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Inheritance of picloram and 2,4-D resistance in wild mustard (*Brassica kaber*)

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The primary goal of this research was to determine the inheritance of cross-resistance to several groups of auxinic herbicides through classical genetic approaches using auxinic herbicide–resistant (R) and –susceptible (S) wild mustard biotypes obtained from western Canada. F1 progeny were raised from crosses between homozygous auxinic herbicide–R and –S wild mustard parental lines. The F1 and F2 populations were assessed for picloram (pyridine group) and 2,4-D (phenoxyalkanoic group) resistance or susceptibility. Analyses of the F1 as well as the F2 progeny indicate that a single dominant gene confers the resistance to picloram and 2,4-D similar to an earlier report of dicamba-based (benzoic acid group) resistance in this wild mustard biotype. Furthermore, analyses of backcross progeny in this species indicate that resistance to all three auxinic herbicides, i.e., picloram, dicamba, and 2,4-D, is determined by closely linked genetic loci. With this information on inheritance of resistance to several auxinic herbicide families, the R biotype of wild mustard offers an excellent system to isolate and characterize the auxinic herbicide–resistance gene.

Nomenclature: 2,4-D; picloram; wild mustard, *Brassica kaber* (DC) L.C. Wheeler SINAR.

Key words: Auxinic herbicides, herbicide resistance, inheritance, near-isogenic lines.

Herbicide resistance in plants is an inherited ability to survive and reproduce after exposure to a dose of herbicide normally lethal to the wild type. Cross-resistance refers to the expression of a mechanism that endows the ability to withstand herbicides from different chemical classes (having a similar mode of action) conferred by a single gene or, in the case of quantitative inheritance, by two or more genes (Hall et al. 1994). On the other hand, multiple cross-resistance refers to the inheritance of resistance to various classes of herbicides because of the action of multiple genes (Peever and Milgroom 1995). Resistance to herbicides results from repeated selection of resistant individuals in field populations, and the resistant plants slowly increase in frequency in the presence of continued herbicide application (Devine and Shukla 2000). A plant is resistant to herbicides either because of differential absorption, translocation, metabolism, sequestration or because of altered target site.

Prolonged use of herbicides has resulted in the development of resistance to several classes of herbicides including the auxinic herbicides, which have been in use for more than 60 yr. However, the incidence of auxinic herbicide resistance is low compared with other herbicide families such as the imidazolinones, sulfonylureas, and triazines because it has been reported in only 23 weed biotypes (Heap 2003). Furthermore, there is no widespread resistance to auxinic herbicides. The rarity of auxinic herbicide resistance has been suggested to be because of their putative multiple sites of action (Gressel and Segel 1982), but this hypothesis has not been tested.

On the basis of their structural and chemical properties, auxinic herbicides have been classified into several groups, viz., phenoxyalkanoic acids (e.g., 2,4-D, MCPA), benzoic acids (e.g., dicamba, chloramben), pyridines (e.g., picloram, clopyralid), and quinolinecarboxylic acids (e.g., quinclorac, quinmerac). Although there are structural differences among the various groups of auxinic herbicides, all these compounds cause similar physiological responses in sensitive species such as cell elongation, epinasty, hypertrophy, and excessive ethylene biosynthesis (Sterling and Hall 1997).

