ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

Estimating Combining Ability and Response of Tomato (*Solanum Lycopersicum l.*) Genotypes to Late Blight (*Phytophthora Infestans*) Disease

Desalegn Negasa Soresa

Wollega University Shambu Campus

*Corresponding author
Desalegn Negasa Soresa, Wollega University Shambu Campus

Submitted: 10 Aug 2022; **Accepted**: 17 Aug 2022; **Published**: 27 Sep 2022

Citation: *Soresa DN. (2022). Estimating Combining Ability and Response of Tomato (Solanum Lycopersicum l.) Genotypes to Late Blight (Phytophthora Infestans) Disease. J Gene Engg Bio Res, 4(3),328-339.*

Abstract

Eight tomato varieties were selected based on all over per se performance and late blight disease reaction. Crosses were made in 8×8 half-diallel mating design to produce 28 F1 single cross hybrids. The experiment was conducted in green-house at Shambu campus research center in 2020. Necessary data for late blight disease were recorded. In this study highly significant genotypic differences were observed indicating the existence of genetic variability among the crosses to late blight. Analysis of variance for the combining ability indicated GCA and SCA mean squares were significant at (P < 0.001) for all disease parameters. The ratios of GCA/SCA variances for all disease parameters were less than unity implying the predominance of non-additive gene actions. Among all parental lines, ARP tomato d2 and Metadel were identified as stable sources of resistant genes for late blight disease resistance with positive days of first disease appearance and negative disease severity index and AUDPC values for GCA effects. From the analysis of epidemiological data and disease progress curves the Logistic model (R2=96.5) better described the disease progress curves than the Gompertz model (R2=92.5) indicating the presence of delays in epidemics and the infection point of the late blight. ARP tomato d2 and Metadel were identified as a good general combiners for late blight disease parameters. Thus, these parents were recommended to be used in breeding programs with a purpose of developing late blight resistant single cross hybrids. In conclusion of the study, ARP tomato d2 and Bishola parents could be used as a source of resistance gene and potential late blight resistant single cross hybrids (ARP tomato d2 x Metadel, Fetan x ARP tomatod2, Bishola x ARP tomatod2, Metadel x Miya and Bishola x Metadel) were identified. Therefore, it is recommended that these hybrids can be used for direct production where this disease is the most prevalent and/or for further breeding programs in generating hybrids for future use.

Keywords: AUDPC, Cross, Disease, Severity

Introduction

Most horticultural crops are generally susceptible to biotic and abiotic stresses. Particularly tomato, among the *solanaceous* family is threatened by biotic stresses like fungal, bacterial and viral diseases. Late blight (*Phytophthora infestans*) which is the common devastating false fungi to tomato and potato crops has been identified as a major disease of tomato and potato and is one of the most devastating plant diseases of all time. An unprotected tomato field can suffer yield losses reaching up to 100% because of late blight infection [1]. *Phytophthora infestans*– literally, "plant destroyer," in Greek – has been traced back to the same origin as tomatoes and potatoes, that is, the Andean region [2, 3]. *Phytophtora infestans* can quickly devastate tomato and potato crops at any time during plant ontogeny. The entire plant may collapse in five to ten days. Late blight can infect all aboveground parts of the plant, causing leaf and stem necrosis, fruit rot, and eventual plant death. The pathogen can also infect tomato seed and potato tubers [4, 5]. Initial infection symptoms, including small lesions on leaf tips and plant stems, are visible only after three to four days, and in some cases reach only 1 to 2 mm in diameter. The purple, dark brown or black water-soaked lesions often have a pale yellowish-green border that blends into the healthy tissue. As the pathogen penetrates the plant tissue, lesions enlarge in size [6]. Fluffy, white sporangia may grow on the lower leaflet surface in moist weather. As the disease progresses, plant leaflets shrivel and die and the disease spreads to the rest of the foliage, leading to extensive defoliation. Dark brown Late blight lesions first appear at the top of the stem or at a node and may progress down the stem. Firm, brown, and greasy tomato fruit lesions are often located at the stem end and sides of green fruit, rendering them unmarketable. Infected tomato fruit may be invaded by secondary pathogens, causing soft-rot disease [7].

Resistance to diseases and pests is one of the common breeding objectives in tomato which is breeding for resistance to the most destructive pests and pathogens. With the release of tomato genome sequences, a significant advance in exploitation of polygenic resistance was obtained using molecular markers to all the known genes for resistance. The development of disease resistant tomato cultivars is perhaps one of the most important contributions of modern plant breeding to tomato improvement

[8]. Resistance to some pests and pathogens has been transferred from the wild into cultivated species. For example, the resistance to *Cladosporium fulvum* was obtained from *S. pimpinelli folium*. Other diseases of interest to breeders include late blight resistances, Fusarium wilt, and tomato spotted wilt virus [9].

Recent observations confirm virulence of the pathogen towards potato, but also tomato: Once an unprotected crop (field, greenhouse, and/or plastic-cover cultures) is infected by *P. infestans*, the whole crop can be devastated within seven to ten days [10]. Economic losses may be in the form of reduced yield, lower quality of the fruit (such as low specific gravity), diminished storability, and increased cost. Total crop failure due to diseases has been common in the region and farmers are sometimes forced to abandon their production due to excessive infection pressure in the field [11]. Despite this fact the identity and relative importance of each disease across locations has not been well profiled and no document information which can be used in developing integrated management strategy against tomato diseases. More over most tomato cultivars are threatened with late blight disease up to total yield loss. According to the use of fungicides to control late blight problem may lead to the emergence of resistant *P. infestans* strains which could result even the worst economic and environmental problem. Moreover, currently in Ethiopia the most adopted commercial tomato varieties by the farmers are becoming susceptible to this disease. This can strongly affect the lively hood of millions of farmers who are partly relying on tomato as income crop leading to failure of their economy and food security envisioned by the country. Thus, searching for late blight resistant hybrids remain the crux of the matter [12].

Disease severity in any plant-path system can be assessed either once at the peak of the epidemic or several times at some intervals starting from disease initiation until the end of the epidemic. The former method of assessment measures the cumulative effects of all the factors operating during the course of epidemic viz. the terminal disease severity scores (TDS), while the latter can be used to estimate different parameters like the area under the disease progress curves (AUDPC), relative area under the disease progress curve (RAUDPC), logistic (r) and Gompertz (k) apparent infection rates, the time required for the disease to reach a specific level of severity in logistic and Gompertz models [13].

