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Resistance to *Leveillula Taurica* in Crosses Involving Resistant and Susceptible Pepper Lines (*Capsicum Annuum L.*)

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ABSTRACT

Leveillula taurica (Lév.) Arn., is one of the plant pathogens that infect many plant species. L. taurica is the only known cause of powdery mildew in pepper. Epidemiology of the disease is highly affected by the climatic conditions, on pepper, disease occurs in both humid and dry regions. Crosses between resistant doubled haploid lines provided by the pepper-breeding laboratory (INRA, Montfavet, France).and susceptible Sudanese hot pepper landraces obtained from the gene bank at the Agricultural Research Corporation (Wad Madani, Sudan). The F1's and F2's together, with the parental lines, were evaluated in open fields under natural conditions of infection for two seasons. Two susceptibility indices, sporulation intensity (Sp) and proportion of infection (Pr) were used to evaluate each plant. A general disease index (DI) was obtained by summing up the two indices: DI=(Sp+Pr) in a range of 0-10. The two susceptible parents were diseased, with mean disease index (DI) 6.6 and 6.3 respectively. While the resistant parents were completely resistant (DI=0). The F1's of the three parents were intermediate in their resistance with mean disease index (DI=1.9- 2.3) the distribution of the F2 individuals in DI classes Showed that most of the plants were distributed in the intermediate class (DI=5) and towards the higher intermediate resistance classes (DI=4, 3 and 2).

A linear relationship between the two susceptible indices Pr and Sp was observed in the susceptible parents (r=0.9) and in the F1's where the correlation coefficient is in the range of 0.8 -0.7. In the F2's this linearity is variable due to the low sporulation on the high level of resistance and those of intermediate level plants. The behavior of the F1's and F2's revealed that the resistance of HV lines to Leveillula taurica is controlled by several genes' of partial effects.

Key words: *Pepper, Capsicum Annuum, Powdery Mildew, Resistance*

Introduction

Leveillula taurica (Lév.) Arn., is one of the plant pathogens that infect many plant species. It is known to infect at least 750 species in 290 genera and 50 families [1]. *L. taurica* is the only known cause of powdery mildew in pepper. The wide host range includes other economically important vegetables, industrial and field crops like tomato, artichoke, cotton, onion, eggplant and potato [2,3]. The disease is prevailing in the Mediterranean region as well as in inter-tropical regions [4-7]. Losses of 30%-40% in pepper production were reported, in addition to losses in quality of the produce due to the sunscald that result from leaf shedding [8,9].

The fungus is not highly specialized and cross-infection can occur between different hosts. In pepper, the first symptoms of the disease are yellow blotches or spots that may become necrotic with time progress. On the older leaves, when lesions are numerous, they may coalesce, resulting in a general chlorosis of the leaves. On the lower leaf surface, the lesions develop a necrotic flecking. The white sporulation showing abundant production of conidia, are generally visible on the lower surface of the infected leaves. The disease progresses from the older to the younger leaves and the abundant to total shedding of the infected leaves is the ultimate symptom [10-14]. However, shedding of symptomless leaves is also possible due to the hormonal imbalance induced by the fungus [15]. In highly susceptible plants, whitish

sporulation is visible on the upper surface of the infected leaves [16]. Contrarily to other powdery mildews, *L. taurica* is an endoparasite, the mycelium grows intercellularly within the host tissue [17-19]. Conidiophores emerge from the stomata and produce cylindrical or egg-shaped conidia 30-80 µ long. The lanceolate shape of the distal conidia is another specific characteristic of *L. taurica*.

Epidemiology of the disease is highly affected by the climatic conditions; several authors have described different environmental conditions favoring the disease epidemics [20]. On pepper, disease occurs in both humid and dry regions. In Mediterranean region, the optimum natural conditions for maximum infection are 85-100% R.H. and 18-25°C. However, similar results were reported in the inter-tropics. Forecasting the disease epidemiology is difficult due to the continuous availability of the inoculum and the possibility of mutual cross infection. Chemical control, if possible, is economically as well as technically difficult to practice and may present some risks for health and environment. This encourages the exploitation of the genetic resistance to control the disease [21].