To elucidate the mechanism of auxinic herbicide resistance in wild mustard, resistant (R) and susceptible (S) biotypes (collected from Gilbert Plains and Minto Manitoba, Canada, respectively) have been extensively characterized at the morphological, physiological, and biochemical level (Debreuil et al. 1996; Peniuk et al. 1993; Webb and Hall 1995). Herbicide dose–response experiments indicate that the R biotype is 104, 18, and 10 times more resistant to picloram, 2,4-D and MCPA, respectively, than the S biotype (Debreuil et al. 1996; Heap and Morrison 1992). Subsequent investigations demonstrated that auxinic herbicide resistance is not due to differential absorption, translocation, or metabolism in this species (Peniuk et al. 1993). However, a possible role of altered target site in auxinic herbicide–induced responses has been suggested in R wild mustard (Webb and Hall 1995). Furthermore, inheritance of dicamba resistance in wild mustard (biotypes from Manitoba, Canada) was shown to be due to a single dominant nuclear gene (Jasieniuk et al. 1995). These data support the hypothesis of altered target site–induced auxinic herbicide resistance in wild mustard because it is believed that a single altered target site may result from single gene mutations (Darmency 1994). Thus, all these investigations (Jasieniuk et al. 1995; Webb and Hall 1995) provide evidence for potential occurrence of a mutation at a putative auxinic herbicide site of action leading to resistance to these compounds in wild mustard.

Although the physiological and biochemical effects of several auxinic herbicides belonging to different structural

groups (e.g., 2,4-D, MCPA, picloram, and dicamba) have been well characterized in wild mustard (Deshpande and Hall 1995; Peniuk et al. 1993; Webb and Hall 1995), except for dicamba (Jasieniuk et al. 1995), the inheritance of resistance to other auxinic herbicides in wild mustard has not been determined. Understanding the inheritance of cross-resistance to several auxinic herbicides in wild mustard will assist in determining the gene or genes controlling the resistance as well as elucidating the mechanism of evolution of resistance in this species. Furthermore, knowledge of the genetics of cross-resistance to herbicides may complement the physiological and biochemical investigations determining mechanisms of herbicide resistance (Jasieniuk et al. 1994).

In this investigation, inheritance of cross-resistance to the auxinic herbicides picloram (pyridine group) and 2,4-D (phenoxyalkanoic group) was determined through classical genetic analyses following conventional breeding methods. In addition, backcross progeny were generated and screened to ascertain whether a single genetic locus confers resistance to several auxinic herbicides (i.e., picloram, dicamba, and 2,4-D).

Materials and Methods

Development of Wild Mustard Parental R and S Lines

Picloram- or 2,4-D–R and –S wild mustard plants were raised from seeds collected from Gilbert Plains and Minto, Manitoba, Canada, respectively (Heap and Morrison 1992). The R biotype was identified in a field that has been repeatedly treated with a combination of MCPA, dicamba, and 2,4-D. The seeds were sown in 1.5-L plastic pots containing Promix[1] and were placed in a growth chamber with a 16-h photoperiod and 22/15 C day/night temperature. The light intensity and the relative humidity were maintained at 350 μmol $s^{-1}m^{-2}$ and 65 to 75%, respectively. Each pot contained one plant, and the plants were irrigated when required and were fertilized weekly with 20:20:20 (N:P:K). To confirm resistance to picloram and 2,4-D, the R plants were treated with either picloram or 2,4-D amine at 100 g ai ha^{-1} at the three- to four-leaf stage of development; all these plants survived the initial herbicide treatment and were used for further experimentation. Also, to ensure homozygosity for resistance or susceptibility of the parental plants to picloram or 2,4-D, several flowers on R and S plants were self-fertilized, and the progeny were tested.

Bud pollination was performed to overcome self-incompatibility. Emasculation (i.e., removal of immature anthers before pollen dehiscence) and pollination were performed at various times of the day. Four to five unopened flower buds (3 to 4 mm in size and ~ 3 d before complete blooming) on 4 to 5 racemes (inflorescences) on each of wild mustard R and S plants were chosen for selfing. The flower buds were emasculated using sharp-edged forceps without damaging the stigma. All the other flower buds on the racemes were removed. The dehisced anthers from other flowers of the same plant were collected, and the pollen was transferred onto the stigmas of the emasculated flower using sterile forceps. Immediately after pollination, the racemes were covered with 20 by 8 cm pollination bags[2] to avoid pollination from other plants. The selfed flowers were labeled appropriately. Silique formation from successful self-pollination could be observed a week after pollination, and subsequently, the pollination bags were removed from the racemes. Four to five weeks after pollination, mature siliques were harvested.

