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Evaluation of the severity and tolerance of a collection Of durum wheat to root rot

Assessment of the severity and tolerance of a Moroccan collection Of durum wheat to root rot disease

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UMMARY

National production of durum wheat falls short of its potential, and By controlling its major diseases, including root rot. These latest induced by *Fusarium culmorum* and *Cochliobolus sativus*, see their Severity worsen with climate change. Genetic improvement work Diseases are more or less discontinuous, and it is from this perspective that this The aim of this work is to evaluate the levels of resistance to root rot National collection of 100 strains of durum wheat. The lines were planted in natural soil Previously inoculated with a conidial suspension of the two fungi. The experience Was repeated twice during two years under glass. The selected variables were The severity index (SI), the percentage of germination (Germ), and the dry weight (PS). The Selection of resistant lines was based on the analysis of variance, and the identification of Tolerant lines was accomplished by integrating main component analysis, And hierarchical classification, all based on (IS, PS, and Germ and yield biological). Seven lines were identified as resistant on both Years: 3012, 9373, 3010, 3191, 9416, 40041 and 3052 with severities ranging from 42 to 52%, On the other hand, lines 3206, 9406, 3017 and 3110 were identified as tolerant with Severity ranging from 41 to 50% but with a biological yield ranging from 3.4 to 5.7 g. The

Implications of our findings are discussed at the level of this paper.

Keywords: root rot, durum wheat, resistance, *Fusarium culmorum*, *Bipolaris sorokiniana*.

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Abstract

National production of durum wheat Has developed a control of its major diseases, especially root rot, which is induced by *Fusarium culmorum* and / or *Cochliobolus sativus*. The severity of root rot disease is Moroccan plant breeding efforts to control this disease by episodic drought Are more or less discontinuous in time. In this perspective, this work aims to evaluate levels Resistance to root rot of a national durum wheat collection of 100 accessions. These lines Were planted in natural soil previously inoculated with a conidial suspension of the two Fungi. The experiment was repeated twice in two years in a greenhouse. Analyzed variables Were severity index (SI), percentage of germination (PG), and biomass dry weight. Analysis Of variance helped in selecting resistant lines, while identification of tolerant lines was Accomplished by the integration of the main component analysis, and hierarchical clustering, Both based on the SI, PG and biological yield. Seven lines were identified as resistant; 3012, 9373, 3010, 3191, 9416, 40041 and 3052 with severities ranging from 42 to 52%. In Contrast, lines; 3206, 9406, 3017 and 3110 were found tolerant with their severity ranging From 41 to 50% and with a biological yield ranging from 3.4 to 5.7 g. The implication of These results are discussed within this document.

Key words: root rot, Durum wheat, Resistance, *Fusarium culmorum*, *Cochliobolus sativus*

INTRODUCTION

Cereal-growing occupies a place Primordial in Moroccan agriculture, Occupying an annual area of 5 Million hectares of which 1.1 million ha Are reserved for durum wheat. The improvement Of the production of this crop is

CM King & H. Bakke and *sorokinianum* Sacc. In Sorokin: Teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (*B. sorokiniana*) (Windels and Wiersma, 1992) that causes Common pedestrian (El Yousfi, 1984; Sjöberg et al., 2007). These two agents are

Subject in the first place to the control of Biotic constraints, including Root rot. These diseases Tackle All cereals Falls (Bockus et al., 2010) there Barley, and are more confined to Arid and semi-arid zones (El Yousfi, 1984; . Smiley et al, 2005 a and b; Dyer and al., 2009; Evans et al., 2010).

The pathogens responsible for *Fusarium* root rot sontle *culmorum* (Wm. G. Sm.) Sacc. (*F. culmorum*) (Windels and Wiersma, 1992) Which induces dry rot of the collar (Backhouse et al., 2004) and *Bipolaris sorokiniana* (*B. sorokiniana*) (Sacc.) Shoemaker: (synonymous *Helminthosporium Sativum* Pammel.

Responsible for the system and seedlings, The appearance of white ears (El Yousfi, 1984; Wahbi, 1989; Bockus et al. 2010). The etiology of this disease has Shown that these two pathogens Can be housed in the same plant (Smiley et al., 2005a) and under the effect of Water stress, the development of The disease can take on the epidemic. Nevertheless, the rots root caused by *B. sorokiniana* and that caused by *F. culmorum* are very good Different biologically and epidemiologically (Duveiller et al. 2007).

In Morocco, the yield losses due to Root rot is not Negligible and can be very

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Important during the episodes of Growth (Zillensky, 1983). These losses Oscillate between 20 to 51% (Ouziki, 1988; Mergoum, 1991), and are at the same level than those Estimated at the scale (Smiley, 2005b).

