Characterization of Powdery Mildew of Snake Melon (Cucumis melo var. Flexuosus L.) And Evaluation of Some Inbred Lines of Snake Melon for Powdery Mildew Resistance

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INTRODUCTION

Snake melon (Cucumis melovar. flexuosus L.) is among the desirable melon groups in the Sudan, it is consumed locally as green salad or pickles. The crop is being affected by major biotic and abiotic stresses which have laid to drastic reduction in yield. One of the major biotic stress is powdery mildew. The disease can reduce yield by decreasing fruit size, number of fruits. Cucurbit powdery mildew is most frequently caused by two obligate fungal pathogens, Podosphaera xanthii and Golovinomyces cichoracearum. The most commonly identified pathogen; particularly in warmer production regions has been P. xanthii, therefore the study was carried out to identify the causal agent of the disease, to screen some inbred lines of snake melon (Cucumis melo var. flexuosus) for resistance to powdery mildew and to determine the physiological races under local field. Nine landraces of snake melon were used, they were sampled for incidence and severity of the disease under studied area. Disease incidence was determined by using rate and severity of the disease by using severity scale of 1- 5. A Complete Randomized Block Design (CRBD), with three replications was used. Although determination of the physiological races by the used differential melon cultivars growing in the field was carried out. Pathogen identification was done microscopy according to morphological characters of conidium, conidiophores, conidia germination, presence or absence of fibrosin bodies and the perfect stage (chasmothecia) of the fungus. Field trial revealed that, incidence ranged from (50.0–80.0%) on leaves. The severity was (2.0–4.8) moderate–very severe infection (40.0–96.0%) on leaves. Significant differences (P= 0.05) were observed in disease severity. The causal agent of powdery mildew disease of snake melon (Cucumis melo var. flexuosus) was conclusively identified as Podosphaera xanthii, microscopic observations of all tested samples revealed hyaline conidia, ellipsoid to ovoid in shape, with fibrosin bodies, also the chasmothecia were exist in a high number, which contained only one ascus with 3-8 ascospores, hayline appendages and globose. At least, the 9 local cultigenes tested they are susceptible to races 1 and 2US of Px under field conditions.

1. INTRODUCTION

The snake melon (Cucumis melo subsp. melo L. var. flexuosus (L.), family Cucurbitaceae) is one of the ancient horticultural crops in many parts in the world including the Middle East and Middle Asia Walters and Thieret, (1993). The crop is among the desirable melon groups in the Sudan, it is consumed locally as green salad, pickles. The yield of the snake melon crop in Sudan has an average of 20 ton/ha Mirghani and El Tahir, (1997). Pests and diseases cause great losses to melon crops around the world. Among the important fungal diseases are Fusarium wilt and powdery mildew. In Sudan powdery mildew is one of the most important limiting production factors on snake melon specifically during winter season (Mohamed et al. 1995). The disease symptoms appear on leaves, petioles, and young stems as white powdery colonies composed of mycelium and countless numbers of spores Agrios, (1997). Two different fungi commonly cause powdery mildew on cultivated cucurbits: Podosphaera xanthii (Castagne) Braun & Shishkoff [syn. Sphaerotheca fuliginea auct. p.p.] and Golovinomyces cichoracearum s.l. (D.C.) V.P.Heluta [syn. Erysiphe cichoracearum auct. p.p] Jahn et al. (2002). The causal agents of cucurbit powdery mildew disease produce identical symptoms and can be difficult to differentiate in the absence of the perfect (sexual or teleomorphic) stage which can be distinguished by the number of asci, ascospores and appendages and presence of the chasmothecia (Block and Reitsma, 2005). Podosphaera xanthii is known to occur in several physiological races. Presently, there are at least seven pathogenically distinct races of P. xanthii and two races of G. cichoracearum (Pitrat et al. 1989). Recently, variability within races 1 and 2 in P. xanthii populations was described using 32 melon cultivens with the potential for the existence of 28 races that include eight variants of race 1 and six variants of race 2 McCreight, (2006). In Sudan, both species are present, races 0, 1 and 2 of P. xanthii were detected as causing powdery mildew on melon Mohamed et al. (1995), Ahmed et al. (2000). This study undertook the following research objectives to:

1-Screen some lines of snake melon bred at NIPHE for resistance to powdery mildew.

