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Evaluation of multiple herbicide resistance in littleseed canarygrass (*Phalaris minor*) populations from Haryana in India

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ABSTRACT

Phalaris minor is seriously affecting wheat productivity and profitability in Haryana. The menace of *P. minor* has worsened after it evolved resistance to herbicides. For rational recommendation and implementation of management strategies, it is imperative to assess and quantify the level of resistance in *P. minor* populations. In dose-response assay, it was found that *P. minor* populations 'Naggal' and 'Kalvehri' exhibited multiple resistance to herbicides from different chemical families. 'Naggal' was 13-, 18-, 26- and 22-fold resistant to clodinafop, pinoxaden, sulfosulfuron and mesosulfuron + iodosulfuron, respectively and 'Kalvehri' was 15-, 29- and 16-fold resistant to pinoxaden, sulfosulfuron and mesosulfuron + iodosulfuron, respectively. All tested populations were resistant to ACCase inhibitors with more number of populations being highly resistant to clodinafop than pinoxaden. However, majority of the populations were susceptible to ALS inhibitors particularly mesosulfuron + iodosulfuron. The evolution of multiple herbicide resistance in *P. minor* is a big challenge for scientists and farmers alike.

INTRODUCTION

In India, wheat (*Triticum aestivum* L.) is the second most important food grain crop after rice, grown in 30 million ha (14% of global area) with 99.7 million tonnes of production (Anonymous 2018). The states of Punjab and Haryana in the North-Western plains are major producers of wheat. Among the many factors, the two most important constraints for declining the total factor productivity of rice-wheat cropping system in these states are *Phalaris minor* (Retz.) and deterioration in soil productivity (Vincent and Quirke 2002). *P. minor* is a highly competitive weed thriving on highly fertile and moist soils (Singh 2007). Grain yield losses of wheat caused by *P. minor* vary between 25 to 50% (Chhokar and Sharma 2008) and may cause the complete failure of wheat in case of severe infestation (2000-3000 plants/m^2). However, greater dependence on herbicides has led to the rapid evolution of herbicide resistance in weeds of wheat. Globally, maximum number of cases of herbicide-resistant weeds *i.e.* 75 has been reported to infest wheat among all crops (Heap 2017). *P. minor* is one such notorious weed, which has evolved different mechanisms to defy many wheat herbicides. The evolution of herbicide resistance in *P. minor* began for the first time against isoproturon during 1992-93 in India which was also the first and the most serious case of herbicide resistance in *P. minor* in the world (Malik and Singh 1995).

To control the spread of isoproturon resistant *P. minor*, a number of new herbicides recommendations with different mode of action were introduced (Punia *et al.* 2008). However, the dreadful *P. minor* evolved biological defense against these new herbicides too. Some *P. minor* populations from Punjab and Haryana were found to be resistant to the newly recommended herbicides eg. pinoxaden without any prior history of exposure, indicating evolution of cross-resistance (Dhawan *et al.* 2010). Similarly, few populations have been found to have very high GR_{50} value for a herbicide, mesosulfuron + iodosulfuron (Dhawan *et al.* 2012). Vincent and Quirke (2002) based on their model suggested that the areas with resistant biotypes of *P. minor* in wheat in North Western India would be approximately 1.2 million ha with more in Haryana state (+70%) than in Punjab state (+20%). Thus, the rapid evolution of multiple resistant *P. minor* populations and their extensive spread is not only threatening the wheat yield of resistance-affected farmers but also nation's food security. Hence, the characterization of resistance in *P. minor* populations with regard to profile (single, cross, multiple) and magnitude (fold level) has become vital for rationale recommendation and implementation of effective management strategies. Keeping this in view, the present study was conducted to confirm multiple herbicide resistance in *P. minor* populations from Haryana and to quantify the resistance profiles of *P. minor* populations for significant implications in designing future resistance management strategies.

