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Breeding for fusarium wilt resistance and some economic characters in cucumber

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Abstract

This study was conducted during 2017 and 2018 at Kaha Vegetable Research Farm, Horticulture Research Institute, Qalyubia Governorate to develop promising hybrids of cucumber (*Cucumis sativus* L.) for yield and fruit quality characters under fusarium wilt (*Fusarium oxysporum*) infection stress in Egypt using diallel mating design. The pathogenicity and host range experiment revealed that *Fusarium oxysporum* isolate No.3 was the most virulent one to the susceptible cucumber cultivar "Beta alpha" and cucumber was the only infected host. The prevalence of the non-additive variance suggested heterosis breeding approach is effective way for improvement of these traits. Most of the traits exhibited significant hybrid vigor for some of crosses based on the better-parent. The analysis of variance showed that all the studied traits were highly significant indicating that both of the parents and hybrids had high variability. Significant general and specific combining ability variances were obtained in all studied traits implying that both additive and non-additive gene effects control genetic expression of these traits. The study showed that lines P_2, P_4 and P_5 had significant positive GCA effects for total yield trait under fusarium wilt stress. Thus, these parents could be successfully used in future breeding programs. Among all the crosses, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_4$ and $P_2 \times P_5$ exhibited significant SCA effects for both early and total yield characters under fusarium wilt stress. So, these hybrids can be used in future breeding program. The genotypes Kaha1×Dokky2 and Kaha1×Kaha2 followed by Kaha2×Dokky3 as well as Kaha1×Dokky3 were the most resistant genotypes decreasing disease severity correlated with increase in total phenol contents and activities of polyphenol oxidase, peroxidase and catalase as compared to susceptible genotypes, *i.e.*, Dokky3, Kaha1×Dokky1 and Dokky1. The scavenging activity was higher in susceptible genotypes as compared with resistant genotypes. Furthermore, there was a high correlation between the total phenol content and the scavenging activity. Results also revealed a noticeable significant correlation between disease severity, total yield and early yield traits. Cluster analysis classified the fifteen genotypes into five clusters with different number of genotypes. Further improvement of fruit yield could be possible through the hybridization and selection in transgressive segregation.

Key words: Cucumber, combining ability, heterosis, fusarium wilt, resistance, antioxidant and oxidative activity, DPPH

Introduction

Cucumber (*Cucumis sativus* L.) belongs to the family *Cucurbitaceae*, which comprises of 117 genera and 825 species with chromosome number 2n=14. It's one of the most important and popular cucurbitaceous vegetable crops grown extensively throughout the tropical and subtropical region of the world.

The crop is characterized by high degree of cross-pollination, wide range of genetic variability in vegetative and fruit traits. Developing a new cucumber variety is a difficult process and limited improved varieties have been developed through efficient breeding program targeting high fruit yield and better quality. The success of any breeding program is determined by collecting favorable gene combination in the form of high yield inbreds and heterosis in their crosses (Mule *et al.*, 2012).

Combining ability and heterosis are useful tools to a breeder in any breeding programme. They provide the desired genetic description concerning the improvement of crop variety or heterotic exploitation for commercial gains. To be adequately informed on the heterosis and combining ability of parents in crosses to produce desirable segregating population for selection, diallel analysis had been used often by many cucumber breeders (Glover *et al.,* 2005; Singh and Asati, 2011; Mule *et al.*, 2012 and Ene *et al.,* 2019). Heterosis is an important tool in providing breeders to increase yield and its components (Hemant and Tiwari, 2018 and Preethi *et al.*, 2019). Hemant and Tiwari (2018) reported that the hybrids with significant heterosis for early yield and the fruit yield per plant is positively associated with vegetative characters particularly number of branches per plant and vine length. Estimates of both general and specific combining ability are useful in determining the breeding value of cucumber lines by suggesting their appropriate use in a breeding program. The GCA is a measure of the additive gene action, while the SCA is assumed to be dominance gene action, if there is no epistasis. Several studies suggested that cucumber can be improved through estimating both general and specific combining abilities (Mule *et al.*, 2012; Malav *et al.,* 2018 and Prashant *et al.,* 2018).

Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *Cucumerinum* (Owen, 1956 and Bedlan, 1986) is a destructive disease accountable for large yield losses. The diagnostic symptoms of Fusarium wilt is a brown discoloration of the vascular system (xylem) when cutting the stems, foliage turn

yellow, wilting and finally leading to plant death (Owen, 1956; Gordon and Martyn, 1997; Izzati *et al.,* 2018). The pathogen which is long-lived in soils, has resulted to use host resistance as the most efficient management to avert economic yield loss caused by the disease. Reactive oxygen species (ROS) are chemically reactive molecules, form as a natural byproduct of the normal metabolism of oxygen and can increase dramatically and accumulates as a result of oxidative stress under biotic and abiotic stress conditions (Apel and Hirt, 2004; Heller and Tudzynski, 2011). Cells are able to defend-itself towards damage resulting from ROS, due to the action of catalases and peroxidases by scavenging free radicals (Torres *et al.,* 2006). Also, activity of polyphenol oxidase (PPO) increased in response to infection by pathogen (Khatun *et al.,* 2009; Madadkhah *et al.,* 2012). Polyphenol antioxidants play a function in inhibiting reactive oxygen species (ROS) harm by scavenging free radicals (Torres *et al.*, 2006), change cell wall structure, and accumulating antimicrobial secondary metabolites which are essential in systemic acquired resistance. Plant resistance to biotic and abiotic stresses is frequently controlled by the metabolic rate of phenolic compounds which are recognized as hydrophilic antioxidants and play a main part of the plants defense (Tripathi *et al.*, 2019). DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to estimate the total phenolic content and antioxidant activities. Important association between the compounds and activites showed that phenolic compounds are the major contributor to the antioxidant properties of these cucumber genotypes (Patel and Patel, 2011; Ahmed and Ali, 2013).

The present study aimed to identify a correlation between differences in the degree of resistance among 15 genotypes of cucumber against Fusarium wilt disease. Also, bio-chemical changes in different genotypes were measured and evaluated. In addition, it aimed to identify breeding lines/varieties having good combining ability effects and best cross combinations for developing promising hybrids with yield and fruit quality characters under fusarium disease stress using diallel mating design and estimating heterosis and phenotypic correlation.

Materials and methods

The study was conducted at research facilities of Horticultural Research Institute and Plant Pathology Research Institute, Agric. Research Center (ARC), Egypt during the period from 2017 to 2018 at Kaha Vegetable Research Farm, Qalyubia Governorate, ARC, Egypt. The genetic materials used in this investigation were selfed seeds of five cucumber (*Cucumis sativus* L) inbred lines. These inbred lines were chosen based on their morphological characters and fusarium wilt disease (*Fusarium oxysporum* f. sp. *cucumerinum*) resistance. The five inbred lines were kindly supported from the Vegetables Breeding Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. These inbred lines were Kaha1(P_1), Dokky1 (P_2), Dokky2 (P_3), Kaha2 (P_4) and Dokky3 (P_5).

