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CONFIRMATION OF RESISTANCE IN LITTLESEED CANARYGRASS (*PHALARIS MINOR* RETZ) TO ACCASE INHIBITORS IN CENTRAL PUNJAB-PAKISTAN AND ALTERNATIVE HERBICIDES FOR ITS MANAGEMENT

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Abstract

Littleseed canarygrass (*Phalaris minor*) infests wheat and other winter crops in Pakistan and many other countries. Studies were conducted in Pakistan to confirm littleseed canarygrass resistance to fenoxaprop-P-ethyl and to appraise the efficacy of other postemergence herbicides against this grassy weed. A field survey was conducted to collect putative fenoxaprop-resistant seeds from various districts of the central Punjab in March 2015. Dose-response assays were conducted in the greenhouse to confirm resistance to fenoxaprop. The response of fenoxaprop-resistant littleseed canarygrass to diverse herbicide molecules like clodinafop-propargyl, metribuzin, pinoxaden, and sulfosulfuron was also evaluated in further dose-response bioassays. All accessions manifested variable resistance to fenoxaprop, which ranged from 2.52- to 6.00-fold. The resistant accessions also showed low-level cross-resistance (two-fold) to clodinafop. Metribuzin, pinoxaden, and sulfosulfuron were still effective in controlling fenoxaprop-resistant canarygrass. This is the first scientific documentation of resistance to ACCase inhibitor herbicides in central Punjab, Pakistan. The use of alternative herbicides in conjunction with other agronomic practices is crucial for sustainable wheat production in the country.

Key words: Cropping systems, Cross resistance, Herbicides, Punjab, Resistance management, Wheat.

Introduction

Littleseed Canarygrass (*Phalaris minor* Retz.) is a troublesome, self-pollinated winter annual grass native to North Africa, Europe, and South Asia. This is a major weed of wheat (*Triticum aestivum* L.) in more than 60 countries worldwide including Pakistan (Hussain *et al.,* 2015), Africa, Australia, Canada, France, India, Iran, Iraq, Mexico and USA (Chhokar *et al.,* 2008; Travlos, 2012). Pre-sowing imbibition for 24 h and 15-20℃ temperatures are favorable for its germination (Om *et al.,* 2004; Yadav and Malik, 2005). It has similar morphology to wheat, but grows taller than the crop at maturity. It produces 300-450 oblong, grayish-green or black seeds per panicle that mature almost 15-18 days ahead of wheat. The introduction of dwarf and input-responsive wheat varieties during the green revolution, and the wide-scale adoption of the rice-wheat crop rotation favored the proliferation of this weed (Chhokar & Malik, 1999; Chhokar *et al.,* 2008; Hussain *et al.,* 2015). This weed causes 30-50% yield loss in wheat depending upon crop conditions, cultural practices, and duration of competition (Chhokar & Sharma, 2008; Ali *et al.,* 2016). Bhan & Sushil (1998) reported wheat yield losses up to 80% because of littleseed canarygrass infestation. With an increased density of this weed (0 to 200 plants m^{-2}), wheat yield loss was increased by 33% (Duary & Yaduraju, 2005). High levels of infestation, i.e., 2000-3000 plants m^{-2} may cause complete crop failure (Chhokar *et al.,* 2006).

The use of herbicides is the most efficient way to control weeds in wheat and littleseed canarygrass is not an exception. Chemical weed control is cheaper and more effective than manual weeding (hoeing/pulling) because littleseed canarygrass mimics the wheat crop during the early vegetative stage (Ranjit *et al.,* 2006). Thus, postemergence grass herbicides are commonly used to control this grassy weed in wheat. Different acetyl-CoA carboxylase (ACCase) inhibitors, including fenoxaprop-P-ethyl, clodinafop–propargyl, and pinoxaden have been used to control littleseed canarygrass in wheat (Yadav *et at*., 2016; Abbas *et al.*, 2016c). ACCase inhibitors are comprised of three chemical families including aryloxyphenoxy-propionate, cyclohexanedione and phenylpyrazoline. Fenoxprop and diclofop belong to aryloxyphenoxy-propionate while pinoxaden is member of phenylpyrazoline family (Cobb & Read, 2010).

