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The Last Barrier for 00-type interspecific rapeseed (*Brassica napus* L.): Glucosinolates

Fatih SEYİS* Emine AYDIN
[a]Department of Field Crops, Faculty of Agriculture and Natural Sciences, Recep Tayyip Erdoğan University, Rize , Turkey
*Corresponding author: fatih.seyis@erdogan.edu.tr

Abstract

The biggest problem of the use of resynthesised rapeseed forms in quality breeding is their high glucosinolate content arising from the same character originating from the *B. oleracea* parent. Glucosinolates are sulphur- and nitrogen containing plant secondary matabolites common in the Brassicaceae and related plant families. The hydrolyzed products of glucosinolates, namely, isothiocyanates and other sulphur-containing compounds, were shown to interfere with the uptake of iodine by the thyroid gland, contribute to liver disease, and reduce growth and weight gain in animals. Consequently, plant breeders realized that if rapeseed (*Brassica napus* L.) meal was to be used in animal feed, the glucosinolate content had to be reduced. Up to now, interspecific rapeseed (*Brassica napus* L.) hybrids displaying low erucic acid quality were developed. But their glucosinolate content are high because of the *B. oleracea* parent. To introduce canola quality in RS-lines crosses with adapted material and subsequent backcrosses to resynthesized material are required, followed by recurrent selection for agronomic performance. A second approach should be the reduction of the glucosinolate content of the *B. oleracea* parent. Possible methods may be the irradiation of *B. oleracea* seeds or interspecific hybridization of *B. oleracea* with related Brassica species,because the selection of cabbage genotypes with low glucosinolate content may be the longer and deficienter way. Another method should be the cultivation of the low erucic acid genotypes *in vitro* since tissue culture cause as well known somaclonal variation, which may led to the breakdown of the high glucosinolate level.

Keywords: rapeseed, interspecific, hybrid, glucosinolates

00 tipi Türler Arası Melez Kolza (*Brassica napus* L.) Önündeki Son Engel: Glikosinolatlar

Özet

Kolza kalite ıslahında türler arası melez formların kullanılmasındaki en büyük problem *B. olearacea* ebeveyneinden gelen yüksek orandaki glukosinolat özelliğidir. Glikozinolatlar Brassicaceae ve akraba bitki familyalarından yaygın olarak bulunan, sülfür ve azot içeren sekonder metabolitlerdir. Glikosinolatların parçalanma ürünleri olan isotiyosiyanatlar ve diğer sülfür içeren bileşiklerin tiroid bezi vasıtasıyla iyot alımı etkilediği ortaya konmuştur, bu da karaciğer hastalığına katkı yapar ve hayvanlarda canlı ağırlık kaybına sebebiyet vermektedir. Sonuç olarak, hayvan yemi içersinde kolza (*Brassica napus* L.) küspesi kullanılacaksa, glikosinolat oranının düşürülmesi gerekmektedir. Şimdiye kadar, bitkisel yağ kalitesine sahip türler arası melez kolza (*Brassica napus* L.) formları geliştirilmiştir. Fakat glikosinolat oranları *B. oleracea* ebeveyninden dolayı yüksektir. Türler arası melz kolza hatlarına kanola kaliteini aktarabilmek için önce adapte edilmiş kolza materyali ile melezleme ve ileriki aşamalarda verim bakımından tekrarlamalı seleksiyona ihtiyaç vardır. İkinci bir yöntem, B. oleracea ebeveynindeki glikosinolat oranının düşürülmesidir. Muhtemel metodlar, *B. oleracea* tohumlarının radyasyona tabi tutulması veya *B. oleracea* 'nın diğer akraba Brassica formları ile melezlenmesidir, çünkü glikosinolat içeriği düşük lahana formlarının selekte edilmesi daha uzun ve zor yoldur. Diğer bir yöntem, düşük erusik asit içeriğine sahip genotiplerin *in vitro* olarak kültüre alınmasıdır, çünkü doku kültüründe somaklonal varyasyon oluşmakta, bu da belki glikosinolat seviyesinin kırılmasına sebebiyet verebilecektir.

Anahtar Kelimeler: Kolza, türler arası, hibrit, glikosinolatlar

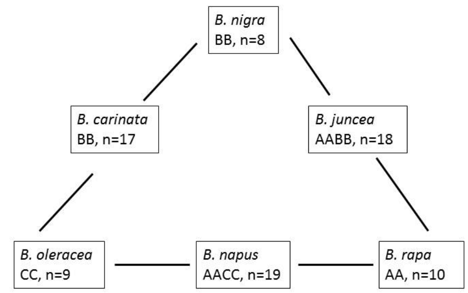
Introduction

The oilseed Brassicas are the world's third most important source of vegetable oils and their production has witnessed a steady upward movement during the past 25 years. During this period, the production share of European countries has also increased considerably, particularly after the introduction of double low (low erucic acid, low glucosinolate) cultivars. Oilseed rape (*Brassica napus* L.) is one of the world's principal oil crops. Its oil is used as a biofuel, for human consumption, for feeding animals, and in the chemical and pharmaceutical industries (Friedt and Snowdon, 2009). This recently developed species (during medieval times or earlier) most likely originated from independent and spontaneous inter-specific hybridizations between genotypes of turnip rape (*Brassica rapa* L.; AA, 2n = 20) and cabbage (*Brassica oleracea* L.; CC, 2n = 18) (Iniguez Luy and Federico, 2011).

