Evaluation of relative resistance of different hull-less seed oil pumpkins to powdery mildew

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ABSTRACT INFO	ABSTRACT
Research Paper **Received: 01 Jul 2023** **Accepted: 20 Nov 2023**	Powdery mildew is one of the most important diseases of cucurbits in the world and Iran. The disease is found in cucumbers, melons, cantaloupes, squash, and watermelons in both farm and greenhouse conditions and causes great damage. Cultivating resistant cultivars or chemical control with fungicides can be used to control this disease. In this study, 28 different genotypes of hull-less seed pumpkin were studied and compared for powdery mildew resistance in potted conditions in a greenhouse. To achieve this, the genotypes in the greenhouse were sprayed with powdery mildew spores, and when signs of powdery mildew appeared through infection on the plants, the severity of the disease was determined by image processing using a program written in MATLAB software. The mean comparison showed that the lowest level of infection was related to lines 11 and 23 and hybrids of 7×16, 2×16, and 2×23. The highest negative general combining ability was related to line 11 and the highest negative specific combining ability was related to hybrids 7×14, 2×16, 2×23, and 11×14. The highest negative heterosis compared to the high parent was related to 11×14, 2×23, and 2×16 hybrids. Due to the significant values obtained for specific combining ability and significant heterosis in hybrids, the role of dominance variance in controlling this trait is prominent, and hybrid production is recommended to improve resistance to powdery mildew. ***Key words***: Diallel, General combining ability, Image processing, Specific combining ability.

INTRODUCTION

The pumpkin genus is from the Cucurbitaceae family and including 12 species spread throughout the American continent (Latin, South, and North America) (Jeffrey, 1990). Hull-less seed pumpkin (*Cucurbita pepo* var. *styriaca*) is an annual and herbaceous plant with straight and strong roots. This type of pumpkin often has unlimited growth and its vegetative and reproductive parts grow simultaneously (Robinson and Decker-Walters, 1997). Pumpkins are one of the plant groups with the most species used as human food (Cohen *et al.*, 2015). Hull-less seeds in this type of pumpkin were created as a result of random and natural mutation and led to morphological changes and the formation of seeds with a thin shell (Habibi *et al.*, 2011). In this pumpkin, each fruit contains 400 to 500 seeds or equal to 150 to 200 grams of seeds. The seeds are dark green or olive green in color (Nomikos *et al.*, 2009). This plant was recently cultivated in Iran and has been noticed as an important medicinal plant (Nikkhah Bahrami *et al.*, 2009).

The powdery mildew fungus (*Podosphaera xanthii*) is one of the most important obligate parasites of plants causing a white coating on their aerial parts, which ultimately leads to yellowness, dryness, and a decrease in quantity and quality of the product (Soudori, 2012; Rashid *et al.*, 2013; Alavilli *et al.*, 2022). The fungus that causes this disease has a host range of more than 60 species belonging to different genera in the Cucurbitaceae family, including important plants such as cucumber, melon, watermelon, and pumpkin (Lebeda and Cohen, 2010). All powdery mildews depend on living organisms and infect a wide range of host plants (Takamatsu, 2004). It is one of the most widespread plant diseases that damage all kinds of plants every year. Fungi that cause powdery mildew are common diseases not only in humid areas but also in dry areas (Agrios, 2005). The symptoms of this disease on leaves are the dense or scattered white powdery coating of mycelia, conidia, and conidiophores of the fungus causing the disease (Habibi and Zamani, 2006). Identifying and planting resistant cultivars, collecting and destroying the remains of diseased plants in the field, and chemical control using fungicides such as sulfur, as soon as the symptoms of the disease are seen, reduces the severity of the disease and increases yield per hectare (Nasr-Esfahani and Karimkhan, 2013).

Various genes are involved in the resistance to different races of fungi. The environment is the second factor that plays a role in the severity of diseases. Temperature, humidity, and rainfall are among the environmental factors affecting powdery mildew severity (Zakeri *et al.*, 2022).