In recent past, herbicide resistance has emerged as the greatest concern of contemporary agriculture which relies primarily on synthetic inputs for its sustainability (Petit *et al.*, 2010). Fenoxaprop-P-ethyl has been used for more than 25 years due to its availability and affordability in Pakistan (Jabbar & Mallick, 1994). Resistance evolution in littleseed canarygrass against ACCase inhibitors has been reported (Owen *et al.*, 2007; Gherekhloo *et al.*, 2012). Resistance of littleseed canarygrass to isoproturon was first reported in India during 1995 (Malik & Singh, 1995). There is ever growing consensus that littleseed canarygrass has evolved resistance to different herbicides including ACCase inhibitors, photosystem II (PS-II) inhibitors, and acetolactate synthase (ALS) inhibitors in many countries including Australia, India, Iran, Israel, Mexico, South Africa, and the United States (California) (Heap, 2016). Multiple resistance (against three and two herbicide sites of action) in littleseed canarygrass against ACCase, ALS, and PS II inhibitors has been confirmed in India and South Africa (Pieterse & Kellerman, 2002; Chhokar & Sharma, 2008).

To date, no scientific study has been undertaken in Pakistan to validate the herbicide resistance of this weed; although, it is an integral part of weed flora of wheat fields throughout the Punjab. In the last few years, growers have encountered ever increasing problem of uncontrolled littleseed canarygrass in wheat fields sprayed with fenoxaprop. Ironically, no confirmed report of resistance to ACCase inhibitors in weeds in central Punjab, Pakistan is available. Early confirmation of herbicide resistance and control of resistant littleseed canarygrass using alternative herbicides is crucial for sustainable wheat production (Burgos, 2015). If resistance do occur, then alternative herbicides are also needed to control fenoxaprop-resistant littleseed canarygrass to reduce or prevent yield losses caused by resistant weed populations. Diverse herbicides molecules with contrasting mode/site of action should be available to be used in rotation, as diversification of management tools strongly reduces selection pressure and delays resistance evolution (Beckie & Reboud, 2009; Burgos, 2015). Classical dose-response assay is a very reliable and informative approach for resistance confirmation and determination of its resistance level (Burgos *et al.,* 2013). Therefore, this study was undertaken with the following objectives: (1) to discover and confirm fenoxaprop (ACCase inhibitor) resistance in littleseed canarygrass biotypes; (2) to determine the level of resistance; and (3) to assess the efficacy of other ACCase inhibitors and herbicides from other groups (contrasting mode of action) commonly used to control littleseed canarygrass in wheat in semiarid climate of Punjab-Pakistan.

Materials and Methods

Survey and collection of littleseed canarygrass seeds: Fields suspected (having poor control and repeated use of single herbicide) to have fenoxaprop-resistant littleseed canarygrass were surveyed during March, 2015 in central Punjab, Pakistan. Farmers of these fields reported consistently poor control of littleseed canarygrass with fenoxaprop in recent years. The facts regarding the history of fenoxaprop use and cropping pattern in surveyed fields are given in Table 1. Seedheads of littleseed canarygrass that survived fenoxaprop application during the current season were collected. From each field, a bulk sample of seeds from 30 randomly selected plants were collected (Burgos, 2015). Five fields were sampled to represent one locality/region. A total of six locations were surveyed and sampled (Table 1). A preliminary trial was conducted which showed that resistance status between fields from the same location was not significantly different. Therefore, these samples were considered as representative of each location (Burgos *et al.,* 2013). Seeds were collected by shaking the spikes when they were fully mature. The collected seeds were dried under shade and stored in craft paper bags at room temperature (25ºC). Subsamples (seeds from 30 plants of same location/region were mixed and approximately 300 seed used for imbibition) of seeds were imbibed in distilled water for 24 h before sowing to promote germination (Om *et al.,* 2004). Fenoxaprop-susceptible littleseed canarygrass seeds (S) were collected from a field known to have consistently 100% control of this weed with fenoxaprop.

