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ORIGINAL ARTICLE

A novel index to estimate the stress-physiological status in wheat and barley against Fusarium head blight pathogens

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Wheat and barley can be massively invaded with Fusarium head blight (FHB) which results in decrease in grain weight and quality. Accurate evaluation of the stress-physiological status including quantitative resistance (QR) and aggressiveness in breeding programs is a key to selecting resistant cultivars. Out of several analyzed components, one or two metric components composing a disease index are commonly employed as a typical methodology to score FHB; however, this resistance index can not describe correctly and accurately the whole resistance in host plants. We have therefore assessed a novel index to estimate the physiological and pathogenic status in a set of diverse bread and durum wheat and barley cultivars under artificial infection with four *Fusarium* species. Eighteen QR components obtained under *in vitro*, growth camber and field conditions were evaluated and nine components at the earliest and latest growth stages were selected and combined in one index to score QR. This novel index was further investigated for its stability in the same fungi-seedling and -adult plants treated with silicon that reduced these nine pathogenic components forming this index. The scores of our index were highly significant and correlated with data of reductions resulted in silicon treatments across the bread and durum wheat and barley cultivars, thus it is suitable to be used for quantification of resistance in host plants and aggressiveness of *Fusarium* isolates. Findings show that the novel index analyzed in the present report captured key components of quantitative wheat and barley resistance. This index can be applied for screening the most aggressive isolates of different FHB species for FHB resistance breeding of wheat and barley. The mean of nine disease components is introduced in this paper as a new index for QR in plants and aggressiveness of fungi. Moreover, the novel index could be a starting point for more correct techniques for assessing on of the most damaging biotic stresses on cereals, FHB.

Key words: Hordeum vulgare, Fusarium pathogens, quantitative resistance, plant breeding, Triticum spp.

Wheat, including bread (*Triticum aestivum*) and durum (*T. durum*), and barley (*Hordeum vulgare* L.) are among the most important cultivated food and feed crops across the globe. Wheat alone contributes ~19% of worldwide human calorie diurnal and protein intake. Barley is largely utilized as animal feed (70%) and for beer production (27%), and successfully grown under a broad array of environments. Annually, they are cultivated over an area of 270 million ha, yielding over 905 million metric tons (FAO, 2022). While wheat and barley are susceptible to a wide range of noxious fungal diseases (physiological stressors), cereals losses due to fungal damages continue to pose an enormous menace to agricultural food and feed sectors, influence economic decisions as well as functional developments. Fusarium head blight (FHB) is a worldwide problem leading to a huge economic burden on the cereal industry because of its significant decreases in kernel yield and quality (Fernando *et al.,* 2021). Disease symptoms encompass head bleaching, necrosis and shriveled grains (Dahl and Wilson, 2018). Several *Fusarium* pathogens are proven to be phytopathogenic fungi, but in bread and durum wheat and barley the most dangerous head blight-causing agents are *F. graminearum* and *F. culmorum* (Xue *et al.,* 2019). FHB pathogens are characterized by low specificity (Sakr, 2022); it makes their spread in plant tissues and aggressiveness changeable and extremely infected by climatic conditions, thus an accurate and precise trail to evaluate the levels of aggressiveness of *Fusarium* fungi is requested. Following invasion, diverse *Fusarium* pathogens produce aggressive secondary metabolites, which result in crop contamination, such as deoxynivalenol, which have negative impacts on human diet, animal growth and fertility (Buerstmayr *et al.,* 2020).

Breeding and planting wheat and barley cultivars with stable resistance to FHB is considered to be the environmentally friendly and most cost-effective policy to decrease the risk of yield and quality losses (Fernando *et al.,* 2021). Wheat and barley resistance to head blight is generally divided into components of resistance with partial overlapping control and is quantitatively (QR) inherited with a complicated genetic architecture (Sakr, 2022), that can be distinguished into several classes (Buerstmayr *et al.,* 2020). Type I resistance, or FHB incidence, refers to the primary resistance to the invasion and it is generally evaluated as the percentage of invaded spikes in a plot as FHB incidence. Type II resistance is the resistance to Fusarium spread and it can be assessed as the percentage of infected florets in a spike as FHB severity. It is widely reported that either Type I or Type II resistance is only a part of the total host resistance. QR affects aggressiveness (i.e., the capacity of a pathogen to cause damage on a susceptible host) in plant pathogens (Van der Plank, 1968); so the experimental measurement of *quantitative* traits in resistance, referred to as *aggressiveness* components (Cowger and Brown, 2019); both QR and *aggressiveness* form the stress-physiological status.

