GENETIC ANALYSIS TO IMPROVE DROUGHT AND LOW NITROGEN TOLERANCE OF CORN IN MONOCULTURE AND IN A KURA CLOVER INTERCROPPING SYSTEM

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DEDICATION

To my loving parents James and Miriam Ziyomo, who highly valued education but never

got the opportunity to get this far.

ABSTRACT

Direct selection for grain yield under stress conditions is often inefficient because the heritability for grain yield is greatly reduced under stress. The objectives of the first study described in this thesis were to determine the efficiency of indirect selection for corn (*Zea mays* L.) grain yield under drought and low N conditions using secondary traits or molecular markers. Testcrosses of 238 intermated B73 x Mo17 recombinant inbreds were evaluated under drought and low N stress conditions. Results indicated that direct selection for grain yield in the targeted stress environment is more efficient than using secondary traits under both drought stress and low N stress. Using significant markers only was not more efficient than direct phenotypic selection for grain yield. The relative efficiency of genomewide selection was significantly greater than 1.0 for grain yield under drought stress but not for grain yield under low N stress. The results suggest that selection based on molecular markers is more efficient than phenotypic selection alone for the improvement of grain yield under drought stress, while for grain yield under low N stress, selection based on markers alone can only be more efficient if gains per unit time and cost are considered. In addition to the genetic improvement of corn for stress tolerance, the use of drought tolerant corn in a kura clover (*Trifolium ambiguum* M. Bieb.) intercropping system can reduce the competition for moisture between the grain crop and the cover crop. The objectives of the second study were to determine if drought tolerant corn can minimize the yield losses incurred when corn is intercropped with kura clover. Results indicate that drought-tolerant corn can maintain high yields and allow

sufficient regrowth of kura clover and therefore significantly reduce the risk associated with intercropping corn with living mulch.

INTRODUCTION

Drought and low soil-N (nitrogen) are two of the major abiotic stresses in corn (*Zea mays* L.) and these stresses often occur together in farmers' fields. The incidence of abiotic stress may increase due to climate change and reductions in soil fertility (FAOSTAT, 2010). The development of corn hybrids that combine high and stable grain yields under abiotic stress with high yields under non-stress conditions leads to sustainable corn production. Direct selection for grain yield under stress conditions has often been considered inefficient because the genetic variance and heritability for grain yield is greatly reduced under stress (Edmeades et al., 1995).

Indirect selection using secondary traits that have a high genetic correlation with grain yield under stress or using molecular markers could significantly improve corn grain yield under abiotic stress. The availability of abundant molecular-marker platforms has made it possible to implement statistical methods that enable the simultaneous estimation of genomewide marker effects as predictors of performance. Such estimations might rapidly accelerate selection gains for quantitative traits (Heffner et al., 2007) although the accuracy of predictions is still a major limitation.

In addition to the genetic improvement of corn for stress tolerance, the use of a leguminous cover crop can reduce the amount of fertilizer N needed. Researchers have developed a living mulch system where corn is grown in band-killed, herbicide suppressed kura clover to mitigate some of the negative environmental impacts of corn production (Albrecht et al., 1999). In this management system, kura clover fixes N and provides permanent soil cover throughout the year. Despite these benefits, one of the

primary challenges with living mulch cropping systems is the competition for moisture between the cover crop and grain crop. Research has shown that with adequate suppression, living mulches can be managed to minimize moisture stress on corn (Zemenchik, 2000), however no studies have been conducted on corn that has been pre-selected for drought and low N stress.

This thesis comprises two studies. The objectives of the study described in the first chapter were to determine (i) the heritability, genetic variance, and genetic correlations for grain yield and agronomic traits for the intermated B73 x Mo17 population under drought and low N conditions and (ii) the efficiency of indirect selection for grain yield under drought and low N conditions using secondary traits or molecular markers. The objectives of the study described in the second chapter were to determine (i) if drought tolerant corn can minimize the grain yield losses incurred when corn is intercropped with kura clover and (ii) if strong suppression of the kura clover living mulch minimizes the loss in grain yield of drought-tolerant and drought-susceptible corn.

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CHAPTER 1

EFFICIENCY OF INDIRECT SELECTION USING MOLECULAR MARKERS AND AGRONOMIC TRAITS TO IMPROVE CORN GRAIN YIELD UNDER DROUGHT STRESS AND LOW SOIL-NITROGEN CONDITIONS

INTRODUCTION

Drought and low soil-nitrogen (N) stress often occur together in the field and their combined effects on corn *(Zea mays L.)* grain yield can be devastating. An estimated 15-20% of corn grain yield is lost each year due to drought stress and such losses may further increase as droughts become more frequent and severe because of climate change (FAOSTAT, 2010). Concerns over the cost and environmental impact of N fertilizers coupled with anticipated reductions in soil fertility (Hirel et al., 2007) necessitate N-use efficient corn. Germplasm that combines high yield potential with a high level of drought and low-N tolerance can lead to more sustainable corn production.

Phenotyping for drought and low soil-N stress tolerance is laborious and resource intensive, requiring multiple locations and years of testing to accurately characterize the traits involved (Bruce, et al., 2002; Collins et al., 2008). Direct selection for grain yield under stress conditions has often been considered inefficient because the genetic variance and heritability for grain yield is greatly reduced under stress (Edmeades et al., 1999). In addition, the correlation between grain yield under stress conditions and non-stress conditions decreases as the level of stress increases (Banziger and Lafitte, 1997, Ribaut et al., 2007). One way to increase the efficiency of selection for tolerance to abiotic stress is

to select for secondary traits that are relatively easy to measure, have high heritability, and are highly correlated with grain yield under stress conditions (Edmeades et al., 1993, Banziger et al., 2000). The efficiency of indirect selection with a secondary trait will be higher than that of direct selection if the heritability of the secondary trait is higher in the indirect selection environment and if the genetic correlation between the direct and indirect selection environments is high. In particular, grain yield under drought stress has been strongly correlated with a high harvest index, more ears per plant, and a short anthesis silking interval (ASI) and moderately correlated with delayed leaf senescence, high leaf chlorophyll content, and plant height (Edmeades et al., 1999). Grain yield under low N stress has been strongly correlated with delayed leaf senescence or "stay green," high leaf chlorophyll content, and a shorter ASI (Hirel, 2007). The correlations of leaf senescence, chlorophyll content, and ASI with grain yield under both drought stress and low soil-N suggest common mechanisms of tolerance and the possibility of breeding simultaneously for tolerance to both types of abiotic stress (Ribaut et al 2007, Banziger et al., 2006).

A second way to improve the efficiency of selection for abiotic stress is to select for molecular markers (Lande and Thompson, 1990) associated with grain yield under stress (Cattivelli, 2008; Messmer, et al., 2009). However, for grain yield under abiotic stress, the complexity of plant response mechanisms across stress levels (Banziger et al., 1999) and high genotype-environment interactions (Rosielle and Hamblin 1981, Tuberosa and Salvi, 2006) have greatly impeded breeding progress. As with secondary traits, QTL analysis has indicated a common genetic basis between tolerance to drought

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and low N (Banziger and Lafitte, 1997; Bruce et al 2002). Common QTLs have been identified for ASI and ears per plant under both drought and low N stress, and QTLs for grain yield under low N stress co-localized with QTLs for ASI (Ribaut et al., 2007).

Despite the initial promise that marker assisted selection held for quantitative traits, QTL information has not directly led to drought and low-N tolerant cultivars (Tuberosa et al., 2002, Collins et al., 2008). This has been partly due to the difficulty in discovering individual QTL with effects large enough to explain a high proportion of the genetic variance (Holland, 2007). The availability of abundant molecular-marker platforms and improvements in computing systems (Bernardo and Yu, 2007; Crossa et al., 2010) has made it possible to implement statistical methods that enable the simultaneous estimation of genomewide marker effects as predictors of performance. Such estimations might rapidly accelerate selection gains for quantitative traits (Heffner et al., 2009) although the accuracy of predictions is still a major limitation.

The intermated B73 x Mo17 (IBM) population is a valuable public resource for genetic studies in corn (Lee et al., 2002). Both B73 and Mo17 have been used extensively as parents in the development of elite corn inbreds (MBS Genetics, 2003). B73 has been sequenced and is also a parent in the Nested Association Mapping population (Yu et al, 2008). The objectives of this study were to determine (i) the heritability, genetic variance, and genetic correlations for grain yield and agronomic traits for the IBM population under drought and low N conditions and (ii) the efficiency of indirect selection for grain yield under drought and low N conditions using secondary traits or molecular markers. The phenotypic and QTL information generated from this study have been deposited in

the maize genetics and genomics database (www.maizegdb.org) for use by researchers

aiming at improving the drought and low N tolerance of corn.