Estimating combining ability, the nature and magnitude of gene action is an important factor in developing an effective breeding program, which can be understood through combining ability analysis. This information is helpful to plant breeders, pathologists and geneticist for formulating hybrid breeding programs for better yield and disease resistance [14]. Currently in Ethiopia the most adopted commercial tomato hybrids by the farmers are becoming Susceptible to late blight disease. This can strongly affect the lively hood of millions of farmers who are relaying on tomato leading to failure of the food self-sufficiency and food security envisioned by the country. Thus, searching for late blight resistant hybrids remain the crux of the matter.

Hence, the objectives of this study was to determine the combining ability and nature of gene action for resistance to late blight and identify resistant hybrids.

Materials and Methods

Description of the Study Area

The experiment was conducted at Shambu campus Research site (in green house) Shambu, in Horo Guduru Wollega Zone, Western Ethiopia. Shambu site is located at 344 km to west of Addis Abeba. The area is characterized by one long rainy season (May to September) with a mean annual rainfall of 1950 mm, and an altitude of 2400 m.a.s.l. According to the report of the maximum average and minimum average air temperature for the area, Shambu, is 22oC and 120C respectively. The soils of the areas predominantly of clay loam type [15]. Late blight test for parents and crosses was carried out in laboratory and the morphology of disease was checked by Ambo Agricultural Research Center (AmbARC).

Experimental Materials

The experiment consisted of 36 tomato genotypes (eight parents and 28 F1 crosses obtained from the parent half diallel crosses). Eight tomato parents or varieties released from Melkasa Agricultural Research Center were used for crossing. The parents were crossed in half diallel cross fashion to obtain 28 F1 cross seeds, the varieties have been selected based on their agronomic performance. Parents with the characteristics of resistance and susceptible to late blight were used in separated pot.

Isolation of the Pathogen and Inoculum Preparation Sample Collection and Isolation

The fresh samples with typical symptoms (lesion and a white mildew under leave) were collected from different farmer's field. The samples kept covered with tissue paper for 24hrs to remove field moisture. A fresh potato tuber free of infection was cut 70% and the disease sample was put inside the cut after rinsed with tap water to remove debris. The cut tuber was sealed with adhesive tape, put into a plastic bag for two to three days for sporangial emergence and taken into incubation at 15 – 18oC for about seven days for sporangial initiation.

The inoculated potato tubers were then cut into 7x7cm2 pieces and each piece was put in the moist petri-dish with artificial carrot media and incubated at 15 – 18ºC.

The artificial carrot media was prepared following procedures [16].

Procedures of Media Preparation

- 220gms of fresh red carrot was washed and cut into small pieces.
- The cut carrot was autoclaved with 500ml of double distilled water.
- By wearing gloves the mixture was blend and squeezed with 3-4 layered muslin cloth.
- 20gms of sucrose and 9gms of agar was added and sterilized by

autoclaving at 15lbs pressure (121 °C) for 20 minutes.
•When the media reaches to 45-50oC and then poured into the petri plates.

After the preparation of media, the previously incubated potato sample was taken and transferred to petri dish containing new media in safe environment (under laminar flow hood and sterilized equipment).The white colony of late blight pathogen was removed from tuber slices then inoculated on plate medium. After culturing for seven to ten days at 15 – 18ºC, the pure colony of Phytophthora infestans was transferred onto slant medium. Fifteen days later, development of sporangia was observed. To check the development of sporangia, the samples were taken from each petri dish and the well developments of sporangia were observed under microscopes. The sporangia were collected from all Petri dishes and mixed together uniformly in sterilized distilled water to get sporangial suspension. To make mixture, nearly 1g of sporangial suspension and 100ml of distilled water was used following the method of then the sporangial suspension was put in the dark for 90 to 100 minutes at 11 to 12°C before inoculation for initiation.

Inoculation

Five plants from each genotype were raised in a 40cm diameter pot in a greenhouse. Seven weeks old tomato plants were then inoculated using hand sprayer by spraying to run-off with sporangial Suspension. For each genotype (five plants per growing container) 50ml of sporangial suspension was used for inoculation. The inoculation was done early in the evening starting at 7:00 pm. The average temperature inside the green house during the inoculation was relatively 14°C and the relative humidity (RH) was 83%. Fifteen days after inoculation, the time which genotypes severely attacked, by visual observation of the plants, estimation of diseased proportion of the plants and estimation of level of resistance was made based on the scale descriptions (Table 2) and method. There were two control containers with one with late blight susceptible variety (Melkasalsa) and the other with late blight resistance variety (ARP tomatod2) to check the natural late blight infestation effect in lath house.

Data Collection

The following disease data were recorded

Days to First Disease Appearance

The days starting from inoculation to the days of first disease symptom appearance was recorded

Disease Severity Index (%)

(Sum of numerical ratings/ total No. plants observed) × (100/9); was recorded from five plants of each pot 15 days after inoculation from area of diseased tissue as described by [17]. To accommodate any variations and peculiarities of disease progression attributed to the stage of plant infection and prevailing weather conditions, disease severity was recorded three times at intervals of seven days. Rating started when obvious genotypic differences for late blight reaction became apparent and continued until the leaves started to senescence (14 DAI to 35 DAI). Disease severity scores were converted into percentage severity index (PSI) using the formula given by Disease observation for PSI was made 15-18 days after inoculations using a 0- 9 scale [18, 19].

Disease Progress Rate

Logistic, ln [(Y/1-Y)] (van der Plank, 1963), and Gompertz, -ln [-ln(Y)] (Berger, 1981) models were compared for estimation of disease progression rate from each treatment, and the logistic model was found fit to the data. The goodness of fit of the models was tested based on the magnitude of the coefficient of determination (R2). The transformed data of disease severity were regressed over time to determine the model. The model was then used to determine the apparent rate of disease increase (r) and the intercept of the curve.

$$Yt=\frac{1}{1+exp-(Yo(1-yo)1+rt}$$ Where, Y=severity, t=time taken

Area under Disease Progress Curve (AUDPC)

It was also computed from PSI values for each plot as described by [20].

$$AUDPC=\sum_{i=1}^{n-1} 0.5(xi + xi + 1)(ti+1-ti)$$

Where, xi = the proportion of host tissue damaged at the ith day
ti = the time in days after appearance of the disease at the ith day

n = the total number of observations or the total number of disease assessments.

