

Full Length Research Paper

Glycosinolate changes in rapeseed varieties in advanced generations

Selim AYTAÇ^{1*}, Şahin GİZLENCİ², Emel Karaca ÖNER³ and Mustafa ACAR²¹Department of Field Crops, Faculty of Agriculture, Ondokuzmayis University, Samsun, Turkey.²Black Sea Agricultural Research Institute, Samsun, Turkey.³Department of Plant and Animal Production, Vocational School of Technical Sciences, Ordu University, Ordu, Turkey.

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The aim of this study was to determine the changes in glycosinolate levels that may arise from cross pollination in the advanced generations in rapeseed. With the greenhouse conditions in addition to two different locations, this study was carried out in 3 locations with two varieties and repeated for 3 years. As a result, the changes in glycosinolate levels in the advanced generations were statistically significant. Although, the increase in the amount of gluconinolate appeared in the 2nd and 3rd generations, it remained below 20 µmol/g.

Key words: Rapeseed, outcrossing, glycosinolate, generations, seed.

INTRODUCTION

Oilseed rape (*Brassica napus*) is an important oilseed crop in most countries and is cultivated for food, feed and non-food uses such as biofuels (Delourme et al., 2013). Rapeseed, is in the third place in the world oil seed production, and mustard cover many species that belong to the Brassicaceae family. Brassica species are important oil crops and several species are cultivated worldwide. *Brassica juncea*, *Brassica napus* and *Brassica campestris* are three widely cultivated species (Alan et al., 2014). It is possible to introgress genetic diversity and specific traits into *B. napus* canola from its progenitor species, *B. oleracea* and *B. rapa* (Bennett et al., 2012).

Glucosinolates are secondary metabolites which are sulphur containing are biosynthesized by plant species in

the order Brassicales (Zelmer et al., 2013). Change in glucosinolate content is principally under control of procreative developmental stages (Bhushan et al., 2013). Actual levels of total glucosinolates depend on variety and range from as low as 25 and up to 200 µmol/gram of rapeseed meal (Mavromichalis, 2013). The glucosinolates were reduced due to their negative impact on palatability and toxic effects in many livestock species (Canola Council of Canada, 2015). Canola meal must contain less than 30 µmoles of glucosinolates (Parsons et al., 2016). Breeding has been successful in reducing the glucosinolate content in meal (Savary, 2013). Canola is the genetically improved rapeseed that contains less than 30 µmoles per gram glucosinolates of seed dry matter

*Corresponding author. E-mail: selim@omu.edu.tr.

after oil extraction (Hashemi and Beiki, 2014).

Rapeseed is a self-pollinated crop; however, it has a 12 to 47% outcrossing rate (Williams et al., 1986; Becker et al., 1992). Beckie et al. (2003) identified the gene flow between commercial areas of varieties with different herbicide resistance properties by evaluating the gene flow data of *B. napus*. Many factors affect outcrossing between plants in fields, these are size of lands, plants fertility, environmental conditions and activity of insect pollinators (Biology Document BIO2007-0, 2012). Several insect pollinators visit canola, especially the honeybee which is one of the most efficient pollinator (Sayed and Teilep, 2013; Mahmoud and Shebl, 2014). Therefore, even if rapeseed cultivation is initiated in an area with "00" type (new type) varieties, using the same seed every year may cause cross pollination with the wild species or with other rapeseed varieties previously cultivated or still being cultivated in the region and lead to changes in the glycosinolate levels. Genetic differences were observed among F2 progenies of *B. napus/B. campestris* and their parents (Fayyaz et al., 2014). In the developed synthetic varieties, the decrease in the yield is lower than that in the hybrids. Therefore, it is possible for the producer to use it for several generations without changing the seed. In the case of deterioration in the quality of the seed in terms of the increase in glycosinolate as a result of outcrossing, the pulp obtained from the produced rapeseed will not be suitable for animal health.

