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IMI resistance associated to crop-weed hybridization in a natural *Brassica rapa* population: characterization and fate

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Abstract Wild turnip (*Brassica rapa*) is a common weed and a close relative to oilseed rape (*Brassica napus*). The Clearfield® production system is a highly adopted tool which provides an alternative solution for weed management, but its efficiency is threatened by gene transfer from crop to weed relatives. Crop-weed hybrids with herbicide resistance were found in the progeny of a *B. rapa* population gathered from a weedy stand on the borders of an oilseed rape (*B. napus*) imidazolinone (IMI)-resistant crop. Interspecific hybrids were confirmed by morphological traits in the greenhouse and experimental field, survival after imazethapyr applications, DNA content through flow cytometry, and pollen viability. The transference of herbicide resistance was demonstrated even in a particular situation of pollen competition between both an herbicide-resistant crop and a non-resistant crop. However, IMI resistance was not found in further generations collected at the same location. These results verify gene transmission from oilseed rape to *B. rapa* in the main crop area in Argentina where resistant and susceptible varieties are found and seed loss and crop volunteers are common. Hybridization, introgression, and herbicide selection would be associated with the loss of effectiveness of IMI technology.

Keywords Off-type plants · Morphology · Flow cytometry · Volunteers · Herbicide resistant

Introduction

Crop-weed hybridization is one of the main evolutionary processes that challenge modern agricultural technologies, due to its unpredictable consequences on weed population dynamics. Modern genetics and biotechnology have developed tools to create new crop varieties with herbicide resistance, as in oilseed rape (*Brassica napus* L.). Oilseed rape crop breeding took place mainly in the USA and Canada, producing transgenic herbicide resistance to glyphosate, ammonium glufosinate and bromoxinyl, and conventional (non-transgenic) cultivars with genetic resistance to imidazolinone herbicides (Beckie et al. 2006).

The genus *Brassica* belongs to the Brassicaceae family, a particular genetic complex integrated by different vegetable crops and weed species (U N, 1935). *Brassica rapa* L. comprises several domesticated biotypes (Polish oilseed rape) used as vegetables or as an oil source and also wild turnip, a cosmopolitan weed biotype. The wild turnip is a persistent weed in different crops in several regions of the word, including Argentina (Simard et al. 2006, Gulden et al. 2008; IBODA, 2014).

At present, only the imidazolinone herbicide-resistant oilseed rape (*B. napus*) is available in

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Argentina (INASE, 2016). Transgenic varieties are not allowed because of the environmental risk of gene flow from crops to noxious weeds, like *B. rapa*. However, the environmental risk of gene flow is similar in genetically modified (GM) and non-GM crops (Ellstrand et al. 2013), since acquisition of herbicide resistance would carry similar consequences to the environment.

To verify introgressive hybridization, wild/weedy relatives and oilseed rape should co-occur, have overlapping flowering, be sexually compatible, and produce viable and fertile progeny, and the crop genes should persist in natural/weedy populations (Ellstrand 2003, Devos et al. 2009). Even if there were a likely hybridization between *B. napus* and a related species growing in the vicinity, high hybrid sterility and poor vigor would generally mean that hybrids and their progeny will not survive in either agricultural or natural habitats. However, some seedlings could survive and develop fertile hybrids (Fitzjohn et al. 2007).

Imidazolinone (IMI) herbicides control weeds by inhibiting the acetohydroxyacid synthase enzyme (AHAS, also called acetolactate synthase, ALS) which is a critical enzyme for the biosynthesis of branched-chain amino acids in plants. These herbicides control a wide spectrum of grass and broadleaf weeds, are effective at low application rates, have low mammalian toxicity, and possess a favorable environmental profile. However, this enzyme has a high mutation rate and, at present, this is the group of herbicides which bear the highest number of resistant species in the world (Heap, 2016). Five AHAS enzyme loci have been reported in oilseed rape; AHAS2, AHAS3, and AHAS4 belong to the A genome, whereas AHAS1 and AHAS5 belong to the C genome. AHAS1 and AHAS3 are the only genes that are constitutively expressed and encode the primary AHAS activities essential for growth and development in *B. napus* (Tan et al. 2005). The highest level of IMI tolerance is obtained when both mutations are stacked and homozygous (Krato and Petersen 2012; Tan et al. 2005).

Oilseed rape is not widely sown in Argentina. In the last decade, the planted area and production was around 36,000 ha and 59,000 t, respectively. This production level is negligible compared with the main production countries: China (11.6 Mt), Canada (15.5 Mt), India (7.9 Mt), Germany (6.2 Mt), France (5.5 Mt), UK (2.5 Mt), and Australia (3.8 Mt). The main oilseed crops in Argentina are soybean (17.7 Mha, 41.7 Mt) and sunflower (1.8 Mha, 3.2 Mt) (www.agroindustria.gob.ar).

The likely transference of herbicide resistance from an IMI-resistant oilseed rape variety to weedy relatives was investigated in cooperation with a private company. The aim of this study was to verify crop-weed gene flow by detecting hybrids with herbicide resistance in the progeny of a *B. rapa* population gathered from a weedy stand on the borders of an IMI-resistant oilseed rape. If the progeny of this weedy population produced morphologically intermediate individuals, with intermediate DNA content between the putative parents and displaying herbicide resistance, the hypothesis of crop-weed gene flow could not be rejected and would indicate environmental risk. We used three approaches for the detection of hybrid plants in the laboratory, greenhouse, and experimental field studies. The F1 fertility and the persistence of IMI resistance in the following generations after hybridization were also evaluated.

