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Frankfurt/Main und Neustadt an der Weinstraße, 14. Juli 2020

Bezug: **Notifizierungsverfahren F 2020/280F**  
„vitro-Mutagenese Verfahren“

Sehr geehrte Frau Perlzweig

Der Conseil d'État (der Staatsrat) hat am 07.02.2020 das EuGH-Urteil (C-528/16) in dem 2015 durch Bauern-, Tier- und Umweltverbänden angestrebten Verfahren zur Einordnung von Mutageneseverfahren umgesetzt. In seinem Urteil (Nr. 388649)<sup>1</sup> folgte der Staatsrat weitgehend der EuGH-Entscheidung nach dem Pflanzen, die aus Mutageneseverfahren hervorgegangen sind, gentechnisch veränderte Organismen (GVO) darstellen und damit der Gentechnik- und Umweltschutzgesetzgebung unterliegen. Der Staatsrat weist die Regierung an, das Umweltgesetz (Art. D 531 innerhalb von 6 Monaten entsprechend anzupassen.

Frankreich hat der EU-Kommission inzwischen den Entwurf eines Dekrets mit dem Titel „über die Änderung der Liste der Verfahren zur Gewinnung genetisch veränderter Organismen, die herkömmlich angewendet wurden, ohne nachweislich die öffentliche Gesundheit oder die Umwelt zu schädigen“ entsprechend der Richtlinie (EU) 2015/1535 unter der Nummer 2020/0280/F<sup>2</sup> zur Notifizierung vorgelegt.

Mit dem Dekret soll Artikel D 531-2 des Umweltgesetzbuches entsprechend den Vorgaben des Staatsrates geändert werden. In Art.2, 2a wird vorgeschlagen:

„ a) Zufallsmutagenese, mit Ausnahme der In-vitro-Zufallsmutagenese, bei der in vitro kultivierte Pflanzenzelle chemischen oder physikalischen Mutagenen ausgesetzt werden.“

Dies würde bei einer Umsetzung des Dekrets bedeuten, dass

- 1.) zwischen *in vivo*- und in *in vitro*-Zufallsmutagenese unterschieden wird und diese Mutagenesen rechtlich unterschiedlich eingeordnet werden

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<sup>1</sup> Urteil: <https://www.conseil-etat.fr/ressources/decisions-contentieuses/dernieres-decisions-importantes/conseil-d-etat-7-fevrier-2020-organismes-obtenus-par-mutagenese>

<sup>2</sup> Notifizierung: <https://ec.europa.eu/growth/tools-databases/tris/en/index.cfm/search/?trisaction=search.de-tail&year=2020&num=280&lang=DE>

2. Pflanzen, die durch Behandlung von pflanzlichen Zellen mit mutagenen Chemikalien oder ionisierenden Strahlen generiert wurden, in Frankreich als gentechnisch veränderte Organismen reguliert werden.

Eine Unterscheidung zwischen *in vivo* und *in vitro* Zufallsmutagenese ist wissenschaftlich nicht vertretbar. Die molekularen Mechanismen, die zu den Veränderungen in der DNA führen, unterscheiden sich in beiden Fällen nicht. Diese sind unabhängig ob der Einsatz der mutagenen Agenzien *in vivo* oder *in-vitro* erfolgt.

**Diese Unterscheidung im französischen Vorschlag erscheint willkürlich und entbehrt einer wissenschaftlichen Grundlage.**

Weder die Freisetzungsrichtlinie 2001/18/EC noch der EuGH unterscheidet bei den Mutageneseverfahren zwischen *in vivo* und *in vitro* Verfahren. Es wird lediglich von Mutageneseverfahren gesprochen, ohne diese genauer zu spezifizieren. Im EuGH-Urteil wird nur zwischen Mutageneseverfahren, die vor und nach Inkrafttreten der Freisetzungsrichtlinie angewandt wurden, unterschieden.

