ROOT ROTS OF WHEAT : INOCULATION TECHNIQUES AND EFFECTS ON GRAIN YIELD AND ITS COMPONENTS UNDER VARYING WATER AND NITROGEN LEVELS IN MOROCCO

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ملخص

اهتمت هذه الدراسة بمفعول تقنيتين للتعفن وآثار أمراض «التعفن الجذري» الذي تسببه فطريات فيزاريوم كلموروم (Fusarium culmprum) وهلمنتوسبوريوم ساتيفوم (Helminthosporium sativium) على ستة أصناف من القمح الصلب والطري وذلك تحت مستويات مختلفة للجفاف والأسمدة الأزوط (N).

لقد أنجزت الدراسة خلال موسمي 88 ـ 1989 و 89 ـ 1990 في ميدانين التجربة لسيدي العايدي (ناحية سطات) وتاساوت (ناحية مراكش) التابعة للمعهد الوطني للبحث الزراعي.

خلال موسم 89 ـ 1990، كانت آثار مرض التعفن الجذري ذات أهمية بالغة مقارنة مع موسم 88 ـ 1989. يرجع هذا من جهة إلى استعمال خليط من الماء وبغات الطفيلي كتقنية للتعفن، ومن جهة أخرى للجفاف الذي عرفته المنطقة خلال هذا الموسم من شهر فبراير إلى أبريل. أما بالنسبة لعامل الأزوط (N) فإنه لم يؤثر بصفة جوهرية على تفاقم المرض.

إن آثار مرض التعفن الجذري، كانت بالغة وخاصة خلال موسم 89 ـ 1990 وتتجلي هذه الآثار في (أ) تقلص محصوص في مردود ووزن حب القمح إلى حدود 34 في المائة و 60 في المائة بالتوالي.

(ب) تكاثر محسوس في عدد السنابل البيضاء التي تكون غالبا خالية من الحب أو تحتوي علي حبات مملوءة جزئيا.

بصفة عامة إن مقاومة القمح الصلب لمرض التعفن الجذري كانت ضعيفة بالمقارنة مع القمح الطري بحيث أظهرت أصناف «مرزاق» و «كوكوريت» (قمح صلب) أكثر حساسير. فيما كان صنف تيكي 32 أكر مقاومة لمرض التعفن الجذري.

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RESUME

Le rendement en grain et certaines de ses composantes de six variétés de blé ont été étudiés au champ avec deux techniques d'inoculation des grains par, ***Fusarium culmorum*** et ***Cochliobolus sativus***, agents de pourriture racinaires, sous différents régimes d'eau et niveaux d'azote.

L'étude a été menée dans les Domaines Expérimentaux de Sidi El Aydi (région de Settat) et Tassaout (région de Marrakech) en 1988-89 et 1989-90. Des effets significatifs sur le développement des pourritures racinaires ont été observés en 1989-90 par rapport à la saison de 1988-89. Ceci est dû à l'inoculation par une suspension de spores des agents de pourritures racinaires et la sécheresse sévère qui a sévi de février à avril 1990.

En général, la fertilisation azotée n'a pas eu d'effet significatif sur la maladie. En 1989-90, l'inoculation artificielle par la maladie des pourritures racinaires des blés a provoqué (1) une diminution significative du rendement et du poids du grain allant jusqu'à 60 et 34% respectivement, et (2) une augmentation significative du nombre d'épis blancs. Les blés durs se sont montrés en général, plus sensibles que les blés tendres. Les variétés de blé dur Marzak et Cocorit se sont révélées les plus sensibles et la variété de blé tendre Teguey-32 la plus résistante.

ABSTRACT

The behavior of wheat grain yield and its caracteristics to two root rot field inoculations (***Fusarium culmorum*** and ***Cochliobolus sativus***) under various levels of nitrogen and water stress were studied for six wheat cultivars at Sidi El Aydi and Tassaout stations in Morocco during 1988-89 and 1989-90. Significant root rot effects coincided with the severe drought of February through April in 1989-90 and seed inoculation with a spore suspension. Nitrogen had no significant effect on the development of the disease at all environments. In 1989-90, inoculation of wheat cultivars significantly reduced grain yield (up to 60%), and kernel weight (up to 34%); and increased white heads. Durum wheats were, in general, more affected by inoculation than common wheats for all studied traits. Marzak and Cocorit durum wheats were the most susceptible and common wheat Teguey-32 was the most tolerant.

