



# Imidazolinone Resistance in Oilseed Rape (*Brassica napus* L.): Current Status, Breeding, Molecular Markers and Prospects for Application in Hybrid Seed Purity Improvement

Elena Victorovna Kozar \* and Elena Alekseevna Domblides \*

Federal State Budgetary Scientific Institution Federal Scientific Vegetable Center (FSBSI FSVC), VNISSOK, 143072 Moscow, Russia

\* Correspondence: koz.leno4ek@gmail.com (E.V.K.); edomblides@mail.ru (E.A.D.)

**Abstract:** Resistance of agricultural crops to herbicides is an important topic that concerns many researchers. One of the most popular groups of herbicides is the imidazolinone group. Resistant forms of crops such as wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.) and oilseed rape (*Brassica napus* L.) have been developed to this group of herbicides. All crops resistant to this group of herbicides have the commercial name Clearfield®. In this review, the information concerning oilseed rape resistance to the imidazolinone group of herbicides is collected and summarized; it will be useful for breeders and researchers engaged in this direction. This review touches upon the topic of mechanisms of oilseed rape resistance to imidazolinones. Mutation variants known to date, which provide resistance to this group of herbicides, are described, and known molecular markers of them are presented. Approaches to the selection of oilseed rape for resistance to the imidazolinone group of herbicides are mentioned. Various methods of utilizing imidazolinone resistance to improve the purity of hybrid seeds are also considered.



**Citation:** Kozar, E.V.; Domblides, E.A. Imidazolinone Resistance in Oilseed Rape (*Brassica napus* L.): Current Status, Breeding, Molecular Markers and Prospects for Application in Hybrid Seed Purity Improvement. *Horticulturae* **2024**, *10*, 553. <https://doi.org/10.3390/horticulturae10060553>

Academic Editors: Xiaowu Wang, Jian Wu and Xu Cai

Received: 27 April 2024

Revised: 20 May 2024

Accepted: 23 May 2024

Published: 24 May 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Keywords:** resistance; herbicide; polyploidy; imidazolinone; Clearfield®; oilseed rape; agricultural crops; oilseeds

## 1. Introduction

Rapeseed (*Brassica napus* L.) is a herbaceous plant of the cabbage family (*Brassicaceae* L.). Rapeseed is an allotetraploid ( $2n = 38$ ) with two genomes A and C. It is derived from an interspecific cross between *Brassica campestris* L., an A-genome donor, and *Brassica oleracea* L., a C-genome donor. The A genome consists of ten chromosomes and the C genome consists of nine chromosomes [1–3]. Rapeseed is an important oilseed crop worldwide. It is used as a raw material for biofuel, and oilseed rape varieties with a reduced content of erucic acid and glucosinolates and improved consumer properties (in particular, lack of unpleasant taste and greenish color) are consumed as food [4,5]. The largest areas of rapeseed cultivation are in countries such as Canada, Australia, EU 28, China and India [6].

As with any other crop, one of the most important parameters determining the profitability of rapeseed cultivation is its yield. The yield of this crop is affected by many factors, including weed infestation of fields by weeds that compete with rapeseed for sunlight, water, soil nutrients and physical space in the field, which is a serious problem and limits the yield of this crop, reducing it by 23–64% compared to weed-free controls [7–10]. In addition, weed infestation affects the permeability of the rapeseed root system, which leads to an increase in moisture in the above-ground space and creates a favorable environment for pathogen growth and spread, increasing yield losses [11]. On the other hand, the presence of weeds deteriorates the quality of the raw material, as the presence of weed seeds among rapeseed reduces the quality of oil and makes it difficult to process [12].

Developing herbicide-resistant canola varieties and hybrids is a priority for breeding, as they are one of the most effective tools for weed control [3,13]. Herbicides to which resistant forms of rapeseed have been developed can be divided into six groups: glufosinate, glyphosate, bromoxynil, imidazolinones, triazinines and sulfonylureas [6]. Rapeseed resistant to glufosinate, glyphosate and bromoxynil has been bred by genetic engineering, while rapeseed resistant to imidazolinones, triazines and sulfonylurea has been bred by conventional breeding [14]. For countries where GMO crops are banned, the fact that it is possible to obtain resistant plants using traditional methods is fundamental when choosing the direction of breeding work. The imidazolinone group of herbicides is attractive not only because resistance to them has emerged without genome editing but also because of the low toxicity of this group of herbicides to mammals and low application rates [15,16]. Imidazolinone herbicides were developed in the 1980s [17,18]. Since then, forms resistant to this group of herbicides have been obtained not only for oilseed rape but also for wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) [3]. Imidazolinone-resistant varieties and hybrids are marketed under the Clearfield® production system label, and the abbreviation “CL” in the names of varieties and hybrids indicates their suitability for the Clearfield® production system [6]. The imidazolinones include imazapyr, imazapic, imazethapyr, imazamox, imazamethobenz-methyl and imazaquin. The imidazolinones belong to one of five chemical families of acetohydroxyacid synthase (AHAS, also called acetolactate synthase or ALS) inhibitor herbicides. The other four families of herbicides are sulfonylureas (SUs), triazopyrimidines (TPs), pyrimidinyl-thiobenzoates (PTBs) and sulfonyl-aminocarbonyl-triazolinones (SCTs) [19]. AHAS is a key enzyme for the biosynthesis of branched-chain amino acids, including valine, leucine and isoleucine [20,21]. Five AHAS loci have been reported in oilseed rape. Three loci, AHAS2, AHAS3 and AHAS4, originate from the A genome, and two loci, AHAS1 and AHAS5, originate from the C genome [2]. Of these, the AHAS1 and AHAS3 genes are expressed constitutively and encode the major AHAS activities required for B. napus growth and development.

