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Evaluation of Maternal Effect and Hybrid Vigorusing Genetic Relationships based on Molecularand Phenotypic Distances between Parents and theirHybrids in Okra

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Abstract

Maternal effect and hybrid vigor using genetic relationships based on molecular distance estimated by RAPD technique and genetic relationships based on phenotypic distance estimated using 14 economic traits among four different parental genotypes of okra with their hybrids and reciprocal hybrids were evaluated. In RAPD technique, out of 19 random primers, only six of them were successful in generating reproducible and reliable 39 amplicons. Among studied genotypes, 29 amplicons were polymorphic with an average of 72.85% polymorphism .The estimated molecular and phenotypic distances ranged between 0.03 to 0.25 and 4.00 to 6.47, respectively. Non-significant positive correlation (r=0.45) between molecular and phenotypic distances was found. The genetic relationships based on molecular distances for all crosses and reciprocal crosses revealed that, some crosses (HK x $Line_2$ and $Line_1$ x $Line_2$) appeared to have maternal effect, while some of the other crosses (HKx$Line_1$ and $Line_2$x$Line_3$) appeared to have hybrid vigor. On the other hand, the genetic relationships based on phenotypic distance revealed that no maternal effect was shown in all crosses while those relationships were able to show hybrid vigor in most crosses. The results suggested the capability of genetic relationships based on molecular distances to clarify the maternal effect and hybrid vigor in some crosses. Besides, these results also indicated the efficiency of genetic relationships based on phenotypic distances using all studied traits to estimate hybrid vigor.

Keywords: Okra, RAPD, maternal effect, hybrid vigor, molecular distance, phenotypic distance

1. Introduction

Okra (*Abelmoschusesculentus* L. Moench.), one of ancient vegetable crops, is economically important in Egypt and very popular in the world. There productive biology of this crop offers goodscope for exploitation of hybrid vigor [1]. Hybrid vigor (heterosis) has a dramatic impact on the development of breeding methods and high yields for many field crops and vegetables [2]. This value can be expected in crosses showing high levels of genetic variability [3].Suitable selection

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for parents in breeding programs is very important and critical for generating heterosis. Therefore, relevant information on genetic variability plays an important role in proper choice of parents in breeding programs of okra [1].

Maternal inheritance is the expression of a trait in a progeny that is due to maternal gene action [4]. This effect in plants was recognized as long as 1909. Evidence showed that maternal influence can contribute substantially to the phenotype of an individual [5]. The study done by Udergwu [6] indicated the existence of maternal effects in okra. In addition, the maternal effect follows the same pattern of heterosis in the F_1, F_2 and subsequent generations, as that arising from nuclear genes. Hence, this phenomenon is desirable in producing superior F_1 hybrids for a given environment. Therefore, adequate knowledge of the maternal inheritance mechanism could be exploited to produce high yielding varieties of okra for a given environment [7].

Molecular and quantitative genetic studies showed the cytoplasmic factors that cause maternal inheritance contribute to heritable variation in both qualitative and quantitative traits in plants [5]. Molecular markers techniques like RAPD (Random amplified polymorphic DNA) were entered for genetic improvement in different fields of agricultural research. Simplicity of RAPD technique makes it ideal for plant breeding programs and genetic diversity studies [8].RAPD is one of the PCR-techniques which use single random primers [9]. Randomly primers designed from short oligonucleotide (8-12 nucleotides) and anneal to many locations on the template genomic DNA. When two primers anneal in the opposite direction to two genomic locations that are reasonably distant from each other, a fragment is amplified, these amplified fragments called amplicons [10]. These amplicons are then analyzed by conventional gel electrophoresis. Subsequent electrophoretic separation of the amplicons has been used to compare the genotypic diversity. RAPD could be effectively used for analyzing genetic relationships and genetic diversity as it is simple, reliable, rapid and superior to those based on pedigree information [11]. This technique needs only small amounts of DNA, suitable for work on anonymous genomes, efficiency and low expense [12]. It is also used to reveal the relationship between molecular distances and heterosis in plants [13-17].On the other hand, phenotypic distances were calculated between different genotypes of plants by applying the Euclidean metric of [18] based on morphological traits which can be used as a measure of genetic diversity.

The molecular and phenotypic distance measurements differ in their ability to predict hybrid vigor and F_1 performance. Phenotypic distance showed a higher correlation with heterosis. Association of phenotypic distances with heterosis was also observed in Canola [19-20]. In some cases, significant correlation between the two types of distances was found [21] but in most cases, no significant correlation was found [22-24]. Thus, this study aimed to evaluate maternal effect and hybrid vigor using genetic relationships based on molecular and phenotypic distance among different genotypes of okra (*Abelmoschusesculentus* L. Moench).

