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Response of the flag leaf for Moroccan bread wheat varieties in the field, highlighting its biochemical composition in its resistance mechanism against yellow rust

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Abstract

This investigation aimed to assess the responsiveness of fourteen Moroccan bread wheat varieties to yellow rust induced by *Puccinia striiformis tritici* (Pst) in comparison with a susceptible variety 'Amal' through natural inoculation in the field, employing a Randomized Complete Block Design (RCBD). A comprehensive evaluation, encompassing 20 variables related to epidemiological, phenological, morphological, and physicochemical parameters, was conducted. In elucidating the role of the leaf in wheat resistance to stripe rust, the mineral and organic composition was scrutinized using sophisticated molecular detection techniques—Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Absorption Spectroscopy (AAS). These two methodologies, recognized for their efficacy, facilitated the measurement of varying concentrations of lipids and carboxylic esters in the epicuticular wax on the adaxial flag leaf. Additionally, the quantity of essential minerals was determined. The outcomes revealed distinct responses among bread wheat varieties to stripe rust, with 'Faiza', 'Bandera', and 'Resulton' demonstrating heightened resistance, manifesting severity values below 15%. Conversely, 'Amal' exhibited the lowest level of resistance. Furthermore, lipid concentration reached its pinnacle in 'Faiza', 'Bandera' and 'Resulton' while 'Amal' displayed the lowest lipid levels. Severity exhibited a very weak correlation with Zn, weak correlations with Cu, and moderate correlations with Ca and K ($p<0.05$). This study provides valuable insights guiding future research endeavours aimed at enhancing the genetic traits of these elite cultivars for integration into Morocco's cereals program.

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Keywords: AAS spectroscopy; bread wheat varieties; chemical composition; FTIR-ATR spectroscopy; natural field inoculation; stripe rust

Introduction

The cereal sector is one of the main agricultural sectors in Morocco and has a significant socio-economic weight. It contributes from 15 to 20% to the agricultural GDP. Wheat is the most consumed cereal in Morocco; its annual consumption is estimated at 216 kg per capita (USDA, 2017) (Khanfri *et al*., 2018). Morocco is one of the world's leading importers of wheat. It is ranked the 11th largest importer, according to the 2017-2018 world map of wheat importers, with 4.9 Mt. European Union countries being the main exporters of bread wheat to Morocco (FAO, 2018) (Camillo and Alborghetti, 2023). However, wheat is vulnerable to attacks by many pathogens that contribute to the decline in its production. Yellow rust (YR), caused by *Puccinia striiformis tritici (*Pst), is one of the most destructive fungal diseases of wheat that appears in cold and humid regions and has led to significant yield reductions in all wheat-producing countries in Australia, New Zealand, China, India, Nepal, Pakistan, Uzbekistan, Yemen, Ethiopia, Kenya, United Kingdom, Chile, Peru, Ecuador, Colombia, Mexico, and the United States (Wellings, 2011; Khanfri *et al*., 2018). In Morocco, previous survey studies have shown that the most devastating diseases of wheat are Septoria, tan spot, Brown rust, yellow rust, and root rots (Ramdani *et al*., 2004). The recent discovery of the alternate host of *P. striiformis* in 2010, *Berberis* spp., has provided an explanation of the sexual life cycle and its role in the disease epidemic and population variation of the pathogen (Wan *et al*., 2017) which can provide an interpretation of the virulence and genetic diversity observed in some of the yellow rust "hotspots" around the world (Jin *et al*., 2010) hence the importance of rust monitoring in barberry areas where conditions conducive to sexual life cycle completion may be present (Rodriguez Algaba *et al*., 2021).

Pst is an obligate biotrophic plant parasite, infecting the main hosts (cereals, grasses) from the deposition of urediniospores by wind or raindrops on leaf surfaces. Germination started within 3 hours of contact with free moisture in a range of temperatures. The cytoplasm of a uredospore moves in the growing germ tube across the leaf surface followed by the formation of an appressorium on a stomatal, until it reaches a balloon-shaped feeding structure, known as a haustorium, which draws its nutrient requirements from the wheat mesophyll cells. Following haustorium formation, infection hyphae are formed, leading to a network of fungal mycelial branches developing inter- and intracellularly in the host tissue. As the mycelium grows, a bed of pustules is established, from which uredinium develops (Chen *et al*., 2014; Wan *et al*., 2017).

Most of the stripe rust resistance genes are from bread wheat (*Triticum aestivum*), but some are from different wild species, such as *Triticum spelta album*, *Triticum dicoccoides*, *Triticum spelta*, *Secale cereale*, *Aegilops comosa*, *Aegilops ventricosa*, *Triticum tauschii*, and *Haynaldia villosa* (Aktar Uz Zaman *et al*., 2017). However, genetic resistance may be limited following changes in the virulence of the Pst pathogen. Over the past decade, new races of Pst have emerged that are able to adapt to warmer temperatures, develop virulence profiles, and be more aggressive than older races. As well as the survey of the diseases allows to have, in a continuous way, information concerning the most severe diseases of wheat in order to take the necessary measures to control them and to optimize the yield. The use of cultural techniques such as crop selection, sowing time, and weed control can provide effective control of yellow rust (Roelfs *et al*., 1992; Wan *et al*., 2007). Removal of volunteer plants that favor yellow rust survival is an effective control measure for epidemics (Roelfs *et al*., 1992). Planting a mixture of wheat varieties with different resistances can significantly reduce disease pressure and may also increase or stabilize wheat yield (Wolfe, 1985). Mechanisms by which varietal mixtures reduce the disease may include dilution of spore density due to the greater distance between susceptible plants. This provides a physical barrier created by the resistant plants that prevents spore movement and causes some resistance (Huang *et al*., 2012; Mundt, 2002).

Disease resistance of the host is its ability to limit the penetration, development, and reproduction of the invading pathogens (Dordas, 2008). The surface of plant leaves is covered by a cuticle composed of cuticular wax and a polymer matrix called cutin (Sajeevan, 2023). Cuticular wax is a complex mixture of very long-chain fatty acids (chain length >C20), including alkanes, alcohols, ketones, aldehydes, esters, and fatty acids (Shaheenuzzamn *et al*., 2019). Its composition varies from one species to another (Fernández *et al*., 2016), and plays an important role in plants, protecting against ultraviolet (UV) radiation, limiting non-stomatal water loss, fungal and bacterial pathogen attack (Bernard and Joubès, 2013; Hwang *et al*., 2016). For example, in wheat, cuticular waxes are composed of diketones, esters, alkanes, aldehydes, and alcohols (Wang *et al*., 2015, 2019). Also, regarding the mineral composition of the leaf, potassium K can encourage the development of thicker outer walls in the epidermal cells, thus preventing disease attacks. Iron Fe can control or reduce the disease severity of several diseases such as rust in wheat leaves (Singh, 2015), while Zn does not affect plant susceptibility to disease (Dordas, 2008). It is necessary to know the concentration of chemical elements in each infected species in order to identify their role in the mechanism of defensive action. To this end, we specifically selected bread wheat varieties of different susceptibility to yellow rust to identify the biochemical composition of epicuticular leaf waxes. Although total leaf wax analyses for several accessions of the genus Hordeum have been reported (Zabka *et al*., 2008) no information was available on the surface composition of epicuticular waxes on the adaxial surfaces of wheat leaves in interaction with fungal infection.

