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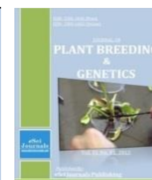

# COMBINATION OF 3 DIFFERENT METHODS OF IDENTIFICATION OF FHB RESISTANT VARIETIES FOR A QUICK SELECTION OF VARIETIES OF INTEREST IN A BREEDING PROGRAM

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**A B S T R A C T**

Fusarium head blight (FHB) caused by Fusarium spp. is one of the major diseases that occurs on wheat in Lubumbashi, in D.R.Congo. Breeding for resistant cultivars is the most used method for controlling the disease although it is laborious, time-consuming and restricted by environmental symptom expression. In this study, 3 different methods of identification of FHB resistant varieties were used: field trials, artificial inoculations and molecular markers. The combination of all results showed that varieties used could be grouped in 4 classes: varieties possessing the major gene Fhb1 which confer a type II resistance to FHB on chromosome 3BS; varieties possessing a major gene of resistance to FHB on the chromosome 3A which confer a type I resistance and a minor gene on the chromosome 3B which contribute weakly to a type II resistance; susceptible varieties and varieties for which resistance were not clearly defined. The simultaneous use of these 3 methods can provide a new approach for a quick selection of varieties of interest in a breeding program.

**Keywords**: wheat, triticale, field trials, artificial inoculation, molecular markers, resistance, FHB.

## INTRODUCTION

Wheat is grown on about 200-250 million hectares throughout the world, which produced approximately 620 million tons of grain annually during the period 2004-2006 (Mac Key, 2005; FAO, 2007). Common wheat (*Triticum aestivum*) and hard wheat (*Triticum durum*) are the most economically important cereals that are adapted to various natural environments (Allan, 1980; Alem *et al.,* 2002). Wheat is grown under latitudes from 3° in Tanzania to 60° in Norway and at elevations as high as 2400 m in Guatemala (Reynolds *et al.*, 2002). It survives extremes of temperature, from under – 35°C in the vegetative phase in Ontario to over 40°C during grainfilling in Sudan (Elahmadi, 1994). Such adaptability reflects the diversity of the wheat genome. When comparing different genotypes, their interaction with the environment is often highly significant (Reynolds *et al.,* 2002; Thomason & Phillips, 2006). Actually, cereal producers are also interested in triticale (*X Triticosecale* Wittmack) which is an intergeneric hybrid between wheat and rye (*Secale cereale* L.). From the former genus it inherited productivity and robustness (Haesaert & De Baets, 1991; Tohver *et al.,* 2005). This cereal was made recently and its production has gained importance. Triticale represents an interesting alternative for wheat in environments where growing conditions are unfavourable or in low-input systems (Martinek *et al.,* 2008; Dogan *et al.,* 2009).

Head blight (FHB, scab) is a widespread fungi disease of wheat and other small grain cereals which can considerably reduce yields and grain quality (Buerstmayr *et al.,* 1999; Champeil *et al.,* 2004; Singh *et al.,* 2005). All species of *Fusarium* can cause FHB but, predominant species are *F. graminearum, F. avenaceum, F. culmorum* and *Microdochium nivale* (Wiese, 1991). FHB occurs after flowering and initial symptoms are generally ring to oval stains with brown edges and clear

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centre on the glumes. The spikes are scalded and spikelets get covered with a white of pink down. Sexual spores produced in perithecia, appear like a dark down at the base of the spikelet. The rachis then necroses, what leads to desiccation of the top or the entire spike. Affected grains are small, light and wrinkled. They can be mummified and covered by white or pink mycelia. These grains have generally reduced test weight and poor seed quality. They can also have low germination capacity, induce root rot and reduce plant strength (Buerstmayr *et al.,* 1999; Champeil *et al.,* 2004; Mascher *et al.,* 2005; Maxime, 2007).

Wheat and triticale have many ways to resist against FHB development. The pathogen needs open flowers to penetrate into the plants. Some varieties are flowering without opening florets, and then avoid infection. Other varieties avoid infection by a very short flowering period, by spike with reduced number of florets or by a weak and opened spike, which allow a rapid drying. A thick epidermis can stop the pathogen penetration and a high stem can reduce spike infection by spores from soil or foliage (Hilton *et al.,* 1996). In addition to these morphological resistances, Schroeder and Christensen (1963) described 2 types of physiological resistance: the type I which is resistance to initial infection and the type II which is resistance to propagation of the pathogenic agent in the tissues. Miller and Arnison (1986) have described the type III resistance which involves the capacity to stop mycotoxins accumulation.

To date, no variety of wheat or triticale has been described which presents an absolute resistance to FHB. The resistance levels known are from moderately resistant to highly susceptible. The abundance of primary inoculum can be reduced by cultural practices that destroy or bury crop residues. Crop rotation with at least one year without cereals, the choice of a sowing date that allow flowering when climatic conditions are not favorable to the pathogen development, a good N fertilization, the reduction of sowing density are agricultural practices that can be effective against FHB. Many fungicides with Tebuconazole, Metconazole and Prothioconazole are also used to reduce primary infection of wheat spikelet but do not prevent accumulation of mycotoxins in grains (Wiese, 1999 ; Champeil *et al.,* 2004 ; Mascher *et al.,* 2005).

The objective of this study was to combine 3 different methods of identification of FHB resistant varieties (field trials, artificial inoculation and molecular markers) to provide to scientist or breeders an easy way to select quickly varieties of interest in a breeding program.

