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Rapid evolution of competitive ability in giant foxtail (*Setaria faberi*) over 34 years

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Research Article

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Abstract

Competition between genotypes within a plant population can result in the displacement of the least competitive by more competitive genotypes. Although evolutionary processes in plants may occur over thousands and millions of years, it has been suggested that changes in key fitness traits could occur in as little as decades, with herbicide resistance being a common example. However, the rapid evolution of complex traits has not been proven in weeds. We hypothesized that changes in weed growth and competitive ability can occur in just a few years because of selection in agroecosystems. Seed of multiple generations of a single natural population of the grassy weed giant foxtail (*Setaria faberi* Herrm.) were collected during 34 yr (i.e., 1983 to 2017). Using a "resurrection" approach, we characterized life-history traits of the different year-lines under noncompetitive and competitive conditions. Replacement-series experiments comparing the growth of the oldest year-line (1983) versus newer year-lines (1991, 1996, 1998, 2009, and 2017) showed that plant competitive ability decreased and then increased progressively in accordance with oscillating selection. The adaptations in competitive ability were reflected in dynamic changes in leaf area and biomass when plants were in competition. The onset of increased competitive ability coincided with the introduction of herbicide-resistant crops in the landscape in 1996. We also conducted a genome-wide association study and identified four loci that were associated with increased competitive ability over time, confirming that this trait changed in response to directional selection. Putative transcription factors and cell wall–associated enzymes were linked to those loci. This is the first study providing direct in situ evidence of rapid directional evolution of competitive ability in a plant species. The results suggest that agricultural systems can exert enough pressure to cause evolutionary adaptations of complex life-history traits, potentially increasing weediness and invasiveness.

Introduction

The need to increase food production to meet future world demands is a major concern for scientists and practitioners. Alongside agronomic constraints, climate change has increased the uncertainty around our ability to meet this goal (van Dijk et al. 2021). Although there has been a strong emphasis on studying factors such as drought and temperature stress on crop productivity, less attention has been paid to changes in biotic dynamics (e.g., pest–crop interactions) in agroecosystems. This is particularly important for changes in weed growth responses to the environment. Weeds are the most important biotic cause of crop yield loss worldwide (Oerke 2006); nevertheless, little is known about the type and magnitude of evolutionary adaptations that may be occurring in these organisms in response to agronomic and environmental factors (Baucom and Holt 2009). Although the idea that weeds can rapidly evolve to adapt to changes in their environments is common in the literature, apart from herbicide resistance, this has not been demonstrated for complex life-history traits.

Historically, herbicide resistance has been the most widely studied evolutionary trait in weeds, partially because herbicides are the greatest cause of mortality for weeds but also due to the ease of testing changes in sensitivity to herbicides. Since the introduction of *Bt* and glyphosate-resistant crops, resistance management has been an important consideration in those crop systems (Duke 2018; Gould 1988). Unlike insect pests and pathogens, most weeds only complete a single life cycle per year. Therefore, evolutionary processes likely occur at a slower pace in

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weeds and take more time to be detected. Furthermore, the study of evolutionary adaptations of complex life-history traits (e.g., competitive ability, growth rate, flowering time, seed production) has been scarcer, because these traits are difficult to measure due to high variation resulting from control by multiple genes, genetic epistasis, and genotype by environment interactions. For this reason, any study of life history traits requires considerable time and large sample sizes. However, adaptive changes beyond herbicide resistance might be key to explaining why most weeds of economic importance have not been driven to local extinction despite experiencing frequent and intense mortality events over extended periods of time (Jordan and Jannink 1997; Kuester et al. 2016).

The study of rapid evolutionary changes in plants has been based on research using phylogenetic characterization of populations that are presumably in different evolutionary stages based on geographic distribution and ecosystem dynamics or by monitoring changes of a lineage over time (Branderburger et al. 2022; Franks et al. 2007; Grant and Grant 2006). Neither of those approaches allows direct assessment of changes in competitive adaptations under the same temporal, spatial, and environmental conditions. More recently, researchers have germinated seeds that were produced and stored in different years to generate plants of a given species with genetic structures from different times, so they can be compared under the same environmental conditions (Franks et al. 2007). These "resurrection" studies have documented decadal changes in life-history traits such as flowering time, self-pollination rates, growth responses to increased atmospheric CO_2, and competitive ability (Cheptou et al. 2022; Franks et al. 2007; Ziska 2017; Ziska et al. 2004). The main limitation of those studies is that they only compare two populations or genotypes (i.e., a single old vs. a single modern genotype). Although phenotypic and genotypic changes have been demonstrated using only two time points, this approach does not enable assessment of whether the changes resulted from a major and sudden alteration of the genetic diversity of the species (e.g., genetic bottlenecks) or from progressive changes in genome architecture (e.g., natural selection, genetic drift) (Lande 1976) in which, over time, new and more fit genotypes can outcompete and displace the older ones, as proposed by Darwin (1859).

We hypothesized that weed success in intensively disturbed agroecosystems is not only the result of weed shifts due to herbicide use patterns and cultural practices (e.g., conservation tillage) but also of changes in growth and competitive ability and that these changes can occur progressively in just a few years.

Materials and Methods

Seed Collection and Increase

Seeds of giant foxtail (*Setaria faberi* Herrm.) were collected annually from the same field under a corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] rotation in Story County, IA (42.005° N, 93.675°W) between 1983 and 2017. Seed collection was done as part of the maintenance of the weed germplasm bank of the Iowa State University Weed Science Program. Each year, at least 20 plants were randomly selected from a 250-m radius (same area every year), and seeds were collected, pooled, and stored in a seed storage vault. In 2018 and 2019, seeds were germinated and grown in 4-L pots in a growing mixture of peat moss and vermiculite (Fafard® 4P Mix, Sun Gro Horticulture, Agawam, MA, USA), and after plants reached maturity, their seeds were harvested for later use in the experiments. This seed increase was done to produce seeds of all year-lines under the same conditions and reduce maternal factors.

