

The Use of Molecular Tools for a Better Management of the French *Daucus* Genetic Resources Network

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Abstract

In numerous countries genetic resources are conserved in centralised gene banks, whereas in France specialised networks do their management. This original organisation allows sharing costs and working between each partner for maintenance and evaluation. Moreover, experts on the considered species manage each network. The French *Daucus* network, focusing on *Daucus carota* L., was created a few years ago. Its goal was to merge the independent *Daucus* germplasm collections belonging to different partners: several French seed companies, some botanical gardens and our institute. In order to be useful for researchers and breeders or other potential users, this national collection needs to be characterised, well conserved to preserve the genetic integrity of each accession, and evaluated.

Traditionally, morphological characters are used to characterise the genotypes, but they can be affected by environmental factors. To obtain a more reliable estimation of the diversity and genetic relationships, we developed molecular markers. They were shown to be useful not only for the characterisation of the accessions but also for genetic drift control during regeneration. To valorise this collection an evaluation of resistance to *Alternaria dauci* was also engaged. The efficiency of molecular markers linked to resistance genes is under evaluation.

INTRODUCTION

Molecular markers are increasingly used to assist the curator in acquisition, maintenance, characterisation and evaluation activities. They may be employed for the accurate identification of germplasm and for the identification of putative duplicates. They can also be useful for the choice of material when establishing a core collection or for the research of genes of interest among conserved germplasm (Ford-Lloyd et al., 1997; Hodgkin et al., 2001). Of course this list is not exhaustive. In our laboratory we already developed numerous molecular techniques to assess the genetic diversity of *Daucus* (Briard et al., 2000; Le Clerc et al., 2000). The present paper describes how molecular markers can be useful to improve the management of carrot and other *Daucus* genetic resources. Before giving some examples we will briefly describe the French organisation for the management of genetic resources and the French *Daucus* network.

SPECIES OR GROUP SPECIES NETWORK, AN ORGANISATION EXCLUSIVE TO FRANCE

In France concern about genetic resources led to the creation, in 1983 of the BRG, the "Bureau des Ressources Génétiques". This governmental organisation has to develop and manage a national policy for animal, plant and microbiological genetic resources, allowing the concertation between all partners. This management instrument, described in the National Charter, has to coordinate scattered actions. Concerning temperate plant species France developed a network organisation instead of creating centralised gene banks (BRG, 1995). Each network is in charge of one species or a group of species. Most of the time those networks associate private and

public partners. Each partner is an expert of the species. Their objective is to coordinate for the long term the setting up and management of an available national collection. This common management makes it possible to share costs and work for characterisation, conservation and regeneration of accessions. Until now about 30 networks were created. The BRG has to supply them with financial and scientific support. It is also in charge of the harmonisation between all the networks.

“Carrot and other *Daucus*” Network, Context of Creation

As for a lot of species, since a few years the demand of the carrot producers, distributors and consumers for more homogeneous and uniform varieties has resulted in an extreme reduction of the varietal types used. The genetic basis used for creation of those modern varieties, most of the time F₁ hybrids, is very narrow. In Europe more than 90 % of the registered new varieties are Nantaise types. To deal with this genetic erosion in France, where various local varieties or landraces were important a few decades ago, public and private partners established numerous collections at the national level. As those collections were established without preliminary consultation between partners, there are unavoidable duplicates. In order to have a common management of those collections under the aegis of BRG, our vegetable and seed crop unit at INH created the “Carrot and others *Daucus*” network. Partners are private societies, scientific organisations and other structures such as botanical gardens. From a practical point of view, in 1996 this group decided to set up a network collection in order to bring together the genetic resources belonging to each partner and to create, later, a national collection available for the general public. The accessions are cultivated carrots, wild types and related species of *Daucus*. In the near future the national collection will consist of 230 accessions. This genetic diversity will offer a great potential for carrot breeders, researchers and other users. However, preceding studies are required. Genetic resources are not sufficiently documented. Indeed, there is an imperative need to characterise and evaluate this material. Genetic resources also need to be well preserved. With this goal the network already achieved numerous projects, using molecular markers, as a help for better management of *Daucus* genetic resources.

MOLECULAR MARKERS FOR CHARACTERISATION AND REGENERATION OF *DAUCUS* GENETIC RESOURCES

Since the introduction of PCR in 1985 (Saiki, 1985) numerous molecular methods were developed offering new perspectives for genetic studies. As mentioned by Hodgkin et al. (2001) they have been used successfully to support work on the conservation and use of plant genetic resources. Therefore, we decided to adapt those techniques for the evaluation of *Daucus* genetic diversity (Le Clerc, 2001).