## MATERIAL AND METHODS

**Field trials:** The FHB symptoms and varieties were followed during the rainy seasons of 2007, 2008 and 2009 at the experimental farm of Kasapa of the University of Lubumbashi (11°35 S, 27°24 E, 1250 m ASL), Katanga province, Democratic Republic of Congo. The soil is a xanthic kandiustox. Over the three growing seasons, experiments were carried out with 9 varieties of Elite Spring Wheat (ESW), 8 varieties of High Rainfall Wheat (HRW) and 11 varieties of Triticale (TTC) from CIMMYT and the common spring wheat cv. Moba from the north of the Katanga province (Table 1). These varieties were selected from a set of 106 varieties of *Triticum sp* in a previous trials made at the same farm for their good expression of resistance to FHB and their yield potential (Mukobo *et al.,* 2008) Experimental design was a completely randomised block with 2, 4 and 5 replications respectively for the 2007, 2008 and 2009 trials.

The previous year grown culture was maize. Elementary plot size was 1 $m^2$ and the sowing rate was 160 seeds $m^{-2}$. Applications of NPK and urea fertilizers at seeding and 40 days after sowing ensure a broadcast supply of 206 kg $ha^{-1}$ N, 90 kg $ha^{-1}$ $P_2O_5$ and 45 kg $ha^{-1}$ $K_2O$. Weeds were removed by hand and Thionex 35 EC (Endosulfan 350 g/l) was used as insecticide at flowering. All plots were harvested manually according to the ripening stage. The severity of the FHB were assessed at growth stage (GS) 69 (Zadok *et al.,* 1974) by using a scoring system based on the percentage of plant presenting symptoms over the entire plot: **1** : no symptom ; **2** : 2.5% ; **3** : 5% ; **4** : 10% ; **5** : 15% ; **6** : 25% ; **7** : 35% ; **8** : 67% ; **9** : 100%).

**DNA extraction and PCR:** DNA extraction was a modified CTAB method (Saghai-Maroof *et al.,* 1984; Doyle & Doyle, 1990). In addition to varieties used for field trials, some crosses and their offspring were added for this part of the experiment. Leaves of three weeks old plants (GS 13) of each variety were clipped, ground in liquid nitrogen and placed in 10 ml centrifuge tubes. The ground samples were incubated for 30 min at 64 °C in 1 ml of extraction buffer (3 mg of proteinase K diluted in 10 ml of CTAB [hexadecyl trimethyl ammonium bromide]). 0.5 ml of the extract was placed into a microcentrifuge tube and 0.5 ml of chloroform:isoamylalcohol (24:1) was added.

Table 1: List of varieties and germplasm lines.

| *Type* | *Variety number* | *Name or pedigree* |
|---|---|---|
| Elite spring wheat | V102 | PBW343 |
| Elite spring wheat | V103 | SERI/RAYON |
| Elite spring wheat | V109 | HD29/2*WEAVER/3/VEE/PJN//2*TUI/4/MILAN |
| Elite spring wheat | V110 | MILAN/S87230//HUITES |
| Elite spring wheat | V116 | KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES |
| Elite spring wheat | V117 | KAUZ//ALTAR 84/AOS/3/PASTOR/4/TILHI |
| Elite spring wheat | V124 | REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES |
| Elite spring wheat | V128 | TOBA97/ATTILA |
| Elite spring wheat | V133 | TILHI/PASTOR |
| High rainfall wheat | V209 | HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI |
| High rainfall wheat | V214 | BAV92/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI |
| High rainfall wheat | V215 | K7680/OTUS |
| High rainfall wheat | V218 | MURGA |
| High rainfall wheat | V221 | CHIBIA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS) /4/WEAVER |
| High rainfall wheat | V223 | ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/CBRD/KAUZ |
| High rainfall wheat | V227 | JUP/ZP//COC/3/PVN/4/TNMU/5/TNMU/6/SITE/7/TNMU |
| High rainfall wheat | V230 | WBLL1*2/TUKURU |
| High rainfall wheat | Moba | MOBA |
| Triticale | V803 | ERONGA 83 |
| Triticale | V806 | DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9 |
| Triticale | V811 | POPP1_2/CAAL//POLLMER_3.5.1 |
| Triticale | V819 | DAHBI/COATI_1/3/CT775.81.ARDI_1//ANOAS_1 |
| Triticale | V834 | DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/4/RONDO/2*ERIZO_11 |
| Triticale | V836 | GAUR_2/HARE_3//JLO 97/CIVET/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/150.83//2*TESMO_1/MUSX 603/7/GAUR_2/HARE_3//JLO 97/CIVET |
| Triticale | V840 | DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/4/2*SONNI_3 |
| Triticale | V842 | DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/4/2*ZEBRA 79/LYNX*//FAHAD_1 |
| Triticale | V843 | ERIZO_10/2*BULL_1-1//CAAL/4/2*PACA_2/COPI_1-1/3/ARDI_1/TOPO 1419//ERIZO_9 |
| Triticale | V844 | ARDI_1/TOPO 1419//ERIZO_9/4/GAUR_1/3/MUSX/LYNX//STIER_12-3/5/LASKO/IBEX//ERIZO_9/6/2*POLLMER_3/FOCA_2-1 |
| Triticale | V846 | POLLMER_2.2.1*2//FARAS/CMH84.4414 |

The mixture was centrifuged for 15 min at 13200 rpm and 0.4 ml of the upper water phase was transferred to a new microcentrifuge tube. To precipitate the DNA, 0.4 ml of isopropanol was added, mixed and centrifuged for 15 min at 13 200 rpm. After washing the samples with 0.2 ml of 70% ethanol, the DNA was dried at room temperature under a fume hood 30 min. The DNA pellet was diluted in 50 µl of TE buffer (Tris + EDTA).

