Genetic Studies of Northern Corn Leaf Blight Disease Resistance in A Yellow Maize Population Using Rapd Markers

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ABSTRACT

Genetic studies were used to determine the genetic nature of the resistance to northern corn leaf blight (NCLB) , using F_1 and F_2 segregating populations obtained by crossing a resistant inbred line (L1021) with a susceptible one (L1002). Bulked segregant analysis (BSA) was used to detect for RAPD markers linked to NCLB resistance genes, using F_2 segregating population of the same cross and its parents.

The genetic studies revealed that the inbred line, L1021, exhibited low disease severity (ranged from less than 5% to 25 % and the inbred line, L1002, exhibited high disease severity (ranged from 26 % to more than 75 %). F_1 severity ranged from less than 5% to 50 %, which means that low disease severity was partially dominant over high severity. The F_2 severity ranged from less than 5% to more than 75 %. The observed ratio (number of plants with high : low disease severity) fitted the Mendelian ratio, 3:1, and suggested the operation of one gene pair in this cross. The degrees of dominance for F_1 (h_1) and F_2 (h_2) gave significant negative values (-0.93 and -0.30, respectively). The heritability value for the tested cross was 68.07 % and the estimated value of genes was 0.52, indicating that there was a difference in resistance genes between the two inbred lines.

Thirty-five primers were screened to identify two RAPD markers; namely, $OPC04_{420}$ linked to NCLB resistance phenotypes and $OPC04_{220}$ linked to NCLB susceptible genotypes. Results from the two RAPD markers fitted the expected Mendalian ratio, 3:1, according to χ^2-test .The regression analysis showed that the relationships between the two markers, $OPC04_{420}$ and $OPC04_{220}$,and the phenotype of the F_2 individuals were significant and they recorded a determination coefficient (r^2) =0.61 and 0.74,for the two markers, respectively.

INTRODUCTION

Maize (*Zea mays L*.) is an important cereal crop for food and feed in many parts of the world. In Egypt, maize is grown for food, feed, fodder and industrial purposes. Egypt imports, approximately, 35 % of its maize need. It is important to develop high-yielding and disease resistant hybrids to meet the country demands.

Leaf blight disease, caused by *Helminthosporium turcicum* (Pass.) (NCLB), is one of the important foliar diseases of maize (*Zea mays L.*) in

temperate areas of the world, including Egypt. The disease is sporadic in occurance , depending on the environmental conditions (Bentolila *et al*. ,1991). It is favoured by moderate temperature and high humidity (Shurtleff, 1980). In Egypt, the disease is mostly found in the northern and north western regions of the Delta in late summer (off-season) maize planting, where favourable weather conditions are prevailing at that time of the year (El-Shafey, 1978; El-Assiuty *et al.*, 1987 and Gouda, 1996). Moreover, sprinkler irrigation systems, that are widely used in the newly reclaimed lands, provide high air moisture in the field throughout the season ,which allows for disease incidence in these areas. When infection becomes severe, high yield losses can occur, especially, when susceptible lines or hybrids are grown in these areas. If the disease is established before silking, grain yield reductions more than 50% may occur (Raymundo and Hooker, 1981 and Ullstrup and Miles, 1957). In U.S.A., in early 1970´s, the disease infections reached a destructive situation where yields were reduced by two-third or more in many areas (Hooker, 1977 and Perkins and Hooker, 1981). Recently, maize leaf blight caused a significant loss in grain yield, estimated by 30% or more in the Northern Delta region, where the climatic conditions favour the disease development (Khalifa and Zein El-Aabedeen, 2000).

The most efficient, effective, environmentally safe and economical means to control NCLB of maize are using resistant cultivars. Most maize breeders, therefore, prefer the use of quantitative NCLB resistance in their cultivar development programs. Several qualitative genes of resistance are known; namely, *Htl, Ht2, Ht3*, and *HtN*, referring to their locus designations (Hooker 1963, 1975, 1977 and Gevers, 1975). *Htl, Ht2* and *Ht3* result in small chlorotic lesions and the amount of necrotic tissue, fungal sporulation and inoculum for secondary infections are all reduced (Hooker and Kim, 1973). The *HtN* gene results in a delay in lesion development until after flowering (Gevers, 1975).

