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Research Article

Evaluation of some cucurbit genotypes for resistance to downy mildew based on AUDPC

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Abstract: Downy mildew is one of the most important diseases of cucurbits in the world and Iran. The development of the disease was investigated in a commercial variety (Sakata® F1 Hybrid Saso), three hybrids and eight pure lines of cucumber, four pure squash lines, and one commercial cultivar of watermelon (Sakata® F1 Charleston Gray 243) in two consecutive years (2017 and 2018 spring and summer) at the experimental field of the University of Guilan, Iran to identify the sources of resistance. Plants were regularly inspected until the downy mildew symptoms appeared. The disease was measured using standard scale and Image J software at five stages in the plant growing season. Comparison of disease progress curves, final severity of the disease, and area under the disease progress curve (AUDPC) showed that cucumber B10 and A12 pure lines were the most susceptible and resistant in both years, respectively. None of the squash lines were infected in the first year, but in the second year, two lines showed the disease symptoms, and the severity of the disease in these lines was close to each other. The commercial cultivar of watermelon was not infected in both years.

Keywords: disease incidence, disease severity, growth rate, *Pseudoperonospora cubensis*

Introduction

One of the plant groups with the most species used as human foods is the family Cucurbitaceae (Cohen *et al*., 2015). Among the diseases that affect cucurbit crops, downy mildew is caused by the Oomycete *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. with global distribution, is economically the most damaging. It has a host range of more than 60 species belonging to 20 genera in the Cucurbitaceae family, including important crops such as cucumber *Cucumis sativus* L., melon *Cucumis melo* L., watermelon *Citrullus lanatus* (Thunb.) Matsum. et Nakai, and squash *Cucurbita* spp. (Lebeda and Cohen, 2011). Field observations and empirical studies indicate that cucurbits respond differentially to infection by *P. cubensis*. For example, cucumber is generally more susceptible to *P. cubensis* than other cucurbitaceous host crops (Urban and Lebeda, 2006; Ojiambo *et al*., 2010). Nowadays, yearly downy mildew epidemics threaten cucumber production in up to eighty countries and muskmelon production in over fifty countries, causing significant economic losses (Lebeda and Urban, 2004; Colucci *et al*., 2006). Up to 100% reduction in cucumber yield is possible when downy mildew strikes early and fungicides are not used. If fungicides are applied one week after symptom appearance, yield is reduced by

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approximately 50% (Holmes *et al*., 2015). Typical symptoms consist of chlorotic lesions between 3-10 mm on upper leaf surfaces that can be irregular or angular depending on the affected host. As the disease develops, the lesions combine to form larger lesions that eventually cover the entire leaf (Lebeda and Cohen, 2010; Cohen *et al*., 2015). During the reproductive phase of the causal agent, a thin layer of dark brown, grey, or violet-black sporangiophores bearing sporangia appears on the leaves' lower surface. Under extremely heavy infection, leaves become necrotic, followed by the death of the whole plant (within 4 to 10 days from first symptoms, depending on weather conditions, inoculum concentration, and host genotype). Heavy infection may significantly reduce yield quantity and quality (Lebeda and Cohen, 2010). Currently, there are no commercially available resistant cucurbit cultivars, and control of cucurbit downy mildew relies heavily on the use of fungicides such as fluopicolide (Presidio), cyazofamid (Ranman), and propamocarb hydrochloride (Previcur Flex). In the absence of any resistance management strategies, heavy use of these fungicides can result in *P. cubensis* developing resistance to these chemicals (Lebeda and Cohen, 2010; D'Arcangelo *et al.,* 2021; Fani *et al.,* 2021). Therefore, many efforts have been made in different countries to identify and introduce resistant cultivars (Call *et al.,* 2012, 2013; Holdsworth *et al.,* 2014; VandenLangenberg and Wehner, 2016; Win *et al.,* 2017; Li *et al*., 2018; Liu *et al.,* 2021). Due to the prevalence of cucurbits downy mildew in the climatic conditions of Guilan province and to optimize the management of the disease, this study was conducted to investigate the resistance of cucurbits available genotypes to downy mildew, including two commercial cultivars and some pure lines locally developed by Department of Horticulture, Faculty of Agricultural Sciences, University of Guilan.