## 2. Mutants and Mutations Conferring Resistance to Imidazolinones

A single-nucleotide polymorphism (SNP) is a type of DNA polymorphism in the genome that results from a change in a single nucleotide in the DNA sequence [22,23]. Since SNPs in coding triplets are capable of making amino acid substitutions, this can lead to changes in the agronomic traits of plants. Therefore, specific single-nucleotide changes are used as an important tool for the genetic improvement of crops [24–27]. The first oilseed rape mutants resistant to imidazolinones were obtained by Swanson et al. in 1989 by chemical mutagenesis of microspores using 20 µM ethyl nitrosourea (ENU) in combination with a selective medium containing 40 µg/L imazethapyr (or a herbicide under the commercial name Pursuit) [28]. These mutants were named P1 and P2 (Table 1). In this study, the mutants were tested for resistance by several herbicides, including testing an imidazolinone group herbicide with the commercial name “Pursuit”. Before describing the results obtained by the researchers in this article, we would like to note that, in our opinion, there is some inconsistency in the data on fertilizer application rates. The point is that the researchers write that they tested 0, 50, 100, 300 and 500 g/ha of “Pursuit” to test the mutants for resistance. However, the herbicide with the commercial name “Pursuit” has an imazethapyr (active ingredient) concentration of about 240 g/L. Therefore, in terms of the active ingredient (for example  $50 \times 0.24 = 12$ ), the treatment concentrations were 0, 12, 24, 75 and 120 g/ha, when the recommended application rate of imazethapyr active ingredient averages between 50 and 100 g per hectare. On the other hand, in the text of the article, the researchers mention an application rate of 50 g/ha and resistance of the P2 mutant at ten times the recommended application rate. These facts indicate that the article lists all herbicide application options in terms of active ingredients and this should be taken into account. Assuming that our conversion of application rates to active ingredient is correct, the P1 mutant was not affected by application of 50 g/ha of imazethapyr and

tolerated rates up to 100 g/ha of imazethapyr (about 90% of plants survived), while the P2 mutant was not affected by application of 100 g/ha of imazethapyr and tolerated rates up to 500 g/ha of imazethapyr (about 80% of plants survived). In comparison, the non-mutant Topas rapeseed died when 20 g/ha of imazethapyr was applied [28,29].

In a study of mutations that cause resistance to imidazolinones, the P1 mutant was shown to have a mutation in the BnAHAS1 gene (C genome) with a single-nucleotide substitution at codon 653 that results in the substitution of serine for asparagin, and the P2 mutant has a mutation in the BnAHAS3 gene (A genome) with a single-nucleotide substitution at codon 574 that results in the substitution of tryptophan for leucine [30]. In addition, the P2 mutant was cross-resistant to SU and TP [30]. Pyramiding the mutations by crossing the P1 and P2 mutants resulted in a mutant that was superior to either of the heterozygous mutants (P1 × Topas and P2 × Topas) individually in imidazolinone resistance, indicating an additive effect of imidazolinone resistance in the presence of both mutations. Testing of the obtained mutants under field conditions and analysis of the inheritability of the trait showed that the mutations conferring resistance to imidazolinones do not possess complete dominance. Thus, two mutations in the homozygous state are required to produce high resistance to imidazolinones. Imidazolinone-resistant canola lines NS738, NS1471 and NS1473 were bred from P1 and P2 mutants [31].

Researchers from China have reported a naturally occurring M9 mutant that is resistant to imidazolinones [32–36]. The mutant carried about 135–180 g/ha of imazethapyr. Surprisingly, the mutation in the M9 mutant was identical to the mutation in the P1 mutant, BnAHAS1R (Ser653Asp), but the researchers are talking about a fully dominant mutation, not a semi-dominant mutation, as the researchers wrote about the P1 mutant [28,30]. This difference in data for the same mutation may be due to the fact that the researchers used different doses of herbicide to determine plant resistance when studying the inheritance of the trait. Researchers Swanson et al., 1989 [28], used 100 g/ha of imazethapyr to test while researchers Hu et al., 2012 [33], used a lower herbicide concentration of 90 g/ha of imazethapyr to test. This may mean that the herbicide dose used may be insufficient to detect the negative effects of herbicide application on plants heterozygous for the reported mutation.

**Table 1.** Information on mutants and mutations of resistance of oilseed rape to herbicides of the imidazolinone group.

| Mutant                                   | P1                           | M9                   | 12WH318                          | P2                           | M342                   | 5N                                             | DS3                                                               |
|------------------------------------------|------------------------------|----------------------|----------------------------------|------------------------------|------------------------|------------------------------------------------|-------------------------------------------------------------------|
| Method of obtaining (chemical substance) | Microspore mutagenesis (ENU) | Spontaneous mutation | Crosses with resistant germplasm | Microspore mutagenesis (ENU) | Seed mutagenesis (EMS) | Gene stacking from mutants M342 and PN19       | Mutagenesis of seeds of a line derived from the M342 mutant (EMS) |
| Resistance                               |                              | IMI                  |                                  | IMI<br>SU<br>TP              | IMI<br>SU<br>(TP)      | (IMI)<br>SU<br>(TP)                            | (IMI)<br>SU<br>(TP)                                               |
| Gene                                     |                              | BnAHAS1              |                                  | BnAHAS3                      |                        | BnAHAS3<br>BnAHAS1                             |                                                                   |
| Genome                                   |                              | C                    |                                  | A                            |                        | A, C                                           |                                                                   |
| Mutated gene (mutation)                  |                              | BnAHAS1R (Ser653Asp) |                                  | BnAHAS3R (Trp574Leu)         |                        | BnAHAS3R (Trp574Leu)<br>BnAHAS1-2R (Trp574Leu) | BnAHAS3R (Trp574Leu)<br>BnAHAS1-3R (Pro197Leu)                    |
| References                               | [28]                         | [32–34]              | [37]                             | [28,30]                      | [35]                   | [9]                                            | [38]                                                              |

In the line with resistance: IMI—imidazolinone, SU—sulfonylurea, TP—triazolopyrimidine; values indicated in brackets “(IMI)” mean that this mutant has not been tested for resistance to this herbicide but is assumed to have it.