Dose-response bioassay for resistance confirmation: Repeated bioassays were conducted in the greenhouse (31.25º N latitude, 73.09º E longitude, and altitude of 184 m) of the University of Agriculture, Faisalabad, Pakistan during winter 2016. Ten seeds each of the susceptible and putative resistant samples were sown in plastic pots (13×10×6 cm) containing sieved and air-dried field soil. The soil was mixed with farmyard manure (2:1, w/w). Since it was difficult to sterilize the soil in bulk, inherent seed bank in the soil was subject to suicidal germination twice before it was used for bioassay studies (Khaliq & Matloob, 2012). Seeds were uniformly spread on the soil surface and covered lightly with the same amount of soil to ensure uniform seeding depth. The pots were placed in a greenhouse with a temperature setting of 20 to 25 ± 2ºC and 14-h photoperiod watered with a sprinkler mist as per need. The relative humidity ranged from 28-55%.

Table 1. Districts, locations, and crop history of different littleseed canarygrass populations collected for fenoxaprop resistance confirmation from Punjab, Pakistan.

Populations	Districts	Locations	Field history	
			Cropping system	Herbicide use (years)
P_1	Mandi Bahauddin	32°19′- 32°34′ N, 73°29′-73°38′ E	Rice-Wheat	>20
P_2	Gujranwala	32°23′-32°29′ N, 74°17′-73° 95′ E	Rice-Wheat	>20
P_3	Jhang	31°24′-31°30′ N, 72°28′-72°35′ E	Cotton-Wheat	15-18
P_4	Sargodha	32°5′-32° 15′ N, 72°44′-72° 61′ E	Maize-Wheat	>20
P_5	Sialkot	32°45′-32° 48′ N, 74°48′-74° 54′ E	Rice-Wheat	>20
P_6	Sheikhupura	32°21′-32°222′ N, 74°19′-72° 89′ E	Rice-Wheat	12-15
Susceptible (S)	Gujranwala	32°10′-32°24′ N, 74°20′-74° 15′ E	Susceptible	0

The experimental units (pots) were arranged in a completely randomized design under two factor factorial arrangement (different accessions of littleseed canary grass sprayed with variable rates of fenoxaprop. The treatments were replicated four times. Fenoxaprop-P-ethyl (Puma Super® 750 EW, Bayer Crop Science, Pakistan) was sprayed at 3 to 4-leaf stage (BBCH scale growth stage 13-14) at eight rates (0, 0.125X, 0.25X, 0.5X, 1X, 2X, 4X, and 8X) for the six putative resistant accessions. The recommended application rate (X) was 93.75 g ai ha^{-1}. Herbicide treatments were prepared using distilled water and applied using a CO_2 pressurized backpack sprayer fitted with TeeJet 8003VS nozzle at 30 psi pressure that sprayed about 187 L ha^{-1}. For a particular application rate of fenoxaprop, different accessions of littleseed canary-grass were sprayed at once. After treatment application, pots belonging to different accessions were separately returned to the greenhouse. Littleseed canarygrass control was evaluated visually at 21 d after treatment (DAT) using a scale ranging from 0% (no control) to 100% (complete control). Percent control was based on injury symptoms including chlorosis, necrosis, stand loss, and stunting of plants as compared with the non-treated control. After visual evaluation, the aboveground tissues were harvested and the dry biomass was recorded after drying the shoot tissues in an oven for 3 d at 70°C.

Cross-resistance and potential control options: The most resistant accession (P2) of littleseed canary-grass was selected to evaluate its cross-resistance pattern and potential control options using a repeated dose-response assay. Experimental conditions and bioassay procedure for this study were similar to those of the resistance confirmation study described earlier. The efficacy of four herbicides including clodinafop–propargyl (ACCase inhibitor), metribuzin (PS II inhibitor), pinoxaden (ACCase inhibitor), and sulfosulfuron (ALS inhibitor) was appraised.

