sparse and largely limited to a few C3 grasses such as rice (Sacks *et al.* 2003, 2006), wheat (Scheinost *et al.* 2001; Murphy *et al.* 2009), and intermediate wheatgrass ‘Kernza’ (Cox *et al.* 2002; Strand 2010).

Among the productive and widely grown grain crops that humans have domesticated is maize (*Zea mays* L.). Genetic/breeding and agronomic improvement of maize production in the United States of America and the rest of the developed world has been incredibly successful, increasing production as much as 800 percent from a century ago; meaning eight times less land is needed to produce the same amount of grain (Brummer *et al.* 2011). While yields continue to increase at 1-2 percent in

areas of the Midwestern United States of America with the heaviest investment, they have remained stable over the last 10 years in lower yielding and lower investment areas such as Texas (Barerro *et al.* 2013). In the developing world, yields have also not increased at the same rate, which is likely due to both lack of agronomic inputs (fertilizer and irrigation) and limited genetic improvement of the crop. Maize could provide the most immediate impact from the development of perennial cultivars if these cultivars would maintain or increase productivity while providing improved ecosystem services and/or decreased costs of production and allow mitigation and improved adaptation to climate change.

PERENNIALISM ACROSS THE GRASSES

From a comparative breeding, physiology and developmental perspective, it is important to note that all C4 grass crops have close perennial relatives within their genus or are perennial themselves. Sorghum (*Sorghum bicolor* L. Moench) is an important grain, forage, sugar and bioenergy crop grown as an annual that is also closely related to maize. *Sorghum propinquum* and *Sorghum halepense* (also known as Johnsongrass) are both perennial. Close relative Sugarcane (*Saccharum officinarum* L.), is already grown and managed as a perennial. Close relative pearl millet (*Pennisetum glaucum*) has perennial relative Napier grass (*Pennisetum purpureum*). Napier grass, miscanthus (*Miscanthus giganteus*), switchgrass (*Panicum virgatum*) and giant reed (*Arundo donax* L.) are undergoing domestication and improvement for forage, restoration, and bioenergy systems specifically because they are perennial (Lewandowski *et al.* 2003; Jessup 2009). Generating and synthesizing information from across all of these species will likely yield the most fruitful understanding of improving perennial phenotypes. Among these species, *Zea* and *Sorghum* provide the most complementary applied and research targets for perennialism with their wild relatives. Maize and sorghum are the 1st and 5th most important cereal crops worldwide, respectively (FAOSTAT, 2013). These crops also have active breeding and genetics programs throughout the world and have extensive genomic information available. Genome annotations have been completed for *Z. mays* (Schnabel *et al.* 2009; Rokhsar *et al.* 2009; Vielle-Calzada *et al.* 2009) and *S. bicolor* (Paterson *et al.* 2009). Because little is known of the molecular mechanisms conditioning the annual/perennial divide across grass species and given the complexity of the trait, molecular dissection would likely be fruitful.

WHAT IS PERENNIAL MAIZE?

Maize has two perennial relatives in its genus; *Zea perennis* (tetraploid – Mexico), *Z. diploperennis* (diploid – Mexico). One of the first reports of crosses within a *Zea* perennial species was that of Emerson and Beadle (1929) who crossed maize to what we now know as *Z. perennis*. Mangelsdorf and Reeves (1939) went further in the investigations of multiple maize crosses with *Z. perennis* and eastern gamagrass, a temperately-adapted, perennial native grass in the genus *Tripsacum* that resides in the tertiary gene pool of maize. Shaver (1964) was one of the first to seriously investigate the possibility of creating perennial domesticated maize. Shaver continued Emerson



and Beadles populations and also crossed *Z. perennis* with colchicine doubled maize to investigate the genetic inheritance. From these crosses, Shaver derived perennial 4N (tetraploid) and 2N (diploid through pollen sieving) populations. The 2N, however, appeared to nearly lose the perennial phenotype, likely because of the complex suite of genes/alleles needed. The 4N and 2N populations have now been selected for over 20 generations each and have been shared with many other investigators (Shaver, pers. comm.). While many investigators have given up on these populations, a few have gone on to report interesting traits such as nodal propagation (Ting and Tran, 1999). Nodal propagation is a trait clearly possible with perennial sugarcane, which is how cultivars of sugarcane are propagated, but not possible in annual maize.