Der französische Vorschlag geht somit weit über das EuGH-Urteil hinaus und führt in Frankreich und den anderen Mitgliedsstaaten zu unterschiedlichen gentechnikrechtlichen Einordnungen von Pflanzen, die über *in vivo*- und *in vitro* Mutageneseverfahren gezüchtet wurden. Pflanzen, die durch Zufallsmutagenese generiert wurden und seit Langem eine „history of safe use“ aufweisen, werden in den Mitgliedsstaaten unterschiedlich reguliert. Die Umsetzung des französischen Vorschlags würde zwangsläufig zu Verwerfungen in der europäischen Gentechnikgesetzgebung führen und den Gedanken eines gemeinsamen Marktes mit gemeinsamen Regeln widersprechen.

Dies hat die EU-Kommission erkannt und deshalb die Europäische Behörde für Lebensmittelsicherheit (EFSA) mit der Erstellung einer wissenschaftlichen Stellungnahme zu *in-vivo* und *in vitro*-Mutageneseverfahren (Mandat-Nr. M-2020-016) beauftragt. Erwartet wird eine detaillierte Beschreibung der *in vivo* und *in vitro* angewandten Zufallsmutagenesetechniken und eine Beurteilung,

- ob die Arten der genetischen Veränderung, die durch zufällige Mutagenesetechniken induziert werden, je nachdem, ob die Technik *in vivo* oder *in vitro* angewandt wird, unterschiedlich sind,
- ob der molekulare Mechanismus, der den Techniken der zufälligen Mutagenese zugrunde liegt, je nachdem, ob die Techniken *in vivo* oder *in vitro* angewendet werden, unterschiedlich sind,
- ob *in vitro*-Zufallsmutagenese-Techniken im Vergleich zu *in vivo*-Zufalls-Mutagenese-Techniken als unterschiedliche Techniken anzusehen sind oder ob sie im Gegenteil als Kontinuum zu betrachten sind.

Die Stellungnahme (EFSA Q-2020-00445) soll bis Ende September 2021 fertiggestellt werden.

Sie wird die wissenschaftlichen Erkenntnisse zu *in vivo* und von *in vitro* Zufallsmutagenesen aufzeigen sowie ihre mögliche Einordnung bzw. Unterscheidung ermöglichen. **Deshalb sollte diese wissenschaftliche Stellungnahme abgewartet werden, bevor das französische Dekret in Kraft gesetzt wird.**

Der Hohe Rat für Biotechnologie wurde aufgefordert, eine Liste von *in vitro* Mutageneseverfahren zusammenstellen, die seit Langem angewandt werden und erfahrungsgemäß mit keiner Schädigung der „öffentlichen Gesundheit oder der Umwelt“ verbunden sind und Pflanzen aufzeigen, die entweder nach *in vivo* oder *in vitro* Zufallsmutagenese generiert und ohne entsprechend Sicherheitsbewertung in Verkehr gebracht worden sind. Diese Liste liegt aber nach unserer Kenntnis bislang nicht vor. Die ersten Versuche zur Erzeugung strahlen-induzierten Mutationen in Pflanzen (Mais) wurden bereits 1928 (Stadler, 1928) publiziert und im Laufe der Jahre weiteten sich die Anwendungen aus. *In vitro* Zufallsmutagenesen werden bei Pflanzen seit Langen erfolgreich und sicher durchgeführt. Erste Publikationen mit Selektion der pflanzlichen Zellen erscheinen bereits Ende der 1960er Jahre (Lescure, 1969). Ab 1973 werden auf diese Weise herbizidresistente Pflanzen generiert. Eine der bekanntesten ist der Imidazolinon-resistente Raps (Swanson, 1989), der seit 1995 in Kanada unter dem Handelsnamen Clearfield kommerziell angebaut wird (Tan et al., 2005). In vergleichbarer Weise wurden ab 1982

mit *in vitro* mutagenisierten Kulturen herbizid-resistenter Mais, Weizen und Reis generiert. In vielen Züchtungsgängen ist heute nicht mehr nachvollziehbar ob die Mutationen *in vivo* oder *in vitro* durchgeführt wurden. Dies sind nur einige Beispiele von Pflanzen, die lange vor Inkrafttreten der Freisetzungsrichtlinie generiert wurden und nun bei Umsetzung des Dekrets als GVO angesehen und vom Markt genommen werden müssen.

