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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 2571-2575

www.thepharmajournal.com
Received: 25-03-2022
Accepted: 03-05-2022

Morphological characterization of resistant and susceptible biotypes of *Phalaris minor* in wheat growing Sub-tropics of Jammu

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Abstract
Pot experiment was conducted during rabi season of 2019-20 and 2020-21. The pot screening bioassay experiments conducted during Rabi season of 2019-20 and 2020-2021 at research farm Division of Agronomy, SKUAST-Jammu, main campus Chatha which were laid out in completely randomized design with six treatments and four replications for each twenty-five *P. minor* biotypes. The *P. minor* biotypes were evaluated against five recommended herbicides (isoproturon, sulfosulfuron, clodinafop, fenoxaprop, and pinoxaden) with their graded doses (0, $^{1/4}$X, $^{1/2}$X, X (Recommended dose), ^{2}X and ^{4}X) *i.e.* isoproturon (0, 187.50, 375, 750, 1500 and 3000 g/ha.), sulfosulfuron (0, 6.25, 12.5, 25, 50 and 100 g/ha.), clodinafop (0, 15, 30, 60, 120 and 240 g/ha.), fenoxaprop (0, 25, 50, 100, 200 and 400 g/ha.) and pinoxaden (0, 10, 20, 40, 80, 160 g/ha.). Sunderbani, Rajouri biotype (33.413" N, 74.284" E, 580 m) was taken as susceptible. Out of 25 biotypes 03 were found resistant which were characterized by morphologically using different parameters and found that morphologically resistant biotypes were higher in plant height, number of tillers, spike length, spike width, leaf blade length, leaf blade width and test weight compared to susceptible biotype of *P. minor* although, seed colour was observed dark brown in susceptible and resistant biotypes.

Keywords: *Phalaris minor*, isoproturon, sulfosulfuron, clodinafop, fenoxaprop, and pinoxaden

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops for the majority of world's populations. It contributed about 760 metric tonnes to the global food grain basket in the year 2020-21, from an area of about 219.70 million hectare. World-wide India is firmly occupying the second position among the wheat producing countries after China, while in India, this crop occupies about 30.79 m ha. area and accounts for a production of about 107.59 metric tonnes with a productivity of 3494 kg/ha (Anonymous, 2021) [4]. The area, production and productivity of wheat in Jammu and Kashmir Union territory is 290.30 thousand ha, 548.5 thousand tonnes and 1890 kg/ha, respectively (Anonymous, 2020) [3]. In Jammu region of J&K Union Territory total area under wheat is 282.53 thousand ha with the production of 542.2 thousand tonnes and an average productivity of about 1919 kg/ha (Anonymous, 2020) [3].

Wheat is a predominant winter *(rabi*) cereal crop of north–western plains, grown in rotation with *kharif* crops. During the period of green revolution, the production of wheat increased by more than five times and the productivity by three times mainly due to evolution of high yielding dwarf wheat varieties, balanced use of fertilizers and assured irrigation facilities. However, higher input requirements of high yielding varieties resulted in dominance of weeds in wheat growing areas of

North-West India. Weeds are plants that compete with crops for natural and induced resources. Weeds reduce the tillering capacity, grain size, spike length, grain weight and harvest index of wheat. Weeds not only lower the market value of the produce but also cause enormous economic losses to the growers. However, among the grassy weeds, little seed canarygrass (*Phalaris minor* Retz.) is a troublesome annual monocot and graminaceous weed which is locally called as Gullidanda and Sitti. It is mostly seen in wheat, barley and oat crop fields, waste and fallow lands, along roads, streets, near water channels, poultry sheds, dairy farms, residential colony parks and on sand dunes. Littleseed canary grass (*Phalaris minor* Retz.) is an annual satellite grassy weed of wheat and barley in India and in many other countries. Globally, *Phalaris minor* has been reported in more than 60 countries.

Each plant produces about 300-400, shiny black, very small, flat seeds, which contaminate wheat grains. The weed is highly competitive with wheat, causes crop lodging and can reduce wheat yields up to 95 per cent (Afentouli and Eleftherohorinos, 1996; Chhokar and Malik, 2002 and Chhokar *et al*., 2006) [1, 7-8].