MATERIALS AND METHODS

Germplasm and marker data

The IBM population is a set of recombinant inbreds developed after five generations of selfing from the cross between B73 and Mo17 (Lee et al., 2002). A total of 238 intermated B73 × Mo17 recombinant inbreds, along with B73 and Mo17, were testcrossed to a proprietary Monsanto inbred tester (LH295) that combined well with both B73 and Mo17. The genotypic data for this population are publicly available from Maize GDB (Lawrence et al., 2008). A total of 998 markers which included simple sequence repeats and restriction fragment length polymorphisms were used to construct a 6240 centiMorgan (cM) linkage map.

Drought stress experiments

Drought stress and low N stress were managed in such a way that stress was enough to identify inbreds that had superior yields under each individual stress, without sacrificing that discriminatory power when stress leads to severe barrenness. For this reason we did not have an experiment where drought stress and low N stress were both present, as this could have been too severe to render selection ineffective.

Drought stress screening was conducted at the Anoka Sand Plain Research station in Becker, MN in 2009 and 2010. The soils at Becker are sandy and have a low-water

holding capacity. The experiments were laid out in an augmented randomized complete block design (Federer, 1961), in eight sets of incomplete blocks consisting of 30 testcrosses and five hybrid checks. The testcrosses and checks were planted in two 6.71 m rows spaced 0.76 m apart at a plant population density of 77,000 plants ha^{-1}.

Drought stress and control experiments were both conducted. The control experiment was included to ensure that the drought-tolerant germplasm developed is not only superior under drought but does not have a yield penalty when grown under well-watered condition (Bolanos and Edmeades, 1993). Drought stress was managed such that it was severe enough to delay silking and cause ear abortion and ultimately reduce yield by about 50% (Banziger et al., 2000). At planting, both the control and the drought stress experiments received recommended amounts of irrigation. However, irrigation was stopped after six weeks in the drought stress experiment. In 2009, the control experiment received a total of 753 mm of water in both rainfall and irrigation while the drought stress experiment received 360 mm of water. In 2010, the control experiment received 745 mm while the drought stress experiment received only 345 mm of water in both rainfall and irrigation.

Nitrogen stress experiments

Low-N and control experiments were conducted in Waseca and Lamberton, MN in 2009 and 2010. The experimental design was the same as in the drought trials. The control experiment received the recommended rate (375kg ha^{-1}) of N for optimal growth

of corn. The low-N experiment received half of the recommended rate (187.5 kg ha^{-1})
with the goal of attaining yield levels that are 65-75% of those obtained under well-
fertilized conditions (Banziger et al., 1997). The fertilizer, which was applied before
planting, had recommended amounts of P and K so that N was the only nutrient limiting
growth.

Traits measured

The same traits were measured in the drought stress, low N, and control
experiments. Days to male flowering (anthesis) and days to female flowering (silking)
were recorded and ASI was calculated as the difference between days to silking and days
to anthesis. Small or negative values of ASI indicate stress tolerance (Vivek et al., 2002).
Chlorophyll content readings were taken using a hand-held MINOLTA 502 chlorophyll
SPAD meter (Minolta Camera Co., 1989) and recorded in SPAD units. Readings were
taken on the third leaf from the tassel and on the ear leaf during the flowering period. The
measurements were taken on five alternating plants from each plot, mid-way between the
stem and leaf tip and between the midrib and the edge of the leaf. Leaf senescence or stay
green score was recorded three weeks after anthesis in both the drought stressed trial and
the low N trial. Leaf senescence was recorded as a visual score on a scale of 0 (best) to 10
(worst).

Plant and ear height were measured as the distance in cm from the soil surface to
the tip of the tassel and the distance from the soil surface to the node of the ear bearing
leaf respectively. Plot means were determined after averaging observations from six

plants. Root lodging for each plot was recorded as the percentage of plants leaning at an angle of 45° or more while stalk lodging was recorded as the percentage of plants with stalks broken below the ear. Grain yield for in the drought experiments for both the control and drought stress plots was determined by hand harvesting the middle 10 feet of the two rows. Grain moisture content was obtained by oven drying a 500g grain sample from each plot. For the low N stress experiments, grain yield and grain moisture were determined by machine harvesting two adjacent rows for each plot. Grain yield for both the drought and low N stress experiments was calculated from shelled grain weight per plot and adjusted to 155g H_2O kg $^{-1}$ moisture content.

Trait means, variances and heritability

SAS PROC GLM (SAS Institute version 9.2, 2009) was used to adjust for set (incomplete blocks) effects based on the performance of the checks in the augmented randomized complete blocks (Scott and Milliken, 1993). Analysis of variance (ANOVA) was conducted using PROC GLM with testcrosses and environments as having random effects. Drought experiments for 2009 and 2010 were combined and analyzed across years, and the data for low N experiments were likewise combined across locations and years. For grain yield, grain moisture, root and stalk lodging plant and ear height in the control experiments, means and variances were estimated across locations and years for each trait. Restricted maximum likelihood estimation (REML) estimates of testcross genotypic and phenotypic variances were used to compute entry- mean heritability (h^2)

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for each trait. Approximate standard errors of the h^2 estimates were derived from the asymptotic distributional properties of REML variance estimates using the delta method (Holland et al. 2003).

Phenotypic correlations among different traits were estimated as simple Pearson correlations in the control, drought, and low N stress experiments and across stress environments for the same trait using SAS PROC CORR procedure (SAS Institute, 2009). Genetic correlations were estimated in two ways: first from genetic variances and covariances using multivariate maximum likelihood estimation in SAS PROC MIXED as outlined by Holland (2006) and second as the correlation between genomewide marker effects for each trait. Procedures for calculating genomewide marker effects are described later.

QTL mapping and analysis of genomewide marker effects

Based on the genetic linkage map and the phenotypic data combined across years for the control, drought and low N stress experiments, QTL mapping analysis was conducted using Windows QTL Cartographer version 2.5 (Wang et al. 2007). Composite interval mapping was used to detect QTL positions, with a scanning interval of 1cM between markers and putative QTL and a window size of 10cM. The default number of five marker cofactors in QTL Cartographer was used for background control. The empirical $\log_{10}$ of odds ratio (LOD) threshold corresponding to a false discovery rate of 0.05 was determined for each trait (Benjamini and Hochberg, 1995). For the

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chromosomal regions with significant LOD scores, genetic effects and percentage of genetic variation were estimated at the significant peak in the region.

Genomewide marker effects were estimated in SAS using PROC IML procedures (SAS Institute, version 9.2, 2009). The model used was $\mathbf{y} = \mathbf{1}\mu + \mathbf{Xg} + \mathbf{e}$, where $\mathbf{y}$ was the mean phenotypic value for each trait in the training set; $\mathbf{1}$ was an N_T x 1 vector of 1s; μ was the overall mean for the trait; $\mathbf{X}$ was an N_T x N_M matrix of marker genotype indicators, which can take values of 1, 0 or -1 for a biallelic marker of a segregating population (Lorenzana and Bernardo 2009); $\mathbf{g}$ was an N_M x 1 vector of effects of the marker alleles from the first parent; and $\mathbf{e}$ was an N_T x 1 vector of residuals. The corresponding mixed model equations were used to solve for $\mathbf{g}$ (Henderson, 1984). Variance of marker effects were calculated as V_g / N_M (Meuwissen, et al., 2001) with V_g being the genetic variance as estimated from the analysis of variance for phenotypic data and N_M being the total number of markers used. A cross validation approach similar to the procedures by Lee et al (2008) and Lorenzana and Bernardo (2009) was used to evaluate the usefulness of the predictions. The data for each of the 238 IBM testcrosses for each trait was randomly divided into five subsets. Four subsets were combined to form the training set and the remainder was the validation set. The correlation between the observed phenotypic values and predicted genotypic values ($r_{MP)}$ was estimated in the validation set with estimates of V_G and V_E being obtained from the training set.

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Indirect responses to selection using secondary-trait data and molecular-marker data

The efficiency of indirect selection (for secondary traits) to improve grain yield under drought and low N stress was estimated as outlined by Falconer (1989). The efficiency of indirect selection was equal to $R_Y^C / R_Y = |r_A| h_X / h_Y$ where R_Y^C was the correlated response in trait Y, R_Y was the direct response to selection for trait Y, $|r_A|$ was the absolute value of the estimated genetic correlation between trait X and trait Y, and h_X and h_Y were the square roots of the heritabilities for traits X and trait Y, respectively.