Twenty-five seeds from each selfed R and S plant were raised (as described before) and screened for picloram and 2,4-D resistance or susceptibility (procedure for screening is described later). One and two weeks after picloram and 2,4-D treatment, respectively, the plants were scored for injury to these compounds. S plants showed epinasty (downward curling of stem and leaf petiole), a typical symptom of auxinic herbicides, and eventually died. Conversely, R plants withstood the injury to these herbicides and survived. Segregation of the 25 self-pollinated progeny into both R and S indicated that the parent was heterozygous and no segregation indicated homozygosity. Only homozygous plants were used for genetic crosses (only one selfed population showed segregation, which was discarded).

Genetic Crosses to Generate F1 and F2 Wild Mustard Populations

The F1 plants were generated from reciprocal crosses between homozygous, wild mustard auxinic herbicide–R and –S plants. The plants were raised in a growth chamber (as described previously). Similar procedures (emasculation and pollination) as described for self-pollination were followed for reciprocal crosses. Four to five weeks after pollination, mature siliques were harvested, and the F1 seed from each cross was harvested separately from R and S plants. After screening the F1 progeny, the F2 population was generated by self-pollinating the F1 plants.

Genetic Crosses to Generate Backcross Progeny of Wild Mustard

Backcross progeny were developed by performing crosses between heterozygous, auxinic herbicide–R F1 hybrid plants (hybrid #20 and #5; F1 hybrid # indicates the randomly assigned numbers for the crosses made between R and S parental plants) and homozygous recessive S parent (S11 and S6; #11 and #6 are the randomly assigned numbers for the original S parents) plants. These F1 (#20 and #5) populations were chosen to be same as the progenitors of the F2 families used for screening picloram and 2,4-D resistance. Similarly, the S plants S11 and S6 were chosen because they represent the S (homozygous recessive) parents used for generating the F1 hybrids #20 and #5, respectively. Ten plants in each of these F1 sibling sets as well as the S parental lines were raised and pollinated as described previously. Mature seed was harvested from the S parent (S11 and S6) plants.

Screening Wild Mustard F1s for Picloram or 2,4-D Resistance

Screening for picloram or 2,4-D resistance or susceptibility was performed as follows. Seeds of the F1, as well as the R and S parental population, were raised as described previously. Ten to fifteen seedlings were treated with either picloram or 2,4-D at three- to four-leaf stage of development using a motorized hood sprayer. The sprayer was

equipped with a flat-fan nozzle (8002 E) and calibrated to deliver 200 L ha^{-1} at 276 kPa. The doses of picloram and 2,4-D were 50, 100, and 200 g ai ha^{-1}. One and two weeks after picloram and 2,4-D treatment, respectively, the seedlings were visually rated for injury and classified as R or S by comparing the injury response with those of seedlings from the R and S parental populations. Susceptibility of the plants to either picloram or 2,4-D was assessed on the basis of epinasty symptoms. The frequencies of the R and S phenotypes after treatment with picloram or 2,4-D were tabulated for the F1 population.

Screening F2 Progeny for Picloram or 2,4-D Resistance

The F2 progeny generated by selfing the F1 plants were also screened for picloram or 2,4-D resistance. Two F2 populations (represented by #20 and #5, which resulted from self-pollinations of F1 hybrids #20 and #5) were chosen for screening purposes. Similar experimental and screening procedures, as well as doses of picloram and 2,4-D, were used as described for screening of F1 progeny. Initially, the plants in both F2 families (#20 and #5) were treated with picloram and 2,4-D separately at 50, 100, and 200 g ai/ha. Upon scoring for herbicidal effects (i.e., R or S), the R plants that survived the initial treatment were tested with the second herbicide in a reverse order, i.e., the plants which survived picloram treatment at 50, 100, and 200 g ai/ha were again treated with respective doses of 2,4-D and vice versa.