Growth and Morphology Characterization

Due to limitations of low seed numbers, many years did not have samples that would properly represent the genetic diversity of the population. Therefore, only the 1983, 1991, 2009, 2013, and 2015 year-lines were used for this study. Seeds germinated from pooled seed from each year-line, and twenty seedlings were randomly selected and grown in an outdoor, pot, common garden experiment. Pots (8-L) with single plants were placed 2 m apart, arranged in a completely randomized design. Pots were watered with a drip irrigation system to ensure conditions close to field capacity during the experiment. Plants were grown to physiological maturity, when they were harvested and measured to determine plant height, width, number of leaves, number of panicles, panicle length, number of tillers, primary leaf length and width, leaf area, plant dry weight, and seed production. The phenotyping study was done twice in the summers of 2019 and 2020.

Data analysis was performed using SAS software v. 9.4 (SAS Institute, Cary, NC, USA). After confirmation of normality and homoscedasticity assumptions, measurements were analyzed with ANOVA, considering year-line as main effect and replication and experimental run as random effects. Means separation was conducted using Tukey's honestly significant difference with a significance level of $\alpha = 0.05$.

Herbicide Sensitivity Study

Year-lines 1983, 1991, 1996, 1998, 2009, and 2017 were selected for the next studies because they had 20 to 81 individual lines (i.e., mother plants) that produced enough seed to characterize the genetic diversity of the lines more adequately and to conduct herbicide sensitivity and competitive ability studies and genotyping. Plants were grown in trays with 5 cm by 5 cm cells in a growing mixture of peat moss and vermiculite under greenhouse conditions: a 14-h photoperiod in which supplemental lighting was provided to extend the daylength to 14 h and temperature maintained at 30/26 C day/night. Each year-line had one plant per cell.

The experiment was arranged as a completely randomized design with 12 single-plant replications per treatment. The experiment was conducted twice. Herbicides used for this study included glyphosate (Roundup PowerMax® II, Bayer CropScience, Research Triangle Park, NC, USA) and imazamox (Raptor®, BASF, Research Triangle Park, NC, USA). Each herbicide had six doses, including a nontreated control (Table 1). Herbicides were applied using a CO_2-pressurized backpack sprayer with four XR 11002 nozzles (TeeJet® Technologies, Spraying Systems, Springfield, IL, USA) calibrated to deliver 187 L ha^{-1} at 206 kPa. Herbicides were sprayed at the 4- to 6-leaf stage, which is the optimum growth stage to maximize control based on herbicide label recommendations. Plants were rated for injury every 5 d by visual estimation on a scale from 0% (no injury) to 100% (plant death). Plants were harvested at the flowering stage, and biomass was collected for fresh and dried samples.

Data analysis was performed using SAS software v. 9.4 (SAS Institute). Herbicide injury and biomass were analyzed with ANOVA using PROC GLIMMIX. Each herbicide was analyzed separately. Herbicide dose, year-line, and their interaction were considered fixed effects, while replication and experimental run were considered random. Means separation was conducted using Tukey's honestly significant difference with a significance level of

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Table 1. Herbicide doses applied to *Setaria faberi* in the dose–response experiment.

Chemical	Trade name	Dose	Manufacturer
Glyphosate	Roundup PowerMax II	g ae ha^{-1}	Bayer CropScience, Research Triangle Park, NC, USA
		0	
		52.5	
		105	
		210	
		420	
Imazamox[a]	Raptor	g ai ha^{-1}	BASF, Research Triangle Park, NC, USA
		0	
		2.19	
		4.38	
		8.75	
		17.50	

[a]Imazamox was applied with nonionic surfactant (NIS; Helena AgriEnterprises, Collierville, TN, USA) at 0.25% vol/vol, as recommended by the manufacturer.

$\alpha = 0.05$. Data were subject to linear and nonlinear regression analysis using SigmaPlot software v. 14.0 (Systat Software, San Jose, CA, USA) with herbicide dose as the independent variable and plant injury and biomass reduction as the dependent variables.

Replacement Series Study

A replacement series study was conducted under greenhouse conditions to assess the competitive ability of each year-line. Plants were grown in 4-L pots filled with a peat moss and vermiculite mix substrate (Fafard® 4P Mix, Sun Gro Horticulture) and watered twice daily and fertilized with a nutrient solution to meet all macro- and micronutrient requirements. Greenhouse conditions consisted of a 14-h photoperiod in which supplemental lighting was provided to extend the daylength to 14 h and temperature maintained at 30/26 C day/night. The experiment was arranged as a randomized complete block design with 25 treatments (5 year-lines and 5 competitive ratios) and 3 replications and was conducted twice. There were four plants per pot using five ratios: 4:0, 3:1, 2:2, 1:3, 0:4 (oldest vs. newer year-line). Due to limited seed availability, a full pairwise comparison of all year-lines was not possible. For this reason, we evaluated how each year-line performed against 1983, the oldest year-line; doing this with multiple newer year-lines allowed us to identify trends in changes in competitive ability over time, if present.

Once seedlings emerged, counts were made to ensure correct ratios. Two plants from each pot were tagged and measured throughout the growing period. These plants were then measured weekly until harvest for height, width, leaf number, index of relative chlorophyll content (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Aurora, IL, USA), and tiller number. In pots with treatments of 4:0 or 0:4, two of the same year plants were chosen, and in pots with treatments including 1:3, 2:2, or 3:1, the two plants chosen included one plant from the oldest year and one plant from the newer year. The two tagged plants were measured weekly for height, width, chlorophyll content, and tiller number. Plant harvest occurred when all lines had initiated flowering. This was done to avoid loss of biomass and leaf area due to senescence. At harvest, the plant height, width, leaf number, tiller number, index of relative chlorophyll content, leaf area, and biomass were collected from all four plants. Leaf area was measured using a leaf area meter (LI-3100, LI-COR Biosciences, Lincoln, NE, USA). Biomass was determined after drying plants at 60 C until constant weight. For simplification purposes, only the most representative and informative variables are presented here.

Data analysis was performed using SAS v. 9.4 (SAS Institute). Treatments were considered fixed effects, and block and experimental run were considered random effects in the analysis due to lack of interactions between the block and experimental run with the treatment effects ($P > 0.05$). Measurements taken throughout the growing period, including height, width, leaf number, tiller number, and index of relative chlorophyll content, were analyzed with repeated measures using PROC GLM, with days after planting as the repeated measure. Biomass and leaf area were analyzed with ANOVA using PROC GLIMMIX, and means separation was conducted using Tukey's honestly significant difference with $\alpha = 0.05$.