Also, in 2015, other researchers reported a new rapeseed germplasm, mutant 12WH318, which was derived from multiple crosses with resistant lines of foreign selection [37]. In the article, the researchers say that they crossed transgenic lines, but the article is written in Chinese, so most likely, we did not translate correctly and the researchers were instead referring to the mutagenic origin of the original lines, because in all the literature we could find, resistance to herbicides of the imidazolinone group was obtained exclusively by conventional breeding through mutagenesis, which is a very big advantage, since in many countries the cultivation of GMO plants is prohibited [37]. Among other things, the researchers mention in the paper that the material they used in the crosses could have come from P1 and P2 mutants. These facts also suggest that the translation “transgenic source lines” was probably incorrect. The 12WH318 mutant possessed the BnAHAS1R (Ser653Asp) mutation, as did mutants P1 and M9 (Table 1), which, according to researchers, is inherited as a fully dominant gene [37]. Regarding the herbicide doses tolerated by this mutant, we did not find a precise formulation in the article that would indicate the level of resistance of this mutant to imazethapyr, possibly also due to translation difficulties. Most likely, this mutant was able to tolerate a maximum of 108.59–190.77 g/ha of imazethapyr.

In 2017, Hu et al. reported an M342 mutant obtained by seed mutagenesis using ethyl methanesulfonate (EMS) [35]. This mutant possessed a mutation similar to the P2 mutant, namely BnAHAS3R (Trp574Leu). It exhibited high resistance to imidazolinones and tolerated imazethapyr at a concentration of 360 g/ha. This mutant also exhibited cross-resistance to sulfonylurea, as did the P2 mutant [35]. Since the mutations in the M342 and P2 mutants are the same, it can be assumed that the M342 mutant is also resistant to TP (studies not performed) like the P2 mutant.

More recently, Guo et al. published data on pyramiding mutations from the M342 and PN19 mutants by crossing them, resulting in the 5N mutant [9]. The researchers sought to obtain a mutant with higher resistance to sulfonylurea than the M342 and PN19 mutants. Indeed, combining the A mutation of the BnAHAS3R gene (Trp574Leu) from the M342 mutant and the C mutation of the BnAHAS1-2R gene (Trp574Leu) from the PN19 mutant in the 5N mutant resulted in its increased resistance. The 5N mutant tolerated 360 g/ha of tribenuron-methyl (TBM) herbicide and 180 g/ha of mesosulfuron (MES) herbicide, whereas M342 tolerated up to 90 g/ha of TBM and 45 g/ha of MES, and the PN19 mutant tolerated 45 g/ha of TBM and 22.5 g/ha of MES. Testing of the 5N mutant for resistance to imidazolinones has not been performed, but since this mutant is derived from the M342 mutant and inherited the BnAHAS3R mutation (Trp574Leu), it can be expected to be cross-resistant to IMI as well as TP.

Another DS3 mutant was obtained by mutagenesis of the M342 mutant using EMS, as reported by researchers Guo et al. [38]. This mutant has two mutations: in genome A, BnAHAS3R (Trp574Leu), inherited from the M342 mutant, and in genome C, BnAHAS1-3R (Pro197Leu), obtained by chemical mutagenesis of seeds using EMS. The calculated 50% phytotoxicity values for the DS3 mutant doses were 753.2 g/ha of TBM and 669.8 g/ha of MES, whereas for the M342 mutant, these doses were 210.6 g/ha of TBM and 179.1 g/ha of MES [38]. The DS3 mutant was not treated with other herbicide groups in the present study. However, since this mutant contains a mutation in the A gene, BnAHAS3R (Trp574Leu), it can be assumed that it is also resistant to the IMI and TP groups of herbicides.

Thus, to date, only two mutations in AHAS genes responsible for resistance to imidazolinones are known: the BnAHAS1R mutation (Ser653Asp) of the BnAHAS1 gene in the C subgenome, which replaced serine with asparagine by single-nucleotide substitution, and the BnAHAS3R mutation (Trp574Leu) of the BnAHAS3 gene in the A subgenome, which replaced tryptophan with leucine by single-nucleotide substitution. It is possible that mutations in mutants 5N and DS3 are synergistic with respect to resistance to imidazolinones, which may mean that other mutations (BnAHAS1-2R (Trp574Leu), BnAHAS1-3R (Pro197Leu)) also contribute to this resistance, but studies on the level of resistance of these mutants to imidazolinones have not been performed.

### 3. Markers for Mutant Forms of AHAS Genes Leading to Resistance to Imidazolinones

Marker-mediated selection greatly facilitates the selection process of plants. Functional markers targeting polymorphic regions within genes are directly related to phenotypic variability. Such markers, due to their complete linkage to alleles of the trait locus, are preferred for selection over DNA markers not located in the gene itself, such as RAPDs, SSRs and AFLPs [27,39–41].

