# EFFECT OF SINGLE QTLs FOR WHEAT FHB RESISTANCE FROM SUMAI 3 AND F 201R ON PHENOTYPIC RESISTANCE TRAITS AND DON CONTENT

Mariana Ittu*, Nicolae. N. Săulescu*, Matilda Ciucă* and Gheorghe Ittu*

## ABSTRACT

**Epidemics of Fusarium head blight (FHB, scab) can result in significant economic losses in terms of yield and food safety. Marker assisted selection is potentially a powerful tool in breeding for quantitative traits, such as resistance of wheat to FHB. The validation of QTLs is a general prerequisite condition before their use for Marker Assisted Selection (MAS) in breeding programs. In this study, a set of bread winter wheat lines derived from crosses with FHB resistant Sumai 3 and Fundulea 201 R, previously genotyped with simple sequence repeat (SSR) markers, were phenotypically evaluated for FHB resistance Type II and DON content, in artificially inoculated field nurseries, at NARDI Fundulea, in two years (2005 and 2006). Differences regarding the mean values and range of variation for the resistance traits among QTLs carriers and non-carriers were observed in derivatives of both donors. FHB severity (%), disease progress (AUDPC), diseased kernels (FDK, %) were reduced on average in QTL carriers derived from crosses with both donors of resistance, as compared with the corresponding non-carrier lines. There was considerable overlapping of distributions for carriers and non-carriers of single QTLs, for all measured traits, and this explains why only the average effect of *Fhb1* on AUDPC was significant. However, for both AUDPC and FHB severity (%), best lines were all carriers of the respective QTLs. High levels of attack were found both among carriers and non-carriers. In contrast, low percentages of diseased kernels were found both among carrier and non-carrier lines. This suggests that the analyzed QTLs have larger effects on AUDPC and FHB severity, than on FDK. This is the first report on effects of the *Fhb1* QTL from Sumai 3 and of the QTL located on chromosome 3A from F 201 R, for resistance to FHB in the Romanian winter wheat breeding materials. Our results prove that, as expected, selecting for only one (even major) QTL cannot guarantee a good level of FHB resistance. However, data on the presence of single FHB resistance QTLs can be useful for choosing parents to increase the level of resistance, by cumulating various QTLs.**

**Key words:** Deoxynivalenol, Fusarium head blight, QTLs, resistance, SSR markers, winter wheat

## INTRODUCTION

Fusarium head blight (FHB, scab) caused by *Gibberella zeae* (Schwein.) Petch. (anamorphs, *Fusarium graminearum* and *F. culmorum*) has become a major constraint of winter wheat production and quality worldwide in recent years. Epidemics of this disease can result in significant economic losses also in terms of food safety because of the grain contamination with several secondary toxic metabolites (mycotoxins) (Leonard and Bushnell, 2003), among which the trichotecene deoxynivalenol (DON) is the most prevalent (Placinta et al., 1999). Storage of cereals under warm and humid conditions may further increase mycotoxin content even when field infections were only light to moderate (Homdork et al., 2000).

Wet weather during flowering and grain filling and some cropping systems (maize as preceding crop and minimum tillage) have favored disease development.

Success with efforts to minimize the impact of Fusarium head blight centered around the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application has been limited (Paul et al., 2005).

Host resistance has been considered a cost-efficient and environmentally sound strategy to control Fusarium head blight (FHB). However, progress in developing FHB-resistant wheat cultivars has been hindered by the complexity of quantitative resistance, a lack of effective sources of resistance and the high importance of genotype x environment interaction.