Primers of 3 STS markers (Sequence Tagged Site) designated as either STS 3B 80, STS 3B 138, STS 3B 142 and 3 SSR markers (Simple Sequence Repeat) designated as either XGWM (Gatersleben Wheat Microsatellite) 148, XGWM 389, XGWM 533 were kindly provided by Invitrogen/Illumina and used to identify microsatellite markers located on chromosome 3BS (Liu & Anderson, 2003; Cuthbert *et al.,* 2006; Wilde *et al.,* 2007; Zwart *et al.,* 2008). All specific primers are listed in Table 2. A Touchdown PCR for molecular markers detection was performed in a total volume of 50 µl containing 2 µl of DNA solution, 10 µl PCR buffer, 2 µl of primer 1, 2 µl of primer 2, 2.5 µl of dNTPs, 0.25 µl of Tag polymerase and 31.5 µl of milli-q water.

DNA amplification was performed in an Applied Biosystems GeneAmp PCR System 9700. After an initial denaturing step for 5 min at 94°C, 10 cycles were performed with 1 min at 94°C, 1 min at 65°C (in each cycle temperature drops by 1°C), 35 cycles with 1 min at 94°C, 1 min at 55°C, 10 min at 72°C. Products of PCR were run on polyacrylamide gels stained with 0.1 µg $ml^{-1}$ ethidium bromide.

Table 2: Primers (5' → 3') used for the FHB resistant varieties determination.

| Marker | Forward primer | Reverse primer | A.T. (*) |
|---|---|---|---|
| STS 3B 80 | AGA AGA AGG AAG CCC CTC TG | GCC ATG TCT TTT GTG CCT TT | 55 °C |
| STS 3B 138 | CAA GAT CAA GAA GGC CAA GC | AGG TAC ACC CCG TTC TCG AT | 55 °C |
| STS 3B 142 | CGA GTA CTA CCT CGG CAA GC | CAT AGA ATG CCC CGA AAC TG | 50 °C |
| XGWM148 2B | GTG AGG CAG CAA GAG AGA AA | CAA AGC TTG ACT CAG ACC AAA | 60 °C |
| XGWM389 3B | ATC ATG TCG ATC TCC TTG ACG | TGC CAT GCA CAT TAG CAG AT | 60 °C |
| XGWM533 3B | AAG GCG AAT CAA ACG GAA TA | GTT GCT TTA GGG GAA AAG CC | 60 °C |

A.T.: Annealing temperature used for analysis with this primer.

According to the presence or absence of molecular markers, all varieties, crosses and their offspring were automatically clustered with the PROC CLUSTER, using the Ward Method in SAS Software (SAS Institute Inc, 2002).

**Inoculum and inoculation procedure:** Plants were grown under greenhouse conditions till anthesis. Macroconidia of *F. graminearum* were obtained and harvested as described by Audenaert *et al.* (2010). A conidia suspension of $10^6$ conidia/ml of the *F. graminearum* strain 8/1 (janssen *et al.,* 2005) was prepared.

Ten ears of each variety at flowering stage (Zadok's stage 60) were infected with 2 droplets of 20 µl of conidia suspension and placed in a growth chamber at 22°C under a relative humidity of 100% for 2 days to guarantee the conidial germination and penetration. After 2 days, the plants were incubated for 12 days in a growth chamber at 22°C under a light regime of 16 h light/8 h dark. Fourteen days after inoculation, the infection was assessed based on the surface of the ear covered with Fusarium symptoms: 1 = healthy; 2 = up to 25%; 3 = 25 to 50%; 4 = 50 to 75%; 5 = 75 to 100% of the ear covered with symptoms (Audenaert *et al.,* 2010). The experiment was repeated twice in time.

**RESULTS**

**Evaluation of FHB symptoms on different varieties:** In general, wheat growing conditions were normal for all the 3 years and, the level of FHB symptom based observations was scored and is shown in figure 1. It appears that the FHB pressure on different populations increase with year but the overall level of the disease remains under 5% for all the varieties. Although they were no artificial infection in this part of the experiment, it's appear that the introduction of small grains varieties in Lubumbashi can lead to high level of FHB infection in the future if the overall surface of cereals increase in the area and if there are no control measures to reduce it.