Screening Backcross Progeny for Picloram, Dicamba, and 2,4-D Resistance

To ascertain whether the loci that are conferring resistance to several auxinic herbicide families are closely linked, 49 plants generated from backcross "S11 × F1 #20", 46 plants generated from backcross "S6 × F1 #5", and 25 parental R and S plants were sprayed sequentially with picloram, dicamba, or 2,4-D at 100 g ai ha^{-1}. Similar experimental and screening procedures were used as described for screening of F1 progeny. Three days after treatment with picloram, the seedlings were scored (on the basis of epinasty symptoms) visually for resistance or susceptibility. Subsequently, 1 wk after picloram treatment, the plants from backcrosses that survived picloram injury (as well as R parentals as control) were sprayed with dicamba at 100 g ai ha^{-1}. Finally, a week after dicamba treatment the plants that survived were treated with 2,4-D at 100 g ai ha^{-1}. The dose of 100 g ai ha^{-1} of each of these herbicides was chosen because their cumulative dose was equivalent to 300 g ai ha^{-1}, and resistance to 2,4-D is only moderate.

Statistical Analyses

Frequencies of R and S phenotypes were tabulated for F1, F2, and backcross populations. Chi-square tests were performed to determine the goodness of fit to specific genetic ratios. In addition, homogeneity chi-square tests (Strickberger 1968) were performed to pool the data across crosses as well as doses.

Results and Discussion

Inheritance of Wild Mustard F1 Progeny for Picloram and 2,4-D Resistance

Selfings as well as reciprocal crosses of the parental R and S lines were successful. Although emasculation and pollinations were performed at various times of the day, the time of pollination did not affect the success of the crosses (data not shown). The F1 seeds from each of these crosses were subsequently germinated to raise plants to test segregation for auxinic herbicide resistance using three doses of picloram or 2,4-D (Tables 1a and 1b). Regardless of the herbicide dose, the F1 plants from several crosses had similar responses to picloram and 2,4-D as those of parental populations. The S plants exhibited epinasty 2 to 3 d after spraying with picloram and eventually died. However, the R plants did not show picloram injury and were able to produce well-developed flowers and subsequently viable seed.

In the case of plants treated with 2,4-D, all plants were initially epinastic 2 d after treatment. Nevertheless, the R plants of both parental populations as well as F1 hybrids recovered 2 wk after treatment and subsequently produced normal flowers as well as viable seeds. These results (Table 1) suggest that resistance to picloram and 2,4-D in wild mustard is a single dominant trait. Conversely, the S plants treated with 2,4-D died.

Chi-Square Test for F2 Progeny

F2 progeny from two self-pollinated F1 hybrid plants (see Tables 2 and 3) segregated 3:1 for R:S after picloram or 2,4-D treatment, as would be expected for a single dominant gene. Chi-square tests for goodness of fit to a 3:1 segregation (R:S) supported our null hypothesis, i.e., the observed frequencies (R or S) after herbicide treatment were in accordance with the expected frequencies for a 3:1 (R:S) segregation ratio. In all cases, segregation of resistance to picloram or 2,4-D did not differ from an expected ratio of 3:1 for R and S phenotypes (Tables 2 and 3). On the basis of the response of F1 hybrids and the segregation ratios observed in the F2 population, resistances to picloram and 2,4-D in wild mustard are conferred, at most, by a single dominant gene for each herbicide.