Genome-wide Association Study

To explore the genetic mechanisms and identify loci explaining the changes in competitive ability, we conducted a genome-wide association study (GWAS) (Risch and Merikangas 1996) and evaluated whether there were changes in the frequency of single-nucleotide polymorphisms (SNPs) that were consistent with the increase in competitive ability of *S. faberi* during the studied period indicating selection. For the genotyping-by-sequencing (GBS) study, we collected fresh leaves from 173 individuals (~30 individuals per year-line) of *S. faberi* grown under optimum temperatures in the greenhouse. Further, DNA was isolated from the fresh leaves with the Macherey-Nagel Plant II DNA extraction kit (Duren, Germany) as per the manufacturer's protocol. DNA concentration was verified using the Quant-iT™ PicoGreen® dsDNA kit (Life Technologies, Grand Island, NY, USA). After confirmation of the DNA quality and integrity, the DNA samples were shipped to CD Genomics (Shirley, NY, USA) for GBS. Libraries were prepared as described in Elshire et al. (2011) with minimal modification; in short, 150 ng of DNA was digested using *Pst*I and *Bfa*I (New England Biolabs, Ipswich, MA, USA), after which bar-coded adapters amenable to Illumina sequencing were added by ligation with T4 ligase (New England Biolabs). The 96 adapter-ligated samples were pooled and amplified to provide library quantities adequate for sequencing, and adapter dimers were removed by SPRI bead purification. Quality and quantity of the finished libraries were assessed using the Agilent Bioanalyzer High Sensitivity Chip (Agilent Technologies, Santa Clara, CA, USA) and Qubit® dsDNA HS Assay Kit (Life Technologies), respectively. Libraries were sequenced targeting at least 300 million reads on a NovaSeq6000 (Illumina Inc., San Diego, CA, USA).

Raw reads were demultiplexed using fastq-multx (Aronesty 2013). Further, Trim Galore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore) was used for adapter trimming and quality control for raw data of individual samples. The clean sequencing data were then used for further downstream processing. We used the *DeNovoGBS* module in NGSEP v. 4 software for individual read mapping and SNP mining (Parra-Salazar et al. 2022). The NGSEP v. 4 software analysis called a total of 526,550 SNPs. Further, for filtering the SNPs, we implemented the software vcftools (Danecek et al. 2011). The parameters for SNP filtering consisted of: (1) ≤30% missing data, (2) read coverage between 10X to 100X, (3) minor allele frequency (MAF) less than 0.01, and (4) multiple SNP alternatives for the same site. The filtering step reduced the number of SNPs to 65,433, and

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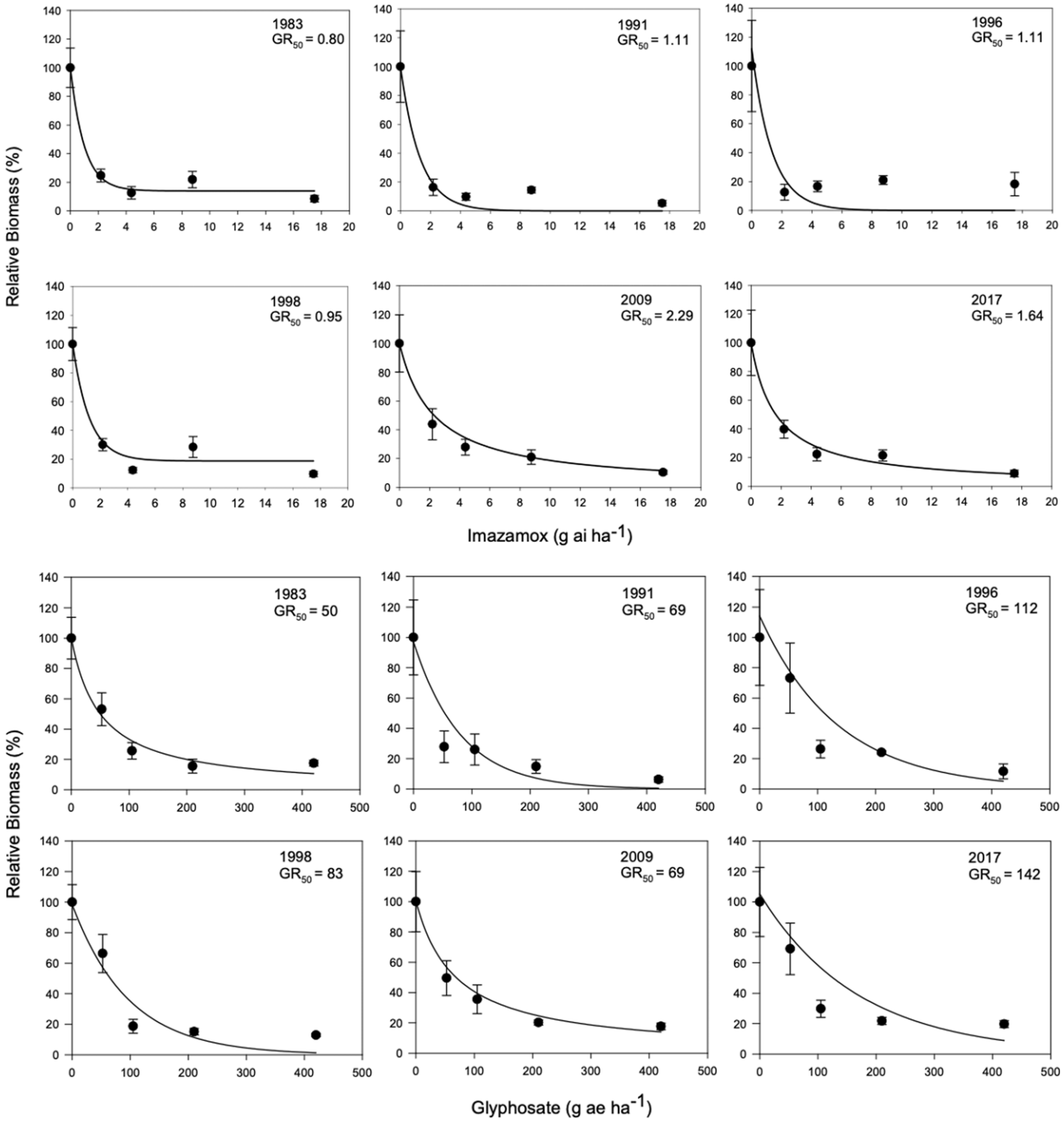