2-Characterization of the causal agent of the disease.
3. Determine the presence of physiological races under the field conditions.

2. MATERIALS AND METHODS

2.1. Description of experiment

The present investigations were carried out under field conditions at the research farm of the University of Gezira-Wad Medani-Sudan (Latitude 14°06', N, Longitude 33°38', E and Altitude 400 m asl), during winter season of 2012/2013 and laboratory investigations at Department of Plant Pathology, College of Agriculture Sciences, University of Gezira. The experiment was setup in a randomization complete block design with three replications (RCBD). The normal agronomic practices (e.g. soil preparation, irrigation, fertilization) were done as recommended. The seeds were sown directly and the necessary thinning was done later.

2.2. Experiment materials

The plant materials used during the study were:

1. The inbred lines
   A total of 9 inbred lines of snake melon (C. melon var. flexuous), developed at the National Institute for Promotion of Horticultural Exports (NIPHE) were subjected to many cycles of inbreeding and selection prior to use in this study for powdery mildew resistances. These were:
   1- Line 4, 2- Multi RB, 3- Multi RA, 4- PI164723 x silka, 5- Line 4 x silka, 6- Silka, 7- Bitter (resulted from a three waycross between PI 414723, 90825 and silka at F3BC6, 8- Farm and 9- F3

2. Differential melon varieties
   Seven differential lines were developed at the National Institute for Agronomy (INRA), France, donated by Dr. Michel Pitrat, (1990). These varieties were Vedrantais, PMR

2.3. Experimental Procedures

Prior to sowing the land was disk, ploughed, harrowed and then divided into 5 plots (6 x 3 m²), seeds were planted in date of 21/11/2012 during winter season of 2012/2013, 6 hills/plot side and 3 seeds/hill, with 50 cm spacing between hills. Plants were irrigated at intervals of 5-7 days, then the interval was extend to 10 days after the first month. Nitrogen fertilizer was applied at a rate of 40 Kg/fedan. Hand weeding was done. The experiment was laid out in randomization complete block design (RCBD) with three replication. Differential lines were included for race identification 3 plants per line. The plants were exposed to naturally occurring powdery mildew inoculum. Incidence of the disease was recorded at weekly interval after appearance of the disease toward the end of the season. The severity of powdery mildew was scored on five randomly selected plants / plot at the last three weeks toward the end of the season by using the rating scale which run from 1-5 developed by Ishii et al., (2001), where 1= 1-5= 0%, 2 = 6 - 25%, 3 = 26 - 50%, 4 = 51 -75% and 5 = >75%. The observations on percent of disease index (PDI) were recorded.

Quantifying Disease
Percent Disease Index (PDI)

The quantity of the disease present was assessed from each line in the field by means of disease intensity, which was measured using severity and incidence. The PDI was calculated with the above scales using the formula given by Wheeler (1969).

Percent Disease Index

\[
PDI = \frac{\text{Sum of numerical values}}{\text{Number of leaves observed}} \times \frac{100}{\text{Maximum disease rating}}
\]

Disease Intensity (x) : Can be Incidence or Severity
Disease Incidence (In) = # of plant units infected as a % of all plant units. Proportion of plant units diseased+ or –, each unit is diseased or not. Expressed as a % of all plant units with disease e.g. 25% incidence of disease on leaves means ¼ of leaves show symptoms at any level.

Number of infected plants

Incidence % = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100

Disease Severity (Se) = area of plant tissue affected as % of total area. Proportional area of plant unit diseased. Expressed as a % of total tissue area e.g. 25% severity of disease on leaves means ¼ of all leaf tissue shows symptoms.

