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Short title: Sensitivity of Pearl millet

Sensitivity of Pearl Millet (*Pennisetum glaucum* [L.] R. Br.) Parental Lines to POST Herbicides: Clethodim, Quizalofop-P-ethyl, Imazamox and Nicosulfuron

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Abstract

Pearl millet is a climate-resilient grain and forage crop. Weeds pose a major constraint to its successful production. Limited herbicide options for grass weed control in pearl millet is a serious problem. The objectives of this study were to 1) evaluate the sensitivity of pearl millet parental lines to POST-applied clethodim (136 g ai ha^{-1}), quizalofop-p-ethyl (QPE) (77 ai g ha^{-1}), imazamox (52 g ai ha^{-1}) and nicosulfuron (70 g ai ha^{-1}), and 2) characterize the sensitivity of selected lines to imazamox and nicosulfuron. A total of 56 parental lines were tested. Three lines with low sensitivity to imazamox (ARCH35R, 45R, and 73R), two to nicosulfuron (ARCH45R and 73R), one line with high sensitivity (ARCH21B), and a susceptible sorghum (SOR) hybrid (P84G62) to both herbicides were characterized. All parental lines were sensitive to clethodim and QPE (only four lines showed 2 to 12% survival with 90 to 95% injury at 21 d after application [DAA]). However, all parental lines showed variable sensitivity to imazamox and nicosulfuron (70 to 100% survival with 5 to 70% visible injury and shoot dry biomass reduction at 21 DAA). Dose-response assays revealed that ARCH35R, 45R, and 49R had 7.7 to 12.2 and 3.2- to 12.2-fold reduced sensitivity to imazamox compared to the ARCH21B and SOR, respectively. Similarly, ARCH45R and 49R had 2.5 to 6.0 and 1.5- to 3.7-fold reduced sensitivity to nicosulfuron compared to ARCH21B and SOR, respectively. These findings confirm the first report of reduced sensitivity to imazamox and nicosulfuron among pearl millet lines, suggesting their potential use for in-season grass weed control.

Nomenclature: Clethodim; imazamox; nicosulfuron; quizalofop; pearl millet, *Pennisetum glaucum* (L.) R. Br.

Keywords: Herbicide Sensitivity; Parental lines.

Introduction

Pearl millet is the 6th most important cereal crop after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* L.) grown with a global production over 31.0 M ha^{-1} (Kumar et al. 2022). It belongs to the Poaceae family and is globally grown for food, feed, and nutritional security (Mishra 2015). In comparison to other major cereals, it has high nutritional values and is a good source of fat (3 to 8%), proteins (8 to 19%), dietary fibers (1.2 g per 100 g), and antioxidants (Uppal et al. 2015). In addition, pearl millet is a rich source of minerals (2.3 mg per 100 g) particularly iron (11 mg per 100 g), zinc (3.1 mg per 100 g), and other micro-nutrients like potassium, phosphorus, and vitamins such as riboflavin, niacin, and thiamine (Uppal et al. 2015). Forage pearl millet can have 12 to 14% crude protein (which is generally higher than corn) with relatively a low lignin concentration and low fiber content (2.8 to 17.6%) (Banks and Stewart 1998; Harinarayana et al. 2005). The development of brown mid-rib mutants with reduced lignin biosynthesis presents a great potential for improving the quality of forage pearl millet (Cherney et al. 1988; Degenhart et al. 1995; Gupta and Govintharaj 2023). Unlike sorghum, pearl millet is genetically free from prussic acid and tannins and hence suitable for grazing for livestock, dairy cows, and horses at any growth stages (Newman et al. 2010).

Pearl millet is grown in arid and semi-arid regions of Asia and Africa (Srivastava et al. 2020). In the United States of America (USA), pearl millet is mainly grown for grazing, hay, cover crops, and forage (southeastern USA), with approximately 0.61 M ha^{-1} in production (Myers 2002). It is recognized as a potential forage and feed crop well-suited for double cropping in the United States (Wilson et al. 1996). It is well adapted to low soil fertility, high pH, low soil moisture, high temperature, high salinity, and limited rainfall areas where other cereals like maize, rice, sorghum, and wheat would fail (Sollenberger et al. 2020). It has a C_4 photosynthetic pathway and can withstand high temperatures and stress up to 42 C during its reproductive phase (Howarth et al. 1996). Due to its ability to produce grain and forage in dry and hot climates and on soils nonsuitable for sorghum and corn, it is a good option for low-input agricultural production systems (Jukanti et al. 2016).

Weed management is one of the most significant challenges in pearl millet production (Kumar et al. 2023a). Weeds compete with crops for nutrients, soil, moisture, sunlight, and space, resulting in yield losses, low-quality grains, and overall low profitability (Diatta et al.

2016). Due to its early slow growth, pearl millet is a relatively poor competitor with weeds that can result in substantial grain yield losses (Cook et al. 2005). The critical period of weed control (CPWC) in pearl millet has been reported, ranging from 28 to 42 days after planting (Chaudhary et al. 2018). Weed competition from both grass and broadleaf species at various densities has been reported to reduce pearl millet grain yield ranging from 16 to 94% (Balyan et al. 1993; Das and Yaduraju 1995; Sharma and Jain 2003). The extent of grain yield loss generally depends on pearl millet cultivar/hybrid, nature and intensity of weeds, duration of weed infestation, environmental factors, and management practices (Mishra 2015). Limited herbicide options with potentially narrow selectivity range between annual grass weeds and pearl millet are major constraints in developing a robust chemical-based weed control program (Dowler and Wright 1995; Mishra 2015). Evolution of herbicide-resistant weed biotypes across various regions further exacerbate the problem of weed control in pearl millet (Heap 2024).