Evaluation for yield and its components and Fusarium resistance under field conditions: This experiment was carried out at Kaha Experimental Research Farm of Horticulture Research Institute, Qalyubia Governorate. This work was done in previously known naturally infested field with fusarium wilt disease. Half diallel cross design for 5 inbred lines to produce 10 hybrids (without reciprocal) was done. The experiment was in randomized complete design with 3 replications. Each plot was 10 plants cultivated by direct seeds in two rows with 0.3×0.3 m space between plant to plant. Plants were randomly chosen from each experimental plot at 60 days after sowing to calculate the disease severity of fusarium wilt.

Disease assessment: Wilt disease severity (DS) was determined in both experiments, pot and field according to the following visual scale (0-5) and description as suggested by Grattidge and O'Brien (1982). Plants were uprooted and stem and root were longitudinally dissected for examination of internal tissue discoloration. The wilt disease severity (%) was determined and calculated using the following formula of Song *et al.* (2004):

$$\text{Wilt Disease Severity \%} = \left(\frac{(n \times v)}{5N}\right) \times 100$$

Where, n = number of infected plants, v= numerical values of symptoms category, N= total number of plants, 5= maximum numerical value of symptom catagories. 0: No discoloration, 1: from 1 to 10 % discoloration, 2: from 11 to 30 % discoloration, 3: from 31 to 50 % discoloration, 4: from 51 to 75 % discoloration and 5: from 76 % to 100 discoloration.

All agronomic cultural practices were applied as recommended for ordinary cucumber fields. At harvest time, all individual plants of five parents and their derived 10 F_1 generation were harvested in order to estimate horticultural traits, *viz.,* plant length (cm), number of branches per plant, internode length, number of nodes, fruit length (cm), fruit diameter (cm), fruit weight (g), early yield, total yield.

Sampling, isolation, purification and morphological identification of the causal pathogen under pot experiment: Cucumber wilted plants samples were collected from Kaha Research Farm, Horticulture Research Institute, Qalyubia Governorate, Egypt. Isolation from lower stem portions of the collected cucumber wilted plants which exhibited different degrees of vascular discoloration were rinsed up with tap water for 3 min, and cut into small pieces (1 cm long). The pieces were surface sterilized by soaking in 70 % ethanol for 30 s, and placed on filter paper for 2 min to dry out. Four sterilized pieces were transferred onto 9 cm diameter Petri dishes containing 10 mL of potato dextrose agar medium (PDA) (Difco, 1985). The plates were incubated at 25±2 °C for 7 days. Pure cultures were obtained by single spore technique. The pure cultures of the growing fungi were examined microscopically and identified (Both, 1971; Nelson *et al.*, 1983; Leslie and Summerell, 2006). The isolates were kept on slants at 4 °C in the refrigerator for further work.

Pathogenicity test: Four isolates of *F. oxysporum* were selected (depending on differences in their culture's colors which varies from white to pink with a purple tinge (Federation of British Plant Pathologists, 1973) to be tested for their pathogenic abilities. In this respect, Koch's postulates were carried out to confirm the virulence of the tested *F. oxysporum* on a susceptible cucumber cultivar (cv. Beta Alpha) under pot experiment at the Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Seeds of cucumber (*cv.* Beta Alpha) obtained from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt were surface sterilized by submersion into solutions of 20-30 % commercial Clorox (5.25 %) sodium hypochlorite for 1 min, then washed several times in sterilized distilled water and dried between two folds of sterilized filter paper. Seeds were sown in plastic trays filled with autoclaved peat-moss substrate.

Fusarium isolates were multiplied on sand corn medium according to Leslie and Summerell (2006). Sand and corn grains were mixed at the rate of 1:3 (25.0 g clean washed sand; 75.0 g corn grain), moistened to 50 percent moisture content, filled in 500 mL glass bottles and then autoclaved at 120 ºC for half an hour. Fungal discs (5 mm) of pure cultures of each of the tested fusarium isolates were used to inoculate the prepared sand corn grain bottles then incubated at 25± 2 °C for 15 days. Pathogenicity test was carried out using autoclaved clay loam soil. Pots (30-cm diameter) were sterilized by immersing in 5 % formalin solution for 15 min and then left for 2 days to insure complete formalin evaporation, the pots containing 5 kg of sterilized soil were infested with the previously prepared inoculum at the rate of 3 % (w/w) watered for 7 days to enhance growth and distribution of the fungal inoculum then, after 30 days of sowing, seedlings were transplanted into the infested potted soil. Each pot was planted by three seedlings. The pots were irrigated two times weekly. Each treatment included five replicates. The treatments were arranged in a completely randomized block design. Plants were observed for cucumber wilt symptoms. After 60 days of inoculation with pathogen the disease severity was recorded (Manzoor *et al.,* 2019).

Host range of highest virulent isolate (*F. oxysporum* No. 3): This trial was done to determine the formae speciales under pot experiment. Plant species belonging to three different families were evaluated against *F. oxysporum* (No. 3). The three different families were: A-Solanaceae: tomato (cv. Super Strain B) and pepper (cv. California). B-Cucurbitaceae: squash (cv. Eskandrane), watermelon (cv. Sugarbaby) and cucumber (cv. Beta Alpha). C-Cruciferaceae: cauliflower (cv. White Magic) and cabbage (cv. Balady). Seeds of cauliflower (cv. White Magic) were obtained from Sakata Seeds Company, while the rest of all seeds were obtained from Horticulture Research Institute, ARC, Egypt. *F. oxysporum* inocula used for inoculation and the transplants of different families were prepared as mentioned before. The reaction of the tested plants was indicated by (+) for the infected host plants and (-) for the non-infected ones.

Evaluation of cucumber genotypes for Fusarium wilt resistance under pots experiment: *F. oxysporum* f.sp *cucumerinum* (isolate No.3) was exploited for evaluating the resistance or susceptibility of cucumber genotypes to fusarium wilt. The test was done in pot experiment at the Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza. The inoculum was prepared as mentioned before in pathogenicity test. Seeds of fifteen cucumber genotypes prepared as mentioned before in pathogenicity test.

Assessment of cucumber genotypes for different defense responding biochemical compounds under pot experiment: Lower stem of cucumber genotypes were taken when cucumber wilt symptoms were observed.

Total phenol content (TPC): Total phenols were measured using spectrophotometer at 520 nm against a reagent blank according to Singleton *et al.* (1999).

Polyphenol oxidase (PPO) and peroxidase (POX) activity: Polyphenol oxidase and peroxidase activity were determined according to Matta and Dimond (1963) as absorbance at 420 and 425 nm/min/g fresh weight, respectively by spectrophotometer .

Catalase (CAT) activity: CAT activity was determined using the method described by Aebi (1984).

Antioxidant activity- DPPH-free radical scavenging (Inhibition %): The free radical scavenging activity (DPPH) was determined by the method described by Begum *et al.* (2018).