Current breeding programs rely predominantly on resistance, which exhibits a quantitative inheritance pattern. This type of resistance is effective against all presently known races of *E. turcicum*, in contrast to loci with qualitative effects (Smith and White, 1988). With quantitative inheritance pattern, resistance seems to be manifested, primarily, by a reduction in lesion number and a broad range of levels of resistance has been observed (Hooker and Kim, 1973). Jenkins and Robert (1961) concluded that general resistance to NCLB was polygenic, highly heritable and, primarily, dominated by additive gene action.

In several previous studies, quantitative trait loci (QTL), associated with general resistance to NCLB, were identified on all ten maize chromosomes (Freymark *et al*., 1993, 1994; Dingerdissen *et al.*, 1996; Welz *et al.*, 1998,1999; Schechert *et al.,* 1999; Welz and Geiger, 2000 and Brown *et al*., 2001).

The use of molecular markers can increase the efficiency of conventional plant breeding by identifying markers linked to the trait of interest, that are difficult to evaluate and/or that are largely affected by the environment (Tanksley *et al*.1989; Young and Tanksley 1989). Hence, there is a need to develop a rapid screening method to select for resistance to NCLB. Several types of DNA markers are generated by a wide variety of techniques, differing greatly in their reliability, difficulty, expense and the nature of the polymorphism that they detect (Karp *et al*.,1997).The simplicity and applicability of the random amplified polymorphic DNA (RAPD) technique has captivated that method in this study.

Tight linkage between molecular markers and genes for disease resistance can be of great benefit to disease resistance breeding programs by allowing the investigator to follow the DNA markers (PCR- based markers) through early generation rather than waiting for phenotypic expression of the resistance genes (Lefebure and Chevre, 1995).

The objectives of the present investigation were to study the genetic nature of the resistance to Northern corn leaf blight and to identify RAPD markers linked to disease resistance genes in a yellow population of maize.

MATERIALS AND METHODS

Plant materials and disease evaluation:

Two yellow maize inbred lines ;i.e. , a resistant line (P_1) (L.1021) and a susceptible line (P_2) (L.1002), to NCLB disease were crossed, during the season of 2005, to produce the F_1 generation (single cross S.C.155Y). The F_1 plants were selfed during the season of 2006 to obtain a set of 139 F_2 plants. The F_1 and F_2 populations and their parents were planted under field conditions, in the late summer of 2007 at the Experimental Farm Station, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, where environmental conditions allow for a uniform disease infection.

The artificial infection was done to enhance the natural infection, using an isolate of *Helminthosporium turcicum* cod T-13AS (obtained from Agriculture Research Center Department of Corn and Sugar Crops Disease Research, Giza) , that was a single spore culture grown in petri-dishes containing potato dextrose agar medium for ten days at 25 ± 2^{o}C. Spore

suspensions were prepared by adding sterilized distilled water over fungal growth, which was scraped off, using a sterilized needle. The suspensions were, then, strained through a sterilized cheese-cloth. Spore concentration was adjusted at 2.5 x 10^3 spore/ml, using sterilized distilled water. Plants were inoculated at the three to five leaf stage of growth, in the evening, using a spore suspension. Severity of NCLB, as a percentage of infected leaf area (% average lesion size), eight weeks after the inoculation and readings were classified, according to Elliot and Jinkins (1946) as follows:

Rating scale	Infected Leaf area (%)	Resistance level
0.5	<5	Highly resistant (HR)
1.0	6-10	Resistant (R)
2.0	11-25	Moderately resistant (MR)
3.0	26-50	Moderately susceptible (MS)
4.0	51-75	Susceptible (S)
5.0	>75	Highly susceptible (HS)

Genetic analysis:

Frequency distribution tables were recorded each for parental, F_1 and F_2 populations for NCLB severity percentage under field conditions and mean $\bar{x}$ and variance S^2 for these populations were calculated.

In respect to mode of inheritance, goodness of fit of the observed to the expected ratios of the phenotypic classes, concerning the NCLB severity and infection types, were determined by χ^2 analysis, according to Steel and Torrie (1980). Moreover quantitative analysis of resistance was calculated using means and variance of the four populations . The following parameters were calculated using these statistics. It was calculated according to the formula derived by Wright (1968) as follows : No. of genes controlling resistance : $n = (\bar{x}_{P1} - \bar{x}_{P2})^2 / 8 (V_{F2} - V_{F1})$

Where:

n = Minimum number of effective genes,

V_{F1}, V_{F2} Variance of F_1 and F_2 ,respectively.