The relative efficiency of selection based on significant molecular markers relative to phenotypic selection of the same intensity was also estimated as $(p/h^2)^{1/2}$, where p was the proportion of the additive genetic variance explained by the marker loci and h^2 was the heritability of the trait (Lande and Thompson, 1990). Standard errors for relative efficiencies were estimated using procedures described in (Gilmour et al., 2006). The relative efficiency of genomewide selection over phenotypic selection was estimated as r_{MP}/h^2 (Dekkers, 2007).

RESULTS AND DISCUSSION

Trait means and heritabilities under stress and non stress conditions

Reductions in corn productivity were more severe under drought stress than under low N stress as indicated by the lower mean grain yield and plant height, longer ASI, and earlier leaf senescence with drought stress than with low N stress. Mean grain yields of the IBM testcrosses were lowest in the drought stress experiments (5.36 Mg ha^{-1}), intermediate in the low N experiments (10.43 Mg ha^{-1}), and highest in the control experiments (12.98 Mg ha^{-1}) with the differences being statistically significant ($P < 0.05$). Drought stress reduced grain yield by 61% while low N stress reduced grain yield by 20%. Although the low N stress imposed in this study did not lead to the 30-50% targeted reduction in grain yield (Banziger and Lafitte, 1997), significant differences for ASI, leaf senescence and leaf chlorophyll content were detected in the low N stress experiments but not in the control experiments. The higher than expected grain yields under low N stress were attributed to high soil nitrogen levels (12-15ppm) in the top 60cm of soil prior to planting.

In the control experiments, the 238 IBM testcrosses showed significant differences ($P < 0.05$) for grain yield, grain moisture, plant and ear height but not for ASI, leaf senescence and leaf chlorophyll. Such lack of significant differences for ASI and leaf senescence in the absence of stress has been previously reported (Banziger et al, 2000; Lafitte et al., 2004). In contrast, under both drought stress and low N stress conditions, there were significant differences for ASI, leaf senescence and leaf chlorophyll content

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among the IBM testcrosses. Generally, means and genetic variances for traits decreased with decreasing mean grain yields except for ASI, and leaf senescence (Table 1).

The heritability on an entry-mean basis for grain yield was 0.60 under control conditions, 0.44 under low N stress conditions and 0.37 under drought conditions (Table 1). The general decline in heritability for grain yield as the level of stress conditions increased corresponded to a decline in mean grain yields. Bolanos and Edmeades (1996) observed this relationship and noted that the decline in heritability is most likely due to a more rapid decrease in genetic variance in comparison to non-genetic variance among plots which consequently limits selection efficiency under abiotic stress. Likewise, the heritability for plant and ear height, leaf chlorophyll content and stalk lodging, was highest in the control experiments and lowest in the drought experiments. In contrast, heritability for ASI and leaf senescence was highest under stress conditions (Table 1). The higher heritability and genetic variance for ASI and leaf senescence with increasing intensity of stress underscores the need for managed stress environments in selecting for these traits.

Indirect selection with molecular markers

The QTL mapping analysis identified a total of eight significant marker loci for grain yield in the control experiments, eight under low N conditions, and five under drought conditions (Table 2). These significant marker loci accounted for 29 to 39% of the total phenotypic variation in each experiment (Table 2). Of the 21 total significant

loci identified for grain yield, none had an R^2 value above 10% suggesting that many loci with undetectable minor effects influenced grain yield. Although the same number of significant loci was observed in the control and under low N stress conditions, the size and effects declined under low N stress, and this could be attributed to the decline in heritability.

The relative efficiencies of marker-based selection (i.e., using significant markers only) observed in this study ranged from 0.59 to 1.16 for traits under drought stress and from 0.71 to 1.18 for traits under low N stress (Table 3). Relative efficiencies for both genomewide selection and marker-based selection were below 1.0 for most traits suggesting that marker based selection can only be more efficient than phenotypic selection when more than one cycle of selection is considered per year. For grain yield, the relative efficiency of marker-based selection was 0.90 for grain yield under drought stress and 0.88 for grain yield under low N stress. Higher efficiencies were obtained with genomewide selection, with an efficiency of 1.24 under drought stress (significantly greater than 1.0) and 0.93 under low N stress (Table 3). It was rather surprising that the relative efficiency for grain yield under drought stress was higher than the relative efficiency for grain yield under low N stress although grain yield under low N stress had a higher heritability. Plant height under drought stress had the highest relative efficiency of 1.42 with genomewide markers, and this trait had the lowest heritability (0.31). However, this was not the same trend for other traits; there was no particular relationship between trait heritability and relative efficiency of using genomewide markers. Marker-

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based selection with only significant markers was more efficient for traits that had lower heritabilities as previously noted by Lande and Thompson, 1990.

Overall, our results indicated that the advantage of marker-based selection and genomewide selection over phenotypic selection would largely be in gain per unit time and cost rather than in gain per cycle (Bernardo and Yu, 2007). Suppose the cost of obtaining testcross performance data under drought stress is US$200 for each inbred (with yield trials under drought stress and control experiments at five locations at the cost of US$20 per location) and the cost of genotyping is 10 cents per data point. When all other associated costs are ignored, the cost of genotyping the 238 testcrosses with the 1339 markers would be about US$32,000 whereas the cost of phenotyping would be about $48,000. Furthermore, gains per unit time can be maximized by conducting up to three generations of genomewide selection in one year in greenhouses or year-round nurseries (Bernardo and Yu, 2007; Eathington et al., 2007; Bernardo, 2008). On the other hand, one cycle of testcross phenotypic selection usually requires two years in temperate regions, i.e., one year for field evaluations, and multiple generations in the second year to recombine the selected individuals or selfed families and to self and testcross for the next cycle of selection making. Therefore, for grain yield under drought stress (relative efficiency of 1.24 with genomewide selection), the cumulative response from three cycles of genomewide selection (in one year) would be about 3.72 times the gain from one cycle of testcross phenotypic selection (in two years), assuming that per-cycle gains from genomewide selection are maintained across three cycles.

The pattern of correlations between estimated molecular marker effects reflected the pattern of genetic correlations observed with the phenotypic data alone for most traits. The sign (positive or negative) of the correlations was the same for all trait pairs except for a few correlations involving plant height and grain moisture content, for which the correlations were positive based on the phenotypic data alone and negative based on the estimated marker effects (Tables 4 and 5). For the drought stress experiments, the absolute values of both the genomewide marker-effect correlations and the genetic correlations ranged from 0.01 to 0.79 (Table 4). For the low N experiments, the genomewide marker-effect correlations ranged from 0.05 to 0.78 whereas the genetic correlations ranged from 0.01 to 0.89 (Table 5).

Genetic correlations and relative efficiency of indirect selection for grain yield using secondary agronomic traits

Genetic correlations were higher under low N stress compared to drought stress as had been observed with trait heritabilities and grain yield means. The highest genetic correlation under low N stress was between leaf senescence and leaf chlorophyll content (-0.89) and this strong correlation was not surprising given that both traits essentially measure the "stay green" trait. Leaf chlorophyll content had a positive and strong correlation with grain yield, underscoring the importance of chlorophyll as an indicator of the N status of the plant. Better nondestructive phenotyping methods for leaf chlorophyll

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content might help in improving results from field experiments and therefore warrant the use of this trait in indirect selection for grain yield.

For both grain yield under drought stress and grain yield under low N stress, there was no single trait that had a relative efficiency significantly greater than 1.0 (Table 6) indicating that direct selection for grain yield alone will lead to larger gains compared to indirect selection. Grain yield in the control had a coefficient of 0.78 under drought stress and a coefficient of 0.85 under low N stress, indicating that selection in high yielding environments where the heritability is higher might not be as efficient as directly selecting for grain yield in the target environment. Therefore control experiments would be grown solely to monitor yield potential but not to select high yielding lines for stress tolerance.   Weber et al. (2012) also concluded that direct selection for grain yield under low N stress in managed stress environments was more efficient than either indirect selection under optimal conditions or random abiotic stress conditions On the other hand it could also mean that the stress was not sufficient enough to reveal a different set of alleles for low N stress tolerance. Atlin (2003) demonstrated that grain yields in stress free environments were not controlled by the same genes as grain yields under stress conditions, therefore rendering indirect selection inefficient. Contrasting results have been reported regarding the most efficient approach in selecting for grain yield under abiotic stress with some authors suggesting indirect selection under stress-free or optimal conditions where the heritability for grain yield is relatively higher (Sinebo et al., 2002; Gallais et al., 2008) while some researchers emphasized the feasibility of direct selection for grain yield in the target environment (Lafitte et al., 2004; Atlin 2003; Venuprasad et

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al., 2007). If yields under stress conditions are to be improved, strategies that utilize information from both environments, with greater emphasis on the target environment could maximize gains from selection by avoiding the selection of genotypes that have high yields under stress conditions but show a huge penalty under stress-free environments.