The development of herbicide-resistant crops [corn, soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), and canola (*Bromus napus* L.)] have transformed agricultural production systems by providing chemical options for weed control (Bajwa et al. 2015). However, no such efforts have been made for the development of pearl millet hybrids resistants. Integration of herbicide-resistant traits combined with drought- and heat-tolerant traits can potentially help pearl millet production rapidly expand across arid and semi-arid regions, even amid changing climates (Kumar et al. 2023a; Todd et al. 2024). Identifying pearl millet parental lines with reduced sensitivity to acetyl-CoA carboxylase (ACCase) (Group 1) and acetolactate synthase (ALS) (Group 2)-inhibiting POST herbicides may help in developing elite herbicide-resistant hybrids that can potentially offer grass weed control options. In this context, we initiated a large-scale herbicide screening of advanced pearl millet parental lines developed by Millet Breeding program at Kansas State University, Agricultural Research Center, Hays (KSU-ARCH), Kansas. We hypothesized that natural variation may exist among advanced pearl millet parental lines with reduced sensitivity to ACCase (clethodim and quizalofop) and ALS- (imazamox and nicosulfuron) inhibiting herbicides. The main objectives of this research were to 1) evaluate the sensitivity of pearl millet parental lines to ACCase (clethodim and quizalofop-p-ethyl - QPE), and ALS (imazamox and nicosulfuron) inhibiting herbicides; and 2) characterize the sensitivity levels of selected lines to imazamox and nicosulfuron.

Materials and Methods

Plant Material. The development of advanced pearl millet parental lines used in this research have previously been described by Ramalingam et al. (2024). In short, by using the recurrent selection method, many selected germplasms were allowed for random mating followed by three selection cycles, and the developed advanced lines were sorted out into seed/female parent (B lines) and pollinator/male (R lines) based on the complete sterility and fertility of the test hybrids evaluation in summer 2016 at KSU-ARCH, Kansas. Backcross breeding was followed to develop new seed parent (A - male sterile and B - male fertile/maintainer) inbred lines and simultaneously pedigree breeding was followed for R - restorer inbred lines development between the summer 2017 and 2020. A total of 56 advanced selected 29B and 27R lines (45 grain and 11 forage types) were used in this study (Table 1).

Single Dose Bioassays. Greenhouse experiments were conducted in summer 2023 and 2024 at the Kansas State University, Agricultural Research Center (KSU-ARC), Hays, Kansas. Seeds of each line were planted in an individual (28 × 53 × 6 cm) 50-cell plastic tray, filled with a commercial potting mixture (Miracle-Gro Moisture Control Potting Mix, Miracle-Gro Lawn Products). Experiments were laid out in a randomized complete block (blocked by herbicides) design with 50 replications (1 tray = 50 replications). The greenhouse conditions during the study periods were maintained at 32/29 ± 5 C day/night with a 15/9 h photoperiod, and plants were watered as needed to avoid any moisture stress. Four herbicides, including clethodim (Select Max®, Valent USA, San Ramon CA) at 136 g ha^{-1}, QPE (Aggressor®, Albaugh LLC, Ankeny, Iowa) at 77 g ha^{-1}, imazamox (Beyond®, BASF Corporation, Research Triangle Park, NC) at 52 g ha^{-1}, and nicosulfuron (Zest™ WDG, Corteva Agriscience, Indianapolis, IN) at 70 g ha^{-1} were separately evaluated on 56 advanced pearl millet parental lines. All selected herbicides were separately applied on all the lines along with crop oil concentrate (1% v/v) at the seedling stage (3- to 4-leaf stage and 8- to 12-cm tall plants) using a cabinet spray chamber (Research Track Sprayer, De Vries Manufacturing) equipped with an even flat-fan nozzle tip (Tee Jet XR8001E, Spraying System). The spray chamber was calibrated to deliver 140 L ha^{-1} of the spray solution at 240 kpa. After herbicide treatment, all the trays were returned to greenhouse and were not watered for at least 24 hours.

Data on survival percentage and visible injury (%) of survived plants were recorded at 7, 14, and 21 d after herbicide application (DAA) on a scale of 0 to 100%, (where 0 = no injury and 100 = complete death). The stunting, chlorosis, and/or necrosis of treated pearl millet plants were compared to nontreated for visible injury evaluation. At 21 DAA, the final number of survived plants were counted from each tray, and the survival percentage was calculated using Equation 1:

$$Survival\ percentage = [\frac{Number\ of\ surviving\ plants}{Total\ number\ of\ plants\ treated}] * 100$$

A treated plant was considered dead if the plant showed chlorosis, necrosis, and no new regrowth at 21 DAA. The height of 12 surviving plants from each tray were measured from the soil surface to the uppermost extended leaf, and the shoot biomass of those plants was collected and dried at 65 C for five days to measure the shoot dry biomass at 21 DAA. The shoot dry biomass reduction (%) was calculated using Equation 2:

$$\text{Shoot dry biomass reduction (\%)} = \left[\frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}}\right] * 100$$

where C is the shoot dry biomass from the nontreated control plants (average of 12 plants), and T is the shoot dry biomass of a treated plant.

Dose-Response Bioassays. Based on results from single dose bioassays, parental lines with relatively higher survival percentage, low visible injury, and low biomass reduction with imazamox (ARCH35R, 45R, and 49R) and nicosulfuron (ARCH45R, and 73R) were selected. In addition, one commercial grain sorghum hybrid (P84G62) and ARCH21B line [based on the highest biomass reduction (% of nontreated)] susceptible to both imazamox and nicosulfuron were also included for comparison. Among these selected lines, ARCH21B, 35R, 45R, and 73R were grain, whereas 49R was forage type. Separate greenhouse dose-response experiments were conducted and repeated in summer 2024 at the KSU-ARCH, KS, to characterize the sensitivity levels of selected parental lines to imazamox and nicosulfuron. Seeds of the selected parental lines were separately planted in 10-by-10 cm squared plastic pots filled with a commercial potting mixture (Miracle-Gro Moisture Control Potting Mix, Miracle-Gro Lawn Products). Experiments were conducted in a randomized complete block (blocked by parental line) design with 12 replications. Greenhouse conditions were the same as followed in the single-dose assay. Actively growing seedlings (3- to 4-leaf stage and 8- to 12-cm tall) from each selected pearl millet line were separately treated with various rates of imazamox (0, 13, 26, 52, 104, 208, 416, and 832 g ha^{-1}) and nicosulfuron (0, 17.5, 35, 70, 140, 280, 560, and 1120 g ha^{-1}) along with 1% COC using the same cabinet spray chamber used in the single dose assay screening. After spraying, all treated parental lines were returned to the greenhouse and watered as needed to avoid soil moisture stress. Percent visible injury (0 to 100%, where 0 = no injury and 100 = complete death) at 7, 14, and 21 d after application (DAA) were collected. At 21 DAA, the shoot biomass of all treated plants was collected and dried at 65 C for five days to measure shoot dry biomass, and the shoot dry biomass reduction (%) was calculated using Equation 2.

