

Introgression of phenoxy herbicide resistance from *Raphanus raphanistrum* into *Raphanus sativus*

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Abstract

Phenoxy herbicides such as 2,4-dichlorophenoxy acetic acid (2,4-D) and 4-chloro-2-methylphenoxy acetic acid (MCPA) are selective herbicides used extensively in agriculture for weed control. Wild radish (*Raphanus raphanistrum*) is a problem weed across the globe and heavily infests crop fields in Australia. Phenoxy herbicides are used to selectively control dicot weeds, including wild radish. As a result of selection, phenoxy-resistant wild radish populations evolved in Western Australia. In this research, introgression of phenoxy resistance from wild radish to cultivated radish (*Raphanus sativus*) was investigated following classical breeding procedures. F₁ progeny were generated by crossing MCPA-resistant *R. raphanistrum* and MCPA-susceptible *R. sativus*. F₁ hybrids were screened for MCPA resistance. The MCPA-resistant F₁ hybrids were used to produce three generations of backcross progeny. Genetic analyses of F₁ and backcross progeny demonstrated introgression of the MCPA-resistant trait from wild radish to cultivated radish. Implications of phenoxy resistance introgression into cultivated radish include potential development of herbicide-tolerant radish cultivars or other members of the Brassicaceae family.

Key words: backcross — introgression — phenoxy resistance — wild radish

The family Brassicaceae comprises several agronomically important crop species, including oilseed rape (*Brassica napus*), Indian mustard (*Brassica juncea*), cabbage (*Brassica oleracea*) and radish (*Raphanus sativus*) (Mithila and Hall 2013). Cultivated radish is a minor crop worldwide and is mainly grown as a vegetable crop; however, in the midwestern United States, oilseed radish is gaining importance as a cover crop and is widely grown in between major crops. Wild radish (*Raphanus raphanistrum*) is a problem weed in agriculture in Eurasia, North America and Australia (Cheam and Code 1995, Holm et al. 1997), and it is one of the 100 most agriculturally important weeds that can cause significant economic loss to growers (Holm et al. 1997). This weed is remarkably successful due to its flexible life cycle, prolific seed production and prolonged seed dormancy (Cheam and Code 1995). In particular, considerable genetic diversity within this weed species (Reeves et al. 1981, Cheam and Code 1995, Kercher and Conner 1996) has enabled wild radish to be successful across several agro-ecosystems.

Brassica crops are among the oldest cultivated plants, and their importance dates back to 1500 BC. This family includes an excellent reservoir of genes for many economically important traits and is receptive to gene transfer techniques (Mithila and Hall 2013). The wild species of Brassicaceae are sources of many agronomically important traits, such as resistance to pests and diseases and tolerance to abiotic stresses. Gene transfer among members of the family

Brassicaceae has been investigated (Prakash and Hinata 1980, Bing et al. 1995, Hu et al. 2002, Snow et al. 2010); for example, resistance to blackleg infection, caused by *Leptosphaeria maculans*, was transferred from *B. kaber* to *B. napus* (Snowdon et al. 2000). Powdery mildew resistance was transferred from *B. oleracea* to *B. carinata* (Tonguc and Griffiths 2004). Furthermore, hybrids between *R. raphanistrum* and *R. sativus* have been successfully produced (Panetsos and Baker 1967, Campbell et al. 2009, Snow et al. 2010), and interspecific hybrids between these two species are vigorous and fertile (Panetsos and Baker 1967). Introgression of traits between wild radish and cultivated radish was investigated extensively (Campbell et al. 2006), and it was reported that the hybridization between these two species may generate new progeny which can possess traits that may help perform well under different environmental conditions. Furthermore, the relationship between wild and cultivated radishes, their breeding behaviour and genomics has been comprehensively reviewed by Kaneko et al. (2011).

Auxinic herbicides have been widely used in agriculture for more than seven decades to selectively control broadleaf weeds. These herbicides are relatively inexpensive and are used extensively in cereal crops and as pre-emergence applications in broadleaf crops. The demand for auxinic herbicides in agriculture has remained consistent, primarily because of their selectivity, efficacy, wide spectrum of weed control and low application costs. Prolonged use of auxinic herbicides resulted in evolution of resistance to these herbicides in 30 weed species (Heap 2013). In general, auxinic herbicide resistance is controlled by a single gene, and in few cases, by two major genes. For example, dicamba, 2,4-D and picloram resistance in *Sinapis arvensis* are determined by a single dominant gene (Jasieniuk et al. 1995, Jugulam et al. 2005), and dicamba resistance in *Kochia scoparia* (biotypes from Henry, Nebraska) is determined by a single allele with a high degree of dominance (Preston et al. 2009). Conversely, a single recessive gene controls clopyralid and picloram resistance in *Centaurea solstitialis* (Sabba et al. 2003) and quinclorac resistance in *Galium spurium* (Van Eerd et al. 2004). However, two additive genes control MCPA resistance in *Galeopsis tetrahit* (Weinberg et al. 2006).