Materials and Methods

Seventeen genotypes of cucurbits including one commercial cultivar (Sakata® F1 Hybrid Saso), three hybrids, and eight pure lines of cucumber (A0, A4, A9, A12, A13, B4, B6, B10, B12 × A13, A4 × B6, and A9 × A4), four lines of squash (*C. pepo* and *C. moschata*) and one commercial cultivar of watermelon (Sakata® F1 Charleston Grey 243) were evaluated for their response to infection by natural populations of *Pseudoperonospora cubensis* in two crop seasons (2017 and 2018). These genotypes had not been screened for downy mildew resistance previously.

In both years, field experiments were conducted on the research farm at the University of Guilan. The farm was plowed, and three ridges were prepared as experimental blocks. Manure was added to the ridges, water tapes were put, and the ridges were covered with black UV plastic mulch to prevent weed growth.

Seeds were germinated in sterile Petri dishes on moist filtered papers and planted in small pots containing perlite + cocopeat and maintained in the greenhouse until the appearance of fourth leaves. Then seedlings were planted in the field in a randomized complete block design (three seedlings per genotype per block). The cultivation time and the evaluations took place one month earlier in the second year than the first year.

Plants were regularly inspected until the downy mildew symptoms were observed through natural infection, and then disease severity was recorded at five stages, 7-10 days apart, in two ways. The percentages of chlorotic and necrotic tissues relative to the healthy tissues of wholly expanded leaves (leaves that had grown enough and were not twisted anymore) were recorded for each leaf using the standard scale presented by Michereff *et al*., in 2009. In this way, the disease severity on the leaves was categorized into 2, 4, 8, 16, 32, 64, 82, to 96% based on the percentage of the infected area. Then the mean of the disease severity was calculated for each plant. Disease severity was measured using *Image J* software for two cucumber genotypes each year and compared with the expected scale results (not for all because it was time-consuming).

Eventually, the severity of the disease was calculated for each plant using the following formula:

$$\%\text{Disease severity} = \frac{\sum (\text{number of leaves} \times \text{infection perce}}{\text{number of total leaves}}$$

These data were used to illustrate the disease progress curve by *Excel* software based on disease severity obtained in two ways for two years.

To make a better comparison of the resistance in the genotypes, the area under the disease progress curve (AUDPC) was calculated for all of the studied genotypes according to the degree of disease severity obtained from the standard scale using the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_{i+} y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right)$$

Where y = disease severity, t = time (day), n = number of evaluations.

At each assessment stage, the amount of sporulation was recorded for each plant in three levels, low, medium, and severe.

To determine the disease index (DI) and based on the diameter of the largest spots on the infected leaves in the third evaluation stage, genotypes were examined for their downy mildew reaction type (RT) and classified on a 1-4 scale of increasing plant resistance, where: RT1 = susceptible, with irregular, chlorotic and necrotic lesions 10 to 15 mm in diameter; RT2 = moderately resistant, with type RT1 lesions mixed with type RT3 lesions; RT3 = resistant, with irregular to circular, chlorotic and necrotic lesions 3 to 4 mm in diameter; RT4 = highly resistant, with circular, chlorotic and necrotic lesions 1 mm in diameter. Then the DI for each genotype was determined using Williams' formula (Thomas, 1999):

$$\textit{Disease index} = \sum \frac{(i \times j)}{n}$$

Where n = total number of plants, i = reaction type, and j = number of plants/reaction type.

Climatic changes and their possible effects on the disease trend were investigated in each crop season.

Some of the infected leaves were checked out for *Pseudoperonospora* sporangiophores in the laboratory to ensure the presence of downy mildew pathogen on diseased leaves.

