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Response to selection in the initial stages of a perennial sorghum breeding program

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Abstract

When developed, perennial grain sorghum could provide an opportunity for more ecologically sound food production. In 2002, we initiated a perennial sorghum breeding program, using *Sorghum bicolor* (L.) Moench X *S. halepense* (L.) Pers. populations. In 2011-13, we evaluated 27 breeding lines from three stages of the program in field experiments to estimate response to selection during the program's first seven years for perenniality and more domesticated phenotype. Regression analysis of mean grain yield and single-grain weight on breeding cycle (0, 1, 2) indicated a significant increase in each of the seeded experiments and for means over all years. For winter survival index, the regression coefficient was small but significant and negative in 2011, while non-significant in 2012. Grain yield was not significantly correlated with survival index, while single grain weight was significant and negatively correlated with survival index, with $r = -0.4923$. We conclude that combined selection for perenniality, a more domesticated phenotype, adaptation, greater grain size, and higher grain yield can result in progress in breeding perennial sorghum.

Key words: grain yield; rhizomes; single grain weight; *Sorghum bicolor*; *Sorghum halepense*; survival index

Introduction

Higher crop yields observed since the start of the Green Revolution resulted from development of new grain cultivars with annual growth habit, along with widespread use of irrigation and extensive application of fertilizers, herbicides, and pesticides. Yields from these conventional farming systems may have already reached their peak ([Gressel 2008](#); [Foley et al. 2011](#); [de Ribou et al. 2013](#)), and may decline further as agricultural regions experience continued soil degradation associated with annual cropping systems ([Glover et al., 2010](#)).

Replacing annual cereal, grain legume, and oilseed crops with improved long-lived perennial crops that produce similar products holds potential not only for increasing food security, but also for enhancing environmental stability and ecosystem resilience ([Cox et al. 2006](#); [Crews 2005](#); [DeHaan et al. 2005](#); [Glover et al. 2010](#); [Piper and Kulakow 1994](#); [Ridley et al. 2001](#); [Sacks et al. 2006](#)). Among the potential benefits of using perennial crops over annual versions is their ability to improve water quality, reduce soil erosion, conserve soil organic matter, and reduce reliance on fertilizers and tillage operations ([Glover et al. 2010](#)). However, perennial, grain-producing crop species currently exist only in the development stage, and much genetic research and breeding remains to be done before perennial grain crops can be deployed in agriculture ([Batello et al. 2014](#)).

Inasmuch as agricultural crops are grown for unique characteristics, two major traits are of primary importance in determining user acceptability and utilization: grain yield and individual grain size ([Cox et al. 2006](#); [Gepts 2004](#)). It has been proposed that during evolution, maximizing fitness required that different hierarchies of phenotypic plasticity be developed, leading to a tradeoff between seed production and vegetative propagation ([Bradshaw 1965](#); [Sadras 2007](#); [Westoby et al. 1992](#)). Were it to exist, such a tradeoff would lead to predictions that

plants are more likely to die after reproducing as observed in annuals ([Law 1979](#)) and that in perennials, large reproductive output in one year would be negatively correlated with growth and survival that year or in the following year ([Sohn and Policansky 1977](#)). Although grain yields and sizes of wild perennial crop species are smaller than those of annual crops ([Piper and Kulakow 1994](#); [Cox et al. 2006](#); [Moffat 1996](#)), this contrast is not evidence for a physiological tradeoff. Rather, it is a trend observed among species produced by natural selection pressures on the one hand and artificial selection carried out in a properly managed agricultural environment on the other hand. It has been shown that the latter type of selection can increase seed yield in perennial crop populations without negative effects on perenniality or other characters; given that, it is argued, artificial selection has the potential to generate perennial crops with acceptable yields, if applied to seed yield and perennial growth habit simultaneously ([DeHaan et al. 2005](#); [Moffat 1996](#)).