Statistical analysis: The data of all the quantitative traits were subjected to analysis of variance (ANOVA) as outlined by Gomez and Gomez (1984). Regression analysis was carried out by using (SPSS software, ver. 22.0, Inc., NY, USA). Also, mean values of parents and crosses were used to estimate heterosis over mid (MP) and better parent (BP) as described by Matzinger *et al.* (1962) and Fonseca and Patterson (1968), respectively. For the traits with significant genotypic variances, Griffing's method 2 model 1 (parents and F_1's without reciprocal) (Griffing, 1956) was used to estimate general combining ability (GCA) for the five parents and specific combining ability (SCA) for their ten hybrids as outlined by Singh and Chaudhary (1985).

Results and discussion

Evaluation of cucumber genotypes for yield and its components and Fusarium resistance under field conditions experiment: Mean performance for some economic traits and disease severity of five parents and their ten F_1's is presented in Table 1. Data showed that the parental lines for plant length ranged from 71.66 cm (P_1) to 180.00 cm (P_3), while plant length of the hybrids ranged from 100.0 cm ($P_1 \times P_4$) to 175.0 cm ($P_2 \times P_3$). Concerning fruit length, inbred lines ranged from 13 cm (P_3) to 20.00 cm (P_5) and hybrids ranged from 11.67 cm ($P_3 \times P_5$) to 18.97 cm ($P_1 \times P_2$). Early yield ranged from 526.2 g (P_1) to1456.7 g (P_2), meanwhile, hybrids ranged from 594.33 g ($P_2 \times P_3$) to 3986.3 g ($P_1 \times P_3$). Parental lines ranged from 2911.7 g (P_1) to 8348.3 g (P_2), while, the hybrids ranged from 4020.3 g ($P_1 \times P_2$) to 10456 g ($P_1 \times P_3$) for total yield. Results in Table 1 show that disease severity for genotypes could be divided into highly resistant inoculated genotypes, *viz.*, $P_1 \times P_3$, $P_1 \times P_4$, $P_4 \times P_5$, $P_1 \times P_5$, P_2 and $P_2 \times P_4$. Three genotypes, *viz.,* $P_2 \times P_5$, $P_2 \times P_3$ and $P_3 \times P_5$ were rated as resistant for this disease. While, P_4, P_3 and $P_3 \times P_4$ were categorized as moderately susceptible genotypes. Genotypes P_5 and $P_1 \times P_2$ were recorded as susceptible and only one genotype (P_1) was highly susceptible.

Correlation among field parameters, economic characters and disease severity under field conditions: Data in Table 2 revealed that disease severity under field condition was negatively correlated with early yield ($r = -0.619$, $P \leq 0.05$) and total yield ($r = -0.988$, $P \leq 0.01$) indicating that total yield is more sensitive to diseases severity. However, disease severity was not correlated with any of the other traits. It is worth mentioning that total yield and early yield were positively correlated ($r = 0.650$, $P \leq 0.01$), which indicates that early yield could be used to predict total yield trait.

Heterosis: Estimates of heterosis over both mid and better parents for some economic characters in 10 cucumber hybrids is illustrated in Table 3. For early yield and total yield traits, heterosis values over mid and better parents were highly positive significant in all studied hybrids except $P_2 \times P_3$ and $P_3 \times P_4$ for early and total yield, respectively, and $P_1 \times P_2$ for both traits. These results indicated that using these materials are suitable for hybridization in developing high yielding hybrids of cucumber that can adapt to the zone. These results are in consonance with the findings of Munshi and Verma (1997) in muskmelon, Chaubey and Ram (2004) in bitter gourd and Sarkar (2003) and Munshi *et al.* (2005) in cucumber. Also, highly negative significant heterosis in most

Table 1. Mean performance for some economic traits and disease severity of five parents and their ten F_1's in cucumber

Genotype	Plant length (cm)	Branch number	Internode length (cm)	Internode number	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Early yield (g)	Total yield (g)	(D.S.) (%)
					Parents					
P_1	71.667 d	146.67 d-f	8.33 ab	6.67 b-e	14.00 c-e	3.00 ab	28.67 bc	526.2 k	2911.7 o	45.06 gh
P_2	102.50 cd	190.67 b-d	6.67 ab	9.07 a-c	17.00 a-d	3.25 ab	28.67 bc	1456.7 fg	8348.3 e	11.55 ab
P_3	180.00 a	221.67 a-c	8.00 ab	9.50 ab	13.00 de	4.00 a	28.67 bc	1100.0 g-i	5742.3 k	27.10 e
P_4	105.00 cd	235.33 ab	8.33 ab	10.67 a	17.33 a-c	2.80 ab	29.67 a-c	1008.5 h-j	6023.0 j	25.12 ef
P_5	150.00 a-c	193.33 b-d	5.00 ab	10.17 ab	20.00 a	3.50 ab	29.33 bc	665.00 jk	5139.0 m	32.18 lm
					Hybrids					
$P_1 \times P_2$	109.33 cd	183.33 b-e	3.67 ab	7.17 a-e	18.97 ab	2.73 ab	31.00 ab	809.67 i-k	4020.3 n	37.45 l
$P_1 \times P_3$	115.33 b-d	181.33 b-e	9.67 a	6.67 b-e	15.58 b-e	2.70 ab	31.67 ab	3986.3 a	10456 a	7.12 ab
$P_1 \times P_4$	100.00 cd	156.67 c-f	4.67 ab	5.33 d-f	14.23 c-e	2.25 b	30.33 ab	2486.3 cd	9877.3 b	7.93 a-c
$P_1 \times P_5$	127.67 a-d	140.67 d-f	3.67 ab	4.00 ef	16.40 a-d	3.47 ab	27.67 bc	3205.7 b	8962.3 d	10.08 ab
$P_2 \times P_3$	175.00 ab	99.00 f	3.67 ab	7.17 a-e	13.00 de	3.75 a	31.00 ab	594.33 k	7831.7 h	18.12 d-f
$P_2 \times P_4$	153.33 a-c	118.67 ef	3.33 b	4.83 ef	15.26 b-e	3.60 ab	21.00 c	1842.0 e	8332.0 f	14.25 b-d
$P_2 \times P_5$	130.00 a-d	279.33 a	4.00 ab	8.50 a-d	17.17 a-c	2.82 ab	38.67 a	2233.7 d	7997.0 g	16.45 c-f
$P_3 \times P_4$	110.67 cd	161.67 c-f	4.33 ab	5.83 c-f	15.17 b-e	3.37 ab	28.33 bc	2706.3 c	5363.0 l	28.15 ef
$P_3 \times P_5$	125.00 a-d	97.00 f	6.33 ab	3.00 f	11.67 e	4.00 a	23.33 bc	1582.3 ef	7241.7 i	19.11 d
$P_4 \times P_5$	152.33 a-c	118.00 ef	2.67 b	3.67 ef	17.77 a-c	2.70 ab	27.33 bc	1196.0 gh	9034.0 c	8.99 a-c

Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. P_1 = Kaha1, P_2 = Dokky1, P_3 = Dokky2, P_4 = Kaha2, P_5 = Dokky3, (D.S.) % = Disease severity Average two seasons

Table 2. Correlation among field parameters economic traits in cucumber under field condition