Phenotyping is important for *Fusarium* research and breeding in wheat, barley and other small grains. All rely on the reliability and quality of phenotyping (Dahl and Wilson, 2018). However, problems in resistance types, the resistance evaluation, and the phenotyping problems in the quantitative trait locus (QTL) experiments and recognition of their function in forming genetic reactions were not in the centrum of the research (Mesterhazy 2020). The expression of QR is not only controlled by the host and the broad range of *Fusarium* species associated host, but also by the environment and their interactions (Sakr, 2022). Spreading of the infection is a common phenomenon in diseases, and during epidemics, steadily increasing numbers are reported (Buerstmayr *et al.,* 2020). Type I resistance has this spreading role, which results in issues. Ma *et al.,* (2019) mentions this problem and stresses the problem of distinguishing of Type I resistance, which was also analyzed by Dill-Macky (2003). Type II resistance (at floret infection) characterizes the spread of FHB from the ovary to the other parts of the spike. Consequently, it cannot solve the resistance issue alone (Mesterhazy 2020). FHB disease (combination of *Fusarium* incidence and *Fusarium* severity) is a typical methodology to record FHB symptoms (Tekauz *et al.,* 2000). Nevertheless, this index does not include the whole head blight development style and thus it can not identify correctly and accurate the entire resistance in

cereal plants (Fernando *et al.,* 2021). In addition, it is known to be partially linked with wheat height and anthesis/flowering date (Buerstmayr *et al.,* 2020). In this concept, recording and comparing head blight resistance across experiments should be conducted carefully (Mesterhazy 2020). In spite of other QR components such as growth rates, latent period, or dwarfing describe mainly biological functions in fungi-host relationships (Mesterhazy *et al.,* 2020); they do not involve successfully in head blight screening nurseries (Sakr, 2022).

Accurate evaluation of the stress-physiological status including quantitative resistance (QR) and aggressiveness in breeding programs is a key to selecting resistant cultivars. Recently, Sakr (2023) analyzed separately several QR components under diverse experimental conditions in a set of wheat and barley cultivars challenged with *Fusarium* fungi at the earliest and latest growth stages, and resistance levels were precisely and correctly identified depending on one QR component. The success of *Fusarium* evaluation for quantitative traits for resistance and aggressiveness relies on a good phenotypic value to properly capture all pathogenic responses caused by *Fusarium* on young and adult parts in the host plants (Mesterhazy 2020). The aims of this research were: 1) to evaluate the feasibility of a novel index based on nine quantitative traits to estimate the stress-physiological status in a set of diverse bread and durum wheat and barley cultivars under artificial infection with four *Fusarium* species; 2) to determine the different levels of QR among eight cereal cultivars and aggressiveness among FHB pathogens by using this index; and 3) to validate this index by correlating the results with the data of reductions resulted in silicon treatments that reduced these nine pathogenic components forming this index across the bread and durum wheat and barley cultivars infected with the same *Fusarium* fungi under *in vitro*, controlled (growth chamber), and field conditions.

MATERIALS AND METHODS

Plant materials, fungal isolates, and inoculum preparation

A set of eight cereal cultivars of Syrian origin covering a wide genetic and resistant variability including six *T. aestivum* and *T. durum* cultivars and two *H. vulgare* cultivars: Arabi Abiad (AB) and Arabi Aswad (AS) was chosen from previous *in vitro*, growth chamber and field experiments (Sakr, 2023) to represent a range of quantitative resistance types to FHB infection. Wheat and barley cultivars AS and Bohoth10 (bread) moderately resistant, AB, Cham4 and Douma4 (bread) moderately susceptible, Cham7 and Cham9 (durum) susceptible to moderately susceptible, and Acsad65 (durum, susceptible) were used.

Sixteen single-spore derived cultures of four *Fusarium* species 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)) chosen for their different aggressiveness levels (established on previous several experimental findings (Sakr, 2023)) were used. The isolates were collected through the 2015 growth season from naturally infected wheat heads over 9 locations in Ghab Plain, one of the principal Syrian wheat production areas, with a FHB history. Single spore cultures on Petri dishes with potato dextrose agar (PDA) with 13 mg/l kanamycin sulphate added after autoclaving, were classified morphologically to species level by using the keys of Leslie and Summerell (2006). By using random amplified polymorphic DNA markers, the 16 *Fusarium* species causing head blight isolates were recently analyzed (Sakr, 2023). The isolates were preserved by freezing at-16°C or in sterile distilled water at 4°C till use (Sakr, 2020).

