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Integrating Sowing Date with Chickpea Genotypes in Managing Fusarium Wilt in Morocco

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Abstract: Fusarium wilt caused by *Fusarium oxysporum* f.p *ciceris* (Foc) is a devastating soil-borne disease of the chickpea. The disease causes crop losses in late-planted chickpeas and no available management option currently exists to recommend to farmers. In order to determine the effect of integrating sowing dates with chickpea genotypes on disease parameters and yield, a field experiment was undertaken in naturally infested soil at Merchouch station during 2017–2018 and 2018–2019. The results showed that significant differences were observed among chickpea genotypes for the three disease parameters, and among sowing dates for final disease incidence. For seed yield, only the sowing dates showed significant differences and all interactions between factors were non-significant for all measured parameters. Late sowing showed high values for the three disease parameters. A high seed yield was obtained from early-planted compared to late-planted chickpeas. In conclusion, the integration of early sowing and chickpea genotypes with good levels of resistance can be recommended for farmers to increase chickpea productivity in Morocco.

Keywords: chickpea; sowing dates; *Fusarium oxysporum* f. sp. *ciceris*; Morocco

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1. Introduction

Food legumes (faba beans, chickpeas, lentils, and field peas) are the major rotational crops in the cereal-based cropping systems in Morocco [1]. Chickpea (*Cicer arietinum* L.) is an important food legume crop occupying over 82,000 ha of arable land with an estimated production of 75,500 tons [2]. The importance of chickpeas for the farming community and consumers resides in its nutritional value, the crop contains high-quality proteins, vitamins, minerals, carbohydrates, and unsaturated fatty acids [3]. Besides its importance in traditional Moroccan food, the chickpea also plays an important role in improving soil fertility through nitrogen fixation [4].

Morocco produces kabuli chickpea in Taza-Al Hoceima-Taounate (27%), Meknès-Tafilalet (16%), Fès-Boulemane (12%), and Gharb-Chrarda-Benihssen (24%) provinces during spring and winter seasons [5]. Local chickpea production is not enough to meet the local demand and the government of Morocco imports to fill gaps. For example, in 2019, the country imported 6270 t of chickpeas worth USD 6.4 million [2]. The low productivity and production of chickpeas are due to insect pests (leaf miners and pod borer), diseases (Ascochyta blight and wilt/root rot), drought, weak seed systems to expand released cultivars, poor agronomic practices, and the growing of landraces with low productivity [6].

Chickpea wilt/root rot disease complex can cause yield losses of 10–15%, and could reach up 100% in highly infested fields [7]. In Morocco, yield losses due to soil-borne diseases are not known and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is the major pathogen in the wilt/root rot complex in chickpea crops in Morocco [8].

F. oxysporum f. sp. *ciceris* is an asexually reproducing fungus that can survive inactive in soil by means of chlamydospores for at least 6 years and can infect the plant during any growth stage. The disease may start only in a few branches but within a few days it occurs on the whole plant causing a dark-brow discoloration of xylem tissues in stems and roots [9]. The genus Fusarium is known to produce mycotoxins that are among the virulence factors and are chemically different. Trichothecenes, diacetoxyscirpenol, fumonisins, zearalenone, and moniliformin are the most important *Fusarium* mycotoxins [10]. These mycotoxins cause major risk to consumer health (neurological damage and are frequently associated with animal and human diseases), agriculture, and the food industry [11].

Various management options are used to mitigate the negative impacts of wilt/root rot diseases including fungicide seed treatment, biocontrol, adjusting sowing dates, seeding depth, crop rotation, intercropping, growing of resistant cultivars, and the integration of two or more control options [12,13]. In India, the integration of intercropping (chickpea with mustard) with November sowing reduced Fusarium wilt incidence and increased the yield of chickpeas [14]. Cultural practices usually influence the development of diseases by affecting the environment to make it favorable to crop growth but not favorable to disease development [15]. Due to land shortages, farmers cannot avoid highly infested soil for a long period since the pathogen can survive for a long time, and hence roles of short period crop rotation in managing wilt/root rot diseases are less effective. The adoption of winter chickpea sowing in Mediterranean countries played a key role in reducing the impacts of Fusarium wilt as compared with spring sowing since the environment in the former sowing is not conducive for wilt development [16].