In an effort to develop perennial sorghum, The Land Institute has generated tetraploid sorghum populations derived from crosses involving a range of *Sorghum bicolor* (L.) Moench and *S. halepense* (L.) Pers. parents, the aim being to combine high seed yield with overwintering ability via rhizome production. Grain sorghum, which belongs to *S. bicolor*, is a diploid ($2n = 2x = 20$) annual species ([Ellstrand and Foster 1983](#); [Doggett 1988](#)). It is one of the world's most important crops based on area sown and production ([FAO 1993](#)). *S. halepense* is a tetraploid ($2n = 4x = 40$) perennial that reproduces by both sexual and vegetative means ([Warwick and Black 1983](#)). It is reported to be one of the world's ten most important weeds ([Holm et al. 1977](#)) and has disseminated naturally worldwide. Grain sorghum hybridizes with *S. halepense*, often called johnsongrass, under field conditions ([Baker 1972](#); [Arriola and Ellstrand 1996](#)). Making *Sorghum*

bicolor X *S. halepense* crosses by hand is facilitated by using a diploid male-sterile or induced tetraploid grain sorghum plant as the female parent ([Hadley 1958](#); [Piper and Kulakow 1994](#)).

Rhizomes and perenniality are considered ancestral and fundamentally related traits in monocots and grasses ([Dahlgren et al. 1985](#)). Production of rhizomes is the sole means by which *S. halepense* and *S. bicolor* X *S. halepense* plants can survive year to year in temperate climates with cold winters ([Piper and Kulakow 1994](#)). Rhizomes are absent in *S. bicolor*. Numerous chromosomal regions affecting rhizome development and perennial growth habit have been mapped in *Sorghum* interspecific crosses ([Paterson et al. 1995](#); [Hu et al. 2003](#)); clearly, perenniality in this genus is a quantitative trait.

By crossing the two species, backcrossing to *S. bicolor*, and selecting for winter survival, we have developed perennial progenies with phenotypes intermediate between those of the parental species. We have not previously evaluated trends in perenniality and grain production under selection during the initial years of this perennial sorghum development program, nor have relationships between perenniality and grain production previously been examined in *S. bicolor* X *S. halepense* populations. Understanding of these mechanisms is crucial when defining new criteria for selection in perennial grains. In this paper we examine (1) the response of grain yield, single grain weight, and survival in *Sorghum bicolor* X *S. halepense* populations to selection for a more domesticated perennial phenotype, and (2) the relationship between survival index and the traits grain yield and single grain weight. The overall goal was to determine whether progress was made in the first seven years of the perennial sorghum breeding program and whether associations exist between degree of perenniality and productivity traits.

Materials and Methods

Germplasm

The perennial parents of the breeding line used in this study were derived from populations developed at The Land Institute, Salina Kansas, USA, during the period 1984–94 by self-pollinating hybrids between *S. bicolor* and *S. halepense* (Piper and Kulakow, 1994). The institute's current perennial sorghum selection program commenced in 2002 when crosses were made between *S. bicolor* inbred lines and perennial plants selected from two bulk seed lots taken from storage, each of which was made up of an unknown number of the 1984–94 populations. Although the parents of those populations belonged to *S. bicolor* and *S. halepense*, the identities of the specific parental accessions are unknown.

Population development

In 2002–04, a large number of crosses were made between diploid, annual, male-sterile inbred lines of *S. bicolor* and perennial tetraploid plants drawn from the populations described in the preceding paragraph. Both nuclear and nuclear-cytoplasmic male-sterile *S. bicolor* lines were used in making crosses. A yield trial comprising a total of 400 F₂-derived lines in the F₃ from 17 of those annual X perennial parental combinations was conducted in the summer of 2005, and approximately 300 plants that survived through the following winter were preserved and self-pollinated in the summer of 2006. In subsequent years, many of these plants were selfed, intercrossed, crossed with perennial plants from the original population, and/or crossed with a range of *S. bicolor* inbred lines. Progenies of these crosses were evaluated in 2008, and surviving plants were selected in the spring of 2009. We refer to the perennial plants used as parents in the 2002–04 crosses as cycle 0, and we refer to the winter-hardy plants selected in the field in the