Materials and methods

Plant materials

Seeds for experiments were collected in a natural *B. rapa* population (BAL) in 2008 (G1), growing near (<10 m) two oilseed rape crop fields in Balcarce county (37° 34′ 41″ S, 58° 32′ 37″ W). One of the fields was sown with an IMI-resistant crop variety and the other with a non-resistant oilseed rape variety. The weedy population occupied an extended patch along the fences and shared the flowering time with the crop. Before 2008, the crop rotation was wheat-soybean, with only one sunflower crop within the ten previous years. The herbicide use register included three sulfonylurea (SU) but no IMI application. The field was under a no-till system since 2001; glyphosate and 2.4-D were applied during fallows. Wheat crop weeds were controlled with dicamba and metsulfuron and glyphosate was applied to RR soybean. There were no repeated applications of SU herbicides in two consecutive years. An IMI herbicide was applied once to canola in 2008. There were no records of oilseed rape crops in the field prior to 2008 and at least since 1996.

Morphological traits from flowering to maturity were used to address the taxonomic status of *B. rapa*. The progeny of a natural population of *B. rapa* (BR) plants from Benito Juarez county (37° 37′ 00″ S, 59° 38′ 27″

W) far from the cultivated area and an oilseed rape *B. napus* IMI-resistant cultivar (BN) were used as controls. Seeds from the BAL population and each control group were grown in trays in the greenhouse up to the four-leaf stage.

Morphology

Morphological traits were measured in the greenhouse and in a common garden experiment. The following traits were recorded on every plant for both experiments: hypocotyl color, seedling leaf color, cotyledon retention, plant height, branching, leaf number, stem pubescence, leaf length, leaf width, leaf insertion angle, leaf shape, leaf margin, leaf division (incision), leaf color (flowering), leaf hairiness, leaf apex shape, leaf base shape, petal color, and floral apex branching pattern, according to IBPGR (http://www.bioversityinternational.org/publications).

Screening and persistence of resistance

Imazethapyr (Pivot, 100 g ai ha^{-1}, SC, BASF Co.) was applied to the BALG1 population at the two- to four-leaf stage with a constant-pressure sprayer with an 8001 flat spray tip calibrated to deliver a commercial dose and 183 l ha^{-1} water volume and a spraying pressure of 345 kPa. Surviving plants were sprayed after 2 weeks with a 2× dose of the same herbicide (200 g ha^{-1}). Resistant individuals were transplanted to pots for morphological characterization. On the other hand, seeds collected in the same *B. rapa* population (BAL) in 2009 (G2), 2011 (G4), 2013 (G6), and 2014 (G7) were sown and treated with imazethapyr, as mentioned before, in order to study the evolution of the frequency of resistant plants. During those further generations, no oilseed rape crops were sown in the vicinity of the BAL population.

Pollen viability

All observed off-type (OT) plants were considered as crop-wild hybrids and evaluated for pollen viability. Five normal plants were chosen randomly within the BALG1 population, and five plants of two natural populations of *B. rapa* (BR) without any contact with rapeseed crops and five plants of *B. napus* cv. Nexera 1700 (BN) were used as controls. Pollen viability was assessed by differential staining (Alexander 1980). Three flowers from each plant were shaken over a clean slide to collect fresh pollen, and then, a drop of stain was added. At least 300 pollen grains were counted on each slide.

Flow cytometry

Flow cytometry was used to determine the DNA content and so estimate the ploidy level in 16 IMI-resistant individuals and the control plants. The fluorescence intensity of DAPI-stained nuclei was analyzed with a Partec PA II (Partec, Münster, Germany) flow cytometer. The DNA content of each individual was determined using samples of fresh leaf tissue following the recommendations of the Partec kit CySatin UV Precise P 05-5002 manual. Briefly, 0.5 cm^2 leaf material was placed in a small Petri dish with a similar amount of tissue from the controls. After adding 0.5 ml extraction buffer, the tissue was chopped with a sharp razor blade. Following a 2-min incubation, the samples were filtered through a 50-lm nylon mesh directly into the sample tube, to which 1.5 ml 4′,6-diamidino-2-phenylindole (DAPI) stain solution (Partec P kit CySatin UV) was added. The mixture was incubated for 2 min at room temperature and analyzed. Ploidy levels were estimated in relation to the DNA peaks in the samples and the internal standard (*B. rapa* from the Benito Juarez population *and B. napus* cv. Gospel). The plants were measured once, but in case of doubt, measurements were repeated two or more times.

Statistical analysis

Metric traits were analyzed by principal component analysis (PCA) and discriminant analysis. ANOVA and Fisher's LSD test were used for comparison between groups. Principal coordinate analysis was performed with categorical data with InfoStat (2011). A hybrid index was calculated based on the numerical scores of the categorical traits: hypocotyl color (1–6), seedling leaf color (1–7), seedling rib-leaf color (1–4), seedling leaf shape (1–7), seedling leaf margin (1–5), seedling leaf pubescence (0–2), reproductive stem pubescence (0–7), reproductive leaf shape (1–7), reproductive leaf margin (1–4), reproductive leaf division (1–4), reproductive leaf color (1–3), reproductive leaf angle (1–3), reproductive leaf tip (1–2), reproductive leaf base (0–2), floral synchrony (0–3), floral disposal (0–2), and seed color (1–10).