Correlation	Early yield/ m^2	Total yield/ m^2	Fruit diameter	Fruit length	Internode number	Internode length	Branch number	Plant length	Fruit weight	D.S. (%)
Early yield\ m^2	1.00	[a]0.65**	[b]-0.23	-0.09	0.10	-0.37	0.07	0.05	-0.18	-0.62*
Total yield\ m^2		1.00	-0.20	-0.11	0.01	-0.37	-0.16	-0.17	0.21	-0.99**
Fruit diameter			1.00	-0.44	-0.48	-0.03	-0.01	-0.30	[c]0.57*	0.15
Fruit length				1.00	0.30	0.38	-0.26	0.44	-0.10	0.09
Internode number					1.00	0.49	0.07	0.69**	-0.13	0.04
Internode length						1.00	0.42	0.77**	0.03	0.37
Branch number							1.00	0.32	-0.35	0.20
Plant length								1.00	-0.13	0.17
Fruit weight									1.00	-0.23
(D.S.) %										1.00

Liner correlation Coefficient (r) is significant at $P \leq 0.01$ (**), [b] Liner correlation Coefficient (r) is significant?, [c] Liner correlation Coefficient (r) is significant at $P \leq 0.05$ (*), (D.S.) % = Disease severity in field

of the studied hybrids was observed for plant length, branch number and internode length traits over mid and better parents. These negative sign is desired for plant breeder. Three ($P_1 \times P_3$, $P_1 \times P_4$ and $P_1 \times P_5$) out of ten hybrids showed the highest values of heterosis over better parent for early yield and total yield traits, meanwhile, the hybrid $P_1 \times P_3$ was the best one in previous traits plus fruit length trait. Similar trend was observed for fruit weight and total yield among the same three hybrids gave the highest values for both mid and better parents, respectively**.** Singh *et al.* (2013) found high positive heterosis for seed yield/ plant in bitter gourd.

General and specific combining ability: Combining ability is useful in successful prediction of genetic capability of parental lines and crosses. General combining ability (GCA) of cucumber growth and fruit yield traits showed that genotype P_1 had positive GCA effect for branch number and early yield traits (Table 4). Furthermore, it was observed that GCA of P_2 was significantly positive for plant length, internode length, fruit length and total yield traits. Genotype P_3 had significantly positive GCA for branch number, fruit diameter, fruit weight and total yield characters. For genotype P_4, significantly positive GCA was observed for only total yield trait. Genotype P_5 showed significantly positive GCA for fruit length, fruit weight and total yield traits. In general, the genotypes P_1 and P_3 were good combiners for early yield traits, meanwhile, the genotypes P_2, P_4 and P_5 were good combiners for total yield traits.

Specific combining ability (SCA) effect on the vegetative growth, fruit quality and yield and yield components traits of studied ten hybrids are presented in Table 5. SCA reveals the best cross combinations which can be useful for developing hybrids with high vigor for the traits. Significant superior SCA effects for all studied traits were not shown by a single hybrid. Data obtained in Table 5 indicated that the F_1 crosses $P_1 \times P_3$ and $P_2 \times P_5$ achieved significant positive SCA effects for plant length. Only one hybrid ($P_1 \times P_3$) showed significant SCA effect for branch number. Also, only one cross ($P_2 \times P_5$) showed significant SCA effect for internode length. Two crosses ($P_1 \times P_3$ and $P_2 \times P_5$) showed significant SCA effects for internode number. Three hybrids exhibited significant SCA effects for fruit length and the cross $P_1 \times P_2$ showed the highest significant value. The hybrids $P_1 \times P_5$ and $P_2 \times P_4$ showed significant SCA effects for fruit diameter. The hybrid $P_2 \times P_4$ showed the highest significant SCA effect for fruit weight. Six crosses ($P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_4$, $P_2 \times P_5$ and $P_3 \times P_4$) had significant positive SCA effects for early yield. For total yield, the SCA effects for eight hybrids ($P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_3$, $P_2 \times P_4$, $P_2 \times P_5$, $P_3 \times P_5$ and $P_4 \times P_5$) were

Table 3. Heterosis values over mid (MP) and better (BP) parents for some economic characters in 10 cucumber hybrids

Hybrids	Plant length	Branch number	Internode length	Internode number	Fruit length	Fruit diameter	Fruit weight	Early yield	Total yield
				Heterosis over mid parents (%)					
$P_1 \times P_2$	8.69	-51.10**	-8.90	8.14	22.30**	-12.53	25.50	-18.30**	-28.50**
$P_1 \times P_3$	-1.54**	18.30*	-17.53**	10.46	15.46	-22.86	-8.35	390.20**	141.60**
$P_1 \times P_4$	-17.97	-43.90**	-38.40**	3.99	-9.15	-22.40**	13.20**	224.00**	121.20**
$P_1 \times P_5$	-17.25**	-44.90**	-52.48**	-4.59	-3.53	6.67	15.19	438.20**	122.60**
$P_2 \times P_3$	-51.90**	-49.90**	-22.80**	8.14	-13.30**	3.45	23.80**	-53.50**	11.10**
$P_2 \times P_4$	-44.20**	-55.50**	-51.01**	-28.00**	-11.10**	19.01	47.70**	49.44**	15.90**
$P_2 \times P_5$	45.40**	-31.43	-11.61*	33.33**	-7.21	-16.54	2.97	110.50**	18.50**
$P_3 \times P_4$	-29.20**	-46.90**	-42.15**	-2.85	0.003	-0.98	-22.30**	156.70**	-8.83**
$P_3 \times P_5$	-53.20**	-2.56	-69.40**	-19.54**	-29.20**	6.67**	-24.24	79.30**	33.10**
$P_4 \times P_5$	-44.90**	-59.90**	-64.80**	-7.35	-4.80**	-14.20**	19.47	42.93	61.80**
				Heterosis over better parents (%)					
$P_1 \times P_2$	-3.85	-55.90**	-20.96**	8.14	11.50**	-15.90	6.66	-44.70**	-51.80**
$P_1 \times P_3$	-18.10**	16.00	-29.82**	10.46	11.30**	-32.50**	-35.90**	262.30**	82.10**
$P_1 \times P_4$	-33.40**	-44.00**	-50.00**	2.25	-17.80**	-25.00	-4.76*	146.50**	63.90**
$P_1 \times P_5$	-27.20**	-55.99	-60.60**	-5.68	-18.00**	-0.95	-14.89	382.10**	74.40**
$P_2 \times P_3$	-55.30**	-54.00**	-24.50**	8.14	-23.53	-6.25	-2.78	-59.40**	-6.19**
$P_2 \times P_4$	-49.50**	-60.00**	-54.69**	-29.21**	-11.90**	10.77**	46.00**	25.70**	-0.20**
$P_2 \times P_5$	44.40**	-40.00	-16.40**	31.8**	-14.10**	-19.50	-13.33	52.40**	-4.20**
$P_3 \times P_4$	-31.30**	-48.00**	-45.31**	-4.50	-12.50**	-92.35	-38.50**	146.00**	-10.90**
$P_3 \times P_5$	-56.20**	-20.83	-70.49**	-20.45**	-41.67	0.00	-30.50**	43.80**	26.10**
$P_4 \times P_5$	-49.80**	-67.90**	-65.63**	-7.87	-11.17	-22.86	1.55**	18.50**	49.90**

* and ** significant at 5 % and 1 % levels of probability, respectively. P_1 = Kaha1, P_2 = Dokky1, P_3 = Dokky2, P_4 = Kaha2 and P_5 = Dokky3.