This formula assumes that there is no linkage, no epistasis, no dominance, all loci have equal effects and all genes controlling resistance are in a single parent of the cross.

Degrees of dominance (h) were calculated ,according to the method suggested by Romero and Frey (1973) as

$h_1 = (\bar{x}_{F1} - \bar{x}_{MP}) / D$,

and $h_2 = 2 (\bar{x}_{F2} - \bar{x}_{MP}) / D$,

Where:

$D = (\bar{x}_{hp} - \bar{x}_{MP})$,

$\bar{x}_{F1}$, $\bar{x}_{F2}$ and $\bar{x}_{hp}$ are the means of F_1 , F_2 and higher parent, respectively, while, $\bar{x}_{MP}$ is the mid-parent value.

The significance of the h_1 and h_2 was calculated using the t-test (Steel and Torrie,1980)

Heritability, in its broad-sense (h^2), was estimated, according to Lush (1949). as follows:

$h^2 = [V_{F2} – 1/3 (V_{F1}+V_{P1}+V_{P2})] / V_{F2} \times 100$

Where: V_{P1}, V_{P2}, V_{F1} and V_{F2} variance of P_1 , P_2, F_1 and F_2 ,respectively.

DNA extraction:

Genomic DNA was extracted from fresh leaves of individual F_2 plants and their parents, using CTAB (Saghai -Maroof *et al*., 1984). RNA was removed from the DNA preparation by adding 10µl of RNAase (10mg /ml) and, then, incubating for 30 min. at 37ºC. DNA sample concentration was quantified by using a spectrophotometer (Beckman Du-65).

PCR amplification:

Thirty-five primers (Table 2) were used in the present investigation to amplify the templated DNA. Each amplification reaction was performed in a 25-µl vol., containing 50 ng of genomic DNA, 1x PCR buffer Mg Cl_2 (60 mM KCl, 10mM Tris- HCl (pH 9.0), 2mM $MgCl_2$ and 1% Triton x-100), 200 mM each of dATP, dCTP, dGTP and dTTP (promega), 50 pM primer, 50ng template DNA and 1.5 µ of Taq DNA polymerase. Amplifications were carried out in an MJ Research PTC-100 thermal cycler with amplification conditions, adopted from Williams *et al*. (1990), DNA denaturation at 94ºC for three minutes and 45 cycles of melting at 94ºC for one min., annealing at 36ºC for one min. and extending at 72ºC for two min. This was followed by a seven min. final extension step at 72ºC, then, the reactions were kept at 10ºC. RAPD fragments were size- fractionated in a 2% agarose gel in 0.5 x TBE buffer, with a 1-kb ladder molecular weight marker. Gels were stained in ethidium bromide solution and, then, photographed.

Bulked segregant analysis:

Bulked–segregant analysis (BSA) was used, in conjunction with RAPD analysis, (Michelmore *et al*., 1991) to find markers linked to genes of resistance to NCLB. Resistant and susceptible bulks were prepared from F_2

individuals by pooling aliquots, containing equivalent amounts of total DNA, approximately, 50 ng/µl from each of fourteen susceptible and fourteen resistant F_2 plants selected, based on phenotypic assessments. RAPD primers were, then, screened on the parents and the two bulk DNA samples, from which some primer combinations revealed bands that were polymorphic, not only between parental genotypes, but also between the pair of the bulk DNA. Based on the evaluations of DNA bulks, individual F_2 plants were analyzed with cosegregating primers to confirm RAPD marker linkage to the NCLB resistance genes.

Data Analysis:

Goodness of fit to a 3:1 ratios was calculated for RAPD marker by Chi-square test. The association between RAPD markers and resistance to NCLB trait was assessed with a simple regression analysis, using PROC REG in SAS version 9.1 software packages (SAS Institute, Cary, NC,2007). Magnitude of the marker associated phenotypic effect was described by the coefficient of determination, R^2, which represented the fraction of variance explained by the polymorphism of the marker.

RESULTS AND DISCUSSION

Genetic analysis:

The qualitative analysis of the resistance to NCLB (Table 1) was carried out, according to the response of the tested inbred lines, F_1 and F_2 populations inoculated with *Helminthosporium turcicum* pathogen at the adult stage, under field conditions, using the single spore culture isolate, T- 13AS .

