УДК 573.6:631.527.8:635.345

MOLECULAR MARKERS IN WHITE CABBAGE BREEDING FOR FUSARIUM WILT RESISTANCE

Radkevich Elena Victorovna, *Postgraduate student of the Department of Botany, Breeding and Seed Production of Horticultural Crops, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, radkevich. elena. vi@gmail. com*

Supervisors: Monakhos Sokrat Grigorievich, *Head of the Department of Botany, Plant Breeding and Seed Technology, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, smonakhos@gmail.com*

Gotovtseva Irina Petrovna, *Head of the Department of Foreign Languages, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, gotovtseva_irina(a)mail. ru*

Abstract: *Obligatory stage in breeding for resistance to phytopathogenic diseases is the selection of resistant plants using an artificial inoculation. Molecular genotyping for resistance loci allows to simplify and accelerate the germplasm screening for resistance and the selection of resistant genotypes in segregating populations. In this paper, the results of Fusarium wilt resistant loci DNA-markers effectiveness and new DNA-marker development are presented.*

Keywords: *molecular marker, resistance, susceptibility, Fusarium wilt, B.oleracea, F.oxysporum.*

Fusarium wilt is one of the three most harmful diseases of white cabbage and other varieties of Brassica oleracea L species [1]. The disease leads to a significant loss of yield (50% or more) and to decrease in product quality in susceptible Fl-hybrids. There is no literary information about the spread of the causative agent of fusarium wilt in Russia, but in practice it is known, that the disease occurs in all fields where vegetable and oilseed cabbage crops are grown. Selection for resistance is the only method to protect plants from this disease, as there are no effective agrotechnical and chemical methods of plant protection against F. oxysporum f. sp. conglutinans. Despite the fact that donors of resistance to F. oxysporum are known and resistant cultivars and hybrids have been developed on their basis, selection of resistant genotypes to develop new resistant F 1-hybrids of cabbage is of great importance. [1]. Increasing the effectiveness of selection of resistant genotypes is possible through the use of molecular markers. However, the DNA markers often offered in open press are ineffective, so the need to develop new markers is urgent.

The aim of our work is to search for and develop molecular markers of the locus of resistance to fusarium wilt of cabbage.

Evaluation of the effectiveness of the markers presented in the literature was carried out using 5 resistant and 5 susceptible fusarium wilting white cabbage pure lines (B.oleracea). A mapping population of BC1 was obtained by crossing a resistant Biib5-103 and a susceptible Ак3-125 line of white cabbage, followed by hybridization of their hybrid progeny FI (Ak3-125 x Byb5-103) with the susceptible line Ak3-125. Assessment of cabbage samples for disease resistance/ susceptibility was carried out on an artificial inoculation infectious background. DNA was isolated from young leaves by the CTAB method [2]. The search for RAPD-markers of the locus of resistance to fusarium wilt was carried out using the method of mass segregation analysis [3]. DNA polymorphism between the resistant and susceptible genotypes of the parental lines and in the segregating backcross BC1 population was detected using 148 RAPD primers. Amplification of DNA fragments was carried out in 15 ц1 of the reaction mixture according to the following program: initial denaturation 92 ^{0} C - 3 min; further 35 cycles - denaturation 92 ^{0} C - 30 **s,** annealing (temperature for RAPD primers was 38 ° C, for STS primers - 55-60 ° C according to the author's instructions) - 30 s, synthesis 72 ^{0} C -1 , 0 min; final synthesis 72 ^{0} C - 7 min. The amplification products were separated by electrophoresis in a *2%* agarose gel and visualized in the transmitted ultraviolet light with GelRed fluorescent. Estimation of the adhesive force of the marker to the locus of resistance was carried out in the BC1 population by calculating the recombination frequency as the ratio of the number of recombinant progeny to the total number of offspring multiplied by 100. The reliability of the assumed segregation was determined using the *y2* criterion.

Analysis of previously published resistance markers S46M48199 [4] and markers of candidate gene FOCI against fusarium wilt R7, R3, S3, Al, V17, S9, M10 in the collection of white cabbage samples has showed that they are monomorphic i.e. do not reveal differences between resistant Арт1-1, Za2-221, Ак 3-12122, Kay3-1252, Dr46alfa and susceptible Lil-12, A611-1, И34МС, Nac2a, АМОI -111 genotypes. Therefore, to implement the marker-mediated selection of cabbage for resistance to fusarium wilt, new markers must be developed. To search for a DNA marker, we created a mapping population BC1, represented by 93 plants segregating for resistance to fusarium wilt, based on the hybridization of resistant Biib5-103 and susceptible Ak3-125 inbred lines of white cabbage. The manifestation of the resistance in their FI-hybrid progeny indicated a dominant character of inheritance of resistance, and the segregation of resistant and susceptible plants of the backcross offspring BC1 (Ak3-125 * Bui) * Ak3-125 1: 1 (*2 = 1.47, P = 0.23) indicated monogenic resistance control.

A small number of 7 polymorphic DNA fragments, potential markers, was detected by mass segregation analysis using 282 decameric RAPD primers, the DNA of the Biib5-103, Ak3-125, their Fl-hybrid progeny and DNA 10 mixtures of resistant and 10 susceptible plants BC1 locus of resistance to Fusarium wilt. By genotyping 93 individual plants of the segregating BC1 population and using the statistical analysis with the *%2* criterion, it was found, that the segregation of the markers 424, 362, 580, 439, 467, 469 corresponds to the monogenic inheritance model. The segregation of the marker 266 deviates from the Mendelian 1: 1.

Estimation of the adhesive force (recombination frequency) of markers with a locus of resistance revealed a weak link between markers 266 (42 cM), 424 (43 cM), 467 (45 cM), 580 (47 cM), 439 (47 cM) and independent marker inheritance, 362 (57 cM). 469 (59 cM).

A small number of polymorphic RAPD loci, among which it was not possible to detect a closely linked fusarium wilt resistance, indicates both the low heterogeneity of the resistant Btib5-103 and the susceptible Ak3-125 lines of white cabbage, and the low effectiveness of RAPD technology in detecting polymorphism. At the same time, the detected linked and unbound RAPD markers in compliance with Mendelian model of segregation and deviating from Mendelian model of segregation will serve as a basis for further development of the fragmentary genetic map of B.oleracea and mapping of the locus of resistance to fusarium wilt.

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