Table 4. General combining ability (GCA) effects for some economic characters in five parental genotypes of cucumber

Parents	Plant length	Branch number	Internode length	Internode number	Fruit length	Fruit diameter	Fruit weight	Early yield	Total yield
P_1	-7.71**	0.77**	-0.63**	0.54	-0.20	-0.29**	-23.92**	197.25**	-538.3**
P_2	7.48**	-0.70**	0.70**	0.70	0.54**	0.03	1.36	-252.37**	280.83**
P_3	-3.86	1.01**	0.11	-0.35	-1.89**	0.38**	17.55**	129.96**	-76.41**
P_4	2.33	-0.18	0.02	-1.11**	0.35	-0.24**	-5.26	12.58	248.63**
P_5	1.76	-0.90**	-0.20	0.22	1.20**	0.12	10.27**	-87.42**	85.88**
S.E. (ĝi- ĝj)	7.31	0.60	0.35	0.89	0.43	0.15	6.21	37.23	0.76

* and ** Significant at 5 % and 1 % levels of probability. P_1 = Kaha1, P_2 = Dokky1, P_3 = Dokky2, P_4 = Kaha2 and P_5 = Dokky3

significant and positive. Among all hybrids, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_4$ and $P_2 \times P_5$ exhibited significant SCA effects for both early and total yield characters. So, these hybrids can be used in future breeding program to improve these traits.

The crosses with high significant specific combining ability effects are useful to obtain high performing hybrids. This could be because they involved parents with high × high, high × low and low × low general combining ability effects which indicated the presence of additive and dominance gene effects for controlling these traits. Also, the superiority of cross combinations involving high × low or low × low general combiners as parents may be attributed to the genetic diversity among parents, in the form of number of heterozygous loci of the parents involved in the cross combinations. Some of the parents with high GCA effects produced hybrids with low SCA effects. This may be due to lack of complementation of the parental genes. On the other hand, parents with low GCA effects produced hybrids with high SCA effects which can be attributed to complementary gene effect. Similar results were reported by Laxuman *et al.* (2012) and Singh *et al.* (2013) in *Momordica charantia* L., Hussien and Hamed (2015) in pumpkin and Reddy *et al.* (2013) in *Abelmoschus esculentus* L. Moench. Furthermore, crosses with good specific combining ability had one of its cultivars as good general combiner of that trait. This was similar to the findings of Choudhary *et al.* (2000) who observed that better performing crosses had at least one of the cultivars with high GCA effect. According to Singh *et al.* (2013), high × low general combining ability combinations are suitable for heterosis breeding, while, high × high general combining ability combinations can be considered for developing superior variants through pedigree method. The estimates of GCA effects (Table 4) revealed that none of the parents exhibited good GCA for all studied characters. So, it was difficult to pick up good combiners for all traits together because the combining ability effects were not consistent for all yield components (Solanki and Shah, 1990). This shows that genes for different desirable characters would have to be combined from different sources (Nehe *et al.*, 2007).

Phenotypic correlation: The phenotypic correlation coefficients among nine cucumber traits are presented in Table 6. Highly positive significant phenotypic correlation was recorded between total yield and early yield. Also, highly positive significant phenotypic correlation were found among plant length and both internode number and internode length. Positive significant phenotypic correlation between fruit weight and fruit diameter was found. These results are in agreement with the findings of Phani *et al.* (2018).

Cluster Analysis: Genetic divergence studies in cucumber revealed some interesting features of differentiation and

adaptability such as cluster analysis which provide additional information for studying interrelationship between genotypes and giving graphical assessment of genetic variability. For this purpose hierarchical cluster analysis, on the basis of average linkage (within groups) and interval Euclidean distance, was applied to investigate genetic distance and diversity between the five parents and their 10 F_1 hybrids. The data matrix of the dissimilarity coefficient on the basis of Euclidean distance is presented in Table 7. Wide range of genetic distance among studied genotypes reflected the presence of wide range of genetic variation and provides an opportunity to improve the genetic basis by implementing crossing. The cluster analysis obtained from UPGMA classified the fifteen (five parents and their 10 F_1 hybrids) cucumber genotypes to five clusters (Fig. 1). The first group (A): consists of four hybrids, *viz.,* $P_2 \times P_4$ (11), $P_3 \times P_5$ (14), $P_1 \times P_4$ (8) and $P_2 \times P_5$ (12). The second group (B): contains five parents plus two hybrids, *viz.,* P_3 (3), $P_1 \times P_2$ (6), P_1 (1), P_2 (2), P_4 (4), P_5 (5) and $P_4 \times P_5$ (15). While, both the third (C) and fifth groups (E) have a unique hybrid for each one $P_2 \times P_3$ (10) and $P_3 \times P_4$ (13), respectively. The fourth group (D) had two hybrids, *viz*., $P_1 \times P_3$ (7) and $P_1 \times P_5$.

Table 5. Specific combining ability (SCA) effects for some economic characters in ten hybrids of cucumber

Hybrids	Plant length	Branch number	Internode length	Internode number	Fruit length	Fruit diameter	Fruit weight	Early yield	Total yield
$P_1 \times P_2$	15.35	-1.89**	0.28	0.73	2.86**	-0.21	4.71	-828.47**	-2873.5**
$P_1 \times P_3$	24.68**	2.40**	0.37	2.44**	1.91**	-0.58**	-5.48	1965.86**	3919.37**
$P_1 \times P_4$	-6.17	-1.41	-0.87**	1.87	-1.6**	-0.42**	1.99	583.24**	3015.65**
$P_1 \times P_5$	-21.60**	-1.70**	-1.99**	-2.13*	-0.36	0.44**	14.13*	1402.57**	2263.41**
$P_2 \times P_3$	-72.84**	-2.13**	-0.46	1.63	-1.4**	0.14	28.90**	-976.52**	475.27**
$P_2 \times P_4$	-59.37**	-1.27	-2.70**	-7.60**	-1.4**	0.61**	30.04**	388.52**	650.56**
$P_2 \times P_5$	101.87**	0.11	1.18**	8.73**	-0.34	-0.53**	-8.82	880.19**	478.32**
$P_3 \times P_4$	-5.03	-1.98**	-1.11**	0.78	0.93*	0.03	-28.8**	870.52**	-1961.2**
$P_3 \times P_5$	-69.13**	0.73	-3.73**	-5.56**	-3.4**	0.31	-30.0**	-153.48**	80.22**
$P_4 \times P_5$	-54.32**	-1.75**	-2.97**	-0.80	0.45	-0.37**	20.14**	-422.43**	1547.51**
$SE_{(Sij-Sik)}$	17.91	1.47	0.85	2.19	1.05	0.36	15.22	91.21	1.86
$SE_{(Sij-Skl)}$	16.35	1.34	0.78	2.00	0.96	0.32	13.89	83.26	1.69

* and ** Significant at 5 % and 1 % levels of probability. P_1 = Kaha1, P_2 = Dokky1, P_3 = Dokky2, P_4 = Kaha2 and P_5 = Dokky3

Table 6. Phenotypic correlation coefficients among some economic characters in cucumber

Traits	Early yield	Total yield	Fruit diameter	Fruit length	No node	Internode length	Branch number	Plant length
Total yield	0.650**							
Fruit diameter	-0.232	-0.203						
Fruit length	-0.086	-0.110	-0.438					
No node	0.104	0.012	-0.482	0.304				
Internode length	-0.370	-0.372	-0.032	0.378	0.493			
Branch number	0.071	-0.155	-0.009	-0.261	0.069	0.419		
Plant length	0.050	-0.170	-0.299	0.438	0.690**	0.766**	0.324	
Fruit weight	-0.178	0.213	0.572*	-0.104	-0.127	0.035	-0.349	-0.132

*and ** Significant at 5 % and 1 % levels of probability.

