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RAPESEED, ITS MODIFICATIONS AND DANGER OF DISSEMINATION IN THE NATURAL ENVIRONMENT

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ABSTRACT

Background. As a result of genetic research and intensive breeding works, rapeseed has become one of the most important oil and protein plants. In cultivation, conventional and genetically modified forms of the plant occur. As a heterogamous plant, it may cross with other related species and get across to the natural environment. Therefore, numerous researches have been carried out in order to evaluate the level of acceptable contamination of conventional rapeseed with the seeds of genetically modified rapeseed. There is a risk of contamination during cultivation, transport, and storage of seeds.
Aim of the study. The aim of the study was to evaluate the condition of rapeseed cultivation and the possibility of its dissemination in the natural environment as a result of cross-fertilization and seed contamination with other related species. In the first part of the work, economic importance and use value of rapeseed seeds is discussed. The main part of the work discusses the condition of genetically modified plant cultivation, with particular emphasis on rapeseed and the possibility of its spread in the natural environment.
Conclusion. On the basis of numerous studies, it was found that there is a high probability of genetically modified rapeseed getting across to the environment, as it crosses easily with related species. Moreover, adulteration of the sowing material of conventional rapeseed seeds with genetically modified seeds was found. The danger of reducing insect population, especially of Lepidoptera butterflies, was also pointed out. Therefore, there is a need to monitor rapeseed cultivation, especially genetically modified rapeseed, with the use of quantitative and qualitative analyses, legal regulations, invasiveness estimation and range of occurrence, as well as risk evaluation of it crossing with other plant species.

Key words: breeding, conventional rapeseed, dissemination in the environment, economic importance, genetically modified rapeseed

INTRODUCTION

In the last few decades, development on breeding works on rapeseed led to important breeding achievements and a rapid increase in the seed production of the species. The above success was reached thanks to the close relation between breeding and research on seed quality. Rapeseed (*Brassica napus* L. ssp. *oleifera* Metzg) belongs to the *Brassicaceae* family, earlier *Cruciferae.* The species came into existence as a result of the crossing in natural conditions of cabbage (*Brassica oleracea* L.) and agrimony (*Brassica comperstris* L.) (Rudko, 2011; Tańska 2003). Rapeseed seeds are important raw material for the production of biofuels, consumer and technical fats, as well as food and fodder proteins. Rapeseed oil is a source of energy in human diet, and deoiled seeds are used as animal fodder. Protein content in rapeseed seeds varies between 43% and

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49%, and crude protein content between 21% and 24% (Rosiak 2014; Rudko 2011) Thanks to intensive research, one- and double-zero rapeseed cultivars were attained, from the seeds of which oil is obtained with very good health-promoting properties. Rapeseed oil is distinguished from other plant oils by the highest concentration of essential fatty acids and has favourable ratio of omega 6 to omega 3 fatty acids (2:1) (Gugała *et al*., 2014; Krzymański *et al*., 2009; Rudko 2011). Rapeseed is also used for energy purposes. Methyl esters obtained from rapeseed oil may be used directly in diesel engines or as a diesel fuel component (Krzymański, 2005; Kupczyk *et al*., 2017; Malarz, 2008).

Economic importance and use

Rapeseed is an oilseed plant, grown and processed on a large scale, well adapted to the agro-climatic conditions of Poland (Wielebski *et al*., 2002; Tańska and Rotkiewicz, 2003; Kapusta, 2015). It constitutes between 95% and 97% of oilseed crops acreage. Accession of Poland to the European Union increased rapeseed production and the supply in the agri-food sector and the demand for the raw material for the production of biocomponents also increased. Rapeseed harvested areas increased from around 1 million (2001–2003) to around 2.5 million (2015–2019) as a result of a double increase in cultivation and harvest areas by about 30% (from 2.2 $t \cdot ha^{-1}$ to 2.9 $t \cdot ha^{-1}$). Also, the number of agricultural farms that grow rapeseed increased to over 90 000 in 2019 (Statistics Poland, 2020). Poland is, at present, one of the greatest rapeseed producers and processors in Europe. From 2007, it ranks third in rapeseed production and is the third greatest producer of rapeseed oil and meal in the European Union (10% of the share) (Rosiak, 2015; 2019). In 2019 in Poland, rapeseed cultivation area reached 875 000 ha, and average seed yields amounted to 2.7 $t \cdot ha^{-1}$ (Table 1).