Size of diseased leaf area

Severity% = \frac{\text{Size of diseased leaf area}}{\text{Total leaf area}} \times 100
Table 1: Rating scale of disease severity

<table>
<thead>
<tr>
<th>Class</th>
<th>Rate</th>
<th>Reaction type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-5  = 0% infection</td>
<td>No infection</td>
</tr>
<tr>
<td>2</td>
<td>6-25 % infection</td>
<td>Mild infection</td>
</tr>
<tr>
<td>3</td>
<td>26-50 % infection</td>
<td>Moderate infection</td>
</tr>
<tr>
<td>4</td>
<td>51-75 % infection</td>
<td>Severe infection</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 75 % infection</td>
<td>Very severe infection</td>
</tr>
</tbody>
</table>

Source: Ishii et al. (2001).

DATA ANALYSIS

The Statistical analysis were carried out using the analysis of variances (ANOVA). Data were analyzed with M-STAT software package. Disease incidence and severity on leaves were measured, followed by Duncan's New Multiple Range Test (DMRT) Steel and Torrie, (1960), to test the significance difference between means. The coefficient of variation was calculated based on the formula suggested by Burton (1952).

\[ CV\% = \sqrt{\text{MSE} / \text{Y}} \]

LABORATORY IDENTIFICATION OF THE SPECIES OF PM

To determine powdery mildew causal agent several laboratory tests were conducted. These were:

1. Morphological examination

Leaf samples were collected from melon (Cucumis melo var. flexuous, the identification of the fungal species associated with powdery mildew was based on the characteristics of the conidia. To verify the shape of the conidia, microscope slides were prepared. One sample was used for each line and examined under the microscope, at 40x magnification.

Conidia with parallel lateral walls and round borders were considered cylindrical, and those with some thinning at the edges were considered ovoid.

2. KOH test

To confirm the presence of fibrosin bodies and their morphology, the conidia from the pustules on each leaf were transferred to one drop, approximately 50 ml of 3% potassium hydroxide solution on a microscope slide, covered with a cover glass and observed under an optical microscope, at 40x magnification after 30 second, for detection of presence or absence of fibrosin bodies, which differentiate between Oidium types of powdery mildew fungi.

3. Conidiophores shape test

Inoculum of powdery mildew was placed on slide and examined under the compound microscopeto observe the conidiophores shape and the spores number along the chain.
4. Sexual stage test

Collect leaves with mature (black) chasmothecia on plants with powdery mildew at the end of the season. The chasmothecia should be visible with eyes, although a hand lens may help. Leaves with abundant chasmothecia were pressed and dried in newspaper and stored in classroom for use throughout the year. Chasmothecia were removed by gently scraping the surface with a moistened, single-edge razor blade, over a white sheet of paper, and transferred it to the water drop on the slide and examined under the microscope. Dried leaves also were observed under binocular, chasmothecia were removed by gently scraping the surface with a moistened, needle, and transferred it to the water drop on the slide and examined under the microscope.

3. RESULTS

Symptomatology

The most common symptoms were found on the lower side of mature leaves as brown sites, which were covered with whitish growth. In severe cases, whitish mass spread over on upper surface of leaf also. In our observation in this study symptoms ranged from little sporulation to 96% coverage of mycelia on plant samples. The diseased leaves curl causing defoliation of heavily infected leaves. The disease later was found on younger leaves, leading to total defoliation. Severely affected plants were completely defoliated. Such defoliation and the presence of numerous shed leaves on the ground, was the most prominent sign of attack, which cannot be missed. In our study the diseased leaves of highly susceptible lines silka and bitter had total disease severity rating of > 4 which means at least 96% mycelium coverage on leaves. Also our observation showed that stem of the two above lines and line 4 were infected; these lines had total stem disease severity rating of > 2 and 50% mycelium coverage on stem (Plate.1). The mean percentage purity is 100.01% and 100.07% for BDT and ND respectively. The percentage deviation was found to be -1.0 to +1.0% and -0.6 to +0.7, for BDT and ND respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

Plate 1. Symptoms on leaves and stem.