Results

Morphology

Among 50 progenies from the Balcarce (BALG1) seed evaluated in the common garden, most plants showed morphological characters similar to those of *B. rapa*. Overall, the BALG1 progeny showed higher variability compared with both controls, *B. napus* (oilseed rape) and *B. rapa* (Table 1, Fig. 1). Principal component analysis showed an overlapping of *B. rapa* control plants and BALG1 progenies, although the former showed a more compact grouping. The detection of *B. napus* traits in BAL progeny provided evidence of hybridization, and this was used to select plants for further evaluation.

Among 1024 BALG1 plants evaluated in the greenhouse, 61 plants (5.9%) were found with intermediate crop-weed morphological traits. These off-type (OT) plants had violet cotyledons which dropped off early, they showed a rosette growth habit at the beginning of the life cycle, and plant height ranged between 0.32 and 1.30 m. They had glabrous or lightly hairy stems. Flowering time was longer than in typical *B. rapa* plants, with yellow flowers, and 75% of individuals possessed buds above the open flowers (similar to *B. napus*). The siliques were erect with an undulating surface, ranging from 2.0 to 3.7 cm in length and 0.35 cm in width, and with beaks of variable length with a mean of 1.1 cm. The number of seeds per silique was variable from four to 21 and seeds were dark brown in color (Fig. 2).

Principal coordinate analysis of categorical data showed separate groups of *B. napus* and *B. rapa* controls while OT plants had an intermediate position, but it was closer to *B. napus* (Fig. 3), showing similarity to the crop that made it possible to identify them in the early development stages.

The hybrid index (Briggs and Walters 1997) based on 17 categorical traits (14 vegetative and three reproductive) showed 54 widely scattered OT plants, although most of them overlapped with *B. napus*. However, three OT individuals were located within the *B. rapa* group (Fig. 4).

Discriminant analysis of metric traits also showed separation between *B. napus* and *B. rapa*, but OT plants were mainly grouped with the latter (Fig. 5). In the analysis, four out of 61 OT plants were assigned as *B. rapa* (8% error).

Screening and persistence of resistance

Only 16 out of 440 plants treated with the herbicide survived (3.6%), and most of them were morphologically similar to the crop. Surviving individuals grouped with *B. napus* plants, and the prediction ellipse included the crop and OT plants (Fig. 6).

The most important variables defining the first component of the PCA analysis were the number of viable seeds per silique, silique length, and beak length. The second component allowed separation based primarily on vegetative characters, such as leaf and basal blade width and length. OT plants split from *B. napus* and *B. rapa* along the first component, whereas the two species separated on the second component (Fig. 7).

Furthermore, when the persistence of resistance was evaluated in G2, G4, G6, and G7, no herbicide resistance was found in any of the progenies (~550 plants treated per year). It is important to point out that these are the results of a single hybridization event under natural conditions. Actually, the progeny of G1 plants (G2), obtained under controlled conditions in an insect-exclusion mesh cage, were evaluated for herbicide resistance and several IMI-resistant plants were found, demonstrating that the trait was transmitted to the following generation (these results will be submitted in a next communication).

Pollen viability

The OT plants showed reduced pollen viability, with a mean (±SE) of 68.6 ± 4% and minimum values near 30%. On the contrary, pollen from normal plants (BR) was completely viable as well as pollen from the *B. rapa* and *B. napus* controls that showed 100% viability.

Flow cytometry

Eleven out of 16 OT individuals analyzed by flow cytometry were confirmed as first-generation hybrids. DNA peaks clearly differentiated between the pure species and off-type samples. OT individuals showed intermediate peaks between *B. napus* ($2n = 38$ AACC) and *B. rapa* ($2n = 20$ AA). The remaining individuals were classified as *B. rapa*, except for one that could not be assigned to any group. Control plants were classified as *B. napus* and *B. rapa* (Fig. 8, Table 2).

Table 1 Morphological traits of flowering plants recorded at the experimental field on BALG1 progenies and controls *B. napus* (oilseed rape) and *B. rapa*

Traits	*B. napus*		BALG1		*B. rapa*	
	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
Plant height (cm)	84.76 ± 7.8 a	0.092	68.96 ± 26.8 b	0.388	57.98 ± 13.0 c	0.220
Leaf length (cm)	13.85 ± 1.6 a	0.115	9.76 ± 3.9 b	0.399	10 ± 2.3 b	0.230
Leaf width (cm)	6.61 ± 1.5 a	0.226	3.94 ± 1.9 b	0.482	4.48 ± 1.1 b	0.245
Silique length (cm)	5.55 ± 0.6 a	0.108	2.98 ± 1.1 b	0.369	3.03 ± 0.8 b	0.264
Silique width (cm)	0.37 ± 0.1 a	0.270	0.31 ± 0.1 b	0.322	0.32 ± 0.1 b	0.312
Beak length (cm)	1.03 ± 0.3 c	0.291	1.4 ± 0.4 a	0.285	1.22 ± 0.4 b	0.327
Beak/silique length	0.20 ± 0.04 c	0.200	0.54 ± 0.15 a	0.270	0.41 ± 0.14 b	0.340

Different letters indicate significant differences according to the LSD Fisher test ($p < 0.05$)