There are abundant amounts of related species of rapeseed (cabbage and turnip) in the Çarşamba and Bafra Plains which have the potential to produce rapeseed in a wide area. It is important to determine whether the changes in glycosinolate levels formed in rapeseeds cultivated in regions where cake and turnip are abundant as a result of indehiscence or natural cross pollination with close-relative varieties reach a level that will threaten the animal health. This study was carried out to investigate whether there is a change in the amount of glycosinolate in advanced generations of rapeseed varieties produced under natural conditions.

MATERIALS AND METHODS

In this study, French originated Bristol synthetic variety and German originated Licrown synthetic variety were used as the materials.

A three-year research was carried out to achieve the aim of the study. The field phase of this three-year study covers the preparation of the material to be tested. The seed production phase of the study was carried out at three different locations. Two of these locations were at the field, whereas one of them was carried out at the greenhouse. Çarşamba and Bafra districts at Samsun city in Turkey, which have a wide range of rapeseed cultivation areas, were selected as the location of the field studies.

The research area soil in Bafra contains middle calcareous (3.9%), unsalted (0.017%), very high phosphorus (19.00 kg/da), middle clay (60%) and organic matter is less (1.70%). The Bafra district has a semi-arid climate with annual average minimum and maximum temperatures of 10.3 and 18.3°C, relative humidity of 74.8% and annual rainfall of 737.4 mm.

The research area soil in Çarşamba has middle clay (44%), mild alkalinity (pH 7.88), medium calcareous (10.50%) and salt-free (0.43%). Phosphorus content is high (16.35 kg/da) and organic matter is low (1.71%). The average annual total precipitation, temperature and relative humidity are 648.7 kg, 15.5°C and 74.4%, respectively

Both locations were carefully selected, ensuring that they contain abundant amounts of *Brassica* species (*B. oleraceae* and *B. rapa*) in the close vicinity. The third was the greenhouse application in order to prevent pollen dust from coming from outside. It was investigated whether the possible exchange of pollens between the rapeseeds planted here would have an effect on the changes in glycosinolate levels. The seeds were planted with four replications. The area of each plot was (5x2) 10 m². However, plot size was determined as 5 m² under greenhouse conditions. Row spacing was 20 cm and each plot consisted of 10 rows.

At the end of three years (three generations), glycosinolate levels were determined in 72 samples taken from 3 different locations, 2 varieties and 4 replications using NIRS device. The obtained results were subjected to analysis of variance according to randomized complete block experimental design (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Glycosinolate levels were determined in rapeseeds produced in different generations varying between 11.90 and 18.88 µmole/g. Although, the differences were statistically not significant, glycosinolate levels in rapeseeds produced under greenhouse conditions were lower than those produced under Bafra and Samsun conditions. This suggests that a small amount of pollen from the environment might be mixed. This result is in line with those stated by Williams et al. (1986) and Biology Document BIO2007-0 (2012).

In the examinations in terms of generations, it was found that 14.03 µmole/g glycosinolate level in the 1st generation had a statistically significant increase in the 2nd generation and reached 15.96 µmole/g. However, the increase ceased in the next generations and determined as 15.86 µmole/g.

Licrown variety (14.49 mmole/g) had a lower glycosinolate content as compared to that of the Bristol variety (16.08 mmole/g) This difference was also statistically significant ($P < 0.05$) (Table 1).

The glycosinolate levels in the seeds of Licrown and Bristol varieties increased parallel to each other in the second generation. However, in the next generation, glycosinolate level slightly decreased in Licrown variety, whereas increasing trend continued in Bristol variety (Figure 1). This difference in the trends was possibly due to the different rates of outcrossing in the varieties.