Statistical Analysis. All collected data on visible injury (%), survival (%), and shoot dry biomass reduction (% of nontreated) in both experiments were subjected to analysis of variance (ANOVA) using the PROC MIXED in SAS 9.4 (SAS Institute, Inc., SAS Campus Drive, Cary, NC). The fixed effects in ANOVA were experimental run, herbicides (four herbicides in single dose bioassay and herbicide dose in dose-response bioassay), parental lines, and their interactions. Replications and all interactions involving replication were considered random effects. The data followed all the ANOVA assumptions as tested by the Shapiro-Wilk (P value = 0.342) and Levene (P value = 0.621) tests with the UNIVARIATE and GLM procedures, respectively, with SAS software. The experimental run-by-treatment interaction for single dose and dose-response bioassays was non-significant (P value > 0.05), therefore, data were pooled across experimental runs for each bioassay. For single dose bioassay, the treatment means were compared using Fisher's protected least significant difference test ($p < 0.05$). Data on shoot dry biomass reduction for each tested pearl millet parental line from dose-response bioassays were regressed over imazamox or nicosulfuron doses using a three-parameter nonlinear log-logistic model in R software (Ritz et al. 2015) using equation 3:

$$Y = \frac{d}{1+\exp[b\,(logx - loge)]}$$

where *Y* is percent shoot biomass reduction, *d* is maximum shoot biomass reduction (upper asymptote, fixed to 100%), *b* is slope, *x* is herbicide dose, and *e* represents imazamox or nicosulfuron dose needed for 50% shoot dry biomass reduction (referred to as GR_{50} values). The Akaike information criterion was used to select the nonlinear three-parameter model. A lack-of-fit test (P > 0.05) was used to confirm that the selected model described the shoot dry biomass reduction of each tested parental line (Ritz et al. 2015). All nonlinear regression parameters and

GR_{90} values (imazamox or nicosulfuron dose required for 90% shoot dry biomass reduction) were estimated using the ‘drc’ package (Ritz et al. 2015) in *R* software (R version 4.3.0 Core Team, 2023). The sensitivity index for each selected pearl millet parental line was calculated by dividing the GR_{50} value by the GR_{50} values of the ARCH21B line and SOR.

Results and Discussion

Single Dose Bioassays

Clethodim. None of the tested pearl millet parental lines survived at 136 g ha^{-1} rate of clethodim with mean visible injury ranging from 95 to 98% and shoot dry biomass reduction from 57 to 95% at 21 DAA (Table 2). These results indicate high sensitivity to clethodim for all screened 56 pearl millet parental lines. Although not reported in pearl millet, clethodim has been found to be highly effective on various grass weed species, including goose grass, bermudagrass, barnyard grass, green foxtail, shattercane, and johnsongrass (Anonymous 2021).

Quizalofop. Among all screened parental lines, only four pearl millet parental lines (ARCH35R, 36R, 50R, and 68R) survived the field use rate of quizalofop (77 g ha^{-1}) with 2 to 12% survival, visible injury of 90 to 95%, and shoot dry biomass reduction of 66 to 95% at 21 DAA (Table 3). The survived plants were transplanted and allowed to set seed in the greenhouse. Further investigations are needed to identify if any quizalofop-resistant trait is present among these lines. However, recently commercialized quizalofop-resistant crops, such as wheat, sorghum, and rice are available in market, and no such trait has been discovered in pearl millet yet. For instance, quizalofop-resistant winter wheat varieties (CoAXium Wheat Production System) allow growers to use POST-applied quizalofop-p-ethyl herbicide (Aggressor®, Albaugh Company, St Joseph, MO) for controlling feral rye and other winter annual grass weed species (Kumar et al. 2021). Similarly, sorghum hybrids (Double Team™ sorghum, S&W Sorghum Partners, Longmont, CO) with resistance to quizalofop-p-ethyl (FirstAct™ herbicide, Adama Agricultural Solutions, Ashdod City, Israel) are commercially available for grass weed control (Kumar et al. 2023b). In addition, quizalofop-resistant rice has also been developed through traditional mutation breeding techniques that allows for postemergence applications of quizalofop for grass weed control (Guice et al. 2015).

Imazamox. All 56 advanced pearl millet parental lines survived imazamox (52 g ha^{-1}) at 21 DAA. The survival percentage among these parental lines ranged from 55 to 100% at 21 DAA (Table 4). All parental lines exhibited a high survival percentage ranging from 89 to 100% except for ARCH09B, 21B, 66R, and 16R which showed a survival percentage of 50 to 86% at 21 DAA (Table 4). These results indicate reduced sensitivity to imazamox in all the 56 parental lines. However, the imazamox surviving plants from the most tested parental lines showed a mean visible injury ranging from 20 to 70% at 21 DAA (Table 4). Only five parental lines (ARCH35R, 03B, 04B, 08B, and 70R) had a mean visible injury of 18 to 19% at 21 DAA (Table 4). Consistent with the visible injury (%), the averaged shoot dry biomass reduction (% of nontreated) of imazamox survived plants ranged from 20 to 76% for most of the lines (Table 4). However, the averaged shoot dry biomass reduction of survived plants from nine parental lines (ARCH35R, 49R, 50R, 60R, 73R, 04B, 12B, 15B, and 25B) ranged from 5 to 19%, indicating reduced sensitivity to imazamox (Table 4). Although not reported in pearl millet, the POST-applied imazethapyr at 50 g ha^{-1} has been found highly effective in controlling wild-proso millet (*Panicum miliaceum* L.) when treated at 1- to 5-leaf stage (Swanton and Chandler 1990).