Increase in rapeseed production caused rapid development in the processing of this raw material. The European Union’s policy on biofuels is responsible for such a dynamic development of the production and processing of the raw material. During the many years of demand for rapeseed oil in the food sector at the level of 400 000 tones, a significant demand was created for the raw material for biocomponents production. The “biofuels” directive specifies the consumption of liquid fuels at the level of 5.75% of the energy value of fuels in 2010 and indicates reaching 10% in 2020 (Rosiak, 2014; 2015; 2019; Kupczyk, 2017).

Table 1. Growth area, yield and harvest of rapeseed in the years 2010-2019 (Statistics Poland, 2020)

Years	Harvested area (thousand ha)	Yield ($t \cdot ha^{-1}$)	Harvest (thousand tons)
2010	946.1	2.36	2228.7
2011	830.1	2.24	1861.8
2012	720.3	2.59	1865.6
2013	920.7	2.91	2677.7
2014	951.1	3.44	3275.8
2015	947.1	2.85	2700.8
2016	822.6	2.70	2219.3
2017	914.3	2.95	2697.7
2018	845.0	2.57	2171.7
2019	875.6	2.71	2373.1
Average	877.3	2.68	2407.2

Rapeseed breeding development

The discovery of new genetic sources, learning how to inherit economically important traits, the practical use of the achievements of genetics and the latest methods of instrumental analysis have become the basis for great achievements in rapeseed breeding, especially thanks to the association of breeding with research. Rapeseed is a relatively young species, which appeared in Western Europe about 500 years ago. It was probably created as a result of the crossing between cabbage and agrimony. Primary forms of rapeseed were characterized by a high content of erucic acid and glucosinolates, which limited the possibility of their use in human nutrition and animal fodder. Crossing of winter rapeseed with spring rapeseed in the 1960s was the basis for qualitative breeding. Offspring of those crossings significantly increased the genetic variability of the starting material for doubly improved cultivar breeding. One of the characteristics of new cultivars was no-erosion of oil and low glucosinolate content in seeds. Oil from the seeds of those cultivars was enthusiastically received by nutritionists, since „...it meets the health requirements formulated by most scientific institutions...” (Krygier and Wroniak, 2002). Canadians pronounced the fatty acids of canola oils as “a mixture perfect for health, nutrition, and application in food” (Ackman, 1990). As a result of further long-time breeding studies in Poland and around the world, cultivars with seeds rich in high quality fat and protein were obtained (Rosiak, 2019). At present, in Poland, the EU countries, and Canada, only doubly improved cultivars are grown, the so-called double-zero „00” or canola, which are characterized by very low (0-2.0%) erucic acid content and harmful sulphur compound (alkene glucosinolates) concentration from 6.9 to 17.1 $\mu M \cdot g^{-1}$ dry fat-free seed matter. Acquiring doubly-improved cultivars made the oil obtained from them one of the most health-promoting in the group of plant oils (Krzymański *et al*., 2009; Gugała *et al*., 2014). Important element in rapeseed breeding is also yield increase through obtaining hybrid cultivars, which give 10-30% higher yield in comparison with the best population cultivars (Bartkowiak-Broda, 1998; 2005).

Genetically modifies rapeseed and the risk of its introduction into the natural environment