Table 7. Genetic distance among five cucumber genotypes and their ten F_1 hybrids based on some economic characters

Genotypes	Rescaled Squared Euclidean Distance														
	P_1	P_2	P_3	P_4	P_5	$P_1 \times P_2$	$P_1 \times P_3$	$P_1 \times P_4$	$P_1 \times P_5$	$P_2 \times P_3$	$P_2 \times P_4$	$P_2 \times P_5$	$P_3 \times P_4$	$P_3 \times P_5$	$P_4 \times P_5$
1 P_1	1.00														
2 P_2	0.01	1.00													
3 P_3	1.00	1.00	1.00												
4 P_4	1.00	1.00	1.00	1.00											
5 P_5	0.02	0.01	0.02	0.01	1.00										
6 $P_1 \times P_2$	1.00	0.01	1.00	0.01	0.03	1.00									
7 $P_1 \times P_3$	0.23	0.23	0.20	0.25	0.35	0.18	1.00								
8 $P_1 \times P_4$	0.04	0.03	0.02	0.04	0.09	0.02	0.09	1.00							
9 $P_1 \times P_5$	0.18	0.18	0.15	0.20	0.29	0.14	1.00	0.06	1.00						
10 $P_2 \times P_3$	0.07	0.05	0.08	0.05	0.02	0.09	0.51	0.17	0.43	1.00					
11 $P_2 \times P_4$	0.02	0.01	0.01	0.02	0.05	0.01	0.14	0.01	0.10	0.11	1.00				
12 $P_2 \times P_5$	0.06	0.06	0.04	0.07	0.12	0.03	0.06	0.01	0.04	0.23	0.02	1.00			
13 $P_3 \times P_4$	0.57	0.59	0.53	0.62	0.77	0.50	0.08	0.35	0.12	1.00	0.44	0.28	1.00		
14 $P_3 \times P_5$	0.02	0.01	0.01	0.02	0.05	0.01	0.14	0.01	0.11	0.11	1.00	0.02	0.45	1.00	
15 $P_4 \times P_5$	0.02	0.01	0.02	0.01	1.00	0.03	0.34	0.08	0.28	0.02	0.04	0.12	0.75	0.04	1.00

Morphological identification of the isolated fungi: Four fungal isolates were selected depending on the differences in their culture's colors (Federation of British Plant Pathologists, 1973). These isolates were identified as *F. oxysporum* (named 1, 2, 3 and 4). Microscopic observations revealed that the mycelia of the isolates were delicate, white to pink with a purple tinge, sparse to abundant than floccose, margins slightly lobed or smooth on PDA. Microconidia formed singly, oval to reniform and without any septation. The size of microconidia ranged from 7.50-10.25 and 2.50-3.50 µm. Conidiogenous cells bearing micro- and macroconidia were monophialides type. Macroconidia were falcate to almost straight, the size of the macroconidia ranged from 20.27-30.50 and 5.00-6.75 µm. Clamydospores were present and formed singly or in pairs (Fig. 2). Isolates were identified as *F. oxysporum* due to occurrence of typical macroconidia with foot-shaped basal cells, microconidia borne in false heads only on monophialides with oval shaped and chlamydospores (Owen, 1956 and Martínez *et al.,* 2003).

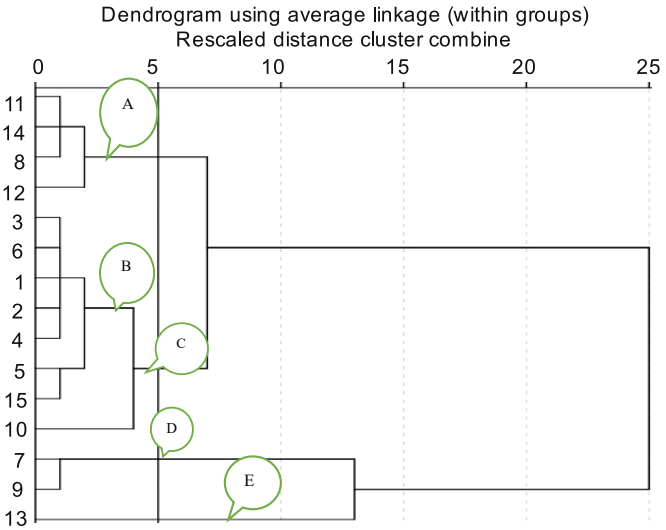

Fig. 1. Dendrogram of five parental and their 10 F_1 hybrids based on some economic traits. Numbers used for five cucumber genotypes and their ten F_1 hybrids are: 1:P_1; 2:P_2; 3:P_3; 4:P_4; 5:P_5; 6:$P_1\times P_2$; 7:$P_1\times P_3$; 8:$P_1\times P_4$; 9:$P_1\times P_5$; 10:$P_2\times P_3$; 11:$P_2\times P_4$; 12:$P_2\times P_5$; 13:$P_3\times P_4$; 14:$P_3\times P_5$; 15:$P_4\times P_5$.

Pathogenicity test: Four isolates of *F. oxysporum* were tested for their pathogenic activities on cucumber susceptible cultivar (cv. Beta alpha) as shown in Table 8. In this respect, the *F. oxysporum* isolate No.3 gave the highest disease severity (52.38 %) followed by isolate No.4 (47.78 %) without significant differences between them. On the other hand,isolate No.1 (29.58 %) and isolate No.2 (32.41 %) recorded the lowest ones without significant differences between them.

Table 8. Pathogenicity test of the four isolates of *F. oxysporum* against the susceptible cucumber cv. Beta Alpha under pots experiments

Isolates code	*F. oxysporum*				Control	LSD (P=0.05)
	1	2	3	4		
Wilt disease severity %	29.58	32.41	52.38	47.78	00.0	6.76

Due to infection with *F. oxysporum*, the host plant fails to absorb water and nutrition from the soil, and the resulting process is called "occlusion of vessels" through the formation of callose, tylose or gels. Finally, the stomata in the leaves will close and wilted, followed by death of the entire plant (Yadeta and Thomma, 2013).