The adoption of rice-wheat system mainly resulted in the initiation of problem of *Phalaris minor* due to maintenance of high soil moisture and the release of phenolic compounds which help in boosting the germination of this weed. The puddling of fields before transplanting rice leads to deep placement of *Phalaris minor* seed and the presence of water in rice fields decreases soil temperature which helps in the survival of the seed. The seed tolerates anaerobic conditions by entering into secondary dormancy and by avoiding aerobic decomposition. The burning of rice straw, high soil moisture, high humidity and low ambient temperature at the time of wheat sowing (20-25 ºC) enhances the *Phalaris minor* germination. Increased *Phalaris minor* density due to straw burning is attributable to the seed germination being stimulated by higher temperature or smoke produced during burning. Early seed dispersal and the ability of the seed to remain dormant in soil for several years are other reasons of its prevalence in the wheat crop field.

There are different methods of weed control which include manual weeding, mechanical weeding, stale seed bed, intercropping and use of herbicides (Mohammad *et al*., 2001) [15]. Morphological similarity of this weed with wheat, increased cost of manual labour for weeding, and its poor efficiency compared to herbicides, and the non-availability of labour during critical periods made chemical weed control method is preferred because it is quick, more effective and relatively cheaper. Among the chemical control, herbicides very attractive tool for weed control in wheat which causes faster knockdown of weeds. Huge amounts of herbicides are used worldwide to manage this weed. However, indiscriminate usage of herbicides has led to the development of resistant biotypes of *Phalaris minor* and required its morphological, biochemical and molecular detailed study for analyzing the difference between resistant and susceptible biotypes.

The genus *Phalaris* belongs to the tribe Phalarideae of the family Gramineae. Hichcock, 1951 and Willis, 1966 listed 20 species in this genus. Of these, five species have been found in India (Twiss, 1969) [24]. One of the major species is *P. minor*. It is self-pollinated (2n = 28, rarely 29) and possesses a C_3 photosynthetic pathway. *Phalaris* seeds have been shown to remain dormant for 3-4 months after maturity (Singh and Dhawan, 1976) [22]. *P. minor* is a native of the Mediterranean region but has been introduced into many other parts of the world. At present, 22 species of *Phalaris* are recognized in the world, of which 11 are native to the Mediterranean including *P. minor* Retz. and 4 in South-Western USA. *P. minor* was reported to be a major weed in Latin America; it was becoming a problem in India by the 1970s (Bhan and Choudary, 1976) [6]. *P. minor* is also reported from, the USA, Canada, Africa, Australia, France, Pakistan, Iran, Iraq and Mexico (Holm *et al*., 1997) [12]. It was reported in every part of the world except Antarctica and the North Pole. It is however, not mentioned in the list of the World's worst weeds by Holm *et al.* (1997) [12]. Earlier publication (Anderson, 1961) [2], indicate *P. minor* infestation in some parts of India. Many farmers believe that the seed of *P. minor* came to India with modern dwarf wheat varieties from Mexico and later became a serious weed pest of wheat. There was no attention for *P. minor* as a weed to be managed in wheat crop in India before 1968. In the past survey reports of Food and Agriculture Organization of the United Nations (FAO, Italy) sponsored survey reports of Parker (1968) [18] and weed surveys of Haryana (Singh *et al*., 1995) also have no mention of this weed in India up to 1968. It was reported to be a major weed in Latin America and probably reached India through the import of Mexican wheat (Lerma rajo and Sonora 64 through PL- 480) which was observed to be a problem by the 1970s (Bhan and Chaudhary, 1976) [6].

Seeds of *Phalaris* are capable of tolerating anaerobic conditions by entering into secondary dormancy which perhaps is one of the reasons of its better adaptation in rice-wheat cropping systems (Parasher and Singh, 1985) [17]. Om *et al.* (2002) observed that dormancy in *P. minor* was lesser than two months under natural field conditions in sandy loam soil as the seeds retrieved from soil of infested field in the last week of May exhibited 80 to 96% germination. Optimum temperature for germination of *Phalaris minor* seeds in laboratory was 15-20 ºC. Dark brown seeds germinated better as compared to light yellow and green ones (Mehra and Gill, 1988) [14]. Bhan and Choudary (1976) [6] found that *Phalaris* germinated well between 10 ºC and 20 ºC and no germination was observed above 30 ºC and below 5 ºC. The root/shoot ratio in little seed canary grass was observed as 1:9 as compared to 1:11 in wheat. Weed species with greater root/shoot ratio are expected to utilize underground resource better than crop plants indicating their greater competing ability. The number of stomata per square millimeter leaf area in *P. minor* was 105 as compared to 65 in wheat, and hence this weed absorbed greater quantities of water from the soil and ultimately offered stiff competition to wheat for water (Rao and Agarwal, 1984) [20].