**Deshalb sollte auch hier der Bericht des Hohen Rates für Biotechnologie abgewartet werden, ehe der französische Vorschlag notifiziert wird.**

Insgesamt gesehen, würde die Umsetzung des französischen Dekrets zu einer Deharmonisierung des europäischen Gentechnikrechtes führen. Eine unterschiedliche Einordnung von Organismen, hier Pflanzen, in einzelnen Mitgliedsstaaten ist zu vermeiden und würde dem europäischen Gedanken eines gemeinsamen Marktes widersprechen.

Die Umsetzung des französischen Vorschlags würde bedeuten, dass Pflanzen, die durch Zufallsmutagenese generiert wurden und seit Langem eine „history of safe use“ aufweisen, in den Mitgliedsstaaten unterschiedlich bewertet werden. Als wissenschaftliche Vereinigung sind wir nicht unmittelbar an dem Marktgeschehen beteiligt, aber wir erwarten aufgrund der unterschiedlichen Einordnung der Pflanzen (Samen und Produkte) Verwerfungen in einem gemeinsamen europäischen Markt und die Entstehung von Handelsbarrieren einerseits im europäischen und andererseits im weltweiten Warenverkehr.

Aus den oben genannten Gründen sind wir, die beiden Vereinigungen, der Auffassung, dass die Notifizierung des französischen Dekrets in dieser Form nicht erfolgen sollte und damit einerseits eine EU-einheitliche Implementierung der Freisetzungsrichtlinie 2001/18/EC erfolgen kann und andererseits inhereuropäische Handelshemmnisse vermeiden werden.

Für Fragen stehen wir Ihnen gern zur Verfügung.

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In Kopie an: EU-Commissioner Stella Kyriakides

Anhang: Literatur

## Anhang 1: Literatur (Auswahl)

Ahloowalia B., Maluszynski M. & Nichterlein K. (2004): **Global impact of mutation-derived varieties**. *Euphytica* 135, 187–204 |

<https://doi.org/10.1023/B:EUPH.0000014914.85465.4f>

During the past seventy years, worldwide more than 2250 varieties have been released that have been derived either as direct mutants or from their progenies. Induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties. The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits, which limit their productivity or enhance their quality value. In this paper, the global impact of mutation-derived varieties on food production and quality enhancement is presented. In addition, the economic contribution of the selected mutant varieties of rice, barley, cotton, groundnut, pulses, sunflower, rapeseed and Japanese pear is discussed. In several mutation-derived varieties, the changed traits have resulted in synergistic effect on increasing the yield and quality of the crop, improving agronomic inputs, crop rotation, and consumer acceptance. In contrast to the currently protected plant varieties or germplasm and increasing restrictions on their use, the induced mutants have been freely available for plant breeding. Many mutants have made transnational impact on increasing yield and quality of several seed-propagated crops. Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture. Future research on induced mutations would also be important in the functional genomics of many food crops.