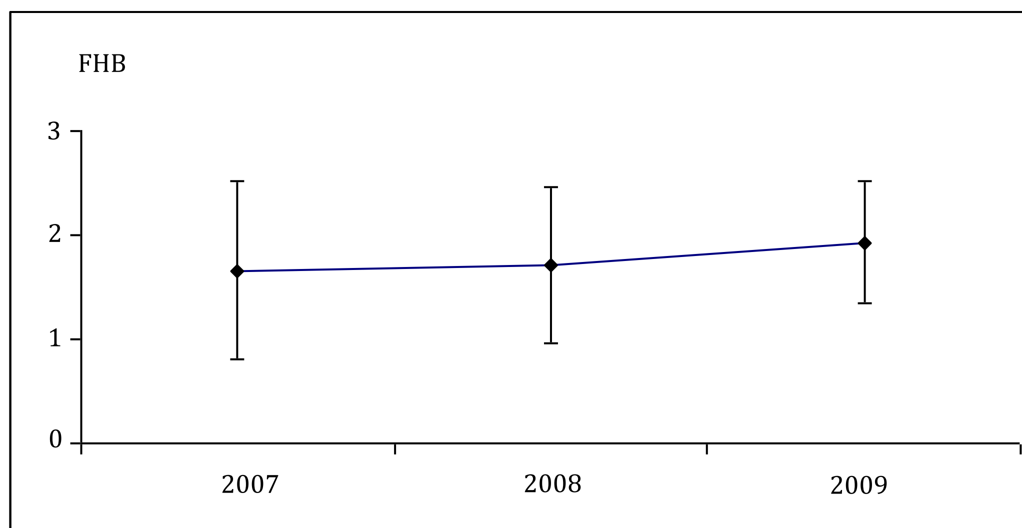


Figure 1: Evolution of Fusarium Head Blight severity during 3 years at Lubumbashi.

When comparing each cereal used in this study, it appears that varieties of HRW are less susceptible to FHB disease than TTC and ESW varieties (Figure 2). Their levels of infection for all the 3 years are also lower than those from TTC and ESW. In 2007, TTC varieties were the most susceptible to FHB but for the 2 years after, ESW varieties were the most susceptible. The common spring wheat cv. Moba and the TTC variety 844 (ARDI_1/TOPO 1419//ERIZO_9/4/GAUR_1/3/MUSX/LYNX//STIER_12-3/5/LASKO/IBEX//ERIZO_9/6/2*POLLMER_3/FOCA_2-1) showed the highest level of FHB in 2007 (score 4) but, they were no diseases on Moba the 2 years after and for the TTC variety 844 (ARDI_1/TOPO 1419//ERIZO_9/4/GAUR_1/3/MUSX/LYNX//STIER_12-3/5/LASKO/IBEX//ERIZO_9/6/2*POLLMER_3/FOCA_2-1), the level of FHB was lower (score 2). They were no FHB in 2008 for HRW and the level of disease scored was the lowest in the 3 years for TTC varieties but at the same time, it was the highest for ESW. Similar results were found by Paul *et al.* (2005) who showed that there is a year to year variability in FHB resistance for the same genotypes. This variability is relative to climatic conditions (temperature, precipitation and relative humidity), especially those recorded 7 days before and 10 days after anthesis, which have an effect on the incidence of FHB (De Wolf *et al.,* 2003). Cowger *et al.* (2003) have also shown that there is a strong correlation between FHB incidence and duration of post-anthesis moisture. In our experiments, the overall low levels of FHB can be due to the reduction of precipitation and relative humidity that happens during the filling which is the period when symptoms appear. Filling always happens in March at Lubumbashi and for that period, precipitation has varied between 180 and 200 mm and, relative humidity has varied between 64 and 77% for all the 3 years.

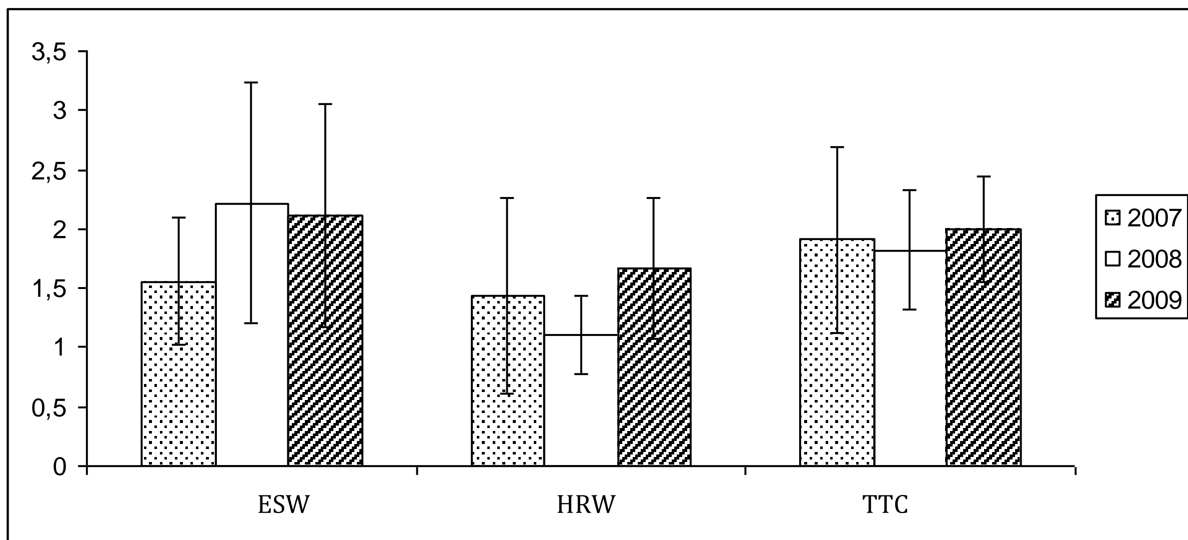


Figure 2: Fusarium Head Blight severity on Elite Spring Wheat (ESW), High Rainfall Wheat (HRW) and Triticale (TTC) varieties at Lubumbashi.