AUDPC was expressed in %-days because severity (x) is expressed in percent and time (t) in days. Table 2. The Scales for late blight assessment of whole plants assay

Rating	Plant assay	Level of resistance category	% of disease index
1	No infections	Immune	0
2	First symptoms as grey-green to brown lesion	Very highly resistant	1-10
3	Symptoms obvious. Yellowing or browning of some leaves or small lesions 50% of plant height	Highly resistant	11-20
4	Increased yellowing or browning, or small lesions to 75% of plant height	Resistant	21-30
5	Plant severely affected, about 50% of the leaves dead	moderate resistant	31-40
6	Yellowing or browning to 50% of plant height	Less moderate resistant	41-50
7	Yellowing or browning to 75% of plant height	susceptible	51-60
8	Entire plant yellow to brown all leaves infected	Highly susceptible	61-70
9	All leaves dead	Very highly susceptible	>71

Data Analysis

The disease data were subjected to analysis of variance (ANOVA) following the standard procedures given by Steel and Torrie (1980) using SAS computer software (SAS, 2011 version 9.3). Prior to analysis, raw data were checked for homogeneity of error variances using Bartlett's test. Statistically significant difference among the genotypes for the character being studied justifies further statistical analysis for that character.

Combining Ability Analysis and Late Blight Epidemics

Diallel cross analysis was carried out using Griffing's (1956) Model I (fixed), Method II (involving parents and one set of F1 hybrids) to estimate components of variance due to general combining ability (GCA) and specific combining ability (SCA). Smaller values of SCA and GCA effects were considered desirable for all disease parameters while higher values were considered desirable for yield and yield components. The variances ratios of GCA and SCA were estimated to identify the nature of gene actions for late blight disease [21]. For F-tests, main effects such as entries and its partitions, GCA and SCA mean squares were tested against their respective error. Significances of GCA and SCA effects were determined by t-test, using standard errors of GCA and SCA effect as in the yield analysis.

Moreover, associations between tomato late blight and yield parameters were examined using simple correlation analyses. Pearson correlation coefficients (r) were used as indices for strength of the associations.

Results and Discussion

Analysis of Variance For Disease

From the analysis of variance it was observed that significant differences were observed for all late blight disease parameters indicating the existence of sufficient genetic variability among evaluated genotypes for resistance against late blight disease. The presented coefficients of determination was greater than 80% for all disease parameters indicates that only more percentage of the variation in the dependent variable, fruit yield is explained by the linear function of the independent variables considered (PSI, FDA, AUDPC and DPR). In other word, little percentage of the variation in fruit yield could not be accounted for by the regression. The present result was in agreement with the previous report of [22].

Mean value of disease parameters

Days to first disease appearance

Among the eight parents, Metadel and ARP tomatod2 had above 14 days of first disease appearance after inoculation compared to Melkaselsa (8.99 days after inoculation) and Miya (9.5 days after inoculation) indicating the susceptibility of these parents in short day after inoculation.(Table 3). This indicates these parents (Metadel and ARP tomatod2) possess significantly different in late blight disease resistance genes as compared to Melkaselsa and Miya which had 9.0 days (Table 3). In the same way, crosses as Fetan x Miya (9 days after inoculation) and Melk shola x Fetan (9 days after inoculation) showed the susceptibility short day after inoculation. In contrast, crosses like Melkashola x Metadel (15 days after inoculation), ARP tomato2 x Metadel (15 days after inoculation) and Chali x ARP tomatod2 (14.5 days after inoculation) had longer days to disease appearance after inoculation. This also revealed as resistant genotypes have longer periods of first disease appearance than susceptible once i.e, disease symptom appears on susceptible varieties earlier than resistant varieties and can be concluded that a line or crosses which had longer DFDA might indicate the existence of resistance genes whereas smaller values of DFDA indicated susceptibility. These results were in agreement with the findings of [23, 24].

Percentage of Severity Index

Based on PSI values categorized tomato genotypes in to four distinct groups. One group included genotypes exhibiting PSI values up to 35% are resistance, the second group included genotypes showing PSI values between 35-40% are moderately resistance. The third and fourth group included genotypes with PSI values of 41-60% susceptible and > 60%, highly susceptible respectively. In this study, none of the tested genotype were completely free from infection. Only two genotypes APR tomatod2 and Metadel x ARP tomatod2 were moderately resistant with 36.25% and 40.3% PSI values, respectively. The level of late blight resistance in these genotypes was relatively higher than most other genotypes suggesting that this may carry resistant gene(s) and can be used as resistance source in breeding for late blight resistance in tomato.

In the present study, among the crosses, only one cross (APR tomatod2 and Metadel) showed less severity value, indicating the crosses were resistant to late blight disease where as some

crosses showed high disease severity values and vulnerability to late blight disease. Most resistant crosses had at least one parent resistant parents; this might be the dominant effect of resistance gene while susceptibility could be the minor effects of resistant parent or dominant effects of susceptible parent gene. The genotypes ARP tomato d2 (36.25%) and cross ARP tomato d2 x Metadel (40.3%) recorded relatively lower infected tissue for late blight infection assessment followed by Bishola (46.25%), Metadel (51.25%) cross Fetan x ARP tomatod2 (48.75%) cross Bishola x ARP tomatod2 (42.5%), crosses Metadel x Miya (47.25%) and crosses Bishola x Metadel (51.2%) revealing their relatively better resistance to late blight infection (Table 3). As this assessment was undertaken at the juvenile stage, those genotypes registered low PIS especially less than 50% PSI has the opportunity to give fruit yield though not sufficient. The result is in agreements with the finding of who reported that tomato is highly susceptible to late blight after flowering stage than during seedling stage.

The records of relatively lower percentage of infected leaves and or whole plant categorized these genotypes under moderate resistant category in compared to others. These genotypes are from high to moderate yielder in marketable fruit yield in relation to other genotypes in the experiment. However, the other genotypes were highly susceptible with PSI values ranging from 61-97.5% Categorized under susceptible and very highly susceptible category to late blight infection. These results were in agreement with the findings.