The aim of this research was devoted to bread wheat lines while studying their agronomic, epidemiological, phenological, and chemical characteristics in order to select the potential candidates with high resistance against yellow rust. This resistance mechanism related to flag leaf chemistry allows the inclusion of promising candidates in a national bread wheat breeding program in Morocco.

Materials and Methods

Plant materials and experimental design

The plant material used in this experiment included 15 different genotypes of bread wheat varieties developed by SONACOS (Société Nationale de Commercialisation des Semences), namely 'Faiza', 'Granota', 'Achtar', 'Amal', 'Kanz', 'Wafia', 'Najia', 'Remax', 'Guadalette', 'Virgile', 'Radia', 'Resulton', 'Rajae', 'Bandera', and 'Arrehane'. Planting took place during the winter wheat sowing period for two successive seasons of 2019/20 and 2020/21 in the experimental field of the National School of Agriculture in Meknes, Morocco, (Latitude of 33°49'55" N, longitude 5°28'18" W, and an elevation of 635 m above sea level). The experimental set-up consisted of 3 complete randomized blocks in which each variety was sown in 5 rows in a 3.23 m^2 microplot with 40 cm of spacing.

Epidemiological responses of the flag leaf in natural inoculation

Disease assessment was performed four times at seven-day intervals. The first reading was in March. Observations consisted of assessing the disease severity on the flag leaf according to the modified Cobb rust severity assessment scale (Wan *et al*., 2017; Yahya *et al*., 2020). Ten leaves were observed and were selected randomly for each variety in each block by calculating the percentage of plant tissue covered by fungal spores according to the following formula:

$$\text{Severity (\%)} = \frac{\text{infected surface of the leaf}}{\text{total surface of the examined leaf}} \times 100 \qquad (1)$$

The area under the disease progress curves (AUDPC) is a quantitative method of assessing disease severity over time. The AUDPC was calculated using the following formula, proposed by (Pandey *et al.*, 1989):

$$\mathrm{AUDPC} = \mathrm{D} \times (\frac{\mathrm{Y_1} + \mathrm{Y_K}}{2} + \mathrm{Y_2} + \mathrm{Y_3} + \cdots \mathrm{Y_{K-1}}) \qquad (2)$$

Where:

D: time interval between severity observations Y1: first severity reading

Yk: last severity reading

Phenology of the plant

Earliness was determined as described by (Deumier *et al.*, 2012). The number of days to heading was counted until half of the ear had emerged from the flag leaf (this corresponds to growth stage 55 according to the Zadocks scale) in 50% of the microplate (Couvreur, 1985). The earliness at flowering refers to the number of days from heading to flowering (Jonard, 1965). The maturity earliness determines the sowing date that should not be exceeded so that the variety reaches maturity before the period when the number of days with maximum temperatures exceeding 25 °C becomes too great (Deumier *et al.*, 2012).

Morphological parameters

Several qualitative and quantitative parameters were measured and are related to the morphology of the ear and the height of the studied plants. Height, length, and number of ears, and length of the beard have been measured from the end of the ear. Nine ears were measured for each variety (Abdelkader and Aissa, 2015; Baye *et al.*, 2020).

Yield parameters

After harvesting, four parameters were measured: Thousand-Grains Weight (TGW), number of grains per ear, yield in grains per hectare (Qx/ha), weight of the total biomass, and harvest index (Abdelkader and Aissa, 2015; Donald and Hamblin, 1976). The plants in each experimental plot were cut at ground level and collected in clusters. Then the weight of the clumps was measured for each variety in each block to determine the total biomass. Then they were threshed to measure grain yield. The harvest index was calculated using the formula proposed by Donald and Hamblin (Donald and Hamblin, 1976):

$$\mathrm{HI} = \frac{\text{Grains yield}}{\text{Total weight of the dry matter}} \times 100 \qquad (3)$$

Spectroscopy FTIR-ATR analysis

Fourier Transform Infrared spectroscopy (FTIR) was used to assess the composition of the upper side of the flag sheet. Three replicates per variety were analysed and five spectra per replicate were recorded at different locations on the leaf. This allowed the detection of the characteristic vibrations of the connections and subsequently performed the analysis of the functions present in the sample analysed (Martin *et al.*, 1986; Stuart, 2004). All the data was collected using the SPECTRUM Software (version 10.6.1, PerkinElmer, Inc., United Kingdom), and measurements were made by irradiating a sample with an infrared beam whose wave number varies between 400 and 4000 cm^{-1}. An infrared spectrum was recorded in wavenumbers ν per cm. The relation between ν and the wavelength λ is:

$$\nu\ (cm^{-1}) = \frac{10^4}{\lambda} \qquad (4)$$

Atomic absorption spectrometry (AAS) analysis

This method is based on the quantification of the energy of the atom that varies during the passage of an electron from one electron orbital to another: $\Delta E = h.v$ where h is Planck's constant and v is the frequency of the absorbed photon (Welz and Vale, 2019; Ritgen, 2023). Since the absorbed photons are characteristic of the absorbing elements, and their quantity is proportional to the number of absorbing element atoms according to Boltzmann's distribution law, absorption allows measurement of the concentrations of the elements to be measured (Welz and Vale, 2019). The analysis by atomic absorption is mainly based on the law of Beer-Lambert (Bertrand, 2002). Five chemical elements were measured: Ca, K, Fe, Zn, and Cu.

Statistical analysis

The analysis of the data from the DBAC device requires a two-way ANOVA without repetition which was carried out using the software SPSS version 25, IBM© to detect the effect of the varieties on the 19 observed parameters. Tukey's posthoc DSH (honest significant difference) test was performed to assess whether the means were significantly different from each other ($P<0.05$) for the different observations made. Normality and homogeneity of variance were confirmed before each analysis.