Figure 1. Biomass of *Setaria faberi* relative to the nontreated control for 6 year-lines in response to increasing doses of imazamox and glyphosate. The dose required to reduce growth 50% (GR_{50}) is indicated for each year-line.

the contigs were then assigned to chromosome and SNP position based on homology with the green foxtail [*Setaria viridis* (L.) P. Beauv.] reference genome downloaded from Ensembl Plants (https://plants.ensembl.org/Setaria_viridis/Info/Index). We aligned the sequences to the *S. viridis* reference genome to call SNPs for the GWAS (Bennetzen et al. 2012), as no reference genome was available for *S. faberi* at the time of the study. After anchoring the contigs on the *S. viridis* genome, we performed a GWAS. We used GAPIT software (Lipka et al. 2012) for the GWAS study, using the GLM model. After SNPs associated with changes in competitive ability were identified, we explored sequences 100 kbp up- and downstream from each SNP and used BLAST to identify putative genes in those regions.

Results and Discussion

To test our hypothesis, we collected seed annually from 1983 to 2017 of *S. faberi*, a weed species of economic importance in many row crops. Although the seed was stored under conditions intended to maintain viability (i.e., low temperature and relative

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humidity), when seed viability was tested in 2018 and 2019, most years had few or no viable seeds. Only year-lines from which there were enough plants to represent the genetic diversity of the population each year (i.e., 20 to 81 plants) were selected. Therefore, we chose a total of 8 year-lines across an expanse of 34 years, including before and after major changes in agroecosystems related to the introduction of acetolactate synthase–inhibiting herbicides and glyphosate-resistant crops. Those lines were allocated to each experiment based on seed availability.

Growth and Herbicide Sensitivity Characterization

We first evaluated the sensitivity of 6 year-lines to imazamox (acetolactate synthase inhibitor) and glyphosate (Figure 1). The dose to reduce growth 50% (GR_{50}) ranged from 0.80 to 2.29 g ai ha^{-1} for imazamox and 50 to 142 g ae ha^{-1} for glyphosate. However, there was no clear trend over time, and in both cases, the GR_{50} was largely below the field use dose (44 to 78 g ai ha^{-1} and 420 to 840 g ae ha^{-1}, respectively). These results suggested that any changes in *S. faberi* growth were not likely the result of herbicide-mediated selection. Next, we characterized the growth of individual plants (noncompetitive conditions) in a common garden experiment. Analysis of variance and Tukey's honestly significant difference tests indicated that plant width ($F_{(4,\ 202)} = 4.16$; $P = 0.0029$), leaf number ($F_{(4,\ 202)} = 2.46$; $P = 0.047$), panicle number ($F_{(4,\ 202)} = 2.68$; $P = 0.033$), biomass ($F_{(4,\ 202)} = 3.6$; $P = 0.0073$), and seed number ($F_{(4,\ 202)} = 2.42$; $P = 0.05$) were higher for plants from the newest year-line than those from the oldest; the rest of the studied years exhibited intermediate values (data not shown).

Competitive Ability

Because of these differences in growth, we hypothesized that more recent year-lines are more competitive than older ones and that competitive ability would increase progressively over time. Therefore, we conducted a replacement series experiment comparing the competitive ability of plants from 5 year-lines against the oldest line (i.e., 1983), which was used as the reference point to identify any evolutionary adaptation. Plants grown in monoculture exhibited the same biomass and total leaf area regardless of the year (Figure 2), but there were differences in height, with the three most recent year-lines being taller. Interestingly, differences in biomass detected in the experiment under noncompetitive interactions were not large enough to be maintained under competitive conditions. Despite the lack of differences in biomass production in monoculture, the presence of differences in plant height could give newer year-lines a competitive advantage in polycultures. We predicted that deviations in the polycultures from predicted relative biomass production under neutral competition would be the result of competitive responses and not constitutive morphological differences.

When plants were placed under 3:1, 2:2, and 1:3 ratios (1983: newer year-line) in polyculture, the relative competitive ability of *S. faberi*, measured as relative biomass accumulation, increased consistently over time in all cases (0.58% to 0.62% per year, $P < 0.0001$ to 0.018), confirming our hypothesis (Figure 3; Table 2). However, in contrast to our prediction, 1983 was not the line with the lowest competitive ability. In fact, 1983 was more competitive than 1991 and 1996, and from that point forward, competitiveness of year-lines increased until plants from 2017 were more competitive than those from 1983. The increase in competitive ability was mainly due to larger leaf area in more recent year-lines than in 1983 (0.60% to 0.82% per year, $P < 0.0001$ to 0.012). Although the differences among year-lines in monoculture were for height, not for total leaf area, this latter factor trended most similarly to biomass increase. These results indicate that individuals from a given year-line were able to modify their growth and produce larger leaf areas, limiting the growth of older year-lines. The decrease (from 1983 to 1991) and increase (from 1996 to 2017) in competitive ability indicate that this *S. faberi* population was possibly undergoing oscillating selection (Gibbs and Grant 1987). Oscillating selection occurs when different selection forces or different levels of intensity of a given selection force act sequentially, resulting in positive and negative (i.e., opposite) changes in the competitive ability of the progeny of the plants under selection (Gibbs and Grant 1987; Grant and Grant 1995;

