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EVIDENCE FOR INCREASED AGGRESSIVENESS IN *FUSARIUM* SPECIES CAUSING HEAD BLIGHT DETECTED USING SERIAL PASSAGE ASSAYS THROUGH BARLEY CULTIVARS OF CONTRASTED QUANTITATIVE RESISTANCE LEVELS *IN VITRO*

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A B S T R A C T

Fusarium head blight (FHB) leads to significant quality and yield losses makes the FHB disease an important threat in all barley-growing areas of the world. Till now, available empirical data are limited on the efficiency of quantitative barley resistance expected to reduce in several generations because of the selection of FHB isolates with a high level of quantitative component of pathogenicity, i.e., aggressiveness, because of the difficulty of conducting such studies in the field. To achieve this goal, the evolutionary aggressiveness response was analyzed in four FHB pathogens faced with selective pressure using *in vitro* serial passage assays on susceptible "S" and moderately resistant "MR" barley. Differences due to the selective effect of a cultivar among non-selected and selected FHB isolates were quantified for traits participating to parasitic (area under disease progress curve (AUDPC) and latent period (LP)) fitness. The pathogen populations adapted quickly to the "MR" cultivar than the "S" cultivar. The selective barley impact on the analyzed FHB pathogens seemed to be species-specific. The results showed that selected isolates on the "MR" cultivar presenting a high level of aggressiveness than selected isolates from "S" cultivar, as they had a shorter LP (21.8%) and a higher level of AUDPC (22.1%). These findings provide the first direct evidence that FHB pathogens evolve rapidly to adapt by increasing aggressiveness to barley, indicating a risk of directional selection (i.e., deployment of a resistance gene) and possible erosion, evolution of aggressiveness due to resistance selection pressure over generations can lead to quantitative resistance erosion, of barely resistance, a vital component for the progress of durable control policies for resistant barley cultivars to FHB infection.

Keywords: area under disease progress curve, directional selection pressure, erosion, FHB fungi, latent period.

INTRODUCTION

Advantage in durable genetic quantitative resistance (QR) to phytopathogenic organisms is growingly being considered as a crucial component in disease management strategy (Mundt *et al.,* 2002). QR results in a reduction in disease can be established on a little to various genes with partial impacts linked to quantitative trait loci (QTLs) and reacted with the quantitative component of pathogenicity, i.e., aggressiveness (Parlevliet, 2002). However, though several QTLs are exhibited to be efficient to a wide range of pathogen isolates, some QTLs are demonstrated to be isolate specific (Krenz *et al.,* 2008). A matching augmentation in pathogen aggressiveness resulting in an erosion of QR has been widely documented (McDonald and Linde, 2002). The functions of recombination, mutation and migration participating to diversities in the aggressiveness of necrotrophic phytopathogenic organisms are widely less completely comprehended compared with biotrophic phytopathogenic organisms (Burdon and Silk, 1997; Wang *et al.,* 2008).

Aggressiveness components directly linked to parasitic fitness can be quantified by evaluating multiple quantitative traits such as sporulation rate, infectious period, infection efficiency, latent period and lesion size (Pariaud *et al.,* 2009). Trait such as growth rate has been used to describe the saprophytic fitness of phytopathogenic fungi. Competition among fungal

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species is crucial over the saprophytic phase; species with weak saprophytic fitness ability is quickly replaced by other competitors. Thus, this permits the compete population to develop and reproduce actively over the parasitic phase (Leach *et al.,* 2001). Several reports have demonstrated that the adaptation of fungal plant pathogens, in terms of parasitic or saprophytic fitness traits, can occur after repeated cycling on the same host (Ahmed *et al.,* 1996; Lehman and Shaner, 1997; Cowger and Mundt, 2002; Akinsanmi *et al.,* 2007; Abang *et al.,* 2006; Wang *et al.,* 2008).The evolution of phytopathogenic organisms when faced with selective pressures in the host may be constrained by fitness costs linked with adaptation to QR (cost of aggressiveness) (Leach *et al.,* 2001).

Fusarium head blight (FHB) is an economically principal disease of barley and other small grain cereals (i.e., triticale, rye, oat and wheat) (Parry *et al.,* 1995). FHB negatively decreases productivity and damages kernel quality due especially to the accumulation of dangerous mycotoxins (DON) which are noxious to the healthy of human and animal beings. As a result, the contamination by DON makes harvested barley grains unsuitable for brewing and malting industry (Xu and Nicholson, 2009). The most worldwide-spread sources of FHB in infected areas are the most aggressive fungi *F. culmorum*, *F. graminearum*, and *F. avenaceum*. Among the less repeatedly FHB pathogens are less aggressive causal agents as *F. equiseti*, *F. cerealis*, and *F. poae*, and, to a lower range, *F. verticillioides*, *F. solani*, and *F. proliferatum* (Bottalico and Perrone, 2002).

Changes in FHB population variation can have a large influence on agriculture, with emergence of novel head blight populations having potentially greater aggressiveness, higher genetic resistance to fungicides or elevated DON production (Puri and Zhong, 2010; Chen *et al.,* 2011). FHB fungi persist saprophytically in debris of small grains and maize (Xu and Nicholson, 2009), however, there is no trade-off between parasitic (aggressiveness) and saprophytic (growth rate and fecundity) fitness in these pathogens (Akinsanmi *et al.,* 2007). Till recently, the presence of head blight pathogens has not documented in Syrian barley fields. However, *Fusarium* pathogens are widely sampled form naturally FHB-diseases wheat kernels (Sakr, 2017). However, head blight isolates from wheat showed an identical range of pathogenicity under *in vitro* conditions on both: Arabi Aswad (AS) and durum wheat plants (Sakr, 2018a). Furthermore, FHB agents are recovered from barley fields in the neighboring countries, i.e., Iraq and Iran (Matny *et al.,* 2012; Chehri and Godini, 2017).