2. Materials and Methods

An experiment involving four parents of Okra (*Abelmoschusesculentus* L. Moench) viz., local cultivar i.e., Cairo Red HK (P_1), genetically divergent parents Line1 (P_2), Line2 (P_3) and Line3(P_4) were developed by El-Gendy [25] and 12 single crosses including reciprocals (6 direct crosses and their reciprocals) were made among these parents according to complete diallel crosses mating design.

In the laboratory of biotechnology, Horticultural Research Center, Agricultural Research Center, Ministry of Agriculture, DNA isolations from Okra seeds were collected separately from parents and their crosses. The bulked DNA extraction was performed using DNeasy Mini Kit (QIAGEN). Bulked DNA from each genotype was used as a template for Polymerase Chain Reaction (PCR) amplification using 19 random 10 mer primers (Operon Technology, USA) from groups A, B, C, E, F, M and Q were used in detecting polymorphism among parents and its F_1

hybrids. Amplification reactions in RAPD technique were performed according to Williams *et al.* [26] using 25µl reaction mixture containing the following; 2.0 µl of bulked DNA (50 ng/µl), 2.5 µl of dNTPs (2.5 mM), 1.5µl of $MgCl_2$ (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 96 ºC for 30 seconds, 37 ºC for 30 seconds each, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. DNA banding patterns generated from RAPD experiments was analyzed by computer program, Gene profiler (version 4.03). Molecular distances between studied genotypes were computed using computational package MVSP version 3.1 by Nei and Licoefficient's [27].

Four parents and 12 F_1 hybrids were sown in Randomized Block Design with four replications each consisted of 16 plots (four parents and 12 F_1 hybrids) during summer season of 2013 at a private farm in KafrSaad, Damietta under the supervision of the Department of Genetics, Faculty of Agriculture, Damietta University. Data were recorded for 14 economic traits on three plants chosen at random from each plot. These traits were: days to first flowering, plant height, number of leaves per plant, number of branches per plant, node number, inter nodal length, fruit length, fruit diameter, number of ridges on fruit, number of seeds per pod, number of fruits per plant, fruit weight and total yield per plant. Mean performances of these data (see Table S1 in the appendix) are presented in previous research by Hamada *et al.* [28] and dissertation by Zaater [29].

Phenotypic distance (PD) between studied genotypes were computed using computational package MVSP version 3.1 from the 14 economic traits by applying the Euclidean metric according the following equation [30].

$$\mathrm{PD}_{ik} = \sqrt{\sum_{i=1}^{n} \frac{(x_{ij} - x_{ik})^2}{sd_i}}$$

Where PD_{ik}=Phenotypic distance between genotypes*j* and *k*; x_{ij} andx_{ik} = phenotypic value of the*i*thtraits for genotype *j* and *k*, respectively; *n* = number of traits; sd_i = standard deviation of all phenotypic traits.

The two types of distances were used to construct dendrograms based on the unweighted pair-group method with arithmetic means (UPGMA) according to Teklewold and Becker [31] using the computational package MVSP version 3.1. The hierarchical cluster analysis [32] was used to investigate patterns of genetic relationships among parental genotypes and also between parents with their hybrids in crosses and reciprocal crosses.

Simple correlations using the computational package Minitab 17 were used to explain relationships between molecular distances (MD) and phenotypic distances (PD) [33-34]. Coefficient of determination (R^2) was used to test the reliability of the regression models. An R^2 closer to 1.0 revealed more reliability.

3. Results and Discussion

After screening, only 6 out of 19 (10-mer) primers were successful in generating reproducible and reliable amplicons as shown in Figures 1-4. The number of polymorphic