Application of MAS to enhance the effectiveness of breeding for FHB resistance it is generally agreed as a valuable alternative, but its implementation on a broad scale has still to be optimized. Several FHB resistance loci have been found in Asian (Shen et al., 2003b; Zhou et al., 2003) and Brazilian spring wheats (Steiner at al., 2004). In the Chinese source Sumai 3 (spring wheat), a major quantitative trait loci (QTL) on chromosome *3BS* explained up to 50% of the phenotypic variation and seems to be primarily associated with Type II resistance to FHB (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001). *Qfhs.ndsu-3BS*, re-designated as *Fhb 1* has been identified and verified by several research groups in several wheat backgrounds and environments. In the same region on chromosome *3BS* between the markers Xgwm 493 and Xgwm 389, three other fungal resistance genes/QTLs have been localized*:* *Sr 2* (durable stem rust resistance), *Stb2* (*Septoria tritici* blotch) and *QSng.sfr.-3BS* (*Stagonospora* blotch) (Uphaus et al., 2005). Additionally, QTLs for FHB resistance on chromosomes *6B* (Waldron et al., 1999), *2A*, and *2B* (Zhou et al., 2002) with minor influence were reported. Recent findings revealed that Sumai 3 has a null *3BS* allele for marker STS3B-256 that is diagnostic for its allele of *Fhb 1* and will be useful in MAS (Liu et al., 2005).

Molecular information on sources of resistance other than the Chinese and Brazilian (Frontana) gene pool is available for *Triticum macha* (Mentewab et al., 2000), *T. dicoccoides*

* National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania

(Otto et al., 2002; Stack et al., 2002), and *Lophopyrum elongatum* (Shen et al., 2004).

In comparison with spring wheat, only a few resistant winter wheat cultivars have been genetically analyzed for FHB resistance to date. Several QTLs associated with FHB resistance localized in different genomic regions were identified in populations derived from crosses *Fundulea 201R/Patterson* (Shen et al., 2003), *Renan/Recital* (Gervais et al., 2003), *Arina/ Forno* (Paillard et al., 2004), *Dream/Lynx* (Schmolke et al., 2005), *Ernie/Mo94-517* (Abate and McKendry, 2005).

Effective MAS for FHB resistance depends on knowledge of the genetic relationship of the germplasm to be improved with identified FHB resistance QTLs. There is also a need for more PCR-based selectable molecular markers that are reliable, predictive and broadly applicable, such as "gene based" (Griffey, 2005). It is expected that recent rapid advancement of high throughput platforms and DNA-based diagnostic assay technologies may contribute to develop and implement in the breeding programs cost-effective genotyping protocol to enhance selection and releasing of lines resistant to FHB (Brady et al., 2005).

This study was conducted to evaluate the effect of QTLs for FHB resistance, localized on chromosomes *3BS*, and *3A* and transferred from Sumai 3 and Fundulea 201R, respectively on some phenotypic resistance traits characteristic for resistance Type II (FHB severity and progress) and DON content in advanced winter breadwheat lines.

## MATERIAL AND METHODS

**Plant materials.** 36 advanced winter breadwheat lines, obtained at the National Agricultural Research & Development Institute Fundulea, were analyzed. The lines were selected from crosses involving F 201R, winter wheat type with improved agronomic traits and Sumai 3, less adapted spring wheat. This germplasm showed various levels of FHB resistance in field tests. These genotypes were previously genotyped with specific SSR markers Xgwm and Xbarc, from regions of the genome where QTLs for FHB resistance have been identified and the presence/absence of corresponding QTLs was documented (Ciucă, 2006). Among the F 201R derivatives, 18 lines were carriers of the QTL on *3A*, while 7 were non carriers. Five lines derived from Sumai 3 carried the *Fhb1* QTL and 6 were non carriers.

**Fusarium isolates.** Single-spore isolates of *F. graminearum* (FG 96) and *F. culmorum* (FC 46) originally isolated from winter wheat in Romania and in The Netherlands, respectively were separately used for inoculation. FC 46 was kindly provided by T. Miedaner, who is intensively using it (Miedaner et al., 2001). Inoculum of the both isolates were produced on Mung bean liquid medium, continuously aerated for seven days under exposure to black UV lamps (Philips HPL-N 400W E40) at room temperature (approximately 24°C).