In 1979, Iltis *et al.* reported a new diploid perennial *Z. diploperennis* had been discovered and since that time it has been crossed by only a few other investigators and generally not for perennialism (Srinivasan and Brewbaker, 1999). J. Holland successfully crossed *Z. diploperennis* to B73 (the maize reference line with the sequenced genome) and NC300 (an elite inbred line important in the southern United States). F_2 progeny of these crosses were investigated both in North Carolina by Holland and by Murray in College Station and Weslaco, Texas. Both Holland and Murray found that combining with B73 was quite poor compared with NC300 in hybrid combination. In Weslaco, the F_1 and F_2 were crossed to late flowering elite Texas maize lines and were also allowed to open pollinate among themselves. From this, 250 progeny were planted in College Station, TX in 2009 and those that demonstrated at least one perennial type trait, (indeterminacy/staygreen, tillering, etc.; described later) were self-pollinated. Shaver's 2N (diploid) and 4N perennial populations (tetraploid) were also late planted in College Station in 2009 and demonstrated a range of perennial phenotypes but due to extreme summer and winter conditions none were found to overwinter. From Shaver's populations out of ~5000 2N plants and ~5000 4N plants, 99 desirable plants from 2N were pollinated, and 34 from 4N were pollinated and planted ear to row in 2010. Plants in this population have been primarily sib-mated but also self-pollinated, crossed with the Holland derived material and crossed with perennial wild relatives by Murray now for a total of nine additional seasons. Germplasm generated and selected has greatly improved for ear size and grain yield which is critical for perennial seed and grain production. A range of other phenotypes has been observed to improve including tillering, totipotency of stem tissue and delayed or eliminated senescence. However, no rhizomes have been observed and plants left in the field have generally died from drought, heat or freezing. Meanwhile, multiple wild *Z. diploperennis* plants have survived for over four years. This continues to suggest that perennial maize could be developed through incorporation of key genes but more work is needed.

GENETICS OF PERENNIALISM

One QTL mapping study of 425 F_2 plants derived from *Z. diploperennis* crossed to the annual teosinte *Z. mays* ssp. *parviglumis* has been reported that used 95 RFLPs (Westerbergh and Doebley, 2004). Very few of the plants had rhizomes and only two QTL for rhizomes were identified but

explained less than 12 percent of the variation. They attributed this low amount of variation to low trait heritability as we might expect in an F_2 study of a threshold trait where the phenotype of interest needs a suite of genes and a favourable environment for screening. However, these findings compare poorly to those in sorghum. Paterson *et al.* (1995) reported a mapping study of 370 F_2 and 378 BC_1 plants in a cross between *S. bicolor* × *S. propinquum* with 78 RFLPs. In this study many more plants had rhizomes and 10 QTL were detected explaining 48 percent of the variance. These findings between perennial maize and perennial sorghum dissection are very consistent with my personal observations having worked with both species. Both the rhizomes and the perennialism observed in *Sorghum* are much more robust than that in *Zea*.

What do we need to make maize perennial?

For a maize plant to act as a perennial a number of conditions must be met: the plant must not senesce at the end of the season; the plant must accumulate energy into structures that can overwinter; the plant must be able to prevent its overwintering structures from both freezing and degradation; and finally the plant must remobilize energy from the overwintering structures into new regrowth in the spring. For perennial maize to actually be grown by farmers this must all be accomplished in a plant that can produce adequate grain yields and can switch back and forth between reproductive and vegetative growth – in addition to the other suites of traits that farmers desire such as disease resistance, nutrient and water use efficiency. The breeding progress for selection on these many different quantitative traits is certain to be slow, but I feel all must be selected simultaneously to avoid breeding into a corner.

There are many quantitative tendencies referred to as perennial-like that do not necessarily result in overwintering. First, stay green, or delayed senescence; from evidence in grain sorghum and maize this is an important trait to maximize grain filling under stress (Thomas and Howarth, 2000; Campos *et al.* 2004). Progeny of perennial by domesticated crosses often show much greater stay green. However, even the most staygreen types of commercial maize senesce completely at the end of grain filling and senescence will be the most important trait to select against in perennial × domesticated *Zea* crosses. Some progeny have had a deceiving staygreen appearance that seems to be due to delayed flowering time and maturity. Domesticated annual sorghum does not naturally senesce and does not appear to be a limiting trait for a perennial phenotype, as many researchers already consider sorghum a weak non-overwintering perennial. This may be the cause of the more heritable perennialism in sorghum. Second, the ability to tiller, ratoon or produce additional stalks from the crown is likely an important component to perennialism. However, basal tillering is also found in many landrace maize cultivars, sweet corn, and many grasses including most annual sorghum; therefore Shaver (1964) cautions against using tillering as a proxy selection criteria alone for perennialism in maize. It has been my observation that often tillering is an underground branching not specifically related to active meristem tissue. Third, ratooning or the ability to regrow tillers when the main stalk is cut is a phenotype expected from perennial plants. However, annual