Objective of this paper is to describe the last barrier in developing double quality resynthesised rapeseed – the glucosinolates.

Cytogenetic relationships between Brassica species

The chromosomal relationships among the A, B and C genomes of the diploid *Brassica rapa* (genome AA, 2n = 20; turnip rape, turnip, Chinese cabbage), *B. nigra* (genome BB, 2n = 16; black mustard) and *B. oleracea* (genome CC, 2n = 18; cabbage, cauliflower, broccoli, kale, kohlrabi, brussel sprouts) and their natural spontaneous amphidiploids *B. carinata* (genome AABB, 2n = 34; Abyssinian or Ethiopian mustard), *B. napus* (genome AACC, 2n = 38; oilseed rape, swede) and *B. juncea* (genome BBCC, 2n = 36; Indian or brown mustard) were elucidated through interspecific crosses and meiotic analyses made by the Asian cytogeneticists Morinaga and U in the early 20th century (Morinaga, 1933, 1934; U, 1935). Because the Brassica amphidiploid species can be generated synthetically with the help of embryo rescue techniques, this complex of the three diploid species and their polyploids (**Fig. 1**) is today one of the most useful model systems for investigations of polyploidy in crop plants (e.g. Song et al., 1995; Lukens et al., 2006). Colchicine treatment can also be used to artificially synthesize autotetraploid Brassicas, which can potentially be used to compare the corresponding effects of gene



Şekil 1: Brassica türleri arasındaki akrabalık ilişkileri

dosage, autoploidy, alloploidy and amphiploidy on gene regulation and expression (Snowdon, 2009).

Glucosinolates

As well known, glucosinolates are secondary plant metabolites: These metabolites are synthesized by species in the family Brassicaceae, including all of the *Brassica* crop species, related mustard crops and the model plant *Arabidopsis thaliana*. İn sixteen families of dicotyledonous angiosperms, including a large number of edible species more than 120 different glucosinolate compounds have been identified (Fahey et al., 2001).

High levels of glucosinolates are commonly present in rapeseed meal. These high levels are reducing feed intake and growth rate, inducing iodine deficiency, goitrogenicity and impairing fertility. Further, they can lead to liver, kidney and thyroid hypertrophy (Burel et al., 2000, Kermanshahi and Abbasi Pour, 2006, McNeill et al., 2004, Mawson et al., 1994a, 1994b; Schöne et al., 1997).

Besides these mentioned negative effects, certain degradation products such as isothiocyanate, exhibit strong anticarcinogenic properties (Keck and Finley, 2004).

To remove or reduce glucosinolate content in rapeseed meal in order to minimize glucosinolate-associated deleterious effects on animal health and production various processing techniques can be applied (Tripathi and Mishra, 2007). However, most of these methodologies include hydrolysis or decomposition of glucosinolate via heat treatment and the high energy costs that is needed mean that it is not economical to generate lowglucoinolate rapeseed meal from cultivars with high glucosinolate content. So it can said that the production of oilseed rape / canola meal is therefore limited to 00 varieties with low concentrations of total seed glucosinolates. In 1969 the Polish spring rape variety 'Bronowski' was identified as a low-glucosinolate form, and this cultivar provided the basis for an international backcrossing program to introduce this polygenic trait into high-yielding erucic acid-free breeding lines. Resultingly in 1974 of the first 00-quality spring rapeseed variety, 'Tower' was released. Today the overwhelming majority of modern spring and winter oilseed rape varieties have 00-quality ("canola"). However, residual segments of the 'Bronowski' genotype in modern cultivars are believed to cause reductions in yield, winter hardiness, and oil content (Sharpe and Lydiate, 2003), therefore finding new allelic sources for low-glucosinolate content are beneficial.

In *B. napus* it is possible to produce 'resynthesized' (Resyn) genotypes via an artificial cross between the parental species B. oleracea and B. rapa. Resyn rapeseed genotypes have been used for many years to broaden the genetic variation of oilseed rape: an overview of this strategy of introgression of single traits is given by Qiong et al. (2009). Becker et al. (1995) suggested the use of Resyn lines to establish a genetically diverse winter oilseed rape gene pool that can be used in hybrid breeding.

We know that only a few B. rapa and B. oleracea genotypes led to the first spontaneous *B. napus* in ancient times (Iniguez- Luy and Federico, 2011). Therefore the use of a broad range of B. rapa and B. oleracea taxa would increase the diversity in Resyn lines, and consequently, in the B. napus gene pool. Diversity in the B. napus gene pool is one requirement for successful hybrid breeding programs, based on the assumption of a positive correlation between heterosis and genetic distance (Falconer and Mackay, 1996). Different approaches have been used to broaden genetic variation in the *B. napus* gene pool with genetically distant material, such as Kebede et al. (2010), who described the introgression of winter rapeseed cultivars into the Canadian spring rapeseed gene pool. Zou et al. (2010) suggested exploiting intersubgenomic heterosis in *B. napus* through the partial introgression of subgenomic components from different Brassica species and Qian et al. (2009) analyzed Chinese semi-winter lines as distant parental lines in European winter oilseed rape hybrid programs.