**Artificial inoculation.** Wheat genotypes were grown in two environments (2005 and 2006) at NARDI Fundulea and artificially point inoculated in the field at Fundulea near Bucharest (geographic location latitude 24°10', longitude 44°30', 68 m above sea level, 10.7°C mean annual temperature, 583 mm mean annual precipitation). Temperature and rainfalls, the critical climatic factors with respect to FHB progress during grain filling were across environments 567 and 686.8°C (sum of centigrade) and 144 and 88.61 mm (precipitation), respectively. For point inoculation, which reveals resistance Type II to FHB, approximately 10 µl droplet was injected by a syringe directly through the glumes in a central floret of each side of 20 arbitrarily chosen heads per plot, that were distinctly marked. Each genotype was inoculated at its respective mid-flowering.

**Resistance traits.** Recording of FHB ratings started in field 10 days post inoculation (dpi) and repeated at 20 dpi in terms of infected spikelets/entry/isolate. The arithmetic mean of the individual successive ratings was used for further calculation of FHB severity (damaged spikelets, % of control at the onset of symptom development, i.e. 20 dpi), and disease progress, area under disease progress curve (AUDPC). Additionally, heading date was recorded on a time scale starting at January 1$^{st}$. At full ripening, inoculated and random non-inoculated main-tiller spikes/entry/isolate were both, harvested and threshed by hand, to save highly infected, shriveled and degenerated kernels. From these samples, percentage of Fusarium diseased kernels (FDK, %) was analyzed and calculated per entry/isolate.

**DON immunoassay analysis.** Grain samples from heads inoculated separately with isolates of *F. graminearum* (FG 96) and *F. culmorum* (FC 46) within each entry were bulked, ground and analyzed for DON content in the laboratory of V. Gagiu at the Institute of Food Bioresources, Bucharest. The concentration of DON was quantified according to the manufacturer's description by ELISA on Ridascreen®FAST DON (ppm = mg/kg) kits (R-Biopharm GmbH, Darmstadt, Germany).

## RESULTS AND DISCUSSION

Field evaluation of FHB resistance revealed differences between donors and their derivatives for most of the resistance traits analyzed. Sumai 3, carrier of the major QTL *Fhb 1*, that explains up to 50% of resistance to FHB Type II, confirmed in this experiment on average across combinations environment / isolate, its high potential of resistance expressed in terms of FHB severity (16 % of damaged spikelets at 20 days post inoculation), FHB progress (AUDPC = 174), Fusarium diseased kernels 17 %, and DON content (4.5 ppm). Fundulea 201R recorded lower values for these parameters, respectively, FHB severity = 29%, AUDPC = 258, FDK = 29% and 6.2 ppm.

Differences regarding the mean values and range of variation for the resistance traits among QTLs carriers and non-carriers have been observed for derivatives groups of both donors. FHB severity (%), disease progress (AUDPC), diseased kernels (FDK, %) were reduced on average in QTL carriers derived from crosses with both donors of resistance, as compared with the corresponding non-carriers lines (Table 1).

These differences cannot be explained by differences in heading date, as average earliness of carrier and non-carrier lines was not significantly different. There was considerable overlapping of distributions for carriers and non-carriers of single QTLs, for all measured traits (Fig. 1-3). This explains why only the average effect of *Fhb1* on AUDPC was significant.

It is interesting to note that for both AUDPC and FHB severity (%), best lines were all carriers of the respective QTLs (Figures 1 and 2). However, high levels of attack were found both among carriers and non-carriers. In contrast, low percentages of diseased kernels were found both among carrier and non-carrier lines (Figure 3). This suggests that the analyzed QTLs have larger effects on AUDPC and FHB severity, than on FDK.