El Yousfi's study in 1984 On varieties of durum wheat, common wheat and Barley has demonstrated that the The most important growth at From which the varieties of cereals may Be differentiated for their sensitivities Is the flowering stage. In addition, the severity Remains an effective means of identifying

Drought during the Representing the genetic resources of The national genebank.

MATERIALS AND METHODS

The collection of durum wheat lines The purpose of this study is to National wheat genetic resources Hard of the Bank of Genes located at the Center Regional Agricultural Research Of Settat.

Due to insufficient seed Supplied by the Bank of Genoa, 100

Most studies in Morocco have shown that That hard wheat is much more Sensitive than tender wheat and barley. Nevertheless, throughout the season, No variety escaped infection (Baye, 1984, Houmairi, 1993). However, All these works remained for the most part Ad hoc studies, which did not A net contribution to the advancement of Genetic improvement of cereals Against root rot.

In order to achieve a stable level of Wheat production in arid zones And semi-arid areas it will be necessary to Solve the problem of rot Roots, and to identify new Genetic resources of resistance. These Resistance levels must be tested In areas known for this purpose. disease. In other words, research Must be carried out under conditions Environmental issues that Growth of the pathogen, which Could reflect the actual resistance of Varieties (Singleton, 2002).

It is in this context that this Study proposes to evaluate the The Rots Roots Of a Collection of durum wheat lines

First, for testing during multiplied, and According to their phenotype, these lines were Coded for the second year of Experimentation. Some lines Are found to be a mixture of 2 Phenotypes. These lines are: 3008, 3009, 3012, 3052, 3054, 3058, 3063, 3069, 3090, 3103, 3110, 3125, 3138, 40032, 389891, 9406, 9402, 9385, 9373, 9331, 9302, 3166, 3162, 3158, 3148, 3147. Other lines such as 3010, 3165 And 9412 Consisted of three Phenotypes.

1. Preparation of the inoculum

1.1. Isolation of mushrooms

The roots or sub-collars of plants Showing symptoms of rot Roots underwent 5 successive washes For 15 min in a solution Soap containing bleach to 10%, then 3 rinses with water Distilled sterile. Then, the roots are Dried sure Filter paper sterile, Cut into small pieces and deposited Immediately on the PDA medium (Potato-Dextrose-Agar). After 4 to 5 days Incubation at a temperature of 20 ° C And at a 12-hour photoperiod, colonies developed *B. sorokiniana* or *Fusarium* sp. Are incubated on medium

PDA, for purification and multiplication, Under the same laboratory conditions, For the production of inoculum.

Homogenized to have a mixture of Ground housing the two Agents Pathogens.

1.2. Inoculum preparation

1.-2.1. *Fusarium culmorum*.

After identifying the fungus as *F. culmorum* (Leslie and *al.*, 2006), the colonies grown on the Have been immersed with water Distilled and scraped with a brush. The Solution obtained is filtered with a Very fine mesh fabric. Concentration Of the conidia of the stock solution was Determined by a conidia count Using a hemacytometer.

1.2.2. *Cochliobolus sativus*.

Petri dishes containing the cultures *B. sorokiniana* are scraped with Brush after adding a Sufficient amount of distilled water. The Suspension is filtered and the concentration of The inoculum East Determined as a result of a Conidia, under binocular lens, twelve (12) drops of 5 µl each were Used for the determination of Concentration of the inoculum in the suspension.

2. Soil Inoculation

The soil used is clayey-silty soil Taken from the Experimental Domain of Sidi El Aidi of INRA. After drying the The soil is sieved and stored in Pots for later use.

To inoculate the soil, a Solution of 100 ml of each agent Pathogen, and each pathogen has Was represented by 5 isolates of Different, for 4 kg of soil in order to have potential inoculum 36.10 6 CFU / g floor *F. culmorum* and 25.10 6 conidia / g soil *B. sorokiniana*. The Similar quantities, taken from each soil Inoculated, mixed and well

The inoculated soil thus prepared and distributed In plastic bins of 11 × 7 holes Having dimensions of 4 × 4 × 4 cm for Each hole. The bins are filled to Half with soil inoculated, then Seeds have been deposited on a layer Of uninfested soil and then covered by Even non-infested soil. The seeds are Thus surrounded by a layer of non- Infested by about 1 cm in height, and the Rest of the hole is filled with soil inoculated. Each hole contained 10 grains And represented a durum wheat line. Of The same way, we sowed in each Tray two control varieties; A sensitive (Ourgh durum wheat) and the other moderately Resistant (soft wheat Amal). Bins Sown are placed in a glasshouse and Watered regularly to Development of plants. Irrigation Fertilization are carried out according to the The needs of the vegetative growth of Lines.