Results

Observations revealed a spectrum of YR severity ranging from 1.20% in the 'Faiza' variety to 77.33% in the 'Amal' variety. Figure 1 depicts the severity distribution among the fifteen bread wheat varieties studied, while Figure 2 illustrates the flag leaves of these varieties arranged in ascending order of severity, from left to right (representing the most resistant to the most susceptible variety). Analysis of variance demonstrated a significant impact of variety on YR severity ($p<0.05$). Conversely, the block factor did not exhibit a significant effect on severity, likely due to block homogeneity concerning fungal development conditions. According to Tukey's test, no significant disparity in severity was discerned among the varieties 'Amal', 'Virgile', 'Remax', 'Guadalette', 'Arrehane' and 'Kanz' ($P<0.05$), all registering severity values surpassing 60%. Severity in the 'Faiza' variety did not significantly differ from that in 'Bandera' and 'Resulton'. Notably, 'Faiza', 'Bandera' and 'Resulton' demonstrated resistance to YR, with severity below 15%, while 'Najia' and 'Achtar' were classified as moderately resistant, exhibiting severity between 15% and 40%. 'Radia', 'Granota' and 'Rajae' manifested moderate susceptibility to the disease (severity between 40% and 60%), whereas 'Kanz', 'Wafia', 'Arrehane', 'Guadalette', 'Remax', 'Virgile' and 'Amal' were deemed susceptible varieties. Area Under Disease Progress Curve (AUDPC) values ranged from 12.13 in the 'Faiza' variety to 1133.67 in 'Amal'. Variance analysis indicated a significant influence of AUDPC across the fifteen varieties examined ($P<0.05$). Tukey's multiple comparison tests revealed no significant discrepancy in AUDPC values among 'Faiza', 'Bandera', 'Resulton' and 'Najia', all recording values not exceeding 220. Similarly, no significant variation was observed in AUDPC values between 'Amal', 'Virgile', 'Remax', 'Arrehane', 'Guadalette', 'Wafia' and 'Kanz' all with values surpassing 780 (Figure 3).

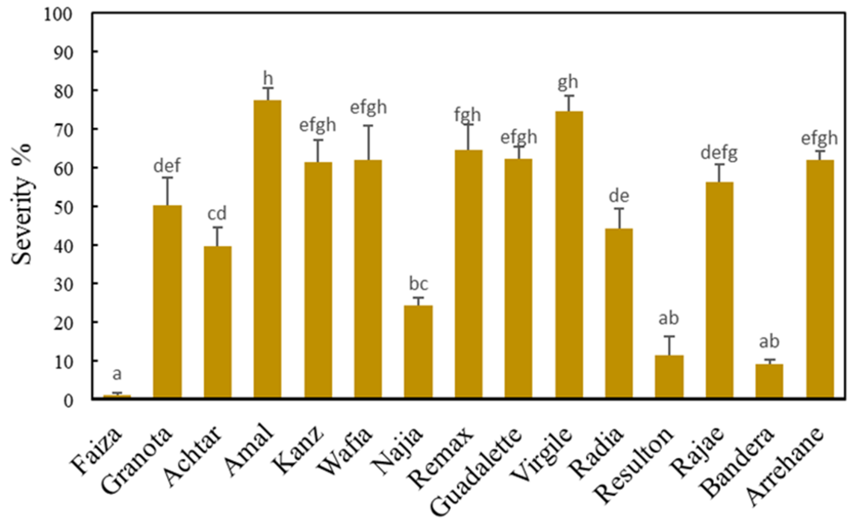

Figure 1. Mean ± (SE) severity observed in the fifteen bread wheat varieties studied. The analysis of variance showed that there was a significant effect on severity between the fifteen varieties studied based on Tukey's HSD test (P < 0.05).

Figure 2. Mean ± (SE) severity observed in the fifteen bread wheat varieties studied. The analysis of variance showed that there was a significant effect on severity between the fifteen varieties studied based on Tukey's HSD test (P < 0.05).

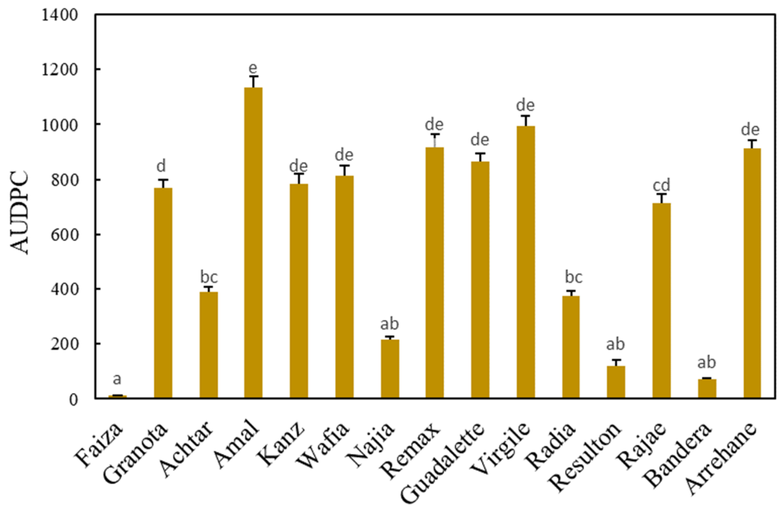

Figure 3. Mean (± SE) AUDPC calculated for the fifteen bread wheat varieties. The analysis of variance showed that there was a significant effect on AUDPC between the varieties studied based on Tukey's HSD test (n= 2; $P < 0.05$).

The results obtained showed that the duration of the sowing-harvesting phase varied from 117 days in the 'Kanz' variety to 134 days in 'Amal' and 'Achtar'. Table 1 shows the number of days from sowing to heading in the 15 varieties studied. The varieties tested can be classified into three main groups; the first group is characterized by a sowing-to-heading time not exceeding 120 days and includes the varieties 'Kanz' and 'Resulton' which are considered semi-early varieties. Then, the second group is characterized by a sowing-epiposition phase lasting between 122 and 130 days and includes 9 varieties considered as semi-late varieties. Then, the varieties 'Radia', 'Granota', 'Achtar', and 'Amal' are characterized by a sowing-epiposition phase exceeding 130 days and are considered late varieties. The number of days to flowering varies from 125 days, recorded in the variety 'Kanz' to 146 days recorded in the variety 'Achtar'. Table 1 shows the duration of the sowing-flowering phase measured for each of the 15 varieties tested. The results obtained allow classifying the studied varieties into three main groups. First, there were the early varieties with several days to flowering ranging from 120 to 130 days which are 'Kanz' and 'Resulton'. Then, the group of semi-late varieties whose sowing and flowering phase lasts from 130 to 140 days, includes 10 varieties. The group of late varieties was characterized by a sowing-flowering phase lasting from 140 to 150 days and includes 3 varieties, 'Granota', 'Amal', and 'Achtar'. The observations made on the 15 bread wheat varieties studied showed that the number of days to maturity varied from 168 days in the variety 'Virgile' to 183 days in 'Amal'. Table 1 shows the number of days from sowing to maturity for each variety. The 15 varieties tested are grouped into three main classes according to earliness at maturity. The class of semi-early varieties, whose number of days to maturity does not exceed 170 days, includes the variety, 'Virgile'. The group of late varieties includes 7 varieties and is characterized by a cycle length ranging from 170 to 179 days. Varieties with a cycle length of more than 180 days are considered very late varieties. One of the most destructive diseases that affect wheat crops globally is stripe rust, which causes significant output losses. The environmentally sound method of fighting the illness and reducing our reliance on fungicides is the development of disease-resistant cultivars. The initial step in identifying possible candidates for future breeding is to screen new lines and current cultivars against yellow rust. In this study, epidemic conditions were similar throughout the observation period for both early and late cultivars. The results obtained show that earliness to heading is positively correlated with severity ($r= 0.27$).