**Markers linked to FHB resistance:** Molecular analysis with STS and XGWM markers led to DNA amplification of the different varieties. For STS markers, only one allele of the *locus* was amplified (Figure 3a) and for the XGWM markers, it was found that they were present on different alleles of the same *locus* (Figure 3b). The amplification of 4 alleles was observed for the XGWM 389 while 5 alleles amplification were observed for XGWM 142 and XGWM 533. Only the Nandu variety amplified 4 alleles for XGWM 533.

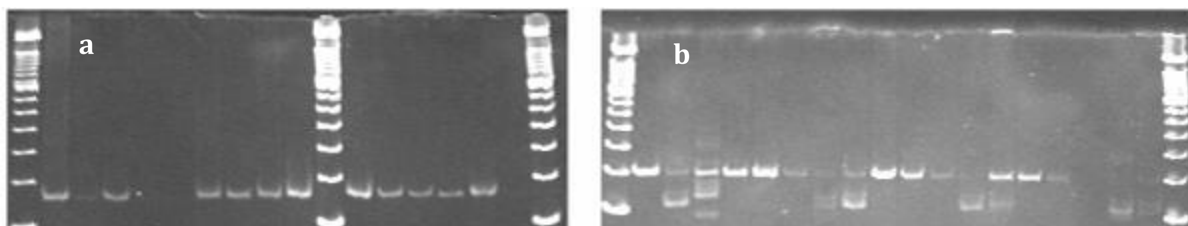


Figure 3: Single amplification of the allele for STS markers (a) and multiple amplifications of alleles for XGWM markers.

All other varieties amplified just 1, 2 or 3 alleles for the different markers. In their study, Liu and Anderson (2003) also found that XGWM 389, XGWM 533 and other SSR markers were highly polymorphic.

By considering presence or absence of markers on polyacrylamide gels, a binary matrix was build which represent all markers combinations for all varieties. There were not much information from the STS 3B 80 and XGWM 142 markers because they were present in all varieties. The Agglomerative Hierarchical Clustering Method of Ward allowed then to make 2 groups which were also subdivided in 2 subgroups each. They then resulted in a total of 4 classes which are represented in the figure 4.

Figure 4: Dendrogram resulting from the cluster analysis of varieties in relation to the presence or absence of molecular markers on chromosome 3BS

The results of classification showed that the first class which is represented by the variety Frontana contains 6 varieties of ESW, 4 varieties of HRW, 2 varieties of TTC, all crosses and their offspring. The second class represented by the variety Nandu contains 1 variety of ESW, 2 varieties of HRW and 2 varieties of TTC. The third class represented by the variety Cadenza contains 2 varieties of ESW and 2 varieties of TTC. The last class represented by the Moba variety contains 2 varieties of HRW and 5 varieties of TTC. The presence of Frontana in the first class allow to consider these varieties as possessing a Type I resistance against FHB (Steiner *et al.*, 2004) while the second class, which contains Nandu, can be considered as possessing a Type II against FHB (Jansen *et al.*, 2005). These 2 classes form the group of varieties which possess genes of resistance to FHB. The 2 other classes form probably a group of susceptible varieties to FHB, especially because of the presence of the Cadenza variety (Haigh *et al.*, 2009) or, possessing a resistance against FHB which is not linked to the molecular markers used in this study on the chromosome 3BS.

**Experimental inoculation:** Results of inoculations by *F. graminearum* for resistance screening were combined to

the 4 classes resulting from the clustering classification (Figure 5). Varieties from the 2 first classes are those which presented the lowest levels of ear infection comparing to the 2 latest classes. The 2 first classes are also those in which there are a great number of varieties without symptoms after inoculation. In the first class, the highest level of infection was for the ESW variety 116 (KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES) and the HRW variety 209 (HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI) but their levels remained less than 25% of the ear surface. All other varieties of the first class didn't present symptoms or very few and, were considered as resistant to FHB. In the second class, the ESW variety 124 (REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES) and the TTC variety 840 (DAHBI_6/3/ARDI_1/TOPO1419//ERIZO_9/4/2*SONNI_3) present few symptoms while all other varieties didn't present symptoms. They were also considered as resistant. All varieties of the third class presented infection levels inferior to 25%. The highest levels of infection (> 25%) were found in the fourth class with the Moba variety and the TTC variety 836 (GAUR_2/HARE_3//JLO97/CIVET/5/DISB5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/150.83//2*TESMO_1/MUSX 603/7/GAUR_2/HARE_3//JLO 97/CIVET). All other varieties of this class presented infection levels inferior to those of the 2 varieties.

In this class, the TTC variety 846 (POLLMER_2.2.1*2//FARAS/CMH84.4414) was an exception because it didn't present any symptom of infection.