Statistical analysis

The experiment was established based on the randomized complete block design. The data were analyzed by SAS software, and the means were compared using Tukey's test at a 5% probability level in both years.

Results

Disease progress curve

Based on the standard scale, the highest severity of the downy mildew disease was observed in A0 and B10 pure lines (respectively 79% and 66%) and the least severity in B6 and A12 pure lines (respectively 35% and 33%) in 2017 (Fig. 1a). In 2018, the highest severity was observed in the B10 pure line and B12 × A13 hybrid (55% and 40%, respectively) and the least severity in A4 and A12 pure lines and A4 × B6 hybrid (12%, 19%, and 14% respectively) (Fig. 1b). The highest amount of the disease severity for the B6 pure line was 30-35% based on two years' data.

Comparison of disease progress curve based on the standard scale and Image J software in two genotypes showed nearly similar results (Fig. 1 and Fig. 2). Both graphs had an uptrend, and disease severity in B10 pure line was continuously higher than A4 pure line and Saso hybrid cultivar. In 2017, minimum and maximum disease severity in B10 pure line were 32 and 66% based on the standard scale and 40 and 72% based on software. Minimum and maximum disease severity in Saso cultivar were 16 and 42% based on the standard scale and 13 and 45% based on software. In 2018, the minimum and maximum disease severity in B10 pure line were 5 and 55% based on the standard scale and 7 and 50% based on software. Minimum and maximum of disease severity in A4 line were 1 and 12% based on the standard scale and 3 and 12% based on software.

None of the squash lines were infected in the first year, but in the second year, two lines showed the disease symptoms, and the disease severity values were close to each other in these lines (Fig. 3). The commercial

watermelon variety was not infected in any of the two years.

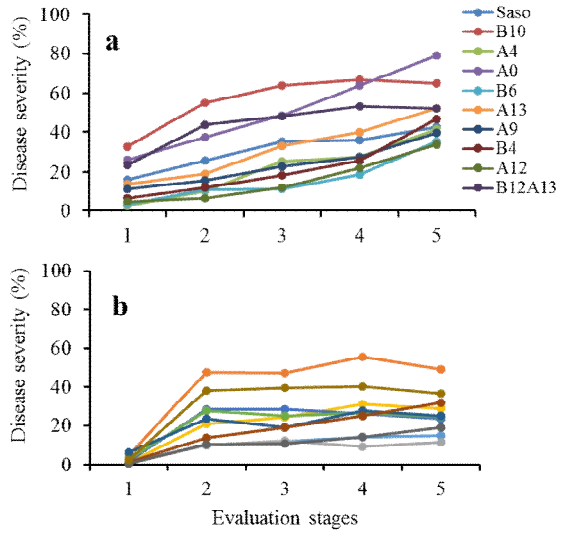

Figure 1 Downy mildew severity in cucumber genotypes at five evaluation stages based on standard scale in 2017 (a) and 2018 (b).

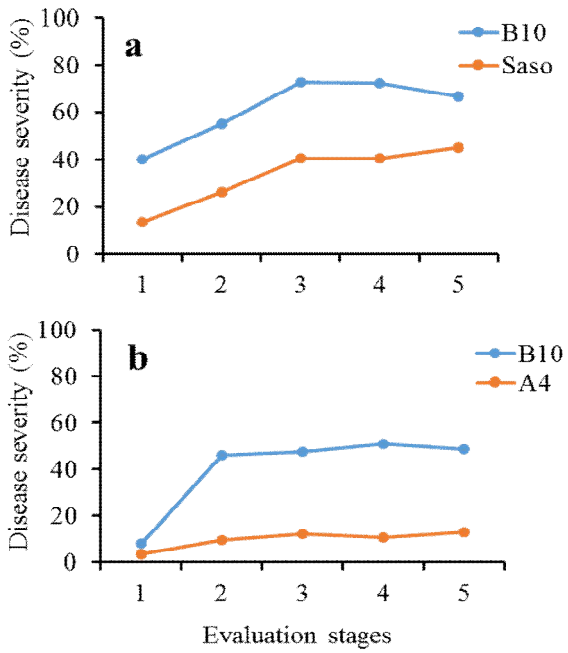

Figure 2 Downy mildew severity in two cucumber genotypes at five evaluation stages based on *Image J* software in 2017 (a) and 2018 (b).