Currently, two markers each have been published in open sources for BnAHAS1R (Ser653Asp) and BnAHAS3R (Trp574Leu) mutations, which are responsible for resistance to imidazolinones (Table 2) [27,34,35,42,43].

**Table 2.** Information on markers to imidazolinone resistance genes in oilseed rape.

| Normal Gene | Mutated Gene (Mutation) | Marker Information |                                                               |                                                                                  |                    |        |           | References     |             |
|-------------|-------------------------|--------------------|---------------------------------------------------------------|----------------------------------------------------------------------------------|--------------------|--------|-----------|----------------|-------------|
|             |                         | No                 | F                                                             | R                                                                                | Restriction Enzyme | Method | Resistant |                | Susceptible |
| BnAHAS1     | BnAHAS1R (Ser653Asp)    | 1                  | AP15F (AHAS1-C):<br>CTTTTCGC-<br>TAGCAGGGGCTAAA               | AP18R (AHAS1-S):<br>CATCTTTGAAAAGT-<br>GCCACAAC                                  | -                  | PCR    | -         | 828 bp         | [33,34]     |
|             |                         |                    | AP15F (AHAS1-C):<br>CTTTTCGC-<br>TAGCAGGGGCTAAA               | AP19R (AHAS1-R):<br>CATCTTTGAAAAGT-<br>GCCACAAT                                  | -                  |        | 828 bp    | -              |             |
|             |                         | 2                  | KBA1R19681913B,<br>Common:<br>CAGGACCATACCT-<br>GTTGGATGTGATA | KBA1R19681913B,<br>X-allele: TATTA-<br>CATCTTTGAAAAGT-<br>GCCACCAC               | -                  | KASP   | -         | -              | [43]        |
|             |                         |                    | KBA1R19681913B,<br>Common:<br>CAGGACCATACCT-<br>GTTGGATGTGATA | KBA1R19681913B,<br>Y-allele: GTTATTA-<br>CATCTTTGAAAAGT-<br>GCCACCAT             | -                  |        | -         | -              |             |
| BnAHAS3     | BnAHAS3R (Trp574Leu)    | 1                  | BsrDI-AHAS3-F:<br>GTTTGC-<br>GAGCAGGGC-<br>TAAGA              | BsrDI-AHAS3-R:<br>GACATC-<br>CAACAGGTACG-<br>GTCCA                               | BsrDI              | PCR    | 766 bp    | 570 and 196 bp | [35]        |
|             |                         | 2                  | KASP-A-1667-COM:<br>TGGCGAACCCCT-<br>GATGC-<br>GATTGTTGTGGAC  | KASP-A-1667-HEX:<br>GAAGGTCGGAGT-<br>CAACGGATT-<br>TAGCTTTGTAGAAC-<br>CGATCTTCCA | -                  | KASP   | -         | -              | [27]        |
|             |                         |                    | KASP-A-1667-COM:<br>TGGCGAACCCCT-<br>GATGC-<br>GATTGTTGTGGAC  | KASP-A-1667-FAM:<br>GAAGGTGAC-<br>CAAGTTCATGCT-<br>GCTTTGTAGAACC-<br>GATCTTCCC   | -                  |        | -         | -              |             |

Researchers Hu et al. in 2013 and in 2015 published markers targeting the Ser653Asp polymorphism in the BnAHAS1 gene that can detect the presence/absence of SNPs associated with resistance or susceptibility to herbicides of the imidazolinone group [34,42]. In the 2013 version, these markers were named AP15F/AP18R (for the susceptibility type) and AP15F/AP19R (for the resistant type); in the 2015 version, these same markers were named AHAS1-C/AHAS1-S (for the susceptibility type) and AHAS1-C/AHAS1-R (for the resistant type). These markers produce bands of the same size (828 bp) after electrophoresis; therefore, two independent electrophoresis experiments with subsequent comparison of the results obtained are necessary to identify the plant genotype A.

In 2017, researchers Hu et al. developed an allele-specific cleaved amplified polymorphic sequence to detect the Trp574Leu polymorphism in the BnAHAS3 gene (Table 2) [35]. And because the mutation in the SNP fell within the BsrDI restriction site (GCAATG), it became possible to distinguish between bands with the mutated and wild-type gene based on the length of the PCR product. The researchers named this marker pair BsrDI-AHAS3-F/BsrDI-AHAS3-R and used it in conjunction with the BsrDI restriction site. In this system, the wild-type allele is cut into fragments of 570 and 196 bp in length, while the mutant allele corresponds to a fragment of 766 bp in length [35].

The markers described above require fragment separation by electrophoresis after PCR amplification, making their application relatively limited due to high cost and low throughput [27]. Mass plant testing is essential for plant breeding programs, so methods are constantly being improved. The development of convenient tools for marker-mediated selection simplifies the mass screening of plants. The KASP method involves a high-throughput competitive allele-specific PCR combined with a fluorescence reporting system for allele identification to detect genetic variation occurring at the nucleotide level (SNPs) [44].

This method can significantly increase throughput and reduce economic costs; as such, KASP technology has been widely used for genetic research [44,45]. In 2020, Hu et al. developed the KASP marker KBA1R19681913B (Table 2), which can accurately detect nucleotide polymorphisms by advising the homozygous and heterozygous states of SNPs in the BnAHAS1R gene responsible for the Ser653Asp substitution [43]. The KASP marker was evaluated in two F<sub>2</sub> populations that met the expected 1:2:1 ratio in genotypes and 3:1 ratio in phenotypes for the resistance trait, confirming the inheritance of the imidazolinone resistance trait by a single gene with complete dominance.