The development and deployment of wilt/root rot-resistant varieties is a preferred method of disease management, and many chickpea breeding programs spend time and resources to develop and release disease-resistant cultivars [17]. In Morocco, breeders developed varieties with resistance to combat multiple biotic and abiotic stresses.

Although resistance breeding is the best option in managing Fusarium wilt, the appearances of races by Foc are reported in many countries and there are reports some races broke the resistance of popular cultivars [18]. Although detailed genetic diversity and race structure of Foc populations are not well studied, the existence of races one and six are reported from a few samples collected from Morocco [7].

Due to the appearance of Foc races and other root rot disease complexes affecting chickpeas, integrating different disease management options is recommended [19,20]. The objective of this study was to determine the effect of integrating chickpea genotypes with sowing dates on Fusarium wilt development and the yields of chickpeas.

2. Materials and Methods

2.1. Experimental Site and Design

Field experiments were conducted in naturally infested soil with soil-borne pathogens at the International Centre for Agricultural Research in the Dry Areas (ICARDA), Merchouch Research Station (Latitude 33.561319 and longitude −6.691883), for two cropping seasons (2017/18 and 2018/19). The experiments were repeated in the same plot with different randomization of the treatments in each season. The research station is characterized by a Mediterranean-type climate, with an average temperature of 28 °C and rainfall of 400 mm in 2017/2018 and 120 in the 2018/2019 cropping season (Figure 1). The soil is clay in texture and has a pH ranging from 6.3 to 7.9.