Table 1. Number of days to heading, flowering, and maturity characterizing the fifteen bread wheat varieties studied

Variety	Days to heading	Days to flowering	Days to maturity	Class of mature wheat
Virgile	127	134	168	Semi-early < 170 days
Wafia	128	137	175	
Achtar	134	146	176	
Bandera	128	137	177	Late
Radia	130	139	177	170-179 days
Kanz	117	125	178	
Guadalette	127	138	178	
Arrehane	124	134	179	
Rajae	125	138	180	
Granota	131	143	180	
Resulton	119	126	182	Very late
Faiza	122	134	182	>180 days
Najia	125	134	183	
Remax	125	139	183	
Amal*	134	145	183	

The observations conducted on the 15 bread wheat varieties unveiled a range of plant heights, with measurements fluctuating from 71.93 ± 3.83 cm in the 'Bandera' variety to 95.47 ± 3.29 cm in 'Arrehane' (Table 2). These varieties were categorized into two primary groups based on their height characteristics. The first group, comprising dwarf varieties with heights ranging from 71.93 ± 3.83 to 79.93 ± 4.76 cm, included 'Bandera', 'Virgile' and 'Rajae'. The second group consisted of semi-dwarf genotypes with heights spanning from 81.13 ± 2.65 to 95.47 ± 3.29 cm, encompassing the majority of the studied varieties. Additionally, beard length measurements conducted on the 15 soft wheat varieties delineated three distinct groups (Table 2). The first group comprised varieties lacking awns, namely 'Virgile' and 'Resulton'. The second group consisted of varieties with short beards, measuring between 3 and 4 cm, including 'Bandera', 'Amal' and 'Faiza'. Finally, the third group, characterized by beard lengths ranging from 4 to 8 cm, encompassed 10 varieties classified as long beard varieties, with 'Arrehane' exhibiting the maximum value of 5.76 ± 0.26 cm. Moreover, ear length measurements unveiled variations among the 15 common wheat varieties, with 'Bandera' exhibiting an average cob length of 8.99 ± 0.04 cm, categorizing it as a short cob variety (Table 2). The class of medium-eared varieties included 11 varieties with ear lengths ranging from 9 to 12 cm, while 'Radia', 'Wafia' and 'Kanz' were classified as long-stalked varieties, with ear lengths between 12 and 15 cm.

Furthermore, observations on grain yield demonstrated variability, ranging from 8.72 ± 1.70 qx/ha in 'Achtar' to 64.85 ± 12.23 qx/ha in 'Najia' (Table 3). The analysis of variance revealed a significant effect of the Variety factor on grain yield ($P < 0.05$), while the Block factor showed no significant effect. Tukey's multiple comparison tests identified 'Najia' as the highest-yielding variety, followed by 'Faiza'. Additionally, there was no significant difference in yield observed among several varieties. Additionally, total dry matter measurements showcased variability, with 'Faiza' exhibiting significantly higher biomass compared to 'Achtar' and 'Virgile' (Table 3). Moreover, the harvest index varied among the varieties, with 'Najia' displaying the highest value of 54% (Table 3). The analysis of variance indicated a significant effect of the variety factor on the harvest index, with 'Najia' showing the highest index compared to other varieties.

Table 2. Mean (± SE) varieties for *Triticum aestivum* physiological parameters; Plant final height (cm), Beard length (cm), and Ear length (cm). Means with a common letter within a column are not significantly different according to Tukey's HSD at (n = 2, P < 0.05)

Cultivar	Final height (cm)	Beard length (cm)	Ear length (cm)
Faiza	87.40 ± 2.59bcd	4.05 ± 0.04bcd	11.10 ± 0.66bcdef
Granota	81.13 ± 2.65abc	4.60 ± 0.40cdef	11.03 ± 0.25bcdef
Achtar	90.47 ± 1.70bcd	4.95 ± 0.50def	10.46 ± 0.08bc
Amal*	81.80 ± 1.96abc	3.75 ± 0.19bc	11.06 ± 0.39bcdef
Kanz	91.60 ± 5.00bcd	4.66 ± 0.50cdef	13.37 ± 0.16g
Wafia	92.27 ± 0.45cd	5.07 ± 0.13def	12.37 ± 0.54fg
Najia	87.67 ± 2.24bcd	4.40 ± 0.13bcde	10.97 ± 0.15bcde
Remax	87.53 ± 1.00bcd	5.23 ± 0.20ef	11.55 ± 0.11cdef
Guadalette	91.40 ± 1.57bcd	4.75 ± 0.16cdef	11.29 ± 0.20bcdef
Virgile	73.67 ± 0.73a	0.00 ± 0.00a	10.78 ± 0.36bcd
Radia	90.07 ± 0.94bcd	5.73 ± 0.21f	12.24 ± 0.14efg
Resulton	87.80 ± 3.98bcd	0.00 ± 0.00a	11.81 ± 0.19def
Rajae	79.93 ± 4.76ab	5.34 ± 0.44ef	10.03 ± 0.46ab
Bandera	71.93 ± 3.83a	3.25 ± 0.17b	8.99 ± 0.04a
Arrehane	95.47 ± 3.29d	5.76 ± 0.26f	11.55 ± 0.19cdef

Table 3. Mean (± SE) varieties for *Triticum aestivum* yield parameters; Number of grains per ear, TWG (g), grain yield (qx/ha), Total biomass, Harvest index (HI). Means with a common letter within a column are not significantly different according to Tukey's HSD at (n = 2, P < 0.05)

