

Protein Profiles of Some Isoproturon Susceptible and Resistant Biotypes of *Phalaris minor* Retz.

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ABSTRACT

Isoproturon resistant biotypes of *P. minor* gained more plant height, leaf area and dry matter as compared to their susceptible counterpart from Karnal area. Susceptible biotypes from Bawal and Rohtak also gained more height as compared to that from Karnal but were slower to grow as compared to the resistant ones. All the resistant ones were dissimilar from their susceptible counterpart in the absence of three protein bands at molecular weights 15.8, 14.1 and 7.9 kdaltons.

INTRODUCTION

Some of the populations of *Phalaris minor* have been seen to show resistance to the isoproturon in Haryana, Punjab and Uttar Pradesh in India (Malik and Singh, 1995; Yadav *et al.*, 1996; Walia *et al.*, 1997). Although the biology of this weed has been reviewed earlier (Singh *et al.*, 1999), a comparative study of the resistant and susceptible biotypes gained importance in evaluating the ecological fitness of the biotypes. An increased germination and faster seedling growth of the resistant biotypes around Karnal area has been observed (Dhawan *et al.*, 2003). A comparative study of the vegetative and reproductive behaviour of isoproturon susceptible and resistant biotypes and their protein profiles was planned to indicate the extent of variability in the biotypes.

MATERIALS AND METHODS

Susceptible seeds were collected from CCS Haryana Agricultural University Regional Research Station, Karnal (S1), Bawal (S2) and Rohtak (S3) in April 2000. Those of the resistant biotypes were collected from villages surrounding Karnal viz. Uchana (R1), Kalwehri (R2) and Nisang (R3) where

isoproturon resistance problem was seen. These had GR₅₀ values of 0.30 (Bawal) and 0.40 (Rohtak and Karnal) and 1.7, 2.25 and 1.2 kg ha⁻¹ for R1, R2 and R3.

Comparative Growth Studies

Twenty seeds in five replicates each of susceptible and resistant biotypes were sown in pots filled with loamy soil. These were thinned to 10 plants per pot 45 days after sowing and five plants per pot 75 days after sowing. Data on plant height, leaf number/plant and fresh/dry matter accumulation were recorded at periodic intervals. Leaf area was measured with a leaf area meter, 211, Systronics. Data on days to spike initiation, spike length and spike weight were also recorded. The experiment was repeated twice. Data presented are the average of two repeated trials.

SDS-PAGE for Proteins

Hundred mg of the tissue in two replicates was taken for protein extraction. The tissue was homogenized with 2 ml phosphate buffer (0.1 mM pH 7.0) for the extraction of proteins. Protein pellet was precipitated by TCA (20%). This was dissolved

in Tris-Cl buffer (pH 6.8) containing sodium dodecyl sulfate. Electrophoretic separation of proteins of each sample was carried out in duplicate by one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS - PAGE) using the Laemmli buffer system (Laemmli, 1970). Gels were stained in coomassie blue for 7-8 h and destained in ethanol-acetic acid for 2-3 days. The appearance of blue distinct bands indicated proteins. The molecular weights of the proteins were determined from a standard graph obtained by running a mixture of proteins of known molecular weights.

RESULTS AND DISCUSSION

The gain in plant height, leaf area and dry matter was the least in the susceptible biotype from Karnal (S1). All the three resistant biotypes gained more plant height, leaf area and dry matter at all the stages measured as compared to the susceptible biotype around Karnal (S1). Plant

height was invariably more in all the resistant biotypes as compared to susceptible ones (Fig. 1). Weight of the spikes was more in the resistant biotypes tested as compared to S1 (Table 1). All these parameters are suggestive of a better ecological fit of the isoproturon resistant biotypes of *Phalaris minor*.