KEY WORDS : Root rot, wheat, water stress, inoculation techniques, nitrogen.

INTRODUCTION

Root and foot rots are among the major diseases of wheat world wide (Weise, 1977). However, the occurrence and frequency of the causal agents vary regionally (Lyamani, 1988). The damage from root rot pathogens varies from year to year as well as from field to field depending upon the amount of inoculum present, cultural practices, soil and climatic factors (Statler and Darlington, 1972; Tinline, 1986; Wildermuth and Mc Namara, 1991). Root rot can be caused by one or several organisms alone or in combination (Statler and Darlington, 1972). The most commonly reported causal pathogens are ***Cochliobolus sativus*** (Verma et al., 1976); ***Fusarium culmorum***; ***Fusarium*** graminearum and ***Fusarium avenaceum*** (Cook, 1980; Dodman and Wildermuth, 1987). Selection for resistant cultivars to root rot pathogens has been hindered by inadequate and inconsistent inoculation methods, and lack of accurate and suitable disease evaluation techniques (Ledingham et al., 1973; Verma et al., 1976; Dodman and Wildermuth, 1987).

In Morocco, root rot occurs throughout the cereal growing areas including the arid and semi-arid zones of the West Central regions of the country (Lyamani, 1988). It is caused mainly by ***Cochliobolus sativus*** and ***Fusarium culmorum*** (Baye, 1984; Lyamani, 1988). Previous studies (Baye, 1984, Mergoum, 1991) have shown the importance of water deficit and nitrogen fertilization effects on the development of the disease. Although, several inoculation techniques have been successfully tried under laboratory and greenhouse conditions, accurate and suitable inoculation methods for screening large number of germplasm under field conditions are urgently needed by breeders. Therefore, this paper reports on a study designed to examine the effects of two techniques of inoculation by ***Cochliobolus sativus*** and ***Fusarium culmorum*** on grain yield and its components using six wheat cultivars under different regimes of water and nitrogen in the west central regions of Morocco.

MATERIALS and METHODS

The experiments were conducted at Sidi El Aydi and Tassaout experiment stations in Morocco during the 1988-89 and 1989-90 growing seasons. Soil type was classified verticalcixeroll at Sidi El Aydi and alfisol at Tassaout. Climatic conditions for these sites during the 1988-89 and 1989-90 growing seasons are presented in Table I.

Seed/soil inoculation : One isolate of ***F. culmorum*** was grown on PDA medium and used to infest autoclaved barley seeds. Two hundred grammes of barley seed were immersed in sterile water in a flask and autoclaved for 30 minutes at 103°C. Cultures of the ***F. culmorum*** on barley substrate were incubated for 3 weeks. The colonized barley seeds were removed from the flasks; dried at room temperature for 4 days; chopped into chunks (1 to 3 mm); sprayed with a spore suspension of ***Cochliobolus sativus*** adjusted to 10^6 spores per ml; and mixed with wheat seed at the rate of 200 g per kg.

Seed inoculation : One isolate of ***Fusarium culmorum*** and one isolate of ***Cochliobulus sativus*** were cultured separetly on PDA for 15 days at room

Table I : **Mean temperature (°C) and rainfall (mm) for the 1988-89 (A) and 1989-90 (B) growing seasons at Sidi El Aydi (SEA) and Tassaout (TST), Morocco.**