Olfati *et al.* (2011) reported that the dominance variance plays a greater role in controlling the powdery mildew resistance trait in cucumbers. Ranjbar *et al.* (2008) stated that the resistance of different cucumber cultivars to powdery mildew is different, and this means that several genes control resistance to different strains and the heritability of this trait (Yundaeng *et al.*, 2020). Ranjbar *et al.* (2008) investigated the resistance of squash and gourd to three physiological strains of the fungus isolated from cucumber and reported that squash was sensitive to these three strains, but gourd was resistant. In a similar work, Thakur *et al.* (2019) introduced the best combination of maternal lines by examining the compatibility and heterosis of different traits in cucumbers.

Evaluation of the ability of hybridization and heterosis are considered important methods in determining superior genotypes for use in cross-breeding programs. Superior parents with high genetic variance effects can be selected by determining the general and specific combining ability and examining the mutual effects of genes, and the maternal effects and the nature of the genes' action in the expression of quantitative traits can be defined in some cases (Khan *et al.*, 2009). According to the mentioned cases, this research was designed and carried out to evaluate the resistance of elite lines and hybrids of hull-less seed pumpkin to powdery mildew to release new hybrids for Iran.

MATERIALS AND METHODS

In this research, seven lines and their direct hybrids (21 hybrids) were investigated in the form of a diallel test in terms of powdery mildew resistance response. Lines and hybrids were received from Negin Bazr Guilan Co. The lines were obtained by crossing between zucchini and hull-less seed pumpkins. Seeds of different varieties of hull-less seed pumpkins were placed between two layers of filter paper (Sohani, 2010). Germinated seeds carrying emerged roots were transferred to small pots containing an equal volume of perlite and cocopeat. Seedlings were transferred to bigger pots (29 cm diameter) following the emergence of second and third true leaves (on September 9, 2022). This experiment was conducted in the form of a completely randomized design (CRD) with three replications in the research greenhouse of the Faculty of Agricultural Sciences of Guilan University. The temperature of the greenhouse was controlled between 25 and 15 °C (day and night)

and the humidity was close to 85% using an air heating fan with a TCL split-type air conditioning unit system (Olfati *et al.*, 2011).

To check the resistance levels of the genotypes to powdery mildew and to prepare the inoculum, about 50 leaves from the cultivated pumpkin plants which grown in the research farm of Guilan University and infected with powdery mildew, were collected and after adding about four liters of distilled water, they were immersed in water for one hour. The resulting suspension was examined by a light microscope and using a hemocytometer slide and the concentration of cell suspension was recorded as 1.5×10^5 (Zakeri *et al.*, 2022). The plants of the examined cultivars were inoculated with five milliliters of the fungal spore suspension under greenhouse conditions, and then each plant was covered with plastic to provide moisture (Ranjbar *et al.*, 2008).

After seven days, symptoms of powdery mildew were observed in several plants, and when powdery mildew appeared in all the plants, photographs were taken of the leaves containing powdery mildew under the same conditions (Figure 1). The amount of infected surface was determined through image processing and by the program written using MATLAB software.

It was checked in terms of infection level and the amount of surface covered with powdery mildew was calculated.