Genetically modified organisms (GMO) are one of the most important products of modern biotechnology. Introduced into cultivation, they gain more and more importance in the global nutrition system. They were created through genome changes as conscious human action. The reason for introducing a foreign gene into a plant was making it resistant to herbicides and diseases caused by bacteria viruses, or fungi. Moreover, such plants are resistant to insect pests. Thanks to the introduced modifications, it is possible to limit the use of chemical plant protection means, eliminate losses in crops, increase the efficiency of plants and food production, as well as decrease production costs and limit CO_2 emission to the atmosphere (Tsatsakis *et al*., 2017; Warmińska, 2017; Brookes, Barfoot, 2020). It is possible to obtain different plant species with improved chemical composition, which absorb poisonous substances from the environment and are a source for biomass or biofuel production, as well as may be used in the pharmaceutical and cosmetic industries (Malepszy, 1995; Mickiewicz *et al*., 2006; Ciepielewska, 2014; Kupczyk *et al*., 2017; Płaza *et al*., 2018). However, cultivation of genetically modified plants raises a lot of controversy (Maciejczak, 2006; Warmińska, 2017; Tsatsakis, 2017; Król *et al*., 2018; Manachini *et al*., 2018). Concerns apply mainly to the effect of those organisms on human and animal health, or their impact on the environment. Lacking proper control and released into the environment, they may cause unpredicted environmental and socioeconomic effects. Therefore, monitoring the environment is an element of biological security system, which is mentioned in the Cartagena Protocol on Biosafety (Journal of Laws, 2004) and in the Convention on Biological Diversity from Nairobi (Journal of Laws, 2002). It especially concerns heterogamous and entomophilous plants that cross easily with related species. According to the data by ISAAA (Global Status of Commercialized Biotech/GM Crops: 2019), in 2018 the cultivation area of genetically modified plants reached 189.8 million ha (Table 2). In comparison with the year 1996 (1.7 million), when first genetically modified (GM) plants were introduced, the area increased to 191.7 million ha in

2018. Among the 26 countries in which GM plants are grown, the greatest area is found in the USA (75 million ha; 40%), Brazil (50.3 million ha), Argentina (23.9 million ha), Canada (12.7 million ha), and India (11.6 million ha). About 51% of GM plants are grown on two continents in South and North America, 33% in Asia, 8% in Europe, and 8% in Africa. GM plants are also grown in the European Union (EU), among others in Spain (0.1 million ha) and Portugal (< 0.1 million ha) (Table 2). In those countries, in comparison with other countries in the world, their share is low. European Union countries, instead of growing genetically modified plants, import about 20 million tons of GM soybean and soybean meal per year (USDA, 2019), including Poland about 2 million tons of soybean meal (Ministry of Agriculture and Rural Development, 2019), which makes 900 thousand tons of pure soybean fodder protein. Import of this raw material in about 70% covers the national demand for protein raw materials. About 60% of the imported soybean is used for fodder production for poultry, 20% for flock, and 10% for cattle (Brzóska, 2016; Dzwonkowski *et al*. 2015). Import of genetically modified raw material should take place under strict control because there are reports of possible adulteration of seeds from conventional cultivation (Manachini *et al*., 2018; Rostoks *et al*., 2019).

In the world, among genetically modified plants, the ones that are grown the most are soybean (50% of the cultivation area), maize (31%), cotton (13%), and rapeseed (5%). In comparison with the growth area of those species in total (conventional and genetically modified plants), GM cultivars make 80% (cotton), 77% (soybean), 32% (maize), and 30% (rapeseed). Mainly plants with herbicide resistance are grown (HT): 47%. The „stack" type cultivars (41%), which have more than one type of trait modification (with resistance to pest and herbicide), are gaining more and more popularity (ISAAA 2018). According to estimate data by ISAAA (2020), an increase in the cultivation of genetically modified plants will still be observed in the world.

Table 2. Cultivation of genetically modified plants in 2018 (Source: ISAAA, 2019)

Country	Area (million hectares)	Plant species
1	2	3
USA	75.0	maize, soybean, cotton, rapeseed, sugar beet, burclover, papaya, squash, potato, apple
Brazil	51.3	soybean, maize, cotton, sugar cane
Argentina	23.9	soybean, maize, cotton
Canada	12.7	rapeseed, maize, soybean, sugar beet, burclover, apple
India	11.6	cotton
Paraguay	3.8	soybean, maize, cotton
China	2.9	cotton, papaya
Pakistan	2.8	cotton
South Africa	2.7	maize, soybean, cotton
Uruguay	1.3	soybean, maize
Bolivia	1.3	soybean