Month	Temperature (°C)				Rainfall (mm)			
	SEA		TST		SEA		TST	
	A	B	A	B	A	B	A	B
September	25	24	26	25	0	0	4	7
October	23	22	20	22	31	33	18	19
November	17	17	18	16	100	80	87	32
December	13	15	11	14	5	53	0	19
January	10	10	10	9	35	54	36	30
February	11	14	12	16	36	0	43	0
March	14	16	15	16	71	49	44	52
Avril	13	15	14	16	61	35	0	18
May	19	21	21	21	0	10	3	41
June	22	27	23	24	0	12	0	4
July	26	27	28	27	0	0	9	9
August	26	28	28	29	0	0	27	1
Total	-	-	-	-	341	326	330	332

temperature (25°C). Distilled sterile water was added to the petri dishes, then spores and mycelia were gently scraped off the plates into a 250 ml flask containing 200 ml distilled water and shaken. This solution was strained through a double layer of sterilized cheesecloth. The strained spore suspensions were adjusted to 106 spores per ml by adding sterile distilled water and were used for wheat seed inoculation. This was done by mixing 200 ml of the fungal suspension and two kg of seeds in a plastic bag for 4 to 5 min. Seeds were then dried at room temperature for 48 h and placed in paper bags for planting.

In 1988-89 and 1989-90, two experiments (one under full and one under partial irrigation) were set up at each of Sidi El Aydi and Tassaout locations. Irrigation was acheived by flooding at Tassaout and sprinkling at Sidi El Aydi. A total of 300 and 100 mm of water was applied in addition to rainfall (Table I) on the full and partially irrigated plots, respectively. Irrigation was stopped at the "boot stage" (Feek's 9) for the partially irrigated experiments. Each experiment included six wheat cultivars (three bread wheats; Marchouch, Jouda and Teguey-32; and three durum wheats; Cocorit, Marzak and Kyperounda); three nitrogen levels (50, 100 and 150/kg ha at Sidi El Aydi; and 0, 50, and 100 kg ha-1 at Tassaout) and two inoculation levels (inoculated and non-inoculated). Seed/soil and seed inoculation as described previously was done in 1988-89 and 1989-90, respectively. Each experiment was planted in a split-split plot design with four replications. Nitrogen levels were the main plots, cultivars the sub-plots and inoculation the sub-sub-plots. Earch plot consisted of 6 rows 0.3 m apart and 5 m long. Furadan (carbofuran) was mixed with seeds at a rate of 20 kg ha-1 at planting time to control Hessian fly (***Meyetiola destructor***).

Data were collected on (1) grain yield (Kg/ha) of the 4 central rows of each plot; (2) 250 kernels weight; and (3) root rot score expressed as the percentage of white heads per plot at grain-filling stage.

RESULTS AND DISCUSSIONS

Root rot evaluation

Percent white heads to evaluate root rot disease development were recorded in both 1988-89 and 1989-90 (Tables II and III). In 1988-89 white head scores were low and not significant compared to 1989-90 (Table III). White head percentages varied significantly only for irrigation regimes and cultivars (Appendix A) and did not exceeded 7% for the susceptible cultivar Marzak for the same season. Practically no disease sysptoms (white head percentages less than 1%) were recorded for most of the cultivars.

During 1989-90 growing season however, white head percentages varied for irrigation regimes, cultivars, and importantly, for inoculation and the cultivar x inoculation interaction. Cultivars showed consistently higher white head percentages under stressed conditions than under irrigation in all 1989-90 field experiments at both locations (Tables II and III). Under stressed conditions, white head percentages were as high as 20% for durum cultivar Cocorit at Tassaout and 31% at Sidi El Aydi on the inoculated plots (Table III).

Inoculation significantly increased white head percentages of Jouda, Marzak, Kyperouda and Cocorit cultivars at Tassaout and Sidi El Aydi in the stressed trials. Under irrigation, however, inoculation did not affect significantly percent white head.

These results showed higher root rot damage on durum wheats compared to common wheat cultivars. Cocorit and Marzak had the highest levels of white head percentages. These results are in agreement with those reported in the same regions by Lyamani (1988) which showed that Cocorit was highly susceptible to ***F. culmorum*** and slightly susceptible to ***C. sativus.*** The same study reported that Marzak was also moderately susceptible to ***F. culmorum*** and fairly resistant to ***C. sativus.***