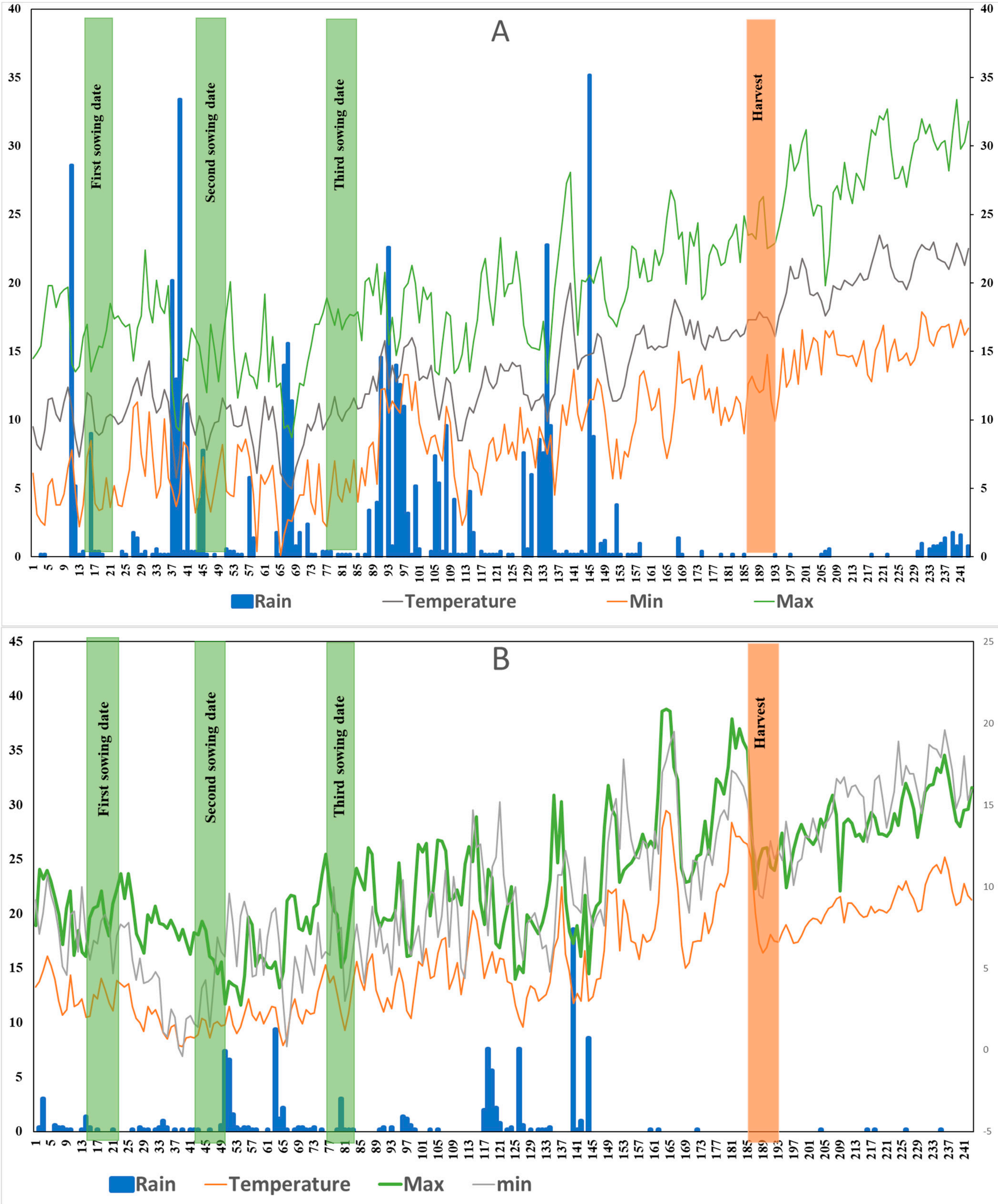

Figure 1. Daily maximum, minimum, average temperatures, and precipitation at the experimental site at Marchouch station during 2017/2018 (**A**) and 2018/2019 (**B**) cropping seasons.

The treatments were released (Arifi: FLIP 98-50C; Farihan: FLIP 84-92C) and elite (FLIP-09-221C and FLIP 07-211) Kabuli chickpea genotypes (Table 1); three sowing dates (mid-December, mid-January, and mid-February). The three sowing dates represented early and late winter sowing and spring sowing practiced by farmers in Morocco. The chickpea genotypes were selected based on their reactions to Fusarium wilt in demonstration plots in the 2016/17 season at Merchouch Research Station where genotype FLIP-09-221 was susceptible and the other three genotypes were moderately resistant. The experimental design was a split-plot with sowing dates as main plots and chickpea genotypes as sub-plots. Each genotype was planted in four rows with 4 m length and 0.45 m row-to-row spacing. The plant to plant spacing was 10 cm and sowing was done by hand. The experiment was replicated three times in both seasons. Ascochyta blight (*Didymela rabiei*) appeared in both cropping seasons and was controlled by spraying the fungicide Curator® (80 g a.i./l of Azoxystrobin and 400 g a.i./l of Chlorothalonil) two times at vegetative and flowering stages. Leaf miner (*Liriomyza cicerina*) was also controlled by spraying Vertigo 018EC (18 g a.i/l of Abamectin) two times in the cropping seasons.

Table 1. Characteristics of the studied chickpea genotype.

Genotype	Type	Pedigree	Days to Flowering	Days to Maturity	Other Traits
ARIFI (FLIP98-50 C)	Kabuli	-	99	150	Erect plant, Aschochyta blight (AB) resistance, large seed size,
FARIHANE (FLIP84-78C)	Kabuli	-	98	153	Erect plant, (AB) resistance, large seed size
FLIP07-211C	Kabuli	X03TH132/FLIP 97-185CXFLIP99-47C	92	144	Erect plant, large seed size
FLIP09-221C	Kabuli	S00789(30 KR)-7/	84	136	Erect plant, small seed size

2.2. Disease Parameters and Seed Yield

Thirteen plants sown in the second and the third rows of each plot were selected for disease and yield assessments. The disease assessments were started when wilting of chickpea plants was observed and continued at 25-day intervals until the crop reached physiological maturity. Three disease parameters were used to evaluate the impacts of the treatments on disease management options. The first one was final disease incidence on each plot calculated using the following formula:

$$Wilt\ incidence\ (\%) = \frac{Number\ of\ wilted\ plants}{Total\ number\ of\ plants} \times 100 \quad (1)$$

The second parameter was the area under the disease progress curve (AUDPC) based on percent wilt incidence collected three times at 25 days intervals. AUDPC was calculated using the following formula [21]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) \times (ti + 1 - ti) \quad (2)$$

Yi_{+1} is the cumulative disease incidence in the i th observation,
ti is the time at the observation and n is the total number of observations.