*Table 1.* Means for Heading date; FHB severity; FHB progress, AUDPC; FDK% and DON (ppm) of donors and corresponding derivatives breeding lines

| Donors / derivatives | Heading date (days after Jan. 1) | FHB Severity (diseased spikelets, %) | FHB progress AUDPC | Diseased kernels (FDK, %) | DON content (ppm) |
|---|---|---|---|---|---|
| Sumai 3 | 141 | 16 | 174 | 17 | 3.8 |
| Carriers of Sumai QTL allele *Fhb 1* | | | | | |
| Average | 141 | 21 | 215 | 20 | 4.5 |
| Range | 138-144 | 9-44 | 138-362 | 3-27 | |
| Non-carriers of Sumai QTL allele | | | | | |
| Average | 143 | 27 | 294 | 24 | 7.8 |
| Range | 143-144 | 18-46 | 216-528 | 10-58 | |
| Effect (Average difference carriers-non carriers) | *-2* | *-6* | *-79* | *-4* | *-3.3* |
| LSD, P≥5% | 3 | 12 | 74.5 | 24.4 | |
| F 201R | 141 | 29 | 258 | 29 | 6.2 |
| Carriers of F 201R QTL allele on *3A* | | | | | |
| Average | 142 | 26 | 240 | 23 | 6.0 |
| Range | 138-145 | 15-79 | 176-598 | 14-34 | 3.4-8.4 |
| Non-carriers of of F 201R QTL allele | | | | | |
| Average | 144 | 29 | 344 | 18 | 6.2 |
| Range | 140-147 | 17-62 | 198-732 | 2-38 | |
| Effect (Average difference carriers-non carriers) | *-2* | *-3* | *-104* | *-5* | *-0.2* |
| *LSD, P≥5%* | *3* | *17.0* | *168.3* | *14.2* | |

The validation of QTLs is a general prerequisite condition before their use for Marker Assisted Selection (MAS) in breeding programs. This is the first report on effects of the *Fhb1* QTL from Sumai 3 and of the QTL located on chromosome 3A from F 201R, for resistance to FHB in the Romanian winter wheat breeding materials.

Our results prove that, as expected, selecting for only one (even major) QTL cannot guarantee a good level of FHB resistance. However, data on the presence of single FHB resistance QTLs can be useful for choosing parents to increase the level of resistance, by cumulating various QTLs.