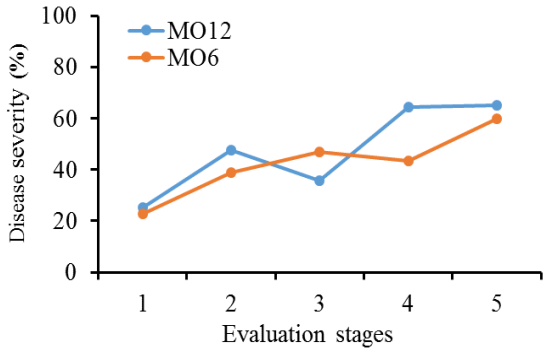

Figure 3 Downy mildew severity in two squash lines at five evaluation stages based on standard scale in 2018.

The area under disease progress curve (AUDPC)

There were significant differences among the genotypes based on AUDPC (Fig. 4). In 2017, the highest AUDPC values were found in B10, A0, and B12 × A13 genotypes (2093.60, 1815.60, and 1128.40, respectively), and the lowest in B6 and A12 pure lines (532.78 and 546.89, respectively) (Fig. 4a). In 2018, the highest AUDPC values belonged to B10, and B12 × A13 genotypes (1257.10 and 962.53, respectively), and the least was observed in A4 pure line (270.81) (Fig. 4b). In squash lines, MO12 had more AUDPC value than MO6 (Fig. 5).

Disease index (DI)

The disease index was calculated for all genotypes based on the reaction type. There were significant differences among the genotypes based on DI (Fig. 6). In 2017, B6 and A9 lines had the highest DI ratings, 2.7 and 2.3, respectively, showing these lines had the smallest spots compared to others. B10 and B12 × A13 genotypes with DI 1.4 had the largest spots (Fig. 6a). In 2018, the highest DI was recorded for A9 × A4 (2.3) and A13 (2.1), and the lowest for A4 (1.6) and B12 × A13 (1.5) genotypes (Fig. 6b). The disease index in MO6 and MO12 lines were 2.28 and 1.59, respectively (Fig. 7).

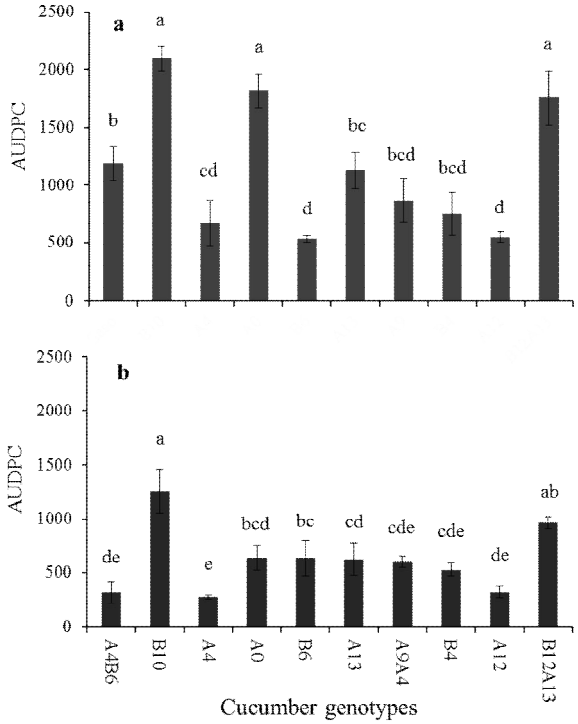

Figure 4 Area under the disease progress curve in cucumber genotypes based on standard scale in 2017 (a) and 2018 (b). Means followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