Table 2 continue

1	2	3
Australia	0.8	cotton, rapeseed
Philippines	0.6	maize
Myanmar	0.3	cotton
Sudan	0.2	cotton
Mexico	0.2	cotton
Spain	0.1	maize
Columbia	0.1	cotton, maize
Vietnam	<0.1	maize
Honduras	<0.1	maize
Chile	<0.1	maize, soybean, rapeseed
Portugal	<0.1	maize
Bangladesh	<0.1	eggplant
Costa Rica	<0.1	cotton, soybean
Indonesia	<0.1	sugar cane
Eswatini	<0.1	cotton
Total	**191.7**	–

In Poland, there is no cultivation of genetically modified plants. The Polish government aims at Poland to be "free from GMO". Such a provision is included in the last amendment to the act on micro-GMO (Act, 22.03.2018, http://orka.sejm.gov.pl/proc8.nsf/ustawy/1424_u.htm. In January 2018, The Ministry of Agriculture prepared a draft of amendments to the Act „on fodder", proposing a further extension of the moratorium on the coming into force of the ban on GM fodder use by further five year, that is until January 1st, 2024. (Ministry of Agriculture and Rural Development, 2018). The Ministry of Agriculture noted that there is no evidence of harmfulness of fodder made from GM plants to human and animal health, or on the natural environment (Report, 2008–2011, http://www.prezydent.pl/prawo/ustawy/podpisane/art,33,kwiecien-2018-r.html. Ban on fodder use Woźniak, Twardowski, 2018, http://www.minrol.gov.pl/Ministerstwo/Biuro-Prasowe/Informacje-Prasowe/O-alternatywie-dlaimportowanego-bialka-GMO.

In Poland in 2018, an act was adopted amending the act on microorganisms and GMO, in which it was decided that "GMO cultivation is only performed on the basis of an entry in the GMO Crops Register", and GM cultivation must be located at a distance of no less than 30 km from the border of a nature protection area (Act, 2018). GM plant cultivation not only in Poland, but also in other countries was and still is a subject of increased interest and control, especially in the context of new breeding techniques (NBT) (Zimny *et al.*, 2019). European Academies' Science Advisory Council (EASAC) introduced the term new breeding techniques as a wide range of techniques, which make precise, targeted changes possible (thus, they are different from previously produced GMO) (EASAC, 2015). Also, these techniques have significant potential for the sustainable intensification of agriculture and for ensuring food security. It is assumed that new breeding techniques can be used to develop a wide range of different products. They are often used in combination with traditional techniques. In the document, it was underscored that new breeding techniques continue to be under debate (European Commission, 2017). However, the application of NBT, for example in food or fodder production, has not been regulated in detail, in both EU and Polish regulations (Bujnicki, 2017). The EU Court of Justice (CJEU, 2018) issued a decision on the legal status of new GMO. It was concluded that all innovative genetic modification techniques lead to the production of GMO and must be subject to the binding EU regulations on GMO (Woźniak and Twardowski, 2018).

Modified rapeseed, like soybean, maize and cotton, is part of a plant group with the highest production of modified seeds (Brookes, Barfoot, 2020). Genetically modified rapeseed is obtained through the introduction to the genome of alien genes which code particular characteristics. Those genes may origin from genetically distant donors (Krzymański 2000; Niemirowicz-Szczytt 2000*;* 2012). In the world, transgenic rapeseed is grown on 24% out or 28 million hectares and adds up to 7.0% of all the cultivation of genetically modified plants (ISAAA Brief, 2018). In Canada, it has been grown since 1996 and constitutes 70% of the total area dedicated to rapeseed cultivation. Canada exports 8.6 million tons of GM rapeseed per year (http://www.canolacouncil.org). Moreover, transgenic rapeseed is approved for cultivation in the USA, Brazil, Argentina, Australia, and Chile (Table 2). Commercial transgenic forms of rapeseed are: lines with an increased content of lauric acid, resistant to bromoxynil, resistant to glyphosate and glufosinate, as well as numerous hybrids resistant to glufosinate and joined with fertility restoration system containing the pat gene, the male sterility gene *barnase*, and the fertility restoring gene *barstar* (Wiśniewska *et al*., 2006). In the European Union, it is not allowed to cultivate genetically modified rapeseed but rapeseed oil obtained from GM rapeseed, tolerant to herbicides, is allowed on the market, as well as products obtained with its use. All the allowed products which contain more than 0.9% of the GM component must be labelled, according to the Regulation number 258/97 (Official Journal of the European Union L 43). Labelling also applies to products derived and produced from GMO, fodder, fodder additives, flour, and seeds (Dzwonkowski *et al*., 2015).