Nicosulfuron. Similar to imazamox, all advanced parental lines survived the field use rate of nicosulfuron (70 g ha^{-1}) at 21 DAA. Application of nicosulfuron resulted in 70 to 100% survival among all tested parental lines (Table 5). Only three parental lines (ARCH13B, 14B, and 15B) tested with nicosulfuron showed the least survival (70 to 80%) at 21 DAA. Interestingly, these results indicated that most of the tested pearl millet parental lines with reduced sensitivity to imazamox also exhibited reduced sensitivity to nicosulfuron. The mean percent visible injury of survived plants from all these tested parental lines ranged from 20 to 79% at 21 DAA. Only two parental lines (ARCH73R and 08B) had mean visible injury of 13 and 16%. Consistent with the percent survival and visible injury, the average shoot dry biomass reduction (% of nontreated) of the survived plants ranged from 22 to 79% (Table 5). Survived plants from ten parental lines (ARCH65R, 68R, 73R, 04B, 08B, 14B, 15B, 25B, 35B, 36B) had an average shoot dry biomass reduction of 0 to 19% at 21DAA (Table 5).

Dose-Response Bioassays

Sensitivity to Imazamox. Three selected pearl millet lines (ARCH35R, 45R, and 49R) had reduced sensitivity to imazamox (Table 6). The imazamox dose needed for 50% shoot dry biomass reduction (GR_{50} values) of these three selected lines ranged from 19.3 to 30.6 g ha^{-1}, which was significantly greater than 6.0 g ha^{-1} (SOR) and 2.5 g ha^{-1} (ARCH21B). Furthermore, imazamox dose needed for 90% shoot dry biomass reduction (GR_{90} values) of these three selected lines ranged from 68.3 to 117.5 g ha^{-1}, which was greater than that of SOR (37.4 g ha^{-1}) and ARCH21B (24.5 g ha^{-1}) and the field use rate of imazamox (52 g ha^{-1}). Based on GR_{50} values, ARCH35R, 45R, and 49R exhibited 3.2 to 12.2 and 7.7 to 12.2-fold reduced sensitivity to imazamox when compared to SOR and ARCH21B, respectively (Table 6; Figure 1). Several studies have previously documented imazamox resistance in wheat, sorghum, rice, and grass weed species. Notably, Kumar et al. (2023b) reported 4.1- to 6.0-fold resistance to imazamox in three shattercane (*Sorghum bicolor*) populations in northwestern Kansas. Domínguez-Mendez et al. (2017) reported 93.7-fold and 43.7-fold resistance to imazamox in wheat cultivars based on Clearfield® technology. Similarly, Kumar and Jha (2017) reported high-level resistance (110.1-fold) to imazamox in downy brome (*Bromus tectorum* L.). Recently, grain sorghum hybrids (igrowth®, Advanta Alta Seeds, Amarillo, TX) resistant to imazamox are commercially available. These hybrids allow PRE and POST emergence applications of imazamox (IMIFLEX™ herbicide; UPL Company, King of Prussia, PA) for annual grass control (Kumar et al. 2023b).

Sensitivity to Nicosulfuron. Results indicated that both SOR and ARCH21B were highly sensitive to nicosulfuron (37 and 40 g ha^{-1} of nicosulfuron for a 90% reduction in shoot dry biomass, although the recommended field use rate is 70 g ha^{-1}). The ARCH45R and 73R had reduced sensitivity to nicosulfuron (Table 7). The nicosulfuron dose needed for 50% shoot dry biomass reduction (GR_{50} values) of these two selected lines ranged from 18 to 42 g ha^{-1}, which was significantly greater than that of SOR (11 g ha^{-1}) and ARCH21B (7 g ha^{-1}) line. Furthermore, the nicosulfuron dose needed for 90% shoot dry biomass reduction (GR_{90} values) of these two selected lines ranged from 132 to 165 g ha^{-1}, which was greater than that of SOR (37 g ha^{-1}) and ARCH21B (40 g ha^{-1}) and the field use rate of nicosulfuron (70 g ha^{-1}) (Table 7). Based on GR_{50} values, ARCH45R and 73R exhibited 1.6 to 3.8 and 2.6- to 6-fold reduced sensitivity to nicosulfuron, compared with SOR and ARCH21B, respectively (Table 7; Figure 2). Altogether, these results revealed that the same selected pearl millet line (45R) with a relatively higher sensitivity index (SI) ranging from 3.2- to 7.7-fold for imazamox had a low SI range (1.6 to 2.6-fold) for nicosulfuron compared to the SOR and ARCH21B, respectively. Recently, grain sorghum hybrids with tolerance to nicosulfuron (Inzen™, Corteva Agriscience, Indianapolis, IN) are commercially available. The Inzen™ sorghum allows producers to use postemergence applications of nicosulfuron (Zest™ WDG herbicide; Corteva Agriscience, Indianapolis, IN) (Abit et al. 2013). However, there is currently no report on pearl millet hybrids with any herbicide-resistance traits.

Practical Implications

This research showed a reduced sensitivity to imazamox and nicosulfuron among advanced pearl millet parental lines screened. It is important to know that both forage and grain-type pearl millet lines were evaluated in this study. This research reports the first case of natural variation of reduced sensitivity to imazamox and nicosulfuron among pearl millet parental lines. However, the underlying mechanism(s) conferring this reduced sensitivity to imazamox and nicosulfuron is unknown and should be investigated. It is important to note that these experiments were conducted in the greenhouse, and the response of the pearl millet lines in a field setting to these herbicides may be different from the results reported here. Future studies should investigate the response of these lines to imazamox and nicosulfuron in field conditions. Furthermore, the growth and reproductive fitness of these pearl millet parental lines with reduced sensitivity to imazamox and nicosulfuron should be evaluated.