Various levels of resistance to powdery mildew in natural infection conditions were reported in capsicum species [22-24]. In *capsicum annuum* several sources of resistance were also characterized Two *C.annuum* accessions PI 372719 'PM 687' and 'PM 807' (H3) were reported as promising donors (Ref Newsletter). However, the resistance from PM 687 was associated with undesirable characters like poor fruit set and blossom end rot but that of PM 807 was shown to be independent of such traits [25]. PM 687 which was reported to have partial resistance in Mediterranean region, was found completely resistant during heavy inoculum pressure in Sudan [26]. Resistance to powdery mildew is incomplete and oligogenically controlled however, it was stable in several infected regions. Daubeze et al., (1995a) reported that the number of genetic factors involved in resistance depends on the infection conditions: two or three genetic factors with additive and partial dominance effects were sufficient to confer resistance at the beginning of the epidemic or under weak infection conditions. However, in severe infection conditions, five to nine factors are necessary to slow the disease progress and secondary infections so that the resistance is considered polygenic and recessive.

This genetic study was conducted in a doubled haploid progeny. The most resistant lines from the progeny were used as donor parents to introduce the resistance into Sudanese varieties and to look further for the stability of the resistance under tropical conditions in the Sudan

Material and Methods

In an attempt to construct a breeding population breed pepper variety to be cultivated in Sudan, Crosses between resistant and susceptible parents were performed.

HV77, HV81 and HV59 are doubled haploid lines resistant to powdery mildew provided by the pepper-breeding laboratory (INRA, Montfavet, France). This resistance originated from PM 807.

HSD 1060 and HSD 1102 were Sudanese hot pepper landraces obtained from the gene bank at the Agricultural Research Corporation (Wad Madani, Sudan) susceptible to powdery mildew.

Each of the susceptible parents HSD 1060 and HSD 1102 was crossed to the three resistant parents. The F1's plants were self-pollinated to obtain F2's.

Crossing and self-pollination were done in glasshouse compartments at INRA, Montfavet, France. The HSD lines were used as susceptible control whereas HV77, HV81 and HV59 were used as resistant control.

The F1's and F2's together, with the parental lines, were evaluated in open fields under natural conditions of infection.

The experiment was conducted in the Sudan for two seasons. Seedlings were raised in the nursery and then transferred to the field in December during the winter season and evaluation was performed at the end of the season, on 12 April.

Two susceptibility indices, sporulation intensity (Sp) and proportion of infection (Pr) were used to evaluate each plant according to Daubeze *et al*., (1995a) (Table 1).

A general disease index (DI) was obtained by summing up the two indices: DI=(Sp+Pr) in a range of 0-10.

The selected F2 plants of high level of resistance were self-pollinated and their progenies were randomly mated to pyramid the resistance genes using Pollen Mixture Technique.

Pollen Mixture Technique

Equal number of fully mature flower buds that were expected to open the next day were chosen in the afternoon from each plant. Those buds should be plumb and white in color. Anthers were collected in a glass container using a

Table 1: A Semi Quantitative Scale For Evaluating Susceptibility To Powdery Mildew.

Index	Sporulation Intensity (Sp)	Proportion of infection (Pr)
0	No visible sporulation	no leaves infected
1	restricted chlorotic spots with weak or no sporulation	lower leaves infected (less than 20% of the leaves)
2	several sporulation sites covering less than 25% of the lower leaf area	from 20% to 40% of the leaves infected
3	numerous sporulation sites covering up to 50% of the lower leaf area	3= from 40% to 60% of the leaves infected
4	numerous coalescent sporulation sites covering up to 75% of the lower leaf area	from 60% to 80% of the leaves infected
5	the whole surface of the leaf covered with dense sporulation	The whole foliage, including the young leaves at the top of the plant are infected

forceps. Then the anthers were left to dry overnight in a desiccator over a layer of calcium chloride. The pollen was extracted by gentle crushing of the anthers in a small plastic mesh on glass board. The pollen was collected on a piece of cotton fixed on a small tube in the pollination bottle with a thin layer of water at the bottom of the bottle.