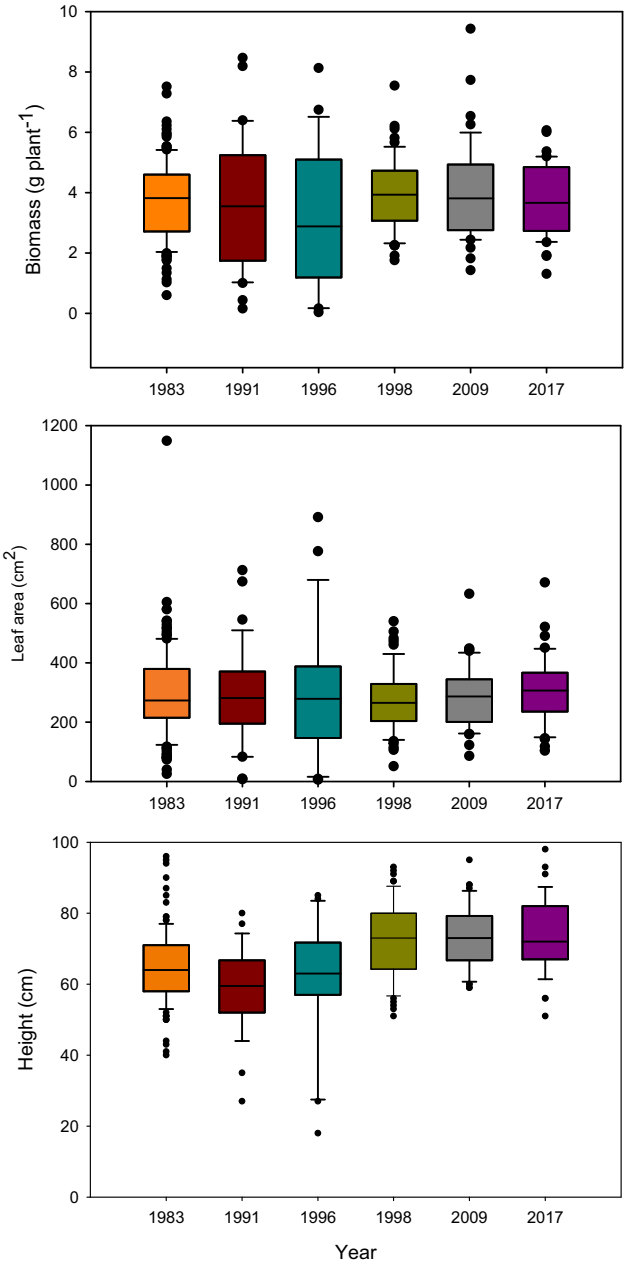

Figure 2. Plant biomass, total leaf area, and height of *Setaria faberi* from 6 year-lines grown in monoculture. No differences in growth potential were found among lines for biomass ($F_{(5,\ 318)} = 1.04$; $P = 0.39$) and leaf area ($F_{(5,\ 329)} = 0.79$; $P = 0.56$), but height differences were detected among year-lines ($F_{(5,\ 308)} = 12.5$; $P < 0.0001$).

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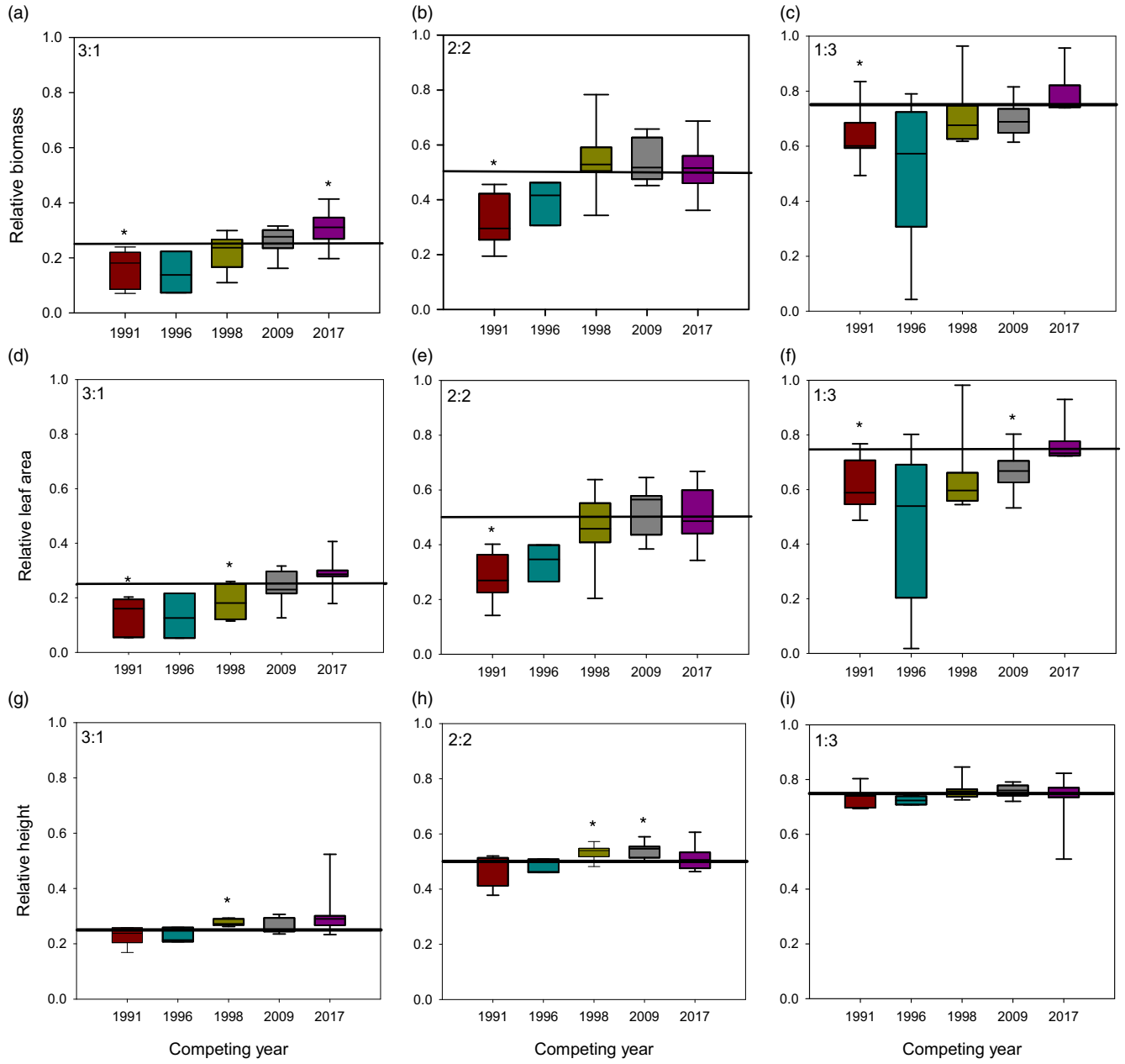