<https://link.springer.com/article/10.1023/B:EUPH.0000014914.85465.4f>

Anderson P.A. & Georgeson M (1989): **Herbicide-tolerant mutants of corn**. *Genome* 31, 994-999 | <https://doi.org/10.1139/g89-173>

Eight imidazolinone herbicide resistant corn cell lines were obtained from *in vitro* cell culture selections. Plants were regenerated from five of the lines and resistant progeny obtained from four. Of the four, one line showed cross resistance to a sulfonylurea herbicide (class A), while three lines were resistant only to imidazolinones (class B). The class A line and one class B line were characterized in detail. Line XA17 possessed a single semidominant gene for resistance and plants homozygous for the trait showed 300-fold resistance to imazaquin and 100-fold resistance to chlorsulfuron. Resistance was due to decreased herbicide sensitivity of acetohydroxy acid synthase (AHAS), the common site of action of the imidazolinone and sulfonylurea herbicides. Resistance was stable following four to six backcrosses to corn inbred lines. Line QJ22 (class B) plants homozygous for tolerance showed 30-fold resistance to imazethapyr and no resistance to imazethapyr and no resistance to chlorsulfuron. The biochemical mechanism of resistance for line QJ22 is presently not clear. *Key words*: corn, maize, herbicide tolerance, acetohydroxy acid synthase, imidazolinone, sulfonylurea.

<https://www.nrcresearchpress.com/doi/abs/10.1139/g89-173?journalCode=gen#.Xwl51OdCR3h>

Broertjes, C., Roest, S. & Bokelmann, G.S. (1976): **Mutation breeding of *Chrysanthemum morifolium* Ram. using in vivo and in vitro adventitious bud techniques**. *Euphytica* 25, 11–19 | <https://doi.org/10.1007/BF00041524>

During experiments, which are being carried out to study the factors which control the process of adventitious bud formation in vivo on detached leaves of *Chrysanthemum morifolium* Ram, adventitious shoots were produced from leaves, irradiated with 500 rad of X-rays. The most important but disadvantageous result was that the majority of the adventitious shoots proved to be of a chimeral nature and obviously developed from more than one cell.

An in vitro adventitious bud technique was developed using different types of explants. Pedicel segments regenerated the highest number of adventitious shoots and, moreover, they developed faster as compared to explants of young flower heads or leaves. The mutants produced by irradiating the various explants were almost exclusively of a solid (non-chimeral) nature. In addition, histological observations suggest that single epidermal cells are involved in the initiation of the adventitious shoot apices.

The optimum dose for mutant production is approximately 800 rad X-rays. Rather often, more than one phenotypically identical mutant was found, which was always derived from the same explant. They could for instance originate from a multi-apical meristem formed by a single mutated cell.

<https://link.springer.com/article/10.1007/BF00041524>

Broertjes C. (1976): **The development of {new} in vivo, and in vitro techniques of significance for mutation breeding of vegetatively propagated crops**, Association Euratom-ITAL, WAGENINGEN, The Netherlands

| [https://inis.iaea.org/search/search.aspx?orig\\_q=RN:6218175](https://inis.iaea.org/search/search.aspx?orig_q=RN:6218175)

Lescure, A.-M. (1969): **Mutagenèse et sélection de cellules d'acer pseudoplatanus L. cultivées in vitro**. *Physiologie végétale* 7: (3) 237-250.

Lescure A.-M. (1970): **Mutagenèse de cellules végétales cultivées invitro. Méthodes et résultats.** Bulletin de la Société Botanique de France, 117:sup2, 353-365, | DOI:10.1080/00378941.1970.10838870

Technics are now available for growing plant cell cultures in liquid suspensions. These methods allow to test the production and the selection of somatic plant cell mutants. Several physical and chemical mutagens have been tested, successfully in some cases. Selection technics of auxotrophic or prototrophic mutants have been studied by the use of selective synthetic media. Experimental production of auxin independent cell lines has been obtained by the use of nitrosoguanidine. For at least one of these lines the impact of the mutation is suggested to result of a molecular modification of an auxin-oxidase.