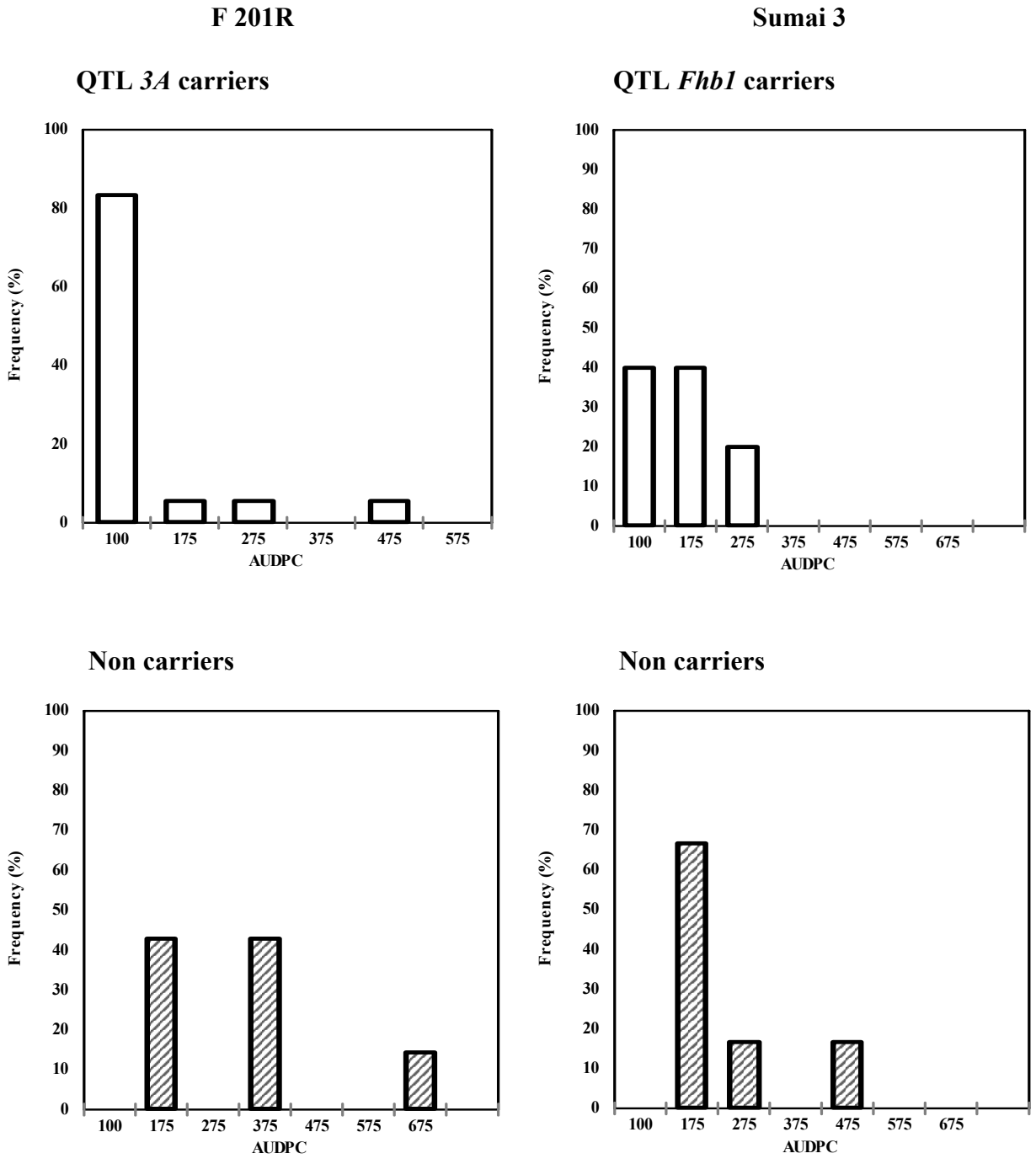


*Figure. 1.* Distribution of QTLs carriers and non carriers lines derived from F 201R and Sumai 3 for AUDPC