Host range: *F. oxysporum* includes a lot of non-pathogenic strain in addition to host specific pathogenic strains (formae speciales). Isolate No.3 was selected in this study due to its high pathogenicity in previous trials. Data in Table 9 indicate that no symptoms were observed on the cruciferaceae (cabbage and cauliflower), solanaceae (tomato and pepper) and cucurbitaceae (Squash and watermelon) hosts. However, only a typical symptom of fusarium wilt was detected on cucumber cv. Beta Alpha.

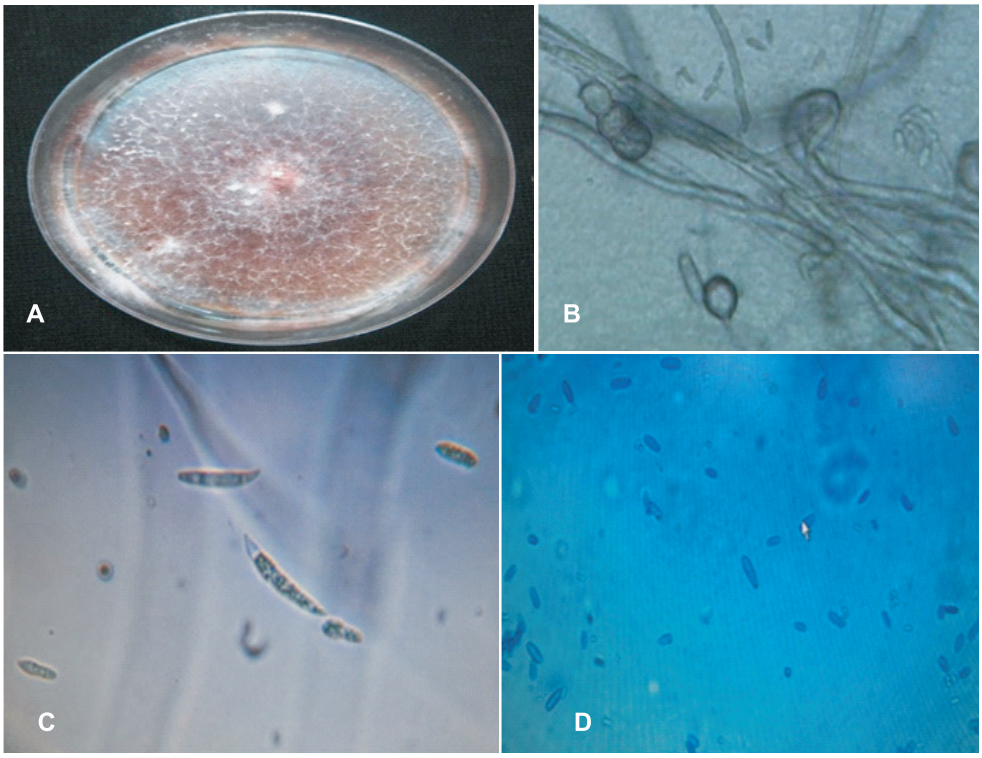

Fig. 2. Morphological identification of *Fusarium oxysporum.* A: Colony morphology of *Fusarium oxysporum* (isolate No. 3) on potato dextrose agar (PDA; 14 days old) showing fluffy growth white to pink aerial mycelia. B: Chlamydospores were present and formed singly or in pairs. C: Macroconidia with foot-shaped basal cells. D: Microconidia oval -shaped

Depending on the results of host range experiment, it is clear that the main causal organism of wilting symptoms observed on cucumber plants is *F. oxysporum* f. sp. *cucumerinum*. Most species belong to the *F. oxysporum* complex with numerous special forms attacking different plant species. Individual special form susually have a very restricted host range (Armstrong and Armstrong, 1981; Ruiz-Roldán and Di Pietro, 2012) .

Enzymatic activities, total phenol contents, DPPH- free radical scavenging activity and disease severity in pot experiment: The values of PPO, POX and CAT activities differed between genotypes as represented in Fig 3. The most susceptible genotypes, *viz.,* P_1, P_3, P_5 , $P_1\times P_2$ and $P_3\times P_4$ had the lowest PPO,

Table 9. Host range of *F. oxysporum* isolate No.3 under pots experiments

Host	Cultivar	Reaction
Tomato	Super Strain B	-
Pepper	California wonder	-
Squash	Eskandrane	-
Cucumber	Beta Alfa	+
Watermelon	Sugarbaby	-
Cauliflower	White Magic	-
Cabbage	Balady	-

POX and CAT activities associated with highest disease severity (%). On other hand, the most resistant genotypes P_2, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$ and $P_4 \times P_5$ had the highest PPO, POX and CAT activities associated with lowest disease severity (%).The results indicated that there were remarkable increase in the enzyme activities in cucumber plants depending on different models of genetic disease resistance. This is in conformity with the finding of Khatun *et al.* (2009) and Huang *et al.* (2019). Peroxidase and polyphenol oxidase are involved in the oxidation of specific host metabolites which in turn act as inhibitors of growth of the phytopathogen (Begum *et al.*, 2018).

The antioxidant enzyme CAT showed contrary activities in resistant and susceptible cultivars. Both CAT and peroxidase are antioxidant enzymes eliminating reactive oxygen species (ROSs). Catalase catalyzes elimination of H_2O_2 during oxidative damages (Luhova *et al.,* 2006). Thus, the patterns and levels of the activity of both enzymes in the resistant cultivar may be correlated with defense responses related to H_2O_2 accumulation. Additionally, H_2O_2 induces cell death and acts as a signal for induction of systemic defense responses (Heller and Tudzynski, 2011).

As can be seen from the results presented in Table 10, infection of cucumber plants with *F. oxysporum* isolate No.3 caused significant decrease in disease severity and significant increases in total phenol contents in case of genotypes $P_1 \times P_3$, $P_1 \times P_4$, $P_4 \times P_5$, $P_1 \times P_5$ and P_2, the percentages of increase in the contents of total phenol were 135.45 , 133.58 , 130.79 , 129.63 and 127.60 %, respectively. whereas, the lines P_3, $P_3 \times P_4$, P_5, $P_1 \times P_2$ and P_1 recorded 85.59, 84.85, 83.31, 71.32 and 70.13 %, respectively.

Table 10. Effect of infested soil with *F. oxysporum* f. sp. *cucumerinum* on total phenol content (TPC), radical scavenging activity (DPPH) and disease severity (D.S. %) of cucumber genotypes

Genotypes	TPC (mg/g fresh weight)	DPPH (IC^{50}mg/mL)	D.S. (%)
P_1	70.13	101.0	68.16
P_2	127.6	63.1	14.12
P_3	85.59	95.53	33.3
P_4	87.49	88.87	32.43
P_5	83.31	100.05	45.23
$P_1 \times P_2$	71.32	102.21	55.2
$P_1 \times P_3$	135.45	51.1	9.15
$P_1 \times P_4$	133.58	54.2	10.23
$P_1 \times P_5$	129.63	61.7	13.43
$P_2 \times P_3$	93.13	79.6	24.7
$P_2 \times P_4$	116.43	67.2	20.21
$P_2 \times P_5$	100.0	78.43	22.72
$P_3 \times P_4$	84.85	96.15	35.4
$P_3 \times P_5$	92.32	84.3	25
$P_4 \times P_5$	130.79	60.1	12.88
L.S.D ($P \leq 0.05$)	0.74	2.01	3.45

P_1 = Kaha1, P_2 = Dokky1, P_3 = Dokky2, P_4 = Kaha2 and P_5 = Dokky3

Total phenol content (TPC) plays an important role in the mechanism of disease resistance. They accumulated rapidly during host pathogen interactions and mediated disease suppression through participating in the oxidation of TPC to quinines, leading to the increase in antimicrobial activity and inhibiting pathogen progression. Then disease severity was negative and highly correlated with the biochemical parameters particularly in phenolic compounds, which can be used to predict disease severity as shown in Fig. 4. This could reduce the amount of work required in screening tests of disease resistance (Khatun *et al.*, 2009).