Furthermore, when plants in both F2 populations (#20 and #5), which survived picloram and 2,4-D treatment at 50, 100, and 200 g ai ha^{-1}, were again treated with the same respective doses of 2,4-D or picloram (in reverse order), they withstood the additional auxinic herbicide treatment (data not shown). These results suggested that the genes controlling resistance to picloram and 2,4-D are either closely linked, or represent the effect of the same gene. Conversely, if the resistances for picloram or 2,4-D were determined by two different genes in wild mustard, we would have observed a further segregation (R:S) of plants among the survivors of the first treatment in the F2 families upon treatment with the second herbicide. On the basis of these observations, backcross progeny were generated to test whether the resistance to these auxinic herbicides (i.e., picloram, dicamba, and 2,4-D) is determined by linked genetic loci (or one gene) in wild mustard.

TABLE 1. Response (R or S phenotype)[a] of F1 hybrids of wild mustard to (a) picloram or (b) 2,4-D.[b]

(a) F1 hybrid cross # (female × male) #	Plant response to picloram at three doses (g ai ha^{-1})					
	50		100		200	
	R	S	R	S	R	S
	No. of plants					
#20: F1 (R5 × S11)	10	0	15	0	10	0
#26: F1 (S11 × R5)	14	0	12	0	15	0
#15: F1 (R2 × S1)	10	0	12	0	10	0
#23: F1 (S1 × R2)	9	0	10	0	11	0
#5: F1 (R13 × S6)	10	0	18	0	9	0
#28: F1 (S6 × R13)	10	0	10	0	11	0
	Plant response to 2,4-D at three doses (g ai ha^{-1})					
(b) F1 hybrid cross # (female × male) #	50		100		200	
	R	S	R	S	R	S
	No. of plants					
#20: F1 (R5 × S11)	11	0	10	0	12	0
#26: F1 (S11 × R5)	10	0	12	0	11	0
#15: F1 (R2 × S1)	10	0	10	0	10	0
#23: F1 (S1 × R2)	10	0	10	0	11	0
#5: F1 (R13 × S6)	11	0	10	0	10	0
#28: F1 (S6 × R13)	10	0	12	0	11	0

[a] Abbreviations: R, resistant; S, susceptible.

[b] Resistance and susceptibility were assessed by comparing the response of F1 seedlings to the responses of seedlings from R and S parental populations after picloram and 2,4-D treatment. The susceptibility of the plants was scored on the basis of epinasty (downward curling of leaf petiole) symptoms. # F1 hybrid cross # indicates the randomly assigned numbers for the crosses made between R and S parental plants. # R5, R2, R13, denote R parents, and S11, S1, S6 denote S parents.

Phenotypic Segregation of Backcross Progeny for Sequential Applications of Picloram, Dicamba, and 2,4-D

The progeny from two backcrosses (BC-1 and BC-2, which resulted from cross-pollination of S plants by F1 hybrids, i.e.,"S11 × F1 #20" as well as "S6 × F1 #5") had similar responses after sequential applications (100 g ai ha^{-1} each) of picloram, dicamba, and 2,4-D (Table 4). When the seedlings were initially scored for picloram injury 3 d after treatment, the S plants of parental population and effectively half of the backcross progeny exhibited epinasty and eventually died. Conversely, the R plants of parental population as well as the remaining backcross progeny showed no picloram injury. Furthermore, these picloram-R plants also exhibited resistance to dicamba as well as 2,4-D. Although the

TABLE 2. Segregation of picloram-resistant (R) and -susceptible (S) phenotypes among F2 populations.[a]

F2 population[b] (female × male)[c]	Picloram dose	Segregation of plants		χ^2 [d]	Probability
		R	S		
	g ai ha^{-1}	No. of plants			
F1 # 20	50	25	7	0.0338	0.8541
(R5 × S11)	100	23	5	0.4284	0.5127
	200	21	10	0.526	0.4682
Test of homogeneity across doses[e]				0.9846	0.6112
Pooled chi-square		69	22	0.0036	0.9521
F1 # 5	50	16	8	0.500	0.4795
(R13 × S6)	100	21	6	0.086	0.7693
	200	18	10	1.190	0.2753
Test of homogeneity across doses[e]				0.8270	0.6613
Pooled chi-square		55	24	0.949	0.3299
Test of homogeneity across crosses[e]				2.482	0.7791
Pooled chi-square		124	46	0.282	0.5953

[a] Resistance and susceptibility were assessed by comparing the response of F2 seedlings to the responses of seedlings from R and S parental populations following picloram treatment with 50, 100, or 200 g ai ha^{-1}.