spring of 2006 and 2009 as cycles 1 and 2, respectively. After field evaluation of selfed progenies of plants from cycles 0 through 2 in 2010, six lines from cycle 0, six from cycle 1, and fifteen from cycle 2 (Table S1) were selected for this study. Lines were selected that had the most crop-like, least “wild” overall phenotype among the lines in their cycle. That is, we selected those having less profuse tillering and nodal branching, less shattering, denser panicles, and larger individual grains than other lines from their selection cycle. We had less information available—only one year of observation—on which to base selections from cycle 2 than on which to base selections from the other cycles; therefore, we included a larger number of lines from cycle 2 to ensure that we were including superior lines from that cycle.

Experimental design

Experiments were sown in late May in 2011, 2012, and 2013. They included the above 27 lines from the three selection cycles along with a local accession of *S. halepense* designated Gypsum 9 and a local commercial grain sorghum hybrid, ‘Phillips 664’. Each experiment was sown on The Land Institute’s research farm on the south edge of Salina, Kansas (lat. 38.84° N, long. 97.61° W, elev: 373 m) in montmorillonitic, mesic pactic Argiustoll soils (Soil Survey of Saline County, Kansas, 1992). In 2011 and 2012, seeds were sown in plot areas that had been under fallow for at least eighteen months. The experiments were conducted using a randomized complete-block design (RCBD) with four replicates. Experimental units were single 3 m rows. Because the genotypes used in the study varied greatly in height and growth pattern, we provided a common border by sowing experimental rows alternately with rows of the grain sorghum hybrid ‘Phillips 664’. Rows were spaced 0.91m apart, with 1.5 m alleys between the ends of rows. Initial densities were approximately 30 plants per row. To permit easier access to the perennial

genotypes with their tall, highly branched growth habit, the rows of ‘Phillips 664’ were mowed at approximately flowering stage, and mowed again after they regrew and before they flowered again. For both 2011 and 2012 experiments, no fertilizer and/or herbicide were used. The 2013 experiment was in a split-plot design with two main-plot treatments: (1) randomized plots newly sown into the spaces formerly occupied by two randomly chosen replicates of the 2012 experiment, after removing regrowing and volunteer plants through tillage, and (2) two regrowing replicates of the 2012 experiment. The subplot treatments were breeding lines, referred to hereinafter as genotypes. In the 2013 experiment, we applied nitrogen fertilizer primarily as urea at a rate of 34 kg/ha.

Data collection

At physiological maturity, grain was hand-harvested from all rows in each experiment. In 2013, plants in the regrowing treatment began emerging in mid-May, three weeks before the newly-seeded treatment was sown. The regrowing plants grew and developed rapidly, initiating flowering in early July and continuing to produce new inflorescences through the summer; therefore, three harvests of ripe grain, in mid-August, mid-September, and mid-October, were required. Total threshed grain from each plot in each year and experiment was dried and weighed. Then from each, approximately 5g sample was removed and mechanically dehulled, and the hullless grain weighed. Pre-dehulling grain yield per linear meter of row (g m^{-1}) was multiplied by the ratio of the corresponding small sample’s hullless grain weight to its pre-dehulling grain weight to estimate grain yield. The number of seed in each hullless grain sample was determined with an electronic seed counter, and mean weight of individual grains was estimated as weight divided by number.

All genotypes under evaluation had experienced inter-row competition from the same border genotype, 'Phillips 664', but that competition had varied in time; it was stronger early in the season and then was reduced by mowing as the season progressed. Therefore, yield data could not reasonably be extrapolated to a land-area basis; instead, we have expressed yield herein as grams per linear meter (g m^{-1}) of row.