Indirect selection based on secondary traits versus molecular markers

Compared to phenotypic selection alone, marker-based selection and genomewide selection would have the greatest impact on grain yield under drought and low N stress because of the greater response per unit time especially for traits with the lowest heritability (Lande and Thompson, 1990; Moreau et al., 2004; Bernardo and Yu, 2007). We conclude that marker based selection and genomewide selection are superior to phenotypic selection and holds great promise for complex traits like grain yield under abiotic stress. Furthermore, with the same amount of resources that are devoted to multi location trials and trait characterization, the same genetic gains could be obtained in half the time with genomewide selection.

In this study, direct selection for grain yield in the target environment (drought stress or low N stress) was predicted to be more efficient than indirect selection using secondary traits. Although ASI is fairly easy to measure, and had a strong genetic association with grain yield, complex trait interactions and response mechanisms under drought stress reduce the reliability of phenotypic data alone therefore necessitating the

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need to incorporate molecular marker information in breeding schemes. Grain yield in the control was not more efficient for indirect selection under drought stress ,therefore control experiments still need to be conducted in order to observe and check the responses of plants to drought stress and therefore accurately phenotype the trait (Banziger et al 2000).

For low N stress, direct selection for grain yield was predicted to be more efficient than using any other secondary trait that was measured. This result implies that somewhat a higher weight should be give to low N stress screening environments than to stress free environments if selection gains are to be maintained. For breeding purposes, this has to be taken with caution because if too much weight is placed on a test environment where the heritability for grain yield is low (0.44), this could potentially reduce if not reverse the power to identify genotypes with high yield potential (Weber et al., 2012).Results from secondary trait data suggests that breeding progress with phenotypic data alone is very limited and therefore use of molecular markers to aid in selection   could   result   in   better   gains   for   such   complex   traits.

Table 1: Trait means and heritabilities for the 238 IBM testcrosses under drought stress, low N stress and control (non-stress) experiments in Minnesota in 2009 and 2010.

Trait	Non stress conditions (Control)				Drought stress conditions				Low N stress conditions			
	Mean	SE$_{(mean)}$[†]	Vg[‡]	h^{2}[§]	Mean	SE$_{(mean)}$[†]	Vg[‡]	h^{2}[§]	Mean	SE$_{(mean)}$[†]	Vg[‡]	h^{2}[§]
Yield (Mg ha^{-1})	12.98	0.04	2.03*	0.60*	5.36	0.09	1.62*	0.37*	10.43	0.04	2.68*	0.44*
Moisture (g H$_2$O kg^{-1})	251	0.20	4.36*	0.76*	232	0.15	12.48*	0.47*	161	0.21	1.73*	0.76*
Plant height (cm)	263	0.32	23.8*	0.87*	197	0.96	12.6*	0.31*	228	0.49	15.1*	0.65*
Ear height (cm)	125	0.26	30.0*	0.83*	96.29	0.73	18.90*	0.46*	106	0.42	16.09*	0.75*
Stalk lodging (%)	21.71	0.18	32.46*	0.61*	7.64	0.23	12.38*	0.53*	33.14	0.14	22.37*	0.55*
Chlorophyll (SPADS)	63.76	0.09	23.21*	0.43*	35.93	0.17	19.19*	0.56*	30.23	0.11	10.02*	0.79*
ASI (days)	1.00	0.01	2.03^{NS}	-[#]	5.00	0.08	8.07*	0.67*	4.00	0.05	4.28*	0.41*
Leaf senescence (%)	7.55	0.14	3.68^{NS}	-	47.95	0.06	30.44*	0.61*	53.27	0.33	15.3*	0.65*

† SE $_{(mean)}$, standard error of the mean

‡ V$_g$, testcross genetic variance

§ h^2, Heritability of trait on an entry mean basis

, Heritability was not estimated under non-stress conditions because there were no significant differences in the means

* , Significance at P = 0.05

Table 2. Significant QTLs identified in the intermated B73 X Mo17 testcrosses for grain yield and secondary traits evaluated under drought and low N stress conditions in Minnesota, 2009 and 2010.

Trait	Chr.[†]	Position	LOD score	Flanking markers		Interval	a[‡]	R^2 %[§]
Yield	1	322	3.4	csu207	ufg43	321-346	3.31	6
Control	1	781	4.5	umc2047	rz630a	751-810	0.12	3
	2	220.8	3.9	bnlg1064	umc1448	204-231	1.60	4
	3	139.9	2.6	bnlg1447	bn18.35a	124-151	2.09	6
	4	331.6	4.8	umc1346	bnlg1137	289-341	-0.36	3
	5	76.4	3.2	rz630f	psr922a	62-109	0.91	5
	9	325.2	3.1	umc1231	umc2134	304-332	2.89	2
	10	406.7	2.8	bnlg2190	umc1084	295-326	-1.32	8
Yield	1	589	3.1	umc1925	lim442	557-615	1.76	3
Low N	1	795	4.2	umc1082	rz630a	782-809	2.30	5
	2	552.7	2.9	bnlg1940	umc36a	410-570	0.87	6
	4	477.1	3.2	bnlg2244	umc2139	426-518	3.62	6
	7	160.7	2.7	bnlg1094	umc1393	142-187	-0.64	5
	9	376.4	2.9	umc1187	umc2238	357-421	1.92	3
	10	287.8	3.1	umc1506	umc1196	251-323	2.12	7
Yield	1	226.5	2.8	umc1403	umc1598	208-262	2.94	5
Drought	1	774	3.4	umc1383	umc1431	714-786	0.62	7
	3	258	2.7	umc1449	umc1501	232-285	-1.13	3
	4	433	3.1	bnlg2244	umc15a	426-484	2.67	4
	5	87	2.7	rz630f	mmp130	62-125	0.26	6
	9	234	2.6	umc1688	umc38c	212-288	1.86	4
ASI	1	229	3.6	umc230	umc13	216-242	-0.62	8
Drought	2	61	2.7	bnlg1297	umc1824	53-80	-0.31	5
	3	129	3.2	bnlg1447	umc1012	124-152	-1.84	10
	4	85	2.9	npi282	bnlg565	52-101	-0.21	6
	4	128	5.3	umc1926	umc1902	105-148	-0.54	8
	6	83	3.3	umc1133	psb108	63-112	-0.31	3
	8	345	4.1	bnlg1065	umc1607	339-371	-0.75	6
	10	403	2.6	umc1366	umc1789	396-436	-0.60	7
ASI	1	551.8	2.6	umc2151	umc239	515-600	-0.19	5
Low N	3	138.6	3.1	bnlg1447	umc1392	124-176	-0.46	8
	4	121.8	3.4	umc1926	umc1902	105-148	-0.43	9
	5	93.5	2.8	rz630f	cd0122b	62-122	-0.52	5
	9	271.4	3.8	bnlg1012	umc2095	251-313	0.23	5
	10	398.7	4.1	npi254b	csu48	387-408	-0.47	6

Chloro	1	551.2	2.8	umc58	csu374b	527-573	1.35	13
Drought	3	232.1	3.2	mmc0132	umc1527	202-245	-0.22	9
	5	112.7	2.6	bnlg565	bcd1072a	101-128	1.94	15
	9	245.4	3.4	psr129a	umc1078	242-293	0.88	3
	10	312.3	3.1	bn17.49a	umc1176	308-359	1.26	14

Chloro	1	537.6	2.6	umc2151	umc1925	515-557	0.77	5
Low N	2	483.8	3.1	umc2085	umc1252	461-516	1.45	4
	3	124.6	2.7	bnlg1647	bn18.35a	100-151	0.64	5
	9	213.5	4.2	bn15.10	bnlg1012	194-251	1.85	6

Senesce	1	487.1	2.5	umc1972	umc1919	455-507	-1.08	9
Drought	1	892.3	3.4	umc161a	umc1331	853-951	0.67	8
	3	224.8	3.7	umc1908	umc1600	209-268	-0.76	12
	4	113.4	2.9	umc870	umc2039	88-165	1.68	7
	7	342.1	3.1	umc1134	umc1029	315-387	0.89	8
	9	246.7	2.6	bn15.04	phi032	224-270	-1.13	9
	10	298.3	4.3	psb365a	umc1196	278-323	-1.28	7

Senesce	1	577.8	4.4	umc1925	mmp189	557-620	-0.85	6
Low N	2	234.3	2.7	umc1024	bnlg121	217-276	0.94	13
	4	565.4	3.2	umc1854	bnl15.07a	539-621	2.38	9
	7	165.2	3.0	bnlg1247	umc1393	153-197	-1.47	16
	9	345.6	3.5	rz574b	csu93a	322-367	-1.86	7
	10	363.1	2.8	umc1196	npi254b	323-382	-0.78	7

[†] Chromosome number
[‡] Testcross additive effect, half the difference between the two homozygous genotypes for the QTL
[§] Percentage of the phenotypic variance explained by the QTL, conditioned on the background markers (Basten et al., 2005)

Table 3. Efficiency of marker based selection with significant markers and with genomewide selection based on all markers for the 238 intermated B73 x Mo17 testcrosses grown in Minnesota in 2009 and 2010 under drought stress, low N stress and non-stress (control) conditions.