Crossing and Self-pollination Methods

The necessary tools include, a bottle of 95% ethanol, sharp pointed forceps, spear needle, adhesive transparent strap and small tags. While crossing or self-pollination can be done at any time during the daylight hours, the best times are those at the early morning for crossing and self-pollination and late in the afternoon for pollen collection, when the flowers are in the mature bud stage. Anthers of the flowers chosen for crossing were removed carefully with the forceps. Several flowers on the same plant were usually emasculated and prepared for pollination at the same time to speed up the process. The pollen was gently transferred to the stigma either from mature indehiscent anther by scooping the pollen out through the lateral sutures with the spear needle, or it was done by touching a freshly dehisced anther to the stigma with the forceps. The flower was then covered with an adhesive transparent strap rolled up in a cylindrical shape and gently stuck to the calyx surface and closed. Parentage was indicated on the small tag, and was loosely tied to the delicate pedicel. Hands and tools were disinfected in-between different crosses and self-pollination operations. Self-pollination was done by closing up the mature flower bud with an adhesive transparent strap before the flower opened.

Result and Discussion

Microscopic observations carried out in the laboratory confirmed that the causal agent of the powdery mildew epidemic was *Leveillula taurica* .The two susceptible parents, HSD 1102 and 1060 were diseased, with mean disease index (DI) 6.6 and 6.3 respectively. While the resistant parents HV77, HV81 and HV59 were completely resistant (DI=0). The F1's of the three parents were intermediate in their resistance with mean disease index (DI=1.9 to 2.3) (Table 2).

As shown in figure 1 the distribution of the F2 individuals in DI classes showed that most of the plants were distributed in the intermediate class (DI=5) and towards the higher intermediate resistance classes (DI=4, 3 and 2). A linear relationship between the two susceptible indices Pr and Sp was observed in the susceptible parents (r=0.9) and in the F1's where the correlation coefficient is in the range of 0.8-0.7. In the F2's this linearity is variable due to the low sporulation on the high level of resistance and those of intermediate level plants. The behavior of the F1's and F2's revealed that the resistance of HV lines to *Leveillula taurica* is controlled by several genes' of partial effects. (Figures 1,2).

Natural epidemics of *Leveillula taurica* vary in severity from year to the other and in locations. In the Sudan, the cool months of the year December-March are favoring the disease development. One evaluation at the end of the season was reported to be useful for screening in the field, since it allows for maximum disease pressure reported Seven genomic regions including additive QTLs and epistatic interactions in doubled-haploid progeny from the cross 'H3' (resistant) by 'Vania' (susceptible) tested for resistance under both natural field infection and artificial inoculation tests they reported Two genomic regions were common to both the evaluation methods, whereas other QTLs were method-specific, reflecting the environment dependence of powdery mildew epidemics [27]. However, reported a single dominant locus, PMR1, which mapped to chromosome 4 of the pepper genome in two independent sources of powdery mildew resistance, *C. annuum* 'VK515R' and 'PM Singang' [28].

Difference in aggressiveness of *Leveillula taurica* isolates was also reported [29]. However, most and least aggressive isolates were collected in the same region where this experiment conducted. The behavior of HV59, HV77 and HV81 lines in Sudan, is similar to that in Montfavet (France) (DI=0) under natural conditions of infection (Daubeze, unpublished data).

Table 2: Showing Mean Disease Index.

	Mean Pro. Of Infection	Mean Int.Of Sporul.	Mean Diease Index
HSD 1060	3.460.52	2.85±0.99	6.31±1.25
HSD 1102	3.31±0.52	2.76±0.67	6.60±0.97
HV 77	0.00	0.00	0.00
HV 81	0.00	0.00	0.00
HV 59	0.00	0.00	0.00
F1 (1102XHV77)	1.300.95	0.80±0.42	2.10±1.29
F1 (1102XHV81)	1.3±0.76	1.00±0.58	2.29±1.25
F1 (1102XHV59)	0.900.57	0.80±0.42	1.70±0.95
F1 (1060XHV77)	1.13±0.83	0.75±0.46	1.88±1.25
F1 (1060XHV59)	1.130.99	0.75±0.46	1.88±1.36
F2 (1102XHV77)	2.63±1.58	1.32±0.96	3.95±2.34
F2 (1102XHV81)	2.671.37	1.25±0.67	3.92±1.77
F2 (1102XHV59)	2.87±1.30	1.33±0.77	4.20±1.79
F2 (1060XHV77)	2.741.47	1.16±0.76	3.90±1.99
F2 (1060XHV81)	*3.03*±1.23	1.13±0.59	4.16±1.52
F2 (1060XHV81)	3.201.19	1.22±0.58	4.42±1.44