In 2023, Shi et al., investigating the 5N mutant, developed functional KASP markers for both mutations of this mutant: BnAHAS3R (Trp574Leu) and BnAHAS1-2R (Trp574Leu) [27]. Table 2 summarizes the markers for the BnAHAS3R(Trp574Leu) mutation since our review focuses on resistance to herbicides of the imidazolinone group. KASP markers KASP-A-1667 (BnALS3) and KASP-A-1676 (BnALS1) were validated in three different populations.

#### 4. Improving Hybrid Seed Purity Using Imidazolinones

Gradually, rapeseed hybrids are replacing varieties due to their higher productivity and other valuable qualities for breeding. Researchers estimate that a 1% reduction in seed purity reduces rapeseed yield by 1%, but it is not easy to obtain hybrid seeds with high purity [34]. An efficient male sterility system is required for the cost-effective production of hybrid seeds on an industrial scale.

At least seven male sterility systems have been used to produce rape hybrids, but they all have a number of drawbacks. These include the following: inheritance of male sterility systems is linked with unfavorable traits, it is time-consuming for cytoplasmic male sterility systems to establish isogenic pairs and other sterility systems can be unstable [46–55].

In 2015, Wu Ni et al. proposed to increase the purity of hybrid seeds using the property of oilseed rape resistance to herbicides of the imidazolinone group [34]. Studies were conducted using the M9 mutant, which carries the BnAHAS1R (Ser653Asp) mutation, conferring resistance to imidazolinone group herbicides. As mentioned above, the resistance trait in this mutant is inherited according to the principle of complete dominance, so for resistance to manifest itself in the phenotype, it is sufficient for the plant to have the mutation in the heterozygous state. Based on this, an experiment was conducted in which the susceptible maternal line 3075R with male sterility was pollinated with the resistant line M9. As a result, all hybrid offspring were heterozygous for the resistance mutation, while the self-pollinated plants did not have this mutation. Thus, when the hybrid was treated postemergence by the herbicide imidazolinone, all true hybrids survived, while plants from self-pollination of the susceptible parental line died. This seed hybridization method increased the purity of hybrids from 83.51% (without herbicide treatment) to 96.92% (after treatment) for one hybrid and from 79.35% to 95.76% for the other hybrid, which increased the yield of the hybrids by 322 and 394 kg/ha, respectively.

Another way to improve hybrid seed purity for systems with unstable male sterility and for double-line hybrids is to use gametocidal chemical treatments. Many different types of gametocides have been developed for oilseed rape, such as gibberellins, etrel/ethephon, methyl arsenate, benzotriazole, etc. [46,56–58], but most of these gametocides have not found practical applications due to high cost, low efficacy, high toxicity, etc. [46]. Therefore, it is relevant to search for gametocides with high efficacy, low toxicity and low cost. Yu et al. 2020 reported their research on testing 27 different AHAS-inhibiting herbicides as

gametocides, among which were herbicides of the imidazolinone group. In this study, the gametocidal effect of herbicides was tested on both fertile lines and unstable male sterile Polima lines (SU only). It has been shown that imidazolinone group herbicides have a gametocidal effect and can induce more than 90% male sterility when 750–1125 mg/ha imazethapyr and 400–800 mg/ha imazamox are applied [46]. The effect of imidazolinone group herbicides on increasing the purity of hybrid seeds when hybridized with Polima male sterile lines was not evaluated in this study.

## 5. Conclusions

Growing crops in herbicide-tolerant cropping systems is undoubtedly the best method of weed control today. But like all technologies, these growing methods have their limitations. On the one hand, since resistance to imidazolinones requires only one or two nucleotide substitutions in AHAS genes, it is not difficult to obtain many imidazolinone-resistant oilseed rape lines by introgression of mutations from resistant mutants. On the other hand, it is this factor that is the most dangerous for the future of Clearfield® technology. This factor is related to the rapid evolution of weeds and their acquisition of resistance. There are many studies highlighting the problem of herbicide resistance transfer from resistant oilseed rape to weeds [11,59–63]. On the other hand, oilseed rape is cultivated in a 3/4-field rotation [28], which reduces the likelihood of resistant forms because pollen viability of interspecific hybrids is inferior to parental forms and resistant forms are unlikely to emerge under unstable selection on herbicide backgrounds [59]. In general, this cultivation technology is promising, but how long it will be effective depends on the correct observance of precautions in agrotechniques.

**Author Contributions:** Conceptualization, investigation, resources, writing, supervision, funding acquisition, E.V.K.; review and editing, E.A.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** The work was funded by a grant from the Russian Science Foundation, grant number 23-76-01070, (<https://rscf.ru/project/23-76-01070/> accessed on 20 April 2024).