The result indicates that most of (>50%) of the tested genotypes were categorized under susceptible, or highly susceptible category to late blight infection. This may be due to the high favoring environments for growth, development and infection of late blight. In addition, P. infestans strain used in this trial may have the ability to infect genotypes having all major late blight resistant genes resulting most genotypes to show great susceptibility to late blight. This result is in line with the findings of whom reported that coldness or heavy wetness increase the development of late blight. In addition, the variation in genotypic difference in response to late blight resistance might be due to the genetic constituents of genotypes. However, this result disagree with the finding of who stated that if more varietal diversity is available for plant resistance against late blight, the disease severity would be reduced if any given mixture or variety is grown [25, 26].

Area under Disease Progress Curve

In the case of AUDPC, parents showed lower ARP tomatod2 (384.05) and Metadel (412.13) AUDPC score values and were considered as resistant while, parents Melkaselsa (556.5), Melkashola (555.52), Chali (565.6) and Miya (551.5) showed higher AUDPC score values and were considered as susceptible to late blight (Table 3).

In progenies, AUDPC for severity ranged from 357.9 (Metadel x Miya) to 570.50 (Fetan x Chali). The crosses which showed lower AUDPC score values were considered as resistant, on the other hand crosses which exhibited higher values were considered as susceptible. Crosses considered as susceptible such as Melkashola x Miya (564.65), Bishola x Miya (545.5), Fetan x ARP tomatod2 (537.85), Bishola x Metadel (539.85) and Melkaselsa x Chali (529.27) had higher values of AUDPC as compare to other crosses while Melkashola x Chali (384.0), Metadel x Miya (357.9), Melkashola x Chali (384.0) and Miya x Chali (408) crosses had consistently lower AUDPC values and they are more resistant to late blight disease. Similarly, higher values of area under disease progress curves on susceptible varieties than resistant varieties were reported. Generally, genotypes which showed resistance were expressed in terms of longer days to disease appearance, lesser disease severity as well as reduced area under disease progress curves (AUDPC) for infected per plant and disease severity.

Disease Progress Rate (DPR)

The rates of late blight progress highly and significantly ($p \leq 0.001$) differed among the tomato parents and crosses (Table 3). In the parent study, the lowest mean DPR was calculated from ARP tomato d2 (0.029) and Metadel (0.031) units per day, respectively. While, the highest mean of DPRs unit per day was registered from parent Melkaselsa (0.045) and Bishola (0.0436) compared to others.

In addition, disease progress rates varied very highly and significantly ($p \leq 0.001$) among treatments in crosses. The highest mean DPRs on different crosses were observed. Those, the range of the rate was varied from 0.0272) in unit per day (ARP tomato d2 x Metadel) and Melkashola x Metadel (0.0274) to 0.0452 in unit per day (Fetan x Chali) and (Melkaselsa x Chali) (Table 3).

Variation in DPRs of late blight among the varieties might be due to the genetic background of the varieties and the importance of environmental conditions, which might have favor or delayed the development of the target pathogen during the growing periods. Reported that resistant gene in the host, environmental condition and frequent application of fungicides could retard the rate of late blight progress on potato (alternate host for the pathogen) in the field.

Table 3: Mean Value Of Late Blight Disease Parameters for Parents and F1 Hybrids Studied In Greenhouse

Gen	AUPDC	DFDA	DPR	PSI
Melkashola	555.25 abcd	10.5 jk	0.0410 abc	82.5 ef
Bishola	509.00 abc	10.5 hij	0.0436 abef	46.25 hi
Metadel	412.75 jkl	14 cde	0.0310 fgi	51.25 lm
Fetan	481.35 ghi	12.5 def	0.0400 defhi	66 ghijkl
Melkaselsa	556.60 cdefgh	8.99 kl	0.0451 ab	91.250 ab
Miya	551.50 abcde	9.5 ijk	0.042 abcd	88.7 abc
Chali	565.60 ab	10.5 hij	0.041 abde	82.50 def
ARP tomatod2	384.05 kl	14.5 ab	0.0291 pq	36.52 mn
Bishola x Chali	494.60 efghi	11.5 fgh	0.0435 abef	66 ghijk
Bishola x Miya	545.50 cdef	11.5 fgh	0.0425 abef	61.2 klm
Bishola x Fetan	524.15 defg	11 .0 ghi	0.0427 abef	54.05 lmn
Metadel x Melkaselsa	480.70 ef	11 .0 jk	0.046 a	92.500 a
Melkashola x Melkaselsa	500.00 defgh	11.5 fgh	0.0418 adeh	59.35 lm
Fetan x Chali	570.50 a	10.0 ijk	0.0420 abcf	86.8 abcde
Melkashola x Miya	564.75 abc	13.0 cde	0.0342 kop	72. fgijk
Bishola x Melkaselsa	419.30 jkl	11.0 abc	0.0296 oq	51.25 mn
Miya x ARP tomatod2	438.95 ijk	10.0 ijk	0.0431 abcf	82.5 abef
Chali x ARP tomatod2	556.25 abcd	14 .5 ijk	0.0427 abef	89.70 abc
Melkashola xMetadel	532.00 abcde	15.0 a	0.0274 q	43.9 n
Melkashola x Chali	384.00 kl	11.5 fgh	0.0392 fijk	77.5cfgh
Metadel x Fetan	538.30 abcde	13.5 bcd	0.0323 mno	55 lmn
Miya x Chali	408.10 jkl	13.5 bcd	0.0333 lop	60.5 klm
Fetan x Melkaselsa	448.30 hij	12 .0 efg	0.0344 jklo	65.5 hkl
Melkaselsa x Miya	484.95 ghi	10.5 hij	0.0410 bcghi	77 cfgh
Fetan x ARP tomatod2	537.85 efg	13. 0 cde	0.0381 jkl	48.75 lm
Metadel x Chali	490.95 fghi	11.5 fgh	0.0392 fghk	78.7 bcfg
Fetan x Miya	508.70 bcdefg	9.0 k	0.0452 abc	93.400 a
Melkashola x Bishola	487.95 fghi	12.0 efg	0.0360 ijmn	81 cdef
Melkashola x ARP tomatod2	421.80 jkl	13.0 cde	0.0317 mn	63.8 ijkl
Bishola x ARP tomatod2	438.90 ijk	12.5 def	0.0394 efghj	42.5 ghijkl
Melkaselsa x Chali	529.70 cde	10.5 hij	0.0452 cdefi	82 cdef
Metadel x Miya	357.90 lmn	9.5 jk	0.0423 abcf	47.2.5 lm
Melkashola x Fetan	554.25 abcd	9.0 k	0.0448 abcd	91.250 ab
Melkaselsa x ARP tomatod2	414.70 jkl	12.5 def	0.0370 hijk	75 efghij
Bishola x Metadel	539.05 fg	9.5 jk	0.0408 bcdef	51.2 abcde
Metadel x ARP tomatod2	369.25 l	15.5 ab	0.0272 q	40.3 n
LSD 5%	58.4	1.47	0.0051	12.45