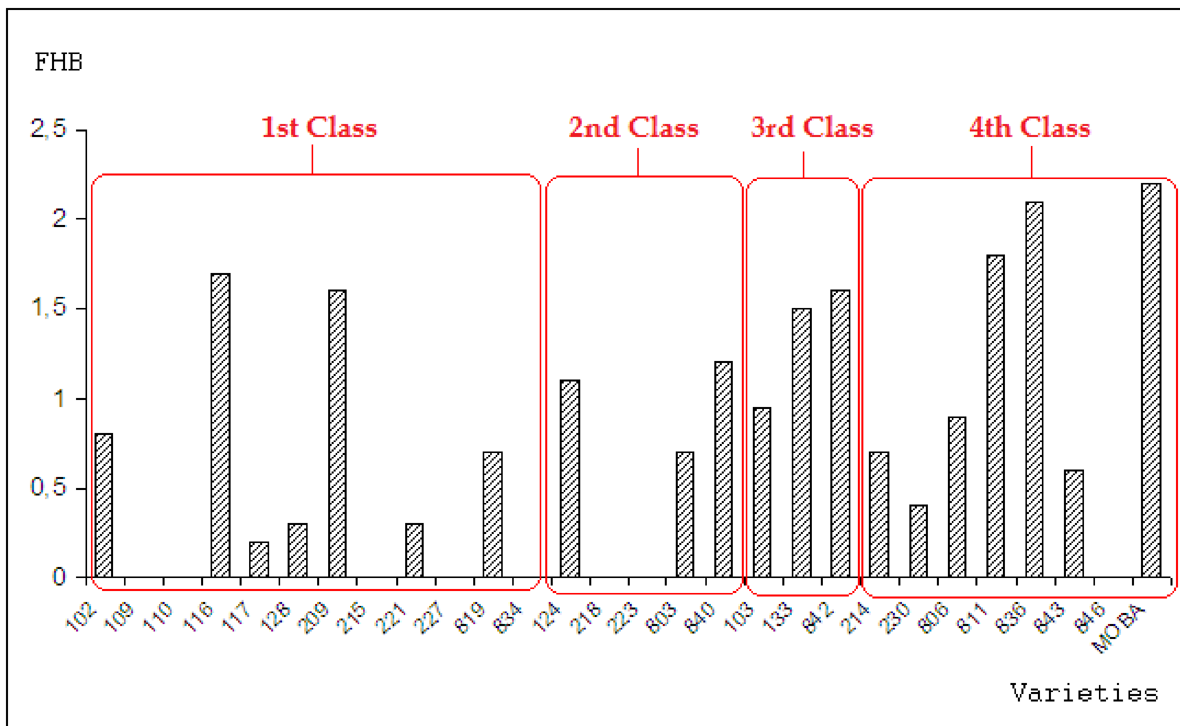


Figure 5: Infection assessment 14 days after inoculation by *F. graminearum*

**DISCUSSION**

Using of a dendrogram on the basis of a binary matrix of presence/absence of molecular markers is an effective mean to establish genetic links between varieties (Bai *et al.*, 2003; Wilde *et al.*, 2007). In this study, all varieties have been clearly grouped in 4 classes, without regarding their species or their types. This grouping in 4 classes showed that only the few varieties associated to the Nandu variety possess the major gene *Fhb1* which confer a type II resistance to FHB on chromosome 3BS (syn. *Qfhs.ndsu-3BS*) (Liu & Anderson, 2003; del Blanco *et al.*, 2003; Zhou *et al.*, 2003; Cuthbert *et al.*, 2006). Snijders (2004) estimated that this gene can explain about 60% of the phenotypic resistance to FHB in varieties which possess it. A same way variation was found in the levels of infection of those varieties after inoculation. It seems that there were no resistances against penetration of the pathogenic but a resistance

against his propagation in the tissues. This explains low infection levels of varieties that showed symptoms in this class.

Varieties from the first class which were associated to the variety Frontana should possess a major gene of resistance to FHB on the chromosome 3A which confer a type I resistance (Wilde *et al.*, 2007) and, a minor gene on the chromosome 3B which contribute weakly (about 10% of phenotypic resistance) to a type II resistance (Steiner *et al.*, 2004). The presence of all crosses and their offspring in this class confirms the character heritable of these genes (Buerstmayr, 1999; Cuthbert *et al.*, 2006). The association of these results with those of artificial inoculations on the same varieties showed that in almost cases, there were no symptoms on their ears. This is probably due to their type of resistance which is against the penetration of the pathogenic agent in the tissues. But, it was not possible to explain the high infection levels of the ESW variety 116 (KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES) and the HRW variety 209 (HD30/5/CNDO/R143//ENTE/MEXI 75/3/AE.SQ/4/2*OCI). However, ear artificial inoculation can make a variety possessing the *Fhb1* gene totally susceptible to the propagation of the FHB (Steiner *et al.,* 2004; Cuthbert *et al.,* 2006). It could be the case for these 2 varieties because, during field trials, in natural infection conditions, they showed good levels of resistance to FHB.

None of all varieties presented a level of infection of 100% at the end of the observing period. Buerstmayr *et al.* (1999) obtained similar results and showed that even susceptible varieties to FHB could not be totally infected after artificial inoculations. However, susceptible varieties associated to the Cadenza variety which doesn't possess the major gene of resistance to FHB were confirmed by artificial infections results that showed high levels of infection in comparison to the first and second classes. Moreover, it can be notice that no HRW variety were in this class. This observation is in concordance with field trials which showed that this type of spring wheat is very less susceptible to FHB.

The great variability of infection levels in the fourth class seems to show that some varieties of this class possess different QTLs of resistance to FHB that are different than those of the 2 first classes. Several studies have shown that major and minor QTLs different than those found on chromosomes 3BS and 3A could be responsible of the resistance to FHB (Li & Gill, 2004; Röder *et al.*, 2004; Bai & Shaner, 2004; Varshney *et al.*, 2006; Edwards & McCouch, 2007; Zwart et al., 2008). The infection levels of varieties of this class like those from ESW variety 116 (KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES) and HRW variety 209 (HD30/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI) showed the necessity to associate molecular markers analyses with fields trials results to confirm the resistance of a variety and also the usefulness of classic breeding methods, although they are laborious, time-consuming and restricted by environmentally dependent symptom expression (Cuthbert *et al.*, 2006; Moullet *et al.*, 2008).