**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

- Poehlman, J.M.; Sleper, D.A. *Breeding Field Crops*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 1995.
- Rutledge, R.G.; Quellet, S.; Hattori, J.; Miki, B.L. Molecular characterization and genetic origin of the *Brassica napus* acetohydroxyacid synthase multigene family. *Mol. Gen. Genet. MGG* **1991**, *229*, 31–40. [[CrossRef](#)] [[PubMed](#)]
- Tan, S.; Evans, R.R.; Dahmer, M.L.; Singh, B.K.; Shaner, D.L. Imidazolinone-tolerant crops: History, current status and future. *Proc. Pest Manag. Sci.* **2005**, *61*, 246–257. [[CrossRef](#)] [[PubMed](#)]
- Ohlrogge, J.B. Design of New Plant Products: Engineering of Fatty Acid Metabolism. *Plant Physiol.* **1994**, *104*, 821–826. [[CrossRef](#)] [[PubMed](#)]
- Thelen, J.J.; Ohlrogge, J.B. Metabolic Engineering of Fatty Acid Biosynthesis in Plants. *Metab. Eng.* **2002**, *4*, 12–21. [[CrossRef](#)] [[PubMed](#)]
- Goncharov, S.V.; Gorlova, L.A. Herbicide tolerance in rapeseed breeding: Results and prospects. *Oilseed Crops* **2018**, *4*, 42–47. [[CrossRef](#)]
- Miki, B.L.; Labbe, H.; Hattori, J.; Ouellet, T.; Gabard, J.; Sunohara, G.; Charest, P.J.; Iyer, V.N. Transformation of *Brassica napus* canola cultivars with *Arabidopsis thaliana* acetohydroxyacid synthase genes and analysis of herbicide resistance. *Theor. Appl. Genet.* **1990**, *80*, 449–458. [[CrossRef](#)] [[PubMed](#)]
- Larue, C.T.; Goley, M.; Shi, L.; Evdokimov, A.G.; Sparks, O.C.; Ellis, C.; Wollacott, A.M.; Rydel, T.J.; Halls, C.E.; Van Scoyoc, B. Development of enzymes for robust aryloxyphenoxypionate and synthetic auxin herbicide tolerance traits in maize and soybean crops. *Pest Manag. Sci.* **2019**, *75*, 2086–2094. [[CrossRef](#)] [[PubMed](#)]
- Guo, Y.; Cheng, L.; Long, W.; Gao, J.; Zhang, J.; Chen, S.; Pu, H.; Hu, M. Synergistic mutations of two rapeseed AHAS genes confer high resistance to sulfonylurea herbicides for weed control. *Theor. Appl. Genet.* **2020**, *133*, 2811–2824. [[CrossRef](#)] [[PubMed](#)]
- Bagherani, N.; Shimi, P. Evaluation of some herbicides for weed control in oilseed rape (*Brassica napus* L.). *J. Agric. Sci. Nat. Resour.* **2001**, *8*, 157–163.