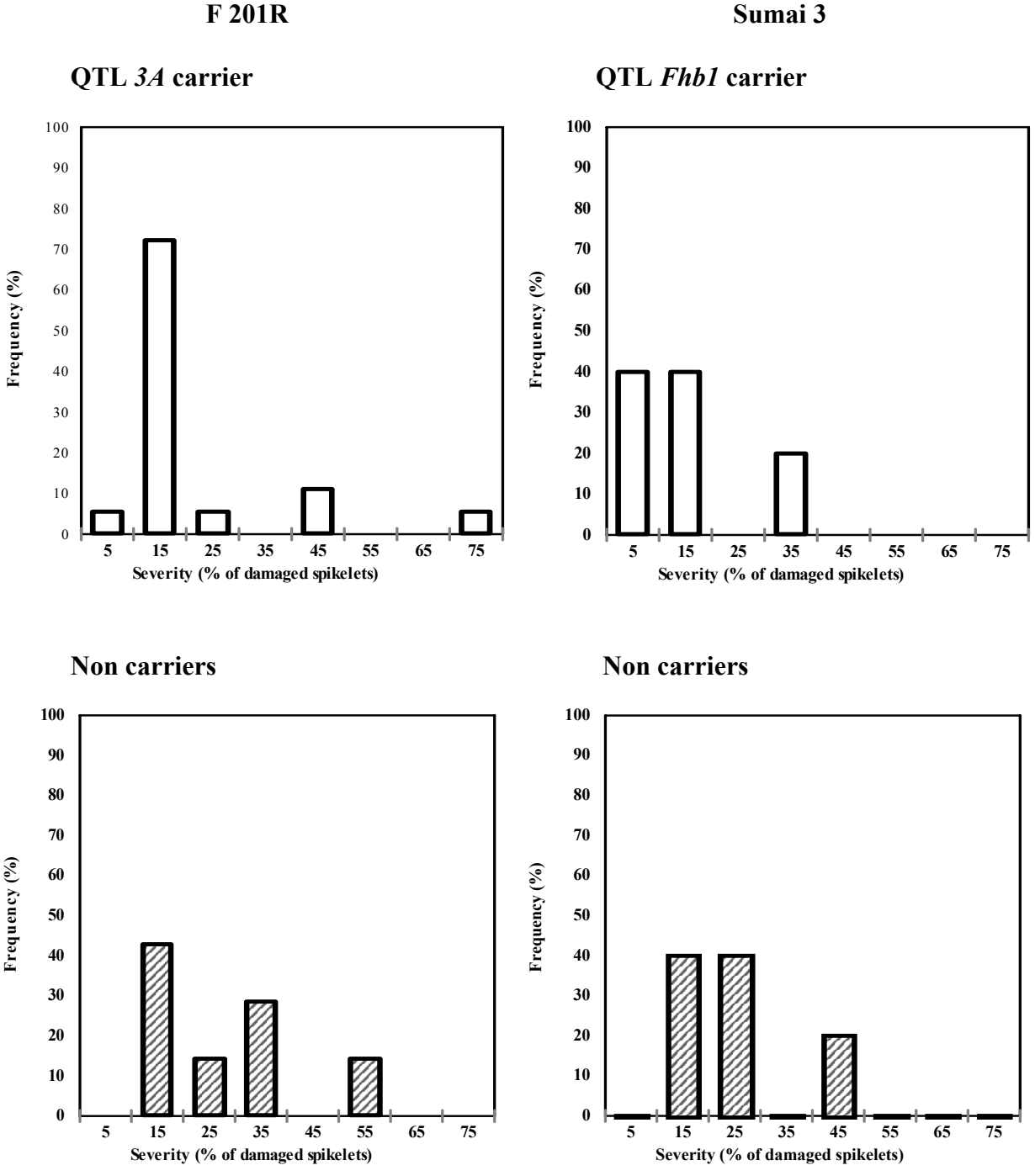


*Figure. 2.* Distribution of QTLs carriers and non carriers lines derived from F 201R and Sumai 3 for the percentage of Fusarium damaged spikelets (severity)