Mulching

After harvest in 2011 and 2012, two randomly chosen replicates were covered with a mulch of chopped wheat straw to a depth of approximately 8 cm. The mulch was intended to insulate the soil and increase overall rhizome survival and spring emergence. Winter survival in 2011-12 was evaluated as number of ramets emerged per linear meter of row by April 9, 2012; in 2012-13, it was estimated as the proportion of plants that produced emerging ramets by May 21, 2013 (the spring of 2013 being colder, and average emergence later than in 2012). The two estimates were converted to a common survival index by dividing each observed value by the mean value of the entry with highest survival and then multiplying by 100.

Data Analysis

Analyses of variance for all traits were performed using JMP version 11 (SAS Institute, Inc.) in a combined analysis for all years. Genotypes and cycles were specified as fixed; year and replicates were considered as random effects. Changes in mean grain yield, single grain weight, and survival per cycle were estimated by linear regression of cycle mean grain yields, mean single grain weight and survival indices on cycle number (0, 1, 2). Regression coefficients were

used to estimate change per cycle in grain yield, single grain weight, and survival per cycle of selection.

In 2013, we compared newly-sown plots with plots in their second year of growth, having been sown in 2012. We performed the analysis of variance as a split-plot design with year of growth as the main-plot treatment and genotypes as the subplot factor. There were two replicates, with main plots and subplots randomized.

To determine the effect of mulch on winter survival, we compared survival indices of mulched and non-mulched plots. Survival index data were analyzed as a split-plot design, with mulch/no mulch as the main-plot treatment and genotypes as subplot treatment.

Simple correlation analyses were carried out using SAS statistical program (SAS Institute version 9.1.3) to examine relationship between the survival index and the traits grain yield and single grain weight.

Results

Grain yield and single-grain weight

Because high-yielding, large-seeded plants as well as winter-hardy plants occur very rarely in annual x perennial sorghum populations evaluated in Kansas, very large populations must be evaluated in order to identify perennial plants with even slight improved productivity.

Analysis of variance showed highly significant ($P < 0.01$) differences among years, breeding cycles, and genotypes-within-cycles for grain yield (Table 1). Mean squares from cycle x year and genotypes-within-cycles x year for grain yield were not significant, indicating that relative mean yields of cycles were consistent across years. Variation among the three selection cycles was further partitioned into a linear component and a residual. In each of the four

experiments (not shown) and in the combined analysis over years (Table 2), mean squares for the linear effect of selection cycle were positive and highly significant ($P < 0.01$), indicating that selection had the effect of increasing grain yield.

The regression coefficients in Table 2 are estimates of the mean response to selection per cycle for grain yield, single grain weight, and survival index. Regression coefficients for grain yield and single grain weight were significant and positive in all three years' experiments grown from seed and over all years. The mean rate of gain across the three seeded experiments was 52.8 g m⁻¹ cycle⁻¹, indicating improvement per cycle equivalent to 56% of the cycle 0 mean. For single grain weight, the highest gain, observed in 2012, was 0.97 mg cycle⁻¹ and the mean gain across seeded experiments was 0.60 mg cycle⁻¹ (Table 2). For survival, the regression coefficient was small and negative in 2011 and non-significant in 2012.

We compared cycle and check yields with those of the *S. halepense* and *S. bicolor* checks in 2011, 2012, and 2013 (Table 3) and for mean grain yield over all years (Table 4). (The low yields in 2012 were largely the result of an unusual early freeze the night of October 4.) Consistent with the positive regression coefficients, rankings were highly consistent year to year, with cycle 0 the lowest and cycle 2 the highest in every case. The significant regression coefficients imply significant differences among all cycles within each year. Furthermore, mean grain yields for cycle 0 were significantly ($P < 0.01$) higher than those of the *S. halepense* check, Gypsum 9, and the means for cycle 2 were significantly lower ($P < 0.01$) than those of the commercial *S. bicolor* hybrid 'Phillips 664' in each year. Therefore, mean grain yields and single-grain weights shown (Tables 3 and 4) of the checks and all three breeding cycles differed significantly within each year. The mean yield of the highest yielding line in each year averaged 54% of the mean yield of Phillips 664 over those years (Table 3).