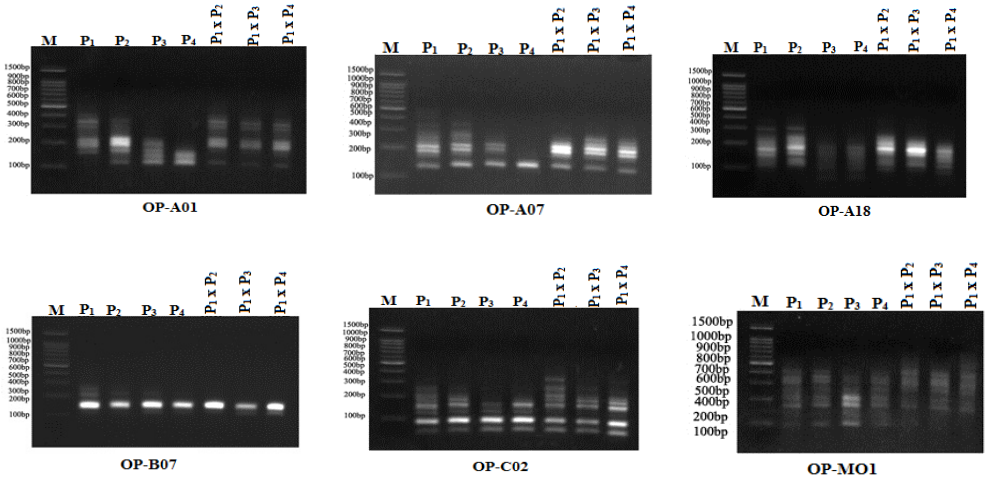

Figure 1. RAPD -PCR products for the four selected parents and its hybrids in which P_1 was where female parent. With six primers OP-A01, OP-A07, OP-A18, OP-B07, OP-C02 and OP-M01

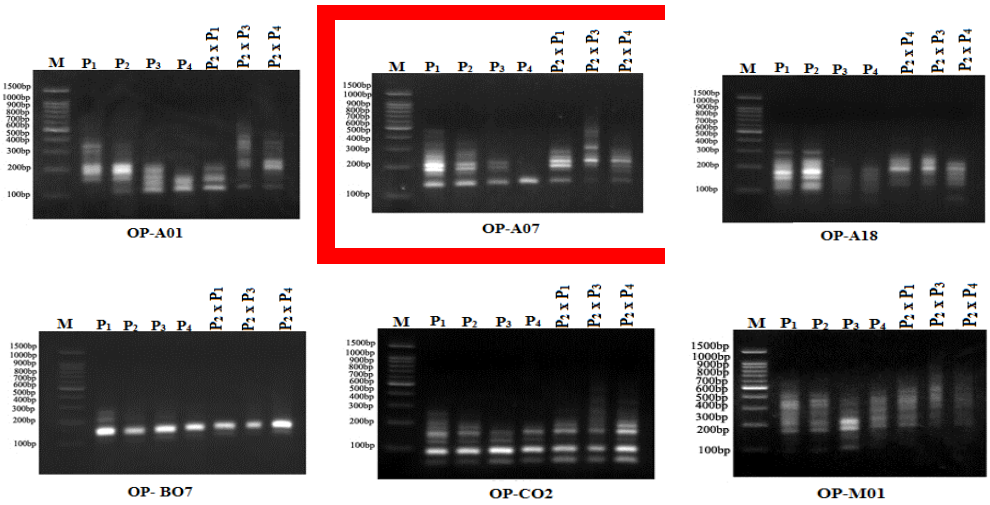

Figure 2. RAPD -PCR products for the four selected parents and its hybrids in which P_2 was where female parent. With six primers OP-A01, OP-A07, OP-A18, OP-B07, OP-C02 and OP-M01

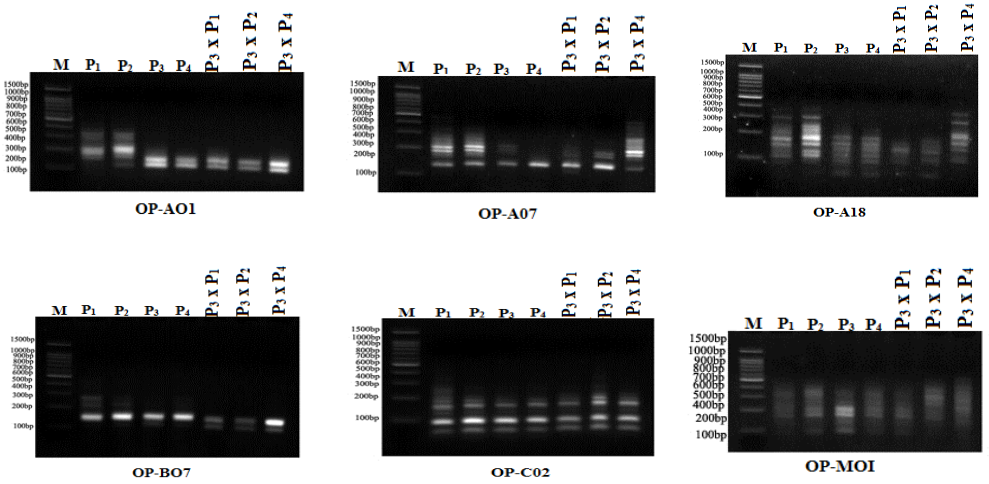

Figure 3. RAPD-PCR products for the four selected parents and its hybrids in which P_3 was where female parent. With six primers OP-A01, OP-A07, OP-A18, OP-B07 , OP-C02 and OP-M01