The use of Resyn lines as hybrid parents and the resulting increase in hybrid yield due to heterosis was previously described for spring *B. napus* (Girke et al., 2001; Udall et al., 2004; Seyis et al., 2006) and winter oilseed rape (Girke et al., 2011). Nearly all of the Resyn lines in these studies originated from interspecific crosses of domesticated *B. rapa* and *B. oleracea* genotypes. However, the domesticated *B. oleracea* vegetable types had been selected for vegetable yield and quality, not for seed yield.

Results and Discussion

Detection of *B. oleracea* genotypes displaying edible oil quality

Low-erucic acid mutants were found in *B. rapa* (AA) (Downey, 1964), *B. napus* (AACC) (Stefansson et al., 1961; Stefansson et al., 1964) and *B. juncea* (AABB) (Kirk and Oram, 1978) considering 'double-low' seed quality (canola). First low erucic acid mutants of amphidiploid *B. carinata* (BBCC) were developed in the 1990's. Low-erucic acid forms of the monogenomic species *B. nigra* have not been detected yet.

B. oleracea genotypes displaying zero erucic acid character were first mentioned by Lühs et al. (2000). Seyis et al. (2004) described two of these genotypes, namely Ladozshkaya and Kashirka. Additionally, Seyis and Friedt (2010a) described the fatty acid composition of three *B. oleracea* genotypes including Kashirka and Ladozskaya; the name of the third accession was Eisenkopf. They identified three groups on the basis of erucic acid content: low, intermediate and high erucic acid group. These material can be used as a genetic resource for quality and yield improvement in oilseed rape breeding.

Resynthesised rapeseed (*B. napus* L.) displaying zero erucic acid content

Seyis et al. (2005) reported the existence of resynthesised rapeseed forms displaying low erucic acid content. The embryo rescue technique was used to develop amphidiploid RS-lines; the parents were the *B. oleracea* genotypes Kashirka and Eisenkopf and the *B. rapa* form Asko. Developed RS lines displayed a erucic acid content between 43.09 and 63.14 %. Additionally, Seyis et al. (2003) crossed two spring type (Reward and apetalous turnip rape) and two winter type *B. rapa* forms (Q3F and SWSP) with the *B. oleracea* genotypes Kashirka and Lazdozshkaya and totally 468 hybrids were obtained. Individual plants of different crossing combinations were analyzed for fatty acid composition and those erucic acid contents displayed near by zero (unpublished data).

Further, during a TUBİTAK project, Seyis et al. (2010b) developed 227 resynthesised rapeseed forms using the two cabbage accessions Kashirka and Ladozshkaya and the two inter *B. rapa* forms 15591 and 15080 displaying 00-quality. The developed RS lines obtained from different crossing combinations were used to develop rapeseed hybrids by crossing them with the male sterile lines MSL 004C, MSL 007C, MSL 501C and MSL 506C. The first two male sterile lines were winter forms and the last two lines were spring forms. Seeds were obtained from 19 hybrids based on the mentioned RS lines. The erucic acid content of the developed hybrids was was near zero and their oleic acid content ranged from 55.56 % to 73.14 %.

Conclusion

The use of resynthesised rapeseed forms in quality breeding is problematically because their high glucosinolate content arising from the same character originating from the *B. oleracea* parent. Crossing with adapted material and subsequent backcrossing to resynthesised material are required, followed by recurrent selection for agronomic performance, to introduce canola quality in RS-lines, (Girke et al*,* 2011).

B. oleracea genotypes displaying low glucosinolate content were not detected up to now. But considering the law of homologous series, existing *B. oleracea* material should screened continuously regarding this character.

Another approach should be the reduction of the glucosinolate content of the *B. oleracea* parent. Applicable methods may be the irradiation of *B. oleracea* seeds or interspecific hybridization of *B. oleracea* with related Brassica species, because the selection of cabbage genotypes with low glucosinolate content may be a long and uncertain approach since no such material has been detected so far.

Yet another method could be the cultivation of the low erucic acid genotypes *in vitro* since tissue culture causes the well known somaclonal variation, which may lead to a breakdown in the high glucosinolate level.

The presence of the three *B. oleracea* genotypes displaying low erucic content offers new possibilities to increase the yield performance of *B. napus*. As found by Seyis et al. (2005) spring rapeseed (*B. napus* L.) hybrids based on RS-lines are higher yielding compared with the presently breeding material.

The present *B. oleracea* material are dsiplaying winter character. Their selection regarding seed yield performance; the use of this material in developing new RS-lines will offer the way for the further yield improvement in *B. napus*. Because assumely the use winter types, will give rise to higher expected yield heterosis.

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