Disease evaluation in the field

Overall powdery mildew incidence in tested lines, on infected leaves was (69%), severity (70%) and (69.5%) percentage disease index. Severity was relatively equal when compared with incidence. The lowest incidence was recorded of PI164723 x silka and Farm (50%) respectively, those lines were less susceptible, (40% severity), than others lines that having severe and very severe infection (69 and 96% severity) respectively. The moderate incidence and severity was recorded on line 4, multi RA, multi RB, line 4 x silka and F3 (67, 70, 74, 74% incidence and 69% severity). On the other hand, both silka and bitter recorded the highest disease intensity, incidence and severity of (80 and 96%) respectively (Table 2). The severity of the disease among inbred lines showed different levels, none was found to be immune. The highest severity was found on silka and bitter, that having disease severity of (4.8%) and classified as very severe infection, while line 4, line 4 x silka, F3, multi RB, and multi RA, showed severe infection having disease severity of (3-4%), farm and PI164723 x silka, showed moderate infection, having disease severity of (2-3%). The ANOVA analysis revealed significant (P = 0.01 and 0.05) differences of disease severity among the nine snake melon inbred lines. Of the 9 lines, 2 lines (22.2%) were moderately resistant to powdery mildew, 5 lines (55.6%) were susceptible, and 2 lines (22.2%) were highly susceptible (Table 2). Disease severity was also showed low values of CV% (11.4%) and ES ± (0.23).
Table 2: Disease intensity. Incidence and severity (%) per weeks and reaction type on inbred lines during winter season of 2012/013

<table>
<thead>
<tr>
<th>Variety</th>
<th>Disease Intensity</th>
<th>Disease Index %</th>
<th>Reaction type</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Incidence % Mean</td>
<td>Severity % Mean</td>
<td></td>
</tr>
<tr>
<td>PI164723 x silka</td>
<td>50.0 2.0= (40%)</td>
<td>45.0</td>
<td>Moderate infection</td>
</tr>
<tr>
<td>Farm</td>
<td>50.0 2.0= (40%)</td>
<td>45.0</td>
<td>Moderate infection</td>
</tr>
<tr>
<td>Line 4 x silka</td>
<td>67.0 3.0= (60%)</td>
<td>63.5</td>
<td>Severe infection</td>
</tr>
<tr>
<td>Multi RA</td>
<td>70.0 3.3= (66%)</td>
<td>68.0</td>
<td>Severe infection</td>
</tr>
<tr>
<td>Multi RB</td>
<td>74.0 3.7= (73%)</td>
<td>73.5</td>
<td>Severe infection</td>
</tr>
<tr>
<td>Line 4</td>
<td>74.0 3.7= (73%)</td>
<td>73.5</td>
<td>Severe infection</td>
</tr>
<tr>
<td>Silka</td>
<td>74.0 3.7= (73%)</td>
<td>73.5</td>
<td>Severe infection</td>
</tr>
<tr>
<td>F3</td>
<td>74.0 3.7= (73%)</td>
<td>73.5</td>
<td>Severe infection</td>
</tr>
<tr>
<td>Bitter</td>
<td>80.0 4.8= (96%)</td>
<td>88.0</td>
<td>Very severe infection</td>
</tr>
</tbody>
</table>

CV% = 11.4% SE ± 0.23

Race identification

From the observation in the field, Golovinomyces cichoracearum and race 0 of Podosphaera xanthii were not considered to be present, since vedrantais was diseased, and PI 414723 and PI124112 were free from infection till the end of the season, vedrantais resistant to P. xanthii race 0, both PI 414723 and PI124112 were free from races 0 and 1 of G. cichoracearum (PMR= 0), thus leading to conclude that, these strains of Px were race 1. Also the laboratory test of samples collected from the field confirmed P. xanthii as the causal agent. Px race 2 in this experiment was absence at the beginning of the season, since PMR 45 was free from infection till the end of evaluation. Also the resistance of PI 414723 decrease the presence of race 2US, that is response for the outbreak of the disease. In our study at the end of the season a trace infection was appeared on PMR 45 (late infection) as yellow spots (Plate 2), this exploded that, race 2 may be arrived later, since race 1 arrived first it is possible to know the presence or absence of race 2, because there are many differential genotypes are resistant to race 1 and susceptible to race 2. At least the 9 local cultigenes tested they are susceptible to at least races 1 and 2US of Px (Plate 2 and Table 5).