The frequency distributions of NCLB severity for the F_1, F_2 and their two respective inbred lines were classified and presented in Table (1). The results revealed that the inbred line ,L1021, was the most resistant to NCLB and exhibited low disease severity, which ranged from less than 5% to 25%. On the other hand, the inbred line ,L1002, expressed high susceptibility to NCLB, with a disease severity ranged from 26% to more than 75%.

The disease severity of F_1 plants ranged from less than 5% to 50%.This result indicated that low disease severity was partially dominant over high disease severity (Table 1).

The F_2 disease severity frequency distribution of the F_2 population ranged from less than 5% to more than 75% (Table 1). Furthermore, number of plants with low: high NCLB severity was 98: 41. This observed

ratio fitted the theoretical expected ratio, 3:1, and suggested that a single locus controlled this trait (Table 1) .

To study the genetic behavior of maize partial resistance to NCLB quantitative scale, the two inbred lines, F_1 and F_2 populations were tested in the adult stage under field conditions. Population means and variances of the inbred lines, F_1 and F_2 were used to estimate the degrees of dominance for F_1 (h_1) and F_2 (h_2), heritability in broad-sense and the number of functioning genes. The F_1 mean value in the cross was 16 %, was significantly lower than its respective mid-parent value, indicating the presence of complete dominance for low disease severity (partial resistance) .The F_2 mean was 31.1 %. Such mean was significantly different from its expected value based on P_1, P_2 and F_1 , revealing the presence of epistasis (Table 1). The obtained result, also, revealed that the estimated values of degree of dominance for the F_1(h_1) and for the F_2(h_2) were -0.93 and -0.3, respectively. The significant negative values of h_1 and h_2 suggested that the average degree of dominance close to complete dominance in the F_1 and supported the F_1 result. The heritability value for the tested cross was 68.07% indicating that the effect of environment on the expression of this trait was small in respect to genetic effect. The deviation of h^2 from 1.0 might be due to the presence of epistasis ,while applying the Wright 's formula to detect the number of genes controlling resistance was small and amounted to 0.52, indicating that there was a difference in resistance genes between the two inbred lines. Several assumptions for the validity of this equation were not fulfilled therefore it is anticipated the No. of genes obtained would be less than expected number.

Resistance to *E. turcicum* is governed in both a race-specific (qualitative) and a race-nonspecific (quantitative) manner (Raymundo and Hooker, 1982; Welz and Geiger, 2000). Presently, five dominant genes were reported to, individually, condition resistance in maize to *E. turcicum* (Leonard, 1993). These genes were designated as Ht1, Ht2, Ht3, Htm1 and Htn1, and were determined from one another by the differential reaction. They sustained to the known physiological races of the pathogen. Race zero was avirulent against lines with any of the Ht genes. Race 1 was virulent against lines with Ht1 gene. Various other combinations of virulence, also, occurred (Leonard, 1993). Maize lines, carrying these genes, were known to express a qualitative form of resistance, characterized by lesions that were chlorotic, rather than necrotic, when challenged by an avirulent race (Raymundo and Hooker, 1982). In contrast to other forms of resistance genes, Htn1 was reported to act quantitatively

by delaying the onset of lesion development until adult plant stages, usually after flowering (Gevers, 1975).

RAPD markers analysis:

Three factors are required for the effective implementation of molecular markers in maize breeding programs; namely, (1) The availability of "user friendly" markers (cheap, easy and reliable), (2) The validation of markers across different genetic backgrounds and (3) The possibility of implementing them within a breeding program (Langridge and Chalmers, 1998). Attempts to generate such markers for maize are neither always successful nor easily achieved. However, when they are found, they represent very robust marker.

Thirty-five primers of arbitrary nucleotide sequence (Table 2) were used to screen the polymorphism between the highly susceptible inbred line, L1002, and the highly resistant inbred line, L1021, to NCLB. Out of 35 primers, twenty RAPD primers (57.14 %), that gave polymorphic bands suitable to differentiate between the two parents. The total 110 bands were amplified ,using 35 RAPD primers, produced an average of three bands per primer. The number of RAPD fragments, that were amplified, ranged from three to eight and the sizes ranged from about 140 to 1400 bp. Of these twenty RAPD primers, OPC04 primer, which produced a single and strong polymorphic band at 420 bp, that was present in only the resistant parent (L1021) , but absent in the susceptible parent (L1002) (Fig. 1) was selected for screening DNA bulks and their parental DNA . On the other hand, the same primer (OPC04) produced a single and strong polymorphic band at 220 bp, that was present in only L1002 the susceptible parent, but absent in L1021 the resistant parent (Fig. 1).The primer ,OPC04 (5'CCGCATCTAC 3'), generated the polymorphic fragment at 420 bp, which was present only in NCLB – resistant bulks and L1021 parent and was missing in NCLB – susceptible bulks and L1002 parent . On the other hand , the primer ,OPC04, generated the polymorphic fragment at 220 bp, which was present only in NCLB-susceptible bulk and L1002 parent and was missing in NCLB-resistant bulk and L1021 parent (Fig. 1). These RAPD markers ($OPC04_{420\ bp}$ and $OPC04_{220\ bp}$) were regarded as candidate markers linked to NCLB resistance gene in maize .

