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Effect of Pinoxaden on the Seedling Growth and Chlorophyll Development of the Fenoxaprop-P-Ethyl Susceptible and Resistant Biotypes of *P. minor* and Wheat

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ABSTRACT

Fenoxaprop-P-ethyl resistant populations of *Phalaris minor* Retz. have been observed in wheat fields in India. A quick and accurate means of confirming resistance is necessary to take timely management decisions. Seedling growth and chlorophyll development as affected by the doses of herbicides in Petri dishes were estimated in this study. ED_{50} values obtained for chlorophyll development compared to the seedling growth, discriminated the two feoxaprop resistant populations from each other and from the susceptible population better and provided a close relationship with the whole plant assay. The efficacy of pinoxaden against these populations was also studied based on this parameter. The parameter has the potential to be utilized for early detection of fenoxaprop resistance in *P. minor* populations, and to screen these on a large scale.

Key words : Chlorophyll, fenoxaprop, pinoxaden, seedling growth, whole plant assay

INTRODUCTION

Continuous use of isoproturon in wheat in Haryana and Punjab resulted in the evolution of resistance to this herbicide in *Phalaris minor* Retz. which was first reported in 1992 (Malik and Singh, 1995). However, in course of time, *P. minor* has developed cross-resistance against herbicides like diclofop (Yaduraju and Ahuja, 1995; Kirkwood *et al.*, 1997), fenoxaprop-P-ethyl (Dhawan *et al.*, 2009) and clodinafop-propargyl (Chhokar and Sharma, 2008), all belonging to Group I i. e. acetyl coenzyme A carboxylase inhibitors. Cross resistance in this weed was also reported against sulfosulfuron belonging to Group II of herbicides i. e. acetolactate synthase inhibitors (Dhawan *et al.*, 2009). A new herbicide pinoxaden (Axial®) was announced as a broad spectrum post-emergence herbicide for control of grassy weeds by Syngenta, Switzerland (Hofer *et al.*, 2006). It is a new chemical belonging to phenylpyrazolin category within the Group 1 (ACCase inhibitors) herbicides. It was speculated that it could be used to manage resistant biotypes of weeds of cyclohexanedione and aryloxyphenoxypropionate categories of Group 1 class of herbicides. Recent surveys of the wheat fields have revealed that cross resistance to fenoxaprop and clodinafop has evolved in small patches in the area and not in the whole belt. Identification of physiological parameters that provide an early indication on the susceptible or resistant nature of a biotype under such situations, will be highly useful to recommend farmers regarding herbicides well before the sowing of wheat. A whole plant bioassay is labour intensive and can be conducted on a limited number of samples. Simple tests like seed germination and seedling growth in the Petri dishes using herbicide solutions have been employed as assays for detecting resistance to different herbicides (Murray *et al.*, 1996; O'Donovan *et al.*, 1996). This investigation was planned to study the seedling growth of fenoxaprop susceptible and resistant biotypes of *P. minor* as affected by fenoxaprop and pinoxaden in Petri plate assay and to compare it with the response in whole plant assay in a bid to establish the use of Petri plate assay in discriminating resistant and susceptible populations. Leaf bioassay which involved chlorophyll development during seedling growth as affected by the herbicides in the medium was also conducted.

MATERIALS AND METHODS

Three populations of *P. minor*, one susceptible and two resistant to fenoxaprop were selected for study. Seeds of the susceptible population were collected from the farms of CCS Haryana Agricultural University, Hisar

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where herbicides had never been sprayed. Seeds of resistant population were collected from the village Uchana in Karnal district and village Chanarthal in Kurukshetra district, Haryana where fenoxaprop had been used continuously for more than five years. Seeds of wheat variety PBW 343 were obtained from Department of Plant Breeding, CCSHAU, Hisar. These were tested for susceptibility and resistance to fenoxaprop and pinoxaden by three methods viz., whole plant assay, seed germination assay and leaf assay for chlorophyll development.

Whole Plant Assay

Seeds were sown in earthen pots (8"dia) filled with clay loam soil and placed in a green house in three replicates per treatment and five plants per pot during **rabi** season of 2008-09. Pots were kept in 10 sq. metre area and seedlings at 2-3 leaf stage (30-35 days after sowing) were sprayed with fenoxaprop (0, 15, 30, 60, 120 and 240 g/ha) and pinoxaden (0, 15, 30, 60 and 120 g/ha) with a knapsack sprayer. Thirty days after spray the number of surviving plants as well as fresh weight for five plants per treatment was recorded. GR_{50} value (herbicide dose required for 50% reduction in growth) was calculated using non-linear polynomial regression line (Dhawan *et al*., 2009). The resistance factor i. e. the ratio of GR_{50} value of the resistant biotype to that of the most susceptible biotype was calculated.