Characterisation of Genetic Diversity

Until now the registration of a new carrot variety by breeders or the description of an accession by curators relies on a morphological description. The characters that are described can be affected by environmental factors, and sometimes the identification of related genotypes is uncertain. Moreover, it is difficult to distinguish accessions with a different genome but showing an identical phenotype due to gene convergence. Therefore, the development of complementary analysis methods represents an interesting cause for the evaluation of diversity. As molecular markers were deemed to be helpful tools we tested the opportunity to use them as a complementary technique. For this reason we decided first to develop powerful tools and methods well adapted to carrot. We tested most of the classical molecular techniques and chose two of them: ISSR (Gupta et al., 1994; Zietkiewicz et al., 1994) and AFLP (Vos et al., 1995). They were chosen to reveal more polymorphism and to be easier to develop than the other molecular markers previously used for genetic diversity studies in carrot (isozymes, RFLP, RAPD, SSR...). They appeared to be

complementary techniques for the analysis of carrot genetic diversity. Indeed, a high diversity was detected between varieties with ISSR, whereas AFLP revealed a high intravarietal variability.

For the analyses of a large number of plant samples we needed to develop reliable and cost-effective analytical procedures. We first developed a satisfying AFLP protocol, based on the use of reduced quantities and on the improvement of silver nitrate staining (Briard et al., 2000). Then we developed an analytical method based on the idea that a precise estimation of intravarietal variability should provide a better estimation of genetic distances between genotypes and, therefore, a better estimation of the genetic diversity conserved in the collection. As recommended by several authors we checked if well-distributed mapped markers on a linkage map could be more efficient than randomly chosen markers to assess varietal identification of carrot genotypes. From our results, we suggested that mapped markers are not more informative than unmapped markers, the efficiency being dependent only on the number of markers investigated. We obtained a reliable estimation of intravarietal variability and, therefore, of genotype distance with 70 AFLP markers. Molecular markers were also useful to quantify the total diversity conserved in the collection. Indeed, the same high level of variability was found within cultivated carrots and within wild carrots. With only morphological markers we were not able to appreciate this level of variability.

Regeneration of Accessions

For efficient conservation of ex-situ diversity the genetic integrity of original accessions must be preserved. Within allogamous species small, finite populations are particularly vulnerable to a loss of diversity during the regeneration process. Carrot is an allogamous species. Most of the time, small mating populations are planted in insect-proof cages, due to lack of space and because regeneration is a cost-effective process. In order to develop a reliable regeneration process we tried to quantify the genetic drift by measuring shifts in allelic frequencies during the regeneration of one accession. Based on a new estimator of recessive allele frequency described by Jorde et al. (1999) we quantified those allele frequency shifts for different sizes of mating populations and two seed-harvesting procedures. On the one hand seeds were harvested from all plants and mixed (bulk sample). On the other hand, an equal number of seeds per plant were collected (balanced sample). This genetic-drift analysis was done with AFLP markers. According to our results, allelic frequency changes were higher for bulk samples than for balanced samples. We also found that a number of 80 equally harvested plants should limit genetic drift and will be effective in maintaining the genetic diversity of each carrot accession. From the present results the members of the network adopted a global strategy for the regeneration of carrot accessions. The regeneration of carrot accessions will be performed with 80 plants equally harvested. Indeed, for each partner the number of regenerations per year is limited (1 to 4). Therefore, the additional work for preparing balanced samples instead of bulk samples seems reasonable.

CONCLUSION

Maintaining collections of genetic resources is only interesting if accessions can be used. As we told previously, their use will be possible if the seed quality is well preserved and if the collection is well documented. This documentation should provide information to characterise the material and also evaluate it for interesting characters. Because *Alternaria dauci* is responsible for a very important foliage disease in carrot a research program was started. With the aim of developing a MAS strategy, ISSR and AFLP will be analysed on a segregant progeny. Each year 50 carrot accessions will be evaluated by our team for resistance to *Alternaria dauci*. If new sources of resistance can be discovered it will be useful for breeders and for our studies. For this reason, we set up a program to analyse the [determinism] determining factors of resistance

developed by some genotypes and we will try to find molecular markers linked to this character. Crosses between resistant and susceptible genotypes have been made to study the segregation in the progenies. Comparison between the levels of resistance observed for each recombinant F₂ and their molecular profiles will be done in order to detect possible linked markers. For this study ISSR and AFLP markers already mapped for carrot in our laboratory will be used.

In conclusion, ISSR and AFLP could be useful tools to assess *Daucus* genetic diversity. Seventy unmapped markers should give sufficiently accurate results for the estimation of the intravarietal variability. After being successfully used to assess the extent of genetic diversity in the collection and to develop a regeneration procedure, these molecular tools will be used for the evaluation of putative duplicates and to characterise all accessions in our collection in order to develop a database. This database should help curators when setting up field trials, by grouping genetically related accessions.

Those different examples show that molecular markers can be a helpful tool for the curator in many tasks but of course, they cannot replace the use of morphological markers. They should be considered a complementary tool for the management of genetic resources.

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