	Marker-based selection				Genomewide selection			
	Drought Stress		Low N stress		Drought Stress		Low N stress	
Trait	V_M/V_G[†]	Efficiency	V_M/V_G	Efficiency	r_{MP}[‡]	Efficiency	r_{MP}	Efficiency
Grain yield	0.30	0.90 ± 0.20	0.35	0.88±0.21	0.46	1.24±0.07*	0.41	0.93±0.12
Grain moisture	0.47	1.01±0.11	0.39	0.72±0.12*	0.51	1.08±0.04*	0.61	0.80±0.14
Plant height	0.42	1.16±0.19	0.49	0.87±0.08*	0.44	1.42±0.11*	0.57	0.88±0.09
Ear height	0.45	0.99±0.13	0.60	0.89±0.06	0.55	0.93±0.04	0.65	0.87±0.06*
Leaf Senescence	0.61	1.00±0.21	0.58	1.18±0.14	0.39	0.64±0.05*	0.51	0.79±0.12
Leaf Chlorophyll	0.53	0.98±0.17	0.53	0.90±0.11	0.47	0.84±0.06*	0.34	0.43±0.15*
ASI	0.50	0.86±0.15	0.38	0.71±0.13*	0.21	0.31±0.05*	0.12	0.29±0.28
Stalk lodging	0.19	0.59±0.18*	0.26	0.69±0.37	0.18	0.33±0.18*	0.26	0.47±0.11*

[†] Proportion of testcross genetic variance (V_G) explained by significant markers (V_M)
[‡] Accuracy of genotypic value predictions expressed as r_{MP} / h^2 where r_{MP} is the correlation between the predicted genotypic values and observed phenotypic values, and h^2 is the heritability of trait on an entry-mean basis.

* Significantly different from a relative efficiency of 1 at P=0.05

Table 4. Genetic correlations estimated from phenotypic data alone (upper diagonal) and correlations based on genomewide marker effects for different traits measured under drought conditions for the 238 intermated B73 x Mo17 testcrosses.

Trait	Yield $_{control}$	Yield $_{Low N}$	Yield $_{Drought}$	ASI	Chlorophyll	Height	Senescence	Moisture
Yield $_{control}$		0.73**	0.61**	-0.16^{NS}	0.14*	0.56*	-0.23*	0.31**
Yield $_{Low N}$	0.78**		0.59*	-0.25***	0.23**	0.13^{NS}	-0.11**	-0.01^{NS}
Yield $_{Drought}$	0.66**	0.55**		-0.77***	0.69**	0.63**	-0.76**	-0.43**
ASI	-0.02^{NS}	-0.31***	-0.79**		-0.28^{NS}	-0.32*	0.32*	-0.21^{NS}
Chlorophyll	-0.06^{NS}	0.27**	0.66**	-0.02^{N}		0.34*	-0.60**	0.25*
Plant height	0.34***	0.09*	0.67***	-0.35***	-0.05^{NS}		-0.15*	-0.14**
Senescence	-0.29**	-0.21**	-0.73***	0.31**	-0.62**	-0.22**		0.12^{NS}
Grain moisture	0.23NS	0.22*	-0.26*	0.24***	-0.28***	-0.07^{NS}	-0.02^{NS}	

Table 5. Genetic correlations estimated from phenotypic data alone (upper diagonal) and correlations between phenotypic data and estimates of marker effects (Lower diagonal) for different traits measured under low N stress conditions for the 238 intermated B73 x Mo17 testcrosses.

Trait	Yield control	Yield Low N	Yield Drought	ASI	Chlorophyll	Height	Senescence	Moisture
Yield control		0.73**	0.76**	-0.06^{NS}	0.23*	0.34**	-0.18*	0.04^{NS}
Yield Low N	0.78**		0.59**	-0.49***	0.76***	0.68**	-0.89***	0.19*
Yield Drought	0.66**	0.55**		-0.12^{NS}	0.17*	0.16*	-0.21*	-0.03^{NS}
ASI	-0.11^{NS}	-0.42**	-0.16*		-0.28*	-0.22*	0.32*	-0.01^{NS}
Chlorophyll	0.06*	0.63**	0.23**	-0.41***		0.34*	-0.69***	0.05^{NS}
Plant height	0.49***	0.75***	0.24*	-0.39**	0.29**		-0.15*	-0.14**
Senescence	-0.32**	-0.71***	-0.34**	0.43**	-0.72***	-0.20**	-0.12^{NS}	-0.13^{NS}
Moisture	0.20*	0.18*	0.17*	0.06^{NS}	-0.01^{NS}	0.12^{NS}	-0.08*	

Table 6. Efficiency of indirect selection for grain yield under drought stress and low N stress with secondary traits for the 238 intermated B73 x Mo17 testcrosses grown in Minnesota in 2009 and 2010.

Trait $_X$	Grain yield Drought$_{(Y1)}$ Relative Efficiency†	Grain yield Low N stress$_{(Y2)}$ Relative Efficiency
Grain yield (drought)	1.00	
Grain yield (Low N)	0.64 ±0.23	1.00
Grain yield (control)	0.78±0.18	0.85±0.23
ASI (drought)	1.04±0.21	
ASI (low N)		0.47±0.16*
Chlorophyll (drought)	0.85±0.14	
Chlorophyll (Low N)		1.01±0.09
Senescence (drought)	0.98±0.17	
Senescence (Low N)		0.47±0.23*
Plant height (drought)	0.58±0.28	
Plant height (Low N)		0.82±0.15
Moisture (Drought)	0.48±0.24*	
Moisture (Low N)		0.04±0.21

†Efficiency of indirect selection expressed as a ratio between the correlated response and the direct response to selection of trait Y_1 (grain yield under drought stress) or trait Y_2 (grain yield under low N stress)

* Significantly different from a relative efficiency of 1 at P = 0.05

CHAPTER 2

DROUGHT-TOLERANT CORN FOR REDUCING GRAIN YIELD LOSSES IN A CORN-KURA CLOVER LIVING MULCH INTERCROPPING SYSTEM

INTRODUCTION

Virtually all of the corn (*Zea mays* L.) in the U.S. Corn Belt is grown in monoculture. An alternative is to grow corn with a living-mulch cover crop that provides ground cover throughout the growing season. A living mulch provides permanent ground cover, reduces soil erosion (Wall et al, 1991), suppresses weed growth (Enache and Iinicki, 1990), and reduces pest incidence (Litsinger and Moody, 1976). In addition to these ecological and environmental benefits, the use of a leguminous living mulch can improve soil nutrient status by releasing symbiotically fixed N to the soil (Scott et al., 1987; Brown et al.,1993) and reducing nitrate leaching (Ochsner et al., 2010).

Kura clover (*Trifolium ambiguum* M. Bieb.) is a perennial, leguminous crop that regrows and spreads rapidly from rhizomes, survives harsh winters, and tolerates frequent defoliation (Kim, 1996) making it an excellent candidate as a living mulch in the U.S. Corn Belt. Researchers at the University of Wisconsin have developed a living mulch system where the kura clover is established for at least one year, bands of the kura clover are suppressed or killed, and corn is planted in the killed or suppressed bands (Albrecht et al., 2000). To facilitate germination and development of corn, suppression of the living mulch should be well managed to sufficiently reduce competition with the corn crop yet still allow for the regrowth and recovery of the living mulch.

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Current strategies of living mulch suppression include tillage, mechanical mowing, thermal suppression, and non-lethal rates of herbicide treatment (Grubinger and Minotti, 1990; Costello, 1994; Bard, 2009). Corn grain yields from studies that use individual or combinations of these suppression methods have been quite variable and seem dependent on the timing and rate of the suppression, species being suppressed, location of the study, N fertilization, temperature, and water availability. Dependable corn yields have been obtained with herbicide suppression of kura clover, particularly with herbicide-resistant corn hybrids (Albrecht et al., 2000; Zemenchik et al., 2000, Ochsner et al., 2010). Under dry conditions, however, increased suppression of the kura clover did not always lead to high corn yields and the living mulch may also fail to recover if the suppression is excessive (Bard, 2009).