Discussion

B. rapa is widely distributed in central and northern Argentina (Martínez-Laborde, 1999; IBODA, 2014). Hybridization between *B. napus* and *B. rapa* has been recorded in different countries under greenhouse and field conditions (Jorgensen and Andersen 1994; Landbo et al. 1996; Halfhill et al. 2002, 2004; Warwick et al. 2003; Wilkinson et al. 2003, Simard et al. 2006). However, there were no previous studies in Argentina, nor any studies that have been published on the transference of imidazolinone resistance between crop-wild *Brassica* species. These results confirm the high risk of gene transmission from oilseed rape to *B. rapa* in the main cropping area of Argentina, comparing different methodologies of hybrid detection. They also demonstrate the transference of IMI resistance from crop to weed. Similar studies on sunflower and its wild relatives were performed earlier by our group, measuring crop-wild gene flow between *Helianthus* species in Argentina (Ureta et al. 2008; Gutierrez et al. 2010; Presotto et al. 2012).

The first assessment on BAL progeny (Table 1) showed that variability was high compared with the pure species, except for the beak length and beak-to-silique length ratio, the latter having taxonomic value. Figure 1 also shows a high dispersion of BAL plants and partial overlapping with *B. rapa*; leaf and silique sizes were

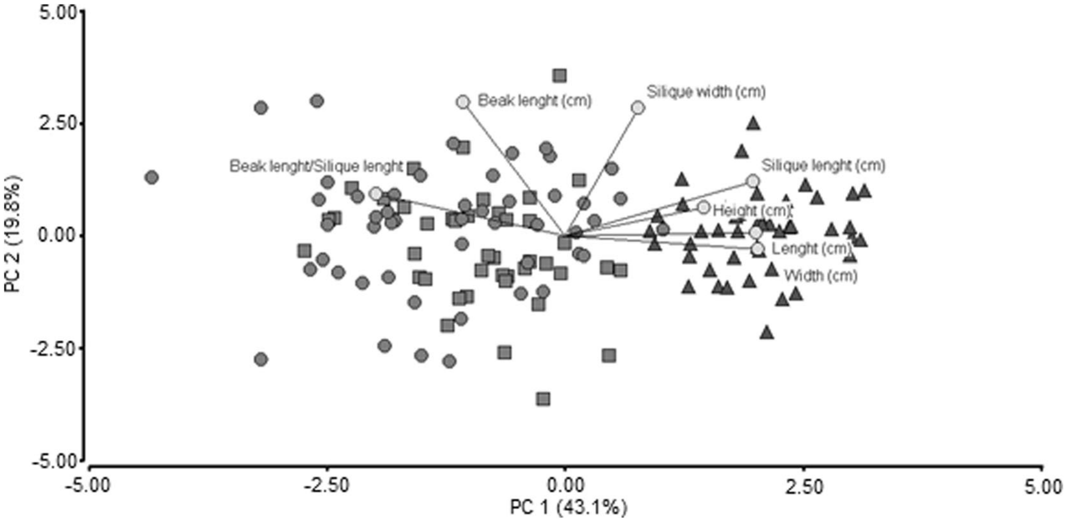

Fig. 1 Principal component for morphological characters of flowering plants of BAL progenies, *B. napus*, and *B. rapa* measured under experimental field conditions. ▲ *Brassica napus*, ■ *B. rapa*, ● Bal G1