Cultivation of modified rapeseed brings about many concerns (Sung *et al*., 2016; Khalid, 2017). Rapeseed is a heterogamous plant which crosses with weed. Numerous discussions have taken place on the level of admissible contamination of conventional rapeseed by the seeds of genetically modified rapeseed. There is a risk of contamination during growth, transport, or storage of rapeseed seeds. Numerous studies carried out by geneticists and breeders demonstrate great easiness of crossing between different rapeseed cultivars within the species, as well as between related species (Bartkowiak-Broda, 2005; Hüsken, Dietz-Pfeilstetter, 2007; https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=29878055). Rapeseed (*Brassica napus*, 2n = 38 chromosomes, genome AACC) originated from the crossing of agrimony (*Brassica rapa*, 2n = 20, AA) with cabbage (*Brassica oleracea*, 2n = 18, CC). It demonstrates relation to the species from the genus *Brassica (B. rapa, B. juncea, B. carinata*, and *B. nigra*) and other related genera. It crosses the most easily with agrimony and Indian mustard. The offspring of those crossings produces fertile seeds which may produce plants that can cross with other numerous species in the fields and cause the creation of the so-called super weeds (Wiśniewska *et al*., 2006). Hybrids *B. napus* x *B. rapa* often occur as contamination caused by over-dusting during seed production. Rapeseed as a partially homo- and heterogamous plant produces high amounts of pollen, which may be transported over long distances by insects (up to several kilometres) and by wind (up to 30-40 meters). Among the insects that carry rapeseed pollen, domestic and wild bees dominate (80–95%), as well as different bumblebee species. Butterflies and larvae are therefore exposed to GM rapeseed plants through nectar, pollen, and other plant parts (for example leaves). The consequence of the above may be a decrease in insect population and biodiversity (Manachini *et al*., 2018). Moreover, rapeseed is regarded as a species which was domesticated relatively recently, which is related to its potentially easy return to wild forms and settlement of ruderal places (Mc Naughton, 1995; Conner *et al.*, 2003). To germinate, rapeseed seeds do not require long dormancy period and may retain the ability to germinate in the soil for a long time: 70% of seeds after 1.5 years and 60% after 5 years. In Canada, already 20 years ago self-seeding rapeseed with concomitant resistance to glyphosate, glufosinate, and imidazolinone was identified in fields, and self-seeding plants were found on 11 different fields (Hall *et al.*, 2000). This demonstrates that crossing occurred between three cultivars tolerant to herbicides. Falling seeds retain the ability to germinate in the soil for a long time and may be a source of self-seeding and contamination of seed and production plantations. The spread of genetically modified rapeseed genotypes may also occur as a result of mechanical intermingling of seeds during harvest, storage, and transport (Rostoks *et al.*, 2018). Presence of genes from transgenic rapeseed in its wild-type counterparts was demonstrated in research carried out in Western Europe, Japan and China (Jorgensen *et al.*, 1999; Hüsken, Dietz-Pfeilstetter, 2007; Liu *et al.*, 2015; Liu *et al.*, 2018). In Poland, the danger is greater due to the fragmentation of agricultural farms. Plant breeders report that getting rid of rapeseed contamination is very difficult. This is evidenced by the persistence for many, even up to 25 years, of the contamination of non-erucic rapeseed with erucic rapeseed plants at the level of 3-4% (Krzymuski 2005, Warwick *et al.*, 2007). In Canada, in the sold conventional certified rapeseed seeds, up to 0.25% of GM products are detected, which demonstrates that it is difficult to maintain seed purity (Friesen *et al.*, 2003).