Resistance of barley to head blight fungi is a quantitative trait governed by a polygenic system (Xu and Nicholson, 2009). Furthermore, isolate-specific resistance in barley to any of the *Fusarium* pathogens involved in the head blight complex has not been detected (Parry *et al.,* 1995). Area under disease progress curve (AUDPC) and latent period (LP) (period from inoculation to sporulation) have been considered as the most main *in vitro* components for analyzing both QR and aggressiveness and in the barley–head blight association (Sakr, 2018b, 2019). FHB isolates having greater AUDPCs and shorter LPs are seen to be more aggressive on barley plants than isolates having lesser AUDPCs and longer LPs (Sakr, 2018b, 2019). These *in vitro* aggressiveness components evaluated on the scale of a particular barley plant might mostly detect the rate of epidemic progress (Sakr, 2018b). As long as AUDPC and LP are *in vitro* indicators of mechanisms of QR and aggressiveness occurring in the adult barely plants through head blight infection (Sakr, 2018b, 2019); the measured changes of evolutionary response of FHB populations faced with resistance selection pressure can be seen to be mostly the same as pathogenic reactions in barley plants planted under field conditions. Recently, Sakr (2022) explored how the deployment of QR in wheat influences modifications of the aggressiveness under *in vitro* conditions which leaded to potential resistance erosion. FHB pathogens evolve rapidly to adapt by increasing aggressiveness to wheat, indicating a risk of directional selection and possible erosion of FHB resistance (Sakr, 2022).

The barley–FHB association is ideal for analyzing the aggressiveness trait evolution in response to quantitative resistance selection pressure. FHB populations possess high levels of aggressiveness variation (Xue*et al.,* 2006; Sakr, 2019, 2020a, 2020b) and thus the basis for rapid adaptation. FHB disease intensity changes quantitatively relying on the resistance of the barley genotypes (Dweba *et al.,* 2015); it is thus helpful to explorer how the deployment of QR impacts variations of the aggressiveness which results in potential resistance erosion. In order to improve profound comprehension of the process of aggressiveness evolution in the barley–head blight association, here we aimed to (i) analyze whether the

aggressiveness (LP and AUDPC) of four FHB pathogens increases after *in vitro* serial passage assays on moderately resistant and susceptible barley cultivars, (ii) explore the pathogen specificity to barley plants, (iii) underline presence of a trade-off between parasitic (aggressiveness) and saprophytic (growth rate) fitness, and (iv) determine potential fitness costs associated with adaptation.

MATERIALS AND METHODS

Head blight isolates, plant materials and inoculum preparation

A set of 16 single-spore derived cultures of four *Fusarium* organisms causing head blight, i.e. (*F. culmorum* (5 isolates), *F. solani* (6 isolates), *F. verticillioides* (synonym *F. moniliforme*) (4 isolates), and *F. equiseti* (one isolate)) was tested in previous *in vitro* studies (Sakr, 2019) to cause head blight disease on two widely grown Syrian barley cultivars previously reported to be quantitatively resistant under *in vitro*, controlled and field conditions (Sakr 2018b, 2019, 2020a, 2020b). Two barley cultivars: cv. Arabi Abiad (AB) (susceptible, "S") with lower values of latent period (LP) and cv. Arabi Aswad AS (moderately resistant, "MR") with higher values of LP were chosen as source of plant materials in LP experiment (Sakr, 2018b). Four highly aggressive pathogens causing shorter latent periods on AB (F30, F7, F16 and F43) and AS (F3, F7, F43 and F21) were utilized for inoculation (Sakr 2019). Two barley cultivars: cv. AB "S" with greater values of area under disease progress curves (AUDPC) and cv. AS "MR" with lower values of AUDPC were chosen as source of plant materials in AUDPC experiment (Sakr, 2018b). Four highly aggressive pathogens for each FHB species causing greater area under disease progress curves on AB (F3, F31, F16 and F43) and AS (F3, F31, F15 and F43) were used for inoculation (Sakr, 2019).

To prepare spores for inoculation, fungal suspension or 4 to 6 agar plugs of each isolate stored at -16 °C were put on the surface of 9-cm Petri plates with potato dextrose agar (PDA) and incubated in the dark for 10 days at 22ºC. Following mycelial development and sporulation, the cultures were flooded with 10 ml of sterile distilled water to dislodge and collect the spores which were filtered through two layers of sterile cheesecloth (SC) to remove mycelia. Under an optical microscope, the resulting spore suspensions were immediately assessed with a hemacytometer and adjusted to 1×10^6 spores per ml for LP and AUDPC experiments. Serial passage trails on barley and aggressiveness comparison assays were both carried out on AB and AS for LP and AUDPC experiments.

Serial passage assays on barley and measurements of parasitic fitness

Metrologies for LP experiment were conducted as reported earlier by Browne (2009) to evaluate *in vitro* QR components and utilized newly by Sakr (2018, 2019) to assess both QR and aggressiveness in the barley–head blight system. LP which corresponds to aggressiveness expression of a given isolate was evaluated as duration in days from inoculation to sporulation. Three replications of each isolate established on notices on 120 detached leaves were arranged in which the plates were set up in a randomized block design (RBD), and the trial was duplicated twice.