Inoculation techniques

Although, The seed/soil inoculation technique gave satisfactory results with ***Fusarium*** **graminearum** in several previous works (Dodman and Widermuth, 1987;), low white head percentages resulted from this technique in 1988-89 plots (Tables II and III). The failure to obtain adequate levels of root rot symptoms could be related to environmental conditions rather than the inoculation technique. At Sidi El Aydi, average air temperatures were simillar in both years and total rainfall was 341 and 326 mm in 1988-89 and 1989-90 respectively (Table I) . However, the distribution of the rainfall differed drastically particularly during Febuary to April period. Whereas 168 mm of rainfall occured in this period in 1988-89, only 84 mm were registred in 1989-90 (Table I). At Tassaout station, average air temperatures and total rainfall were comparable and had simillar distribution pattern to that registred at Sidi El Aydi station in both seasons (Table I). The inoculation technique was modified in 1989-90 by treating wheat seeds with a spore suspension of the pathogens. The results obtained at both sites were satisfactory since average white head percentages reached 31% on durum wheat Cocorit at Tassaout under non irrigated regime (Table II). This technique was also successfully used in a previous work (Lyamani, 1988).

Effects of nitrogen

Nitrogen fertilization had no effects on inoculation techniques and development of root rot in general (Appendix A). At Sidi El Aydi this might have been due to the high level of residual nitrogen in the soil from heavy application during previous years (soil test levels were 23 to 27 ppm of NO3-N). At Tassaout, however, the soils are relatively deficient in nitrogen (soil test levels were 3 to 9 ppm of NO3-N). These results are in agreement with certain previous reports (Sims et al., 1961; Broscious an Frank, 1986) but in conflict with previous work in Morocco (Baye, 1984) and elsewhere (Smiley et al., 1972) which demonstrated that root rot severity was enhanced by excess nitrogen. The excess nitrogen normally promotes growth and increases transpiration of plants resulting in depletion of soil moisture (Papendick and Cook, 1974; Cood, 1980).

Effects on wheat grain

Special interest was given to the effects of inoculation by ***F. culmorum*** and ***C. sativus*** on grain yield and kernel weight since these are the final components for wheat grain yield production.

Table II : **White head percentages (%) caused by root rot pathogens in plots of six wheat cultivars grown at Tassaout and Sidi El Aydi, Morocco, under two water regimes in 1989-90 season.**

Trait1	Tassaout				Sidi El Aydi			
	Irrigated		Non irrigated		Irrigated		Non irrigated	
	Non-Inoc.1	Inoc.1	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.
	%							
Marchouch	0	0	4	8	0	0	4	6
Jouda	0	3	4	9*	0	0	4	7*
Teguey-32	0	0	2	5	0	0	1	2
Marzak	1	6	5	28**	0	3	4	18**
Kyperouda	0	2	3	9*	0	0	3	9*
Cocorit	0	5	6	31**	0	1	4	20**
Means	0	3	5	15	0	1	3	10
LSD (0.05)	4	4	5	5	3	3	4	4

*, ** significant as compared to Non-Inoc. treatments at the 0.05 and 0.01 probability levels, respectively.
1Cont. = control and Inoc. = inoculated with root rot.

Table III : **Means of kernel weight and yield, and white head percent of six wheat cultivars, as affected by root rot pathogens, grown at Tassaout and Sidi El Aydi, Morocco, under two water regimes in 1988-90.**

Trait[1]	Tassaout				Sidi El Audi			
	Irrigated		Non irrigated		Irrigated		Non irrigated	
	Non-Inoc.[2]	Inoc.[2]	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.
1989-90 (inoculation with a spore suspension)								
K. weight	49	43*	27	20*	39	32**	36	33*
G. yield	4526	3864**	1850	1180**	3140	2311**	3536	2236**
W. heads	2	3	5	15**	0	2	3	10**
1988-89 (inoculation with infected barley seed)								
K. weight	36	35	34	33	44	43	47	46
G. yield	2691	2579	2462	2423	4096	4016	3784	3852
W. heads	2	3	3	4	2	4	1	3

*, ** significant as compared to Non-Inoc. treatments at the 0.05 and 0.01 probability levels, respectively.

[1] K. weight = 1000 Kernels weight (g); G. yield = grain yield (Kg ha^{-1}) and W. heads = white heads percentage (%)

[2] Non-Inoc.= Non inoculated and Inoc. = inoculated with root rot pathogens.