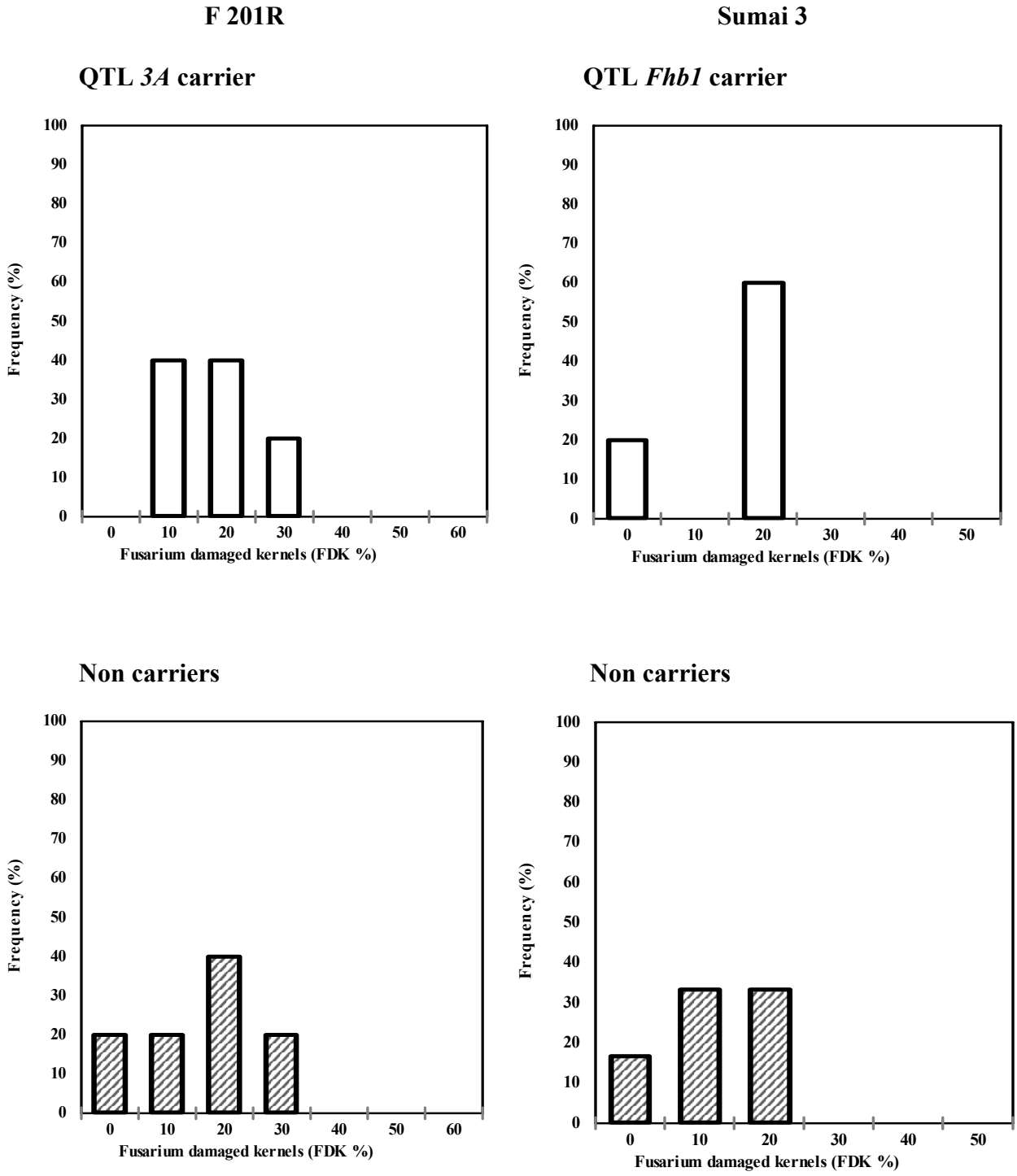


*Figure. 3.* Distribution of QTLs carriers and non carriers lines derived from F 201R and Sumai 3 for the percentage of Fusarium damaged kernels (FDK)

## CONCLUSIONS

The first investigation on effects of the *Fhb1* QTL from Sumai 3 and of the QTL located on chromosome *3A* from F 201R, for resistance to FHB in a set of Romanian bread winter wheat lines derived from crosses with FHB resistant Sumai 3 and Fundulea 201R, previously genotyped with simple sequence repeat (SSR) markers, show under field artificial inoculation at NARDI Fundulea, differences regarding the mean values and range of variation for resistance traits, among QTLs carriers and non-carriers derivatives of both donors.

FHB severity (%), disease progress (AUDPC), diseased kernels (FDK, %) were reduced on average in QTL carriers derived from crosses with both donors of resistance, as compared with the corresponding non-carrier lines, but only the average effect of *Fhb1* on AUDPC was significant**.**

Results suggests that the analyzed QTLs have larger effects on AUDPC and FHB severity, than on FDK, and based on this it is possible to select the best lines, beeing carriers of the respective QTLs.

Our results prove that, as expected, selecting for only one (even major) QTL cannot guarantee a good level of FHB resistance, but this approach can be useful for choosing parents to increase the level of resistance, by cumulating various QTLs.

## ACKNOWLEDGMENTS

This research was partially funded by the Romanian Center for Programs Management (CNMP), BIOTECH project 4545/2004-2006.

## REFERENCES

Abate, Z. and McKendry, A.L., 2005. Quantitative trait loci associated with reduced deoxynivalenol in the soft red winter wheat 'Ernie'. In: Canty, S. M., Boring, T., Wardwell, J., Siler, L., and Ward, R. W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 3-5.

Anderson, J.A., Stack, R. W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Mitchell Fetch, J., Song, Q.J., Cregan, P.B., Frohberg, R.C., 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102: 1164-1168.

Bai, G., Kolb, F.L., Shaner, G., Dornier, L.L., 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89: 343-347.

Brady, L., Anderson, J., Smith, K., and Chao, S. 2005. A cost-effective high throughput genotyping method. In: Canty, S.M., Boring, T., Wardwell, J., Siler, L., and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 18.