Three factor factorial experiment comprising of four different herbicides, and their eight variable application rates were evaluated against most resistant accession (P2) and fenoxaprop-susceptible littleseed canarygrass seeds (S) in a completely randomized design. Experiment was replicated four times. Herbicides were sprayed at 3-4 leaf stage of test species at eight rates (0, 0.125X, 0.25X, 0.5X, 1X, 2X, 4X, and 8X) of Clodinafop–propargyl (1X = 55 g a.i. ha $^{-1}$), metribuzin (1X = 425 g a.i. ha $^{-1}$), pinoxaden (1X = 45 g a.i. ha $^{-1}$), and sulfosulfuron (1X = 50 g a.i. ha $^{-1}$). These herbicides were sprayed using a CO_2 pressurized backpack sprayer fitted with TeeJet 8003VS nozzle at 207 kPa. Percent control and shoot biomass were recorded 21 DAT following the procedure described previously.

Resistance patterns of various accessions: All the remaining accessions were subjected to 1X and 2X doses of clodinafop–propargyl, metribuzin, pinoxaden, and sulfosulfuron to evaluate the resistance patterns against these herbicides, without reporting resistance levels. The objective was to ascertain whether these herbicides can still be used to control fenoxaprop-resistant littleseed canarygrass. A completely randomized design with the factorial arrangement was used in this study. Experimental conditions, sowing procedures, and herbicides application methods were same as described in previous section. Mortality and dry biomass were recorded 21 DAT.

Data analyses: There was no significant difference between the two experimental runs; therefore, the data were pooled for statistical analysis. Biomass data were subjected to regression in nonlinear sigmoid curves by using inverse prediction of logistic 3P in JMP 13 to predict the dose needed to reduce biomass by 50% (GR_{50}).

$$\mathbf{GR50} = \frac{c}{1 + \exp\{-a(dose - b)\}}$$

where *a* is the growth rate, *b* is an inflection point, and *c* is an asymptote.

Resistance level (RL) for different populations was calculated as the ratio of the GR_{50} of the suspect accession relative to the GR_{50} of the susceptible population (Travlos & Chachalis, 2010; Travlos *et al.,* 2011; Burgos *et al.,* 2013). Data were analyzed using Fisher's Analysis of Variance technique and means were separated using Tukey's honest significant difference (HSD) test at the 5% probability level.

Results

Resistance confirmation in littleseed canarygrass populations: Herbicide-susceptible littleseed canarygrass showed yellowing of leaves from younger to older, stunted growth and eventually plant death. Accessions with low-level resistance were symptomatic, but mortality was not observed in such case. High-level resistant populations showed minimal injury. Six accessions showed different levels of resistance to fenoxaprop (Table 2). The recommended rate of fenoxaprop (1X) killed the susceptible standard (S) by 100%. The putative resistant accessions had 32-71% mortality at this specific dose. The 2X dose killed 48.50- 83.75 % of putative resistant accessions. Significantly higher doses, 4X (for P3 and P4) and 8X (for P1, P2, P5, P6), were required to attain 100% mortality of these accessions (Table 2). Based on GR_{50} and RL, the populations in each location showed different levels of resistance to fenoxaprop. The P2 showed greater GR_{50} (1.62X) and RL (6.00) compared to other accessions. The GR_{50} values for the other accessions were 0.68-1.22 kg a.i. ha^{-1} with 2.5-4.5-fold resistance levels (Table 2).

Cross-resistance and potential control options: The fenoxaprop-resistant littleseed canarygrass was controlled 100% by 1X of metribuzin and sulfosulfuron (Table 3). It was moderately resistant to the 1X dose clodinafop (76.25% control), but still susceptible to pinoxaden (97.50% control). Doubling the dose of clodinafop achieved 100% control of this accession, but this is not a practical and economically viable solution. This accession showed a 2-fold resistance to clodinafop; it is low level resistance, but because the field use rate could control only about 75% of this population, we would classify this as cross-resistant to clodinafop.

Table 2. Percent mortality, $GR_{50,}$ and resistance level (RL) of different littleseed canarygrass populations three weeks after application of fenoxaprop.