FHB inoculum used for inoculation for the *in vitro*, growth chamber and field trials was normally performed as following: fungal suspension or four to six agar plugs out of each stored single-spore culture were put over the surface of Petri dishes PDA and incubated under continuous darkness at 22ºC for 10 days to allow sporulation and fungal development. Following incubation, isolates were covered with 10 ml of sterile distilled water and conidia were dislodged. Fungal suspensions were filtered through 2 layers of sterile cheesecloth to remove the pieces of mycelia and agar and directly quantified with a Neubauer chamber under an optical microscope and diluted to a desirable concentration as inoculum sources.

Selecting of quantitative components for resistance and aggressiveness

In order to select the appropriate, accurate and correct quantitative components for resistance and aggressiveness that form the index, two criteria were used and should be applied together: (1) cereal cultivars/*Fusarium* isolates should represent a substantial variation in resistance/aggressiveness for a given quantitative component, and (2) the scores of a given quantitative component for resistance and aggressiveness that showed a significant variability should significantly correlate among the values of other quantitative component that also exhibit a significant variation. So, the reaction of eight wheat and barley cultivars to a set of 16 fungal isolates was evaluated by measuring eighteen quantitative components at the seedling and adult plant stages (Table 1). Pathogenic reactions of all cultivars infected with *Fusarium* fungi were previously evaluated according to methods described by Sakr (2023). Then, all quantitative components were subjected to the two criteria to select accurate and correct quantitative components that compose the novel index, and the following components were selected (Table 1): latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a detached head test (DHT) under controlled conditions, disease incidence (DI^{CC}, Type I) detected using a head artificial inoculation and disease severity (DS^{CC}, Type II) detected using a floret artificial inoculation under controlled conditions in a growth chamber, and disease incidence (DI^{FC}, Type I) and disease severity (DS^{FC}, Type II) detected using a head artificial inoculation under field conditions (FC) over the three growing seasons. Since no significant interaction year × fungus/cultivar was observed (climatic data for the station were somewhat similar during the three growing seasons (Sakr, 2023)), field data were shown as the averages of the three growing seasons. Analysis of variance of bio-experiments revealed significant cultivar-by-isolate interactions for these nine quantitative components (Table 2); however, QR stability in cultivars to FHB infection was fulfilled over years as well as several experimental conditions, suggesting that QR of wheat and barley to *Fusarium* is mainly explained by major quantitative trait loci that confer resistance to all FHB isolates (Sakr, 2023). The constancy of QR resistance ratings of cultivars is consistent with a hypothesis that wheat- and barley-*Fusarium* interactions for QR were of reduced magnitude (Sakr, 2023).

A novel index to estimate the resistance of cereals and the aggressiveness of Fusarium fungi

Following the selection of the appropriate, accurate and correct quantitative components for resistance and aggressiveness that compose the index, a novel index to estimate the physiological and pathogenic status in wheat and barley challenged with FHB pathogens could be formed.

Resistance index (R index) was introduced (Eq. 1). This index combined nine components linked with resistance of wheat and barley cultivars.

$$R\ index = LP/(AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC}) \quad \text{(Eq. 1)}$$

Where:

A wheat/barley cultivar with higher value of LP and lower values of AUDPC, CD, DI^{DHT} (Type I), DS^{DHT} (Type II), DI^{CC} (Type I), DS^{CC} (Type II), DI^{FC} (Type I) and DS^{FC} (Type II) was considered as more resistant than a wheat/barley cultivar with lower value of LP and higher values of AUDPC, dwarfing, DI^{DHT} (Type I), DS^{DHT} (Type II), DI^{CC} (Type I), DS^{CC} (Type II), DI^{FC} (Type I) and DS^{FC} (Type II).

Aggressiveness index (A index) was introduced (Eq. 2). This index combined nine components linked with aggressiveness of fungal isolates.

$$A\ index = (AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC})/\ LP \quad \text{(Eq. 2)}$$

Where:

A *Fusarium* isolate with higher values of AUDPC, CD, DI^{DHT} (Type I), DS^{DHT} (Type II), DI^{CC} (Type I), DS^{CC} (Type II), DI^{FC} (Type I) and DS^{FC} (Type II) and lower value of LP was considered as more aggressive than a *Fusarium* isolate with lower value of AUDPC, CD, DI^{DHT} (Type I), DS^{DHT} (Type II), DI^{CC} (Type I), DS^{CC} (Type II),

DI^{FC} (Type I) and DS^{FC} (Type II) and higher values of LP.