maize and many types of sorghum harvested green for silage can also demonstrate ratooning ability under the rare proper environmental conditions (Coors *et al.* 2007; Livingston and Coffman, 1997). These annual ratooning plants, however, are not able to overwinter because they often senesce and also do not produce rhizomes. Fourth, the ability to produce rhizomes, modified underground storage stems that allow vegetative reproduction, is an important and heritable phenotype that is found in many perennial plants but no annual ones. Rhizomes are totipotent, can overwinter underground and produce new plantlets (rammets) in the same seasons or in following years from nodes that grow tillers. This totipotency of rhizomes makes them important in perennial growth habit. Perennialism in *S. halepense* (Johnsongrass) has been found to require rhizomes and to be primarily, but not solely, a function of rhizome depth (Warwick *et al.* 1987; Washburn 2012). While the goal is to produce a high-yielding perennial crop and not a noxious weed difficult to kill, vigorous regrowth alone is not a desired criterion. As an alternative to rhizomes, many temperate plants (switchgrass, cordgrass, bahiagrass, etc.) regrow from the crowns via carbohydrate reserves in short, knotty proaxes occasionally mistaken for rhizomes (Haferkamp and Copeland, 1984; Boe *et al.* 2009). This is the overwintering mechanism in close *Zea* relative eastern gamagrass (*Tripsacum dactyloides* L.) (Jackson and Dewald, 1994), its interspecific hybrids with maize (Jatimlinsky *et al.* 2004). This may also be the case for *Z. diploperennis*, in which rhizomes are clearly but only occasionally produced. Regrowth from the crown proaxes in combination with rhizomes would provide an avenue for developing robust perennial maize with multiple overwintering mechanisms. Finally, overwintering, which is the most important component of true perennials will be conditioned by all of these traits and their interaction with the environment. Because of this complexity perennialism is a threshold trait, or one that if even one component or gene/allele is missing, or if the material is screened in a poor environment, we will not be able to observe any plants as perennial. Selection on a complex quantitative trait like perennialism will not be as efficient as selection on a simple additive trait. In both maize and in sorghum we have already developed and/or evaluated genetic resources that improve the chances of rescuing a perennial plant.

CHALLENGES AND INTERESTING TRAITS

While perennialism is the primary goal of this research, a number of other traits of interest have arisen in the germplasm that could be useful for farmers, environment or society. *Later canopy cover* – At the end of an annual maize growing season, not only the nutrients but also the sunlight is typically striking bare ground and not being turned into useful biomass for humans or agro-ecosystems. We observe that the perennial derived material has much greater green leaf tissue at the end of the season, whether through branching, tillering, and/or delayed senescence. *Delayed/non-senescence* – the wild species and many of the crosses do exhibit staygreen or delayed senescence, this is believed to be associated with increased stress tolerance, grain filling period and increased yield. Many breeders look for increased staygreen, especially in hot, arid, tropical climates. *Prolificacy* – the ability to produce many ears on the same plant has been a long

selected trait by maize breeders. These plants tend to produce ears under even the most stressful condition. Many plants derived in the perennial maize crossing program have multiple ears on each stalk or multiple stalks with at least one ear. *Indeterminant regrowth* – the ability to produce biomass after initial flowering and seed set would be very advantageous for making the most of environmental resources. This biomass would be useful for preventing soil erosion, capturing carbon, and providing additional silage or cellulosic biofuel feedstocks. *Ear forest* – some of the germplasm continues to produce a succession of ears that flower at the base of the plant until drought, heat or freeze kills the plant. A potential application for this trait is to graze livestock that can digest starch. *Deeper rooting* – We observe the root systems of perennial *Zea* to typically be much larger and deeper than annual maize. This suggests that they can access additional water and nutrients and may contribute additional biomass to the soil. *Increased sinks* – In the case of sorghum and sugarcane it has been clearly shown that increased diversity of sink tissue results in an increase of potential harvestable energy and that “tradeoffs” are unlikely to be a problem if cultivars are selected for multiple sinks (Murray *et al.* 2012). This is in large part because photosynthesis has been shown to be sink as opposed to source limited.

CONCLUSIONS

The domestication of crops was a long-term proposition and it has taken over 100 years and billions of research dollars to get maize to reach its current productivity. Developing high-yielding perennial maize is likely to take an additional 10 to 40 years, at which point hypotheses of yield and eco-system service comparisons can be formally tested. This long-term high risk research is most appropriate for the public sector. This is concerning given the erosion of public research funds, especially in agriculture. Substantial commitments and investments will be needed to make perennial maize a reality.

FIGURE 1. *ZEA DIPLOPERENNIS*

Fig1A-B: Division of part of a *Z. diploperennis* rhizome from a pot.
Fig1C: Appearance of *Z. diploperennis* in late summer / fall (College Station, 2010). This has not started flowering while everything else is long dead.

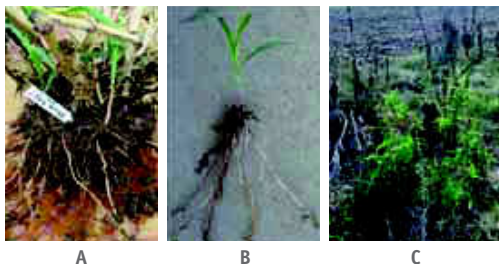


FIGURE 2. DIFFERENCES IN ROOTS

Weslaco, December 2013





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