Pearl millet parental lines with reduced SI for imazamox and nicosulfuron can potentially be utilized for introgression in developing elite hybrids resistant to ALS-inhibiting herbicides. Development of such elite pearl millet hybrids with reduced sensitivity to ALS-inhibiting herbicides can allow POST applications of imazamox and nicosulfuron for in-season grass weed control. In this context, the breeding program at KSU-ARCH, KS, focuses on developing high-yielding pearl millet hybrids with tolerance to ALS-inhibiting herbicides. These hybrids with reduced sensitivity to ALS-inhibiting herbicides may facilitate the adoption and expansion of grain and forage pearl millet by providing POST herbicide options for weed control and can fit into the existing cropping and livestock production system in the central Great Plains drylands.

Based on the dose-response bioassay results, four fresh crosses (Grain: ARCH21B x ARCH35R and ARCH21B x ARCH73R; Forage: ARCH41B x ARCH49R and ARCH41B x ARCH65R) of parental lines showing reduced sensitivity for imazamox and nicosulfuron were made in summer 2024 at KSU-ARCH, KS. The main purpose of developing these new crosses is to focus on further development of four bi-parental mapping (mini-nested association mapping) populations by forwarding these four crosses separately from F_1 to F_8 generations to develop recombinant inbred lines (RILs: each cross with 200-250 lines), tag the genomic regions for herbicide tolerance and execute the marker-assisted selection (MAS). This approach is integral to accelerating classical breeding efforts for developing high-yielding pearl millet hybrids with tolerance to ALS-inhibiting herbicides for effective weed control.

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Table 1. List of 56 advanced parental lines of pearl millet used for herbicide screening.

Parent	Type	Lines
Female	Grain	ARCH-01B, 03B, 04B, 05B, 08B, 12B, 13B, 14B, 15B, 16B, 21B, 22B, 24B, 25B, 30B, 31B, 32B, 33B, 35B, 36B, 42B, 44B, 45B, and 47B
	Forage	ARCH-09B, 27B, 37B, 41B, and 46B
Male	Grain	ARCH-26R, 35R, 36R, 45R, 46R, 50R, 60R, 01R, 16R, 21R, 22R, 61R, 62R, 64R, 66R, 67R, 68R, 69R, 73R, 75R, and 76R
	Forage	ARCH-30R, 49R, 63R, 65R, 70R, and 78R

Table 2. Percent survival, visible injury, and shoot dry biomass reduction (% of nontreated) of pearl millet parental lines treated with clethodim at 21 d after application (DAA).

Parental lines	Survival[1]	Visible injury[2]	Shoot dry biomass reduction[2, 3]
	%	%	% of nontreated
Female			
ARCH01B	0	100	82 b
ARCH03B	0	100	75 c
ARCH04B	0	100	83 b
ARCH05B	0	100	83 b
ARCH08B	0	100	90 a
ARCH09B	0	100	92 a
ARCH12B	0	100	87 b
ARCH13B	0	100	89 ab
ARCH14B	0	100	89 ab
ARCH15B	0	100	91 a
ARCH16B	0	100	93 a
ARCH21B	0	100	95 a
ARCH22B	0	100	84 b
ARCH24B	0	100	94 a
ARCH25B	0	100	81 b
ARCH27B	0	100	40 e
ARCH30B	0	100	89 a
ARCH31B	0	100	90 a
ARCH32B	0	100	90 a
ARCH33B	0	100	89 ab
ARCH35B	0	100	85 b
ARCH36B	0	100	89 ab
ARCH37B	0	100	92 a
ARCH41B	0	100	83 b
ARCH42B	0	100	79 b
ARCH44B	0	100	86 b
ARCH45B	0	100	88 ab
ARCH46B	0	100	95 a
Male			
ARCH26R	0	100	86 b
ARCH30R	0	100	89 ab
ARCH35R	0	100	79 b
ARCH36R	0	100	88 ab
ARCH45R	0	100	89 ab
ARCH46R	0	100	93 a

ARCH49R	0	100	83 b
ARCH50R	0	100	95 a
ARCH60R	0	100	82 b
ARCH01R	0	100	89 ab
ARCH16R	0	100	57 d
ARCH21R	0	100	96 a
ARCH22R	0	100	95 a
ARCH61R	0	100	97 a
ARCH62R	0	100	76 c
ARCH63R	0	100	91 a
ARCH64R	0	100	92 a
ARCH65R	0	100	95 a
ARCH66R	0	100	75 c
ARCH67R	0	100	93 a
ARCH68R	0	100	86 b
ARCH69R	0	100	91 a
ARCH70R	0	100	95 a
ARCH73R	0	100	80 bc
ARCH75R	0	100	86 b
ARCH76R	0	100	94 a
ARCH78R	0	100	73 c

[1] Percent survival for each parental line was calculated based on 50 seedlings tested.

[2] Percent visible injury and shoot dry biomass reduction (% of nontreated) were recorded from 12 representative seedlings in each parental line.

[3] Means followed by the same letters within a column are not significantly different using the Fisher’s protected least square difference at $\alpha = 0.05$.

Table 3. Percent survival, visible injury, and shoot dry biomass reduction (% of nontreated) of pearl millet parental lines treated with quizalofop at 21 d after application (DAA).