Continuous exposure to phenoxy herbicides (2,4-D and MCPA) in rotation over 17 years in Western Australia resulted in evolution of phenoxy-resistant populations of wild radish (Walsh et al. 2004). Phenoxy-resistant wild radish populations have been well characterized (Walsh et al. 2004). Classical genetic studies have demonstrated that MCPA resistance in a Western Australian wild radish population was determined by a single incompletely dominant gene (Mithila et al. 2013). The

single gene inheritance facilitates transfer of genes among related species. In this research, we investigated introgression of MCPA resistance from wild radish to *R. sativus* using conventional breeding procedures. Introgression of phenoxy resistance from wild species to cultivated species will have several implications in agriculture, such as availability of herbicide-tolerant cultivars, which will facilitate effective weed control, encourages less tillage and also provides herbicide rotation options to growers.

Materials and Methods

Production of hybrids between *R. raphanistrum* and *R. sativus*:

Phenoxy-resistant *R. raphanistrum* seeds were collected from a field where it was exposed to frequent auxinic herbicide applications over at least a 17-year period (Walsh *et al.* 2004). Phenoxy-resistant *R. raphanistrum* and phenoxy-susceptible *R. sativus* plants were raised from seed. The seeds were sown in 10-cm plastic pots (1 seed per pot) containing Promix and were placed in a growth chamber with a 16-h photoperiod and 22/15°C day/night temperature. The light intensity and relative humidity were maintained at 350 μ moles/s/m and 65–75%, respectively. Plants were irrigated when required and were fertilized weekly with 20 : 20 : 20 (N:P:K). When plants were flowering, crosses were performed between phenoxy-resistant *R. raphanistrum* and phenoxy-susceptible *R. sativus* following the procedure described by Jugulam *et al.* (2005). Silique formation from successful crosses 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, and F₁ hybrid seed was collected.

Assessment of transfer of MCPA resistance in hybrids: Whole-plant screening was performed to determine the transfer of phenoxy resistance from *R. raphanistrum* into F₁ hybrids. Phenoxy-resistant *R. raphanistrum*, phenoxy-susceptible *R. sativus* as well as F₁ hybrid seedlings were grown in a growth chamber (as described earlier) to test their response to MCPA. The recommended field use dose of MCPA is 500 g ae/ha. We performed dose–response experiments as follows to determine the response of hybrids to MCPA. Ten to fifteen seedlings of parents and hybrid plants were treated with a range of MCPA doses: 0, 50, 100, 250, 500 and 750 g ae/ha at the three- to four-leaf stage of development with a motorized hood sprayer. The sprayer was equipped with a flat-fan nozzle (8002 E) and calibrated to deliver 200 L/ha at 276 kPa. Two and three weeks after MCPA treatment, the seedlings were visually rated for injury and classified as resistant or susceptible by comparing the injury response with those of MCPA-resistant *R. raphanistrum* and MCPA-susceptible *R. sativus* parents. Susceptibility of plants to MCPA was assessed based on epinasty (downward curling of plant parts) symptoms and plant death.

Repeated backcrosses to introgress MCPA resistance from F₁ hybrid into *R. sativus*:

The first generation of backcross progeny (BC₁F₁) was produced by performing crosses between MCPA-resistant F₁ hybrids and *R. sativus*. Cross-pollinations were performed (as described earlier) between *R. sativus* and F₁ hybrids to obtain BC₁F₁ seed. *R. sativus* was used as seed parent, whereas the F₁ hybrids were chosen as the pollen parent. Mature seeds (BC₁F₁) were harvested from *R. sativus*. BC₁F₁ seeds were raised in a growth chamber (as described previously), and seedlings at the 3- to 4-leaf stage of their development were screened with MCPA (250 g ae/ha) and scored for MCPA resistance or susceptibility. The survivors of MCPA were used as the pollen parent and backcrossed to *R. sativus* (the recurrent parent) to obtain BC₂F₁ progeny. Three backcrosses were performed using this procedure, and in every generation, the progeny were screened with MCPA (250 g ae/ha).