Mean survival indices of the three selection cycles (0, 1, 2) were lower than those of Gypsum 9, but the experimental line with highest survival had a higher survival index than Gypsum 9 (Table 4).

In 2013, comparing newly-sown plots with plots in the second year of growth (i.e., having been sown in 2012 and regrowing from rhizomes in 2013), the analysis of variance showed a highly significant ($P < 0.01$) linear effect of selection cycle, and a significant ($P < 0.05$) cycle by year interaction (Table S2). Mean grain yields of genotypes in selection cycles 1 and 2 in regrowth plots were not significantly lower than those of their corresponding first year counterparts (Table 3). Because of the early emergence, rapid growth rate, and early flowering observed in the second year of growth, plants were harvested three times, in mid-August, mid-September, and mid-October, with 16 %, 37 % and 47 % of the mean total grain weight harvested on the first, second and third date of harvesting, respectively.

Survival index

When we compared survival index of entries under mulch and those with no mulch, there was a highly significant ($P < 0.01$) difference in winter survival for genotypes, mulch, cycles, genotypes within cycle, and mulch x genotypes within cycle interaction in 2011-12 evaluations; however, in 2012-13 only genotypes, mulch and genotypes within cycle were significant (Table S3). Mean survival index in mulched blocks was 32 on a 1-to-100 scale, while that of non-mulched was 7, and the mean survival index in nonmulched plots was zero for several individual lines. Because of that, and because of the nonsignificant genotype x mulch interaction, we analyzed relationships between survival index and other traits using data from mulched plots only, and used the 2012 mulched plots as the “regrowing” treatment in 2013.

Pearson's correlation analysis showed that grain yield was not significantly correlated ($r = -0.2637$, $P > 0.15$) with survival index, and a scatter plot shows a wide range of survival and yield in all three selection cycles (Fig. 1). Single grain weight was significant and negatively correlated with survival index ($r = -0.4923$, $P < 0.01$).

Discussion

Like grain yield and single-grain weight, rhizomatousness and winter survival in the genus *Sorghum* are highly complex traits, affected by genetic factors on almost every chromosome pair (Paterson et al. 1995). In a region with cold winters and often little snow cover, such as the U.S. Central Great Plains, we find that winter-hardy perennial plants occur at very low frequencies in populations derived from crosses between annual and perennial plants—even from *S. bicolor* x *S. halepense* crosses. In the southern United States with its mild winters, frequency of winter survival is much higher. Paterson et al., (1995), studying a population of 370 F₂ progeny derived from a cross between *S. bicolor* and *S. propinquum* (a non-winter-hardy diploid perennial) and grown in a field near College Station, Texas, USA (lat. 30.58° N, long. 96.36° W, elev: 97 m) found 92.2% of progeny surviving the region's mild winter. In a trial of F₂-derived lines in F₃ derived from the same population, when grown in 2006 in Salina, Kansas, we observed no ramets emerging from rhizomes before the onset of winter and observed no emergence at all the following spring.

Selection in both cycles was based chiefly on gross morphological differences among plants and their progenies in perenniality, grain size, and domestication traits, and in the second cycle this was based only on individual plant phenotypes. Although grain yield had not been a selection criterion in any cycle, we found improvements in grain yield and and single-grain

weight that were consistent across years of growth and years of evaluation. However, even the highest-yielding lines from cycle 2 did not approach the commercial hybrid check in grain yield or single grain weight, and all lines had phenotypes intermediate between those of *S. halepense* and *S. bicolor*; that is, none would be acceptable as a crop cultivar at this stage of development even if they had demonstrated higher yields. Since 2009, we have practiced additional cycles of hybridizing perennial lines with a broader range of *S. bicolor* parents and have been selecting among segregating progenies for perenniality, traits of domestication, grain yield, and single-grain weight. We will continue doing so.