Plate 2. Yellowing spots on PMR 45
Table 5: Observed reaction of differential hosts to infection of powdery mildew during winter season of 2012-013 in the field

<table>
<thead>
<tr>
<th>Season</th>
<th>Vedrantais</th>
<th>PMR45</th>
<th>PMR 5</th>
<th>WMR 29</th>
<th>MP-1</th>
<th>PI414723</th>
<th>EDIS.47</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012-013</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

R=resistant, S=susceptible

Laboratory identification

For all samples examined the conidia have ovoid shape. The conidiophores were of oidiunm type, unbranched, bearing conidia in chain 3-6. Fibrosin bodies were present in the interior of the conidia after using one drop of KOH solution, presence of fibrosin bodies clearly differentiated P. xanthii from G. cichoracearum, the former have fibrosin bodies. Also the chasmothecia were exist in a high number on more susceptible cultivars (silka and bitter), which contained only one ascus with 3-8 ascospores, unforked and hayline appendages. It was globose, white in color when immature and then brown and black when mature. The chasmothecium is generally spherical with no natural opening; asci with ascospores are released when a crack develops in the wall of the fruiting body. This type of fruiting body is unique among the Ascomycota. A variety of appendages may occur on the surface of the chasmothecia. Also in our study the two above lines exhibited white colonies containing chasmothecia, that covered the stem, this is related to a bigger incidence and severity of powdery mildew at the end of the season. In this study, only fungal structures corresponding to P. xanthii were found, but not those of G. cichoracearum. Among differential melon lines, the conidia obtained from vedrantais and PMR 45 had ovoid shape. At least all these characteristic fit with those of P. xanthii.