Validation of stability of "R index" and "A index"

To verify the stability of the novel index among eight wheat and barley cultivars infected with a set of 16 fungal isolates of four *Fusarium* pathogens, the correlation analyses were assessed between data of novel index and silicon (Si) treatments including the same plant and fungal materials. Since the decrease in FHB severity due to silicon application was not affected by the pathogenicity of the disease isolate (Sakr and Kurdali, 2023a,b), R index and A index were further investigated for their stability in the same fungi-seedling and -adult plants treated with silicon that reduced these nine pathogenic components forming this index.

Statistical analyses

The experimental data were subjected to analysis of variances (ANOVA) using DSAASTAT add-in version 2011. Before statistical analysis, the percentages were transformed using the angular transformation to stabilize variances. ANOVA incorporating the Fisher's LSD test at $p \leq 0.05$ was used to (1) determine the host cultivar × isolate interactions, and (2) compare the means of resistance of cultivars and aggressiveness of fungal isolates. The simple correlation (Pearson r) at $p<0.05$ was used to test for correlations among the scores of R index and A index and data of reductions resulted in silicon treatments across the same tested bread and durum wheat and barley cultivars infected with the similar *Fusarium* isolates.

RESULTS

Relative to the water-negative control, wheat/barley plants growing in the presence of 16 *Fusarium* culrtres causing FHB under several experimental conditions exhibited typical head blight symptoms, showing a strong impact of *Fusarium* fungi on the growth of wheat and barley plants across the earliest and latest development stages. Table 3 shows values of RI among the tested wheat and barley cultivars. NOVA detected significant differences between cultivars for scores of R index that ranged from 10.8 to 23.2 among the tested cultivars. Results indicated that AS and Bohoth10 were the most resistant cultivars with a value of R index of 26.1; followed by other tested cultivars, and Acsad65 was the most susceptible one with a value of R index of 10.8.

Values of AI among the tested fungal isolates were shown in Table 4. ANOVA detected significant differences among FHB isolates for scores of A index that ranged from 5.9 to 7.5 among the tested *Fusarium* isolates. Results shown in Table 4 indicated that the isolates F16 of *F. verticillioides* and F29 of *F. solani* were the most aggressive with a value of A index of 7.4; followed by other tested FHB isolates, and isolate F35 of *F. solani* was the less aggressive one with a value of R index of 5.9. Aggressiveness index did distinguish isolates within and among species.

The values of our index were highly significant and correlated with data of reductions resulted in silicon treatments across all the tested bread, durum and barley cultivars for quantitative traits related for resistance (Table 5) and aggressiveness (Table 6).

DISCUSSION

A main and continuous challenge for the breeders in wheat and barley producing countries is to supply cultivars with elevated and durable level of resistance to head blight (Dahl and Wilson, 2018), which significantly decreases yield and quality losses (Fernando *et al.,* 2021). Because the severity of *Fusarium* species communally changes from region to region and from year to year resulting in alternation in disease epidemics and aggressiveness shifts of head blight population that leads to potential erosion in resistance (Sakr, 2022), researches to describe a compressive index to assess the stress-physiological status in wheat and barley challenged with *Fusarium* pathogens are of great importance (Mesterhazy, 2020). Since climatic and environmental conditions infect *Fusarium*/cereal reactions because of isolate/cultivar by environment interaction (Buerstmayr *et al.,* 2020; Fernando *et al.,* 2021), FHB responses have been successfully analyzed *in vitro*, in climatic growth chamber and field conditions using young plant parts and adult heads (Sakr, 2023). In order to assess the accurate and correct stress-physiological status including both: QR in wheat and barley and aggressiveness of FHB populations; the novel index tested in this study for the first time captured key and appropriate QR and pathogenicity components at the earliest and latest growth stages representing a

wide range of several experimental and natural conditions.

Table 1. Selecting of quantitative components for resistance and aggressiveness on eight wheat and barley cultivars of Syrian origin infected with a set of 16 fungal isolates of four Fusarium head blight species

Experimental assay	Quantitative components	First criterion	Second criterion
***In vitro* detached leaf inoculation**	**Incubation period**	No	No
	Latent period	Yes	Yes
	Lesion length	No	No
***In vitro* Petri-dish assay**	**Germination rate reduction**	No	No
	Area under disease progress curve	Yes	Yes
	Coleoptile length reduction	No	No
***In vitro* seedling assay**	**Percentage of infected seedlings of spraying inoculation assay**	No	No
	Percentage of infected seedlings of pin-point inoculation assay	No	No
	Lesion length of clip-dipping inoculation assay	No	No
***In vitro* coleoptile infection assay**	**Seed germination**	Yes	No
	Coleoptile dwarfing	Yes	Yes
	Coleoptile weight	Yes	No
	Root weight	Yes	No
A detached head assay under controlled conditions	**Disease incidence**	Yes	Yes
	Disease severity	Yes	Yes
An artificial inoculation assay of adult plants under controlled conditions	**Disease incidence**	Yes	Yes
	Disease severity	Yes	Yes
An artificial inoculation assay of adult plants under field conditions	**Disease incidence**	Yes	Yes
	Disease severity	Yes	Yes
	Fusarium damaged kernels	No	No