The prepared images were received in the image processing toolbox of MATLAB software, and the image processing operation was performed to detect the spots infected with the disease on the leaf surface. Figure 2 shows the images obtained from different stages of the image processing operation. The primary images were in color and RGB color space (Figure 2A). First, to determine the surface area of the leaf, segmentation of the leaf was carried out from the background of the image. For this purpose, the images were converted from the RGB color space to the gray level image (Figure 2B), and by applying the appropriate threshold limit on the gray level image, the areas related to the leaf were separated from the areas related to the background of the image, and the original black and white image was obtained (Figure 2C). To remove unwanted white points in the image field (possible effects) successive contraction and expansion operations were performed on the black-and-white image. Also, the unwanted black points inside the areas related to the leaf surface were filled. The final black and white image representing the leaf surface was obtained, where the white points represented the areas
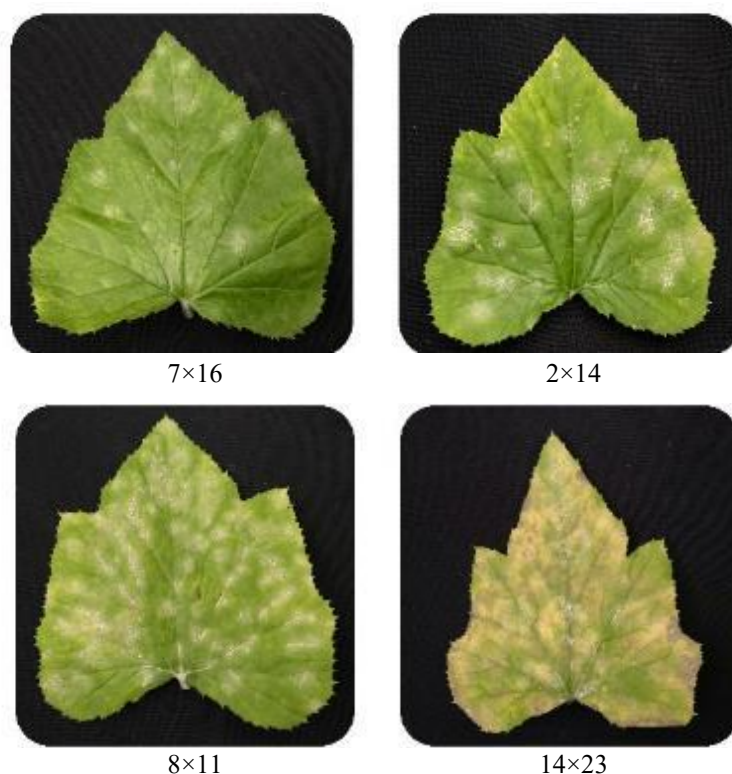

Figure 1. Images prepared from the leaves of genotypes (7×16, 2×14, 8×11 and 14×23) infected with powdery mildew for image processing.
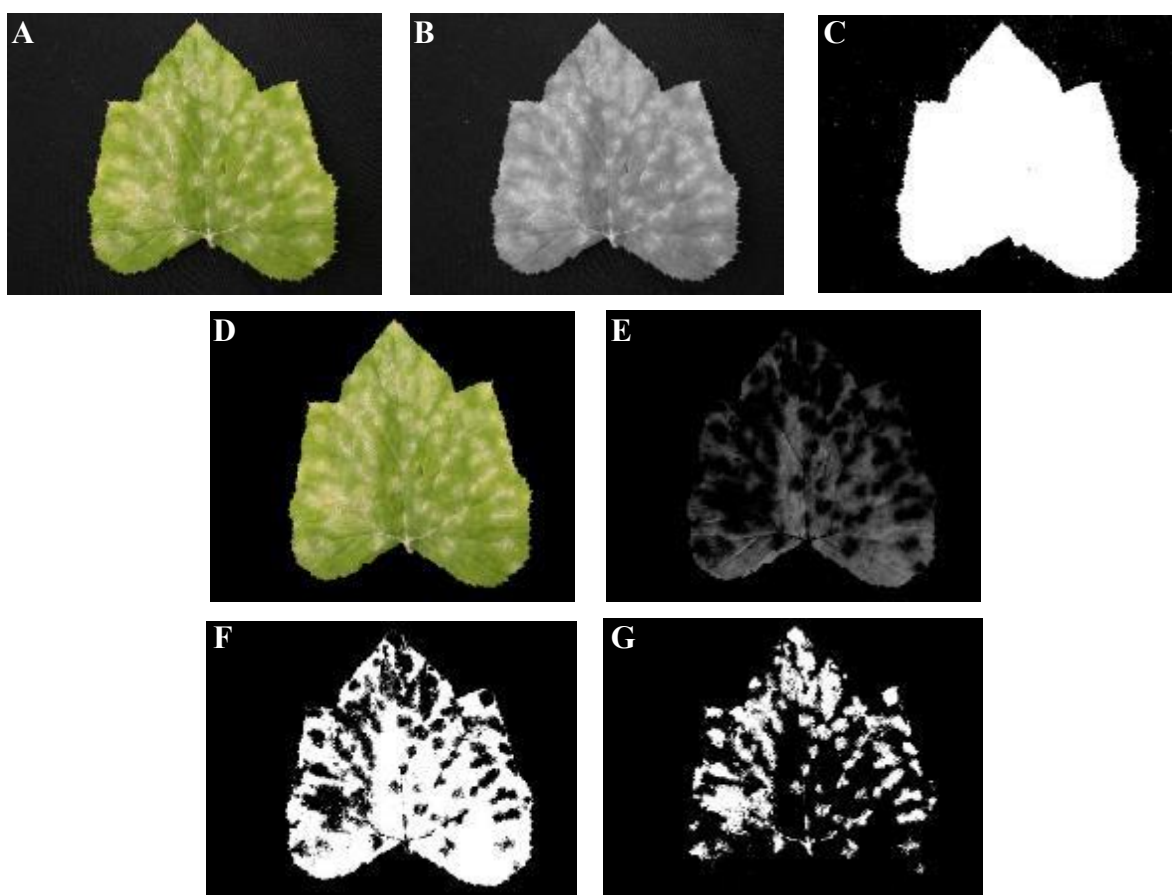
Figure 2. Gallery of images resulting from image processing steps, **A:** initial color image, **B:** gray level image, **C:** initial black and white image of leaf surface, **D:** color image of leaf surface with zero value in the image background, **E:** image of excess greenness, **F:** black and white image of healthy spots on the leaf surface, and **G:** black and white image of infected spots on the leaf surface.

