CMS/RF HYBRID SEED PRODUCTION SYSTEM FOR TURNIP RAPE

Niemelä Tarja[1], Seppänen Mervi[1], Jauhiainen Lauri[2] and Tulisalo Unto[1]

[1]Department of Agriculture, University of Helsinki, PO Box 27, 00014 Helsinki, Finland
e-mail: tarja.niemela@helsinki.fi
[2]MTT Agrifood Research Finland, Research Services, 31600 Jokioinen, Finland

Abstract

In spring turnip rape (*Brassica rapa* L.), one of the most promising F1 hybrid system, is the Ogu-INRA cytoplasmic male sterility and fertility restoring (cms/Rf) system. The Ogura cms has been introduced into spring turnip rape and the sterility has been stable. However, the Ogura fertility restoring gene (*Rfo*) is introgressed into C genome of oilseed rape (*Brassica napus* L.) and that has complicated its transferring into A genome of turnip rape. To establish a specific hybrid system for turnip rape, the Kosena fertility restoring gene *Rfk1*, homologue of the Ogura restorer gene *Rfo*, was transferred successfully from oilseed rape into turnip rape by traditional backcross method. The transmission rate of the *Rfk1* gene was 35% through the pollen cells and 33% through the egg cells. For the selection of homozygous (Rfk1/Rfk1) turnip rape plants, both testcrosses to male sterile (Ogura cms) turnip rape line and TaqMan based real-time qPCR method were used simultaneously. The TaqMan based qPCR analysis proved a useful method to select homozygous plants out of heterozygous ones before flowering stage. This enabled the interpollination among the selected plants instead of inbreeding, which is a benefit with cross-pollinating crops like turnip rape. During the subsequent selection of homozygotes, the instability of the *Rfk1* gene was observed. Before the commercial exploitation of turnip rape hybrids the *Rfk1* gene needs to stabilized.

Introduction

Spring turnip rape is the most important oilseed crop cultivated for production of vegetable oil and protein rich meal for animals in Finland. Turnip rape is early maturing and shows better yield stability in Northern climatic conditions than oilseed rape. To compete in higher seed yields with oilseed rape, the hybrid breeding has proved to be effective way in turnip rape to increase seed yields without lengthening the growing time (Niemelä et al. 2006). Turnip rape will also be an important choice of species in the future if the climate change lengthens the growing period in Northern parts of Scandinavia and enables the cultivation of oilseed crops in further North.

Several functional hybridization systems have been found in genus *Brassica*. One of the main systems used commercially is the Ogu-INRA cytoplasmic male sterility and fertility restoring (cms/Rf) system. The Ogura cms has been introduced earlier into spring turnip rape (Delourme et al. 1994) and the sterility has been stable. In F1 hybrids the pollen production has to be restored with a functional restorer gene. However, the introgression of the Ogura fertility restoring gene (*Rfo*) in C genome of oilseed rape (*Brassica napus* L.) has challenged its transferring into A genome of turnip rape and the commercial hybrids in turnip rape has not been produced. The proposed different location of the Kosena fertility restoring gene (*Rfk1*), a homologue of the Ogura fertility restoring gene (*Rfo*) (Brown et al. 2003), in Japanese oilseed rape breeding lines enables its possible introduction from spring oilseed rape into spring turnip rape. Both the Ogura and Kosena cms/Rf systems have been originally found and introduced from Japanese radish (*Raphanus sativus* L.) to oilseed rape.

Interspecific crosses and addition of alien chromosomes have been utilized by plant breeders in order to transfer desirable genes from one species to another especially in *Brassica*. To utilize alien chromosome additions in practice, stable introgression lines should be produced. Possible potential ways to achieve stable introgression are disomic additions, where two copies of a chromosome have been added to the genome of another species (Budahn et al. 2008) or homologous/homeologous recombination between *Brassica* genomes (Leflon et al. 2006). Crossing between oilseed rape (AACC allopolyploid) and turnip rape (AA diploid) is known to be usually successful and fertility of the hybrid is in most cases high (Leflon et al. 2006; Metz et al. 1997).

The aim of this study was to transfer the Kosena *Rkf1* gene from oilseed rape into turnip rape and to evaluate its potential as a functional cms/Rf hybrid system in turnip rape. Also the advantage of the

TaqMan based qPCR method in selecting homo- and heterozygotes in cross-pollinating crop was studied.

Material and methods

Transferring of the *Rfk1* gene from oilseed rape into turnip rape was done by backcrossing the homozygous Rfk donor plants (Plantech Res. Inst. Japan) with turnip rape breeding lines having Ogura cms. Reciprocal crossings were done during the backcross to test the transmission rate of the *Rfk1* gene. The presence of the Ogura cms and *Rfk1* gene were verified in every backcross generation by PCR. After six backcross generations inbreeding was carried out and the TaqMan based qPCR together with testcrosses to cms line were used to identify homozygous plants out of heterozygous ones.

Results and discussion

The transfer of the *Rfk1* gene from oilseed rape into turnip rape was successful. The transmission rate of the gene was not significantly affected weather it was done through the pollen or egg cells. The segregation ratio of fertile and sterile plants was approximately 30:70 in every backcross generation, instead of expected 1:1 ratio in a normal single dominant gene situation. This is most likely a result of an aneuploidy situation, where the additional radish chromosome having the *Rfk1* gene, has stayed unaltered in A genome background. During the subsequent selection of homozygous (Rfk1,Rfk1) plants testcrosses to Ogura cms plants were done. Testcrosses gave even 100% fertility results, which was a good indication, that turnip rape with Ogura cms can be restored with the *Rfk1* gene. However, during the subsequent selection of homozygotes, the instability of the *Rfk1* gene was observed. During the three generation of selecting homozygotes using the TaqMan based qPCR the proportion of heterozygotes settled in 10% and homozygotes in 90% (Table 1.). The TaqMan based qPCR analysis proved a useful way to select homozygous plants out of heterozygous ones before flowering stage. This enabled us to interpollinate the selected plants instead of inbreeding, which is a benefit with cross-pollinating crops like turnip rape. Before the commercial exploitation of turnip rape hybrids the *Rfk1* gene needs to be stabilized. Another possibility is to utilize this somewhat unstable restorer line in hybrid production with intensive nursing through the official seed production chain. Turnip rape has a small seed size, which makes the seed production very efficient and the generations of commercial seed production categories are only few. The preliminary hybrid yield trials where the commercial aspects of hybrid seed production is evaluated, are on-going. So far the hybrid system has been promising, since no negative effects can be found in seed quality parameters. Further research to reveal the instability problem has also been carried out.

Table 1. Fertility and zygosity results of turnip rape restorer line 4021-2 during three generation of selecting homozygous plants using TaqMan qPCR. In every generation 60-80 plants were analysed and the selected homozygous plants were interpollinated.

4021-2 Rf	1. generation of qPCR	2. generation of qPCR	3. generation of qPCR
Fertility %	93	100	100
Homozygotes %	67	90	90
Heterozygotes %	26	10	10
Steriles %	7		

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