ABSTRACT

Due o it many uses, rapeseed (*Brassica napus* L) is considered a plant with high economic importance and now is the third oil source after soy and palm (Snowdon şi colab., 2007).

The PhD thesis titled **"Use of the molecular markers to identify some germplasm sources of rapeseed (*Brassica napus L.*) with genetic resistance to *Verticillium sp.*"** is divided into two parts and comprises seven chapters.

In the first part, it is presented the bibliographic study which comprises the description of the species *Brassica napus* and of the pathogen *Verticillium longisporum* and general aspects regarding the modern methods used in the oilseed rape breeding programs.

The second part presents an overview of natural and institutional condition of the researches, the description of the biological material, method used in the research and the obtained results.

Chapter I - **General aspects regarding the importance and the cultivation of winter oilseedseed** - includes an extensive documentary on the history, origin, importance, morphology and rapeseed cultivation technology.

Rapeseed (*Brassica napus* L) is a amphidiploid species that appeared a few hundred years ago by spontaneous hybridization between wild cabbage (*Brassica oleracea* L.) and turnip (*Brassica rapa* L.). Due to the various use of the oil in different was such us people feed, as an alternative source of biofuel or as a raw material in the chemical industry, oilseed rape has become one of the most cultivated oilseed crop. After oil extraction, the remaining residues are used in animal nutrition being an important sources of protein for the animal.

Chapter II - **Description of the *Verticillium longisporum* pathogen** – comprises an comprehensive documentation on the pathogen morphology, life cycle, mode of transmission of disease symptoms and yield loss produces in the rape culture.

Chapter III - **Use of modern methods in oilseed rape breeding** - is dedicated to a short presentation of the main modern methods used in the rape breeding programs. Also in this chapter is a comprehensive documentary regarding the rape resistance to the pathogen *V. longisporum*.

In chapter IV are presented the description of natural and institutional conditions where were conducted the researches. This chapter includes information on topography, vegetation and soil in the area of SCDA Secuieni research station, the characterization climatic conditions from the experimentation year and the structure of the LECOM laboratory.

Chapter V describes the objectives, the biological material and methods used in the experiments.

The aim of the study was to to identify sources of rape germplasm (*Brassica napus* L.*)* with genetic resistance to *Verticillium longisporum* pathogen, using molecular markers techniques.

For this purpose, there were established some objectives:

- the phenotypic evaluation of biological material by analyzing the main morphological characters in the field: plant height, the number of branches, the number pods per plant and MMB (mass of 1000 seeds);
- genetic evaluation of the 130 winter rape provided Dutch Centre for Genetic Resources (Centre for Genetic Resources Netherlands - CGN) using RAPD and SSR molecular markers and generate the dendrogram in order to determine the genetic similarity between these lines;
- identify cultivars sensitive/tolerant to the disease caused by *V. longisporum* pathogen;
- establishing correlations between the genetic structure and phenotypic evaluation of the genotypes using SSR molecular markers, genetic mapping and QTL identification (Quantitative Traits Loci);

In this chapter it has been also presented the protocols and the techniques used in the field and the laboratory work. There it can be read, the protocols used in the molecular analysis and the inoculation methods used to test the resistance of oilseed rape cultivars to the pathogen *V. longisporum*.

Chapter VI - **Results and Discussion** presents the results of research conducted during doctoral studies. Is divided into several chapters and presents the results of phenotypic evaluation of studied material by studying the main morphological characters, the genetic evaluation using molecular markers, artificial inoculation with the pathogen *V. longisporum*,

correlations between phenotypic data and the genotype.

- The obtained results after the phenotypic evaluation of the 130 rapeseed cultivars are:

- The plant height ranged between 144.97 cm and 59.99 cm with a mean value of 84.98 cm;

- The mean number of pods per plant varied between 1283 and 115 pods per plant with an average value of 394;

- The number of branches per plant ranged between 13.67 and 4 branches per plant with an average of 8.49 branches;

- MMB ranged between 7.29 g and 3.24 g with an average of 4.95 grams.

The genotypic evaluation of the biological material wad made using RAPD and SSR techniques For the RAPD analysis we used 20 decamer primers which amplified 301 fragments. The level of polymorphism in this case was between 29 and 90%.

For the SSR analysis, we used a total of 51 markers which amplified 139 fragments, the level of polymorphism being 100%.

Based on data obtained from each method it was calculated the genetic similarity (GS) between analyzed genotypes that concluded in two matrices which led to the generation RAPD and SSR dendrograms. Next, using Mantel test the two matrices were correlated in order to generate the third dendrogram (RAPD + SSR).

Analyzing the three dendrogram obtained it was observed that the genetic diversity between the studied cultivars was high.

In the RAPD dendogram, the 130 cultivars were grouped into six clusters, and genetic similarity coefficient ranged between 0.51 and 0.89;

In the SSR dendrogram , the genotypes were grouped into ten clusters, with a similarity coefficient between 0.61 and 0.89;

The dendogram RAPD and SSR formed four clusters, and the similarity coefficient had values between 0.61 and 0.89. It was interesting that in this dendrogram the cultivars "Libritta", "Skiverskii", "Lingora" and "Blagodatny", formed a separate sub cluster. This cultivars proved to be moore resistant to the infection test with *V. longispoum* this why there may be useful in future studies in choosing the parents for hybridization.

After the artificial inoculation of the studied cultivars with *V. longisporum,* we identified 21 genotypes which proved to be more resistant than the control „Express” (tolerant).

Based on the data obtained from artificial infection and the molecular data from the SSR analysis, we applied the ANOVA calculation method in order to identify those markers that can be associated with the resistance level to *V. longisporum*. In this manner, we identified 18 SSR markers associated with resistance to *V. longisporum.*

Using the molecular SSR data constructed a genetic map comprising 19 linkage groups with a distance of 520.3 cM. The distance between two chromosomes is 26 cM and the average distance between molecular markers is 10 cM.

The largest linkage group from this map is LG1 that has five SSR markers and a size of 57.7 cM and the smallest linkage group is LG4 with a size og 12.6 cM and two SSR markers.

Based on the molecular data and on the phenotypic data represented by AUDPC values calculated after the infection with *V..longisporum* we identified a QTL for resistance . This QTL is localized on chromosome 1 and has a LOD score of 3.4 and an R^2 value of 11.4%.

The last chapter of the thesis present the general conclusions and the references.

Keywords: **molecular markers, *Verticillium longisporum*, resistance, QTL, genetic map.**