[b] F2 populations resulted from self-pollinations of F1 hybrids from indicated cross numbers given in Table 1.

[c] R5 and R13 denote the original R parents, whereas S11 and S6 denote original S parents.

[d] Chi-square values are the result of tests of goodness of fit to a 3:1 (R:S) segregation model. The null hypothesis, i.e., the observed frequencies are in accordance with expected frequencies, is accepted in all cases.

[e] Homogeneity chi-square tests (Strickberger, 1968) were performed before pooling the data across doses as well as crosses, and then the pooled chi-square tests were performed.

TABLE 3. Segregation of 2,4-D-resistant (R) and -susceptible (S) phenotypes among F2 populations.[a]

F2 population[b] (female × male)[c]	Picloram dose	Segregation of plants		$\chi^{2(4)}$ [d]	Probability
		R	S		
	g ai ha^{-1}	No. of plants			
F1 # 20	50	23	10	0.252	0.6156
(R5 × S11)	100	24	7	0.010	0.9203
	200	18	9	0.603	0.4374
Test of homogeneity across doses[e]				0.423	0.8093
Pooled chi-square		65	26	0.4424	0.5059
F1 # 5	50	20	5	0.120	0.7290
(R13 × S6)	100	16	10	1.845	0.1743
	200	15	8	0.709	0.3995
Test of homogeneity across doses[e]				1.522	0.4671
Pooled chi-square		51	23	1.1523	0.2829
Test of homogeneity across crosses[e]				1.837	0.8712
Pooled chi-square		116	49	1.698	0.1925

[a] Resistance and susceptibility were assessed by comparing the response of F2 seedlings to the responses of seedlings from R and S parental populations after 2,4-D treatment with 50, 100, or 200 g ai ha^{-1}.
[b] F2 populations resulted from self-pollinations of F1 hybrids from indicated cross numbers given in Table 1.
[c] R5 and R13 denote the original R parents, whereas S11 and S6 denote original S parents.
[d] Chi-square values are the result of tests of goodness of fit to a 3:1 (R:S) segregation model. The null hypothesis, i.e., the observed frequencies are in accordance with expected frequencies, is accepted in all cases.
[e] Homogeneity chi-square tests (Strickberger, 1968) were performed before pooling the data across doses as well as crosses, and then the pooled chi-square tests were performed.

R plants (from backcrosses as well as parental) tolerated the effects of all three herbicides, the seed set was poor in these plants, which is understandable considering that the cumulative dose applied was equivalent to 300 g ai ha^{-1}.

Chi-Square Test for Backcross Progeny

Chi-square tests were performed to determine the goodness of fit to a 1:1 segregation (R:S) among the backcross

TABLE 4. Segregation of resistant (R) and susceptible (S) plants among backcross progeny after sequential treatment with 100 g ai ha^{-1} each of picloram, dicamba, and 2,4-D.[a]

Backcross[b] (female × male)[c]	Picloram, dicamba, and 2,4-D dose	Segregation of plants		χ^2 [d]	Probability[e]
		R	S		
	g ai ha^{-1}	No. of plants			
BC-1	100 (picloram)	26	23	0.0816[e]	0.775[f]
(S11 × F1 # 20)	100 (dicamba)	26	0	26	0[g]
	100 (2,4-D)	26	0	26	0[g]
BC-2	100 (picloram)	21	25	0.1956	0.6582[f]
(S6 × F1 # 5)	100 (dicamba)	21	0	21	0[g]
	100 (2,4-D)	21	0	21	0[g]
Test of homogeneity across backcrosses				0.277	0.8205[f]
	Pooled (picloram)	47	48	0.00	1.00[f]
	Pooled (dicamba)	47	0	47	0[g]
	Pooled (2,4-D)	47	0	47	0[g]
Parents					
R	100	25	0	[h]	
S	100		25	[h]	