<https://doi.org/10.1080/00378941.1970.10838870>

Magha M.I, P. Guerche P., Bregeon M., M. Renard M. (1993): **Characterization of a spontaneous mutant tolerant to sulfonylurea and imidazolinone herbicides.** Plant Breeding 111, 132-141. | <https://doi.org/10.1111/j.1439-0523.1993.tb00619.x>

A spontaneous chlorsulfuron tolerant variant, RCS-5, was isolated from protoplast culture of rapeseed, *Brassica napus* L. The tolerance level of the mutant was due to mutation of a single dominant gene resulting in an alteration of the acetolactate synthase enzyme. Seedling growth bioassays applied *in vitro* or after seed-soaking treatment showed that the mutant was 250—500 more tolerant to chlorsulfuron than the sensitive control. Field tolerance was evaluated with commercial formulations of chlorsulfuron (Glean T), triasulfuron (Kéos), and metsulfuron methyl (Allié), and an imidazolinone imazamethabenz (Méganet). The results indicated that the mutant could tolerate a lower dose rate of sulfonylureas than the dose recommended for cereal culture. In contrast, the mutation provided a good protection against imazamethabenz. The possibility of using the protoplast-derived mutation in crop rotation and F1 hybrid seed purity assessment is also discussed. However, isolated lines of the mutant were found to be less productive and one week later than the sensitive parent.

<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0523.1993.tb00619.x>

Newhouse, K., Singh, B., Shaner, D. et al. (1991): **Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides.** Theoret. Appl. Genetics 83, 65–70 |

<https://doi.org/10.1007/BF00229227>

Three corn (*Zea mays* L.) lines resistant to imidazolinone herbicides were developed by *in vitro* selection and plant regeneration. For all three lines, resistance is inherited as a single semidominant allele. The resistance alleles from resistant lines XA17, XI12, and QJ22 have been crossed into the inbred line B73, and in each case homozygotes are tolerant of commercial use rates of imidazolinone herbicides. All resistant selections have herbicide-resistant forms of acetohydroxyacid synthase (AHAS), the known site of action of imidazolinone herbicides. The herbicide-resistant phenotypes displayed at the whole plant level correlate directly with herbicide insensitivity of the AHAS activities of the selections. The AHAS activities from all three selections have normal feedback regulation by valine and leucine, and plants containing the mutations display a normal phenotype.

<https://link.springer.com/article/10.1007/BF00229227>

Newhouse K., Smith W.A., Starrett M.A., Schaefer T.J., Singh B.K. (1992): Tolerance to imidazolinone herbicides in wheat. Plant Physiol 100:882–886.

An imidazolinone-tolerant wheat (*Triticum aestivum* L. em Thell) mutant in the winter wheat cultivar Fidel has been identified and characterized. The mutant was isolated from a population derived through seed mutagenesis of the variety with an aqueous solution containing sodium azide. Imidazolinone-tolerant wheat seedlings were selected from the M<sub>2</sub> generation of the population in the presence of imazethapyr herbicide and identified as herbicide-insensitive individuals. The trait is inherited as a single semidominant gene and confers high levels of tolerance to imazethapyr. Acetohydroxyacid synthase activity in extracts from imidazolinone-tolerant plants was less inhibited by imazethapyr than the enzyme from the wild type. The herbicide-tolerant plants have a completely normal phenotype and display no negative effects on growth and yield in either the absence or presence of imazethapyr.

<http://www.plantphysiol.org/content/plantphysiol/100/2/882.full.pdf>

Stadler, L. J. (1928): **Genetic Effects of X-Rays in Maize.** PNAS, 14 (1), 69-75

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1085350/pdf/pnas01813-0080.pdf>

Stadler, L. J.(1928): **Mutations in Barely induced by X-rays and radium.** Science 68, Issue 1756, 186-187 | DOI: 10.1126/science.68.1756.186

<https://science.sciencemag.org/content/68/1756/186>

Pfenning M., Palfay G. & Guille T. (2008): **The CLEARFIELD® technology – A new broad-spectrum post-emergence weed control system for European sunflower growers.** Journal of Plant Diseases and Protection, Special Issue XXI, ISSN 1861-4051.