Conidia, germinated conidia and fibrosin bodies of the fungus

Conidiophores and chasmothecia under binocular and microscope at 40 x

4. DISCUSSION

In this study, all tested materials were infected at various levels non was found to be immune. However, Farm and PI164723 x silka are more tolerance to the disease under field conditions than other tested plants. The present study clearly identify P. xanthii as the most prevalent cause the powdery mildew on cucurbits in Sudan, these results is agree with
Lebeda et al. (2009) who stated that P. xanthii grows in tropical and sub-tropical areas and in greenhouses, and G. cichoracearum needs temperate or cooler temperatures in open fields. In contrast in Southern Italy Miazzi et al. (2011) and the Southeastern coast of Spain Del Pino et al. (2002), only P. xanthii was found on cucurbits reinforcing the hypothesis of Lebeda et al. (2009). Also PMR45 as previous study was reported resistant to G. cichoracearum, thus can be used as a field indicator of Px if races other than race 1 are present. The presence of the powdery mildew pathogen on leaves and stems of infected plants in this study agrees with reports presented by Jim (2006), Kavanagh (2005) and Kaye et al. (2006), who stated that the pathogens of the order Erysiphales are known to develop only on leaf and stem surfaces of plants. The incidence of the disease in this study ranged from (50 – 80%). These values could attain epidemic levels in the absence of timely application of fungicides and appropriate cultural control measures. Powdery mildew on C. flexuosis a rapidly evolving disease with serious impact on their production worldwide. Therefore, resistance screening to emerging races and pathotypes and development of differential lines that can be used to detect these different forms of fungus are of great importance. The severity of the disease during this study was higher thus, due to varied agroclimatic conditions in the months of Nov to Feb which coincides with cool and dry weather, also cultural practices and host susceptibility were affected the severity of the disease. The findings are in conformity with the reports of Koren (1978) and Palit (1971) who observed that, intensive cultivation of chilli resulting from continuous cropping, wherein proximity of infected crops and the amount of inoculum present undoubtedly affect the severity of infection besides creating the favourable environmental conditions. Also such variations in powdery mildew severity and wide spread nature have been reported by earlier workers (Jhooting and Munshi, 1990 and Palit, 1988). The differences in reaction of the snake melon lines to the disease may be attributed to the physiologic races of the fungus Mohamed et al. (1995), have stated that different melon (Cucumis melo L.) cultivars reacted differently to the different races of P. xanthii, these findings might explain to the differences observed in the reaction of these lines to P. xanthii when they are were tested under natural conditions. In our study race 1 was appeared at the beginning of the season and race 2 was arrived later it is evident that P. xanthii race prevalence is changing within one growing season, and from one season to another. This result agree with Mohamed et al. (1995), have reported that, race 1 and 2 prevails during winter season. For confirming the causal agent in the field for all samples examined the conidia have ovoid shape, these results agree with Bojorques-Ramosetal.(2011) have reported that, conidia were ellipsoid-ooid to doliform-shaped and had fibrosin bodies present. Vakalounakis et al. (1994) and Stadnik et al. (2001), according to these authors, P. xanthii has ovoid conidia, whereas G.cichoracearum, in general, has cylindrical conidia. The conidiophores were of oидium type, unbranched, bearing conidia in chain 3–6, these results agree with Bojorques-Ramosetal. (2011) have stated that, conidiophores of the euoidium type (conidia in chains), with crenated margins; conidiophores basal cells were straight or slightly bent, and were slightly constricted at the base. Presence of fibrosin bodies clearly differentiated P. xanthii from G. cichoracearum, the former have fibrosin bodies, this result agrees with Yawood, (1978) and Stadnik et al. (2001), have reported that, the presence or absence of fibrosin bodies inside the conidia of Oidium spp. is an indicator of the teleorphic forms of P. xanthii and G. cichoracearum, respectively. The existence of chasmothecia in a high number on more susceptible cultivars was detected in this study. These results agree with Agrios, (1978) and Heffer et al. (2006) have reported that, at the end of the growing season, powdery mildew fungi produce sexual spores, known as ascospores, in a sac-like ascus (pl.asci) enclosed in a fruiting body called a chasmothecium (pl. chasmothecia) (cleistothecium is a former term for this structure that is still widely used). The presence and abundance of chasmothecia was related to plant susceptibility and coincided with temperatures, ranging from 13.3 to 26.4, averaging 19.3°C. Smith (1970) observed maximum ascocarp production of Erysiphe pisi (as E. polygoni) on Pisum sativum, E. ludent var lathyr (as Microsphaera penicillata) on Lathyrus ochroleucus and Golovinomyces cichoracearum (as Erysiphe cichoracearum) on Aster laevis, when temperatures ranged from 10 to 20°C, while in North America and Europe ascocarps of P. xanthii (= S. fuliginea s. lat.) on cucurbits have been observed mainly in fall and winter time (McGrath et al. 1996).

5. CONCLUSIONS AND RECOMMENDATIONS

- The present study has demonstrated a high incidence and intensity of powdery mildew disease on melon found in the area.
- Resistance for the disease does not exist in tested lines.
- Farm and PI164723 x silka lines (50%) may be good source of resistance to powdery mildew and adaptable for winter growing conditions.
- Podosphaera xanthii was identified as the most prevalent cause of powdery mildew on snake melon in the Gezira area.
- Both races 1 and 2 of Podosphaera xanthii were found on melon during winter of growing seasons.
- The variability of physiological races of powdery mildew and the suddenly appearance of new physiological races in the world and the capability of the powdery mildews to generate resistance to commercial fungicides (Coffey et al.2007), justifies the continuous research on the prevailing physiological races of powdery mildew in order to better manage cucurbit crops and to prevent major damages by unexpected pathogen break throughs. This information is important for cucurbit breeders, plant pathologists and extensionists, as well as cucurbit grower.

REFERENCES


Jim, C. 2006. Powdery Mildew on ornamental plants Ohio State University Extension Ohio USA.