P. minor with greater root growth and more number of stomata utilized the available NPK in greater quantities at the expense of the associated wheat crop (Vengris *et al*., 1953) [25]. Different biotypes of *P. minor* showed different anatomical and morphological variations in stomata structure, leaf thickness, shape of silica bodies, guard cells and the presence of different kinds of hairs was reported in Iran (Keshavarzi *et al*., 2007) [13]. On an average, *P. minor* has six leaves and produced 300-450 seeds per panicle (Bansal and Singh, 1984) [5] and had a test weight of 2 gm (Bhan and Choudary, 1976) [6]. *P. minor* seeds, after dispersal, underwent true dormancy for about four months (Singh and Dhawan, 1976) [22]. The presence of chemical-inhibitors in the seeds is a likely possibility for true dormancy in *Phalaris* seeds (Rost, 1975) [21]. Root exudates of *P. minor* decreased shoots and ear length and dry matter production by wheat (Porwal and Gupta, 1986) [19]. *Phalaris* spp. contains alkaloids which have been associated with poisoning in cattle (Gallagher *et al*., 1966) [9] In morphological characterization research experiment we will study the morphological difference among the identified susceptible and resistant biotypes of *P. minor* in wheat growing subtropics of Jammu.

Materials and methods

The pot screening bioassay experiments conducted during *Rabi* season of 2019-20 and 2020-2021 which were laid out incompletely randomized design with six treatments and four replications for each twenty-five *P. minor* biotypes. The *P.*

minor biotypes were evaluated against five recommended herbicides (isoproturon, sulfosulfuron, clodinafop, fenoxaprop, and pinoxaden) with their graded doses (0, $^{1/4}$X, $^{1/2}$X, X (Recommended dose), ^{2}X and ^{4}X) *i.e.* isoproturon (0, 187.50, 375, 750, 1500 and 3000 g/ha.), sulfosulfuron (0, 6.25, 12.5, 25, 50 and 100 g/ha.), clodinafop (0, 15, 30, 60, 120 and 240 g/ha.), fenoxaprop (0, 25, 50, 100, 200 and 400 g/ha.) and pinoxaden (0, 10, 20, 40, 80, 160 g/ha.). Sunderbani, Rajouri biotype (33.413” N, 74.284” E, 580 m) was taken as susceptible. The soil for the pot experiment was taken from SKUAST-J research farms where there was no application of herbicide form last two years so that there was no residual effect of any herbicide in pot soil and free from *P. minor* infestation. The soil was air-dried, crushed, and well-grounded passes through a 2 mm sieve. The pots (holes at the bottom for drainage and aeration) were watered thoroughly and left for a week to let the soil attain workable conditions and to exhaust the soil seed bank. Pots were filled with 500 g soil: coco-peat material in the ratio of 4:1. Morphological characterization were taken from control lines only for resistant biotypes and compared with susceptible biotype Sunderbani, Rajouri. Morphological parameters Plant height (cm), Spike length (cm), Spike width (mm), Leaf blade length (cm), Leaf blade width (mm), Test weight (g), No. of tillers/plant and Seed colour were taken during the two years of experimentation

Results

The data given in Table 1 pertaining to morphological characterization of resistant and susceptible biotypes of *P. minor* reveled that susceptible biotype recorded spike length of 7.71 cm and spike length of 8.03 cm was observed in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant *P. minor* and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded spike length to the tune of 8.20 cm and 8.76 cm, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 11.98% increase in spike length as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, spike width of 11.49 mm was observed in susceptible biotype and 12.86 mm in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant

P. minor and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded spike width to the tune of 13.18 mm and 13.36 mm, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 14% increase in spike width as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, leaf blade length of 7.04 cm was observed in susceptible biotype and 8.50cm in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant *P. minor* and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant