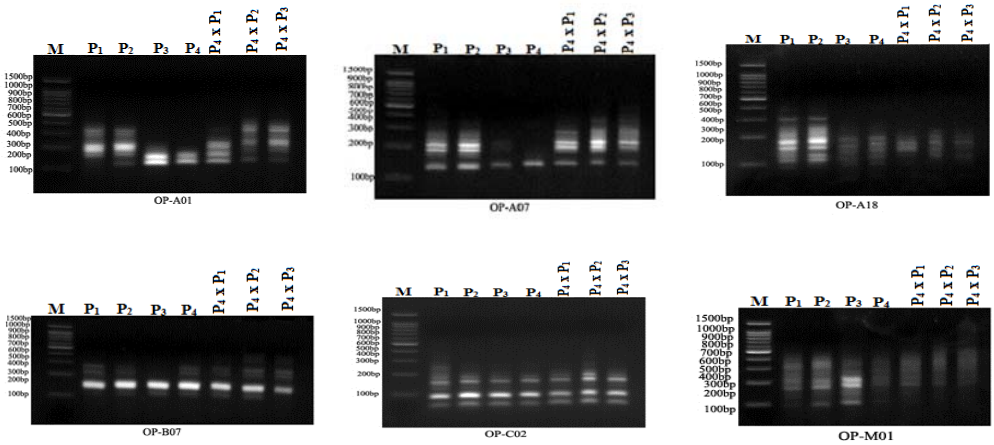

Figure 4. RAPD -PCR products for the four selected parents and its hybrids in which P_4 was where female parent. With six primers OP-A01, OP-A07, OP-A18, OP-B07 , OP-C02 and OP-M01

bands and percentage of polymorphism obtained by analyzing four parental varieties and 12 single crosses from 6 direct crosses of okra were presented in Table 1.

The six primers detected a total of 39 fragments, with an average of 6.5 fragments per primer. The percentage of polymorphism ranged from 50% (OP-B07) to 100% (OP- A01). Among these, 29 amplicons were polymorphic with an average of 4.83 polymorphic bands per primer. This technique was efficient in detecting polymorphism with an average of 72.85%.

The RAPD data which were used to estimate the molecular distance (MD) among the four selected okra parents are presented in Table 2. The highest value of MD was recorded as (0.25) between HK and $Line_3$ parents. The results showed that the lowest value was detected as (0.03) between HK and $Line_1$ parents. These results showed disparity in MD between parents. The phenotypic distance (PD) of parental lines were calculated using 14 phenotypic traits in okra and

presented in Table 3. The highest value of PD was recorded as (6.47) between $Line_1$ and $Line_3$ parents. While, the lowest value was recorded as (4.00) between HK and $Line_1$ parents. These results showed disparity in PD between parents. These disparity in distances between the parents indicates the presence of molecular and phenotypic diversity that can be counted on to achieve the aim of this study.

The UPGMA dendrograms representing genetic relationships between parental lines based on MD and PD are shown in Figures 5 and 6, respectively. Clustering based on MD established the four selected parents in two distinct groups, the first group included HK and $Line_1$ parents. Classification of parental genotypes based on PD separated them in three and groups, the first group included HK and $Line_1$ while the second group include $Line_2$ and the third group included $Line_3$.

Estimated values of correlations between MD according to Vaillancourt *et al.* [35] and PD according to Sneath and Sokai [30] showed non-significant positive correlation with value (r= 0.45) and coefficient of determination R^2%= 20.3% (Figure 7).This result was in agreement with those obtained by Teklewold and Becker [31]. They noticed that non-linear relationship between the two types of distances exacerbated as the number of loci that contribute to the variation of the traits used to estimate PD increases. The relationship is further complicated by linkage disequilibrium. With no linkage disequilibrium between DNA marker and QTL (Quantitative Trait Loci) for phenotypic traits, the two types of distances vary independently and both high and low MD can correspond to similar PD.