Metrologies for AUDPC experiment were conducted as described earlier by Purahong *et al.,* (2012) to quantify *in vitro* aggressiveness criteria and used newly by Sakr (2018, 2019) to analyze both QR and aggressiveness in the barley–head blight system. AUDPC which corresponds to aggressiveness expression of a given isolate was evaluated as disease development during 6 days post inoculation (dpi) and its estimate was varied from 0 (not aggressive) to 1 (very aggressive). Three replications of each isolate were arranged in which the plates were set up in a RBD, and the trial was duplicated twice.

Re-isolation in each plate was conducted by flooding the sporulated detached leaves or seeds completely covered with mycelium with adequate amounts of SDW depending on the intensity of sporulation for LP and AUDPC experiments, respectively. Subsequently, the resulting conidia of one cycle of selection were dislodged, collected, filtered via two layers of SC to remove mycelia and adjusted to 1×10^6 spores per ml. Thus, these offspring isolates were separately preserved and utilized to begin the next serial passage utilizing the same method on barley for forty-nine asexual generations of selection giving a total of fifty serial generations. The variation of aggressiveness of selected isolates from passage assays on barley was measured in each monocyclic infection experiment by comparing AUDPC or LP of the offspring isolates to their ancestral isolate. Once evolution of aggressiveness achieved, a portion of the resulting spores of selected isolates was multiplied for saprophytic fitness and fitness cost analyses and another portion was used to start the next

serial passage in order to analyze a potential increase of aggressiveness until the achievement of the fifty selection generations. FHB ancestral and offspring isolates were maintained at 4°Cin sterile distilled water or at -16°C by freezing till used (Sakr, 2020c).

Serial passage assays on PDA

To complete the serial passage with barley, all the parental isolates were serially passaged on PDA. This was conducted by multiplying all old isolates on PDA under continues darkness at 22°C for ten days, after which the resulting conidia of one cycle of selection were transferred separately into novel plates. These were considered as the offspring isolates and the total proceeding was then repeated forty-nine times giving a total of fifty serial generations on PDA. Three replications of each isolate were arranged in which the plates were set up in a RBD, and the trail was duplicated twice. Once evolution of aggressiveness achieved for a given selected isolate in a given monocyclic experiment during passage assays on barley, a portion of the resulting spores of offspring isolate in the corresponding monocyclic experiment from passage assays on PDA was multiplied for aggressiveness (LP and AUDPC) analyses by comparing the changes of aggressiveness between ancestral and offspring isolates on both moderately resistant and susceptible cultivars and another portion was used to start the next serial passage on PDA in parallel with passage assays on barley until the achievement of the fifty selection generations.

Measurements of saprophytic fitness

The growth rate of ancestral and offspring isolates was evaluated *in vitro* on PDA plates in five replicates of each isolate and the plates were set up in a RBD. The trial was duplicated twice. A 7 mm agar plug was taken from the edge of actively growing 10 day old culture plate of each ancestral and offspring isolate and placed in the centre of a PDA plate and incubated the same way as that for inoculum preparation. Radial growth was obtained by measuring two perpendicular axes at 72 h.

Measurements of fitness cost

Two trials were carried out under *in vitro* and growth chamber conditions to determine fitness cost on barley plants of a very susceptible cultivar to FHB infection (cv. Furat-7 (Sakr, unpublished data)) between ancestral isolates and offspring isolates generated from passage assays on two barley cultivars with varying quantitative resistance. In trial I, differences in aggressiveness traits (LP and AUDPC) between ancestral isolates and offspring isolates were analyzed *in vitro* using the same methodology described previously. In trial II, variations in aggressiveness traits (disease incidence, DI and disease severity, DS) between ancestral isolates and offspring isolates were distinguished using spray and floret artificial inoculations in the growth chamber, respectively using the same methodology described previously by Sakr (2020a). DI (% symptomatic heads) was evaluated as the proportion of heads exhibiting head blight symptoms. DS (% symptomatic spikelets/head) was measured as the proportion of diseased spikelets on the inoculated heads with visually identified head blight symptoms on a nine grade scale as described by Xue*et al.,* (2006), where 0 referred no disease and 9 referred dead or gravely diseased.

Statistical analyses

An analysis of variances (ANOVA) treated the experimental findings using DSAASTAT add-in version 2011. Before statistical analysis, the proportions were transformed utilizing angular transformation to stabilize variances. A Fisher's least significant difference test compared the differences between ancestral isolates and offspring isolates generated from passage experiments with a significant level of $P<0.05$.

RESULTS

Serial passage assays on barley for LP and AUDPC experiments: Regarding LP and AUDPC trials, all highly aggressive pathogens successfully passaged through 50 monocycles on barley, suggesting that FHB isolates kept their capacity to confer *in vitro* symptoms on juvenile plant parts at several stages during the serial passage process, whereas the control plants did not show any disease symptoms. Generally, it was observed that LP decreased with increasing AUDPC in selected FHB isolates irrespective of the inoculated barley cultivar (moderately resistant "MR" versus susceptible "S" cultivars). Diversities in LP and AUDPC (% of non-selected isolate) between selected isolates of both (*F. culmorum* and *F. equiseti*) and (*F. solani* and *F. verticillioides*) were not significant regardless of the inoculated barley cultivar (data not shown). Once evolution of aggressiveness achieved for a given selected isolate in a given monocyclic experiment from passage assays on barley, no significant differences were detected for LP and AUDPC in the

successive selected isolates till the end of the fifty passage assays.