Parental lines	Survival[1]	Visible injury[2]	Shoot dry biomass reduction[2, 3]
	%	%	% of nontreated
Female			
ARCH01B	0	100	82 cd
ARCH03B	0	100	90 ab
ARCH04B	0	100	86 bc
ARCH05B	0	100	89 bc
ARCH08B	0	100	88 bc
ARCH09B	0	100	91 ab
ARCH12B	0	100	89 b
ARCH13B	0	100	93 a
ARCH14B	0	100	92 ab
ARCH15B	0	100	86 bc
ARCH16B	0	100	95 a
ARCH21B	0	100	86 bc
ARCH22B	0	100	85 bc
ARCH24B	0	100	98 a
ARCH25B	0	100	72 d
ARCH27B	0	100	94 a
ARCH30B	0	100	83 bc
ARCH31B	0	100	92 a
ARCH32B	0	100	90 ab
ARCH33B	0	100	84 bc
ARCH35B	0	100	90 ab
ARCH36B	0	100	88 bc
ARCH37B	0	100	95 a
ARCH41B	0	100	66 e
ARCH42B	0	100	88 bc
ARCH44B	0	100	90 ab
ARCH45B	0	100	77 d
ARCH46B	0	100	94 a
ARCH26R	0	100	89 bc
Male			
ARCH30R	0	100	81 cd
ARCH35R	3	95	83 bc
ARCH36R	2	95	85 bc
ARCH45R	0	100	88 bc
ARCH46R	0	100	94 a

ARCH49R	0	100	86 bc
ARCH50R	12	90	87 bc
ARCH60R	0	100	90 ab
ARCH01R	0	100	84 bc
ARCH16R	0	100	77 d
ARCH21R	0	100	95 a
ARCH22R	0	100	95 a
ARCH61R	0	100	85 b
ARCH62R	0	100	79 d
ARCH63R	0	100	91 a
ARCH64R	0	100	70 de
ARCH65R	0	100	89 b
ARCH66R	0	100	76 d
ARCH67R	0	100	92 a
ARCH68R	5	95	86 b
ARCH69R	0	100	92 a
ARCH70R	0	100	90 ab
ARCH73R	0	100	95 a
ARCH75R	0	100	87 bc
ARCH76R	0	100	95 a
ARCH78R	0	100	94 a

[1] Percent survival for each parental line was calculated based on 50 seedlings tested.

[2] Percent visible injury and shoot dry biomass reduction (% of nontreated) were recorded from 12 representative seedlings in each parental line.

[3] Means followed by the same letters within a column are not significantly different using the Fisher's protected least square difference at α = 0.05.

Table 4. Percent survival, visible injury, and shoot dry biomass reduction (% of nontreated) of pearl millet parental lines treated with imazamox at 21 d after application (DAA).

Parental lines	Survival[1]	Visible injury[2]	Shoot dry biomass reduction[2, 3]
	%	%	% of nontreated
Female			
ARCH01B	95	40	61 b
ARCH03B	100	19	20 g
ARCH04B	100	17	19 g
ARCH05B	98	38	40 de
ARCH08B	100	19	25 f
ARCH09B	55	25	50 c
ARCH12B	98	28	15 h
ARCH13B	92	25	27 f
ARCH14B	98	40	41 de
ARCH15B	98	26	10 h
ARCH16B	100	33	39 e
ARCH21B	80	50	76 a
ARCH22B	97	28	20 g
ARCH24B	100	42	28 f
ARCH25B	98	33	14 h
ARCH27B	98	38	30 f
ARCH30B	90	44	53 c
ARCH31B	93	49	49 c
ARCH32B	100	37	47 cd
ARCH33B	95	50	58 bc
ARCH35B	90	30	36 ef
ARCH36B	98	28	25 fg
ARCH37B	89	25	41 de
ARCH41B	86	56	57 bc
ARCH42B	94	33	40 de
ARCH44B	100	28	20 g
ARCH45B	94	72	62 b
ARCH46B	100	45	39 e
ARCH47B	92	55	62 b
Male			
ARCH26R	100	26	66 b
ARCH30R	100	28	46 cd
ARCH35R	100	18	14 h
ARCH36R	100	20	26 g
ARCH45R	100	36	31 f

ARCH46R	100	21	20 g
ARCH49R	100	20	5 i
ARCH50R	100	29	10 hi
ARCH60R	98	30	19 g
ARCH01R	100	27	36 ef
ARCH16R	86	33	42 de
ARCH21R	91	36	45 d
ARCH22R	100	37	57 bc
ARCH61R	98	31	26 fg
ARCH62R	98	28	39 e
ARCH63R	96	37	45 d
ARCH64R	100	37	24 g
ARCH65R	100	38	28 fg
ARCH66R	84	43	33 ef
ARCH67R	94	34	46 d
ARCH68R	97	64	60 bc
ARCH69R	93	37	65 b
ARCH70R	89	19	25 fg
ARCH73R	100	33	18 g
ARCH75R	98	51	56 bc
ARCH76R	98	44	53 c
ARCH78R	96	43	54 c

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[1] Percent survival for each parental line was calculated based on 50 seedlings tested.

[2] Percent visible injury and shoot dry biomass reduction (% of nontreated) were recorded from 12 representative seedlings in each parental line.

[3] Means followed by the same letters within a column are not significantly different using the Fisher’s protected least square difference at $\alpha = 0.05$.

Table 5. Percent survival, visible injury, and shoot dry biomass reduction (% of nontreated) of pearl millet parental lines treated with nicosulfuron 21 d after application (DAA).