Seed Germination Bioassay

Seed germination experiments were conducted in the months of December and January when the day/ night temperature was most congenial for the growth of the weed. Twenty seeds of each population of *P. minor* and 10 seeds of wheat were placed in 9-cm dia Petri dishes lined with two layers of filter paper saturated with 10 ml of the herbicide solution in three replicates per treatment. The concentrations of the herbicide used were 0, 0.01, 0.1, 1, 10 and 100 µM fenoxaprop and pinoxaden. Distilled water without herbicide was used as control. The Petri dishes were covered with lids and left in the laboratory at room temperatures. Germination percentage, shoot and root length of seedlings were determined at 7 and 15 days. Length was measured from the point of seed attachment to the shoot or root tip. Data were calculated as percentage of untreated controls. A non-linear regression line was drawn on the data obtained to describe the response of the populations and dose required for 50% reduction in the parameter (GR_{50}).

Leaf Bioassay

For a study on chlorophyll development during seedling growth, 50 seeds of each population of *P. minor* and 10 seeds of wheat were placed on double layer of Whatman No. 1 filter paper in a 15 cm Petri dish and moistened with 10 ml of distilled water and then covered with a lid. These were grown in dark in an incubator maintained at $15\pm2^{O}C$. After 10 days when the etiolated leaves had emerged from the coleoptile sheath, these were exposed to photoperiodic conditions in the laboratory and floated on 10 ml of the herbicide solution at different concentrations of fenoxaprop and pinoxaden as mentioned above in the seedling growth assay. After one week, chlorophyll developed was extracted by the dimethyl sulfoxide method (Gunes *et al.*, 2007). Fifty mg of the leaf tissue was placed in a vial containing 3 ml DMSO (Dimethyl sulfoxide) at room temperature till the tissue became chlorophyll free (12-16 h). The extract was transferred to a graduated tube and absorbance was read at 665 and 645 nm as described by Kaloyereas (1958) on a computer aided spectrophotometer. DMSO was used as blank. Calculations for different pigments were made according to formula given below (Lichtenthaler, 1987).

$$\text{Chlorophyll a (mg/g FW)} = (11.75 \times A_{665} - 2.35 \times A_{645}) \times \text{dilution factor}$$

$$\text{Chlorophyll b (mg/g FW)} = (18.61 \times A_{645} - 3.96 \times A_{665}) \times \text{dilution factor}$$

Data for different doses were calculated as percentage of controls. Dose response curves were plotted. A non-linear regression line was drawn on the data obtained to describe the response and ED_{50} (Equivalent dose to cause 50% reduction) values were estimated. Since the data for chlorophyll 'b' did not provide any clues regarding the susceptible or resistant nature of a biotype to a herbicide the data are not included in the tables.

RESULTS AND DISCUSSION

P. minor biotype from CCSHAU, Hisar is susceptible to fenoxaprop with a GR_{50} value of 28 g/ha (Table 1), while that from Uchana and Chanarthal are

Table 1. The response of *P. minor* biotypes and wheat to fenoxaprop in whole plant assay, seed germination assay and leaf assay

Biotype	GR_{50} (g/ha)	Resistance factor	Regression equation	R^2
Whole plant assay				
HAU, Hisar	28	-	$Y=159.11 - 0.29x + 10.36x^2$	0.82
Uchana	180	6	$Y=54.63+76.89x -16.96x^2$	0.69
Chanarthal	240	8	$Y=94.8 + 4.74x + 11.14x^2$	0.97
Wheat	>240	>8	$Y=78.16 +12.20x -0.91x^2$	0.18
Seed germination assay				
Coleoptile length GR_{50}/ED_{50} (µM)				
HAU, Hisar	0.7		$Y=120.4-17.04x-0.538x^2$	0.96
Uchana	0.7	1	$Y=121.6-18.7x-0.28x^2$	0.97
Chanarthal	40	57	$Y=86.5+8.98x-2.89x^2$	0.81
Wheat	40	57	$Y=76.4+21.36x-4.69x^2$	0.86
Radicle length				
HAU, Hisar	0.04	2	$Y=146.2 -50.26x + 4.31x^2$	0.96
Uchana	0.02	-	$Y=161\ 71.8x +7.67x^2$	0.97
Chanarthal	0.2	10	$Y=140-33.60x +1.63x^2$	0.95
Wheat	1.5	75	$Y=89.7-0.46\ x-2.14x^2$	0.80
Leaf assay				
Chlorophyll 'a' development				
HAU, Hisar	0.5	-	$Y= 111.9 -16.39x -0.061x^2$	0.94
Uchana	1.0	2	$Y= 113.1 -14.82x -0.274x^2$	0.99
Chanarthal	9.0	18	$Y= 103.2 -4.13x -1.46x^2$	0.82
Wheat	70	140	$Y= 118.2+0.84x -2.34x^2$	0.71

