Indian Journal of Weed Science **45**(4): 296–298, 2013

Short communication

Pollen germination assay for detection of cross resistance against acetyl coenzyme-a inhibiting herbicides in littleseed canary grass

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Received: 17 October 2013; Revised: 20 December 2013

Key words: Herbicde resistance, Cross resistance, Clodinafop-propargyl, Fenoxaprop-p-ethyl, Pinoxaden, *Phalaris minor*, Pollen germination

Phalaris minor is an annual weed infesting winter crops across several continents. In India, the weed evolved insensitivity to urea herbicides (isoproturon), ACCase (fenoxaprop-p-ethyl, clodinafop-propargyl, pinoxaden) and ALS (sulfosulfuron) inhibiting herbicides (Malik and Singh 1995, Dhawan *et al.* 2009, Dhawan *et al*. 2010). An existence of multiple resistance in *P. minor* populations (Chokhar and Sharma 2008) makes the decision making task regarding use of alternate herbicides more difficult. A resistant population at a particular population could be resistant to one or more herbicides. Laboratory methods like seed germination assays (Murray *et al*. 1996, O' Donovan *et al*. 1996) and pollen germination assays (Richter and Powles 1993, Letouze and Gasquez 1999) have been employed for early detection of herbicide resistance and management options. While the utility of seed germination assay in *P. minor* is established in an earlier communication (Dhawan *et al*. 2010) the utility of pollen germination assay is explored in this investigation. A medium for germination of *P. minor* pollen has been identified to be raffinose, 7.5% + boric acid 20 ppm + calcium chloride, 300 ppm (Dhawan *et al.* 2013). The response of herbicides like fenoxaprop-p-ethyl, clodinafop-propargyl and pinoxaden was evaluated in pollen germination assay and a comparison was made with whole plant assay.

Six populations, *viz*. Karnal-Uchana, Karnal-Sagga, Kurukshetra-Neemwali, Kurukshetra-Chanarthal, Jind-Raseedan and Jind–Pipaltha were selected for study. Their response to fenoxaprop, clodinafop and pinoxaden was studied in a pot trial during 2012-13. Seeds were sown in pots filled with sandy loam soil in the month of November. These were thinned to 5 plants per pot after one week of emergence. For herbicide spray, the pots (4 replicates per treatment) were kept in 10 m^2 area and sprayed with a knapsack sprayer at 2-3 leaf stage. Per cent mortality was recorded after 30 days. The dose range for herbicide spray was: fenoxaprop-p-ethyl - 0, 60, 120, 240, 480 and 960 g/ha; clodinafop-propargyl - 0, 30, 60, 120 and 240 g/ha and pinoxaden - 0, 25, 50, and 100g/ha

Plants of different populations were raised in November in pots filled with sandy loam soil. Anthesis in the inflorescence proceeded basipetally with the terminal flowers of each panicle opening first followed by next flower down shortly thereafter. Pollen viability was estimated by employing TTC (triphenyl tetrazolium chloride) test. The test solution was prepared by boiling 0.5% TTC in a solution of 30% sucrose and 0.1% sodium succinate. After cooling, the pollen grains were put in a drop of the solution and kept in an oven at 30^{o}C for 15 minutes. The red pollen grains indicated viable pollen. Pollen germination was expressed as a percentage of viable grains.

To study the response of herbicides, stock solutions of 4000 µM each of fenoxaprop, clodinafop and pinoxaden were prepared and diluted to give final concentrations of 0.1, 1, 10, 100, 1000 1nd 2000 µM when added to the germination medium. 100µL solution was taken on a glass slide and pollen grains from one anther were tapped on to it with the help of a needle and left to germinate. These were observed under the microscope. Germinating pollen grains with long pollen tubes could be observed within 10-30 min. The number of germinated and no germinated pollen was counted in a field and percentage calculated. Observations under 10 fields per treatment were recorded and the means calculated. Data for different doses was calculated as percentage of controls. Dose response curves were drawn and ED_{50} (equivalent dose required for 50% inhibition of germination) values calculated by polynomial regression on dose response curves as described in an earlier communication (Dhawan *et al*. 2009).

None of the populations was susceptible to fenoxaprop, since the populations showed GR_{50} (herbicide dose for 50% reduction of growth) values in the range of 120-240 g/ha higher than the recommended dose (100 g/ha). The most resistant populations was identified to be Kurukshetra-Chanarthal with GR_{50} value of 240g/ha. The populations showed resistance against clodinafop-

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propargyl too. The range in GR_{50} value was found to be 50-180 g/ha. Populations from Kurukshetra- Chanarthal and Jind–Raseedan showed highest GR_{50} values of 120 and 180g/ha, respectively. These populations also showed resistance against pinoxaden with GR_{50} values of 60 and 85 g/ha (Table 1).

Pollen germination assay showed parallel results. The most resistant population against fenoxaprop was seen to be Kurukshetra-Chanarthal with ED_{50} value of 3000 µM. The most resistant populations against clodinafop were seen to be Kurukshetra-Chanarthal and Jind-Raseedan with ED_{50} value of 1000 µM each as compared to ED^{50} value of 75-100 µM in other populations. Similarly these populations showed ED^{50} value of 2100 µM against pinoxaden as compared to 500-1000uM in other populations (Table 2).