Glycosinolate ratios were found to be acceptable when Licrown and Bristol varieties were left to open pollination for three years. According to Stewart (2002), the pollination time of *Brassica* species is close to that of related species, which poses a risk of pollination with each other. However, it is clear that the probability of natural hybridization of *B. napus* ($2n = 36$ or 38) due to *B. rapa* ($2n = 20$), *B. oleraceae* ($2n = 18$) of different species and chromosome numbers is very low.

Table 1. The changes in erucic acid levels in rapeseeds produced in Central Black Sea Region which might be formed as a result of outcrossing with each other or the close-relative species in the flora*.

Generations	Varieties	Locations			Average
		Samsun	Bafra	Greenhouse	
1 st	Bristol	14.82	13.57	14.32	14.24
	Licrown	15.03	14.56	11.90	13.83
	Average	14.92	14.07	13.11	14.03 ^b
2 nd	Bristol	16.77	16.13	17.28	16.73
	Licrown	14.86	15.88	14.87	15.20
	Average	15.82	16.00	16.07	15.96 ^a
3 rd	Bristol	17.42	18.42	16.00	17.28
	Licrown	12.84	18.88	14.59	14.44
	Average	15.13	17.15	15.29	15.86 ^a
Average of locations		15.29	15.74	14.82	
Bristol					16.08 ^a
Licrown					14.49 ^b

LSD_{0.05} for generations =1.60

*The difference between the averages indicated by the same letter is statistically insignificant within the group.

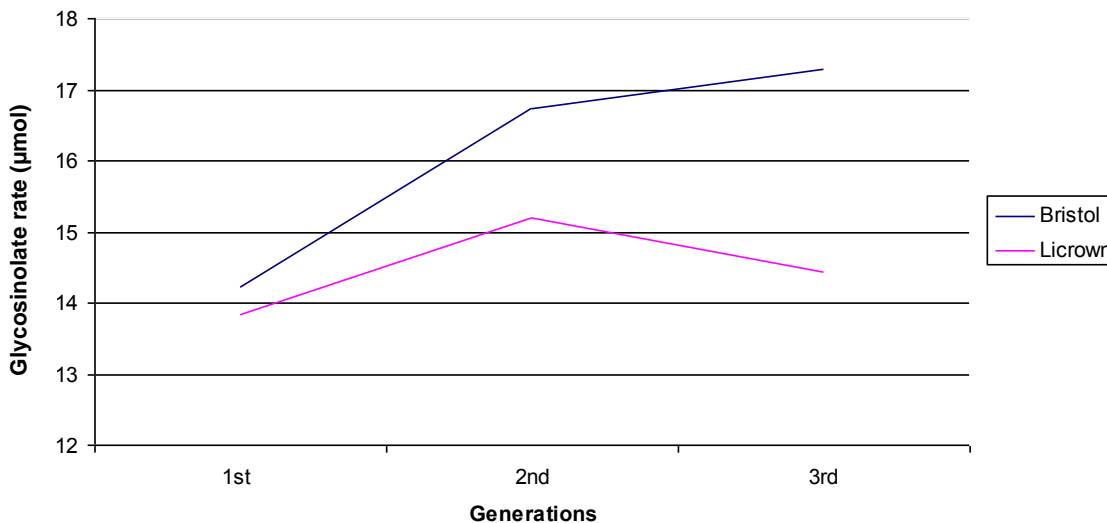


Figure 1. The effect of generation x species interactions on glycosinolate levels.

Conclusion

This study was carried out based on the fact that synthetic varieties have been used unchanged for a couple of generations by some farmers. Glycosinolate levels in the seeds in the advanced generations showed a slight increase; however, did not reach a level in the 3rd generation that will threaten the animal health. Therefore, these synthetic varieties can be used for 2 to 3 years. However, the necessity of changing the seed every year

in modern agriculture should not be overlooked.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alan MM, Nahar K, Hasanuzaman M, Fujita M (2014). Alleviation of