Table IV : **Grain yield reductions (%) caused by root rot pathogens in plots of six wheat cultivars grown at Tassaout and Sidi El Aydi, Morocco, under two water regimes in 1988-89 and 1989-90.**

Cultivars	1989-90				1988-89			
	Tassaout		Sidi el Aydi		Tassaout		Sidi el Aydi	
	Ir.1	N. Ir.	Ir.	N. Ir.	Ir.	N. Ir.	Ir.	N. Ir.
	%							
Marchouch	10*	26**	12**	25**	1	3	2	2
Jouda	1	14**	23**	20**	3	2	0	3
Teuey-32	19**	31**	36**	48**	0	4	1	2
Marzak	33**	50**	40**	51**	3	6	5	4
Kyperouda	12*	60**	27**	40**	4	4	3	5
Cocorit	11*	43**	20**	41a**	2	5	4	6
Means	14	37	26	38	2	4	2	4
LSD (0.05)	8	9	8	10	4	6	6	7

*, ** significant as compared to Non-Inoc. treatments at the 0.05 and 0.01 probability levels, respectively.
[1] Ir = irrigated and N. Ir. = non irrigatad.

Table V : **Kernel weight reduction (%) caused by root rot pathogens in plots of six wheat cultivars grown at Tassaout and Sidi El Aydi, Morocco, under two water regimes in 1989-90 season.**

Cultivars	Tassaout		Sidi el Aydi	
	Irrigated	Non Irrigated	Irrigated	Non Irrigated
	.....%			
Marchouch	1	20**	1	2
Jouda	2	12*	0	2
Teguey-32	4	8	3	9
Marzak	8*	34**	10*	14**
Kyperouda	2	28**	3	12**
Cocorit	2	29**	11*	17**
Means	3	26	4	11
LSD (0.05)	7	11	6	10

*, ** significant as compared to Non-Inoc. treatments at the 0.05 and 0.01 prob ability levels, respectively.

Grain yield : while in 1988-89 grain yield differed significantly only for cultivars and nitrogen (Appendix A). Inoculation treatments did not affect significantly yield in this season. In 1989-90 however, inoculation, cultivars, and inoculation x cultivar interaction effects were significant were higher under limited water regimes than under irrigated (Tables III and IV). Under water stress of 1989-90 trials, inoculation significantly reduced average grain yield ofr all cultivars by 38 at Sidi El Aydi and 37% at Tassaout (Table IV). However, under irrigation the inoculation reduced yield by only 26 and 14%, respectively. Except for Jouda at Tassaout irrigated, inoculation greatly reduced grain yield of all cultivars without regard to water stress at both locations in all field experiments in 1989-90. Yield losses as high as 51 and 60% were recorded for durum wheats Marzak and Kyperounda, respectively.

Appendix A : **Source of variance and mean squares significance of four wheat traits studied during 1988-89 and 1989-90 in Morocco.**

Source	1988-89				1989-90		
	DF[1]	GY	KW	WH	GY	KW	WH
Location (L)	1	**	**	*	*	*	*
Irrigation(IR)	1	ns	ns	*	**	**	**
L x IR	1	ns	ns	ns	**	**	ns
Block (LxIR) (Error a)	12						
Nitrogen (N)	2	*	**	ns	ns	*	ns
L x N	2	ns	ns	ns	ns	ns	ns
IR x N	2	ns	ns	ns	ns	ns	ns
L x IR xN	2	ns	ns	ns	ns	ns	ns
NxBlk (LxIR) (Error b)	24						
Cultivar(C)	5	**	**	*	**	**	*
L x C	5	**	**	ns	**	*	ns
IR x C	5	ns	ns	ns	**	**	ns
L x IR x C	5	ns	ns	ns	**	**	ns
N x C	10	ns	**	ns	ns	ns	ns
L x N x C	10	ns	*	ns	ns	ns	ns
IR x N x C	10	ns	ns	ns	ns	ns	ns
L x IR x N x C	10	ns	ns	ns	ns	ns	ns
BLKxV (LxIRxN) (Error c)	180						
Inoculation(I)	1	ns	ns	ns	**	**	**
L x I	1	ns	ns	ns	**	ns	ns
IR x I	1	ns	ns	ns	*	**	ns
L x IR x I	1	ns	ns	ns	ns	**	ns
N x I	2	ns	ns	ns	ns	ns	ns
L x N x I	2	ns	ns	ns	ns	ns	ns
IR x N x I	2	ns	ns	ns	ns	ns	ns
C x I	5	ns	ns	ns	**	ns	**
L x C x I	5	ns	ns	ns	ns	ns	ns
IR x C x I	5	ns	ns	ns	ns	ns	ns
N x C x I	10	ns	*	ns	ns	ns	ns
Residual	253						