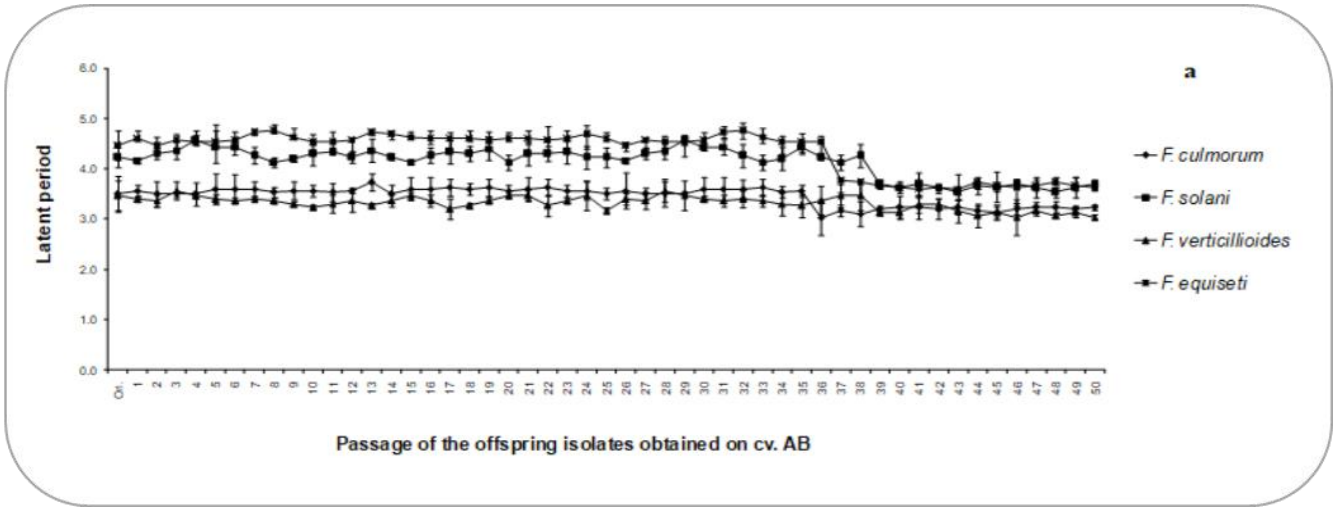
The pathogen populations evolved most slowly on "S" cv. AB (Figure 1, a and b), no significant differences in aggressiveness were indicated in offspring isolates generated till the 35 passage assays. Furthermore, offspring isolates generally showed higher aggressiveness than that of ancestral isolates on this "S" cultivar. From the ~ 36th passage assay on, offspring isolates of *F. culmorum* and *F. equiseti* had a shorter LP (24.6%) and a higher level of AUDPC (31.7%) compared with offspring isolates of *F. solani* and *F. verticillioides*, from the ~ 39th passage assay on.

Evolution was faster on "MR" cv. AS (Figure 1, c and d), no significant differences in aggressiveness were observed in offspring isolates generated till the 31 passage assays. In addition, offspring isolates generally exhibited higher aggressiveness than that of ancestral isolates on this "MR" cultivar. From the ~ 32th passage assay on, offspring isolates of *F. culmorum* and *F. equiseti* had a shorter LP (32.1%) and a higher level of AUDPC (33.0%) compared with offspring isolates of *F. solani* and *F. verticillioides*, from the ~ 35th passage assay on. Selected isolates from "MR" cultivar were more aggressive than selected isolates from "S" cultivar, as they had a shorter LP and higher levels of AUDPC. The mean LP and AUDPC of selected populations from "MR" of *F. culmorum*, *F. equiseti*, *F. solani* and *F. verticillioides* was shorter (21.8%) and higher (22.1%), receptively than that of selected populations from "S" cultivar of the four tested species.

Serial passage assays on PDA: All highly aggressive isolates of *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* successfully passaged through 50 cycles on PDA. It was observed the absence of contamination by bacteria or other fungi which affect the purity of selected isolates and the viable offspring cultures maintained their morphological features corresponded to their ancestral origin.

In parallel with serial passage trials for LP on AB, LP of offspring isolates generated from the passage assays on PDA: F3-C36, F31-C39, F16-C39 and F43-C37 was compared to their ancestral isolates. In parallel with serial passage trials for AUDPC on AB, AUDPC of F30-C37, F7-C40, F16-C39 and F43-C37 was compared to their ancestral isolates.

In parallel with serial passage trials for LP on AS, LP of F3-C33, F31-C35, F15-C35 and F43-C33 was compared to their ancestral isolates. In parallel with serial passage trials for AUDPC on AS, AUDPC of F3-C32, F7-C36, F21-C35 and F43-C33 was compared to their ancestral isolates. Seedlings of the four tested barley cultivars growing in the presence of FHB offspring isolates generated from the passage assays on PDA showed typical FHB symptoms according to *in vitro* LP and AUDPC assays. No significant differences in LP and AUDPC were observed on barley plants between non-selected and selected isolates generated from the passage assays on PDA (Table 1).