Table 1. List of RAPD primers, the number of amplified products, the number of polymorphic bands and percentage of polymorphism obtained by analyzing parents and its hybrids of Okra

NO.	Name	Primer sequence (5′→ 3′)	Mol. wt Range (bp)	Total Number of bands	Number of polymorphic bands	Polymorphism (%)
1	**OP- A01**	5′ CAGGCCCTTC3`	115-360	7	7	100.0
2	**OP-A07**	5′ GAAACGGGTG3 `	160-325	5	4	80.0
3	**OP-A18**	5′ AGGTGACCGT3`	115-340	8	6	75.0
4	**OP-B07**	5′ GGTGACGCAG3 `	145-270	4	2	50.0
5	**OP- C02**	5` GTGAGGCGTC3`	75-320	8	6	75.0
6	**OP- M01**	5` GTTGGTGGCT3`	115-430	7	4	57.1
Total				39	29	---
Mean				6.5	4.83	72.85

Table 2. Molecular distance (MD) among four selected Okra parents based on RAPD data.

MD	HK	Line1	Line2	Line3
HK	0.00			
Line1	0.03	0.00		
Line2	0.23	0.22	0.00	
Line3	0.25	0.24	0.14	0.00

Table 3. Phenotypic distances (PD) among four selected Okra parents based on data of all studied traits data.

PD	HK	Line1	Line2	Line3
HK	0.00			
Line1	4.00	0.00		
Line2	4.80	4.86	0.00	
Line3	5.09	6.47	6.14	0.00

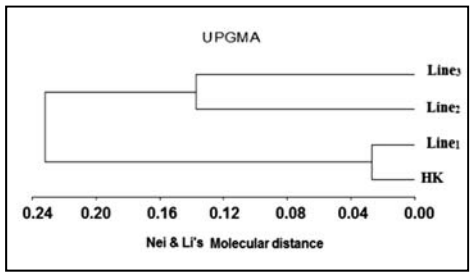

Figure 5. UPGMA clustering for selected Okra parents based on MD

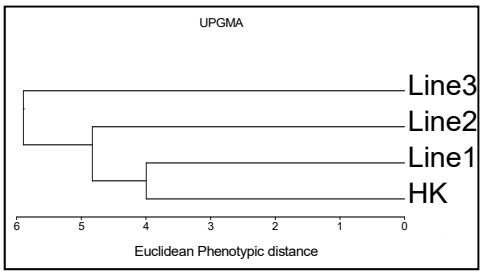

Figure 6. UPGMA clustering for selected Okra parents based on PD

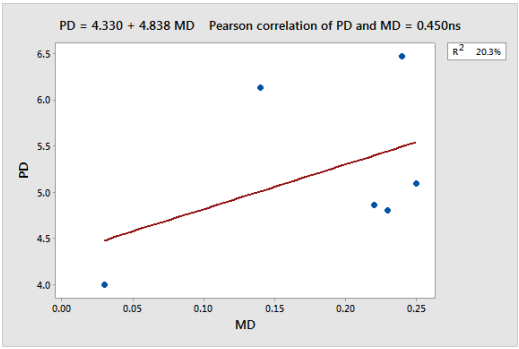

Figure 7. Relationship between molecular distance MD and phenotypic distances (PD)

Dendrograms of UPGMA (Figures 8 -13) indicated the genetic relationships among any two parents with their hybrid and with their reciprocal hybrid for each cross based on MD and PD.

3.1 The genetic relationships based on MD took three ways as follows:

In the first way, dendrograms for crosses (HKx$Line_2$) and ($Line_1$x$Line_2$) as shown in Figures $9_{a\&b}$ and $11_{a\&b}$ respectively, were separated into two major groups: the first group includes the hybrid and female parent, the second group included the male parent both in cross and reciprocal cross. This result could illustrate that these crosses appeared maternal effect.

In the second way, dendrograms for crosses (HKx$Line_1$) and ($Line_2$x$Line_3$) as shown in Figures $8_{a\&b}$ and$13_{a\&b}$ respectively, were separated into two major groups, the first group included the hybrid alone and the second group included the two patents both in cross and reciprocal cross. This result could illustrate that these crosses appeared hybrid vigor.

In the third way, dendrograms for cross (HKxLine$_3$) and (Line$_1$xLine$_3$) as shown in Figures 10$_{a\&b}$ and 12$_{a\&b,}$ respectively, were separated into two major groups, the first group included the hybrid and the same parent in cross and reciprocal cross, the second group included the other parent. This result could illustrate that hybrid is genetically closer to one of his parents compared to the other one and this case didn't show neither maternal effect nor hybrid vigor.