The study substantiated the earlier findings of existence of resistance against ACCase inhibiting herbicides. It also provided an indication of an expression of ACCase resistance in pollen germination behaviour of the populations. Haughen and Somerville (1986) reported that herbicide resistant trait conferred by single nuclear encoded genes could be expressed in pollen due to an insensitive target site and resistance due to detoxification mechanism may not be expressed in pollen. Since a parallel could be observed in pot culture assay and pollen assay, it may be inferred that evolution of resistance to ACCase inhibiting herbicides in these populations is due to target site alterations. This is in contrast to evolution of resistance to isoproturon which was reported to be due to metabolic detoxification of the herbicides (Singh *et al.* 1998a,b). Further work on molecular analysis of the populations could provide leads towards the mutations involved at the target site.

Table 1. The response of *Phalaris minor* populations to fenoxaprop, clodinafop and pinoxaden in whole plant assay

Herbicide / population	GR_{50} (g/ha)	Regression equation	R^2
Fenoxaprop			
Karnal-Uchana	150	$143.5 - 55.9x + 6.5x^2$	0.90
Karnal-Sagga	120	$164 - 81.1x + 10.9x^2$	0.85
Kurukshetra-Neemwali	120	$158 - 72x + 9.3x^2$	0.92
Kurukshetra-Chanarthal	240	$126 - 38.6x + 4.2x^2$	0.79
Jind-Raseedan	120	$150 - 65.3x + 8.2x^2$	0.87
Jind-Pipaltha	200	$131 - 46x + 6.2x^2$	0.64
Clodinafop			
Karnal-Uchana	95	$122.7 - 26.3x + 1.8x^2$	0.89
Karnal-Sagga	90	$141.3 - 26.8x - 0.42x^2$	0.88
Kurukshetra-Neemwali	50	$143.1 - 42.8x + 2.9x^2$	0.99
Kurukshetra-Chanarthal	120	$137.5 - 43x + 5x^2$	0.98
Jind-Raseedan	180	$130.1 - 29.1x + 2.6x^2$	0.93
Jind - Pipaltha	50	$131.7 - 32.9x + 1.6x^2$	0.94
Pinoxaden			
Karnal-Uchana	24	$177.2 - 96.7x + 14.7x^2$	0.85
Karnal-Sagga	22	$188.3 - 106x + 16.7x^2$	0.89
Kurukshetra-Neemwali	20	$210.3 - 134.1x + 21x^2$	0.95
Kurukshetra-Chanarthal	60	$93.2 + 25.6x - 12.2x^2$	0.89
Jind-Raseedan	85	$-8.8 + 176.7x - 44.2x^2$	0.62
Jind-Pipaltha	20	$187 - 101x + 140x^2$	0.99

SUMMARY

Pollen germination was tested in six *Phalaris minor* populations in a germination medium consisting of raffinose (7.5%) + boric acid (20 ppm) + calcium chloride (300ppm) supplemented with 0.1–2000 µM of fenoxaprop-p-ethyl, clodinafop-propargyl and pinoxaden. The populations were also tested in a pot culture assay. A parallel response was observed with regard to the level of resistance in the two systems. The method showed the possibility of a rapid screening test for resistant populations.

Table 2. The response of *Phalaris minor* populations to fenoxaprop, clodinafop and pinoxaden in pollen

Herbicde /population from different places	GR_{50} (µm)	Regression equation	R^2
Fenoxaprop			
Karnal-Uchana	900	108.1 – 8.5x -1.7 x^2	0.90
Karnal-Sagga	950	97.1 -9.2 x -0.55 x^2	0.57
Kuruukshetra-Neemwali	700	125.1 – 31.2 x + 2.4 x^2	0.81
Kurukshetra-Chanarthal	3000	76.1 + 26.8 x - 4.3 x^2	0.77
Jind-Raseedan	750	118.4 – 16.9 x -0.46 x^2	0.93
Jind- Pipaltha	1100	123.1 – 13.8 x -0.86 x^2	0.93
Clodinafop			
Karnal-Uchana	75	122.7 – 26.3 x + 1.8 x^2	0.62
Karnal-Sagga	90	141.3 – 26.8 x -0.42 x^2	0.82
Kuruukshetra-Neemwali	80	143.1 – 42.8 x + 2.9 x^2	0.92
Kurukshetra-Chanarthal	1000	137.5 - 43 x +5 x^2	0.68
Jind-Raseedan	1000	130.1 – 29.1 x + 2.6 x^2	0.96
Jind-Pipaltha	100	131.7 – 32.9 x + 1.6 x^2	0.97
Pinoxaden			
Karnal-Uchana	500	132.8 – 39.2 x + 3.7 x^2	0.95
Karnal-Sagga	1000	125.6 – 25.6 x +2.1 x^2	0.95
Kuruukshetra-Neemwali	600	119.3 – 28 x + 2.2 x^2	0.83
Kurukshetra-Chanarthal	2100	93.7 + 5.5 x -1.9 x^2	0.82
Jind-Raseedan	2100	71.2 + 42 x – 8.1 x^2	0.93
Jind-Pipaltha	900	98.6 – 10 x – 0.75 x^2	0.78

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