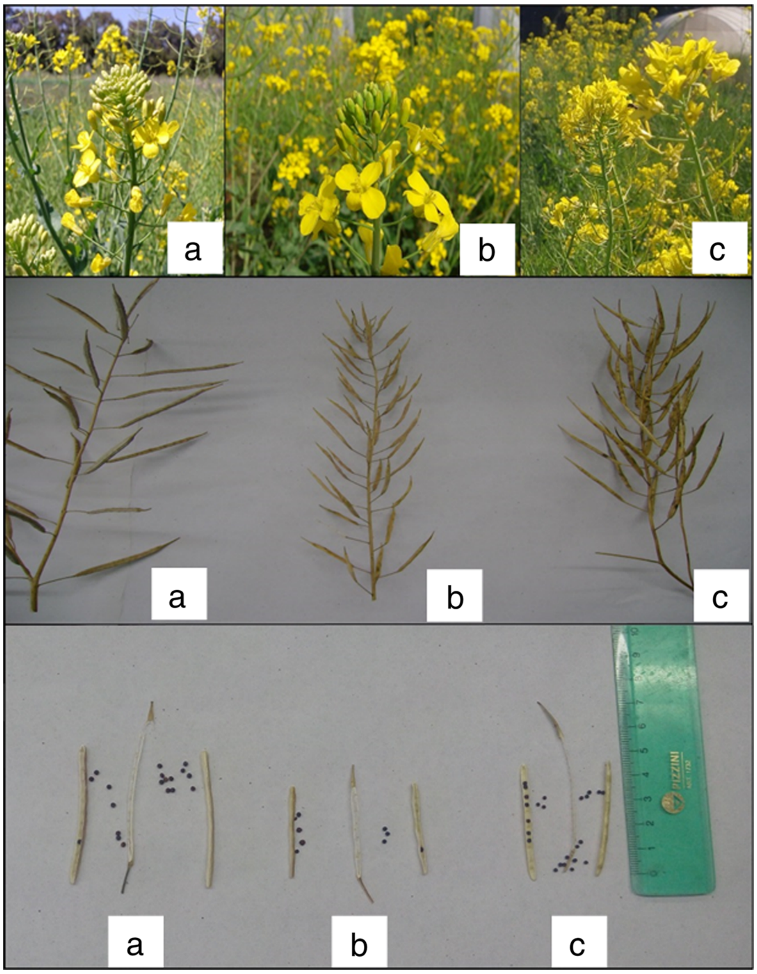

Fig. 2 Flower position, inflorescence, and siliques from **a** *Brassica napus*, **b** off-type plants, and **c** *B. rapa.* **a** *Brassica napus* and **b** off-type flowers with yellow petals and buds above the open flowers and **c** *B. rapa* flowers with buds below the open flowers. **a** *Brassica napus* long siliques with short beaks, **b** off-type short siliques with intermediate-length beaks, and **c** *B. rapa* long siliques with long beaks

larger for *B. napus* plants, while the beak length and the beak-to-silique ratio were larger for *B. rapa* and OT plants. This was interpreted as evidence for crop-weed hybridization and gene flow. The second study focused on those OT plants. Categorical trait analyses placed OT plants close to *B. napus* (Fig. 3). The glaucous leaves with a rosette disposition seen in the early growth stage of BAL progeny are characteristic of oilseed rape. Also,

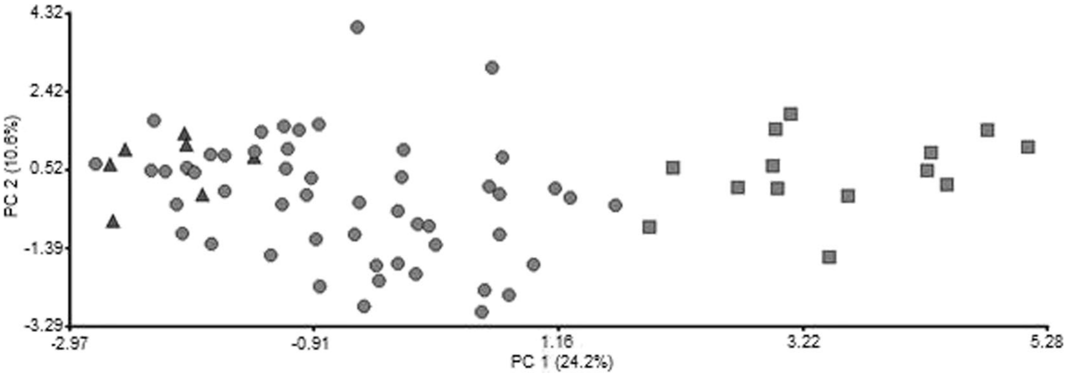

Fig. 3 Principal coordinate analysis of *B. napus*, *B. rapa*, and off-type (OT) individuals based on qualitative data recorded in the greenhouse. ▲ *Brassica napus*, ■ *B. rapa*, ● OT

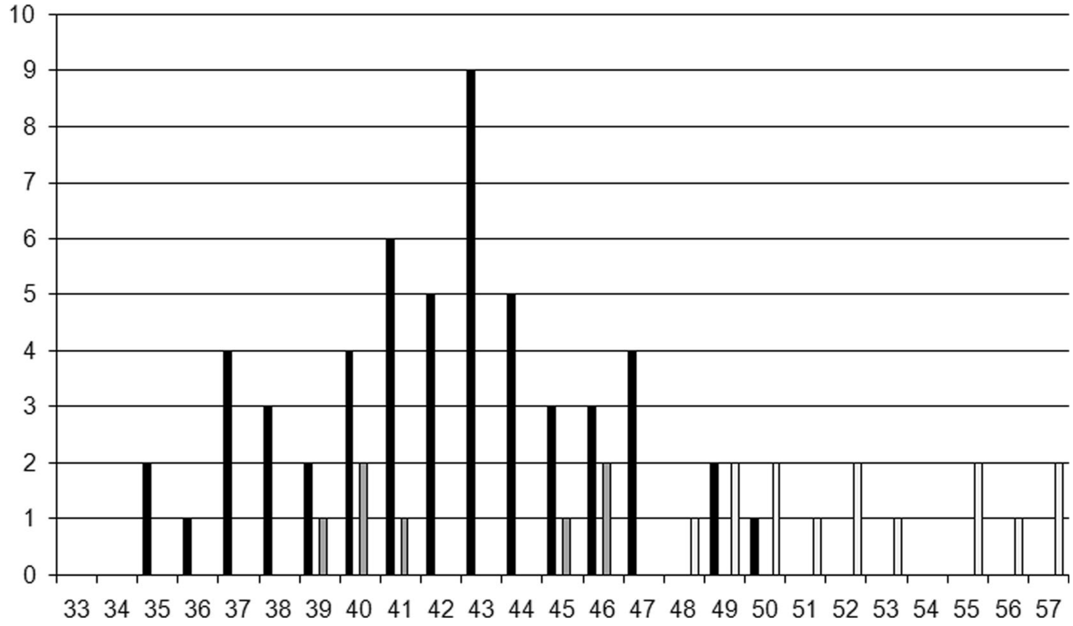

Fig. 4 Hybrid index based on categorical traits of *B. napus*, *B. rapa*, and off-type (OT) plants from BAL progenies. ■ *Brassica napus*, ■ *B. rapa*, ■ OT