Figure 3. Relative biomass, total leaf area, and height of *Setaria faberi* from 5 year-lines, in competition with year-line 1983 in 3:1, 2:2, and 1:3 proportions (1983:newer year-line). The horizontal bar indicates the expected relative biomass under neutral competition. Values under the expected neutral competition indicate that plants from 1983 were more competitive than the respective alternative year-line, indicating uneven competitive ability between year-lines. Conversely, when the alternative year-line surpasses the neutral competition threshold, this indicates that it was more competitive than the 1983 year-line In all three competitive conditions, competitive ability increased over time. Asterisks indicate when a year-line was different from the predicted relative biomass based on neutral competition ($P < 0.05$).

Kellogg 1975). Furthermore, these results provide evidence that not only can weeds evolve rapidly and vary their competitive ability, but they do so by a progressive process that might result in the continuous displacement of older genotypes.

Identification of Loci Associated with Competitive Ability Evolution

Previous studies have taken advantage of population and quantitative genetics to detect evolutionary changes in life-history traits in weeds (Kuester et al. 2016; Leon et al. 2006; Leon and van der Laat 2021), although genome mapping of the loci responsible for those changes has been lacking. The modifications in biomass and leaf area production under polyculture provided evidence that the differences in competitive ability were not just due to evolution of structural morphological traits (e.g., plant height), but instead a physiological mechanism, likely controlled genetically, evolved to respond to the presence of other plants. To characterize the evolutionary nature of those changes, we sequenced the genomes of 30 randomly selected individuals of each year-line and aligned the sequences to the *S. viridis* reference genome to call SNPs for the GWAS (Bennetzen et al. 2012), as no reference genome was available for *S. faberi* at the time of the study. *Setaria faberi* is an allotetraploid ($n = 18$) closely related to diploid *S. viridis* ($n = 9$), and both share an A genome. The second genome of *S. faberi* seems to be from a common ancestor to both species (Benabdelmouna et al.

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Table 2. Linear regression of relative changes in biomass, leaf area, and height (y) of *Setaria faberi* under different competitive conditions over time (x = year) as presented in Figure 3.

Variable	Competitive condition 1983: recent year-line	Linear regression equation	P-value
Biomass	3:1	$y = -11.3241 + 0.0058x$	<0.0001
	2:2	$y = -11.4306 + 0.0059x$	0.0083
	1:3	$y = -11.7763 + 0.0062x$	0.0180
Leaf area	3:1	$y = -11.8634 + 0.0060x$	<0.0001
	2:2	$y = -15.9652 + 0.0082x$	0.0003
	1:3	$y = -13.4497 + 0.0070x$	0.0123
Height	3:1	$y = -5.0113 + 0.0026x$	0.0089
	2:2	$y = -2.2776 + 0.0014x$	0.1088
	1:3	$y = 0.7619 - 0.00001x$	0.9922

Table 3. Chromosome-wise distribution of putative single-nucleotide polymorphisms (SNPs) in *Setaria faberi* based on the *Setaria viridis* reference genome.

Chromosome	Chromosome length bp	Putative coding genes	No. of SNPs
1	41,732,233	4,241	7,143
2	47,849,963	4,970	8,283
3	50,382,502	4,611	7,709
4	39,677,845	3,308	5,792
5	46,702,114	5,170	8,874
6	36,371,416	2,903	4,626
7	35,460,007	3,771	6,460
8	40,988,899	2,983	5,416
9	56,381,885	6,373	11,130
Total	395,546,864	38,330	65,433

2001; Kellogg 2017; Layton and Kellogg 2014). Therefore, using *S. viridis* reference genome allowed us to better align the sequencing reads and generate more informative and reliable SNPs. A total of 65,443 SNPs were identified for which frequencies could be tracked across the 6 year-lines. Those SNPs provided an extensive coverage of the nine reference chromosomes. The average distance between SNPs was 6,045 bp. In our analysis, we found maximum SNPs in chromosome 5 (Table 3). The data were analyzed to identify SNPs that increased or decreased in frequency as competitive ability increased over time. Therefore, the directionality of the change was considered to be part of the selection criteria for SNP calling, making this process more rigorous than identifying SNPs based on comparisons of pairs of year-lines. The results confirmed that several SNPs were associated with changes in competitive ability under the three competitive conditions (i.e., 3:1, 2:2, and 1:3; Figure 4). In total, we found about 15 SNPs showing strong correlation with the phenotypic data. Importantly, chromosomes 3, 5, and 8 had SNPs that were consistently associated with competitive ability changes in both 3:1 and 1:3 conditions, strongly indicating that their locations are in close proximity to loci that were under directional selection during the last three decades.

Several of the putative genes in the vicinity of the loci associated with competitive ability had domains frequently found in transcription factors, such as RRM, YABBY, AP2/ERF, and bZIP in chromosome 9, MYB in chromosome 5, and WRKY in chromosome 3 (Table 4). Additionally, several protein kinase domain–containing genes were associated with loci in chromosomes 5, 8, and 9. The involvement of putative transcription factors and protein kinases indicates that selection likely acted upon the genetic structure of signal-transduction pathways, presumably affecting competitive ability (Pierik et al. 2013). Also, the detection of genes related to cell metabolism and growth, including genes coding for cellulases, pectin esterases, glycosyltransferases, and glycine-rich cell wall structural proteins, suggests that genetic determinants of cell growth responses are part of the evolutionary changes in competitive ability documented here, which agrees with the role of cell wall proteins in stress responses in plants (Ezquer et al. 2020; Le Gall et al. 2015).