Pathogenic reactions of all cultivars infected with *Fusarium* fungi were previously evaluated according to methods described by Sakr (2023). Two criteria were used and should be applied together: (1) cereal cultivars/*Fusarium* isolates should represent a substantial variation in resistance/aggressiveness for a given quantitative component, and (2) the scores of a given quantitative component for resistance and aggressiveness that showed a significant variability should significantly correlate among the values of other quantitative component that also exhibit a significant variation. Yes: a variation was detected for a given quantitative component for the first criterion/a significant correlation was detected between a given quantitative component and all quantitative components that showed a significant variation for pathogens and cultivars; No: No significant variation was detected for a given quantitative component for the first criterion/no significant correlation was detected between a given quantitative component and all quantitative components that showed a significant/no significant variation for pathogens and cultivars.

Table 2. Analyses of variance for latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DICC, Type I) detected using a head artificial inoculation and disease severity (DSCC, Type II) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a head artificial inoculation under field conditions (FC) over the three growing seasons (F-test values)

Source of variation	***df***	**LP, AUDPC, CL, DI^{DHT}, Type I, DS^{DHT}, Type II, DI^{CC}, Type I, DS^{CC}, Type II, DI^{FC}, Type I, DS^{FC}, Type II**
Cultivar (C)	7	$^{++}$
Isolate (I)	15	$^{++}$
C × I	105	$^{++}$
Error	256	

$^{++}$ – significant at 1% level; *df* – degree of freedom.

Resistance of the eight wheat and barley cultivars infected with *Fusarium* fungi was earlier analyzed and presented by Sakr (2023).

Table 3. Scores of resistance index composing from nine quantitative resistance components on eight wheat and barley cultivars of Syrian origin infected with a set of 16 fungal isolates of four Fusarium head blight species

Cultivars	Nine quantitative resistance components									Resistance index (value × 10)
	LP (days × 10)	AUDPC (value × 100)	CD (%)	DI^{DHT}(%)	DS^{DHT}(%)	DI^{CC}(%)	DS^{CC}(%)	DI^{FC}(%)	DS^{FC}(%)	
Acsad65, durum	43	49	44	57	51	55	50	53	40	10.8
Cham7, durum	45	46	42	52	46	48	46	48	37	12.3
Cham9, durum	48	44	41	53	43	47	47	47	36	13.4
Cham4, bread	60	38	38	46	36	36	35	42	31	19.9
Douma4, bread	62	40	37	48	34	36	36	43	32	20.3
Bohoth10, bread	66	35	32	32	28	32	31	36	28	26.0
Arabi Abiad, barley	63	39	39	46	35	36	34	42	33	20.7
Arabi Aswad, barley	68	34	33	34	29	33	30	38	29	26.2
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}, Type I) detected using a head artificial inoculation and disease severity (DS^{CC}, Type II) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a head artificial inoculation under field conditions. R index = LP/(AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC}).

Table 4. Scores of aggressiveness index composing from nine aggressiveness components of a set of 16 fungal isolates of four Fusarium head blight species infecting eight wheat and barley cultivars of Syrian origin