The experiment described Repeated for two successive years (2010-2011 and 2011-2012). The device Experimental study and the randomized Complete with four repetitions. For the The first three blocks, the rate was Germination, severity and dry weight Of each lineage including the witnesses, While at maturity the fourth block Has been assessed only for the Germination and biological yield.

3. Methods devaluation and Observations

Germination rate and emergence.

Fifteen days after sowing, the percentage Of germination is estimated by counting Of plants raised in each hole, the Value is divided by the number of Total of grains (10 grains) and then multiplied Per 100.

Evaluation of severity. At the stadium Flowering, the plants of each line have Washed and rinsed under a stream of water To clear the root system, and The severity assessment focused on the Description of the attack at the level of The root system, namely the collar, the Sub-collar and seminal roots (El Yousfi, 1984). The severity of the disease At the level of the root system was Evaluated according to the class scale from 0 to 5 according to **Table 1**. The plants of Each line were classified according to 5 The severity classes, then the index of Severity was calculated according to the formula (Cooke, 1998):

$$\text{Severity Index (\%)} = \left(\frac{\sum (N_i \times S_i)}{N_t \times 5} \right) \times 100$$

N_i : Number of Plants in the Class of severity i , i ranging from 1 to 5.
 S_i : Class severity number.
 N_t : Total number of plants Observed by lineage.

Dry weight. Once the evaluation of the Severity is complete, samples are Dried in the open air at the level of Until it dries out, then the Dry weight of each line is measured For the three blocks mentioned above.

Biological yield. At maturity, the Biological yield is evaluated in Cutting the aerial part of the plant Of each line, including controls. This parameter was evaluated only for the fourth block of each experimentation.

Statistical analysis

For each year of experimentation, An analysis of variance (ANOVA) With the Waller option for comparison Averages has been adopted to treat The severity indices of the tested lines According to the experimental block arrangement Complete random with 3 repetitions. The Lines showing less severity Were found to be resistant to this disease.

Table 1. Scale for estimating the severity of root rot.

Class of Severity	Degree of infection of the plant
0	No symptoms
1	Small necrotic lesions scattered at collar, sub-collar and roots Seminars.
2	Distinct and clear necrotic lesions in the root system.
3	Large necrotic lesions on collar, sub-collar and seminal roots.
4	Severe rot of the root system and chlorosis of the plant.
5	Dead plant.

From the data of the three blocks, for each Experimentation, Averages of germination rates, Dry weight of biomass, indices of Severity, and which are explored by a test Of variance homogeneity (Levene, 1960).

These averages were Biplot analysis (Gabriel, 1971), referring to Two years, which focused on the Matrix whose columns represented The means of the variables, the dry weight, The germination rate, the index of Severity and lines by the wheat lines hard. The matrix is centered and standardized By columns. The residual matrix is Decomposed into value and vector "Single Value decomposition" According to dimensions. Graphic representation In two dimensions projects columns (Variables) as vectors and lineages As points.

Two analyzes of classification Hierarchy of lines, one for each Year, were made and Option to analyze the Ward and the Distances Euclidean. Before The analysis, Variables have been Standardized (average = 1 and Variance = 0), and the results were Represented under form of an Two - dimensional dendrogram. The First dimension is the distance from Connection between the different lines Durum wheat for group formation And the second presents the lines

And hierarchical classification Is SAS (SAS, 2002).

RESULTS

Table 2 presents the means of The index of severity, germination, Dry weight and biological Lines tested during the two years, And that only the variance between the Two years, for the severity index Was homogeneous.

In the first year, Germination index, severity index and Dry weight were lower than those For the second year. In Effect, the index of severity Was 43% and 69% respectively for the First and second year; the Germination was 78 and 83%, as well as The dry weight which exhibited averages 1.03 and 2.88 g. While the The biological yield Average of 3.22 g in the first year and Of 2.46 g in the second year.

Figure 1 reflects the linear relationship Decreasing between the average of the weights And indices of severity. The Lines with a severity index Higher yielded a low dry weight, Whereas those with an index of Lower severity produced a dry weight high. This had happened for both Years of experimentation, except that The effect of the disease on dry weight was Much more important on the reduction of

Have been tested. This analysis of variables Biomass (was on the order of 16
 Classification were; The dry weight, the While that of the second year was
 Germination index, severity index and Of -6.15).
 Biological yield from the fourth
 Block (Ghosh and Rao, 1996, Izenman,
 2008).
 The software used for the analysis of
 Variance, the analysis in Component

Table 2. Means, standard errors, homogeneity of variance test, the Probability of severity index, germination, dry weight and Biological yield of durum wheat lines inoculated with Root rot during the two years.