Parental lines	Survival[1]	Visible injury[2]	Shoot dry biomass reduction[2, 3]
	%	%	% of nontreated
Males			
ARCH26R	100	22	44 de
ARCH30R	93	23	39 ef
ARCH35R	100	21	22 h
ARCH36R	100	31	26 gh
ARCH45R	100	79	76 a
ARCH46R	92	61	59 bc
ARCH49R	97	23	29 g
ARCH50R	100	30	31 g
ARCH60R	100	52	80 a
ARCH01R	100	46	77 a
ARCH16R	94	27	45 de
ARCH21R	100	66	77 a
ARCH22R	100	50	22 h
ARCH61R	98	22	23 h
ARCH62R	100	33	29 g
ARCH63R	100	20	47 d
ARCH64R	97	39	35 fg
ARCH65R	98	29	12 j
ARCH66R	98	44	41 e
ARCH67R	100	25	39 ef
ARCH68R	95	23	8 j
ARCH69R	97	26	48 d
ARCH70R	96	20	28 g
ARCH73R	100	13	0 k
ARCH75R	97	41	62 b
ARCH76R	98	30	43 de
ARCH78R	100	52	59 bc
Females			
ARCH01B	96	37	26 gh
ARCH03B	100	23	42 de
ARCH04B	98	36	11 j
ARCH05B	94	23	47 d
ARCH08B	96	16	10 j
ARCH09B	94	39	24 gh
ARCH12B	100	32	27 g

ARCH13B	80	34	23 h
ARCH14B	70	26	15 ij
ARCH15B	77	35	10 j
ARCH16B	98	70	47 d
ARCH21B	90	39	79 a
ARCH22B	91	35	24 gh
ARCH24B	100	20	27 g
ARCH25B	100	22	0 k
ARCH27B	96	48	32 g
ARCH30B	96	37	56 c
ARCH31B	91	20	60 bc
ARCH32B	96	28	30 g
ARCH33B	98	23	43 de
ARCH35B	94	20	10 j
ARCH36B	95	43	19 hi
ARCH37B	92	45	64 b
ARCH41B	95	38	64 b
ARCH42B	97	28	46 d
ARCH44B	96	40	41 e
ARCH45B	98	37	62 bc
ARCH46B	94	39	40 e
ARCH47B	90	37	44 de

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[1] Percent survival for each parental line was calculated based on 50 seedlings tested.

[2] Percent visible injury and shoot dry biomass reduction (% of nontreated) were recorded from 12 representative seedlings in each parental line.

[3] Means followed by the same letters within a column are not significantly different using the Fisher's protected least square difference at $\alpha = 0.05$.

Table 6. Regression estimates of the 3-parameter log-logistic equation fitted to shoot dry biomass reduction (% of nontreated) of selected pearl millet parental lines sprayed with different imazamox doses 21 d after application (DAA).

	Parameter estimates (± SE)[2]							
Parental lines[1]	*d*	*b*	GR_{50} (g ha^{-1})	95% CI	SI (SOR)[3]	SI (21B)[4]	GR_{90}[5] (g ha^{-1})	95% CI
ARCH45R	75 (1.6)	2.1 (2.6)	19.3	16-22	3.2	7.7	79.3	45-113
ARCH35R	74 (1.6)	1.5 (2.1)	24.7	22-28	4.1	9.8	68.3	48-89
ARCH49R	71 (1.9)	1.6 (2.1)	30.6	26-35	12.2	12.2	117.5	66-169
ARCH21B	85 (2.4)	9.7 (5.7)	2.5	0-8	-	-	24.5	12-36
SOR	88 (2.0)	1.2 (2.8)	6.0	2-10	-	-	37.4	30-44

[1] ARCH21B = highly sensitive pearl millet line; ARCH45R, 35R, and 49R = least sensitive pearl millet parental lines SOR = commercial sorghum check hybrid.

[2] *d* is maximum shoot biomass reduction (upper asymptote, fixed to 100%), *b* is the slope of each dose-response curve with standard error in parentheses, and GR_{50} is the effective dose of imazamox needed for 50% shoot dry biomass reduction (% of nontreated) for each tested line

[3] SI (SOR) is the ratio of the GR_{50} value of each least sensitive pearl millet line relative to that of the GR_{50} value of the sorghum check hybrid

[4] SI (21B) is the ratio of the GR_{50} value of each least sensitive pearl millet line relative to that of GR_{50} value of highly sensitive ARCH21B line

[5] GR_{90} is the effective dose (g ha^{-1}) of imazamox needed for 90% shoot dry biomass reduction (% of nontreated) for each parental line; CI, confidence interval.

Table 7. Regression estimates of the 3-parameter log-logistic equation fitted to shoot dry biomass reduction (% of nontreated) of selected pearl millet lines sprayed with different nicosulfuron doses 21 d after application (DAA).

	Parameter estimates (± SE)[2]							
Parental lines[1]	*d*	*b*	GR_{50} (g ha^{-1})	95% CI	SI (SOR)[3]	SI (21B)[4]	GR_{90}[5] (g ha^{-1})	95% CI
ARCH45R	81.1 (2.0)	1.1 (0.1)	18	15-22	1.6	2.6	132	50-213
ARCH73R	69.5 (1.7)	1.6 (0.1)	42	36-49	3.8	6	165	98-231
ARCH21B	82.4 (1.4)	1.2 (0.4)	7	2-12	-	-	40	31-49
SOR	98.2 (1.1)	1.8 (0.3)	11	9-15	-	-	37	26-48

[1] SOR = commercial sorghum hybrid; ARCH21B = highly sensitive pearl millet line; ARCH45R and 73R = least sensitive pearl millet parental lines

[2] *d* is maximum shoot biomass reduction (upper asymptote, fixed to 100%), *b* is the slope of each dose-response curve with standard error in parentheses, and GR_{50} is the effective dose (g ha^{-1}) of nicosulfuron needed for 50% shoot dry biomass reduction (% of nontreated) for each tested line

[3] SI (SOR) is the ratio of GR_{50} value of each least sensitive pearl millet line relative to that of GR_{50} value of the commercial sorghum check hybrid

[4] SI (21B) is the ratio of GR_{50} value of each least sensitive line relative to that of GR_{50} value of highly sensitive ARCH21B line

[5] GR_{90} is the effective dose (g ha^{-1}) of nicosulfuron needed for 90% shoot dry biomass reduction (% of nontreated) for each line; CI, confidence interval

