AGRONOMY SCIENCE
wcześniej – formerly
Annales UMCS sectio E Agricultura

VOL. LXXIV (2) 2019

 http://dx.doi.org/10.24326/as.2019.2.1

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Cisgenesis as a novel prospect for crop improvement. A review

Cisgeneza jako perspektywa dla hodowli roślin. Praca przeglądowa

Summary. Nowadays, the development of new biotechnological methods is necessary to satisfy requirements of market to produce enough good-quality food. Application of novel scientific approaches can be of great importance for improving the quality and quantity of plant crops. However, the most efficient strategies are based on genetic modification, which is still very controversial issue. GMO opponents do not accept the use of genetic engineering in crop improvement and production of new varieties suited for organic agriculture. Major discussion among various scientific and social issues concerns the possibility of existence of unintended effects of GMO both on human and world safety. Political, ethical, and social fears are related mostly to the best known transgenic approach, which is 'transgenesis'. Novel strategies and techniques are therefore required in the development of genetically engineered crops of the future. Nowadays, a new plant breeding technique, called 'cisgenesis' is intensively studied. In this paper, we review the most common strategies for crops improvement and describe cisgenesis as an alternative to transgenesis for safe and eco-friendly agriculture.

Key words: cisgenesis, genetically modified plants, plant crops improvement, transgenesis

INTRODUCTION

Present development of biotechnology brings a number of new useful tools in crop breeding. However, most of them are based on genetic modification [Jacobsen and Schouten 2009]. The best known and the most frequently rejected by society technique is transgenesis, which involves incorporation of sequence derived from a non-crossable species and might lead to creation of new gene pool. Formation of new plants that have combinations of genes from different organisms that cannot be crossed by natural means is a major reason of public concern. Recently, a new transformation concept that belongs to NBT (New Breeding Techniques), known as cisgenesis, has been proposed in response to this controversial public opinion. Contrary to transgenesis, cisgenesis implies that plants are transformed only with their own genetic material or genetic material from closely related species capable of sexual hybridization. It is possible that cisgenesis will be significantly more acceptable to the public as it is more 'natural' than the creation of transgenic organisms [Russell and Sparrow 2008, Edenbrandt et al. 2018].

CISGENESIS

Nielsen [2003] was the first who put GM (genetically modified) plants in different categories based on the phylogenetic distance between recipient organism and DNA donor. He suggested that ecological evaluation of genetically modified plants should be undertaken according to these categories. In 2006, three Dutch researches (H.J. Schouten, F.A. Krens and E. Jacobsen) introduced cisgenic concept with high expectation that cisgenic crops will be more acceptable to the public [Schouten et al. 2006]. However, the first idea of cisgenesis appeared few years earlier, in 2000 and was published in the book ”Toetsen en begrenzen. Een ethische en politieke beoordeling van de moderne biotechnologie” by Jochemsen and Schouten [Jochemsen and Schouten 2000]. The proposed main cisgenesis principle was that the genes or gene elements should be derived from the species that was to be genetically modified itself. Additional sequences, including introns or the regulatory sequences originating from the same gene as the coding sequences, were not necessary. However, in 2006, Schouten et al. published a new definition that became internationally established and is valid till today. According to this, the cisgene is a naturally existing gene derived from a gene pool of sexually compatible species and it is an identical copy of the endogenous gene in a sense orientation, including introns and flanking regions such as promoter and terminator. Although, for cisgenesis *in vitro* rearrangements are not permitted, if transformation is based on *Agrobacterium* method, T-DNA border sequences can also be introduced [Holme et al. 2013a]. Along cisgenic approach, intragenic concept exists, which also implies the use of DNA that is derived from the sexually compatible gene pool; however, sequence rearrangements are allowed, meaning that regulatory and encoding sequences may originate from different genes [Holme et al. 2013b]. In some cases cisgenesis meets barriers that limit its wider application. Variability in gene expression could be observed due to random insertion of the cisgene in the host genome. Negative position-dependent epigenetic regulation may take place [Cardi 2016]. Further, random integration of cisgenes can potentially cause the interruption and silencing of resident genes or other relevant sequences. For these reasons, the selection of regenerated plants having insertion sites with the best expression of the cisgene and minimal side effects is required. Random cisgene integration is comparable with random insertion that occurs in transgenic GM varieties, natural transposons and induced translocations [Keller et al. 2001, Schmidt 2002]. Additional issue is the number of gene copies and vector backbone sequences transferred into the recipient plant. Schouten et al. [2006] indicated that cisgenic transformation through *Agrobacterium* may lead to introduction of small, non-coding sequences from the vector such as T-DNA borders. In fact, up to 80% of plants regenerated from cisgenic transformation experiments indicated the integration of vector backbone sequences [Jo et al. 2014, Vanblaere et al. 2014]. However, the T-DNA borders comprise of non-coding sequences and are unlikely to have a phenotypic effect. Furthermore, some studies indicated that several DNA sequences identical to T-DNA borders have been identified within plants [Rommens et al. 2004, Conner et al. 2006]. For the purpose of obtaining 'cleaner' genetically modified plants, selection for the backbone free plants or the use of minimal linear cassettes and biolistic gene delivery may be applied [Cardi 2016].