In 2013, mean grain yields of cycle 1 and 2 genotypes were not significantly different from those in regrowing plots. That was despite our experience that, thanks to its longer growing season, with other things being equal, a perennial plant produces a higher grain yield in its second year of growth than in the first year. However, the second-year yield of a plot depends in part on the survival rate. In a plot with less than 100% winter survival, a single plant regrowing from rhizomes has the opportunity to occupy a larger area both within and beside the previous year's row than does a plant growing from seed in a dense stand the first year. Nevertheless, in the second year of growth, there were blank spots in rows that were not completely filled in, and that reduced their total yield.

A previous study evaluating overwintering in *S. bicolor* $\times$ *S. halepense* population indicated that after two backcrosses seed yield was similar or even exceeded the yield of the cultivated parent ([Kraus 1990](#)). However, after the first backcross, rhizome production dropped markedly ([Kraus 1990](#); [Anderson 1989](#); [Jones 1991](#)), to the point of not being sufficient for overwintering. To overcome this challenge, [Jones \(1991\)](#) suggested for stronger selection for rhizome traits before backcrossing, and use of higher-yielding BC₂F₄ parents instead of

tetraploid cultivated sorghum in order to increase the chance of recapturing important agronomic traits while retaining sufficient rhizome production. Results from our backcrossing and selection program support the conclusions of Jones (1991), and we backcross only winter-hardy perennial plants to *S. bicolor*.

There was no large change in mean survival index across cycles of selection. Furthermore, degree of perenniality as signified by survival index was not associated with grain yield in this collection of 27 genotypes. There was a moderately negative correlation with single-grain weight, which would not preclude development of larger-seeded perennial sorghum cultivars but could add to the time required.

Our findings demonstrate that continuous improvement in perennial sorghum can be accomplished with traditional plant breeding techniques, provided selection for more domesticated phenotype, adaptation, and higher grain yield and grain weight are practiced simultaneously.

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1 Table 1. Analysis of variance for grain yields (g m^{-1}) of 27 lines from cycles 0, 1, and 2 of a
2 perennial sorghum selection program evaluated in 2011, 2012, and 2013.
3

Source of variation	Degrees of freedom	Mean square ^a
Year Evaluated	2	91397**
Cycle	2	11678**
Linear	1	150580**
Residual	1	4827*
Genotype(Cycle)	24	6176**
Cycle*Year	4	2001
Genotype(Cycle)*Year	48	1107
Pooled Error	131	1077

4 ^a * indicates $p < 0.05$; ** indicates $p < 0.01$; all other mean squares were not significant.

Table 2. Coefficients of regression of mean grain yield and mean single-grain weight on cycle number (0, 1, 2) for 27 lines from a perennial sorghum selection program.

	Year	Grain yield (g m ⁻¹ cycle ⁻¹)	Single grain weight (mg ⁻¹ cycle ⁻¹)	Survival Index
Seeded plots	2011	65*** ^a	0.51**	-5.4*
	2012	38.4**	0.97**	1.6 ns
	2013	52.5**	0.52 ns	
	Mean	52.8**	0.60**	-2.48 ns
Plots regrowing from rhizomes	2013	13.2 ns	0.29 ns	

^a * indicates $p < 0.05$; ** indicates $p < 0.01$; ns indicates values are non-significant.

Table 3. Mean grain yield of selection cycles 0, 1, and 2 in newly seeded plots (2011-2013), and in second-year plots sown in 2012 and evaluated when regrowing from rhizomes in 2013 (“2013-regrowth”).

Genotype or Group	Number of genotypes	2011	2012	2013	2013- regrowth
<i>S. halepense</i>	1	43	49	38	119
Cycle 0	6	109	45	125	188
Cycle 1	6	205	107	191	165
Cycle 2	15	250	126	233	206
Highest Line	1	357	200	316	312
Commercial hybrid	1	547	432	640	---

Table 4. Three-year mean grain yield, single-grain weight, and survival index of lines in cycles 0, 1, and 2 of a perennial sorghum selection program, along with a *S. halepense* check, a commercial hybrid check, and the experimental line with the highest mean for the respective trait (which in each case belonged to cycle 2).