resistant with a GR_{50} value of 180 and 240 g/ha, respectively. Wheat showed GR_{50} values of more than 240 g/ha. ED_{50} values in the seed germination assay for coleoptile length were 0.7 µM for biotype from Hisar and Uchana, and 40 µM for biotype from Chanarthal as well as also for wheat. While GR_{50} value for coleptile length could discriminate between susceptible biotype from Hisar and resistant biotype from Chanarthal, it could not discriminate the resistant biotype from Uchana. GR_{50} values for radicle length were 0.02 µM for Hisar biotype, 0.04 µM for biotype from Uchana, 0.2 µM for biotype from Chanarthal and 1.5 µM for wheat. The GR_{50} values for radical length being lowest in the resistant biotype from Uchana rather than the susceptible biotype from Hisar. ED_{50} values as calculated in chlorophyll development assay were 0.5 µM for biotype from Hisar, 1.0 µM for biotype from Uchana, 9 µM for biotype from Chanarthal and 70 µM for wheat. The resistance factor could differentiate the two resistant biotypes as also wheat as in case of whole plant bioassay.

GR_{50} values for pinoxaden for biotype from Hisar were 10 and 28 g/ha for biotype from Uchana, 120 g/ha for biotype from Chanarthal and more than 120 g/ha for wheat. This indicated that biotype from Hisar was the most susceptible to pinoxaden one from Uchana was medium resistant and that from Chanarthal was highly resistant to pinoxaden with a resistance factor of 12. This pattern was apparent in seed germination assay in GR_{50} values obtained with coleoptile length, the values being 0.05, 0.5, 1.0 µM for *P. minor* biotypes and 5.0 µM for wheat. The GR_{50} values obtained with radical length did not match with whole plant bioassay (Table 2). The GR_{50} values obtained with chlorophyll development also provided a match with whole plant assay clearly indicating Hisar biotype as the most susceptible and Chanarthal biotype as the most resistant to pinoxaden and the biotype from Uchana possessing intermediate values.

The data, therefore, indicate that pinoxaden provided control for the fenoxaprop susceptible and medium resistant biotypes. However, the biotypes highly resistant to fenoxaprop showed high level of resistance to pinoxaden as seen by all the three bioassays. A weak level of resistance to pinoxaden in *P. minor* had been indicated by Chhokar and Sharma (2008). Some level of resistance to pinoxaden has been reported in *Alopecurus myosuroides* and *Lolium multiflorum* populations even when these had not been earlier exposed to this herbicide (Kuk *et al.*, 2008).

An expression of resistance to fenoxaprop and

Table 2. The response of *P. minor* biotypes and wheat to pinoxaden in whole plant assay, seed germination assay and leaf assay

Biotype	GR_{50} (g/ha)	Resistance factor	Regression equation	R^2
Whole plant assay				
HAU, Hisar	10	-	$Y= 172.4 - 0.18x + 11.39x^2$	0.94
Uchana	28	2.8	$Y= 148.7 -42.42x + 2.79x^2$	0.85
Chanarthal	120	12	$Y= -12.4+275.43x -48.75x^2$	0.80
Wheat	>120	>12	$Y= 13.6+105.2x - 14x^2$	0.40
Seed germination assay				
Coleoptile length GR_{50}/ED_{50} (µM)				
HAU, Hisar	0.05	-	$Y= 156.3-55.41x+4.90x^2$	0.97
Uchana	0.5	10	$Y= 121.6-18.7x-0.28x^2$	0.97
Chanarthal	1.0	20	$Y= 102.9-0.525x -3.04x^2$	0.90
Wheat	5.0	100	$Y= 81.39+12.66x -4.1x^2$	0.89
Radicle length				
HAU, Hisar	0.01	-	$Y= 155.8-71.3x +7.7x^2$	0.94
Uchana	0.01	1	$Y= 163-68.17x +6.96x^2$	0.94
Chanarthal	0.8	80	$Y= 108.3-8.36x -1.81x^2$	0.92
Wheat	0.5	50	$Y= 116.1-17.91x -0.38x^2$	0.92
Leaf assay				
Chlorophyll 'a' development				
HAU, Hisar	0.03	-	$Y= 147.3-54.54x + 5.15x^2$	0.96
Uchana	0.50	16	$Y= 113.08-15.61x -0.48x^2$	0.98
Chanarthal	0.75	25	$Y= 114.17-14.47x-0.74 x^2$	0.99
Wheat	5.0	166	$Y= 109.6-7.08x -1.39x^2$	0.96

pinoxaden in the seed germination assay as assessed by coleoptile length and chlorophyll development in the seedlings could be exploited as a method for early detection of resistance to these herbicides in the laboratory on a large scale.

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