Populations	Mortality %								GR_{50}	Resistance
	0	0.125X	0.25X	0.5X	1X	2X	4X	8X	(X)[a]	level
P_1	0 ± 0.00	2.75 ± 2.64	9.5 ± 3.01	33.75 ± 1.68	71.25 ± 2.76	83.75 ± 3.02	98.75 ± 2.76	100 ± 0.00	0.68	2.52
P_2	0 ± 0.00	0 ± 0.00	4.25 ± 2.67	12.5 ± 3.01	32.5 ± 2.58	48.5 ± 2.67	87.5 ± 1.54	100 ± 0.00	1.62	6.00
P_3	0 ± 0.00	1 ± 1.65	11 ± 2.19	25 ± 2.56	49.25 ± 3.43	60 ± 2.55	100 ± 0.00	100 ± 0.00	0.92	3.41
P_4	0 ± 0.00	0 ± 0.00	12 ± 3.02	27.5 ± 1.89	64 ± 1.68	79 ± 1.78	100 ± 0.00	100 ± 0.00	0.74	2.74
P_5	0 ± 0.00	0 ± 0.00	5.5 ± 2.78	20.75 ± 2.75	32.5 ± 2.67	64.5 ± 3.03	97.5 ± 2.46	100 ± 0.00	1.17	4.33
P_6	0 ± 0.00	0 ± 0.00	5 ± 2.54	15 ± 2.75	38.75 ± 3.65	61.5 ± 2.66	92.25 ± 2.73	100 ± 0.00	1.22	4.52
S	0 ± 0.00	15 ± 2.88	36.25 ± 1.67	93.75 ± 3.23	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.27	1.00

[a]GR_{50} was predicted by using inverse prediction of logistic 3P in JMP 13
[b] RL was calculated by dividing the GR_{50} dose (g a.i. ha^{-1}) of resistant biotype by the GR_{50} dose of susceptible biotype
The data are the means ± standard error

Table 3. Percent mortality, $GR_{50,}$ and resistance level (RL) of fenoxaprop resistant littleseed canarygrass to clodinafop, pinoxaden, metribuzin and sulfosulfuron according to dose response experiments.

Herbicides	Populations	Mortality %								GR_{50}	Resistance
		0	0.125X	0.25X	0.5X	1X	2X	4X	8X	(X)[a]	level
Clodinafop	P_2	0±0.00	2.5±3.04	19.5±1.65	53.75±2.64	76.25±3.01	100±0.00	100±0.00	100±0.00	0.44	2.10
	S	0±0.00	20.12±2.76	39.25±2.05	94.75±1.68	100±0.00	100±0.00	100±0.00	100±0.00	0.21	1.00
Pinoxaden	P_2	0±0.00	18±1.78	68.5±1.87	95±1.98	97.5±2.45	100±0.00	100±0.00	100±0.00	0.20	1.05
	S	0±0.00	13±2.89	65.25±1.86	95.80±2.75	100±0	100±0.00	100±0.00	100±0.00	0.19	1.00
Metribuzin	P_2	0±0.00	15±2.56	76.25±2.56	97.5±2.45	100±0.00	100±0.00	100±0.00	100±0.00	0.17	0.94
	S	0±0.00	15±3.05	75.50±1.45	96.75±1.67	100±0.00	100±0.00	100±0.00	100±0.00	0.18	1.00
Sulfosulfuron	P_2	0±0.00	9.25±2.79	51.25±2.54	95±3.05	100±0.00	100±0.00	100±0.00	100±0.00	0.24	1.14
	S	0±0.00	14.25±1.67	55.25±2.66	92.75±2.76	100±0.00	100±0.00	100±0.00	100±0.00	0.21	1.00

[a]GR_{50} was predicted by using inverse prediction of logistic 3P in JMP 13
[b] RL was calculated by dividing the GR_{50} dose (g a.i. ha^{-1}) of resistant biotype by the GR_{50} dose of susceptible biotype
The data are the means ± standard error

Resistance patterns to alternative herbicides: The remaining populations including P1, P3, P4, P5, P6 and the susceptible standard were tested against 0X, 1X and 2X application rates of clodinafop, pinoxaden, metribuzin and sulfosulfuron. Clodinafop controlled P1, P3, P4 and the susceptible standard at 1X, however, P5 and P6 showed less than 80% mortality and produced 15-18% dry biomass than that of the 0X (Fig. 1a). Pinoxaden controlled all populations except P5 and P6 at 1X. At 2X all populations were controlled by 100% (Fig. 1b).