[a] Resistance and susceptibility were assessed by comparing the response of progeny from backcrosses to the responses of seedlings from R and S parental populations after sequential treatment with picloram, dicamba, and 2,4-D treatment (100 g ai ha^{-1} each, respectively).
[b] Backcross populations resulted from cross-pollination of S plants with F1 hybrids. F1 hybrids were heterozygous siblings from individual cross numbers given in Table 1.
[c] S11 and S6 denote S plants used as female parents; #20 and #5 denote the F1 hybrid population from which a heterozygous sibling was chosen as male parents.
[d] Chi-square values are the results of tests for goodness of fit to a 1:1 (R:S) segregation model.
[e] Probability of accepting or rejecting the null hypothesis.
[f] Accept the null hypothesis that backcross progeny segregate 1:1 (R:S).
[g] Reject the null hypothesis that picloram resistance, as well as dicamba and 2,4-D resistance are not linked (i.e., backcross progeny resistant to picloram segregate 1:1 [R:S] for dicamba or 2,4-D resistance).
[h] Not subjected to χ^2 because of zero expected value for one class.

progeny upon treatment with picloram. The null hypothesis was stated as "the observed ratios were in accordance with the expected ratios for a 1:1 segregation" (R:S; Table 4). When the two backcross progeny were treated with picloram (100 g ai ha^{-1}), initially the plants segregated 1:1 (R:S); therefore, the null hypothesis in this case was accepted (Table 4). However, the plants, which survived picloram effect upon treatment with dicamba and 2,4-D (100 g ai ha^{-1} each), did not show further segregation. Chi-square tests were performed on these results as well. The null hypothesis was stated as "picloram resistance, as well as dicamba and 2,4-D resistances, are not linked (i.e., backcross progeny resistant to picloram should segregate 1:1 [R:S] for dicamba and 2,4-D resistance) in wild mustard." Because there was no further segregation in plants treated with dicamba followed by 2,4-D, the null hypothesis was rejected in these two cases (Table 4). These results suggested that resistances to picloram, dicamba as well as 2,4-D in wild mustard are closely linked or possibly represent the effect of one locus.

The incidence of auxinic herbicide resistance is relatively low, and the reason for this phenomenon has been attributed to the proposed multiple mechanisms of action of these herbicides (Gressel and Segel 1982; Morrison and Devine 1994). Among the 23 auxinic herbicide–R weed biotypes discovered to date, inheritance of resistance has been determined in only a very few species, such as dicamba resistance in wild mustard (Jasieniuk et al. 1995), quinclorac resistance in false clever (Van Eerd et al. 2003), as well as picloram and clopyralid resistance in yellow starthistle (Sabba et al. 2003). This study provides the first report of inheritance of cross-resistance to several groups of auxinic herbicides in a single weed species. Furthermore, genetic analyses of progeny from two backcrosses in wild mustard demonstrate that the genetic loci that are conferring resistance to several groups of auxinic herbicides, which belong to different chemical families, are closely linked; these results further suggest that a single gene may be responsible for these effects.