Cultivar	No grain/ear	TWG (g)	Yield (qx/ha)	T. biomass	HI
Faiza	53.56 ± 1.42cd	42.75 ± 0.10j	43.21 ± 0.39c	4100 ± 70.71c	0.341 ± 0.006i
Granota	63.70 ± 2.28e	15.03 ± 0.88b	11.34 ± 1.26a	2600 ± 308.22abc	0.141 ± 0.002abc
Achtar	66.25 ± 1.57c	26.55 ± 0.41ef	8.72 ± 1.70a	1833 ± 294.39a	0.153 ± 0.005bc
Amal*	37.01 ± 1.94a	9.84 ± 1.08a	11.62 ± 1.43a	2367 ± 294.39abc	0.159 ± 0.002cd
Kanz	64.64 ± 2.81e	22.15 ± 0.54cd	16.69 ± 0.97ab	2800 ± 244.95abc	0.193 ± 0.006e
Wafia	48.79 ± 1.54bcd	21.79 ± 0.17c	23.22 ± 3.19ab	3500 ± 533.85abc	0.215 ± 0.003f
Najia	65.08 ± 1.63e	25.65 ± 0.27ef	64.85 ± 12.23d	3867 ± 742.74bc	0.543 ± 0.006j
Remax	51.12 ± 1.37bcd	29.72 ± 0.46gh	14.82 ± 2.63ab	2700 ± 463.68abc	0.177 ± 0.001de
Guadalette	44.88 ± 2.24b	21.86 ± 0.57c	32.06 ± 4.83bc	3133 ± 470.81abc	0.330 ± 0.002i
Virgile	53.65 ± 2.62cd	24.29 ± 0.41de	15.32 ± 1.55ab	2100 ± 212.13ab	0.236 ± 0.002g
Radia	47.33 ± 1.54bc	34.57 ± 0.23i	10.09 ± 1.78a	2467 ± 318.85abc	0.131 ± 0.006a
Resulton	46.61 ± 1.41bc	27.95 ± 0.00fg	15.92 ± 2.00ab	3600 ± 509.90abc	0.143 ± 0.003abc
Rajae	49.65 ± 0.84bcd	26.31 ± 0.52ef	12.13 ± 1.31a	2900 ± 212.13abc	0.135 ± 0.006ab
Bandera	55.55 ± 1.73d	31.54 ± 0.34h	25.67 ± 5.62abc	3033 ± 697.61abc	0.275 ± 0.004h
Arrehane	49.29 ± 1.12bcd	28.45 ± 0.22g	31.78 ± 1.41bc	3500 ± 122.47abc	0.293± 0.006h

Data from Fourier transform infrared spectroscopy (FTIR) allowed a characterization of the fifteen bread wheat varieties according to the chemical bonds and functional groups that constitute the epicuticular surface of the upper face of the flag leaf. The results illustrated in (Figure 4) show a clear difference between the varieties in the two absorption regions. Region 1 of the spectrum, whose wavenumber ranges from 2840 to 2970 cm^{-1}, includes three peaks (Figure 4a). This band represents the vibrations of the aliphatic chains of lipids (asymmetrical and C-H symmetric). Region 2 of the spectrum, shown in (Figure 4b), includes a spectral band ranging from 1730 to 1740 cm^{-1} corresponding to the >C=O group of carboxylic acids. The estimated concentration of aliphatic lipids is higher in the 'Faiza' variety (Figure 4c). Tukey's multiple comparison tests showed that there is no significant difference between 'Faiza', 'Resulton', 'Najia', 'Kanz', 'Remax', 'Rajae', 'Arrehane' and 'Achtar'. The 'Radia' variety presents the lowest lipid concentration, with an average significantly equal to that recorded in 'Virgile', 'Amal', 'Guadalette', and 'Granota'. The estimated concentration of carboxylic esters (Figure 4d) is higher in the 'Bandera' variety with an average significantly equal to those observed in the 'Najia', 'Wafia', 'Virgile' and 'Remax' varieties. The other varieties do not show any significant difference in concentration. The lowest value is observed in 'Guadalette'. In the present study, differences in the wax composition as evaluated by the difference in peak height of asymmetrical and symmetrical C-H and C=O groups (vibrational peaks in mid-infrared) were estimated as well as aliphatic chains of lipids and carboxylic esters respectively. The most significant changes between cultivars grown under natural conditions and severity were related to changes in the aliphatic lipid chain and carboxylic acids. The concentration of aliphatic lipid chains observed on the adaxial surface of 'Faiza', 'Resulton','Wafia, and 'Najia' flag leaves was greater than that of 'Radia', and 'Virgile' under natural conditions, while the estimated concentration of carboxylic esters is higher in the 'Bandera' with an average significantly equal to those observed in the 'Najia', 'Wafia', 'Virgile' and 'Remax'. The lowest value is observed in 'Guadalette'.