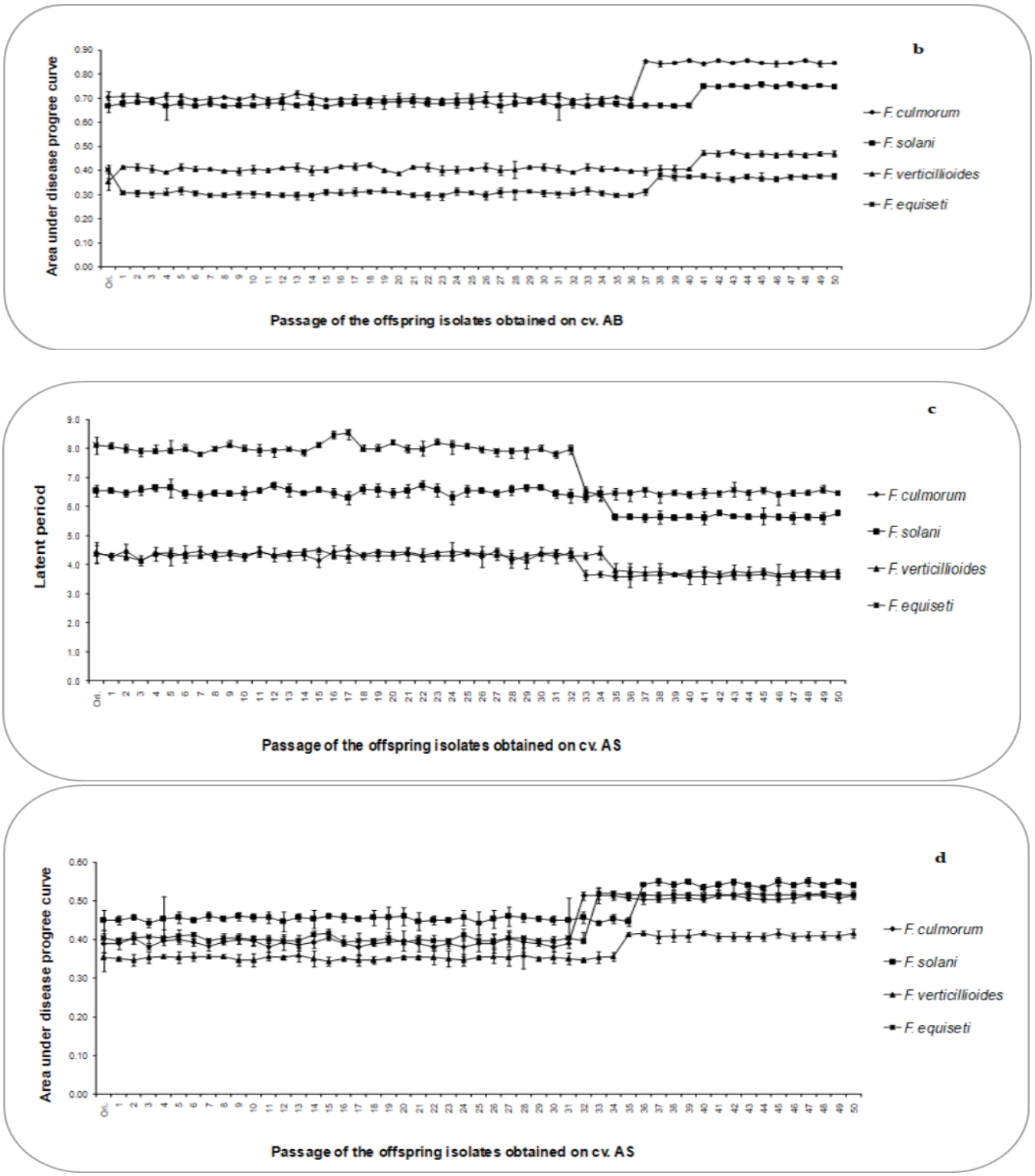


Figure 1. Variation of parasitic (aggressiveness) fitness in selected isolates of the four non-selected Fusarium head blight pathogens generated from serial passages on susceptible (Arabi Abiad, AB, a and b) and moderately resistant (Arabi Aswad, AS, c and d) barley cultivars. Two parasitic (aggressiveness) fitness traits are presented: latent period and area under disease progress curve. The values are mean of six replicates from two repeated experiments. Ori. = original. The bars are standard deviations.

Table 1. Comparisons in parasitic (aggressiveness) fitness measured on susceptible (Arabi Abiad, AB) and moderately resistant (Arabi Aswad, AS) barley cultivars between selected and non-selected isolates of four Fusarium head blight pathogens generated from serial passages on potato dextrose agar. Two parasitic (aggressiveness) fitness traits are presented: latent period (LP) and area under disease progress curve (AUDPC)

Fitness traits	cvs.	*F. culmorum*		*F. solani*		*F. verticillioides*		*F. equiseti*	
		S	N-S	S	N-S	S	N-S	S	N-S
LP	AB	3.5±0.2a	3.6±0.2a	4.2±0.2a	4.4±0.2a	3.4±0.2a	3.4±0.2a	4.6±0.1a	4.5±0.1a
	AS	4.3±0.2a	4.4±0.2a	6.6±0.2a	6.5±0.2a	4.4±0.1a	4.3±0.2a	8.0±0.2a	7.9±0.2a
AUDPC	AB	0.70±0.02a	0.69±0.01a	0.68±0.01a	0.67±0.02a	0.41±0.02a	0.41±0.01a	0.30±0.01a	0.30±0.02a
	AS	0.39±0.02a	0.38±0.01a	0.45±0.02a	0.46±0.01a	0.35±0.02a	0.36±0.02a	0.41±0.02a	0.40±0.02a

cvs. = cultivars, S = selected, N-S = non-selected. The values are mean ± standard deviation of six replicates from two repeated experiments. Values of aggressiveness between two selected and non-selected isolates of a given FHB pathogen in same linage with the same letter were not significantly different based on Fisher's LSD at P<0.05.

Measurements of component of saprophytic fitness: Growth rate of the offspring isolates (F3-C36, F31-C39, F16-C39 and F43-C37) for LP trial generated from the passage assays on AB, (F30-C37, F7-C40, F16-C39 and F43-C37) for AUDPC trial generated from the passage assays on AB, (F3-C33, F31-C35, F15-C35 and F43-C33) for LP trial generated from the passage assays on AS and (F3-C32, F7-C36, F21-C35 and F43-C33) for AUDPC trial generated from the passage assays on AS were compared to their ancestral isolates. Growth rates were the same for non-selected and selected pathogen populations (Table 2).