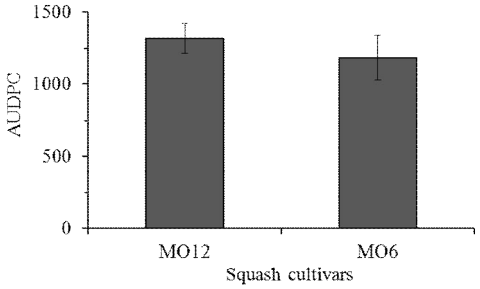

Figure 5 Area under the disease progress curve in squash lines based on standard scale in the second crop season (2018).

Sporulation

In every evaluation stage, sporulation for each leaf was recorded in three levels (low, medium, and severe) by a magnifying glass and in a visual way. Sporulation usually increased as the infected leaf area enhanced. However, with increasing temperature, sporulation decreased and then stopped altogether. In 2017, sporulation stopped from the third stage of assessment, and in 2018, it stopped from the fourth stage of assessment. Sporulation in the first year was more severe than in the second year. The highest sporulation was observed in the B10 line, and the least was found in the A4 line. In the second year, severe sporulation was not observed in A12 and B4 lines.

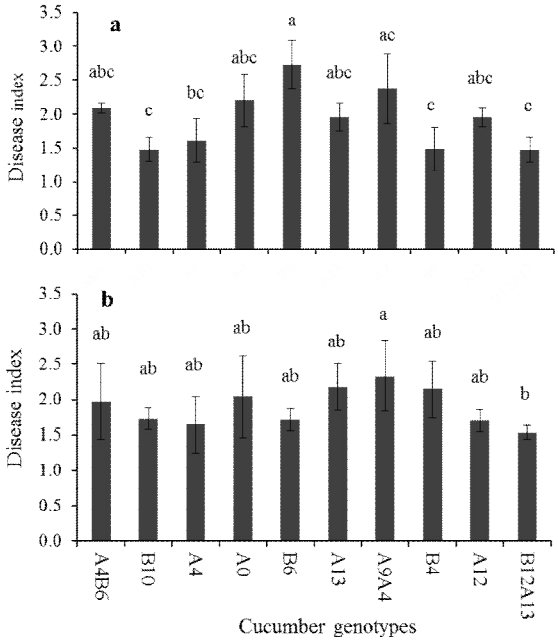

Figure 6 Disease index in cucumber genotypes based on reaction type at third stage of evaluation in 2017 (a) and 2018 (b). Means followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

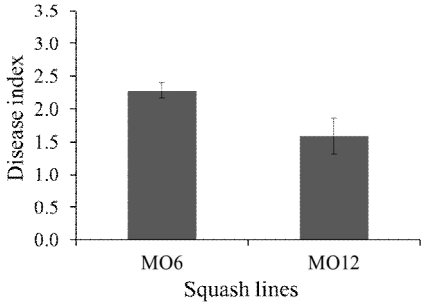

Figure 7 Disease index in squash lines based on reaction type at third stage of evaluation in the second crop season (2018).

Discussion

The causal agent of cucurbit downy mildew is *P. cubensis*. The disease is commonly spread in warm and humid areas such as the northern part of Iran. Since the most effective way to cope with this disease is planting resistant cultivars, some researchers worldwide have tried to identify the resistant genotypes and then examined the effect of different fungicides on this disease. So, identifying resistant and high-yielding genotypes can be the first step to reduce the cost of damages, especially in Guilan province, where downy mildew naturally contaminates most cucurbits plants in field conditions and causes significant damages every year. The use of old fungicides is no longer effective. In this research, the resistance of 17 genotypes of cucurbits to the downy mildew was evaluated. The severity of the disease was significantly different among genotypes. In general, the level of AUDPC in 2018 was lower than in 2017 due to changes in planting dates and climatic conditions.