Nineteen protein bands could be seen in the Karnal biotype (S1) at Rfs 0.02, 0.03, 0.06, 0.11, 0.13, 0.18, 0.21, 0.23, 0.24, 0.29, 0.31, 0.33, 0.36, 0.42, 0.44, 0.58, 0.61, 0.66 and 0.73 at the plant growth stage at 65 days after sowing (Fig. 2). In S2, the bands of Rfs 0.58, 0.66 and 0.73 corresponding to molecular weights 15.8, 14.1 and 7.9 Kdaltons, respectively, were seen in lesser intensity. In S3 and R1, R2 and R3 these bands were missing altogether. Although S2 was distantly located, its protein profile matched with S1. S3, however, was different (Fig. 2). It was apparent from the data that the protein profiles also showed variability within the susceptible populations. S1 and S2 had similar banding pattern, while S3 was different in the absence of three bands. The resistant biotypes differed from the susceptible biotype from Karnal and Bawal in the absence of these three protein bands and did not show any variability in the protein profiles amongst themselves. This may be because of the fact that the resistant biotypes have evolved from a population like S1. Susceptible population is expected to be a more heterogenous lot from which the resistance populations have originated. The

Table 1. Final leaf number and spike weight of susceptible and resistant biotypes of *Phalaris minor*.

Biotype	Final leaf No.	Spike weight (mg)
S1	18	432±42
S2	16	475±48
S3	17	450±42
R1	16	500±48
R2	17	525±50
R3	17	467±42

±Standard error of three spikes in two replicates.

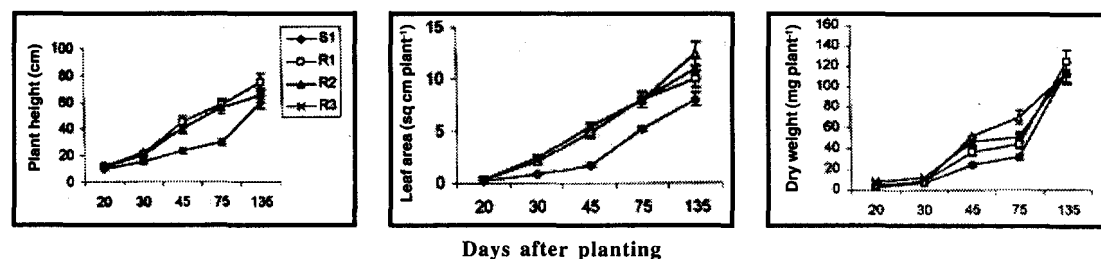


Fig. 1. Plant height, leaf area and total dry weight of three isoproturon resistant (R1, R2 and R3) as compared with susceptible biotype (S1) of *Phalaris minor* around Karnal. Data represent means (n=5)±standard error.

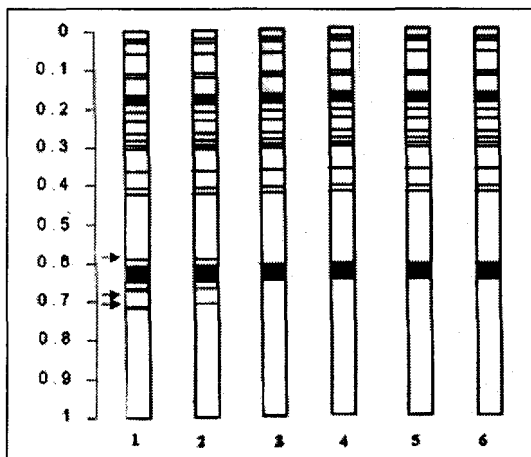


Fig. 2. Protein profiles of three isoproturon susceptible S1 (1), S2 (2) and S3 (3) and three resistant biotypes R1 (4), R2 (5) and R3 (6) of *Phalaris minor*. The arrows indicate bands specific to the S1 and S2 biotypes missing in the resistant ones.

absence of these protein bands could not be rated as an exclusive trait of the resistant biotypes. Further studies on random amplified polymorphic DNA profiles (RAPD) of these biotypes are in

progress to look for markers exclusive to the resistant biotypes.

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