Table 2. Comparisons in saprophytic fitness between selected and non-selected isolates of four Fusarium head blight pathogens generated from serial passages on susceptible (Arabi Abiad, AB) and moderately resistant (Arabi Aswad, AS) barley cultivars for latent period (LP) and area under disease progress curve (AUDPC) trials. A saprophytic fitness trait is presented: growth rate (mm)

Saprophytic fitness trait	cvs.	*F. culmorum*		*F. solani*		*F. verticillioides*		*F. equiseti*	
		S	N-S	S	N-S	S	N-S	S	N-S
Growth rate	$AB^{LP\ trial}$	40.4±0.8a	40.7±1.3a	49.3±0.8a	39.0±0.8a	63.8±0.4a	63.6±0.7a	48.6±0.7a	48.0±0.9a
	$AS^{LP\ trial}$	40.5±0.7a	41.7±0.9a	49.4±0.8a	49.2±0.8a	56.2±0.9a	56.3±0.8a	48.8±0.8a	48.2±0.6a
	$AB^{AUDPC\ trial}$	30.1±0.9a	30.0±0.8a	43.8±0.8a	43.9±0.7a	63.6±0.5a	63.8±0.4a	48.8±0.7a	48.3±0.5a
	$AS^{AUDPC\ trial}$	40.6±0.7a	41.0±0.8a	43.9±0.7a	44.0±0.7a	43.3±1.2a	43.0±1.1a	48.5±0.7a	48.4±0.5a

cvs. = cultivars, S = selected, N-S = non-selected. The values are mean ± standard deviation of ten replicates from two repeated experiments. Values of saprophytic fitness between two selected and non-selected isolates of a given FHB pathogen in same linage with the same letter were not significantly different based on Fisher's LSD at P<0.05.

Measurements of fitness cost: Aggressiveness of the offspring isolates, previously mentioned, generated from the passage assays on AB and AS, respectively were compared to their ancestral isolates on barley plants of a very susceptible cultivar to FHB infection (cv. Furat-7) in two trials under *in vitro* and growth chamber conditions. In trial I, seedlings of cv. Furat-7 growing in the presence of FHB offspring isolates showed typical FHB symptoms according to *in vitro* LP and AUDPC assays. In trial II, cv. Furat-7 did not escape from head blight disease. Typical FHB symptoms induced by the offspring isolates were clear and easy to score in the inoculated spikes and spikelets. Generally, the ancestral and offspring isolates caused more severe symptoms on cv. Furat-7 compared with the two tested barley cultivars under *in vitro* and controlled conditions, indicating that cv. Furat-7 is a very

susceptible barley cultivar to FHB infection. For the two trials I and II, no significant differences were observed between non-selected isolates and selected isolates for LP and AUDPC *in vitro* traits (Table 3) and DI and DS traits obtained from head and floret artificial inoculation assays in growth chamber (Table 3), whereas the control plants did not show any FHB symptoms.

Table 3. Comparisons in fitness cost measured on a very susceptible (Furat-7) barley cultivar between selected and non-selected isolates of four Fusarium head blight pathogens generated from serial passages on susceptible (Arabi Abiad, AB) and moderately resistant (Arabi Aswad, AS) barley cultivars for latent period (LP) and area under disease progress curve (AUDPC) trials. Four parasitic fitness traits are presented: in trial I, latent period (LP) and area under disease progress curve (AUDPC) measured under *in vitro* conditions and in trial II, disease incidence (DI) and disease severity (DS) (%) measured using spray and point artificial inoculations, receptively under controlled conditions

cvs.	Parasitic fitness traits (trials I+II)	*F. culmorum*		*F. solani*		*F. verticillioides*		*F. equiseti*	
		S	N-S	S	N-S	S	N-S	S	N-S
$AB^{LP\ trial}$	LP	3.2±0.2a	3.1±0.1a	3.7±0.2a	3.6±0.1a	2.9±0.1a	2.8±0.2a	3.9±0.1a	4.0±0.1a
	AUDPC	0.71±0.01a	0.70±0.02a	0.43±0.01a	0.42±0.02a	0.51±0.02a	0.50±0.01a	0.41±0.02a	0.42±0.01a
	DI (%)	74.7±0.8a	75.0±0.6a	41.3±0.8a	41.7±1.1a	52.3±1.0a	51.8±1.3a	30.7±0.8a	30.2±0.8a
	DS (%)	68.2±1.0a	68.0±0.8a	46.8±0.7a	47.2±1.1a	34.2±0.8a	34.5±1.0a	44.8±0.8a	45.2±1.0a
$AS^{LP\ trial}$	LP	3.9±0.2a	3.9±0.1a	5.9±0.1a	6.0±0.1a	3.8±0.1a	3.9±0.1a	6.6±0.1a	6.7±0.1a
	AUDPC	0.42±0.01a	0.41±0.01a	0.45±0.01a	0.44±0.02a	0.32±0.02a	0.32±0.01a	0.54±0.03a	0.55±0.01a
	DI (%)	44.3±1.2a	43.8±1.5a	55.3±0.9a	55.7±1.0a	33.7±1.1a	34.0±0.8a	62.3±0.5a	62.7±0.5a
	DS (%)	37.7±0.5a	38.0±0.8a	51.0±0.7a	51.3±1.1a	31.2±0.5a	31.3±0.5a	74.3±0.5a	74.2±0.4a
AB^{AUDPC} trial	LP	4.7±0.1a	7.5±0.1a	8.0±1.0a	8.1±0.2a	2.6±0.1a	2.7±0.1a	3.8±0.1a	3.6±0.2a
	AUDPC	0.81±0.02a	0.83±0.03a	0.75±0.02a	0.74±0.03a	0.49±0.02a	0.50±0.03a	0.43±0.01a	0.43±0.02a
	DI (%)	90.0±0.9a	90.5±1.0a	71.8±0.5a	72.0±0.8a	51.5±0.8a	51.2±1.1a	30.8±0.8a	31.2±1.3a
	DS (%)	83.3±1.2a	82.7±1.6a	76.3±1.6a	77.0±1.2a	31.7±0.5a	31.8±0.9a	42.0±0.8a	42.3±0.8a
AS^{AUDPC} trial	LP	3.6±0.1a	3.7±0.2a	7.2±0.2a	7.1±0.1a	5.9±0.2a	5.9±0.1a	6.5±0.1a	6.4±0.1a
	AUDPC	0.45±0.02a	0.45±0.01a	0.52±0.02a	0.53±0.02a	0.46±0.01a	0.47±0.01a	0.53±0.01a	0.54±0.01a
	DI (%)	44.2±0.8a	44.3±1.0a	56.3±0.8a	56.8±0.8a	41.2±0.5a	41.5±0.8a	62.2±1.0a	61.7±0.9a
	DS (%)	34.3±0.5a	34.0±0.8a	43.8±0.8a	44.3±1.1a	25.0±0.7a	25.2±0.8a	74.7±0.5a	74.2±1.0a