Based on the disease severity and AUDPC, B10 and B12 × A13 were the most susceptible genotypes among cucumbers. B10 line showed the highest susceptibility, and the highest sporulation occurred on it. A12, A4, and A4 × B6 were the most resistant genotypes. The A12 line, in addition to its resistance, showed low sporulation. The highest disease severity for the B6 line was 30-35% based on two years of data, nearly the same in both years. There was no significant difference between genotypes based on disease index. However, the least DI was found in B12 × A13 in both years, which shows it had the largest spots among the genotypes. Disease severity and AUDPC values in MO12 were higher among winter squash lines than in MO6. In both years, the commercial variety of watermelon (Sakata F1-Charleston Gray 243) did not get infected with downy mildew.

Based on the previous studies, the pathogen can infect the plants at temperatures between 10 and 27 °C, with an optimum day temperature of 25-30 °C and a night temperature of 15-21 °C. Sporulation and infection are arrested above 35 °C, but the fungus can survive for several days at that temperature. Relative humidity of more than 75% is conducive to disease development. Disease severity was positively correlated with rainfall at 7 and 8-14 days before disease occurrence but negatively correlated with average RH. Disease progress was highest between mid-August and September when the maximum temperature was 32-35 °C, and the minimum temperature was 21-25 °C and RH 75-93% (Awasthi, 2015).

In the current study, disease progress curves generally rose during evaluation weeks (except the last week) in 2017, as the minimum and maximum temperatures were relatively favorable for the development of the disease. However, with the increase in temperature between the second and third evaluation stages to 34.4 ^{o}C, sporulation almost stopped. In all weeks, although the minimum relative humidity was low and could be a good reason for the early cessation of sporulation, the maximum relative humidity was mainly recorded as 100%. In the last week, the minimum and maximum relative humidity decreased significantly. The highest amount of rainfall was two weeks before the first assessment, with some rainfall between the first and second stages of assessment, which can justify the rate of disease progression in this period. There was no rain in the later stages of assessment.

In 2018, the rate of disease progression was the highest from the first to the second

assessment. During this week, the temperature was optimum for the growth of the pathogen. The minimum temperature was 23.7 °C, and the maximum was 33.7 °C. The minimum relative humidity was 47% which increased significantly in the following days. The maximum relative humidity was always more than 90%, which had the most critical effect on the development of the disease. One week before the first evaluation, 11 mm, and in between the first and second evaluations, 19.7 mm precipitation were recorded, all of which justified the rapid trend of disease development in this period. From the second to third evaluations, the disease trend was almost constant. In some genotypes, it decreased, which could be due to rising temperatures up to 34.5 °C, rainfall interruption, and reduced minimum relative humidity. Between the third and fourth evaluation stages, the disease had increased slightly. At the same time, the minimum temperature was favorable for the disease development, but the maximum temperature caused the sporulation to stop. The minimum and maximum relative humidity had decreased compared with the previous week, and the rainfall was only 0.3 mm. After the fourth assessment, the disease had slightly reduced in some genotypes and was fixed in some cases. Even though the minimum and maximum temperatures and relative humidity were suitable for the development of the disease during this time, and rainfall had been recorded during that week, the disease decline on cucumber plants could be due to the plant's loss of turgidity at the end of the growing season (Fig. 8). The weather conditions were also suitable for the disease occurrence on the squash lines at the end of the growing season in 2018. There was 19.7 mm rainfall about ten days before the first symptoms appeared on the squash plants, and the average RH was 79.63% in this period. Then the squash lines with young and fresh, expanded leaves showed disease symptoms in 2018. As the results of the current study show, the level of resistance to downy mildew was different among studied cucurbit genotypes. Still, it was also dependent on climatic conditions in the growth season, and it should be considered in genotype resistance evaluation programs.