Historical Context

Individuals from 1991 were less competitive than those from 1983 (i.e., lower relative biomass than the neutral competition prediction), and it was after 1996 that competitive ability shifted and started increasing in the newer year-lines. The present study does not allow us to determine the cause of this shift. However, it is worth noting that it was in 1996 that glyphosate-resistant crops were introduced to the area where the studied *S. faberi* population was located (Duke 2018). It is unlikely that the changes in competitive ability in this weed were the direct result of the increased use of glyphosate, especially considering that no major change in sensitivity to this herbicide was observed. Also, field commercial doses were high enough to ensure the same selection pressure in all year-lines. Therefore, we propose that the uniformity in the agricultural landscape resulting from the widespread adoption of glyphosate-resistant crops created conditions favoring the evolution of more competitive biotypes (Bravo et al. 2017; Leon and van der Laat 2021; Owen 2008). Landscape fragmentation and diversity favor genetic diversity and dispersal-related traits in human-disturbed landscapes. Conversely, uniform, stable, less diverse landscapes tend to favor competitive ability (Cheptou et al. 2017; Legrand et al. 2017; Urquhart and Williams 2021). In the present case, it is likely that the reduction in the use of integrated management practices decreased the diversity of factors driving the mortality of weeds, and this occurred at an unprecedented scale in the U.S. Midwest with the advent of glyphosate-resistant crops. Thus, competition between individuals surviving or avoiding glyphosate applications would become more important to maintain and increase reproductive output. The progressive changes in morphological traits due to competition for food has been robustly demonstrated in long-term studies such as those of Darwin's finches (Gibbs and Grant 1987; Grant and Grant 2002, 2006). The fact that weeds are at the same trophic level as crops makes competitive ability particularly important in agroecosystems, because niche differentiation via evolution can only occur if the weed adapts. The crop, due to human breeding and agronomic management, will not evolve to optimize its access to available resources, in contrast to plants experiencing coevolutionary processes in natural systems (Luescher and Jacquard 1991).

Evolution of Increased Competitive Ability

The findings of the present study provide a different perspective on the evolution of increased competitive ability (EICA) hypothesis (Blossey and Notzold 1995), which has been used to explain the observation that invasive plant species tend to grow more and faster in alien environments than in their original environments. This hypothesis has been predominantly tested under the premise that those species do not encounter natural enemies in invaded areas. However, there have been contradictory results (Cripps et al. 2009; Felker-Quinn et al. 2013; Siemann et al. 2017; van Kleunen and Schmid 2003), and researchers have proposed

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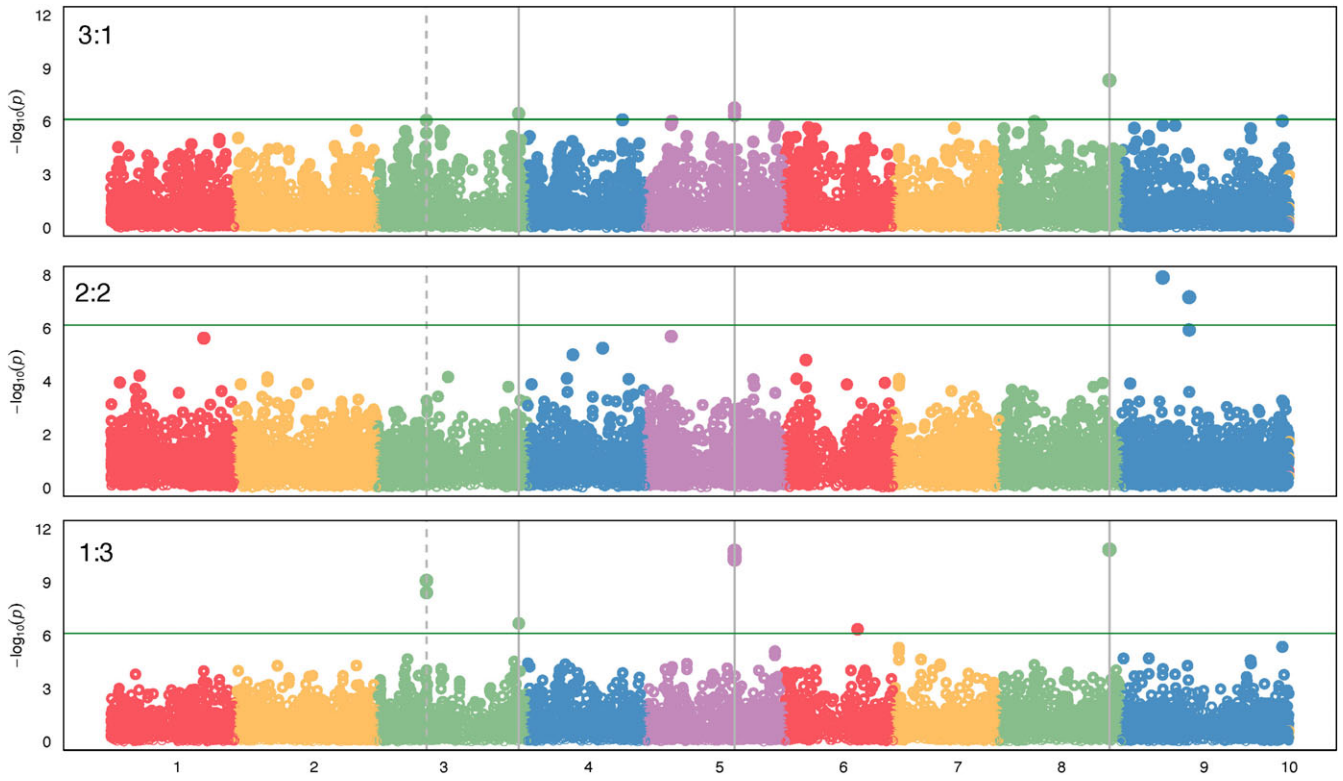

Figure 4. Genome-wide association study (GWAS) results indicating single-nucleotide polymorphisms (SNPs) associated with competitive ability in a *Setaria faberi* from year-lines 1991, 1996, 1998, 2009, and 2017 in competition with year-line 1983 in 3:1, 2:2, and 1:3 proportions (1983:newer year-line). The analysis was done using the *Setaria viridis* reference genome consisting of nine chromosomes (*x* axis). Only SNPs that changed over time in either of the three competitive conditions are shown. SNPs above the green horizontal bar were significant and were considered strongly associated with increase in competitive ability over time. Vertical, solid bars indicate SNPs that were significant under the extreme competitive conditions 3:1 and 1:3. The dashed line shows SNPs that were significant in the 1:3 condition and were at the limit of significance in the 3:1 condition. The horizontal line indicates the minimum level of significance for SNP association with competitive ability.

alternatively the evolution of reduced competitive ability (ERCA) hypothesis (Bossdorf et al. 2004). Although *S. faberi* is a weed, and not an invasive species of natural systems per se, our results demonstrated that there can be evolutionary bidirectional changes in competitive ability within the same plant population (Gibbs and Grant 1987; Grant and Grant 2002), and those can occur, or at least be expressed, without the influence of phytophagous insects or pathogen infections. Therefore, invasive biology must consider the fact that evolution can occur by kin competitive relations (File et al. 2012), and the natural enemy release effect might act independently from or in conjunction with this process to ultimately yield the commonly reported rapid evolution of competitive ability (Siemann et al. 2017).