related to the leaf surface in the image (Figure 2B). By counting the white pixels in the image, the leaf surface area was obtained based on pixels.

In the next step, infected areas were detected on the leaf surface (Lin *et al.*, 2021). For this purpose, to eliminate the effect of the background of the image in the subsequent calculations, by performing a logical and operation between the primary color image and the black and white image of the leaf surface, an image was obtained in which the areas related to the leaf surface were in color and the areas related to the background had a value of zero (Figure 2D). The color components of red (R), green (G), and blue (B) were extracted from this image, and by using equation 1 the image representing the green points of the leaf was obtained (Bakhshipour and Jafari, 2018).

(1)   EGI=2G-R-B

Where, EGI is called the excess greenness index image, and pixels with a value greater than zero

represent green points (Figure 2E). Therefore, by selecting the pixels with an intensity greater than zero in the EGI image, a black and white image representing the leaf healthy (green) points was obtained, and the white points represented the healthy points (Figure 2F). By differentiating this image from the black-and-white image of the whole leaf, the black-and-white image representing the infected spots was obtained (Figure 2G). By counting the number of white pixels in this image, the areas related to the infected leaf spots were obtained. Finally, by dividing the area of the infected spots by the area of the whole leaf, the severity of the spread of the disease on the leaf surface was calculated (Wang *et al.*, 2021).

After normalization, the obtained data were examined and statistically analyzed, and means were compared. After the difference between genotypes was significant, to determine the general and specific combining ability of the examined parents, the fixed model of the second method of Griffing was used

Table 1. Results of variance analysis effect of treatment on the disease severity in the *Cucurbita pepo* var. *styriaca*.

| Source of variation	df	Mean squares
Genotype	27	2153.53**
Error	56	58.98
Coefficient of variation (%)		17.23

**: Significant at *P*≤0.01.

Table 2. Analysis of variance of the general and specific combining ability of lines and hybrids of hull-less pumpkin for powdery mildew infection percent.