Scavenging activities of cucumber genotypes varied from 51.1 to 101.0. Genotype $P_1 \times P_3$ gave the highest content of total phenolic contents (TPC) (135.45mg/100 g), which causes its

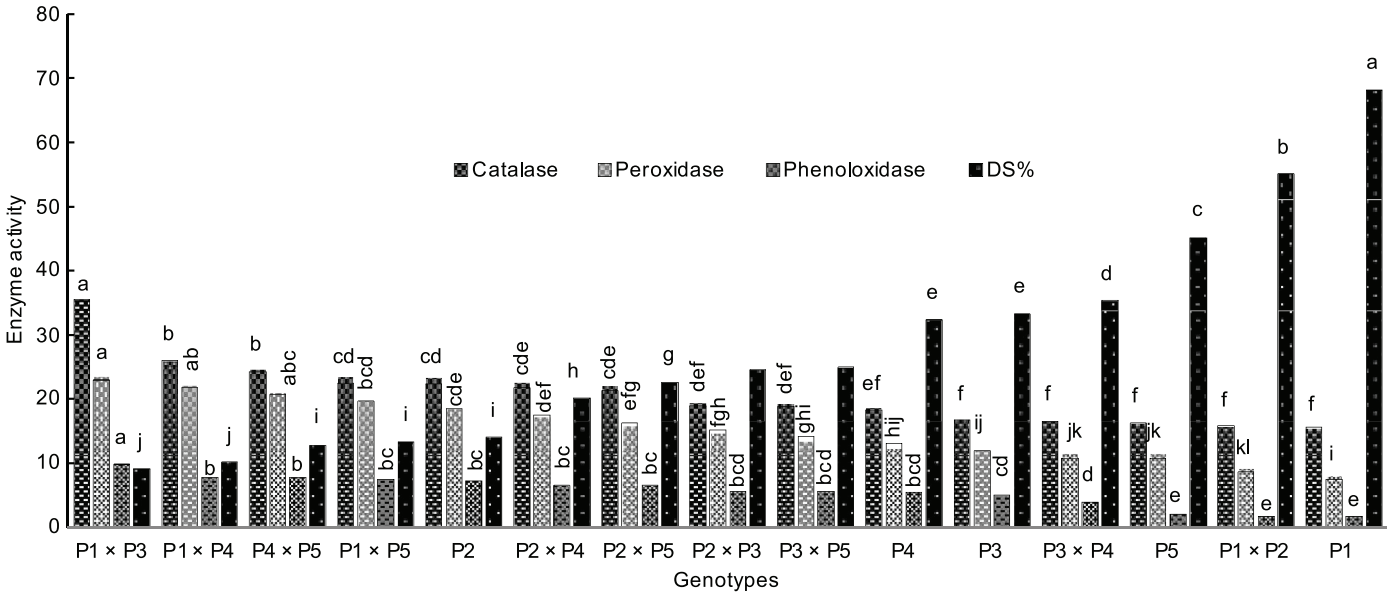

Fig. 3. Effect of infested soil with *Fusarium oxysporum* f. sp. *cucumerinum* on catalase (CAT), peroxidase (POX), poly phenol oxidase (PPO) activities and disease severity (D.S. %) of cucumber genotypes.

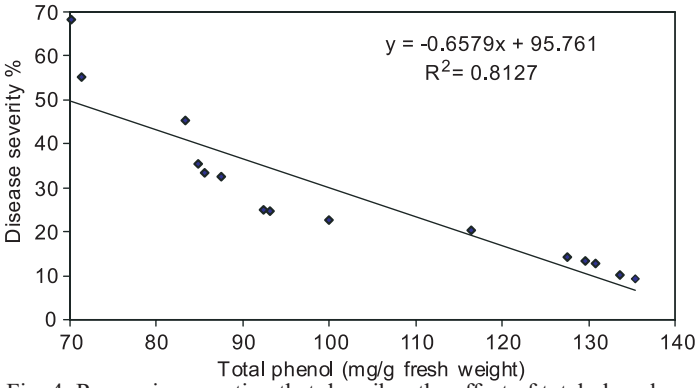

Fig. 4. Regression equation that describes the effect of total phenol on disease severity in cucumber genotypes

stronger antioxidant ability as DPPH radical scavenging activity (IC_{50} 51.1 mg/mL) followed by $P_1 \times P_4$ (IC_{50}54.2 mg/mL) and $P_4 \times P_5$ (IC_{50}60.1 mg/mL), whereas P_1 caused weak antioxidant ability as DPPH radical scavenging activity (IC_{50}101.0 mg/mL). Furthermore, there was a high correlation between the TPC and the scavenging activity. Therefore, the presence of phenolic compounds in different genotypes contributes significantly to their antioxidant potential as shown in Fig. 5. Antioxidant properties of phenolic compounds are directly linked to their structure. Indeed, phenolics are composed of one (or more) aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals (Rice-Evans *et al.*, 1996; Bors and Michel, 2002).

From this study, it can be concluded that the lines P_2, P_4 and P_5 showed significant positive GCA effects for total yield trait under fusarium wilt stress. So, these parents could be successfully used in future breeding programs. Also, among all crosses, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_4$ and $P_2 \times P_5$ exhibited significant SCA effects for both early and total yield characters under fusarium wilt stress. So, these hybrids can be used in future breeding program.

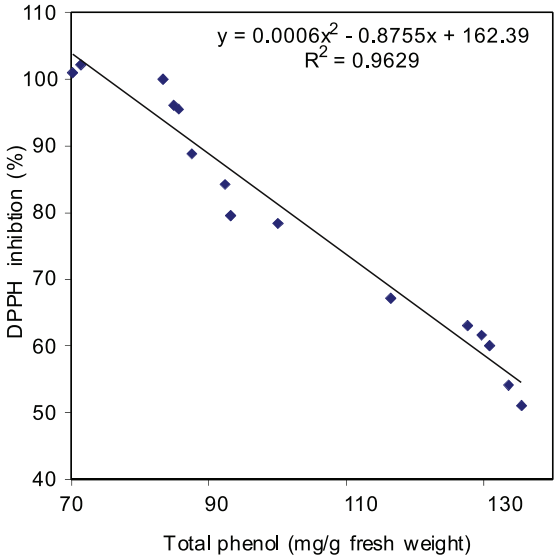

Fig. 5. Regression equation that describes the effect of total phenol on radical scavenging activities (DPPH).

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