These polymorphic markers, $OPC04_{420\ bp}$ and $OPC04_{220\ bp}$, were further used to check their linkage to the NCLB resistance gene, using a segregating F_2 population, derived from the cross between the resistant parent L1021 and the susceptible parent ,L1002. When analyzing the individual plants of F_2 population, the $OPC04_{420\ bp}$ and $OPC04_{220\ bp}$

fragments were amplified in the DNA obtained only from F_2 resistant plants and F_2 susceptible ones , respectively. The PCR amplification of resistant parent (L1021), susceptible parent (L1002), resistant bulk, susceptible bulk , five F_2 resistant and five F_2 susceptible individuals, using primer, OPC04 are shown in Fig. 2.

For the RAPD marker OPC04 $_{420\ bp}$, 96 of 139 (69.1 %) individuals, in the F_2 population , exhibited the amplified polymorphic fragments (420 bp) , while , the remaining did not (Fig. 2) . The ratio fitted the expected Mendalian ratio, 3:1 ($\chi^2 = 2.6$, $P < 0.1$). In the RAPD marker OPC04 $_{220\ bp}$, 37 of 139 (26.6 %) individuals, in the population, exhibited the amplified polymorphic fragment (220 bp), while, the remaining did not (Fig. 2). The ratio fitted the expected Mendalian ratio, 3:1 ($\chi^2 = 0.06$, $P<0.75$).

To check for potential co-segregation of DNA fragments and NCLB resistant phenotypes, simple regression analysis was carried out in order to confirm an association between the $OPC04_{420}$ markers and the $OPC04_{220}$ markers resistance to NCLB in all 139 F_2 progenies. The results showed that the regression analysis for the relationship between the two markers, $OPC04_{420}$ and $OPC04_{220}$, and the phenotypes of F_2 individuals were significant and they recorded $r^2 = 0.61$ and 0.74, respectively . This indicates that the two markers were linked with the NCLB resistant gene.

The PCR technique has proved to be a powerful tool for the identification of polymorphism in cereals.In, Barakat *et al*., (2008 A,B andC) detected several molecular markers linked to wheat rust resistance regions (Lr34/Yr18 and Lr46/Yr29).. Recently, identification of RAPD and SCAR markers, linked to NCLB in waxy maize, have been reported by Khampila *et al* (2008).They used bulked segregant analysis (BSA) to search for RAPD markers linked to NCLB resistance genes, using F_2 population. Four RAPD markers had been identified. These markers were converted into dominant SCAR markers.

The present study indicated that RAPD markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to NCLB resistance gene in maize. Once these markers are identified, they can be used to detect the QTLs linked to NCLB resistance in breeding programs, as a selection tool in early generations.

Table 1: Leaf blight severity classes, Chi - square analysis of F_2 population generated from the cross, L1021 X L1002, degree of dominance, heritability in its broad sense (%) and number of genes.

Genotype	No. of tested plants	Leaf blight severity						Means ($\bar{x}$)	Variance (S^2)	Phenotypes	Expected ratio	X^2	Degree of dominance		Heritability	No. of genes
		HR	R	MR	MS	S	HS			R : S	R : S		h_1	h_2		
L1021	60	20	25	15				14.5[c]	166.9							
L1002	60				10	30	20	53.5[a]	171.7				-0.93	-0.3	68.07	0.52
F_1	50	10	25	10	5			16.0[c]	226.0							
F_2	139	12	52	34	3	15	23	31.1[b]	588.9	98 : 41	3 : 1	1.68[ns]				

Means followed by the same letter are not significant according to t-Test at 0.05 level.

Table 1: Number of amplifications and polymorphic products of thirty- five primers used to screen the two inbred lines (L1021 and L1002).