Discussion

Fenoxaprop was registered in Pakistan in 1993 (Jabbar & Mallick, 1994). Continuous use of fenoxaprop over a long period imposed a persistent selection pressure resulting in resistance evolution (Owen *et al.*, 2007) as is evident from the results of this study. Resistance in littleseed canarygrass to fenoxaprop has also been reported in other countries (Heap, 2017; Travlos, 2012). Variable resistance among the littleseed canarygrass populations might be due to a combination of factors. Selection pressure could vary across fields and regions, depending on crop rotation, other cultural practices, intensity and duration of herbicide usage, and how farmers use the herbicide (i.e. timing, and method and rate of application). The genetic makeup could vary slightly between populations across regions; thus, resistance could evolve faster in some regions than others. Dissimilar cropping history and herbicide use can be accounted for differences in resistance levels (Beckie & Reboud, 2009; Travlos, 2012). These differences may also be due to different resistance mechanisms involved (Maneechote *et al.*, 1994; Travlos *et al.*, 2011). Resistance to ACCase inhibitors have been attributed to either increased detoxification or target site mutation (Kaundun, 2014). The former could result in different levels of resistance simply because of differing capacities of each resistant plant to detoxify the herbicide. Low resistance level and endowed by nontarget-site mechanisms usually indicate that the population is at an early phase of resistance evolution. Moreover, a population that is yet heterogeneous and heterozygous with respect to the resistance genes would exhibit low-level resistance. The resistance level increases as the population is purified further (through sustained selection pressure) toward higher homogeneity of resistant individuals. The majority of resistance cases to ACCase inhibitors is endowed by mutation(s) of the *ACCase* gene (Kaundun, 2014). These mutations do not confer the same level of resistance as their impact on herbicide binding differs (Warner *et al.*, 2008).

The cross resistance in *P. minor* might be due to continuous use of pinoxaden. Pinoxaden belongs to phenylpyrazoline family of ACCase inhibitors which is the most recent chemistry discovered in this mode-of-action group (Linda *et al.*, 2010). Farmers are also using clodinafop for more than 10 years in Pakistan. This pattern of being cross-resistant to a herbicide from the same family of aryloxyphenoxypropionate (AOPP) is common (Bourgeois *et al.*, 1997; Délye *et al.*, 2008; Petit *et al.*, 2010). It has also been reported in Italian ryegrass (*Lolium multiflorum* L.) that the majority (about 75%) of diclofop-resistant *L. multiflorum* are susceptible to pinoxaden (Kuk *et al.*, 2008; Salas *et al.*, 2013). It does appear that the same cross-resistance profile applies to littleseed canarygrass. Littleseed canarygrass has evolved multiple resistances across three modes of actions: PSII site, ACCase and ALS inhibitors in India (Chhokar and Sharma, 2008). Chhokar & Sharma (2008) reported that clodinafop-resistant littleseed canarygrass in India showed a high level of resistance against fenoxaprop with low-level cross-resistance pinoxaden. It was susceptible to other herbicides. Effective control of isoproturon-resistant littleseed canarygrass with alternate herbicides have been reported by Chhokar & Malik (2002).