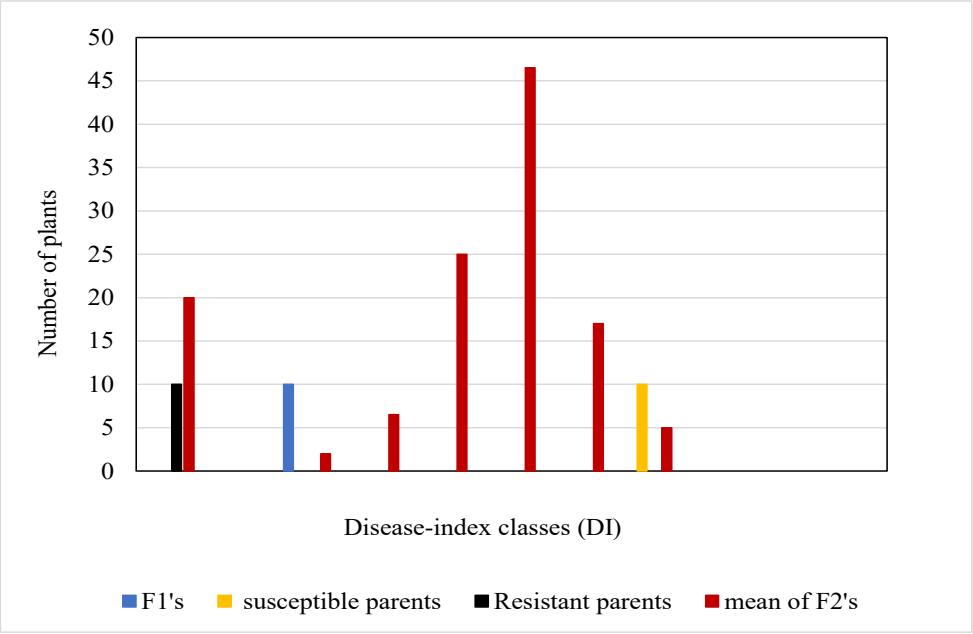

Figure 1: Distribution of the F2, F1 individuals in susceptibility classes (DI) (mean of the six F2's) to Leveillula taurica with mean class of susceptible and resistant parents.

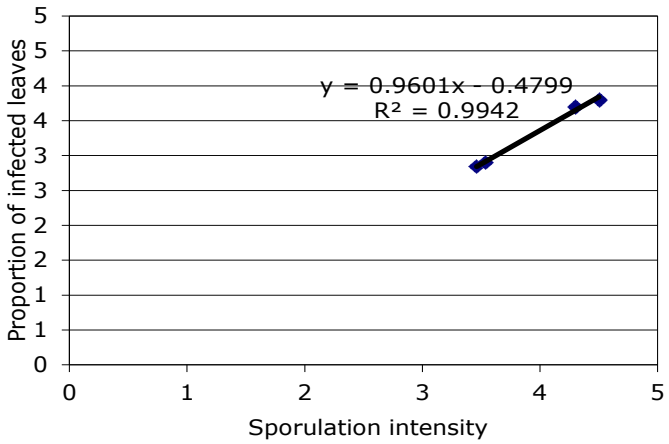

Figure 2: Relationship between mean sporulation intensity(Sp) and proportion of infected leaves (Pr) for the susceptible parents.

Conclusion

The stability of the resistance in these sources in Sudan is evident, since F2 plants with high level of resistance could be recovered. Thus, the exploitation of powdery mildew resistance from the HV lines is possible. Thus, the F2 plants with high level of resistance were selected and self-pollinated and their progenies were randomly mated to pyramid the resistance genes using the pollen mixture technique as C0 cycle for the recurrent selection program to breed resistant hot pepper varieties.

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