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in Europe with a total planted area of about 9.2 million hectares in 2006. Weeds are a major production problem in sunflower cultivation. Sunflower is a poor competitor during the early growth stages until canopy closure. Therefore, weeds compete successfully during these growth stages for light, water and nutrients. Limitation of available herbicides, especially herbicides to control broadleaf weeds, causes considerable yield losses to sunflower producers. The CLEARFIELD technology has been developed in sunflower to allow the use of imidazolinone herbicides as a post-emergence weed control option. The mode of action of imidazolinone herbicides is the

inhibition of the enzyme acetohydroxyacid synthase (AHAS). While conventional sunflower is sensitive to imidazolinone herbicides, CLEARFIELD sunflower hybrids have been modified to survive an otherwise lethal application of these herbicides. The trait for tolerance to imidazolinone herbicides in CLEARFIELD sunflower goes back to a naturally occurring mutation in the AHAS gene detected in a wild population of *Helianthus annuus*. This technology does not involve the introduction of foreign genetic material from other sources and thus is characterized as a non-GMO (genetically modified organism) process. CLEARFIELD herbicides provide exceptional foliar and soil activity to control a broad spectrum of weeds occurring across regions and cropping systems where sunflowers are produced.

<http://www.ask-force.org/web/HerbicideTol/Pfenning-CLEARFIELD-technology-Sunflower-2008.pdf>

Predieri, S. (2001): **Mutation induction and tissue culture in improving fruits.** *Plant Cell, Tissue and Organ Culture* 64, 185–210 | <https://doi.org/10.1023/A:1010623203554>

This review describes *in vitro* mutation induction methods in fruits and the *in vitro* selection procedures available for early screening. Results obtained through *in vitro* mutation techniques, including somaclonal variation, are reviewed and compared with the current achievements and future prospects of transgenic breeding. Plant improvement based on mutations, which change one or a few specific traits of a cultivar, can contribute to fruit improvement without altering the requirements of fruit industry. Induced mutations have well defined limitations in fruit breeding applications, but their possibilities may be expanded by the use of *in vitro* techniques. Tissue culture increases the efficiency of mutagenic treatments for variation induction, handling of large populations, use of ready selection methods, and rapid cloning of selected variants. Molecular techniques can provide a better understanding of the potential and limitations of mutation breeding e.g. molecular marker-assisted selection, which can lead to the early identification of useful variants. The relatively high number of research reports compared with the low number of cultivars released suggests that mutagenesis in combination with tissue culture is either ineffective or has yet to be exploited in fruits. Positive achievement recorded in other species seem to support the hypothesis that *in vitro* mutation induction has high potential also for fruit improvement. The possible contribution of a well-pondered and coordinated use of the numerous mutation induction, mutant selection, and field validation procedures available to advances in fruit breeding is discussed.

<https://link.springer.com/article/10.1023/A:1010623203554>

Radin D.N. and Carlsson P.S. (1978): **Herbicide-tolerant tobacco mutants selected in situ and recovered via regeneration from cell culture.** *Genet. Res. Camb.* 32, 85-89.

The herbicides Bentazone and Phenmedipham kill the leaves of intact tobacco plants but do not affect callus cultures. Tolerant mutants were isolated by treating leaves of previously  $\gamma$ -irradiated haploid plants with herbicide then excising and culturing the green herbicide-resistant cell clones on the otherwise yellowed leaves. Among plants subsequently regenerated were a total of ten stable independently isolated mutants. Sexual crosses show these ten represent four Bentazone and two Phen-medipham loci; all mutants were recessive to wild type.

[https://www.cambridge.org/core/services/aop-cambridge-core/content/view/583BFC4080A1C14DE2417ADC6392C66D/S0016672300018553a.pdf/herbicide\\_tolerant\\_tobacco\\_mutants\\_selected\\_in\\_situ\\_and\\_recovered\\_via\\_regeneration\\_from\\_cell\\_culture.pdf](https://www.cambridge.org/core/services/aop-cambridge-core/content/view/583BFC4080A1C14DE2417ADC6392C66D/S0016672300018553a.pdf/herbicide_tolerant_tobacco_mutants_selected_in_situ_and_recovered_via_regeneration_from_cell_culture.pdf)

Sung Z.R. (1976): **Mutagenesis of cultured plant cells.** *Genetics* 84, 51-57.

