MOLECULAR CHARACTERISATION OF *FUSARIUM* HEAD BLIGHT RESISTANCE IN THE BKT9086-95/MV MAGVAS WHEAT POPULATION

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Abstract: The aim of the experiments was to detect and analyse the genetic factors responsible for the FHB resistance of old Hungarian wheat varieties. The resistant 'BKT9086-95' line developed from the variety 'Bánkúti 1201' was crossed with the moderately FHB-resistant variety 'Mv Magvas' to create a single seed descent population for the purpose of studying the genetic background of resistance. Based on the results of artificial inoculation, 15 resistant and 15 susceptible genotypes were selected for the purpose of bulked segregant analysis (BSA). The bulk samples and the parents were analysed using the amplified fragment length polymorphism (AFLP) method. The two bulk samples and the parents were tested with a total of 81 primer combinations, and an average of 5.02 deviations per reaction was found between the parents. AFLP patterns similar to that of the resistant parent were found in a further 16 cases. On the basis of the BSA results the testing of the whole population using the AFLP and simple sequence repeat methods has been commenced.

Keywords: Fusarium head blight, QTL

Introduction

Wheat (*Triticum aestivum*) is the most important staple crop world-wide, with a total production of over 600 million tons annually. Since the green revolution in the 1960s the total yield quantity has increased by around 40%. However, consumption is also increasing at an accelerating rate, so the fight against biotic stress factors endangering yield quantity and quality is of key importance. Fusarium head blight (FHB) is one of the most important wheat diseases, leading to yield reduction and poor grain quality as well as contamination with deoxynivalenol (DON), which poses serious health risks when inhaled as dust or ingested as food by humans and livestock (Hornok et al. 2005, Reddy et al. 2008, Szabó-Fodor et al. 2008). Recent studies have demonstrated that the prevalence of *F. graminearum* is increasing in Central Europe and is now responsible for 90% of the losses, followed in importance by *F. culmorum* (Tóth et al. 2005). The presence of pathogenic fungus species is indicated by the discoloration of the spikelets and the spindle and by the whitening of the spikes. In infected spikes the grains are typically white or pink and have lower thousand kernel weight compared with healthy grains, leading to substantial yield losses.

The efficiency of chemical control depends greatly on the time of application (Prigozliev et al. 2008) and the technology (Lehoczki-Krsjak et al. 2008). The greatest level of resistance is possessed by varieties from Asia ('Sumai 3' and its derivatives) and South America (e.g. 'Frontana'; Bai and Shanner 2004). The agronomic properties of these resistance sources differ considerably from those of Hungarian varieties (thus lengthening the time required to breed resistant commercial varieties), while the European sources reported as resistant have proved to belong to at most the moderately resistant category (e.g. Arina; Ruckenbauer et al. 2001).

Fusarium head blight (FHB) was first detected in Hungary in the 1920s, but the first nation-wide epidemic did not occur until 1970. This could be attributed not only to the introduction of more intensive cultivation techniques (soil cultivation, forecrops,

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nutrient supplies, sowing date, seed quantity) and to weather conditions favourable for infection, but also to the susceptibility of the varieties (Kükedi 1988). The variety 'Bezostaya 1' was registered in Hungary in 1960, after which its sowing area increased rapidly until it occupied almost 80% of the total area (Koltay and Balla 1982). 'Bezostaya 1' is susceptible to FHB (Mesterházy 1986) and many scientists held this variety responsible for the epidemic in 1970 (Szunics and Szunics 1992). It was partly this that later caused the variety to disappear rapidly from general cultivation (Bedő et al. 2001). It was impossible to determine, however, what role the change of variety played in the spread of FHB. As the old Hungarian wheat varieties did not become infected in farmers' fields, the question arises of whether this was due simply to a favourable constellation of various factors, or whether they had genetically coded resistance. The resistance of 'Bánkúti 1201' has been investigated in artificially inoculated trials for several decades and the variety is consistently one of the most resistant genotypes to this disease (Szunics and Szunics 1992).

The homogeneity within populations of old Hungarian varieties is characterised by the fact that while they can be distinguished more or less on the basis of morphology, they are more heterogeneous for quality traits than modern plant varieties, on the basis of both biochemical and molecular markers (Vida et al. 1998, Rakszegi et al. 2000, Juhász et al. 2000).