The third parameter was the rate of disease development calculated from disease incidence by regressing wilt incidence over time. Seed yield was measured from the two middle rows of each plot [22].

2.3. Data Analysis

Disease parameters (final disease incidence, AUDPC, and rate of disease development) and seed yield of the two seasons were combined and analyzed using restricted maximum likelihood (REML) in generalized linear mixed models using GenStat software (GenStat Release 20.1 (PC/Windows 10)) developed by Rothamsted Research in the United Kingdom. Cultivars were considered as fixed factors whereas sowing dates were considered as a random factor in the data analyses. The means of percent wilt incidence are treated as binomial counts. For seed yield, covariance analysis was done since the number of plants at harvest (co-variate) was not uniform. Correlation analyses were performed between final percent wilt incidence, AUDPC, rate of disease development, and yield.

3. Results

Significant differences ($p \leq 0.05$) were observed among sowing dates for mean percent final wilt incidence and seed yields of chickpeas (Table 2). Highly significant differences ($p \leq 0.05$) were observed for the mean final disease incidence and rate of disease development, as well as significant differences for AUDPC among chickpeas. All interactions were not significant to all disease parameters measured (Table 2).

Table 2. Analysis of variance for three disease parameters and seed yield of chickpea.

Sources of Variation	Final Percent Wilt Incidence	AUDPC	Rate of Disease Development	Seed Yield
Season (S)	NS	NS	*	NS
Dates (D)	*	NS	NS	*
Genotypes (G)	***	*	***	NS
D*G	NS	NS	NS	NS
S*D	NS	NS	NS	NS
S*D*G	NS	NS	NS	NS

Significance code: "***" 0.001; "*" 0.05; "NS" not significant.

For mid-December and mid-January sowing, no disease was developed 45 days after sowing in all genotypes, expecting the susceptible genotype (FLIP-221C), while 45 days after sowing the disease appeared in all genotypes sown in mid-February. Disease progress on the two released cultivars and one elite genotype (FLIP-211C) was slow in all three sowing dates. The disease progress on the susceptible genotype (FLIP-221C) was high and fast on all sowing dates.

Disease progress was high in the last sowing date for all chickpea genotypes. However, the released cultivars showed a high wilt incidence in the last sowing date in both seasons of sowing (Figure 2).