Caveats

It is possible that in the present study there were limitations in the identification of SNPs associated with competitive ability. Calling SNPs without having an assembled reference genome for *S. faberi* likely reduced the resolution of our analysis, so it is conceivable that there are other loci that were under selection, and we were not able to detect them, especially for the B genome. Even under these constraints, the level of significance of association of the SNPs identified across the studied years and the fact that they were consistently detected under different competitive conditions provide robust evidence that the detected loci were under directional selection. It is also important to recognize that studying a single population still leaves open the question of whether the results are unique to that population and the conditions under which it evolved or whether the phenomenon documented here might be more widespread within this and perhaps other species. Regardless of this limitation, the results clearly illustrate the potential for very rapid evolution of polygenic, complex traits that can indeed increase weediness and invasiveness. Another limitation of our study is that we did not have year-lines between 1983 and 1991, so it was not possible to describe how the decrease in competitive ability during this period occurred or could be explained. The simplest explanation is that selection pressure of processes other than intraspecific competitive ability were more important for the fitness of *S. faberi* during the 1980s than in the 1990s. However, we recognize that the present study is more informative for changes that occurred after 1991.

This is the first report of rapid directional evolution of competitive ability in a natural (nonexperimental) plant population determined by comparing multiple year-lines under the same environmental conditions. Our results help explain the contradictory results reported for the EICA hypothesis and seem to indicate that, given current trends, weeds might be evolving and becoming better competitors at a rate unknown until now. This should motivate researchers to pay more attention to how evolutionary processes in weeds and pests can modify biological interactions, ultimately making crop production in agroecosystems more difficult. Whether the shift in competitive ability in *S. faberi* is the result of homogenization of selection pressure is yet to be determined. However, having variable or oscillating selection might prevent the progressive increase in competitive ability (Gibbs and Grant

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Table 4. Chromosome-wise distribution of select putative genes associated with single-nucleotide polymorphisms (SNPs) under selection for competitive ability in *Setaria faberi* based on the *Setaria viridis* reference genome.

Gene ID	Chromosome	SNP position	Gene position	Distance from SNPs bp	Annotation
TKV92970	9	13921194	13917345	3,849	Methionine aminopeptidase, 3.4.11.18
TKV92974	9	13921194	13932069	−10,875	RRM domain–containing protein
TKV92976	9	13921194	13953856	−32,662	YABBY protein
TKV94066	9	22820419	22756611	63,808	Protein kinase domain–containing protein
TKV94071	9	22820419	22789332	31,087	AP2/ERF domain–containing protein
TKV94074	9	22820419	22827136	−6,717	Cellulase domain–containing protein
TKV94078	9	22820419	22860696	−40,277	Transcription factor bZIP family
TKV94079	9	22820419	22877245	−56,826	Glycosyltransferase, 2.4.1.
TKW02172	8	37067267	37051193	16,074	Protein kinase domain–containing protein
TKW02173	8	37067267	37064809	2,458	PK_Tyr_Ser-Thr domain–containing protein
TKW02174	8	37067267	37071843	−4,576	Mediator of RNA polymerase II transcription subunit 1
TKW02177	8	37067267	37118012	−50,745	Protein kinase domain–containing protein
TKW02180	8	37067267	37163787	−96,520	AAI domain–containing protein
TKW10071	6	24317743	24290032	27,711	Nucleobase-ascorbate transporter 2
TKW10074	6	24317743	24350739	−32,996	RING-CH-type domain–containing protein
TKW10078	6	24317743	24368029	−50,286	Cytochrome b5 heme-binding domain–containing protein
TKW10080	6	24317743	24373893	−56,150	Hexosyltransferase, 2.4.1.
TKW10081	6	24317743	24381151	−63,408	Mitochondrial carrier protein MTM1
TKW15575	5	29757870	29722920	34,950	MYB transcription factor
TKW15577	5	29757870	29729986	27,884	Glycosyltransferase, 2.4.1
TKW15579	5	29757870	29736204	21,666	3Beta_HSD domain–containing protein
TKW15586	5	29757870	29766320	−8,450	Protein kinase domain–containing protein
TKW15589	5	29757870	29791502	−33,632	Carbonic anhydrase, 4.2.1.1
TKW26875	3	16443816	16397576	46,240	Membrane-associated kinase regulator 4
TKW26879	3	16443816	16480648	−36,832	Glycine-rich cell wall structural protein 1
TKW26886	3	16443816	16494141	−50,325	Metal tolerance protein 5
TKW26888	3	16443816	16491557	−47,741	Glycine-rich cell wall structural protein 1
TKW26890	3	16443816	16504041	−60,225	AT-hook motif nuclear-localized protein
TKW29415	3	47394496	47384031	10,465	Amino_oxidase domain–containing protein
TKW29418	3	47394496	47388506	5,990	RING-type domain–containing protein
TKW29421	3	47394496	47402802	−8,306	Pectinesterase
TKW29423	3	47394496	47415854	−21,358	RNA-binding KH domain–containing protein
TKW29426	3	47394496	47423387	−28,891	WRKY domain–containing protein

1987; Schluter et al. 1991). This might be more likely to be achieved by diversification of crops and agronomic and ecological management, rather than by the current trend of relying on simple, more aggressive, but still uniform, control tools (Jordan and Jannink 1997; Mortensen et al. 2012; Owen 2008; Owen et al. 2015).

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