CISGENESIS VS. CLASSICAL BREEDING

Both traditional breeding and cisgenesis are restricted to gene transfer within the same or between evolutionarily close species (Fig. 1). Therefore, cisgenes belong to the gene pool of traditional breeding [Jacobsen and Schouten 2009]. However, cisgenic approach is characterized by a number of advantages when compared to conventional breeding methods. Mainly, classical backcross breeding is time-consuming and the process of gene transfer can lead to introduction of additional genetic material (linkage drag). If the gene of interest is genetically tightly linked to one or more undesired genes, the breeding process can be significantly delayed. Plant breeders try to reduce linkage drag usually through backcrossing with the cultivated plant and subsequent selection to generate a genotype, in which the gene of interest is no longer linked to any deleterious genes [Schouten et al. 2006]. However, linkage drag is sometimes so tightly linked to the gene of interest that recombination between this gene and linkage drag is almost impossible. In consequence, undesired genetic material encoding for inferior properties may cause the backcrossed line useless [Holme et al. 2012]. In contrast, cisgenesis is faster and more precise tool for genes transfer between related species than classical backcross breeding. In cisgenic approach, only the gene of interest originating from the donor plant is inserted into the recipient plant. Therefore, this one-step process avoids the linkage drag problem. This can be perceived as a "clean" introgression breeding with insertion of only the target gene [Schouten et. al. 2006, Jacobsen and Schouten 2009].

Cisgenesis is a particularly valuable tool in situations, where more than one gene from different relatives must be introduced into recipient plant in order to obtain e.g. durable multi-gene resistance [Schouten et. al. 2006]. Another potential advantage of cisgenesis compared to conventional breeding is the higher knowledge of transferred sequences [Cardi 2016]. Moreover, a higher expression level of a trait can be achieved by insertion of an additional copy of a cisgene – the new sequence may be inserted in recipient's genome not once, but several times. That might cause changes in gene expression and, therefore, plant phenotype. Thus, cisgenesis can be compared to gene duplication, which is a common natural event, for instance in the case of resistance genes [Bergelson et al. 2001].

CISGENESIS VS. TRANSGENESIS

Recombinant DNA technology gives an opportunity to modify plants with one or more genes to induce the expression of additional valuable traits, but also transfer alleles between compatible species or between species that are impossible or difficult to cross. Depending on the nature and origin of the transferred DNA, it is possible to obtain transgenic or cisgenic plants [Lombardo and Zelasco 2016]. Cisgenic organisms are clearly different from transgenic ones. The crucial difference is the source of the gene [Schouten et al. 2006]. In transgenic approach, the incorporated genes and control sequences are exogenous. Transgenes usually originate from an alien species that is neither the recipient species nor a close, sexually compatible relative. Many transgenes derive from viruses, bacteria and other non-crossable plant species. Transgenic approach may involve the introduction of sequences in both sense and anti-sense orientation. Any artificial combination of encoding sequence and a regulatory sequence (e.g. promoter from another gene) is permitted as well as the utilization of a synthetic gene. Transgenes might provide new agricultural traits such as resistance to herbicides, insects and viruses. Moreover, transferred genes are also used as selection markers (e.g. antibiotic and herbicide resistance genes) during transformation process. Transgenes are the oldest type of molecularly isolated genes available for GM plant breeding [Jacobsen and Schouten 2009]. In contrast, by definition, cisgenesis concerns introduction of natural gene from a sexually compatible plant. Such a gene includes all its native exons and introns as well as its native regulatory sequences (promoter and terminator) in the normal sense orientation. Cisgenic plants might have one or more cisgenes, but they cannot contain any exogenous sequences. Therefore, unlike transgenesis, cisgenesis respects species barriers [Schouten et al. 2006]. A further issue is a genetically modified pollen flow. This phenomenon and the constant increase of GM acreage [James 2014] will make it difficult to ensure the absolute (100%) purity of organic crops in the future, which is a mandatory requirement of organic farming [Lombardo and Zelasco 2016]. Fundamental difference between cisgenic and transgenic approach is the change in the gene pool of the recipient species (Fig. 1).