in the hybrid index figure, most OT individuals were close to *B. napus*, though some plants were morphologically similar to *B. rapa*. In contrast, analysis of metric traits placed OT plants close to *B. rapa*, but they also showed the highest variability in the BAL population compared with both pure species (Fig. 5). The greater variability found in the OT individuals is characteristic of hybrid populations, while pure populations form more uniform blocks. The flowering time in OT, plus the low fertility of interspecific hybrids, generated highly variable yields, which were lower on average compared to those of the pure species. These results indicate that morphological traits are a good hint, but inconclusive, for detecting crop-wild hybrids in *Brassica*, because it depends on the life stage and the trait under consideration. Additional studies are needed to determine the hybrid origin of OT plants; flow cytometry, chromosome counting, molecular markers, and the realized acquisition of a particular trait, i.e., herbicide tolerance, are recommended as tools for this purpose.

Considering only the morphological traits for detecting crop-wild hybrids, 5.9% of individuals of the

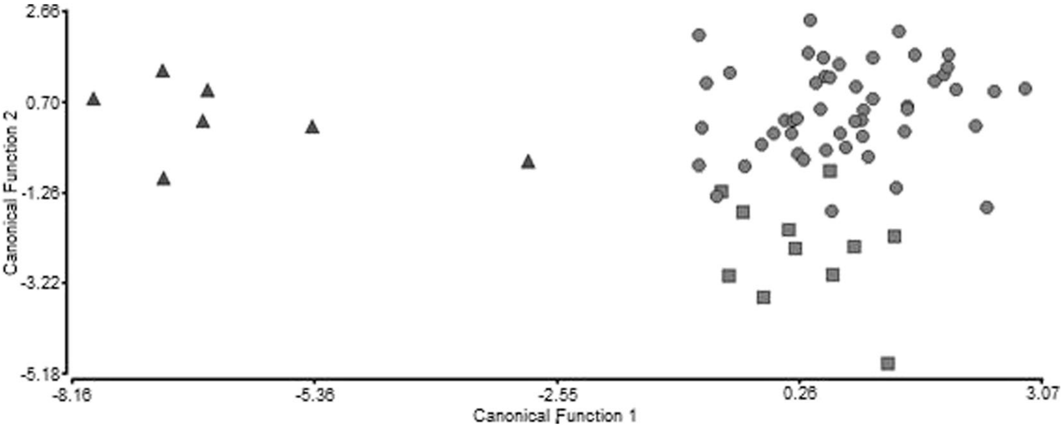

Fig. 5 Discriminant analysis of *B. napus*, *B. rapa*, and off-type (OT) individuals based on quantitative data. ▲ *Brassica napus*, ■ *B. rapa*, ● OT

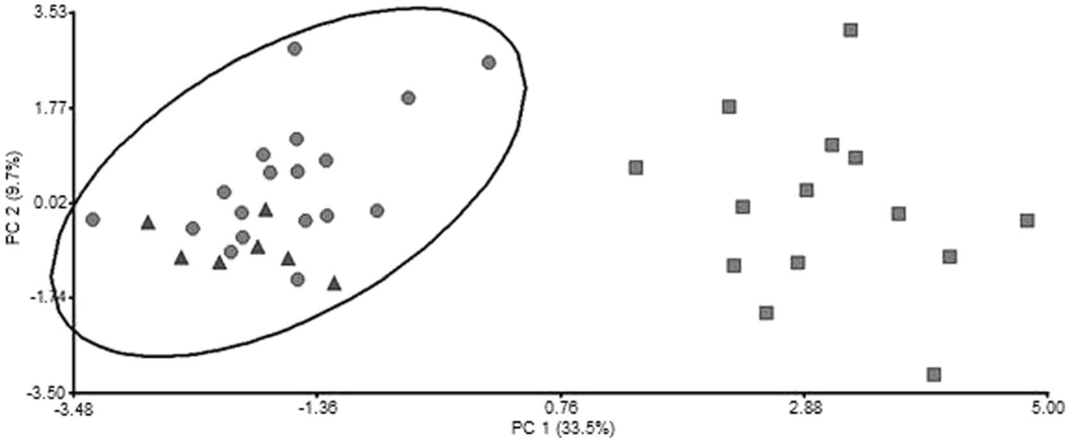

Fig. 6 Principal coordinate analysis of *B. napus*, *B. rapa*, and herbicide-surviving off-type (OT) individuals based on qualitative data. The *prediction ellipse* includes crop (*B. napus*) and OT plants. ▲ *Brassica napus*, ■ *B. rapa*, ● OT

BAL progeny showed evidence of crop hybridization in this study. However, when herbicide resistance was used as a criterion for hybrid detection, the percentage fell to 3.6% of the BAL progeny. This reduction in the hybrid rate of herbicide resistance could be explained by the presence of an herbicide-susceptible oilseed rape cultivar in the same area. It is possible that some hybrids detected by morphology were in fact first-generation hybrids originating from pollen from a non-resistant crop. The BAL population was growing near an oilseed rape crop field, and according to the farmers, it was the first time that the crop had been sown in the area. This could explain the high number of off types found given that they would mostly be first-generation hybrids. Moreover, recall that oilseed rapeseed is not a common crop in Argentina, and in 2008, there was only 1500 ha sown with this crop in Balcarce county; pollination from further lots by bees or other insects would be unlikely. A few backcross generations are enough for introgressed plants to morphologically resemble the wild/weedy parent (Halfhill et al. 2002, 2004). Therefore, the hybrid rate could be underestimated in morphological studies.