Results

Evaluation of MCPA Resistance in F₁ Hybrid and Backcross Progeny

Cross-pollinations between *R. sativus* and MCPA-resistant *R. raphanistrum* were successful, and hybrid seed was obtained

Table 1: Response (resistant [R] or susceptible [S] phenotype) of F₁ hybrids of *Raphanus sativus* x *R. raphanistrum* 3 weeks after MCPA treatment*

MCPA dose (g ae/ha)	F ₁ hybrid plants (<i>R. sativus</i> x <i>R. raphanistrum</i>) Response to MCPA	
	R	S
0	–	–
50	10	0
100	13	0
250	15	0
500	12	0
750	10	0

*Resistance and susceptibility were assessed by comparing the response of F₁ seedlings to response of seedlings from R and S parental populations following MCPA treatment.

from *R. sativus*. All F₁ plants survived MCPA application at all doses tested (Table 1). After treatment with MCPA, *R. sativus* plants showed epinasty (downward curling of leaf petiole) and eventually died (Fig. 1a), but F₁ hybrids exhibited little or no epinasty (Fig. 1b). The response of F₁ hybrids to herbicide application was similar to MCPA-resistant *R. raphanistrum* plants (Fig. 1b and c). The progeny from backcrosses (BC₁F₁ to BC₃F₁) showed similar responses following MCPA treatment; that is, upon scoring for MCPA injury 3 weeks after treatment (WAT), MCPA-susceptible *R. sativus* and effectively half of the backcross progeny exhibited epinasty and eventually died. Conversely, the MCPA-resistant *R. raphanistrum* and the remaining backcross progeny showed no MCPA injury. Chi-square tests performed to determine the goodness of fit to a 1 : 1 segregation (resistant:susceptible) among the backcross progeny (3WAT with MCPA) is presented in Table 2. The 1 : 1 segregation of resistant:susceptible in backcross progeny (BC₁F₁ to BC₃F₁) suggests that the gene controlling MCPA resistance was likely to have introgressed into *R. sativus*.

Discussion

Interspecific crosses for transferring agronomically important traits are routinely performed among agricultural crops. The family Brassicaceae consists of diploids as well as allotetraploids. Natural crossing among the Brassica species was reported to occur with varying degrees of difficulty (Myers 2006). In this research, we report successful production of hybrids and transfer of phenoxy resistance from *R. raphanistrum* to *R. sativus* for the first time. Based on backcross segregation data (Table, 2), the MCPA resistance from wild radish likely introgressed into cultivated radish. Introgression of traits between these two species was reported earlier by several groups (Panetsos and Baker 1967, Snow *et al.* 2010); however, these studies reported transfer of transgenes from cultivated radish to wild radish. Based on cytogenetic analyses of Brassica members by Mizushima (1980), the *R. sativus* genome can form up to three bivalents with *B. rapa*, which possesses the AA genome, and hence, there is a possibility that MCPA resistance can also be transferred to economically important Brassica crops possessing AA genome via interspecific crosses. Although availability of herbicide-tolerant cultivars has several advantages, *viz.* facilitates effective weed control and encourages less tillage, there is a possibility of excessive use of herbicides with the same mode of action leading to herbicide selection. Therefore, it is extremely



Fig. 1: Plant response to MCPA (250 g ae/ha) 3 weeks after treatment. (a), (b) and (c) represent *R. sativus*, F₁ hybrid and *R. raphanistrum*, respectively

important to educate growers about diversifying weed management strategies to minimize evolution of herbicide-resistant weed populations.

Table 2: Segregation into resistant (R) and susceptible (S) phenotypes in backcross progeny 3 weeks after treatment with 250 g ae/ha of MCPA. Chi-square values are the results of tests for goodness of fit to a 1 : 1 (R:S) segregation model*

Backcross	Backcross progeny response to MCPA (250 g ae/ha) Segregation of plants		χ^2 ⁽¹⁾	P ⁽²⁾
	R	S		
BC ₁ F ₁	16	13	0.31	0.58 ³
BC ₂ F ₁	18	15	2.72	0.01 ³
BC ₃ F ₁	17	20	0.11	0.74 ³

*Resistance and susceptibility were assessed by comparing the response of backcross seedlings to response of seedlings from R and S parental populations following MCPA treatment. The null hypothesis was stated as 'the observed ratios were in accordance with the expected ratios for a 1 : 1 segregation' (resistant:susceptible).

¹Chi-square values are the results of tests for goodness of fit to a 1 : 1 (R:S) segregation model.

²Probability of accepting or rejecting the null hypothesis.

³Accept the null hypothesis that backcross progeny segregated 1 : 1 (R:S).

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