Currently, in environmental monitoring, various methods of detecting genetic modifications in rapeseed are used. These are qualitative PCR methods, methods based on the ELISA test, which allow for the identification of protein products of specific transgenes, or real-time PCR techniques. The development of methods that make it possible to determine the presence of genetic modification in a seed sample is of particular importance in the case of seed control in field cultivation, where plantations often become contaminated (Wiśniewska *et al.*, 2006, Warwick *et al*. 2007, Tsatsakis *et al.*, 2017, Rostoks *et al.*, 2019).

CONCLUSIONS

Rapeseed is one of the most important oil and protein plants in the world. Poland is in the group of countries with the highest production and processing of rapeseed seeds, which are an important raw material for the production of biofuels, consumer and technical fats, as well as food and fodder proteins. At present, rapeseed oil is considered to be one of the best and most health-promoting vegetable oils. It is distinguished by the highest concentration of essential unsaturated fatty acids and has a very favourable ratio of omega-6 to omega-3 acids. Methyl esters derived from rapeseed oil may be used directly in diesel engines or as a component of diesel fuel. In breeding stations, research is carried out in order to create cultivars with high quality values, resistant to environmental stress and with high yield-forming potential. In addition to traditional rapeseed cultivars, also its genetically modified cultivars are grown. Growing such cultivars brings some benefits to the global agriculture, although it also raises many doubts and concerns. Concerns result mainly from the possibility of releasing modified rapeseed into the environment and adulterating conventional crops of this plant. In addition, rapeseed has a high potential to hybridize with sexually compatible crops and its wild relatives. Possible adulteration of seeds during harvest and transport is also of concern. Therefore, the available quantitative and qualitative analyses should be used, which make it possible to monitor genetically modified rapeseed cultivars in the environment and may be useful in the implementation of biosafety projects and studies on the coexistence of different genetically modified and unmodified species.

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RZEPAK, JEGO MODYFIKACJE I NIEBEZPIECZEŃSTWO ROZPOWSZECHNIENIA W ŚRODOWISKU PRZYRODNICZYM

Streszczenie

W wyniku badań genetycznych i intensywnych prac hodowlanych rzepak stał się jedną z najważniejszych roślin oleisto-białkowych. W uprawie występują formy konwencjonalne tej rośliny oraz zmodyfikowane genetycznie. Jako roślina obcopylna może krzyżować się z innymi gatunkami pokrewnymi i przedostawać do środowiska naturalnego. Dlatego prowadzone są liczne badania nad oceną poziomu dopuszczalnych zanieczyszczeń rzepaku konwencjonalnego przez nasiona rzepaku genetycznie modyfikowanego. Istnieje bowiem niebezpieczeństwo zanieczyszczeń podczas uprawy, transportu czy przechowywania nasion. Celem pracy była ocena stanu uprawy rzepaku oraz możliwości jego rozpowszechnienia w środowisku przyrodniczym na skutek zapylenia krzyżowego, a także zanieczyszczenia nasion innymi gatunkami pokrewnymi. W pierwszej części pracy omówiono znaczenie gospodarcze i wartość użytkową nasion rzepaku. W zasadniczej części pracy przedstawiono stan upraw roślin modyfikowanych genetycznie, ze szczególnym uwzględnieniem rzepaku i możliwości jego rozprzestrzenienia w środowisku przyrodniczym. Na podstawie licznych badań wykazano, że istnieje duże prawdopodobieństwo przedostania się rzepaku modyfikowanego do środowiska, który łatwo krzyżuje się z gatunkami pokrewnymi. Stwierdzono ponadto zafałszowania materiału siewnego nasion konwencjonalnych rzepaku – nasionami genetycznie zmodyfikowanymi. Wskazano także na niebezpieczeństwo ograniczenia populacji owadów, głównie motyli Lepidoptera. Istnieje zatem konieczność monitorowania upraw rzepaku, szczególnie genetycznie modyfikowanego, za pomocą analiz ilościowych i jakościowych, regulacji prawnych, oszacowania jego inwazyjności oraz zasięgu występowania, a także oceny ryzyka na skutek przekrzyżowania z innymi gatunkami roślin.

Słowa kluczowe: hodowla, rozprzestrzenianie się w środowisku, rzepak konwencjonalny, rzepak modyfikowany genetycznie, znaczenie gospodarcze