P. minor recorded leaf blade length to the tune of 8.66 cm and 8.90 cm, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 20.90% increase in leaf blade length as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, leaf blade width of 8.21 mm was observed in susceptible biotype and 8.27mm in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant *P. minor* and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant

P. minor recorded leaf blade width to the tune of 8.53mm and 9.68mm, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 15.19% increase in leaf blade width as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, test weight of 1.83 g was observed in susceptible biotype and 2.20g in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant

P. minor and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded test weight to the tune of 2.23 g and 2.79g, respectively. *Phalaris minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 34.41% increase in test weight as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, number of tillers per plant of 2 was observed in susceptible biotype which was similar to isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant *P. minor* and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded number of tillers per plant to the tune of 3 and 4, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 50% increase in number of tillers per plant as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.

Among the susceptible and resistant biotypes, plant height of 46.03 cm was observed in susceptible biotype and 59.08 cm in isoproturon resistant biotype. Similarly, isoproturon, sulfosulfuron, clodinafop and fenoxaprop resistant *P. minor* and isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded plant height to the tune of 61.75 cm and 67.75 cm, respectively. *P. minor* biotype resistant to isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden recorded 32.06% increase in plant height as compared to susceptible biotype. Almost a similar trend was observed in the second year of experimention.Seed colour was observed dark brown in susceptible and resistant biotypes during both the years of experimentation.

Discussion

Isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* recorded numerically higher plant height (32.06%), number of tillers (50%), spikelength (11.98%), spike width (14%), leaf blade length (20.90%), leaf blade width (15.19%) and test weight (34.41%) compared to susceptible biotype of *P. minor* during 2019-20 and similar trend was observed in 2020-21. Resistant biotypes were found higher in morphological characters when compare with susceptible biotype may be due to its robust performance and earlier germination. Seed colour was observed dark brown insusceptible and resistant biotypes during both the years of experimentation. It may be possible that the resistance had been developed independently in the biotypes and not that resistance had been developed solely from an initial population if they were originated from a single resistant population, biotypes are expected to be similar morphologically due to its genetic closeness (García-Franco *et al*., 2014) [10]. *P. minor* were not reported different anatomical and morphological variations in leaf thickness, leaf blade length, leaf blade width, shape of silica bodies, guard cells and the presence of different kinds of hair (Keshavarzi *et al*., 2007) [13]. Morphologically resistant biotypes were more robust and higher in growth due to its early germination than susceptible biotype. These results are in close conformity with those of Bhan and Choudary, (1976) [6], Mehra and Gill, (1988) [14] and Tripathi and Gaur (2014) [23].

Table 1: Morphological characterization of resistant and susceptible *P. minor* biotypes

Morphological characters	Susceptible		Resistant (isoproturon)		Resistant (Isoproturon, sulfosulfuron, clodinafop and fenoxaprop)		Resistant (Isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden)	
	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21	2019-20	2020-21
Spike length (cm)	7.71	7.50	8.03	7.70	8.20	8.13	8.76	8.73
Spike width (mm)	11.49	12.12	12.86	12.59	13.18	12.83	13.36	12.83
Leaf blade length (cm)	7.04	6.97	8.50	8.63	8.66	8.63	8.90	8.67
Leaf blade width (mm)	8.21	7.68	8.27	8.14	8.53	8.34	9.68	9.66
Test weight (g)	1.83	1.67	2.20	2.20	2.38	2.23	2.79	2.77
No. of tillers/plant	2	1	2	3	3	4	4	3
Plant height (cm)	46.03	42.57	59.08	57.53	61.75	61.34	67.75	64.35
Seed colour	Dark Brown							

Conclusion

Isoproturon, sulfosulfuron, clodinafop, fenoxaprop and pinoxaden resistant *P. minor* were recorded numerically higher plant height, number of tillers, spike length, spike width, leaf blade length, leaf blade width and test weight compared to susceptible biotype of *P. minor* during both the year. Seed colour was observed dark brown in susceptible and resistant biotypes during both the years of experimentation.
Morphologically resistant biotypes were found higher in plant height, number of tillers, spike length, spike width, leaf blade length, leaf blade width and test weight compared to susceptible biotype of *P. minor* although, seed colour was observed dark brown in susceptible and resistant biotypes.

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