Fungal isolates (identification)	Nine aggressiveness components									Aggressiveness index (value × 10)
	LP (days × 10)	AUDPC (value × 100)	CD (%)	DI^{DHT}(%)	DS^{DHT}(%)	DI^{CC}(%)	DS^{CC}(%)	DI^{FC}(%)	DS^{FC}(%)	
F1 (*F. culmorum*)	59	40	59	47	47	48	49	45	34	6.3
F2 (*F. culmorum*)	55	40	61	50	46	50	47	46	34	6.8
F3 (*F. culmorum*)	55	49	51	52	52	53	52	48	36	7.2
F28 (*F. culmorum*)	53	44	59	42	39	43	39	45	33	6.5
F30 (*F. culmorum*)	57	44	60	43	42	43	43	45	34	6.2
F7 (*F. solani*)	61	50	51	46	45	46	46	48	36	6.1
F20 (*F. solani*)	58	46	52	63	44	62	39	54	43	7.0
F26 (*F. solani*)	52	44	62	52	38	53	35	48	37	7.1
F29 (*F. solani*)	52	41	59	52	44	52	47	53	40	7.4
F31 (*F. solani*)	54	41	59	43	39	44	44	41	31	6.3
F35 (*F. solani*)	64	48	52	51	47	49	47	47	36	5.9
F15 (*F. verticillioides*)	46	34	67	35	29	36	28	36	28	6.4
F16 (*F. verticillioides*)	45	40	65	43	37	45	38	41	32	7.5
F21 (*F. verticillioides*)	57	40	63	46	37	46	36	46	37	6.2
F27 (*F. verticillioides*)	49	35	67	39	31	39	29	36	28	6.2
F43 (*F. equiseti*)	54	43	59	44	44	43	48	44	34	6.7
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI) and disease severity (DS) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}) detected using a head artificial inoculation and disease severity (DS^{CC}) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI and disease severity (DS) detected using a head artificial inoculation under field conditions. A index = (AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC})/LP.

Table 5. Correlation coefficients determined by Pearson correlation among scores of resistance index and data of reductions resulted in silicon treatments across eight wheat and barley cultivars of Syrian origin infected with a set of 16 fungal isolates of four Fusarium head blight species

Resistance index for cultivars	Nine quantitative resistance components for cultivars								
	LP	AUDPC	CD	DI^{DHT}	DS^{DHT}	DI^{CC}	DS^{CC}	DI^{FC}	DS^{FC}
Acsad65, durum	**	***	***	***	**	***	**	***	***
Cham7, durum	**	**	***	**	**	**	**	***	**
Cham9, durum	*	***	**	**	*	***	**	**	**
Cham4, bread	*	*	*	*	*	***	***	**	**
Douma4, bread	***	*	*	*	**	**	*	*	***
Bohoth10, bread	**	***	**	***	**	**	*	*	*
Arabi Abiad, barley	*	**	**	**	*	**	*	**	*
Arabi Aswad, barley	**	*	***	**	*	**	***	**	**
	*	***	**	*	***	**	**	***	***

Latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}, Type I) detected using a head artificial inoculation and disease severity (DS^{CC}, Type II) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI, Type I) and disease severity (DS, Type II) detected using a head artificial inoculation under field conditions. R index = $LP/(AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC})$.
(p<0.05)*, (p<0.01)**, (p<0.001)***.

Table 6. Correlation coefficients determined by Pearson correlation among scores of aggressiveness index and data of reductions resulted in silicon treatments of a set of 16 fungal isolates of four Fusarium head blight species infecting eight wheat and barley cultivars of Syrian origin

Aggressiveness index for fungal isolates	Nine aggressiveness components								
	LP	AUDPC	CD	DI^{DHT}	DS^{DHT}	DI^{CC}	DS^{CC}	DI^{FC}	DS^{FC}
F1 (*F. culmorum*)	***	**	**	***	**	**	**	**	**
F2 (*F. culmorum*)	**	**	**	**	**	**	**	**	**
F3 (*F. culmorum*)	**	***	***	**	*	***	*	**	***
F28 (*F. culmorum*)	*	**	***	**	*	***	*	***	***
F30 (*F. culmorum*)	*	*	***	***	*	**	***	***	**
F7 (*F. solani*)	**	*	**	**	***	***	**	*	**
F20 (*F. solani*)	***	***	**	**	***	***	**	*	*
F26 (*F. solani*)	**	**	***	**	**	*	***	**	*
F29 (*F. solani*)	**	**	**	***	**	*	***	***	*
F31 (*F. solani*)	**	***	***	***	***	**	***	**	***
F35 (*F. solani*)	***	***	**	**	**	**	**	***	**
F15 (*F. verticillioides*)	***	**	***	**	***	***	*	**	**
F16 (*F. verticillioides*)	**	*	***	***	**	***	*	***	**
F21 (*F. verticillioides*)	**	***	**	**	**	***	**	**	**
F27 (*F. verticillioides*)	**	**	*	***	***	**	**	**	*
F43 (*F. equiseti*)	***	***	***	**	***	**	***	***	***

Latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI) and disease severity (DS) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}) detected using a head artificial inoculation and disease severity (DS^{CC}) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI and disease severity (DS) detected using a head artificial inoculation under field conditions. A index = $(AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC})/LP$.