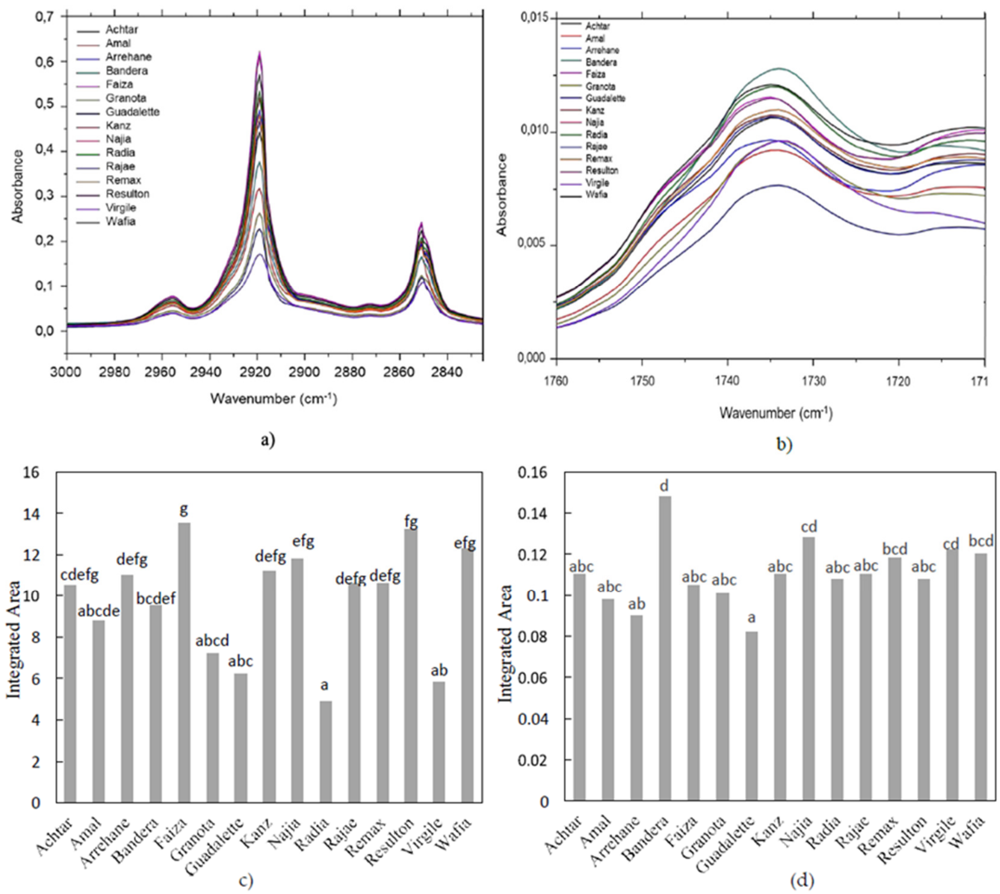

Figure 4. FTIR-ATR spectrum of two regions of the adaxial surface of the flag leaf of fifteen varieties of *Triticum aestivum* was quantified; region I (2840-2970 cm^{-1}), corresponding to the aliphatic chain of lipids (a) and region II (1730-1740 cm^{-1}), corresponding to carboxylic esters (b). Mean (± SE) Area of lipid regions and carboxylic regions represented by bars c and d respectively. Means with a common letter within a column are not significantly different according to Tukey's HSD at (n = 2, P < 0.05)

The results of the chemical analysis of calcium by atomic absorption spectrometry are illustrated in (Table 4). The Ca contents vary from 14.28 ± 0.20 mg/g, recorded at 'Guadalette', up to 22.35 ± 0.06 mg/g recorded at 'Resulton'. The two-factor variance analysis indicates a significant effect of the variety factor on calcium levels (P < 0.05) while that of the block factor is not significant. The multiple comparisons using the Tukey test showed that the Ca content in the 'Resulton' variety is significantly higher than those of the 'Bandera', 'Radia', 'Virgile', 'Guadalette', 'Remax', 'Amal', 'Achtar' and 'Granota' varieties. The potassium concentrations for the varieties studied are illustrated in (Table 4). The values of the concentration vary from 6.28 ± 0.36 mg/g, recorded at 'Arrehane', up to 18.82 ± 1.28 mg/g recorded at 'Bandera'. The analysis of variance shows that the two factors, variety, and block, have a significant effect on potassium levels (p<0.05). Multiple comparisons using Tukey's test indicated that there is no significant difference in K concentration between 'Bandera', 'Radia'and 'Achtar'. The 'Arrehane', 'Resulton', 'Virgile', 'Guadalette', 'Remax', 'Wafia', 'Kanz', 'Amal', 'Granota' and 'Faiza' varieties have significantly non- different potassium contents. The results of the analysis of iron by atomic absorption spectrometry are illustrated in (Table 4). The analysis of variance showed a significant variety effect. The multiple comparisons carried out using the Tukey test showed that the iron concentration in 'Kanz' 225.60 ± 78.80 ppm is significantly higher than that observed in 'Faiza' 77.44 ± 12.34 ppm. The iron contents in the rest of the varieties are not significantly different. The concentrations of zinc measured for the wheat varieties vary from 9.51 ± 0.24 ppm in 'Rajae' up to 35.55 ± 12.95 ppm in 'Achtar' (Table 4). The analysis of the variance shows that the variety effect on the zinc content is significant while that of the block is not significant. The concentration of zinc in the 'Achtar' variety is significantly higher than that observed in the other varieties. The rest of the varieties have significantly equal zinc content. The results of the chemical analysis of copper Cu by atomic absorption spectrometry are shown in (Table 4). The analysis of variance indicates that neither the variety factor nor the block has a significant effect on the copper contents.

Table 4. Statistical comparison of the main elemental composition of the epicuticular wax of the flag leaf of fifteen bread wheat cultivars affected by stripe rust. Means with a common letter within a column are not significantly different according to Tukey's HSD at (n = 2, P < 0.05)

Variety	Concentration of elements determined by atomic absorption spectrometry				
	Ca (mg/g)	K (mg/g)	Fe (ppm)	Zn (ppm)	Cu (ppm)
Faiza	17.72abcd	9.95abc	77.44 a	13.14 a	27.62c
Granota	15.52abc	10.78abc	175.36 ab	13.83a	43.58e
Achtar	16.54abc	14.76cde	192.64 ab	35.55b	9.16a
Amal*	16.83abc	9.56abc	125.60 ab	15.12a	10.28a
Kanz	20.57cd	7.69ab	225.60b	13.86a	27.68c
Wafia	19.72bcd	11.34abc	125.76ab	10.98a	19.78b
Najia	20.34bcd	11.96bcd	97.92ab	12.12a	8.80a
Remax	15.83abc	10.39abc	177.12ab	14.37a	22.02b
Guadalette	14.28a	8.79ab	123.84ab	16.08a	7.48a
Virgile	15.44abc	11.56abc	203.84ab	12.45a	34.58c
Radia	15.28ab	17.16de	159.20ab	17.70a	15.16b
Resulton	22.35d	11.15abc	127.36ab	14.10a	7.92a
Rajae	18.72abcd	11.90bcd	120.16ab	9.51a	16.56b
Bandera	16.72abc	18.82e	100.64ab	13.62a	14.96b
Arrehane	18.87abcd	6.28a	195.04ab	12.09 a	38.12a

The detailed numerical values of the correlations are comprehensively presented in the correlation matrix (Table 5). Severity exhibits negative correlations with various factors such as TGW (r= - 0.65), total biomass (r= - 0.54), grain yield (r= - 0.43), harvest index (r= - 0.32), the number of grains per ear (r= - 0.28), calcium content (r= - 0.32), and potassium content (r= - 0.44). A weak positive correlation is observed between severity and number of days to heading (r= 0.28). In terms of agronomic traits, the correlation matrix (Table 5) delineates that grain yield demonstrates positive correlations with total biomass weight (r= 0.76), harvest index (r= 0.97), TGW (r= 0.30), number of grains per ear (r= 0.21), and calcium concentration (r= 0.28). Conversely, yield exhibits negative correlations with the duration of the entire phenological cycle (r= -0.36), which in turn is negatively correlated with the harvest index (r= - 0.33), the weight of the total biomass (r= - 0.41), and the number of grains per ear (r= - 0.31).