Genotype or Group	Number of genotypes	Grain Yield (gm ⁻¹)	Single Grain Weight (mg)	Survival Index
<i>S. halepense</i>	1	43	4.2	72
Cycle 0	6	93	7.1	35
Cycle 1	6	168	8.3	32
Cycle 2	15	203	8.9	31
Highest line	1	272	10.5	79
Commercial hybrid	1	540	10.6	---

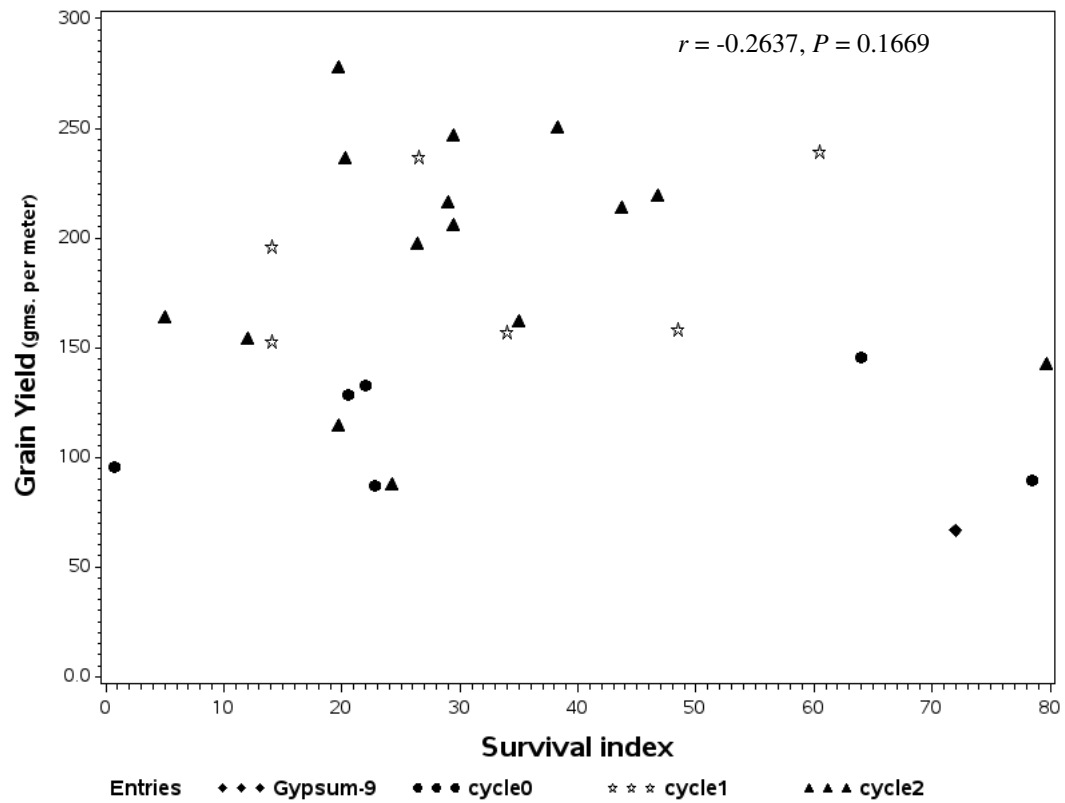
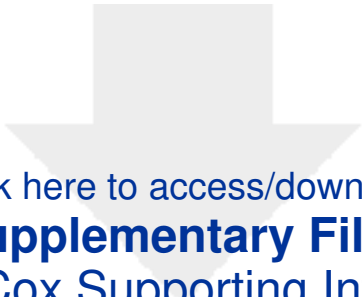


Fig. 1. Scatter diagram showing relationship between mean grain yield and mean survival index for selection cycles 0, 1, and 2.

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Supplementary Files

NabukaluAndCox Supporting Information.docx