Year	Severity Index (%)		Germination (%)		Dry weight (g)		Yield Biological (g)	
	MOY	ES	MOY	ES	MOY	ES	MOY	ES
2010-2011	43	0.011	78	0.733	1.03	0.091	3.22	0.153
2011-2012	69	0.010	83	0.638	2.88	0.079	2.46	0.134
Homog.	1.299	-	238,352	-	58.279	-	11,688	-
Probability	0.256	-	<.0001	-	.0001	-	0.001	-
MOY: mean, ES: Standard Error, Homog. : Test for homogeneity of the variance (test of Levene).								

1 FIG. Relationship between severity indices and dry weights of lines tested during The 2010-2011 and 2011-2012 campaigns.

The analysis of the ANOVA variance Was based solely on the indices of Severity showed that the lines were Significantly different in the course of The first ($p = 0.002$) and very highly Significantly different at the Second year ($p < 0.0001$) according to their Sensitivity to root rot. In the course of the 2010-2011, the resistant lines were: 9338, 3005, 2995, 3012, 9412, 9411, 9373, 3123, 9389, 3010, 40032, 3191, 9331, 9323, 16563, 9416, 3162, 40041, 3124, 3009, 9417, 3207, 3151, 9409,

3071, 3052, 9415, 3064, 3205, and had a severity ranging from 14 to 35% (**Table 3**). On the other hand, in the second Of 2011-2012, the severity of the Resistant to know 3112, 9380, 3010/1, 3147/2, 3010/2, 9416, 3117, 3095, 39891/1, 3108, 9373/1, 9406/2, 16549, 3010/3, 3114, 3052/1, 3107, 3057, 2963, 3085, 3052/2, 3082, 3007, 3017, 3012/2, 4001, 3118, 3125/1, 3191, 9406/1 and Amal varied between 39% and 63%. Finally, resistant strains for Two years of experiments were: 3012, 9373, 3010, 3191, 9416, 40041 and

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3052. In general, and over the two years Experiments, the strains judged Resistant were: 3012, 9373, 3010, 3191, 9416, 40041 and 3052, while the Lines 3206, 9406, 3017 and 3110 were Tolerant.

The Biplot graph for the first year Accounts for 86% of the variability and That the rate of germination of Seeds and the dry weight of plants are More or less positively correlated. By Against, the severity index is highly

And negatively correlated with dry weight Plant. Given that our interest Related to dry weight lines High germination rate and a high Severity index, the Possessing these characteristics were: 100 (40041), 96 (16549), 36 (3097), 7 (3010), 42 (3110), 34 (3095) and 99 (40032) (**Figure 2**). The first value and That of the value of the line on the Biplot Whereas the second value is relative to the Lineage code at the Bank level Of Gene.

Table 3. The durum wheat lines resistant to the year 2010-2011 and 2011-2012

	Year 2010-2011	Year 2011-2012
Dendrogramme	3206 , 9358, 9377, 16549,	3017 , 9411, 9406/1 , 3118, 9409,
(Germination rate,	40041, 3064, 9385, 40032,	3206 , 3125/1, 3191, 3012/2
Severity index, weight	3097, 3110, 3017 , 9406 , 3074,	
Dry and yield	3007, 9417, 3151, 3157	
biological)		
Biplot	3097, 3110 , 3010, 40032,	9406/1, 3110/1 , 9411, 3012/2,
(Germination rate,	16549, 40041, 3095	3191, 3017 and 9409
Severity index and		
Dry weight)		
	9338, 3005, 2995, 3012 , 9412, 3112, 9380, 3010/1 , 3147/2,	
	9411, 9373 , 3123, 9389, 3010/3 , 3010/2 , 9416 , 3117, 3095,	
	40032, 3191 , 9331, 9323,	39891/1, 3108, 9373/1 , 9406/2,
ANOVA	16563, 9416 , 3162, 40041 ,	16549, 3010/3 , 3114, 3052/1 ,
(Severity Index)	3124, 3009, 9417, 3207, 3151, 3107, 3057, 2963, 3085, 3052/2 ,	
	9409, 3071, 3052 , 9415, 3064, 3082, 3007, 3017, 3012/2 ,	
	3205	40041 , 3118, 3125/1, 3191 ,
		9406/1 and Amal

Figure 2. Biplot of EMG analysis of the germination rate, dry weight and The severity index of the lines tested for the year 2010-2011.