*, ** significant at the 0.05 and 0.01 probability levels, respectively

[1] DF = degree of freedom, GY = grain yield, KW = kernel weight, and WH = white heads.

Large yield losses due to inoculation by ***F. culmorum*** and ***C. sativus*** observed in 1989-90 (Tables III and IV) consistently showed that durum wheat cultivars Marzak, Cocorit, and Kyperounda were more damaged than bread wheat cultivars Jouda, Marchouch, and Teguey-32. The lowest grain yield reductions were recorded for Jouda and Marchouch whereas the highest yield loss was always attributed to one of the durum wheat cultivar. These findings are in agreement with previous works in the USA (Hill and Fernandez, 1983; Stack, 1982), Canada (Ledingham et al., 1973; Verma et al., 1976) and Australia (Wearing and Burgess, 1977).

Kernel weight : Cultivars and nitrogen showed significant differences for kernel weight under all environments in both seasons (Appendix A). Inoculation had no effects on kernel weight of wheat cultivars during the 1988-89 crop season Table III).

In 1989-90, however, inoculation, and its interaction with irrigation showed significant effects on kernel weight (Appendix A). Cultivars differed significantly for kernel weight in all field experiments at both locations (Table V). Similarly, inoculation significantly reduced kernel weight of most cultivars under all environments. At Sidi El Aydi, kernel weight of most cultivars under all environments. At Sidi El Aydi, kernel weight of most cultivars under all environments. At Sidi Al Aydi, kernel weight was significantly reduced by inoculation for durum wheats Marzak, Kyperounda and Cocorit by 14, 12, and 17% under a limited water regime and by 10, 3 and 11% under irrigation, respectively. At Tassaout, reductions in kernel weight of 12, 20, 28, 29, and 34% were recorded on Jouda, Marchouch, Kyperounda, Cocorit, and Marzak cultivars, respectively under limited water conditions. Under irrigation however, kernel weight of all other cultivars was not significantly affected except for Marzak (Table V).

Effect of root rot pathogens inoculation on kernel weight and volume has been intensively investigated in areas where it is a major problem (Sims et al., 1961; Ledingham et al., 1973; Verma et al., 1976). These workers agreed that reduction of kernel weight was a major component in yield losses due to root rot. The 1989-90 results in Morocco showed similar effects on kernel weight under all environments after inoculation. reduction of up to 34% of kernel weight was recorded on durum wheat Marzak and in general, kernel weight of durum wheat cultivars was reduced more than bread wheats. In Colorado, USA, however, there were no significant effects of ***F. acuminatum*** and ***C. sativus*** inoculation on grain volume weight of CO840186 and Vona winter wheats (Mergoum, 1991).

CONCLUSIONS

In Morocco, field studies conducted in the West Central regions in 1988-89 and 1989-90 on root rot inoculation with ***F. culmorum*** and ***C. sativus*** of Moroccan wheats showed that; (1) high root rot severity (up to 31% white heads in 1989-90) coincided with the severe dry period of February to April and seed inoculation with a spore suspension rather with infected barley seed used in 1988-89; (2) nitrogen fertilization had no effect on disease development; (3) in

1989-90, inoculation of spring wheat cultivars had significantly reduced grain yield and weight, spike number; and increased white head percentage and (4) durum wheats were, in general, more affected by rot inoculation than bread wheats for all characteristics studied (Marzak and Cocorit durum wheats were the most susceptible and common wheat Teguey-32 was the most tolerant to root rot in Moroccan field trials).

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