Flow cytometry is the current method for measuring nuclear DNA content (Shapiro 2003). Intermediate peaks were identified in 15 of the 16 samples analyzed. The hybrid ploidy level was easily detected by this technique since *B. rapa* ($2n = 20$ AA) has nearly half of the DNA content compared to *B. napus* ($2n = 38$ AACC) (U N, 1935). Flow cytometry should be strongly recommended for this species in cases where hybrid detection becomes difficult.

Pollen viability in OT plants was reduced, less than 70% on average, with the lowest values of about 30% of viable pollen. Low male fertility in OT plants is consistent with that observed in hybrids between *B. napus* and

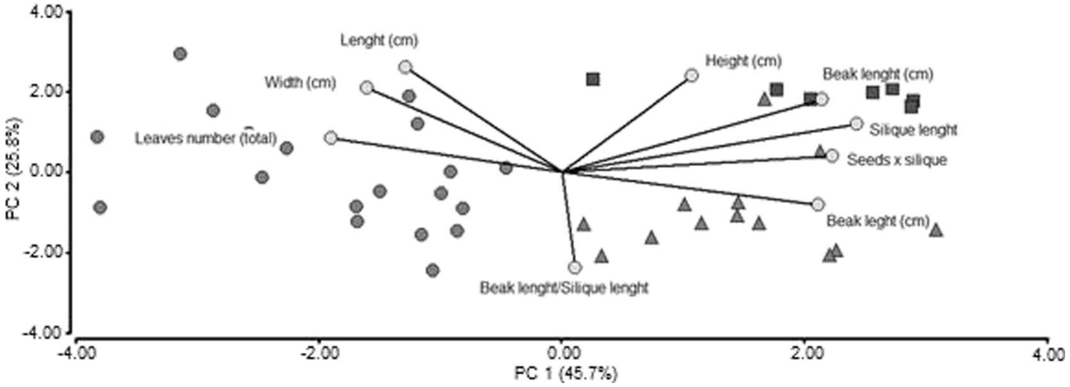

Fig. 7 Principal component analysis of *B. napus*, *B. rapa*, and herbicide-surviving off-type (OT) individuals based on quantitative data. ■ *Brassica napus*, ▲ *B. rapa*, ● OT

Fig. 8 Relative DNA content in *B. napus* (**a**), *B. rapa* (**b**), and off-type plants (**c**) assessed by flow cytometry