Primer	Nucleotide sequence (5' ⟶ 3')	No. of amplification products	No. of polymorphic products
Pr_1	CAGGCCCTTC	0	0
Pr_2	TGCCGAGCTG	0	0
Pr_3	AGTCAGCCAC	8	3
Pr_4	AATCGGGCTG	4	1
Pr_5	AGGGGTCTTG	6	0
Pr_6	GGTCCCTGAC	0	0
Pr_7	GAAACGGGTG	4	1
Pr_8	GTGACGTAGG	0	0
Pr_9	GGGTAACGCC	0	0
Pr_{10}	GTGATCGCAG	0	0
Pr_{11}	CAATCGCCGT	6	1
Pr_{12}	TCGGCGATAG	4	3
Pr_{13}	CAGCACCCAC	4	1
Pr_{14}	TCTGTGCTGG	3	3
Pr_{15}	TTCCGAACCC	5	2
Pr_{16}	AGCCAGCGAA	5	0
Pr_{17}	GACCGCTTGT	3	1
Pr_{18}	AGGTGACCGT	0	0
Pr_{19}	CAAACGTCGG	4	0
Pr_{20}	GTTGCGATCC	4	2
UBC321	ATCTAGGGAC	0	0
UBC475	CCAGCGTATT	4	3
UBC532	TTGAGACAGC	5	1
OPA02	TGCCGAGCTG	4	4
OPA06	GGTCCCTGAC	0	0
OPA07	GAAACGGGTG	4	1
OPB8	GTCCACACGG	7	1
OPB9	TGGGGGACTC	4	1
OPB13	TTCCCCCGCT	5	3
OPC04	CCGCATCTAC	6	4
OPC15	GACGGATCAG	8	7
OPE20	AACGGTGACC	0	0
OPF15	CCAGTACTCC	0	0
OPJ 04	CCGAACACGG	0	0
OPJ10	AAGCCCGAGG	5	5

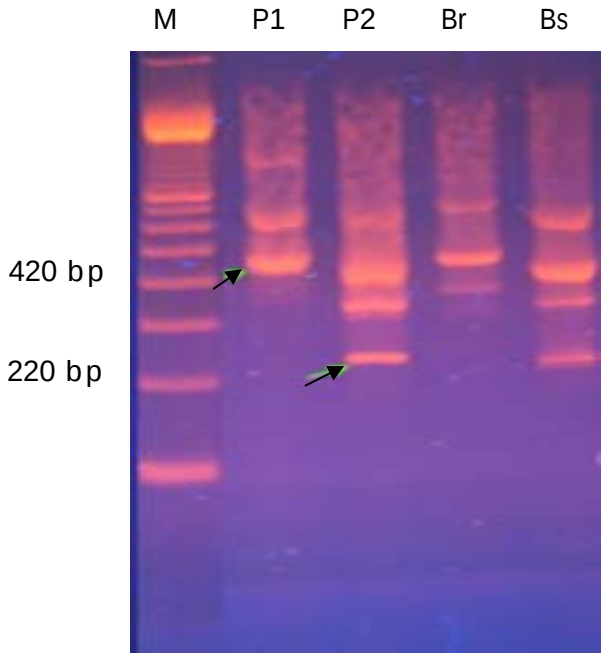

Figure 1 .RAPD fragments, produced by primer OPC04 , RAPD fragments produced by primer OPC04, M: Molecular weight, followed by PI and P2 parents, L1021 and L1002 ,respectively. Br, bulk resistance; Bs, bulk susceptible.