**CONCLUSION**

This research can provide a new approach in the using of molecular markers. The Marker Assisted Selection is a tool which arouse interest in the breeding domain (Najimi *et al.*, 2003). This interest is confirmed in our study by a clear identification of a group of varieties that can be used as a gene source for resistance to FHB. But a great revolution can be the combination of the 3 methods (field trials, artificial inoculation and molecular markers) simultaneously to identify and to select quickly varieties of interest in a breeding program, especially for scientist from developing countries who, almost time, have not enough time and mean to wait 6 to 8 years to popularize a variety.

It also open a window for those who want to integrate in a same genotype different QTLs which confer FHB resistance from different varieties by a quick identification of parents varieties that shall be crossed together and a check in their offspring. Unfortunately Marker Assisted Selection is expensive and can't be used by any research centre, especially those from developing country in which it could allow a rapid verification of the presence of gene in offspring plants and to eliminate systematically genotypes which doesn't possess it. But the challenge remains open and the combination of these 3 methods can be recommended each time it is possible to do it for an accurate result.

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**REFERENCES**


Alem C., Labhilili M., Brahmi K., Jlibene M., Nasrallah N. & Filali-Maltouf A. 2002. Adaptations hydrique et photosynthétique du blé dur et du blé tendre au stress salin, © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS, C. R. Biologies 325: 1097–1109.

Allan R. E. 1980. Wheat in W. R. Fehr and H. H. Hadley, H. H. (eds.) Hybridization of crop plants, Amer. Soc. Agron., Madison, Wis., US.

Audenaert K., Callewaert E., Höfte M. De Saeger S. & Haesaert G. 2010. Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum.* BMC Microbiology, 10:112.

Bai G. & Shaner G. 2004. Management and Resistance in Wheat and Barley to Fusarium Head Blight, Annu. Rev. Phytopathol. 42: 135-161.

Buerstmayr H., Lemmens M., Berlakovich S. & Ruckenbauer P. 1999. Combining ability of resistance to head blight caused by *Fusarium culmorum* (W.G. Smith) in the F1 of a seven parent diallel of winter wheat (*Triticum aestivum* L.), *Kluwer Academic Publishers, Euphytica* 110: 199–206.

Champeil A., Doré T. & Fourbet J.F. 2004. *Fusarium* head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains, Elsevier, Plant Science 166: 1389–1415.

Cowger C., Patton-Özkurt J., Brown-Guedira G. & Perugini L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat, Phytopathology 99: 320-327.

Cuthbert P.A., Somers D.J., Thomas J., Cloutier S. & Brulé-Babel A. 2006. Fine mapping Fhb1, a major gene controlling fusarium head blight resistance in bread wheat (Triticum aestivum L.). Springer-Verlag, Theor Appl Genet 112: 1465–1472.

De Wolf E.D., Madden L.V. & Lipps P.E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on withinseason weather data, Phytopathology 93: 428-435.

del Blanco I.A., Frohberg R.C., Stack R.W., Berzonsky W.A. & Kianian S.F. 2003. Detection of QTL linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat lines, Springer-Verlag, Theor Appl Genet 106: 1027–1031.

Dogan R., Kacar O., Coplu N. & Azkan N. 2009. Characteristics of new breeding lines of triticale, Academic Journals, African Journal of Agricultural Research Vol. 4 (2).

Doyle J.J. & Doyle J.L. 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus 12: 13-15.

Edwards J.D. & McCouch S.R. 2007. Molecular markers for use in plant molecular breeding and germplasm evaluation in Elcio P. Guimarães, John Ruane, Beate D. Scherf, Andrea Sonnino and James D. Dargie Editors. Marked Assisted Selection - *Current status and future perspectives in crops, livestock, forestry and fish*, FAO, Rome.

Elahmadi A.B. 1994. Development of wheat germplasm tolerant to heat stress in Sudan *In Saunders D.A., Hettel G.P. (Eds.), Wheat in Heat-stressed Environments: Irrigated, Dry Areas and Rice-Wheat Farming Systems*, CIMMYT, Mexico DF.

FAO (Food and Agriculture Organization). 2007. FAO Production Statistics, Published online at: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567.

Giunta F. & Motzo R. 2004. Sowing rate and cultivar affect total biomass and grain yield of spring triticale (*X Triticosecale* Wittmack) grown in a Mediterranean-type environment, Field Crops Research 87: 179–193.

Haesaert G. et De Baets A. 1991. Génétique et amélioration du triticale, Bull. Rech. Agron. Gembloux 26 (4), 481-506.

Haigh I.M., Jenkinson P. & Hare M.C. 2009. The effect of a mixture of seed-borne *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale* infection on Fusarium seedling blight severity and subsequent stem colonisation and growth of winter wheat in pot experiments, Springer, Eur J Plant Pathol 124: 65–73.

Hilton A.J., Jenkinson P., Parry D.W. & Hollins T.W. 1996. Relationship between plant morphology and severity of fusarium ear blight in eight cultivars of winter wheat, *Brighton Crop Protection Conf. Pests and Diseases* 1, 419-420.