The graphical representation of PCA results is illustrated through correlation circles between the variables (Figure 5). The PCA conducted on the "PC1-PC2" factorial plane highlights predominant trends. Specifically, the PC1 factor, accounting for 27.7% of the variance, is positively correlated with group 1 variables including Yield, HI (harvest index), Total biomass, Ca, TGW, and earliness to maturity. Conversely, the PC2 factor, representing 21.3% of the variance, exhibits positive correlations with group 2 variables such as "Number of grains per ear", "Earliness to heading", "the number of days in the entire cycle", "K", and "Zn". Conversely, the negative part of the PC2 factor is correlated with group 3 variables encompassing "Head length", "Height", "Beard length", "Early to flowering", "Fe", "Cu", "Severity", and "AUDPC". These findings underscore the intricate relationships between morphological, phenological, and chemical parameters elucidated through PCA analysis.

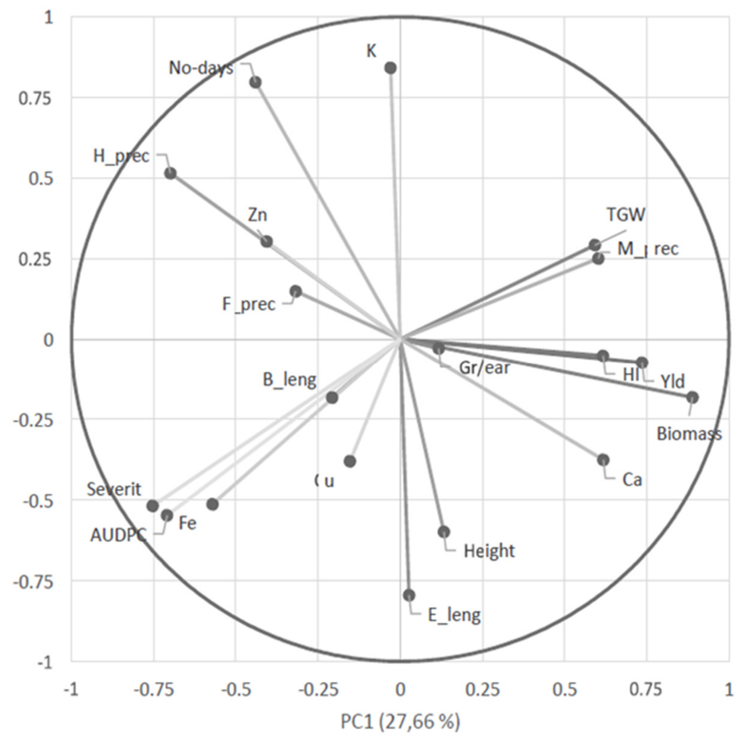

Figure 5. PCA correlation circle of morphological, phenological, chemical, and epidemiological parameters of studied soft wheat varieties on the PC1 × PC2 factorial design

Discussion

The bread wheat lines examined differed significantly in their resistance to yellow rust and in their early maturity (r=0.28). We would expect higher severity in early genotypes because the upper leaf of these lines had longer exposure to the pathogen at a given observation time. This expected trend may exist as the r between HD and AUDPC was 0.23 (almost significant at P>0.05), but is quite low at best. For example, cv. 'Amal' was indeed the least precocious and most affected, but 'Remax', 'Arrehane', 'Virgile', and 'Guadalette' were among the most precocious, also ranging in severity from high to fairly low. This is easily explained by genotypic differences in resistance independent of the effect of earliness, as shown by Danial (1994). HDs vary between

2 and 2.5 weeks, and the confounding effect of earliness on resistance assessment seems small enough, if not even existing to justify special corrective measures. However, if HD has extreme differences, the disruptive effect of HD may be too great. In such cases, the breeder is recommended to divide the genotypes into earliness groups for a representative comparison. The high correlation coefficients between HD and AUDPC suggest that HD is a reliable epidemiological parameter and can be used as a parameter for field resistance assessment instead of the laborious AUDPC.

Table 5. Correlation matrix of morphological, phenological, chemical, and epidemiological parameters of studied soft wheat varieties

	Yld	HI	Biomass	TGW	Gr/ear	E_leng	B_leng	Height	H_prec	F_prec	M_prec	No_day	Ca	K	Fe	Zn	Cu	Sever	AUDPC
Yld	1.00																		
HI	0.97	1.00																	
Biomass	0.76	0.60	1.00																
TGW	0.30	0.22	0.40	1.00															
Gr/ear	0.20	0.23	-0.08	0.10	1.00														
E.leng	-0.07	-0.14	0.12	-0.09	-0.05	1.00													
B_leng	0.07	0.02	0.02	0.03	0.10	0.15	1.00												
Heigh	0.19	0.10	0.31	0.11	-0.00	0.70	0.50	1.00											
H_prec	-0.30	-0.21	-0.59	-0.38	-0.11	-0.42	0.22	-0.24	1.00										
F. prec	-0.11	-0.14	-0.12	0.02	-0.01	-0.29	0.60	0.06	0.32	1.00									
M. prec	0.02	-0.09	0.45	0.58	-0.25	-0.15	-0.46	-0.28	-0.56	-0.22	1.00								
No_day	-0.35	-0.32	-0.41	-0.06	-0.31	-0.61	-0.04	-0.48	0.82	0.24	-0.01	1.00							
Ca	0.28	0.13	0.56	0.05	0.12	0.32	-0.19	0.29	-0.64	-0.41	0.29	-0.58	1.00						
K	-0.17	-0.14	-0.28	0.31	0.14	-0.49	-0.07	-0.46	0.42	-0.10	0.15	0.62	-0.25	1.00					
Fe	-0.52	-0.42	-0.62	-0.26	0.30	0.41	0.04	0.18	-0.02	-0.12	-0.37	-0.28	-0.13	-0.25	1.00				
Zn	-0.30	-0.24	-0.54	0.02	0.32	-0.11	0.12	0.22	0.48	0.19	-0.45	0.27	-0.28	0.32	0.29	1.00			
Cu	-0.11	-0.10	-0.04	-0.01	0.26	0.15	0.04	-0.09	-0.14	0.04	0.03	-0.14	-0.13	-0.37	0.48	-0.32	1.00		
Sever	-0.43	-0.31	-0.54	-0.64	-0.28	0.30	0.21	0.08	0.27	0.12	-0.56	-0.04	-0.32	-0.43	0.58	-0.07	0.24	1.00	
AUDPC	-0.38	-0.28	-0.44	-0.68	-0.32	0.26	0.16	0.05	0.23	0.17	-0.50	-0.06	-0.30	-0.55	0.53	-0.16	0.33	0.97	1.00

In correlation analysis, significant relationships between different morphological traits were identified, consistent with previous findings (Arama *et al.*, 1999). Screening of diverse wheat germplasm revealed lines/varieties with complete immunity to yellow rust (Khodadadi *et al.*, 2011; Waqas, 2018). Immune and highly resistant varieties could prolong the green state of leaves, enhancing photosynthetic activity and, consequently, grain yield (Wang *et al.*, 2015), as was noticed in Faiza and Najia varieties. The flag leaf emerged as the primary source of photosynthate during grain filling, thereby regulating yield (Rehman *et al.*, 2022).