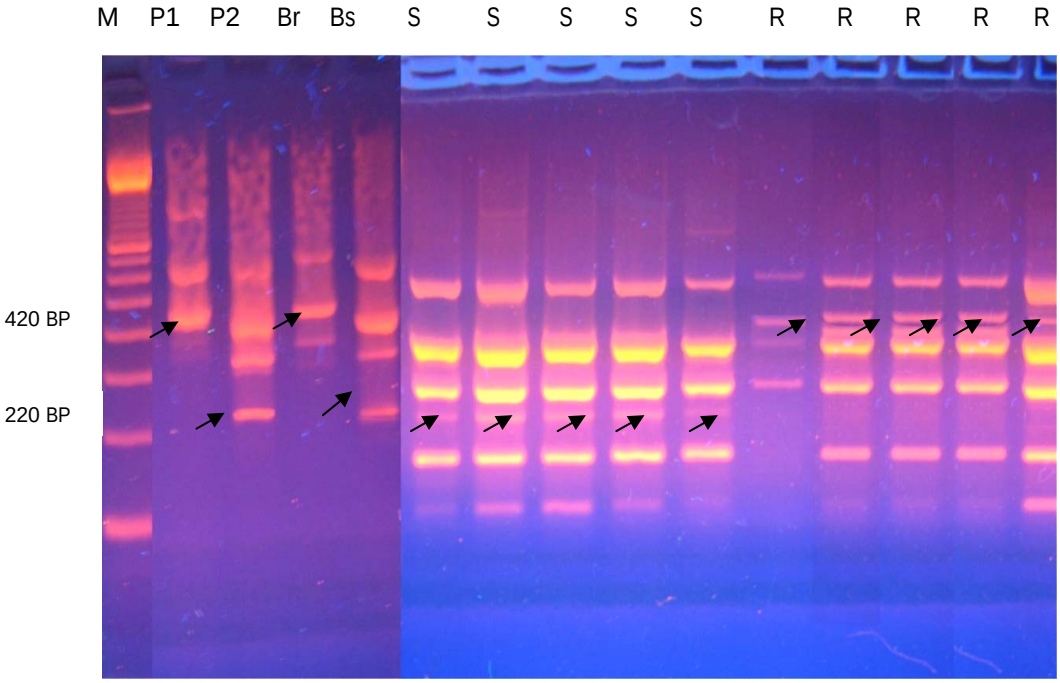

Figure 2. RAPD fragments, produced by primer OPC04 , RAPD fragments produced by primer OPC04, M: Molecular weight, followed by PI and P2 parents, L1021 and L1002 ,respectively. Br, bulk resistance; Bs, bulk susceptible, F_2 individuals in the cross, L1021 X L1002 (R : resistant; S: susceptible).

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الملخص العربي

دراسات وراثية و دلائل ال RAPD المرتبطة بالمقاومة لمرض اللفحة الشمالية لأوراق الذرة في عشيرة ذرة صفراء

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استخدمت دراسات وراثية لتقدير الطبيعة الوراثية لمقاومة مرض الفحة الشمالية للأوراق في عشيرة ذرة الصفراء و ذلك باستخدام F_1 و عشيرة انعزالية من F_2 الناتج من التهجين بين السلالة النقية المقاومة (L1021) مع السلالة النقية المصابة (L1002) و الآباء . استخدم تحليل الBSA للبحث عن دلائل RAPD المرتبطة بجينات المقاومة لمرض الفحة الشمالية للأوراق و ذلك باستخدام العشيرة الانعزالية لل F_2 لنفس الهجين مع الآباء.

أوضحت الدراسات الوراثية أن السلالة النقية "L1021" أظهرت نسبة إصابة منخفضة (أقل من 5 % إلى 25 %) و السلالة النقية "L1002" أظهرت نسبة عالية (26 % إلى أكبر من 75 %) . تراوحت نسبة الإصابة في الجيل الأول أقل من 5 % إلى أكبر من 50 % هذا يعني أن الإصابة المنخفضة كانت سائدة جزئيا على الإصابة المرتفعة . تراوحت نسبة الإصابة في ال F_2 ما بين أقل من 5 % إلى أكبر من 75 % .وباستخدام تحليل مربع كاي و جد أن النسبة المشاهدة للأفراد السليمة : المصابة تنطبق على النسبة المندلية المتوقعة 1:3 و هذا يعني أن زوج واحد من العوامل الوراثية يتحكم في صفة المقاومة في هذا الهجين.و أعطت درجة السيادة في ال F_1 و ال F_2 قيماً معنوية (-0,93 و -0,30،على الترتيب).و كانت درجة التوريث لهذا الهجين 68,07% .

وباستخدام خمسة وثلاثين بادئاً لتحديد دلائل ال RAPD قد تم تحديد دليلين و هما: $OPC04_{420}$ و مرتبط بالمقاومة المظهرية و $OPC04_{220}$ و مرتبط بالإصابة المظهرية و بالنسبة للدليلين فإن النسبة المشاهدة انطبقت على النسبة المتوقعة 1:3 و ذلك باستخدام اختبار مربع كاي. و اثبت اختبار الارتداد أن العلاقة بين الدليلين و الشكل المظهري لأفراد ال F_2 كانت قوية و سجلا قيما لل R^2= 0,61 و 0,74 ، على الترتيب .