Despite the ecological benefits of intercropping corn with a living mulch, yield losses due to competition between the living mulch and the grain crop have prevented the widespread use of such a cropping system (Duiker and Hartwig, 2004). Grain yield reductions have been mostly attributed to poor corn emergence and moisture stress as the living mulch competes with the grain crop for water (Dehaan et al., 1997, Box et al., 1980). In Wisconsin, reductions in corn grain yield when grown with kura clover have ranged from 8 to 30% (Zemenchik et al., 2000; Ochsner et al., 2010). The key to a successful corn-kura clover cropping system lies in reducing water demands of both the cover crop and the grain crop at critical developmental stages of the grain crop. Previous research has also indicated that kura clover delays corn growth and physiological

development through reduced soil temperatures at the time of planting (Heyland and Werner, 1988).

The use of drought-tolerant corn is one way to combine the environmental and economic benefits of a kura clover living mulch with high corn yields and to minimize the risk associated with this cropping system. This could potentially accelerate the adoption of living mulches in corn production and may allow for increased stover removal for cellulosic ethanol production. If the drought-tolerant corn is competitive enough with the living mulch early in the season, the amount of herbicide required to suppress the living mulch could be reduced and non-herbicide mulch suppression strategies might become feasible. No published information is currently available on the impact of drought-tolerant corn in a kura clover living mulch system. The objectives of this study were to determine (i) if drought tolerant corn can minimize the grain yield losses incurred when corn is intercropped with kura clover and (ii) if strong suppression of the kura clover living mulch minimizes the loss in grain yield of drought-tolerant and drought-susceptible corn.

MATERIALS AND METHODS

Plant materials and drought stress evaluation

The intermated B73 x Mo17 population is a set of recombinant inbreds developed after six generations of selfing seed from a random mated F_2 population (Lee et al., 2002). Both B73 and Mo17 have been used extensively in the development of commercial hybrids in the U.S. Corn Belt. A total of 240 intermated B73 × Mo17 recombinant inbreds, along with the inbred parents B73 and Mo17, were testcrossed to a proprietary Monsanto inbred tester (LH295) that performed well when crossed with both B73 and Mo17.

The testcross hybrids were evaluated for drought tolerance at the Anoka Sand Plain Research station (45°35'N, 93° 10'W), MN in 2009 and 2010. The soils at Anoka are light textured loamy sand that has poor natural drainage hence a low water holding capacity. Drought stress was managed by withholding irrigation such that moisture stress was severe enough to delay silking and cause ear abortion and ultimately reduce yield by approximately 50% (Banziger et al., 2000). Control experiments, which received supplementary irrigation, were planted alongside the drought experiments. The control experiments were included to ensure that the drought-tolerant germplasm developed is not only superior under drought but also do not have a yield penalty in well-watered conditions (Bolanos and Edmeades, 1993).

The drought and control experiments were conducted in an augmented block design experiment with five check hybrids. The hybrids were grown in two-row plots, each row 6.6 m long and spaced 0.76m apart, and at a plant population density of 72 000 plants ha^{-1} in the control experiments. In the drought experiments, the plots were 4.5m long and had a plant population density of 66 000 plants ha^{-1}. At planting, both the control and the drought stress experiments received recommended amounts of irrigation. However, irrigation was stopped after six weeks in the drought stress experiment. In 2009, the control experiment received a total of 753 mm of water in both rainfall and irrigation whereas the drought stress experiment received 360 mm of water. In 2010, the control experiment received 745 mm whereas the drought stress experiment received 345 mm of water in both rainfall and irrigation.

Drought stress has been known to delay silk emergence (Edmeades and Bolanos, 1993), and anthesis-silking interval (ASI) was recorded as the difference, measured in days, between silking and pollen shed. Leaf senescence or stay green was recorded two weeks from flowering as a visual score on a scale of zero (low senescence) to 10 (high senescence). Plant and ear heights were measured at physiological maturity as the visually determined average for each plot. Plant height was measured as the distance from the soil surface to the tip of the tassel whereas ear height was measured as the distance from the soil surface to node of the ear leaf. At physiological maturity, root lodging was measured as a percentage of plants that were inclined at an angle greater than 45^0 and stalk lodging was measured as a percentage of plants with stalks broken below the ear. Grain yield was determined by hand harvesting the ears from three meters

of each row at maturity. Final grain yields were estimated from shelled grain weight per plot after adjusting to 155 g H_2O kg^{-1} moisture content.

Selection of drought-tolerant and drought-susceptible hybrids

A selection index was used to combine information from different traits related to drought tolerance. First, means for each trait of each of the 240 hybrids were calculated across the 2009 and 2010 drought stress experiments, and were likewise calculated across the 2009 and 2010 control experiments. For each hybrid, the means from the drought stress experiments were used to construct a Yield Index as I = (grain yield in t/ha) – 0.028(grain moisture in g H_2O/kg) – 0.059 (stalk lodging percentage) – 0.036(root lodging percentage), where the weights were retrospective weights that have been used by commercial corn breeders (Bernardo, 1991). A Yield Index for each hybrid was likewise calculated with the means from the control experiments.

Second, a Drought Index was constructed by combining information from the (1) Yield Index in the drought experiments; (2) Yield Index in the control experiments; (3) ASI from the drought experiments; and (4) leaf senescence from the drought experiments. Based on empirical data from CIMMYT (Edmeades et al., 1997), we assigned the following weights to each component of the Drought Index: 4 for the Yield Index in the drought experiments; 2 for ASI; 1 for the Yield Index in the control experiments; and 1 for leaf senescence. The Drought Index was calculated as the sum of the ranks (Kang, 1988) multiplied by their respective weights. For example, hybrid MO093 had the highest yield (rank of 240) in the drought experiments, had moderately

high yield (rank of 227) in the control experiments, and had the best ASI and leaf senescence (ranks of 240). The Drought Index of hybrid was then (4)240 + (1)227 +2(240) +1(240) = 1907. The five hybrids with the highest Drought Index were identified as the drought-tolerant hybrids for evaluation in the kura clover living mulch experiments. Likewise, the five hybrids with the lowest Drought Index were identified as the drought-susceptible hybrids for further evaluation.

Kura clover experiments

The ten hybrids chosen for their drought tolerance and drought susceptibility were evaluated in 2011 at the three locations: University of Wisconsin Arlington Agricultural Research Station (43° 18'N, 89° 21'W); University of Wisconsin Lancaster Agricultural Research Station (42° 49'N, 90° 47'W); and University of Minnesota Rosemount Research and Outreach Center (44°71'N, 93°07'W). At each location, the kura clover had been established two years beforehand and was approximately 15cm tall at the time of corn planting. Corn was planted in four-row plots, each 6.6 m long and spaced 0.76 m apart, at a plant population density of 80 000 plants ha^{-1}. At each location, the 20 treatments comprised a factorial combination of the 10 hybrids and two levels of kura clover suppression (killed or living). The experiments were conducted in a randomized complete block design with four replications. No supplementary irrigation was supplied and the total rainfall received at the locations was 452mm at Rosemount, 367mm at

Arlington and 529mm at Lancaster. At each location, a recommended total of 230kgha^{-1} N fertilizer was applied at planting to ensure that N was not limiting corn growth.

In both the living mulch and killed mulch treatments, an initial preplant mulch suppression using glyphosate at a rate of 1.26 kg a.e ha^{-1} was applied to the entire field two weeks before corn was planted. The living mulch treatment and the killed mulch treatment differed in the subsequent dose and placement of herbicide. In the living mulch treatment, herbicide was only applied in 25cm bands where the corn was to be planted. In the killed mulch treatment, herbicide was applied to the entire plot area to kill all aboveground clover in the plot. Specifically, a combination of glyphosate (0.37kg a.e. ha^{-1}) and clopyralid (0.42 kg a.e. ha^{-1}) was applied on each plot in the killed mulch treatment. Corn was hand planted at Arlington into kura clover that was 10 to 15 cm tall whereas a four-row White model 6104 (White Manufacturing, Coldwater, OH) no-till planter was used for planting at Lancaster and Rosemount. Four weeks after planting, glyphosate (0.37 kg a.e. ha^{-1}) was applied for post emergence kura clover suppression and weed control in both the killed and living mulch plots.