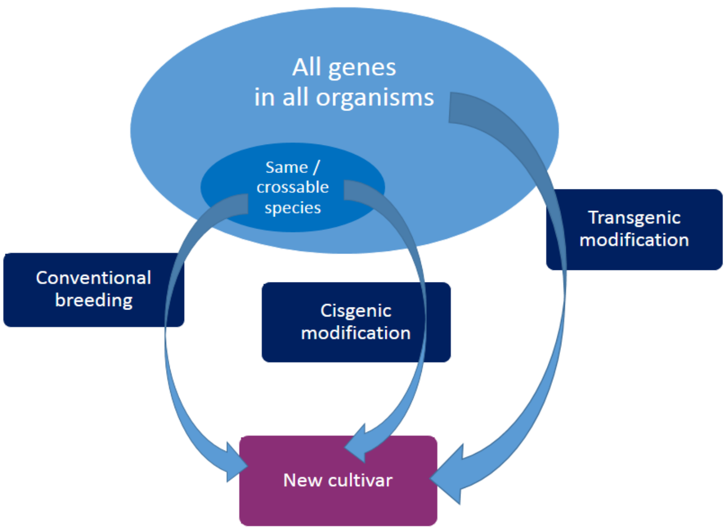

Fig. 1. Major crop improvement concepts

Transgenesis can extend this gene pool – it may lead to formation of a novel gene, which might provide new trait that does not occur in the recipient species in nature or cannot be introduced through traditional breeding. These novel traits might affect the usefulness of the recipient species in various ways. A major concern respecting transgenic plants is the unintentional spread of the new genes from cultivated plants to their wild relatives and its subsequent impact on the ecology of wild plants and their associated flora and fauna, potentially creating shifts in natural vegetation [den Nijs et al. 2004]. Contrary, cisgenes do not alter the gene pool of the recipient species and provide no additional traits such as herbicide or antibiotic resistance. These genes have undergone natural gene evolution. Cisgenesis causes no changes other than those, which can be introduced through traditional breeding or natural gene flow. Moreover, there is no risk of side effects on non-target organisms or soil ecosystems [Schouten et al. 2006, Jacobsen and Schouten 2009]. Considering transformation techniques, the same methods for both cisgenesis and transgenesis are applied. For this reason, the risk linked to transfer technology similar for both cisgenesis and transgenesis as random introduction of one or more genes into a plant genome, might be a reason of unintended effects occurrence [Hou et al. 2014]. To overcome negative side effects of transformation and/or regeneration, precise selection followed by breeding programs similar to traditional strategies need to be done [Lusser et al. 2011].

ACHIEVMENTS OF CISGENIC APPROACH

There are several examples of successful use of cisgenesis in crop improvement. Among plants subjected to cisgenic approach, cereals, potato, grapevine, apple or poplar can be mentioned (reviewed in Holme et al. 2013b, Cardi 2016). However, one should bear in mind that in order to be correctly classified as cisgenic, the plant should not contain any foreign genetic elements (such as CaMV promoter or *nptII* selectable marker gene) [Cardi 2016].

