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Assessment of Downy mildew Resistance (*Peronospora farinosa*) in a Quinoa (*Chenopodium quinoa* Willd.) Germplasm

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***Abstract*—**Seventy-nine accessions, including two local wild species (*Chenopodium album* and *C. murale*) and several cultivated quinoa lines developed through recurrent selection in Morocco were screened for their resistance against *Peronospora farinose,* the causal agent of downy mildew disease. The method of artificial inoculation on detached healthy leaves taken from the middle stage of the plant was used. Screened accessions showed different levels of quantitative resistance to downy mildew as they were scored through the calculation of their area under disease progress curve and their two resistance components, the incubation period and the latent period. Significant differences were found between accessions regarding the three criteria (Incubation Period, Latent Period and Area Under Diseases Progress Curve). Accessions M2a and S938/1 were ranked resistant as they showed the longest Incubation Period (7 days) and Latent Period (12 days) and the lowest area under diseases progress curve (4). Therefore, M24 is the most susceptible accession as it has presented the highest area under diseases progress curve (34.5) and the shortest Incubation Period (1 day) and Latent Period (3 days). In parallel to this evaluation approach, the accession resistance was confirmed under the field conditions through natural infection by using the tree-leaf method. The high correlation found between detached leaf inoculation method and field screening under natural infection allows us to use this laboratory technique with sureness in further selection works.

***Keywords*—**Detached leaf inoculation, Downy mildew, Field screening, Quinoa.

I. Introduction

QUINOA (*Chenopodium quinoa* Willd.) is an important pseudo-cereal crop for the twenty first century. It has an excellent nutritional value and has the capacity for growth under several abiotic stresses such as drought and heat [1]-[3]. Improving quinoa productivity is a central goal to comfort the food security and the future human wellbeing. In quinoa production, downy mildew is a major constraint; it is the most widespread and potentially destructive global disease [4]-[5]. *Peronospora farinosa*, the causal agent of downy mildew disease on many *chenopodiaceous* host plants (*Atriplex*, *Beta*, *Spinacia*, *Chenopodium*) including quinoa, is an obligate parasite and is regarded as a single species [6].

The occurrence of this severe disease on economically important hosts, such as spinach and *Chenopodium*, has been reported in almost all countries where these crops are cultivated [7]. Many research carried on *Chenopodium quinoa* indicate that quinoa varieties show significant variation in their resistance to downy mildew.

Quinoa resistance against powdery mildew has been evaluated in both the field and in the laboratory. For the field screening works, quinoa is usually either artificially inoculated or, if the natural conditions are favorable, quinoa is left untreated to allow the disease developing naturally

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Reference [4] have found that the *Three-leaf* method calculated by the mean disease severity of three leaves per plant selected randomly from the lower, middle and upper part of the plant, showed the highest negative correlation to yield (r = - 0.736) and is regarded as the best method to predict yield loss caused by downy mildew. In addition, this method proved to be the quickest and easiest method to assess the disease. However, it is influenced by field condition, the microclimate humidity and the distribution of the spore hotspots across the experiment field.

Most resistance studies are generally performed in the laboratory under controlled conditions. Currently, the detached leaf technique is thought to be the most reliable for assessing resistance to downy mildew in the laboratory. This technique has the advantage to be more efficient in terms of time of execution and the number of revealed components of partial resistance, such as the incubation period (IP), the latent period (LP) and the disease intensity (DI). In addition, the inoculation on detached leaf has been used successfully in many plant-pathogen systems [8]. It is also convenient since it is carried under controlled temperature and humidity conditions and presents the advantage of having homogenous distribution of the inoculum.

The main objectives of this study were; (1) to identify some sources of resistance to downy mildew among the quinoa collection; (2) to compare between both evaluation methods as a rapid evaluation on whole plant without reference to sporulation; and (3) to determine whether the germplasm accessions show variation in their level of resistance when using the three resistance criteria.

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II. Materials and Methods

A. Plant Material and Peronospora Isolate

The 79 quinoa accessions used in this study was developed under Moroccan environmental conditions through several recurrent selection cycles from a collection introduced to Morocco in the year 2000 [9]. This germplasm includes 14 advanced breeding lines and 2 cultivars from Denmark (Puno and Titicaca).