Plant and ear height, ASI, root and stalk lodging, leaf senescence, and grain yield and moisture content in the kura clover plots were measured from the middle two rows of each plot in the same manner as in the drought stress experiments. In addition, kura clover regrowth was measured as a percentage of ground cover on the plot area, with zero indicating no ground cover and 100 indicated total ground cover. SAS PROC MIXED (SAS Institute version 9.2, 2008) was used for analysis of variance and to calculate least significant differences at the $P = 0.05$ significance level. Effects of locations and

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location-treatment interactions were considered as random while effects of the 10 hybrids and the two levels of kura clover suppression were considered as fixed. Single degree-of-freedom contrasts at the $P = 0.05$ significance level were used to compare the levels of kura clover suppression as well as the different corn hybrids (drought tolerant and drought susceptible).

RESULTS AND DISCUSSION

Corn grain yields and agronomic traits in the drought experiments

In the experiments at Becker, MN in 2009 and 2010, mean grain yields of the 240 B73 x Mo17 hybrids were 12.36 Mg ha^{-1} in the control experiments and 6.49 Mg ha^{-1} in the drought stress experiments. Drought stress therefore reduced grain yields by about 47%, which was close to the target reduction of 50% (Banziger et al., 2000). The yield losses were incurred at later developmental stages as supplementary irrigation was provided in both the drought stress and the control experiments at planting. Mean grain yields for the five drought tolerant hybrids (identified as those with the highest Drought Index values) were 14.22 Mg ha^{-1} in the control experiments and 9.66 Mg ha^{-1} in the drought stress experiments and the difference between these two means was significant (Table 1). The five drought tolerant hybrids were among the 20 highest yielding hybrids in both the drought stress experiments and the control experiments suggesting that high yield potential can be combined with a high level of drought stress tolerance. Similar results have been reported in both tropical and temperate corn where improvements to abiotic stresses have been associated with high yields under non stress growing conditions (Edmeades et al., 1993a; Duvick, 2005).

Mean yields of the drought susceptible hybrids were 9.01 Mg ha^{-1} in the control experiments and 4.82 Mg ha^{-1} in the drought stress experiments (Table 1). As was the case for drought tolerant hybrids, significant differences were observed between the

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control and the drought stress experiments for the drought susceptible hybrids. Relative to the control, drought stress therefore reduced grain yields by 37% for the drought tolerant hybrids and by 45%. A linear contrast revealed a significant difference ($P \leq 0.05$) between these 37% versus 45% yield reductions (Table 3), indicating that drought stress was more severe on the drought susceptible hybrids. In the Becker drought experiments, the five drought susceptible hybrids were not necessarily the lowest yielding when either drought stress experiments or control experiments were considered alone.

Drought stress can affect corn growth and development in a variety of ways depending on the timing, duration and intensity of stress. In the control experiments, no significant differences were detected for ASI, leaf senescence, and leaf chlorophyll content. Similar results for these traits have been consistently observed in previous studies where corn was grown in different environments with varying levels of stress intensity (Ribaut et al., 1996; Betran et al., 2003). The lack of significant differences for these traits in the control experiment implies they are of adaptive value and assume greater importance under the targeted stress than in environments where water is not limiting (Banziger et al., 2000).

In the drought experiments, mean silking date was delayed by 2 days among the drought tolerant hybrids and by 4 days among the drought susceptible hybrids (Table 1). Mean leaf senescence under drought stress was significantly lower among the drought tolerant hybrids (10%) than among the drought susceptible hybrids (44%) and conversely, chlorophyll content was higher in the drought tolerant hybrids (59.62) that in the drought susceptible hybrids (45.64). Mean grain moisture content, plant height, and

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ear height decreased with drought stress among both the drought tolerant and the drought susceptible hybrids. The lower grain moisture for the drought susceptible hybrids suggested a shorter grain filling period in the drought susceptible hybrids which in turn contributed significantly to the lower grain yields.

Corn grain yields and agronomic traits in the living mulch experiments

Drought tolerant hybrids and drought susceptible hybrids differed in their response to the kura clover, with the drought susceptible hybrids showing extremely low yields in the living mulch treatment. Grain yields for the drought tolerant hybrids ranged from 12.37 to 14.84 Mg ha^{-1} in the killed treatment and from 12.75 to 14.86 Mg ha^{-1} in the living mulch treatment (Table 2). Mean grain yields of the five drought susceptible hybrids ranged from 3.37 Mg ha^{-1} to 5.25 Mg ha^{-1} in the killed treatment and from 2.14 Mg ha^{-1} to 4.76 Mg ha^{-1} in the living mulch treatment (Table 2). Unlike the drought tolerant hybrids, the mean grain yields of the drought susceptible hybrids differed significantly ($P \leq 0.05$) between the killed and living mulch treatments. This result for the drought susceptible hybrids was consistent with the results from the monoculture drought stress experiments in Becker in 2009 and 2010.

Reduced corn yields in living mulch have been attributed to poor seedling emergence and delayed corn development due to restricted root development at the early stages which has often led to moisture stress coinciding with critical periods (Ochsner et al., 2010; Liedgens et al., 2004). Furthermore, most living mulch crops are C3 species

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which have higher photosynthetic rates under cool spring conditions (Tiaz and Zeiger, 2002) and therefore quickly regain competitiveness after suppression early in the season when corn is most vulnerable to frost. In this study, final plant density was 71000 plants ha^{-1} for the drought tolerant hybrids and 68 000 plants ha [-1] the drought susceptible hybrids. The difference between the mean reductions in plant populations was not significant(Table 3) and therefore reduction in population density alone was not the primary cause for the extremely low yields in the drought susceptible hybrids. In a similar study to investigate the effect of living mulch on corn grain yield, Zemenchik et al., 2000, reported a 12% corn plant population reduction for band killed kura clover and a 28% corn plant population reduction in suppressed kura clover.

For the drought tolerant hybrids, the nonsignificant 6% reduction in mean yield between the killed and living mulch treatments was generally similar to the 8 to 14 % reductions in yield for hybrids not selected for drought tolerance or susceptibility (Zemenchik et al., 2000; Affeldt et al., 2004). However, the 22% reduction in mean yield for the drought susceptible hybrids was larger than the yield reductions previously reported by Zemenchik et al. (2000) and Affeldt et al. (2004). These results suggest that drought tolerance is indeed a key factor for maintaining corn grain yields in a kura clover living mulch system.

Three weeks after planting, differences in plant height were apparent between the drought tolerant and the drought susceptible hybrids suggesting that drought tolerant hybrids had a competitive advantage which enabled better resource capture. The ability of the drought tolerant hybrids to suppress the living mulch early in the growing season

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may have helped minimize the yield penalty associated with intercropping corn in living mulch. The presence of living mulch has been previously found to reduce temperatures by up to 2°C in relation to bare soil, resulting in significant delays in corn growth and development (Heyland and Werner, 1988). Band suppression in the corn row was sufficient enough to kill all the above-ground clover and therefore suppress clover growth. However, prevailing weather conditions at planting could have likely impacted on corn development and facilitated faster reestablishment of the kura clover. In the Midwestern United States, spring conditions are highly unpredictable and therefore time of planting and the lag time between suppression of living mulch and planting of corn still need to be carefully managed to reduce the negative impact that unsuppressed living mulch can have on corn grain yields.

In addition to higher yields, drought tolerant hybrids had a significantly shorter ASI, taller plants, lower senescence scores, and higher grain moisture contents for both the living mulch treatment and the killed treatment compared to the drought susceptible hybrids (Table 2). Early leaf senescence score for the drought susceptible hybrids was highest in the living mulch treatment where the scores ranged from 25 to 40% (Table 2). No significant difference in mean leaf senescence between the killed and the living mulch treatment was found for drought tolerant hybrids. This result may have been due to drought tolerant hybrids also being tolerant to low soil N. In particular, the 10 hybrids selected for their drought tolerance and susceptibility in this study have also been evaluated for their performance under low N conditions. The five drought tolerant hybrids were among the top 10 highest yielding hybrids under low N conditions, and the

genetic correlation between grain yield under drought stress and grain yield under low N among all 238 hybrids was 0.65 (significant at P = 0.05). Zemenchik et al. (2000) found that kura clover can meet the N requirements during the first year of corn production, and the enhanced low-N tolerance of drought tolerant hybrids could lead to minimal N fertilizer requirements in the kura clover living mulch system.

For the drought tolerant hybrids, mean grain moisture was 317g H_2O kg $^{-1}$ in the killed mulch treatment and 284 g H_2O kg $^{-1}$in the living mulch treatment (Table 2). For the drought susceptible hybrids, mean grain moisture was 210 g H_2O kg^{-1} in the killed mulch treatment and 236 g H_2O kg^{-1} in the living mulch treatment. A contrast of grain moisture content between the tolerant and the susceptible hybrids was significant (P $\leq$ 0.05). As in the drought experiments in Becker, the lower moisture content for drought susceptible hybrids in both the killed and living mulch treatment could have been due to a shorter grain filling period as the plants shorten their life cycle to avoid prolonged exposure to drought stress.