The dendrograms using PD for all crosses and their reciprocal crosses except direct cross (HKxLine$_1$) indicated the parents separated in major group out of their hybrid which was alone in a second group as shown in Figures 8$_d$, 9$_{c\&d}$, 10$_{c\&d}$, 11$_{c\&d}$, 12$_{c\&d}$ and 13$_{c\&d}$. This result illustrated that most crosses appeared hybrid vigor.

These results suggested the capability of genetic relationships based on RAPD technique to clarify the maternal inheritance and hybrid vigor in some crosses. This capability may be better using many more numbers of primers or using other techniques. On the other hand, these results indicated the efficiency of relationships based on phenotypic distances to estimate hybrid vigor for all studied traits together. These results were in agreement with those obtained by El-Zanaty *et al.* [34] who indicated the ability to predict heterosis levels using phenotypic or genetic distance between the parents.

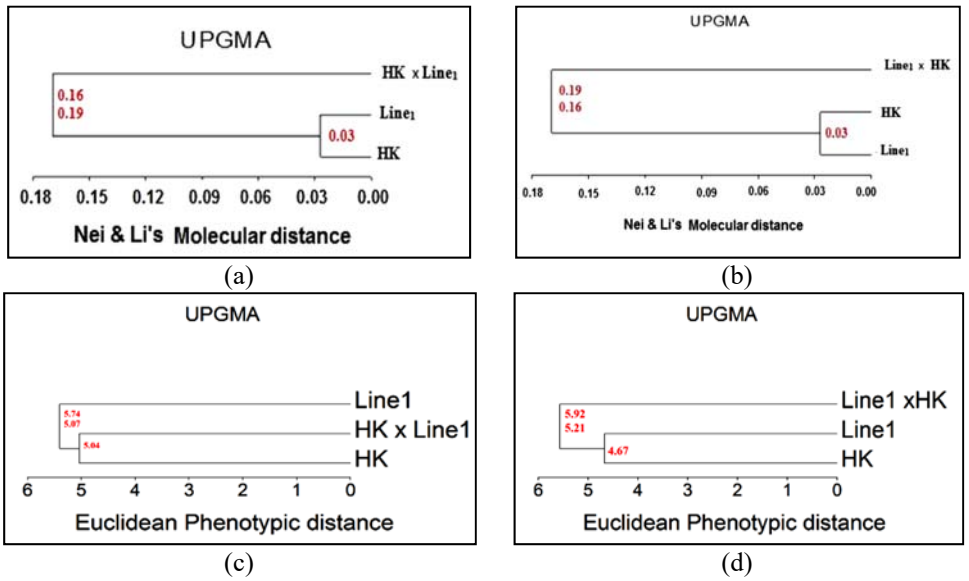

Figure 8. UPGMA dendrograms (a), (b), (c) and (d) indicating the genetic relationships among parents HK and Line$_1$ with their hybrid in cross and reciprocal cross using MD and PD, respectively

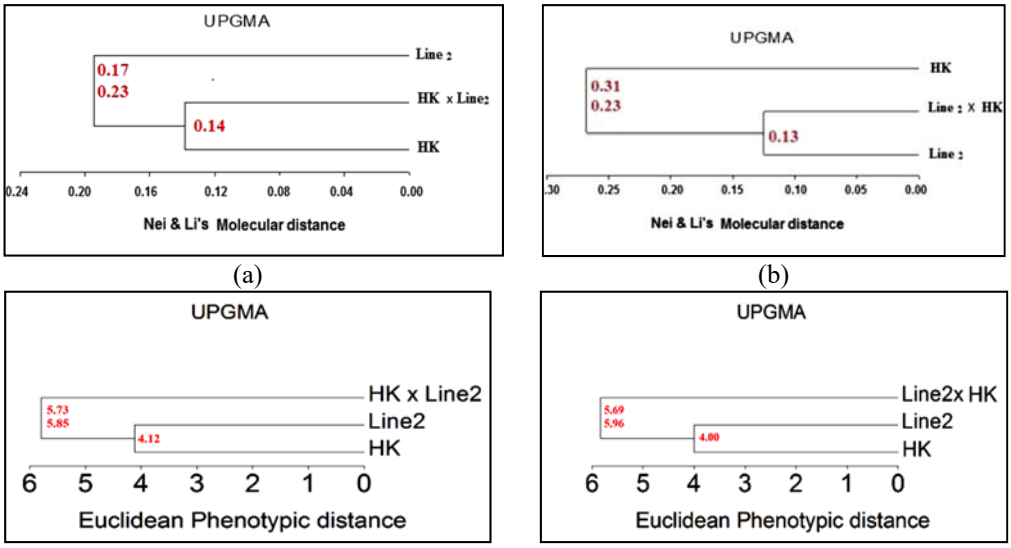

Figure 9. UPGMA dendrograms (a), (b), (c) and (d) indicating the genetic relationships among parents HK and Line2 with their hybrid in cross and reciprocal cross using MD and PD, respectively