Where AUDPC-area under disease progress curve, PSI-percentage of severity index, FDA-first day disease appearance, and DPR-disease progress rate

Analysis of Late Blight Disease Progress Rates Using The Gompertz And Logistic Models For 28 F1 Hybrids

Results of late blight disease development and yield in comparison with Logistic model and Gompertz model were presented in (Table 4). All rates and adjusted coefficients of determination were significant at P≤0.01. The highest disease progress rates were at late times after of inoculation. Rate of late blight disease development was greater at the latest time after inoculation for both model even though their value is close to each other (Table 4).

Analyses of epidemiological data for late blight disease development revealed that the logistic model (R2=96.5) described the disease progress curves more accurately than the Gompertz model (R2=92.5) (Table 4). Large differences have been noted between epidemic progresses in larger field experiments whereas, such small difference could be observed in laboratories, green house and other controlled environments (Berger, 1981). This variation might have happened due to the differences in epidemiological factors of the experimental locations, seasonal variation, and cultivar differences and the nature of the patho system prevailed during the epidemic periods [27, 28].

All intercept values with the logistic and Gompertz models were almost similar. In addition, all rate and adjusted coefficient of determination values are significant at P ≤ 0.01 revealing large differences have been noted between epidemic progresses. The present result is in agreement with the finding of [29].

Table 4: Disease Progress Rates, Intercepts and Adjusted Coefficients of Determination for Late Blight Disease of Tomato Tested For Logistic and Gompertz Model

Days after inoculation	Logistic			Gompertz		
	Rates	Intercept	AdjR2 (%)	Rates	Intercept	AdjR2 (%)
15 days AI	0.021	-12.3	92.8	0.024	-9.71	91.5
22 days AI	0.035	-13.23	89.3	0.032	-10.25	88.12
29 days AI	0.043	-15.65	82.6	0.045	-12.16	81.4
Lsd 5%	**	ns	**	**	ns	**

Ns= non-significant, *, **= significant at 5% and 1 %

Model equations were y = 1/ (1 + exp (− [a + rt]) for the logistic model and y = exp (−B exp (−kt)) for the Gompertz model, in which r and k represent the rate parameters for logistic and Gompertz models, respectively.

Estimation of GCA Effects

The estimates of GCA effects of parents for late blight disease parameters are shown in Table 6. The GCA effects for days to first disease appearance ranged from 0.873 values for ARP tomatod2 to -0.732 values for Chali. This indicates that Chali was infected very soon after inoculation date by late blight disease while the ARP tomatod2 was very resistant exhibiting longer latent period. In addition, Melkaselsa and Fetan had negative and significant GCA effects, whereas, Miya, Bishola, Metadel and Melkashola showed positive and significant GCA effects (Table 5).

The GCA effects for area under disease progress curve ranged from -45.3 values for ARP tomatod2 to 23.7 values for Bishola. This indicates that Bishola was infected very highly by late blight disease while the ARP tomatod2 was found more resistant exhibiting less area under disease progress curve. In addition, Melkaselsa, Chali, Miya and Fetan had positive and significant GCA effects, whereas Metadel showed negative and significant GCA effects (Table 5).

The range of general combining ability effect for percentage of severity index was ranged from (- 9) value for ARP tomatod2 and (-8.9) for Metadel to (6.8) value for Melkaselsa. In addition Miya, Chali, Bishola and Melkashola were parents that showed positive general combining ability effect (Table 5). In other case, parent ARP tomatod2, Metadel and Chali showed negative and desirable Direction for disease progress rate whereas, other parents registered positive and significant value for disease progress rate on late blight disease.

On the other hand the negative and significant GCA effects of ARP tomatod2 and Metadel for, disease severity index, disease progress rate and AUDPC indicated that these parents would be useful sources of resistance to produce hybrids with reduced late blight disease severity whereas Melkaselsa, Chali and Fetan had positive and significant GCA effects suggesting that they contribute high disease pressure in their crosses. Therefore, parents ARP tomatod2 and Metadel are the best general combiners due to having negative and significant GCA effects whereas Melkaselsa, Chali and Fetan were the poorest combiners due to positive and significant GCA effects. Over all, parents such as ARP tomatod2 and Metadel were the best combiners to develop late blight disease resistance in all disease parameters whereas Melkaselsa, Chali and Fetan were the poorest combiners and contribute susceptibility to their crosses (Table 5). Except days of first disease appearance, a line which exhibited negative GCA effects in all disease parameters for late blight disease would be expected to be a useful source of resistance gene and may contribute resistance in their crosses.

Table 5: Estimates of GCA Effect for Four Disease Parameters of Eight Tomato Parents Genotypes

Parents	DFDA	AUDPC	DPR	PSI
Melkashola	0.325*	10.7	0.0027	2.6*
Bishola	0.423*	23.78*	0.0026	1.1*
Metadel	0.47*	-24.8*	-0.0025*	-8.9*
Fetan	-0.375	1.64	-0.00078	-0.9*
Melkasels	-0.672*	12.95*	0.0012	6.3*
Miya	0.175	16.84*	0.00063	4*
Chali	-0.723*	4.3	-0.00064	3*
ARP tomatod2	0.873*	-45.3*	-0.0038*	-9*
SE(gi)	0.26	10.4	0.00089	0.8
SE(gi-gi)	0.35	11.21	0.0019	0.2

Where AUDPC-area under disease progress curve, PSI-percentage of severity index, DFDA- day to first disease appearance, and DPR-disease progress rate

Estimation of Nature of Gene Action For Late Blight

Analysis of variance for GCA and SCA components of resistance to late blight disease traits are presented in (Table 6). Mean squares of GCA revealed highly significant ($p < 0.01$) difference for all disease parameters except for disease progress rate. Mean square of SCA also showed significant difference at ($P < 0.01$). Traits which showed significant difference of GCA indicated the presence of additive gene action while of SCA indicated non-additive gene action. This result indicated both GCA and SCA contributed for late blight disease resistance (Table 6). Studies by other researchers using different tomato populations have indicated similar findings where additive gene action was more important than non-additive gene action [30]. In contrast, studies conducted in the USA using collected germplasms reported 100% GCA contribution to the variation for late blight resistance implying that it would be possible to determine progeny performance for late blight disease resistance for those materials based on GCA alone. However, since the results in each case apply to the specific reference populations used, the variations observed amongst different researchers are therefore a result of the different tomato parents, environments used and possibly late blight isolates. For disease resistance, negative GCA and SCA effects are desirable. Several studies have shown that, late blight disease is mainly controlled by additive and non-additive gene interaction in tomato and potato. However, other studies observed late blight disease resistance were controlled only by additive, while dominance and epistasis contributions are normally non-significant [31].