Experiments were designed to study the effectiveness of the chemical mutagens ethylmethane sulfonate and nitrosoguanidine on plant cells growing in liquid suspensions. Mutation frequency was defined as the number of colonies appearing on selective plates divided by the number of colonies growing on non-selective plates. The compounds tested usually increased mutation frequency by one order of magnitude over the spontaneously occurring rate, although the increase ranged from one to 140-fold. Cell killing was found to be directly correlated with mutation frequency.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1213564/>

Swanson E.B. et al. (1989): **Microspore mutagenesis and selection: Canola plants with field tolerance to the imidazolinones.** *Theoretical Applied Genetics*, 78: 525-530.

In vitro microspore mutagenesis and selection was used to produce five fertile double-haploid imidazolinone-tolerant canola plants. The  $S_2$  plants of three of the mutants were resistant to at least the field-recommended levels of Assert and Pursuit. One mutant was tolerant to between five and ten times the field-recommended rates of Pursuit and Scepter. Two semi-dominant mutants, representing two unlinked genes, were combined to produce an  $F_1$  hybrid which was superior in imidazolinone tolerance to either of the heterozygous mutants alone. Evaluation of the mutants under field conditions indicated that this hybrid and the original homozygous mutants could tolerate at least two times the field-recommended rates of Assert. The field results indicated the mutants were unaffected in seed yield, maturity, quality and disease tolerance. These genes represent a potentially valuable new herbicide resistance system for canola, which has little effect on yield, quality or maturity. The mutants could be used to provide tolerance to several imidazolinones including Scepter, Pursuit and Assert.

<https://link.springer.com/article/10.1007/BF00290837>

Tan S. et al. (2005): **Imidazolinone-tolerant crops: history, current status and future.** *Pest Management Science*, 61: 246-257 - DOI: 10.1002/ps.993.

Imidazolinone herbicides, which include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin, control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS). AHAS is a critical enzyme for the biosynthesis of branched-chain amino acids in plants. Several variant AHAS genes conferring imidazolinone tolerance were discovered in plants



through mutagenesis and selection, and were used to create imidazolinone-tolerant maize (*Zea mays*L), wheat (*Triticum aestivum*L), rice (*Oryza sativa*L), oilseed rape (*Brassica napus*L) and sunflower (*Helianthus annuus*L). These crops were developed using conventional breeding methods and commercialized as Clearfield\* crops from 1992 to the present. Imidazolinone herbicides control a broad spectrum of grass and broadleaf weeds in imidazolinone-tolerant crops, including weeds that are closely related to the crop itself and some key parasitic weeds. Imidazolinone-tolerant crops may also prevent rotational crop injury and injury caused by interaction between AHAS-inhibiting herbicides and insecticides. A single target-site mutation in the AHAS gene may confer tolerance to AHAS-inhibiting herbicides, so that it is technically possible to develop the imidazolinone-tolerance trait in many crops. Activities are currently directed toward the continued improvement of imidazolinone tolerance and development of new Clearfield\* crops. Management of herbicide-resistant weeds and gene flow from crops to weeds are issues that must be considered with the development of any herbicide-resistant crop. Thus extensive stewardship programs have been developed to address these issues for Clearfield\* crops.

<https://naldc.nal.usda.gov/download/6812/PDF>

Tan S. and Bove S.J. (2012): **Herbicide-tolerant crops developed from mutations.** *In* Plant mutation breeding and biotechnology, Eds Shu QY et al, ISBN 9781780640853

<https://www.cabi.org/VetMedResource/ebook/20123349362>