cvs. = cultivars, S = selected, N-S = non-selected. The values are mean ± standard deviation of six replicates from two repeated experiments. Values of aggressiveness between two selected and non-selected isolates of a given FHB pathogen in same linage with the same letter were not significantly different based on Fisher's LSD at $P<0.05$.

DISCUSSION

Our findings demonstrated that selected isolates serially passaged on barley were significantly more aggressive than non-selected isolates, while no increasing aggressiveness was found in these obtained on PDA, indicating that the evolution of aggressiveness in FHB agents is associated with the presence of barley. Our findings are in parallel with those presented by Wang *et al.,* (2008) for Fusarium wilt pathogen, *F. oxysporum* f. sp. *vasinfectum*, serially passaged on water agar and cotton. The current report presents experimental evidence that the evolution of both aggressiveness components: area under disease progress curve (AUDPC) and latent period (LP) in FHB populations occurs in a continuous evolution. As highly aggressive isolates were chosen to serially passage on barley, LP and AUDPC was observed to gradually increase in these isolates irrespective of the inoculated barley cultivar (moderately resistant "MR" versus susceptible "S" cultivars). This type of continual evolution in aggressiveness has earlier shown in other *Fusarium* pathogens (Wang *et al.,* 2008).

Because of the linkage between both aggressiveness criteria: AUDPC and LP and head blight progress under field conditions (Sakr, 2019), comprehension the diversity of AUDPC and LP for *Fusarium* populations on quantitatively barley cultivars and the response of these populations to selection should be useful in predicting durability of barley resistance. Here, we observed that

selected isolates from "MR" cultivar were more aggressive than selected isolates from "S" cultivar. These data agree with other reports (Cowger and Mundt, 2002; Abang*et al.,* 2006; Delmas*et al.,* 2016). The adapted isolates had a shorter LP associated with a higher level of AUDPC. Despite FHB pathogens are generally restricted to one infection cycle per season because of short duration of vulnerability to infection in anthesis stage (Xu and Nicholson, 2009); these traits may present various advantages on these isolates influencing population fitness, encompassing a greater ability for transmission to healthy resistant barley plants which results in faster rates of FHB disease development.

In theory, selection for maximal pathogen aggressiveness could be impeded by trade-offs between aggressiveness features (Leach *et al.,* 2001). We observed that LP was negatively linked with irrespective of the inoculated barley cultivar. An identical phenotypic linkage has been observed between spore production and LP in a study investigating selective effect of quantitative host resistance on the pathogen of grapevine downy mildew, *Plasmopara viticola* (Delmas *et al.,* 2016). Adapted isolates of FHB populations exhibiting more AUDPC are as long as more infective in the following generation of the pathogen life cycle and possibly have a selective feature. Besides, high levels of AUDPC were linked with a short LP, which would exhibit a competitiveness feature on aggressive isolates.

Our findings show that FHB pathogens showed the most rapid evolution on "MR" barley plants compared with slow evolution on the "S" cultivar. Adapted FHB isolates on "MR" plants were selected five serial cycles sooner compared with adapted isolates on "S" plants. Also, adaptation to quantitative resistance was correlated by an augmentation in aggressiveness, indicating that pathogen populations changeably adapted depending on selective pressures imposed by barley plants with contrasted resistance levels. Such observation has been supported by a study investigating quantitative resistance selection in barley on *Rhynchosporium secalis*, a scald pathogen (Abang *et al.,* 2006).