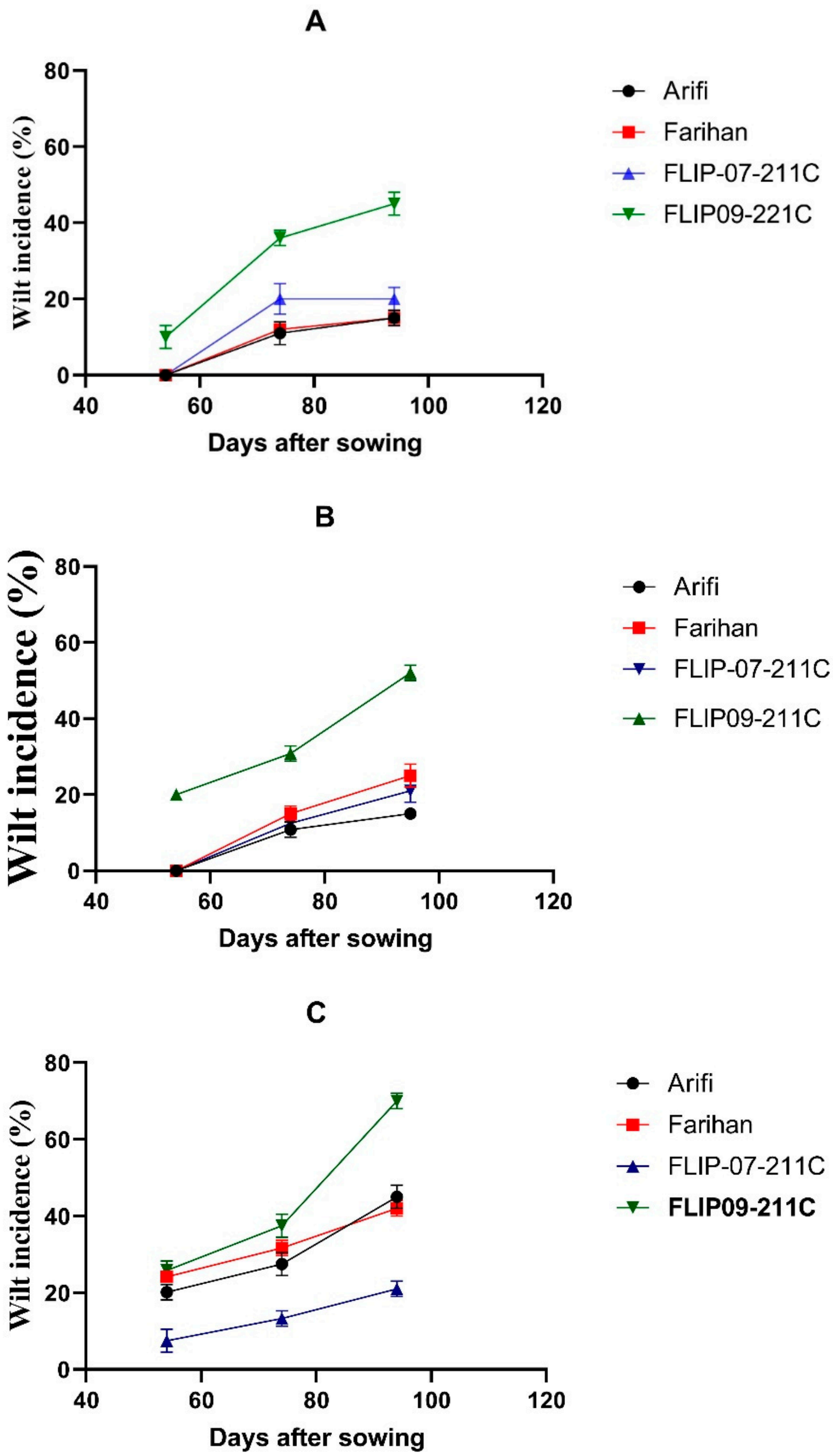

Figure 2. Disease progress wilt on the four chickpea genotypes as affected by sowing dates (**A**) mid-December, (**B**) mid-January, and (**C**) mid-February. The vertical bars represent standard deviation with three replicates.

The moderately resistant genotypes showed less than a 30% final wilt incidence and the susceptible genotype showed about a 56% wilt incidence. The susceptible genotype (Figure 3) showed a high wilt incidence in all planting dates while the other three genotypes showed less than 30% in the mid-December and Mid-January planting dates. The two released cultivars showed high wilt incidence when they were planted in February (Table 3).

Figure 3. Partial view naturally of the field experiment at Merchocuh Research station, 2018.

Table 3. Effects of sowing date and chickpea genotypes on mean percent final Fusarium wilt incidence ±SD, 2017–2018 cropping seasons.

Sowing Date	**Chickpea Genotypes**				
	Arifi	**Farihan**	**FLIP-07-211C**	**FLIP-09-221C**	**Mean**
Mid December	15.8 (±5.01)	15.3 (±3.61)	20.8 (±6.12)	45.0 (±10.09)	24.2
Mid-January	15.8 (±2.80)	25.7 (±2.71)	21.0 (±9.66)	52.7 (±14.01)	28.8
Mid-February	45.0 (±2.65)	48.0 (±7.87)	21.3 (±2.00)	70.7 (±7.33)	46.3
Mean	25.5	29.7	21.0	56.1	

LSD ($p \leq 0.05$) for genotypes = 15.79 and for sowing dates = 13.67.