Field studies have shown that resistant varieties tend to have a thicker epicuticular wax layer than susceptible cultivars when grown under the same field conditions (Rehman *et al.*, 2022). Comparison of the results of FTIR spectroscopy with those of severity assessment shows that the low severity varieties, in particular, 'Faiza' and 'Resulton', are characterized by a more or less dense epicuticular wax on the upper surface of the flag leaf. As confirmed by (Johnson *et al.*, 1983), who studied the chemical composition of the cell walls of the epidermis of barley leaves inoculated with powdery mildew pathogen (*Blumeria graminis*), changes in the composition of the cell walls were characterized in a resistant barley variety 'CDC Silky'. Spectroscopy results showed peaks representing asymmetric and symmetric stretching vibrations corresponding to fatty acid groups, phenols, lignin, cellulose, hemicellulose, pectins, and glucans. The FTIR spectroscopy data made it possible to divide the inoculated samples (showing the formation of papillae) and the non-inoculated controls into two groups. In fact, changes in the composition of the wall, especially cellulose, hemicellulose, pectin and glucans, correspond to the formation of papillae and cellulose networks associated with the defense reaction of barley against powdery mildew. Increases in phenolic compounds help strengthen the cell wall, in addition to acting directly on pathogens (Johnson *et al.*, 1983; Zabka *et al.*, 2008). The analyses carried out by FTIR spectroscopy make it possible to study plant-pathogen interactions and in particular to determine the role of the cell wall in the response to fungal infection.

Analysis of leaf chemical composition using atomic absorption spectrometry (AAS) revealed a significant increase in Ca, K, and Fe in flag leaves presented in high severity wheat varieties. This shows that the chemical could implicate the resistance of wheat varieties to stripe rust. In the case of K deficiency, the synthesis of high-molecular-weight compounds (proteins, starch, and cellulose) is hundred, and low-molecular-weight organic compounds accumulate. K can also help thicken the outer wall of epidermal cells, preventing disease outbreaks (Lahlali *et al.*, 2019). It has been shown that K fertilization can reduce the intensity of several infectious diseases of obligate and facultative parasites. K has been widely observed to cause the occurrence of various foliage diseases such as sesame leaf spot in rice, bacterial leaf spot in cotton, *Cercospora* leaf spot in cassava, Tikka leaf spot in peanut, *Cercospora* leaf spot in mung bean and *Helminthosporium* leaf spot (Dordas, 2009; Marschner *et al.*, 1996; Sharma *et al.*, 2005). Although Ca plays an important role in plant disease resistance as it maintains the selective permeability and structural integrity of biomembranes (Awasthi, 2015), promotes cell wall strengthening (Legge *et al.*, 1982), and inhibits pectin enzymes secreted by pathogens (Conway, 1991), is required for phytoalexin synthesis (Biggs *et al.*, 1997), and regulates defense-related gene expression (Stäb and Ebel, 1987). The underlying mechanisms of Ca- mediated disease suppression, particulary at the molecular level, remain largely unknown (Dordas, 2009). The blast severity caused by *Pyricularia oryzae* in wheat decreased from 36.8 to 8.2% when the Ca concentration in the nutrient solution increased from 0.26 to 5 mM. The expression levels gene markers of salicylic (SA) and jasmonic acid (JA) signaling pathways as well as chitinase, β-1,3-glucanase, phenylalanine ammonia lyase, peroxidase and polyphenol oxidase were lower in inoculated plants grown with 5 mM, Ca higher than in those cultured with 0.26 mM (Arfaoui *et al.*, 2016).

Conclusions

The experimental investigation conducted herein offers valuable insights into the diverse responses of bread wheat varieties to stripe rust, shedding light on pivotal agronomic parameters and underlying biochemical mechanisms. Our findings underscore substantial variability among the studied varieties, highlighting 'Faiza' as exhibiting the lowest severity of stripe rust infection, while 'Amal' emerges as the most susceptible cultivar. These observations are pivotal for breeding programs aiming to develop resilient wheat cultivars against this devastating disease. A comprehensive analysis of agronomic parameters revealed nuanced distinctions in morphology, phenology, yield, and chemical composition among the studied varieties. Notably, the 'Najia' variety exhibited superior performance in terms of yield, boasting the highest harvest index among the tested cultivars. These results hold significant implications for wheat breeding strategies geared towards enhancing yield potential and agronomic resilience.

Further elucidation of leaf surface characteristics through FTIR spectroscopy unveiled intriguing insights into the biochemical defense mechanisms against stripe rust. Varieties such as 'Faiza', 'Bandera', and 'Resulton', known for their resistance to the disease, showcased denser epicuticular wax layers compared to their counterparts. This observation suggests a potential role of lipid content in leaf surfaces in impeding infection, potentially by obstructing spore penetration into leaf tissues. These findings provide valuable insights into the molecular basis of disease resistance in wheat and pave the way for targeted breeding efforts aimed at enhancing crop resilience. Correlation analyses yielded valuable insights into the interplay between agronomic traits and disease severity. We observed moderately weak negative correlations between severity and traits such as thousand-grain weight, grain yield, aerial biomass weight, harvest index, number of grains per ear, as well as calcium and potassium concentrations. These findings underscore the multifaceted nature of disease resistance mechanisms in wheat, highlighting the need for a holistic approach towards breeding for enhanced resilience.

In light of these observations, we advocate for further genotypic studies aimed at unraveling the genetic determinants of stripe rust resistance and their associations with broader agronomic traits. Such endeavors hold immense promise for accelerating the development of resilient wheat varieties capable of withstanding the challenges posed by stripe rust and other prevalent diseases. By leveraging cutting-edge genomic tools and interdisciplinary approaches, we can unlock the full potential of wheat breeding and pave the way toward sustainable agricultural practices.

Authors' Contributions

Conceptualization, I.D., H.M. and R.L.; methodology, I.D., K.B., H.D., and R.L.; software, I.D. and C.E.H.; validation, R.L., and H.M.; formal analysis, R.L., and H.M.; investigation, R.L., and H.M.; resources, R.L., and H.M.; data curation, I.D., S-E. L., R.E., and C.E.H.; writing—original draft preparation, I.D.; writing—review and editing, R.L., Z.B., H.E., H.M., H.E.H. and M.E.J; supervision, R.L., and H.M.; project administration, H.M., and R.L.; funding acquisition, R.L. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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