MATERIALS AND METHODS

Plant materials

In April 2013, seeds of fourteen populations of *P. minor* were collected from locations where farmers reported poor control of the weed with recommended alternate herbicides (clodinafop, sulfosulfuron, fenoxaprop) after the failure of earlier recommended isoproturon to control these *P. minor* populations. Seeds from Hisar-CCSHAU Research Farm were also collected from plots, which had never been sprayed with the herbicides. This population has earlier been confirmed as sensitive and therefore used as standard susceptible check for comparison. The information with respect to history of herbicide use followed in different locations from where the *P. minor* populations were collected is mentioned (**Table 1**).

Dose-response studies

Greenhouse experiments for dose-response bioassays were conducted during the winter seasons of 2014-15 and 2015-16 to determine the levels of resistance in selected *P. minor* populations to recommended alternate herbicides *i.e.,* clodinafop, pinoxaden, sulfosulfuron and mesosulfuron + iodosulfuron at registration doses in screen house of Department of Agronomy, CCSHAU, Hisar. Dose ranges were generated according to registered field rates for post-emergence application of these herbicides. These doses correspond to 0.5, 1.0 and 2.0 times the recommended field rate of the products in India. Recommended dose for different herbicides is: clodinafop 60 g, pinoxaden 50 g, sulfosulfuron 25 g and mesosulfuron + iodosulfuron 14.4 g/ha. Dose-response experiments for each herbicide were conducted using a completely randomized design with four replications per treatment. One untreated control was also kept for each population for comparison. Seeds of different *P. minor* populations were sown by November end during both the years in pots (93 diameter) filled with sandy loam soil. Soil for filling the pots was taken from the field in Agronomy research area, which was not sprayed with herbicides during the last two years and was not infested with *P. minor*. The soil was air-dried, crushed, well ground to pass through a sieve of 2 mm pore size before filling in the pots along with vermin-compost in the ratio of 4:1. The pots were watered before sowing in order to maintain optimum soil moisture and to exhaust the soil weed seedbank. Seeds of *P. minor* populations (50 each) were sown in four replicates at a depth of 0.5-1.0 cm. Two weeks after the emergence of *P. minor* plants, thinning was done and 10 plants per pot were maintained for the spray of herbicide. The plants were sprayed at 3-4 leaf stage with clodinafop 30, 60, and 120 g/ha; sulfosulfuron 12.5, 25 and 50 g/ha; mesosulfuron+ iodosulfuron 7.2, 14.4 and 28.8 g/ha and pinoxaden 25, 50 and 100 g/ha for assessment of the herbicidal efficacy. Herbicides were sprayed with a knapsack sprayer fitted with flat fan nozzle delivering 375 l/ha spray volume at 40 psi pressure. The plants that remained unsprayed with herbicide served as control. Dry weight data of *P. minor* populations at 30 DAT were regressed over herbicide treatments using the three-parameter log-logistic model (Seefeldt *et al.* 1995).

$$y = d/1 + \exp\,[b - (\log(x) - \log(e))]$$

Where, y is the response variable (dry weight g/plant), d is the upper limit, b is the slope of the line, e is the dose resulting in a 50% dry weight control (known as GR_{50}) and x is the herbicide dose. Analysis of dose–response curves were performed separately for each *P. minor* population and GR_{50} values were determined using the *drc* package (drc 1.2, Christian

Table 1. Origin and herbicide use history of different *P. minor* populations collected for confirmation of multiple herbicide resistance

Location	Population	Herbicide use history
Saanch, Kaithal	Saanch	Clodinafop and sulfosulfuron
Pundri, Kaithal	Pundri	Clodinafop and sulfosulfuron
Geong, Kaithal	Geong	Clodinafop and fenoxaprop
Sola, Kurukshetra	Sola	Clodinafop
Rampur, Kurukshetra	Rampur	Clodinafop
Morthali, Kurukshetra	Morthali	Clodinafop
Naggal, Ambala	Naggal	Clodinafop and fenoxaprop
Naranga Majra, Ambala	Naranga Majra	Clodinafop and pinoxaden
Kalvehri, Karnal	Kalvehri	Clodinafop
Takhana, Karnal	Takhana	Clodinafop, fenoxaprop and sulfosulfuron
Kachwa, Karnal	Kachwa	Clodinafop
Danauda, Jind	Danauda	Clodinafop
Rania, Sirsa	Rania	Clodinafop and sulfosulfuron
Silani, Jhajjar	Silani	Clodinafop and sulfosulfuron
Hisar, CCSHAU Farm	Hisar	No herbicide