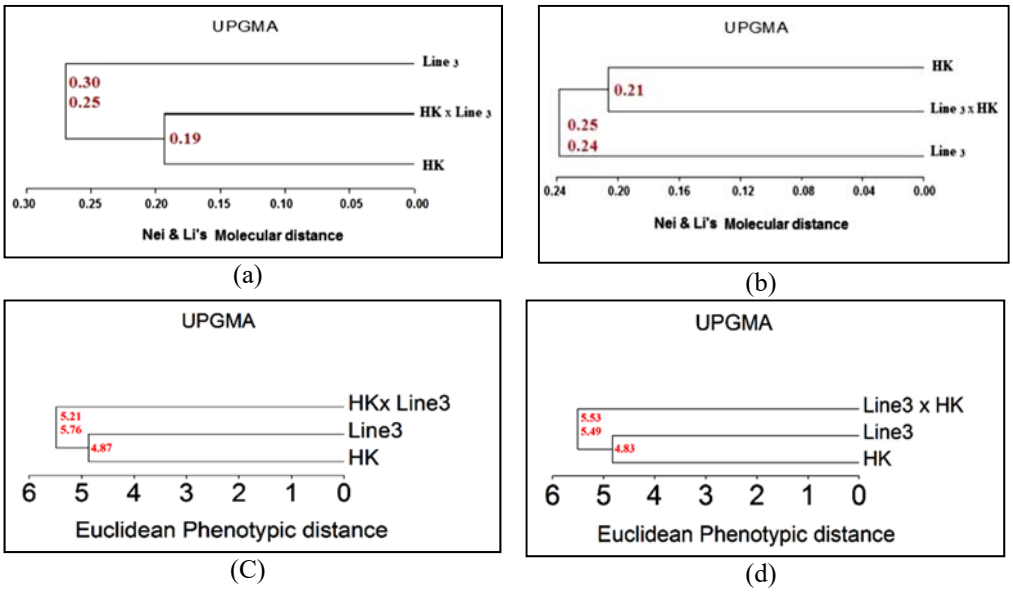

Figure 10. UPGMA dendrograms (a), (b), (c) and (d) indicating genetic relationships among parents HK and Line3 with their hybrid in cross and reciprocal cross using MD and PD, respectively

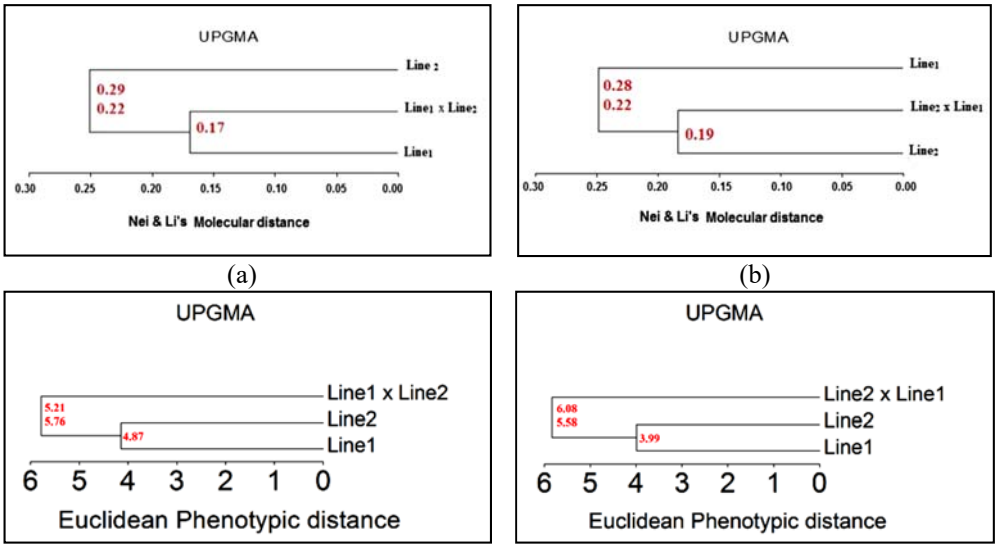

Figure 11. UPGMA dendrograms (a), (b), (c) and (d) indicating genetic relationships among parents Line1 and Line2 with their hybrid in cross and reciprocal cross using MD and PD, respectively