Hierarchical classification analysis
Based on germination, the index of
Severity, dry weight and yield
biological as Variables of
Classification, has made it possible to differentiate
Lined in Several groups of
Sensitivity, and to situate the group of
The target lines, it was based on
The identification of lines at the level of
Biplot (**Figure 2**) to select the distance
Which is 6 at the level of the
Dendrogramme (**Figure 3**).
The location of these Lineages,
Previously identified at the level of
Biplot, in the dendrogramme we
Facilitates the identification of the
Targeted lines. Indeed, this group
Was composed of resistant lines:
3064, 40041, 40032, 9417, 3151, and
Tolerant lines: 16549, 3097, 3110,
3206, 9358, 9377, 9385, 3017, 9406,
3074, 3007, and 3157 (**Figure 3**).

For the second year, the graph
Biplot accounts for 84% of the variability and
Shows that the severity index is
Highly and negatively correlated with the
dry weight of plants. The lines 114
(9406/1), 54 (3110/1), 117 (9411), 13
(3012/2), 92 (3191), (3017) and 116
(9409) had a high dry weight,
Of germination and an index of
low severity (**Figure 4**).

Similarly, and using the dendrogram of
The hierarchical classification analysis,
Based on the same variables as
Of the first year and following the same
Methodology, has enabled us to
differentiate the group (distance
Cutting edge of 7) of target lines having
As resistant lines: 3017, 9406/1,
3118, 3125/1, 3191, 3012/2, whereas the
Tolerant lines were: 9411, 9409,
3206 (**Figure 5**).

3. FIG Dendrogram analysis of the hierarchical classification of wheat lines
For the year 2010-2011. Rated: Hierarchical tree using the Ward method.

Figure 4. Biplot of EMG analysis of the germination rate, dry weight and the index
Of the severity of the lines tested for the year 2011-2012.

5. FIG Dendrogram analysis of the hierarchical classification of wheat lines
For the year 2011-2012. Note: Hierarchical tree using distance from Ward
(Combination distance of resized classes).

D ISCUSSION AND CONCLUSIONS

The significant relationship between
The severity index of the disease and the
Dry weight of plants shows that
Roots Roots Influence
Negatively on plant vigor
By the impediment of the assimilation of
Nutrients through the system
Root. Similarly, Miedaner (1988)
Reported that the dry weight of the roots of

Local lines of durum wheat a very
Great genetic variability with regard to
Their reaction to root rot
(Figure 3 and 5). In the past two
Years, the analysis of variance has
Identified the resistant lines 3012,
9373, 3010, 3191, 9416, 40041
Had indices of severity
Weak compared to the whole of the

Resistant varieties was significantly higher than that of susceptible varieties. Therefore, and based only on the severity index and the dry weight of the plants evaluated at the flowering stage, could easily identify the lines resistant (**Figure 1**).

The analytical methodology level of this study allowed us to highlight the existence between

Collection tested. These lines can be used in the program for the genetic improvement of hard wheat in order to develop suitable varieties and resistant to root rot, caused by *Bipolaris* complex *sorokiniana* and *Fusarium culmorum*.

The biological yield was used in this study to differentiate resistant lines of those which are

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Tolerant. The assessment of severity occurs at the bloom stage (El Yousfi, 1984) prior to the installation of grain at level of ears, whereas the evaluation of biological yield is only maturity.

However, for both years experiments, the combination of the analysis ANOVA, The analysis in main component and classification hierarchical we at allowed of select the resistant lines having of biological yields and of satisfactory germination.

These lines were: 3012, 9373, 3010, 3191, 9416, 40041 and 3052. Resistance had had repercussions on their production performance (**Table 3**). On the other hand, four lines were tolerant: 3206, 9406, 3017 and 3110.

Previous studies have shown that agents of roots roots

Tolerant and resistant lines identified in this study are sought after by improvers, since they are resistant to necrosis root system (severity index low) in addition to their relatively high germination and consequently better resistance to melting of seedlings. These same lines showed a production of dry matter relatively high and yield biological growth with grains of mass.

The integration of the analysis of variance made on the indices of severity, Principal Component Analysis and the analysis of the hierarchical classification has enabled us to select both the resistant lines and lines tolerant of the national collection of durum wheat kept at the level of national genebank. These lines are available to improvers

Germination, emergence and
Also induced the death of seedlings
(Verma and Spurr, 1987, Lyamani, 1988;
Khabouze, 1988; Mergoum, 1991;
Wagacha and Muthomi, 2007). Thereby,

National and international geneticists
Durum wheat for the development of
Varieties resistant and tolerant to
Root rot.

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