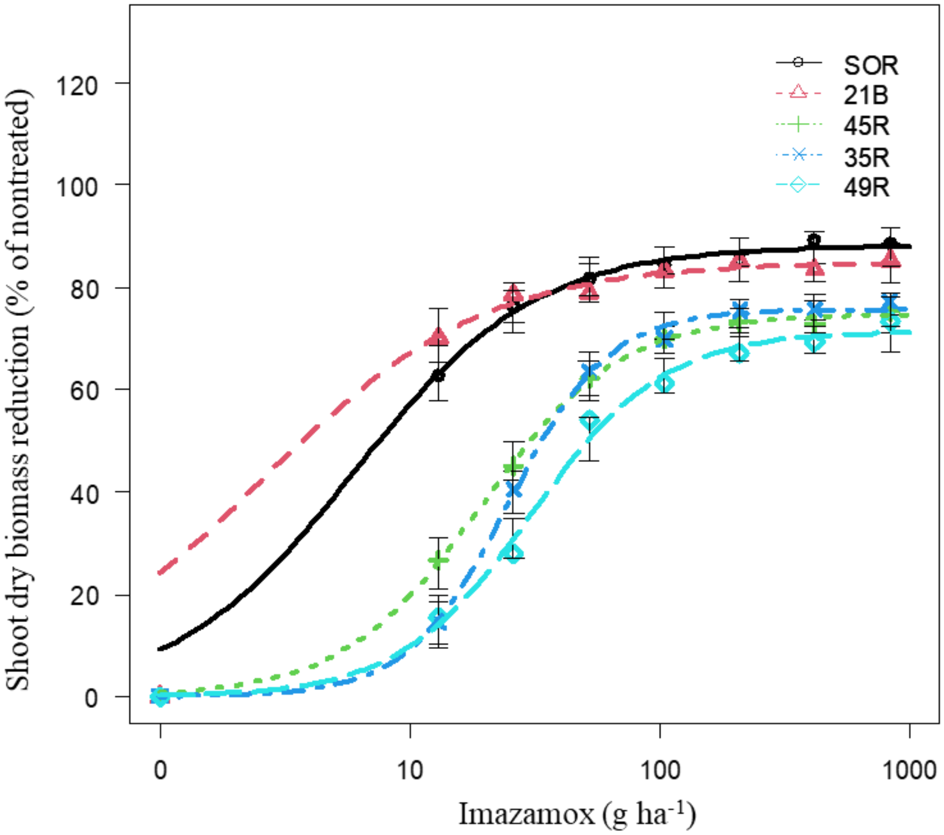

Figure 1. Shoot dry biomass reduction (% of nontreated) of pearl millet parental lines and commercial sorghum hybrid treated with different doses of imazamox at 21 d after application (DAA). Symbols indicate actual values of shoot dry biomass (% of nontreated), and lines indicate predicted values of shoot dry biomass (% of nontreated) obtained from the three-parameter log-logistic model. Vertical bars indicate model-based standard errors (plus and minus) of the predicted mean. SOR = commercial sorghum hybrid; ARCH21B = highly sensitive line; ARCH45R, 35R, and 49R = least sensitive lines.

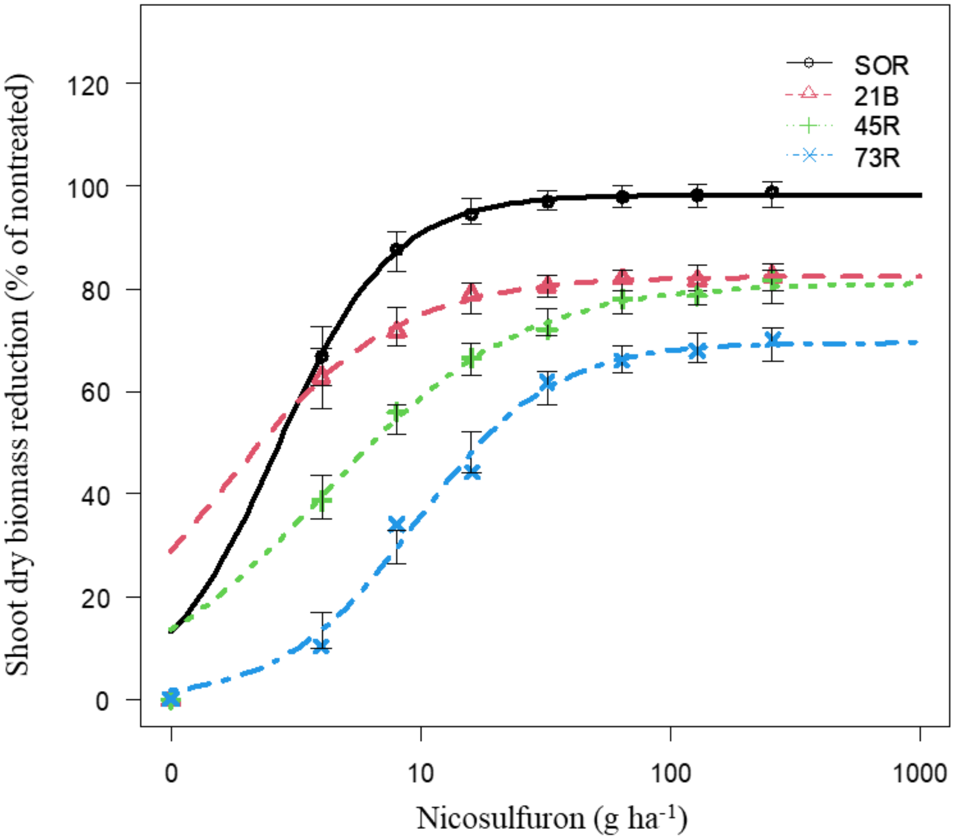

Figure 2. Shoot dry biomass reduction (% of nontreated) of selected pearl millet parental lines and conventional sorghum hybrid treated with various doses of nicosulfuron at 21 d after application (DAA). Symbols indicate actual values of shoot dry biomass reduction (% of nontreated), and lines indicate predicted values of shoot dry biomass reduction (% of nontreated) obtained from the three-parameter log-logistic model. Vertical bars indicate model-based standard errors of the predicted mean. SOR = commercial sorghum hybrid; 21B = highly sensitive ARCH21B line; 45R and 73R = least sensitive ARCH45R and 73R lines.