The first truly cisgenic plant was reported in 2011 by Vanblaere et al., and it was a cisgenic apple carrying a resistance gene against apple scab – a disease caused by pathogen *Venturia inaequalis.* Apple scab greatly impairs the worldwide production of apples. Protection against it requires frequent fungicides applications. More than 15 different resistance genes to this disease have been detected in apple species. Genes conferring high level of disease resistance are often found in wild *Malus* accessions [Kost et al. 2015]. These resistance genes can be introduced into modern cultivars by means of traditional breeding. However, conventional breeding of apple species is a very long process. It took 50 years to introduce the apple scab resistance gene into modern varieties and to remove the majority of unwanted genes (alleles) from *M. floribunda* by traditional methods. Obtaining resistant plants using cisgenesis is much faster. Vanblaere et al. [2011] generated cisgenic apple plants by introducing the endogenous apple scab resistance gene *Rvi6* (formerly *HcrVf2*) under the control of its own regulatory sequences into scab susceptible cultivar Gala. There were some earlier studies of genetically modified apples that carried native target genes, however, as they contained selectable marker genes (for example *nptII*) and/or regulatory sequences such as CaMV (cauliflower mosaic virus) 35S promoter, they are/should be correctly referred to as 'transgenics' [Barbieri et al. 2003, Belfanti et al. 2004, Szankowski et al. 2009, Joshi 2010]. More recently, molecular characterization of truly cisgenic apple plants previously produced and expressing the

Rvi6 scab resistance genes has been reported in detail [Vanblaere et al. 2011], while new cisgenic apples with the same trait have been developed using an alternative recombinase system [Würdig et al. 2015].

Furthermore, marker-free cisgenic potato (*Solanum tuberosum*) plants with blight resistance genes from *S. stoloniferum* (*Rpi-sto1*) and *S. venturii* (*Rpi-vnt1.1*) have been obtained by *Agrobacterium*-mediated transformation. In mentioned study, no marker gene was used. The selection of transformed plants was performed only by the PCR. Due to the activity of both R genes that were introduced, cisgenic plants showing broad-spectrum of resistance to late blight could be selected [Jo et al. 2014]. Moreover, cisgenesis has been used in improvement of cereals. For instance, a marker-free cisgenic variety of barley (*Hordeum vulgare*) with improved phytase activity has been developed. It contained an extra phytase gene in order to enhance phosphate bioavailability by degrading the phytic acid enzymatically [Holme et al. 2012]. Improvement of nitrogen utilization was achieved in cisgenic barley overexpressing the cytosolic glutamine synthetase gene (*GS1*) [Gao et al. 2018]. Maltseva et al. [2018] reported cisgenic common wheat lines carrying class I chitinase gene expressing moderate resistance to leaf rust. Furthermore, Tamang et al. [2018] found a way to incorporate the rice blast disease resistance gene *Pi9* into exclusive rice variety *via* cisgenesis with no evidence of a selectable marker gene in the final product.

CONCLUSION

As we showed in this review, cisgenesis holds a great promise for crop improvement. There is a number of examples indicating great potential of cisgenic concept for increasing the plant resistance and crop quality. Based on presented knowledge, we consider that the cisgenic plants are more similar to plants derived by traditional breeding methods rather than transgenic plants. Cisgenic food and feed pose no danger of toxicity or allergy risk, other than those that can occur through classical breeding [Schouten et al. 2006]. Furthermore, cisgenic products may be even more safe than some products obtained by traditional breeding as in cisgenesis no linkage-drag occurs and no undesired deleterious genes are introduced [Jacobsen and Schouten 2009]. Since the same regulation system is being applied for cisgenic plants as for transgenic plants, the crop improvement *via* cisgenic approach is hindered. Researchers expect that in the future, cisgenic crops might be subjected to less rigid regulatory measures than transgenic crops [Holme et al. 2012]. Given a chance for further development, cisgenesis can greatly enhance the economic and environmental prospects of agriculture.

REFERENCES

Barbieri M., Belfanti E., Tartarini S., Vinatzer B.A., Sansavini S., Silfverberg-Dilworth E., Gianfranceschi L., Hermann D., Patocchi A., Gessler C., 2003. Progress of map-based cloning of the Vf-resistance gene and functional verification: preliminary results from expression studies in transformed apple. HortScience 38, 329–331.

Belfanti E., Silfverberg-Dilworth E., Tartarini S., Patocchi A., Barbieri M., Zhu J., Vinatzer B.A., Gianfranceschi L., Gessler C., Sansavini S., 2004. The *HcrVf2* gene from a wild apple confers scab resistance to a transgenic cultivated variety. Proc. Natl. Acad. Sci. U.S.A. 101, 886–890, https://doi.org/10.1073/pnas.0304808101.

Bergelson J., Kreitman M., Stahl E. A., Tian D., 2001. Evolutionary dynamics of plant R-genes. Science 292, 2281–2285, https://doi.org/10.1126/science.1061337.