Analysis of variances due to GCA and SCA effects were highly significant for both characters indicating differences among hybrids (Table 6). Significant GCA and SCA mean squares were also reported. The ratio of GCA to SCA variances was less than unity for percentage of severity, AUDPC, days to first disease appearance indicating that non-additive type of gene action was playing a greater role in the inheritance of these traits (Table 6). In general, non-additive genes had greater importance for all disease parameters to control late blight disease. Similar findings were reported by. In trying to establish, the relative importance of GCA and SCA in determining progeny performance, the ratio of 2 σ2 g/ (2 σ2g+σ2 s) was found close to unity in the case of late blight indicating that late blight reaction of hybrids should be predictable on the basis of both GCA SCA effects.

The GCA effects accounted for a greater proportion of the sum of squares than the SCA effects for late blight disease severity (89%) and fruit yield (t ha-1) (78%), SCA for late blight disease severity (34%) and for fruit yield (t ha-1) (32%), not presented in table, revealed additive gene action is more important. Resistance to late blight is inherited quantitatively by genes that act primarily in an additive manner and is expressed as the rate disease reducing resistance. Quantitative resistance to late blight leads to prolonged latent and incubation periods, reduced infection rates and sporulation capacity. Thus, both additive and dominance effects are important in the expression of partial resistance traits in tomato. The importance of additive effects from breeders' point of view is essential for predictability in gene expression as genes contribute to traits in additive manner especially for quantitative ones while with dominance effect can easily identify hybrids with promising performance because of dominance nature of the traits [32, 33].

Table 6: Analysis Of Variance for Combining Ability and Gene Action

variables	Mean square		Error	Variance		Ϭ²GCA/ SCA
	GCA	SCA		Ϭ²GCA	Ϭ²SCA	
	DF=7	DF=28				
DFDA	14.45**	10.99**	0.47	1.39	10.43	0.13
AUPDC	25125.7**	14708.9**	744.5	2438	13964.4	0.17
DPR	0.00024*	0.000098*	0.000056	1.8	4.2	0.042
PSI	1521**	600**	22.7	149.8	573.3	0.26

Where AUDPC-area under disease progress curve, PSI-percentage of severity index, DFDA- days to first disease appearance, DPR-disease progress rate, Ϭ2GCA-GCA variance and Ϭ2SCA- SCA variance

Estimation of Specific Combining Ability Effect

Estimates of specific combining ability (SCA) effects for the crosses were presented in (Table 7).Crosses Metadel x Chali (3.12), Metadel x ARP tomatod2 (0.93), Fetan x Melkaselsa (1.22), Chali x Melkaselsa (1.67), Bishola x ARP tomatod2 (2.01), Melkashola x Fetan (0.92), Melkashola x Melkaselsa (0.93) and Bishila x Chali (0.92) had positive and significant SCA effects for days to first disease appearance (DFDA). This result indicated that the generated crosses exhibited longer days of disease appearance. This is owing to the manifestation of additive gene action in the good

Crosses complementing each other in favoring late blight disease resistance. On the contrary, crosses ARP tomatod2 x Fetan (-2.03), Melkaselsa x ARP tomatod2 (-1.41), Bishola x ARP tomatod2 (-2.01), Fetan x Chali (-0.87), Metadel x Miya (-1.76), Metadel x Melkaselsa (-2.77) and Bishola x Miya (-1.41) had showed negative and significant SCA effects (Table 7), indicating that these crosses exhibited shorter days of late blight appearance than the predicted values based on their parental performance. This result indicated that the combination of these parents showed lower disease severity as compared to the average value of their respective parents. The reason could be the dominant effect or the interaction of non-allelic effects between parents. The most desirable genotypes would be those that have delayed onset of the disease having specific or vertical resistance [34].

In the case of AUDPC values for disease severity, crosses Mlkashola x Fetan (-69.1), Mlkashola x Melkaselsa (-27.3), Bishola x Metadel (-49), Bishola x Cahli (-36.6), Metadel x Fetan (-29.9), Mlkashola x ARP tomatod2 (-53) and Metadel x ARP tomatod2 (-28) had showed negatively significant SCA effects for infected plant. On the other hand crosses, Melkashola x Bishola (37.8), Mlkashola x Metadel (28.4), Mlkashola x Chali (27.9), Mlkashola xMiya (47.9), Mlkashola x Fetan (50.5), Metadelx Miya (47.8) and Melkaselsa x Chali (37) had showed positively significant SCA effects for infected plant (Table 8). The result was inagreement with the reports of [35].

For percentage of severity index, 16 crosses registered negative and desirable crosses viz., Melkashola x ARP tomatod2 (-4.7), Melkashola x Metadel (-6.8), Bishola xMetadel (-15), Bishola xFetan (-10.3), Metadel x Fetan (-11), Miya x Metadel (-18), Metadel x ARP tomatod2 (-7) and Bishola x ARP tomatod2 (-3.2) recorded high and negative SCA effects for reaction to late blight. In contrast, 14 crosses positive and from that six crosses viz., Fetanx ARP tomatod2 (21), Metadel x Melkaselsa (11), Bishola xChali (12), Bishola x Melkaselsa (16), Melkashola x Melkaselsa (19) and Melkashola x Fetan (14). Among these crosses one of the parents had significant negative GCA effects, (Metadel and ARP tomatod2) revealing that non-additive gene effect and the other parents (Melkashola, Melkaselsa and Miya) had significant positive GCA effects, played predominant role in their expression and is worthwhile for exploitation of heterosis. This result gets support from the finding of in the same way 50% of the crosses had showed positive and from these six crosses were significant and expressed the early time for the infestation of inoculum from one plant to other or from one parts to other part. This can facilitate the early invention of the pathogen all the plant in the pot. In contrast, the rest 50% of the crosses expressed negative and from which seven crosses had registered significant. Crosses that showed negative correlation for days to first disease appearance had significant negative SCA effect for disease progress rate. The result of this experiment showed positive relation.