The accessions were screened for their resistance against downy mildew by using both techniques, the artificial inoculation on detached leaves in the laboratory and the *Three-leaf screening method* under field natural infection.

The inoculum was prepared from *Peronospora farinosa* previously isolated from leaves collected in the IAV experiment plot. The spores were dislodged from the surface of the leaves in an Erlenmeyer. The water suspension thus obtained was filtered through a filter paper and diluted with tap water. The spore concentration was adjusted to 5.10^6spores ml^{-1} with a Malassez haemacytometer slide (OSI, LAB'85, Paris, France).

B. Three-Leaf Screening Method

Three-leaf screening method is based on the evaluation of the intensity of the disease symptoms in the field on three leaves at the top, the median and base of the plant using the 0-5 scale. In this method, the scored leaves are maintained on the plant stem.

C. Detached Leaf Essay

In the laboratory method of artificial inoculation, detached leaves are taken at the flowering stage before the natural infection begun.

Leaves were inoculated by spreading 0.10ml of the prepared suspension on each leaf with a sprayer. The Petri dishes with the material inoculated were immediately placed in a growth chamber at 20±2°C under 12-hour photoperiod.

Disease progress was assessed daily after the inoculation by evaluating the severity of the attack during 12 days by using the 0-5 scale (Table I).

TABLE I
Scoring Scales of Disease Intensity

Point	Description
0	No lesion
1	Small and disperse lesions with a diameter less than 1 mm without sporulation on the lower side of the leaf
2	Clearly individualized lesions increasing in number and size with a diameter between 0.5 and 1 cm without showing any sporulation on the lower side of the leaf
3	Brown lesions, covering less than 50 % of the leaf surface with a beginning of sporulation at the lower side
4	Lesions of larger size, covering more than 50 % of the leaf area
5	Lesions covering more than 90% of leaf area, with a high sporulation rating on the lower and the upper area

Each leaf was daily examined to determine (1) the incubation period (IP) defined as the time necessary for first symptom appearance, (2) the latent period (LP) which is the period before the beginning of the production of sporangia (dissemination form of *Peronospora farinosa*), (3) the disease intensity (DI) that was scored five times through the 0-5 scale at 2days, 4 days, 7 days, 9 days and 11 days. Regenerated data allowed a rapid disease assessment without considering the cycle of *Peronospora farinosa* and they were used to calculate the area under disease curve progression (AUDPC).

The AUDPC was computed for the disease scores according to the following formula (1):

$$AUDPC = \sum_{i=1}^{Ni-1} \frac{(yi+yi+1)}{2} (ti+1 - ti) \quad (1)$$

where *ti* = time (days); *yi* = disease severity at the day i; and n = number of observations.

D. Statistical Analysis

In the *Three-leaf* screening method, the experimental design was three blocks; accessions were randomly planted in a 2 m row of 0.50 m inter-row. Five measurements were taken per accession per block. Trait variation was analyzed through frequency distributions and descriptive statistics. For the detached leaf essay, the experimental design was three blocks and accession's leaves were randomly placed in Petri dishes.

The analysis of variance was performed based on a completely randomized design with one factor (the genotype) and three replicates. Multivariate analysis was carried out using the following methods: (1) the ANOVA analysis by R software [10], (2) Pearson coefficient of correlation between the variables (Clifford and Stephenson, 1975) and (3) the k-mean clustering.

III. Results

A. Symptoms

Plants showed typical downy mildew symptoms: Chlorotic lesions on the upper leaf surface and grayish spore masses on the lower side of the leaf. Microscopic examination of the lesions showed the presence of dichotomously branched sporangiophores, which tapered to a blunt point and produced ellipsoidal, light brown sporangia.

B. Component of Partial Resistance

1. Incubation Period and Latent Period

When the whole collection data was analyzed, the IP and the LP were not significantly different between accessions. Indeed, when a group of accessions that are late maturing, having a high IP and LP (M10, M17, M17bl, M17n and M214) were excluded from the analysis, the differences between the remaining accessions became highly significant.