Quantitative resistant wheat/barley cultivars are identified by long LP and low AUDPC, CD and FHB disease spike and spikelet values of the fungus compared with the susceptible ones (Sakr, 2022, 2023). In the current experiment, the differences in resistance/susceptibility levels among the eight cereal cultivars were recognized for resistance index. Overall, AS, AB and bread wheat cultivars, Bohoth10, Cham4, Douma4, showed lower infection FHB levels than did durum cultivars, Acsad65, Cham7 and Cham9, indicating that barley and bread wheat provided broad, though incomplete, resistance to the four *Fusarium* species causing FHB examined compared to durum wheat. The values of R index rating for eight wheat and barley cultivars (Table 3) reflects the ability of the same isolate of the pathogen to distinguish different levels of resistance as observed for the same pathosystem (Xue *et al.,*, 2019).

It seems that R index included these key and accurate QR traits measured in the work are indicators of mechanisms of resistance occurring in the whole plant during FHB infection (Buerstmayr *et al.,* 2020; Fernando *et al.,* 2021). The different wheat and barley cultivars were shown to have highly differing responses depending on the scores of R index measured, which may be a sign of different genes and gene interactions impacting the resistance. Generally, the level of susceptibility to *Fusarium* invasion in plant heads reduces from durum wheat to bread wheat to barley (Miedaner *et al.,* 2021). It is widely accepted that QR of the cereal plant to head blight infections does not confer absolute protection (Mundt, 2014), but is considered to be effective against all known races/isolates of the pathogen (Van der Plank, 1968). In the present research, no wheat and barley cultivar was completely resistant to FHB as shown by scores of R index in harmony with Van der Plank's (1968) concept about QR.

High AUDPC, CD and FHB disease spike and spikelet and long LP values of the fungus represent high aggressiveness (Sakr, 2022, 2023). The significance of differences in our aggressiveness index was indications of pathogenicity of individual isolates (Xue *et al.,* 2019; Mesterhazy 2020). All tested wheat and barley cultivars showed a qualitative pattern in which FHB isolates expressed either high aggressiveness or low aggressiveness (Sakr, 2023). The wide range of variability of pathogenicity among FHB isolates in our study has been supported by other studies investigating pathogenicity of several FHB species (Xue *et al.,* 2019).

Aggressiveness of *Fusarium* is possibly the outcome of timely expression of several genes, governing release of cell-wall-degrading enzymes, mycotoxins, specific metabolites, and hormones that modify the host's resistance response (Fernando *et al.,* 2021). An aggressiveness test is useful to select the best inocula for infection. Our investigation supported the view that earlier experimental findings are useful in the selection of the best inocula for experimentation (Mesterhazy, 2020). The earlier aggressiveness test of *Fusarium* isolates for employment proved to be a good technique (Toth *et al.,* 2020). As experiments of aggressiveness at the seedling/adult stage exhibit a significant linkage with head blight symptoms (Mesterhazy *et al.,* 2020), tests to choose more aggressive isolates for field utilization can be conducted. The same is correct to screen inocula for field infections. It is significance to characterize the resistance variations for the distinguishing of resistance level in cultivars or parental lines and for variety registration (Buerstmayr *et al.,* 2020). For phenotyping in QTL, this technique was acceptable, but further issues coming from the largely broad flowering duration and other problems should be resolved in future investigation (Dahl and Wilson, 2018). For mass selection, one aggressive isolate or mixture of isolates varying in aggressiveness following an aggressiveness test can ensure the disease pressure to perform an efficient negative selection (Xue *et al.,* 2019). Here, the A index can be used for screening the most aggressive isolates of several *Fusarium* pathogens for head blight resistance breeding of wheat and barley. The mean of nine disease components is introduced in this paper as a new index for aggressiveness of *Fusarium* fungi.

The question is, how completely can the visual scoring identify *Fusarium* resistance? FHB infection can be conducted in several ways (Mesterhazy, 2020). Head blight can be distinguished by visual examination of the proportion of invaded spikes, i.e. resistance to primary