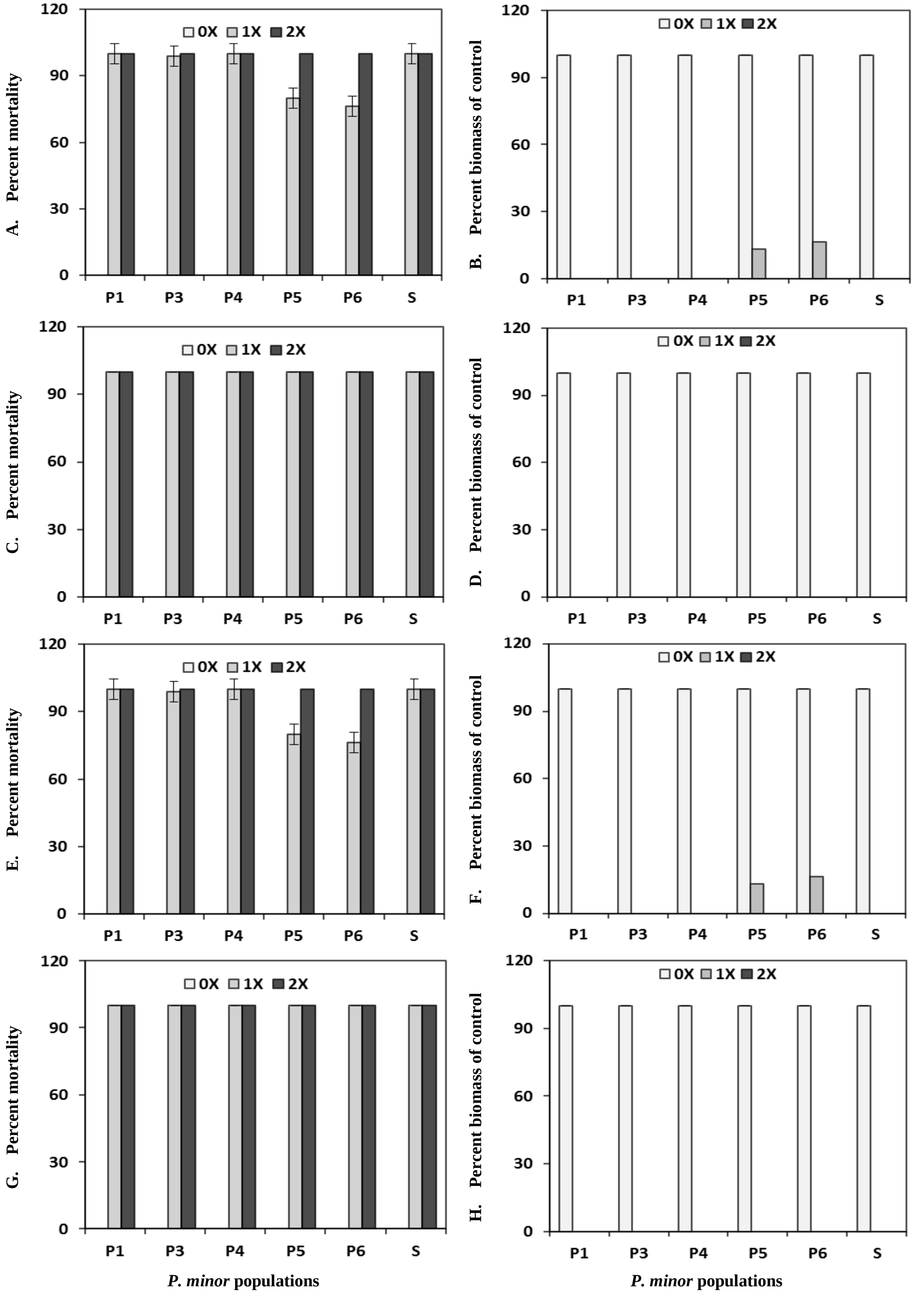

Fig. 1. Efficacy of clodinafop (A-B), pinoxaden (C-D), metribuzin (E-F) and sulfosulfuron (G-H) to control fenoxaprop resistant littleseed canarygrass. Vertical bars with cap indicate standard error of mean.