11. Bijanzadeh, E.; Naderi, R.; Behpoori, A. Interrelationships between oilseed rape yield and weeds population under herbicides application. *Aust. J. Crop Sci.* **2010**, *4*, 155–162.
12. Haukkaää, A.L.; Junnila, S.; Eriksson, C.; Tulisalo, U.; Seppänen, M. Efficacy of imazamox in imidazolinone-resistant spring oilseed rape in Finland. *Agric. Food Sci.* **2005**, *14*, 377–388. [\[CrossRef\]](#)
13. Green, J.M. Current state of herbicides in herbicide-resistant crops. *Pest Manag. Sci.* **2014**, *70*, 1351–1357. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Monaco, T.J.; Weller, S.C.; Ashton, F.M. *Weed Science: Principles and Practices*; John Wiley & Sons: Hoboken, NJ, USA, 2002; ISBN 0471370517.
15. Mazur, B.J. Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. *Plant Physiol.* **1987**, *85*, 1110–1117. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Bernasconi, P.; Woodworth, A.R.; Rosen, B.A.; Subramanian, M.V.; Siehl, D.L. A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *J. Biol. Chem.* **1996**, *271*, 13925. [\[CrossRef\]](#)
17. Los, M. *Synthesis and Biology of the Imidazolinone Herbicides*; Blackwells Scientific Publications: Oxford, UK, 1987.
18. Shaner, D.L. *Mechanisms of Resistance to Acetolactate Synthase/Acetoxyacid Synthase Inhibitors*; Western Society of Weed Science: Newark, CA, USA, 1991.
19. Yu, Q.; Powles, S.B. Resistance to AHAS inhibitor herbicides: Current understanding. *Pest Manag. Sci.* **2014**, *70*, 1340–1350. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Duggleby, R.G.; McCourt, J.A.; Guddat, L.W. Structure and mechanism of inhibition of plant acetoxyacid synthase. *Plant Physiol. Biochem.* **2008**, *46*, 309–324. [\[CrossRef\]](#)
21. Garcia, M.D.; Wang, J.; Lonhienne, T.; Guddat, L.W. Crystal structure of plant acetoxyacid synthase, the target for several commercial herbicides. *FEBS J.* **2017**, *284*, 2037–2051. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Drenkard, E.; Richter, B.G.; Rozen, S.; Stutius, L.M.; Angell, N.A.; Mindrinos, M.; Cho, R.J.; Oefner, P.J.; Davis, R.W.; Ausubel, F.M. A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. *Plant Physiol.* **2000**, *124*, 1483–1492. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Vignal, A.; Milan, D.; SanCristobal, M.; Eggen, A. A review on SNP and other types of molecular markers and their use in animal genetics. *Genet. Sel. Evol.* **2002**, *34*, 275–305. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Henikoff, S.; Comai, L. Single-nucleotide mutations for plant functional genomics. *Annu. Rev. Plant Biol.* **2003**, *54*, 375–401. [\[CrossRef\]](#) [\[PubMed\]](#)
25. You, Q.; Yang, X.; Peng, Z.; Xu, L.; Wang, J. Development and applications of a high throughput genotyping tool for polyploid crops: Single nucleotide polymorphism (SNP) array. *Front. Plant Sci.* **2018**, *9*, 297846. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Lv, J.; Huang, Q.; Sun, Y.; Qu, G.; Guo, Y.; Zhang, X.; Zhao, H.; Hu, S. Male sterility of an AHAS-mutant induced by tribenuron-methyl solution correlated with the decrease of AHAS activity in *Brassica napus* L. *Front. Plant Sci.* **2018**, *9*, 1014. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Shi, J.; Yu, H.; Fu, Y.; Wang, T.; Zhang, Y.; Huang, J.; Li, S.; Zheng, T.; Ni, X.; Zhao, J. Development and validation of functional kompetitive allele-specific PCR markers for herbicide resistance in *Brassica napus*. *Front. Plant Sci.* **2023**, *14*, 1213476. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Swanson, E.B.; Herrgesell, M.J.; Arnoldo, M.; Sippell, D.W.; Wong, R.S.C. Microspore mutagenesis and selection: Canola plants with field tolerance to the imidazolinones. *Theor. Appl. Genet.* **1989**, *78*, 525–530. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Mcvetty, P.B.E.; Zelmer, C.D. Breeding Herbicide-Tolerant Oilseed Rape Cultivars. *Adv. Bot. Res.* **2007**, *45*, 233–270.
30. Hattori, J.; Brown, D.; Mourad, G.H.; Labb, N.; Ouellet, S.; Sunohara Robert Rutledge, G.; King-Brian Miki, J.; Schell J Hattori, C.J.; Labb, H.; Ouellet-B Miki, T.; et al. An acetoxy acid synthase mutant reveals a single site involved in multiple herbicide resistance. Key words Acetoxy acid synthase Feedback inhibitors-Herbicides Multiple resistance. *Mol. Genet. Genom.* **1995**, *246*, 419–425. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Canadian Food Inspection Agency (CFIA) Decision Document DD 95–03. Determination of environmental safety (and feed safety) of Pioneer Hi-Bred International Inc.'s imidazolinone-tolerant canola. Available online: <https://inspection.canada.ca/en/plant-varieties/plants-novel-traits/approved-under-review/decision-documents/dd1995-03> (accessed on 20 April 2024).
32. Pu, H.; Gao, J.; Long, W.; Hu, M.; Zhang, J.; Chen, S.; Chen, X.; Chen, F.; Gu, H.; Fu, S.; et al. Studies on inheritance of imidazolinones resistance in *Brassica napus* and its utilization. *Chin. J. Oil Crop Sci.* **2011**, *33*, 15–19.
33. Hu, M.; Pu, H.; Gao, J.; Long, W.; Qi, C.; Zhang, J.; Chen, S. Inheritance and Gene Cloning of an ALS Inhabiting Herbicide-Resistant Mutant Line M9 in *Brassica napus*. *Sci. Agric. Sin.* **2012**, *45*, 4326–4334. [\[CrossRef\]](#)
34. Hu, M.; Pu, H.; Kong, L.; Gao, J.; Long, W.; Chen, S.; Zhang, J.; Qi, C. Molecular characterization and detection of a spontaneous mutation conferring imidazolinone resistance in rapeseed and its application in hybrid rapeseed production. *Mol. Breed.* **2015**, *35*, 46. [\[CrossRef\]](#)
35. Hu, M.L.; Pu, H.M.; Gao, J.Q.; Long, W.H.; Chen, F.; Zhou, X.Y.; Zhang, W.; Peng, Q.; Chen, S.; Zheng, J.F. Inheritance and molecular characterization of resistance to AHAS-inhibiting herbicides in rapeseed. *J. Integr. Agric.* **2017**, *16*, 2421–2433. [\[CrossRef\]](#)
36. Hu, M.L.; Pu, H.M.; Long, W.H.; Gao, J.Q.; Qi, C.K.; Zhang, J.F.; Chen, S. Enzymatic characteristics of acetolactate synthase mutant S638N in *Brassica napus* and its resistance to ALS inhibitor herbicides. *Acta Agron. Sin.* **2015**, *41*, 1353–1360. [\[CrossRef\]](#)
37. Li, X.; Wu, L.; Jia, Y.; Li, K.; Chen, Y.; Lu, C. Inheritance of imidazolinone resistance in rapeseed 12WH318 and BnALS gene cloning. *Chin. J. Oil Crop Sci.* **2015**, *37*, 269–276.