Ritz and Jens Strebig, R2.5, Kurt Hornik online) in software R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http:// www.R-project.org) (Ritz and Streibig 2005). The level of resistance (resistance index) for different populations was determined by dividing the GR_{50} value of the populations with the GR_{50} value of the susceptible population. The values >2 were considered to be resistant.

RESULTS AND DISCUSSION

The GR_{50} values (dose required to cause 50% reduction in dry weight) for the *P. minor* populations for different herbicides are presented in **Table 2**. Majority of the populations tested were not sensitive to the application of clodinafop. The GR_{50} values for clodinafop for all the populations was more than the recommended dose (60 g/ha) except for Kalvehri, Rania, Silani and Hisar which recorded GR_{50} values less than the recommended dose. The highest GR_{50} value (≥4 times the recommended dose) for clodinafop was observed for Kachwa (245-298 g/ha) followed by Morthali (226-267 g/ha), Naggal (215-244 g/ha), Rampur (177-228 g/ha) and Geong (187-205 g/ha) which had GR_{50} values ≥3 times the recommended dose of clodinafop. *P. minor* populations Pundri, Sola and Danauda also recorded higher GR_{50} values. However, Saanch, Naranga Majra, Takhana showed moderate resistance to clodinafop and had GR_{50} values in the range of 60-85 g/ha, which were slightly above the recommended dose of clodinafop.

The GR_{50} values for pinoxaden for different *P. minor* populations varied in the range of 10-209 g/ha. The lowest GR_{50} values were recorded for Silani (12 g/ha) and Hisar (10-11 g/ha) while the highest GR_{50} values (2.9 to 4.2 times the recommended dose) for pinoxaden were recorded for Naggal (161-209 g/ha) followed by Morthali (160-189 g/ha) and Kalvehri (143-157 g/ha). Saanch, Pundri also recorded GR_{50} values higher than the recommended dose. Geong, Sola, Rampur, Naranga Majra, Takhana, Kachwa, Danauda and Rania had lower GR_{50} values varying from 26-62 g/ha. The *P. minor* populations showed a differential response to the application of sulfosulfuron and mesosulfuron + iodosulfuron. The GR_{50} values for sulfosulfuron for majority of the *P. minor* populations remained below the recommended dose (25 g/ha) except for Naggal and Kalvehri which showed much higher GR_{50} values of 86-92 and 84-113 g/ha, respectively *i.e.* 3.4 to 4.5 times the recommended dose for sulfosulfuron whereas for Takhana, Kachwa and Danauda, GR_{50} values (17-26 g/ha) were around the recommended dose of sulfosulfuron.

Likewise, for mesosulfuron + iodosulfuron, the GR_{50} values for all the populations were in the range of 3-8 g/ha, much below the recommended dose (14.4 g/ha) except for Naggal and Kalvehri which showed GR_{50} values 2.5 to 4.9 times the recommended dose.

The resistance index for population Hisar which was used as the standard susceptible *P. minor* population was 1.0 for all the herbicides (**Table 3**). The resistance index for clodinafop was between 2.4 and 5.0 for Kalvehri, Takhana, Saanch and Naranga Majra, while for Pundri, Geong, Sola, Rampur, Morthali, Naggal, Kachwa and Danauda the resistance indices ranged from 7.9-16.2. *P. minor* populations Rania and Silani had resistance indices in the range of 0.8-1.5. For pinoxaden, lower resistance indices in the range of 2.6-5.7 were observed for Geong, Sola, Rampur, Naranga Majra, Takhana, Kachwa, Danauda and Rania whereas Saanch, Pundri, Morthali, Naggal and Kalvehri showed higher

Table 2. GR_{50} (g/ha) values of *P. minor* populations for different herbicides based on per cent reduction in dry weight 30 DAT