The IP varied from 24 h in M24, S108, S114/2, W151 and S137/2 to eight days in M10, M17, M17bl, M17n and M214 (Table II).

TABLE II
Mean, Standard Deviation, Minimum and Maximum of the Incubation Period, Latent Period and AUDPC

	IP (day)	LP (day)	AUDPC
Mean±SD	3,38±1,82	7,39±2,18	22,58±7,31
Min	1	3	3
Max	8	12	37

The LP ranged from 3 days to 12 days (Table II). The two susceptible genotypes, M24 and S108, had the shortest LP of three days; the resistant accessions M2a and S938/1 took 9 days more; they show the symptoms only after 12 days.

2. Disease Progression and AUDPC Calculation

The analysis of the variance showed that the difference within the germplasm collection using the AUDPC values were highly significant (P=0.001).

Fig. 1 shows that differences between accessions for the disease intensity became highly significant on day 9 after inoculation. The germplasm was significantly separated into five groups describing each level of resistance. Therefore, the highly sensitive accessions grouped in the group 5 and the highly resistant accessions grouped in the group 1 were significantly separated from the other accessions during the entire infection period (Table III).

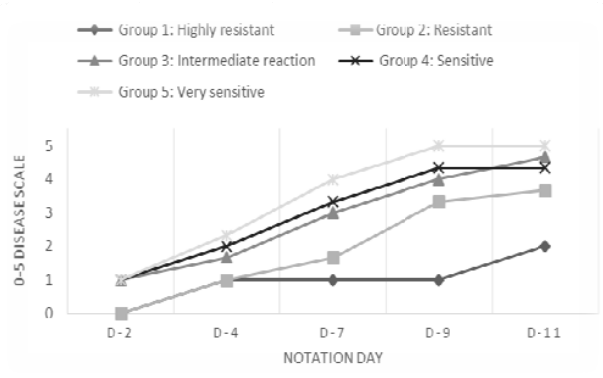

Fig. 1 Disease progression from the second day after the artificial inoculation until the day

TABLE III
GROUPS BASED ON K-MEAN CLUSTERING USING THE AUDPC VALUE

Group name	Mean	AUDPC Interval	Example of accessions names
Group 1: Highly resistant	11,04	5,50 - 14,83	S114/5, PUNO, S314/2, M18
Group 2: Resistant	19,26	18,50 - 20,67	S66/1, S66/2, W150, W6/2, W142/2, W6/1, S137/1, M138/2, M143/2J, M143/2r, S318/2
Group 3: Intermediate reaction	23,08	22,00 - 24,00	S119b/1, S314/1, W119/2, S122, M143, W5/2, W6/3, W11/1
Group 4: Sensitive	27,41	25,50 - 28,83	W4/2, TITICACA, W3/2, M138/1, W119/1, W151, W5/1, S114/4, M1, W12, S11
Group 5: Very sensitive	31,72	30,00 - 33,00	S137/2, S310, W143/2, S100, W16, S108, M24

C. Correlation

Table IV shows the Pearson correlation coefficients between the components of partial resistance. The IP and LP were not correlated with any other component. This was mainly due to the behavior of M17, M17n, M17bl, M214 and M10 that is a relatively an extreme expression of resistance to this isolate. Indeed, when these accessions were excluded from the analysis, negative correlation coefficients shows up between IP and AUDPC (0.76***) and between LP and AUDPC (r = -0.87***).

The highly significant negative correlation between the “IP and AUPDC” and the “LP and AUPDC” shows that accessions having a long IP and LP are more resistant.

Significant positive correlation was observed between the AUDPC and the disease intensity evaluated by the three leaves method (0.28**).

TABLE IV
CORRELATION TABLE

	AUDPC	IP	LP	DI	Leaf1	Leaf2
IP	-0.75***					
LP	-0.87***	0.61***				
DI	0.28**	-0,10	-0.33***			
Leaf1	0.39***	-0.25**	-0.37***	0.68***		
Leaf2	0,15	-0,00	-0.21*	0.89***	0.48***	
Leaf3	0.23**	-0,07	-0.28**	0.87***	0.39***	0.66***

IV. DISCUSSIONS

Quinoa is an invaluable crop, highlighted by the Food and Agriculture Organization of the United Nations (FAO) as one of the world's main crops for future food security. Downy mildew was the main disease that shows up in most of the quinoa fields at the different climatic regions.