Cardi T., 2016. Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding. Plant Breed. 135(2), 139–147, https://doi.org/10.1111/pbr.12345.

Conner A.J., Barrell P.J., Baldwin S.J., Lokerse A.S., Cooper P.A., Erasmuson A.K., Nap J.P.H., Jacobs J.M.E., 2006. Intragenic vectors for gene transfer without foreign DNA. Euphytica 154(3), 341–353, https://doi.org/10.1007/s10681-006-9316-z.

den Nijs H.C.M., Bartsch D., Sweet J., 2004. Introgression from genetically modified plants into wild relatives. CABI, Wallingford.

Edenbrandt A.K., House L.A., Gao Z., Olmstead M., Gray D., 2018. Consumer acceptance of cisgenic food and the impact of information and status quo. Food Qual. Prefer. 69, 44–52, https://doi.org/10.1016/j.foodqual.2018.04.007.

Gao Y., de Bang T.C., Schjoerring J.K., 2018. Cisgenic overexpression of cytosolic glutamine synthetase improves nitrogen utilization efficiency in barley and prevents grain protein decline under elevated CO_2. Plant Biotechnol. J. 1–13, https://doi.org/10.1111/pbi.13046.

Holme I. B., Dionisio G., Brinch-Pedersen H., Wendt T., 2012. A cisgenic approach for improving the bioavailability of phosphate in the barley grain, Isb News Report, Vol. March, 8–11.

Holme I. B., Wendt T., Holm P. B., 2013a. Current Developments of Intragenic and Cisgenic Crops. Isb News Report (July), http://www.isb.vt.edu/news/2013/Jul/HolmeWendtHolm.pdf.

Holme I.B., Wendt T., Holm P.B., 2013b. Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol. J. 11(4), 395–407, https://doi.org/10.1111/pbi.12055.

Hou H., Atlihan N., Lu Z.X., 2014. New biotechnology enhances the application of cisgenesis in plant breeding. Front. Plant Sci. 5, 389, https://doi.org/10.3389/fpls.2014.00389.

Jacobsen E. and Schouten H. J., 2009. Cisgenesis: an important sub-invention for traditional breeding companies. Euphytica 170:235–247, https://doi.org/10.1007/s10681-009-0037-y.

James C., 2011. Global Status of Commercialized Biotech/GM Crops: 2011. ISAAA Brief 43. ISAAA, Ithaca, NY.

Jo K.R., Kim C.J., Kim S.J., Kim T.Y., Bergervoet M., Jongsma M.A., Visser R.G., Jacobsen E., Vossen J.H., 2014. Development of late blight resistant potatoes by cisgene stacking. BMC Biotechnol. 14, 50, https://doi.org/10.1186/1472-6750-14-50.

Jochemsen H., Schouten H.J., 2000. Ethische beoordeling van genetische modificatie. In: Jochemsen, H. (ed.), Toetsen en Begrenzen. Een Ethische en Politieke Beoordeling van de Moderne Biotechnologie. Buijten and Schipperheijn, Amsterdam, , 88–95.

Joshi S.G., 2010. Towards durable resistance to apple scab using cisgenes. Wageningen University, Wageningen, PhD Thesis.

Keller B., Feuillet C., Messmer M., 2001. Genetics of disease resistance. Basic concepts and application in resistance breeding. In: Slusarenko A.J., Fraser R.S.S., van Loon L.C. (eds), Mechanisms of Resistance to Plant Diseases. Kluwer Academic Press, 101–160.

Kost T.D., Gessler C., Jänsch M., Flachowsky H., Patocchi A., Broggini G.A.L., 2015. Development of the first cisgenic apple with increased resistance to fire blight. PLoS ONE 10(12), 1–17, https://doi.org/10.1371/journal.pone.0143980.

Lombardo L., Zelasco S., 2016. Biotech approaches to overcome the limitations of using transgenic plants in organic farming. Sustainability (Switzerland) 8(5), 1–7.

Lusser M., Parisi C., Plan D., Rodríguez-Cerezo E., 2011. New plant breeding techniques: State-of-the-art and prospects for commercial development (JRC IPTS Report EUR 24760 EN). Seville: JRC, Institute for Prospective Technological Studies (IPTS).