Population	Clodinafop		Pinoxaden		Sulfosulfuron		Mesosulfuron + iodosulfuron	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Saanch	60	71	76	93	6	13	6	4
Pundri	119	214	83	116	6	9	3	4
Geong	187	205	37	45	7	8	8	7
Sola	138	197	42	28	7	7	3	5
Rampur	177	228	30	35	5	13	2	5
Morthali	226	267	160	189	3	10	3	5
Naggal	215	244	161	209	92	86	46	70
NarangaMajra	76	68	37	53	7	15	5	8
Kalvehri	37	54	143	157	84	113	36	47
Takhana	67	85	38	42	22	26	5	7
Kachwa	245	298	36	62	17	23	5	4
Danauda	134	206	26	27	20	20	3	5
Rania	22	15	26	32	5	10	5	3
Silani	22	22	12	12	5	6	3	3
Hisar	15	20	10	11	3	4	3	3

resistance indices in the range of 8.0-19.5. Silani had resistance index value fairly close to that of Hisar. For sulfosulfuron, the highest resistance index values were observed for Naggal (27.9-29.7) and Kalvehri (30.5-22.7). Populations Takhana, Kachwa and Danauda had resistance indices of 5.2-7.2 while remaining populations showed much lower resistance indices. Resistance indices for mesosulfuron + iodosulfuron was d”3 for all the populations except for Naggal and Kalvehri which showed highest resistance indices of 18.5-25.9 and 14.3-17.4, respectively.

The GR_{50} values of *P. minor* populations for different herbicides showed intriguing trends (**Table 2**) and variation in GR_{50} values over the two years could be due to variation in dry weight data over two seasons. The GR_{50} values of all populations were greater than the susceptible population (Hisar) with clodinafop. However, Rania and Silani with clodinafop had GR_{50} values similar to susceptible population and with pinoxaden, Rania had greater GR_{50} value than both Silani and Hisar populations. The dose of sulfosulfuron required for 50% growth reduction of Naggal, Kalvehri, Takhana, Kachwa and Danauda was much higher than that of the susceptible Hisar population while all other populations had similar dose requirement for reducing their growth by 50% as that for Hisar. Mesosulfuron + iodosulfuron caused 48-90% mortality of the test populations and the same was reflected in the GR_{50} values except for Naggal and Kalvehri, which required 12 to 23.3 times higher dose of mesosulfuron + iodosulfuron compared to susceptible Hisar population for the same level of effect. Based on the GR_{50} and resistance index, *P. minor* populations were found to have variable levels of resistance and resistance pattern to applied herbicides. From the herbicide resistance profiles of *P. minor* populations, it was observed that majority of the tested *P. minor* populations were resistant to acetyl-CoA carboxylase (ACCase) inhibitors (clodinafop and pinoxaden) whereas majority of the populations were found to be susceptible or having low level of resistance to acetolactate synthase (ALS) inhibitors (sulfosulfuron and mesosulfuron + iodosulfuron). The level of resistance to clodinafop was found to be high (10.4- to 16.2-fold) for Geong, Rampur, Morthali, Naggal and Kachwa; medium (7.9- to 10.8-fold) for Pundri, Sola and Danauda; low (2.4- to 5.0-fold) for Saanch, Naranga Majra, Kalvehri and Takhana. *P. minor* populations Morthali, Naggal and Kalvehri displayed high level of resistance (14.6- to 19.5- fold), Saanch and Pundri had medium level of resistance (8.0- to 10.8-fold) and Geong, Naranga Majra, Takhana, Kachwa and Danauda were low in resistance (2.6- to 5.7-fold) against pinoxaden. When treated with sulfosulfuron, Naggal and Kalvehri revealed very high level of resistance (22.7- to 30.5-fold); Takhana, Kachwa and Danauda indicated medium level of resistance (5.2- to 7.2- fold) and whereas rest of the populations showed low level of resistance. Very high level of resistance against mesosulfuron + iodosulfuron was shown by Naggal and Kalvehri populations (14.3- to 25.9- fold) whereas other populations were low in resistance or susceptible.