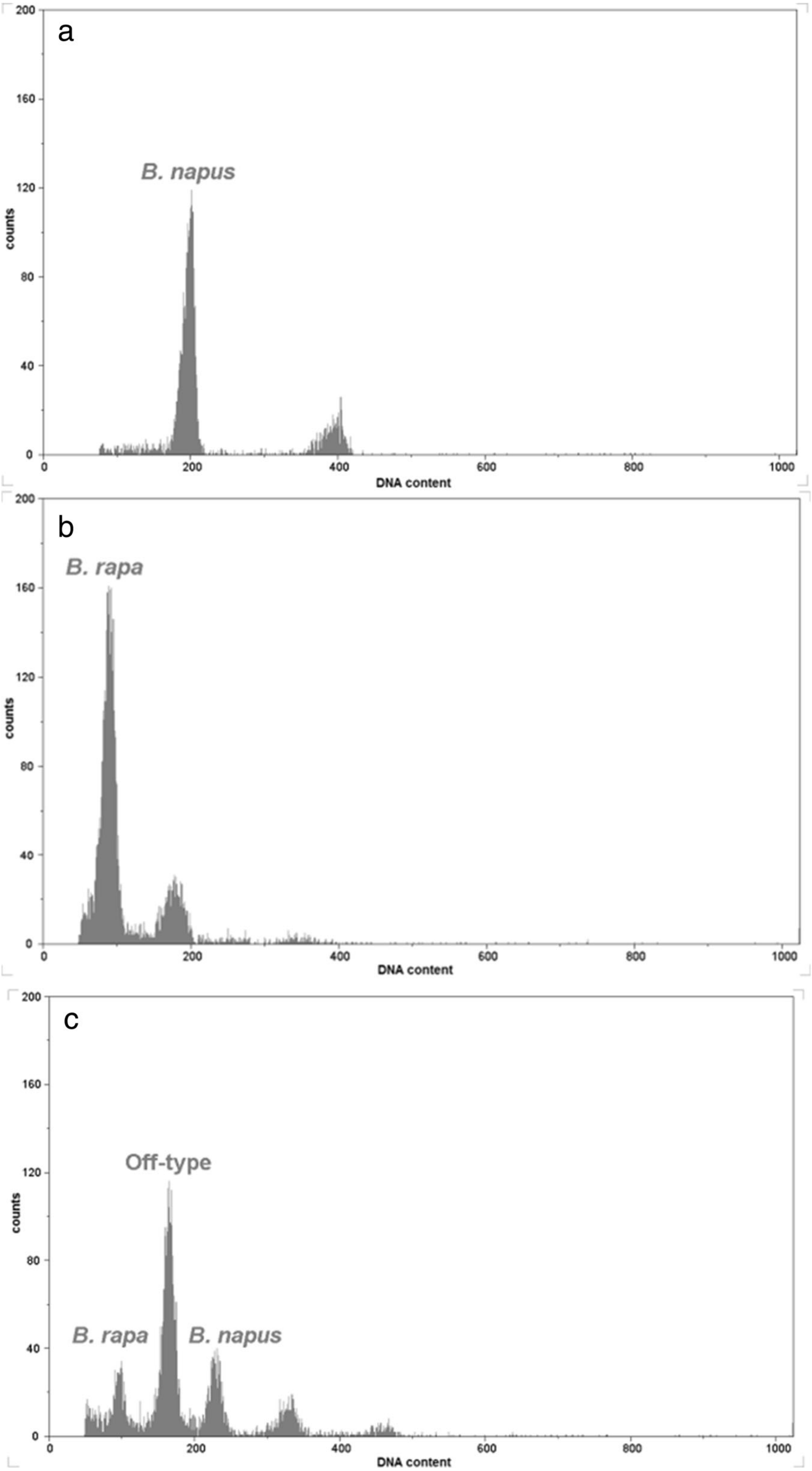

Table 2 Off-type plants (OT) identified as first-generation (F1) hybrids and putative parents (BR, BN) classified by discriminant analysis based on morphology and DNA content by flow cytometry

Samples	Flow cytometer classification	Discriminant analysis classification
OT 1	F1	OT
OT 4	F1	OT
OT 5	F1	OT
OT 10	R	OT
OT 11	F1	OT
OT 12	F1	OT
OT 13	F1	OT
OT 16	n/a	OT
OT 17	F1	OT
OT 18	R	OT
OT 20	F1	OT
OT 22	F1	OT
OT 23	R	OT
OT 24	F1	OT
OT 25	F1	OT
OT 26	F1	OT
B. rapa	BR	BR

n/a Not assigned classification by the flow cytometer

B. rapa (Hauser et al. 1998; Warwick et al. 2003; Allainguillaume et al. 2006). This would confirm that the OT individuals were in fact hybrids between the two species.

This study demonstrates the transfer of resistance, even in a particular situation of pollen competition, between a herbicide-resistant and a non-resistant crop. This could be assumed as a decrease of allele frequency transmitted by gene flow. Despite the fact that the BAL *B. rapa* population was receiving pollen from a non-resistant cultivar, a herbicide-resistance level was found in its progeny. The appearance of herbicide resistance in a population is an example of rapid weed evolution. However, resistance was not found in the following progenies collected from 2009 to 2014, which shows that in this scenario where a single hybridization event occurred, the persistence of IMI resistance is unlikely. Several features cooperate for this effect, namely the low frequency of the resistant allele, the lower fitness of hybrids because of chromosome imbalance, the likely pollination of those hybrids by surrounding *B. rapa* plants, and the absence of herbicide-resistant oilseed rape varieties in the following cropping years.

The IMI-resistance trait has apparently a negligible fitness cost in the absence of herbicide selection (Tranel and Wright 2002; Yu et al. 2010). Previous studies have shown that the introgression event is not only related to hybrid fitness but also dependent on local frequencies of the parental species and different hybrid classes (F1, BC, and advanced generations). Therefore, gene transmission from F1 plants to *B. rapa* via pollen is biologically much more difficult and only likely if F1 plants occur in higher frequencies than *B. rapa* (Pertl et al. 2002; Hauser et al. 2003), which is far from the BAL field situation. The extensive *B. rapa* population was found along the field margins in a long, undisturbed patch of 800 m^2, and included other Brassicaceae species like *Hirschfeldia incana* and *Raphanus sativus*. The presence of such habitat strongly contributes to the selection against the spread of an advantageous allele such as resistance. The fate of a resistance allele may depend on the balance between herbicide treatment favoring the resistance and the capacity to maintain the resistance gene in untreated areas or during years without treatment (Roux et al. 2008).

It is possible that some first-generation hybrids in the BAL progeny population could have only one copy of AHAS (AHAS3, from the A genome), leading to a reduced number of resistant individuals. The copy number of the resistant allele could vary in the population, and some crop-weed hybrids would not be detected because of the lack of or incomplete resistance, due to a heterozygous rather than homozygous condition. The highest level of IMI resistance is obtained when both mutations are stacked and homozygous (Krato and Petersen 2012; Tan et al. 2005).

Conclusion

These results verified gene transmission from oilseed rape to *B. rapa* in the main cropping area of Argentina where resistant and susceptible rapeseed cultivars were found. However, resistance was not found in the following 6 years, which shows that in this scenario, after a single hybridization event and the absence of herbicide selection, the persistence of IMI resistance is unlikely. The IMI technology has become available in the last

5 years for this crop, and an increase in the resistant oilseed rape crop is expected in the cropping area increasing the risk of transfer of herbicide resistance. Hybridization and introgression would be associated with the loss of effectiveness of IMI technology under the latter conditions, generating noxious weeds, and this effect will increase with the continuous use of herbicides that have the same mode of action. Gene flow would limit the effectiveness of IMI herbicide-resistance technology in the field. Moreover, IMI technology has been widely used in other crops, meaning that if the resistance spreads in *B. rapa* populations, it could turn into a problem for oilseed rape crops and for other crops, like sunflower, maize, and wheat. Thus, crop rotation is strongly recommended in the management of herbicide-resistant weeds, which will reduce the risk of developing herbicide-resistant weeds.

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