Components of variation	df	Mean squares
G.C.A.	6	207382.65**
S.C.A.	27	123037989**
M'e	56	19.66

M'e=MSe/r (MS: Mean of square, e: Error, r: replications).
**: Significant at *P*≤0.01.
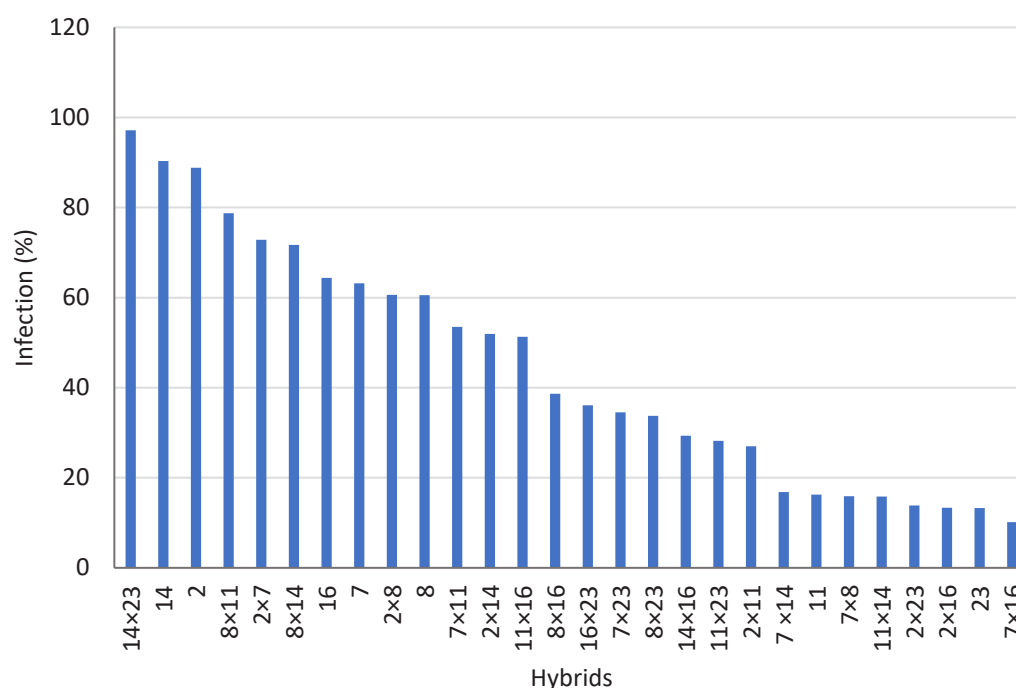
Figure 3. Mean comparison of genotypes in terms of the amount of surface infected with powdery mildew.

(Griffing, 1956). DIALLEL 1.1 software was used to check the specific and general combining ability and the heterosis. SAS 9.1 software was used to analyze variance and compare mean data by Tukey's method.

RESULTS AND DISCUSSION

Analysis of variance showed a significant difference between genotypes for the infection levels of the genotypes tested (Table 1). Based on the results of the comparison of the means of the lowest level of infection related to lines 23 and 11 and hybrids 7×16, 2×16, 2×23, 11×14, 7×8, 7×14, 2×11, 11×23, 16×14 (Figure 3). The results of the investigation by Thakur *et al.* (2019) showed that the cucumber hybrids studied had a higher resistance to diseases than the studied lines. However, some of the lines studied in this research showed resistance similar to or better than hybrids, due

to the high parent dominance effect in hybrids.

General and specific combining abilities (Table 2) were significant for the amount of surface infected with powdery mildew. The highest positive general combining ability for this trait was related to line 14 and the highest negative general compatibility was related to lines 11 and 23 (Table 3). The negative combining ability of parents caused the reduction of the infected level in breeding populations, and therefore, they should be considered when creating breeding populations to increase resistance to this disease. Previous genetic mapping in some resistant sources has enabled the identification of 25 dominant genes, two recessive genes, and seven QTLs conferring powdery mildew resistance, as well as eight dominant genes and 11 QTLs for downy mildew resistances (Cui *et al.*, 2022). These genes are affected additively in the progeny of lines.