Jansen C., von Wettstein D., Schäfer W., Kogel K.-H., Felk A. & Maier F.J. 2005. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium*

*graminearum*. PNAS, vol. 102, No. 46, 16892–16897.

Li W. & Gill B.S. 2004. Genomics for cereal improvement in *P.K. Gupta and R.K. Varshney (eds.), Cereal Genomics*, *Kluwer Academic Publishers.*

Liu S. & Anderson J.A. 2003. Marker Assisted Evaluation of Fusarium Head Blight Resistant Wheat Germplasm. Crop Sci. 43: 760–766.

Mac Key J. 2005. Wheat: Its Concept, Evolution, and Taxonomy in Royo C., Nachit M.M., Di Fonzo N., Araus J.L., Pfeiffer W.H. and Slafer G.A. Editors. Durum Wheat Breeding – *Current Approaches and Future Strategies*, Vol. 1, Food Products Press.

Martinek P., Vinterová M., Burešová I. et Vyhnánek T. 2008. Agronomic and quality characteristics of triticale (*X Triticosecale* Wittmack) with HMW glutenin subunits 5+10, Elsevier, Journal of Cereal Science 47: 68–78.

Mascher F., Michel V. & Browne R. A. 2005. Sélection de variétés de blé et de triticale résistantes à la fusariose sur épi, Agroscope RAC Changins, Revue suisse Agric. 37 (5): 189-194.

Maxime T. 2007. Incidence des contaminations tardives du blé tendre par *Fusarium culmorum* sur l'accumulation des TCT dans les grains - *Incidence of inoculations during grain filling by* Fusarium culmorum *on the accumulation of TCT in kernels*, Symposcience, Colloque Fusariotoxines des Céréales, Arcachon.

Miller J.D. & Arnison P.G. 1986. Degradation of deoxynivalenol by suspension cultures of the *Fusarium* head blight resistant wheat cultivar Frontana, Can. J. Plant Pathol. 8 : 147–150.

Moullet O., Fossati D., Mascher F., Schori A. & Guadagnuolo R. 2008. Les marqueurs moléculaires comme outils dans la sélection des céréales, Revue suisse Agric. 40 (3): 133-138.

Mukobo R.P., Ngongo L.M. & Haesaert G. 2008. Diseases on *Triticum sp.* under the growing conditions of Lubumbashi (Congo RD), Gent Symposium on plant pathology, June 2008.

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

Reynolds M.P., Trethowan R., Crossa J., Vargas M. & Sayre K.D. 2002. Physiological factors associated with genotype by environment interaction in wheat, Elsevier, Fields Crops Research 75: 139-160.

Röder M.S., Huang X.-Q. & Ganal M.W. 2004. Wheat Microsatellites: Potential and Implications in H. Lörz and G. Wenzel (Eds), Biotechnology in Agriculture and Forestry, Vol. 55, Springer-Verlag, Molecular Marker Systems.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. & Allard R.W. 1984. Ribosomal DNA spacer length polymorphism in barley - *Mendalian inheritance, chromosomal location and population dynamics*. Proc Natl Acad Sci 81: 8014-19.

SAS Institute Inc. 2002. SAS/STAT Guide for Personal Computer. Version Learning Edition 4.1.

Schroeder H.W. & Christensen J.J. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*, *Phytopathology* 53: 831-838.

Singh R.P., Huerta-Espino J., Fuentes G., Duveiller E., Gilchrist L., Henry M. et Nicol J.M. 2005. Résistance to Diseases in Royo C., Nachit M.M., Di Fonzo N., Araus J.L., Pfeiffer W.H. and Slafer G.A. Editors. Durum Wheat Breeding – *Current Approaches and Future Strategies*, Vol. 1, Food Products Press.

Snijders C.H.A. 2004. Resistance in wheat to *Fusarium* infection and trichothecene formation, Elsevier, Toxicology Letters 153: 37–46.

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

Thomason W.E. & Phillips S.B. 2006. Methods to evaluate wheat cultivar testing environments and improve cultivar selection protocols, Elsevier, Field Crops Research 99: 87–95.

Tohver M., Kann A., Täht R., Mihhalevski A. & Hakman J. 2005. Quality of triticale cultivars suitable for growing and bread-making in northern conditions, Elsevier, Food Chemistry 89: 125–132.

Varshney R.K., Balyan H.S. & Langridge P. 2006. Wheat (Book chapter) in C. Kole (Ed.), Genome Mapping and Molecular Breeding in Plants, Volume 1, Cereals and Millets, Springer-Verlag.

Wiese M.V. 1991. Compendium of wheat diseases, 2nd Edition, The American Phytopatholgical Society.

Wilde F., Korzun V., Ebmeyer E., Geiger H.H. & Miedaner T. 2007. Comparison of phenotypic and marker-based selection for Fusarium head blight resistance and DON content in spring wheat.

Springer Science+Business Media B.V., Mol Breeding 19: 357–370.

Zadoks J.C., Chang T.T. & Konzak C.F. 1974. A decimal code for growth stages of cereals. Weed Research 14: 415–421.

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, Blackwell Verlag, Berlin, Plant Breeding 122: 40-46.

Zwart R.S., Muylle H., Van Bockstaele E. & Roldán-Ruiz I. 2008. Evaluation of genetic diversity of Fusarium Head Blight resistance in European winter wheat. Springer-Verlag, Theor Appl Genet 117: 813–828.