Ciucă, M., 2006. Detection of QTLs linked to Fusarium head blight resistance in Romanian winter wheat. Romanian Agricultural Research 23:7-12.

Gervais, L., Dedryver, F., Morlais, J.Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C., Trottet, M., 2003. Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theor. Appl. Genet. 106: 961-970.

Griffey, C.A., 2005. Identification and incorporation of FHB resistance in winter wheat: an overview of progress and strategies. In: Canty, S.M., Boring, T., Wardwell, J., Siler, L., and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 31-34

Homdork, S.H., Fehrmann, H., Beck, R., 2000. Influence of different storage conditions on the mycotoxin production and quality of Fusarium-infected wheat grain. J. Phytopathology 148:7-15.

Leonard, K.J. and Bushnell, W.R., 2003. Fusarium Head Blight of Wheat and Barley, St. Paul, Minnesota, USA: APS Press.

Liu, S., Zhang, X., Pumphrey, M.O., Stack, R.W., Gill, B.S. and Anderson J.A., 2005. New DNA markers for the chromosome 3BS Fusarium head blight resistance QTL in wheat. In: Canty, S. M., Boring, T., Wardwell, J., Siler, L., and Ward, R. W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 55

Mentewab, A., Rezanoor, H.N., Gosman, N., Worland, A.J., Nicolson, P., 2000. Chromosomal location of Fusarium head blight resistance genes and analysis of the relationship between resistance to head blight and brown foot rot. Plant Breed 119:15-20.

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., Geiger, H. H., 2001. Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. Plant Breeding 120: 97-105.

Otto, C.D., Kianian, S.F, Elias, E.M, Stack, R.W, Joppa, L.R., 2002. Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. Plant. Mol. Biol. 48:625-632.

Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., Schachermayr, G., 2004. QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L). Theor. Appl. Genet. 109: 323-332.

Paul, P.A., El-Allaf M., Lipps, P.E., and Madden, L.V., 2005. Relationship between incidence and severity of Fusarium head blight on winter wheat in Ohio. Phytopathology 95: 1049-1060.

Placintă, C.M., D'Mello, J.P.F. and Macdonald, A.M.C., 1999. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Anim Feed. Sci. Techn. 78: 21-37.

Schmolke, M., Zimmermann, G., Buerstmayr, H., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., Hartl, L., 2005. Molecular mapping of Fusarium head blight resistance in winter wheat population Dream/Lynx. Theor. Appl Genet 111: 747-756.

Shen, X., Zhou, M., Lu, W., Ohm, H., 2003 b. Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor. Appl. Genet. 106: 1041-1047.

Shen, X., Ittu, M., Ohm, H., W., 2003 a. Quantitative trait loci conditioning resistance to Fusarium head blight in wheat, Crop Sci. 43: 850-857.

Shen, X., Kong, L. Ohm, H.W., 2004. Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum* L.) - *Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. Theor. Appl. Genet. 108: 808-813

Stack, R.W., Elias, E.M., Mitchell-Fetch, J., Miller J.D., Joppa L.R., 2002. Fusarium head blight reaction of Langdon Durum *Triticum dicoccoides* chromosome substitution lines. Crop Sci. 42: 637-642.

Steiner, B., Lemmens, M., Griesser, M., Scholtz, U., Schondelmaier, J., Buerstmayr, H., 2004. Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor. Appl. Genet. 109: 215-224.

Uphaus J., Shen X., Goodwin S., Buechley G., Breeden J. and Ohm H., 2005. Developing multiple disease resistance linkage block on wheat chromosome 3 BS. In: Canty, S. M., Boring, T., Wardwell, J., Siler, L., and Ward, R. W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 94.

Waldron, B.L., Moreno-Sevilla, B., Anderson, J.A., Stack, R. W., Frohberg, R.C., 1999. RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci. 39: 805-811.

Zhou W.C., Kolb F. L., Bai G. H., Shaner G., Domier L. L. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45:719-727.

Zhou, W.C., Kolb, F.L., Bai, G.H., Domier, L.L., Boze, L.K., Smith, N.J., 2003. Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breed 122: 40-46.