In the majority of herbicide-R weed biotypes, a single dominant gene determines resistance (Shaaltiel et al. 1988; Yamasue et al. 1992). However, in certain weed species, single recessive gene inheritance of herbicide resistance has also been reported (Jasieniuk et al. 1994; Sabba et al. 2003, Van Eerd et al. 2003). Furthermore, in *Arabidopsis*, a member of the Brassicaceae family, several 2,4-D–R mutants were isolated, and genetic analyses of these indicate that resistance is controlled by a single recessive gene (Estelle and Sommerville 1987). In any population, a recessive resistance trait will establish in only the homozygous recessive condition, which would arise in highly self-pollinating weed species such as false cleavers and *Arabidopsis*. On the other hand, in weed species that are primarily out-breeders (e.g., wild mustard), a dominant resistance trait will spread rapidly if resistance alleles are present in the population (Jasieniuk et al. 1995). Although auxinic herbicide resistance in weed species (investigated so far, including the current research) is a single gene trait, the low frequency of R biotype occurrence may be due to mutations that are lethal at this locus. The pattern of inheritance of picloram and 2,4-D resistance observed in this study, as well as previously reported dicamba resistance (Jasieniuk et al. 1995), suggests that alteration of a single locus is sufficient to cause resistance to several auxinic herbicides in wild mustard.

Genetic mutations associated with responses to several physiological stresses, including herbicide resistance, are generally associated with a fitness cost. Differences between auxinic herbicide–R and –S wild mustard biotypes regarding factors influencing fitness such as seed return, physiological, and morphological parameters have been studied previously. In the absence of herbicide treatment, the R biotype accumulated shoot biomass more slowly than the S biotype, and also, the seed return of the R biotype was slightly lower compared with the S biotype in a 2 yr experiment (Debreuil et al. 1996). Furthermore, Hall and Romano (1995) reported several morphological differences between the two biotypes. For example, the R biotype had a smaller root system with more branches and higher leaf chlorophyll content than the S biotype. In addition, the percentage of seed germination at 24, 30, and 35 C was higher in the R than in the S biotype (Hall and Romano 1995).

Earlier physiological studies in wild mustard suggest that dicamba, picloram, and 2,4-D resistance is not due to herbicide absorption, translocation, or metabolism (Peniuk et al. 1993). Subsequently, the auxin-binding protein (ABP) was proposed as a potential target site of auxinic herbicides whereby an altered ABP would result in auxinic herbicide resistance (Webb and Hall 1995). Furthermore, studies on the role of ethylene biosynthesis, calcium, and H^+ efflux also indicate that auxinic herbicide resistance may be due to an altered target site, possibly ABP in wild mustard (Deshpande and Hall 1995; Hall et al. 1993; Wang et al. 2001). Thus, the single nuclear gene inheritance of dicamba (Jasieniuk et al. 1995) as well as picloram and 2,4-D resistance in wild mustard may support the hypothesis of an altered target site in auxinic herbicide resistance.

In conclusion, knowledge about the inheritance of herbicide resistance will help in the development of viable management strategies, prudent use of herbicides as well as preventing the spread of resistance. Furthermore, because resistance is in all likelihood based on a single gene in wild mustard, this study offers several avenues for future investigations toward understanding the precise mechanism of auxinic herbicide resistance in this weed species. Molecular analyses of the R biotype will ultimately demonstrate whether it is the same gene that is conferring resistance to auxinic herbicide family. Thus, isolation and characterization of the auxinic herbicide–resistance gene in wild mustard has potential for elucidating both the genetic and molecular basis of resistance as well as mode of action of these herbicides.

To isolate the genes determining the qualitative traits (controlled by a single or a few major genes), such as the auxinic herbicide resistance in wild mustard, it is important to develop near-isogenic lines for the trait of interest. Near-isogenic lines are two identical lines, differing only for the trait of interest. These lines are also excellent tools for molecular characterization of the genes. Therefore, experiments are in progress in our laboratory to develop these lines for auxinic herbicide resistance in wild mustard through a repeated backcross procedure. Furthermore, functional and genomic analyses of the auxinic herbicide–resistant gene have potential in development of auxinic herbicide–resistant crops in agriculture through gene transfer techniques.

Sources of Materials

[1] Plant Products, 314 Orenda Road, Brampton, ON L6T 1G1, Canada.

[2] Lawson no. 217, Lawson Bags, 480 Central Avenue, Northfield, IL 60093.

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