38. Guo, Y.; Liu, C.; Long, W.; Gao, J.; Zhang, J.; Chen, S.; Pu, H.; Hu, M. Development and molecular analysis of a novel acetohydroxyacid synthase rapeseed mutant with high resistance to sulfonylurea herbicides. *Crop J.* **2022**, *10*, 56–66. [\[CrossRef\]](#)
39. Andersen, J.R.; Lübberstedt, T. Functional markers in plants. *Trends Plant Sci.* **2003**, *8*, 554–560. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Varshney, R.K.; Graner, A.; Sorrells, M.E. Genomics-assisted breeding for crop improvement. *Trends Plant Sci.* **2005**, *10*, 621–630. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Li, L.; Sun, Z.; Zhang, Y.; Ke, H.; Yang, J.; Li, Z.; Wu, L.; Zhang, G.; Wang, X.; Ma, Z. Development and utilization of functional kompetitive allele-specific PCR markers for key genes underpinning fiber length and strength in *Gossypium hirsutum* L. *Front. Plant Sci.* **2022**, *13*, 853827. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Hu, M.L.; Long, W.H.; Gao, J.Q.; Fu, S.X.; Chen, F.; Zhou, X.Y.; Peng, Q.; Zhang, W.; Pu, H.M.; Qi, C.K.; et al. Development and Application of Allele-Specific PCR Markers for Imidazolinone-Resistant Gene BnALS1R in *Brassica napus*. *Acta Agron. Sin.* **2013**, *39*, 1711. [\[CrossRef\]](#)
43. Hu, M.L.; Cheng, L.; Guo, Y.; Long, W.H.; Gao, J.Q.; Pu, H.M.; Zhang, J.F.; Chen, S. Development and application of the marker for imidazolinone-resistant gene in *Brassica napus*. *Acta Agron. Sin.* **2020**, *46*, 1639–1646.
44. He, C.; Holme, J.; Anthony, J. SNP genotyping: The KASP assay. *Crop Breed. Methods Protoc.* **2014**, *1145*, 75–86.
45. Semagn, K.; Babu, R.; Hearne, S.; Olsen, M. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Mol. Breed.* **2014**, *33*, 1–14. [\[CrossRef\]](#)
46. Yu, C.Y.; Lian, J.L.; Gong, Q.; Ren, L.S.; Huang, Z.; Xu, A.X.; Dong, J.G. Sublethal application of various sulfonylurea and imidazolinone herbicides favors outcrossing and hybrid seed production in oilseed rape. *BMC Plant Biol.* **2020**, *20*, 69. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Xia, S.; Wang, Z.; Zhang, H.; Hu, K.; Zhang, Z.; Qin, M.; Dun, X.; Yi, B.; Wen, J.; Ma, C. Altered transcription and neofunctionalization of duplicated genes rescue the harmful effects of a chimeric gene in *Brassica napus*. *Plant Cell* **2016**, *28*, 2060–2078. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Frauen, M.; Noack, J.; Girke, A.; Paulmann, W. Ten years experience of development and cultivation of winter oilseed rape hybrids in Europe based on the MSL system. *Proc. 12th Int. Rapeseed Congr.* **2006**, *1*, 39–41.
49. Levings III, C.S. The Texas cytoplasm of maize: Cytoplasmic male sterility and disease susceptibility. *Science* **1990**, *250*, 942–947. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Chakrabarty, S.K.; Maity, A.; Yadav, J.B. Influence of cyto-sterility sources of female line on seed quality of Indian mustard (*Brassica juncea* L. Czern & Coss.) in relation to storage period. *Plant Breed.* **2015**, *134*, 333–337.
51. Dey, S.S.; Bhatia, R.; Parkash, C.; Sharma, S.; Dabral, M.; Mishra, V.; Bhardwaj, I.; Sharma, K.; Sharma, V.K.; Kumar, R. Alteration in important quality traits and antioxidant activities in *Brassica oleracea* with Ogura cybrid cytoplasm. *Plant Breed.* **2017**, *136*, 400–409. [\[CrossRef\]](#)
52. Lobos-Sujo, V.; Duncan, R. Evaluation of the Rfo introgression following recombination and mutation. In Proceedings of the Saskatoon: Proceedings of 14th International Rapeseed Congress, Saskatoon, AB, Canada, 5–9 July 2015.
53. McVetty, P.B.E. *Cytoplasmic Male Sterility*; Cambridge University Press: Cambridge, UK, 1997.
54. Liu, X.-Q.; Liu, Z.-Q.; Yu, C.-Y.; Dong, J.-G.; Hu, S.-W.; Xu, A.-X. TGMS in rapeseed (*Brassica napus*) resulted in aberrant transcriptional regulation, asynchronous microsporocyte meiosis, defective tapetum, and fused sexine. *Front. Plant Sci.* **2017**, *8*, 1268. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Fan, Z.X.; Lei, W.X.; Hong, D.F.; He, J.P.; Wan, L.L.; Xu, Z.H.; Liu, P.W.; Yang, G.S. Development and primary genetic analysis of a fertility temperature-sensitive polima cytoplasmic male sterility restorer in *Brassica napus*. *Plant Breed.* **2007**, *126*, 297–301. [\[CrossRef\]](#)
56. Banga, S.S.; Labana, K.S. Production of F1 hybrids using ethrel-induced male sterility in Indian mustard (*Brassica juncea* (L.) Czern.). *J. Agric. Sci.* **1983**, *101*, 453–455. [\[CrossRef\]](#)
57. Singh, V. Benzotriazole-A New Chemical Hybridizing Agent for *Brassica Juncea* L. *J. Cytol. Genet.* **2001**, *2*, 81–83.
58. Guan, C.Y.; Stringam, G.R. The effect of ZMA on inducing male sterility on spring canola. *Crucif. Newsl.* **1998**, *20*, 55–56.
59. Torres Carbonell, F.; Ureta, S.; Pandolfo, C.; Presotto, A. Molecular characterization of imidazolinone-resistant *Brassica rapa* × *B. napus* hybrids. *Environ. Monit. Assess.* **2020**, *192*, 746. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Saari, L.L.; Cotterman, J.C.; Thill, D.C. Resistance to acetolactate synthase inhibiting herbicides. In *Herbicide Resistance in Plants*; CRC Press: Boca Raton, FL, USA, 2018; pp. 83–140.
61. Powles, S.B.; Yu, Q. Evolution in action: Plants resistant to herbicides. *Annu. Rev. Plant Biol.* **2010**, *61*, 317–347. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Warwick, S.I.; Xu, R.; Sauder, C.; Beckie, H.J. Acetolactate Synthase Target-Site Mutations and Single Nucleotide Polymorphism Genotyping in ALS-Resistant *Kochia* (*Kochia scoparia*). *Weed Sci.* **2008**, *56*, 797–806. [\[CrossRef\]](#)
63. Tranel, P.J.; Wright, T.R. Review Resistance of weeds to ALS-inhibiting herbicides: What have we learned? *Weed Sci.* **2002**, *50*, 700–712. [\[CrossRef\]](#)

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.