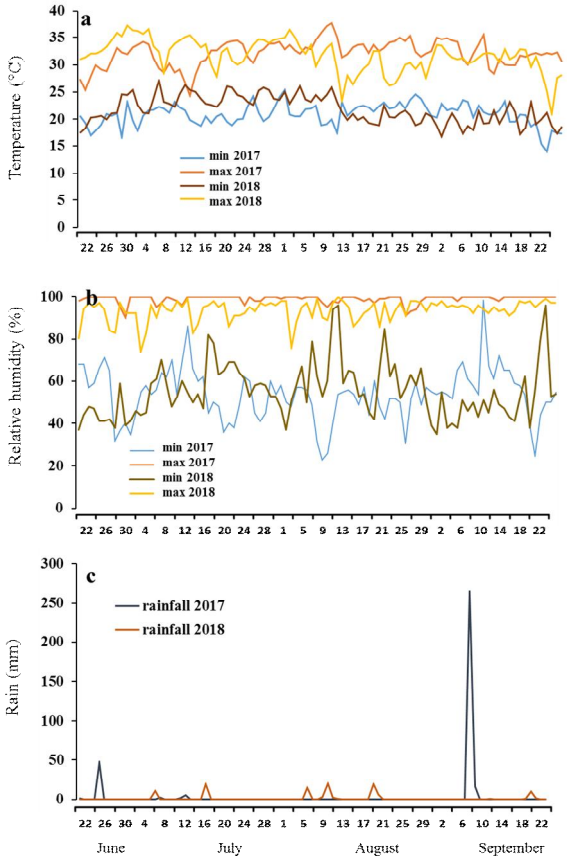

Figure 8 Temperature (a), relative humidity (b) and rainfall (c) fluctuations as recorded in the weather station near the research farm in 2017 and 2018.

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ارزیابی مقاومت تعدادی از ژنوتیپ‌های کدوئیان به سفیدک دروغی براساس سطح زیرمنحنی پیشرفت بیماری

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دریافت: ۱۳ بهمن ۱۳۹۸؛ پذیرش: ۵ آبان ۱۴۰۰

چکیده: سفیدک دروغی یکی از مهم‌ترین بیماری‌های کدوئیان در ایران و جهان است. به‌منظور شناسایی منابع مقاومت به این بیماری در استان گیلان، پیشرفت بیماری در تعدادی از ژنوتیپ‌های کدوئیان ازجمله یک رقم تجاری (Sakata® F1 Hybrid Saso)، سه هیبرید و هشت لاین خالص خیار، چهار لاین خالص کدو و یک رقم تجاری هندوانه (Sakata® F1 Charleston Gray 243) در دو سال متوالی (۱۳۹۶ و ۱۳۹۷) در مزرعه آزمایشی دانشگاه گیلان بررسی شد. آزمایش در قالب طرح بلوک‌های کامل تصادفی انجام شد. گیاهان به‌طور منظم بررسی شدند تا زمانی‌که علائم سفیدک دروغی از طریق آلودگی طبیعی ظاهر شدند. شدت بیماری با استفاده از مقیاس استاندارد و هم‌چنین نرم‌افزار *Image J* در پنج مرحله در فصل رشد اندازه‌گیری شد. مقایسه منحنی پیشرفت بیماری، شدت نهایی بیماری و سطح زیرمنحنی پیشرفت بیماری در ژنوتیپ‌ها نشان داد که لاین‌های خالص B10 و A12 خیار به‌ترتیب حساس‌ترین و مقاوم‌ترین ژنوتیپ در هر دو سال بودند. هیچ‌یک از لاین‌های کدو در سال اول آلوده نشدند، اما در سال دوم دو لاین علائم بیماری را بروز دادند و شدت بیماری در هر دو لاین تقریباً نزدیک به‌هم بود. رقم تجاری هندوانه در هیچ‌یک از دو سال زراعی آلوده نشد.

واژگان کلیدی: شیوع بیماری، شدت بیماری، نرخ رشد، *Pseudoperonospora cubensis*