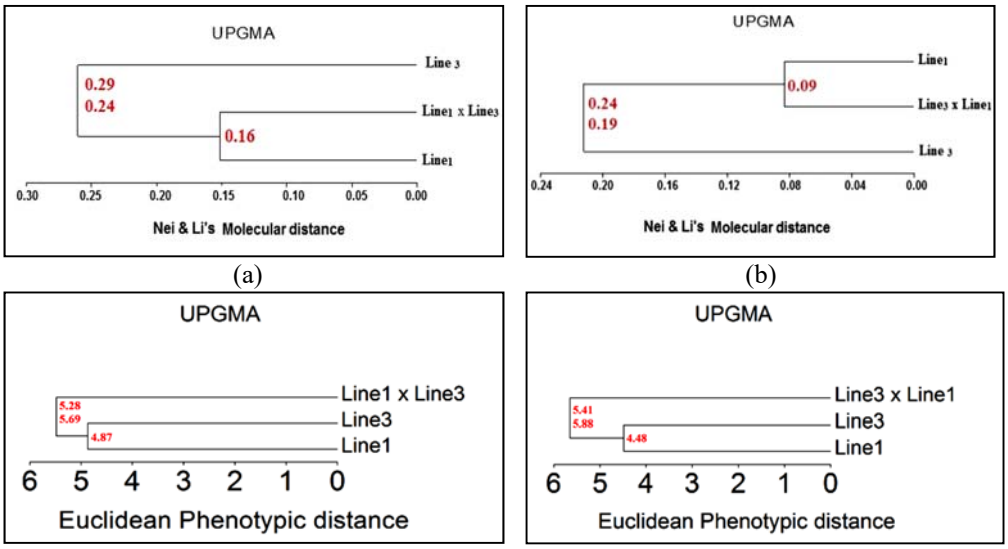

Figure 12. UPGMA dendrograms (a), (b), (c) and (d) indicating genetic relationships among parents Line1 and Line3 with their hybrid in cross and reciprocal cross using MD and PD, respectively

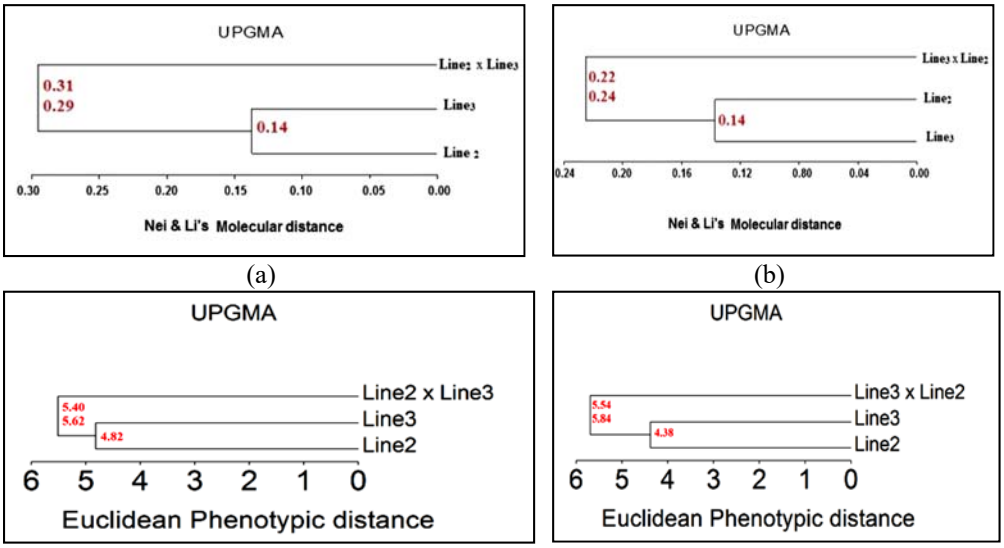

Figure 13. UPGMA dendrograms (a), (b), (c) and (d) indicating genetic relationships among parents Line2 and Line3 with their hybrid in cross and reciprocal cross using MD and PD, respectively

4. Conclusions

It could be concluded that non-significant positive correlation between molecular and phenotypic distances were found. The genetic relationships based on molecular distances for all crosses and reciprocal crosses revealed that, some crosses appeared to have maternal effect, while some of the other crosses showed hybrid vigor. On the other hand, the genetic relationships based on phenotypic distance revealed that no maternal effect was shown in all crosses while those relationships were able to show hybrid vigor in most crosses. These results suggested the capability of genetic relationships based on molecular distances to clarify the maternal effect and hybrid vigor in some crosses.

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