The AUDPC ranged from 442 in early sowing to 702 in the last sowing dates (Table 4). Concerning chickpea genotypes, the highest AUDPC (878) was obtained with the susceptible variety FLIP-09-221 C, while the lowest AUDPC (477) was observed by FLIP-07-211C.

Table 4. Effects of sowing date and chickpea genotypes on AUDPC ± SD, 2017–2018 cropping seasons.

Sowing Date	**Chickpea Genotypes**				
	Arifi	**Farihan**	**FLIP-07-211C**	**FLIP-09-221C**	**Mean**
Mid-December	277.5 (±96.21)	311.0 (±133.56)	319.0 (±32.90)	860.0 (±60.86)	441.9
Mid-January	505.0 (±31.16)	900.3 (±66.30)	323.3 (±35.44)	807.5 (±39.17)	634.0
Mid-February	649.3 (±38.79)	840.0 (±46.77)	352.8 (±31.34)	966.7 (±53.65)	702.2
Mean	477.3	683.8	331.7	878.1	592.7

LSD ($p \leq 0.05$) among genotypes, 303.3 and sowing dates is 262.7.

The lowest mean rate (0.33/day) of disease development was observed in the December sowing date as compared with the January and February sowing dates. The lowest rate of disease development was observed for Arifi (0.23) followed by FLIP07-221C (0.34), Farihan (0.48), and FLIP09-221C showed the highest rate of disease development (Table 5).

Table 5. Effects of sowing date and chickpea genotypes on the mean rate of diseases progress ±SD, 2017–2018 cropping seasons.

Sowing Date	Arifi	Farihan	FLIP-07-211C	FLIP09-221 C	Mean
Mid-December	0.26 (±0.16)	0.21 (±0.20)	0.33 (±0.28)	0.51 (±0.19)	0.33
Mid-January	0.19 (±0.10)	0.62 (±0.35)	0.32 (±0.18)	0.72 (±0.40)	0.46
Mid-February	0.25 (±0.14)	0.61 (±0.29)	0.38 (±0.18)	0.77 (±0.25)	0.50
Mean	0.23	0.48	0.34	0.66	0.43

LSD ($p \leq 0.05$) among varieties = 0.18 and sowing dates = 0.21.

Significant differences ($p < 0.05$) in seed yield were observed among sowing dates where the lowest yield (1.5 t/ha) was recorded from February sowing and the highest seed yield was obtained from December sowing. The Arifi genotype showed the highest seed yield with a mean of 1.8 t/ha, the lowest seed yield was obtained with the susceptible genotype with a mean of 1.4 t/ha (Table 6).

Table 6. Effects of sowing date on mean seed yield of chickpea genotypes (t/ha), 2017–2018 cropping seasons.

Sowing Date	Chickpea Genotypes				
	Arifi	**Farihan**	**FLIP-07-211C**	**FLIP-09-221 C**	**Mean**
Mid-December	1.8 (±0.93)	2.2 (±0.27)	1.8 (±0.25)	1.8 (±0.63)	1.9
Mid-January	1.7 (±0.29)	1.6 (±0.48)	1.6 (±0.10)	1.5 (±0.45)	1.6
Mid-February	1.9 (±0.27)	1.5 (±1.1)	1.5 (±0.18)	1.1 (±0.13)	1.5
Mean	1.8	1.7	1.6	1.4	

LSD ($p \leq 0.05$) among genotypes = 481.0 and sowing dates: 416.5. LSD ($p \leq 0.05$) among genotype 0.481 and sowing dates is 0. 416.

For the three sowing dates, a positive correlation was observed between the three diseases parameters (wilt incidence, AUDPC, and rate of disease progression). A low negative correlation was observed between the seed yield and the three disease parameters (Figure 4).