invasion (referred to as Type I), and proportion of infected spikelets, i.e., resistance to spread of FHB symptoms within a head (referred to as Type II) (Fernando *et al.,* 2021). It is well-known that Type II resistance is only a part of the total host resistance (Sakr, 2022). However, Type I is usually neglected as researchers do not see exactly what is it and how it should be worked with (Dill-Macky 2003). This can result in susceptibility (Buerstmayr *et al.,* 2020). Contrary, head blight symptoms in barley normally do not spread internally from initially invaded florets to neighboring spikelets (Mesterhazy *et al.,* 2020; Toth *et al.,* 2020). Consequently, Type II resistance, in spite of mentioned for barley (Mesterhazy 2020), has little meaning. Type I resistance is more significance for barley. In addition, the spreading following spray artificial infection cannot be identified as Type II resistance as reported by Ma *et al.,* (2019). The spreading role is also observed in Type I resistance (area under disease progress curve) (Dahl and Wilson, 2018). For this and other reasons, we have to rethink the problem of the resistance components/types. Therefore, distinguishing in this matter was hardly possible (Mesterhazy 2020). Ma *et al.,* (2020) describe the situation "The inherent differences or relationships of diverse resistance types are far from clear". On the field, however, the proportion of diseased spikes and spikelets characterized *Fusarium* development at the latest stage can be employed to assess an FHB index (% incidence × % severity) (Tekauz *et al.*, 2000).

In the present investigation, the novel index for wheat and barley covers besides disease incidence (Type I) and disease severity (Type II), detected on adult plants following artificial head and floret infections under controlled climatic conditions and field conditions, several *in vitro* quantitative traits generated on young plant parts and described pathogen development at the earliest stage. The advantageous features of *in vitro* method reside in its predictive ability of adult plant disease; the results from the *in vitro* assay are considerably correlated and comparable with the results of screening under greenhouse and field conditions in FHB-wheat and barley intersections (Sakr, 2023). Latent period (LP) is the duration interval between infection and the onset of sporulation from that infection. It determines the time of epidemic cycles and thus largely controls the rate of epidemic development (Mundt *et al.,* 2002). The area under the disease progress curve (AUDPC) is a quantitative rating to quantify disease progress over time entailing repeated disease evaluations over pathogen progression (Simko and Piepho, 2012). Coleoptile dwarfing (CD) is a symptom characteristic of plants systemically infected by fungal pathogens and is characterized by a reduction in the concentration of growth hormone in invaded tissue. This reduction in size can be notified at a very early stage (Van der Plank, 1968). It seems that the novel index obtained under a board range of controlled and normal climatic conditions and included quantitative traits described *Fusarium* development at the earliest and latest stages is more compressive than disease index based only on DI and DS. In harmony with our findings, Nyanapah *et al.,* (2020) compared eight components of disease resistance to gray leaf spot on maize which include latent period, sporulation rate, disease incidence, disease severity and other QR components.

Silicon (Si) has been proven to be advantageous for the healthy development and growth of several plants exposed to biotic and abiotic constraints. The existence of soluble Si in plant shoots has been shown to decrease the intensity of several economically noxious fungal diseases (Guo-Chao *et al.,* 2018) including FHB on wheat and barley under *in vitro*, climatic controlled and field condition (Sakr and Kurdali, 2023a,b). The stability of the novel index was proved using Si applications on the same plant and fungal materials. *Since Si does not exert selective pressure* on the four tested *Fusarium* pathogens and enhances resistance of the analyzed eight wheat and barley cultivars (Sakr and Kurdali, 2023a,b); this novel index was further investigated for its stability in the same fungi-seedling and -adult plants treated with silicon that reduced these nine pathogenic components forming this index. The scores of our index were highly significant and correlated with data of reductions resulted in silicon treatments across the bread and durum wheat and barley cultivars for quantitative traits related for resistance and aggressiveness, thus it is suitable to be

used for quantification of resistance in host plants and aggressiveness of *Fusarium* isolates.

CONCLUSION

The stress-physiological status in small-grains crops, i.e., wheat and barley, covering both QR and *aggressiveness* is the foundation of breeding for FHB disease resistance especially to achieve stable and durable resistance. Taken into account that efficient use of favourable resistance in breeding programs requires that QR in host and aggressiveness of *Fusarium* should be correctly and accurately evaluated. In agreement with susceptibility ranking to fungus development in the heads/florets of small-grain cereals, results of our resistance index showed that barley and bread wheat consistently showed very low mean levels of FHB disease compared to durum wheat, among the three tested crop species. Resistance index showed that the cultivars AS and Bohoth10 showed very stable and remarkable resistance in almost all experimentations and give the lowest susceptibility rates, thus could be very promising sources of genetic resistance to FHB in breeding programs, and an alternative for farmers to *Fusarium* susceptible cultivars. The novel index can also aide the breeder to monitor the disease pressure in different environments as well as to monitor the level of resistance to the tested plant materials. Aggressiveness index showed significant differences among fungal isolates of different species, consequently, it can be used to select the most aggressive isolates or fungal mixture for breeding purposes. Further research to understand the interaction between wheat/barley and *Fusarium* pathogen will be useful for characterizing additional factors participating to resistance in host plants and aggressiveness of head blight populations.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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