Materials and methods

In the course of field testing, detailed examinations were made on several lines of 'Bánkúti 1201' origin. Known resistance sources and a susceptible control variety were also sown in the experiment and given the same treatment as the 'Bánkúti 1201' lines. Testing was carried out with single isolates of *F. graminearum* or *F. culmorum* in three replications. Bunches of spikes in the same stage of development were inoculated at flowering using a spore suspension with a concentration of $5*10^5$ macroconidia/ml. Inoculation was repeated two days later. The degree of spike infection was evaluated on the 26th day.

Among the 'Bánkúti 1201' lines included in the experiment, 'B9086-95' proved to be the most resistant, with an FHB infection level of 10–20%. By comparison, the level was 1% for the resistant genotype 'Sumai 3' and 70–80% for the susceptible control. This line was crossed with several Martonvásár varieties. Among the combinations the FHB resistance of the parental genotypes differed to the greatest extent for 'B9086-95'/'Mv Magvas', so this combination was chosen for further analysis. Uniform lines developed from the progeny population using the SSD method were used to identify chromosome regions connected with the FHB resistance of 'Bánkúti 1201'.

The resistance of 250 SSD lines derived from this combination was tested in the greenhouse and field for the spread of *Fusarium* within the spike (Type II resistance). The 'IFA-104' *F. culmorum* isolate was used for artificial inoculation. Conidia were washed off the surface of infected seeds, after which the spore concentration was adjusted to 1 million/ml. Spikelets on the upper 2/3 of the spike were inoculated with 5µl conidium suspension on four plants of each line. The extent of *Fusarium* spike infection (severity %) was determined on the 21st day after inoculation.

Results and discussion

The level of spike cover was 36.7% in 2005 and 31.7% in 2006, averaged over the lines. Averaged over these two years, the infection levels of the lines, parents and control varieties ranged from 5.0 to 72.3%. Based on the mean data for 2005 and 2006 the 'BKT9086-95' parent had the lowest rate of infection (5.0%), followed by the resistance source 'Sumai 3' (6.37%). The infection severity of 36 lines did not differ significantly from that of the better parent ($LSD_{5\%}$=16.8). The difference between the rate of infection of 'Mv Magvas' and that of the resistant parent was more than double the significant difference (44.8%). Spike cover significantly greater than that of the susceptible parent was observed for six lines (Fig 1). The distribution of the lines in terms of spike infection did not differ from normal distribution (Kolmogorov-Smirnov test, D = 0.048^{ns}). As there was a large interval between the lines with the best and worst FHB resistance and the population exhibited normal distribution, this line population was suitable for analysis at the molecular level, aimed at identifying DNA sequences related to FHB resistance.

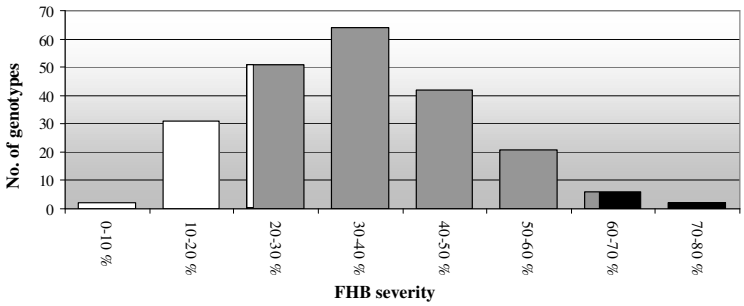

Figure 1. Distribution of FHB infection in'BKT9086-95'/'Mv Magvas' lines (Martonvásár, 2005–2006)

DNA was isolated from the plants examined in the greenhouse (Qiagen DNeasy Plant Mini kit), after which bulk samples were made from the DNA solutions of plants with extreme values. Based on the ratio of infected spikelets and the Xu-Fan scale (field data for 2005 and 2006, greenhouse data for 2007), 15 resistant and 15 susceptible plants were selected for bulk segregant analysis. The bulk samples and the parents were analysed using the amplified fragment length polymorphism (AFLP) method. The reaction products were separated on 6% polyacrylamide gel using the Li-Cor 4300 DNA sequencing gel documentation system.

The DNA of the four bulked samples and the parents was tested using a total of 81 primer combinations. An average of 5.02 diverse patterns per reaction was observed between the

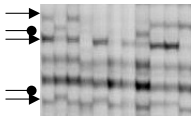

Figure 2. Polymorphic AFLP products

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parents. In the better parent and the samples formed from resistant plants, reaction products of the same size were found in 16 cases, which could indicate that *Fusarium* resistance has a genetic background. Averaged over the population, 8.7 different products per reaction were identified with markers giving patterns of the maternal type, and 40% of the polymorphisms observed were of this type (Fig 2.). The results confirm the presence of the genetic markers detected by BSA at the level of the whole population.

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