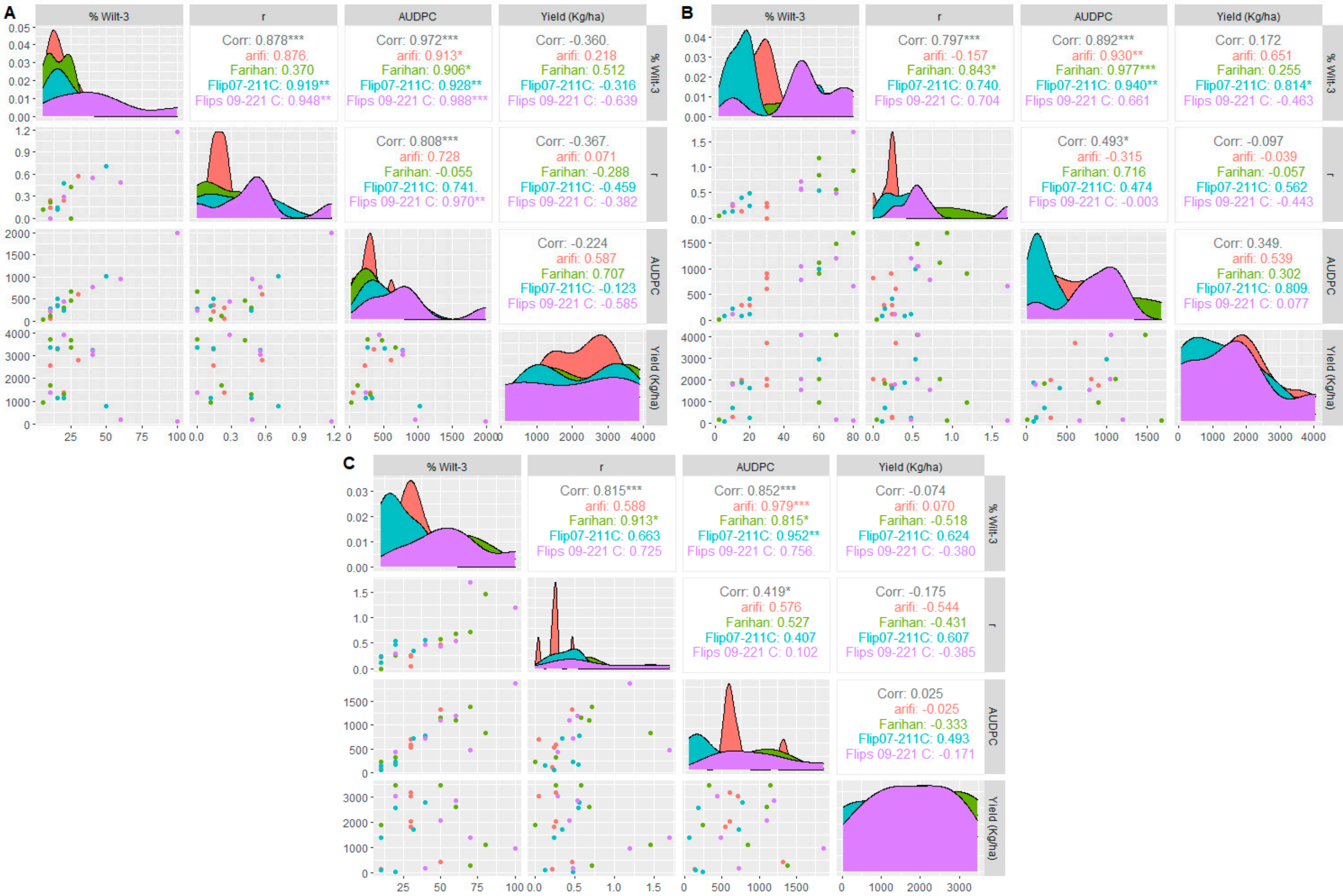

Figure 4. Correlogram showing the correlation between disease parameters and seed yield in the three sowing dates (**A**) mid-December, (**B**) mid-January, and (**C**) mid-February. Significance code: "***"0; "**" 0.001; "*" 0.01; "." 0.05.