Table 3. General and specific combining ability of lines (on diagonal) and hybrids (out of diagonal) for infection percent according to Griffing's method 2.

Parents	2	7	8	11	14	16	23
2	7.093*	25.193**	2.630ns	-16.166*	-11.576ns	-32.901**	-28.154**
7		-3.618ns	-31.346**	21.071*	-35.929**	-25.367**	3.244ns
8			6.695*	29.279**	8.580ns	-7.212ns	-7.872ns
11				-8.117*	-32.450**	20.257*	1.381ns
14					12.221**	-22.039*	50.012**
16						-5.022ns	6.211ns
23							-9.252**

ns, *, and **: non-significant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Among the hybrids, the highest negative private compatibility was related to 16×7, 16×2, and 2×23 hybrids, while the parents of these crosses had an average status in terms of these traits. This shows the importance of hybrid production for increasing resistance to this quality. These results were consistent with the results of Olfati *et al.* (2011) reporting that the role of dominance variance in the control of resistance to powdery mildew is strong in cucumber. Due to the high rate of specific combining ability of this trait, selection-based methods can be used to improve this trait (Moradipour *et al.*, 2016; Alavilli *et al.*, 2022), and at the same time, due to the significant negative heterosis in 14×11, 14×7, 23×2, and 2×16 hybrids were observed (Table 4). It is related to both dominant and additive effects on this trait inheritance. This means that the prevalence rate is probably high and hybrid production is also recommended. Downy mildew resistance was found to be polygenically controlled with dominance of resistance over susceptibility (Olfati *et al.*, 2011; Rashid *et al.*, 2013).

The results obtained from the research in the world showed the difficulty of obtaining resistant cultivars with favorable growth characteristics, where different species of pumpkins show different levels of sensitivity or resistance to new pathotypes of the fungal pathogens (Ranjbar *et al.*, 2008).

Genetic resistance continues to be the most economical and effective means for the management of disease in cucurbits (George *et al.*, 2004; Rashid *et al.*, 2013). Resistance of the released hybrids and lines to downy mildew might break in the future years, and again screening is needed (Rashid *et al.*, 2013). Downy mildew resistance breakdown has been common in breeding lines and hybrids, which is largely attributed to the evolution of new virulence in the pathogen populations (Thakur *et al.*, 2009). The durability of

Table 4. Heterosis by the cross, relative to mid-parents or high-parent for traits.

Parent1	Parent2	Mean	Het (mid-parents)	Het (high-parent)
2	7	72.856	-0.833	-16.004
2	8	60.605	-14.098	-28.255
2	11	26.998	-25.566	-61.862
2	14	51.925	-37.670	-38.405
2	16	13.357	-63.407	-75.503
2	23	13.874	-37.191	-74.986
7	8	15.918	-43.615	-44.629
7	11	53.523	16.131	-4.995
7	14	16.860	-57.563	-73.469
7	16	10.179	-51.414	-54.489
7	23	34.561	-1.333	-23.957
8	11	72.044	33.637	11.497
8	14	71.683	-3.755	-18.647
8	16	38.647	-23.961	-26.021
8	23	33.757	-3.152	-26.790
11	14	15.841	-37.456	-74.488
11	16	51.304	10.837	-13.363
11	23	28.199	13.430	11.932
14	16	29.346	-48.153	-60.984
14	23	97.168	45.368	6.838
16	23	36.123	-2.846	-28.545

downy mildew resistance in most commercial hybrids was only about five years because of the robust genetic variability in the pathogen and rapid selection of virulence against specific host cultivars (Thakur *et al.*, 2004).

Considering the significant values of specific combining ability and significant heterosis obtained in hybrids, the role of dominance variance in the control of this trait is strong and the production of hybrids is recommended to improve resistance to powdery mildew. At the same time, image processing with high accuracy is effective in identifying the infected surface and is recommended for future research.

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