As observed among Italian ryegrass populations, some cross-resistance (about 25%) is expected to occur with AOPP herbicides and pinoxaden (Kuk *et al.,* 2008; Salas *et al.,* 2013). Whenever this occurs, the resistance level to penoxaden would lower than to the selector AOPP herbicide. The 1X dose of metribuzin and sulfosulfuron controlled all populations (Fig. 1c-d). Thus, fenoxaprop-resistant little seed canarygrass can still be controlled with metribuzin and sulfosulfuron, corroborating the findings of Chhokar *et al.* (2008). Wheat cultivars might have differential tolerance to metribuzin (Runyan *et al.,* 1982); thus, varietal testing is necessary. Most fenoxaprop-resistant populations should still be susceptible to clodinafop and pinoxaden as was reported by Chhokar *et al.* (2008); however, the continued use of other AOPP herbicides is risky because of low-level resistance has already been observed in some accessions P5 and P6. The use of other ACCase herbicides should be avoided as much as possible in locations where resistance is confirmed. Weed populations in these locations have already been preselected for resistance to one type of ACCase inhibitors; hence, are expected to evolve resistance to other ACCase inhibitors rapidly (Chokkar *et al.,* 2008). The use of such herbicides in non-affected locations should be done with utmost vigilance and adherence to best recommended practices for herbicide use. Weeds escaping applications of ACCase herbicides should be removed by other means and the cycle of using ACCase herbicides should be interrupted by planting other crops that are more competitive with grasses and not dependent on ACCase herbicides for production. Metribuzin and sulfosulfuron are currently the best options to control littleseed canarygrass in central Punjab of Pakistan to reduce wheat yield losses.

Resistant populations are expected to spread quickly due to sharing of wheat seeds among farmers, some of which may be contaminated with herbicide-resistant seeds. To mitigate further resistance evolution, farmers in Pakistan need to be educated about weed resistance evolution and assisted in the adoption of integrated weed management systems including crop rotation, rotation of herbicides with different modes of action, use of herbicide mixtures at recommended doses (Abbas *et al.,* 2016c), competitive wheat varieties, optimum seeding rate, hand weeding where applicable, and sanitation practices to avoid resistant seed dispersal. Accurate dose and application method is crucial for optimum efficacy, as low doses of fenoxaprop were found to cause hormesis (growth enhancement) in littleseed canarygrass (Abbas *et al.,* 2016a).

Manual removal of weeds surviving the herbicide application should be done before seed production to reduce the weed seed bank. Littleseed canarygrass emerge at the end of December (favorable temperature 10-20℃) while in November due to comparatively high temperature it does not emerge and grow well. Sowing wheat at the time that would not coincide with grass weed germination and zero tillage practice in the rice-wheat cropping system are also effective in reducing the infestation of this grass (Chhokar & Malik, 1999; Chhokar *et al.,* 2007; Hassan & Bano, 2016) Furthermore, allelopathic crop mulches can also be used to control little seed canarygrass. Abbas *et al.* (2016b) reported that mulches of allelopathic crops including sorghum, maize, sunflower and rice at 12 tons ha^{-1} provided good control of herbicide-resistant littleseed canarygrass. Comprehensive research on allelopathic weed control in field crops has been undertaken in Pakistan (Farooq *et al.,* 2011). The extension of knowledge and application of technology arising from such research endeavors need to be accelerated.

Rotating wheat with dicot crops and multi-cut fodders can reduce the weed seed bank significantly (Maxwell *et al.,* 1990). However, replacing wheat with other crops cannot be done across a large area at any one time because it will be detrimental to the food supply of the country. It will threaten food security. Alternative crops that are currently grown in Pakistan have less economic value than wheat; thus, reducing wheat production area on a massive scale will be detrimental economically. Development of economically viable alternative crops and markets should be undertaken for the long-term health of agricultural production in Pakistan. The immediate option to curtail resistance evolution is to use alternative herbicides in rotation with other agronomic practices to attain sustainable wheat production.

In conclusion, littleseed canarygrass is the main threat to the sustainability of wheat production especially in the rice– wheat cropping system of central Punjab, Pakistan. This research confirmed resistance to fenoxaprop in littleseed canarygrass in this region. Some fenoxaprop-resistant populations have low-level cross resistance to clodinafop and reduced senstivity to pinoxaden, although the latter was still effective on almost all populations. The use of other ACCase herbicides should be minimized and used with extreme caution. Metribuzin and sulfosulfuron are the immediate best options to manage fenoxaprop-resistant littleseed canarygrass. Long term resistance management and avoidance or developing strategies for this grass in wheat should consist of crop rotation, zero tillage, stale seedbed, timely planting of wheat, use of allelopathic mulches, and use of competitive cultivars together with the manual/mechanical control of weeds that survive the herbicide application.

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