Kura clover growth and recovery after herbicide suppression

Kura clover is a weaker competitor for limiting resources than other perennial legumes (Albrecht et al., 2000). As a cover crop, this characteristic is favorable because the kura clover will impose a less severe moisture stress on the grain crop. But from an ecological perspective, this characteristic is unfavorable because it will allow weeds to thrive. Differences in growth habit and phenology between corn and kura clover make

them highly compatible because corn requires fewer nutrients as it matures (Richie et al., 1996) and more resources can be allocated to the recovery of the mulch to provide full ground cover. This compatibility also allows for flexibility in timing of kura clover management or suppression to reduce competition during critical periods.

Dependable corn yields in living mulch systems are directly related to the amount of ground cover retained after suppression, with less groundcover resulting in higher yields. Little clover was maintained in all the plots until corn flowering as light availability became limited under the dense corn canopy. However, the kura clover began to spread between the corn rows during grain filling. As expected, mulch recovery was higher and more rapid in the living mulch plots than in the killed plots where suppression was severe for both drought tolerant and drought susceptible plots. Similar results have been previously reported (Affeldt et al., 2004). Overall, mulch recovery was higher with the drought susceptible hybrids (maximum of 80% ground cover at harvest) than with the drought tolerant hybrids (maximum of 50% ground cover at corn harvest; Table 2). Reduced ground cover in the drought tolerant hybrids could be attributed to poor solar radiation for the kura clover due to shading by the corn once it was fully established. In a study of sod maintenance in row crop systems, favorable yields were maintained with up to 60% living mulch ground cover (Elkins et al., 1983). The 50% groundcover observed for the drought tolerant hybrids is evidence that a living mulch system can be successfully managed to combine high yields with the ecological benefits in a living mulch system. For the drought tolerant corn, the lack of significant yield response to mulch suppression treatments corn suggested that drought tolerant corn can be sown in an

established kura clover stand with suppression of only the kura clover bands where the corn will be planted. This strategy could significantly reduce dependence on herbicide application in temporarily inhibiting living mulch growth and suppressing weeds.

In addition to reduced kura clover ground cover, weed growth and establishment was minimal in the drought tolerant plots. Weed pressure was most critical at the onset of tasseling stage in the Rosemount location, necessitating the need for a second application of herbicides to reduce the penalty on corn yield. Although variable results have been obtained from studies that focus on critical time of weed management in a living mulch system, there is a consensus that weed pressure is more detrimental to corn grain yield if it is early in the season than at later developmental stages (Bosnic and Swanton, 1997; Abdin et al., 2000).

CONCLUSIONS

Drought-tolerant corn can improve the economic productivity and sustainability of living mulch cropping systems by maintaining high corn yields and allowing the regrowth and survival of the living mulch. Although less kura clover was maintained in the drought tolerant corn plots during the growing season, the living mulch's rhizomatous nature should allow it to fully recover after the corn is harvested. The lack of significant differences in grain yields between the living mulch and the killed treatment for the drought tolerant hybrids suggests that light band suppression with herbicides is sufficient to reduce competition from the living mulch and maintain high yields. This finding opens up opportunities to evaluate alternative mulch suppression strategies that do not rely heavily on herbicides. We speculate that high yields should be attainable with mechanical

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mulch suppression strategies, therefore making the system more amenable to corn organic production. Further research regarding the biological N fixation of kura clover could help in understanding whether kura clover living mulch can meet all the N requirements of the drought tolerant corn and therefore increase the practical significance of this intercropping system. Whilst drought tolerant corn provides an additional option to reduce the risk associated with this cropping system, severity and time of suppression of the kura clover needs to be appropriately managed to maintain high yields.

Table 1: Trait means for the drought tolerant and drought susceptible corn hybrids under irrigated and non-irrigated (drought stress) experiments at Becker, MN in 2009 and 2010.

Hybrid	Yield mg ha⁻¹	Moisture g kg⁻¹	ASI days	Plant & Ear height cm	cm	Sen %	Chlorophyll SPADS	Yield mg ha⁻¹	Moisture g kg⁻¹	ASI days	Plant & Ear height cm	cm	Sen %	Chlorophyll SPADS
Tolerant hybrids					Control Experiments							Drought experiments		
MO011	14.55	266	1	280	125	0	63.24	9.42	263	3	265	100	10	48.75
MO080	12.67	287	-1	275	120	10	57.24	8.64	248	2	270	110	15	45.23
MO093	16.04	276	0	290	120	5	54.38	10.73	255	3	275	115	10	50.14
MO114	14.57	284	1	290	130	10	62.47	9.88	243	0	260	100	15	42.36
MO179	13.26	259	0	285	125	5	60.78	9.67	261	3	270	120	0	41.73
Mean	14.22	274	0	284	124	6	59.62	9.66	254	2	268	110	10	45.64
Susceptible hybrids														
MO008	6.25	264	0	265	110	10	51.47	3.25	232	5	245	90	45	33.25
MO054	8.37	278	0	275	115	5	53.24	5.94	224	4	240	95	35	43.67
MO062	7.29	257	1	270	105	5	48.95	5.78	236	6	250	100	45	37.29
MO104	9.38	269	1	280	125	10	54.87	4.38	218	5	255	95	50	45.82
MO198	7.76	256	1	270	120	0	56.72	4.76	221	5	240	80	50	38.24
Mean	7.81	265	1	272	115	6	53.05	4.82	226	5	246	92	44	39.65
LSD (0.05)	2.42	6.23	NS	9.28	5.64	NS	NS	2.87	NS	2	10.67	6.32	7.21	4.92

Table 2: Trait means for the drought tolerant and drought susceptible corn hybrids at two different levels of kura clover living mulch suppression at Rosemount, MN and Arlington and Lancaster, WI in 2011.

Hybrid	Yield mg ha^{-1}	Moisture g kg^{-1}	ASI days	Plant cm	Ear height cm	Sen %	Regrowth* %	Yield mg ha^{-1}	Moisture g kg^{-1}	ASI days	Plant cm	Ear height cm	Senesce %	Regrowth* %
Tolerant hybrids				Killed treatment							Living mulch treatment			
MO011	12.37	284	3	285	130	10	40	13.26	297	0	290	135	5	50
MO080	13.25	321	1	270	120	20	50	12.75	310	1	275	125	10	55
MO093	14.84	395	2	290	130	5	40	13.78	357	1	285	130	5	50
MO114	13.41	306	2	280	120	10	30	14.86	345	2	290	130	0	40
MO179	14.63	277	3	275	125	15	45	13.82	322	0	280	120	10	55
Mean	12.90	317	2	280	625	12	41	13.69	326	1	284	127	6	50
Susceptible hybrids														
MO008	4.12	216	4	265	95	25	50	2.88	227	6	235	80	35	65
MO054	5.25	255	5	250	90	20	75	4.76	267	4	240	85	40	75
MO062	5.46	241	4	260	100	15	60	4.10	274	5	240	90	30	70
MO104	4.47	194	4	255	100	25	65	3.53	231	4	245	85	25	75
MO198	3.37	147	6	260	85	10	55	2.14	183	7	230	80	40	80
Mean	4.53	210	5	258	470	19	61	3.48	236	5	238	84	34	73
LSD (0.05)	1.21	5.63	NS	10	6	NS	15	2.47	21	NS	8	5	NS	6

Regrowth* is the percentage of kura clover ground cover at corn harvest

Table 3: Linear contrasts for corn agronomic traits for the drought experiments in Becker, MN in 2009 and 2010 and for living mulch experiments at Rosemount MN, Arlington and Lancaster ,WI in 2011.

	Trait							
Contrast	Yield	Moisture	ASI	Senescence	Plant height	Ear height	Regrowth	Plant popn
Drought experiments				Significance at P = 0.05				
Control vs. drought (Drought tolerant)	*	NS	NS	NS	*	NS		
Control vs. drought (Drought susceptible)	*	*	*	*	NS	NS		
Mean reduction of tolerant vs. susceptible	*	*	*	*	*	NS		
Mean reduction of control vs. drought	*	*	*	*	*	*		
Kura clover experiments								
Living mulch vs. killed (Drought tolerant)	NS	NS	NS	NS	NS	NS	*	NS
Living mulch vs. killed (Drought susceptible)	*	*	NS	*	NS	*	*	*
Mean reduction of killed vs. living mulch	*	*	*	*	*	*	*	*
Mean reduction of tolerant vs. susceptible	*	*	*	*	*	*	*	NS

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