P. minor populations Naggal and Kalvehri were found to be highly resistant to both group of herbicides. Their high resistance indices indicated towards target-site resistance. Kaundun (2007) previously reported the first case of target-site resistance in one biotype of *P. minor* from India. The alteration of target enzyme in resistant weed populations confers a very high level of resistance to ACCase and ALS inhibitors similar to observed in the present investigation. Gherekhloo *et al.* (2008)

Table 3. Resistance index of *P. minor* populations for different herbicides

Population	Clodinafop		Pinoxaden		Sulfosulfuron		Mesosulfuron + iodosulfuron	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Saanch	4.0	3.6	8.0	8.7	2.0	3.4	2.4	1.4
Pundri	7.9	10.8	8.7	10.8	1.9	2.3	1.1	1.5
Geong	12.4	10.4	3.9	4.2	2.3	2.1	3.2	2.6
Sola	9.1	10.0	4.4	2.6	2.4	1.8	1.3	1.7
Rampur	11.7	11.6	3.2	3.3	1.7	3.3	0.7	1.9
Morthali	14.9	13.6	16.8	17.6	0.9	2.5	1.3	2.0
Naggal	14.3	12.4	17.0	19.5	30.5	22.7	18.5	25.9
NarangaMajra	5.0	3.4	3.9	4.9	2.4	3.9	1.8	2.9
Kalvehri	2.4	2.7	15.1	14.6	27.9	29.7	14.3	17.4
Takhana	4.4	4.3	4.0	4.0	7.2	6.9	2.1	2.6
Kachwa	16.2	15.1	3.7	5.7	5.7	6.0	2.0	1.6
Danauda	8.9	10.5	2.8	2.6	6.6	5.2	1.3	1.8
Rania	1.5	0.8	2.8	3.0	1.7	2.8	2.0	1.3
Silani	1.5	1.1	1.2	1.1	1.8	1.7	1.4	1.2
Hisar	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Resistance index: GR_{50} of resistant population/GR_{50} of susceptible population (Hisar, CCSHAU Farm) reported very high resistance in three *P. minor* populations to fenoxaprop due to an altered ACCase enzyme. Likewise, very high level of resistance in *Lolium multiforum* to diclofop and in *Setaria faberi* and *Digitania sanguinalis* to sethoxydim has been documented due to an altered ACCase enzyme (Volenberg and Stoltengerg 2002). Greater than 10-fold resistance conferred by modification of target site has been documented in several weed species that are resistant to herbicides inhibiting ALS enzyme (Eberlein 1999). Resistance to isoproturon in these populations has earlier been documented due to non-target site mechanism *i.e.* enhanced metabolism/ detoxification of isoproturon (Singh 1998). The prevalence of multiple resistance in *P. minor* populations has been reported earlier by Singh (2015) and Chhokar and Sharma (2008). These results were in conformity with the earlier findings of Dhawan *et al.* (2009) and Dhawan *et al.* (2010) who reported high or medium level of resistance in most of the populations tested against clodinafop. The occurrence of resistance (high to medium) against pinoxaden in some *P. minor* populations showing resistance to clodinafop indicated towards cross-resistance to this herbicide. While, sulfosulfuron and mesosulfuron + iodosulfuron remained effective on the majority of the tested *P. minor* populations. Similarly, Travlos (2012) found that about half of the diclofop resistant *P. minor* populations were at least equally or more sensitive to mesosulfuron + iodosulfuron than the sensitive population used as standard for comparison. However, contrasting results were reported by Dhawan *et al.* (2012). They found that none of the *P. minor* population was susceptible to sulfosulfuron and only one population was susceptible to mesosulfuron + iodosulfuron.

It was concluded that *P. minor* populations in Naggal, Ambala, Kalvehri and Karnal have evolved multiple herbicide resistance. Furthermore, the dominance of *P. minor* populations resistant to ACCase inhibitors chiefly clodinafop in present investigation undoubtedly specifies the extended selection pressure for resistant *P. minor* from the unabated use of clodinafop in farmers' fields.

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