4. Discussion

The disease progress in the two seasons was started in February and its development was increased in subsequent months. Delaying chickpea sowing favors disease development, and more plant mortality was observed in chickpea genotypes depending on their levels of resistance. The increase in air and soil temperature that favors *Fusarium* infection could explain the higher values of wilt incidence obtained in the late sowing [21,23].

The shifts from spring to winter planting being promoted in some Mediterranean countries doubled the productivity of the crop [24]. Winter season chickpea planting in the region was able to avoid the negative effects of Fusarium wilt and leaf miner that are critical in limiting the productivity of spring chickpeas. Chickpea cultivars released in Mediterranean regions are mainly resistant to Ascochyta blight and tolerance to cold and no effort was made to combine Fusarium wilt resistance since the disease development is not favored during winter sowing.

In this study, early sowing dates reduced the mean percent of Fusarium wilt for all genotypes with varying levels of resistance to the disease. However, late sowing, which is practiced by many farmers for spring planting, favored disease development and the level of mortality was higher on the released varieties as compared to early planting. The two released cultivars though were not tested for their reaction to Fusarium wilt pathogen in Morocco.

Many studies showed that Fusarium wilt development on chickpea is favored by spring (March) planting compared with winter planting (November and December) [16]. The integration of January sowing with partially resistant cultivars decreased fusarium wilt incidence and increased seed yield [20]. In Spain, Navas-Cortés et al. [25] demonstrated that advancing chickpea planting from early spring to early winter can decrease Fusarium wilt epidemics and increase seed yield. In Lentil wilt, the percent final wilt and AUDPC were low in November sowing under North Syrian conditions [26].

In our study, the increase in disease parameters in late-planted chickpeas could be due to an increase in temperature starting in February (Figure 1) that favored Fusarium wilt development [27]. Due to a decrease in rainfall in spring, the late-planted chickpea plots were exposed to the drought that can pre-dispose the crop to *Fusarium* infection [28]. An increase in temperature was reported to favor some races of Foc to cause high wilt incidence [28], but no attempt was made in this study to identify the prevalent races in the Foc population at the testing station.

Since a high correlation among the three disease parameters was observed, the final disease incidence evaluation is recommended for the evaluation of integrated wilt management and breeding lines.

Concerning the productivity, early sowing gave a higher yield than late sowing because early plants utilize available moisture and cool weather for high biomass and seed yield. However, late sowing exposes plants to drought and reduces photosynthetic efficiency [29]. The productivity of genotypes varies according to sowing dates, indicating that some genotypes can be planted early in the season to increase production. The absence of significant differences between genotypes and sowing date interactions suggested that the ranking of the genotypes was not changed due to sowing dates. In Spain, Lopez et al. [30] showed that fusarium wilt decreased both seed yield and seed weight.

The management of Fusarium wilt through short-term rotation is not helpful to chickpea growers since the pathogen can survive for approximately 6 years [18] and farmers are forced to practice short-term rationing to keep their wheat production sustainable through legume rotation that reduces weeds, chemic uses, and other benefits. So, developing resistant cultivars and early sowing can help to manage Fusarium wilt constraints in Morocco.

5. Conclusions

Fusarium wilt is an important constraint of chickpea production in spring-planted chickpeas in Morocco. Farmers don not have effective soil-borne disease management options other than treating their seeds with fungicides with low effectiveness and experiencing crop losses every year. Although Morocco's chickpea breeding program released cultivars with a high yield, their adoption is minimal due to the small seed size that is not preferred by consumers. Moreover, wilt/root rot diseases are not a priority for breeding programs. Hence farmers who are planting chickpeas in the spring are experiencing annual yield losses.

An integrated management of chickpea wilt can reduce yield losses so that farmers can have a good harvest for their home consumption and market. The early sowing of cultivars with good levels of fusarium wilt resistance is the best approach that can be recommended to farmers in this region based on the two seasons evaluated. This result could be extrapolated in Morocco since the observed conditions are representative of actual conditions in the country.

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