Except, days to first disease appearance, all crosses that showed significant and negative SCA for disease severity and AUDPC were more resistant while those with significant and positive SCA were more susceptible than their parental GCA effects. Negatively significant SCA effects of most crosses were produced by crossing two inbred parents with high negative general combining ability or could be good general combing ability with poor general combing ability. Sometimes both parents might have high negative general combing ability effects but their crosses might be poor, for instance, Melkashola x Metadel exhibited poorer specific combining effects while their parents had good general combing ability effects, this indicates that both of these parents may have the same resistance genes and are not able to take advantage of any additive gene action. Fetan (poorer GCA) x Melkashola (better GCA) cross combination was found to be highly resistant to late blight; this implies that resistance is controlled by dominant gene. On the contrary, Metadel x Miya cross showed positive SCA effect in most of the disease parameters and being susceptible to late blight disease. This cross was developed from better general combiner (Metadel) and poorer general combiner (Miya) inbred parent combinations, indicating that resistance is controlled by recessive genes. Although it has been well known that most plant resistant genes are dominant, the presence of recessive ones has been recognized in many plant and pathogen relationships. The significant genetic varia-

tions among crosses in different components that were consistent in both locations suggest possible involvement of several genes in the expression of the components that confer partial resistance to late blight of tomato and that maximization of expression of a trait depends on the specific combination of parental genotype.

Table 7: Estimates of specific combing ability effects for disease parameters of 28 hybrids in 8 x 8 half-diallel cross of tomato grown 2019/20

Crosses	DFDA	AUDPC	DPR	PSI
Melkashola x Bishola	-1.37**	37.8**	0.002*	-2.2
Melkashola x Metadel	-0.27	28.4**	-0.001	-6.9*
Melkashola x Fetan	0.92*	-69.1**	0.0012	14*
Melkashola x M.selsa	0.93*	-27.3**	-0.0013	19*
Melkashola x Miya	-0.02	-13.0*	0.0008	9*
Melkashola xChali	-0.67*	27.9**	0.0024*	-7.6*
Bishola x Metadel	0.82*	-49**	-0.0013	-15*
Bishola x Fetan	-0.47	50.5**	0.0031*	-10.3
Bishola x M.selsa	-0.48	2.6	0.0025*	16*
Bishola x Miya	-1.42*	47.9**	0.0027	5.6
Bishola x Chali	0.92*	-36.6**	-0.0007	12*
Metadel x Fetan	0.73*	-26.9**	-0.0045*	-11*
Metadel x M.selsa	-2.77**	8.3*	0.0071*	11*
Metadel x Miya	-1.72**	47.8**	0.004*	-18*
Metadel x Chali	3.12**	-10.4*	-0.0099*	-7.3
Fetan x M.selsa	1.22**	5.1*	-0.0043*	8.7*
Fetan x Miya	0.72*	-2.5	-0.0052*	-5.3*
Fetan x Chali	-0.87*	2.33*	0.0019	-6.8*
Melkaselsa x Miya	-1.17**	4.85*	0.0038*	4.2
Melkaselsa x Chali	1.67**	37**	-0.0051*	-8.5*
Miya x Chali	0.02	-0.3	0.00013	-5*
Melkashola x ARP tomatod2	0.51	15*	-0.0046*	-4.7*
Bishola x ARP tomatod2	2.01**	-53**	-0.0076*	-3.2
Metadel x ARP tomatod2	0.93*	-28**	-0.0034	-7
Fetan x ARP tomatod2	-2.03**	12.2*	0.0061*	21*
Melkaselsa x ARP tomatod2	-1.42**	16.3*	0.0024*	-4
Miya x ARP tomatod2	0.005	13.3*	-0.0011	2.1
Chali x ARP tomatod2	-0.64*	6.2	-0.0003	2.8
SE+	0.001	0.6	0.000001	3.6

Where AUDPC-area under disease progress curve, PSI-percentage of severity index, FDA-first day disease appearance, DPR-disease progress rate and SE- standard error

Conclusion

After the past few decades, breeding for tomato late blight resistance has been a major focus for the resistance breeding programs in tomato. Therefore, breeding efforts have been made to combine significant resistance to late blight with important fruit quality and yield traits. The dialed mating design is a popular choice as it helps in the identification of parents with good GCA effects and hybrids with good SCA effects. Additionally, it provides the important information on gene action and inheritance of the traits. In the study, we evaluated on fruit yield and disease traits of interest for tomato breeding under late blight pressure. The high diversity in the material was confirmed by GCA and SCA values for all traits. This showed the significance of both the additive and the non-additive effects in the inheritance of the traits evaluated. Furthermore, it was shown that total yield was more dependent on additive and non-additive variance. Overall, this information will be useful to design and develop breeding programs aiming to improve late blight resistance along with a suitable combination of important traits. The moderately resistant and high yielding parents (ARP tomatod2 and Metadel) and hybrids (Melkashola x Fetan, Metadel x ARP tomatod2, and Bishola x ARP tomatod2) could be used for resistance breeding in tomato.