It has been mainly accepted that highly aggressive FHB isolates may induce more grave symptoms in moderately resistant barley cultivars than do less aggressive isolates; thus, isolate-specific resistance has not been observed (Xu and Nicholson, 2009). However, other studies reported that FHB isolates show some degree of host specialization (Xue *et al.,* 2006; Sakr, 2020a, 2020b). Here, we found that the selective barley effect seemed to be species-specific; offspring isolates of some FHB populations evolved faster and had a shorter LP and a higher level of AUDPC compared with other populations. Adapted isolates of *F. equiseti* and *F. culmorum* pathogens are more aggressive than adapted isolates of *F. verticillioides* and *F. solani* and thus they have a selective advantage on any of barley cultivars whether it is resistant or susceptible. Our data propose that cultivation of either resistant or susceptible barley cultivar with the presence of isolates of *F. culmorum* and *F. equiseti* could select for specifically adapted pathogen isolates and lead to erosion of FHB resistance, making the surveillance of extent FHB pathogens is needed for improved control. LP and AUDPC aggressiveness traits appear to be conferred additively by genes with partial effects, *F. culmorum* and *F. equiseti* pathogens may possess quantitative traits which are conferred by a several genes and those for *F. solani* and *F. verticillioides* are conferred by a few genes on the same barley cultivar. Other possibility is that the frequency of the tested FHB pathogens is highly variable in barley fields (Bottalico and Perrone, 2002); greater selection pressure incited on *F. culmorum* and *F. equiseti* (widely distributed FHB species in most infected barley areas) compared with minor selection pressure incited on *F. solani* and *F. verticillioides* pathogens (less frequently FHB species) making these pathogens are more adaptive to be selected. Contrary to our results, Delmas *et al.,* (2016) found that *P. viticola* adaptation faced with grapevine quantitative resistance selection pressure, with the progress of higher aggressiveness, to be nonspecific and global.

Our findings demonstrated the absence of differential selection during the saprophytic phase among selected (more aggressive) and non-selected (less aggressive) FHB isolates, indicating that parasitic and saprophytic fitness traits may be independently controlled in four FHB pathogens and novel pathogens do not have a selective saprophytic advantage. Of key importance is the reason behind this observation that old and novel pathogens have an equal saprophytic fitness and competition among these selected and non-selected pathogens cannot exist; they thereby have the similar ability to infect barley plants in the next growing season. Our data are in accordance with those reported by Akinsanmi *et al.,* (2007); no trade-off were detected between parasitic (aggressiveness) and saprophytic

(growth rate and fecundity) fitness in two *Fusarium* pathogens (Akinsanmi *et al.,* 2007). Nevertheless, Tunali *et al.,* (2012) found a significant correlation between some saprophytic and pathogenic fitness components in three *Fusarium* species.

Fitness cost linked with pathogen adaptation to plant hosts is one of the most crucial factors affecting the extent and rate of adapted isolate emergence (Leach *et al.,* 2001). No apparent differences in aggressiveness traits assessed under *in vitro* conditions and in a growth chamber between non-selected and selected isolates on barley plants of a very susceptible cultivar, indicating that no fitness cost was linked with aggressiveness. In parallel to our results, Delmas *et al.,* (2016) observed that no differences between naive and adapted *P. viticola* populations. Nevertheless, the deficiency of evidence for fitness costs should be explained with care. The probability of fitness costs detectable only for features depending on the sexual part of the life cycle of FHB pathogens cannot be excluded, as these two fitness criteria were not evaluated here. Furthermore, the current report was conducted in optimal conditions for FHB pathogen progress, which may have reduced the opportunities of revealing few variations in aggressiveness features on the highly susceptible Furat-7 cultivar. Finally, fitness costs may have been hampered by compensative mutations. Indeed, second-position compensative mutations have been exhibited to decrease the damaging impact of another mutation on fitness (McDonald and Linde, 2002).

Fungal pathogens can bypass plant resistance barriers due to pathogenicity shifts owing to mutation, immigration or genetic recombination (McDonald and Linde, 2002). In our investigation, the potential role of immigration and sexual recombination contributing to changes in aggressiveness was not assessed. Thus, novel FHB adapted isolates arose through mutation here. In the genome of *F. oxysporum*, transposons produce spontaneous mutations resulting in variations in aggressiveness (Wang *et al.,* 2008). It has been proposed that newly emerging population of *F. graminearum* was more aggressive than the old population based on artificial floret inoculation assay in a greenhouse experiment (Puri and Zhong, 2010). The authors demonstrated that the newly population is different from the prevalent population and might have emerged more recently (Puri and Zhong, 2010). Our findings show that mutation, as the most significant evolutionary force in asexually reproducing pathogens (McDonald and Linde, 2002), is the definitive origin of genetic variability that increases aggressiveness of exciting FHB isolates to erode quantitative barley resistance.

In summary, our results provide the first direct evidence that FHB pathogens adapt to barley by increasing aggressiveness, suggesting a risk of directional selection and possible erosion of FHB resistance. Such adaptation is consistent with the high evolutionary possibility of FHB pathogens observed earlier for fungicide resistance and DON production. Rapidly evolving pathogens extremely complex the function of marinating the durability of plant resistance genes. This is especially correct for FHB pathogens in which we observed no trade-off between parasitic and saprophytic fitness, pathogen specificity to barley plants, no apparent fitness cost associated with this adaptation and potential role of mutation contributing to aggressiveness evolution. This information has implications for the development of FHB-resistant barley cultivars and disease management. First, it is postulated that use of barley quantitative resistance drive FHB populations to shift to novel adapted pathogens with greater aggressiveness. Thus, sampling and monitoring of FHB populations periodically on local and national scales are still necessary. Second, either “MR” or “S” barley cultivars can exert a selection pressure on FHB populations. Thus, if a mixture of “MR” and “S” barley cultivars were grown in a given region, evolution of FHB pathogens might be slowed owing to reduced exposure to the pathogen, disruptive selection on FHB populations and subsequently reduced fitness of *Fusarium* spp. This would stabilize the pathogen population and contribute to the durability of FHB resistance.

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Nachaat Sakr : Design experiment, conduct research and writing manuscript