Maltseva E.R., Iskakova G.A., Rsaliev A.S., Skiba Y.A., Naizabaeva D.A., Ismagulova G., Ismagul A., Eliby S., 2018. Assessment of cisgenic bread wheat lines carrying class I chitinase gene to leaf rust. J. Biotechnol. 280:S80-S81, https://doi.org/10.1016/j.jbiotec.2018.06.264.

Nielsen K.M., 2003. Transgenic organisms – time for conceptual diversification? Nat. Biotechnol. 21(3), 227–228, https://doi.org/10.1038/nbt0303-227.

Rommens C.M., Humara J.M., Ye J., Yan H., Richael C., Zhang L., Perry R., Swords K., 2004. Crop improvement through modification of the plant's own genome. Plant Physiol. 135, 421–431, https://doi.org/10.1104/pp.104.040949.

Russell A.W., Sparrow R., 2008. The case for regulating intragenic GMOs. J. Agric. Environ. Ethics 21(2), 153–181, https://doi.org/10.1007/s10806-007-9074-5.

Schmidt R., 2002. Plant genome evolution: lessons from comparative genomics at the DNA level. Plant Mol. Biol. 48, 21–37, https://doi.org/10.1007/978-94-010-0448-0_2.

Schouten H.J., Krens F.A., Jacobsen E., 2006. Cisgenic plants are similar to traditionally bred plants: international regulations for genetically modified organisms should be altered to exempt cisgenesis. EMBO Rep. 7(8), 750–753, https://doi.org/10.1038/sj.embor.7400769.

Szankowski I., Waidmann S., Degenhardt J., Patocchi A., Paris R., Silfverberg-Dilworth E., Broggini G., Gessler C., 2009. Highly scab-resistant transgenic apple lines achieved by introgression of *HcrVf2* controlled by different native promoter lengths. Tree Genet. Genomes 5, 349–358, https://doi.org/10.1007/s11295-008-0191-8.

Tamang T., Park J., Kakeshpour T., Valent B., Jia Y., Want G., Park S., 2018. Development of selectable marker-free cisgenic rice plants expressing a blast resistance gene *Pi9*. World Congress on *In vitro* Biology, 54, 544.

Vanblaere T., Szankowski I., Schaart J., Schouten H., Flachowsky H., Broggini, G.A. L., Gessler C., 2011. The development of a cisgenic apple plant. J. Biotechnol. 154, 304–311, https://doi.org/10.1016/j.jbiotec.2011.05.013.

Würdig J., Flachowsky H., Saß A., Peil A., Hanke M.V., 2015. Improving resistance of different apple cultivars using the *Rvi6* scab resistance gene in a cisgenic approach based on the Flp/FRT recombinase system. Mol. Breed. 35(3), 1–18, https://doi.org/10.1007/s11032-015--0291-8.

Financing source: RGH/DS4.

Streszczenie. W dzisiejszych czasach rozwój nowych technik biotechnologii jest konieczny, aby zaspokoić oczekiwania rynku dotyczące produkcji odpowiedniej ilości żywności dobrej jakości. Zastosowanie innowacyjnych metod skutkuje zwiększeniem wydajności oraz jakości plonu. Najbardziej efektywne programy hodowlane oparte są na modyfikacjach genetycznych, co w dalszym ciągu jest bardzo kontrowersyjną kwestią. Przeciwnicy GMO nie akceptują użycia inżynierii genetycznej w podwyższaniu plonu roślin oraz wytwarzaniu nowych odmian dostosowanych do rolnictwa ekologicznego. Kwestią sporną pomiędzy środowiskiem naukowym a mediami jest możliwość występowania niezmierzonych konsekwencji stosowania GMO zarówno dla zdrowia człowieka jak i środowiska naturalnego. Obawy polityczne, medialne oraz etyczne są głównie związane z transgenezą. Nowe strategie i techniki są wymagane w rozwoju genetycznie modyfikowanych roślin przyszłości. Obecnie dokładniejszym badaniom poddawana jest metoda zwana „cisgenezą". W tym artykule skupiono się na najbardziej powszechnych strategiach zwiększania plonu roślin oraz przedstawieniu cisgenezy jako bezpiecznej alternatywy dla transgenezy.

Słowa kluczowe: cisgeneza, rośliny genetycznie modyfikowane, zwiększanie upraw, transgeneza

Received: 3.03.2019
Accepted: 5.06.2019