- osmotic stress in *Brassica napus*, *B. campestris*, and *B. juncea* by ascorbic acid application. *Biol. Plant.* 58(4):697-708.
- Becker HC, Karle R, Han SS (1992). Environmental variation for outcrossing rates in rapeseed (*Brassica napus*). *Theor. Appl. Genet.* 84:303-306.
- Beckie HJ, Warwick SI, Nair H, Séguin-Swartz G (2003). Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecol. Appl.* 13:1276-1294.
- Bennett RA, Seguin-Swartz G, Rahman H (2012). Broadening Genetic Diversity in Canola Using the C-Genome Species *Brassica oleracea* L. *Crop Sci.* 52:2030-2039.
- Bhushan G, Mishra VK, Iqbal M A Singh YP (2013). Effect of genotypes, reproductive developmental stages, and environments on glucosinolates content in rapeseed mustard. *Asian J. Plant Sci. Res.* 3(1):75-82.
- Biology Document BIO2007-01 (2012). The Biology of *Brassica juncea* (Canola/Mustard). A companion document to the Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits. <http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/brassica-juncea/eng/1330727837568/1330727899677>
- Canola Council of Canada (2015). Canola Meal Feeding Guide. 5th Edition. Canola Council of Canada, Winnipeg, MB. http://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf
- Delourme R, Falentin C, Fomeju BF, Boillot M, Lassalle G, André I, Duarte J, Gauthier V, Lucante N, Marty A, Pauchon M (2013). High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. *BMC Genomics* 14(1):120.
- Gomez AG, Gomez AA (1984). *Statistical Procedures for Agricultural Research*. 2nd Edition. A Wiley-Interscience Publication, Singapore.
- Hashemi SM, Beiki M (2014). The effect of canola meal processing by heat, moisture and ammonium bicarbonate on metabolisable energy and nitrogen retention in broiler chicken. *J. Anim. Poult. Sci.* 3(4):110-116.
- Fayyaz L, Rabbani F, Iqbal MA, Kanwal SM, Nawaz I (2014). Genetic Diversity Analysis of *Brassica napus/Brassica campestris* Progenies Using Microsatellite Markers. *Pak. J. Bot.* 46:779-787.
- Mahmoud MF, Shebl M (2014). Insect fauna of canola and phenology of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) as a key pest. *Redia* 97:125-132.
- Mavromichalis I (2013). Rapeseed meal in pig, poultry feeds, Geraadpleegd op 12 April 2013 via <http://www.wattagnet.com/articles/14938-rapeseed-meal-in-pig-poultry-feeds>.
- Parsons CE, Kelly J, Bacon R, Slaton N, Lorenz G, Kring T, Cartwright R (2016). Canola Production in Arkansas. <http://www.uaex.edu/publications/pdf/FSA-2154.pdf>
- Savary R (2013). Evaluating Canola (*Brassica napus*) Meal and Juncea (*Brassica juncea*) Meal With or Without Supplemental Enzymes for Two Commercial Strains of Laying Hens. Submitted in partial fulfillment of the requirements for the degree of Master of Science. Dalhousie University Halifax, Nova Scotia March 2013.
- Sayed AMM, Teilep WMA (2013). Role of natural enemies, climatic factors and performance genotypes on regulating pests and establishment of canola in Egypt. *J. Basic Appl. Zool.* 66:18-26.
- Stewart AV (2002). A review of Brassica species, cross-pollination and implications for pureseed production in New Zealand. *Agron. New Zealand.* 32:63-81.
- Williams IH, Martin AP, White RP (1986). The pollination requirements of oil-seed rape (*Brassica napus*). *J. Agric. Sci.* 106(1):27-30.
- Zelmer CD, McVetty PBE, Asif M, Goyal A (2012). *Molecular Genetics of Glucosinolate Biosynthesis in Brassicas: Genetic Manipulation and Application Aspects*, Crop Plant, Dr Aakash Goyal (Ed.), ISBN: 978-953-51-0527-5, Chapter 9.