The present study analyzed three of *in vitro* epidemiological parameters that characterize partial resistance in plant-pathosystems. The *Chenopodium quinoa* Willd -*Peronospora farinosa* pathosystems epidemiological parameters were analyzed to characterize the partial resistance of *Chenopodium quinoa* accessions against *Peronospora farinosa*.

In the hours immediately following contact between the pathogen and the leaves, the incubation period (IP) represents a crucial criterion for evaluating the plant resistance; it could be prioritize, before the latent period.

Spores of *Peronospora farinosa* colonize quinoa tissue soon after penetration. Differences in the incubation period between accessions are probably due to the host plant early resistance to fungal penetration. Resistance in plant may take the form of a reduction in the number of appressoria, or in the number and the size of germinating tubes, or even in the mass of hyphae, as described on oat varieties infected with *Erysiphe graminis* [11].

The AUDPC is another pertinent criterion that represents the speed by which the pathogen progress in the plant tissues; AUDPC allows differentiating with trust between resistant and susceptible genotypes [12]. Pathogen progression is generally slower in the resistant genotypes which usually delay and limit (IP, LP) or ban pathogen colonization and the disease symptoms expansion.

Accessions M2a and S938/1 were ranked resistant as they showed both the longest IP (7 days) and LP (12 days) and the

lowest AUDPC (9). Accession M24 as the most susceptible accession, presented the shortest IP (1 day) and LP (3 days) and the highest AUDPC (34.5).

The AUPDC significant variation indicates that the accessions have different level of tolerance and reaction to the *P. farinosa* infection; some accessions allow fast fructification of the pathogen, others compete against its proliferation. This variation in the reaction to the pathogen suggests that there are intrinsic factors involved in the tolerance mechanism. Quick disease spreading is generally proper to short latency period and negatively correlation to plant tolerance.

Accession S306/2 has presented the lowest AUDPC, the shortest IP and LP; it has the fastest fructification, however the expansion of the infection on the leaves remained limited to small lesions. This type of reaction called hypersensitivity induces the nearest cells deaths that ban the pathogen dissemination in the tissues [13].

It is evident that M2a, S938/1 and S114/2, prevent the apparition of the first spots and diminish the rate of the disease dissemination, delay their IP and reduce the AUDPC. However, LP did not seem to be a reliable parameter by itself for disease resistance screening. For example, resistant accessions M143/2B and M18 had the same LP (6 days) to the susceptible ones.

This study revealed an extensive diversity within the quinoa collection. The evaluation of the response to downy mildew diversity through univariate and multivariate analysis pointed out the most performing genotypes. Accessions belonging to the same cluster and having desirable traits (genes) could be crossed to accessions from other clusters emanating from other studies to gather favorable genes in one elite variety.

The detached leaves screening technique is an appropriate tool for analyzing the partial resistance components to *P. farinosa* in quinoa. It allows evaluating the plant resistance without environmental effects including the interaction between microorganisms and the heterogeneity of pathogen density.

V. Conclusion

Quinoa (*Chenopodium quinoa* Willd) is exposed to several diseases and pests with different intensity depending on the environment. Downy mildew caused by *Peronospora farinosa*, is probably the most widespread disease in the quinoa fields; injure mainly the foliage and can affect significantly the yield.

Detached leaves technique, besides showing genotypic variability in the resistance to downy mildew ; it has allowed analyzing a large number of accessions and repeating the test as many times as there were plant leaves available.

Quinoa breeders over the world should add the resistance to downy mildew (*Peronospora farinosa)* to their priority beside the tolerance of the abiotic stresses like salinity and drought. So, more studies should be done on the pathogen and its life cycle and also the way it penetrate the leaf so as to develop new cultivars that resist the damage caused by the pathogen.

The detached leaves screening technique provides the quinoa breeders community with highly informative parameters and allowed them to establish a diversified quinoa germplasm and to enhance breeding program efficiency.

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