References

1. Nowicki, M., Foolad, M. R., Nowakowska, M., & Kozik, E.

U. (2012). Potato and tomato late blight caused by Phytophthora infestans: an overview of pathology and resistance breeding. Plant disease, 96(1), 4-17.
2. Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J.H., Champouret, N., Oliva, R. and Segretin, M.E.(2011). Understanding and exploiting late blight resistance in the age of effectors, PMID: 21663437.
3. García-González, T., Sáenz-Hidalgo, H. K., Silva-Rojas, H. V., Morales-Nieto, C., Vancheva, T., Koebnik, R., & Ávila-Quezada, G. D. (2018). Enterobacter cloacae, an emerging plant-pathogenic bacterium affecting chili pepper seedlings. The plant pathology journal, 34(1), 1.
4. Eatough Jones, M., & Paine, T. D. (2015). Effect of chipping and solarization on emergence and boring activity of a recently introduced ambrosia beetle (Euwallacea sp., Coleoptera: Curculionidae: Scolytinae) in Southern California. Journal of economic entomology, 108(4), 1852-1859.
5. Foolad MR. and Panthee DR. (2012). Marker-assisted selection in tomato breeding. Critical Reviews in Plant Sciences 31: 93-123.
6. Hanson, P., Lu, S. F., Wang, J. F., Chen, W., Kenyon, L., Tan, C. W., & Yang, R. Y. (2016). Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. Scientia Horticulturae, 201, 346-354.
7. Foolad, M. R., Merk, H. L., & Ashrafi, H. (2008). Genetics, genomics and breeding of late blight and early blight resistance in tomato. Critical Reviews in Plant Sciences, 27(2), 75-107.
8. Desta, M., & Yesuf, M. (2015). Efficacy and economics of fungicides and their application schedule for early blight (Alternaria solani) management and yield of tomato at South Tigray Ethiopia. J Plant Pathol Microbiol, 6(5), 1-6.
9. Broers, L. H. M., Cuesta Subias, X., & Lopez Atilano, R. M. (1996). Field assessment of quantitative resistance to yellow rust in ten spring bread wheat cultivars. Euphytica, 90(1), 9-16.
10. Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. Science, 327(5967), 818-822.
11. Fenta W., D. Gadisa and C. Tesfa, (2018). Scio- Economic Profile of Horo Guduru Wollega one, Western Oromiya. Wollega University, Center of the Studies of Environment and Society. Wollega University, Ethiopia. Pp12 in Plant Breeding. Biom Biostat Int J 4(1): 00085.
12. Horneburg, B., & Becker, H. C. (2011). Selection for Phytophthora field resistance in the F2 generation of organic outdoor tomatoes. Euphytica, 180(3), 357-367.
13. McNeal, F. H., Konzak, C. F., Smith, E. P., Tate, W. S., & Russell, T. S. (1971). A uniform system for recording and processing cereal research data (No. REP-10904. CIMMYT.).
14. Berger, R. D. (1981). Comparison of the Gompertz and Logistic Equations to Describe Plant Disease Progress. Phytopathology, 71(7), 716-719.
15. Campbell and Madden. (1990). Modelling Plant Disease Epidemics September 2003. European Journal of Plant Pathology 109(7):669-682.
16. Steel, R. G. D., & Torrie, J. H. (1980). Principles and procedures of statistics, a biometrical approach (No. Ed. 2). McGraw-Hill Kogakusha, Ltd.
17. Griffing, B. R. U. C. E. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Australian journal of biological sciences, 9(4), 463-493.
18. Akhtar, K. P., Saleem, M. Y., Iqbal, Q., Asghar, M., Hameed, A., & Sarwar, N. (2016). Evaluation of tomato genotypes for late blight resistance using low tunnel assay. Journal of Plant Pathology, 421-428.
19. Solankey, S. S., Singh, R. K., Baranwal, D. K., & Singh, D. K. (2017). Genetic expression of tomato for heat and drought stress tolerance: An overview. International Journal of Vegetable Science, 21(5), 496-515.
20. Dobhal, S., Olson, J. D., Arif, M., Suarez, J. A. G., & Ochoa-Corona, F. M. (2016). A simplified strategy for sensitive detection of Rose rosette virus compatible with three RT-PCR chemistries. Journal of virological methods, 232, 47-56.
21. Worku M., and Sahela S. (2018). Review on Disease Management Practice of Tomato WiltCaused Fusarium oxysporum in case of Ethiopia. J Plant Pathol Microbiol 9: 460.
22. Almeida, R. P., & Nunney, L. (2015). How do plant diseases caused by Xylella fastidiosa emerge?. Plant disease, 99(11), 1457-1467.
23. Ajal, M. O., & Ajani, O. O. (2014). Variation in fruit yield and correlations between seed quality components and fruit yield of tomato (Lycopersicon esculentum Mill). Tanzania Journal of Agricultural Sciences, 8(2).
24. Ayda, T. T. (2015). Effect of fungicides and resistant genotypes on severity of potato late blight [Phytophthora infestans (Mont.) de Bary] and yield and yield components at Haramaya, Eastern Ethiopia (Doctoral dissertation, Harmaya University).
25. Getachew Gudero., Temam Hussien Mashilla Dejene., and Birhanu Biazin. (2018). Integrated Management of Phytophthora infestans (Mont.) de Bary] Through Host Plant Resistance and Reduced Frequency of Fungicide in Arbaminch Areas, Southern Ethiopia (PCTOC) 120: 881-902.
26. Girma, F., Ayalew, A., & Dechassa, N. (2011). Management of late blight (Phytophthora infestans) of potato (Solanum tuberosum) through potato cultivars and fungicides in Hararghe Highlands, Ethiopia. International Journal of Life Sciences, 2(3), 130-138.
27. Debela, K. B., Belew, D., & Jima, N. E. G. O. (2016). Evaluation of Tomato (Lycopersicon esculentum Mill.) varieties for growth and seed quality under Jimma Condition, South Western Ethiopia. International Journal of Crop Science and Technology, 2(2).
28. Kumbar, B. (2017). Standardization of specific media for Phytophthora infestans. Glob. J. Bio-Sci. Biotechnol, 6, 374-376.
29. Moricca, S., Linaldeddu, B. T., Ginetti, B., Scanu, B., Franceschini, A., & Ragazzi, A. (2016). Endemic and emerging pathogens threatening cork oak trees: Management options for conserving a unique forest ecosystem. Plant Disease, 100(11), 2184-2193.

30. NPPO of Germany. (2016).
31. Ram, B. K., Singh, D., Lalchan, C. P., & Joshi, B. K. (2016). Participatory F1 hybrid seed production of tomato and its economic benefit. Journal of Agriculture and Environment, 17, 46-55.
32. SAS Institute, Inc. (2013). SAS/STAT user's guide. Version 9.3, 4th edition. Cary, NC.
33. Singh, S. P., Thakur, M. C., & Pathania, N. K. (2010). Reciprocal cross differences and combining ability studies for some quantitative traits in tomato (Lycopersicon esculentum Mill.) under mid hill conditions of Western Himalayas. Asian Journal of Horticulture, 5(1), 172-176.
34. Stuber, C.W., (1999). Biometry, molecular biology, and physiology of heterosis. Pp.173-183.
35. Wheeler, B.E.J. (1969). An Introduction to Plant Disease. Wiley, London, pp. 617-619